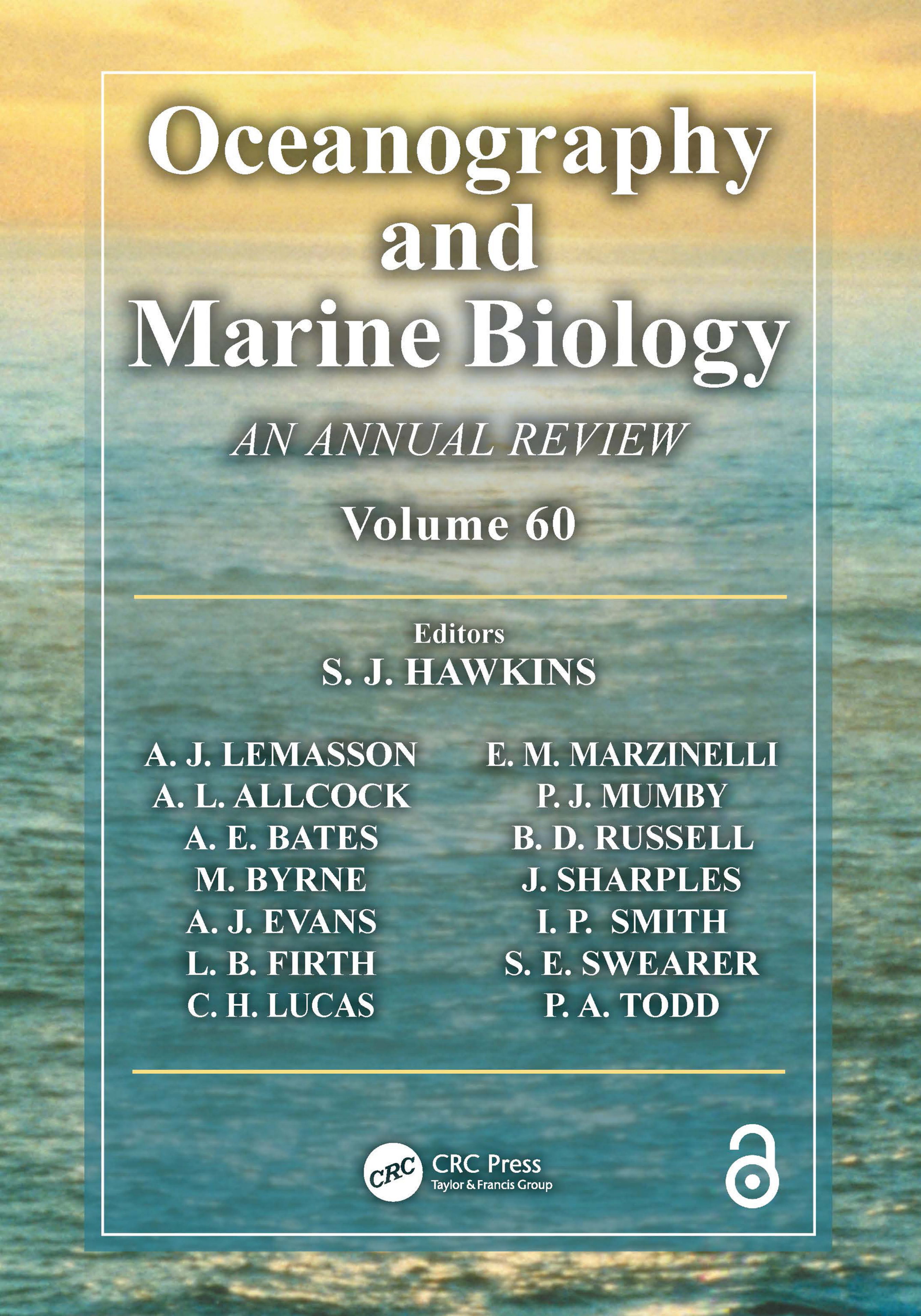

Oceanography and Marine Biology

AN ANNUAL REVIEW

Volume 60

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Oceanography and Marine Biology

Oceanography and Marine Biology: *An Annual Review* remains one of the most cited sources in marine science and oceanography. The ever-increasing interest in work in oceanography and marine biology and its relevance to global environmental issues, especially global climate change and its impacts, creates a demand for authoritative refereed reviews summarizing and synthesizing the results of both historical and recent research.

This volume celebrates 60 years of OMBAR, over which time it has been an essential reference for research workers and students in all fields of marine science.

The peer-reviewed contributions in Volume 60 are available to read Open Access via https://www.taylorfrancis.com/books/9781003288602 and on OAPEN. If you are interested in submitting a review for consideration for publication in OMBAR, please email the Editor-in-Chief, Stephen Hawkins, at S.J.Hawkins@soton.ac.uk for volume 61, and for volume 62 onwards, please email the new co-Editors in Chief, Dr Peter Todd (dbspat@nus.edu.sg) and Dr Bayden Russell (brussell@hku.hk).

Volume 60 features an editorial on the UN Decade of Ocean Science and goes on to consider such diverse topics as Cenozoic tropical marine biodiversity, blue carbon ecosystems in Sri Lanka, marine litter and microplastics in the Western Indian Ocean, and the ecology and conservation status of the family Syngnathidae in southern and western Africa. This volume also contains a retrospective Prologue on the evolution of OMBAR and pays tribute to one of its early Editors-in-Chief, Margaret Barnes, by providing an update on her review in OMBAR of the stalked barnacle *Pollicipes*.

An international Editorial Board ensures global relevance and expert peer review, with editors from Australia, Canada, Hong Kong, Ireland, Singapore and the UK. The series volumes find a place in the libraries of not only marine laboratories and oceanographic institutes, but also universities worldwide.

Oceanography and Marine Biology

An Annual Review, Volume 60

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Contents

CONTENTS

Oceanography and Marine Biology: An Annual Review, 2022, **60**, 1-14

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SIXTY YEARS OF *OCEANOGRAPHY AND MARINE BIOLOGY*: *AN ANNUAL REVIEW* (OMBAR) – A BRIEF RETROSPECTIVE AND PROSPECTIVE

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OMBAR: From the beginning

The vision of Harold Barnes – the founding editor-in-chief of *Oceanography and Marine Biology: an Annual Review* (OMBAR) – 60 years ago was that the journal would produce timely reviews by experts in the field of marine biology and oceanography. New areas would be highlighted, and previous reviews revisited from time to time to provide updates. This ethos was strongly maintained by Margaret Barnes, who succeeded Harold as editor-in-chief after he passed away in 1978, with Margaret being involved up until 2002 (see appreciation by Robin Gibson in the epilogue of this volume). Participation was generally by invitation and occasionally by recommendation.

Both Harold and Margaret were fine scientists, prolifically publishing on barnacles, but also very aware of the wider marine science landscape. The PhD thesis of the current editor-in-chief (SJH), written in the 1970s, was 40% on barnacles, and SJH has read and cited many of the Barnes' papers over the years. A particular favourite is Barnes & Powell (1950) on what would become called 'intense density-dependent processes' in a more jargonized age. In parallel with OMBAR, Harold Barnes founded the *Journal of Experimental Marine Biology and Ecology* (JEMBE) in 1967 and its active editing by both Harold and subsequently Margaret (until 1999) contributed to keeping them abreast of the field, aiding identification of emerging fields and experts, and hence invitations to contribute reviews to OMBAR. They were also avid attenders of conferences, particularly the European Marine Biology Symposia series which they helped establish – a good talk on an exciting topic could result in an invitation to submit a review to OMBAR.

In the last decade of her stewardship of OMBAR, Margaret as lead editor involved various colleagues from the Scottish Marine Biological Association/Scottish Association for Marine Science Laboratory at Dunstaffnage in co-editorial roles from volume 26 onwards (Alan Ansell, Robin Gibson); Ansell, Gibson and Barnes edited volumes 31–37; Gibson and Barnes edited volumes

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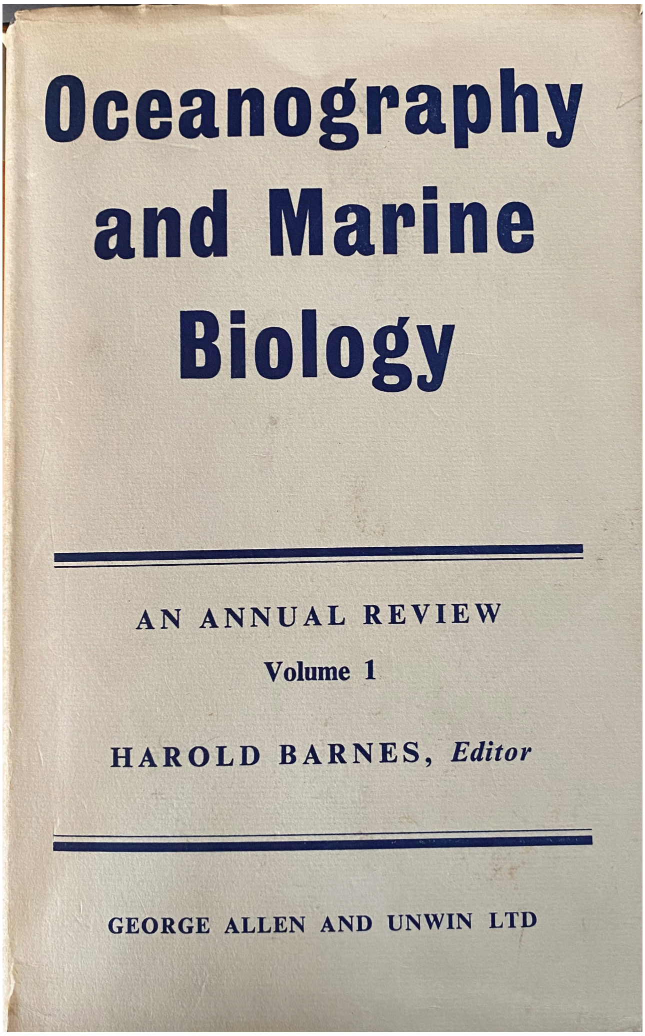
38–40 when Margaret eventually stepped down in 2002 after 40 years association with the journal. Robin Gibson was involved as an editor-in-chief along with colleagues from both Dunstaffnage (John Gordon, David Hughes) and Millport (Jim Atkinson involved from Vol. 39, Philip Smith) until volume 50 (Vol. 41: Gibson, Atkinson; Vol. 42–48: Gibson, Atkinson, Gordon; Vol. 49: Gibson, Atkinson, Gordon, Hughes, Smith). Roger Hughes joined the editorial team from volume 50 (Gibson, Atkinson, Gordon, D.J. Hughes, Smith, R.N. Hughes), taking over as editor-in-chief after volume 51 (Vol. 52 R.N. Hughes, D.J. Hughes, Smith; Vol. 53–54 R.N. Hughes, D.J. Hughes, Smith, Dale), in turn handing over to SJH in 2016 (Vol. 55), just before sadly passing away.

SJH has tried to establish a larger and more diverse international editorial board, including some mid- and early-career scientists, to facilitate peer review of all manuscripts. This has fluctuated with work commitments of the team (Vol. 55: Hawkins, Dale, Evans, Firth, Hughes, Smith; Vol. 56: Hawkins, Dale, Evans, Firth, Smith; Vol. 57: Hawkins, Allcock, Bates, Firth, Smith, Swearer, Todd; Vol. 58: Hawkins, Allcock, Bates, Evans, Firth, McQuaid, Russell, Smith, Swearer, Todd; Vol. 59: Hawkins, Lemasson, Allcock, Bates, Byrne, Evans, Firth, Marzinelli, Russell, Sharples, Smith, Swearer, Todd; and finally this volume 60: Hawkins, Lemasson, Allcock, Bates, Byrne, Evans, Firth, Lucas, Marzinelli, Mumby, Russell, Smith, Swearer, Todd). Philip Smith is its longest serving member. Despite expanding to increase diversity, the editorial board retains a strong British, Irish and Commonwealth base.

The success of OMBAR stems very much from Harold's initiative, taken forward by Margaret's scholarship, wide knowledge, and supportive role as editor over many years. Cruz et al. (2022 – this volume) revisit Margaret Barnes' classic 1996 review in OMBAR of the stalked barnacle *Pollicipes* as a tribute to her research and scholarship.

The inaugural volume of OMBAR published in 1963 reads like an international Who's Who of marine science at the time (Figure 1). There was a very strong input from the physical, chemical and biological oceanographers as well as marine biologists covering topics of very broad scope: Tides (by Rossiter), The Geology of some Continental Shelves (by Stride), Optical Oceanography (by Jerlov); Underwater Television (by Harold Barnes himself), Chemical Oceanography (by Hood), Primary Production (by Yentsch), The Rhodophyta (by Dixon), Heterotrophic Micro-organisms (by Wood), Microdistribution of Plankton (by Cassie); Ecology and Functional Morphology of Molluscs (by Allen), Parasitic Copepods (by Boquet and Stock – the only review not by a single author), The Effects of Temperature and Salinity on Marine and Brackish Water Animals; Part 1 Temperature (by Kinne), The Biogeography and Intertidal Ecology of Australian Coasts (by Knox), Pogonophora (by Eve Southward), and finally Lisitzin contributed reviews on both Mean Sea Level and The Hydrography of European Arctic and Sub-arctic Seas. To save space and cram in more articles, the titles of cited references were omitted in earlier volumes, but they were still invaluable for keeping abreast of the literature (see Figure 1 – photographed in Eve Southward's office in 2021, who was still publishing on Pogonophora at 91 years of age).

It is interesting to reflect on the diversity of topics reviewed in OMBAR publications, and on what has been highly cited. A look back at the 20 most cited reviews published in OMBAR is given in Table 1. These cover a broad range of subjects from authors all over the world. Some older reviews may pre-date bibliometric information retrieval and not feature in this list. Interestingly and coincidentally, members of the current (and recently expanded) editorial board feature in this list (Hawkins & Hartnoll 1983, Byrne 2011). The current board also has several members who have contributed to OMBAR in the past and/or in this volume. Looking back over the top-cited papers over the last 10-year period (2009–2019, Table 2), the current board (Vol. 60) features again with Firth et al. (2016), Todd via Neo et al. (2017), Byrne again in addition to Byrne (2011) via Purcell et al. (2016). It is clear from Table 2 that a diversity of topics are covered in OMBAR reviews, including historical ecology (Lotze 2010). It is gratifying that both applied and pure research topics feature in these highly cited papers.



Oceanography
and Marine
Biology

AN ANNUAL REVIEW
Volume 1

HAROLD BARNES, *Editor*

GEORGE ALLEN AND UNWIN LTD

Figure 1 The first volume of OMBAR photographed in 2021 in the office of one of the original review authors. (Eve Southward of the Marine Biological Association of the UK.)

In the early days, OMBAR was published by Allen and Unwin followed by Aberdeen University Press, and then later by University College London. Since 1999 (Vol. 37), it has been published by various parts of Taylor and Francis, who also republished older volumes under their imprints. Physical sales of OMBAR peaked at the turn of the century, each hardcover volume selling 500–700 copies throughout the 2000s. Today, OMBAR has an excellent Impact Factor of over 6 (based on publications up to volume 58, published in 2020), with an outstanding h-index of 49 (Figure 2). Its 2020 CiteScore was 7.2 with Scopus, ranking OMBAR 9/224 journals in the field of Aquatic Science, and 7/128 in the field of Oceanography (in the 96th and 94th percentile, respectively). Figure 1 is based on data from Clarivate as this gives a complete record for the last 20 years, since Scopus has missed some years. The impact factor has fluctuated considerably in the last decade, reflecting the limited number of articles each year, but in most years it is over 4 with occasional higher peaks depending on its content that year (11 in 2014). Interestingly, Clarivate gives it an impact factor of 8

Table 1 Twenty most cited *Oceanography and Marine Biology: An Annual Review* (OMBAR) reviews of all time as shown by Scopus in early 2022

Reference	Number of citations
Diaz, R.J. & Rosenberg, R. 1995. Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. OMBAR, **33**, 245–03.	1,590
Heip, C.H.R., Vincx, M. & Vranken, G., 1985. The ecology of marine nematodes. OMBAR, **23**, 399–489.	906
Pawlik, J.R. 1992. Chemical ecology of the settlement of benthic marine invertebrates. OMBAR, **30**, 273–335.	735
Snelgrove, P.V.R. & Butman, C.A., 1994. Animal sediment relationships revisited: Cause versus effect. OMBAR, **32**, 111–177.	722
Airoldi, L. & Beck, M.W., 2007. Loss, status and trends for coastal marine habitats of Europe. OMBAR, **45**, 345–405.	696
Decho, A. 1990. Microbial exopolymer secretions in ocean environments: Their role (s) in food webs and marine processes. OMBAR, **28**, 73–153.	680
Carlton, J.T. 1985. Transoceanic and interoceanic dispersal of coastal marine organisms: the biology of ballast. OMBAR, **23**, 313–371.	635
Butman, C.A. 1987. Larval settlement of soft-sediment invertebrates: the spatial scales of pattern explained by active habitat selection and the emerging role of hydrodynamical processes. OMBAR, **25**, 113–165.	625
Shannon, L.V. 1985. The Benguela ecosystem, Evolution of the Benguela physical features and processes. OMBAR, **23**, 105–182.	579
Levin, L.A. 2003. Oxygen minimum zone benthos: adaptation and community response to hypoxia. OMBAR, **41**, 9–9.	558
Hicks, G.R. 1983. The ecology of marine meiobenthic harpacticoid copepods. OMBAR, **21**, 67–175.	542
Hawkins, S.J & Hartnoll, R.G. 1983. Grazing of intertidal algae by marine invertebrates. OMBAR, **21**, 195–282.	528
Byrne, M. 2011. Impact of ocean warming and ocean acidification on marine invertebrate life history stages: Vulnerabilities and potential for persistence in a changing ocean. OMBAR, **49**, 1–42.	507
Ballesteros, E. 2006. Mediterranean coralligenous assemblages: a synthesis of present knowledge. OMBAR, **44**, 123–195.	487
Elsdon, T.S., Wells, B.K., Campana, S.E., Gillanders, B.M., Jones, C.M., Limburg, K.E., Secor, D.H., Thorrold, S.R. & Walther, B.D., 2008. Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations and inferences. OMBAR, **46**, 297–330.	460
Thiel, M., Castilla, J.C., Fernández Bergia, M.E. & Navarrete, S. 2007. The Humboldt current system of northern and central Chile. OMBAR, **45**, 195–344.	449
Levin, L.A. 2005. Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes. OMBAR, **43**, 11–56.	445
Smith, C.R. & Baco, A.R. 2003. Ecology of whale falls at the deep-sea floor. OMBAR, **41**, 311–354.	439
Doherty, P.J. & Williams, D.M. 1988. The replenishment of coral reef fish populations. OMBAR, **26,** 487–551.	439
Andrew, N.L. & Mapstone, B.D. 1987. Sampling and the description of spatial pattern in marine ecology. OMBAR, **25**, 39–90.	434

NB early contributions may be missed by bibliometric searches.

Table 2 Twenty most cited OMBAR reviews for the period 2009–2019 (Scopus, early 2022)

Reference	Number of citations
Byrne, M. 2011. Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential for persistence in a changing ocean. OMBAR, **49**, 1–42.	507
Stella, J.S., Pratchett, M.S., Hutchings, P.A. & Jones, G.P. 2011. Coral-associated invertebrates: diversity, ecology importance and vulnerability to disturbance. OMBAR, **49**, 43–104.	209
Firth, L.B., Knights, A.M., Bridger, D., Evans, A., Mieskowska, N., Moore, P.J., O'Connor, N.E., Sheehan, E.V., Thompson, R.C. & Hawkins, S.J. 2016. Ocean sprawl: challenges and opportunities for biodiversity management in a changing world. OMBAR, **54**, 193–269.	171
Pratchett, M.S., Caballes, C.F., Rivera-Posada, J.A. & Sweatman, H. 2014. Limits to understanding and managing outbreaks of crown-of-thorns starfish (*Acanthaster* spp). OMBAR, **52**, 133–200.	152
Mineur, F., Cook, E.J., Minchin, D., Bohn, K., Macleod, A. & Maggs, C.A., 2012. Changing coasts: marine aliens and artificial structures. OMBAR, **50**, 189–233.	140
Pratchett, M.S., Anderson, K.D., Hoogenboom, M.O., Widman, E., Baird, A.H., Pandolfi, J.M., Edmunds, P.J. & Lough, J.M. 2015. Spatial, temporal and taxonomic variation in coral growth—implications for the structure and function of coral reef ecosystems. OMBAR, **53**, 215–295.	138
Bonaldo, R.M., Hoey, A.S. & Bellwood, D.R. 2014. The ecosystem roles of parrotfishes on tropical reefs. OMBAR, **52**, 81–132.	112
Morrisey, D.J., Swales, A., Dittmann, S., Morrison, M.A., Lovelock, C.E. & Beard, C.M. 2010. The ecology and management of temperate mangroves. OMBAR, **48**, 43–160.	107
Thiel, M., Penna-Díaz, M.A., Luna-Jorquera, G., Salas, S., Sellanes, J. & Stotz, W. 2014. Citizen Scientists and Marine Research: Volunteer participants, their contributions, and projection for the future. OMBAR, **52**, 257–314.	106
Purcell, S.W., Conand, C., Uthicke, S. & Byrne, M. 2016. Ecological roles of exploited sea cucumbers. OMBAR, **54**, 375–394.	103
Tsounis, G., Rossi, S., Grigg, R., Santangelo, G., Bramanti, L. & Gili, J.M. 2010. The exploitation and conservation of precious corals. OMBAR, **48**, 161.	96
McMahon, K.W., Hamady, L.L. & Thorrold, S.R. 2013. Ocean ecogeochemistry: a review. OMBAR, **51**, 327–374.	76
Pillay, D. & Branch, G.M. 2011. Bioengineering effects of burrowing thalassinidean shrimps on marine soft-bottom ecosystems. OMBAR, **49**, 137–192.	69
Lotze, H.K., 2010. Historical reconstruction of human-induced changes in US estuaries. OMBAR, **48**, 267–338.	62
Graham, N.A., Ainsworth, T.D., Baird, A.H., Ban, N.C., Bay, L.K., Cinner, J.E., De Freitas, D.M., Diaz-Pulido, G., Dornelas, M., Dunn, S.R. & Fidelman, P.I. 2011. From microbes to people: tractable benefits of no-take areas for coral reefs. OMBAR, **49**, 105–136.	61
Peck, L. 2018. Antarctic marine biodiversity: adaptations, environments and responses to change. OMBAR, **56**, 105–236.	60
Neo, M., Wabnitz, C., Braley, R., Heslinga, G., Fauvelot, C., van Wynsberge, S., Andréfouët, S., Waters, C., Tan, S., Gomez, E. & Costello, M. 2017. Giant clams (Bivalvia: Cardiidae: Tridacninae): a comprehensive update of species and their distribution, current threats and conservation status. OMBAR, **55**, 87–387.	60
Lucrezi, S. & Schlacher, T.A. 2014. The ccology of ghost crabs. OMBAR, **52**, 201–256.	60
Bright, M. & Lallier, F.H. 2010. The biology of vestimentiferan tubeworms. OMBAR, **48**, 213–266.	60
Benjamins, S., Dale, A.C., Hastie, G., Waggitt, J.J., Lea, M.A., Scott, B. & Wilson, B. 2015. Confusion reigns? A review of marine megafauna interactions with tidal-stream environments. OMBAR, **53**, 1–54.	58

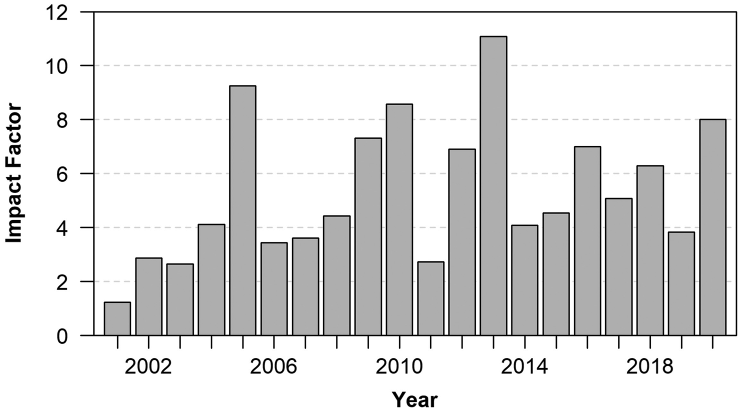


Figure 2 *Oceanography and Marine Biology: An Annual Review*: Clarivate's impact factor.

in 2020. Impact factors paint only a partial picture of the value of OMBAR, with reviews continuing to be read and cited for many years with long citation half-lives (OMBAR >10 years).

OMBAR: an evolution through time

Publishing and retrieval of information has changed greatly in the 60 years of *Oceanography and Marine Biology: An Annual Review* (OMBAR), and especially so in the 45 plus years that the current editor-in-chief (SJH) has been researching marine biology.

When SJH started as a research student in 1976, keeping up with the literature involved visiting the library at the Marine Biological Station in Port Erin, UK, on Friday afternoons (except when doing fieldwork), scanning any new journal issues arriving on the shelves that week, writing down the publication on a reference card, reading it and sometimes making a few notes on the back of the card. The references cited in the back of relevant papers were also looked at and more cards filled out, and if not in the Port Erin library nor available via the main library at the University of Liverpool, requests were made from inter-library loans via the British Library. These would arrive two to three weeks later as photocopies. It was too expensive and time-consuming to photocopy any articles of interest on the shelves as each page had to be individually copied with two pages costing around the same as a half pint of beer at the time (10–12 pence then, >£1.75 now) and was strongly discouraged.

Getting to grips with the wider literature and concepts as a new graduate student in the 1960s and 1970s involved first reading more advanced textbooks, then specialist books and research monographs, edited volumes with chapters by invited experts, and then proceeding to reading review articles by experts in the field, as well as in parallel reading of individual journal articles. Reviews were extremely important as they attempted to cover the literature comprehensively as well as synthesising it. If they were good reviews, new directions and research gaps would be identified. Most importantly, the references cited provided an integration of the field up to about one year or so before publication. The gold standards in marine biology at the time were *Advances in Marine Biology* and its rival *Oceanography and Marine Biology: An Annual Review*. In the wider field of ecology and evolution *Annual Review of Ecology, Evolution and Systematics* was excellent, with *Biological Reviews* providing a broader view of biological sciences. Trends journals were yet to emerge and proliferate. When SJH wrote his PhD thesis and subsequent papers from it, several reviews were highly influential on his thinking (Southward 1958 in *Biological Reviews*; Southward 1964 in special issue on grazing from a British Ecological Society Symposium; Connell 1972 in *AREES*; Underwood 1979 in *Advances in Marine Biology*) including two in *OMBAR* (Lewis 1976 and Branch 1981), plus of course Lewis (1964) – the most comprehensive book on British and Irish rocky shores. Several early seminal papers on experimental ecology on rocky shores published in *Ecological Monographs* (Dayton 1971, Menge 1976, Lubchenco & Menge 1978) also had comprehensive introductions which greatly helped. Fortunately, the volume of literature in SJH's field of rocky shore ecology was not huge historically, making it possible to keep on top of new publications and to actually read nearly all of them from end to end. Thus, greater scholarship was possible, but often with a lag phase of a year or two given the time from submission to print publication.

SJH's first paid job in December 1979 was as a Research Fellow at the Marine Biological Association of the UK (MBA) in Plymouth. The National Marine Biological Library (NMBL), located at Citadel Hill within the MBA building, was well funded by the Natural Environment Research Council and represented at the time (and still to this day) a huge national and international resource, which is also much valued by the members of the Association. The NMBL was an abstracting hub for Aquatic Science and Fisheries Abstracts (ASFA). In the 1970s, a literature search involved using hardcopy abstracting volumes such as Zoological Record, Current Contents and ASFA. As a new scientist at the MBA, SJH was introduced to one of the 'information scientists' who ascertained his research interests, and every week a neat stack of reference cards magically appeared on SJH's office desk (no one locked offices in those days). SJH's visits to the library became much more focused, but probably missed out on more whimsical tangential reading on other topics that sometimes happened when browsing the contents page of journals.

SJH's active links with OMBAR (apart from perusing the volumes as a PhD student) started in 1981, when George Russell was approached by Margaret Barnes to write a review on grazing on seaweeds for OMBAR. George had helped SJH's algal identifications and advised on seaweed ecology during his PhD and was aware of his work on grazing, and thus suggested that SJH would be better placed to write such a review. Hence, SJH embarked on this review on grazing with his PhD supervisor, Richard Hartnoll, which is still cited (Hawkins & Hartnoll 1983, see Table 1). Margaret Barnes was an excellent editor, and SJH learnt much from the exceedingly detailed neatly handwritten edits of the submitted typescript. Adjectival nouns have been largely avoided since her feedback. Much of the grazing review was done at the NMBL during research visits to Plymouth while on vacations from the University of Manchester where he was then based. He also liaised with Alan Varley, a pioneering Head Librarian of the NMBL, in using a computer-based search of the literature using keywords – the output arriving on teleprinter paper 40 cm wide with holes down the side (also known as 'tractor paper'). This was state-of-the-art information retrieval at the time.

Throughout his career, OMBAR would remain a constant resource for SJH and his PhD students, especially when preparing lectures on subjects away from his immediate research interests or stumbling into new fields. Having retired from university administration at the beginning of 2016, SJH took on the editor-in-chief role at OMBAR from volume 55 at the request of Roger Hughes. SJH intends to step down in a phased manner over the next year or so, working with two new co-editors-in-chief from the existing board.

OMBAR: current and future contribution

Information technology and bibliometric approaches exploded in the 1980s and 1990s. Abstract journals initially started issuing searchable CDs and then swiftly moved online as the Internet took off. Journals moved to hybrid print and online publication, with online-only journals beginning to appear about 10–15 years ago. Nowadays, many traditional print journals have switched to online-only. Reprints have become obsolete as PDFs have emerged. The literature can now be searched using bespoke platforms such as Web of Science, Scopus, PubMed or Google Scholar. A quick Google search on a smartphone can deliver a literature search in a few moments that once required a trained information scientist and a mainframe computer occupying a dedicated large building. OMBAR publications are easily found on these platforms or using these search engines. To keep with the times, OMBAR has had to adapt. While the volumes remain printed, as per tradition, all volumes and their contents are listed online, and some of the publications are downloadable directly from the Internet (those that are Open Access – discussed further below).

So, is there still a place, and a need, for annual volumes of reviews 60 years on from the first publication of OMBAR? And, if so, how can OMBAR keep up with the ever-evolving field of scientific reviews?

There certainly is a need – and both the contributing authors' and the readers' benefit. In some academic settings, reviews have sadly become downplayed. The UK's Research Assessment Exercise and its successor the Research Excellence Framework certainly discourage reviews in both the physical and biological sciences, as they are not considered to be reports on individual pieces of new research. Reviews do still count if new ground or concepts are developed and meta-analytical and systematic review approaches are adopted – but it is a risk that scientists do not want to take when submitting their best outputs for assessment. Fortunately, synthetic and synoptic reviews are still valued in some research cultures where periodic assessments are made (e.g. Australia and New Zealand). Authors who write reviews generally benefit from the necessity to carefully read and digest the literature. Certainly, SJH spent the 20 years or so following his 1983 grazing review working on topics identified as gaps – including the role of biofilms, the importance of mucus, behaviour of grazers and the need for broad-scale geographic comparisons using replicated experiments.

The format and content of reviews have also changed with new literature searching and cataloguing technologies, and the ability to deposit appendix material in online supplementary files such as large tables, videos or interactive maps and figures. The intellectual rigour of reviews has also greatly improved, with the increasing demand for robust, reliable, transparent and repeatable reviews from the academic community. There has been a clear spread in recent years of Cochrane-style systematic reviews pioneered in medicine and infused into other academic disciplines, such as environmental science and biodiversity conservation. Statistical syntheses through meta-analyses are now routinely used to complement systematic reviews as well as other more traditional reviews – although their quality can vary and there is a general need for consistency in methodology when it comes to systematic syntheses (Haddaway & Macura 2018, Haddaway et al. 2020, Christie et al. 2021). Re-analysis and reuse of freely available data are now commonplace. Good systematic work will also allow the identification of research clusters and research gaps, which can then inspire researchers to write further reviews and/or undertake new research. Bibliometric techniques can also be used to trace the development of a field. Workshops are convened to explore particular topics and often lead to reports that can be subsequently condensed into agenda-setting reviews (Wolfe et al. 2020 in Vol. 58).

Postgraduate student training has become more formalised, and in many countries, a formal literature review has become a key part of the process. Some recent reviews in OMBAR fall into that category (Chen et al. 2021 in Vol. 59; Martinez et al. 2017 in Vol. 55; and Veenhof et al. 2022 and Leeuwis & Gamperl 2022 – both in this volume), but when approached by younger authors, the editorial team usually advise involvement of a senior colleague with a broad view of the field. Recently, retired scientists often with no or limited access to funding or laboratories, but usually with a good amount of free time on their hands, often write valuable reviews drawing on years of expertise. When approached by such prospective authors, the editorial team often advise involving a younger colleague to ensure an up-to-date approach. Such reviews can also give a strong feel for the historical foundations of a particular subject area. In this regard, OMBAR has recently been pleased to publish reviews on seminal scientific activities such as the Great Barrier Reef expedition of the 1920s (Spencer et al. 2021 in Vol. 59). Most reviews these days have more than a sole author, but there have been some recent excellent exceptions published in OMBAR (Peck 2018 (see Table 2), McQuaid 2018, both in Vol. 56). There are also some where authorship is much greater and diverse, reflecting a multidisciplinary work stemming from a workshop (Morris et al. 2019).

The days when Harold or Margaret Barnes could single-handedly edit the volume and give scientific criticism akin to refereeing are gone. Now, the editor-in-chief reads a penultimate draft of the review before final submission, which is then refereed usually by two external referees plus expert input from one of our team of associate editors. We hope these steps have raised the quality of the published reviews, by constructive formative criticism and advice.

From the publishers' (Taylor and Francis) perspective, OMBAR forms an important part of CRC Press marine science portfolio by enabling in-depth annual reviews. As mentioned above, more recently, it has been possible to publish a mix of traditional subscription-only papers and Open Access articles in each volume, allowing authors yet another level of flexibility. Take-up of the Open Access option has increased in the last five years allowing access to OMBAR articles by a wider readership.

In its 60th year, the Editorial Board has been expanded to broaden its discipline base, have a global outlook and embrace a greater diversity of ages, locations and genders. Looking forward to the next few years, OMBAR will retain and expand on its large and diverse editorial board drawn from across the world and from a variety of research fields, especially reaching out to the physical and chemical oceanographic sciences. It will continue to publish its traditional longer monographic reviews, but will also welcome short reviews. Contributions which can also include new data (which can be especially important to give a long-term view by building on past studies), papers on historical ecology and on the historical development of the field up to its present cutting edge, as well as Cochrane-style systematic reviews and meta-analyses are all encouraged. It will also welcome brief opinion pieces and mini-review articles on emerging topics, from time to time drawing on its editorial board for these. Manuscripts, both invited by the editorial board and unsolicited works submitted by the marine science community, will be considered for publication. All articles will continue to be peer-reviewed (OMBAR publications have been peer-reviewed from Vol. 55 onwards), and also edited by one of the board with appropriate expertise.

We hope that Harold and Margaret Barnes would have approved of these developments. After an editorial on the challenges set by the UN Decade of Ocean Science for Sustainable Development, the first review in the present volume is a tribute to Margaret Barnes by Cruz et al. revisiting her comprehensive 1996 review of the biology of the stalked barnacle *Pollicipes* in OMBAR. Then follows a series of reviews: one by Claassens et al. (2022) which covers the diversity, distribution, ecology and conservation of Syngnathidae in Africa, one on hotspots of Cenozoic tropical marine biodiversity by Yasuhara et al. (2022), a review discussing blue carbon ecosystems in Sri Lanka by Gorman et al. (2022), a review of kelp gametophyte ecology by Veenhof et al. (2022), one systematically reviewing the use of stable isotopes for coral reef studies by Skinner et al. (2022), a review of echinoderm larval settlement by Doll et al. (2022), one presenting the relationship between boxer crabs and sea anemones by Schnytzer et al. (2022), a topical review of marine litter and microplastics in the Indian Ocean by Honorato-Zimmer et al. (2022), one discussing the deep source-sink hypothesis by Mercier et al. (2022) and finally a review of adaptations and responses of marine animals to the high intertidal zone by Leeuwis & Gamperl (2022).

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Taylor & Francis

EDITORIAL: AN *OMBAR* PERSPECTIVE ON THE UNITED NATIONS' DECADE OF OCEAN SCIENCE FOR SUSTAINABLE DEVELOPMENT

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The year 2021 saw the start of the UN Decade of Ocean Science for Sustainable Development. Ocean and coastal ecosystems play crucial roles in supporting our planet and our lives, from regulating climate and protecting shorelines, to providing food and employment. The UN estimates that billions of people rely on the ocean for their livelihoods. The First Global Integrated Marine Assessment (World Ocean Assessment I), published by the United Nations in 2016 (UN 2016), flagged that much of the ocean was severely degraded. The second (WOAII), released last year, suggests that the situation has not improved (UN 2021). The UN Climate Change Conference (COP26) in Glasgow demonstrated that the world's oceans and their species and ecosystems are under immense pressure from climate change. The UN Decade of Ocean Science is a call to arms for scientists and others to deliver research to enable societal outcomes, which include a clean ocean, a healthy and resilient ocean, a predicted ocean, a safe ocean, a sustainably harvested and productive ocean and a transparent and accessible ocean. These are lofty ambitions, but can they be realised in a 10-year programme that has no funding attached to it? This question is particularly relevant when one considers the starting point and how little progress has been made in addressing other major global environmental issues such as the climate crisis.

The research and development (R&D) priority outcomes for the Decade of Ocean Science are likely to be achievable, especially when considering the enormous scientific progress made during the 10-year Census of Marine Life programme (www.coml.org) that showed what can be achieved when scientists are brought together with a common purpose. Global science programmes during the Census of Marine Life demonstrated the real impact that could be made through international

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coordination and cooperation. The UN Decade of Ocean Science aspires to produce a georeferenced digital atlas of the oceans and a comprehensive ocean observing system – data systems that can be used in research and prediction. Achieving this will require progress in data standards, particularly for biological data which lag behind their physical and chemical counterparts, such that data from individual studies are interchangeable, enabling science to move away from local studies to a more global understanding. To make such an atlas truly global will also require capacity building among less developed countries and accelerated technology transfer. National funding needs to be aimed at all aspects of marine science, and to ensure that the R&D outcomes are translated to societal outcomes will require increased participation and better integration of social science within national frameworks. Pan-national funding schemes such as those driven by the European Union and European Science Foundation have a key role to play in facilitating integrated approaches and effective sharing of platforms such as satellites and research vessels. Major international charities or foundations can also contribute to this effort, as the Sloane Foundation did with the Census of Marine Life.

Foundations have an important role to play in global science. For example, the global Ocean Health Index is supported by a diversity of philanthropic organisations (oceanhealthindex.org), including the Pacific Life Foundation; the Tara Ocean Foundation[i] is currently supporting public-private-citizen partnerships focused on ocean exploration.

Some fields of marine science are better prepared to embrace the global challenges presented by the UN Decade of Ocean Science. For example, the International Ocean Discovery Programme (known as the Integrated Ocean Drilling Programme 2003–2013, and the Ocean Drilling Programme prior to that) that has been operating internationally in some form since 1983. Expensive, large-scale research requiring expensive infrastructure such as research vessels tends to drive international cooperation through shared platforms. Thus, the open and deep-water oceanographic science community may be in a better position to deliver a fast response to the UN Decade than the more fragmented coastal marine science community. This is particularly the case for coastal marine science given the regional uniqueness of coastal fauna and flora, and the context dependency of many important processes influenced by sharp local environmental gradients as well as mesoscale oceanographic and coastal features. There are, of course, many international networks linking scientists, managers and policy-makers working on particular systems, including the International Coral Reef Society, the World Seagrass Association and the Global Mangrove Alliance, who could play critical roles in coordination and stimulating collaboration. Many of the most pressing issues are global – especially the rapid alteration of the coastal zone by development leading to much habitat loss or modification and the proliferation of artificial habitat (Firth et al. 2016, Bugnot et al. 2021), on top of rising and stormier seas. Clearly, the interaction of global change with regional- and local-scale impacts will influence biodiversity, ecosystem structure and functioning and continued delivery of critical ecosystem services to society. Some of the impacts will be the direct result of societal mitigation of, and adaptation to, climate change, such as offshore renewable structures and defences of vulnerable coastal property and infrastructure and peoples' lives.

Nonetheless, scientists appear to be rising to the challenges that the UN Decade of Ocean Science presents. The first tranche of endorsed Decade Actions[ii] includes projects that aim to observe and predict the coastal ocean globally, and to monitor estuaries globally. Other projects are focusing on the deep sea and open ocean, and some are specifically addressing capacity building – especially early-career researchers and women. But none of these programmes come with guaranteed funding. Project leads, and all marine scientists globally, now have the challenge of pressing at national and regional (e.g. EU) levels to ensure that policy-makers open sufficient funding calls to make the UN Decade of Ocean Science for Sustainable Development a success. To ensure that research funding translates to actionable improvements in protecting and restoring oceanic and coastal ecosystems, it will be important to include end-users when designing such research opportunities. Failure to do so may yield a proliferation of cutting-edge science that continues to overlook key barriers to operationalisation. For example, while automated, AI-based monitoring approaches require sufficient technological innovation to appeal to many donors, the resulting data have limited impact without a parallel investment in ecological interpretation and the identification of thresholds that might trigger remediative management action.

Going forward from its 60th year, Oceanography and Marine Biology: an Annual Review (OMBAR) will play its role in the UN Decade of Ocean Science for Sustainable Development by providing an integrated view of marine science embracing physical, chemical, geological, biological and social science for a wide readership.

Notes

i https://fondationtaraocean.org/en/foundation/

ii https://www.oceandecade.org/resource/166/Announcement-of-the-results-of-the-first-endorsed-Decade-Actions-following-Call-for-Decade-Actions-No-012020

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PEDUNCULATE CIRRIPEDES OF THE GENUS *POLLICIPES*: 25 YEARS AFTER MARGARET BARNES' REVIEW

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Abstract Twenty-five years ago, Margaret Barnes reviewed the genus *Pollicipes* published in *Oceanography and Marine Biology: An Annual Review.* Our review complements and updates Barnes (1996). An endemic species of *Pollicipes*, *P. caboverdensis*, from Cape Verde Islands, has since been described, joining the three previously known extant species (*P. polymerus*, north-eastern Pacific Ocean*, P. elegans,* tropical eastern Pacific Ocean, and *P. pollicipes,* north-eastern Atlantic Ocean). Most research has been on *Pollicipes polymerus* and *P. pollicipes*. We provide a georeferenced map of the worldwide distribution of *Pollicipes*. All *Pollicipes* species are harvested throughout their geographic distributions with varying intensity and levels of management. Phylogeography and population genetics are new areas developed since Barnes (1996). We update systematics and morphological studies (adult descriptions, cirral form and function, and adhesion). Various aspects of the life history of *Pollicipes* (reproduction, larval phase, settlement, recruitment and growth), the biological assemblages associated with *Pollicipes* and post-settlement population processes are reviewed. Pollution and geochemical studies are outlined before a detailed appraisal of Atlantic and Pacific fisheries. Considerable progress has been made in emerging areas, particularly phylogeography, adhesion and cement, fisheries management and aquaculture. Research gaps are highlighted, despite the much progress in the last quarter-century.

Keywords: Stalked barnacles, *Pollicipes,* Systematics, Phylogenetics, Ecology, Fisheries

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Introduction

Twenty-five years ago, Margaret Barnes made an extensive and thoughtful review of the genus *Pollicipes,* published in *Oceanography and Marine Biology: An Annual Review* (Barnes 1996). In the penultimate paragraph, she stated that "The commercial exploitation of *Pollicipes,* particularly in Europe, has encouraged a renewed interest in these pedunculate cirripedes and hopefully it will also increase some fundamental research on their biology and ecology". She ended the review with a general description of the challenges of living in the wave-swept intertidal zone.

The species of *Pollicipes* have many common names (e.g. percebe in Spanish and Portuguese), but the one that best illustrates the way of life of these species is the name given by the First Nations in Canada. They call it '*caʔinwa*', which means 'playing with the waves'. Twenty-five years after Barnes (1996), what have we learned about those who play with the waves? What have been the scientific advances and what are the research gaps and challenges? Answering these questions is the main objective of the present review.

We use the structure of the sections of Barnes' (1996) review wherever deemed appropriate. Thus, some sections have been retained with the same titles, such as 'Geographical distribution' or 'Description of adults'. We have chosen to coalesce other topics that were scattered in several sections of Barnes (1996) into unifying sections, such as 'Settlement and recruitment' or 'Post-settlement processes of distribution and abundance'. Moreover, there are completely new sections on emerging topics, such as phylogenetics and population genetics, which have led to the recent recognition of a new species, *Pollicipes caboverdensis*, or aquaculture because of the recent progress in addressing the challenges it poses. In contrast, some sections of Barnes (1996) are not included in this review, such as various aspects of functional morphology or physiology, as they have not developed much in the last 25 years. On these topics, Barnes (1996) should be consulted. Thus, the order roughly follows Barnes (1996). In turn, we consider the following: systematics and taxonomy, evolution and population genetics, geographic distributions, habitat along with local distribution patterns and associated biota, description of adults, cirral morphology and feeding, and adhesion and cement. We then review various aspects of life history: reproduction, larval biology, settlement and recruitment, growth, post-settlement processes determining population processes and community structuring. Pollution and geochemical studies are then outlined, before giving a detailed consideration of fisheries and then aquaculture. The cultural importance of *Pollicipes* is highlighted. We conclude by outlining research gaps and making some final remarks.

In each section, we begin with briefly recapping Barnes (1996) and then describe new knowledge, before ending with research gaps and challenges. In a few sections, we have integrated studies prior to Barnes (1996) and cited in Barnes (1996), to better contextualize the section (e.g. 'Ecological habitat, and patterns of distribution and abundance'). Barnes (1996) predicted that the commercial interest in the European species, *Pollicipes pollicipes*, would drive more studies of its biology and ecology. It has, but it has also brought about considerable development of the knowledge and the management of these fisheries. All species of the genus *Pollicipes* (*Pollicipes polymerus*, *P. elegans*, *P. pollicipes* and *P. caboverdensis*) are harvested. In fact, the 'Fisheries, management and conservation' section of this review is the longest.

All sections of this review 'play with the waves'. The very exposed shores where the species of *Pollicipes* occur can be considered as some of the most extreme habitats on our planet, as well as being some of the most difficult to make a living as a fisher or study as an ecologist. These habitats modulate the biology of these species and their interactions with other species. They also challenge those exploiting *Pollicipes* species and constrain scientific research. Our review aims to complement and update Barnes (1996). It is also a tribute to Margaret, a leading light who guided and edited OMBAR for many years, as well as being an expert barnacle biologist, despite never having a paid position at the research institutes in which she worked.

Systematics and taxonomy

Barnes (1996) used the classification proposed by Anderson (1994). Here, we endorse the revised classification proposed by Chan et al. (2021), currently recognized in the World Register of Marine Species (WoRMS; Table 1). A major change in this classification is the new order Pollicipedomorpha (Chan et al. 2021). Both classification systems and the extant and fossil species, including invalid species names and generic reassignments, are listed in Table 1. We also include in the list the extant species and the two fossil species of the genus *Capitulum*, formerly placed in the genus *Mitella*, along with species now in the genus *Pollicipes*. Since the Barnes' (1996) review, a new living species (Fernandes et al. 2010) and two new fossil species of *Pollicipes* have been described (Gale & Sørensen 2015).

Table 1 Taxonomical classification used in Barnes (1996) and in the present review, with a list of extant and fossil species of the genus *Capitulum* and the genus *Pollicipes*, including invalid species names and generic reassignments

Classification proposed by Anderson (1994) and used by Barnes (1996)	Classification proposed by Chan et al. (2021), used in WoRMS (2021) and in the present review
Class Thecostraca	Class Thecostraca Gruvel, 1905
Subclass Cirripedia	Subclass Cirripedia Burmeister, 1834
Superorder Thoracica	Infraclass Thoracica Darwin, 1854
Order Pedunculata	Superorder Thoracicalcarea Gale, 2015
Superfamily Scalpelloidea	Order Pollicipedomorpha Ord. nov. Chan, Dreyer, Gale, Glenner, Ewers-Saucedo, Pérez-Losada, Kolbasov, Crandall, & Høeg, 2021
Family Scalpellidae	
Subfamily Pollicipedinae	Family Pollicipedidae Leach, 1817
Pollicipes	*Anelasma* Darwin, 1852
Capitulum	*Capitulum* Gray, 1825
	(formerly placed in the genus *Mitella* with current *Pollicipes* species)
	One living species:
	Capitulum mitella (Linnaeus, 1758)
	Two fossil species:
	Capitulum sklenari Veselská, Kočí, Collins & Gale, 2015
	Capitulum caelatum (Withers, 1935)

(*Continued*)

Table 1 (*Continued*) Taxonomical classification used in Barnes (1996) and in the present review, with a list of extant and fossil species of the genus *Capitulum* and the genus *Pollicipes*, including invalid species names and generic reassignments

Classification proposed by Anderson (1994) and used by Barnes (1996)	Classification proposed by Chan et al. (2021), used in WoRMS (2021) and in the present review
	Pollicipes Leach, 1817
	Four living species:
	Pollicipes caboverdensis Fernandes, Cruz, & Van Syoc, 2010
	Pollicipes elegans (Lesson, 1831)
	Pollicipes pollicipes (Gmelin, 1791 [in Gmelin, 1788–1792])
	Pollicipes polymerus Sowerby, 1833
	Seven fossil species:
	Pollicipes davisi (Withers, 1953)
	Pollicipes italica (Withers, 1953)
	Pollicipes (?) lailae (Withers, 1953)
	Pollicipes toombsi (Withers, 1953)
	Pollicipes (?) striatum Gale & Sørensen, 2015
	Pollicipes vansyoci Gale & Sørensen, 2015
	Pollicipes venablesi (Withers, 1953)
	Invalid species names and generic reassignments:
	Pollicipes aboriginalis Buckeridge, 1983, accepted as *Pachyscalpellum glauerti* (Withers, 1935) (listed as synonym by Gale & Sørensen, 2015)
	Pollicipes cornucopia Leach, 1824, accepted as *Pollicipes pollicipes* (Gmelin, 1791 [in Gmelin, 1788–1792]) (listed as synonym by Zevina, 1981)
	Pollicipes darwini Hutton, 1879 accepted as *Smilium spinosa* (Quoy & Gaimard, 1834) (listed as synonym by Foster, 1978)
	Pollicipes darwini Quinteiro, Rodríguez-Castro, López, López-Jurado, González-Henríquez & Rey-Méndez, 2011, junior synonym of *Pollicipes caboverdensis* Fernandes, Cruz, & Van Syoc, 2010
	Pollicipes mitella (Linnaeus, 1758), accepted as *Capitulum mitella* (Linnaeus, 1758) (generic reassignment)
	Pollicipes mortoni Conrad, 1837 accepted as *Pollicipes polymerus* Sowerby, 1833 (listed as synonym by Young, 2007)
	Pollicipes rigidus Sowerby, 1839 accepted as *Pollicipes elegans* (Lesson, 1831) (listed as synonym by Young, 2007)
	Pollicipes ruber Sowerby, 1833 accepted as *Pollicipes elegans* (Lesson, 1831) (listed as synonym by Young, 2007)
	Pollicipes sertus Darwin, 1851 accepted as *Calantica spinosa* (Quoy & Gaimard, 1834) accepted as *Smilium spinosa* (Quoy & Gaimard, 1834) (generic reassignment)
	Pollicipes sinensis Chenu, 1843 accepted as *Capitulum mitella* (Linnaeus, 1758) (listed as synonym by Young, 2007)
	Pollicipes smythii Leach, 1818, accepted as *Pollicipes pollicipes* (Gmelin, 1791 [in Gmelin, 1788–1792]) (listed as synonym by Young, 2007)
	Pollicipes spinosus (Quoy & Gaimard, 1834), accepted as *Smilium spinosa* (Quoy & Gaimard, 1834) (generic reassignment)
	Pollicipes villosus Leach, 1824 accepted as *Calantica villosa* (Leach, 1824)

References to taxonomical authorities are only stated in the list of references for the descriptions of new taxa. See Chan et al. (2021) and WoRMS (2021) for the missing references concerning taxonomic authorities

Phylogeny, evolution and population genetics

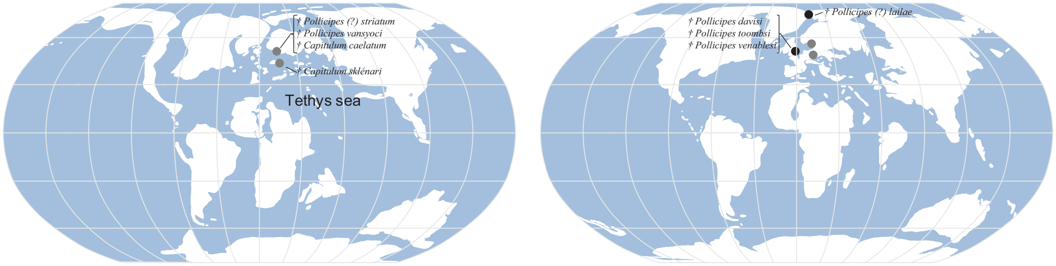
Fossil record and biogeography

The distribution of the four living species in the genus *Pollicipes* is best explained as the relicts of Pollicipedidae that evolved along the margins of what was once the Tethys Sea (Figure 1). The closest living relative to *Pollicipes*, *Capitulum mitella*, inhabits the western Pacific Ocean, on what was the eastern margin of the ancient Tethys Sea (Newman 1992, Van Syoc 1995). The known fossil record of these two genera has expanded somewhat since Barnes (1996). At that time, the only known fossils of *Pollicipes* were *Pollicipes aboriginalis* from Western Australia in deposits aged at 85 Ma (Buckeridge 1983), the four species Withers (1953) described from England and Norway (55–60 Ma) and one species from younger deposits in Italy (Withers 1953; 15–20 Ma) (see Figure 1 for map).

Since then, Gale & Sørensen (2015) considered *Pollicipes aboriginalis* to be a junior synonym of *Pachyscalpellum glauerti* (Withers 1935) and unrelated to *Pollicipes*, and fossils of two *Pollicipes* species have been described from the Late Cretaceous (80 Ma) in rocky shoreline deposits of Sweden (*Pollicipes vansyoci*; *Pollicipes (?) striatum;* Gale & Sørensen 2015). In addition, one

(A) 90 Ma. Late Cretaceous – Late Tethys

(B) 53 Ma. Eocene – Fragmented Tethys with widening of Atlantic



(C) 22 Ma. Miocene – Further fragmented Tethys, maturing Atlantic and Caribbean

(D) 0 Ma. Holocene

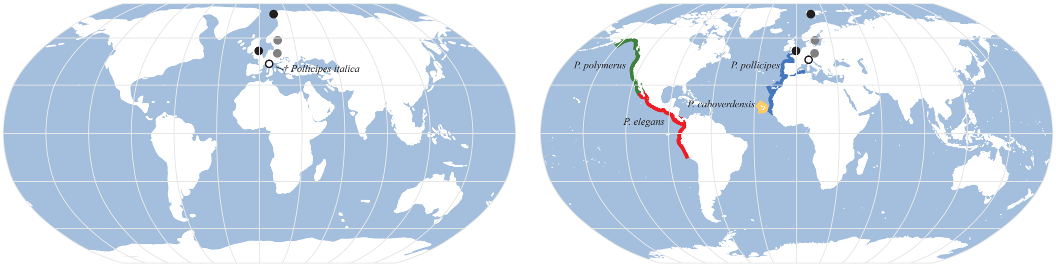


Figure 1 Maps of land masses and oceans showing the evolution of the Tethyan relict distribution for the Pollicipedidae (*Pollicipes* and *Capitulum*). Continental reconstructions were based on Matthews et al. (2016). (A) The Tethys Sea with the Atlantic Ocean forming, 90 Ma, with Pollicipedidae fossil records in areas that will become Sweden and Czech Republic. *Pollicipes* in Sweden (*Pollicipes vansyoci* Gale & Sørensen 2015 and *Pollicipes (?) striatum* Gale & Sørensen 2015; 80 Ma). *Capitulum sklenari* Veselská, Kočí, Collins & Gale, 2015 in the present-day Czech Republic (94 Ma). *Capitulum caelatum* (Withers 1935) Gale & Sørensen 2015 in the present-day Sweden (80 Ma). (B) Fragmented Tethys with a widening Atlantic, 53 Ma. Four additional records of species of *Pollicipes* from England and Norway. (C) Evolving Atlantic, 22 Ma. Emergent Cape Verde Islands, hypothesized date of divergence of *Pollicipes caboverdensis*, 25 Ma. One additional record of species of *Pollicipes* in Italy (*Pollicipes italica* (Withers 1953), 15–20 Ma). (D) Present time, showing the four living *Pollicipes* species distributions after the Eocene/Oligocene boundary radiation of the current species.

fossil species of the closely related genus *Capitulum* is known from Sweden (*Capitulum caelatum*; 80 Ma; Gale & Sørensen 2015) and a second species from similar, but slightly older (94 Ma), deposits in the Czech Republic (*Capitulum sklenari*; Veselská et al. 2015) (Figure 1).

Based on morphological similarities of the three species of *Pollicipes* known prior to the description of *Pollicipes caboverdensis* in 2010, it has been hypothesized that *Pollicipes pollicipes* and *Pollicipes elegans* are more closely related than either is to *Pollicipes polymerus*, with all of the living *Pollicipes* species representing relict elements of this Tethys Sea fauna that became restricted to the eastern boundary conditions of the Atlantic and Pacific Oceans (Newman & Killingley 1985, Newman & Foster 1987, Newman 1992).

The present biogeography of the living species, in conjunction with the fossil records for *Pollicipes* and *Capitulum* coupled with molecular phylogenetics of the four living species of *Pollicipes* (Van Syoc et al. 2010), supports a hypothesis of a radiation of pollicipedine species during the Tethys Sea era (see maps, Figure 1).

Molecular phylogenetics: relationship among living species of Pollicipes

Barnes (1996) did not include any molecular-level analyses in her review of *Pollicipes*. Polymerase chain reaction (PCR) and DNA sequencing were first applied to *Pollicipes* in the early 1990s (Van Syoc & Newman 1992, Van Syoc 1993, 1994a, b, 1995) shortly before Barnes published her review. These early papers on molecular relationships among barnacles relied on comparative analyses of the nucleotide sequences of fragments of the mitochondrial DNA cytochrome oxidase subunit 1 gene (CO1). Since that time, sequences from additional mitochondrial DNA genes (e.g. ribosomal subunits 12S and 16S) and nuclear DNA genes (e.g. H3 and 5.8s rDNA) have been analysed in various barnacle taxa, including *Pollicipes* (e.g. Van Syoc et al. 2010, Quinteiro et al. 2011, Seoane-Miraz 2015). More recently, entire mitochondrial genome sequences have added to our knowledge of barnacle molecular phylogenetic relationships (e.g. Lim & Hwang 2007, Tsang et al. 2017, Tian et al. 2020).

Several recent molecular-based studies (Tsang et al. 2017, Kim et al. 2018, 2019b, Tian et al. 2020) have generally confirmed the suggestion, from earlier morphologically based taxonomic and paleontological studies, that the extant genera of pollicipedines, *Pollicipes* and *Capitulum,* may have diverged from each other 100 or more Ma (e.g. Newman 1987, Withers 1953).

The first study of the genus *Pollicipes* to use DNA sequence data (Van Syoc 1995) analysed nucleotide sequence data from CO1, along with a suite of traditional and new morphological characters, that supported the suggested morphology-based relationship among the then three known species of *Pollicipes* identified from prior studies (see section 'Description of adults'). The tree generated from the DNA sequence data placed *Pollicipes pollicipes* and *P. elegans* together in a branch, with *P. polymerus* occupying another branch.

Subsequently, additional molecular-level studies of the relationships of species within the genus *Pollicipes* (using DNA sequences from more individuals and more gene fragments) have also confirmed this general relationship among the extant species, also leading to the discovery of a new species, *P. caboverdensis*, endemic to the Cape Verde Islands (Fernandes et al. 2010, Van Syoc et al. 2010, Quinteiro et al. 2011). This new species was previously considered a genetically differentiated population of *Pollicipes pollicipes* (Quinteiro et al. 2007). Using mtDNA (CO1 and 16S) and nDNA (H3) genes, Van Syoc et al. (2010) supported the hypothesis that the two eastern Atlantic species, *Pollicipes pollicipes* and the Cape Verde Islands *Pollicipes* species (described as *P. caboverdensis* in a subsequent paper by Fernandes et al. 2010), are most closely related to *P. elegans*, the tropical eastern Pacific species. They found the northern Pacific species, *Pollicipes polymerus*, to be the outlying species within the genus. Quinteiro et al. (2011), using the sequence data from mtDNA (CO1) and nDNA (5.8s rDNA and two flanking interspacer regions), produced a similar tree topology. However, the relationships among the four species were not strongly supported by their data using neighbour-joining distance or parsimony-calculated bootstrap values. The topology of a phylogenetic tree for the extant *Pollicipes* species will continue to be elusive until additional molecular-level data can be obtained.

A broad-stroke phylogenetic tree for the Cirripedia generated by Pérez-Losada et al. (2008, Figure 3), using morphological and multi-gene molecular data in the context of the fossil record, aged the ancestral node of *Pollicipes polymerus* and *P. pollicipes* at about 65 Ma. This correlates somewhat with Van Syoc's (1995) estimate of about 55 Ma for the same node in his early CO1 tree.

Using similar estimates of lineage divergence times, Van Syoc et al. (2010) concluded that *Pollicipes caboverdensis*, *P. pollicipes* and *P. elegans* are the most recently diverged species in the genus, some time after the Eocene/Oligocene boundary radiation of *Pollicipes*, about 25–34 Ma. *Pollicipes polymerus* emerged much earlier at around 55–65 Ma, on the north-western margin of the Tethys, in what is now the north-eastern Pacific. Sal Island, the first of the Cape Verde Islands to emerge, dates to about 25 Ma (Ramalho 2011). Therefore, that would be the earliest time of divergence for *Pollicipes caboverdensis*.

Producing a well-supported molecular phylogenetic tree will continue to be challenging, due to the apparently short time of divergence among the extant species of *Pollicipes* and the relatively long time since those times of divergence. Future research using additional genes, complete mitochondrial genomes or chromosome-level assembled genome analyses may yield data more suitable to resolving the various branch patterns.

Phylogeography and population genetics

The four species of the genus *Pollicipes* have received different levels of attention regarding phylogeographic and population genetic studies, with a stronger effort and more publications regarding *Pollicipes pollicipes*. In contrast, there are no published studies on the genetic structure of populations of *Pollicipes caboverdensis*.

With the advancement of molecular techniques, different DNA markers have been used to analyse the genetic structure of *Pollicipes* species (see Table 2 with summary of studies and methods). A few older studies have used allozymes (Van Syoc 1994a, Miner 2002), but the majority have used fragments of the mitochondrial DNA gene cytochrome oxidase c subunit 1 (CO1) (e.g. Van Syoc 1994a – *Pollicipes polymerus*, Van Syoc 1994b – *P. elegans*, Campo et al. 2010 – *P. pollicipes*), the mitochondrial hypervariable non-coding control region (Quinteiro et al. 2007, Barazandeh 2014) or

Table 2 Summary of phylogeographic/population genetic studies and respective methods (molecular markers and sampling design) of *Pollicipes* species

Molecular marker	Number of loci/ fragment length	Sampling regions	Number of sites	Sample size	Reference
Pollicipes polymerus					
Allozymes	3	California (the USA)	2	43–62	Van Syoc (1994a)
CO1	403 bp	Vancouver Island (Canada)	1	8	
		San Diego (California, the USA)	1		
Allozymes	8	South California (the USA)	5	27–40	Miner (2002)
CO1	550 bp	North-eastern Pacific (57.05°N–135.33°W–34.43°N –119.71°W)	6	12–26	Kelly & Palumbi (2010)
CO1	658 bp	North-eastern Pacific (45.75°N–123.97°W–30.47°N –116.05°W)	32	14–15	Dawson et al. (2014)

(*Continued*)

Table 2 (*Continued*) Summary of phylogeographic/population genetic studies and respective methods (molecular markers and sampling design) of *Pollicipes* species

Molecular marker	Number of loci/ fragment length	Sampling regions	Number of sites	Sample size	Reference
CO1/mtDNA control region	773 bp/601 bp	Vancouver Island (Canada)	8	24	Barazandeh (2014)
SNPs	16	Vancouver Island (Canada)	2	48–50	
Pollicipes elegans					
CO1	312 bp	Mexico	1	7	Van Syoc (1994b)
		Peru	1		
CO1/6 nuclear genes	590 bp/109–803 bp	Mexico	3	Unknown	Marchant (2014)
		El Salvador	3		
		Peru	2		
Microsatellites	11	Mexico	1	47–48	Plough & Marko (2014)
		Peru	1		
CO1	590 bp	Mexico	3	15–31	Marchant et al. (2015)
		El Salvador	3		
		Peru	2		
Pollicipes pollicipes					
mtDNA control region	505 bp	Brittany (France)	1	9–56	Quinteiro et al. (2007)
		Asturias (Spain)	1		
		Galicia (Spain)	9		
		Portugal	1		
		Morocco	1		
		Canary Islands	1		
		Cape Verde Islands[a]	1		
CO1	444 bp	Brittany (France)	2	31–128	Campo et al. (2010)
		Basque Country (Spain)	2		
		Asturias (Spain)	2		
		Galicia (Spain)	1		
		Portugal	2		
		Morocco	1		
		Mauritania	1		
Microsatellites/ CO1	11 loci/609 bp	Brittany (France)	1	13–54/13–32	Fernandes et al. (in prep.)
		Galicia (Spain)	1		
		Portugal	6		
		Morocco	3		
		Western Sahara	1		
		Mauritania	1		
		Senegal	3		
Microsatellites	20	Asturias	5	42–50 (juveniles)/ 44–50 (adults)	Parrondo et al. (2022)
		Galicia	5		
		Portugal	5		

[a] In this study, individuals from Cape Verde Islands were still considered as a population of *Pollicipes pollicipes*, although they were later described as *Pollicipes caboverdensis* (Fernandes et al. 2010).

nuclear genes (Marchant 2014). More recently, nuclear microsatellite markers have been developed and used with success for *Pollicipes elegans* (Plough & Marko 2014) and *P. pollicipes* (Parrondo et al. 2022, Fernandes et al. in prep.). In addition, single nucleotide polymorphisms (SNPs; 16 loci) have been developed for *Pollicipes polymerus* (Barazandeh & Davis 2012).

Regarding *Pollicipes polymerus*, most studies have found high gene flow and genetic homogeneity across the geographic range of this species (Van Syoc 1994a, Miner 2002, Kelly & Palumbi 2010, Dawson et al. 2014). Van Syoc (1994a), using both allozymes and a fragment of CO1 gene, found genetic homogeneity among two populations of the north-eastern Pacific coast. A similar result was obtained by Miner (2002) when using allozymes to analyse five populations from the southern California coast (the USA). More recently, several populations of *Pollicipes polymerus* were sampled across its entire geographic range in multi-species comparative genetic studies using CO1 data (Kelly & Palumbi 2010, Dawson et al. 2014). In these studies, gene flow and genetic diversity were high and population genetic structure was very low, although Kelly & Palumbi (2010) did find a mild differentiation of a population from Monterey (California) from the other sampled populations.

Small-scale spatial genetic structure in *Pollicipes polymerus* was also analysed using the CO1 gene, the mitochondrial control region and 16 SNPs, when testing the effects of wave action and tidal height in genetic patterns of this species, but again, genetic homogeneity was found (Barazandeh 2014).

Contrary to *Pollicipes polymerus*, genetic studies of *P. elegans* found strong genetic structure among populations of this species. Early work by Van Syoc (1994b) using CO1 data showed significant genetic divergence between populations in Mexico and Peru, suggesting limited gene flow across the two localities. This is probably due to higher seawater temperatures in the tropical zone north of the equator (Van Syoc 1994b) where the distribution of this species is fragmented (see section ‘Geographical distribution’). In this study, genetic diversity was higher in Peru than in Mexican populations and the time of divergence of these two populations was estimated to have occurred between 2.36 Ma and 590 Ka. This estimated time window coincides with: (1) a general warming of the tropical eastern Pacific during the Pliocene that could have caused an initial vicariant event of a once continuous trans-tropical population; (2) periods of cooling and warming during the Pleistocene that could have allowed a series of expansions and contractions and intermittent genetic exchange of *Pollicipes elegans* populations (Van Syoc 1994b). However, *Pollicipes elegans* populations from the middle of the species range (El Salvador and Costa Rica) were not sampled in this early study. More recently, Marchant et al. (2015) analysed *Pollicipes elegans* populations using the CO1 gene and, in addition to several Mexican and Peruvian populations, three populations from El Salvador. The populations from El Salvador had higher genetic diversity and older estimated population ages than those from Mexico and Peru. Consequently, these authors proposed that tropical populations from El Salvador could be relicts of a once continuous ancestral distribution, supporting the theory that *Pollicipes elegans* had a tropical origin. The decline and extinction of tropical populations and the current disjunct distribution of *Pollicipes elegans* (see section ‘Geographical distribution’) might be explained by Pleistocene glacial cycles and present-day sea surface temperature and habitat availability (Marchant et al. 2015). Moreover, similar to the results obtained by Van Syoc (1994b), two highly differentiated genetic clusters were found, one consisting of Mexican populations and the other including all the southern populations of El Salvador and Peru. Considering this clear phylogeographic break, Marchant (2014) analysed CO1 data plus additional data from six nuclear genes, proposing an ongoing cryptic speciation process within *Pollicipes elegans* between the Mexican and the southern populations. The time since the divergence of these populations was estimated around 150–350 Ka, with negligible gene flow since separation. Supporting these findings, a significant genetic differentiation between Peruvian and Mexican populations of *Pollicipes elegans* was also revealed in preliminary analyses using microsatellite loci, as well as a markedly reduced genetic diversity in Peru (Plough et al. 2014).

Population genetic studies of *Pollicipes pollicipes* have revealed high gene flow, but some genetic differentiation of populations across the Atlantic distribution range of the species, although the patterns of genetic structure have not always been concordant among studies (Quinteiro et al. 2007, Campo et al. 2010, Parrondo et al. 2022, Fernandes et al. in prep.).

Quinteiro et al. (2007), using the mitochondrial control region, found the following differentiated populations/groups of populations of *Pollicipes pollicipes*: (1) Brittany, (2) Asturias, (3) Galicia, Portugal and Morocco, (4) Canary Islands and (5) Cape Verde Islands. However, populations from Cape Verde were later described as a new species (*Pollicipes caboverdensis*; Fernandes et al. 2010). In the study by Campo et al. (2010) using CO1 data, only populations from Brittany were genetically differentiated from the southern populations of Iberia/north-western Africa (Basque Country, Asturias, Galicia, Portugal, Morocco and Mauritania), but the Canary Islands were not sampled. Contrastingly, Fernandes et al. (in prep.), when analysing CO1 data, found genetic homogeneity among populations from Brittany as far south as Mauritania (again the Canary Islands were not included in that study), and a differentiated genetic group of populations from Senegal, the southern range of the species, which were not sampled in the previous studies. However, the lack of separation between Brittany and Iberia found by these authors might be explained by the low sample size used with the CO1 marker (Fernandes et al. in prep.). Moreover, in this study, more discriminatory analyses of microsatellite data (11 loci) confirmed the divergent group of populations from Senegal and revealed two other genetic clusters, corresponding to Brittany/Iberian populations and north-western African populations (Morocco, Western Sahara and Mauritania), although considerable admixture occurred among these latter two clusters. Parrondo et al. (2022), using microsatellites (20 loci) and sampling fifteen populations of adult individuals from three regions in Iberia (Asturias, Galicia and Portugal), also observed the same pattern of genetic homogeneity along the Iberian coast.

Several different explanations were given for the genetic patterns of *Pollicipes pollicipes* observed in the above studies, related to both historical and contemporary processes (Quinteiro et al. 2007, Campo et al. 2010, Fernandes et al. in prep.). According to Quinteiro et al. (2007), the differentiated populations found by these authors are exclusively related to the main oceanographic features (e.g. the gyres and eddies in the Bay of Biscay and in the Cantabrian shelf) in the region that may constitute barriers to larval dispersal.

In contrast, the two genetic clusters (Brittany versus all southern populations) observed by Campo et al. (2010) were suggested to be the result of a past fragmentation of *Pollicipes pollicipes* populations into three refugial areas (Brittany; north-western Iberia; and North Africa) during Pleistocene glaciations, with a subsequent demographic expansion and rapid homogenization of populations from North Africa and Iberia. This process could have been slower between Iberia and Brittany, explaining the genetic differentiation between these populations detected using CO1 data, since the lack of suitable rocky habitat between these regions may constitute a partial barrier to larval dispersal (Campo et al. 2010) (see section ‘Geographical distribution’).

Based on these previous studies and on the combined results obtained with CO1 and microsatellites, Fernandes et al. (in prep.) suggested the following overall patterns and processes of genetic structure for *Pollicipes pollicipes*. Genetic homogeneity between Brittany and Iberian populations occurs due to contemporary larval dispersal and high gene flow between these regions (Fernandes et al. in prep.), although a signal of a past fragmentation during Pleistocene glaciations can still be detected with mitochondrial data (Quinteiro et al. 2007, Campo et al. 2010). The differentiation of north-western African populations from those in Iberia is caused by a contemporary oceanographic barrier to larval dispersal and gene flow located at the Gulf of Cadiz/Strait of Gibraltar (Fernandes et al. in prep.). The differentiation of the Canary Islands is the result of contemporary oceanographic processes (Quinteiro et al. 2007). The differentiation of Senegal populations result from the lack of suitable habitat along the 750 km sandy coast from Mauritania to Dakar (Senegal), which, together with the main currents in the region, may constitute an old and established barrier to larval dispersal (Fernandes et al. in prep.).

In the study by Parrondo et al. (2022) using microsatellites, besides analysing the populations of adults across the Iberian coast, juveniles of the same size were also analysed, which revealed less diversity and higher relatedness than adults, showing a significant spatial genetic differentiation. Several populations of juveniles from Galicia were genetically differentiated from those of Asturias and Portugal, with a mild differentiation also occurring between juveniles of these last two regions. The spatial patterns of genetic homogeneity in adults *versus* complex patterns of genetic heterogeneity in juveniles (chaotic genetic patchiness) could be caused by the aggregation of related dispersing larvae, by sweepstakes reproductive success or by self-recruitment events (Parrondo et al. 2022).

There are no published population genetic studies for *Pollicipes caboverdensis*. This is a major research gap that should be addressed in the future, especially given the extent and scattered nature of the Cape Verde archipelago and that these are exploited populations.

Overall, in future studies, efforts should be directed to sampling potential phylogeographic discontinuity regions and to developing new molecular markers in *Pollicipes* species. For example, it would be important to sample *Pollicipes pollicipes* from the Mediterranean coast, as the Strait of Gibraltar and the Almeria/Oran front are well-known phylogeographic barriers for other marine invertebrate species including barnacles (e.g. Pannacciulli et al. 1997, 2017). More intensive sampling of *Pollicipes elegans* along the Pacific north and central coast of Mexico between Mazatlán, Sinaloa (23°10′N) and the state of Oaxaca (~15°N) (see section 'Geographical distribution') would also allow more exact location of the strong phylogeographic break found by Marchant et al. (2015) in this species. More genetic data on multiple loci are needed, especially for *Pollicipes polymerus*, as most of the inferences for this species were based on a single mitochondrial DNA gene or allozymes. Cross-amplification of microsatellite markers already developed for *Pollicipes pollicipes* and *P. elegans* in congeneric species should be investigated, together with the development of new microsatellites for *P. polymerus* and *P. caboverdensis*. Moreover, the development of SNP loci for all of the *Pollicipes* species should be addressed in the future. The recent availability of the complete genome of *Pollicipes pollicipes* (RefSeq GCA_011947565.3) opens new possibilities for research. Genome-wide markers can detect subtle genetic differentiation that may exist among populations, thereby helping to identify adequate fishery management units in commercially exploited *Pollicipes* species.

Geographical distribution

The four extant species of *Pollicipes* have an East Pacific/East Atlantic longitudinal disjunct distribution that is considered to be a Tethyan relict pattern (see section 'Phylogeny, evolution and population genetics', Figures 1 and 2). *Pollicipes polymerus* lives in the north-eastern Pacific Ocean. *Pollicipes elegans* inhabits the tropical eastern Pacific Ocean. *Pollicipes pollicipes* occurs in the north-eastern Atlantic Ocean. *Pollicipes caboverdensis* occurs in the Cape Verde Islands only (tropical eastern Atlantic Ocean) (Figure 2). *Pollicipes elegans* and *P. caboverdensis* are warm-water species, with *P. pollicipes* being mostly a warm temperate species and *P. polymerus* being a warm and cold temperate species (Southward 2008, and Figure 6 of Briggs & Bowen 2012).

In the Barnes' (1996) review, the geographical distributions of *Pollicipes polymerus*, *P. elegans* and *P. pollicipes* were thoroughly reviewed, mostly based on published articles. Herein, we have built a georeferenced map of the worldwide distribution of *Pollicipes* species (Figure 2 and georeferenced database in supplementary material). Using *Pollicipes* records in Barnes (1996), we mapped their occurrence data ('Barnes points', Figure 2). In addition, we mapped the sites where the presence of these species and *Pollicipes caboverdensis* has been detected, based on an extensive review of the literature published after Barnes (1996), locations where these species have been recorded based on our personal observations and those of colleagues, personal communications and websites with geographic information (Figure 2 and Table 3). We used only the presence data for *Pollicipes* species taken from websites in regions corresponding to range limits and gaps in the distribution, as was the case in Alaska (the USA) and Baja California (Mexico) for *P. polymerus*; Mexico, Costa Rica and northern Ecuador for *P. elegans*; and the British Isles, southern coast of

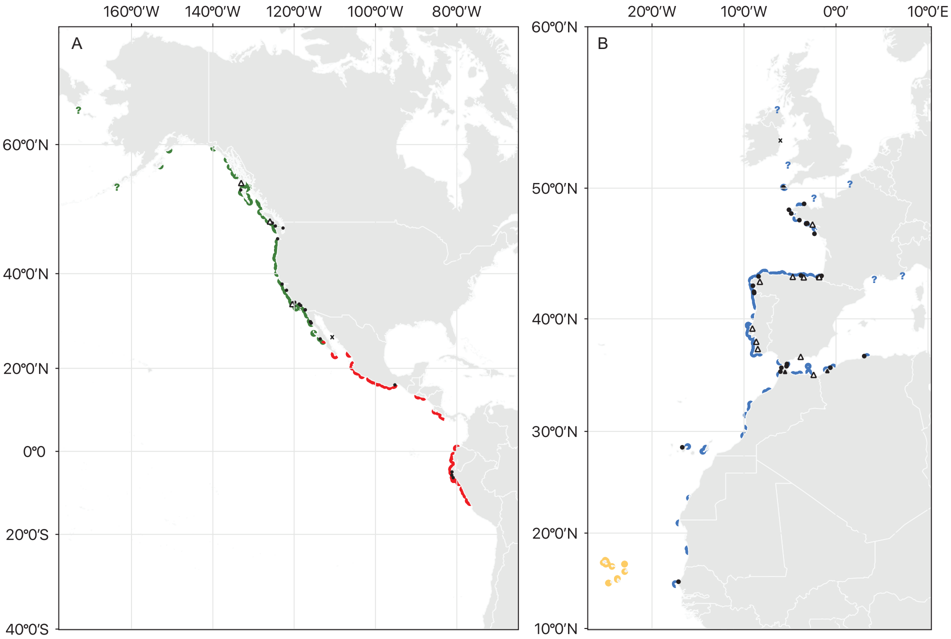


Figure 2 Global distribution of *Pollicipes*. Maps with georeferenced sites ($n = 664$) where *Pollicipes* species were sampled or detected, based on published information after Barnes (1996) or not referred to in Barnes (1996), in personal observations and communications and on websites with geographical information (see Table 3 and georeferenced database in supplementary material). Symbols (colour scheme according to each species): ● *P. polymerus*; ● *P. elegans*; ● *P. pollicipes*; ● *P. caboverdensis*; ● sites mentioned in Barnes (1996); × sites mentioned in Barnes (1996) that were considered an error; "?" dubious records of *Pollicipes*; ▲ Pre-historic shell middens where *Pollicipes* were found and cited by Barnes (1996); Δ Pre-historic shell middens where *Pollicipes* were found after Barnes (1996). Projected coordinate system used: WGS 84/Pseudo-Mercator (EPSG: 3857).

Table 3 Published and unpublished data on the presence of *Pollicipes* species by country after Barnes (1996) that were used for building the georeferenced map of Figure 2

Species/Range	Country	References
P. polymerus NE Pacific 59°N–26°N	Alaska, the USA	Kelly & Palumbi (2010), Alaskafisheries.noaa.gov (2021), MARINe (2021), Inaturalist.org (2021).
	Canada	Van Syoc (1994a), Jamieson et al. (2001), Lessard et al. (2003), Marchinko & Palmer (2003), Marchinko et al. (2004), Quinteiro et al. (2011), Barazandeh et al. (2013), Barazandeh (2014), Schiller (2015), Gagne et al. (2016), Inaturalist.org (2021), MARINe (2021).
	The USA	Carrington-Bell & Denny (1994), Pineda (1994), Wootton (1994), Van Syoc (1994a), Wootton (1997), Harris et al. (2000), Miner (2002), Helms (2004), Marchinko et al. (2004), Pérez-Losada et al. (2004), Phillips (2005), Tapia (2005), Phillips (2006), Dudas et al. (2009), Shanks & Shearman (2009), Tallis (2009), Kelly & Palumbi (2010), Morgan & Fisher (2010), Tapia et al. (2010), Van Syoc et al. (2010), Wootton (2010), Menge et al. (2011), Perina et al. (2011), Dawson et al. (2014), Seoane-Miraz (2015), Seoane-Miraz et al. (2015), Bingham (2016), Morgan et al. (2017), Perina (2018), Romersa (2018), Hagerty et al. (2019).

(*Continued*)

Table 3 (*Continued*) Published and unpublished data on the presence of *Pollicipes* species by country after Barnes (1996) that were used for building the georeferenced map of Figure 3

Species/Range	Country	References
	Mexico	Ladah et al. (2005), Van Syoc et al. (2010), Dawson et al. (2014), Hagerty et al. (2019), Rani Gaddam (pers. comm.); MARINe (2021), Benthic Invertebrate Collection of Scripps Institution of Oceanography (C6703).
P. elegans Tropical E Pacific 26°N–8°N	Mexico	Van Syoc (1994a), Van Syoc et al. (2010), Walther et al. (2013), Plough & Marko (2014), Plough et al. (2014), Crickenberger et al. (2015), Marchant et al. (2015), Gutiérrez & Cabrera (2019), Pedro Flores (pers. comm. 2014) in Marchant et al. (2015), Inaturalist.org (2021).
0°–6°/12°S	El Salvador	Van Syoc (1994a), Walther et al. (2013), Marchant et al. (2015), Inaturalist.org (2021).
	Costa Rica	Mora-Barboza & Sibaja-Cordero (2018), Inaturalist.org (2021).
	Ecuador	Ladines (2018), Cárdenas-Calle et al. (2020), youtube.com/watch?v=9zkNYlOucLQ, Inaturalist.org (2021).
	Peru	Tarazona et al. (1985), Arntz et al. (1987), Kameya & Zeballos (1988), Van Syoc (1994a), de la Cruz et al. (2001), Samamé & Quevedo (2001), Stucchi & Figueroa (2006), Van Syoc et al. (2010), Zapata et al. (2010), Perina et al. (2011), Quinteiro et al. (2011), Walther et al. (2013), Plough & Marko (2014), Crickenberger et al. (2015), de la Cruz et al. (2015), Marchant et al. (2015), Ramírez & de la Cruz (2015), Seoane-Miraz (2015); Seoane-Miraz et al. (2015), Alemán et al. (2016), Alegre (2017), Ramírez et al. (2017), Ibanez-Erquiaga et al. (2018); Perina (2018); Valqui et al. (2021), Leonardo Romero (pers. comm.).
P. pollicipes	The UK	Southward (2008), Stephen J. Hawkins, Keith Hiscock and Nova Mieszkowska (pers. comm.), marlin.ac.uk 2021.
NE Atlantic 50°N–14°N	France	Quinteiro et al. (2007), Southward (2008), Campo et al. (2010), Van Syoc et al. (2010), Seoane-Miraz et al. (2015), Aguión et al. (2022b), Fernandes et al (in prep), Eric Thiebaut (pers. comm.).
	Spain	de la Hoz & Garcia (1993), Mas et al. (1996), Pérez-Losada et al. (2004), Candeias (2005), Macho et al. (2005), Borja et al. (2006a, b), Macho (2006), Quinteiro et al. (2007), Campo et al. (2009), Álvarez-Fernández et al. (2010), Macho et al. (2010), Perina et al. (2011), Sestelo & Roca-Pardiñas (2011), Parada et al. (2012), Álvarez-Fernández et al. (2013), Parada et al. (2013), Gofas et al. (2014), Perina et al. (2014), Rivera et al. (2014), Bidegain et al. (2015), Seoane-Miraz (2015), Seoane-Miraz et al. (2015), Höfer et al. (2016), Bidegain et al. (2017), Perina (2018), Aguión et al. (2022a, 2022b), Román et al. (2022), Parrondo et al. (2022), Inaturalist.org (2021), Fernandes et al (in prep).

(*Continued*)

Table 3 (*Continued*) Published and unpublished data on the presence of *Pollicipes* species by country after Barnes (1996) that were used for building the georeferenced map of Figure 3

Species/Range	Country	References
	Portugal	Van Syoc (1994a), Cardoso & Yule (1995), Kugele & Yule (1996), Norton (1996), Cardoso (1998), Cruz & Hawkins (1998), Cruz & Araújo (1999), Cruz (2000), Kugele & Yule (2000), Jesus (2004), Candeias (2005), dos Santos et al. (2007), Quinteiro et al. (2007), Cruz et al. (2008), Valente (2008), Campo et al. (2010), Cruz et al. (2010), Fernandes et al. (2010), Jacinto et al. (2010), Van Syoc et al. (2010), Jacinto et al. (2011), Costa (2012), Reis et al. (2012), Rodharth (2013), Sousa et al. (2013), Albuquerque (2014), Franco (2014), Ramos et al. (2014), Almeida et al. (2015), Figueira (2015), Jacinto et al. (2015), Rocha (2015), Seoane-Miraz (2015), Albuquerque et al. (2016), Jacinto (2016), Jacinto & Cruz (2016), Darras (2017), Lobo-da-Cunha et al. (2017), Mateus (2017), Pedro (2017), Belela (2018), Fernandes (2018), Torres (2018), Machado et al. (2019), Tilbury et al. (2019), Domínguez-Perez et al. (2020), Aguión et al. (2022b), Fernandes et al. (2021), Parrondo et al. (2022); Inaturalist.org (2021), Fernandes et al. (in prep).
	Gibraltar	Inaturalist.org (2021).
	Algeria	Kallouche et al. (2014), Bachetarzi et al. (2016).
	Morocco	Quinteiro et al. (2007), Bazairi (2010), Campo et al. (2010), Perina et al. (2011), Boukaici et al. (2012, 2015), Seoane-Miraz (2015), Seoane-Miraz et al. (2015), Perina (2018), Bourassi et al. (2019), Fernandes et al (in prep), Laura Palacín-Fernández (pers. comm.).
	Canary Islands, Spain	Marín & Luengo (1998), Quinteiro et al. (2007), González et al. (2012).
	Western Sahara	Fernández de Larrinoa & Cedenilla (2003), Van Syoc et al. (2010), Inaturalist.org (2021), Fernandes et al. (in prep).
	Mauritania	Campo et al. (2010), Fernandes et al. (in prep).
	Senegal	Fernandes et al. (2010). Fernandes et al. (in prep).
P. caboverdensis Tropical E Atlantic 17°N–14°N	Cape Verde	Quinteiro et al. (2007), Fernandes et al. (2010), Van Syoc et al. (2010), Quinteiro et al. (2011), Baessa (2015), Seoane-Miraz (2015), Seoane-Miraz et al. (2015), Soares (2018).
P. polymerus middens	Canada	Moss & Erlandson (2010), Efford (2019).
	The USA	Erlandson et al. (2004).
P. pollicipes middens	Spain	Álvarez-Fernández et al. (2010), Aristu et al. (2011), Gutiérrez-Zugasti (2011), Gibaja et al. (2012), Álvarez-Fernández et al. (2013), Fernández-Rodríguez et al. (2014), García-Escárzaga et al. (2017).
	Portugal	Valente (2008), Bicho (2009), Dean (2010), Bicho et al. (2013), Valente (2014), Callapez & Pimentel (2018).

Georeferenced database in supplementary material.

Portugal and Spain, Gibraltar and the Western Sahara for *P. pollicipes*. Occasionally, publications before, but not included in, Barnes (1996) were considered (e.g. Tarazona et al. 1985, Kameya & Zeballos 1988, Laguna 1990 and Van Syoc 1994a for *P. elegans*), particularly where it was important to define the limits of each species. We have considered species limits records as dubious when there was contradictory information, or when references were old and not possible to confirm with recent observations ('?' in Figure 2). As *Pollicipes* species have been exploited since prehistoric times, we also mapped the locations of the presence of these species in shell middens (Figure 2, and see section ‘Fisheries, management and conservation’). In total, we mapped 664 points of presence (61 points were taken from websites) of *Pollicipes* species recorded after Barnes (1996), including points of shell middens and overlapping points (Figure 2).

When compared with Barnes (1996), the main differences found in the geographical distribution of *Pollicipes* species were as follows: changes in the northern limit of *P. polymerus*; the detailed description of the geographical distribution of *P. elegans*, including the southward extension of its southern limit and relationship with El Niño Southern Oscillation events; and the description of the geographical distribution of the newly described *P. caboverdensis* (see sections ‘Systematics and taxonomy’ and ‘Description of adults’, Figure 2).

Pollicipes polymerus

According to several references in Barnes (1996), the latitudinal range of *Pollicipes polymerus* was considered to be from 64°N to ~27°N. This northern limit was defined at Plover Bay, Bering Strait, but its presence was associated with other barnacles on a humpback whale (Pilsbry 1907). There is also a reference in Barnes (1996) to the presence of *Pollicipes polymerus* on wave-exposed rocky shores at Sakhalin Island (Tarasov & Zevina 1957 cited in Bernard 1988). However, in the original publication (Tarasov & Zevina 1957), the specimens were described as included in a museum collection and associated with an unknown substrate, probably the bottom of a vessel. Since neither reference is on rocky coasts, these records are difficult to verify and thus we consider them to be doubtful. The northernmost rocky coast regions described in Barnes (1996) are the Aleutian Archipelago (the USA) (Bernard 1988) and further south in British Columbia (Canada) (see several references in Barnes 1996). There are no recent references to the presence of *Pollicipes polymerus* in the Aleutian Archipelago, and one of us (Robert J. Van Syoc) has not found this species at these islands; therefore, we also consider the presence of this species in these islands doubtful. However, since Barnes (1996), there are records of this species at several locations on the rocky coasts of Alaska (the USA) (Figure 2). Hence, we consider the northern limit of this species to be Kenai Peninsula (59° 18′N) and the north-western limit to be Kodiak Island (57° 15′N 152°56′W) (Alaskafisheries.noaa.gov 2021).

In addition, there are several recent records of this species on the coasts of British Columbia (Canada), Washington, Oregon and California (the USA), as far south as Baja California (Mexico) (Figure 2).

Regarding the southern limit of *Pollicipes polymerus*, Barnes (1996) mentioned that the most southern records are Punta Abreojos (26° 42′N) (Newman & Abbott 1980, Newman & Killingley 1985) and Punta Santa Domingo (26° 31′N; Bernard 1988), both in Baja California, Mexico. Bernard (1988) also noted that this species is found sporadically south of Cedros Island (~28°N), in areas cooled by upwelling currents as far south as Punta Santa Domingo, but did not present any data regarding its presence in these locations. However, we found a record of specimens of *Pollicipes polymerus* at Punta Santa Domingo in the Benthic Invertebrate Collection of Scripps Institution of Oceanography (catalogue number C6703, collected in 1979 by R. Cimberg). After the 1980s, there is apparently no further record of the presence of *Pollicipes polymerus* at this location. Based on the information from the Multi-Agency Rocky Intertidal Network (MARINe 2021), the southern record of *Pollicipes polymerus* is from Punta Abreojos, but no sites south of this location were sampled (Rani Gaddam pers. comm.). In conclusion, we consider the historical record in Punta Santa Domingo to be the southern limit of *Pollicipes polymerus*, but further sampling in this location is needed.

In a 1953 record that was subsequently found to be incorrect, *Pollicipes polymerus* specimens attached to floating wood near Madras, India, were described as a new subspecies, *Pollicipes polymerus madrasensis* (Daniel 1953). However, Newman & Killingley (1985) used ^{18}O fractionation in calcite from the capitular plates of the putative subspecies to discover that these specimens had grown in seas much cooler than the Indian Ocean. Therefore, the specimens Daniel (1953) described are *Pollicipes polymerus*, but are not a distinct subspecies and did not live and grow in the Indian Ocean (Trivedi et al. 2021). It is unclear how they arrived there.

Pollicipes elegans

Citing several authors (Darwin 1852, Gruvel 1905, Pilsbry 1909), Barnes (1996) considered that the northern limit of the distribution of *Pollicipes elegans* was Lobos Island located in the Gulf of California, Mexico. We consider that this record is probably a misidentification of Lobos Island, as the reference of Lobos Island in Darwin (1852) and in Pilsbry (1909) is in Peru, not in Mexico, and the location is not clear in Gruvel (1905). There are several recent references to the presence of *Pollicipes elegans* in Lobos Islands, Peru (~6°S–7°S, Islas Lobos de Tierra, e.g. de la Cruz et al. 2015b; Islas Lobos de Afuera, e.g. Ramírez & de la Cruz 2015), so we consider the northern limit of Lobos Island, Mexico, suggested in Barnes (1996) as incorrect. Based on this misidentification of Lobos Island, Barnes (1996) noted that the southern record of *Pollicipes polymerus* (Punta Abreojos and Punta Santa Domingo, Newman & Killingley 1985, Bernard 1988) were at approximately the same latitude as Lobos Island (26°N–27°N).

Although the northern boundary of Lobos Island for *Pollicipes elegans* is incorrect, Newman & Killingley (1985) (based on a personal communication with R. Brusca and R. Cimberg) and Laguna (1990) indicated a region of sympatry between *P. polymerus* and *P. elegans* at 26°N. In addition, Van Syoc (1994a) mentioned specimens of *Pollicipes elegans* collected at Punta Santa Domingo (26° 31′N) based on the database of records in the Benthic Invertebrate Collection of Scripps Institution of Oceanography. *Pollicipes elegans* has, however, never been observed at Punta Abreojos (26° 42′N) (Rani Gaddam pers. comm., based on Multi-Agency Rocky Intertidal Network, MARINe 2021). In more recent studies, samples of *Pollicipes elegans* have been taken from sites located south of Punta Abreojos and Punta Santa Domingo – Cape San Lucas, 22° 53′N (Van Syoc 1994a), and Punta Gasparino, 23° 10′N (Walther et al. 2013, Plough & Marko 2014, Plough et al. 2014, Crickenberger et al. 2015, Marchant et al. 2015). In conclusion, we consider that the northern limit of *Pollicipes elegans* is Punta Santa Domingo, based on Van Syoc (1994a), with a possible region of sympatry between *P. elegans* and *P. polymerus* at Punta Santa Domingo.

In addition to Lobos Island, Mexico, Barnes (1996) reported the presence of *Pollicipes elegans* from the west coast of Mexico to Peru, indicating its presence in Tehuantepec, Mexico (~16°N), citing Darwin (1852), and in Paita, Peru (several references in Barnes, 1996, ~6°S), which she considered to be the southern limit of its distribution (Figure 2).

Barnes (1996) did not mention the fragmented nature of the distribution of this species with several gaps, which is characteristic of *Pollicipes elegans* and different from the more continuous distributions of the other *Pollicipes* species. Marchant et al. (2015) have described the fragmented distribution of *Pollicipes elegans* as including extra-tropical populations in north and central Mexico and northern Peru and populations within the Intertropical Convergence Zone (El Salvador and Costa Rica), the warmest waters of the eastern Pacific (see Figure 2). According to Manuel Ixquiac-Cabrera (pers. comm. in Marchant et al. 2015), *Pollicipes elegans* was not detected on the coast of Guatemala. Apparently, the coast of Nicaragua has never been sampled for the presence of *Pollicipes elegans*. The southern limit of *Pollicipes elegans* in the northern hemisphere is Punta Burica (~8°N) on the southern Pacific coast of Costa Rica (Mora-Barboza & Sibaja-Cordero 2018), close to the border of Panama, since the presence of *P. elegans* in Panama (Marchant et al. 2015) and in Colombia (Lozano-Cortés & Londoño-Cruz 2013) has not been detected. This species appears again near the equator, its most northern record being at Punta Tortuga, Ecuador (0° 46′N, Inaturalist.org 2021). In the southern hemisphere, *Pollicipes elegans* has also been found in Manabi and Santa Elena provinces of Ecuador (e.g. Isla Salango, ~1° 35′S, Inaturalist.org 2021; Chocolatera, 2° 11′S, Cárdenas-Calle et al. 2020), which are far north of the previous locations mentioned for South America that were in Peru (Figure 2). *Pollicipes elegans* is not present in the Galápagos Islands (Ecuador). The disjunct distribution of *Pollicipes elegans* was considered by Marchant et al. (2015) as paramphitropical, as Newman & Foster (1987) had already suggested, a trans-tropical distribution in which a species is more abundant towards the periphery than at the centre of its latitudinal range.

Regarding the southern limit of *Pollicipes elegans*, Tarazona et al. (1985) and Kameya & Zeballos (1988) detected the presence of this species at several sites in Peru south of the southern limit considered by Barnes (1996) (Figure 2). The southernmost site sampled by Kameya & Zeballos (1988) was Playa Yaya, Chilca (Lima) (12° 29′S). This can be considered the southern limit of the distribution of this species, as we are unaware of any studies further south in Peru where it has been found, and the species is not distributed along the coast of Chile (Juan Carlos Castilla pers. comm.).

The distribution and abundance of *Pollicipes elegans* in Ancon (~11° 46′S) by Tarazona et al. (1985), and between Yasila (Paita, ~5°S) and Chilca (~12°S), recorded in Kameya & Zeballos (1988), was associated with the warm episode of El Niño of 1982/1983 (Paredes et al. 2004, Arntz et al. 2006). Kameya & Zeballos (1988) considered that there was an unusual increase in *Pollicipes elegans* in response to this warm episode. We are not aware of any study with the same territorial coverage, but several have been made in Islas Lobos de Tierra and Islas Lobos de Afuera (~6° 26′S –6° 57′S), where the presence of *Pollicipes elegans* was recorded (e.g. Samamé & Quevedo 2001, de la Cruz et al. 2002, Ramírez et al. 2008, de la Cruz et al. 2015a, b, Ramírez & de la Cruz 2015), with some observations further south: Ancon (~11° 46′S) (in 1997/1998 and 2001, Leonardo Romero pers. comm.), and Isla San Lorenzo, Callao, Lima (~12° 09′S) in 1997/1998 (Alegre 2017). This last record was also associated with an El Niño episode (1997/1998). In conclusion, the southern distribution of this species is Punta Aguja, Piura (5° 47′S) and the Lobos de Tierra (~6° 26′S) and Lobos de Afuera (~6° 57′S) islands in Peru, but during El Niño or warm conditions, it may extend as far south as Chilca (12° 29′S), as had been reported by Kameya & Zeballos (1988) (Carmen Yamashiro pers. comm.), persisting for a few years after a warm episode. The dynamic nature of the southern limit of *Pollicipes elegans* merits investigation in the future in the context of global change, as does the extent of the zone of overlap with *P. polymerus* in the north.

Pollicipes pollicipes

The northern and southern limits of the geographic distribution of *Pollicipes pollicipes* are similar to those considered in Barnes (1996) (Figure 2). Some earlier records in Ireland and Scotland mentioned in Barnes (1996) are considered to be erroneous or doubtful, including the British records of *Pollicipes* quoted by Darwin (1852) that were considered misidentifications by other authors according to Southward (2008). However, there is a vague mention of *Pollicipes pollicipes* in Ireland by Fischer-Piette (1936) cited by Barnes (1996) and there are two recent observations in Ireland and Northern Ireland (Darrynane Bay, Kerry 51°45′ in 2016, and White Park Bay, 55°13′ in 2015, in records.nbnatlas.org). Nevertheless, we were able to confirm that the reference from Kerry in Ireland was wrong, so it cannot be considered and has already been removed from this website. Regarding the reference to Northern Ireland, as well as another reference recorded on this website with respect to the presence of *Pollicipes pollicipes* in Wales, the UK (the Dakotian, Dale, 51°42′N in 2016, in records.nbnatlas.org), we were unable to confirm that these are indeed locations where this species occurs, so the presence of this species in Wales and Northern Ireland was considered doubtful (Figure 2). In south-western England, on the other hand, there are observations of occasional individuals found around the Land's End peninsula, from Lamorna to Sennen Cove, dating back to the late nineteenth century (specimens in the Natural History Museum, London). On this peninsula, there is a solitary record from the 1980s, which is mentioned in Barnes (1996), with several recent observations of scattered individuals since 2005 (e.g. Porthcurno, Tator Du Lighthouse) with animals found in most years just west of Sennen Cove, with a maximum of eight individuals being found in a suitable 20×20 m area in 2021 (Southward 2008, Stephen J. Hawkins, Keith Hiscock and Nova Mieszkowska pers. obs.; Barnes 2021). In the Channel Islands, there are nineteenth century observations of *Pollicipes pollicipes* at Guernsey and Sark (Ansted & Latham 1865 in le Mao et al. 2020), but no recent observations. Therefore, we have considered that the northern limit of the distribution of this species in Europe is Sennen Cove, Land's End peninsula, the UK (50°4′N) (Figure 2), and that the distribution of *Pollicipes pollicipes* in Ireland, Northern Ireland and Wales (the UK) should be further investigated.

On the European and African Atlantic continental coast, this species is found from north Brittany (France) southwards down the coasts of France, Spain, Portugal, Morocco, Western Sahara, Mauritania and Senegal (Figure 2). In mainland Europe, the northern limit of distribution of *Pollicipes pollicipes* is plateau de la Méloine (48°43′N –03°47′W; Eric Thiébaut pers. comm.) in the north-east of the Bay of Morlaix, although there is a nineteenth century record from Berck-sur-Mer (50°24′N, Musée des Confluences, in gbif.org) that was considered doubtful, as it is a very sandy coast (Figure 2). The most southern location where this species has been observed is Cape Manuel, Dakar (14°38′N, Fernandes et al. 2010), which can be considered the southern limit of this species. Stubbings (1967) had already mentioned that there was no doubt in considering Dakar as the southern limit of *P. pollicipes.*

In Macaronesia, *P. pollicipes* only occurs in the Canary Islands, at Tenerife (Marín & Luengo 1998) and Fuerteventura (González et al. 2012), and there are no records of its presence in the Azores (Southward 2008), as Barnes (1996) had already mentioned, or in Madeira (Wirtz et al. 2006).

Regarding the distribution of this species in the Mediterranean, there is no doubt about its presence on the North African coast, with recent records along this coast (e.g. Kallouche et al. 2014) as far east as Aguelli Island, Algiers, Algeria (36°47′N –3°21′E) (Bachetarzi et al. 2016). Barnes (1996) had also noted the presence of *Pollicipes pollicipes* in Algiers. In addition, there is a record of this species on Isla de Alborán in the Alboran Sea, part of the western Mediterranean Sea (Mas et al. 1996). However, there is more doubt regarding its presence on the northern Mediterranean coast of Spain and France. Barnes (1996) mentioned its presence in Catalan Bay, Gibraltar, and considered that this species extended along the southern Spanish and French coast to the "Mer de Nice" (Caziot 1921). There is also a nineteenth century record of *Pollicipes pollicipes* near Montpellier (Sète) (Museum of Comparative Zoology, Harvard University in gbif.org). As there are no recent observations of the presence of *Pollicipes pollicipes* on the southern Spanish and French coasts, we consider these records from the environs of Nice and near Montpellier (Sète) as doubtful and are in need of a sampling effort directed at this species and the type of habitat where it occurs (wave-exposed shores).

Pollicipes caboverdensis

The description of *Pollicipes caboverdensis* came after the review of Barnes (1996) (Fernandes et al. 2010). According to personal observations and studies (Fernandes et al. 2010, Quinteiro et al. 2007, 2011, Baessa 2015, Soares 2018), and information provided by local fishermen and local restaurant employees, *Pollicipes caboverdensis* is present on all the islands of Cape Verde (Santo Antão, São Vicente, Santa Luzia, São Nicolau, Sal, Boavista, Santiago, Fogo and Brava), with the exception of Maio where it was not possible to confirm its presence (Figure 2).

Considering all *Pollicipes* species and their respective geographical limits, observations should be made on the exposed rocky coastline of some regions, where there are doubts, or need of more recent observations, about the presence of these species: the Aleutian Archipelago and the Pacific coast of Baja California for *P. polymerus*; the Pacific coast of Baja California, the coast of Nicaragua and the central and southern coasts of Peru for *P. elegans*; and the coast of Ireland and of Northern Ireland and South West Wales (the UK), the south Mediterranean coast of Spain and the north Mediterranean coast of Africa from Algiers eastwards for *P. pollicipes.* The most intriguing question is the extent of overlap in the distributions of *Pollicipes polymerus* and *P. elegans* in Baja California at Punta Santa Domingo.

Ecological habitat, and patterns of distribution and abundance

Barnes (1996) described the ecological habitat of *Pollicipes* species by reviewing the knowledge of its physical features and noting a few abundant species or taxa that co-occurred with *Pollicipes polymerus* and *Pollicipes pollicipes*, as well as other barnacles that were more abundant close to the upper and lower limits of these species. We have reviewed the literature concerning the abiotic and biotic environment of the habitats inhabited by each *Pollicipes* species, as well as their patterns of distribution and abundance.

Physical habitat and distribution

The physical habitat features that we identified from subsequent, more recent publications are similar to those described by Barnes (1996). We have also updated information regarding *Pollicipes elegans* and *Pollicipes caboverdensis*.

All species occur on wave-exposed coasts (*Pollicipes polymerus* Gagne et al. 2016; *P. pollicipes* Sousa et al. 2013; *P. elegans* Oliva 1995 in Monsalve 2016; *P. caboverdensis* Fernandes et al. 2010), usually on vertical or steep-sided rocks where wave action is strong (*P. polymerus* Barnes & Reese 1960, Bingham 2016; *P. pollicipes* Macho 2006, Boukaici et al. 2015; *P. elegans* Samamé & Quevedo 2001, Barraza et al. 2014, Ramírez et al. 2017). They also occur in localized areas protected from the direct force of waves, but that are characterized by constant water turbulence, or by strong currents, such as surge gullies and channels (*Pollicipes polymerus* Barnes & Reese 1960 and *P. pollicipes* Barnes 1996). Passages with fast tidal currents (*Pollicipes polymerus* Lamb & Hanby 2005) are also colonized, as well as crevices, rock fissures and caves (*P. pollicipes* Cruz 2000, Fernandes et al. 2010; *P. elegans* Kameya & Zeballos 1988, Monsalve 2016, Ladines 2018). *Pollicipes polymerus* is perhaps the most opportunistic species, being found on rocky substrata of different geologies, or attached to mussels and acorn barnacles (Austin 1987 in Lauzier 1999b), colonizing surfaces cleared by storm disturbance such as gaps in mussel beds (Paine & Levin 1981, Meese 1993), overhangs and boulders (Barnes 1996), as well as diverse artificial structures (Barnes & Reese 1960), including buoys (Austin 1987 in Lauzier 1999b), seawater intakes (Newman & Abbott 1980) and the supports connected to offshore oil platforms (Page 1986). In contrast, there are very few records of the other *Pollicipes* species on substrata other than rock – a population of *P. pollicipes* on a ship stranded on a beach in Nouakchott, Mauritania (Fernandes et al. in prep.), and the presence of *P. elegans* on buoys (Ladines 2018).

All *Pollicipes* species seem to be mostly intertidal, although there are references to their occurrence in the shallow subtidal zone (*P. polymerus* McDaniel 1985 in Lauzier 1999b, Austin 1987 in Lauzier 1999b; *P. pollicipes* Cruz 2000, Borja et al. 2006a, b; *P. elegans* Alegre 2017). Barnes (1996) described the presence of *Pollicipes pollicipes* in channels that may be 100 m deep, and Lamb & Hanby (2005) outlined the lower limit of the vertical distribution of *P. polymerus* as 30 m water depth. The interest of recreational divers about the large aggregations of *Pollicipes polymerus* at Nakwakto Rapids, Slingsby Channel, British Columbia (Lamb & Hanby 2005) has provided consistent depth records of 12–15 m for this subtidal population (north-east Pacific diving websites, e.g. Rogers 2016). Besides these observations, the actual measurements of the maximum depth of occurrence of *Pollicipes* species are rare. Exceptionally, Borja et al. (2006a) stated maximal depths of 1.5 or 2 m below the Lowest Astronomical Tide for *Pollicipes pollicipes* within the Gaztelugatxe Marine Reserve (north-west Spain). In the intertidal zone, all *Pollicipes* species commonly occur in the mid-shore (*P. polymerus* Paine 1974, Jamieson et al. 2001; *P. pollicipes* Cruz 2000, Macho 2006, Fernandes et al. 2010; *P. elegans* Kameya & Zeballos 1988, Ramírez et al. 2017; and *P. caboverdensis* Fernandes et al. 2010). Their intertidal distributions have been also described to include the low-shore level for *Pollicipes pollicipes* (e.g.

Cruz 2000, Macho 2006, Sousa et al. 2013, Jacinto et al. 2015), *P. polymerus* (Menge et al. 2011, Bingham 2016, Gagne et al. 2016) and *P. elegans* in Ecuador (Ladines 2018). The upper limit of their vertical distribution has been described to reach 4.1 m above chart datum for *Pollicipes polymerus* in British Columbia (Jamieson et al. 1999) and 4–5 m above chart datum for *P. pollicipes* in SW Portugal (Cruz 2000).

Abundance

We have reviewed the literature regarding quantitative population assessments of abundance for species of the genus *Pollicipes*. To our knowledge, there are no studies on the abundance of *Pollicipes caboverdensis*. Generally, abundances of the other three species have been locally assessed, either by measuring their percentage cover or by quantifying their number and/or weight of individuals per unit area. While percentage cover has widely been used as a proxy of abundance of *Pollicipes polymerus* (Dawson et al. 2014 "Appendix B", Menge et al. 2011) and *P. pollicipes* (Spain – Borja et al. 2006a, Parada et al. 2012, Bidegain et al. 2017; Portugal – Sousa et al. 2013, Jacinto & Cruz 2016, Neves 2021), this variable has never been used in the case of *P. elegans*. On the other hand, density and/or biomass quantifications are currently widespread in the literature for *Pollicipes polymerus* (e.g. Austin 1992 in Jamieson et al. 1999, Dawson et al. 2014 "Appendix B", Gagne et al. 2016), *P. pollicipes* (Spain – Borja et al 2006a, b, Bidegain et al. 2017; Portugal – e.g. Sousa et al. 2013, Cruz et al. 2015c, Neves 2021; Morocco – Boukaici et al. 2012, Bourassi et al. 2019) and *P. elegans* (Costa Rica – Mora-Barboza & Sibaja-Cordero 2018; Ecuador – Ladines 2018; Peru mainland – e.g. Pinilla 1996, Ordinola et al. 2010, Alemán et al. 2016; Lobos de Tierra Island and Lobos de Afuera Islands – e.g. Samamé & Quevedo 2001, Ramírez & de la Cruz 2015). The following examples refer to estimates of biomasses per area of three species: *Pollicipes polymerus* ranged from 0 to 39 kg/m^2 across the total estimated area of intertidal distribution of this species within 19 rocks off the west coast of Vancouver Island (Gagne et al. 2016); *P. pollicipes* ranged from 1.3 to 7.7 kg/m^2 on mid-shore and from 0.5 to 2.4 kg/m^2 on low-shore populations within three regions in Portugal (Sousa et al. 2013); and *P. elegans* averaged 21.1 kg/m^2 across the total estimated area of intertidal distribution of this species within ten sites in Lobos de Afuera Islands (Ramírez & de la Cruz 2015).

This wealth of research was, however, undertaken with a diversity of objectives and methodologies, hindering the combined analysis of data from multiple studies and precluding interspecific comparisons. Several studies were part of stock assessment surveys and protocols for monitoring local *Pollicipes* species fisheries (e.g. Ramírez & de la Cruz 2015, Bingham 2016, Bidegain et al. 2017), aiming to provide baseline data on temporal or spatial comparisons of abundance (e.g. de la Cruz et al. 2001, Borja et al. 2006a, Menge et al 2011), which can support the evaluation of the resource status (e.g. Cruz et al. 2015c, de la Cruz et al. 2015b) and the effects of management strategies (e.g. Borja et al. 2006b, Cruz et al 2008). Due to the role of *Pollicipes* as habitat-forming species, qualitative or quantitative information on abundance has also been important for the conservation of priority areas (Rubidge et al. 2020, Neves 2021).

The more standardized methodologies (*Pollicipes polymerus* – Lauzier 1999a; *P. pollicipes* – Sousa et al. 2013; *P. elegans* – Kameya & Zeballos 1988, Samamé & Quevedo 2001) were based on the relative density/biomass per size class, taken from a series of destructive samples, and then corrected by the total area of *Pollicipes* species spatial coverage, allowing calculation of the overall size of a population, or the proportion of its harvestable fraction. As an alternative approach, the integration of local ecological knowledge (LEK) on scientifically based assessments and management plans has been proposed for the First Nations' fishing territories of *Pollicipes polymerus* in British Columbia, namely through the inclusion of estimates of harvestable biomass provided by experienced harvesters (Lessard et al. 2003, Gagne et al. 2016). In fact, experienced harvesters of *Pollicipes* species possess much relevant empirical knowledge and wisdom about traditional issues related to harvesting locations, conditions and techniques. They often develop visual skills to estimate the availability of the resource and detect its population changes within localities where they usually operate; thus, their involvement in management decisions and compliance should be encouraged (Gagne et al. 2016 and references therein). Therefore, by adapting knowledge transfer procedures in the context of other *Pollicipes* species fisheries, LEK can be acquired as quantifiable information to supplement scientific data. LEK could be a potentially useful tool for assessing abundance in fragmented three-dimensional habitats (such as crevices/caves) and for overcoming the challenges of surveying subtidal areas or remote and risky intertidal areas.

Recently, other promising approaches have been developed to estimate the extent of intertidal areas occupied by *Pollicipes* species using advanced technological tools, namely GPS mapping handheld devices (Gagne et al. 2016) or unmanned aerial vehicles (drones) (Neves 2021). The feasibility of using drones equipped with high-resolution digital cameras to quantify the abundance of *Pollicipes pollicipes* has been tested on several shores in central and SW Portugal (see Neves 2021 for the Berlengas Nature Reserve). Drone imagery has proven successful in covering large spatial extents in a timely manner, with sufficient resolution to assess *Pollicipes pollicipes* populations in extreme environments, such as wave-swept rocky shores located in islets of difficult and dangerous access (TC and DJ pers. obs.). Stalked barnacle percentage cover was estimated from images collected through a low-altitude (<5 m) drone (DJI phantom 4+) and compared with estimates derived from photo-quadrats (25 × 25 cm and 50 × 50 cm). These two methods described similar spatial patterns of abundance of *Pollicipes pollicipes*, demonstrating the suitability of drone imagery as an alternative method to study the intertidal distribution of this species (Neves 2021). In other regions and for the other *Pollicipes* species, this new technology-based approach should be tested, ground-truthed with *in situ* sampling, and, where appropriate, validated with LEK-based approaches, to obtain multiple abundance estimates at relevant scales, which could hopefully inform stock assessment and management, as well as allow adequate intra- and interspecific comparisons.

Associated taxa

We have reviewed the biological assemblages associated with each of the *Pollicipes* species, considering taxa (nomenclature following WoRMS Editorial Board (2021)) that have been described as co-occurring with *Pollicipes* species in the same habitat (i.e. every taxon whose presence was observed in overlapping distribution ranges, or forming an ecological interaction with a *Pollicipes* species – see Table 4 and associated taxa table in supplementary material which presents bibliographic references and geographic region of records for each taxon). The following co-occurring categories were defined: (1) “Accompanying” – taxa interspersed with specific *Pollicipes* species at the same vertical intertidal level(s) and/or microhabitat(s), with the symbol (+) applied to those accompanying taxa registered in greatest abundance and/or referred to as “dominant” in the source texts; (2) “Near limits – Upper and Lower intertidal limit or Adjacent microhabitats” – taxa, respectively, present at or near the vertical or horizontal limits of *Pollicipes* species and interspersed among *Pollicipes* species at those limits; (3) “Endozoic and Epizoic” – taxa, respectively, found to be resident on the interior or the exterior of a *Pollicipes* species; (4) “Predators” – taxa found to prey upon a *Pollicipes* species (Table 4).

The inclusion of taxa in Table 4 required information, either qualitative or quantitative, concerning the association of those taxa with *Pollicipes* species. Consequently, studies presenting just lists of taxa for a given shore/region, with inclusion of a *Pollicipes* species but lacking any other information on co-occurrence, were not included. We have included the references from the “Ecological Habitat” section of Barnes’ (1996) review, studies made since Barnes (1996) and some relevant references prior to Barnes (1996) (some of these also considered in other sections of Barnes 1996). The references presented in Table 4 were all based on non-destructive sampling procedures. The presence of every taxon was visually recorded within the same sampling areas where a *Pollicipes* species was found, but information on abundance was not provided among the whole set of taxa references.

Table 4 Co-occurring category, taxonomic group and taxa associated with *Pollicipes* species (scientific names following WoRMS Editorial Board 2021), considering non-destructive surveys of biological assemblages

Accompanying	Near distribution limits	Endozoic/epizoic and predators
Pollicipes polymerus		
Rhodophyta (Red algae): *Callithamnion pikeanum, Corallina vancouveriensis* (+)*, Endocladia muricata,* genus *Lithophyllum,* genus *Polysiphonia,* genus *Porphyra, Halosaccion glandiforme, Mastocarpus papillatus, Neorhodomela larix, Plocamium violaceum, Pyropia pseudolanceolata* Ochrophyta (Brown algae): genus *Alaria/Alaria marginata, Hedophyllum sessile, Postelsia palmaeformis* Chlorophyta (Green algae): genus *Ulva* Porifera (Sponges): genus *Haliclona, Halichondria (Halichondria) panicea* Cnidaria, Anthozoa (Anemones): *Anthopleura elegantissima, Anthopleura xanthogrammica* Bryozoa: Phylum *Bryozoa* Mollusca, Polyplacophora (Chitons): *Dendrochiton flectens, Katharina tunicata, Tonicella lineata* Mollusca, Gastropoda (Limpets): *Lottia austrodigitalis, Lottia digitalis, Lottia pelta, Lottia strigatella* Mollusca, Bivalvia (Mussels and other bivalves): *Mytilus californianus* (+)*, Mytilus trossulus, Penitella penita* Echinodermata, Echinoidea (Urchins): *Strongylocentrotus purpuratus* Echinodermata, Asteroidea (Seastars): *Leptasterias hexactis* Arthropoda, Cirripedia (Barnacles): *Balanus glandula* (+)*, Balanus nubilus, Chthamalus dalli, Chthamalus fissus, Megabalanus californicus, Tetraclita rubescens, Semibalanus cariosus* (+)	Rhodophyta (Red algae): *Endocladia muricata* (*A*) Ochrophyta (Brown algae): genus *Fucus* (*U*), genus *Pelvetiopsis* (*U*)*, Postelsia palmaeformis* (*U*) *Egregia menziesii* (*A*)*,* genus *Alaria/Alaria marginata* (*A*)*, Nereocystis luetkeana* (*A*)*, Postelsia palmaeformis* (*A*) Tracheophyta (Seagrasses): *Phyllospadix scouleri* (*A*) Cnidaria, Anthozoa (Anemones): *Anthopleura xanthogrammica* (*A*) Mollusca, Bivalvia (Mussels and other bivalves): *Mytilus trossulus* (*L*) Echinodermata, Echinoidea (Urchins): *Strongylocentrotus purpuratus* (*A*) Echinodermata, Asteroidea (Seastars): *Pisaster ochraceus* (*L, A*) Arthropoda, Cirripedia (Barnacles): *Balanus glandula* (*U*)*, Chthamalus dalli* (*U*)*, Chthamalus fissus* (*U*) *Semibalanus cariosus* (*L*)*, Tetraclita rubescens* (*L*)	*Endozoic* Ascomycota, Dothideomycetes (Fungi): *Pharcidia balani* *Epizoic* Chlorophyta (Green algae): Unidentified filamentous green alga(e) Mollusca, Gastropoda (Limpets): *Lottia austrodigitalis, Lottia digitalis, Lottia pelta, Lottia strigatella, genus Notoacmaea* Arthropoda, Cirripedia (Barnacles): *Chthamalus fissus* *Predators* Mollusca, Gastropoda, Dogwhelks: *Nucella canaliculata, Nucella emarginata, Nucella lamellosa* Nemertea, Hoplonemertea (Ribbon worms): *Emplectonema gracile* Echinodermata, Asteroidea (Seastars): *Leptasterias hexactis, Pisaster ochraceus* Chordata, Aves (Birds): *Aphriza virgata, Corvus brachyrhynchos*/genus *Corvus, Haematopus bachmani, Larus glaucescens, Larus occidentalis*

References: Feder (1959) *in* Paine (1980), Giesel (1968), Jobe (1968), Giesel (1969, 1970), Dayton (1971), Paine (1974), Murphy (1978), Brym (1980), Paine (1980), Hartwick (1981), Paine & Levin (1981), Vermeer (1982), Hoffman (1984), Palmer (1984), Mercurio et al. (1985), Marsh (1986), Page (1986), West (1986), Austin (1987) in Lauzier (1999b), Bernard (1988), Byers (1989), Hoffman (1989), Lindberg & Pearse (1990), Wootton (1992), Meese (1993), Wootton (1994), References *in* Barnes (1996), Wootton (1997), Sanford (1999), Jamieson et al. (2001) (Appendix A – Site descriptions), Kay (2002), Crummett & Eernisse (2007), Wootton (2010), Menge et al. (2011), Murphy (2014), Bingham (2016).

(+): accompanying taxa registered in greatest abundance.

(*U, L, A*): taxa, respectively, present at or near the upper/lower limits of *Pollicipes* species or in adjacent microhabitats.

(*Continued*)

Table 4 (*Continued*) Co-occurring category, taxonomic group and taxa associated with *Pollicipes* species (scientific names following WoRMS Editorial Board 2021), considering non-destructive surveys of biological assemblages

Accompanying	Near distribution limits	Endozoic/epizoic and predators
Pollicipes pollicipes		
Rhodophyta (Red algae): *Asparagopsis armata, Callithamnion granulatum, Chondrus crispus, Corallina officinalis* (+), *Ellisolandia elongata, Gelidium corneum,* genus *Ceramium,* genus *Polysiphonia,* "*Lithothamnia*" *sensu Hawkins & Jones 1992/Lithophyllum incrustans* (+), *Lomentaria articulata, Mastocarpus stellatus, Nemalion elminthoides, Osmundea pinnatifida, Plocamium cartilagineum, Pterocladiella capillacea, Tenarea tortuosa* Ochrophyta (Brown algae): *Caulacanthus ustulatus/genus Caulacanthus, Ralfsia verrucosa* Chlorophyta (Green algae): genus *Ulva* Porifera (Sponges): genus *Hymeniacidon* Cnidaria, Hydrozoa: class Hydrozoa Cnidaria, Anthozoa (Anemones): *Actinia equina* Mollusca, Gastropoda (Periwinkles, Dogwhelks, Limpets): *Melarhaphe neritoides, Nucella lapillus,* genus *Patella, Patella depressa, Patella ulyssiponensis* (+), *Siphonaria pectinata* Mollusca, Bivalvia (Mussels): *Mytilus galloprovincialis* (+), *Perna perna* Echinodermata, Echinoidea (Urchins): *Paracentrotus lividus* Echinodermata, Asteroidea (Seastars): *Marthasterias glacialis* Arthropoda, Cirripedia (Barnacles): *Chthamalus montagui, Chthamalus stellatus, Megabalanus tintinnabulum, Perforatus perforatus*	Rhodophyta (Red algae): *Corallina officinalis* (*U, L*), "*Lithothamnia*" *sensu* Hawkins & Jones 1992/ *Lithophyllum incrustans* (*U, L*), genus *Ceramium* (*U, L*) *Nemalion elminthoides* (*U*), genus *Polysiphonia* (*U*) *Asparagopsis armata* (*L*), *Plocamium cartilagineum* (*L*), *Tenarea tortuosa* (*U*) Ochrophyta (Brown algae): *Caulacanthus ustulatus* (*U*), *Ralfsia verrucosa* (*U*) genus *Cystoseira* (*L*), genus *Fucus* (*L*), genus *Laminaria* (*L*), *Saccorhiza polyschides* (*L*) Chlorophyta (Green algae): *Codium tomentosum* (*L*) Cnidaria, Anthozoa (Anemones): *Anemonia viridis* (*A*) Mollusca, Gastropoda (Limpets and Periwinkles): *Patella ulyssiponensis* (*U, L*) *Melarhaphe neritoides* (*U*), *Patella depressa* (*U*), *Siphonaria pectinata* (*U*) Mollusca, Bivalvia (Mussels): *Mytilus edulis* (*U*), *Mytilus galloprovincialis* (*U*) Echinodermata, Echinoidea (Urchins): *Paracentrotus lividus* (*L, A*) Arthropoda, Cirripedia (Barnacles): *Chthamalus montagui* (*U*), *Chthamalus stellatus* (*U*) *Megabalanus tintinnabulum* (*L*)	*Endozoic* Rhodophyta (Red algae): Unidentified endolithic alga, *Porphyra* sp. (*Conchocelis* phase) *Epizoic* Rhodophyta (Red algae): *Corallina officinalis,* "*Lithothamnia*" *sensu* Hawkins & Jones 1992, *Tenarea tortuosa* Ochrophyta (Brown algae): *Caulacanthus ustulatus, Ralfsia verrucosa* Chlorophyta (Green algae): genus *Ulva* Bryozoa: Phylum *Bryozoa* Mollusca, Bivalvia (Mussels): *Mytilus galloprovincialis* Arthropoda, Cirripedia (Barnacles): *Chthamalus dentatus, Chthamalus montagui, Perforatus perforatus* Mollusca, Gastropoda (Limpets): *Patella ulyssiponensis* *Predators* Mollusca, Gastropoda (Dogwhelks): *Nucella lapillus* Annelida, Polychaeta (Worms): *Eulalia viridis* Echinodermata, Asteroidea (Seastars): *Marthasterias glacialis* Chordata, Aves (Birds): *Larus michahellis* Chordata, Pisces (Fishes): genus *Diplodus/Diplodus sargus* *Balistes capriscus*

References: Broch (1927), References in Barnes (1996), Cruz (2000), Macho (2006), Fernandes et al. (2010), Moreno et al. (2010), Boukaici et al. (2012, 2015), Pedro (2017), Neves (2021), Personal observations.

(+): accompanying taxa registered in greatest abundance.

(*U, L, A*): taxa, respectively, present at or near the upper/lower limits of Pollicipes species or in adjacent microhabitats.

(*Continued*)

Table 4 (*Continued*) Co-occurring category, taxonomic group and taxa associated with *Pollicipe*s species (scientific names following WoRMS Editorial Board 2021), considering non-destructive surveys of biological assemblages

Accompanying	Near distribution limits	Endozoic/epizoic and predators
Pollicipes elegans		
Chlorophyta (Green algae): genus *Chaetom*orpha (+) Mollusca, Polyplacophora (Chitons): *Acanthopleura echinata, Chiton (Chiton) granosus, Enoplochiton niger* Mollusca, Gastropoda (Limpets and Trochids): genus *Fissurella* *Tegula atra* Mollusca, Bivalvia (Mussels): *Modiolus capax* (+)*, Perumytilus purpuratus* (+), *Semimytilus algosus* (+) Annelida, Polyc`haeta (Worms): *Nereis grubei* Arthropoda, Decapoda (Crabs): *Acanthocyclus gayi* Echinodermata, Holothuroidea (Sea cucumbers): *Pattalus mollis*	Rhodophyta (Red algae): *Gymnogongrus durvillei* (*U*) Mollusca, Gastropoda (Periwinkles): *Echinolittorina peruviana* (*U*) Arthropoda, Cirripedia (Barnacles): *Austromegabalanus psittacus* (*L*) Arthropoda, Decapoda (Crabs): *Acanthocyclus gayi* (U) Echinodermata, Asteroidea (Seastars): *Heliaster helianthus* (*L*)*, Stichaster striatus* (*L*)	*Epizoic* Chlorophyta (Green algae): genus *Chaetom*orpha *Predators* n/a
References: Kameya & Zeballos (1988) (Figure 3, adapted from Paredes 1974), Barraza et al. (2014).		
Pollicipes caboverdensis		
Chlorophyta (Green algae): genus *Chaetom*orpha (+)	n/a	*Epizoic* Chlorophyta (Green algae): genus *Chaetom*orpha *Predators* n/a

References: Fernandes et al. (2010).

(+): accompanying taxa registered in greatest abundance.

(*U, L, A*): taxa, respectively, present at or near the upper/lower limits of *Pollicipes* species or in adjacent microhabitats.

n/a: information not available.

Information organized by taxon (with bibliographic references and geographic region of records) is presented in supplementary material (associated taxa table).

From the total of 126 taxa mentioned in Table 4, the great majority were associated with *Pollicipes polymerus* (45%) and *P. pollicipes* (40%), with only 13% recorded with *P. elegans* and 1% with *P. caboverdensis*. This reflects the higher number of ecological studies on the two former species and their associated assemblages, compared with much less research effort expended on the other two species. The overall biological composition of *Pollicipes* species assemblages comprised the following categories: 1% fungi, 40% macrophytes, 2% Porifera, 4% Cnidaria, 2% Bryozoa, 1% Nemertea, 2% Annelida, 25% Mollusca, 12% Crustacea, 6% Echinodermata and 6% Chordata.

Apart from *Pollicipes caboverdensis* habitat, mussels were invariably described as the dominant species, and accompanying mussel clusters are a characteristic element of these communities (Table 4, Figure 3). Acorn barnacles were also consistently present as major co-occurring filter-feeders in the habitats of *Pollicipes* species (Table 4, Figure 3). Calcareous turfs and crusts were both abundant in association with *Pollicipes polymerus* and *P. pollicipes*, and a diverse assemblage

Figure 3 *Pollicipes* species in natural habitats. (A) *Pollicipes polymerus* (courtesy of Jesús Pineda); (B) panoramic view of *Pollicipes pollicipes* in Berlenga (Portugal); (C) *Pollicipes elegans* (Jicalapa, La Libertad, El Salvador, www.inaturalist.org/observations/69057855); (D) *Pollicipes caboverdensis* (Santiago Island, Cape Verde). Approximate scale presented when appropriate.

of foliose red and brown algae is also present in the habitats of both species (Table 4). Thus, not surprisingly, the assemblages described are typical of wave-exposed and very wave-exposed steep rocky shores in the regions where *Pollicipes* species occur. Remarkably, the green alga, *Chaetomorpha*, was recorded as a very conspicuous element of the habitat of both *Pollicipes elegans* in El Salvador and *P. caboverdensis* in Sal and Santiago islands (Table 4). Several sessile taxa, usually abundant in these assemblages, have been frequently observed as epizoics of large specimens of *Pollicipes* (Table 4). Brym (1980) recorded several biofilm and algal items when scrutinizing the surfaces of *Pollicipes polymerus* capitula, while Hoffman (1989) recorded "epiphytic algae" attached to their peduncles; both studies were carried out in California. In *Pollicipes pollicipes* from Morocco, Broch (1927) noted external colonization by "algae, bryozoans and lamellibranchs" on the peduncle, and by *Perforatus perforatus* on the capitulum.

North-eastern Pacific limpets, mostly belonging to the genus *Lottia*, have frequently been recorded living on the capitular plates (usually, the scutum is the limpet's home scar) of *Pollicipes polymerus* and on nearby rock surfaces (Table 4). The association of *Pollicipes polymerus* with the fingered limpet, *Lottia digitalis,* and its sibling species, the southern finger limpet, *L. austrodigitalis,* has been widely documented along the west coast of North America (associated taxa table in supplementary material). The patterns of shell colour and shape of these two limpet species, when living epizoically on *Pollicipes polymerus,* were described as identical to those of the barnacle's plates (see Section 'Post-settlement processes of distribution and abundance'). North-eastern Atlantic limpets, mostly belonging to the genus *Patella*, have also been described as common inhabitants on rocky intertidal habitats where *Pollicipes pollicipes* occurs (Table 4) and can be seen moving on *P. pollicipes* capitula (Cruz 2000, Figure 12). Limpet populations, surveyed within areas comprising *Pollicipes pollicipes* clumps in the Cape of Sines (SW Portugal), were mostly found on rock surfaces, located underneath or adjacent to *P. pollicipes* peduncles, and were mainly composed of *Patella ulyssiponensis* (95% of 383 individuals), with approximately half of the total number of limpets (52%) being juveniles (maximum shell length lower than or equal to 10 mm) (Cruz et al. unpublished data).

Predation of *Pollicipes polymerus* and *P. pollicipes* by birds (mostly gulls) and dogwhelks (muricid snails of the genus *Nucella*) has been clearly documented (Table 4). Starfish were found to prey upon *Pollicipes polymerus* (*Pisaster ochraceus*: references *in* Lauzier 1999b and Jamieson et al. 2001; *Leptasterias hexactis*: Wootton 1994) and *Pollicipes pollicipes* in SW Portugal (*Marthasterias glacialis*, TC pers. obs., see Figure 11), and to occur close to the lower vertical limit of *Pollicipes elegans* in Peru (*Stichaster striatus* and *Heliaster helianthus*, Table 4).

The infection of *Pollicipes polymerus* by the ascomycete *Pharcidia balani* (Table 4), also known as *Didymella conchae*, might be dubious, as it was reported by Giesel (1968) apparently based only on the texture of capitular plates and on previous references to parasitized limpets in California (Test 1945), but the genus *Pollicipes* was indeed described as one of many hosts of this endozoic fungus (Kohlmeyer & Kohlmeyer 2013). Endolithic algae have been also reported to infect peduncular scales and capitular plates of *Pollicipes pollicipe*s (Table 4, Drew & Richards 1953).

The faunal diversity associated with *Pollicipes* species is not fully considered in Table 4. In fact, the infauna, as well as part of the epifaunal community, living within *Pollicipes* species and their accompanying mussel clusters is characterized by many invertebrates, particularly inconspicuous species of small size and/or cryptic behaviour. Barnes & Reese (1960) stated that dense aggregates formed by *Pollicipes polymerus* and *Mytilus californianus* provide shelter for a great number of animals, in particular crustaceans, flat worms and annelids. A similar richness of invertebrate groups (crabs, amphipods and polyclad flatworms, as well as opisthobranch molluscs and nemerteans) residing within *Pollicipes polymerus* clumps was found by Hoffman (1989), who emphasized their possible role as predators of young barnacles. When disturbed, numerous specimens of an unidentified isopod, possibly belonging to the genus *Dynamenella*, emerged from their hiding places within the Nakwakto *Pollicipes polymerus* clusters (Lamb & Hanby 2005). Likewise, huge numbers of amphipods are also commonly encountered while collecting *Pollicipes pollicipes* clumps in SW Portugal (TC pers. obs.). Extensive faunal lists have been presented in two studies, where destructive samples were collected from *Pollicipes polymerus* (Vancouver Island, Canada – Jamieson et al. 2001 "Appendix B") and *P. elegans* (Yasila to Chilca, Peru – Kameya & Zeballos

1988 "Tabla 2") assemblages. Both studies recorded many polychaete taxa (Kameya & Zeballos 1988, Jamieson et al. 2001), previously described as potentially active predators on newly settled *Pollicipes polymerus* (Bernard 1988). The following taxa were recorded by Jamieson et al. (2001) as "species predominating numerically" (>1000 individuals/species collected, 85% of all individuals found) within the *Pollicipes polymerus-Mytilus californianus* matrix: the small holothurid *Cucumaria pseudocurata*; the snail *Lacuna vincta*, the eelgrass limpet *Lottia alveus* and other small-sized gastropods of the genera *Amphissa* and *Margarites*; the isopod *Cirolana harfordi*, the amphipod genera *Corophium* and *Hyale*, and porcelain crabs such as *Petrolisthes cinctipes* and the genus *Pachycheles*. Small crabs were also observed by Bernard (1988), who suggested them as potential predators of *Pollicipes polymerus*. Additional taxa associated with *Pollicipes elegans* were the predatory gastropod *Concholepas concholepas*, the anemone *Phymactis clematis* and the brittle star *Ophiactis kroeyeri* (Kameya & Zeballos 1988).

It should be noted that observations on the accompanying fauna of *Pollicipes elegans* in Peru by Kameya & Zeballos (1988) were made in 1985, following an El Niño event (1982–1983), and included locations where this species was previously absent (the more southern surveyed sites). A remarkable population increase in *Pollicipes elegans* and a major change in marine community composition were documented along this coast during the El Niño of 1982–1983 (e.g. Tarazona et al. 1985, references in Arntz et al. 2006), possibly affecting local occurrence patterns and hence ecological relationships. Finally, all taxon references that are presented in Table 4 regarding Jamieson et al. (2001) and Kameya & Zeballos (1988) were, respectively, retrieved from "Appendix A – Site descriptions" and "Figure 3, adapted from Paredes 1974", or from the text of methods or discussion sections, and not based on results derived from destructive sampling procedures.

Further information on the co-occurring species of *Pollicipes pollicipes* is expected in the near future, as relevant data (e.g. destructive samples of aggregates and time-series photographs of habitat area) were recently collected from intertidal shores in SW Portugal, Galicia, Asturias and Brittany, under the scope of the European project PERCEBES promoted by BiodivERsA research network (Acuña et al. 2020).

Description of adults

At the time of the Barnes (1996) review, the genus *Pollicipes* included three species (*P. polymerus*, *P. elegans* and *P. pollicipes*) (Foster 1979, Newman & Killingley 1985, Newman 1987). These three species were those that, according to Darwin (1852), "form one thoroughly natural genus" from his list of six originally described species (see section 'Systematics and taxonomy'). Surprisingly, it was only in 2010 that a fourth species of *Pollicipes* was described as an addition to Darwin's original list – *Pollicipes caboverdensis,* endemic to the Cape Verde Islands (Fernandes et al. 2010). This surprise was even greater given that *Pollicipes caboverdensis* is an exploited species with commercial value (Fernandes et al. 2010, Cruz et al. 2015a). Moreover, Darwin visited Cape Verde in 1832 (Darwin 1839), at the beginning of the voyage of the Beagle. Darwin spent 23 days on the island of Santiago in Cape Verde and walked along its coastline (Darwin 1839, Vala 2009), but apparently only near the port of Praia In the coastal zone of the city of Praia, *Pollicipes caboverdensis* does not seem to occur today (TC and JNF pers. obs.). This species is mainly abundant in the northern part of Santiago Island (Baessa 2015). Moreover, Darwin's great interest in barnacles only began later, after he collected an empty 'loco' shell (*Concholepas concholepas*), covered with hundreds of millimetre perforations, on a beach in Chile in 1835 (Stott 2003, Castilla 2009). The small organisms living inside those orifices were shell burrowing barnacles (*Cryptophialus minutus*, 'Mr. Arthrobalanus'), considered to mark Darwin's taxonomical interest in Cirripedia (Stott 2003, Castilla 2009). After returning to England, specimens of barnacles from around the world were posted to Darwin by an army of collectors, friends, missionaries, naturalists, mineralogists and shell collectors (Stott 2003), but probably, no *Pollicipes* were sent to him from the Cape Verde Islands. Given Darwin's extraordinary observation skills, we can speculate that, if he had seen specimens of *Pollicipes caboverdensis*, he would have realized it was a different species.

We have illustrated the four species of the genus *Pollicipes* together for the first time (Figure 4). Externally, as in all pedunculate cirripedes, the capitulum can be distinguished from the peduncle, which has an uncalcified basis. The capitulum is formed by a series of plates composed of calcite: the paired plates – scutum and tergum; the unpaired plates – carina and rostrum; and several other plates of different sizes which may be paired (e.g. lateral or median latus, rostrolatus, carinolatus) or unpaired (subcarina, subrostrum) (nomenclature of plates according to Newman 1987 and Fernandes et al. 2010, see Figure 5). We follow the definition of axes and sides of Anderson (1994): the long axis of the animal is baso-apical; the axis perpendicular to this is rostrocarinal; capitular valves can be identified as right and left relative to carina (dorsally) and the peduncle (anteriorly). The peduncle is narrower and generally longer than the capitulum, consisting of small calcareous scales or spicules arranged in an elastic organic matrix (Chaffee & Lewis 1988). The size of these calcareous scales varies along the peduncle, due to the addition of new scales occurring from the apical end of the peduncle (Chaffee & Lewis 1988).

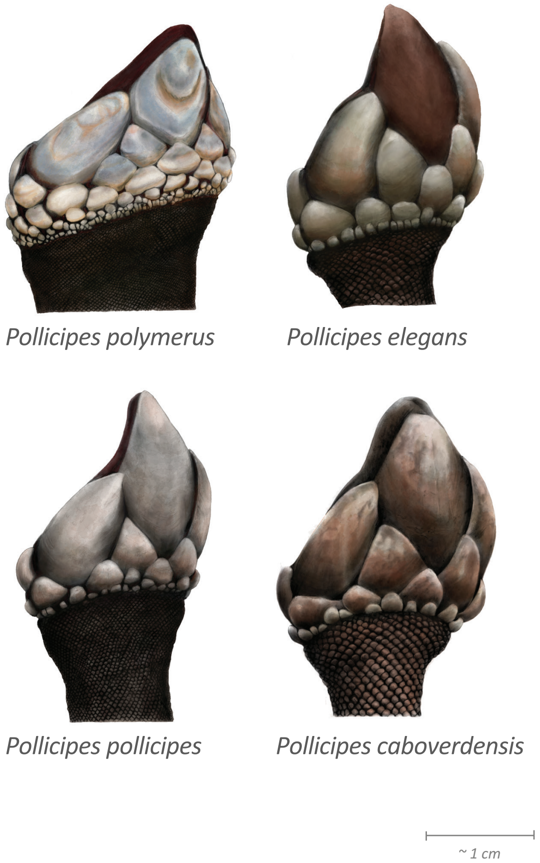


Figure 4 Colour drawing of the four species of *Pollicipes* by Cristina Espírito Santo. Specimens preserved in alcohol of *Pollicipes elegans* (Ñuro, Peru) and of *Pollicipes caboverdensis* (Tarrafal, Santiago, Cape Verde), frozen specimen of *Pollicipes pollicipes* (Berlengas, Portugal) and a photo of a fresh specimen of *Pollicipes polymerus* (California, the USA) were used for the drawings.

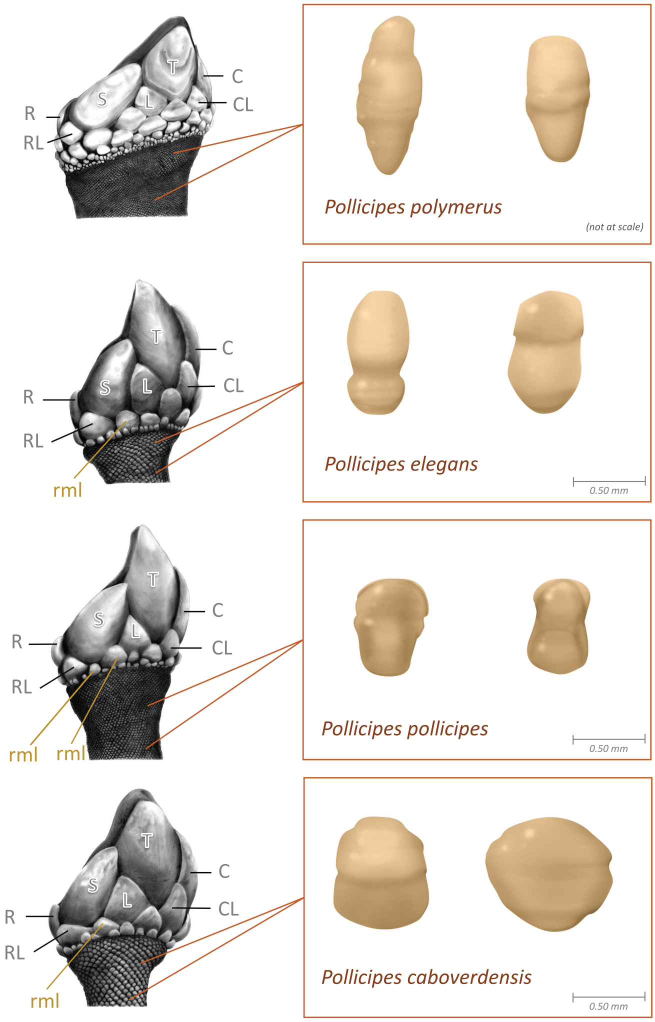


Figure 5 Illustration of the scales of the peduncle of the four species of *Pollicipes* by João Tiago Tavares (based in photos), and of capitular plates of the four species of *Pollicipes* (drawing by Cristina Espírito Santo). Plates: R – rostrum; C – carina; L – median latus; S – scutum; RL – rostrolatus; CL – carinolatus; RML – rostral median latus. Nomenclature of Newman 1987 and Fernandes et al. 2010.

Based on Darwin (1852) and other studies carried out mainly in the first half of the twentieth century (e.g. Gruvel 1905, Pilsbry 1909), Barnes (1996) described in detail the external and internal morphological characteristics of the three *Pollicipes* species known at that time. Herein, we review the diagnostic characteristics of the new order Pollicipedomorpha and the family Pollicipedidae according to Chan et al. (2021); the diagnostic characteristics of the genera *Capitulum* and *Pollicipes* based on Van Syoc (1995) (not cited by Barnes 1996), and the diagnostic characteristics of the four *Pollicipes* species taken from Van Syoc (1995), Fernandes et al. (2010) and Quinteiro et al. (2011) (Table 5, Figures 4 and 5). These diagnostic characteristics are mostly related to external morphology. Regarding internal morphology, besides Barnes' (1996) review of *Pollicipes polymerus*, *P. elegans* and *P. pollicipes*, Fernandes et al. (2010) and Quinteiro et al. (2011) described some aspects of the internal morphology of *P. caboverdensis*. However, no comparative analysis of the internal morphology of the four species has been made.

Table 5 Morphological diagnosis of the four *Pollicipes* species within the new order Pollicipedomorpha (Chan et al. 2021)

Taxa	Diagnosis	Reference
Order Pollicipedomorpha	Capitulum includes a large number (20– 40+) of imbricating, secondary lateral plates, which decrease in size towards the basal margin.	Chan et al. (2021)
Family Pollicipedidae	Small accessory lateral plates present on basal lateral surfaces of capitulum.	Chan et al. (2021)
Genus *Pollicipes*	Filamentary processes.	Van Syoc (1995)
	Lower latera of different sizes.	Van Syoc (1995)
	Tuft of spines at the end of caudal appendages.	Van Syoc (1995)
	Scutum is not triangular.	Van Syoc (1995)
	Carina with sub-equal diamond shape.	Van Syoc (1995)
Pollicipes polymerus (Figure 5)	More than a single row/whorl of capitulum plates below sublatera/subrostrum.	Van Syoc (1995) & TC pers. observations
	Uni-articulate caudal appendages.	Van Syoc (1995) & Barnes (1996)
	Peduncle scales pointed outwards rather than up towards capitulum.	Van Syoc (1995)
	Peduncle scales spine-like or spindle shaped.	Van Syoc (1995) & Barnes (1996)
Pollicipes pollicipes, *Pollicipes elegans* & *Pollicipes caboverdensis* (Figures 5 and 6)	A single row/whorl of capitulum plates below sublatera/subrostrum.	Van Syoc (1995) & Fernandes et al. (2010)
	Multi-articulate caudal appendages.	Van Syoc (1995), Barnes (1996) & Fernandes et al. (2010)
	Peduncle scales pointed up towards capitulum.	Van Syoc (1995) & Fernandes et al. (2010)
	Peduncle scales not spine-like or spindle shaped.	Van Syoc (1995) & Fernandes et al. (2010)
Pollicipes pollicipes (Figures 5 and 6)	Rostrolatus and carinolatus are more separated from median latus than in *P. elegans* and *P. caboverdensis*.	Newman (1987) & TC pers. observations
	Two plates between each median latus and each rostrolatus named as rostral median latus by Fernandes et al. (2010) (named "r" plates by Newman 1987, imbricating plates added between rostrum and median latus).	Fernandes et al. (2010)
	Colour of capitular plates more whitish-grey than in *P. elegans* and *P. caboverdensis*.	Fernandes et al. (2010)
	Peduncle scales narrower than in *P. caboverdensis,* sub-equal oval shaped, with a smaller width than height.	Fernandes et al. (2010)
Pollicipes elegans (Figures 5 and 6)	Rostrolatus and carinolatus are less separated from median latus than in *P. pollicipes*.	Newman (1987)
	A single pair of rostral median latus.	Fernandes et al. (2010)
	Colour of capitular plates more reddish-orange than in *P. pollicipes.*	Fernandes et al. (2010)
	Peduncle scales narrower than in *P. caboverdensis,* sub-equal oval shaped, with a smaller width than height.	Fernandes et al. (2010)

(Continued)

Table 5 (*Continued*) Morphological diagnosis of the four *Pollicipes* species within the new order Pollicipedomorpha (Chan et al. 2021)

Taxa	Diagnosis	Reference
Pollicipes caboverdensis (Figures 5 and 6)	Rostrolatus and carinolatus are less separated from median latus than in *P. pollicipes.*	TC and JNF pers. observations
	A single pair of rostral median latus.	Fernandes et al. (2010)
	Colour of capitular plates more reddish-orange than in *P. pollicipes.*	Fernandes et al. (2010)
	Peduncular scales about the same width as height or peduncle scales wider than long.	Fernandes et al. (2010) Quinteiro et al. (2011)

The following basic description of the internal morphology of *Pollicipes* is based on Anderson (1994), Molares (1994) and Barnes (1996). The capitulum is formed by a bivalved carapace that encloses the body and limbs (cirri) of the barnacle. The opening of the capitular valves along one margin is the aperture of the capitulum. The cavity enclosed by the capitular valves and housing the body and cirri is the mantle cavity. The inner lining of the capitular valves is a respiratory surface (the mantle). The body consists of the prosoma, the thorax and a vestigial abdomen. The prosoma is an extension of the thorax and contains the stomach and the mouth parts (labrum, mandibular palps, mandibles, maxillule and maxillae). The thoracic limbs are biramous (see discussion on section 'Cirral morphology and feeding behaviour' regarding the identification of the maxillipeds), with the first pair modified into a maxilliped, the function of which is to transfer food from the cirri to the mouth. Associated with these structures are several groups of salivary glands. The prosoma is confluent with the capitular plates at the level of the inner face of the scutum, through an adductor muscle. Behind the prosoma, the remaining five pairs of biramous thoracic limbs, cirri II–VI, form the captorial feeding apparatus (see section 'Cirral morphology and feeding behaviour'). The digestive tract extends from the mouth to the anus, the latter situated between the bases of cirri VI. Parallel and posterior to the digestive tract paired seminal vesicles extend, to which numerous testicles are attached. The testicles are scattered in the connective tissue of the prosoma and thorax, and in the filamentary appendages. The filamentary appendages are projections of the prosoma and thorax. The seminal vesicles join at their dorsal ends to form the penis. On each side of the anus, just above the penis, are two small projections, the caudal appendages, which may be uni- (*Pollicipes polymerus*) or multi-articulate (*P. elegans*, *P. pollicipes* and *P. caboverdensis*) (Table 5). The caudal appendages, anus and penis form the vestigial abdomen. The interior of the peduncle consists externally of layers of circular and longitudinal muscles. The adhesive gland (see section 'Adhesion and cement') and the ovary are located central to these muscles. A pair of oviducts pass apically along the rostral side of the peduncle and mantle cavity, before entering the prosoma and opening at the bases of the first pair of cirri.

Considerable intraspecific morphological variation can be observed in *Pollicipes*, such as in the colour and form of the capitular plates, the colour of the capitular aperture, the colour and shape of the peduncle, and the morphology of the cirri (see section 'Cirral morphology and feeding behaviour'). Regarding intraspecific colour variation of the capitulum, in *Pollicipes caboverdensis*, the reddish-orange colour of the capitular plates, which easily separates *P. caboverdensis* from *P. pollicipes* and *P. polymerus* (Figure 4, Table 5), is not always evident in small individuals (Fernandes et al. 2010). Additionally, in *Pollicipes caboverdensis* and *P. elegans*, some plates such as the tergum (see illustration of *P. elegans* in Figure 4) may, in some specimens, have a brighter orange colour than the other plates (TC pers. obs.). Finally, alterations in the morphology of juvenile *Pollicipes pollicipes* maintained in laboratory conditions and mostly fed with *Artemia* sp. nauplii, compared with wild individuals, were observed (e.g. pink capitular plates, plate decalcification and plate deformation) (Franco 2014). These changes may have been caused by diet (Franco 2014).

The capitular aperture may also show considerable intraspecific colour variation. Darwin (1852) noted that the edges of the capitular aperture (mantle edges) in *Pollicipes pollicipes* “are widely bordered by membrane, coloured fine crimson red”. The red-coloured mantle edge of *Pollicipes pollicipes* is common in individuals from the low shore and subtidal zone, whereas barnacles from the upper shore have mantle edges with a brownish colour (Cardoso 1998, TC pers. obs.). The red mantle edge was also noted in *Pollicipes polymerus* by Darwin (1852) and in subtidal specimens of *P. polymerus* found in Nakwakto Rapids (British Columbia, Canada) (Nakwakto gooseneck barnacles), where their large and dense clumps form “spectacular formations” that display a “glorious red colour” (Lamb & Hanby 2005). This “Nakwakto variety” of *Pollicipes polymerus* has been reported in two other subtidal areas in British Columbia, namely a sea cave on Calvert Island (Brietzke et al. 2013) and Race Rocks (Fletcher 2011), with the red colour seemingly caused by the lack of black pigments that are present in sun-exposed barnacles (Lamb & Hanby 2005). Barnes & Reese (1960) noted that the development of the deep purple colour of the peduncle of *Pollicipes polymerus* seems to be, in part, dependent upon exposure to light, as the more basal parts of crowded peduncles are coloured light orange, turning black within a few days after removal of surrounding animals. According to these authors, whether the pigment is formed under the influence of light, or whether migration to the epidermis of pre-formed pigment or its precursor (the peduncular fluid is strongly pigmented) is involved, is not yet known. More research is needed to unravel the processes behind these phenotypical variations in colour.

The most obvious phenotypic variability in *Pollicipes* is probably the variation in peduncle length and water content, and the corresponding variation in condition and food quality of the barnacles. In wave-exposed situations, the peduncles of *Pollicipes polymerus* are strong, relatively short and attached over a considerable basal area, while in less wave-exposed situations, as well as among the more sheltered mussel beds, the peduncles are often greatly elongated and attached to a relatively smaller basal area (Barnes & Reese 1960). Chaffee & Lewis (1988) described a morphological variation in *Pollicipes polymerus* between stouter barnacles and slender animals. Stouter barnacles on the periphery of clusters showed marked thickening of the exoskeleton at the base of the stalk, while slender animals in the centre of clusters showed no thickening at all (Chaffee & Lewis 1988). Thicker stalks have been associated with a higher product quality in the fishery of *Pollicipes polymerus* on the west coast of Vancouver Island, Canada (Lessard et al. 2003).

Phenotypic variation of peduncular shape was also detected in *Pollicipes pollicipes* from the Iberian Peninsula, with two extreme forms recognized: large and short barnacles (Portugal, Cruz et al. 2016b), barnacles with a standard form with a smooth peduncle (Galicia, Spain, Parada et al. 2012), barnacles with a greater amount of muscle in the peduncle (Asturias, Spain, Rivera et al. 2014); versus an elongated form of thin and long barnacles (Portugal, Cruz et al. 2016b), with a wrinkled peduncle (Galicia, Spain, Parada et al. 2012). In Portugal, thin and long barnacles are called ‘*percebe mijão*’ (‘pissing’ barnacles), due to their high water content and the fact that they can squirt water when caught or eaten. The elongated form is considered to be of a low quality by the fishers and the market, having a lower commercial value (Parada et al. 2012, Sousa et al. 2021). The knowledge of the fishers to be able to classify different stretches of coast based on the quality of the barnacles has been used in a few studies (Galicia, Parada et al. 2012; Asturias, Rivera et al. 2014), as well as being used in the co-management system of *Pollicipes pollicipes* in West Asturias (Rivera et al. 2014). The commercial quality of stalked barnacles depends on the relationship between the length, width and weight of the barnacle (Molares et al. 1987) and has been measured by the ratio of the capitular base diameter to total height (Parada et al. 2012) or by the ratio between maximal rostral-carinal length and total height (Cruz et al. 2016b, Sousa et al. 2021) (lower values in low-quality barnacles). The biochemical composition of low- and high-quality *Pollicipes pollicipes* collected along the Portuguese coast was significantly different, with low-quality barnacles showing higher values of water content and lower values of fat, total protein content and energetic value (Cruz et al. 2016b). No genetic or epigenetic differences have been found to date between these two morphotypes (Sousa et al. 2021).

Thin and long barnacles tended to be at higher densities, although density may have been confounded with other factors such as hydrodynamics or predation (Cruz 2000). More research is needed to understand and disentangle the factors responsible for the phenotypic variation associated with the elongation of the peduncle and the quality of *Pollicipes pollicipes*.

Cirral morphology and feeding behaviour

Morphology and function

As in other Cirripedia Thoracica, *Pollicipes* are suspension-feeders employing six pairs of biramous thoracic appendages (cirri I–VI) to capture food items from the water (Anderson 1994). In the Thoracica, up to three of the anterior pairs of cirri can be modified to generally shorter maxillipeds that are specialized for transferring food to the mouth, with the long and slender posterior cirri forming a fan (captorial cirri), for capturing food from the water column (Chan et al. 2008). Feeding relies on the action of the captorial cirri, maxillipeds, mouth parts and the oral cone. Barnes (1996) provided a brief description of the cirri of the three species of *Pollicipes* known at that time, based on Darwin (1852) (Table 2 in Barnes 1996). She did not, however, include the detailed cirral morphology of *Pollicipes polymerus* presented by Barnes & Reese (1959). Subsequent to Barnes (1996), Norton (1996) described the morphology of the cirri of adults and juveniles of *Pollicipes pollicipes* and Pérez-Losada et al. (2004) classified the degree of similarity of cirrus III of *Pollicipes* (*P. polymerus* and *P. pollicipes*) to cirrus II and to cirrus IV. Chan et al. (2008) examined the cirral setation and setal morphology of *Pollicipes polymerus*, describing cirri I and II. To our knowledge, there has been no further description of the morphology of the cirri of *Pollicipes elegans* other than that by Darwin (1852). There is no description of the morphology of the cirri of *Pollicipes caboverdensis*.

Each cirrus has two rami that are multi-articulated and supported by a two-segmented protopod (or pedicel), the anterior ramus being designated as the exopod and the posterior as the endopod (Anderson 1994). The anterior side of a cirrus corresponds to the side of its greater curvature, while the posterior to the side of lesser curvature (Chan et al. 2008). Cirri carry setae that may be diverse and of various types (Chan et al. 2008). Based on the descriptions of Barnes & Reese (1959) (*Pollicipes polymerus*) and Norton (1996) (*P. pollicipes*), cirri IV–VI are described as similar, although the length of the cirri increases from IV to VI, each cirrus consisting of 16–21 (*P. polymerus*) or 13–19 (*P. pollicipes*) laterally flattered segments, protuberant on their anterior faces, with the two rami of each cirrus equal in length. Distribution of the setae (called spines by Barnes & Reese 1959) on the segments of both rami of cirri IV–VI is similar in both species, but the number of pairs of setae per segment seems to differ (five in *Pollicipes pollicipes*, usually six in *P. polymerus*). Moreover, the degree of setal overlap in *Pollicipes pollicipes* has been considered lower than in *P. polymerus*, which may indicate a higher degree of carnivory in *P. pollicipes*, associated with a larger setal mesh size (Norton 1996). In both species, the endopods of cirri III and of cirri II have fewer segments (cirrus III, 12–15, cirrus II, 10–14, in *Pollicipes pollicipes;* not specified for *P. polymerus*), but otherwise are similar to cirri IV–VI. In both species, the distal segments of the exopods of cirri II and III are also similar to the segments of the endopods and, consequently, to cirri IV–VI. In contrast, the proximal segments of the exopods of cirrus III (three proximal segments) and of cirrus II (four proximal segments) are highly modified in both species in terms of the number, types and distribution of setae. Furthermore, Norton (1996) described that in *Pollicipes pollicipes,* the rami of cirrus III are approximately equal in length, while the exopod is longer than the endopod in cirrus II. In a study on the evolution of Cirripedia Thoracica using molecular and morphological evidence, cirrus III of *Pollicipes* (*P. polymerus* and *P. pollicipes*) was considered as resembling cirrus IV more than cirrus II (Pérez-Losada et al. 2004, appendix 1 and 2). Cirrus I in both *Pollicipes polymerus* (Barnes & Reese 1960) and *P. pollicipes* (Norton 1996) is described as arising from the side of the mouth and being much modified in all segments of both rami, with dense setation. In both species, it was observed that the rami of cirrus I arise from the pedicel at a sharper angle than taken by those of the other cirri. Regarding *Pollicipes pollicipes*, Norton (1996) described the exopod of cirrus I as being longer than the endopod.

The definition of which cirri in *Pollicipes* species serve as maxillipeds is not completely clear. Anderson & Southward (1987) considered that cirri I of *Pollicipes polymerus* are the maxillipeds, while Norton (1996) considered that cirri I–III in *P. pollicipes* are the maxillipeds. According to Chan et al. (2008), in *Pollicipes polymerus*, cirrus I and the exopod of cirrus II serve as maxillipeds to transfer the food to the mouth. In this study, it was found that cirrus I of *Pollicipes polymerus* carries serrulate setae only, while cirri II–VI carry serrulate, pappose and multicuspidate setae (terminology of Garm 2004a, b in Chan et al. 2008). The functions of these types of setae, assuming similar functions as those of the various types of setae that have been defined for decapods (Garm 2004b in Chan et al. 2008), are the following: serrulate setae are used for gentle prey handling; pappose setae are for water current generation and filter-feeding purposes; and multicuspidate setae are for rough prey handling. In comparison with species from the orders Iblomorpha, Lepadomorpha and Balanomorpha, Chan et al. (2008) considered that *Pollicipes polymerus* as well as *Capitulum mitella* (both now included in the new order Pollicipedomorpha, Chan et al. 2021) had a more diverse diet than the species of Iblomorpha and Lepadomorpha (by having more types of setae than in these orders). However, they were more limited regarding food manipulation behaviour compared with balanomorphan species, by having only the first pair of cirri and one of the rami of the second cirri serving as maxillipeds. Balanomorphan species have two or three pairs of maxillipeds and a highly complex setation. Considering that the number, types and distribution of setae on the cirri reflect adaptations to the various feeding modes that have emerged throughout barnacle evolution (Chan et al. 2008), it would be very interesting to compare in detail the cirral morphology within the genus *Pollicipes*, and also to clarify the function of cirri II–III.

Cirral activity and feeding

Barnes (1996) described in detail the captorial feeding mode in adults of *Pollicipes polymerus*, based on Barnes & Reese (1959, 1960), showing that the extension of the cirri and their reaction to stimuli depended on water flow above a certain critical level. Anderson & Southward (1987) revised the various types of cirral behaviour in several species of Cirripedia and considered that adults of *Pollicipes polymerus* exhibit captorial extension of the cirri outside the capitulum in a prolonged manner (Barnes & Reese 1959, 1960). They also considered that juveniles of this species (Lewis 1981) and of *Pollicipes pollicipes* (Hui 1983) displayed a pumping beat (curled cirri protruded and retracted in a rhythmic manner). However, as Barnes (1996) had already pointed out for juvenile *Pollicipes polymerus*, this pumping beat appeared to be more associated with still water conditions, as in higher flow laboratory conditions, the juveniles of *P. pollicipes* also exhibited prolonged cirral extension (Norton 1996). In *Pollicipes pollicipes*, a critical velocity, when beating ceases and extension takes over, is apparent at flow rates between 8 and 14 cm/s under laboratory conditions (Norton 1996). Cirral extension was still exhibited at the higher flow rate examined (~48 cm/s). As well as these extension and pumping beat behaviours, Norton (1996) also described two more types of cirral activity in addition to those identified by Anderson & Southward (1987) in *Pollicipes pollicipes*. These were testing (the capitular aperture open, but the cirri not extended) and very slow normal beat (cirri unrolled and spread out as a fan, then curled up and withdrawn into the mantle cavity). All the rhythmic behaviours were at much lower rates and hence not equivalent to the cirral beating of balanomorph species, with rhythmic activity occurring only in laboratory conditions of very low flow or no flow (Norton 1996). Rhythmic cirral activity was considered to have a respiratory function that might be more important in low flow conditions. As such hydrodynamic conditions would be rarely experienced by adult *Pollicipes pollicipes* in the wild, Norton (1996) considered that cirral extension should predominate in natural conditions. In laboratory conditions, the most common activity for *Pollicipes pollicipes* of various sizes in conditions of moderate to high flow was cirral extension of various types, with the in-curling of at least one cirrus to the mouth to transport and ingest captured food (Norton 1996). The observations made by Norton (1996) do not support the hypothesis of Lewis (1981) for *Pollicipes polymerus*, and Hui (1983) for *P. pollicipes*, for a juvenile to adult switch in feeding strategies from cirral beating to cirral extension in *Pollicipes*.

Barnes (1996) reviewed the available studies on the diet of *Pollicipes* species (Barnes 1959, Howard & Scott 1959, Lewis 1981), which all concerned *P. polymerus*. With the exception of Norton (1996) (*Pollicipes pollicipes*), there have been no further studies on this subject. Based on Lewis (1981), the following patterns have been identified for *Pollicipes polymerus*: (1) little inorganic material (< 12% of total volume), namely a few grains of sand, which seems to be associated with a capacity to reject this kind of material (Barnes & Reese 1959); (2) higher relative percentage of organic particulate food items (< 10 μm diameter) in small barnacles (1–6 mm rostrocarinal length, RC) (e.g. detritus and diatoms) than in larger barnacles (15–28 mm RC); (3) higher percentage of large organic material (e.g. copepods, barnacle moults, polychaetes) in larger barnacles than in smaller ones. Howard & Scott (1959) also detected the usual presence of cyprids, amphipods, small clams and hydroids in the gut of *Pollicipes polymerus*. In *Pollicipes pollicipes*, Norton (1996) found a similar pattern of the relative importance of the different types of food items (inorganic, organic particulate and large organic material) in relation to size. In this study, the most common food types identified were diatoms, other unicellular phytoplankton, large algae, crustaceans and their remains, cirripede larvae (nauplii and cyprids) and moults. According to Norton (1996), *Pollicipes pollicipes* from the high shore had more homogeneous gut contents, with less identifiable material, than the lower shore animals, and smaller barnacles appeared to have more thoroughly digested gut contents than larger animals.

Phenotypic variation of the cirri and behaviour

In the last 20 years, several studies on the phenotypic variation of the cirri of several cirripede species, including *Pollicipes polymerus,* have been carried out: *Pollicipes polymerus* (Marchinko & Palmer 2003, Marchinko et al. 2004), *Chthamalus dalli* (Marchinko & Palmer 2003, Marchinko et al. 2004), *Chthamalus fissus* (Miller 2007), *Tetraclita japonica* (Chan & Hung 2005), *Balanus glandula* (Arsenault et al. 2001, Marchinko 2003, Marchinko & Palmer 2003, Li & Denny 2004, Marchinko et al. 2004, Marchinko 2007, Kaji & Palmer 2017) and *Semibalanus cariosus* (Marchinko & Palmer 2003). The most commonly used response variables in these studies were the ramus length and the ramus diameter of cirri IV–VI, namely of cirri VI, corrected for body size, as cirral dimensions increase with increased body size (Marchinko & Palmer 2003). The most common environmental predictor related to phenotypic variation of the cirri is the degree of wave exposure. In general, barnacles growing on less exposed shores have longer, thinner cirri than conspecifics growing on wave-exposed sites, and these cirral traits can be altered between moults in response to changing flow patterns (Marchinko 2003). However, there may be a threshold water velocity above which barnacles cease responding plastically to flow (Li & Denny 2004, Miller 2007). It was predicted that variation in feeding behaviour compensates or substitutes for further morphological variation (Marchinko & Palmer 2003, Li & Denny 2004), a hypothesis supported by field observations of the feeding behaviour of *Chthamalus fissus* (Miller 2007). *Chthamalus fissus* has the ability to withdraw and avoid individual breaking waves that generate high flow speeds (Miller 2007). However, the high peak flows of breaking waves are extremely transient (often lasting <1 s), and the turbulent bore that continues up the shore and eventually washes back down moves much more slowly than the peak flows. *Chthamalus fissus* reacted quickly to the decelerating flows and began feeding again shortly after withdrawing to avoid the initial breaking wave (Miller 2007). Although there are no field observations of the cirral behaviour of *Pollicipes* species in response to hydrodynamic conditions (e.g. wave impact, wave velocity), field observations on the orientation of the capitular aperture of *P. polymerus* suggest that it feeds after the initial wave impact (Barnes & Reese 1960). As Barnes (1996) described in detail, it appears that *Pollicipes polymerus* faces the backwash and not the incoming wave. In contrast, Norton (1996), from field observations on the orientation of the capitular aperture in *Pollicipes pollicipes*, did not find such a clear pattern, recording much small-scale variability, probably related to microtopographic variation.

The only known studies of phenotypic variability of the cirri of species of *Pollicipes* are related to *P. polymerus* (Marchinko & Palmer 2003, Marchinko et al. 2004). The lengths of the rami of cirrus VI of *Pollicipes polymerus* specimens were significantly longer in semi-exposed sites (2.66 m/s) in comparison with high wave exposure sites (4.41 m/s) (Marchinko & Palmer 2003). Compared with other barnacle species, the proportional difference in cirral length between exposed and semi-exposed sites was 75%–80% in *Balanus glandula*, 47%–68% in *Chthamalus dalli*, 29%–37% in *Semibalanus cariosus*, 7%–12% in *Pollicipes polymerus* (all data from Marchinko & Palmer 2003) and ~4% in *Tetraclita japonica* (Chan & Hung 2005). The lower relative plastic variation exhibited by *Pollicipes polymerus* was associated with a narrower range of hydrodynamic conditions where this species naturally occurs (Marchinko & Palmer 2003). Chan & Hung (2005) suggested that *Tetraclita japonica* and *Pollicipes polymerus* do not have much longer cirri in semi-exposed sites, as their feeding mode is prolonged extension of the cirral net. Consequently, shorter cirri might reduce the chance of being damaged and of being preyed upon.

Adhesion and cement

Despite excellent progress in understanding barnacle adhesion, advances since Barnes (1996) have mainly been restricted to acorn barnacles. Description of adhesion in stalked barnacle species is much less common and, of these, only *Lepas anatifera* and *Pollicipes pollicipes* have received much attention, with no progress on the remaining species within the genus *Pollicipes*. Nevertheless, it is possible to draw some parallels from the better-described groups, which is the approach adopted in the following sections.

The adhesive gland

Three types of adhesion are generally recognized in barnacles: larval temporary adhesion, larval permanent adhesion and adult permanent adhesion, with a metamorphosis step between larval and adult adhesion events (Liang et al. 2019). Each life-history event, or phase, has a corresponding adhesive. In keeping with the structure of Barnes (1996), we mainly consider adult adhesives (but see Liang et al. 2019, for a recent review on larval adhesives). The understanding of adhesion specific to *Pollicipes* has forged ahead, in several ways, since the review of Margaret Barnes (1996). At the time of her review, most of what was known regarding the biochemistry of the *Pollicipes* adhesive was extrapolated from balanomorph barnacles (e.g. Yule & Walker 1987), or from preliminary analyses in stalked barnacles, such as *Lepas anatifera* (Walker & Youngson 1975) and *Dosima (=Lepas) fascicularis* (Barnes & Blackstock 1974, 1976). But structural studies of the adhesive interface in *Pollicipes* (*polymerus*) go back to Darwin (1852) who showed a drawing of canals or 'ducts' terminating in pores at the base of the peduncle, for the delivery of adhesive to the substratum (Figure 6). Notwithstanding this very early knowledge, the *Pollicipes* adhesive gland was still unstudied at the time of Barnes (1996), so the description of the gland given at that time was extrapolated from observations made in *Lepas* (Lacombe & Ligouri 1969, Lacombe 1970; the latter have since been updated by Jonker et al. 2012 and Power et al. 2010).

The adhesive gland in *Pollicipes pollicipes* was finally described by McEvilly (2011) and Lobo-da-Cunha et al. (Figure 1 therein, 2017). Its structure follows the same scheme as in other stalked barnacle species, with some minor variations. Gland tissue is located in the peduncle, immediately beneath the capitulum and somewhat interspersed with the ovarian tissue (not in the 'basal' portion of the peduncle, as suggested in Koehler 1888, cited in Barnes 1996). Unicellular in structure, the gland comprises a series of 'giant' cells, which are identical and responsible for synthesising all of the adhesive components. This differs markedly from other adhesive-producing organisms such as

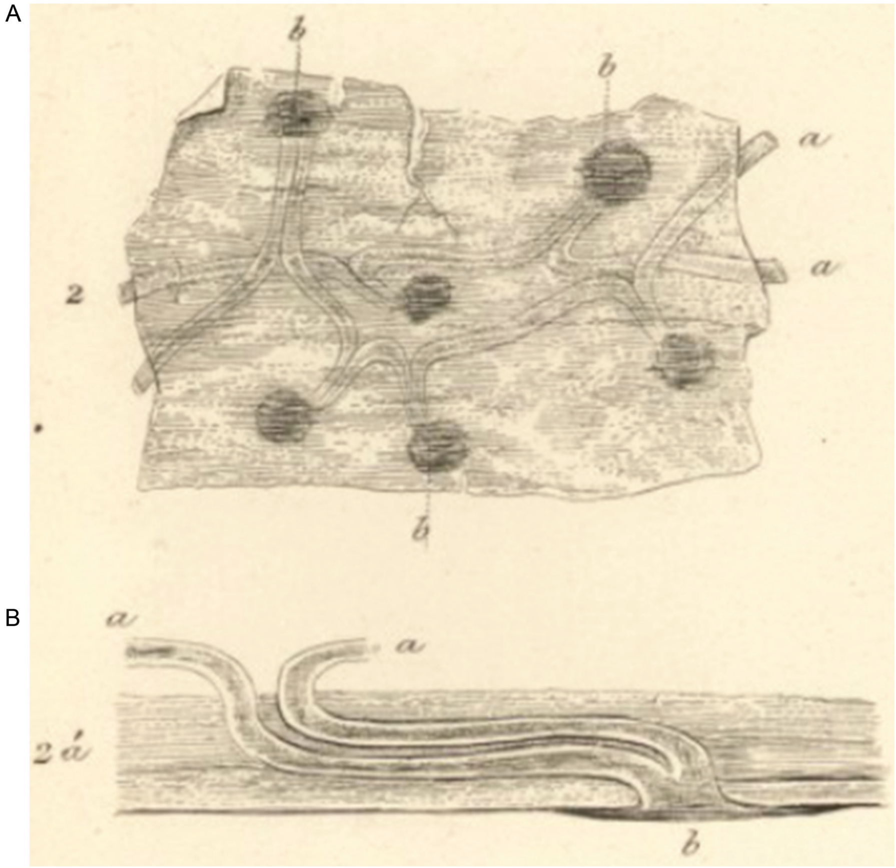


Figure 6 (A) “A portion (about 1/10th of an inch square) of the surface of the peduncle of *Pollicipes polymerus*, seen from the outside, greatly magnified, showing the small circular (bb) patches of cement, poured out from the cement ducts (aa) which lie within the peduncle”. (B) “A secretion, still more magnified, through the basal membrane of the peduncle, through one of the loops of the cement ducts (aa), and through one of the circular patches (b) of cement.” Both drawings are reproduced from Darwin (1852).

mytilids (mussels) (Wiegemann 2005), sabellariid worms (sandcastle worms or honeycomb worms) (Wang et al. 2010) and echinoderms such as *Asterias rubens* (Flammang et al. 1998). These organisms have several different gland cell types, each producing unique components that are clearly separated prior to secretion, which is a possible strategy to prevent premature polymerization of the adhesive. The lack of any such strategy, in the adhesion system of barnacles, is probably due to a much slower ‘curing’ (hardening) process in adult barnacle adhesives, compared with other systems. In marked contrast, larval barnacle adhesives possess a subdivided glandular system and a much faster-acting adhesive (Walker 1971). Indeed, this is one of several differences setting barnacles apart from other adhesion systems – unlike the adhesives of mussel byssus and reef-building sabellariid adhesives, barnacle cements do not contain L-dopa (L-3,4-dihydroxyphenylalanine) and, therefore, must contain molecular novelties compared to those systems (Wiegemann 2005, Jonker et al. 2012). Within the giant gland cells of barnacles, adhesive components are packaged into vesicles, which congregate in intracellular canals. They leave the gland cell in extracellular canals, which are drained by larger secondary canals, before being delivered to the substratum by two principal canals that run down the remaining length of the peduncle (Figure 7). McEvilly (2011) noted that intracellular canals may or may not be present in *Pollicipes pollicipes*. According

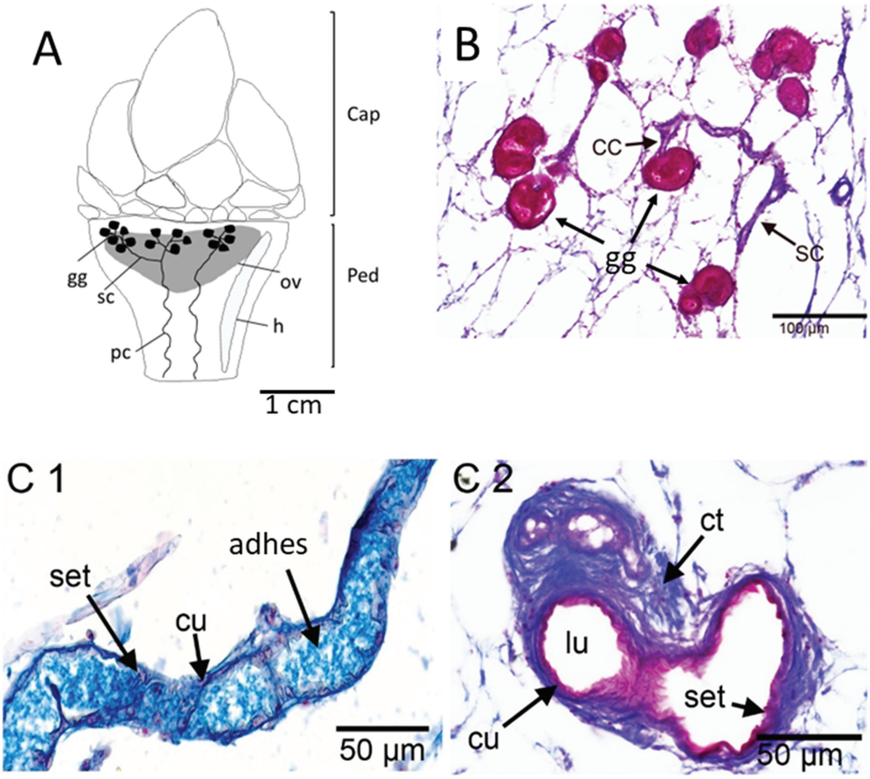


Figure 7 (A) Schematic of *Pollicipes pollicipes* showing the position of the giant adhesive gland cells that are drained by a series of canals in the peduncle. (B) *Pollicipes pollicipes* rosette of gland cells. (C1) *Dosima fascicularis* principal canal containing adhesive. (C2) *Pollicipes pollicipes* principal canal with cuticle lining. Cap, capitulum; Ped, peduncle; gg, glue gland; h, haemolymph; ov, ovary; pc, principal canal; cc, collecting canal; sc, secondary canal; set, squamous epithelial tissue; cu, cuticle; lu, lumen; adhes, adhesive; and ct, connective tissue. Adhesive secretion can be seen inside the lumen in C1. All sections were stained using AZAN. Scale bars are indicated in each image. Images are adapted from McEvilly (2011).

to McEvilly (2011), the gland cells in *Pollicipes pollicipes* were 40–70 μm in diameter (described by Lobo-da-Cunha et al. (2017) as up to 100 μm long) and were arranged in distinct circular arrangements, 'rosettes', of 5–10 gland cells, rather than singly or in groups as seen in other barnacle species. The giant cells possess a large-lobed nucleus, numerous nucleoli and a high concentration of heterochromatin, along with large amounts of rough endoplasmic reticulum, indicating exceptionally high protein synthesis levels within these cells. Fine details of the gland cells and secretory canals are given in McEvilly (2011) and Lobo-da-Cunha et al. (2017), including the evidence that adhesive proteins in *Pollicipes pollicipes* may be secreted through the endoplasmic reticulum secretory pathway (Lobo-da-Cunha et al. 2017).

According to Barnes (1996), the adhesive glands are 'always active' to maintain adhesion, but the inner tegumental glands (i.e. the thin lining of the mantle cavity) are only active during moulting, while the outer tegumental glands (i.e. the ones supplying the calcareous 'scales' of the peduncle) may also be active at all times, to repair damage and produce new scales. However, regarding the adhesive glands, this is far from clear. Early studies suggested secretion of glue into the canal system coincided with moulting of the exoskeleton, at least in some species (Fyhn & Costlow 1976). A link between adhesive production and moulting was further supported by molecular studies, which showed the expression level of adhesive protein mRNA increases towards moulting and is at its highest immediately after moulting (Kamino 2006). However, Wang et al. (2015) found no major differences in adhesion gene expression (i.e. adhesion transcriptome) in pre- and post-moult tissues. They did, however, find upregulated genes in the pre-moult sub-mantle tissues functioning in cuticular development, bio-mineralization and proteolytic activity.

Movement on the substratum

Despite being known for their ‘permanent’ adhesive, adult barnacles move slightly (Davey et al. 2021), particularly species with membraneous bases, and this is taken to extremes in *Pollicipes*. Adult *Pollicipes* can voluntarily detach and relocate along the substrate at a mean rate of 50 µm per day (Kugele & Yule 2000). Also noteworthy is the high degree of movement in *Pollicipes* juveniles along the substratum as they grow (i.e. along the peduncle of an adult, due to settling as larvae on conspecific adults). Barnes (1996) described cement ‘tracks’ in *Pollicipes pollicipes* adults, which got wider in the direction of movement. This suggested that movement could be brought about during growth, via a series of directed extensions of the peduncle base (Kugele & Yule 1993). The question remained open as to the degree of muscular involvement in this process, for instance *via* hydrostatic pressure, created through a combination of haemolymph pressure and the three muscle layers within the peduncle. However, Kugele & Yule (2000) have since ruled out involvement of muscular activity in the movement of *Pollicipes pollicipes*, deeming that this was unnecessary. They also provided evidence that the initial larval fixation points (i.e. the larval antennules that become embedded in the adhesive plaque and are usually visible even in adults) are lost in *Pollicipes*, due to sloughing off of the trailing edge of the basis following movement. However, these initial antennule attachment points can still be seen in species that move much less, such as *Lepas anatifera* (Kugele & Yule 2000). Although Barnes (1996) described the *Lepas* adhesive as being delivered through a canal (duct) system, leading to an opening through each antennule in the base of the peduncle (citing Lacombe & Ligouri 1969, Lacombe 1970), this is not quite correct. The adhesive is delivered via new pores, which are created off the principal canal, and the antennules are only used for the initial attachment (Jonker et al. 2012). Returning to *Pollicipes*, it was observed that directed movement in response to gravity, or a unidirectional water flow, was absent in adults and was only observed in juveniles, which always moved down the peduncle of their adult host, towards the substratum (Kugele & Yule 2000).

Biochemistry of barnacle adhesives

According to Barnes (1996) citing Naldrett (1993), the only clue to the *Pollicipes* adhesive mechanism was that saltwater had a part in curing its adhesive. This was based on observations that the addition of Tris buffer without salt inhibited the adhesive changing from liquid to opaque (‘cured’ form). Beyond this limited information, all knowledge of the adhesive biochemistry came from other barnacle species. Early studies ignored *Pollicipes pollicipes*, although Barnes & Blackstock (1974, 1976) outlined how stalked barnacle (*Dosima fascicularis*) adhesive mostly comprised protein (along with <2% carbohydrate, ~8% lipid and some ‘ash’). These two studies also described the proportion of amino acids in the bulk cement. That the adult barnacle adhesive is primarily protein-based is important, from an experimental point of view, as this means that the adhesive is accessible to proteomic and gene expression profiling (Davey et al. 2021). A year after Barnes (1996), Naldrett & Kaplan (1997) solubilized the bulk cement in acorn barnacles (*Balanus* species), separating this complex into individual proteins, a process which itself offered clues as to the adhesive characteristics. This was also the first study to describe partial amino acid sequence information for individual adhesive proteins, underlining how the adhesive mechanism in barnacles differed from other adhesive animals, without polyphenolic chemistries involving L-dopa, as seen in mussels and tubeworms. Since it was only possible to render the bulk barnacle cement soluble using denaturants with heating and the addition of a strong reductant, the authors concluded that hydrophobic interactions (and possibly sulfur cross-links) were important in conferring insolubility, and hence strength, in the hardened barnacle adhesive (Naldrett & Kaplan 1997). Meanwhile, the rubbery nature of the barnacle adhesive, which confers flexibility and prevents cracking, was suggested to be associated with abundant small amino acids (alanine, serine and glycine) (Naldrett & Kaplan 1997). The absence of L-dopa was later confirmed histochemically (Arnow's assay) in other species – *Lepas anatifera* (Jonker et al. 2012) and *Dosima fascicularis* (Zheden et al. 2014) – and, although this has not been confirmed in *Pollicipes pollicipes*, L-dopa is almost certain to be absent in the latter case as well. The only post-translation modification (i.e. changes which take place in a protein after it is translated in the ribosome) identified on a barnacle protein was O-glycosylation (Naldrett & Kaplan 1997). This was later found on the N-terminal of a 52 kilodalton (kD) barnacle adhesion protein (Kamino 2013; see below). No other evidence of the post-translation modifications, common to other marine bioadhesives, such as hydroxylation of tyrosine residues to form L-dopa (see above) or phosphorylation of serines (Jonker et al. 2012) has been documented to date.

Individual adhesive proteins in barnacles

Next, it is important to look in detail at individual adhesive proteins in barnacles, what is known about these and how adhesion in *Pollicipes* fits inside that framework. Beginning with Naldrett & Kaplan (1997), a number of studies on individual adhesive proteins in various barnacle species have been made in the last 25 years. Most prominent is a series of pioneering studies by Kamino and colleagues, working on acorn barnacles. They identified five main proteins that were characterized by their apparent molecular weight (i.e. 19, 20, 52, 68 and 100 kD) and by particular amino acid biases, which resulted in a protein being either hydrophobic (52 and 100 kD) or hydrophilic (19 and 68 kD) (Kamino et al. 2000, Kamino 2013). Markers, developed against the relevant mRNA sequences in these proteins, showed that their expression was localized at or near the location of the adhesive gland. Each had different empirical functionality; for example, the 20 kD protein from *Megabalanus rosa* was recombinantly expressed in *E. coli* and bound to calcite, something which might be relevant for binding a calcium carbonate baseplate to the surface (Mori et al. 2007). Meanwhile, the 19 and 68 kD proteins were rather similar, with strong biases towards the amino acids serine, threonine, glycine, alanine, lysine and valine. The recombinantly expressed 19 kD protein adsorbed to more varied material surfaces (Urushida et al. 2007). No enzymes were identified in the adhesive with reactivity against these proteins. Thus, the mechanism underpinning adhesion and strength (i.e. 'cohesion') within the protein complex remained a matter of speculation.

Kamino et al. (2000, 2012) described the 52 kD protein, which, with the 100 kD protein, is together responsible for the insoluble nature of barnacle cement, being hydrophobic. Another important avenue of research was protein folding into amyloids, which are particular secondary structures made of cross-β-sheets that take a fibrillar form in certain proteins. Nakano & Kamino (2015) identified certain 'amyloidogenic motifs' in the 52 kD protein using a ThT assay on various peptides, showing that environmental factors (pH and ionic/salt conditions) could induce self-assembly of synthetic peptides derived from these motifs into a β-sheet structure (i.e. a precursor of amyloid fibre formation). These authors suggested that proteins are transformed to the cross-β-sheet conformation by environmental conditions and are fibrillated simultaneously or subsequently (Nakano & Kamino 2015). Hence, the two smaller proteins in acorn barnacles (19 and 20 kD) were proposed to have surface coupling functions, while the larger proteins (52 and 100 kD) were suggested to be involved in more bulk cohesion functions within the cement (Kamino 2013). Repeated peptide sequences were evident in some of the proteins (e.g. the 20 and 52 kD cases), but strong molecular 'motifs', as seen in mussel byssus, were absent. No means of intermolecular cross-linking of proteins were found; rather, the characteristics of the proteins suggested non-covalent mechanisms of curing the adhesive into cement. These included molecular conformation (folding) with intensive hydrogen bonding and hydrophobic interactions. These processes would contribute to self-assembly of protein into sheets and, in some cases, ultimately into fibrils. Thus, the adhesive, which takes hours to harden into a cement (Cheung et al. 1977), has an action that involves protein folding triggered by changes in pH and ionic environment.

Biochemistry of Pollicipes *adhesive*

Although many gaps remain, much progress has been made in understanding the mode of adhesion in *Pollicipes*. Despite low sequence similarities, homology was evident, on the basis of histochemical staining, between the 52 and 68 kD adhesive proteins from acorn and stalked barnacles, with homologues for the 19 and 100 kD proteins from acorn barnacles being identified bioinformatically in *Pollicipes pollicipes* (Jonker et al. 2014).

The full sequences of three adhesive proteins for *Pollicipes pollicipes* are now verified and available – 19, 52 and 100 kD (Rocha et al. 2019). A principal component analysis (i.e. statistical similarity exploration) of 23 adhesive proteins (various barnacle species including *Pollicipes pollicipes*) showed no significant differences, between acorn and stalked barnacles, in residue composition of homologous adhesive proteins (Rocha et al. 2019). The gene sequence encoding the 19 kD adhesive protein in *Pollicipes pollicipes* was identified from an expressed sequence tag (EST) database of arthropods published by Meusemann et al. (2010), later being verified by RACE-PCR sequencing and proteomic analysis using LC-MS/MS (Rocha 2015, Rocha et al. 2019). This protein was expressed in an *E. coli* recombinant expression system and purified, and its nanomechanical properties were characterized using surface plasmon resonance (Tilbury et al. 2019). The results of nanomechanical tests, on surface types that included hydrophobic, hydrophilic, charged and neutral surfaces, showed that the adsorption of the *Pollicipes pollicipes* 19 kD protein (i.e. 'Ppolcp19k') was not elevated, compared with the controls, in conditions designed to mimic either the barnacle cement gland or seawater (Tilbury et al. 2019). Hence, this small protein did not demonstrate enhanced binding to diverse surface types described for the homologous protein in *Megabalanus rosa* (Urushida et al. 2007). Tilbury et al. (2019) noted that, while the same amino acid bias in the 19 kD protein was seen in all barnacle species, including *Pollicipes pollicipes* (where serine, threonine, glycine, alanine, lysine and valine made up 70.5% of all amino acids), the isoelectric point (pI) in *Megabalanus rosa* was lower (pI = 5.8) than in *Pollicipes pollicipes* and several other barnacle species (pI = 9.26–9.80).

Unpublished results (AMP pers. obs.) show that the *Pollicipes pollicipes* 19 kD adhesive protein self-assembles into fibrils, which stain positively for amyloid at given pH and salt concentrations, behaving in a similar fashion to the 52 kD acorn barnacle protein outlined above. This agrees with the analysis of Rocha et al. (2019), who predicted the secondary structure of three *Pollicipes pollicipes* adhesive proteins, based on sequence information. Of these, the 19 kD protein in *Pollicipes pollicipes* was the protein with the highest predicted percentage of β-sheets encompassing 26.4% of its amino acid residues (Rocha et al. 2019). The predicted secondary structure of the 52 and 100 kD proteins from *Pollicipes pollicipes* were, respectively, classified as mixed (52 kD) and α-helix (100 kD). Not all β-sheets form amyloid; however, Barlow et al. (2010), working on the cements of *Amphibalanus amphitrite*, showed that these are highly hydrated (20%–50%) and comprised nanofibrillar matrices. Using various spectroscopic techniques, these authors showed that the nanofibrils are consistent with amyloid (as opposed to non-amyloid β-sheets) and that amyloid made up a large proportion (perhaps 30%) of the cement, with globular protein components also present (Barlow et al. 2010). Amyloid may form in some proteins due to conserved β-motifs, which act as nuclei in amyloid formation, or due to the alternating polar and non-polar residues in the 100 kD *Megabalanus rosa* protein (e.g. Davey et al. 2021). Overall, research in *Pollicipes pollicipes* showed no major differences from acorn barnacle proteins, apart from minor features, such as a shorter than usual form of the 52 kD protein, which at 356 residues is 209–274 residues smaller than the homologous protein in *Megabalanus rosa* or *Amphibalanus amphitrite* (Rocha et al. 2019). *Pollicipes pollicipes* joins only a handful of barnacle species for which recombinant expression of an adhesive protein has been attempted and surface dynamics tested (see also Liang et al. 2015 and Liu et al. 2017 working on *Balanus albicostatus* and Mori et al. 2007 and Urushida et al. 2007 working on *Megabalanus rosa*).

Current and future research in bioadhesion

The most recent research on barnacle bioadhesion has suggested additional proteins may be involved. A proteomic analysis found ~ 50 new proteins active at the barnacle adhesive interface, including new 114 kD (Wang et al. 2015) and 43 kD proteins (So et al. 2016; note that this may be homologous to the 68 kD protein from *Megabalanus rosa* – see Lin et al. (2021), and also that the molecular weight from SDS-PAGE gel migration and the predicted molecular weight from the protein sequence do not agree for 'AaCP43', as is common with adhesion proteins). Of most interest among these were enzymes that could have activity upon previously described adhesion proteins, including lysyl oxidases, peroxidases, peroxinectins and proteases. The former could mediate cross-linking in proteins with prominent abundances of lysine amino acids (So et al. 2016, 2017, Davey et al. 2021). Further work is required in this area since Cheung et al. (1977) claimed that inhibitors of enzymes, such as lysyl oxidase, did not prevent polymerization. While all these new proteins were being added into the mix, one may potentially be removed. The 20 kD protein has since been suggested to be a shell protein rather than an adhesive protein (Fears et al. 2019, Davey et al. 2021). That this protein had never been described from membraneous-based barnacles, including *Pollicipes pollicipes*, was consistent with that view (Lin et al. 2014, Jonker et al. 2015). However, new research described 20 kD protein homologues from three membraneous-based species, *Capitulum mitella*, *Conchoderma hunteri* (of which both are stalked barnacle species, as well as being pollicipedid, in the case of *C. mitella*) and *Chthamalus malayensis* (Lin et al. 2021). Fresh perspectives from all of this new information include a new way of categorising barnacle adhesive proteins into those that are rich in glycine/serine/alanine/threonine and those that are rich in leucine/valine/isoleucine (So et al. 2016, Rocha et al. 2019). This would place the 19 kD protein and a new 57 kD protein into the first group, with polar and hydrophilic side chains and a tendency to display domains otherwise seen in silk-producing arthropods. Meanwhile, the 52 and 100 kD proteins fall into the second group, which is hydrophobic with aliphatic groups (So et al. 2016). The glycine-serine-rich category above is a key component in the nanofibrillar structures observed in barnacle cement (So et al. 2016).

The future of bioadhesion research, including in *Pollicipes*, seems assured, given an explosion in the quantity and quality of data available in the era of 'omics' (Davey et al. 2021). Machado et al. (2019) presented the first assembled transcriptome for adhesive glands of *Pollicipes pollicipes* and *Lepas anatifera* (150 bp paired-end reads on the Illumina HiSeq 4000 platform) and deposited raw RNA-Seq data, transcriptome shotgun assemblies and final *de novo* assembly contigs on publicly available databases. In addition, Perina et al. (2014) added to the existing EST database of Meusemann et al. (2010), with genes specific to the body and foot tissues of adult individuals of *Pollicipes pollicipes*, discovering two 100 and 52 kD cement protein transcripts in the process. Finally, Domínguez-Pérez et al. (2020) provided a high-throughput proteome of the *Pollicipes pollicipes* adhesive gland and cement. This showed that, although the 52 kD protein was abundant in the cement, known adhesive proteins only made up a minor portion of the proteome. The remainder comprised several undescribed 19 kD-like proteins, along with 12 unannotated proteins, enzymes, chemical cues and protease inhibitors within the cement proteome (Domínguez-Pérez et al. 2020). All in all, these resources should greatly add to a fuller understanding of barnacle adhesion, including that of *Pollicipes pollicipes* in the decade to come. Finally, and remarkably, there has until recently been no good-quality barnacle genome available, despite this being a prerequisite for functional studies and for properly assembling and annotating a burgeoning number of transcriptomes (Rosenblad et al. 2021). Recently, two acorn barnacle genomes (with N50 > 100kbp) have been made available (*Balanus* (*Amphibalanus*) *improvisus*, Rosenblad et al. 2021, and *Balanus* (*Amphibalanus*) *amphitrite*, Kim et al. 2019a), along with one stalked barnacle genome (*Lepas anserifera*, Ip et al. 2021). Unpublished genome assemblies are also available for *Pollicipes pollicipes* (see Rosenblad et al. 2021 and Schultzhaus et al. 2021 for details).

Reproduction

The section on reproduction in Barnes (1996) is the longest of the whole review. The probable reasons for its length were not only the considerable knowledge that was available at that time, but also the interest and effort that Margaret Barnes devoted to this field of research, including two reviews on reproduction in Cirripedia published in *Oceanography and Marine Biology: An Annual Review* (Barnes 1989, 1992). Barnes' (1996) review of reproduction in the genus *Pollicipes* encompassed the following topics: size at maturity, number of broods and number of eggs, breeding season, spermatogenesis and spermatozoa, oogenesis, fertilization, ultrastructural investigations of fertilization and embryo development and chemical composition of ovary and egg lamellae. The only known studies on the reproduction in *Pollicipes* at that time were on *P. polymerus* and *P. pollicipes*.

Twenty-five years after Barnes' review, studies on the reproduction in *Pollicipes* are still primarily on these two species, especially *P. pollicipes*, but we also have some information on *P. elegans* and *P. caboverdensis*. All species are hermaphrodites. However, one of the most surprising advances in the study of reproduction in *Pollicipes* species, and one that challenges our knowledge of barnacle reproductive biology, came from the studies of Barazandeh et al. (2013) and Barazandeh & Palmer (2015) on the modes of fertilization in *P. polymerus*. Additionally, the first study to demonstrate high levels of multiple paternity in barnacles was in a *Pollicipes* species, *P. elegans*, by Plough et al. (2014).

Fertilization and mating

At the time of Barnes' (1996) review, it was assumed that there was no self-fertilization in *Pollicipes polymerus* based on the studies of Hilgard (1960) and Lewis & Chia (1981), although cases of self-fertilization had been identified in several species of Thoracica (see Barnes 1989). At that time, evidence of self-fertilization came from the presence of isolated barnacles carrying egg masses. On the other hand, Margaret Barnes stated that in many thousands of *Pollicipes polymerus* examined by her over the years,

> …the penis has never been found to vary in length (it is about half the length of the cirral net) or in the position it occupies in the mantle cavity. It is heavily pigmented, always very rigid and always lies over the body of the animal with its tip near the base of the first cirri, that is the atrium of the oviduct
>
> *Barnes (1992).*

In addition, she stated that "Animals have often been found with blobs of spermatozoa over the ends of the oviduct…", but "Copulation has, however, never been seen" (Barnes 1992) "…even after hundreds of hours of observing animals in aquaria" (C.A. Lewis pers. comm.in Barnes 1992). Margaret Barnes made the same kind of observations and considerations regarding *Pollicipes pollicipes* (Barnes 1992), but in this case, she questioned whether this species was capable of self-fertilization, as there were no studies that refuted self-fertilization in that species at that time. She also cited the question posed by Gruvel (1893): "How does this species fertilize?" and noted the hypothesis that Gruvel (1893) had suggested, in which spermatozoa might be passed into seawater and be transported by the cirri of adjacent functional females into their mantle cavities (Barnes 1992). However, this hypothesis was considered unlikely by Gruvel (1893) and by Barnes (1989, 1992), and self-fertilization was suggested as a possible mode of fertilization as "*P. pollicipes* has never been seen to cross-fertilize" (Barnes 1992). In parallel to these observations and considerations, Barnes (1996) noted that species of *Pollicipes* can manoeuvre themselves by means of the peduncle (Barnes & Reese 1960) and an extendable penis may not be so vital to copulation as it is in acorn barnacles. Therefore, at the time of Barnes' (1996) review, it was thought that species of *Pollicipes* could (i.e. *P. pollicipes*) or most likely could not (i.e. *P. polymerus*) self-inseminate and probably would be able to copulate as most thoracican barnacles do, although copulation in *Pollicipes* had never been observed.

In contrast, it is common to observe copulation, also called pseudo-copulation (release of sperm into a functional female mantle cavity), in balanomorphan species (see Anderson 1994 for a review). Mating in hermaphrodite thoracicans was described by Anderson (1994) as the extension of the penis of a 'functional male' by turgor pressure, followed by searching movements of the penis to detect a 'functional female' leading to penetration and ejaculation. For pseudo-copulation to take place, a barnacle must be within the penis range of at least one neighbour. According to Anderson (1994), several functional males may copulate with a functional female simultaneously, but copulation is never reciprocal.

Over the last 25 years, observations of pseudo-copulation in *Pollicipes polymerus* (observations in the field by Barazandeh & Palmer 2015; see online videos in supplementary material) and in *P. pollicipes* (observations in aquaria from Aquarium Finisterrae, Spain, https://youtu.be/Wuqz4fC9gLk, plus field observations in Portugal, TC and DJ pers. obs., https://youtu.be/EEN-Rq9klXc) have been made. Based on the film from Aquarium Finisterrae, we have made an illustration of the pseudo-copulation in *Pollicipes pollicipes* (Figure 8), in which mating between a functional female and two functional males is represented. Conversely, evidence has also been obtained that self-fertilization in *Pollicipes pollicipes* does not appear to occur, since barnacles with a carina-to-carina distance of >5 cm (after pushing them towards each other), hence regarded as isolated, did not contain egg lamellae (60% of non-isolated barnacles sampled at the same site and sampling date had eggs) (Cruz & Hawkins 1998).

Therefore, there is no longer any doubt that both *Pollicipes pollicipes* and *P. polymerus* copulate, and probably, self-fertilization does not seem to occur in *P. pollicipes*, as in *P. polymerus*. Surprisingly, however, a third mode of fertilization has been described in *Pollicipes polymerus* that

Figure 8 Illustration of pseudo-copulation in *Pollicipes pollicipes* by João Tiago Tavares, based on a film from Aquarium Finisterrae, Spain (https://youtu.be/Wuqz4fC9gLk).

had never been observed in barnacles: sperm-cast mating – where sperm released into the water by males fertilize eggs retained in the body of a female (Barazandeh et al. 2013). This discovery began with the field observation of occasional *Pollicipes polymerus* individuals leaking a foamy white liquid (confirmed to be sperm in a subsequent study by Barazandeh & Palmer 2015) between capitular plates, namely at the junction between the scutal and tergal plates, during low tide (see Figure 1b in Barazandeh et al. 2013 and Figure 1 in Barazandeh & Palmer 2015), and of isolated (outside penis range) yet fertilized individuals (Barazandeh et al. 2013). In comparison with other thoracican barnacles, species of *Pollicipes* have short penises, shorter than the cirri (Barnes 1992). Barnacles are sessile and most have a long penis that can extend multiple body lengths to reach neighbours and cross-fertilize (Neufeld & Palmer 2008), but *Pollicipes polymerus* has a relatively short penis (~twice body length after full extension) in comparison with acorn barnacles such as *Chthamalus dalli* and *Balanus glandula* (~seven times body length after full extension) (Barazandeh et al. 2013, 2014, Barazandeh & Palmer 2015). By using 16 single nucleotide polymorphism (SNP) markers, Barazandeh et al. (2013) found that sperm capture (presence of non-parent SNP alleles) occurred in 100% of the eggs from isolated individuals/mothers and, remarkably, even in 24% of the eggs from individuals/mothers that had just one adjacent partner. Therefore, these authors suggested that sperm capture might be a common supplement to pseudo-copulation in *Pollicipes polymerus*. At the time of their study (Barazandeh et al. 2013), observations of the pseudo-copulation in *Pollicipes polymerus* were not available. However, several observations of pseudo-copulation in this species were made in a later study by Barazandeh & Palmer (2015), by recording the behaviour of this species during flood and ebb conditions on short videos. Furthermore, in this study, the mantle cavity of individuals leaking sperm was inspected. Among the 13 leaker individuals examined, eight individuals had no eggs or sperm in the mantle cavity, four had newly released egg sacks or sperm blobs in the mantle cavity, and one had fully mature egg lamellae (Barazandeh & Palmer 2015). According to these authors, the observations might indicate that sperm leakage is an active process. Rates of copulation in the field were about four times those of sperm leakage, which was classified as an infrequent event, suggesting that it might be a secondary mode of fertilization in *Pollicipes polymerus* (Barazandeh & Palmer 2015). In this study, aerial copulation of *Pollicipes polymerus* was observed, a unique mode of mating among barnacles, as pseudo-copulation occurred mainly when barnacles were partially emersed, shortly after contact with breaking waves on an incoming tide. In Portugal, field observations of pseudo-copulation in *Pollicipes pollicipes* were also made in these conditions (TC and DJ pers. obs.). An extraordinary observation made by Barazandeh & Palmer (2015) was the occasional reciprocal copulation in *Pollicipes polymerus* (11% of 106 individuals), which had never been recorded for barnacles in general (Anderson 1994).

In parallel to these studies, multiple paternity in a natural population of *Pollicipes elegans* (416 nauplius II larvae cultivated in the laboratory from 14 broods and their respective mothers) was investigated using microsatellite markers (Plough et al. 2014). Multiple paternity was considered common (over 70% of broods and up to five fathers), and the number of fathers contributing to a brood was positively related to the field density of conspecifics in a cluster. In the three observed cases of single paternity, offspring inherited at least one allele that was not present in the mother's genotype, showing that self-fertilization is improbable (Plough et al. 2014). The upper limit to multiple paternity (five fathers) might be apparent, due to the lack of power of this study, but it might also be real and set by the number of neighbours within reach, or by the discarding of sperm, or the rejection of copulation by females (Plough et al. 2014). In this study, no evidence of long-distance sperm-casting was found. In fact, most broods had microsatellite alleles that matched those found in adults sampled from their mother's cluster, showing that fertilization occurs primarily by physically proximal males (Plough et al. 2014). Consequently, these authors suggested that if sperm-casting occurs in *Pollicipes elegans*, it is only effective over short distances, or within clusters. Nevertheless, some caution is needed when comparing the genetic studies of Barazandeh et al. (2013) and Plough et al. (2014). In addition to the different methods used (SNPs and microsatellites markers, respectively), the sampling units were also different: mothers (barnacles with eggs) and portions of egg lamellae in different stages of development and holding an undetermined number of individuals (Barazandeh et al. 2013); mothers and individual nauplius II larvae that were cultivated in laboratory conditions after taking the egg lamellae from the mothers (Plough et al. 2014). In addition, in *Pollicipes elegans* (Plough et al. 2014), determining the genetic structure of eggs from isolated individuals and mothers was not possible, because no isolated barnacles with eggs were found.

In summary, now we know that *Pollicipes polymerus* exhibits sperm-cast mating (Barazandeh et al. 2013) and pseudo-copulation (Barazandeh & Palmer 2015) and that there are still doubts regarding the possibility of partial self-fertilization (Barazandeh et al. 2013). Sperm capture in this species occurred in isolated individuals and in individuals that had only one adjacent partner (Barazandeh et al. 2013). Sperm-cast mating in *Pollicipes polymerus* might be an active process (Barazandeh & Palmer 2015), but this needs further research, namely by investigating the genetic structure of leaking sperm and leaking individuals. Field observations of sperm leaking during low tide and of copulation activity during flood and ebb suggested that pseudo-copulation is the major mode of sperm transfer in *Pollicipes polymerus* when potential mates are nearby, but observations under water are lacking (Barazandeh & Palmer 2015). The aerial copulation and reciprocal copulation that have been described for *Pollicipes polymerus* correspond to the first descriptions of these processes in barnacles (Barazandeh & Palmer 2015). Although there are no observations of pseudo-copulation in *Pollicipes elegans*, multiple paternity in this species was common (up to five fathers), fertilization occurred primarily by physically proximal males, but there is no evidence of long-distance sperm-cast mating and self-fertilization seems unlikely.

Gametogenic cycles

Gametogenic cycles of *Pollicipes* species have been described based on macroscopical observations of the female gonad and seminal vesicles of *P. polymerus* by Hilgard (1960) in California (the USA) and of *P. pollicipes* by Cardoso & Yule (1995) in SW Portugal. Histological observations of both male and female gonads and of seminal vesicles of *Pollicipes pollicipes* have been made by Molares et al. (1994b) in Galicia (Spain), Cruz & Hawkins (1998) in SW Portugal, Pavón (2003) in Asturias (Spain) and Boukaici et al. (2015) in SW Morocco.

The following description is based on the study of gametogenic cycles in *Pollicipes pollicipes* in SW Portugal by Cruz & Hawkins (1998). From October to January, most female gonads were resting and egg lamellae were absent. From March to August, the joint observation of the stage of the female gonad and the stage of development of the eggs (when egg lamellae were present) permitted the identification of the following reproductive conditions: (1) beginning of gonad maturation (a few mature oocytes and absence of egg lamellae); (2) pre-fertilization (gonad dominated by mature oocytes and frequent presence of egg lamellae, 22%, $n=64$); (3) fertilization (disintegrated ovarioles with no mature oocytes and presence of egg lamellae in all individuals); (4) post-fertilization (ovarioles dominated by residual material, atretic oocytes and immature oocytes and a few mature oocytes, and common presence of egg lamellae, 71%, $n=45$); and (5) recovery (ovarioles dominated by mature oocytes, some presence of residual material and atretic oocytes, and common presence of egg lamellae, 82%, $n=33$). In September, most gonads were in a fertilization or post-fertilization condition, while in October, most of the gonads were resting (a few empty and/or degenerated ovarioles, or sometimes with a few oogonia inside). The joint observations of the female gonad and stage of egg development suggested that, at the beginning and in the middle of the reproductive season (March to August), the ovary became disrupted after fertilization and recovered at the same time as, or before, the complete development of the eggs. However, most female gonads in pre-fertilization condition did not have egg lamellae, suggesting a delay before fertilization of the new batch. At the end of the season (September and October), the recovery of the ovary was slower than egg development and, by the time of larval hatching, the ovary had still not recovered in most of the cases. It was suggested that the pre-fertilization condition of the ovary that was common in September might change to the resting condition without fertilization occurring, as most of the female gonads were resting in October and just a few barnacles with eggs were observed (41% of barnacles with eggs in September versus 8% in October).

A similar pattern, of synchrony between female gonad and egg development during most of the reproductive period and of delayed ovary recovery relative to embryonic development at the end of this period, was observed in *Pollicipes polymerus* (Hilgard 1960). This pattern of female gonad functioning should allow *Pollicipes pollicipes* and *P. polymerus* to produce several sequential broods during the reproductive season. Hilgard (1960) suggested the existence of a time lag between broods in *Pollicipes polymerus*, as she had never observed the entire population sexually active (at most, 60% of animals with eggs). In Cruz & Hawkins (1998), similar observations were made. Most animals whose ovaries were full of mature oocytes did not contain eggs and seemed to be awaiting fertilization. These observations suggest that in *Pollicipes pollicipes*, egg production also does not seem to be continuous.

Cruz & Hawkins (1998) identified three reproductive conditions of the male gonad gametogenic cycle of *Pollicipes pollicipes* in SW Portugal: (1) the resting period (absence of testes or presence of a few small testes without sperm); (2) the beginning or end of the reproductive period (small testes present, some with a mass of sperm); and (3) the main reproductive period (numerous big testes filled with sperm). Additionally, the storage of sperm was assessed by the examination of the size of seminal vesicles using the method of Molares et al. (1994b). Throughout the year, spermatozoa were stored in the seminal vesicles, although testes were mostly absent or without sperm (resting condition) from October to December, while in the rest of the year, testes were numerous and filled with masses of sperm.

In Galicia, Spain, Molares et al. (1994b) also observed this male reproductive pattern and suggested that the duration of the reproductive period in *Pollicipes pollicipes* is primarily dependent on the development of the female gonad. The same pattern was observed in Asturias (Pavón 2003). Although no observations were made of the seminal vesicles, the results presented by Boukaici et al. (2015), regarding the development of the male gonad of *Pollicipes pollicipes* in SW Morocco, show a less pronounced seasonality between the resting period and the main reproductive period than that observed on the Iberian Peninsula by Molares et al. (1994b), Cruz & Hawkins (1998) and Pavón (2003). In other studies on male reproductive activity in *Pollicipes* (*P. polymerus* – Hilgard 1960, Lewis & Chia 1981; *P. pollicipes* – Cardoso & Yule 1995), only macroscopic observations were made on the relative size of the seminal vesicles and, as in the above Iberian studies, no seasonal pattern of variation was detected.

Regarding the female gonad, histological studies of *Pollicipes pollicipes* in Asturias (Pavón 2003), Galicia (Molares et al. 1994b), SW Portugal (Cruz & Hawkins 1998) and SW Morocco (Boukaici et al. 2015) seem to indicate an increase in the length of the reproductive period from north to south, controlled by the development of the female gonad. The ovary is dominated by mature oocytes between April and September in Asturias (Pavón 2003) and between March and September in Galicia (Molares et al. 1994b) and SW Portugal (Cruz & Hawkins 1998). In these regions, a resting period of the female gonad was identified between October and February (March in Asturias). On the contrary, in SW Morocco, ovaries with mature oocytes were observed throughout the year, although the relative abundance of individuals with mature oocytes was higher between March and October (Boukaici et al. 2015). No histological studies have been made on the development of the female gonad on the other species of *Pollicipes*.

Cruz & Hawkins (1998) did not find differences in gametogenic patterns of *Pollicipes pollicipes* at two intertidal levels (mid- and low shore), but found differences in gonad development of isolated individuals and individuals in groups, both sampled on the upper-shore. At the peak of the reproductive period, the ovaries of the isolated animals showed signs of advanced degeneration that was not a consequence of recent fertilization (as they had no eggs in the mantle cavity), while in the grouped animals, the ovaries were dominated by mature oocytes (pre-fertilization condition) or, in post-fertilization and recovery conditions, with egg lamellae in the mantle cavity. No sperm were observed in the testes of most of the isolated animals, although sperm storage was observed in their seminal vesicles, while all grouped barnacles had numerous big testes filled with sperm. These observations suggest that, in *Pollicipes pollicipes*, the presence or absence of adjacent mature animals of the same species is an important factor, through triggering or interrupting gametogenesis, respectively.

Breeding patterns

The breeding cycles of *Pollicipes* species have been studied by describing the spatial and temporal variation in the percentage of barnacles with eggs (see Table 6). The paired egg lamellae inside the mantle cavity are conspicuous and easily collected for further observations, such as grading the stage of egg development (e.g. Cruz & Araújo 1999, Macho 2006, Román et al. 2022), measuring individual egg size (e.g. Hilgard 1960, Lewis 1975a, Barnes 1989) and counting eggs (e.g. Hilgard 1960, Lewis & Chia 1981, Barnes 1989, Cruz & Araújo 1999). All studies of breeding patterns in *Pollicipes polymerus* preceded Barnes' (1996) review and were cited therein. By contrast, most studies concerning *Pollicipes pollicipes* have been made in the last 25 years (Table 6). We are not aware of any published studies on the breeding cycle of *Pollicipes elegans*, and for *P. caboverdensis*, there is a single study (Cruz et al. in prep). In most of these studies, values of the monthly variation of seawater temperature were presented (see Table 6 for range values during the main breeding season). There are several examples of cyclic breeding activities in marine invertebrates associated with seawater temperature (Orton 1920), temperature being a major environmental factor influencing physiology and ecology of marine species (Kinne 1970). Table 6 summarizes studies on the breeding patterns of the three species, including those presented in Barnes (1996). Although some caution is needed when comparing these studies, as they relate to observations in different years, different habitats and different sizes of individuals sampled and sampling size, we can identify some patterns. Moreover, reproductive phenology can alter with climate change, as shown in other intertidal species such as *Patella* (e.g. Moore et al. 2011).

One pattern is the apparent greater variation between intertidal levels that were detected at several locations for *Pollicipes polymerus* (higher values of percentage of barnacles with eggs at lower intertidal levels), whereas this variation was considered negligible in most studies of *P. pollicipes*. In the only study on the breeding pattern conducted other than under natural conditions (sea-water system of the Marine Laboratory, UC Santa Barbara, SWS, Page 1984), it was observed that most animals had egg lamellae throughout the year. Barnacles were fully submerged in the seawater system, leading Page (1984) to investigate in the laboratory whether the period of submergence had an influence on breeding activity. The percentage of *Pollicipes polymerus* with eggs was significantly greater in the continuously submerged treatment than in the exposed treatment after 6 weeks, but not after 3 weeks (Page 1984). In contrast, observations over 1 year on *Pollicipes pollicipes* in a subtidal environment (Cape of Sines, SW Portugal) revealed no variation between the breeding patterns found in intertidal and subtidal conditions (Cruz & Araújo 1999). A general pattern common to both *Pollicipes polymerus* and *P. pollicipes* is a positive relationship between barnacle size and the percentage of barnacles with eggs (Page 1986, Cruz & Araújo 1999). In *Pollicipes pollicipes*, brooding activity in small adults (12.5–15.0 mm rostrocarinal length, RC) was significantly less than in large animals (RC > 15 mm) (average of two times less in the peak of the breeding season) (Cruz & Araújo 1999).

Breeding patterns of *Pollicipes polymerus* have been studied at several locations along the American western coast, between San Juan Island, Washington (~47°N) (Lewis & Chia 1981), and Santa Catalina Island, California (~33°N) (Cimberg 1981), building on earlier observations made in the 1950s (Hilgard 1960) and 1970s (Cimberg 1981, Lewis & Chia 1981, Page 1984). Twelve degrees of latitude separate San Juan Island (Lewis & Chia 1981) from Monterey, the USA (Hilgard 1960). There was greater seasonal variation between the main breeding season (>50% of animals with eggs) and the period of lower breeding activity (<5% of animals with eggs) at the northernmost site compared with the less seasonal southernmost site, with a shorter main breeding season at San Juan Island (Hilgard 1960, Lewis & Chia 1981) (Table 6). In both studies, the main breeding

Table 6 *Pollicipes* species. Breeding patterns (% of barnacles with eggs) according to species, location and habitat (when available and when there are differences), period of observation, seawater temperature in the main breeding season, size of barnacles and sampling size

Species/latitude/location/habitat		% Of barnacles with eggs/month												Period of observation	Seawater temperature (main breeding season)	Size of barnacles and sampling size	References
Pollicipes polymerus		**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**				
48°N	San Juan Island, Washington, the USA (mean 2 sites, 2 tidal levels)	░	░	░	░	▓	■	■	■	▓	░	░	░	1971–1973	9°C–10°C	>14 mm (RC), n = ?	Lewis & Chia (1981)[a]
36°N	Mussel Point, Monterey Bay, California, the USA (mid-shore)	░	░	░	■	■	■	■	■	■	▓	▓	■	1956–1957	14°C–17°C	>27.5 mm (RC), n = 10–25	Hilgard (1960)[a]
35°N	Pismo Beach, California, the USA (low shore)	▓	░	□	▓	▓	■	▓	■	■	■	■	■	1972–1974	14°C–17°C	n = 10–80	Cimberg (1981)[a]
34°N	Goleta Point, California, the USA (low shore)	■	■	■	■	■	▓	▓	▓	▓	▓	▓	■	1978–1979	12°C–14°C	>15 mm (CH), n = 45–80	Page (1984)[a]
34°N	Latigo Point, California, the USA (low shore)	■	░	░	░	▓	▓	▓	▓	▓	■	▓	▓	1976–1977	15°C Jan; 18°C Oct	n = 10–80	Cimberg (1981)[a]
33°N	Santa Catalina Island, California, the USA (low shore)	■	■	■	■	▓	▓	▓	▓	▓	▓	■	■	1973	12°C–16°C	n = 10–80	Cimberg (1981)[a]
33°N	Santa Catalina Island, California, the USA (low shore)	■	▓	▓	▓	▓	▓	▓	▓	▓	▓	▓	■	1976	13°C–17°C	n = 10–80	Cimberg (1981)[a]
34°N	Sea-water system, Marine Laboratory UC Santa Barbara, the USA	■	■	■	■	■	■	■	■	■	■	■	■	1978–1979	12°C–17°C	>15 mm (CH), n = 20–35	Page (1984)[a]
35°N	Pismo Beach, California, the USA (upper shore)	░	░	□	▓	░	▓	▓	▓	▓	▓	▓	▓	1972–1974		n = 10–80	Cimberg (1981)[a]
34°N	Goleta Point, California, the USA (upper shore)	▓	▓	▓	▓	▓	▓	▓	░	░	▓	▓	▓	1978–1979		>15 mm (CH), n = 20–25	Page (1984)[a]
34°N	Latigo Point, California, the USA (upper shore)	░	░	░	░	░	▓	░	░	▓	▓	░	░	1976–1977		n = 10–80	Cimberg (1981)[a]
33°N	Santa Catalina Island, California, the USA (upper shore)	▓	▓	■	■	░	░	░	░	░	░	▓	▓	1973	13°C	n = 10–80	Cimberg (1981)[a]
33°N	Santa Catalina Island, California, the USA (upper shore)	▓	▓	▓	░	░	░	░	▓	▓	░	▓	▓	1976		n = 10–80	Cimberg (1981)[a]

(Continued)

Table 6 (*Continued*) *Pollicipes* species. Breeding patterns (% of barnacles with eggs) according to species, location and habitat (when available and when there are differences), period of observation, seawater temperature in the main breeding season, size of barnacles and sampling size

Species/latitude/location/habitat		% Of barnacles with eggs/month	Period of observation	Seawater temperature (main breeding season)	Size of barnacles and sampling size	References
Pollicipes pollicipes						
47°N	Brittany, *Belle-Ile*	□□□□▒██▒□□□□	1982	ND	>15 mm (RC), *n*>70	Girard (1982)
47°N	Brittany, *Saint-Guénolé* (mid-shore)	□□□□░▒█▒█▒□□	2005	ND	>15 mm (RC), *n*>170	Joncourt (2005)
47°N-48°N	Brittany, France (mid-shore, 1 or average of up to 3 sites)	□░░░░███▒░░□	2018	16.5°C–20.1°C	>15 mm (RC), *n*=40 per site	Aguión et al. (2022a)
43°N	Biarritz, France (sublitorally)	░░░▒█████▒░░	<1992	ND	ND	Barnes (1992)
43°N	Asturias (1–2 sites, mostly different among dates)	░□▒▒█████□□□	1990–1991	ND	>15 mm (AC), *n*=35–150 per site	de la Hoz & Garcia (1993)
43°N	*Punta la Cruz*, Asturias, Spain (low shore)	░□░▒██▒██▒░░	1999	14.5°C–19.5°C	>12.14 mm (LRT), *n*=50	Pavón (2003)
43°N	Asturias, Spain (mid-shore, 1 or average of up to 6 sites)	░░░▒█████▒░▒	2018	14.8°C–21°C	>15 mm (RC), *n*=40 per site	Aguión et al. (2022a)
42°N	*Cabo Home*, Galicia, Spain	░░░▒█████▒░░	1998–2001	15.1°C–17.2°C	>10 mm (LBC), *n*=70	Macho (2006)
42°N-43°N	Galicia, Spain (mid-shore, 1 or average of up to 6 sites)	▒▒▒▒▒████▒▒▒	2018	14.5°C–17°C	>15 mm (RC), *n*=40 per site	Aguión et al. (2022a)
37°N	SW Portugal (average low and mid-shore, 2 sites)	░░░▒████▒▒░░	1990–1992	15°C–20°C	>15 mm (RC), *n*>80 per site	Cruz & Araújo (1999)
37°N	Castelejo, Algarve, Portugal	░░░▒▒▒█▒▒░░░	1992–1993	17°C	> 8 mm (CH), *n*=?	Cardoso & Yule (1995)[a]
37°N	SW Portugal (mid-shore, 1 or average of up to 5 sites)	░▒▒█████▒▒░░	2018	15.5°C–16.8°C	>15 mm (RC), *n*=40 per site	Aguión et al. (2022a)
Pollicipes caboverdensis						
15°N	Tarrafal, Santiago Island, Cape Verde	▒▒▒▒▒▒▒▒▒▒▒▒	2014–2016	22°C–28°C (all year)	>15 mm (RC), *n*=40	Cruz et al. (in prep.)

Light grey stands for the non-breeding season (<5%), middle grey for 5%–49%, and black for the main breeding season (>50%). Data for blank cells are missing.

CH, capitulum height; LBC, length of capitulum base; ND, no data; RC, rostrocarinal length.

[a] Studies cited in Barnes 1996.

season occurred when seawater temperatures were highest (summer, 9°C–10°C in San Juan Island; spring and summer, 14°C–17°C in Monterey). The results of studies conducted south of Monterey (33°N–35°N) by Cimberg (1981) and Page (1984) are more intriguing and have even led Cimberg (1981) to propose that there are two physiological races of *Pollicipes polymerus*: a northern race with maximum brooding activity at cold water temperatures (14°C or less, Pismo Beach and Santa Catalina Island) and a southern one which broods in warmer temperatures (20°C, Latigo Point). According to Cimberg (1981), these races correspond, respectively, to the cold and warm temperate zones located north and south of Point Conception (34°N 27′N). However, Santa Catalina Island is located south of Point Conception, and Cimberg (1981) proposed that animals at Santa Catalina Island were derived from populations north of Point Conception. We are not aware of any subsequent genetic studies that have supported the existence of these two physiological races.

Revisiting the studies of breeding patterns in *Pollicipes polymerus* in California (see Table 6), and considering only the animals sampled at low shore in the studies of Cimberg (1981) (Pismo Beach, Latigo Point and Santa Catalina Island) and Page (1984) (Goleta Point), we can identify the following patterns (Table 6): (1) 'summer breeders', sites north of Point Conception (Monterey and Pismo Beach), with low breeding activity in winter at both sites and higher breeding activity in spring and summer (Monterey) and in summer and autumn (Pismo Beach) (in both sites, the higher breeding activity is observed when seawater temperature is relatively high, ranging from 14°C to 17°C), and (2) 'winter breeders', sites south of Point Conception (Goleta Point and Santa Catalina Island), with less pronounced seasonality (>5% of animals with eggs all year), relatively lower breeding activity in summer and higher breeding activity from late autumn, through winter, until early spring (higher breeding activity when seawater temperature is relatively lower, from 12°C to 14°C at Goleta Point, and from 12°C to 16°C at Santa Catalina Island). The location that departs from this pattern is Latigo Point, located south of Point Conception, with lowest breeding activity in late winter and early spring when seawater temperatures range between 12°C and 15°C (data from Cimberg 1981). At this site, the highest breeding values (~50%) were observed in January and October, when seawater temperatures were ~14.5°C and 18.5°C, respectively, but with much small-scale variation of the percentage of animals with eggs (error bars in Figure 2B in Cimberg 1981). More studies are needed on the reproductive patterns of the southernmost populations of *Pollicipes polymerus*, including California (the USA) and Baja California (Mexico) (no published studies) where this species reaches its southern limit of distribution (see section 'Geographical distribution'). The existing studies are more than 40 years old, and there are patterns that require further concurrent investigation in order to infer the processes that may cause them and to inform the management of the exploited populations of Baja California (see section 'Fisheries, management and conservation').

The only reference found concerning the breeding pattern of *Pollicipes elegans* is from Peru (Yacila, Paita), where the percentage of animals with eggs was higher in November (Villena 1995 in Pinilla 1996). It was suggested that the percentage of eggs varied depending on the density of the groups of this species.

Several studies have been made since Barnes (1996) of the breeding patterns of *Pollicipes pollicipes*, namely in Brittany (France), Asturias and Galicia (Spain) and SW Portugal (Table 6). The latitudinal patterns of biological processes are sometimes difficult to compare because the same methodology has not been used throughout the geographical distribution of a species. In a recent study, populations located near the northern limit of distribution of *Pollicipes pollicipes* (Brittany, France) were compared with Iberian populations (Asturias, Galicia and SW Portugal) using a standardized protocol (Aguión et al. 2022a). Brittany populations had a significantly lower reproductive effort characterized by a shorter main brooding season (3 months, July to September) than Iberian populations (5 months, May to September in Spain, and April to August in SW Portugal) (Aguión et al. 2022a). With the exception of SW Portugal, the main breeding period corresponded to the period when the sea temperature was at its highest (16.5°C–21°C in Brittany, 14.8°C–21°C in Asturias and 14.5°C–17°C in Galicia) (Aguión et al. 2022a). The average values during this period were 2°C–4°C higher than in autumn (Aguión et al. 2022a). On the contrary, the average seawater temperature between April and August in Portugal was 16.3°C, slightly lower than that recorded between September and December (17.2°C) (based on data from Aguión et al. 2022a). As suggested in a previous study in SW Portugal (Cardoso & Yule 1995), breeding patterns of *Pollicipes pollicipes* in this region seem to be positively associated with air temperature that is lower during autumn. Breeding patterns identified in this recent study (Aguión et al. 2022a) match previous observations made in France, Spain and Portugal, although a 1-month earlier start to the main breeding season was recorded in SW Portugal (Table 6). In studies made in the early 1990s, the majority of the population in SW Portugal were only observed to have ≥ 50% of barnacles with eggs from May onwards (e.g. Cruz & Araújo 1999), while in 2018, it was from April onwards (Table 6). The breeding patterns of populations to the south of Portugal are not known. However, based on the study of gametogenic cycles of *Pollicipes pollicipes* in SW Portugal and SW Morocco (see subsection above, Cruz & Hawkins 1998, Boukaici et al. 2015), we can predict a similar pattern in SW Morocco to that observed in Portugal, with a likely decline in seasonality and a potential reduction in the length of the non-breeding period. In a study rearing adults of *Pollicipes pollicipes* in the laboratory, it was observed that adults, reared for 28 days when the temperature was gradually increased from 16°C to 24°C, had a percentage of eggs (~27%) about twice as high as that observed for adults kept at 16°C (~12%) (the percentage of animals with eggs at the beginning of the experiment was ~5%) (Franco et al. 2015).

The breeding pattern of *Pollicipes caboverdensis* appears to be very different from that of its Atlantic congener, *P. pollicipes*. In the sole study conducted on the breeding of *Pollicipes caboverdensis,* monthly observations of the percentage of barnacles with eggs over 3 years (2014–2016) in Tarrafal, Santiago Island, Cape Verde, revealed that a value above 50% was recorded only in March 2016 (Cruz et al. in prep.). Although no pronounced seasonality was observed and there was variation among sampling years, the lowest values were recorded in May and June and the highest values in February and March (Cruz et al. in prep.). Seawater temperature in Santiago Island is in general lower in winter (~22°C–24°C) and higher from August to November (~26°C–28°C) (Cruz et al. in prep.). Consequently, it seems that there is a tendency in *Pollicipes caboverdensis* to breed at the coldest time of the year, while its congener, *P. pollicipes*, breeds more in warmer waters.

Size at maturity, number of broods and fecundity

The minimum size of female sexual maturity in *Pollicipes* corresponds to the minimum size at which an individual contains egg lamellae in the mantle cavity. Most studies use the rostral-carinal length (RC) as the variable to measure this size, with the following values being found: *Pollicipes polymerus* – 11 mm (Barkley Sound, British Columbia, Canada, Barazandeh et al. 2013); 14 mm (San Juan Island, Washington, the USA, Lewis & Chia 1981); 17 mm (Monterey, California, the USA, Hilgard 1960); *P. elegans* – 17 mm (Yacila, Peru, Villena 1995 in Pinilla 1996); *P. pollicipes* – 12.5 mm (SW Portugal, Cruz & Araújo 1999), ~12 mm (Galicia, Spain, from Figure 7 in Sestelo & Roca-Pardiñas 2007); *P. caboverdensis* – 13.9 mm (Tarrafal, Santiago Island, Cape Verde, Baessa 2015). Other studies have used capitular height (*Pollicipes polymerus* – 12 mm, Goleta Point, California, the USA, Page 1986; *P. pollicipes* – 8 mm, SW Portugal, Cardoso & Yule 1995) or the diameter of the base of the capitulum (13.4 mm, Parada et al. 2013). Estimates of the minimum age of *Pollicipes polymerus* for reaching female maturity, varied between: 175 days at Goleta Point, California, the USA, Page (1986); 1 year at San Juan Island, Washington, the USA, Lewis & Chia (1981); 13 months at Santa Catalina Island, the USA, Cimberg (1981); to 2 years at Vancouver Island, Canada, Bernard (1988). Most individuals of *Pollicipes pollicipes* in Galicia and SW Portugal reach female maturity within 1 year (Cruz & Hawkins 1998, Cruz & Araújo 1999, Parada et al. 2012, Parada et al. 2013).

The minimum size of male sexual maturation is not as easy to estimate as the minimum size of female maturity. Based on histological observations, Cruz & Hawkins (1998) observed that individuals of *Pollicipes pollicipes* smaller than 10 mm (RC) did not contain sperm, and thus, 10 mm (RC) was defined as the minimum size of male sexual maturation in Cape of Sines, SW Portugal. As the minimum size of female maturity was 12.5 mm (RC) at the same site, this species can be considered a simultaneous hermaphrodite with a slight protandric tendency (Cruz & Hawkins 1998).

For both *Pollicipes polymerus* and *P. pollicipes*, the number of broods per individual per year is likely to be greater than one. The number of broods has been estimated according to methods proposed or adapted from Hilgard (1960), Page (1984), Burrows et al. (1992), Aguión et al. (2022a) or Román et al. (2022). In all cases, it is necessary to assume a value of the time for complete development of embryos, from oviposition to release. Larval hatching in *Pollicipes polymerus* reared in vitro occurred on average 25.4 days (13°C–15°C, Lewis 1975b) and 30 days (13°C, Hilgard 1960) after fertilization. Molares et al. (1994a), taking egg lamellae from the mantle cavity of *Pollicipes pollicipes* and culturing them in vitro at 20°C, observed that their incubation period varied from 1 to 25 days, depending on their initial developmental stage. Larval hatching does not appear to be simultaneous for all eggs present on a single lamella. Hatching began in the peripheral eggs and ended 2 or 3 days later in the central eggs (Molares et al. (1994a). Based on a study in which adults of *Pollicipes pollicipes* were reared in the laboratory, Franco et al. (2015) observed that a low number of nauplii were released daily and that embryos at the periphery of the egg lamellae were often several developmental stages ahead of the central embryos, hatching between 2 and 10 days earlier. These authors also suggested that, in nature, the hatching process may be extended over several days, rather than occurring in a single event. Traditionally, to calculate the number of broods, a fixed embryo time of 25 days (Lewis & Chia 1981, Cardoso & Yule 1995, Cruz & Araújo 1999, Macho 2006) or 1 month (Girard 1982, Hilgard 1960) is assumed. However, a novel approach was used recently, which considered embryo development time as a function of seawater temperature (Aguión et al. 2022a, Román et al. 2022), based on Patel & Crisp (1960). Table 7 summarizes the various estimates of the number of broods per individual per year that were calculated for *Pollicipes polymerus* and *P. pollicipes*. As these estimates also use in their calculation the values of the percentage of animals with eggs, the number of broods will also be positively associated with the size of the individuals. In SW Portugal, while the estimated number of broods for animals with RC between 14 and 16 mm ranged between one and two broods, for animals with RC between 18.5 and 21 mm, it was four (Cruz & Araújo 1999). Broods of *Pollicipes pollicipes* in SW Portugal are apparently produced asynchronously, as several stages of embryonic development were always observed in individuals collected on a same sampling date (fortnightly samples taken during the breeding season) (Cruz & Araújo 1999).

The few studies that estimate the number of eggs per brood (partial fecundity) in *Pollicipes* are as follows: *Pollicipes polymerus* – 144,000–288,000 eggs (San Juan Island, Lewis & Chia 1981), 104,000–240,000 eggs (barnacles with RC between 27.5 and 32.5 mm, Monterey, Hilgard 1960); *P. pollicipes* – 16,229 eggs (average per adult with RC between 14 and 16 mm), 34,172 eggs (average per adult with RC between 18.5 and 21 mm) and a maximum of 130,000 eggs for a barnacle with RC of 25 mm (all estimates from SW Portugal, Cruz & Araújo 1999); and *P. caboverdensis* – 29,000 eggs (average per adult with RC between 18.5 and 21 mm) (Cruz et al. in prep.). Attention must be paid to the size of individuals when comparing fecundities estimated in different studies and species. Individual size (RC) was considered to positively affect the fecundity of *Pollicipes pollicipes* in SW Portugal (see estimates above), but other factors (e.g. age and density) might also be important (Cruz & Araújo 1999). For example, it was estimated that animals with RC 23–25 mm produced from 30,000 to 130,000 eggs (Cruz & Araújo 1999). Total fecundity is calculated as the product of partial fecundity and the number of broods. As an example, using the data from the study by Cruz & Araújo (1999), it can be estimated that an adult *Pollicipes pollicipes* with RC between 23 and 25 mm can produce a total of 120,000–520,000 eggs/year, while one with RC of ~15 mm will produce ~32,500 at most.

More studies are needed to estimate embryo development time (from oviposition to release). This is a key value to use in the calculation of number of broods and to describe the patterns of

Table 7 *Pollicipes* species. Estimates of number of broods per individual per year with notes on period of observation, methods used, embryo development time and study locations

Species/location	Methods	Period of observation	Number of broods and size (RC)	Embryo development time	References
Pollicipes polymerus					
San Juan Island, the USA	Adapted from Hilgard (1960)	1971–1973	2–4 (mostly < 27.5 mm)	25 days	[a]Lewis & Chia (1981)
Monterey, the USA	Hilgard (1960)	1956–1957	4–7 (27.7–32.5 mm)	30 days	[a]Hilgard (1960)
Santa Barbara, the USA	Adapted from Hilgard (1960)	1956–1957	1 (upper shore) 6.6 (low shore) 11 (seawater system)	25 days	[a]Page (1984)
Pollicipes pollicipes					
Brittany, France	Hilgard (1960)	1982	2–3	25 days	Girard (1982)
Brittany, France	Hilgard (1960)	2018	2.6 (mean 3 sites) (RC > 15 mm)	25 days	Aguión et al. (2022a)
Brittany, France	Aguión et al. (2022a)	2018	2.3 (mean 3 sites) (RC > 15 mm)	Function of seawater temperature	Aguión et al. (2022a)
Asturias, Spain	Page (1984) and Burrows et al. (1992)	2001	2.1–2.4	25 days	Pavón (2003)
Asturias, Spain	Hilgard (1960)	2018	4.5 (mean 6 sites) (RC > 15 mm)	25 days	Aguión et al. (2022a)
Asturias, Spain	Aguión et al. (2022a)	2018	3.7 (mean 6 sites) (RC > 15 mm)	Function of seawater temperature	Aguión et al. (2022a)
Galicia, Spain	Hilgard (1960)	1998–2001	3.4–4.9	25 days	Macho (2006)
Galicia, Spain	Page (1984) and Burrows et al. (1992)	2006–2007	1.73	25 days	Sestelo & Roca-Pardiñas (2007)
Galicia, Spain	Hilgard (1960)	2018	5.5 (mean 6 sites) (RC > 15 mm)	25 days	Aguión et al. (2022a)
Galicia, Spain	Aguión et al. (2022a)	2018	3.7 (mean 6 sites) (RC > 15 mm)	Function of seawater temperature	Aguión et al. (2022a)
Galicia, Spain	Román et al. (2022)	2017–2019	3.6 to 9.6 (RC > 15 mm)	25 days	Román et al. (2022)
Galicia, Spain	Román et al. (2022)	2017–2019	2.7 to 6.6 (RC > 15 mm)	Function of seawater temperature	Román et al. (2022)
SW Portugal	Hilgard (1960)	1992–1993	1–3	25 days	Cardoso & Yule 1995
SW Portugal	Page (1984) and Burrows et al. (1992)	1990–1992	1–2 (14–16 mm, RC) 4 (18.5–21 mm, RC)	25 days	Cruz & Araújo 1999
SW Portugal	Hilgard (1960)	2018	4.9 (mean 5 sites) (RC > 15 mm)	25 days	Aguión et al. (2022a)
SW Portugal	Aguión et al. (2022a)	2018	3.7 (mean 5 sites) (RC > 15 mm)	Function of seawater temperature	Aguión et al. (2022a)

[a] Studies cited in Barnes (1996).

variation in fecundity in *Pollicipes* and understand the processes causing this variation. Given that these species are exploited, estimating reproductive output and describing its variation is important, particularly for use in population dynamic models, such as that of Bald et al. (2006).

Larval phase and metamorphosis

The review by Barnes (1996) considered only three studies of larval development in *Pollicipes*, based on laboratory-reared larvae of *Pollicipes polymerus* (Lewis 1975a, b) and *Pollicipes pollicipes* (Molares et al. 1994a). Four other studies had been published prior to Barnes (1996): the descriptions of laboratory-reared larval stages of *Pollicipes pollicipes* (Coelho 1990) and its cyprid carapace shape and lattice organs, based on scanning electron microscopy (SEM) images (Jensen et al. 1994), and the descriptions and comparisons of several barnacle larvae of the Oregon and California coasts (the USA), including those of *P. polymerus* (Standing 1981, Miller & Roughgarden 1994).

Since that time, several laboratory studies of larval development, settlement and metamorphosis, and the optimization of culture conditions of larvae of *Pollicipes pollicipes* have been published (Kugele & Yule 1996, Candeias 2005, Rocha 2015, Franco 2014, Franco et al. 2016, 2017). In contrast, there have been no studies on larval rearing of *Pollicipes polymerus* since Lewis (1975a, b). Moreover, only two studies concerning the physiological temperature tolerance of nauplius II larvae of *Pollicipes elegans* have been published (Walther et al. 2013, Crickenberger et al. 2015).

The emphasis on supply-side ecology has led to several field studies that analysed the spatial and temporal patterns of larval occurrence in the wild, for both *Pollicipes polymerus* (Pineda 1999, Dudas et al. 2009, Shanks & Shearman 2009, Morgan & Fisher 2010, Tapia et al. 2010, Morgan et al. 2017, Hagerty et al. 2019) and *P. pollicipes* (Macho et al. 2005, dos Santos et al. 2007, Macho et al. 2010, Figueira 2015, Höfer et al. 2017, Mateus 2017, Fernandes in submission). Moreover, the development of physical oceanographic models enabled the estimation of larval dispersal distances and predictions of connectivity patterns in *Pollicipes pollicipes* (Rivera et al. 2013, Nolasco et al. 2022).

To our knowledge, there is no published laboratory or field research on larval phases of *Pollicipes caboverdensis*.

Description and identification of the nauplii

Laboratory-reared naupliar stages of *Pollicipes pollicipes* were first described in a preliminary study by Coelho (1990), followed by Molares et al. (1994a) and Kugele & Yule (1996). Kugele & Yule (1996) gave a detailed description of naupliar larvae, with illustrations of morphological characteristics of the naupliar stages I–VI (shape of the carapace, caudal thoracic spine and thoracic process, antennules, antennae and mandibles), including setation formulae. In *Pollicipes pollicipes*, size measurements (total length and carapace width; Table 8) are considered good predictors to distinguish naupliar stages, except for stages II and III, for which morphological characteristics are also needed, as differentiation based on size alone is not possible (Kugele & Yule 1996, Candeias 2005). Based on Molares et al. (1994a) and Kugele & Yule (1996), Candeias (2005) developed a dichotomous key for the identification of naupliar stages of *Pollicipes pollicipes*, including recognizable features in fast-moving larvae. Moreover, Macho (2006) presented a key with diagnostic characters for quick and easy identification of naupliar stages, and another to distinguish larvae of *Pollicipes pollicipes* from other intertidal barnacle larvae found in plankton samples from the NW Spanish coast.

Based on Lewis (1975a) and Miller & Roughgarden (1994), Shanks (2001) published an identification guide to the invertebrate larvae of the Pacific Northwest, including larvae of *Pollicipes polymerus*.

Effects of different culture conditions on naupliar development

Several studies have tested the effects of different environmental factors, such as temperature (Coelho 1990, Rocha 2015, Franco et al. 2017), salinity and photoperiod (Franco et al. 2017) or rearing diets (Coelho 1990, Candeias 2005, Franco et al. 2017), on the development time, survival and size of *Pollicipes pollicipes* naupliar larvae.

Table 8 Mean sizes (µm) of naupliar stages of *Pollicipes pollicipes*

	Coelho (1990)		Molares et al. (1994a)		Kugele & Yule (1996)			Candeias (2005)		
Stage	TL	W	TL	W	TL	W	CL	TL	W	CL
N I	235	149	245 (225–295)	151 (125–180)	212 (163–265)	118 (97–163)			218 (143–255)	
N II	238	235	370 (325–395)	218 (200–225)	349 (311–383)	205 (189–219)		379 (285–459)	255 (194–296)	
N III	292	296	406 (395–415)	245 (235–250)	372 (326–408)	224 (194–255)		424 (364–459)	289 (235–337)	
N IV	351	351	437 (405–490)	284 (265–300)	429 (347–469)	302 (286–337)	295 (255–367)	442 (408–479)	338 (255–398)	289 (235–326)
N V	411	403	485 (450–505)	321 (320–325)	490 (434–531)	354 (316–388)	344 (286–393)	472 (347–530)	386 (347–469)	330 (275–398)
N VI	414	407	558 (550–580)	367 (360–380)	561 (490–612)	403 (352–454)	392 (337–449)	542 (490–612)	218 (143–255)	390 (326–439)

Size ranges are given in parentheses, when available in the literature.
N I, nauplii stage I; N II, nauplii stage II; N III, nauplii stage III; N IV, nauplii stage IV; N V, nauplii stage V; N VI, nauplii stage VI; CL, carapace length; TL, total length; W, width.

Table 9 Larval development time in culture for *Pollicipes pollicipes,* according to temperature, and corresponding development stages reached in each study

Larval development time	Development stages	Reference
20 days at 15°C	NI to NVI	Coelho (1990)
9 days at 22°C		
23–28 days at 20°C	NI to C	Molares et al. (1994a)
11–24 days at 15°C–24°C	NI/II to C	Kugele & Yule (1996)
9–14 days at 17.5°C	NI to NVI	Candeias (2005)
24 days at 16°C	NI to NVI	Rocha (2015)
21 days at 22°C	NI to C	
17 days at 24°C	NI to NVI	
25 days at 11°C	NI to C	Franco et al. (2017)
18 days at 15°C		
15–16 days at 20°C		
13 days at 22°C		
10 days at 24°C		

C, cyprid; NI, nauplii stage I; NII, nauplii stage II; NVI, nauplii stage VI.

Temperature has a significant effect on the development time of *Pollicipes pollicipes* from naupliar stages I–VI to the cyprid stage (Table 9), with cultures having shorter development times with higher temperatures (Coelho 1990, Rocha 2015, Franco et al. 2017). Optimum growth and survival rates were accomplished at 15°C–20°C (Franco et al. 2017). Salinity (20–40 psu) did not affect growth rates or survival. A full-day photoperiod (24:0 L:D) provided the highest growth and shortest development time through the naupliar stages (Franco et al. 2017). Although the nutritional requirements of *Pollicipes pollicipes* nauplii appear to change during naupliar development (Candeias 2005), several tested algal monodiets provided successful development of naupliar larvae to the cypris stage, such as the flagellates *Rhinomonas reticulata* (Candeias 2005), *Isochrysis galbana* (Coelho 1990) and *Tetraselmis suecica* (Coelho 1990, Franco et al. 2017), or the diatom *Skeletonema marinoi* (Franco et al. 2017). However, higher survival rates and high-quality larvae occurred when using mixed diets of *Tetraselmis suecica/Skeletonema marinoi* or *Isochrysis galbana/Skeletonema marinoi* (Franco et al. 2017).

Two recent studies of *Pollicipes elegans* have tested population-specific, physiological temperature tolerance of stage II naupliar larvae in the laboratory (Walther et al. 2013, Crickenberger et al. 2015). Both studies found that larvae from three different populations of *Pollicipes elegans* (Mexico, El Salvador and Peru) had distinct thermal tolerance windows, with larvae from El Salvador being the most thermally tolerant of the three populations (Walther et al. 2013). Moreover, larvae from Mexico had higher thermal tolerances when collected in the northern hemisphere summer compared with the winter, an indication of seasonal acclimatization that was not seen in larvae from Peru (Crickenberger et al. 2015).

Occurrence of nauplii in the plankton

A few studies have analysed nauplii of *Pollicipes pollicipes* in plankton samples (Macho et al. 2005, Macho 2006, Macho et al. 2010, Höfer et al. 2017). In these studies, most sampled larvae were early-stage nauplii (I–III) in low numbers compared with total cirripede larvae (nauplii and cyprids): 0.1% (Macho et al. 2010), 1.5% (Macho 2006) and 4.3% (Macho et al. 2005). Macho et al. (2005) sampled early-stage nauplii (I and II) of *Pollicipes pollicipes* in NW Spain, finding that larval release of *P. pollicipes* occurred mainly during waxing and full moon and at morning high tide. In a long-term study over 3 years in estuaries (‘*rias*’) of NW Spain (Macho 2006), abundance of *Pollicipes pollicipes* nauplii was always <30 individuals/m^3 and was higher during summer and autumn, with no larvae present from December to March. By contrast, at another location (30 km to the south) closer to adult populations, larvae reached a maximum of 145 individuals/m^3 and were present from the middle of spring through summer, with very few larvae detected in autumn (Macho 2006). In the southern Bay of Biscay (Spain), Höfer et al. (2017) found consistently high densities (maximum of 300 individuals/m^3) of *Pollicipes pollicipes* nauplii inside a river plume. In this study, naupliar abundance was strongly correlated with chlorophyll concentration. These authors suggested that river plumes may act as nurseries for *Pollicipes pollicipes* naupliar larvae, due to local enhanced food supply and convergent currents that promote the aggregation of larvae.

Regarding *Pollicipes polymerus* naupliar larvae, while assessing the vertical and horizontal distribution of nauplii (II–VI) collected on the Oregon coast (the USA), Shanks & Shearman (2009) found that all larval stages remained close to the shore (within 5 km) and that upwelling/downwelling events had no influence on their distribution. In this study, larvae were rare in surface waters, in contrast to the study of Tapia et al. (2010), in which nauplii of *Pollicipes polymerus* were mainly found near the surface in a fixed nearshore station in Southern California (the USA). Tapia et al. (2010) also tested day/night patterns of larval vertical distribution, but no differences were found. In their study, nauplii of *Pollicipes polymerus* represented 15% of the total barnacle larvae.

Description and identification of the cyprids

Cypris larvae of *Pollicipes pollicipes* reared in the laboratory were first described by Coelho (1990) and Molares et al. (1994a). However, in the latter study, cyprids were probably in a poor nutritional condition and the authors incorrectly used the absence of oil droplets as a diagnostic characteristic of *Pollicipes pollicipes* larvae (Kugele & Yule 1996, Cruz 2000). Kugele & Yule (1996) presented detailed descriptions and illustrations of the thoracic and caudal appendages of *Pollicipes pollicipes* cyprids. Furthermore, studies based on SEM images of *Pollicipes pollicipes* cyprids have provided detailed descriptions of their carapace shape and lattice organs (Jensen et al. 1994) and the morphology of their attachment organs (Al-Yahya et al. 2016).

Table 10 Mean sizes of cyprid larvae of *Pollicipes pollicipes* and *P. polymerus*, either reared in laboratory conditions (L) or collected in the field (F)

Species	Length (µm)	Height (µm)	Width (µm)	Lab/field	Field location	Reference
Pollicipes pollicipes	299	153		L		Coelho (1990)
	402 (390–420)	231 (210–250)		L		Molares et al. (1994a)
	433 (403–455)	215 (170–248)		L		Kugele & Yule (1996)
	487	252	164	F[a]	Cape of Sines, SW Portugal	Cruz (2000)
		200 to 212		L		Franco et al. (2017)
	503 (487–525)	250 (237–262)	158 (147–175)	F	Cape of Sines, SW Portugal	Fernandes (in submission.)
Pollicipes polymerus	425	232		L		Lewis (1975a)
	622	320		F	Monterey Bay, CA, the USA	Miller & Roughgarden (1994)
	494 (420–520)	256 (220–260)	177 (160–200)	F	Bodega Harbour, CA, the USA	Standing (1981)

Size ranges are given in parentheses, when available in the literature.

[a] Settled cyprids collected from the peduncle of conspecifics, not from plankton.

A combination of size measurements (carapace length, height and width; Table 10) and morphological characteristics can be used to distinguish cyprids of *Pollicipes pollicipes* from those of other cirripede larvae found in Atlantic Iberian waters (Cruz 2000, O'Riordan et al. 2001, Fernandes in submission). Carapace length alone can separate cyprids of *Pollicipes pollicipes* and of *Chthamalus montagui* from the other cirripedes. Although both the length and the height of the cyprid carapace of *Pollicipes pollicipes* and *Chthamalus montagui* overlap, the width in dorsal view can be used to distinguish cyprids of these two species (Cruz 2000, Fernandes in submission). Moreover, several morphological characteristics can be used to clearly and easily distinguish *Pollicipes pollicipes* cyprids collected in the plankton: (1) the carapace shape, with a broadly rounded anterior end visible in side view and a very narrow profile detected in dorsal view, (2) the carapace ornamentation, with rounded or polygonal contiguous depressions, and (3) the size of the compound eyes, which are bigger than in *Chthamalus montagui* cyprids (Fernandes in submission; Figure 9).

Cyprids of *Pollicipes polymerus* have been described in detail by Standing (1981) and Miller & Roughgarden (1994), based on plankton samples collected along the California coast (the USA). Lamont & Emlet (2018) also described the morphology of plumose setae and their relationship with swimming motions in cyprids of *Pollicipes polymerus* and detected that setules are permanently fused, as in other cirripede groups. Cyprids of *Pollicipes polymerus* are morphologically similar to those of *P. pollicipes* (Kugele & Yule 1996) and are also similar in size (Table 10) to cyprids of *Chthamalus* species from the Oregonian province (Standing 1981).

The main characteristics distinguishing cyprids of *Pollicipes polymerus* from those of other cirripedes found in the plankton of the NE Pacific coast are as follows: the broadly rounded anterior end in side view, the narrow carapace profile in dorsal view, a carapace sculptured with small papillae, a break in the posterodorsal margin and the greater depth of the carapace located at the level of the compound eyes (Standing 1981, Miller & Roughgarden 1994). This last characteristic is not present in *Pollicipes pollicipes* cyprids (Fernandes in submission), and the break in the posterodorsal margin is also not as clear (Cruz 2000, Fernandes in submission).

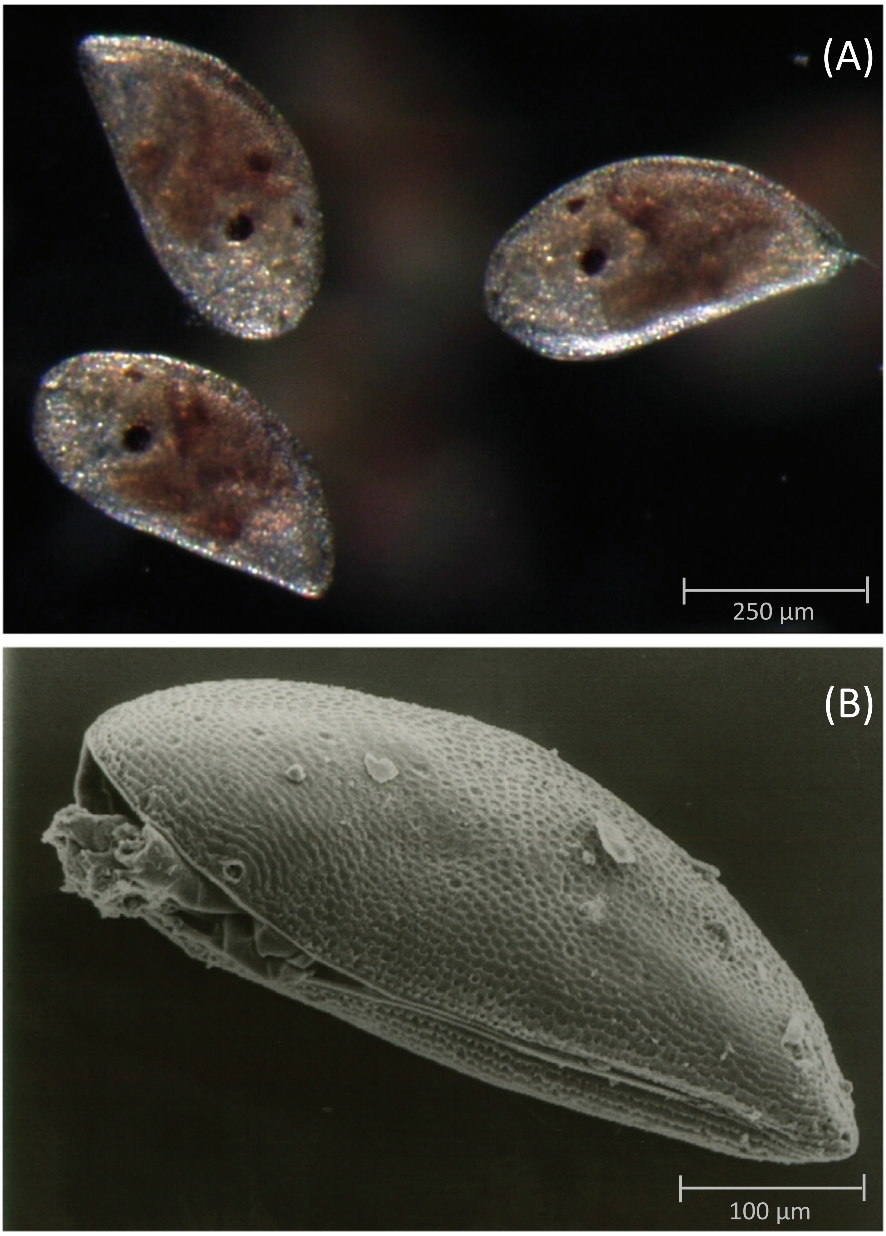


Figure 9 Cyprids of *Pollicipes pollicipes* collected (A) in plankton samples from SW Portugal and (B) from the peduncle of an adult *Pollicipes pollicipes* individual, where the cyprid was starting to metamorphose (SEM image).

Settlement and metamorphosis in culture

The conditions necessary to promote settlement and metamorphosis of cyprids of *Pollicipes* species in culture are still poorly understood, with very few studies to date (Lewis 1975a, Kugele & Yule 1996, Franco 2014, Franco et al. 2016). The work by Lewis (1975a) regarding *Pollicipes polymerus* settlement in culture was reviewed by Barnes (1996). Overall results from these studies suggest high cyprid selectivity in these species.

Settlement experiments with *Pollicipes pollicipes* cyprids in laboratory conditions compared several different natural and artificial substrata, with the majority of total settled cyprids (93%, Kugele & Yule 1996; 82%–97%, Franco 2014) consistently being found on live adults. Nevertheless, settlement rates of cypris larvae on conspecifics were low in these studies and varied between less than 1% (Kugele & Yule 1996) and 30%–40% (Franco 2014, Franco et al. 2016). Kugele & Yule (1996) found that only cyprids aged less than 10 days settled and metamorphosed and 78% of these were aged 4 days or less. Nevertheless, cyprids older than 17 days (Kugele & Yule 1996) or 20 days (Franco et al. 2016) were still alive in cultures. Franco et al. (2016) showed that preferential environmental conditions needed to maximize settlement and metamorphosis rates include natural salinities (30–40 psu), a temperature of 20°C, illumination, water circulation and cyprid age not exceeding 3 days.

Cyprid metamorphosis is a complex process that involves a series of morphological, physiological and biochemical events. To our knowledge, there are no published studies of this process in *Pollicipes* species. However, a description of cyprid metamorphosis of the closely related species, *Capitulum mitella* (Family Pollicipedidae), was published recently (Lin & Rao 2016), where a timeline and a detailed description of the morphological events are given, based on light microscopy and SEM analyses.

Occurrence of cyprids in the plankton

Several field studies have investigated the spatial and temporal patterns of the natural distribution and abundance of larvae of *Pollicipes* species, namely *P. pollicipes* and *P. polymerus*. These studies used neustonic and water-column plankton trawls (dos Santos et al. 2007, Shanks & Shearman 2009, Macho et al. 2010, Morgan & Fisher 2010, Höfer et al. 2017, Morgan et al. 2017, Fernandes in submission), pump sampling (Pineda 1999, Macho et al. 2005, Tapia et al. 2010, Hagerty et al. 2019), cyprid traps (Dudas et al. 2009, Figueira 2015, Mateus 2017) or a combination of several of these methods.

Studies where cypris larvae of *Pollicipes pollicipes* were collected in the plankton are scarce. In a study of spatial distribution of cypris larvae in the central Portuguese coast, dos Santos et al. (2007) only considered the cypris carapace length as a criterion for species distinction. Consequently, these authors were unable to distinguish cyprids of *Pollicipes pollicipes* from those of *Chthamalus montagui*. These authors found that cyprids of these species (*Chthamalus montagui* and *Pollicipes pollicipes*) were close to the coast and mainly in the neuston during the day. However, some doubts remain as to whether these data can be attributed to *Pollicipes pollicipes* larvae. Indeed, a very small proportion of *Pollicipes pollicipes* larvae (1%–3%) was reported out of the combined total of *Chthamalus montagui* and *Pollicipes pollicipes* larvae in a study on the temporal and spatial distribution of cyprid larvae in SW Portugal (Fernandes in submission). In this study, cyprids of *Pollicipes pollicipes* reached a maximum of 126 larvae/m^3 at the neuston layer and 33 larvae/m^3 in the water column. Also, in the same region (SW Portugal), but inside the Port of Sines where adults of *Pollicipes pollicipes* are absent or rare, Figueira (2015) and Mateus (2017) found cypris larvae of this species using neustonic trawls (maximum densities 36 cyprids/m^3 during summer and autumn). Moreover, in a study over 3 years in NW Spain, cyprids of *Pollicipes pollicipes* were found in very low numbers (maximum of 5 cyprids/m^3) during summer (Macho 2006).

Besides using plankton trawls, Figueira (2015) and Mateus (2017) also used cyprid traps (adapted from those used in Dudas et al. 2009) to study the spatial patterns of *Pollicipes pollicipes* cyprid distribution. However, cyprids were not efficiently retained in the traps (Mateus 2017). The number of cyprids found in the cyprid traps deployed during either a 4-day or a 12-hour period was consistently much lower than the number of cyprids collected during 1-minute plankton trawls for the same location and dates (Mateus 2017).

Several studies in the NE Pacific coast have analysed patterns of abundance of invertebrate larvae in the plankton, including the cyprids of *Pollicipes polymerus*, relating those patterns to physical transport processes to the shore. In the nearshore of southern California (the USA), Pineda (1999) showed that *Pollicipes polymerus* cypris larvae accumulated in internal tidal bore warm fronts. Accumulation of these larvae was also found in the offshore warmer side of these features, but not in the onshore colder side of the fronts (Pineda 1999). It was suggested that, if these fronts propagate all the way to the shore, larvae will be transported onshore. In this study, the first to analyse the vertical distribution of *Pollicipes polymerus* cyprids, larvae were not strictly neustonic, but their abundance peaked at the sea surface (~56% of total cyprids). In contrast, a study by Tapia et al. (2010), in the same location, found cyprids of *Pollicipes polymerus* (6.8% of total barnacle larvae) located mainly within the mid-depth and bottom layers, suggesting that changes in their mean depth of distribution may be correlated with the pycnocline depth. Moreover, the distribution of cyprids of *Pollicipes polymerus* on the Oregon coast (the USA) and its relationship with upwelling/downwelling events was investigated by Shanks & Shearman (2009). This study found that cypris larvae always remained close to the shore (less than 2 km) during both sets of oceanographic conditions. Conversely, on the same coast, Dudas et al. (2009) did not find any differences in the abundance of *Pollicipes polymerus* cyprids along shore, across shore or at different depths. The importance of surf-zone hydrodynamics to onshore larval transport was studied by Morgan et al. (2017), but total barnacle cyprids were analysed as a group, with few details about the distribution and abundance of *Pollicipes polymerus* cyprids. On reflective beaches, and contrary to most zooplankters, barnacle cyprids were mainly located inside the surf zone, and in those situations, *Pollicipes polymerus* represented 12.1% of total barnacle cyprids (Morgan et al. 2017). Finally, barnacle cyprid distribution in the nearshore of southern California was analysed before, during and after the 2015–2016 El Niño event (Hagerty et al. 2019). In summer 2016, after the El Niño event, Hagerty et al. (2019) documented a large peak in the density of *Pollicipes polymerus* cyprids, which were found to be closer to shore during fall-winter than during spring-summer sampling periods.

Larval dispersal and connectivity

Biophysical models of larval dispersal have long been the dominant approach for estimating contemporary marine larval dispersal (see the review of Swearer et al. 2019). According to this review, there is a need for greater emphasis on the validation of model assumptions, as well as testing of dispersal predictions with empirically derived data. For the first time, Nolasco et al. (2022) have described and validated a biophysical model of larval dispersal with *Pollicipes pollicipes*. Furthermore, in the latter study, predictions of supply (from the model) have been compared against observations of recruitment on conspecifics at monthly intervals (see next section) in three regions: Asturias and Galicia (Spain) and SW Portugal. The main results were the following: estimates of average realized dispersal were –73 to +63 km in the S/W and N/E directions and of larval retention were 2.0 to 2.4%; extensive larval exchange occurred within regions; Galicia and Asturias regularly exchanged larvae; and SW Portugal does not seem to be directly connected to Galicia and Asturias through larval dispersal (Nolasco et al. 2022).

A more simplistic biophysical model of *Pollicipes pollicipes* larval dispersal, based on current profiles of a single nearshore location on the Cantabrian coast, was also developed by Rivera et al. (2013). These authors predicted high recruitment success, with a peak at 56 km west of the emission point, in a year of high upwelling activity. In contrast, in a year of low upwelling, theoretical recruitment success was low and peaked 13 km east of the emission point (Rivera et al. 2013). Furthermore, estimates of population migration rates, obtained in a reanalysis of genetic data for five populations of the Cantabrian coast, pointed to a net long-term, westward larval transport along this coast (Rivera et al. 2013).

Lewis (1975a) made a theoretical estimation of *Pollicipes polymerus* larval dispersal from Bodega Bay (California, the USA) of 187–933 km, based on current speeds in that area and on a larval development time of 42 days.

The main shortcomings in knowledge of the larval biology of *Pollicipes* species are the absence of studies concerning larval phases, and metamorphosis of *P. caboverdensis* and *P. elegans*, either in the field or in laboratory conditions. The clear identification of the various larval stages of these two species, estimates of larval development time in culture, and the study of their distribution and abundance in the plankton are essential for a better understanding of the mechanisms of larval release, dispersal and transport back to the coast.

Biophysical models of larval dispersal, such as that developed for *Pollicipes pollicipes* in the study by Nolasco et al. (2022), should be pursued for the other three *Pollicipes* species. These are powerful models and, when coupled with empirical data such as larval distribution in the plankton, or settlement data, can give us important new insights into ecological questions or management strategies of *Pollicipes* species.

Settlement and recruitment

Barnes (1996) had no specific section on settlement and recruitment within the genus *Pollicipes*. The knowledge available at that time regarding these processes was reviewed and integrated into the sections 'Orientation and movement of peduncle' and 'Moulting and growth'. This knowledge was mainly related to the spatial variation of these processes in *Pollicipes polymerus*, namely the distribution and abundance of cyprids and juveniles on peduncles of conspecifics and on other habitats (Barnes & Reese 1960, Lewis 1975b, Hoffman 1984, 1988, Bernard 1988, Hoffman 1989, Satchell & Farrell 1993), and on the ability of juveniles to move along the peduncle (Kugele & Yule 1993).

The concepts of settlement and recruitment that Barnes (1996) used ("the settlement period begins when an animal first lands on a substratum, that is when a cyprid has cemented itself to the surface" and "recruitment is a measure of recently-settled juveniles that have survived for a period of time after settlement") are the same as those previously defined by Connell (1985), which are particularly suitable for both acorn and stalked barnacles after metamorphosis. Other definitions of recruitment are also widely used: in many studies of population biology, recruitment is defined as entry to the adult population (see Pineda et al. 2006); in fisheries research, recruitment is generally considered to be to the exploited population, which is usually also the adult breeding population. As juvenile barnacles occur on or among the peduncles of adults which are harvested, they can certainly be considered to have entered the exploited population, albeit as by-catch and although not yet adult.

As Connell (1985) noted, however, the measurement of settlement of planktonic propagules of marine benthic organisms is very complex (with a need for observations at very frequent intervals to avoid missing any larvae that attach and then become detached within a short period), since most larvae or algal propagules either are very small when they attach, or attach in cryptic habitats (e.g. crevices, among algae). There is some consensus that daily sampling of settlement is a good compromise between logistical difficulty and accuracy of estimating settlement (Connell 1985, Pineda et al. 2010). In the case of *Pollicipes*, the measurement of settlement rate is even more complex due to the very hydrodynamic locations where these species live, hindering observations, especially those with high temporal resolution, such as on a daily basis. To our knowledge, Pineda (1991, 1994) made the only field studies of *Pollicipes* in which daily settlement rates were measured. Pineda (1991, 1994) used white polyvinyl chloride semi-pipes, with grooves in their inner face, to measure daily settlement of *Pollicipes polymerus* (Dike Rock, La Jolla, California, the USA). These studies were not cited in Barnes (1996). Thus, we can consider that all other field studies regarding settlement and recruitment of *Pollicipes* species have effectively measured recruitment as defined by Connell (1985) (e.g. Hoffman 1989, Satchell & Farrell 1993, Cruz et al. 2010a, Fernandes et al. 2021).

Do cyprids of Pollicipes *prefer to settle on conspecifics?*

A major paradigm that exists in relation to the settlement of *Pollicipes polymerus*, and that has also been stated for *P. pollicipes*, is that cyprids settle preferentially on adult peduncles. The references commonly cited to support this paradigm are Barnes & Reese (1960), Lewis (1975b) and Hoffman (1989). Barnes (1996) did not specifically refer to the term 'preference', although she mentioned that "cyprids and young spat are almost always found on peduncles of older *Pollicipes* (*P. polymerus*) as this provides an ideal substratum for settlement". However, none of the studies cited above tested specific hypotheses regarding preference behaviour of cyprids for the peduncles.

Barnes & Reese (1960) observed that in the field, solitary individuals of *Pollicipes polymerus* were rare, stating that "…neither cyprids nor young spat (which are more easily detected) are found in numbers on the rocks. By contrast, the peduncles of adults, both small and large, are covered with cyprids and young spat" and that "it is curious that this restriction to the adult as a settling surface is virtually limited to the peduncle; very rarely are cyprids found on the capitulum". In the study by Lewis (1975b), laboratory observations were made, from fertilization through to settlement of *Pollicipes polymerus*. Very few cyprids were successful in settling: two cyprids settled on mudstone rock adjacent to the base of an adult *Pollicipes polymerus*; one cyprid settled on the base of the peduncle of a healthy adult *P. polymerus* (Lewis 1975b). Based on this study, no cyprids were observed to settle on the epidermis of the peduncle of *Pollicipes polymerus*, on slides dipped in *P. polymerus* extract and/or on slides with primary films. In parallel with this laboratory study, based on field observations, Lewis (1975b) noted that settled juveniles were never found far from established adult clusters and stated that "From 37 adult clusters, an average of 81% of the associated juveniles preferred the adult peduncle to any other available substrate", but no detail on these observations was given. The conclusion of Lewis' (1975b) study was that "it appears that both chemosensory and tactile responses are necessary for the specific settling behaviour of *Pollicipes polymerus* cyprids". Hoffman (1989) assumed preferential attachment of cyprids of *Pollicipes polymerus* on conspecifics, having observed very high recruitment on conspecifics (recently settled spat reaching very high densities on the peduncular surface, >300 cm^2). He also considered that there was little evidence that *Pollicipes polymerus* had established new aggregates on open hard substrates, although he did observe recruitment on primary substrata (the under surface of a rock that had been cut by a storm, and on a denuded rock).

On the other hand, there are several observations of recruitment of *Pollicipes polymerus* onto substrates other than conspecific adults. Artificial substrates on which settlement occurred were terra-cotta tiles (intake seawater system, La Jolla, California, the USA, Hoffman 1988), grooved polyvinyl chloride plates (intertidal, La Jolla, California, the USA, Pineda 1991, 1994), fibre glass plates covered by safety walk tape (intertidal, Monterey, California, the USA, Satchell & Farrell 1993) and Plexiglas plates covered by safety walk tape (intertidal, four sites along the central coast of Oregon, the USA, Dudas et al. 2009). Natural substrates included cleared rock, laminarian fronds, coralline algae in tide pools, any solid substrate (Amphitrite Point, British Columbia, Canada, Bernard 1988); primary substrate (intertidal, La Jolla, California, the USA, Hoffman 1989, Pineda 1994); and mussels and acorn barnacles (Clayoquot Sound, western Canada, Gagne et al. 2016).

Field recruitment of *Pollicipes pollicipes* on artificial substrates is apparently more difficult to observe, as there are descriptions of several failed attempts of observation on various substrates: Tufnol, plastic net, rubber and sisal ropes with or without extracts of crushed *P. pollicipes* (Coelho 1991); grooved marble stone, grooved rectangular PVC plates, PVC threaded cylinders similar in size to an adult *P. pollicipes* (Cruz 2000); epoxy biomimetics, calcium silicate, Tufnol, glass epoxy, carbon epoxy, glass vinyl ester, nylon and slate (Franco 2014). However, recently, recruitment *of Pollicipes pollicipes* has been observed on artificial substrates: marine epoxy (Cape Sardão, SW Portugal, Franco 2014); PVC half-pipes covered by safety walk tape with the concavity facing the rock surface (Cape of Sines, SW Portugal, Jacinto 2016); and a specifically designed settlement substratum, the 'barticle'(Cape of Sines, SW Portugal, Fernandes 2018, European Patent nr. EP3372073B1, see Figure 15). Recruitment of Pollicipes pollicipes on natural substrates other than conspecifics (rock, calcareous algae and acorn barnacles, Coelho 1991, Cruz 2000, Franco 2014; mussels GM, DJ and AS pers.obs.) has also been observed.

In conclusion, we are not aware that the theory of preferential settlement of cyprids of *Pollicipes* species on adult peduncles has ever been tested directly and clearly by addressing specific hypotheses. Differences in settlement and post-settlement mortality of *Pollicipes* on conspecifics versus other natural substrates have never been tested in the field. What we do know today is what Margaret Barnes stated in 1996: "There is little doubt that settlement is heavy among adults of the same species". Challenging questions to answer in the future are the following: 'Is there preferential settlement of cyprids of *Pollicipes* species on adult peduncles?' and 'Is heavy recruitment on conspecifics the result of post-settlement processes (e.g. more benign physical habitat and/or lower predation on conspecifics)?'.

Settlement and recruitment on artificial substrates: small-scale spatial patterns

Satchell & Farrell (1993) observed that *Pollicipes polymerus* consistently settled in a strongly aggregated pattern, since typically three to eight cyprids settled in contact with each other (settlement on plates collected every 8 days, Monterey, California, the USA). These authors also observed a positive association between densities of barnacle settlers and the degree of aggregation among cyprids. Contrasting spatial patterns among sites and vertical levels (intertidal, La Jolla, California, the USA) of daily settlement of *Pollicipes polymerus* and *Chthamalus* species on artificial plates prompted Pineda (1994) to suggest that settlement is intensified by behaviour in *Pollicipes polymerus*, but much less so in *Chthamalus* species. In relation to *Pollicipes pollicipes*, a higher intensity of recruitment was detected on artificial substrates placed at sites where this species naturally occurs than at sites located relatively close (from metres to 100s metres), but where the abundance of this species is much lower or absent (sites relatively less exposed) (Jacinto 2016, Mateus 2017, Cruz et al. unpublished observations).

Recruitment indices

As recruitment of *Pollicipes polymerus* and *P. pollicipes* is high on conspecifics, Barnes (1996) considered that the recruitment rate could be determined with relative ease. Different types of indices of recruitment on conspecifics have been used: the number of cyprids and/or juveniles of different sizes attached to adults (Hoffman 1989, de la Hoz and Garcia 1993, Cruz 2000, Pavón 2003, Cruz et al. 2010a, Fernandes et al. 2021, Aguión et al. 2022a), and percentage of adults with attached barnacles (Molares 1994) or juveniles (Macho 2006). Artificial substrates have also been used to describe the temporal and spatial variation of settlement and recruitment in *Pollicipes polymerus* (Pineda 1991, 1994, Dudas et al. 2009), but were not compared with indices of recruitment on conspecifics. Unlike indices of recruitment on conspecifics, which measure recruitment to a certain size of juveniles (typically juveniles with maximum distance between the rostrum and the carina, RC of <0.6 mm or RC < 1 mm), indices that use artificial substrates can measure recruitment over an exact period corresponding to time of field deployment of the substrates. Recently, a new recruitment index for *Pollicipes pollicipes* has been developed for monitoring purposes in ecological or fisheries studies, in which the recruitment rate of *P. pollicipes* on an artificial substratum ('barticles', European Patent nr. EP3372073B1, see Figure 15) was measured over a given period (Mateus 2017). A comparison was made among the index of recruitment on 'barticles' and indices of recruitment of *Pollicipes pollicipes* on conspecifics (see above) based on observations in SW Portugal (Mateus 2017). The conclusion from this study was that the mean number of cyprids and juveniles (RC < 0.6 mm) attached to conspecific adults (*n* = 15–20 adults) represents the best estimate of recruitment on conspecifics that occurred 15 days or 1 month before sampling. The use of artificial substrates ('barticles' or others) can enable comparisons of recruitment rates of *Pollicipes* among different locations, periods or experimental situations, making them less confounded, and avoiding destructive sampling (Mateus 2017).

Recruitment on conspecifics: small-scale spatial patterns

Small-scale spatial patterns of settlement and recruitment of *Pollicipes polymerus* and *P. pollicipes* on conspecifics (adults) have been described in relation to: position along the adult (both species, Hoffman 1984, Cruz et al. 2010a, Helms 2004), position around the peduncle (*P. polymerus*, Helms 2004), location within the cluster of conspecifics (edge, middle and centre) and cluster size (small – 4.7 cm diameter, large – 8–14 cm diameter) (*P. polymerus*, Helms 2004), intertidal levels (both species, Hoffman 1989, Pavón 2003, Cruz et al. 2010a), subtidal versus intertidal level (*P. pollicipes*, Cruz 2000), and horizontally among places (*P. pollicipes*, Cruz 2000).

The distribution of juveniles along the adult (capitulum and peduncle) is not random, with *Pollicipes polymerus* and *P. pollicipes* showing opposite patterns of distribution along the peduncle. In both studies conducted with *Pollicipes polymerus* (La Jolla, California, the USA, Hoffman 1984; Cape Arago, Oregon, the USA, Helms 2004), a gradient was observed, from the smallest and most abundant juveniles on the peduncle near the junction with the capitulum ('growth zone', see section 'Growth and size') to the largest and fewest near the base of the peduncle. In *Pollicipes pollicipes*, most small juveniles (RC < 0.6 mm) were located on the basal half of the peduncle and only a few (<5%) were located on the 'growth zone' (SW Portugal, Cruz et al. 2010a). These patterns might be explained by differential settlement and post-settlement processes between species and along the peduncle. As already noted in Barnes (1996), we know that individuals of the species *Pollicipes polymerus* and *P. pollicipes* are able to move along the peduncle towards the base. This mobility was first suggested by Hoffman (1984. in *Pollicipes polymerus*) and later confirmed by Kugele & Yule (1993, 2000 in *P. pollicipes*). The active relocation of juveniles along the peduncle might explain the pattern of larger juveniles being more abundant near the base. The higher abundance of small juveniles of *Pollicipes polymerus* on the growth zone might be not only due to a potential greater attractiveness of this zone (new cuticle) for cyprid settlement (Hoffman 1984), but also due to the suppression of juvenile growth in this zone, as a result of intraspecific competition for food with adult conspecifics ('shading effect') (Cimberg unpublished observations in Hoffman 1984). The discrepancy in the patterns observed in the two species may also result from possible differences in the density of *Pollicipes* clumps and, consequently, in the availability of settlement substrate (Cruz et al. 2010a). If there are differences in adult density between California/Oregon and SW Portugal (no data available), namely more dense and packed clumps in California/Oregon since *Pollicipes pollicipes* is heavily exploited in Portugal (see section 'Fisheries, management and conservation'), the basal area of the peduncles might be less accessible for settlement in California/Oregon (Cruz et al. 2010a). Recruitment on the capitulum of the adults was considered to occur rarely (*Pollicipes polymerus*, Hoffman 1984, 1989) or at a significantly lower rate than on the peduncle (*P. pollicipes*, <5% on the capitulum, Cruz et al. 2010a). Another explanation for the lower recruitment on the capitulum might be due to higher mortality of cyprids and settlers due to grazing/bulldozing of limpets that can be abundant on the capitular plates (Hoffman 1984, unpublished observations, see section 'Post-settlement processes of distribution and abundance' and Figure 12), or by higher selectivity of cyprids for the scales of the peduncle than the capitulum (Barnes & Reese 1960, Chaffee & Lewis 1988).

Juveniles of *Pollicipes polymerus* on adults, in clusters, were observed to aggregate more frequently below the rostrum and carina of the adult conspecific than at other locations around the peduncle (Cape Arago, Oregon, the USA, Helms 2004). Previously, this pattern had been described by Hoffman (1989). Helms (2004) suggested that individuals in a cluster might act as a filter, creating dead spaces around their peduncles below the rostrum and carina, or that abrasion (and consequent mortality) by adjacent adults is less intense below the rostrum and carina.

The distribution of juveniles of *Pollicipes polymerus* on adults within clusters is not random (Cape Arago, Oregon, the USA, Helms 2004), and, according to this study, there was a significantly higher abundance of juveniles on adults at the edges of the clusters that in the centre of the clusters. Helms (2004) suggested that settlers on adults at the edges of clusters might have higher survival if cluster edges have higher food availability, reduced crowding by adults, or reduced predation, namely from within-cluster predators (e.g. flatworms and crabs). The cluster size of *Pollicipes polymerus* was not as important in determining the abundance of recruits as location within the cluster (Helms 2004).

Regarding variation in recruitment of species in *Pollicipes*, at different tidal levels, no consistent patterns have been found. Higher recruitment of *Pollicipes pollicipes* occurs on the low shore of SW Portugal than higher on the shore (two sites in SW Portugal, Cruz et al. 2010a). In contrast, no consistent patterns were observed between tidal levels regarding the recruitment of *P. pollicipes* in Figueras, Asturias, Spain (Pavón 2003) or of *P. polymerus* in La Jolla, California, the USA (Hoffman 1989). In a unique study of *Pollicipes* in which recruitment on adults of *P. pollicipes* collected in the intertidal and subtidal was compared, no consistent patterns were found between the two habitats (Cruz 2000).

Concerning horizontal patterns in recruitment, several observations were made of *Pollicipes pollicipes* in SW Portugal. This species is abundant on very exposed shores, but even on these shores, abundance can drop dramatically between seaward rock walls directly facing the swell compared with the landward walls of those same rocks (Cruz 2000). No differences in the recruitment on conspecifics were observed in adults collected in the centre of their horizontal distribution or in adults located at the edge of that same horizontal distribution, in slightly less exposed locations (Cruz 2000). However, when transplanting adults of *Pollicipes pollicipes* in cages to areas where barnacles were much less abundant, or absent (rock walls not directly facing the swell on very wave-exposed shores, less-exposed shores), less recruitment on transplanted adults was observed than on adults collected from naturally occurring areas (Cruz et al. unpublished observations).

Settlement and recruitment: large-scale spatial patterns and temporal patterns

The only study mentioned by Barnes (1996) on the temporal variation of recruitment of species of *Pollicipes* was the study by Hoffman (1989) (La Jolla, California, the USA), of the monthly recruitment on conspecifics by *P. polymerus* over 1 year (Table 11). At the time of Barnes' (1996) review, two studies of the daily variation in the settlement of *P. polymerus* were also published (artificial substrates, April to July 1989, La Jolla, California, the USA, Pineda 1991, 1994), but were not cited by Barnes (1996).

Twenty-five years after Barnes (1996), there have been various studies investigating the temporal variation of recruitment of *Pollicipes polymerus* during periods of 3–14 days (artificial plates, July to September 1998 and 1999, four sites along the central coast of Oregon, Dudas et al. 2009), of the monthly recruitment of *P. pollicipes* on conspecifics at various sites in Europe and over various years (Pavón 2003, Macho 2006, Cruz et al. 2010a, Fernandes et al. 2021, Aguión et al. 2022a) and of the monthly recruitment of *P. caboverdensis* on conspecifics (Tarrafal, Santiago, Cape Verde, 2014–2016, Cruz et al. in prep.) (see Table 11). At the time of Barnes' (1996) review, there is no published information on spatial or temporal variation in the recruitment of *Pollicipes elegans*.

The description and comparison of large-scale spatial patterns and temporal patterns of recruitment in *Pollicipes* is hampered when not using the same methods, namely the same recruitment index. However, since many of these studies did use the same recruitment index (average number of cyprids

Table 11 Recruitment in *Pollicipes* species

Species/latitude/location/habitat		Recruitment on conspecifics/month												Period of observation	Index of recruitment (size of juveniles)	Size of adults and sampling size	References
		J	F	M	A	M	J	J	A	S	O	N	D				
Pollicipes polymerus																	
32°N	Dike Rock, La Jolla, California, the USA (low/mid-shore)	█	█	█	█	█	█	█	█	█	█	█	█	1983–1985	RC < 1 mm	> 9 mm (RC), $n=30–120$	Hoffman (1989)[a]
Pollicipes pollicipes																	
47°N–48°N	Brittany, France (mid-shore, 1 or average of up to 3 sites)		░	░	░	░	░	▓	░	░	▓	░		2018	Cyprids + RC < 0.6 mm	>15 mm (RC), $n=20$ per site	Aguión et al. (2022a)
43°N	*Punta la Cruz*, Asturias, Spain (low shore)		░	░	░	░	░	░	▓	▓	▓	▓	▓	2000	Cyprids + RC < 1 mm	>12.14 mm (LRT), $n=50$	Pavón (2003)
43°N	*Punta la Cruz*, Asturias, Spain (low shore)	░	░	░	░	░	░	░	█	█	█	▓	█	2001	Cyprids + RC < 1 mm	>12.14 mm (LRT), $n=50$	Pavón (2003)
43°N	Asturias, Spain (mid-shore, 1 or average of up to 6 sites)	▓	░	░	░	░	░	█	█	█	█	▓	▓	2018	Cyprids + RC < 0.6 mm	>15 mm (RC), $n=20$ per site	Aguión et al. (2022a)
42°N–43°N	Galicia, Spain (mid-shore, 1 or average of up to 6 sites)	░	░	░	░	░	░	░	█	▓	▓	░	░	2018	Cyprids + RC < 0.6 mm	>15 mm (RC), $n=20$ per site	Aguión et al. (2022a)
37°N	Cape of Sines, SW Portugal (mid-shore)										█	▓	░	1990	Cyprids + RC < 0.6/1 mm	>15 mm (RC), $n=50$	Cruz et al. (2010a)
37°N	Cape of Sines, SW Portugal (mid-shore)	░	░	░	░	░	░	█	█	█	█	█	█	1991	Cyprids + RC < 0.6/1 mm	>15 mm (RC), $n=50$	Cruz et al. (2010a)
37°N	Cape of Sines, SW Portugal (mid-shore)	█	▓	░	░	░	░	░	░	█	█			1992	Cyprids + RC < 0.6/1 mm	>15 mm (RC), $n=50$	Cruz et al. (2010a)
37°N	Cape of Sines, SW Portugal (mid-shore)							░	▓	█	█	█		2007	Cyprids + RC< 1 mm	>15 mm (RC), $n=20–50$	Fernandes et al. (2021)
37°N	Cape of Sines, SW Portugal (mid-shore)	▓					░	▓	▓	█	█			2008	Cyprids + RC < 1 mm	>15 mm (RC), $n=20–50$	Fernandes et al. (2021)
37°N	Cape of Sines, SW Portugal (mid-shore)					░	░	▓	█	█	█	█		2009	Cyprids + RC < 1 mm	>15 mm (RC), $n=20–50$	Fernandes et al. (2021)
37°N	Cape of Sines, SW Portugal (mid-shore)	█		▓	░	░	░	▓	█	█	█	█		2010	Cyprids + RC < 1 mm	>15 mm (RC), $n=20–50$	Fernandes et al. (2021)
37°N	Cape of Sines, SW Portugal (mid-shore)	▓		▓		░	░	█	█	█	█	█	█	2011	Cyprids + RC < 1 mm	>15 mm (RC), $n=20–50$	Fernandes et al. (2021)

(*Continued*)

Table 11 (*Continued*) Recruitment in *Pollicipes* species

Species/latitude/location/habitat		Recruitment on conspecifics/month	Period of observation	Index of recruitment (size of juveniles)	Size of adults and sampling size	References
37°N	Cape of Sines, SW Portugal (mid-shore)	■■▨░░■■■■■■■	2012	Cyprids + RC < 1 mm	>15 mm (RC), $n = 20–50$	Fernandes et al. (2021)
37°N	Cape of Sines, SW Portugal (mid-shore)	■■▨▨░▨■■■■■■	2013	Cyprids + RC < 1 mm	>15 mm (RC), $n = 20–50$	Fernandes et al. (2021)
37°N	Cape of Sines, SW Portugal (mid-shore)	□□▨░░░■■■■■■	2014	Cyprids + RC < 1 mm	>15 mm (RC), $n = 20–50$	Fernandes et al. (2021)
37°N	Cape of Sines, SW Portugal (mid-shore)	▨▨▨▨░░■■■■■■	2015	Cyprids + RC < 1 mm	>15 mm (RC), $n = 20–50$	Fernandes et al. (2021)
37°N	Cape of Sines, SW Portugal (mid-shore)	■■▨▨░▨■■■■■■	2016	Cyprids + RC < 1 mm	>15 mm (RC), $n = 20–50$	Fernandes et al. (2021)
37°N	Cape of Sines, SW Portugal (mid-shore)	■■▨▨░■■■■■■■	2017	Cyprids + RC < 1 mm	>15 mm (RC), $n = 20–50$	Fernandes et al. (2021), Aguión et al. (2022a)
37°N	Cape of Sines, SW Portugal (mid-shore)	▨□□□░░░■■■■■	2018	Cyprids + RC < 1 mm	>15 mm (RC), $n = 20–50$	Aguión et al. (2022a)
37°N	SW Portugal (mid-shore, 1 or average of up to 5 sites)	▨░░░░░▨■■■■■	2018	Cyprids + RC < 0.6 mm	>15 mm (RC), $n = 20$ per site	Aguión et al. (2022a)
Pollicipes caboverdensis						
15°N	Tarrafal, Santiago Island, Cape Verde	□□░▨░▨░▨▨▨▨▨	2014	Cyprids + RC < 1 mm	>15 mm (RC), $n = 40$	Cruz et al. (in prep.).
15°N	Tarrafal, Santiago Island, Cape Verde	░░▨■▨■□░░▨░□	2015	Cyprids + RC < 1 mm	>15 mm (RC), $n = 40$	Cruz et al. (in prep.).
15°N	Tarrafal, Santiago Island, Cape Verde	▨░░▨▨▨░□░░□░	2016	Cyprids + RC < 1 mm	>15 mm (RC), $n = 40$	Cruz et al. (in prep.).

Seasonal patterns of recruitment on conspecifics (average number of cyprids and juveniles (RC < 0.6 mm or RC < 1 mm) per adult) according to species, location and habitat (when available and when there are differences), period of observation, index of recruitment and size of juveniles, size of adult conspecifics and sampling size. Light grey stands for the non-recruitment season (<1 recruit per barnacle), middle grey for 1–3 recruits per adult, and black for the main recruitment season (>3 recruits per adult). Data for blank cells are missing.

LRT, length between rostrum and tergum; RC, rostrocarinal length.

[a] Study cited in Barnes (1996). For a few dates in Fernandes et al. (2021) (Nov 2012, Jan 2013, Nov 2014 and Dec 2015), we did not have data, but as these months were in the main recruitment season, we considered them as part of the main recruitment season.

and juveniles with RC < 0.6 mm or RC < 1 mm per adult), we have compiled Table 11, summarizing the information on the timing and intensity of recruitment in species of *Pollicipes.* The main recruitment season was defined when this index was greater than 3. This threshold has been used in previous studies on *Pollicipes pollicipes* (Cruz et al. 2010a, Fernandes et al. 2021, Aguión et al. 2022a), but it is arbitrary and only serves to compare the patterns exhibited by the various species at the various sites and years of observation. The non-recruitment season was defined when this index was lower than one.

Although there is only one study on recruitment in *Pollicipes polymerus* based on observations made approximately 35 years ago (La Jolla, California, the USA, Hoffman 1989), it appears that the intensity of recruitment is much higher than that observed in the two Atlantic *Pollicipes* species (Table 11). Hoffman (1989) described the recruitment of *Pollicipes polymerus* as occurring year-round, with peak recruitment in early spring (March–April, 74–190 recruits per adult). From September to February, recruitment values were lower (8–22 recruits per adult) (Hoffman 1989). This continuous recruitment pattern observed in La Jolla may be directly related to the reproductive pattern that has been observed at some sites in Southern California, identified as ‘winter breeders’ (see reproductive pattern in Goleta Point and Santa Catalina Island, Table 6 and section ‘Reproduction’).

At the other extreme of recruitment intensity is *Pollicipes caboverdensis*, which showed very low recruitment values throughout the year, with only 2 months in 3 years of observations with recruitment values above three (April and June) (Cruz et al. in prep. a). Again, this recruitment pattern appears to be directly related to the reproductive pattern of this species (see Table 6). Reproduction of *Pollicipes caboverdensis* did not show pronounced seasonality, and the highest values were observed in late winter and early spring (Cruz et al. in prep.).

Regarding *Pollicipes pollicipes*, there are several studies, in several European locations, and in several years (see Table 11). In a recent study, populations located near the northern limit of distribution of *P. pollicipes* (Brittany, France) were compared with Iberian populations (Asturias, Galicia and SW Portugal) by using a standardized protocol (Aguión et al. 2022a). Using the criterion of three recruits per adult for the definition of the lower limit of the main recruitment season, there is no main recruitment season in Brittany comparable to that occurring with other locations (Table 11). The highest values of recruitment (1–3 recruits per adult) in Brittany were observed in July and October (based on 2018 data, Aguión et al. 2022a). The low recruitment rates of *Pollicipes pollicipes* observed in Brittany in comparison with most locations in Iberia might be explained by: lower reproductive effort (see Table 6), and potential lower larval supply due to Brittany’s more ‘insular’ situation (absence of *P. pollicipes* north of Brittany and on most of the French coast south of Brittany) (Aguión et al. 2022a).

Considering the studies conducted to date on large-scale spatial patterns and temporal patterns of recruitment on conspecifics of *Pollicipes pollicipes* in Iberia, using the same index (see Table 11), we can define the following main patterns:

1. interannual variation of recruitment patterns, but months with the highest recruitment intensity occurred in summer and autumn;
2. the sole month that was classified as belonging to the non-recruitment season (<1 recruit per adult), in all locations and years, was May;
3. although there is interannual variation, the main recruitment season in SW Portugal is longer than in Asturias and Galicia, and the non-recruitment season in SW Portugal is shorter than in Asturias and Galicia;
4. in 12 consecutive years of recruitment observations in SW Portugal (2007–2018), there were some years in which the duration of the main recruitment season was longer than in others (i.e. 9 months in 2012 and 2017 compared with < 5 months in 2007 and 2010).

A positive relationship between recruitment and seawater temperature was suggested in the study of recruitment of *Pollicipes pollicipes* in Europe (Brittany, Asturias, Galicia and SW Portugal, Aguión et al. 2022a). This had been previously detected and found to be significant in a 10-year study monitoring the recruitment of *P. pollicipes* at Cape of Sines (SW Portugal) (Fernandes et al. 2021). In this study, a clear association between recruitment and both relaxation of upwelling and seawater warming was detected.

However, this association has not been detected in other studies with *Pollicipes pollicipes* or *P. polymerus*. In a previous study at Cape Home, Galicia (Spain), Macho (2006), using a different recruitment index (percentage of adults with one or more recruits), observed a decline in the recruitment rate of *Pollicipes pollicipes* during a 5-month period of strong downwelling. In relation to *Pollicipes polymerus*, Dudas et al. (2009) did not find any significant correlation between recruitment (3–14 days recruitment on artificial plates, Oregon) and several physical variables (across-shore and alongshore currents, seawater temperature and wind intensity). Pineda (1991) found a significant negative correlation between daily settlement of *Pollicipes polymerus* and surface water temperature (La Jolla, California). In that study, internal tidal bores were suggested as the mechanism that was driving decreases in water temperature and larval transport onshore. These contradictions may be related to the different scales at which recruitment was measured, to the different indices of recruitment used, or to different processes acting at different locations, and also differences between species, which, in turn, could reflect local coastal oceanographic context. Recruitment, measured on the scale of 1 month at a particular site, may not be related to the settlement that has occurred at that site. On the other hand, the settlement rate can underestimate or overestimate larval supply (e.g. due to predation, behaviour). Thus, as noted by Pineda et al. (2010), "knowledge of physical transport processes derived from settlement time series may be flawed". Further studies are needed to describe and understand the patterns and processes of settlement and recruitment of species of *Pollicipes*, namely *P. polymerus* and *P. elegans*. Further studies are also needed to understand the relative importance of settlement and post-settlement processes in the recruitment to the reproductive (and exploitable) populations.

Growth and size

Growth in *Pollicipes* is continuous throughout the life of the individual. Upon settlement onto an appropriate substrate and subsequent metamorphosis into the juvenile form, stalked barnacles grow by increasing the length of peduncle and by calcareous accretion of the plates in the capitulum (thoroughly described in Anderson 1994). Unlike most crustaceans, complete moulting of the exoskeleton does not occur in stalked barnacles. The outer integument (i.e. the outer covering of the peduncle and capitulum) is not shed at a moult, as is the inner integument (i.e. covering of the mantle cavity and the soft parts of the body) (Anderson 1994).

Barnes (1996) described the post-settlement development and growth of *Pollicipes* species based on the work by Darwin (1852), Broch (1922), Mahmoud (1959b, 1960), Koehler (1889, Chaffee & Lewis (1988) and Anderson (1994). This included a description of the appearance of the primordial valves of the capitular and peduncular scales and of the development of secondary capitular plate development, addition of new scales in the 'growth zone' (i.e. the transition zone between the capitulum and the peduncle), the growth of the stalk exoskeleton and embedded spicules, and some species-specific differences within the genus *Pollicipes*, namely of *P. polymerus* and *P. pollicipes*. In 1996, studies reporting estimates of the growth rates of *Pollicipes* were relatively scarce and most concerned *P. polymerus* (8 out of 9 references in Table 15 in Barnes 1996; see also Table 12), with only one concerning *P. pollicipes* (see Table 12). Most estimates were based on field measurements of marked individuals (*in situ* or transplanted), or barnacles that had recruited on cleared surfaces on ambient intertidal rocky shores (Barnes & Reese 1960, Paine 1974, Newman & Abbot 1980, Lewis & Chia 1981, Page 1986, Bernard 1988, Cruz 1993). There were also references to growth rate estimates observed in ambient subtidal conditions (Hoffman 1989) and artificial conditions (an intertidally located offshore oil platform and on a submerged intake seawater system) (Page 1986, Hoffman 1988). These studies suggested that the growth rates of *Pollicipes* species were variable at different multiple spatial and temporal scales. Barnes (1996) addressed such variation, discussing how it might be affected by abiotic and biotic interacting factors, such as water temperature, light, water flow, food availability and consumption, season, intra- and interspecific competition, and ecological context.

Herein, we compile and summarize the main findings of work concerning *Pollicipes* growth rates subsequent to Barnes' review. In Table 12, we compile ranges of estimates of average growth rate (mm/month; min-max) observed in ambient intertidal conditions, irrespective of intertidal height, season and methods used in different studies cited in Barnes (1996) as well as those published thereafter. Two size classes were considered: small (RC < 15 mm) and large (RC > 15 mm). Data are listed per species and country, with notes on methods used, study location and data source. Some progress has been made during the last 25 years, mainly regarding *Pollicipes pollicipes* (e.g. Cardoso 1998, Cruz 2000, Cruz et al. 2010a, Boukaici et al. 2012, Jacinto et al. 2015, Neves 2021) and, to a lesser extent, *P. polymerus* (Helms 2004, Phillips 2005) and *P. elegans* (e.g. Pinilla 1996, Samamé & Quevedo 2001). To date, no studies have been carried out on the growth of *Pollicipes caboverdensis*, despite being an endemic species with increasing economic importance. In general, growth in the various *Pollicipes* species is highly variable at multiple temporal and spatial scales. Mean growth rates are higher and more variable in juveniles, gradually decreasing with age and/or size (Table 12).

Table 12 Growth rates of *Pollicipes* spp.

Species	Methods and location	Growth rate (mm RC/ month) Small	Large	References
Pollicipes polymerus				
Canada	Size increments of marked individuals (physical tags). Size structure analysis of recruits on cleared surfaces British Columbia	0.80–1.25	0.54	Bernard (1988)[a]
The USA	Size increments of marked individuals (physical and calcein tags) on natural or transplanted clumps. Size structure analysis of recruits on cleared surfaces. Washington State, Oregon and California.	0.03–4.07 0.20–0.60	0.04–0.67	Barnes & Reese (1960)[a], Lewis & Chia (1981)[a], Paine (1974)[a], Page (1984)[a] Helms (2004), Phillips (2005)
Pollicipes elegans				
Peru	Size structure analysis of natural population; size increments of marked individuals (physical tags) on natural clumps. Piura.	3.00	2.34 (CH)	Pinilla Garcia (1996), Samamé & Quevedo (2001)
Pollicipes pollicipes				
Spain	Size structure analysis of natural population. Galicia.	-	0.34	Sestelo & Roca-Pardiñas (2007, 2011)
Portugal	Size increments of marked individuals (physical and calcein tags) on natural or transplanted clumps and artificial substrata; size structure analysis of recruits on cleared surfaces and artificial substrata. SW Portugal; RNB Portugal	0.17–0.66 0.18–5.20	0.08–0.48 0.11–0.47	Cruz (1993)[a] Cruz (2000), Cruz et al. (2010a), Figueira (2015), Jacinto et al. (2015), Mateus (2015), Cruz et al. (2016a,b), Darras (2017), Mateus (2017), Belela (2018), Fernandes (2018), Santos (2019), Cruz et al. (2020), Neves (2021)
Morocco	Size structure analysis of natural population; SW Morocco	1.03	0.20–0.45	Boukaici et al. (2012)

Range of estimates on average growth rate observed in ambient intertidal conditions (mm RC/month; min - max) per size class (small: RC < 15 mm; large: RC > 15 mm; RC: rostrocarinal length) with notes on methods used and study locations.

[a] Studies cited in Barnes (1996).

Field studies on the growth of Pollicipes *species*

Field studies on the growth of *Pollicipes* species have been based on population size structure analyses (Cardoso 1998, Cruz 2000, Samamé & Quevedo 2001, Sestelo & Roca-Pardiñas 2007, Cruz et al. 2010a, Sestelo & Roca-Pardiñas 2011, Boukaici et al. 2012), monitoring size increments of barnacles that have recruited on cleared natural surfaces or artificial substrata (Cruz 2000, Cruz et al. 2010a, Mateus 2015, Cruz et al. 2016a, b, Mateus 2017, Belela 2018, Santos 2019, Cruz et al. 2020) and estimates of growth rates of marked individuals (Pinilla 1996, Helms 2004, Phillips 2005, Cruz et al. 2016a, b, Figueira 2015, Jacinto et al. 2015, Neves 2021).

The most widely used biometric variable for growth and size structure studies in *Pollicipes* species (e.g. Phillips 2005, Cruz et al. 2010a, Boukaici et al. 2012) and the one that best represents linear growth (Cruz 1993) is the maximum rostrocarinal length (RC). However, other variables such as total length (Hoffman 1984, Boukaici et al. 2012), capitular height (Page 1986, Cardoso & Yule 1995, Pinilla 1996) and capitular base diameter (Parada et al. 2012) have also been used.

Growth estimates in all studied *Pollicipes* species suggest a rapid increase in size (up to 11–17 mm RC) during the first year, with most individuals reaching maturity within 1 year (Lewis & Chia 1981, Hoffman 1989, Bernard 1988, Pinilla 1996, Samamé & Quevedo 2001, Cruz 2000, Cruz et al. 2010a, Boukaici et al. 2012, Parada et al. 2012, 2013, see section 'Reproduction'), followed by a decline in growth rates in subsequent years. For example, Boukaici et al. (2012) estimated growth rates of *Pollicipes pollicipes* on the intertidal rocky shores of SW Morocco as approximately 1.0 mm RC/month during the first year, decreasing to 0.5 RC/month in the second year and 0.2 RC/month in the fifth year.

Age determination

A major limitation in stalked barnacle growth studies is that age determination is difficult to obtain through methods such as length frequency, or shell band analyses. Studies that have tried to establish size:age relationships, based on growth curve analyses, have estimated maximum ages of 7 or more years (*Pollicipes pollicipes*, 26 mm RC; Boukaici et al. 2012), 12 years (*P. polymerus*, 31 mm RC; Bernard 1988) and 15–20 years (*P. polymerus* Barnes & Reese 1960). Several studies considered that length frequency data should not be used to determine age and growth parameters in *Pollicipes pollicipes*, due to the constant settlement of larvae throughout the summer months and the difficulties of successfully following cohorts through time, namely for more than 1 year after settlement (Cardoso 1998, Cruz 2000, Cruz et al. 2010a, Sestelo & Roca-Pardiñas 2011, Parada et al. 2012). Likewise, the use of shell bands in age determination in *Pollicipes pollicipes* is prone to error (Mahmoud 1959a, Broch 1922 in Barnes 1996, Cardoso 1998). Despite the successful identification of shell bands and several environmental influences on banding periodicity (e.g. tidal periodicity), Cardoso (1998) questioned the applicability of such technique in barnacle age determination, since band widths were very similar throughout the shell length, with no regular 'check' marks that could provide age estimation. Also, fracturing of the capitular plates is quite common in *Pollicipes* (Cornwall 1925 in Barnes 1996, Wootton 1993, Cruz 2000) and sections through capitular plates show that the number of laminae is variable, even in the same plate, as well as in different plates of the same animal (Mahmoud 1959a in Barnes 1996). Analysing the banding on polished and etched cross sections of the carina of *Pollicipes polymerus*, Bernard (1998) observed a reasonable correlation with capitular width and estimated age in years, but considered that age estimation by such a method was not reliable, except during the active growth phase. Growth and age determination of field populations of *Pollicipes pollicipes* seems attainable only by mark and recapture experiments.

Individual marking techniques

Individual marking techniques in *Pollicipes* studies include mapping individuals in relation to marks made in adjacent substrata, or using marks, such as insect tags glued to the capitular plates of suitably sized individuals (e.g. Phillips 2005, Cruz et al. 2010a). Despite the valuable data obtained, such individual physical marking techniques are very difficult to implement in the field, often resulting in low numbers of marked individuals and size constraints in marking efficiency. A major advance in this field was the successful use of chemical marking with calcein in *Pollicipes polymerus*, pioneered by Helms (2004), and later applied in *P. pollicipes* by Jacinto et al. (2015) and several others thereafter (Figueira 2015, Mateus 2015, Cruz et al. 2016a, b, Mateus 2017, Belela 2018, Santos 2019, Cruz et al. 2020, Neves 2021). Such techniques have a great potential in stalked barnacle growth studies, mainly because they allow mass marking of individual barnacles of different size cohorts within a short period (e.g. less than 1 day of manipulation) and involve less fieldwork time (Jacinto et al. 2015), which is a major advantage since this species lives on extremely exposed rocky shores. Immersion of *Pollicipes* individuals in a calcein solution leaves a fluorescent mark in calcified structures. Calcein is incorporated into the growing calcium carbonate of the capitular plates of the barnacles. Because these plates grow in thickness and in area, through laminar accretion or basal marginal accretion (Anderson 1994), the entire original plate is stained. When marked animals are returned to the field, new and unmarked plate material is added during growth. Calcein marks are visible under epifluorescent light and may be used to identify marked individuals and to estimate growth rates in *Pollicipes* species, mostly by estimation of the marginal growth of the capitular plates (Figure 10). Consequently, calcein marking has been applied with great success in several studies, both in field and laboratory conditions, to address spatial and temporal variability in stalked barnacle growth, namely of *Pollicipes pollicipes* (Figueira 2015, Jacinto et al. 2015, Mateus 2015, Cruz et al. 2016a, b, Mateus 2017, Belela 2018, Santos 2019, Cruz et al. 2020, Neves 2021).

Spatial and temporal variability in growth

Variability in patterns of growth in *Pollicipes* has been addressed at multiple spatial and temporal scales. At minute spatial scales, the effect of location within the cluster and cluster size, on the growth rates of juvenile *Pollicipes polymerus*, was studied *in situ* over a 2-month period at Cape Arago, Oregon, the USA (Helms 2004). Findings suggest a negative effect of density on juvenile stalked barnacle growth, namely that the location of juvenile barnacles within the cluster significantly influenced their growth rates. The average growth rates of juveniles located at the edges of the clusters were 1.4 higher than those of juveniles located on the inside of the clusters. Such an effect of location within the cluster on juvenile growth rates was discussed in the context of intraspecific competition for space and food between juveniles and adults. Helms (2004) also noted that cluster size was less important in explaining differences in the growth of juvenile barnacles than was location within the cluster, because the growth rates of juveniles in small and large clusters were similar. However, the growth rates of juveniles on solitary adults were almost twice as fast as those of juveniles in clusters. These results indicate that there may be important differences in growth between clusters and solitary adults, which may help explain how clusters are formed and maintained, and how *Pollicipes polymerus* may compete with other intertidal organisms (e.g. the mussel, *Mytilus californianus*) (Helms 2004). Increased knowledge of hydrodynamics and water flow at the cluster scale, along with experiments testing the effects of the location of the cluster and cluster size on food capture by adults and larger juveniles, would enable questions regarding competition among adults or between juveniles and adults to be tackled.

Variation in growth rate with intertidal height has been hypothesized as a driver of size structure differences found between low-shore and high-shore barnacles in SW Portugal (Cruz 2000,

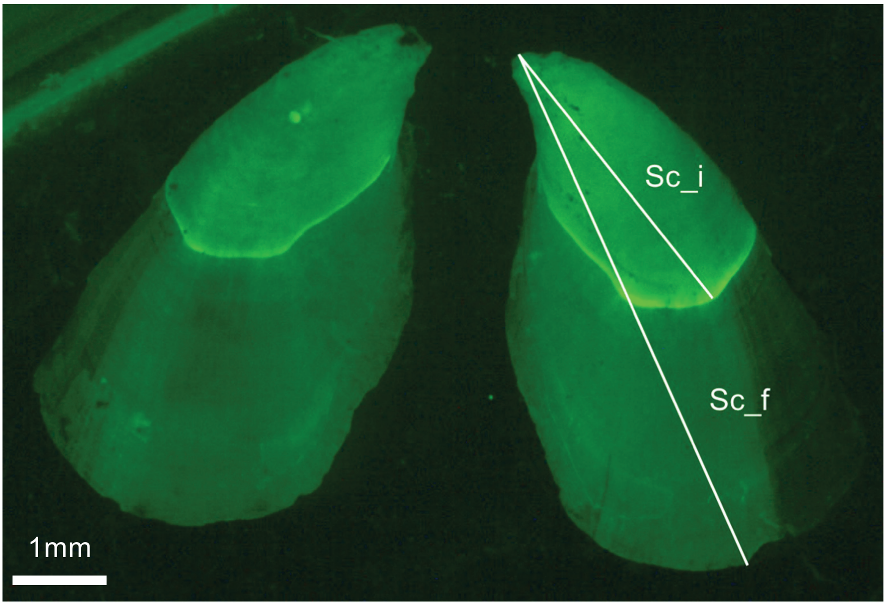


Figure 10 *Pollicipes pollicipes. Scuta* with visible fluorescent calcein mark (Sc_i and Sc_f are initial and final maximal lengths, respectively).

Cruz et al. 2010a). Barnacles at low tide levels reach a higher maximum size, possibly indicating that growth at this level is higher than at the high shore (Cruz 2000, Cruz et al. 2010a). This hypothesis has been supported by studies carried out at the Cape of Sines and at the Berlengas Nature Reserve (Portugal), where growth rates of juvenile *Pollicipes pollicipes* in the low intertidal were about twice those at the mid-intertidal (Figueira 2015, Neves 2021). Other studies, however, did not find an effect of intertidal height on *Pollicipes pollicipes* growth rates (Pavón 2003, Jacinto et al. 2015). Growth rate variability with intertidal height may be the result of less intraspecific competition, due to lower barnacle density and/or increased feeding time on the low shore. Further support, for the positive effect of increased feeding time on growth rates, comes from observations of *Pollicipes pollicipes* growth in permanently submerged conditions. Recently settled *Pollicipes pollicipes* individuals (RC < 5 mm) on artificial substrata were reallocated to permanently submerged conditions on an experimental aquaculture raft, located inside the Port of Sines (SW Portugal), and then compared with growth rates on the nearby natural intertidal shores of the Cape of Sines (SW Portugal) on several occasions (Mateus 2015, Cruz et al. 2016a, Darras 2017, Belela 2018, Santos 2019, Cruz et al. 2020). In general, juvenile growth rates were 1.5–3.0 times higher in permanently submerged conditions compared with ambient intertidal conditions. Caution should be exercised when interpreting these results, however, as the ecological context in which these permanently submerged barnacles were growing may have been quite different from the shallow subtidal, rocky shores where *Pollicipes pollicipes* naturally occurs.

Seasonal variation in stalked barnacle growth rates has also been observed in natural intertidal habitats, but patterns are inconsistent. Growth rates in recently settled *Pollicipes pollicipes* juveniles on artificial substrata were ca. 1.5 times larger during summer than during winter (Cruz et al. 2016a), ca. two times larger during winter, spring and summer than during autumn (Belela 2018) and ca. 3.4 times larger during autumn compared with spring/summer (Cruz et al. 2020). No effect of season (summer vs winter) was found in the growth rates of juveniles in transplanted barnacle clumps (Cruz et al. 2016a). Likewise, field measurements of the maximum size of barnacles recruiting onto cleared surfaces do not reveal any seasonal variation in juvenile growth, but in adult barnacles, growth rates were ca. 4.3 times larger in winter and spring than in summer (Cruz 2000). Pavón (2003) also did not find significant differences in growth rates of *Pollicipes pollicipes* between sampling seasons, but did observe a positive relationship between growth rates and wave period, wind velocity and direction. It is still not clear weather *Pollicipes pollicipes* growth rates vary with season, or wether these observations mainly reflect the immense variability between individual growth rates, and other site-specific factors.

Phillips (2005) also observed inconsistent seasonal variation (spring vs summer) in growth rates of *Pollicipes polymerus* individuals (6.6–15.4 mm RC), marked with insect tags and planted out on intertidal rocky shores of California (the USA). He described contrasting patterns strongly affected by regional oceanographic effects (e.g. upwelling intensity). During spring, growth was similar at all studied sites, while during summer, growth rates were higher at southern sites (relative to Point Conception, California), but lower at northern sites. Other previous observations carried out by Bernard (1988) on Vancouver Island (BC, Canada), based on individuals with capitular length 15 mm in the active growth phase, suggested that maximum growth occurs between May and July. Given these observations, it seems that growth in *Pollicipes* might be affected by seasonal changes, but patterns of variation are site dependent and probably related to specific nearshore oceanographic processes that affect the local barnacle population.

The growth rate variability in *Pollicipes* has been linked to the oceanographic and ecological contexts of its populations. A striking geographic pattern of growth has been observed by Phillips (2005), who examined growth rates of intertidal benthic filter-feeders, *Pollicipes polymerus* included, over a geographic region that contains sites where upwelling is typically weak (south of Point Conception, California, the USA) and sites where upwelling is often strong and frequent (at and to the north of Point Conception). Growth rates of *Pollicipes polymerus* at southern sites were relatively high and declined moving around Point Conception and to northern sites. Although the author was not able to isolate the underlying cause, there was clear evidence for a geographic gradient in growth rates (higher at southern sites where upwelling is weaker) for other species of common and abundant benthic filter-feeders (e.g. *Mytilus californianus* and *Balanus glandula*). Spatial variation in food availability and quality, and tidal height, did not seem to explain these patterns of growth. On the other hand, water temperature varied in a pattern consistent with growth rates (i.e. mean temperatures were warmer in the south). This study suggests that temperature may be an important driver of large-scale differences in benthic filter-feeder growth rates.

In conclusion, there has been a significant increase in knowledge on *Pollicipes* growth since Barnes' review, except for the lack of studies on *Pollicipes caboverdensis*. However, the different approaches to growth studies that have been applied worldwide make it difficult to meta-analyse the available results. It is important to establish a standard methodology in *Pollicipes* growth studies. The use of calcein marking techniques to estimate growth rates, and the RC length as a standard biometric variable to report growth estimates are good contenders and should be adopted in future work regarding all *Pollicipes* species. Such approaches could be used to estimate von Bertalanffy growth parameters, by measuring growth increments in differently sized individuals, as recently utilized for intertidal limpets (e.g. Oróstica et al. 2021).

The ecological and economical importance of *Pollicipes* species makes it extremely important to have good estimates of growth rates which may be incorporated into ecological and fisheries management models. All evidence gathered to date regarding growth rates in *Pollicipes* species suggests that these are highly variable at multiple temporal and spatial scales. It is, however, difficult to untangle the relative importance of different abiotic and biotic factors that influence growth in natural populations of *Pollicipes*, especially when they seem to covary and interact at multiple scales affecting growth. Observations suggest a rapid increase in size during the first year, with most individuals reaching maturity within the first year after settlement, followed by a gradual decrease with barnacle age and/or size. More observations are needed at suitable spatial and temporal scales and must include ecologically relevant covariates, in order to explain the high intraspecific variability in growth rates observed in *Pollicipes* species. Additionally, while RC may be the best indicator of linear growth in *Pollicipes*, other biometric variables, such as peduncular length, which have been linked to stalked barnacle quality (see section 'Description of adults'), are highly variable at different spatial scales and should be addressed in future work. Age estimation in *Pollicipes* is still unattainable, and longevity estimates are scarce and prone to error.

Post-settlement processes of distribution and abundance

The main ecological processes determining the patterns of distribution and abundance of *Pollicipes* species after settlement were not placed in a single section in the review by Barnes (1996), but were considered as separate sections ("Effect of temperature change and desiccation" and "Predation"). Furthermore, other abiotic or biotic factors (wave action, or intraspecific and interspecific competition), as well as their relationship to behavioural, functional, demographic and life-history traits of *Pollicipes* species, were treated in various other places in Barnes (1996).

We have reviewed the relevant literature on the post-settlement processes affecting the occurrence of *Pollicipes* recruits, juveniles and adults. This was mostly based on observational and experimental evidence acquired after Barnes (1996), but work carried out prior to Barnes (1996) was also integrated. Although an outline on the sole effects of physical factors is included, herein we have mostly focused on the role of biological factors on *Pollicipes* assemblages, particularly on species interactions. This section mainly reports on *Pollicipes polymerus* and *P. pollicipes*, due to the scarcity of ecological studies on the other two species, for which information has been included when available and appropriate.

Physical factors

Barnes (1996) reviewed the work on the eco-physiology of *Pollicipes polymerus,* namely on its tolerance to body temperature changes, desiccation and osmotic stress (Fyhn et al. 1972), on the temperature sensitivity of heart activity and high pressure of pulsating haemolymph (Fyhn et al. 1973) and on the dynamics of aquatic and aerial respiration (Petersen et al. 1974). Barnes (1996) considered *Pollicipes polymerus* as a eurythermal species and "more permeable to water than many balanids", enabling cooling by evaporation from the peduncle, leading to high rates of water and body weight losses during low tide, and compensating rehydration rates during the next submersion at high tide. Physiological regulation is a likely adaptive trait for the survival of *Pollicipes polymerus* in the intertidal zone, driving the metabolism and allocation of energy of barnacles, and allowing them to adjust to periods of submersion and emersion, fluctuating air and water temperatures and gaseous exchange under changing environmental conditions (Barnes 1996 and references therein).

Despite the recognisable tolerance of *Pollicipes* species to adverse intertidal conditions, desiccation has been suggested as physiologically challenging during low-tide periods for *Pollicipes* recruits and adults living at higher tidal levels, possibly setting their upper limit of vertical distribution (Barnes 1996). Moreover, the vertical and local-scale distribution of intertidal invertebrates has often been found to be strongly influenced by lethal and sub-lethal stress associated with heat and/or water loss while exposed to air (e.g. Somero 2002, Miller et al. 2009). To our knowledge, the potential role of thermal and/or desiccation stress during low tide on the intertidal patterns of distribution and abundance of *Pollicipes* species has yet to be investigated. Since *Pollicipes* live in wave-exposed conditions on oceanic, swell-dominated coastlines, considerable swash and spray will ameliorate stresses when the tide is out, except during calm conditions.

A positive relationship with seawater temperature has recently been demonstrated across most of the life cycle of *Pollicipes pollicipes*, including larval survival and growth (Franco et al. 2017), cyprid metamorphosis into juveniles (Franco et al. 2016), recruitment of cyprids and juveniles onto conspecific adults (Fernandes et al. 2021), adult feeding behaviour (Franco 2014) and reproduction (Cruz & Hawkins 1998, Cruz & Araújo 1999, Cruz et al. 2010a). In turn, these processes are known to strongly affect the occurrence of benthic life stages of *Pollicipes* species on the shore. Therefore, seawater temperature might have indirect effects on the patterns of distribution and abundance of *Pollicipes pollicipes* after settlement, with consequences for density-independent population processes. During the El Niño 1982–1983, Tarazona et al. (1985) and Kameya & Zeballos (1988) reported major increases in the abundance of *Pollicipes elegans* on the mid-shore of several locations in Peru associated with conditions of seawater warming, abrupt changes in salinity and increased dissolved oxygen. Conversely, in the same region after the end of the El Niño 1982–1983, a decrease in the abundance of *Pollicipes elegans*, in parallel with increases in dominant native species, such as mytilids (‘choritos’ *Semimytilus algosus* and *Perumytilus purpuratus*) and large acorn barnacles (‘picos de loro’ *Austromegabalanus psittacus*), were both described and suggested to follow changes in abiotic conditions (Kameya & Zeballos 1988).

As described in the section ‘Ecological habitat, and patterns of distribution and abundance’, *Pollicipes* species are associated with habitats with strong wave action throughout their geographical ranges. The restriction of *Pollicipes polymerus* to shores exposed to heavy surf and strong water flows has long been related to a suggested need for a certain hydrodynamic stimulation, to initiate activity of the cirri and to maintain an effective macro-feeding behaviour (Barnes & Reese 1959). Additionally, turbulent flow and water mixing after wave breaking were also suggested to facilitate settlement of *Pollicipes polymerus* (Lauzier 1999b). Likewise, greater water movement in the laboratory was found to stimulate *Pollicipes pollicipes* cyprid attachment (Franco et al. 2016), as well as feeding behaviour of adults (Franco 2014) and their survival and growth (Cribeiro 2007).

Two studies have shown a clear, positive relationship between wave exposure and abundance of *Pollicipes pollicipes*: Borja et al. (2006a) in the Gaztelugatxe Marine Reserve (Basque Country, Spain) and Jacinto & Cruz (2016) in a stretch of coast in SW Portugal. Both studies provided numerical models, based on locally derived wave exposure indices, with strong predictive power of barnacle distribution patterns across the studied regions. These can be used as simple and cost-effective tools to resource assessment and management in different areas, with direct application in the fishery and conservation of this species. *Pollicipes pollicipes* mostly occurs at wave-exposed coastal features, such as capes and headlands (Barnes 1996), and within these coastal features, its abundance varies at small spatial scales (metres apart), depending on the orientation of the site to the prevailing wave direction (Borja et al. 2006a, Jacinto 2016, Jacinto & Cruz 2016). Additionally, another model based on landscape metrics (distance to the coast, convexity at a 25 km scale and wave exposure at 1 km scale) predicted that higher quality (shorter and more robust morphology) *Pollicipes pollicipes* in Asturias would be present in areas that are relatively more distant to the coast, more convex and highly exposed (Rivera 2015).

Among other effects of physical disturbance, abrasion by drift logs has been documented to knock sea mussel clumps free (Dayton 1971), as well as clusters of *Pollicipes polymerus* (Jamieson et al. 1999). Despite these destructive effects on intertidal organisms, the battering by these logs, as well as storm damage, is crucial for the provision of space in exposed intertidal areas and, consequently, for the structure and dynamics of rocky-shore communities (Dayton 1971). These physical factors create patchiness in north-eastern Pacific and Atlantic *Pollicipes* assemblages, often resulting from an initial clearing in *Mytilus* beds, which is then enlarged by wave shock ripping newly vulnerable mussels from the surrounding substratum. These scattered patches and mussel gaps can be subsequently colonized by *Pollicipes* individuals (Dayton 1971).

Competition

Competition between individuals of the same or different species occurs when resources are limited, leading to a change in fitness (survival and reproductive success), which can result in competitive exclusion (elimination of weaker competitors by superior competitors) and set the population carrying capacity of a given area over time (e.g. Hardin 1960, Sommer & Worm 2002). In stable conditions with limiting resources, coexistence between species will only occur if they differ in niche characteristics (e.g. Paine 1984, Sommer & Worm 2002 and references therein). Fluctuating environmental conditions influencing relative competitive ascendency of co-occurring species, and variable recruitment due to supply-side processes and disturbance can all promote coexistence on rocky shores (Worm & Karez 2002 and references therein).

Competitive interactions involving *Pollicipes* species include a variety of forms and mechanisms, as they occur between members of the same species (intraspecific, i.e. among conspecific individuals of a *Pollicipes* species, e.g. Page 1986, Hoffman 1989, Barnes 1996) and of different species (interspecific, i.e. between a *Pollicipes* species and mussels or acorn barnacles or coralline algae, e.g. Wootton 1994, 2010). The ultimate limiting resource is space to settle, grow and have access to food, similar to most intertidal species (Connell 1961, Dayton 1971, Connell 1972, Paine 1980, Worm & Karez 2002). For several intertidal species, intraspecific competition can be more intense than interspecific competition (e.g. Creese & Underwood 1982, Boaventura et al. 2002), although this has been largely tested in grazing molluscs using factorial designs that segregate effects of intra- versus interspecific competition (e.g. Underwood 1978, 1984). Less experimental work has been done on the relative importance of intraspecific competition in sessile species, but self-thinning (i.e. competitively induced mortality within a cohort of organisms growing on shared substratum) has long been recognized in mussels and acorn barnacles (Hughes & Griffiths 1988). In acorn barnacles, density-dependent effects of intraspecific competition and facilitation have been both assessed, particularly concerning their configuration of packing and structural morphological modifications of individuals within hummocks (e.g. Bertness 1989, Bertness et al. 1998, López et al. 2007). In the case of *Pollicipes* species, manipulative studies on the effects of conspecific density have rarely been published (but see Page 1986 and Helms 2004 on individual growth rate response). Given that *Pollicipes* recruitment is high on conspecifics (see section 'Settlement and recruitment'), intraspecific competition for both space and food might be important and should be investigated in the future. Thus, as the role of intraspecific competition on the distribution and abundance of *Pollicipes* species is still to be determined, the account below focuses on interspecific interactions in the context of community structure.

Competition can occur through a direct type of struggle to attain resources (i.e. interference, when one individual stops the access of another individual to mutually desired resources). The following examples of interference competition involving *Pollicipes* species have been documented: (1) interactions by which canopy algae may overgrow *Pollicipes* individuals and possibly kill them, as observed in the case of the annual macroalga, *Postelsia palmaeformis* (sea palm), relative to *Pollicipes polymerus* (Carefoot 1977 in Jamieson et al. 1999); (2) large-sized individuals of *Pollicipes polymerus* and the mussel *Mytilus californianus* are frequently seen overgrowing *Semibalanus cariosus* acorn barnacles (Wootton 1994); (3) *Pollicipes polymerus* individuals can directly decrease recruitment of *Mytilus californianus*, by filtering out mussel larvae before the latter can settle (Wootton 1993); and (4) *Mytilus californianus* can directly subdue *Pollicipes polymerus*, through the growth of its rigid shell, by crushing *P. polymerus* individuals between mussel shells, or by abrasion of the barnacle body wall by the mussel shell edge (Wootton 1993).

An alternative mechanism of competition is through dominance, in which organisms consume common resources without direct confrontation (i.e. exploitation, when the use of a resource by one individual will decrease the amount available for others). The most emblematic example of exploitation competition concerning *Pollicipes* species is the strongly hierarchical dominance of *Mytilus californianus* in the rocky intertidal community of the north-eastern Pacific, which is mainly achieved by monopolizing space, rendering it unavailable for all other animals and algae (e.g. Paine 1966, 1974, Connolly & Roughgarden 1999) and hence outcompeting and displacing *Pollicipes polymerus*, especially on horizontal mid-intertidal surfaces (e.g. Dayton 1971, Paine 1974, Paine & Levin 1981, Wootton 1993, 1994, 2010). In contrast, on very exposed steep vertical cliffs or overhangs, *Pollicipes polymerus* dominates and outcompetes *Mytilus californianus* by holding space, possibly due to the stronger adherence of the goose barnacles to the substratum (Paine 1974, Barnes 1996). Additionally, Wootton (1993) demonstrated that *Pollicipes polymerus* dominates vertical walls, mainly because loose *Mytilus californianus* adults are not able to recruit onto these habitats where the time of contact with the substratum is insufficient for their reattachment, hindering *M. californianus* invasion.

Additionally, apparent competition can occur when two individuals affect each other indirectly by being prey for the same predator. This was detected by Wootton (1994) in the interaction between *Pollicipes polymerus* and *Nucella* sp. Regarding this interaction, reciprocal negative effects (increased density of one species when the density of the other is reduced), as well as depletion by avian predation, were experimentally demonstrated (Wootton 1994).

Space on which to live is likely critical in *Pollicipes*-associated assemblages composed of a matrix of sessile organisms, specifically among dense turfs of coralline/foliose algae or beds of dominant filter-feeders (Table 4). In these assemblages, competition for space can be intense (e.g. Dayton 1971, Paine 1974, Worm & Karez 2002) and the availability of primary substratum (such as bare-rock or algal crusts) might be scarce and dependent on localized events of disturbance (e.g. Paine 1974, Paine & Levin 1981, Sousa 1984). Disturbance has been experimentally demonstrated to modulate competitive interactions in ecosystems characterized by high diversity, such as exposed rocky intertidal shores, where physical (drift logs and winter storm damage, e.g. Dayton 1971) or biological (predation, e.g. Paine 1974) factors often renew space, by adding patches of cleared substratum and gaps in aggregations of dominant competitors. Heavy catastrophic, physical damage to mussel beds of *Mytilus californianus* creates unique opportunities for colonization by competitively inferior species, such as *Pollicipes polymerus* and *Semibalanus cariosus* (Paine & Levin 1981). Similarly, keystone predation by the seastar (starfish), *Pisaster ochraceus*, on competitively dominant species (*Mytilus californianus* or *M. trossulus*) strongly influences the community structure on shores in California and the Pacific Northwest, potentially shaping the distribution and abundance patterns of a variety of coexisting species, including *Pollicipes polymerus* (e.g. Paine 1974, Menge et al. 1994, Sanford 1999). Rates and patterns of succession in areas subject to disturbance and subsequent recovery result in a heterogeneous progression of species invasion and occupancy, enhancing interspecific facilitation and coexistence while preventing monopolization of substrata and competitive exclusion (Menge et al. 2011).

Competition for food in interactions between *Pollicipes* species and co-occurring filter-feeders could also be possible, due to potentially similar planktonic diets. Common detrital food sources (kelp, intertidal macroalgae, seagrasses and particulate organic matter) assimilated by four common, sympatric, rocky intertidal filter-feeders (*Mytilus californianus*, *Balanus glandula*, *Semibalanus cariosus* and *Pollicipes polymerus*), living near river mouths in the Pacific Northwest, were identified by Tallis (2009) using a dual stable isotope approach, showing that all species shared a common resource base. However, these species occupied distinct trophic positions, with estimated trophic level increasing with barnacle size, being lowest in *Mytilus californianus* and highest in *Pollicipes polymerus* (Tallis 2009). Therefore, interspecific competitive interactions for food including *Pollicipes* species seem improbable given trophic partitioning among abundant filter-feeding species, as well as the flexible peduncle adjustments during feeding and the longer cirri of *Pollicipes polymerus* relative to the two other barnacle species (Barnes 1996, Tallis 2009 and references therein).

The coexistence of *Pollicipes* and *Mytilus* species is found on the Pacific coasts of North, Central and South America, in Europe and the west coast of Africa (Kameya & Zeballos 1988, Barnes 1996, Barraza et al. 2014). *Pollicipes polymerus* is often abundantly interspersed with the mussel complex *Mytilus californianus* and *M. trossulus* to form the North American distinctive *Pollicipes*-*Mytilus* community (Barnes & Reese 1960, Hoffman 1989). Numerous studies have been carried out on the influence of competition and disturbance on biological succession in this community (Dayton 1971, Paine 1974, 1980, Paine & Levin 1981, Wootton 1992, 1993, 1994, 2010, Menge et al. 2011). Despite among-region variability in natural rates of change on rock patches available for colonization, the basic successional sequence is generally an early establishment of *Pollicipes polymerus* with a subsequent increase in the abundance of the dominant species, *Mytilus californianus* (Paine & Levin 1981, Paine 1974, Menge et al. 2011). Wootton (1993, 1994, 2010) clearly demonstrated that while experimentally induced changes of the competitive hierarchy of the *Pollicipes polymerus*-*Mytilus californianus* interaction can affect the ecological dynamics of intertidal succession, they do not affect its end point (a mussel-dominated community). Much of this work has consisted of species removal experiments, where the ecological role of a species is inferred by comparing controls versus manipulative treatments with reduced abundance. One lesson that can be learnt from these experiments is that the role of each species on communities, and the strength of interactions, varies locally, depending on the timing, habitat and geographical location.

Image analysis of time-series photographs of intertidal habitat areas of *Pollicipes pollicipes*, taken of fixed plots for up to 2 years in the scope of the European project PERCEBES promoted by BiodivERsA (Acuña et al. 2020), will provide information with unprecedented latitudinal coverage on the temporal variation of *P. pollicipes* assemblages. In particular, the abundance patterns of *P. pollicipes* and other accompanying sessile species potentially competing with stalked barnacles will give further insight into the biological processes governing the observed patterns.

Predation

Predation requires that predators kill and eat prey, and predator-prey interactions can be complex due to adaptations of both organisms. Barnes (1996) stated that predation was "sometimes not considered as seriously as it should be" as a process driving distribution and abundance of intertidal organisms, and thoroughly reviewed the studies of predation upon *Pollicipes* carried up to the early 1990s.

These pioneering studies were entirely concerned with the predation of *Pollicipes polymerus*, except for one reference to predation of *P. pollicipes* by crabs (Hui 1983). Initial evidence of *Pollicipes polymerus* as prey included: (1) observations of its predation by many invertebrates – *Pisaster ochraceus* with *P. polymerus* individuals in their hollow oral region (Feder 1959, Paine 1980, Bernard 1988); *Nucella* species drilling between *P. polymerus* capitular plates (Palmer 1984, West 1986, Bernard 1988); small pagurid crabs and polychaetes feeding on newly settled *P. polymerus* (Bernard 1988); *Emplectonema gracile* nemerteans wrapped around small *P. polymerus* juveniles (Hoffman 1989); (2) signs of its predation by gulls i.e. presence in faecal pellets and regurgitations (Vermeer 1982, Marsh 1986); and (3) the first experimental study addressing the effects of predation by seabirds – western gull *Larus occidentalis*, glaucous-winged gull *L. glaucescens* and surfbirds *Aphriza virgata* (Meese 1993). Meese (1993) reported on a bird-exclusion caging experiment within the Bodega Marine Reserve in North California, finding that predation by birds was both spatially and temporally patchy, with a relatively large impact in reducing the local percentage cover of *Pollicipes polymerus* during the wintertime.

Further work was carried out on avian predation of *Pollicipes polymerus* (Wootton 1993, 1994, 1997) and of *P. pollicipes* (Moreno et al. 2010). These studies highlighted: (1) the generalist nature of gulls' diets, whose temporal and spatial shifts in *per capita* interaction strength and species impacts were associated with changes in community composition, and (2) the relative importance of *Pollicipes* species, being dependent on their abundance and/or on the availability of other prey. Moreno et al. (2010) considered *Pollicipes pollicipes* as "occasional prey" of the yellow-legged gull (*Larus michahellis*) in north-west Spain. Conversely, Wootton (1997) found that *Pollicipes polymerus* accounted for 88% of the intertidal organisms taken by *Larus glaucescens* on wave-exposed shores of Washington. However, the importance of *Pollicipes polymerus* in the diet of gulls was much lower in nearby wave-sheltered shores (Wootton 1997). A similar spatial pattern was found previously by Vermeer (1982), who reported that the percentage of regurgitated pellets of *Larus glaucescens* containing *Pollicipes polymerus* was much higher on the west than on the east coast of Vancouver Island. *Pollicipes polymerus* was also preyed upon by other actively foraging intertidal birds, the American black oystercatcher (*Haematopus bachmani*) and north-western crows (*Corvus caurinus*) (Wootton 1994, 1997), but was only a minor component of their diets (Wootton 1997).

In the intertidal community of Tatoosh Island (Washington), both direct and indirect effects of *Pollicipes polymerus* consumption by birds were assessed by examining populations of *P. polymerus* and of other invertebrate species (Wootton 1992, 1993, 1994). When birds were experimentally excluded by cages, the percentage cover of *Pollicipes polymerus* increased by more than 5.5 times (Wootton 1994). By feeding on *Pollicipes polymerus*, gulls were found to indirectly enhance the cover of *Mytilus californianus* by releasing it from space competition (Wootton 1992), promoting recovery of mussel beds in gaps created by physical disturbance (Wootton 1993). Using a combined experimental and path analysis approach, Wootton (1994) demonstrated that the decrease in abundance of *Pollicipes polymerus*, due to avian predators, was followed by an increase in the abundance of its major spatial competitors (*Semibalanus cariosus* and *Mytilus californianus*), with a consequent rise in the density of *Nucella* predatory snails, due to the increased abundance of *Semibalanus cariosus*, its major food resource. However, in contrast to avian predation, *Nucella* predation on *Pollicipes polymerus* was considered a weak direct interaction, occurring at relatively low rates and causing no indirect effects on the rest of the community (Wootton 1994).

Predation upon *Pollicipes polymerus* by seastars has been systematically observed when examining the wide variety of the diet of *Pisaster ochraceus* (Feder 1959, Paine 1980, Sanford 1999), the principal predator on rocky intertidal communities of central California and the Pacific Northwest (Paine 1966, 1974, Menge et al. 1994, Connolly & Roughgarden 1999 and references therein). Spatial variation has been recognized in the overall trophic patterns of *Pisaster ochraceus* (Feder 1959, Paine 1980), with the importance of *Pollicipes polymerus* as a food species for this large starfish also apparently varying with local ecological conditions. An exceptionally high importance was found within Neptune State Park in Oregon, where 41.8% of actively feeding *Pisaster ochraceus* individuals were recorded preying on *Pollicipes polymerus*, which was the second most common prey item (Sanford 1999). According to Paine (1980), the percentage of *Pollicipes polymerus* consumption by *Pisaster ochraceus* decreased with increasing latitudes, when comparing three geographic regions with similar species composition of intertidal communities (Punta Baja in Mexico, Monterey Bay in California and Outer coast in Washington: 10%, 4% and 3%, respectively). In the Monterey Bay area, Feder (1959) found that, while both mussels and acorn barnacles were preyed upon by *Pisaster ochraceus* in proportion to their relative abundance, the relative frequency with which *Pollicipes polymerus* was eaten by *Pisaster ochraceus* was lower than expected when considering its local availability in sampled habitats. Lauzier (1999b) and Jamieson et al. (1999) stated that the incidence of *Pollicipes polymerus* predation by *Pisaster ochraceus* was "fairly low" and occurred "in the lower midtidal", since only the upper distribution of the starfish coincides with the lower distribution of the barnacle. Similarly, the middle intertidal assemblage, where *Pollicipes* *polymerus* occurs on Tatoosh Island in Washington, was described to be located "above the effective feeding range of *Pisaster ochraceus*" (Wootton 1994).

Observational evidence of predation of *Pollicipes pollicipes* has been frequently recorded on the Portuguese coast (Figure 11): partially ingested *P. pollicipes* individuals observed in the oral region of the spiny starfish, *Marthasterias glacialis*, while preying upon clumps of stalked barnacles (Figure 11A, TC pers. obs.); large specimens (> 0.5 kg) of sea bream, *Diplodus vulgaris*, captured

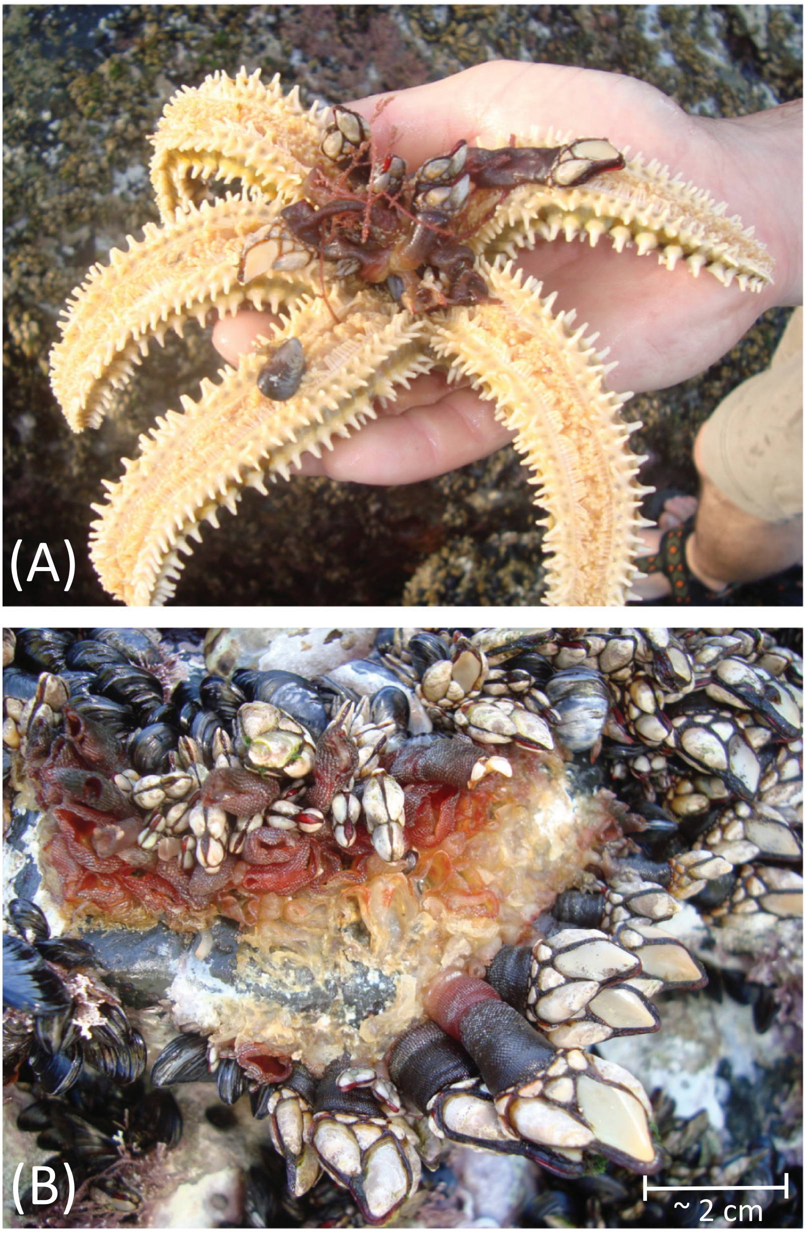


Figure 11 Predation of *Pollicipes pollicipes* in the Portuguese coast. (A) A seastar (*Marthasterias glacialis*) observed while eating several *Pollicipes pollicipes* individuals. (B) Predation signs on a *P. pollicipes* clump. Approximate scale presented when appropriate.

with the stomach holding high quantities of *P. pollicipes* individuals (Cruz 2000, JJC pers. obs.); trigger fish, *Balistes capriscus*, observed biting and probably ingesting *P. pollicipes* (TC and JJC pers. obs.); and cleared areas within *P. pollicipes* clumps denoting signs of recent losses of stalked barnacles from the rock and partially eaten peduncles (Figure 11B). Furthermore, the restricted occurrence of *Pollicipes pollicipes* to very wave-exposed rocky shores, as well as the variation of its distribution and abundance at small scales, suggested that predation on these barnacles might vary along hydrodynamic exposure gradients, being less intense at exposed sites (Sousa 2007, Jacinto 2016). The hypothesis of higher predation in sheltered sites was supported in a series of manipulative experiments, undertaken in different years and locations of Central and SW Portugal (Sousa 2007, Jacinto 2016, Cruz et al. unpublished observations). When kept in cages, transplanted stalked barnacles were able to survive in places where they normally do not occur (e.g. inside the Port of Sines) (Cruz et al. unpublished observations). These results have provided experimental evidence of predation as a cause of post-settlement mortality of *Pollicipes pollicipes*, and as a highly variable process in space that might play a major role shaping the abundance patterns of *P. pollicipes* and limiting this species' distribution (Sousa 2007, Jacinto 2016, Cruz et al. unpublished observations).

Associations between Pollicipes *species and limpets*

As described in the section 'Ecological habitat, and patterns of distribution an abundance' (see Table 4), limpets are one of the taxonomic groups commonly associated with *Pollicipes* species assemblages (e.g. *Pollicipes polymerus* – genus *Lottia, Pollicipes pollicipes* – genus *Patella,* and *Pollicipes elegans* – genus *Fissurella*).

A considerable amount of literature has been published on *Pollicipes*-limpet interactions, despite predominantly being restricted to the species pair formed by *Pollicipes polymerus* and the fingered limpet *Lottia digitalis* (associated taxa table in supplementary material). Barnes (1996) referred to the classical work of Giesel (1969, 1970) by mentioning the influence of the *Pollicipes polymerus-Lottia digitalis* interaction on the growth form and shell pattern of limpets involved in the interaction, in comparison with conspecific limpets inhabiting intertidal rock. Overall, Giesel (1968, 1969, 1970) and subsequent studies on *Pollicipes*-limpet interactions have been mostly focused on issues concerning the biology of limpet populations, specifically their morphology and/or behaviour (e.g. Brym 1980, Byers 1989, Lindberg & Pearse 1990), or their genetics and evolution (Murphy 1978, Crummett & Eernisse 2007, Murphy 2014). In contrast, there is much less information on the effects of interactions with limpets on *Pollicipes* themselves. Nevertheless, some ecological evidence reveals that the association of *Pollicipes* species with limpets may provide mutual benefits for both interacting organisms.

Limpets can act as surface cleaners of *Pollicipes* species while feeding on algal spores, algal propagules and biofilms present on the capitula. Limpets of the genus *Lottia* (*L. digitalis, L. pelta* and *L. strigatella*) have been observed scraping *Pollicipes polymerus* plates in Monterey Bay (California), both in the field and in the laboratory (Brym 1980). Similarly, patellogastropods of the genus *Patella* and the false limpet, *Siphonaria pectinata*, have often been sighted on the plates of *Pollicipes pollicipes*, mostly foraging when awash by waves in the south-west Portuguese coast (Figure 12, MIS pers. obs.). Moreover, the major food types ('*Hildenbrandia*, *Ectocarpus*, blue green algae, fungi, *Ulva* and diatoms'), identified in the stomach contents of limpets (*Lottia* species) that occurred within *Pollicipes polymerus* clusters, were found to grow on barnacle capitula, being only conspicuous in the grooves between the plates (Brym 1980). This constrained distribution of epizoic algae on *Pollicipes polymerus*, mostly visible in-between capitular plates, has also been observed in the other *Pollicipes* species in nature (*P. caboverdensis*: Figure 5 in Fernandes et al. 2010; *P. elegans*: photograph on p.71, Barraza et al. 2014; *P. pollicipes*: Figure 12, MIS pers. obs.). The same spatial pattern has been also found for acorn barnacles that are natural epizoans on *Pollicipes* species (e.g. *Chthamalus fissus* on *Pollicipes polymerus*: Figure 15 in Barnes 1996). In

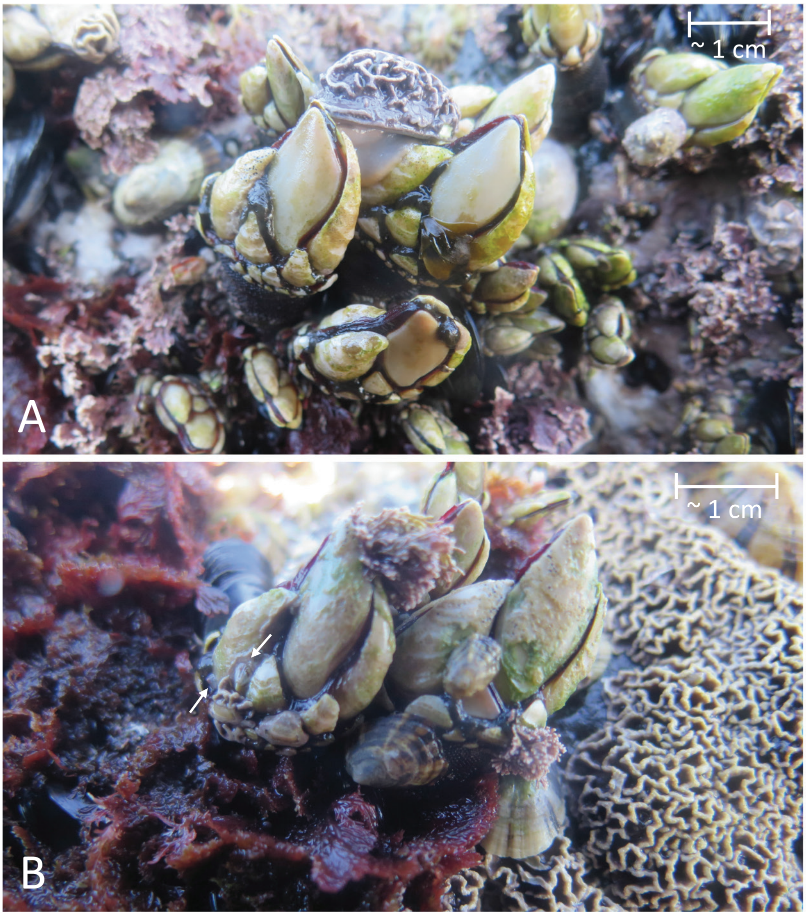


Figure 12 Limpets observed on *Pollicipes pollicipes* capitula in SW Portugal, during low-tide periods while awash by waves. (A) An actively foraging *Patella ulyssiponensis*. (B) Arrows indicate two juveniles of *P. ulyssiponensis* attached to a *Pollicipes pollicipes* individual. Approximate scales presented.

fact, the overgrowth of large areas of the capitulum by sessile organisms (macroalgae, acorn barnacles and mussels) was shown to be lethal for *Pollicipes pollicipes* growing on artificial substrata deployed in an extensive system of production, where grazers were nearly absent (Fernandes 2018). Therefore, the anti-fouling effect potentially produced by the grazing activity of limpets on the capitula of *Pollicipes* species can play a vital role in the maintenance of their feeding and respiratory capacities and hence on their survival and growth.

In turn, *Pollicipes* species offer a hard substratum for settling limpet larvae (Kay 2002) or for the fixation of juvenile and adult limpets (e.g. Giesel 1968, Jobe 1968, Hartwick 1981, Hoffman 1984). Giesel (1969, 1970) showed that specimens of *Lottia digitalis*, naturally occurring within *Pollicipes polymerus* clusters, are behaviourally confined to a single cluster, being resident on the capitulum and commonly fixed to an individual home scutum. In SW Portugal, *Pollicipes pollicipes* clusters have been observed to be used by limpets, primarily during low-shore wave-splash periods, as possible feeding grounds, being climbed on by many actively foraging *Patella* individuals (moving foot across the capitulum, Figure 12) that have their home scars on neighbouring substrata (patches of rock and algal crusts) (MIS pers. obs.). Less frequently, small juveniles of *Patella ulyssiponensis* can be found firmly attached to the barnacle's capitulum (Figure 12), even in non-splash conditions at low tide, suggesting a possible use of *Pollicipes pollicipes* as a microhabitat for recruitment and early-shore residency of this limpet species (MIS pers. obs.). Furthermore, *Pollicipes* species can act as a protective living host by providing limpets: (1) ameliorated physical conditions compared with other microhabitats, mainly by absorbing the force of waves and by offering moisture and shade for limpets (Giesel 1969, Brym 1980, Crummett & Eernisse 2007), and/or (2) a hiding place from visual predators, mainly for avoiding avian predation through cryptic mimesis (Giesel 1970, Frank 1982, Byers 1989, Mercurio et al. 1985, Wootton 1992). Given all of these benefits for limpets, the association between *Pollicipes polymerus* and the limpet taxonomic complex formed by *Lottia digitalis* and *L. austrodigitalis* has become a classic case study on the adaptive value of polymorphism and crypsis (e.g. Giesel 1970, Murphy 1978), phenotypical plasticity (Lindberg & Pearce 1990), habitat partitioning and the role of ecotypes in speciation (Crummett & Eernisse 2007, Murphy 2014). Thus, there is some evidence of the symbiotic nature of these associations.

Despite these mutual gains, some disadvantages of *Pollicipes*-limpet interactions have also been discussed. While grazing by limpets is likely to have a positive effect on keeping *Pollicipes* species free from encrusting organisms, a negative effect can also arise since limpets can account for the dislodgment, ingestion or bulldozing of *Pollicipes* cyprids and juveniles from the capitula of their conspecific adults. The putative influence of limpets on the distribution of *Pollicipes* recruits on conspecifics, particularly on the higher abundance of recruits onto the peduncle than onto the capitulum, has been suggested for *Pollicipes polymerus* (Hoffman 1984) and *P. pollicipes* (Cruz 2000). Moreover, a reduction in barnacle recruitment by limpets has been confirmed for other limpet-barnacle interactions (Dayton 1971, Denley & Underwood 1979, Hawkins 1983). Manipulative experiments in which limpet density would be effectively controlled would be important to understand the effects of limpets on *Pollicipes* species, especially on their fouling and recruitment patterns.

Pollution, contamination and geochemical structure

Barnes (1996) addressed the sparse literature on the effects of pollution on *Pollicipes* by recounting the effects on *Pollicipes polymerus* of an oil spill in 1969 that occurred in Santa Barbara (California, the USA), and on the effects of radiation by tritiated seawater on larval development. Intertidal stalked barnacle populations in oil-polluted areas suffered higher mortality (due to smothering from thickened oil covering capitular plates), reduced breeding and reduced space for settlement (Straughan 1969a, b, 1971, Foster et al. 1971 in Barnes 1996). Negative effects on larval development, namely a reduced moulting index, were observed with increasing tritiated seawater concentration (Abbott & Mix 1979 in Barnes 1996).

In the years that followed Barnes' review, *Pollicipes pollicipes* was used to assess the accumulation of petroleum hydrocarbons in commercial shellfish from the Galician coast (NW Spain) affected by the *Prestige* oil spill (Viñas et al. 2009) and more chronic coastal contamination in NW Portugal (Reis et al. 2012, 2013, Ramos et al. 2014, 2016, Reis et al. 2017, 2019) (see Table 13). Biotoxin uptake of putative phytoplankton and bacterial origin (associated with toxic algal blooms) was reported in *Pollicipes pollicipes* from NW Morocco (Silva et al. 2015, 2018, 2020) (see Table 13) and in *Pollicipes polymerus* from California (Sharpe 1981, cited by Austin 1987 in Lauzier 1999b). Geochemical trace element signatures have also been used as tools to discriminate harvesting origin (Albuquerque et al. 2016).

On November 13, 2002, a major oil spill occurred off Cape Finisterre (NW Spain), when the oil tanker *Prestige* suffered hull damage, due to a heavy sea and high winds, and eventually sank. After the accident, a monitoring programme was established (January 2003–October 2004) to assess the spatial distribution and temporal persistence of petrogenic hydrocarbons in the affected area for regulatory purposes regarding public health (see Viñas et al. 2009). This involved water and sediment sampling, plus monthly sampling of bivalves and other species with high commercial value,

Table 13 Contaminant markers assessed (chemical and biological, biotoxins) and ranges of values observed in *Pollicipes pollicipes* from the Iberian Peninsula and NW Morocco

Contaminant marker	Ranges of observed values	
Chemical markers	*NW Portugal (July 2010)*	*NW Portugal (2011)*
Metals (mg/kg)	Reis et al. (2012)	Reis et al. (2013, 2017)
Cd: Cadmium	0.70–2.22	0.35–3.75
Cr: Chromium	0.49–1.40	0.25–1.79
Cu: Copper	2.4–3.3	0.76–6.09
Fe: Iron	134–578	55–614
Mn: Manganese	5–59	2.89–48.33
Ni: Nickel	1.37–2.07	-
Zn: Zinc	728–1854	413–976
	NW Spain (2003–2004)	*NW Portugal (2011)*
PAH (µg/kg)	Viñas et al. (2009)	Reis et al. (2019)
A: Anthracene	0–1	0.24–15.47
Ace: Acenaphthylene	-	0.07–2.03
AcP: Acenaphthene	-	0.78–5.63
BaA: Benz[a]anthracene	0.1–5	-
BaPy: Benzo[a]pyrene	0.2–9	-
BbFl: Benzo[b]fluoranthene	0.9–39	-
BePy: Benzo[e]pyrene	0-6–35	-
BkFl: Benzo[k]fluoranthene	0.2–12.5	-
BPer: Benzo[ghi]perylene	0.1–8	-
C: Chrysene	0.5–38	-
DBA: Dibenz[ah]anthracene	0–7.5	
F: Fluorene	-	0.08–3.34
Fl: Fluoranthene	5–20	0.26–26.42
IPy: Indeno[1,2,3-cd]pyrene	0.5–7	-
N: Naphthalene	-	0.10–1.79
P: Phenanthrene	6–18	0.24–16.03
Py: Pyrene	1.2–18	0.28–10.56
Biological markers	*NW Portugal (2010–2011)*	*NW Portugal (2013–2014)*
Indicative of oxidative stress	Ramos et al. (2014)	Ramos et al. (2016)
GSTs – isoenzymes glutathione-S-transferases (mmol thioether produced/min/mg protein)	20–1600	50–1000
Indicative of neurotoxicity		
Che – inhibition of cholinesterase activity AChE activity (nmol/min/mg protein)	1.5–4.5	10–125
Indicative of oxidative damage		
TBARS – lipid peroxidation (nM/MDA equivalents mg protein)	5 ($\times 10^{-8}$) to 1.2 ($\times 10^{-6}$)	0.025–0.22
Indicative of stress		
Glycogen levels (µg/mg protein)	-	1–6
Haemocyte counts ($\times 10^{3}$ mL/haemolymph)	-	3–4.5
Biotoxins	*NW Morocco (July 2013)*	
Paralytic shellfish toxins (µg/kg)	Silva et al. (2015, 2018, 2020)	
Saxitoxin and its analogues	17.4–17.6	
Diarrhetic shellfish toxins (µg/kg)		
Okadaic acid	Not detected	
Lipophilic toxins (µg/kg)		
Azaspiracids (AZA-2)	0.83	

such as *Pollicipes pollicipes*. Polyaromatic hydrocarbons (PAHs) obtained from petroleum oil are considered as environmental pollutants and carcinogens, and their concentrations in *Pollicipes pollicipes* tissues were high after the spill (430 µg/kg dry weight of the sum of 13 PAHs: phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, benzo[ghi]perylene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene; Viñas et al. 2009). However, ~6 months after the accident, these PAH concentrations were already below the threshold level proposed by the Spanish Agency for Food Safety and Nutrition for commercial exploitation of these organisms (200 µg/kg dry weight of the sum of 6 PAHs: benzo[a]anthracene, benzo[b] and benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene). One year after the accident, the median values in PAH concentrations were 26 µg/kg for *Pollicipes pollicipes* and 74 µg/kg for mussels (Viñas et al. 2009). Physiological factors, including the rates of uptake and elimination (metabolism, diffusion and excretion), also determine PAH accumulation in tissues of different marine organisms. Therefore, species occupying the same habitat, such as *Pollicipes pollicipes* and mussels, may be accumulating different hydrocarbons at different rates during normal feeding and other biological activities (Viñas et al. 2009). Despite being sampled on the same heavily impacted shores, the PAH accumulation in soft tissues of *Pollicipes pollicipes* was generally lower than that found in mussels (Viñas et al. 2009). Nevertheless, this study demonstrated that *Pollicipes pollicipes* can also be a suitable species for monitoring the biological effects of oil spills in wave-exposed areas (Viñas et al. 2009) where such spills often occur (see Hawkins et al. 2017 for a review).

Reis et al. (2019) also showed that *Pollicipes pollicipes* might be an adequate species to assess spatial and seasonal variations of PAHs, since there were always significant positive correlations between PAH concentrations in seawater and in *P. pollicipes* on the NW coast of Portugal. Maximum concentrations of PAHs were found in locations in the proximity of an oil refinery plant and an industrial wastewater treatment plant. *Pollicipes pollicipes* accumulation rates varied for different PAHs dissolved in the seawater, which might reflect the physico-chemical properties of these compounds, the feeding strategy and the physiological inability to regulate and/or eliminate them (Reis et al. 2019). Based on the total PAH concentrations accumulated by *Pollicipes pollicipes*, and following the OSPAR guidelines for PAHs in shellfish (mussels and oysters – OSPAR 461/2009) as a reference, Reis et al. (2019) suggested that the NW coast of Portugal did not have significant contamination of PAHs during the four seasons of 2011. Since the bioaccumulation of PAHs differs between stalked barnacles and mussels (e.g. Viñas et al. 2009), Reis et al. (2019) noted that future adaptations to the OSPAR guidelines for PAHs in shellfish might be required to accommodate reference values for other important benthic marine resources, such as stalked barnacles.

Different pedunculate and acorn barnacle species have also been shown to bioaccumulate different amounts of metals in their soft tissues, reflecting both short- and long-term metal level environmental variations (reviewed by Reis et al. 2011). Subsequently, Reis et al. (2012, 2013, 2017) used the stalked barnacle, *Pollicipes pollicipes,* to monitor metal contamination on the NW coast of Portugal. The metals that *Pollicipes pollicipes* bioaccumulated more efficiently were Fe, Cd and Zn, reflecting the species' high sensitivity to these elements (Reis et al. 2012, 2013, 2017). Spatial variability observed in metal contamination levels in the soft tissues of *Pollicipes pollicipes* in NW Portugal revealed potential metal anthropogenic contamination, with the most metal-contaminated locations near to, and the least contaminated locations away from, the Oporto metropolitan area (Reis et al. 2012, 2013, 2017). Reis et al. (2013) suggested that, due to the high Cd concentrations bioaccumulated in *Pollicipes pollicipes* from the most contaminated locations (above the 2.50 mg/kg dry wt maximum concentration of Cd allowed in soft tissues of crustacean species by the European Community Commission Regulation No. 629/2008 for food consumption safety), the collection of barnacles for human consumption in these locations should be banned, or individuals collected should be depurated. The results of these studies showed that soft tissues of *Pollicipes pollicipes* can be used for monitoring metal contamination in coastal seawater.

Ramos et al. (2014, 2016) used a biomarker-based approach to assess the spatial and seasonal variation in the physiological responses of *Pollicipes pollicipes*, prompted by anthropogenic compounds (e.g. metals, hydrocarbons, pesticides and several other contaminants) that contaminate coastal waters in NW Portugal. Sources of contamination in coastal waters may include agriculture, industrial and urban run-off, released directly in the coastal area or into the adjacent environment. In these studies, several biochemical parameters (e.g. oxidative stress, peroxidative damage, neurotoxicity and general fitness) were quantified in different tissues (such as cirri and peduncle, but also haemolymph as a non-lethal source tissue for the determination of biomarkers). Fluctuations in these biomarkers were strongly related to seasonality, but also influenced by patterns of chemical contamination, confirming the use of *Pollicipes pollicipes* in biomonitoring coastal pollution.

Stalked barnacles of the genus *Pollicipes* have been found also to accumulate biotoxins (e.g. as a result of exposure to harmful algal blooms). Tests for paralytic shellfish poisoning (PSP) in *Pollicipes polymerus* and California mussels (*Mytilus californianus*) carried out in California (the USA) showed an accumulation of 85 µg toxin/100 g tissue in stalked barnacles, compared with 6400 µg toxin/100 g tissue in mussels (Sharpe 1981, cited by Austin 1987 in Lauzier 1999b). Biotoxin uptake by *Pollicipes pollicipes* was also observed in NW Morocco (Silva et al. 2018, 2020) at concentrations well below limits that represent serious threat to public health. Samples of *Pollicipes pollicipes* collected in July 2013 in NW Morocco tested positive for the presence of PSP toxins (saxitoxin and its analogues; 17.6 µg/kg; Silva et al. 2018), lipophilic toxins (azaspiracid, AZA-2; 0.83 µg/kg; Silva et al. 2020), but not of diarrhetic shellfish poisoning toxins such as okadaic acid and its analogues (Silva et al. 2015). Despite the low levels of biotoxins detected in *Pollicipes* species in these studies, the authors stress the importance of these types of studies in different invertebrates, especially in edible species such as *Pollicipes* species, and the need for revision and update of legislative policies.

Pollicipes pollicipes barnacles are, thus, sufficiently sensitive to several chemical and biological markers commonly used to evaluate contamination caused by human activities (Table 13). However, the species is not commonly used as a bioindicator (compared to mussels) due to its restricted occurrence on highly hydrodynamic shores, which are often less polluted, which present difficulties for routine sampling and which usually do not enable comparisons between sites in different ecological contexts, or along contamination gradients. *Pollicipes pollicipes*, however, is a widely consumed, high-value, coastal marine resource (see section 'Fisheries, management and conservation'). For this reason, it should be monitored for contaminants at different spatial and temporal scales, especially widely dispersed contaminants such as persistent organic pollutants.

The geochemical structure of *Pollicipes pollicipes* capitular plates was studied by Albuquerque et al. (2016) as a potential tool to discriminate their origin along the Portuguese coast, based on trace elemental signatures (Ba, B, Cd, Cr, Li, Mg, Mn, P, Pb, S and Zn). In stalked barnacles, capitular plates are not shed during the moult, but are maintained, increasing in size through peripheral accretion over time (see section 'Growth and size'), putatively preserving imprinted trace elemental signatures over their lifespan. Significant differences were recorded between locations for all elements (Albuquerque et al. 2016), demonstrating that the geochemical structure of the capitular plates of *Pollicipes pollicipes* can be used to assign individuals to their harvesting site at different geographic scales along the Portuguese coastline. This approach has potential implications for the management of stalked barnacle fisheries, enforcement of conservation *policies* and labelling, such as Marine Stewardship Council accreditation.

Fisheries, management and conservation

Worldwide fisheries of marine crustaceans mainly focus on the order Decapoda, with barnacle fisheries generally considered as marginal and only exploited at smaller scales by artisan fishers and shore harvesters (López 2020). Only a few barnacle species are commercially exploited for human consumption: the stalked barnacles *Capitulum mitella* or 'kamemoto' in southern Japan (López 2020) and China (Lin & Rao 2016), plus the four species of *Pollicipes*, along with eight species of acorn barnacles, three of which support the most relevant fisheries (*Austromegabalanus psittacus* or '*picoroco*' in Peru, Chile and Argentina; *Balanus rostratus* or '*mine fujit subo*' in Russia and northern Japan; and *Megabalanus azoricus* or '*craca*' in the Azores, Portugal) (López et al. 2010, López 2020).

In Barnes' (1996) review, commercial exploitation of *Pollicipes* was described mainly for *Pollicipes polymerus* and *Pollicipes pollicipes*, since only a small localized fishery of *Pollicipes elegans* in Costa Rica was mentioned. Regarding *Pollicipes polymerus*, Barnes (1996) noted the traditional use by native people living in the coastal areas of North America of this species as food, also describing the interest of the Iberian market for this species in the late 1980s and the consequent export from British Columbia to Iberia. In relation to *Pollicipes pollicipes*, Barnes (1996) mentioned the probable use of this species as food since the Neolithic period in North Africa, the great and growing commercial interest in this resource in Spain and Portugal in the late 1980s and early 1990s, the depletion of stocks in Spain in the early 1990s and the importation of this species from France and Morocco to Spain.

Twenty-five years after Barnes' review, we know that all *Pollicipes* species (including *Pollicipes. caboverdensis*, first described in 2010) are exploited throughout their geographic distributions (see section 'Geographical distribution' and Figure 2). The common names differ depending on the region, language and history: '*caʔinwa*' in Canada; 'gooseneck barnacle' or 'goose barnacle' in Canada and the USA; '*pouce-pied*' or '*pied de biche*' in France, Morocco and Senegal; '*percebe*' in Portugal, Spain, Mexico, Costa Rica and Cape Verde Islands; '*percebe*' or '*manitas*' in El Salvador; and '*percebe*' or '*uña de cabra*' in Ecuador and Peru. All *Pollicipes* species are exploited, although with different intensities. Despite the lack of local or national data for some of these fisheries, the available data and our overall perception show that the most intensively exploited species is *Pollicipes pollicipes* in Portugal and Spain.

Pollicipes harvesting is a risky activity since the species inhabit very wave-exposed rocky shores (see Figure 13), with reports of serious accidents and deaths of fishers. Most of this harvesting is carried out at low tide (Figure 13, either by hand (only evidence *for P. caboverdensis*, Baessa 2015, Soares 2018; and *P. elegans*, YouTube video on this fishery in Salango Island is available online) or using a scraper or similar equipment (all fisheries)). There is also tradition as well as permission to harvest *Pollicipes pollicipes* in Portugal (Jacinto et al. 2011) and *P. caboverdensis* in Cape Verde (Soares 2018) by free diving/snorkelling (see Figure 14).

Based on a literature review, personal communications, online information, personal observations and our own expertise, we have identified that *Pollicipes* species are harvested in the following regions/countries: *P. polymerus* in Vancouver Island (Canada), Washington and Oregon (the USA) and Baja California (Mexico); *P. elegans* in Guerrero (Mexico), Costa Rica, Ecuador and Peru; *P. pollicipes* in Brittany (France), Basque Country, Cantabria, Asturias and Galicia (Spain), in three marine protected areas in Portugal ('*Parque Natural do Litoral Norte*', '*Reserva Natural das Berlengas*' – RNB and '*Parque Natural do Sudoeste Alentejano e Costa Vicentina*' – PNSACV) and in the rest of mainland Portugal, Morocco, Western Sahara and Mauritania and Senegal; and *P. caboverdensis* in Cape Verde Islands (see Table 15). Table 15 presents a summary of the general characteristics of each fishery, including the following aspects (when information was available): type of harvesting (recreational and professional), main management measures, management level, access type (according to Hilborn et al. 2005, see Aguión et al. 2022b), number of fishers, official annual landings and respective references. We have considered the following five management levels: co-management (when some sort of consultation with the fishers exists) divided into three levels, (1) co-management high level – consultative-cooperative regime and interactive or functional participation of users (see Aguión et al. 2022b), (2) co-management mid-level – consultative or instructive-consultative regime and functional or consultation participation of users (see Aguión

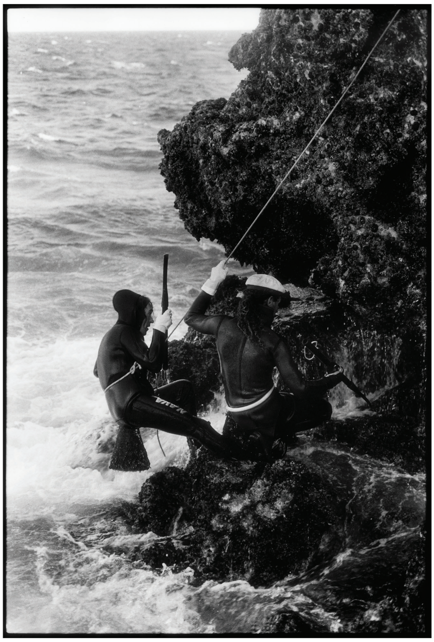


Figure 13 Exploitation of *Pollicipes pollicipes* in SW Portugal during low tide. Photos by João Mariano from the book “Warriors of the sea” © João Mariano.

et al. 2022b, scale modified from Sen & Nielsen 1996) and (3) incipient co-management – when there is a mechanism for the administration to consult users, but all decisions are taken by the administration; (4) top-down – absence of mechanisms for dialogue with users, imposed by the administration, and when we have found or received information specific to this fishery (e.g. licences, landings, stock status and fishery measures); and (5) mainly unregulated and unreported (hereinafter referred to as unregulated) – when we did not find or receive any specific information regarding this fishery (e.g. licences, landings, stock assessments and fishery measures). To provide context, below we start by reviewing archaeological evidence for their use in prehistoric times and their record in shell middens. We then consider each species in turn.

Figure 14 Exploitation of *Pollicipes pollicipes* by free diving in the Berlengas Nature Reserve, Portugal.

Prehistoric harvesting

Calcareous plates of *Pollicipes* capitula were found in several archaeological sites, mostly of prehistoric periods and in Iberian or Northern African regions (Table 14; Figure 2). The identification of these plates was made according to where they were found, with the northern American specimens being assigned to *Pollicipes polymerus* and the European and Northern African ones to *P. pollicipes*. One plate was found with features indicating a possible human use as an adornment, shown in Table 14 as one of the oldest findings (Upper Palaeolithic). Lateral notches, abrasion and polishing of a large *Pollicipes pollicipes* carina plate indicate its use as a suspended object (Aristu et al. 2011). All of the other *Pollicipes* plates from excavations listed in Table 14 were found in deposits as middens, together with molluscan shells or other skeletal remains of fish or shellfish, indicating that these stalked barnacles were intentionally collected, transported to the (archaeological) site and eaten by humans, raw or after burning (e.g. Álvarez-Fernández et al. 2010).

Most of those findings were from archaeological sites, used during Mesolithic and/or Neolithic times in the Iberian Peninsula and Northern Africa, and were assigned to *Pollicipes pollicipes*

Table 14 Archaeological findings (calcareous plates) of *Pollicipes* used by humans in prehistory and history

Prehistoric/historic	Date (years)	Archaeological site	Pollicipes relative abundance (%)	References
Pollicipes polymerus				
?	9400–8000 BP	Busted Balls Cove, San Miguel Is., CA, the USA	0.8–0.9 (MNI); <0.1–0.1 (WMSR)	Erlandson et al. (2004)
?	5600–2500 cal. BP	Kit'n'Kaboodle Cave, Dall Is., AK, the USA	0.014 (WMSR)	Moss & Erlandson (2010)
?	2900–1700 cal. BP	Kit'n'Kaboodle Cave, Dall Is., AK, the USA	0.005 (WMSR)	Moss & Erlandson (2010)
Neolithic-XIXth cent.	5300–150 BP	Several sites, Vancouver Is., BC, Canada	72 ('ubiquity'); 0.094 (WMSR)	Efford (2019)
?	1130-1080 BP	Obsidian Cove, Suemez Island, AK, the USA	0.2 (WMSR)	Moss & Erlandson (2001)
Pollicipes pollicipes				
Upper Palaeolithic (Gravettian)	30211-26288 cal. BP	*Vale Boi*, Algarve, Portugal	0.3–1.3 (MNI of marine shellfish)	Bicho et al. (2013)
	30180-28550 cal. BP	Nerja Cave, Malaga, Spain		Aristu et al. (2011)
Epipaleolithic (Azilian)	13400-10800 cal. BP	*La Fragua*, Cantabria, Spain	0.01 (MNI of shellfish)	Gutiérrez-Zugasti (2011)
Epipaleolithic	11000-7632 cal. BP	*Ifri Oudadane*, NE Morocco	0.59 (MNI of marine shellfish)	Hutterer et al. (2021)
Mesolithic	10800-6800 cal. BP	Several caves, Cantabria, Spain	0.01–3.1 (MNI of shellfish)	Gutiérrez-Zugasti (2011)
	9200-7800 BP	*Toledo*, Lourinhã, Portugal	1.23 (MNI of marine shellfish)	Dupont (2011)
	9020-8360 BP	*B. das Quebradas*, Algarve, Portugal	0.028–1.1 (MNI); 0.007–1 (WMSR)	Bicho (2009); Valente (2008)
	8900-7600 cal. BP	*El Mazo*, Asturias, Spain		Gutiérrez-Zugasti et al. (2016)
	9009-8724 cal. BP		0.05 (MNI of marine shellfish)	García-Escárzaga et al. (2017)

(*Continued*)

Table 14 (*Continued*) Archaeological findings (calcareous plates) of *Pollicipes* used by humans in prehistory and history

Prehistoric/historic	Date (years)	Archaeological site	Pollicipes relative abundance (%)	References
	7839-7607 cal. BP	*El Toral III*, Asturias, Spain	0.5 (MNI of marine shellfish)	Bello-Alonso et al. (2015)
	?	Several caves, Cantabria, Spain		Álvarez-Fernández et al. (2010); Álvarez-Fernández (2011)
	?	*Kobeaga*, Basque Country, Spain		López (1998-2000) in Álvarez-Fernández et al. (2010)
	7520 BP	*Samouqueira I*, Alentejo, Portugal	1.54 (WMSR)	Silva & Soares (1997)
	7450 BP	*Castelejo*, Algarve, Portugal		Silva & Soares (1997)
Mesolithic/ Neolithic	10000-5600 BP	Several sites, Algarve, Portugal		Bicho (2009), Dean (2010), Valente (2014)
	8965-6820 BP (Mesolithic)	*Rocha das Gaivotas*, Algarve, Portugal	Mesolithic – 17.2 (MNI of shellfish)	Dean (2010), Valente (2008)
	6801 BP (Neolithic)		Neolithic – 40 (MNI of shellfish)	
	?	*Jaizkibel*, Basque Country, Spain	0.31–3.95 (NR of marine fauna)	Álvarez-Fernández et al. (2013)
Early Neolithic	7610-6700 cal. BP	*Ifri Oudadane*, NE Morocco	0.48–0.55 (MNI of marine shellfish)	Hutterer et al. (2021)
	6820 BP	*Medo Tojeiro*, Alentejo, Portugal		Silva & Soares (1997)
	6800-5700 cal. BP	Several caves, Cantabria, Spain	0.1–7.5 (MNI of shellfish)	Gutiérrez-Zugasti (2011)
	5600-5280 BP	*El Zafrín*, Chafarinas Is., Spain		Gibaja et al. (2012)
	?	*Padrão I*, Algarve, Portugal	1.47 (MNI of marine shellfish)	Callapez & Pimentel (2018)
Neolithic	6900-4800 BP	*Cueva de los Gitanos*, Cantabria, Spain		Álvarez-Fernández et al. (2010)
	?	Near Oran, Algeria		Doumergue (1921) in Barnes (1996)
	?	Cape Spartel, Morocco		Doumergue (1921) in Barnes (1996)
	?	*Vale Santo I*, Algarve, Portugal	30.2 (NR)	Carvalho & Valente (2005) in Dean (2010)

(*Continued*)

Table 14 (*Continued*) Archaeological findings (calcareous plates) of *Pollicipes* used by humans in prehistory and history

Prehistoric/historic	Date (years)	Archaeological site	Pollicipes relative abundance (%)	References
Chalcolithic	4550 BP	*Montes de Baixo*, Alentejo, Portugal	2.57 (WMSR)	Silva & Soares (1997)
	4500 BP	ETAR, V. N. Milfontes, Portugal	5.3 (WMSR)	Silva & Soares (1997)
	?	*Cueva de los Gitanos*, Cantabria, Spain		Álvarez-Fernández (2011)
Early Bronze Age	?	*Catalão*, Algarve, Portugal	'Dominant in the assemblage'	Carvalho (2007) and Carvalho & Valente (2005) in Dean (2010)
Middle Bronze Age	3460 BP	*P. da Oliveirinha*, Alentejo, Portugal	0.24 (WMSR)	Silva & Soares (1997)
Bronze Age	4200-2800 BP	Several sites, Channel-Atlantic, France		Mougne & Dupont (2020)
Iron Age	?	*Port Blanc*, Höedic Is., Morbihand, France		Dupont et al. (2008)
	2400-2100 BP	*Queiruga*, Galiza, Spain	2.7 (MNI of marine shellfish)	Fernández-Rodríguez et al. (2014)
	2200-1900 BP	*Facho de Donón*, Galiza, Spain	26.1 (MNI of marine shellfish)	Fernández-Rodríguez et al. (2014)
Roman Age	2000-1700 BP	*Punta Atalaia*, Galiza, Spain	12.8 (MNI of marine shellfish)	Fernández-Rodríguez et al. (2014)
	2000-1600 BP	*Santa Trega*, Galiza, Spain	11.1 (MNI of marine shellfish)	Fernández-Rodríguez et al. (2014)

BP, before present; cal., calibrated; MNI, minimum number of individuals; NR, number of remains; WMSR, weight of marine shellfish remains; pers. comm., personal communication; ?, information not provided by cited authors.

(Table 14; Figure 2). Some of these sites are located on the Mediterranean coast, but most are Atlantic, probably due to the occurrence of this species on very exposed shores. According to Álvarez-Fernández et al. (2010), Álvarez-Fernández (2011) and Gutiérrez-Zugasti (2011), the absence of remains of these crustaceans at archaeological sites in the Upper Palaeolithic in south-west Europe could be explained: (1) by the flooding of the archaeological deposits nearer the prehistoric coastline (which potentially may have contained the remains of this resource) owing to the Flandrian transgression, (2) by the absence of this species in a cold climate (the most likely explanation) and/or (3) because human groups did not gather them (the least likely hypothesis). The following Holocene climatic conditions enabled an increase in the diversity and abundance of several marine species, and intensification in the gathering strategies of human groups resulted in enlarged gathering areas during the Mesolithic, especially in the Neolithic, favouring an increase in the diversity of taxa exploited and introducing the use of resources such as stalked barnacles (Gutiérrez-Zugasti 2011). In the western coast of the Algarve, Portugal, *Pollicipes* collection in the early Neolithic replaced Mesolithic economies focused on larger shellfish such as limpets (*Patella*) and mussels (*Mytilus*) (Dean 2010, Valente 2008, 2014). According to Dean (2010), this change indicated a reduction in foraging efficiency, through resource overexploitation, which may have preceded the introduction of agriculture into the region, and may have been a reason for the adoption of new economic adaptations, as happened in other European coastal regions (Schulting et al. 2004, Bonsall et al. 2009, Dupont et al. 2009, Gutiérrez-Zugasti 2011).

The relative abundance of *Pollicipes* plates is low in the remains found in most sites referred to in Table 14, due to the dominance of molluscan shells, mostly of marine or estuarine species of gastropods and bivalves. In sites close to Atlantic and Mediterranean shores, the main molluscan genera found in such shell middens are *Patella* (limpets), *Littorina* (periwinkles), *Phorcus* and *Steromphala* (topshells), *Stramonita* (whelk), and *Mytilus* and *Perna* (mussels), possibly collected on marine rocky shores, although bivalves such as oysters (*Ostrea*), clams (*Ruditapes* and *Scrobicularia*), cockles (*Cerastoderma*) and razor clams (*Solen*) were also found in sites close to estuaries or coastal lagoons (Silva & Soares 1997, Valente 2008, Bicho 2009, Álvarez-Fernández et al. 2010, Álvarez-Fernández 2011, Gutiérrez-Zugasti 2011, Valente 2014, Bello-Alonso et al. 2015, Callapez & Pimentel 2018, Hutterer et al. 2021). Skeletal remains of marine fishes, birds, reptiles and mammals also occur in some of these deposits (Silva & Soares 1997, Valente 2008, Álvarez-Fernández 2011, Gibaja et al. 2012). The same pattern of relative abundance regarding *Pollicipes polymerus* remains was observed in the prehistoric north-eastern Pacific sites listed in Table 14 (Erlandson et al. 2004, Moss & Erlandson 2010, Efford 2019). While the occurrence in middens of several barnacle species indicated reduced use over time, Efford (2019) considered that *Pollicipes polymerus* has remained a significant resource, despite its low proportional weight, across several sites studied on the west coast of Vancouver Island, Canada. Ubiquity-based analyses from this study showed that *Pollicipes polymerus* is far more abundant in shell midden remains than previously appreciated.

As stated by several authors (Silva & Soares 1997, Álvarez-Fernández et al. 2010, Álvarez-Fernández 2011, Álvarez-Fernández et al. 2013, Gutiérrez-Zugasti 2011, Valente 2014, Gutiérrez-Zugasti et al. 2016), it appears that molluscan resources formed an important part of the diet of Mesolithic/Neolithic human groups of hunter-fisher-gatherers, contrasting with the relativily limited importance of other marine shellfish resources collected on oceanic shores, such as *Pollicipes*, sea urchins and crabs. Although the continuity in their exploitation pattern observed at several sites suggests that they were a regular food source, at most sites, their collection was probably opportunistic and sporadic, possibly due to the difficulties of exploitation in the habitats where they occur (low intertidal or shallow subtidal levels, and/or very wave-exposed rocky shores) and their low food value (Álvarez-Fernández et al. 2010, Álvarez-Fernández 2011, Álvarez-Fernández et al. 2013, Gutiérrez-Zugasti 2011, Gutiérrez-Zugasti et al. 2016). Supporting these observations of relatively small fishing intensity, results of biometric analyses indicate that these barnacles were not overexploited by human groups in a northern Spain Mesolithic/Neolithic site (Álvarez-Fernández et al. 2013), although the opposite trend has been suggested by Valente (2008, 2014) in a study of southern Portugal sites of the same periods. However, given the pattern of continuous exploitation of these species in northern Iberia and other areas of Atlantic Europe, Gutiérrez-Zugasti et al. (2016) suggested that they can be interpreted, from a qualitative perspective, as stable resources with a significant social value, and proposed that they could have been sought after as delicacies involved in the celebration of social activities (at group and/or inter-group level), which would increase their social significance and general importance.

Pollicipes polymerus

The only regulated professional fisheries of *Pollicipes polymerus* are along the west coast of Vancouver Island (British Columbia, Canada), Oregon (the USA) and in Baja California (Mexico). The former is also a unique example of a co-managed *Pollicipes* fishery on the Pacific Ocean. In 2015, the *Pollicipes polymerus* fishery in Vancouver Island was considered to be sustainable by the Monterey Bay Aquarium Seafood Watch (Schiller 2015).

Vancouver Island, British Columbia, Canada

In this region, *Pollicipes polymerus* or '*caʔinwa*' (First Nations name, which means 'playing with the waves or in the waves') is a traditional food source for the Nuu-chah-nulth First Nations, who have harvested these barnacles along the north-west coast of North America for millennia (Gagne et al. 2016). A modern commercial fishery started in 1978 (Gagne et al. 2016), and six phases can be identified. The first (1978–1985) and second phases (1985–1999) of this fishery are similar (unlimited entry, open year-round, no size limits), but have one fundamental difference, which was the reporting of landings since 1985. During the second phase, this fishery peaked in 1988 (467 licences and 49 t landed) (Lauzier 1999b). From 1995 to 1997, landings decreased to 8-12 t per year and were associated with a high economic value for fishers (> Can$9 per kg, ~€6.1) (Lauzier 1999b). At that time, barnacles were harvested by both commercial and First Nation harvesters (Jamieson et al. 2001). The third phase corresponded to the closure of this fishery in 1999 due to various reasons, including a lack of information for performing stock assessments (Lauzier 1999a, b). The fourth phase (2000–2002) was characterized by two experimental fisheries with low catches (1.3–1.8 t/year) and the collection of information to develop a management plan (DFO 2005 in Gagne, 2016). In the fifth phase (2003–2005), an experimental multi-stakeholder (including administration, First Nations, fishers, buyers, NGOs) co-management fishery was implemented, which included the assessment and management of the fishery based on the local ecological knowledge (Schiller 2015). However, this first co-management attempt failed, due to management costs and the increasing market competitiveness of cheaper South American stalked barnacles in the European market (Gagne et al. 2016).

The current (sixth) phase (from 2009) of this fishery followed the implementation of *T'aaq-wiihak* (harvesting with permission of the *Ha'wiih* (hereditary chiefs)), after the recognition in 2009 by the British Columbia Supreme Court of the rights of five Nuu-chah-nult Nations to catch and sell species traditionally harvested within their territories, including *Pollicipes polymerus* (T'aaq-wiihakfisheries.ca 2021). This second experimental co-management attempt between the Nuu-chah-nult Nations and the Department of Fisheries and Oceans Canada (DFO) was initiated in 2013 and is exclusively a First Nations' fishery (Gagne et al. 2016). The general rules adopted are described in Table 15 and also include submission of harvest log data and stock assessment (Schiller 2015, Gagne et al. 2016, Fisheries and Oceans Canada 2021). The monitoring assessment techniques have been

Table 15 *Pollicipes* species. Fisheries identified by country, main management measures (for recreational and professional harvesting), management level, access type, number of fishers and official annual landings

Species/country/ fishery	Recreational harvesting? Main management measures	Professional harvesting? Main management measures	Management level	Access type	Number of fishers	Official annual landings (tonnes)	References
Pollicipes polymerus							
Canada							
Vancouver Island	Yes IQ-day (2 kg) Type of gear – handpicking	Yes IQ-month (453 kg, but never achieved), rotation (rock-by-rock basis), TAC (5,4 t)	Co-management High level	TURF	2–6 (On average from 2013 to 2016)	1,1 (On average from 2013 to 2016)	British Columbia Sport Fishing Regulations (1996), Lauzier (1999a, b), Edwards (2020), Jamieson & Levings (2001), Jamieson et al. (2001), Lessard et al. (2003), Schiller (2015), Bingham (2016), Gagne et al. (2016), T'aaq-wiihak (2016), T'aaqwiihakfisheries.ca (2021)
The USA							
Washington	*Yes?* TC in open coast (Apr–Oct), NTZs (Olympic National Park & several MPAs in Puget Sound), IQ-day (4,5 kg)	*No* NA	Top-down	NA	NA	NA	Washington Department of Fish and Wildlife (2021)
Oregon	*Yes* NTZs (large MPAs network), IQ-day (ten individuals)	*Yes* IQ-day (50 individuals – any size), on man-made structures such as rock jetties	Top-down	ND	ND	0,2 (On average from 2015 to 2019)	ZAGAT (2014), Bingham (2016), Eater (2020), Oregon Department of Fish and Wildlife (2021), Julia Bingham & Alan Shanks (pers. comm.)
Mexico							
Baja California	*No* NA	*Yes* ND	Top-down	ND	Three companies	28[a] (On average from 2014 to 2018)	Rogelio Cano (pers. comm.), Mario J. Escobedo (pers. comm.)

(*Continued*)

Table 15 (*Continued*) *Pollicipes* species. Fisheries identified by country, main management measures (for recreational and professional harvesting), management level, access type, number of fishers and official annual landings

Species/country/ fishery	Recreational harvesting? Main management measures	Professional harvesting? Main management measures	Management level	Access type	Number of fishers	Official annual landings (tonnes)	References
Pollicipes elegans							
Mexico							
Guerrero	*?* ND	*Yes* Mainly unregulated	Mainly unregulated and unreported	ND	ND	ND	Gutiérrez & Cabrera (2012, 2019)
Costa Rica	*?* ND	*?* ND	Mainly unregulated and unreported	ND	ND	ND	Mora-Barboza & Sibaja-Cordero (2018), Restaurante Costa del Sol Puntarenas (2018)
Ecuador	*?* ND	*Yes* Mainly unregulated	Mainly unregulated and unreported	ND	ND	ND	Cadena et al. (2008), Guilelrmo9111 (2015), Ladines (2018), López (2018), Cárdenas-Calle et al. (2020), Restaurant '*Delfim Mágico*', Restaurant '*Sabor Españo*l', Maritza Cárdenas-Calle (pers. comm.)
Perú							
Tumbes	*?* ND	*?* ND	Top-down	ND	ND	ND	Ordinola et al. (2010), Alemán et al. (2016)
Piura	*No* NA	*Yes* IQ-month (3–100 kg) 100 kg in 2017 and 3 kg in 2019	Top-down	Individual quotas	ND	ND	Kameya & Zeballos (1988), Pinilla (1996), '*Resolución Directoral Regional Nº 166-2019-GORE Piura*', '*Resolución Directoral Regional Nº 026.2017-GORE Piura*', '*Resolucion Ministerial 319-2007 PRODUCE*', '*Resolucion Ministerial 031-2002 PRODUCE*', '*Resolucion Ministerial 197-2002 PRODUCE*', Villena (1995), Oliva (1995)

(*Continued*)

Table 15 (*Continued*) *Pollicipes* species. Fisheries identified by country, main management measures (for recreational and professional harvesting), management level, access type, number of fishers and official annual landings

Species/country/ fishery	Recreational harvesting? Main management measures	Professional harvesting? Main management measures	Management level	Access type	Number of fishers	Official annual landings (tonnes)	References
Islas Lobos	*No* NA	*Yes* But closed since 2007	Top-down	ND	ND	6,6 (On average from 1997 to 2006)	Kameya & Zeballos (1988), de la Cruz et al. (2001), Samamé & Quevedo (2001), Ramírez et al. (2008), de la Cruz et al. (2015a,b), Ramírez & de la Cruz (2015), de la Cruz et al. (2016), Ramírez et al. (2017), Ladines (2018), Ramírez et al. (2019)
Pollicipes pollicipes							
France							
Finistère	*Yes* NTZs (areas with total and partial protections: Cap Sizun Special Protection Area), IQ-day (3 kg)	*Yes* TC (Jul-Aug), NTZs (areas with total and partial protections: Cap Sizun Special Protection Area), IQ-day (90 kg)	Co-management Mid-level	Limited entry	18 (In 2020)	5,6^a (Annual average estimated)	Aguión et al. (2022b), '*Comite Regional des Peches Maritimes et des Elevages Marins de Bretagne* (161-2020)', Dominique Davoult pers. comm.
Morbihan	*Yes* NTZs (areas with total and partial protections), IQ-day (3 kg)	*Yes* TC (Jul-Aug), NTZs (2 no-take areas in Groix Island), IQ-day (120 kg)	Co-management Mid-level	Limited entry	30 (On average from 2013 to 2016)	50^a (On average from 2013 to 2016)	Aguión et al. (2022b), '*Comite Regional des Peches Maritimes et des Elevages Marins de Bretagne (181-2020)*'
Spain							
Orio and Bakio	*No* NA	*Yes* MLS (TL > 40 mm = RC > 17 mm), TAC (4 t)	Co-management Mid-level	Limited entry	<10 (On average from 2013 to 2016)	0,1 (On average from 2013 to 2016)	Aguión et al. (2022b)

(*Continued*)

Table 15 (*Continued*) *Pollicipes* species. Fisheries identified by country, main management measures (for recreational and professional harvesting), management level, access type, number of fishers and official annual landings

Species/country/ fishery	Recreational harvesting? Main management measures	Professional harvesting? Main management measures	Management level	Access type	Number of fishers	Official annual landings (tonnes)	References
Basque Country General	*Yes* MLS (40 mm TL = 17 mm RC), TC (closed May-September), NTZs (Gaztelugatxe Marine Reserve), IQ-day (0,5 kg)	*Yes* MLS (TL > 40 mm = RC > 17 mm), NTZs (Gaztelugatxe Marine Reserve)	Top-down	Open access	ND	ND	Bald et al. (2006), Borja et al. (2006a, b), Aguión et al. (2022b)
Cantabria	*No* NA	*Yes* MLS (RC > 18 mm), TC (May–Sep), NTZs (three types of protection regimes; permanently open, seasonally closed and permanently closed – Sonabia)	Top-down	Limited entry	ND	4,6 (On average from 2019 to 2020 – before 2019, landings were very low due to misreporting)	Gutiérrez-Cobo & Bidegain. (2012), Bidegain et al. (2015, 2017), '*Orden MED/15/2020, de 20 de julio*'
Asturias East	*No* NA	*Yes* MLS (RC > 18 mm), TC (Oct–Apr), IQ-day (5–8 kg)	Top-down	Limited entry	234 (On average from 2013 to 2016)	11 (On average from 2013 to 2016)	Rivera et al. (2013, 2014), Rivera (2015), Rivera et al. (2016a, b, c, 2017, 2019), Aguión et al. (2022b)
Asturias West	*No* NA	*Yes* MLS (RC > 18 mm C), TC (May–Sep), Rotation, IQ-day (5–8 kg), SE	Co-management High level	TURF	204 (On average from 2013 to 2016)	44 (On average from 2013 to 2016)	Aguión et al. (2022b)

(*Continued*)

Table 15 (*Continued*) *Pollicipes* species. Fisheries identified by country, main management measures (for recreational and professional harvesting), management level, access type, number of fishers and official annual landings

Species/country/ fishery	Recreational harvesting? Main management measures	Professional harvesting? Main management measures	Management level	Access type	Number of fishers	Official annual landings (tonnes)	References
Galicia	*No* NA	*Yes* MLS (DBC > 15 mm = RC > 18.3 mm), rotation, IQ-day (3–10 kg), SE	Co-management High level	TURF	1308 (On average from 2013 to 2016)	333 (On average from 2015 to 2019)	Freire & Garcia-Allut (2000), Molares & Freire (2003), Molares et al. (2008), Navarrete (2009), García-Negro et al. (2009), Parada et al. (2012, 2013), Macho et al. (2013), Vázquez-Rowe et al. (2013), Pita et al. (2019), Aguión et al. (2022b)
Canary Islands	*No* NA	*Yes* But closed since 2011	Top-down	ND	ND	ND	Marín & Luengo (1998), '*Orden 2 de mayo de 2011*'
Portugal							
RNB	*No* NA	*Yes* MLS (RC ≥ 23 mm – at least in 50% of the volume), TC (Jan-Mar & Aug-Sep), NTZs, IQ-day (20 kg)	Co-management Mid-level	Limited entry	40 (Maximum allowed)	16 (On average from 2015 to 2019)	Jacinto et al. (2010, 2011), Sousa et al. (2013), Albuquerque (2014), Cruz et al. (2015c), Albuquerque et al. (2016), Sousa et al. (2020), Aguión et al. (2022b), Neves (2021)
PNSACV	*Yes* MLS (R C ≥ 20 mm – at least in 75% of the volume), TC (15 Sep–15 Dec), NTZs, IQ-day (2 kg)	*Yes* MLS (RC ≥ 20 mm – at least in 75% of the volume), TC (15 Sep–15 Dec), NTZs, IQ-day (10–15 kg)	Incipient co-management	Limited entry	80 (Maximum allowed)		Cruz (2000), Castro & Cruz (2002), Castro (2004), Jesus (2004), Penteado (2011), Costa (2012), Sousa et al. (2013), Stewart et al. (2014), Cruz et al. (2015c), Jacinto (2016), Jacinto & Cruz (2016), Carvalho et al. (2017), Diogo et al. (2020), Aguión et al. (2022b)

(*Continued*)

Table 15 (*Continued*) *Pollicipes* species. Fisheries identified by country, main management measures (for recreational and professional harvesting), management level, access type, number of fishers and official annual landings

Species/country/ fishery	Recreational harvesting? Main management measures	Professional harvesting? Main management measures	Management level	Access type	Number of fishers	Official annual landings (tonnes)	References
Portugal General	*Yes* MLS ($RC \geq 20$ mm – at least in 75% of the volume), TC (15 Sep–15 Oct), IQ-day (2 kg), NTZs (Marine Park – Parque Marinho Luíz Saldanha)	*Yes* MLS ($RC \geq 20$ mm – at least in 75% of the volume), TC (15 Sep–15 Oct), IQ-day (20 kg), NTZs (Marine Park – Parque Marinho Luíz Saldanha)	Top-down	Limited entry	456	136 (On average from 2015 to 2019 – including RNB & PNSACV)	Sousa et al. (2013), Cruz et al. (2015c), Aguión et al. (2022b)
Morocco	? ND	*Yes* MLS ($RC > 25$ mm), TC (Jun–Oct)	Top-down	Limited entry	ND	ND	Boukaici et al. (2012, 2015), Boukaici (2015), Bourrassi et al. (2019), Hakima Zidane (pers. comm.)
Western Sahara & Mauritania	? NA	*Yes* Mainly unregulated	Mainly unregulated and unreported	ND	ND	ND	Fernández de Larrinoa & Cedenilla (2003), Pablo Fernández de Larrinoa (pers. comm.)
Senegal	? NA	*Yes* Mainly unregulated	Mainly unregulated and unreported	ND	ND	ND	'*Direction des pêches maritimes – Rapport statistique 2002 – La mer: richesse et avenir du Sénégal*'; online information of selling barnacles
Pollicipes caboverdensis							
Cape Verde	? NA	*Yes* Mainly unregulated	Mainly unregulated and unreported	ND	ND	ND	Fernandes et al. (2010), Baessa (2015), Cruz et al. (2015a), Soares (2018)

References in this table include references used for building this table and other references found related to each fishery.

DBC, diameter of the *capitulum* base; IQ-day, individual quota per day; IQ-month, individual quota per month; MLS, minimum legal size; MPAs, marine protected areas; NA, not applicable; ND, no data; PNSACV, '*Parque Natural do Sudoeste Alentejano e Costa Vicentina*'; RC, maximum distance between the *carina* and *rostrum* plates; Rotation, rotation of the harvesting areas; RNB, '*Reserva Natural das Berlengas*'; SE, self-enforcement; TAC, total allowable catch per year; TC, temporal closure; TL, total length; TURF, Territorial User Rights for Fishing; ? – We have doubts on the existence of a type of harvesting, or we have a recent reference (personal communication, online information) that the species is exploited or sold, but no further data were obtained.

[a] Based on personal communications.

updated from Lauzier (1999a) and now include new techniques, such as the use of GPS technology, and the inclusion of local ecological knowledge (Gagne et al. 2016). The amount of barnacles sold ranged from 659 lb (299 kg) in 2013 to 6279 lb (2848 kg) in 2015 (T'aa q-wiihak 2016). These values are lower than the DFO *caʔinwa* harvest allocation of 12,000 lb per year (5443 kg), due to a limited market and a limited number of harvesters (T'aa q-wiihak 2016). According to Edwards (2020), the small available quota makes it difficult to attract buyers.

A profound change in the commercial market has taken place from the 1970s and 1980s to the present day. By the time of Barnes' (1996) review, the main market of the British Columbia barnacles was Spain (Jamieson et al. 2001). Barnes (1996) described in detail the difficulties and logistical problems associated with the export of live animals to Europe, as it would be unlikely that the European market would pay the prices for a frozen product. Currently, approximately 75% of the barnacles harvested at Vancouver Island are sent to the USA, while the remainder is sold domestically (Schiller 2015). The annual amount earned ranged between Can$4400 (~€2987) in 2013 and Can$51,000 (~€34,630) in 2015 (T'aa q-wiihak 2016), fetching about US$9–12 per kg (~€7.6–10.1), with a maximum price of US$22 per kg (~€18.5) (Schiller 2015).

In British Columbia, *Pollicipes polymerus* can also be harvested recreationally, although participation is assumed to be negligible (Gagne et al. 2016). A recreational management plan allows the collection by hand of 2 kg by fishers who must have a recreational licence (British Columbia Sport Fishing Regulations 1996).

Washington and Oregon, the USA

Despite having extensive populations in the three Pacific states of the USA and the historic use of this species as food (Bingham 2016), no professional harvesting of *Pollicipes polymerus* is allowed in Washington and California (Washington Department of Fish and Wildlife 2021, California Department of Fish and Wildlife 2021). In Oregon, in the last decade, several attempts have been made to develop a professional fishery on man-made structures (jetties) (Bingham 2016). Despite some general regulations being in place, there is only an incipient commercial fishery (Oregon Department of Fish and Wildlife 2021, Alan Shanks pers. comm., see Table 15). In California, *Pollicipes polymerus* is not allowed to be harvested recreationally (California Department of Fish and Wildlife 2021); in the states of Oregon and Washington, licensed recreational harvesting is allowed, although under minimal regulations, and focused on a daily quota and a strong network of MPAs where shellfish harvesting is banned (Oregon Department of Fish and Wildlife 2021, Washington Department of Fish and Wildlife 2021).

Baja California, Mexico

In this region, *Pollicipes polymerus* recreational harvesting is not allowed, but a small and fluctuating professional fishery has been developed (Rogelio Cano, pers. comm., Mario J. Escobedo, pers. comm.). Most of the harvesting takes place around the coast of Ensenada (Baja California). These barnacles are mainly sold to local tourist restaurants, but can also be sent to Mexico City, where the product can be found in the main markets (e.g. *'Mercado de San Juan'*) and restaurants, or even exported to the USA.The *Pollicipes* fishery in Baja California is managed through a special prospective fisheries permit ('*pesca de fomento*'), granted to three companies by the Federal Government through CONAPESCA ('*Comisión Nacional de Acuacultura y Pesca*'). Since 2009, professional harvesting has only taken place in some years (2009, 2011, 2014-16 and 2018, while in 2019 and 2020 no professional harvesting took place). Annual landings were very variable, ranging from 0.02 t in 2011 to 88 t in 2016 (average of 28 t, 2014-2018).

Pollicipes elegans

Pollicipes elegans is harvested in Mexico, Costa Rica, Ecuador and Peru. Regarding its geographical distribution area (see section ‘Geographical distribution’, Figure 2), only in El Salvador could no information be found on this species being fished. With the exception of Peru, all of these *P. elegans* fisheries can be considered as unregulated small-scale fisheries.

Guerrero, Mexico

In Mexico, the only accessible information on the fishing of *Pollicipes elegans* is the reference to its exploitation on the coast of the state of Guerrero (Gutiérrez & Cabrera 2012, 2019). Here, this species has been classified as having local and regional economic importance, being commercially important for consumption in tourist areas (Gutiérrez & Cabrera 2019). This fishery is seasonal, namely in December (Gutiérrez & Cabrera 2012) and in the months before the rainy season, reaching first sale values between Mex$85.00 and 100.00 per kg (~3.5€–4.0€/kg) and restaurant sale values between Mex$180.00 and Mex$260.00 per kg (~7.4€–10.7€/kg) (Gutiérrez & Cabrera 2019).

Costa Rica

The small-scale fishery of *Pollicipes elegans* in Costa Rica was identified by Bernard (1988) and cited in Barnes (1996) and appears to remain small-scale. Mora-Barboza & Sibaja-Cordero (2018) reported that *Pollicipes elegans* is not a very common species on the Pacific coast of Costa Rica and is not a traditional source of food as in Peru. However, it was found as an appetizer in a restaurant menu in the province of Puntarenas (price in 2021 of ₡12,000, ~€16 per portion, restaurant “Costa del Sol”, online information).

Ecuador

In Ecuador, *Pollicipes elegans* has limited commercial interest (Cárdenas-Calle et al. 2020), is exploited in an unregulated manner and is not in great demand, being harvested mainly in the province of Manabi (Maritza Cárdenas-Calle, pers. comm.). In Manabi, this species seems to be of special interest in Puerto López canton (Cadena et al. 2008, López 2018), namely in Salango, in front of Salango Island where it is harvested (a YouTube video on this fishery in Salango Island is available online). In Salango, *Pollicipes elegans* is consumed as an appetizer in restaurants and can reach a considerable price, ranging from US $10 (~€8, 2021 price in restaurant “Sabor Español” per ~0.5 kg, online information) to US $25 (~€21, 2021 price in restaurant “Delfin Mágico” per portion, online information). In a survey of people of both sexes in Guayaquil (Guayas province) aged between 18 and 50 ($n = 164$), the vast majority (85%) did not consume this species, indicating a lack of knowledge of these barnacles (López 2018), but in Puerto Engabao (Guayas), *Pollicipes elegans* is harvested locally (Ladines 2018).

Peru

Historically, the main commercial fishery of *Pollicipes elegans* in Peru took place in Isla Lobos de Tierra and Islas Lobos de Afuera (Lambayeque), in the north of Peru. In the 1990s and early 2000s, these islands supported a fluctuating fishery, focused on exporting fresh and frozen stalked barnacles to Spain and Portugal (IMARPE 2005), taking advantage of the overfishing in many Spanish fisheries, such as Galicia (Molares & Freire 2003). The fishery in these islands had two main harvesting periods, the first during 1992–1995, with annual landings between 14 and 34 t, and the second in 2001–2002, with landings between 19 and 28 t, while in the other years, the fishery was below 4 t (IMARPE 2005, and Jaime de la Cruz, IMARPE – *Instituto del Mar del Perú*, pers. comm.). The lack of regulations and enforcement resulted in massive overexploitation of the resource and collapse of the stock of both Islas Lobos by the mid-2000s (Jaime de la Cruz – IMARPE, pers. comm.). The IMARPE monitoring reported an abundance of 39.5 million individuals at the Islas Lobos in 1995, dropping to 2.5 in 2002 and 0.4 in 2010 and 2015 (the biomass in 2015 was only 3 t) (Ramírez & de la Cruz 2015, Ladines 2018). Consequently, the fishery in Islas Lobos was closed in 2007 (‘*Resolución Ministerial n° 319-2007-PRODUCE*’), with no recovery observed to date (Ladines 2018, and Jaime de la Cruz – IMARPE, pers. comm.). Currently, some small, subsistence harvesting is done by local fishers (Jaime de la Cruz – IMARPE, pers. comm.). Some commercial fishing still takes place along the continental coast of Piura. It seems that *Pollicipes elegans* populations in this region are also overexploited based on the individual quotas allowed to be harvested and their recent drop from 100 kg/month per fisher in 2017 to only 3 kg in 2019, based on the administrative decisions of the regional government of Piura (‘*Resolución Directoral Regional Nº 026, 2017*’ and ‘*Resolución Directoral Regional Nº 166-2019*’). At Tumbes, IMARPE monitors *Pollicipes elegans* populations on the shore. A slight decrease in size was found compared with Ordinola et al. (2010) by Alemán et al. (2016), but it is not clear if these populations are currently supporting any fishery. *Pollicipes elegans* exports to the Iberian Peninsula no longer take place, following the closure of the Islas Lobos fishery (Jaime de la Cruz – IMARPE, pers. comm.).

Pollicipes pollicipes

Pollicipes pollicipes is the only *Pollicipes* species that is heavily harvested throughout its range, wherever significant populations are present (i.e. France, Spain, Portugal and Morocco). The species has long been considered a seafood delicacy in Spain and Portugal, where it is the most important fishery in the rocky intertidal (Cruz et al. 2010a, Aguión et al. 2022b). The main fishery is located in Galicia (Spain) (average of 333 t and 8.9 million € per year between 2015 and 2019), which is larger in terms of volume harvested and market value than all of the other *Pollicipes pollicipes* fisheries combined (Aguión et al. 2022b). In Brittany (France) and Morocco, this species is also extensively harvested, but, since it is rarely sold locally, most catches are exported to the Iberian countries, where it costs much less than the locally fished species. In Western Sahara, Mauritania and Senegal, harvesting appears to be residual.

Brittany, France

The *Pollicipes pollicipes* fishery in Brittany is the third largest in the world, after Galicia and Portugal, in terms of landed weight (around 55 t in recent years, but with peaks over 100 t in the early 2000s), although its socio-economic relevance is much smaller than in the Spanish and Portuguese fisheries (Aguión et al. 2022b). Around 90% of the landings in Brittany come from the department of Morbihan, with the rest from Finistère (Dominique Davoult, pers. comm.). In Morbihan, a co-management system has been implemented with around 50 harvesters involved. Fishers can harvest large amounts of barnacles per day (120 kg), the highest in any *Pollicipes* fishery, that nevertheless fetch a very low market value (5–8 €/kg) (Table 15) due to the lack of a local market, with almost everything being exported to Spain and Portugal. A similar system in terms of governance and management measures is found in Finistère, although at a much smaller scale (Table 15 and Dominique Davoult, pers. comm.). Despite the strong tradition in French cuisine for seafood (e.g. bivalves, gastropods and decapods), *Pollicipes pollicipes* has never been locally appreciated, which has prevented the development of a more locally significant fishery. In the 1970s, Spanish middlemen went to France, attracted by the amount of unharvested stock and the low prices, and today, the fishery is driven by Spanish demand. Consequently, this strong link with Spanish markets has created a trans-national poaching system, from France to Spain, due to the large respective differences in governance, control and surveillance, in the social structure of the fishery, and in the demand and market prices (Geiger et al. 2022).

Spain

In Spain, only commercial harvesting of *Pollicipes pollicipes* is allowed, while recreational fishing is forbidden, with the exception of a residual recreational fishery in the Basque Country. The species is mainly harvested in Galicia, but also in Asturias, Cantabria and residually in the Basque Country, while in the Canary Islands, its small fishery has been closed by the regional government since 2011, due to overfishing (‘*Order 2 Mayo, 2011, Gobierno de Canarias*’). *Pollicipes pollicipes* is a highly appreciated seafood in Spain, with an average first-sale price of 17–32 €/kg, which is much higher in premium areas (e.g. 65 €/kg in Cangas, Galicia), with record prices at Christmas up to 250–350 €/kg (Pescadegalicia.gal 2021).

Historically, this species has been commercially harvested in the NW of Spain since at least the 1930s (Dirección General de la Marina Civil y Pesca, 1935), without much regulation until its collapse in the 1970s and 1980s (Molares & Freire 2003), despite initial measures being introduced, such as a summer reproductive closure in Galicia (Goldberg 1984). Since the 1970s, and while the local stocks were becoming depleted, the large Spanish market demand was partly met through importation from France, Portugal, Morocco, and even Canada and Peru (Molares & Freire 2003). Imports to Spain from Canada and Peru continued until the 2000s, when they stopped for a combination of reasons: the difficulty of importing fresh product from so far away, the collapse of the Peruvian stocks and the recovery of the Spanish stocks. Nevertheless, importation from France, Portugal and Morocco was consolidated during the last two decades and continues until the present.

Barnes’ (1996) review concluded with the depletion of the Spanish populations of *Pollicipes*, and she noted the recent implementation of “strict conservation measures”. A profound change has occurred since that time, not merely through the implementation of new management measures, but mainly due to a totally new governance approach. This required the strengthening and empowerment of the fisher’s associations, who were granted exclusive access to the fishing beds under a co-management approach (Molares & Freire 2003, Macho et al. 2013, Rivera et al. 2014, 2016a, Aguión et al. 2022b). The most prominent examples are Galicia and Asturias, with steps in this direction also taking place in other regions (Cantabria and Basque Country).

In Spain, several professional *Pollicipes pollicipes* fisheries currently operate in place in Galicia, Asturias, Cantabria and the Basque Country. The main management measures for each fishery are summarized in Table 15 and include a maximum number of harvesting licences (limiting access to the fishery), minimum sizes, temporal and spatial closures (including no-take zones), and even individual daily quotas, fishing bed rotation and self-enforcement in the most developed fisheries in Galicia and Asturias, based on exclusive access to fishing grounds (*i.e.* Territorial User Rights for Fishing – TURF), which are granted to the fishers’ organizations locally known as *cofradías* (Aguión et al. 2022b).

Galicia supports the main and oldest regulated *Pollicipes pollicipes* Spanish fishery. Historically, despite the secular tradition of the *cofradías* since the Middle Ages, shellfishing was mostly a *de facto* open access system until the 1990s (Macho et al. 2013). In 1992, the first co-management system in Spanish fisheries started in Galicia using TURF, where the responsibility for the exploitation was shared between the cofradías (fishers’ guilds supervised by the regional government) and the fishery authorities (Molares & Freire 2003). This change opened new opportunities for innovation and improvement in the management system, following an adaptive process necessary to design and implement fishery management plans that have become mandatory since 1992 (Molares & Freire 2003). The management plans specify annually (triennially for the future 2022–2024 period) the different components of the management system: authorized fishers, fishing grounds, general objectives, state of the fishery and stock assessment analyses, harvesting and trade plans, actions for stock enhancement, and a financial plan (Macho et al. 2013). The *cofradías* have to design the management plan and seek approval from the regional fishery administration, who evaluates them. The introduction of management plans was a key step in the management of this fishery, and their numbers quickly grew, from 12 plans in 1992, to 29 in 2001 and to 37 in 2021 (Molares & Freire 2003, Aguión et al. 2022b), now covering all the fishing beds. The performance of the fisheries managed by the *cofradías* using the plans was generally positive, and the production (both in biomass and in economic value) showed an increasing trend, despite some isolated cases of overexploitation (Molares & Freire 2003). One key aspect when developing these plans, and in general for the management of the fishery, is the role of the biologist, directly working for the *cofradías* with government funding. This role matches the 'barefoot ecologist' concept (Prince 2003, 2010), who gives management advice and facilitates communication between stakeholders. Formally known as technical assistants, these biologists enable the provision of good-quality and organized fisheries data, to facilitate and support decision-making processes. They also build robust social capital, by acting as knowledge collectors and translators between fishers, managers and scientists (Macho et al. 2013). In 2018, there were 41 technical assistants in Galician cofradías, overseeing almost all *Pollicipes pollicipes* fishery management plans (our unpublished data). Another key aspect is that most of the *cofradías* have their own surveillance service, co-paid by the fishers. This effectively enforces the management measures internally, and externally promotes collaboration with the government fishery inspection service to avoid poaching by illegal fishers (Molares & Freire 2003). Stalked barnacle harvesters also participate in the enforcement activities personally, in coordination with the surveillance service (Aguión et al. 2022b). Furthermore, the *cofradías* also have the capacity to commercialize the catch as they generally manage the first-sale markets (Molares & Freire 2003), giving them strong economic status.

The stalked barnacle fishery is one of the most important artisanal fisheries in Galicia, from a socio-economic point of view (~1300 harvesters, 333 t and ~9 millions of € per year), although still far from the clam fishery, the largest artisanal fishery in Spain (~7100 fishers, ~7.900 t and ~74 millions of € per year) (Domínguez et al. 2021). Around 80% of the harvesters access the intertidal fishing grounds by boat, and the rest by land (*i.e.* by car, on foot). The latter specialize in harvesting stalked barnacles, but the boat fishers also use other gear during the year, mainly octopus traps, depending on the market. Harvesters actively participate in all aspects of the management and share responsibilities with the administration in decision-making. The key decisions deal with the rotation scheme between fishing beds and the daily individual quotas allowed for each harvester, although the system is very flexible and adaptive to accommodate changes regarding new and unforeseen circumstances. The Galician stalked barnacle fishery has a very strong governance framework, focused on promoting participation by harvesters, which has rendered a very high number of sustainability attributes in a recent European stalked barnacle fisheries review (Aguión et al. 2022b).

In Asturias, there are two very different stalked barnacle fisheries, a co-management system in the west and a top-down system in the east. The former, as in Galicia, is a highly participatory system, based on adaptive management plans and exclusive access rights to the fishing beds (TURF) granted to the *cofradías*, who share responsibilities with the administration in the decision-making (Rivera et al. 2014). In Asturias-West, the eight management plans are subdivided into 250 zones, according to resource quality, and catch monitoring is done at this micro-/patch scale (from single rocks 3 m long up to 3.3 km extents of coastline) (Rivera et al. 2014). Such a detailed spatial scale is only possible due to the close collaboration between harvesters and managers (Rivera et al. 2014). Another key attribute of this fishery is the strong monitoring and control system (MCS) at various scales: (1) the official control and surveillance system from the regional government, (2) the presence of one enforcement officer on each of the cofradías with TURF, who are mainly focused on this fishery and (3) the direct involvement of the stalked barnacle harvesters in the surveillance and control activities (Rivera et al. 2014). Before the early 1990s, stalked barnacles in Asturias were only harvested sporadically, but in 1994, and led by the fisheries administration, a pilot TURF programme started in the *cofradía* of Ortiguera, which was expanded to seven *cofradías* by 2001 (Rivera et al. 2014). The system has received public approval, where 73% of the stakeholders indicated that the only way to maintain a sustainable stalked barnacle fishery in Asturias is through the current management regime (Rivera et al. 2016a). Recently, the TURF system in Asturias has also been found to achieve high sustainability scores (Aguión et al. 2022b), where social factors (e.g. conflict resolution mechanisms and strong leadership) are the key drivers for the sustainability of this bottom-up management system (Rivera et al. 2019).

The other *Pollicipes pollicipes* fishery of Asturias, on the east coast, is a top-down limited-entry system. It has similar management measures (size limit and daily individual quotas), except that the open harvesting period is set from May to September, but with much less involvement of the harvesters in the decision-making, a much broader spatial scale of management and a much weaker MCS (Aguión et al. 2022b). This fishery has much less socio-economic significance (Table 15).

In Cantabria, a small top-down limited-entry system is in place in the stalked barnacle fishery. It is not clear how many fishers are involved, since it is not mandatory for the harvesters to be associated with any *cofradía*, but a regional census was established in 2018 ('*Orden MED/25/2018, Gobierno de Cantabria*'). Since 2016, a daily reporting system requires all catches to go through official landing points so that catch statistics are available (annual landings of 4.6 t and average price of 22 €/kg) (Gorka Bidegain, pers. comm.). The fishery is managed based on three measures: a minimum size, a temporal closure and a spatial harvesting system with areas permanently open, seasonally closed and permanently closed (Bidegain et al. 2015, '*Orden MED/7/2021, Gobierno de Cantabria*'). In 2017, the regional government of Cantabria promoted a pilot co-management plan with some harvesters, but the lack of a united harvesters' association led to failure (Gorka Bidegain, pers. comm.).

Finally, in the Basque Country, a residual stalked barnacle fishery takes place. There are two management plans in Orio and Bakio under a co-management approach, with fewer than 10 harvesters involved and annual catches of only 0.1 t (Aguión et al. 2022b). In the rest of the region, a top-down open access system is in place for the small fishing beds available. The Basque Country is also the only region in Spain where recreational harvesting of stalked barnacles is allowed, although not in the areas of Bakio, Orio and the MPA Biotopo Protegido de San Juan de Gaztelugatxe (Borja et al. 2006b).

Portugal

In Portugal, there is a long tradition of exploiting *Pollicipes pollicipes* by professional and recreational fishers. The fishery is regulated differentially along the Portuguese mainland coast. The first Portuguese legislation relating specifically to this fishery was in 1989, when the '*Reserva Natural da Berlenga*' (called '*Reserva Natural das Berlengas*' after 1998, RNB, an archipelago in the central coast of Portugal) was created. At that time, the *Pollicipes pollicipes* fishery was totally banned in the RNB area. In 2000, the first specific regulation for this fishery in a marine protected area (RNB) was published, together with the first Portuguese general fisheries legislation with specific reference to *Pollicipes pollicipes* (Sousa et al. 2013). After 2000, several changes were made to these professional fishing regulations (RNB, modified in 2011; general legislation modified in 2006 and 2011). Specific regulation for this professional fishery was also created in another marine protected area, the '*Parque Natural do Sudoeste Alentejano e Costa Vicentina*' (PNSACV) in 2006, and modified in 2008 and 2011 (Sousa et al. 2013). Additionally, recreational harvesting with specific reference to *Pollicipes pollicipes* has been regulated since 2006 and changed in 2009, 2011 and 2014 (Cruz et al. 2015c). Consequently, three main *Pollicipes pollicipes* fisheries can be identified in Portugal: RNB and PNSACV, corresponding to two marine protected areas, and the rest of the mainland coast outside the marine protected areas (referred to as Portugal General, Aguión et al. 2022b). A large marine protected area where *Pollicipes pollicipes* harvesting is prohibited is the '*Parque Marinho Professor Luiz Saldanha*', a 38 km area of coast near to Cape Espichel, central Portugal (Sousa et al. 2013). The current management measures for each fishery are summarized in Table 15. They include temporal and spatial closures, individual quotas, minimum size (maximum distance between the carinal and rostral plates in relation to a given catch volume), a ceiling of harvesting licences (in all fisheries) and catch reporting in logbooks (in RNB and PNSACV) (Sousa et al. 2013, Aguión et al. 2022b). In Portugal, and in contrast to Spain, *Pollicipes pollicipes* is harvested both professionally and recreationally, with the exception of RNB and a few small areas in PNSACV, where recreational harvesting is prohibited (Table 15).

Official statistical data from the exploitation of *Pollicipes pollicipes* in Portugal are centralized and managed by the '*Direção Geral de Recursos Naturais, Segurança e Serviços Marítimos*' (DGRM). Most of the catches of this species are sold directly to intermediaries or final consumers, and not in official auctions. Until 2006, the official data did not include catches sold outside of auctions, which meant that the official statistics could not be considered representative of the amounts caught. Since 2006, professional fishers have also been required to report what they sell outside of auctions. Thus, the most recent official estimates are more representative of the professional fishing effort. However, it is known that there are many unreported catches, and there are also no records of the recreational fishing effort. The most recent statistical data available on this fishery in Portugal (2015–2019, unpublished data from DGRM) report an average of 456 licensed professional fishers and 136 t of annual catches (maximum of 146 t in 2016). This corresponds to a mean annual value of €1,622,131 and reveals a slight positive trend in the price of €10.1 kg^{-1} in 2015 to €11.3 kg^{-1} in 2019. Nevertheless, the average first-sale prices charged by fishers, based on surveys conducted in 2013 (and 2018 only for RNB), was higher than these official data, being higher in the RNB than in other fisheries. The variation reported is as follows: RNB, €23.3 kg^{-1} (2013), €28.8 kg^{-1} (2018) (maximum of €173 kg^{-1} in 2013 and €100 kg^{-1} in 2018) ($n=32$ in 2013, $n=39$ in 2018); central coast, €14.4 kg^{-1} (maximum of €70 kg^{-1}) ($n=26$); PNSACV, €13.1 kg^{-1} (maximum of €168 kg^{-1}) ($n=49$) (Cruz et al. 2016b; unpublished observations).

In a study of European *Pollicipes pollicipes* fisheries, of the three main Portuguese fisheries identified (RNB, PNSACV and Portugal General), the RNB fishery showed the highest levels of governance and sustainability attributes (based on Gutiérrez et al. 2011) (Aguión et al. 2022b). RNB was considered a bottom-up harvester-governed fishery at an intermediate sustainability level, while PNSACV and Portugal General scored low in sustainability, despite PNSACV being subjected to bottom-up governance. The rest of Portugal (Portugal General) has governance that was considered to be top-down. The classification of bottom-up versus top-down governance was based on a governance score obtained by summing the levels of four governance elements: spatial scale of management, co-management, access structure and participation of fishers (Aguión et al. 2022b). Several factors contribute to RNB having the highest sustainability classification among Portuguese fisheries: no recreational harvesting, being a marine reserve and being the first area in Portugal with a managed *Pollicipes pollicipes* fishery (Sousa et al. 2013), low accessibility (i.e. it is a group of islands), long-term professional licences granted in this fishery and a constant number of licences through time (Jacinto et al. 2011). Furthermore, several scientific projects and studies, which monitor the state of the resource and the state of management, have the participation of fishers (e.g. Sousa et al. 2013, Cruz et al. 2015c, Sousa et al. 2020, Neves 2021). In the RNB, a higher biomass of stalked barnacles (mid-shore, 7.7 kg/m^2) and a higher proportion of adults with commercial value were observed when compared to other Portuguese fisheries (PNSACV and the central coast, data from 2011, Sousa et al. 2013). Recently, Portuguese commercial fisheries legislation has changed and now includes the possibility of implementing co-management ('*Decreto-Lei n.º 73/2020*'). Consequently, a formal co-management system for the *Pollicipes pollicipes* fishery in RNB was implemented in 2021 ('*Portaria n.º 309/2021*'). This is the first case of co-management of a fishery in Portugal. Consequently, we have classified the management level of this fishery in Table 15 as 'co-management mid-level', but consider that it will probably progress to 'co-management high level' in the very near future.

An assessment of the state of the fishery and the management of *Pollicipes pollicipes* in RNB, PNSACV and the coastal area from Cape Carvoeiro to Cape Raso (in the central coast of Portugal, regulated by Portugal General legislation) was made in 2013 using different approaches (independent observations, enquiries to the fishers and logbook information) (Cruz et al. 2015c). This assessment has not been repeated in the PNSACV or the central coast, but there have been recent monitoring studies in RNB (Sousa et al. 2020, Neves 2021). An overall decline in the state of the fishery and conservation of this resource was observed in all regions in 2013, with the exception of a stable tendency detected in the PNSACV when using the enquiries approach. The worst situation was observed in the central coastal area. Reasons for this include the following: not being part of a marine protected area; less management measures in practice; no specific licences for exploiting barnacles in this area (Cruz et al. 2015c); and the fact that the maximum number of licences available for this coast has not yet been reached ('*Direção Geral de Recursos Naturais, Segurança e Serviços Marítimos – DGRM*' information, 2021). This diagnosis was also identified by Aguión et al. (2022b), where the Portugal General fishery, which includes the central Portuguese coast, was classified as low in sustainability. In the PNSACV, although also scoring low on sustainability (Aguión et al. 2022b), the *Pollicipes pollicipes* fishery is more regulated and prospects are more promising, as there is bottom-up involvement through consultative participation of the fishers in the management of the fishery (Aguión et al. 2022b). Consequently, the management level of the PNSACV was considered as 'incipient co-management' (Table 15). Furthermore, there are several characteristics of this fishery that might favour improvement in the current management and promote the sustainability of this activity, such as the existence of specific professional licences, a constant number of licences over time, and several associations that represent the fishers of this area. Studies conducted in the PNSACV recommend greater involvement of fishers and the local community in the management of *Pollicipes pollicipes* (Castro & Cruz 2009, Stewart et al. 2014, Cruz et al. 2015c, Carvalho et al. 2017). Based on public debates, surveys and information from professional fishers, the main problems of the RNB fishery are poaching and poor surveillance (Sousa et al. 2020, Geiger et al. 2022), while in the PNSACV (Stewart et al. 2014, Cruz et al. 2015c, b, Carvalho et al. 2017) and the central coast (Cruz et al. 2015c, 2016b), there is excessive exploitation, poaching, unorganized harvesting, lack of association and union among fishers, and insufficient surveillance.

Morocco

Of the fisheries that exist in Africa, a regulated fishery of *Pollicipes pollicipes* exists only in Morocco. According to Hakima Zidane from the laboratory '*Prospections des Ressources Littorales*', *Institut National de Recherche Halieutique* (INRH), Morocco (June 2021), the exploitation of this species is not a traditional activity in this country and local consumption of these barnacles is very limited. Boukaici (2015) described this fishery in the Mirleft region, southern Morocco. Hakima Zidane (pers. comm.) added that *Pollicipes pollicipes* is harvested all along the Atlantic coast, namely in Mansouria, Sidi Abed and Souiria Kdima, and that there are no fisheries on the Mediterranean coast. This fishery is regulated by several ministerial decrees (Bourassi et al. 2019), which include the establishment of the following management measures: seasonal closure (exploitation is allowed from 1st November to 31st May and prohibited from 1st June to 31st October), size limit (RC of 2.5 cm, since 2015) and licences for professional fishmongers (Hakima Zidane pers. comm.). Hakima Zidane (pers. comm.) stated that these professionals mainly sell barnacles for export to Spain and Portugal and to a few five-star hotels in the Casablanca region. These professionals hire the services of an intermediary, who in turn sub-contracts the services of several fishers who collect the barnacles (Hakima Zidane pers. comm.). According to Hakima Zidane (pers. comm.), this fishery has increased in the last 10 years. The price charged by fishers at first sale is around €3 to €7 kg^{-1}, depending on the quality and the size of the barnacles, while the price charged by professional fishmongers is, on average, 60–80 DH/kg (~€6–€8 kg^{-1}) and can reach 120 DH per kg (~€12 kg^{-1}). Boukaici (2015) presented photographs of large quantities of barnacles stored in burlap sacks in the intertidal zone of the Bay of Agadir, illustrating the intermediate step of the sales circuit which precedes their export, carried out by professional fishmongers. According to Boukaici (2015) and Hakima Zidane (pers. comm.), poaching is the biggest threat to the fishery of *Pollicipes pollicipes* in Morocco.

Western Sahara and Mauritania

In the Western Sahara and Mauritania, there is indication of disturbance by *Pollicipes pollicipes* fishers at the Cape Blanco Monk Seal Colony (Fernández de Larrinoa & Cedenilla 2003). These fishers descend from the clifftops to harvest the barnacles in the intertidal zone, and although they do not interact negatively with the seals, they do cause disturbance in the locations occupied by these animals. Fernández de Larrinoa & Cedenilla (2003) determined through interviews with these fishers that this activity originated at a time when the territory was still a Spanish colony. At present, Pablo Fernández de Larrinoa (pers. comm.) considers that this unregulated fishery is not important in the Cape Blanco peninsula and that these barnacles are not consumed locally, being sold abroad. According to this researcher, it is currently forbidden to harvest *Pollicipes pollicipes* in the seal reserve.

Senegal

Although Senegal corresponds to the southern limit of distribution of *Pollicipes pollicipes* (see section ‘Geographical distribution’), this species is considered to be an exploited species in this country (‘*Direction des pêches maritimes*’, Senegal, 2002). Although this fishery is not regulated, there are records of the sale of these barnacles in Senegal to foreigners (informal online information in 2021 of the sale at 3000 West African CFA franc per kg (~4.6 euros)).

Pollicipes caboverdensis

In 2010, populations of *Pollicipes* from the Cape Verde Islands were described as a new species, *Pollicipes caboverdensis* (Fernandes et al. 2010). This species, endemic to Cape Verde, is exploited in an unregulated manner, and no statistical data regarding this small-scale artisanal fishery exist (Cruz et al. 2015a, b). Based on personal observations, enquiries to fishers (Baessa 2015, Soares 2018) and contacts with restaurants, we found that this fishery occurs on all Cape Verde Islands, with the exception of the island of Maio, where this activity does not seem to exist.

Two studies have been carried out on the fishery of *Pollicipes caboverdensis* in Cape Verde: on the island of Santiago in the Sotavento Islands (leeward) (Baessa 2015, Cruz et al. 2015a) and on the Barlavento Islands (windward) of Santo Antão, São Vicente and São Nicolau (Soares 2018). According to these studies, this fishery appeared to be more important in São Nicolau, where most of the fishers interviewed considered it to be their main activity and very important for their family income. On the other islands, all, or the vast majority of the fishers interviewed, had other main activities, although they considered the fishery of *Pollicipes caboverdensis* important for their family income. The fishing effort on Santiago was found to be very variable: 4–10 kg of barnacles per day and per fisher; from 3 to 4 days of harvesting per week to 2 days every 3 months (Baessa 2015, Cruz et al. 2015a). On the islands of Santo Antão, São Vicente and São Nicolau, the fishing effort appeared to be higher than that reported for Santiago, although harvesting is apparently restricted between May and October (Soares 2018). In these islands, the amounts harvested per day varied between a maximum of 50 kg when fishers were alone or with a partner, to a maximum of 300 kg when fishers were in a group of 3–4 (Soares 2018). Most of the fishers interviewed sell the barnacles to the general population and tourists, intermediaries (fishmongers) and owners of bars, restaurants and hotels. The selling price varied between 200 CVE/kg (~€2) in Santo Antão (in 2017, Soares 2018) to 1000 CVE/kg (~€9) in Santiago (in 2015, Cruz et al. 2015a) and São Nicolau (in 2017, Soares 2018). Regarding the abundance of *Pollicipes caboverdensis*, the fishers perceived a decline at Santiago (Baessa 2015, Cruz et al. 2015a) and Santo Antão (Soares 2018), most considering that the amount and size of the barnacles had decreased over time ($n = 12$ enquiries performed in 2014 in Santiago and $n = 7$ enquiries performed in 2017 in Santo Antão). On the contrary, the vast majority of fishers from São Vicente and São Nicolau had a positive perception regarding the state of the resource, considering that the quantity of *P. caboverdensis* has increased or remained similar and that their size has not decreased ($n = 9$ enquiries performed in São Vicente and n = 4 enquiries performed in São Nicolau, both in 2017) (Soares 2018). Considering that *Pollicipes caboverdensis* is an endemic species of Cape Verde, and that there is little knowledge of its fishery, biology and ecology, this species should be considered for special status with regard to its conservation, with further studies recommended.

Integrative summary and critical view

When comparing *Pollicipes*’ fisheries worldwide, a clear contrast shows up among countries. In Spain, Portugal, France and Canada, co-management systems (with different degrees of fisher participation) are in place or in development, mostly under limited-entry systems, as well as under TURF systems (Galicia and Asturias in Spain), although some top-down systems still exist (e.g. in Portugal). In the USA, there is a top-down system, but apparently there is not much interest in harvesting *Pollicipes*. In Morocco, Baja California-Mexico and Peru, top-down approaches are the norm. Fisheries are still mostly unregulated in many countries (Western Sahara & Mauritania, Senegal, Cape Verde, Guerrero-Mexico, Costa Rica and Ecuador). In Canadian and in some European fisheries, the focus has been on the social component of the fishery promoting the bottom-up participation of the harvesters in decision-making, by empowering the fishers’ associations. By contrast, in Latin American countries (Peru and Mexico) and Morocco, the focus has been more top-down, with scientific institutions (IMARPE in Perú, INAPESCA in Mexico and INRH in Morocco) conducting stock assessments on which to base the management of the fishery. In this stock assessment-driven approach, fisheries management depends on monitoring the stock status, through periodic direct assessment, which has been found to be unsuitable for most small-scale fisheries in developing countries (see Berkes et al. 2001 for a review). Moreover, the tendency for managers of fisheries of small stocks in developing countries to believe that stock assessment is essential for successful fishery management often leads to disproportionate allocation of resources to stock assessment, rather than other critical components of management (Mahon 1997). More focus should be placed on top-down and incipient co-management fisheries on the social component, mainly by strengthening and empowering the associations of fishers, and promoting their participation in the MCSs and the decision-making. The case of Islas Lobos in Peru should be carefully analysed. It has been closed since 2007, but the stalked barnacle populations are still depleted. MCSs have to be strengthened, incorporating the role of the fishers as stewards of their marine resources. The analysis of the European *Pollicipes pollicipes* fisheries clearly shows that strong governance frameworks, in the form of exclusive property rights, nested spatial scales of management, fishers’ participation and co-management, all promote sustainability. By reinforcing the social dimension, the system achieves both socio-economic and ecological sustainability (Aguión et al. 2022b). However, independent observations about the state of these resources, as well as on the ecological impacts of harvesting on biodiversity, are needed. The development of local markets for stalked barnacles, and a new wave cuisine that exalts the local products (e.g. Peru, Mexico and the USA) could help foster new co-management systems around this fishery in Latin America. In Peru, where there was an important export-driven stalked barnacle fishery in the past, a new law, approved in July 2021 for benthic marine invertebrates (‘*Decreto Supremo 018-2021-PRODUCE*’), sets a new framework and opportunity to develop co-managed plans with the active participation of the local fishing communities. In NW Africa, in both the endemic *Pollicipes caboverdensis* fishery and the *P. pollicipes* fishery, more steps have to be taken in order to start with a simple regulation of the fishery and the empowerment of the fishers associations, which we hope to see in a future review of the genus *Pollicipes*.

Aquaculture

In Barnes’ (1996) review, there was no section devoted to the aquaculture of *Pollicipes* species. However, in the subsection “*Pollicipes pollicipes*” under “Commercial exploitation”, Barnes (1996) stated that there was “…a great desire in Spain, and in Portugal particularly, to encourage the cultivation of *P. pollicipes*…”. López et al. (2010, 2012) presented a list of barnacles with commercial value, including *Pollicipes* species, and discussed the potential for and challenges of their cultivation. *Pollicipes* species have several features that give them high interest and potential for aquaculture. They are harvested species that can fetch a high price (see section ‘Fisheries, management and conservation’). They occur on very wave-exposed shores, where exploitation activity is dangerous and can lead to damage to the rest of the community on rocky shores, prompting conservation concerns. They are low trophic level species with low energy requirements. They can potentially be cultured in extensive systems in an environmentally sustainable way.

In the 25 years since Barnes (1996), there are no known cases of successful commercial aquaculture of *Pollicipes* species. However, several research projects and development trials have attempted cultivation, particularly of *Pollicipes pollicipes* (e.g. Goldberg 1984, Norton 1996, Franco 2014, Franco et al. 2015, 2016, 2017, Bingham et al., 2017, Darras 2017, Belela 2018, Fernandes 2018, Romersa 2018, Torres 2018, Santos 2019) (see Table 16). The aim of this section is to summarize the current state of these efforts.

The following types of potential production of *Pollicipes* species can be identified: (1) production based on collection of wild cyprids/juveniles and subsequent transfer to extensive systems of grow-out of juveniles and adults; (2) production based on collection of wild cyprids/juveniles and subsequent transfer to semi-intensive or intensive systems of grow-out of juveniles and adults; (3) production based on collection of egg lamellae from wild adults, followed by production phases in an intensive system (larval rearing, settlement and early juvenile rearing) and subsequent transfer to systems of grow-out of juveniles and adults; and (4) production based on reproduction by adults in captivity – with initial production phases in an intensive system (broodstock conditioning, larval release, larval rearing, settlement and early juvenile rearing) followed by transfer to systems for grow-out of juveniles and adults. The main results achieved to date and the main problems and challenges for each type of production and production phase are summarized in Table 16.

The first attempt to cultivate *Pollicipes* species was in Santander (Spain) in the 1980s with *P. pollicipes* (Goldberg 1984). This was based on the collection of wild juveniles (pieces of rock with barnacles) and subsequent transfer to an extensive system of grow-out of juveniles and adults, suspended on a floating platform. Besides the impact of destruction of the natural habitat, this trial was neither successful (75% of the structures were lost), nor had any follow-up. The current only known case of aquaculture of a barnacle species (giant barnacle, ‘*picoroco*’, *Austromegabalanus psittacus,* Chile) uses the same production method (López et al. 2010, 2012). Artificial substrata in the wild are used for larval settlement and metamorphosis of *Austromegabalanus psittacus*, followed by a grow-out system of juveniles in suspension (long lines) (López et al. 2010, 2012). According to these authors, the productivity of such farming systems is very high in relation to the productivity from the artisanal fishery of this resource, but there is spatial and temporal variability and, consequently,

Table 16 Aquaculture of *Pollicipes* species

Species/location or laboratory conditions/ production type and production phase		Methods and main achievements	Problems and challenges	References
Pollicipes polymerus				
Laboratory conditions, the USA	(2) Production based on wild cyprids/juveniles and transfer to semi-intensive systems *Collection of wild barnacles* *Rearing of juveniles in a semi-intensive system*	Collection of wild barnacles off the University of Oregon Institute of Marine Biology boathouse breakwater. Development of a novel aquaculture system with vertical tubes, low flow and air supply. Low cost. Use of unfiltered seawater. *P. polymerus* glued to plates. Effect of type of diet (no additional food, microalgae, *Artemia*) on growth. High survival in general. Highest growth in *Artemia* treatment. Settlement and recruitment during the experiment. Success of cultivation despite the low water velocity used. Barnacles can be stimulated to feed using aeration.	Need for research on dietary enhancement and for increasing the barnacle capacity of the system.	Bingham et al. (2017)
Laboratory conditions, the USA	(2) Production based on wild cyprids/juveniles and transfer to semi-intensive systems *Collection of wild barnacles* *Rearing of juveniles in a semi-intensive system*	Collection of wild barnacles at Drakes Point, South Cove, Oregon, the USA. Development of a novel infrastructure to cultivate *Pollicipes*: vertical aquaculture tanks and flow system based on ABS (acrylonitrile butadiene styrene) tubes (ABS tanks, adapted from Bingham et al. 2017). Low cost of ABS tanks. Effect of type of diet (cultured rotifers and seafood industrial by-product and waste material) on growth. No consistent effect of diet on growth. Settlement and recruitment on cultivated barnacles. Low cost of ABS tanks and of feeding. Use of upcycled fish is promising to enable sustainable aquaculture.	Development of more efficient infrastructure to increase feeding time.	Romersa (2018)
Oregon, the USA	(1) Production based on wild cyprids/juveniles and transfer to extensive systems *Collection of wild barnacles* *Extensive system of grow-out of juveniles*	Collection of wild barnacles at Drakes Point, South Cove, Oregon, the USA. Use of ABS tanks adapted to be moored to docks in Charleston Boat Basin, Oregon. 29% survival after 2.5 months. No increment of size/no growth.	Lack of water movement. Feeding limitation. Potential predation. Fouling problems, namely of air flow supplementary system. Need for scaling up air flow. ABS tanks are a unique system specific to *P. polymerus*, not ideal for a more complex farming system. Need to investigate the polyculture (e.g. abalone, mussels) potential of *P. polymerus* in a well-aerated system.	Romersa (2018)

(*Continued*)

Table 16 (*Continued*) Aquaculture of *Pollicipes* species

Species/location or laboratory conditions/ production type and production phase		Methods and main achievements	Problems and challenges	References
Pollicipes pollicipes				
Santander, Spain	(1) Production based on wild cyprids/juveniles and transfer to extensive systems *Collection of wild barnacles and extensive system of grow-out of juveniles and adults*	Pieces of rock with barnacles transplanted to an extensive system of grow-out of juveniles and adults (floating platform with ropes, *Baía de Santander)*. Higher growth rates in the floating system than in the intertidal zone.	Destruction of the natural habitat. Loss of 3 of the 4 cultivation structures due to vandalism, accident or rough sea.	Goldberg (1984)
Sines, SW Portugal	(1) Production based on wild cyprids/juveniles and transfer to extensive systems *Collection of wild juveniles*	Development of a device (‘barticle’) and process for fixation of larvae and growth of juveniles. European Patent. Choice of mid-shore as the most suitable intertidal level for the deployment of ‘barticles’. Optimization of the method of deployment. Collection of wild juveniles attached to ‘barticles’ (Cape of Sines, SW Portugal).		Patent nr. EP3372073B1, Fernandes (2018)
	Extensive system of grow-out of juveniles	Development of an extensive system of grow-out of juveniles: use of a floating platform in a less exposed area (Port of Sines). Plates within individual cages chosen as the best option for suspension in the floating platform. Optimization of the transfer method. High rates of survival and growth after transfer of ‘barticles’/barnacles to floating platform during first months (winter).	Fouling problems. Total mortality of barnacles after one year.	Fernandes (2018)
		Test of several anti-fouling methods (e.g. manual cleaning, water jet). Survival after one year after the transfer of ‘barticles’/ barnacles to floating platform. Biological viability with the application of anti-fouling methods.	Do not reach commercial size after one year. Quality of barnacles not measured. Not economically viable (e.g. human resources costs). Need for alternative anti-fouling methods.	Darras (2017), Belela (2018), unpublished data

(*Continued*)

Table 16 (*Continued*) Aquaculture of *Pollicipes* species

Species/location or laboratory conditions/ production type and production phase		Methods and main achievements	Problems and challenges	References
Laboratory conditions, the UK	(2) Production based on wild cyprids/juveniles and transfer to intensive systems *Collection of wild juveniles* *Rearing of juveniles in an intensive system*	Collection of wild animals in SW Portugal. Individuals were glued to Velcro that was stuck to the inside walls of a plastic container. Effect of type of diet (several species of microalgae, *Artemia*, rotifers) on ingestion rate, growth and biochemical variables. Highest growth rates, faecal production, food capture efficiency and energy and digestion efficiency when barnacles are fed with *Artemia*. Protein digestion is high.	Possibility to culture *P. pollicipes* with several artificial diets. Need for research into lipid and carbohydrate digestion to aid diet formulation. Fouling problems and fungal infection indicate that culturing in tanks is not ideal. Bottleneck of settlement to sustainable farming.	Norton (1996)
Laboratory conditions, Spain	(2) Production based on wild cyprids/juveniles and transfer to intensive systems *Collection of wild juveniles* *Rearing of juveniles in an intensive system*	Collection of wild animals in Galicia, Spain. Preliminary experiments on rearing juveniles and adults and on effects of diet on feeding behaviour and growth. Acceptability of prey higher for live foods (*Artemia*). Inert diets not consumed. Turbulent conditions essential to stimulate feeding.	Need for control of culture conditions.	Cribeiro (2007)
Laboratory conditions, the UK	(2) Production based on wild cyprids/juveniles and transfer to intensive systems *Collection of wild juveniles* *Rearing of juveniles in an intensive system*	Collection of wild juveniles attached to pieces of rock from Cape Sardão, SW Portugal. Experiments in aquaria. Effects of different conditions of feeding, temperature, tidal cycle and photoperiod on growth, survival and external morphology. High survival rates. Food quantity variation and photoperiod did not affect growth. Higher growth was observed in individuals kept without a tidal cycle. Higher growth at 15°C than at 10°C or 20°C.	Changes in *P. pollicipes* juvenile morphology during growth in intensive system (plate colouration, deformation and decalcification).	Franco (2014)
	Finishing in an extensive system of grow-out of juveniles	Transfer of juveniles cultured in the intensive system (see above) to the wild (pieces of rock with the cultivated juveniles glued to rock, intertidal of Cape Sardão, SW Portugal). Control barnacles. Monitoring after 6 months. Low survival of transferred juveniles. Reversal of morphological changes.	High mortality of transferred juveniles. Need for the development of extensive systems of grow-out of juveniles (onshore or suspended culture systems).	Franco (2014)

(*Continued*)

Table 16 (*Continued*) Aquaculture of *Pollicipes* species

Species/location or laboratory conditions/ production type and production phase		Methods and main achievements	Problems and challenges	References
Laboratory conditions, Portugal	(2) Production based on wild cyprids/juveniles and transfer to intensive systems *Collection of wild juveniles* *Rearing of juveniles in an intensive system*	Collection of wild juveniles attached to 'barticles' deployed at Cape of Sines, SW Portugal. Experiments in aquaria. Effect of hydrodynamic conditions, food type, food density and frequency of feeding on survival, size and growth. High rates of survival. No effect of factors on survival. Growth rate is higher in more hydrodynamic conditions. Highest growth rates when barnacles are daily fed with *Artemia*.	Production costs (e.g. feeding) are potentially high. Quality of barnacles not measured.	Torres (2018), Santos (2019), unpublished data
Laboratory conditions, the UK	(3) Production based on wild egg lamellae *Collection of wild egg lamellae from adults* *Larval rearing*	Mature egg lamellae taken from adults collected at SW Portugal. Experiments in aquaria. Preliminary experiments of the effect of diet on larval development, survival and ingestion rate. Indication of preferred diets.	Poor knowledge on the effects of environmental conditions in larval development. No cultivation to cyprid stage.	Candeias (2005)
Laboratory conditions, the UK	(3) Production based on wild egg lamellae *Collection of wild egg lamellae from adults* *Larval rearing*	Mature egg lamellae taken from adults collected at Cape Sardão, SW Portugal. Experiments in aquaria. Effects of temperature (11°C–24°C), diet, photoperiod and salinity on larval development and survival. No salinity effect. Best growth and survival using rearing temperatures of 15°C–20°C, daily feeding with *Tetraselmis suecica*/*Skeletonema marinoi* or *Isochrysis galbana*/*S. marinoi* and a photoperiod of 24:0L:D. High-quality cyprids were obtained.	No cultivation to settlement. Need to investigate the implications of larval culture conditions (e.g. density) towards larval quality, competence to settle and ability to complete metamorphosis. Use of recirculating systems should be examined.	Franco (2014), Franco et al. (2017)

(*Continued*)

Table 16 (*Continued*) Aquaculture of *Pollicipes* species

Species/location or laboratory conditions/ production type and production phase		Methods and main achievements	Problems and challenges	References
Laboratory conditions, the UK	(3) Production based on wild egg lamellae *Collection of wild egg lamellae from adults* *Larval rearing and settlement*	Mature egg lamellae taken from adults collected at Cape Sardão, SW Portugal. Cultivation of larvae until cyprid stage. Design and use of a new device to study the settlement of cyprids in the presence of an individual conspecific adult. Effects of hydrodynamics, temperature, light, salinity, larval age and batch on % of attached cyprids, % of metamorphosed cyprids and survival of cyprids. Maximum attachment on adults of 30%–35%, with a one-week metamorphosis rate of 70%–80%. Cyprid attachment stimulated by light conditions and circulating water. Temperature did not affect attachment rate. Metamorphosis rate decreased at 14°C (compared with 17°C or 20°C). Higher settlement rates than in previous studies. Cyprids survived (in the absence of settlement substrata) for prolonged periods (20 days; 40% survival). First laboratory investigation about factors affecting settlement of *P. pollicipes*.	Need of further research on the importance of substrata-related biotic and abiotic factors on settlement, namely the relative importance of settlement-inducing protein complex (SIPC). Need for further observations on the maximum survival duration of cyprids in the absence of settlement substrata. Settlement phase remains the largest limitation to culture.	Franco (2014), Franco et al. (2016)
Laboratory conditions (the UK)	(4) Production based on reproduction by adults in captivity *Collection of wild adults* *Broodstock conditioning*	Barnacles collected at Cape Sardão, SW Portugal. Experiments in aquaria. Effects of rearing temperature (16°C–24°C) on reproductive condition (% of barnacles with eggs, egg lamellae development, larval release, nauplius size and survival over 24 h). Increasing temperature treatment led to greater maturation of egg lamellae and higher % of barnacles with eggs. Low nauplius release daily, but production of continuous supply of nauplius during most of the experiment. First working protocol for *P. pollicipes* reproduction and larval collection in laboratory conditions.	Need for further optimization of cultivation protocol and study of the effects of food quality (including alternative diets) and photoperiod. Need for development of larval release induction protocols.	Franco (2014), Franco et al. (2015)

Summary of main achievements, problems and challenges in the context of aquaculture of four production types: (1) production based on collection of wild cyprids/juveniles and subsequent transfer to extensive systems of grow-out of juveniles and adults; (2) production based on collection of wild cyprids/juveniles and subsequent transfer to semi-intensive or intensive systems of grow-out of juveniles and adults; (3) production based on collection of egg lamellae from wild adults followed by production phases in an intensive system (larval rearing, settlement and early juvenile rearing) and subsequent transfer to systems of grow-out of juveniles and adults; (4) production based on reproduction by adults in captivity – with initial production phases in an intensive system (broodstock conditioning, larval release, larval rearing, settlement and early juvenile rearing) followed by transfer to systems for grow-out of juveniles and adults. Information on larval rearing is only provided in an aquaculture context (more information in section ‘Larval phase and metamorphosis’).

a certain degree of unpredictability of production. In the Azores, experimental studies on the settlement of *Megabalanus azoricus* (‘*craca*’) on artificial substrata were successful, and the species has been considered as having high potential for aquaculture (Pham et al. 2011).

Regarding the aquaculture of *Pollicipes* species (Table 16), the studies and progress can be divided into three lines of research: (1) projects with *P. polymerus* mostly devoted to the development of cultivation infrastructure that address the challenge of cultivating a species described as having high water flow requirements (high energy, high costs) (Bingham et al. 2017, Romersa 2018); (2) projects with *P. pollicipes* in intensive systems, motivated by the limited knowledge of the cultivation of *Pollicipes*, to optimize production in captivity, and to investigate fundamental production phases such as larval cultivation and settlement (e.g. Norton 1996, Franco 2014, Franco et al. 2015, 2016, 2017); projects with *P. pollicipes* initiated with the discovery of a device (‘barticle’) and process for settlement of larvae and growth of juveniles (European Patent nr. EP3372073B) (Figure 15), which resolved the major bottleneck of finding an artificial substrate where cyprids

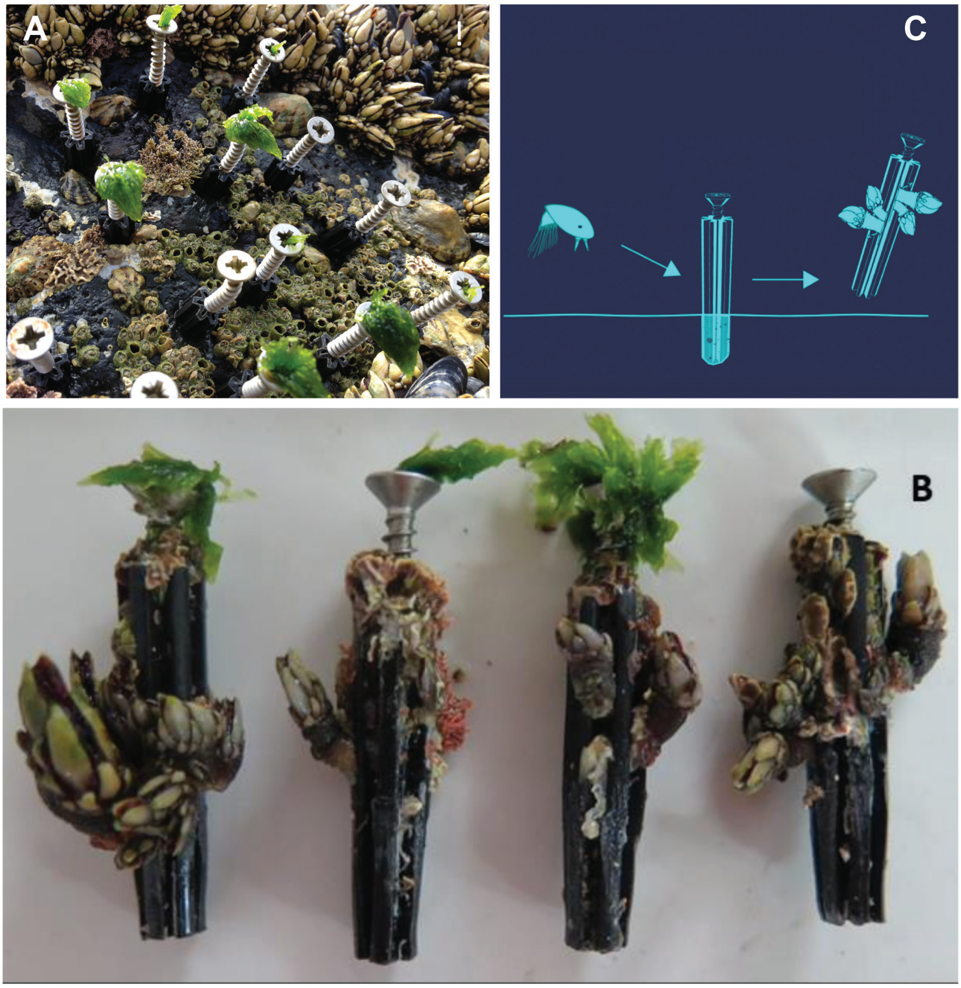


Figure 15 Artificial substrate designated as ‘barticle’ used to collect cyprids and juveniles *of Pollicipes pollicipes* in SW Portugal. (A) ‘Barticles’ deployed in the intertidal where *P. pollicipes* naturally occurs. (B) ‘Barticles’ taken from the field with juveniles attached. (C) Illustration of the process of settlement on ‘barticles’ and collection of juveniles.

in the wild would settle, and which was easy to deploy and remove (Darras 2017, Belela 2018, Fernandes 2018, Torres 2018, Santos 2019).

From the summary in Table 16, and as Barnes (1996) predicted, considerable progress has been made during the last 25 years in the development of aquaculture of *Pollicipes* species. However, there are still several problems and challenges, such as fouling problems in extensive systems of grow-out of juveniles and adults. These problems, the new bottleneck of this type of production, will have to be solved by alternative anti-fouling methods that are environmentally friendly and low cost. The biological viability of the system tested in SW Portugal was partially achieved, but economic viability was not, due to the high personnel costs associated with the anti-fouling methods applied (Cruz et al. 2020). In theory, an extensive farming system is expected to have the lowest costs. However, the extensive experimental system tested was found to be non-viable. Another important challenge is to resolve the bottleneck of the larval settlement and metamorphosis phases in intensive cultivation systems. Progress has been made, but this phase still needs to be investigated and optimized (Franco et al. 2016). Diversifying aquaculture and ensuring that aquaculture is environmentally sustainable are global challenges. The next 25 years will show whether we can overcome the old and new barriers to the cultivation of *Pollicipes* species.

Pollicipes in local cultures

In addition to the socio-economic importance that *Pollicipes* species have worldwide, due to their exploitation and consumption (provisioning services), these barnacles also have been extensively used culturally. They provide iconic inspiration for cultural heritage in various ways: gastronomy, painting, sculpture, photography, graphic and fashion design, handicrafts, and local festivals dedicated to *Pollicipes pollicipes* (e.g. in Aguiño and Corme, both in Galicia, Spain; in Vila do Bispo and Aljezur, both in Algarve, Portugal).

In Figure 16, we show some examples of these uses and representations: a traditional alcoholic beverage from Cape Verde (‘*grogue*’), made with *Pollicipes caboverdensis* that is locally considered to have aphrodisiac properties (Figure 16A); in jewellery, such as the silver earrings shown in Figure 16B; a painting used on a stamp from Senegal (Figure 16C, note that the taxonomy is not that currently used); diverse forms of handicrafts, such as a *P. pollicipes* princess created by a Portuguese artisan (Figure 16D); and public sculptures in several localities in SW Portugal (Rogil and Carrapateira, both in Algarve) and Spain (Aguiño, Galicia, Figure 16E). These demonstrate the important cultural services provided by *Pollicipes* around the world.

Research gaps and challenges

In each section, we identified the main research gaps and challenges regarding the genus *Pollicipes*. Herein, we summarize and integrate this information.

Considering the imbalance of the current knowledge of the four known *Pollicipes* species, it is important to study the basic aspects of the biology and ecology of the less studied species: *Pollicipes elegans* and *P. caboverdensis*. Information is also needed on the fishing effort associated with these resources. In the case of *Pollicipes caboverdensis*, there is no official information on this fishery, and this is also lacking for *P. elegans* in various regions and countries (e.g. Guerrero (Mexico), Costa Rica, Ecuador). Considering that *Pollicipes caboverdensis* is an endemic species of Cape Verde, this species should be considered for designation of special conservation status.

Despite the various molecular-level studies of the relationships of the species within *Pollicipes* that have been developed over the last 25 years, the topology of a phylogenetic tree for living *Pollicipes* will continue to elude us until additional molecular-level data can be obtained. Future research, using additional genes, may yield data more suitable to resolving the various different branch patterns that have been detected.

Figure 16 Examples of uses and representations of *Pollicipes* in local cultures: (Top left) traditional alcoholic beverage from Cape Verde (‘grogue’) with *Pollicipes caboverdensis*. Photo by Correia PM; Public Domain; (Top right) silver earrings by Tânia Gil (© Tânia Gil Jewelry, Portugal); (Middle left) stamp from Senegal. Photo by Wim Decock; (Middle right) handmade doll by Paula Estorninho (Portugal); (Bottom) public sculptures in Aguiño (Spain). Photo by Carmela Queijeiro, La Voz de Galicia.

Based on the new map of *Pollicipes* species worldwide produced in this review, observations should be made on the exposed rocky coastlines of regions where there are doubts, or where more recent observations are needed regarding the presence of these species e.g. Aleutian Archipelago and Pacific coast of Baja California for *P. polymerus*; Pacific coast of Baja California, coast of Nicaragua and central and southern coast of Peru for *P. elegans*; and British Isles, south Mediterranean coast of Spain and France and north Mediterranean coast of Africa, from Algiers eastwards, for *P. pollicipes*. The most intriguing question is whether there is overlap in the distributions of *Pollicipes polymerus* and *P. elegans* in Baja California. Furthermore, the dynamic nature of the southern limit of *Pollicipes elegans* merits future investigation in the context of global change.

Regarding population genetics, an effort should be directed to sampling potential phylogeographic discontinuity regions (e.g. Mexican Pacific coast for *Pollicipes polymerus*, Mediterranean coast for *P. pollicipes*) and to the development of new molecular markers in *Pollicipes* species. Cross-amplification of microsatellite markers, already developed for *Pollicipes pollicipes* and *P. elegans* in congeneric species, should be investigated, together with the development of new microsatellites for *P. polymerus* and *P. caboverdensis*. Moreover, the development of SNP loci for all *Pollicipes* species should be addressed in the future. Finally, there is a need to clarify the patterns of genetic structure of *Pollicipes pollicipes* across the north-east Atlantic.

Pollicipes species appear to be mostly intertidal, although there are references to their occurrence in the shallow subtidal zone. However, actual measurements on the maximum depth of occurrence of *Pollicipes* species are rare, due to difficulties associated with sampling the shallow subtidal zone of extremely exposed shores by snorkelling or diving. Quantitative population assessments of abundance for species of *Pollicipes* are available, but have been undertaken with a diversity of objectives and methodologies, hindering the combined analysis of data from multiple studies and precluding interspecific comparisons. Classical approaches, using a standardized protocol, or using new technology-based methods (e.g. drones), are needed to obtain multiple abundance estimates at relevant scales, important for stock assessment, management and conservation, as well as for allowing adequate intra- and interspecific comparisons.

The four species of *Pollicipes* can be identified on the basis of diagnostic features of the capitular plates and peduncular scales. However, the species also show considerable phenotypic variation in colour and peduncular length. Variations in peduncular length, and of water content, are associated with variations regarding the condition and food quality of the barnacles, (e.g. in *Pollicipes pollicipes*, longer peduncles, higher water content, lower quality). More research is needed to understand and disentangle the factors responsible for this phenotypic variation.

In the last 25 years, less importance has been given to the study of functional morphology and physiology of *Pollicipes* species than in the past. We still do not know the basic aspects of the physiology of these species, given the extreme habitats and very exposed shores on which they live. Regarding functional morphology, exceptions have been studies on cirral morphology and feeding of both *Pollicipes polymerus* and *P. pollicipes*, and on adhesion and the cement of *P. pollicipes*. Considering that cirral morphology reflects adaptations to the various feeding modes that have emerged throughout barnacle evolution, it would be very interesting to compare, in detail, the cirral morphology within *Pollicipes*. Moreover, the hypothesis that there is a juvenile-to-adult shift in feeding strategies, from cirral beating to cirral extension, in *Pollicipes* (originally suggested for *P. polymerus*) needs further investigation, namely whether it also applies *to P. pollicipes*, as there are contradictory observations. Additional adhesive proteins remain to be discovered in *Pollicipes pollicipes*, and the detection of a range of enzymes that are active at the adhesive interface in acorn barnacle species also makes this an important direction for future research. The recent publication of the genomes of acorn barnacle species and of *Pollicipes pollicipes* will make the task of adhesive protein discovery and characterization easier in future.

The major research gaps to determine and investigate life-history traits of *Pollicipes* species are listed as follows: (1) investigate whether the newly described mode of fertilization in *P. polymerus*, sperm-cast mating, is an active process; (2) investigate the occurrence of sperm-cast mating in other *Pollicipes* species; (3) investigate whether self-fertilization or partial self-fertilization occurs in *Pollicipes* species; (4) update information on the breeding patterns of *P. polymerus* in southern California and Baja California, taking into account that observations were made more than 40 years ago and that reproductive phenology might alter with climate change; (5) describe the breeding patterns of *P. pollicipes* populations to the south of Portugal; (6) estimate embryo development time (from oviposition to release); (7) describe the patterns of variation in fecundity in *Pollicipes;* (8) investigate the contrasting patterns of vertical distribution of cyprids of *P. polymerus* observed in California (more on the surface or nearer to the bottom); (9) describe the temporal and spatial patterns of distribution of cyprids of *P. pollicipes*; (10) develop and apply biophysical models of larval dispersal to estimate larval dispersal; (11) test specific hypotheses about preferential settlement of cyprids of *Pollicipes* species on adult peduncles; (12) describe and investigate the patterns and processes of spatial and temporal variation of recruitment of *Pollicipes* species at different scales; (13) understand the relative importance of settlement and post-settlement processes (especially intraspecific competition and predation) in the recruitment to adult (and exploitable) populations; and (14) unravel the processes causing the high intraspecific variability in growth rate observed in *Pollicipes* species.

All *Pollicipes* species are exploited throughout their geographic distributions. However, knowledge about these fisheries is uneven and more information is needed from several fisheries (e.g. Mexico, Ecuador, Morocco and Cape Verde). Additionally, there are fisheries that remain largely unregulated and unreported (e.g. Ecuador and Cape Verde), contrasting strongly to those managed through high-level, co-management systems (British Columbia, Canada, and Galicia and Asturias, Spain). More focus should be placed on the social component of top-down and incipient co-management fisheries, mainly by strengthening and empowering the associations of fishers, and promoting their participation in MCSs and decision-making. Moreover, independent observations regarding the state of these resources are needed, as well as on the ecological impacts of harvesting on biodiversity. These could benefit from studies within marine protected areas. Finally, further studies are needed on the use of geochemical structure as a potential tool to discriminate harvesting sites and *Pollicipes* populations. This approach has potential implications for the management of *Pollicipes* fisheries, enforcement of conservation policies, and labelling.

Despite the considerable progress made in developing the cultivation of *Pollicipes* species, there are two main challenges: to develop alternative anti-fouling methods, which are environmentally friendly and low cost, in extensive systems of production of *Pollicipes*; and to resolve the bottleneck of the larval settlement and metamorphosis phase in intensive cultivation systems.

While much has been done since Barnes (1996), more still needs to be done.

Concluding remarks

After the review of *Pollicipes* by Barnes (1996), a new species, *Pollicipes caboverdensis*, was described, joining the three previously extant species (*Pollicipes polymerus*, *Pollicipes elegans* and *Pollicipes pollicipes*). Knowledge about *Pollicipes caboverdensis*, endemic to the Cape Verde Islands, is scarce. This is also the case for *Pollicipes elegans*, which inhabits the tropical eastern Pacific Ocean. Most of the research conducted on *Pollicipes* is based on *P. polymerus* (north-eastern Pacific Ocean) and *P. pollicipes* (north-eastern Atlantic Ocean). We have built a new georeferenced map of the worldwide distribution of *Pollicipes* species. Twenty-five years after Barnes' (1996) review, we know that all *Pollicipes* species are harvested throughout their geographic distributions, with varying levels of intensity. There is archaeological evidence for their use in prehistoric times and their record in shell middens. The oldest record, assigned to *Pollicipes pollicipes,* is from the Upper Palaeolithic, but most findings are from archaeological sites used during the Mesolithic and/or Neolithic, in the Iberian Peninsula and Northern Africa. At the present time, the most intensively exploited species is *Pollicipes pollicipes* in Portugal and Spain. Some fisheries are mainly unregulated and unreported (e.g. Mexico, Costa Rica, Ecuador, Cape Verde), while others are subject to high levels of co-management (e.g. Galicia and Asturias in Spain). Although we have more information on these fisheries, we still lack information on the real fishing effort applied to these resources. Poaching is a common problem across several fisheries. Strengthening and empowering the associations of fishers, to promote their participation in monitoring, in control systems and in decision-making, is highly recommended.

All living *Pollicipes* species are considered to represent relict elements of a Tethys Sea fauna, which became restricted to the eastern boundary conditions of the Atlantic and Pacific Oceans. The present biogeography of the living species, in conjunction with fossil records and coupled with molecular phylogenetics, supports a hypothesis of radiation of pollicipedine species during the Tethys Sea era. Currently, all *Pollicipes* species are placed in a new order, the Pollicipedomorpha Chan et al. 2021. Population genetics is a new area of knowledge in development since Barnes' (1996) review, and considerable progress has been made, with genetic studies leading to the discovery of the new species *Pollicipes caboverdensis*.

Since the extensive review by Barnes (1996) of the functional morphology of *Pollicipes* species, there has been little further development in the last 25 years. However, structural studies of the adhesive interface in *Pollicipes* are an important exception, highlighted in the present review. Although many gaps remain, much progress (e.g. description of adhesive proteins) has been made in understanding the mode of adhesion, namely of *Pollicipes pollicipes*.

Pollicipes species are mostly intertidal and inhabit very exposed shores. *Pollicipes polymerus* is perhaps the most opportunistic species, being found on the greater diversity of substrata. We have made an extensive review of the biological assemblages associated with each *Pollicipes* species. In the last 25 years, our knowledge of life history has increased considerably, particularly for *Pollicipes pollicipes*. All species are hermaphroditic. There is now evidences of pseudo-copulation in *Pollicipes,* which was not available at the time of Barnes' (1996) review. The most surprising advances relate to the study of reproduction in *Pollicipes polymerus*, as two main aspects challenge the accepted wisdom of barnacle reproductive biology: (1) evidence of a novel mode of fertilization, sperm-cast mating, where sperm, released into the water by males, fertilize eggs retained in the body of a female, and (2) the observation of occasional reciprocal copulation, previously never observed in barnacles. Breeding and recruitment patterns were extensively reviewed. The description of recruitment and reproductive phenology are particularly important in the context of climate change. Our knowledge of patterns and processes of growth in *Pollicipes* has progressed considerably due to the use of calcein marking techniques. Although much remains to be studied, there is growing evidence of the importance that post-settlement processes might play in the distribution and abundance of *Pollicipes* species (e.g. the role of species interactions) and in the recruitment (e.g. the role of intraspecific competition and predation) to adult (and exploitable) populations.

In the 25 years since Barnes (1996), there are no known cases of commercially successful aquaculture with *Pollicipes* species. However, several research projects and development trials have attempted to cultivate *Pollicipes* species. The most relevant advance was the discovery of a device ('barticle') and process for the settlement of larvae and the growth of juveniles of *Pollicipes pollicipes* (European Patent nr. EP3372073B1). This solved the major bottleneck of the quest for an artificial substrate, where cyprids could settle in the wild, and which could be easily deployed and removed. This discovery opens the possibility of developing extensive and sustainable cultivation systems and complementary experiments of optimal conditions in the laboratory.

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Nearly 30 years ago, William (Bill) Newman advised Margaret Barnes that Teresa Cruz was studying *Pollicipes* in Portugal. At that time, Margaret was gathering material for her 1996 review.

She wrote to Teresa Cruz asking for information – “I was talking to Dr Newman (Scripps) recently and he mentioned your possible work on the cirripeds of the Portuguese coast…”, “…I think there are so few people in Europe working on cirripedes that it is good to know one who is interested. I also think *Pollicipes* is an intriguing genus and I often wish I had access to living material on a regular basis…” (letters from Margaret, 1992). This was a time when information for a review article came by letter and with a different speed – “…My *Pollicipes* notes go very slowly; there has been so much editing to do recently…” (letter to Teresa Cruz from Margaret, 1994).

In addition to the collective gratitude we have for the role Margaret played in the study of cirripedes, we also want to emphasize the support and care Margaret gave to younger researchers. I (Teresa Cruz) felt this support in those early days studying these amazing living creatures. Later, in 2008, we had the opportunity to organize the “*Pollicipes* international conference”, the first and only conference dedicated to the genus *Pollicipes*, which was dedicated to Margaret Barnes (Cruz et al. 2010b) and several papers included in “The Biology of Barnacles” (*Journal of Experimental Marine Biology and Ecology*, 392). Like that conference, our review, 25 years after Barnes (1996), is a tribute to Margaret Barnes.

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DIVERSITY, DISTRIBUTION, ECOLOGY AND CONSERVATION STATUS OF THE FAMILY SYNGNATHIDAE IN SUB-SAHARAN AFRICA AND ADJACENT ISLANDS

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Abstract The charismatic Syngnathidae occur in coastal and freshwater environments across the globe and play an important role by acting as flagship species for conservation. Despite this status, many syngnathids are threatened by a range of anthropogenic impacts including exploitation and habitat destruction. In addition, the cryptic nature and generally low population densities of syngnathids make research and related conservation action difficult, which can lead to data gaps. The gap in knowledge of the biology and status of syngnathids is especially acute within Africa. This review provides the first synthesis of syngnathid diversity, biogeography, ecology, threats and conservation in sub-Saharan Africa and adjacent islands. Research focus and effort are biased towards the southeast coast of Africa, with limited research specifically on syngnathids. A total of 63 species of syngnathids in 26 genera are recorded in Africa, with higher syngnathid diversity on the east coast of Africa. Ecological research focused on population trends and diversity is identified as priorities, specifically for those species listed as Data Deficient on the IUCN Red List. Other priorities identified include research on the extent and impact of illegal catch and trade and the development of local capacity and expertise. These findings provide an important resource that can be used for the future conservation of this iconic group of fishes.

Keywords: seahorse, pipefish, pipehorse, synganthids, South Africa, ecology, diversity, conservation

Introduction

Syngnathidae (seahorses, pipefish, pipehorses and seadragons) are charismatic fishes and, as such, are effective flagship species for the conservation of threatened habitats (Shokri et al. 2009, Vincent et al. 2011). Yet, many species are harvested for use in traditional Chinese medicine and as curios, caught as by-catch in industrial and artisanal fisheries, and threatened by habitat loss (Pollom et al. 2021). Unfortunately, little is known about the biology and ecology of the majority of syngnathid species, and this is especially true of those inhabiting the waters of sub-Saharan Africa and adjacent

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islands. If syngnathid populations in sub-Saharan Africa and adjacent islands are to be conserved and sustainably managed, a better understanding of the status of this group of fishes is needed. Available data on the diversity, distribution, threats and conservation status of syngnathids in this region are scattered and need to be synthesized to provide a current overview, which will help identify research and conservation priorities.

Syngnathids, comprising over 300 species in 57 genera, are widely distributed in temperate and tropical habitats, predominantly among shallow coastal areas of the Atlantic and Indo-Pacific Oceans, including soft sediment habitats, seagrass beds, estuaries, coral and rocky reefs, and mangroves (Foster & Vincent 2004, Kuiter 2009). Members of this family are uniquely characterised by male pregnancy, cryptic morphology and behaviour, and a fused jaw that allows for suction feeding. All 45 extant species of seahorses currently belong to the genus *Hippocampus* (IUCN 2021). There are over 300 species of pipefish in 50 genera, with only three species of seadragon in two genera and eight species of pipehorse in four genera (IUCN 2021). Seahorses are found between latitudes 50° north and 50° south (Lourie et al. 2004, 2016), pipefish occur circumglobally in nearshore habitats (Dawson 1985), whilst seadragons are confined to southern Australia (Stiller et al. 2015). Pipehorses are less commonly observed and occur in both the Atlantic and Indo-Pacific, including in the western Indian Ocean (Dawson 1985, Kuiter 2004, 2009). Mitochondrial and nuclear sequence data from a broad diversity of syngnathid genera strongly support the geographic origin of the seahorse genus *Hippocampus* in the Indo-Pacific and its sister clades as a grouping of morphologically diverse Indo-Pacific genera, including the Indo-Pacific pygmy pipehorses (Hamilton et al. 2017). The data also revealed speciose clades that originated in southern Australia and the western Atlantic, with no large clades originating in Africa (Hamilton et al. 2017).

Syngnathids are generally found in complex habitats that provide suitable cover and protection from predators. The habitats include seagrasses (e.g. Choo & Liew 2003, Dias & Rosa 2003, MasonJones et al. 2010, Choi et al. 2012, Filiz & Taskawak 2012, Correia et al. 2015a, Otero-Ferrer et al. 2015, Manning et al. 2018), algal beds (e.g. Moreau & Vincent 2004, Curtis & Vincent 2005), mangroves (e.g. Dias & Rosa 2003), muck habitats (de Brauwer & Burton 2018) and coral reefs (Marcus et al. 2007, Vincent et al. 2011). In addition to protection against predators, the availability of holdfasts (structures that a seahorse is able to curl its tail around for support) is a critical component in seahorse habitats. Various studies have found that seahorse presence and abundance are positively associated with the number of available holdfasts (Curtis & Vincent 2005, Aylesworth et al. 2015, Lazic et al. 2018). A decrease in available holdfasts has been linked to population declines of the long-snouted seahorse, *Hippocampus guttulatus*, in the Ria Formosa, Portugal (Correia et al. 2015a), as well as White's seahorse, *H. whitei*, in Port Stephens, Australia (Harasti 2016). In addition to natural habitats, many syngnathids use, and in some instances even prefer, artificial structures (Dias & Rosa 2003, Correia et al. 2015b, Gristina et al. 2015, Otero-Ferrer et al. 2015, Lazic et al. 2018), such as wall- and mattress-type gabions (Claassens 2016, Munro 2017, Claassens et al. 2018), swimming nets (Harasti et al. 2010) as well as structures specifically designed for seahorse conservation, e.g. 'seahorse hotels' (Simpson et al. 2019, 2020).

Estuaries are particularly important coastal systems for some syngnathids (Rosa et al. 2007, MasonJones et al. 2010, Aylesworth et al. 2015, Whitfield et al. 2017). According to Lourie et al. (2016), the Knysna seahorse *Hippocampus capensis* is the only known true estuarine seahorse species, found in only three South African estuaries (Bell et al. 2003, Lockyear et al. 2006). However, *H. whitei* also occurs exclusively in estuarine habitats in eastern Australia, including Sydney Harbour and Port Stephens, New South Wales and Moreton Bay, Queensland (Harasti et al. 2012, Short et al. 2019). Members of the Indo-Pacific *Hippichthy*s move between rivers and estuaries (Dawson 1985, Ishihara & Tachihara 2009, Lim et al. 2011, Paller et al. 2011, Jayaneththi et al. 2014, Moore et al. 2014), whilst pipefish in the west African genus *Enneacampus* occur exclusively in freshwater systems (Dawson 1985).

Syngnathids are vulnerable to anthropogenic impacts because they occur in shallow, coastal systems and within threatened habitats (Lim et al. 2011, Vincent et al. 2011). In addition, syngnathids have various characteristics (uneven distribution, low mobility, small home-ranges, monogamy and low fecundity) that leave them susceptible to habitat destruction and overexploitation (Foster & Vincent 2004, Lim et al. 2011, Vincent et al. 2011). Within the Syngnathidae, research examining threats has been biased towards seahorses (Foster & Vincent 2004, Vincent et al. 2011), and only species of *Hippocampus* is listed in CITES Appendix II (Vincent et al. 2013, Foster et al. 2016). Regardless of this bias, many threats faced by seahorses also apply to pipefish, pipehorses and seadragons, as these groups are found in similar habitats to seahorses and are used for similar purposes by humans (Martin-Smith & Vincent 2006, Lim et al. 2011, Vincent et al. 2011). The three most important anthropogenic threats to syngnathids are as follows: overexploitation by targeted fisheries, incidental capture in non-selective fishing gear (by-catch) and habitat degradation and loss (Martin-Smith & Vincent 2006, Lim et al. 2011, Vincent et al. 2011, Harasti 2016).

The overarching aim of this review is to evaluate the current state of knowledge of syngnathids in sub-Saharan Africa and adjacent islands. Here, all available literature on southern African syngnathids and adjacent islands has been collated to investigate research effort and focus across this part of the African continent. This is followed by a detailed review of syngnathid diversity in sub-Saharan Africa and adjacent islands to verify occurrence records and distributions. Ecological information on syngnathids within the region is synthesized on habitat use, life histories, reproduction, feeding and predation, and behaviour. Major threats and conservation actions will be reviewed within an African context. Lastly, research gaps and future priorities for African syngnathids will be identified.

Geographic scope and literature review

Whilst the focus of this review is on sub-Saharan African syngnathids, we have extended the geographic boundaries north of the equator to include countries located south of Western Sahara on the Atlantic coast, and south of Somalia on the Indian Ocean coast. In addition, several neighbouring western Indian Ocean islands (Europa, Madagascar, Réunion, Mauritius, Seychelles, Comoros and Zanzibar) are also covered. Many syngnathid species that occur in the Mediterranean Sea and the Red Sea range across Europe (with a suite of resources and information available on these species), and to maintain the focus on Africa, those countries bordering the Red Sea and the Mediterranean Sea were excluded from this review. Most of the area under review lies within the tropics and subtropics. However, around the coast of South Africa, there are four distinct biogeographic regions: a tropical north-east region on the east coast close to the border with Mozambique; a subtropical east coast; a warm temperate south coast; and the cold temperate west coast that extends into Namibia.

Extent and type of syngnathid research

To determine the geographic extent and type of research on syngnathids in southern Africa, a literature search using online databases was conducted that included specific syngnathid-focused search terms including generic, species and common names, as well as more general marine-related searches. Key word searches were also conducted using Google Scholar and Rhodes University

Library Catalogue searching words such as “syngnathids”, “seahorse”, “pipefish”, “pipehorse”, “estuary” as well as variations of these words. The literature search was focused on research from sub-Saharan Africa and adjacent islands, syngnathid ecology, reproduction, conservation and taxonomy, in which the literary sources included both peer-reviewed articles and grey literature. In addition to sub-Saharan African-focused research, general searches were conducted on those syngnathid species that are found in Africa, but also occur in other regions globally.

Scuba divers and citizen scientists can provide important information on species diversity and distributions, especially in areas where scientific research is limited, on social platforms designed for observational input via photographic records of species and associated habitat. One such network, iNaturalist (2021), is a social network for naturalists, citizen scientists and biologists and is built on the concept of mapping and sharing observations of biodiversity across the globe. To gain a better understanding of the distribution patterns of African syngnathids, the lead author created a project entitled “Syngnathids of Africa” on iNaturalist (https://www.inaturalist.org/projects/syngnathids-of-africa) with the aim of collating citizen science syngnathid observations from Africa. However, such data do have limitations. For example, it is extremely difficult to identify many syngnathid species correctly, both *in situ* and from photographs, owing to morphological similarities across species and their cryptic nature. In addition, owing to the threatened status of many syngnathid species, only general locality information is provided for most observations on iNaturalist.

A total of 147 scientific publications and unpublished reports, dating from 1900 to the present, were found that referenced, recorded, listed or mentioned syngnathids in Africa, with a clear geographic bias to the south-eastern coast and in particular South Africa. The earliest publication found was the description of *Hippocampus capensis* in 1900 (Boulenger 1900).

A similar pattern in terms of geographic focus was found for ecological publications that included specific locality information. Various species descriptions and a detailed overview of Indo-Pacific pipefish by Dawson (1985) provided extensive information on this group.

The majority of articles (71%) on syngnathids had an ecological focus (Ecological in Figure 1), ranging from field surveys to genetics research; studies which targeted a specific syngnathid species were only found for *Hippocampus capensis* and *Syngnathus watermeyeri* in South Africa, and *Hippocampus hippocampus* and *H. algiricus* along the west coast of Africa. Only 41 publications (28%) were exclusively focused on syngnathids, whilst the rest consisted of general fish surveys, biodiversity assessments and species lists. Species lists (Lists in Figure 1) and reviews (Review in Figure 1) mostly depend on other primary sources for the inclusion of syngnathid-related data and were considered secondary sources. The remaining publications included species descriptions (Figure 1) using specimens originating from sub-Saharan Africa and adjacent islands and management-focused publications (Figure 1). The dependence on unverified historic sources can lead to the perpetuation of misinformation, owing to incorrect species identification. For example, in various reports (Cyrus 2001, Weerts & Cyrus 2002, Whitfield 2005, Kleynhans 2007, Perera et al. 2011, Weerts et al. 2014, Máiz-Tomé et al. 2018, Cutler et al. 2020), the pipefish *Microphis millepunctatus* is mistakenly recorded as *M. brachyurus*, which does not occur in Africa (Dawson 1985). In addition, members of the pipefish genus *Corythoichthys*, as well as larval syngnathids, are extremely difficult to identify to species. Misidentification to species level in these instances is thus likely (Harris et al. 1999, Pattrick & Strydom 2008, Mwaluma et al. 2010, Jaonalison et al. 2016).

Hippocampus capensis, *Syngnathus watermeyeri*, *S. temminckii* and *Hippichthys spicifer* were the species with the highest number of ecological publications, whilst *Microphis millepunctatus* had the greatest number of records in species lists and reviews (Figure 1). Genetic research on syngnathids in Africa is limited to a few studies from South Africa, mostly focused on *Hippocampus capensis* and studies investigating the evolutionary history of *Hippocampus* spp. (Toeffie 2000, Teske et al. 2003, 2004, 2005, Galbusera et al. 2007, Mkare et al. 2017, 2021), *Syngnathus temminckii* and *S. watermeyeri* (Mwale et al. 2013).

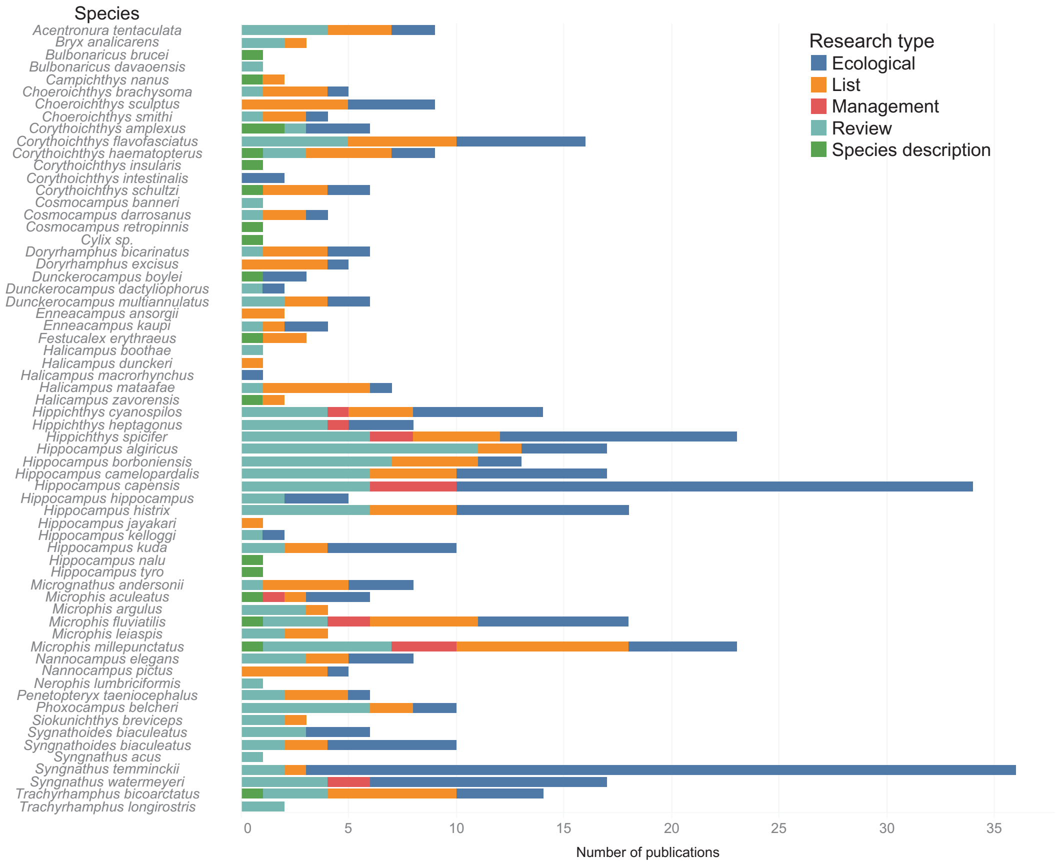

Figure 1 Number of publications relating to different species: publications are divided into ecological research, species lists and reviews, and species descriptions using specimens that originated from Africa. Note that one publication or report can provide a record for multiple species.

A total of 668 syngnathid observations were logged from the region on iNaturalist between April 2002 and March 2021. Of these records, 58% of observations were Research Grade (an observation is considered to be "Research Grade" when the community agrees on species-level identification, i.e. when more than two-thirds of identifiers agree on the identification) and 32% of species required identification. The observations from iNaturalist provided data for Cameroon (*Hippocampus algiricus*), which were not available in the published literature, as well as a first record for *Halicampus macrorhynchus* in Kenya.

Diversity and biogeography

To determine the species richness of African syngnathids, data from the literature review, observations from iNaturalist and information provided by diving schools, divers and non-governmental organisations (NGOs) were used. In addition, collection records from the South African Institute of Aquatic Biodiversity (SAIAB), Grahamstown, and Iziko Museum, Cape Town, were reviewed.

High species richness, with a total of 63 species of syngnathids in 26 genera (Table 1), is recorded in African waters (excluding the African countries bordering the Mediterranean and Red Sea) and the nearby island countries and territories of São Tomé and Príncipe, Zanzibar, Comoros, Mayotte, Madagascar, Europa Island, Seychelles, Mauritius and Réunion Island. The total includes

Table 1 All syngnathid species and the countries in which they are found within sub-Saharan Africa and adjacent Indian Ocean islands

Species	Country	Reference
Acentronura tentaculata Günther, 1870	Madagascar	Dawson (1985), McKenna & Allen (2006), Fricke et al. (2018)
	Mozambique	Smith (1963), Dawson (1985), De Boer et al. (2001), Pereira (2008)
	Kenya	iNaturalist (2021)
	Tanzania	iNaturalist (2021)
	Comoros	Smith (1963)
Bryx analicarens (Duncker, 1915)	Madagascar	Fricke et al. (2018)
	Seychelles	Dawson (1985)
	Zanzibar	Dawson (1985)
Bulbonaricus brucei Dawson, 1984	Tanzania	Dawson (1984a)
Bulbonaricus davaoensis (Herald, 1953)	Kenya	Dawson (1985)
Campichthys nanus (Dawson, 1977)	Mozambique	Dawson (1977a), Pereira (2008)
Choeroichthys brachysoma (Bleeker, 1855)	Madagascar	Fricke et al. (2018)
	Mauritius	Smith (1963), Arndt & Fricke (2019)
	Réunion	Letourneur et al. (2004), Fricke et al. (2009)
Choeroichthys sculptus (Günther, 1870)	Madagascar	McKenna & Allen (2006), Weis et al. (2009), Jaonalison et al. (2016), Fricke et al. (2018)
	Mozambique	Pereira (2000), De Boer et al. (2001), Pereira (2008)
	Réunion	Letourneur et al. (2004), Fricke et al. (2009)
Choeroichthys smithi Dawson, 1976	Europa Island	Fricke et al. (2013)
	Madagascar	McKenna & Allen (2006)
	Mozambique	Dawson (1985), Pereira (2008)
Corythoichthys amplexus Dawson & Randall, 1975	Kenya	Huxham et al. (2008), Mwaluma et al. (2010)
	Seychelles	Dawson & Randall (1975), Dawson (1977b)
	Zanzibar	Tyler et al. (2009), Berkström et al. (2012)
Corythoichthys flavofasciatus (Rüppell, 1838)	Madagascar	McKenna & Allen (2006), Jaonalison et al. (2016), Fricke et al. (2018)
	Mauritius	Arndt & Fricke (2019)
	Mozambique	Smith (1963), Gell & Whittington (2002), Pereira (2008)
	Réunion	Letourneur et al. (2004), Fricke et al. (2009)
	Zanzibar	Smith (1963), Tyler et al. (2009), Berkström et al. (2012), Kloiber (2013), Palmqvist (2013)
	Kenya	Smith (1963)
	Seychelles	Smith (1963)
Corythoichthys haematopterus (Bleeker, 1851)	East coast of Africa/ Seychelles	Dawson (1977b)
	Madagascar	McKenna & Allen (2006), Fricke et al. (2018)
	Mozambique	Smith (1963), Gell & Whittington (2002), Pereira (2008)
	Mozambique	Gell & Whittington (2002)
	Seychelles	Smith (1963)
	Réunion	Letourneur et al. (2004), Fricke et al. (2009)

(*Continued*)

Table 1 (*Continued*) All syngnathid species and the countries in which they are found within sub-Saharan Africa and adjacent Indian Ocean islands

Species	Country	Reference
Corythoichthys insularis Dawson, 1977	Comoros	Dawson (1977b)
Corythoichthys intestinalis (Ramsay, 1881)	Madagascar	Ory (2008)
	Mozambique	Fordyce (2016)
Corythoichthys schultzi Herald, 1953	Kenya	Cowburn et al. (2018)
	Mozambique	Gell & Whittington (2002), Pereira (2008)
	Réunion	Letourneur et al. (2004), Fricke et al. (2009)
	Seychelles	Dawson (1977b)
Cosmocampus banneri (Herald & Randall, 1972)	South Africa	Dawson (1985)
Cosmocampus darrosanus (Dawson & Randall, 1975)	Madagascar	McKenna & Allen (2006), Fricke et al. (2018)
	Mozambique	Pereira (2008)
Cosmocampus retropinnis Dawson, 1982	Gambia	Dawson (1982)
Cylix sp.	South Africa	G. Short, unpublished data.
Doryrhamphus bicarinatus Dawson, 1981	Madagascar	Fricke et al. (2018)
	Mauritius	Arndt & Fricke (2019)
	Mozambique	Pereira (2008)
	Réunion	Fricke et al. (2009), Pinault et al. (2013)
	South Africa	Dawson (1981, 1985)
Doryrhamphus excisus Kaup, 1856	Kenya	Cowburn et al. (2018)
	Madagascar	Fricke et al. (2018)
	Mozambique	Pereira (2000, 2008)
	Réunion	Letourneur et al. (2004)
Dunckerocampus boylei Kuiter, 1998	Mauritius	Forget et al. (2020)
	Seychelles	Daly et al. (2018)
	South Africa	Kuiter (1998)
Dunckerocampus dactyliophorus (Bleeker, 1853)	South Africa	Dawson (1985)
	Mozambique	Fordyce (2016)
Dunckerocampus multiannulatus (Regan, 1903)	Mauritius	Forget et al. (2020)
	Réunion	Letourneur et al. (2004), Fricke et al. (2009), Tea et al. (2020)
	South Africa	Dawson (1985)
	Mauritius	Smith (1963)
Enneacampus ansorgii (Boulenger, 1910)	Angola	Skelton (2019)
Enneacampus kaupi (Bleeker, 1863)	Angola	Skelton (2019)
	Côte d'Ivoire	Kamelan et al. (2013)
	Gabon	Mamonekene et al. (2006)
	Western and central African tropical estuaries	Whitfield (2005)
Festucalex erythraeus (Gilbert, 1905)	Mozambique	Dawson (1977a), Pereira (2000), Pereira (2008)
Halicampus dunckeri (Chabanaud, 1929)	Madagascar	Fricke et al. (2018)

(*Continued*)

Table 1 (*Continued*) All syngnathid species and the countries in which they are found within sub-Saharan Africa and adjacent Indian Ocean islands

Species	Country	Reference
Halicampus macrorhynchus Bamber, 1915	Kenya	Ewout Knoester pers. comm., iNaturalist (2021)
	Madagascar	Alain Rassat pers. comm.
Halicampus mataafae (Jordan & Seale, 1906)	Madagascar	Fricke et al. (2018)
	Mauritius	Arndt & Fricke (2019)
	Mozambique	Pereira (2000, 2008)
	Réunion	Letourneur et al. (2004), Fricke et al. (2009)
	South Africa	Dawson (1985)
Halicampus zavorensis Dawson, 1984	Mozambique	Dawson (1984b), Pereira (2008)
Halicampus boothae (Whitley, 1964)	Kenya	Dawson (1985)
Hippichthys cyanospilos (Bleeker, 1854)	Eastern African tropical estuaries	Whitfield (2005)
	Kenya	Okeyo (1998), Crona & Rönnbäck (2007)
	Madagascar	McKenna & Allen (2006), Fricke et al. (2018)
	Madagascar and Indian Ocean islands	Máiz-Tomé et al. (2018)
	Mauritius	Arndt & Fricke (2019)
	Mozambique	Dawson (1985), De Boer et al. (2001), Pereira (2008)
	South Africa	Forbes et al. (2013), Van Niekerk et al. (2019a)
Hippichthys heptagonus Bleeker, 1849	South Africa	Dawson (1985), Skelton et al. (1989), Harris et al. (1995, 1999), Van Niekerk et al. (2019a)
	South-eastern African subtropical estuaries and eastern African tropical estuaries	Teugels et al. (1994), Whitfield (2005)
	Kenya	Dawson (1985)
Hippichthys spicifer (Rüppell, 1838)	Kenya	Van der Velde et al. (1995), Seegers et al. (2003), Mirriam (2010)
	Madagascar	Smith (1963), Fricke et al. (2018), Máiz-Tomé et al. (2018)
	Mauritius	Arndt & Fricke (2019)
	Mozambique	Pereira (2008)
	South Africa	Cyrus & McLean (1996), Mbande (2003), Harrison & Whitfield (2006), O'Brien et al. (2009), Forbes et al. (2013), Van Niekerk et al. (2019a)
	South-eastern African warm-temperate estuaries and south-eastern African subtropical and eastern African tropical estuaries	Teugels et al. (1994), Whitfield (2005)
	Mozambique	Smith (1963)

(*Continued*)

Table 1 (*Continued*) All syngnathid species and the countries in which they are found within sub-Saharan Africa and adjacent Indian Ocean islands

Species	Country	Reference
	Tanzania	Smith (1963), Mwandya et al. (2009), Mwandya (2019)
	Zanzibar	Lugendo (2007), Berkström et al. (2012), Palmqvist (2013)
Hippocampus algiricus Kaup, 1856	Gabon	Mamonekene et al. (2006)
	São Tomé	Afonso et al. (1999), Lourie et al. (2004), Wirtz et al. (2007)
	Senegal	West (2012), Lourie et al. (2004), Cisneros-Montemayor et al. (2016)
	Angola	Lourie et al. (2004)
	Benin	Lourie et al. (2004)
	Côte d'Ivoire	Lourie et al. (2004)
	Gambia	Lourie et al. (2004), Cisneros-Montemayor et al. (2016)
	Ghana	Lourie et al. (2004)
	Guinea	Lourie et al. (2004)
	Liberia	Lourie et al. (2004)
	West Africa	Otero-Ferrer et al. (2017)
	Nigeria	Lourie et al. (2004)
	Western and central African tropical estuaries	Whitfield (2005)
Hippocampus borboniensis Duméril, 1870	Madagascar	Lourie et al. (2004), Fricke et al. (2018)
	Mozambique	Lourie et al. (2004), Pereira (2008), Warnell et al. (2013), Fordyce (2016)
	Réunion	Smith (1963), Lourie et al. (2004), Fricke et al. (2009)
	Mauritius	Lourie et al. (2004)
	South Africa	Lourie et al. (2004)
	Tanzania	Lourie et al. (2004), McPherson & Vincent (2004)
Hippocampus camelopardalis Bianconi, 1854	Eastern African tropical estuaries	Whitfield (2005)
	Madagascar	McKenna & Allen (2006), Fricke et al. (2018)
	Mozambique	Smith (1963), Almeida et al. (1999, 2001), De Boer et al. (2001), Lourie et al. (2004), Teske et al. (2004), Pereira (2008), Warnell et al. (2013), Fordyce (2016)
	Réunion	Letourneur et al. (2004)
	Mauritius	Smith (1963)
	Tanzania	Lourie et al. (2004), McPherson & Vincent (2004)
	South Africa	Lourie et al. (2004)

(*Continued*)

Table 1 (*Continued*) All syngnathid species and the countries in which they are found within sub-Saharan Africa and adjacent Indian Ocean islands

Species	Country	Reference
Hippocampus capensis Boulenger, 1900	South Africa	Boulenger (1900), Smith (1963), Riley (1986), Whitfield (1989), Russell (1994), Teugels et al. (1994), Grange & Cretchley (1995), Whitfield (1995a), Le Cheminant (2000), Toeffie (2000), Bell et al. (2003), Teske et al. (2003), Lourie et al. (2004), Teske et al. (2005), Whitfield (2005), Harrison & Whitfield (2006), Lockyear et al. (2006), Galbusera et al. (2007), Teske et al. (2007), Claassens (2016), Claassens & Hodgson (2018a), Mkare et al. (2017), Western Cape Government (2017), Claassens & Hodgson (2018b), Claassens et al. (2018), De Villiers et al. (2019), Van Niekerk et al. (2019b), Claassens & Harasti (2020), Claassens et al. (2020), Arendse & Russell (2020), SANParks (2020), Mkare et al. (2021)
Hippocampus hippocampus (Linnaeus, 1758)	Senegal	Lourie et al. (2004), West (2012), Cisneros-Montemayor et al. (2016)
	The Gambia	Cisneros-Montemayor et al. (2016)
	Guinea	Lourie et al. (2004)
	West Africa	Otero-Ferrer et al. (2017)
Hippocampus histrix Kaup, 1856	Kenya	Van der Velde et al. (1995), McPherson & Vincent (2004), Cowburn et al. (2018)
	Mozambique	Almeida et al. (1999), Pereira (2000), Almeida et al. (2001), Gell & Whittington (2002), Pereira (2008), Warnell et al. (2013), Fordyce (2016)
	Réunion	Letourneur et al. (2004)
	Kenya	McPherson & Vincent (2004)
	Zanzibar	Smith (1963), Lugendo (2007), Berkström et al. (2012)
	South Africa	Lourie et al. (2004)
	Tanzania	Lourie et al. (2004)
	Mauritius	Lourie et al. (2004)
Hippocampus jayakari Boulenger, 1900	Réunion	Fricke et al. (2009)
Hippocampus kelloggi Jordan & Snyder, 1901	Tanzania	Lourie et al. (2004), Teske et al. (2005), McPherson & Vincent (2004)
Hippocampus kuda Bleeker, 1852	Madagascar	McKenna & Allen (2006)
	Mozambique	Smith (1963), Almeida et al. (2001), Teske et al. (2005), Pereira (2008), Warnell et al. (2013), Fordyce (2016)
	South Africa	Teske et al. (2004, 2005)
	Zanzibar	Smith (1963)

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Table 1 (*Continued*) All syngnathid species and the countries in which they are found within sub-Saharan Africa and adjacent Indian Ocean islands

Species	Country	Reference
Hippocampus nalu Short, Claassens, Smith, De Brauwer, Hamilton, Stat & Harasti, 2020	South Africa	Short et al. (2020)
Hippocampus tyro Randall & Lourie, 2009	Seychelles	Randall & Lourie (2009)
Micrognathus andersonii (Bleeker, 1858)	Kenya	Sindorf et al. (2015), Cowburn et al. (2018)
	Madagascar	Dawson (1985), McKenna & Allen (2006), Fricke et al. (2018)
	Mozambique	Pereira (2000, 2008)
Microphis aculeatus (Kaup, 1856)	Angola	Dawson (1984c), Skelton (2019)
	Benin	Adite et al. (2013)
	Côte d’Ivoire	Kamelan et al. (2013)
	Gabon	Cutler et al. (2020)
	Nigeria	Ukaonu et al. (2011)
	Senegal	Dawson (1984c)
Microphis argulus (Peters, 1855)	Comoros	Smith (1963), Dawson (1985)
	Madagascar	Dawson (1985), Fricke et al. (2018)
Microphis fluviatilis (Peters, 1852)	Kenya	Dawson (1985), Okeyo (1998), Seegers et al. (2003)
	Madagascar	Dawson (1985), Fricke et al. (2018), Máiz-Tomé et al. (2018)
	Mozambique	Smith (1963), Dawson (1985), Desai et al. (2019)
	South Africa	Cyrus (2001), Weerts & Cyrus (2002), Kyle (2002), Du Preez et al. (2007), Kleynhans (2007), Perera et al. (2011), Weerts et al. (2014), Evan (2017), Van Niekerk et al. (2019a)
	South-eastern African subtropical estuaries and eastern African tropical estuaries	Whitfield (2005)
Microphis leiaspis (Bleeker, 1854)	Madagascar	Smith (1963), Dawson (1985), Fricke et al. (2018), Máiz-Tomé et al. (2018)
Microphis millepunctatus (Kaup, 1856)	Réunion	Dawson (1984c, 1985), Letourneur et al. (2004), Fricke et al. (2009)
	Gabon	Cutler et al. (2020)
	Madagascar	Smith (1963), Dawson (1984c, 1985), Keith (2002), Fricke et al. (2018), Máiz-Tomé et al. (2018)
	Mauritius	Dawson (1984c, 1985), Arndt & Fricke (2019)
	Mozambique	Pereira (2008)
	São Tomé	Wirtz et al. (2007)

(*Continued*)

Table 1 (*Continued*) All syngnathid species and the countries in which they are found within sub-Saharan Africa and adjacent Indian Ocean islands

Species	Country	Reference
	South Africa	Cyrus (2001), Weerts & Cyrus (2002), Perera et al. (2011), Kleynhans (2007), Weerts et al. (2014), Evan (2017), Van Niekerk et al. (2019a)
	Kenya	Dawson (1985)
	South-eastern African subtropical estuaries and eastern African tropical estuaries and western and central African tropical estuaries	Whitfield (2005)
Nannocampus elegans Smith, 1953	Mozambique	Smith (1963), Pereira (2000, 2008)
	South Africa	Smith (1963), Christensen & Winterbottom (1981), Bennett (1987), Pattrick & Strydom (2008)
Nannocampus pictus (Duncker, 1915)	Mauritius	Arndt & Fricke (2019)
	Mozambique	Pereira (2000, 2008)
	South Africa	Dawson (1985)
	Réunion	Letourneur et al. (2004), Fricke et al. (2009)
Nerophis lumbriciformis (Jenyns, 1835)	Western Sahara	Dawson (1986a)
Penetopteryx taeniocephalus Lunel, 1881	Madagascar	Dawson (1985), McKenna & Allen (2006), Fricke et al. (2018)
	Mozambique	Pereira (2008)
	Mauritius	Smith (1963)
	Réunion	Letourneur et al. (2004)
Phoxocampus belcheri (Kaup, 1856)	Madagascar	McKenna & Allen (2006), Fricke et al. (2018)
	Mauritius	Arndt & Fricke (2019)
	Kenya	Dawson (1985), Smith (1963)
	Mozambique	Smith (1963), Pereira (2000)
	Zanzibar	Smith (1963)
	Mafia Island	Smith (1963)
	Seychelles	Smith (1963)
Siokunichthys breviceps Smith, 1963	Mozambique	Smith (1963), Dawson (1985), Pereira (2008)
Syngnathoides biaculeatus (Bloch, 1785)	Kenya	Van der Velde et al. (1995), Crona & Rönnbäck (2007), Mirriam (2010), Gajdzik et al. (2014)
	Madagascar	McKenna & Allen (2006), Fricke et al. (2018)
	Mozambique	Smith (1963), Almeida et al. (1999, 2001), Gell & Whittington (2002), Pereira (2008)
	Eastern African tropical estuaries	Whitfield (2005)

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Table 1 (*Continued*) All syngnathid species and the countries in which they are found within sub-Saharan Africa and adjacent Indian Ocean islands

Species	Country	Reference
	Comoros	Smith (1963)
	Seychelles	Smith (1963)
	Zanzibar	Lugendo (2007), Berkström et al. (2012)
Syngnathus acus Linnaeus, 1758	Western Sahara	Dawson (1986)
Syngnathus temminckii Kaup, 1856	Namibia	Mwale et al. (2013)
	South Africa	Beckley (1984), Wallace et al. (1984), Bennett (1989), Whitfield (1989), Bennett & Branch (1990), Ter Morshuizen & Whitfield (1994), Teugels et al. (1994), Clark et al. (1996), Whitfield & Bruton (1996), Harris et al. (1999), Paterson & Whitfield (2000), Strydom (2003), Teske et al. (2004), Strydom & Wooldridge (2005), Harrison & Whitfield (2006), Pattrick et al. (2007), Pattrick & Strydom (2008), Wasserman et al. (2010), Sheppard et al. (2011), Becker et al. (2012), Mwale et al. (2013, 2014), Strydom (2015), Whitfield et al. (2017), Ntshudisane et al. (2021)
Syngnathus watermeyeri Smith, 1963	South Africa	Smith (1963), Dawson (1985), Ter Morshuizen & Whitfield (1994), Teugels et al. (1994), Whitfield & Bruton (1996), Cowley & Whitfield (2001), Whitfield (2005), Harrison & Whitfield (2006), Vorwerk et al. (2007), Sheppard et al. (2011), Mwale et al. (2013, 2014), Whitfield et al. (2017), Van Niekerk et al. (2019b), Ntshudisane et al. (2021)
Trachyrhamphus bicoarctatus (Bleeker, 1857)	East Africa	Dawson (1985)
	Madagascar	Dawson (1985), McKenna & Allen (2006), Weis et al. (2009), Fricke et al. (2018)
	Mozambique	Almeida et al. (1999), Pereira (2000, 2008), Fordyce (2016)
	Réunion	Letourneur et al. (2004), Fricke et al. (2009)
	Mauritius	Dawson (1985)
	South Africa	Dawson (1985)
	Zanzibar	Nordlund et al. (2013)
Trachyrhamphus longirostris Kaup, 1856	Zanzibar	Dawson (1985)
	Madagascar	Dawson (1985)

Species highlighted in grey are endemic either to a specific country or to the region.

11 species of seahorses, 50 pipefish and two pygmy pipehorse (Table 1). The syngnathids occurring in Africa represent 40% and 20% of the world's known genera and species, respectively. Countries with the highest number of syngnathid species are located along the south-east African coast and include South Africa, Mozambique, Tanzania and Madagascar, with a total of 40 species (Figure 2). Countries with nine syngnathid species or more include Seychelles, Mauritius, Réunion, Tanzania, Kenya, South Africa, Madagascar and Mozambique. In contrast, the lowest numbers of syngnathid species per country occur mostly along the west African coast, including Namibia, Benin, Ghana and Liberia, and in Europa Island in the Mozambique Channel off the east African coast, with only one species recorded in each of these countries (Figure 2). When considering the number of syngnathid species per kilometre of coastline, east African countries dominate. An exception to this trend is The Gambia on the west coast, which has the fourth highest species density out of 23 countries (Figure 2).

South Africa currently exhibits a high degree of endemism, which may be due to higher research efforts in the country, relative to all other African countries that were reviewed (Table 1). This endemism comprises 4 out of 63 species of syngnathids occurring nowhere else in Africa: the temperate *Hippocampus capensis* and *Syngnathus watermeyeri*, and two new tropical species from Sodwana Bay, a pygmy pipehorse belonging to the new genus *Cylix* (Short, unpublished data; https://www.inaturalist.org/observations/11120680) and the pygmy seahorse *Hippocampus nalu* (Short et al. 2020, https://www.inaturalist.org/observations/31815098). The two new species of syngnathids from Sodwana Bay are remarkable for South African syngnathid endemism and overall African syngnathid biodiversity in that they were the first newly described species since *Syngnathus watermeyeri* in 1963 (Smith 1963). Other single-country endemics include *Bulbonaricus brucei* from Tanzania, *Campichthys nanus* from Mozambique, and *Hippocampus tyro* from the Seychelles, all of which are currently found nowhere else in eastern Africa and offshore islands.

African syngnathid endemicity can be also be assessed regionally on a broader scale with respect to marine provinces and ecoregions. For example, the pipefish *Choeroichthys smithi* has been recorded only in Réunion Island, Mauritius, the Seychelles, Madagascar, Tanzania, Mozambique and South Africa, yet this range spans the subtropical-tropical Agulhas and western Indian Ocean marine provinces in eastern Africa (Table 1; Spalding et al. 2007). Similarly, the pipefish *Doryrhamphus bicarinatus*, *Microphis millepunctatus*, *M. fluviatilis* and *Nannocampus elegans* have all been recorded in eastern Africa and nowhere else in the Indo-Pacific (Table 1). Additionally, the seahorse *Hippocampus algiricus* and the freshwater pipefish *Enneacampus ansorgii, E. kaupi* and *Microphis aculeatus* occur only in the western Africa and Gulf of Guinea provinces (Spalding et al. 2007) in the tropical eastern Atlantic (Table 1). In contrast, the temperate pipefish *Syngnathus temminckii* occurs only in the temperate marine environments of Namibia and South Africa.

Seahorses

Eleven species of seahorses are recorded in sub-Saharan Africa and adjacent islands, three of which are endemic to Africa, including *Hippocampus algiricus*, *H. capensis* and *H. nalu* (Table 1). The West African *H. algiricus* occurs from north to south in the following eastern Atlantic countries: the Canary Islands (Spain), Senegal, The Gambia, Guinea, Sierra Leone, Liberia, Ivory Coast, Ghana, Benin, Nigeria, São Tomé and Príncipe and Angola. The Knysna seahorse *H. capensis* is endemic to the temperate south coast of South Africa, where it has been recorded from three estuaries: Keurbooms, Knysna and Swartvlei estuaries (Bell et al. 2003, Lockyear et al. 2006). Lastly, *H. nalu* is the first recorded species of pygmy seahorse for continental Africa and is currently known only from Sodwana Bay in KwaZulu-Natal, South Africa (Short et al. 2020). This species, however, may occur further north into Mozambique and Tanzania, and further ichthyofaunal surveys, diver

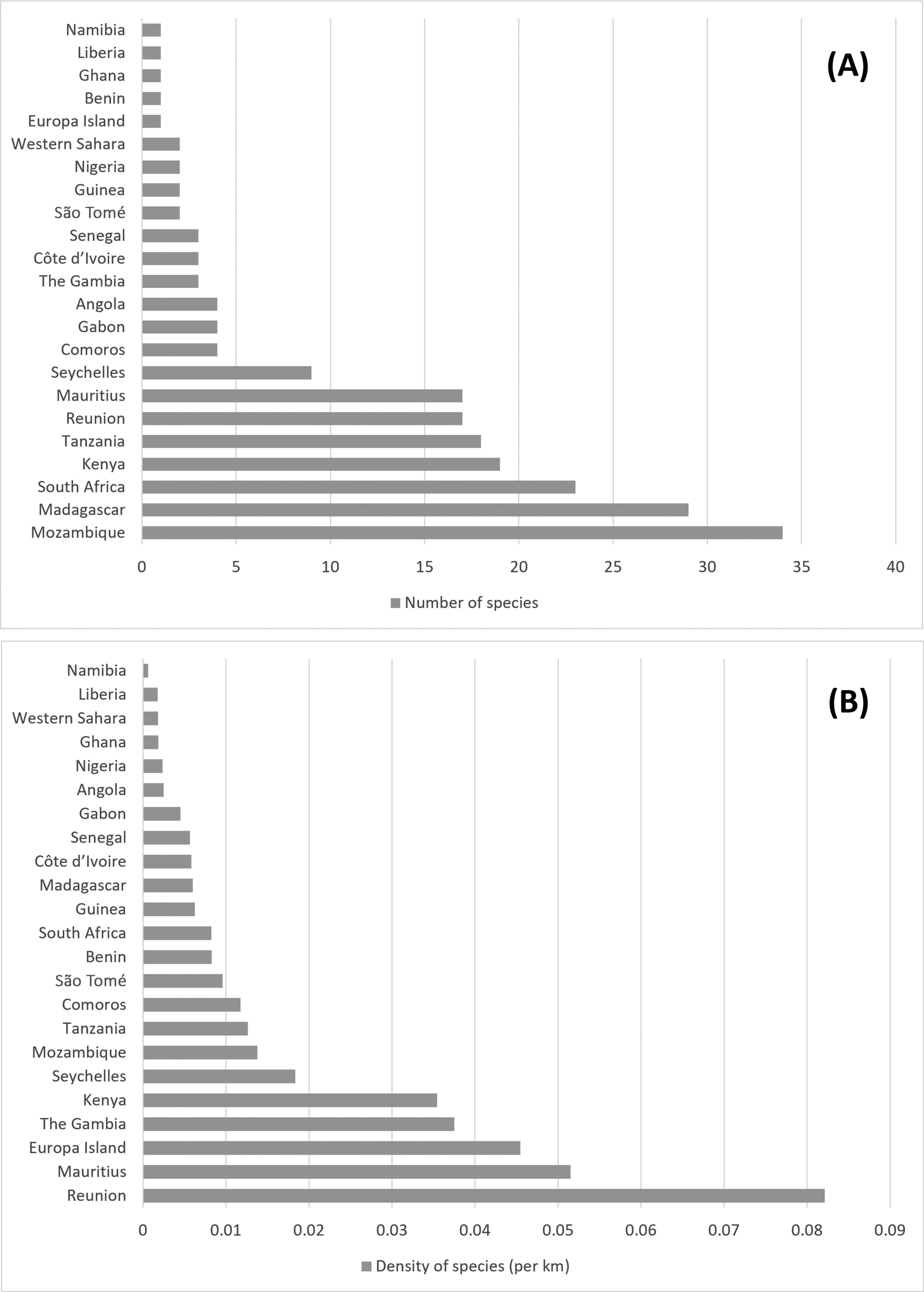

Figure 2 (A) Total number of syngnathid species per country and (B) number of species per kilometre of coastline for each country.

observations and a better understanding of the distribution of sandy coral reefs, which it inhabits, are needed.

The broadly distributed Indo-Pacific species, *H. histrix*, *H. kelloggi*, *H. kuda* and *H. jayakari*, have been recorded in South Africa, Mozambique, Madagascar, Mauritius, Tanzania and Kenya (Table 1) (Lourie et al. 2004). The exception is *H. hippocampus*, which has a distribution that includes the Mediterranean, British Isles, Wadden Sea, the Gulf of Guinea, the Azores and the Canary Islands. The Réunion seahorse *H. borboniensis* and the giraffe seahorse *H. camelopardalis* exhibit a regional East African distribution that covers South Africa, Mozambique, Tanzania, Madagascar, Mauritius and Réunion Island (Table 1); however, new distributional records for these seahorses in Indian waters have recently been published (Krishnan et al. 2011, Subburaman et al. 2014). The description of *H. borboniensis* was based on seven individuals taken as by-catch from the Gulf of Mannar, India, indicating that there may be a local breeding population of this species. In contrast, the description of *H. camelopardalis* is based on the occurrence of a single specimen collected in fishing by-catch in the vicinity of the Mithapur reef in the Gulf of Kachchh Marine National Park, Gujarat, India (Subburaman et al. 2014). The collected individual is presumed to have drifted in the prevailing equatorial currents to the eastern Arabian Sea (Subburaman et al. 2014).

In a revision of the genus *Hippocampus*, *H. borboniensis* has been synonymised with *H. kuda* due to a lack of distinguishable morphological, genetic or geographic differences (Lourie et al. 2016). However, we find the results of the revisional study inconclusive as preliminary examinations of specimens of *H. borboniensis* and *H. kuda* by Graham Short and Louw Claassens revealed several morphological differences in the presence of diagnostic characters of the eye and snout spines. Similarly, the partial cytochrome c oxidase subunit I (COI) DNA sequences generated from the single individual of *H. borboniensis*, which were sourced from the Barcode of Life Data (BOLD) and used to calculate genetic divergences in the study, were also examined by the co-authors and deemed to be of low quality due to messy chromatogram data. Therefore, it appeared that the low-quality DNA sequences were not suitable for meaningful genetic analyses. A phylogenomic study of the family Syngnathidae based on next-generation sequencing of ultra-conserved elements (UCE) is currently underway, and the results strongly support *H. borboniensis* as a distinct evolutionary unit from *H. kuda* (Josefin Stiller pers. comm.).

Several seahorse specimens collected in Mozambique and South Africa between 1954 and 1989, and housed in the fish collections at the South African Institute for Aquatic Biodiversity (SAIAB), have been identified as *H. whitei*, the south-west Pacific seahorse endemic to New South Wales and Queensland, Australia (Short et al. 2019) (catalogue numbers 5631, 12285, 12286, 12287 and 36140, and identified as *H. novaehollandae* or *H. whitei*). However, this species designation has often been given to members of *Hippocampus* of unknown identity at the time based on superficial similarity in appearance to *H. whitei*, including *H. camelopardalis* in Mozambique and South Africa, *H. kelloggi* in Papua New Guinea and Solomon Islands, and *H. breviceps* in South Australia (Lourie et al. 2016).

Pipefishes

Fifty species of pipefish are recorded in Africa, eight of which are endemic to western, southern and eastern Africa, respectively (Table 1): the freshwater pipefishes *Enneacampus ansorgii* and *E. kaupi*, and the brackish water pipefish *Microphis aculeatus* from western Africa; the obligate *Galaxea* sp. coral dweller *Bulbonaricus brucei* from Tanzania; *Campichthys nanus* from Mozambique; *Nannocampus elegans* from Mozambique and South Africa; and the temperate estuarine pipefishes *Syngnathus temminckii* and *S. watermeyeri* from Namibia and South Africa. The distribution records of Pereira (2008) and Mwaluma et al. (2010) that record *S. temminckii* in Mozambique and Kenya, respectively, are probably based on misidentification, as this is a temperate species. The non-endemic pipefish species listed in Table 1 have an Indo-Pacific distribution and have been recorded in eastern Africa, including Kenya, Madagascar, Mozambique, Mauritius, Réunion, Seychelles and South Africa. The exceptions are *Nerophis lumbriciformis*, a species recorded in Western Sahara, and in the north-eastern Atlantic, the Baltic, Mediterranean and Black Seas (Dawson 1986a, 1986b); and *Syngnathus acus*, which is recorded in Western Sahara and in the eastern Atlantic (British Isles, Norway, and the Faroe Islands) (Dawson 1986a). The wide-ranging coral rubble- and sand-associated Indo-Pacific winged pipefish, *Halicampus macrorhynchus*, has recently (2020) been recorded in south-eastern Kenya and Madagascar (Alain Rassat, pers. comm., Ewout Knoester, pers. comm., https://www.inaturalist.org/observations/41930021, https://www.inaturalist.org/observations/81180257).

Some taxonomic authorities show the distributions of *Syngnathus acus* extending into Namibia and South Africa (Dawson 1985, 1986a, Kuiter 2009); however, recent studies revealed that *S. acus* is replaced by the temperate *S. temminckii* in these countries (Mwale et al. 2013). Morphological data show that *S. temminckii* is distinct from the broadly distributed European pipefish *S. acus*, and a molecular phylogeny reconstructed using mitochondrial DNA recovered *S. temminckii* and *S. watermeyeri* as sister taxa to the North Atlantic members of *Syngnathus* (Mwale et al. 2013). Similarly, the flagtail pipefish, *Doryrhamphus bicarinatus*, which occurs in Mozambique, South Africa and Réunion Island, was recorded in the Maldives (Anderson et al. 1998) with identification based on meristic and diagnostic characters of the snout spine of one individual. However, we regard the individual of flagtail pipefish observed in the Maldives as an undescribed species of *Doryrhamphus*. Although morphologically similar to *D. bicarinatus* in meristic characters and the number and placement of spines present on the snout, it has a colour pattern on the tail that is highly distinct from that observed in *D. bicarinatus* from South Africa (Dawson 1981, 1985). The distinct coloration patterns present on the tail in members of *Doryrhamphus* appear to distinguish populations with large genetic distances among them throughout the Indo-Pacific (Dawson 1981, Lessios & Robertson 2016, Rudie Kuiter 2020 pers. comm.). Therefore, we retain *D. bicarinatus* as a regional eastern African species.

Pygmy pipehorses

Only one species of pygmy pipehorse has been definitively identified in African waters, the Indo-Pacific *Acentronura tentaculata*, which has been recorded in Kenya, Madagascar, Mozambique, South Africa and Tanzania (Table 1). Observations of an undescribed species, provisionally identified as a member of *Hippocampus*, were recorded in Sodwana Bay, South Africa, in 2009 on iNaturalist.org (https://www.inaturalist.org/observations/11120680, https://www.inaturalist.org/observations/11120683). Subsequently, further examinations of preserved specimens by the co-authors placed them in the genus *Cylix*, which has recently been described from New Zealand (Short & Trnski 2021). Pygmy pipehorses superficially resemble seahorses and share many morphological synapomorphies, including the head at an angle to the body axis, fully enclosed brood pouch and prehensile tail, and hence are often misidentified as seahorses by recreational scuba divers. There are currently eight described species of pygmy pipehorses that occur in the Indo-Pacific in the genera *Acentronura*, *Cylix*, *Idiotropiscis* and *Kyonemichthys* (Short & Trnski 2021). It therefore seems a matter of time before new genera and species are discovered in eastern and southern Africa.

Ecology

Data from the literature search and information from diving schools, divers and NGOs across Africa were used to review the ecology of African syngnathids. iSeahorse, a seahorse-focused citizen science initiative developed by Project Seahorse, is available from within iNaturalist and provides an opportunity to log additional ecological data with a seahorse record, such as depth, abundance and habitat. Empirical data from field surveys conducted by the lead author are also included.

Habitats

Most syngnathids occurring in sub-Saharan Africa and adjacent islands inhabit shallow coastal environments in water depths of 1–30 m (Table 2), and several species are recorded from intertidal rock pools, such as *Micrognathus andersonii* (Sindorf et al. 2015, Cowburn et al. 2018), *Nannocampus elegans* (Christensen & Winterbottom 1981), *N. pictus* (Brian Sellick pers. comm.), *Cosmocampus darrosanus* (Dawson 1985), *Choeroichthys sculptus* (Fricke et al. 2009), *C. smithi* (Fricke et al. 2009, 2013), *Doryrhamphus bicarinatus* (Fricke et al. 2009), *Halicampus zavorensis* (Adrian Pearton pers. comm.) and *Phoxocampus belcheri* (Arndt & Fricke 2019).

Some species are known to occur in deeper, offshore environments: *Hippocampus algiricus* (West 2012), *Syngnathus acus* (Dawson 1986), *Hippocampus tyro* (only known from a dredged sample at 48 m depth, Randall & Lourie 2009), *Trachyrhamphus longirostris* (Dawson 1985) and *Dunckerocampus multiannulatus* recorded at 80–90 m depth on mesophotic reefs at Réunion (Tea et al. 2020), although these species also occur in shallow coastal environments. *Dunckerocampus multiannulatus* has also been observed in caves in reef habitats in South Africa at 25 m and in Madagascar at 16 m (Brian Sellick pers. comm., Adrian Payton pers. comm., Alain Rassat pers. comm.). Kuiter (1998) concluded that *D. boylei*, a sister species to *D. multiannulatus*, only occurs in depths greater than 25 m. However, Daly et al. (2018) recorded the first observation of this species in the Seychelles at 18 m. Furthermore, a study on fish aggregation device (FAD) arrays in the western Indian Ocean recorded these two species together on a FAD in Mauritius at a depth of 15 m (Forget et al. 2020). It should be noted; however, it is difficult to distinguish between these two species, and there is a possibility that the fish were misidentified.

The majority (61%) of syngnathids (39 species) that occur in sub-Saharan Africa and adjacent islands inhabit shallow reef habitats, ranging from smooth volcanic reefs (Pinault et al. 2013) to coral rubble and boulders (Dawson 1985, Fricke et al. 2009, Mwaluma et al. 2010). In most instances, syngnathids are found in complex habitats, although some species occur in less complex habitats such as sandy or muddy bottoms (Goran & Spanier 1985, Golani & Lerner 2007, Ali et al. 2020). *Hippocampus histrix*, in particular, is commonly found in sandy habitats with sparse coral, sponge and sea pen cover (Adrian Pearton pers. comm., Alain Rassat pers. comm., Ewout Knoester pers. comm.).

Limited information is available on habitat specialisation by African species. The type specimens of *Bulbonaricus brucei* were collected from the coral *Galaxea astreata* in Tanzania (Dawson 1984a), and species in this genus are known to live in obligate associations with dendrophyllid corals, including those of the genus *Galaxea*, which provide refuge from predators (Araki et al. 2020). *Bulbonaricus brucei* is, however, only known from its type specimen, and no published observations of this species could be found other than its initial description in 1971. *Corythoichthys flavofasciatus* has been recorded associating with the corals *Acropora formosa*, *A. pulchra*, *Echinopora mammiformis* and *Heliopora coerulea* in the Indo-Pacific; it seems likely that this species inhabits similar habitats in Africa (Coker et al. 2014).

Seagrass is another important habitat for African syngnathids, with 15 species recorded in seagrass beds along the east African coast (Van der Velde et al. 1995, Almeida et al. 2001, De Boer et al. 2001, Gell & Whittington 2002, Bell et al. 2003, Vorwerk et al. 2007, Fricke et al. 2009, Mwaluma et al. 2010, Berkström et al. 2012, West 2012) (Table 2). Species that depend on seagrass habitat consequently occur in areas suitable for development of seagrass beds, especially in bays and estuaries (De Boer et al. 2001, Gell & Whittington 2002, Weerts & Cyrus 2002, Mamonekene et al. 2006, Lugendo 2007, Mwandya et al. 2009, Palmqvist 2013, Mwandya 2019). For some species,

Table 2 A synthesis from the literature of the general environment, depth ranges and habitats used by syngnathid species in sub-Saharan Africa and adjacent islands

Species name	General environment	Depth range	Habitats (southern and western Africa specific)	General habitat information
Acentronura tentaculata	Coastal	1 m (De Boer et al. 2001)	Seagrass (*Halodule wrightii*, *Cymodocea serrulata*, *Zostera capensis*) in Inhaca Island, Mozambique (De Boer et al. 2001)	No global habitat data found
		<5 m (Louw Claassens unpublished data)	Weeds in Inhaca Island, Mozambique (Smith 1963) Seagrass beds in Vilankulo, Mozambique (Louw Claassens unpublished data)	
Bryx analicarens	Coastal	Shallow tide pools up to 45 m (Dawson 1985)	No habitat data found for sub-Saharan Africa and adjacent islands	First record of *B. analicarens* in India found underneath a dead coral boulder overgrown with macroalgae, in a pool on an intertidal reef flat (Chandran et al. 2020) Shallow tide pools with macroalgal cover (including the brown alga *Cystoseira* spp.), but has also been captured by trawl at 45 m depth (Dawson 1985)
Bulbonaricus brucei	Coastal	1 m (Dawson 1984a)	Type specimen collected from the coral *Galaxea astreata* in Tanzania (Dawson 1984a)	Endemic to Tanzania
Bulbonaricus davaoensis	Coastal	1–8 m (Dawson 1985) 6–15 m (Araki et al. 2020)	East African specimens found among the coral *Galaxea fascicularis*, and planktonic specimens collected from upper 200 m to depths of 610–7120 m (Dawson 1985)	*Galaxea* sp. coral polyps in Japan (Araki et al. 2020)
Campichthys nanus	Coastal	3–11 m (Dawson 1985)	Coral knolls (Dawson 1985)	Endemic to Africa
Choeroichthys brachysoma	Coastal	Found up to 25 m deep, but mostly <5 m (Dawson 1985)	Tide pools in northern Mozambique (Smith 1963) Coral reef and rocky shore habitats in Réunion (Letourneur et al. 2004)	Seagrass, reef and coral reef habitats (Dawson 1985)
Choeroichthys sculptus	Coastal	<1 m (Weis et al. 2009)	Shallow mangrove (*Avicennia*, *Sonneratia*, *Rhizophora*, *Bruguiera*) pools in western Madagascar (Weis et al. 2009)	Intertidal reef flats and seagrass beds up to a few metres in depth (Kuiter 2009)

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Table 2 (*Continued*) A synthesis from the literature of the general environment, depth ranges and habitats used by syngnathid species in sub-Saharan Africa and adjacent islands

Species name	General environment	Depth range	Habitats (southern and western Africa specific)	General habitat information
		<3 m (Dawson 1985)	Coral reefs in southwest Madagascar (Jaonalison et al. 2016)	Shallow rock pools in Japan (Murase 2015)
		1.4 m (De Boer et al. 2001)	Tide pools in East Africa (Smith 1963)	Shallow reefs (Dawson 1985)
		0–9 m (Fricke et al. 2009)	Mud flats and beds of seagrass (*Zostera capensis*) in Inhaca Island, Mozambique (De Boer et al. 2001)	
			Reef flats and seagrass areas, including tidal pools, in Réunion (Fricke et al. 2009)	
Choeroichthys smithi	Coastal	<1 m (Fricke et al. 2013)	Reef flat with tidal pools in Europa Island (Fricke et al. 2013)	No global habitat data found
		0–25 m (Fricke et al. 2009)	Coral reefs and seagrass areas, including tidal pools, in Réunion (Fricke et al. 2009)	
Corythoichthys amplexus	Coastal	Recorded depth range is 0–30 m, and 15 of 32 collections are from confirmed water depths greater than 9 m; only 7 are from less than 5 m (Dawson 1977b)	Coral reefs in Zanzibar (Berkström et al. 2012)	Associated with hard substrata in Australia (Moore et al. 2014)
		3–14 m (Berkström et al. 2012)	Mangroves (*Sonneratia alba*) in Kenya (Huxham et al. 2008)	Mangrove habitat in New Caledonia (Thollot 1996)
		<2 m (Huxham et al. 2008)	Larvae found in reef lagoons along the Kenyan coast, characterised by coral outcrops interspersed with seagrass beds, sand and coral rubble of varying cover (Mwaluma et al. 2010)	Shallow coastal waters in Malaysia (see Lim et al. 2011)
		<3 m (Mwaluma et al. 2010)	Coral reefs in Zanzibar (Tyler et al. 2009)	
		<30 m (Moore et al. 2014)		
		<15 m (Tyler et al. 2009)		

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Table 2 (*Continued*) A synthesis from the literature of the general environment, depth ranges and habitats used by syngnathid species in sub-Saharan Africa and adjacent islands

Species name	General environment	Depth range	Habitats (southern and western Africa specific)	General habitat information
Corythoichthys flavofasciatus	Coastal	Depth range of 0–24 m; 25 of 36 collections were from 0 to 5 m, six were from 5 to 10 m and only five lots were from confirmed depths exceeding 10 m (Dawson 1977b)	Coral reefs in southwest Madagascar (Jaonalison et al. 2016)	*Acropora formosa*, *A. pulchra*, *Echinopora mammiformis*, *Heliopora coerulea* in the Indo-Pacific (Coker et al. 2014)
		3–14 m (Berkström et al. 2012)	Coral reefs in Zanzibar (Berkström et al. 2012)	Sandy bottom habitat with low knolls consisting of coral in the Red Sea (Goran & Spanier 1985)
		2–5 m (Palmqvist 2013)	*Thalassodendron ciliatum* in Zanzibar (Palmqvist 2013)	Associated with hard substrata in Australia (Moore et al. 2014)
		<30 m (Moore et al. 2014)	Seagrass beds dominated by *Enhalus acoroides*, *Thalassodendron ciliatum* and *Cymodocea serrulata* in Mozambique (Gell & Whittington 2002)	Shallow coastal waters in Malaysia (see Lim et al. 2011)
		2–5 m depth (Gell & Whittington 2002)		
Corythoichthys haematopterus	Coastal	2–5 m depth (Gell & Whittington 2002)	Seagrass beds dominated by *Enhalus acoroides*, *Thalassodendron ciliatum* and *Cymodocea serrulata* in Mozambique (Gell & Whittington 2002)	Found in mangrove habitats in Australia (Blaber 1986)
		7–9 m (Matsumoto & Yanagisawa 2001)	Reefs in East Africa (Smith 1963)	Rubble bottom in Japan (Matsumoto & Yanagisawa 2001)
		2–10 m; <1 m; 0–7 m (Sogabe & Takagi 2013)		Steep boulder and bedrock slopes, shallow seagrass beds and on a vertical wharf structure in Japan (Sogabe & Takagi 2013)
		0.5–2 m (Nakamura et al. 2003)		*Enhalus acoroides*-dominated seagrass beds in Japan (Nakamura et al. 2003)

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Table 2 (*Continued*) A synthesis from the literature of the general environment, depth ranges and habitats used by syngnathid species in sub-Saharan Africa and adjacent islands

Species name	General environment	Depth range	Habitats (southern and western Africa specific)	General habitat information
		Data from 27 collections indicate a 0–19 m depth range; 18 samples were from 0 to 3 m, four were from 3 to 10 m, and five were from SCUBA collections in 10–19 m (Dawson 1977b)		
Corythoichthys insularis	Coastal	Depths of 20–42 m (Dawson 1977b)	No habitat data found for sub-Saharan Africa and adjacent islands	No global habitat data found
Corythoichthys intestinalis	Coastal	<30 m (Moore et al. 2014)	Shallow reefs in Madagascar (Ory 2008)	Associated with hard substrata in Australia (Moore et al. 2014)
		0–3 m depth (Dawson 1977b)		Sand, coral or seagrass (Dawson 1977b)
Corythoichthys schultzi	Coastal	2–5 m (Gell & Whittington 2002)	Seagrass beds dominated by *Enhalus acoroides*, *Thalassodendron ciliatum* and *Cymodocea serrulata* in Mozambique (Gell & Whittington 2002)	Sandy, trench habitat with coral knolls and branched corals (Goran & Spanier 1985)
		9–12 m (Goran & Spanier 1985)	Reefs in Mozambique (Cowburn et al. 2018)	Associated with hard substrata in Australia (Moore et al. 2014)
		<30 m (Moore et al. 2014)		Shallow, coastal waters in Malaysia (Lim et al. 2011)
		Depth range of 0–30 m; 15 collections were in 0–9 m, eight in 10–16 m and seven from confirmed depth greater than 16 m (Dawson 1977b)		Seagrass beds in Jordan (Khalaf et al. 2012)
Cosmocampus banneri	Coastal	<30 m (Dawson 1985)	No habitat data found for sub-Saharan Africa and adjacent islands	Shallow coastal waters in Malaysia (Lim et al. 2011)
		<30 m (Moore et al. 2014)		Associated with hard substrata in Australia (Moore et al. 2014)
				Coral reef habitats (Dawson 1985)

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Table 2 (*Continued*) A synthesis from the literature of the general environment, depth ranges and habitats used by syngnathid species in sub-Saharan Africa and adjacent islands

Species name	General environment	Depth range	Habitats (southern and western Africa specific)	General habitat information
Cosmocampus darrosanus	Coastal	<3 m (Dawson 1985)	No habitat data found for sub-Saharan Africa and adjacent islands	Reef flats and tide pools (Dawson 1985)
				Shallow coastal waters in Malaysia (Lim et al. 2011)
Cosmocampus retropinnis	Coastal	Depths to 79 m (Dawson 1982)	No habitat data found for sub-Saharan Africa and adjacent islands	No global habitat data found
Cylix sp.	Coastal	Still being described	Still being described	Still being described
Doryrhamphus bicarinatus	Coastal	<5 m (Louw Claassens unpublished data)	Rocky reefs and artificial reefs in Vilankulo, Mozambique (Louw Claassens unpublished data)	No global habitat data found
		0–20 m (Fricke et al. 2009)	Shallow lagoon and coral and rocky reef areas, including tidal pools in Réunion (Fricke et al. 2009)	
		5–30 m (Pinault et al. 2013)	Compact lava substrata with high algal cover and *Pocillopora verrucosa*, *P. eydouxi*, *P. damicornis* and *P. meandrina* corals in Madagascar (Pinault et al. 2013)	
		<28 m (Dawson 1985)		
Doryrhamphus excisus	Coastal	45–49 m (Dawson 1985)	No habitat data found for sub-Saharan Africa and adjacent islands	Rock or coral bottoms (Dawson 1985)
		<30 m (Moore et al. 2014)		Associated with hard substrata in Australia (Moore et al. 2014)
		1.5 m (Goran & Spanier 1985)		Mangrove habitat in New Caledonia (Thollot 1996)
				Sandy bottom habitat with low knolls consisting of coral in the Red Sea (Goran & Spanier 1985)
Dunckerocampus boylei	Coastal	18 m (Daly et al. 2018)	Recorded on a chain of 0.2 m diameter rigid buoys as part of a FAD array in Mauritius (Forget et al. 2020)	Deeper water and associated with reef habitats and caves (Kuiter 1998)

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Table 2 (*Continued*) A synthesis from the literature of the general environment, depth ranges and habitats used by syngnathid species in sub-Saharan Africa and adjacent islands

Species name	General environment	Depth range	Habitats (southern and western Africa specific)	General habitat information
		20–95 m (Kuiter 1998)		
		<15 m (Forget et al. 2020)		
Dunckerocampus dactyliophorus	Coastal	<30 m (Moore et al. 2014)	Coral reefs in Mozambique (Fordyce 2016)	Associated with hard substrata in Australia (Moore et al. 2014)
		Maximum recorded depth of 56 m with records from tide pools and intermediate depths (Dawson 1985)		Shallow coastal waters in Malaysia (Lim et al. 2011)
Dunckerocampus multiannulatus	Coastal	Depths to 45 m (Dawson 1985)	Recorded within a chain of 0.2 m diameter rigid buoys as part of a FAD array in Mauritius (Forget et al. 2020)	Coral and rocky reefs and associated caves (Dawson 1981, 1985)
		<15 m (Forget et al. 2020)	Mesophotic reefs in Réunion (Tea et al. 2020)	Flat coral reefs and artificial structures in the Red Sea (Rilov & Benayahu 2000)
		25 m (Brian Sellick pers. comm.)		
		<35 m (Kuiter 1998)		Oil terminal jetties in the Red Sea (Rilov & Benayahu 1998)
		20–30 m (Rilov & Benayahu 2000)		
		80–90 m (Tea et al. 2020)		
Enneacampus ansorgii	Freshwater	<6 m depth (Mamonekene et al. 2006)	Rivers in Angola (Skelton 2019)	Endemic to western Africa
			Estuaries in Nigeria (Kone et al. 2021)	
			Ndogo Lagoon in Gabon (Mamonekene et al. 2006)	
Enneacampus kaupi	Freshwater	No depth information	Rivers in Angola (Skelton 2019)	Endemic to western Africa
			River systems in the Democratic Republic of the Congo (Walsh et al. 2014)	
			Estuaries in Nigeria (Kone et al. 2021)	

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Table 2 (*Continued*) A synthesis from the literature of the general environment, depth ranges and habitats used by syngnathid species in sub-Saharan Africa and adjacent islands

Species name	General environment	Depth range	Habitats (southern and western Africa specific)	General habitat information
			Tidal flats in Mauritania (Wolff et al. 2006)	
			Estuarine lake in Ivory Coast (Ecoutin et al. 2005)	
			Mangrove creek in Nigeria (Allison et al. 1997)	
			River delta in Nigeria (Onwuteaka 2015)	
Festucalex erythraeus	Coastal	18–43 m (Dawson 1985)	No habitat data found for sub-Saharan Africa and adjacent islands	Reef habitats (Dawson 1985)
				Shallow coastal waters in Malaysia (Lim et al. 2011)
Halicampus dunckeri	Coastal	<30 m (Moore et al. 2014)	No habitat data found for sub-Saharan Africa and adjacent islands	Associated with hard substrata in Australia (Moore et al. 2014)
		<13 m (Dawson 1985)		Sand, rubble and reef habitats (Dawson 1985)
Halicampus mataafae	Coastal	Generally found at depths of <9 m, but have been recorded as deep as 15 m (Dawson 1985)	No habitat data found for sub-Saharan Africa and adjacent islands	Rock and coral habitats and reef pools (Dawson 1985)
Halicampus zavorensis	Coastal	15 m (Ziyadi et al. 2018)	Holotype collected from a tide pool in Mozambique (Dawson 1984b)	Two specimens collected from Oman in sandy rocky reef over rocky sand with some coral (Dawson 1984b)
		4 m (Dawson 1984b)	Shallow reefs and also in intertidal rock pools in Durban (Adrian Pearton pers. comm.)	
Halicampus macrorhynchus		3–25 m (Dawson 1985)	Sandy area with isolated patches of algae and sponges in Kenya (Ewout Knoester pers. comm.)	Seagrass, coral rubble and algae-covered rocks (Dawson 1985)
	Coastal	10–15 m (Ewout Knoester pers. comm.)	Sandy area with patches of algae in Madagascar (Alain Rassat pers. comm.)	Shallow coastal habitats in Malaysia (Lim et al. 2011)
Halicampus boothae	Coastal	3–30 m (Dawson 1985)	No habitat data found for sub-Saharan Africa and adjacent islands	Rock and coral habitats (Dawson 1985)

(*Continued*)

Table 2 (*Continued*) A synthesis from the literature of the general environment, depth ranges and habitats used by syngnathid species in sub-Saharan Africa and adjacent islands

Species name	General environment	Depth range	Habitats (southern and western Africa specific)	General habitat information
Hippichthys cyanospilos	Coastal and estuarine	1 m (De Boer et al. 2001)	*Sonneratia alba* mangroves in Gazi Bay, Kenya. Juveniles found in estuaries; adults found further upstream (Crona & Rönnbäck 2007)	Coastal and brackish water (Dawson 1985)
		<1 m (Weerts & Cyrus 2002)	Of 81 fish sampled in Inhaca Island, Mozambique, most were found within channel habitat covered by old coral debris and rocks, with patches of *Halodule wrightii* seagrasses. Most fish caught at night and during summer. Also recorded within sandflat, mudflat, mangrove, sandbank and *Zostera capensis* habitats (De Boer et al. 2001)	Larvae found in mangrove habitats in Malaysia (Azmir et al. 2017)
		<30 m (Moore et al. 2014)	Recorded within *Z. capensis* within the Mhlathuze Estuary, South Africa (Weerts & Cyrus 2002)	*Halophila ovalis* and *H. uninervis* beds in Malaysia (Jani et al. 2019)
			Associated with moderate to large, closed estuaries and predominantly open estuaries (Harrison & Whitfield 2006)	Associated with mangroves and estuaries in Australia (Moore et al. 2014)
				Rivers and estuaries within seagrass in Malaysia (Lim et al. 2011)
Hippichthys heptagonus	Coastal and estuarine	<1 m (Jayaneththi et al. 2014)	Associated with predominantly open estuaries (Harrison & Whitfield 2006)	Estuarine seagrass beds and mangroves in Australia (Blaber 1986)
			Lower swamp habitat in the Mkuze swamps, South Africa (Skelton et al. 1989)	Found resting underneath overhanging roots of Ficus benghalensis in a Sri Lankan lake (Jayaneththi et al. 2014)
			South African estuaries (Harris et al. 1995, 1999, Harris & Cyrus 2000)	Rivers in the Philippines (Paller et al. 2011)
				Lower reaches of rivers and estuaries (Dawson 1985)

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Table 2 (*Continued*) A synthesis from the literature of the general environment, depth ranges and habitats used by syngnathid species in sub-Saharan Africa and adjacent islands

Species name	General environment	Depth range	Habitats (southern and western Africa specific)	General habitat information
Hippichthys spicifer	Coastal	3–14 m (Berkström et al. 2012)	Mangroves in Zanzibar, but also recorded in seagrass (Berkström et al. 2012)	Shallow coastal waters and estuaries and sometimes in mangroves (Dawson 1985)
		<1 m (Mirriam 2010)	Mangrove creek (*Rhizophora mucronata* and *Avicennia marina*) in Kenya (Mirriam 2010)	Abandoned aquaculture ponds in the Philippines (Ikejima et al. 2006)
		2–5 m (Palmqvist 2013)	*Thalassodendron ciliatum* in Zanzibar (Palmqvist 2013)	Mangroves in Japan (Ishihara & Tachihara 2009)
		Average depth of 3 m (Lugendo 2007)	Mangrove creeks and channels (*Rhizophora mucronata*) with a muddy substratum with prop roots as well as in Enhalus acoroides interrupted by small patches of *Thalassodendron ciliatum* and the calcareous algae *Halimeda* spp. in Kenya (Lugendo 2007)	
		<1 m (Mwandya et al. 2009)	Found within mangrove creeks (*Avicennia marina* and *Xylocarpus granatum*) in Kenya. Substrate varied between mud, seagrass and sand (Mwandya et al. 2009, Mwandya 2019)	
			Recorded in estuaries in South Africa (O'Brien et al. 2009, Forbes et al. 2013)	
			Recorded in mangroves and seagrass beds in Kenya (Van der Velde et al. 1995)	
Hippocampus algiricus	Coastal and estuarine	1–50 m (West 2012)	Ndogo Lagoon, Gabon (Mamonekene et al. 2006)	Endemic to western Africa
		1–25 m (Wirtz et al. 2007)	Found frequently holding onto sponges (Wirtz et al. 2007)	
		<6 m (Mamonekene et al. 2006)	Seagrass beds and also on soft bottom habitats in deeper water on the west African coast (West 2012)	

(*Continued*)

Table 2 (*Continued*) A synthesis from the literature of the general environment, depth ranges and habitats used by syngnathid species in sub-Saharan Africa and adjacent islands

Species name	General environment	Depth range	Habitats (southern and western Africa specific)	General habitat information
		2–24 m (Afonso et al. 1999)	Mixed bottom and muddy plain in São Tomé Island (Afonso et al. 1999)	
Hippocampus borboniensis	Coastal	<5 m (Louw Claassens unpublished data)	Shallow seagrass beds in Vilankulo, Mozambique (Louw Claassens unpublished data)	Indian record only with no habitat data
		5–60 m (McPherson & Vincent 2004)	Associated with seagrass, soft bottom, sponges on the east African coast (McPherson & Vincent 2004)	
Hippocampus camelopardalis	Coastal	2 m (Subburaman et al. 2014)	Seagrass beds (*Thalassodendron ciliatum/Cymodocea serrulata* and *Thalassodendron ciliatum/Cymodocea serrulata*) in Inhaca Island, Mozambique (Almeida et al. 2001)	Reefs in India (Subburaman et al. 2014)
		<5 m (Louw Claassens unpublished data)	Seagrass beds in Mozambique (Louw Claassens unpublished data, Teijema 2020)	
		Up to 45 m (McPherson & Vincent 2004)	‘Weeds’ from Inhaca Island and Inhambane estuary, Mozambique (Smith 1963)	
			Seagrass beds, algal beds and shallow reefs (McPherson & Vincent 2004)	
Hippocampus capensis	Estuarine	<1 m (Arendse & Russell 2020)	Seagrass (*Zostera capensis*, *Ruppia cirrhosa*, *Halophila ovalis*) and macroalgae (*Caulerpa filiformis*, *Codium extricatum*) – predominantly *Zostera capensis* (Lockyear et al. 2006, Teske et al. 2007)	Endemic to South Africa
		1–3 m (Claassens 2016)	Bare sediment habitats and among vegetation (*Z. capensis*, *Halophila ovalis*, *Caulerpa filiformis*) (Bell et al. 2003)	

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Table 2 (*Continued*) A synthesis from the literature of the general environment, depth ranges and habitats used by syngnathid species in sub-Saharan Africa and adjacent islands

Species name	General environment	Depth range	Habitats (southern and western Africa specific)	General habitat information
		1–2 m (Claassens & Hodgson 2018)	*Codium tenue* (De Villiers et al. 2019)	
		<1.5 m (Whitfield 1989)	Reno mattress, *Zostera capensis*, *Caulerpa filiformis*, *Asparagopsis taxiformis*, *Codium tenue* (Claassens 2016, Claassens et al. 2018, Claassens & Hodgson 2018b, Claassens & Harasti 2020)	
Hippocampus histrix	Coastal	2–5 m (Gell & Whittington 2002)	Seagrass beds dominated by *Enhalus acoroides*, *Thalassodendron ciliatum* and *Cymodocea serrulata* in Mozambique (Gell & Whittington 2002)	*Euplexaura* sp. gorgonian fan in Port Stephens, Australia (Harasti 2015)
		Up to 20 m (McPherson & Vincent 2004)	Seagrass beds, weedy rocky reefs, sponges and sea squirts in areas of sparse or no seagrass, soft bottoms with soft corals and sponges (McPherson & Vincent 2004)	Mangroves in New Caledonia (Thollot 1996)
		Average depth of 3 m (Lugendo 2007)	Mangrove creeks and channels (*Rhizophora mucronata*) with a muddy substratum with prop roots in Kenya (Lugendo 2007)	
		30 m depth (Dave Harasti unpublished data)	Shallow reefs in Mozambique (Fordyce 2016)	
			Mangrove and seagrass habitats in Kenya (Van der Velde et al. 1995)	
		25–30 m (Adrian Pearton pers. comm.)	Deeper reef habitat in South Africa (Adrian Pearton pers. comm.)	
		22 m (Alain Rassat pers. comm.)	Sandy substrata and among macroalgae, hard or soft coral, dead corals, sponges in Madagascar (Alain Rassat pers. comm.)	

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Table 2 (*Continued*) A synthesis from the literature of the general environment, depth ranges and habitats used by syngnathid species in sub-Saharan Africa and adjacent islands

Species name	General environment	Depth range	Habitats (southern and western Africa specific)	General habitat information
		3–14 m (Berkström et al. 2012)	Seagrass beds (*Thalassia hemprichii/Halodule wrightii* and *Thalassodendron ciliatum/Cymodocea serrulata*) in Mozambique (Almeida et al. 2001)	
			Mainly found in seagrass in Zanzibar, but also recorded in coral reefs (Berkström et al. 2012)	
Hippocampus hippocampus	Coastal	12–15 m (Kitsos et al. 2008)	No habitat data found for sub-Saharan Africa and adjacent islands	Found in habitats with less complexity and using various holdfasts (artificial structures, the small tuft-forming bryozoan *Bugula neritina*, sea urchins and small or tuft-forming macroalgae) in Portugal (Curtis & Vincent 2005, Correia et al. 2015a)
		>3 m (Caldwell & Vincent 2012)		Found in habitats with less complexity in Italy (Gristina et al. 2015)
		2 m (Spinelli et al. 2020)		*Posidonia oceanica* meadows in the Aegean Sea (Kitsos et al. 2008)
		4–13 m (Otero-Ferrer et al. 2015)		Found holding on to various holdfasts in the Canary Islands, including *Cystoseira abies-marina, Sargassum* spp. or *Asparagopsis taxiformis* and artificial structures (Otero-Ferrer et al. 2015)
Hippocampus jayakari	Coastal	2–30 m (Fricke et al. 2009)	Rubble-algae habitats and on soft bottoms on sponges in Réunion (Fricke et al. 2009)	Reef habitat in India (Parmar et al. 2015)
				Sandy beach habitat in the Red Sea (Golani & Lerner 2007)
Hippocampus kelloggi	Coastal	Up to 90 m (McPherson & Vincent 2004)	Soft bottoms and gorgonians (McPherson & Vincent 2004)	Recorded to use sponges and soft coral *Dendronephthya australis* as holdfasts in Port Stephens, Australia (Harasti 2017)

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Table 2 (*Continued*) A synthesis from the literature of the general environment, depth ranges and habitats used by syngnathid species in sub-Saharan Africa and adjacent islands

Species name	General environment	Depth range	Habitats (southern and western Africa specific)	General habitat information
		65–90 m (Choo & Liew 2003)		Associated with muddy bottoms and gorgonids (Murugan et al. 2008)
		25–30 m (Perry et al. 2020)		Sandy and rocky habitats, floating with seaweed in India (Perry et al. 2020)
		10–20 m (Murugan et al. 2008)		
		5–75 m (Balasubramanian & Murugan 2017)		
Hippocampus kuda	Coastal	<5 m (Louw Claassens unpublished data)	Seagrass beds (*Thalassia hemprichii*/*Halodule wrightii*) in Mozambique (Almeida et al. 2001)	Seagrass and macroalgae in estuaries and shallow coastal waters in Malaysia (Lim et al. 2011)
		0–4 m (McKenna & Allen 2006)	Seagrass beds in Mozambique (Louw Claassens unpublished data, Teijema 2020)	Shallow estuarine habitats and shallow reef flats in Malaysia (Choo & Liew 2003)
		1–3 m (Choo & Liew 2003)	Coral reefs in north-west Madagascar (McKenna & Allen 2006)	Seagrass, dead coral and sponges in India (Murugan et al. 2008)
		3–10 m (Murugan et al. 2008)		
Hippocampus nalu	Coastal	12–17 m (Short et al. 2020)	Flat sandstone-based coral reefs comprising low pinnacles, shallow drop-offs and sandy gullies, the latter being exposed to wave action and strong (e.g. tidal) currents. Associated with short algae turf (Short et al. 2020)	Endemic to South Africa
Hippocampus tyro	Coastal	43–48 m depth (Randall & Lourie 2009)	Found in a dredge grab with fragments of various coral species (*Stylophora pistillata*, *Montipora digitata* and *Dendrophyllia* sp.) from the Seychelles (Randall & Lourie 2009)	Endemic to Seychelles
Micrognathus andersonii	Coastal	Rock pool depths of an average of 14 cm (Sindorf et al. 2015)	Intertidally within rocky tide pools in Mozambique (Cowburn et al. 2018)	Rock or coral tide pools within vegetation, or from reef and sand flats (Dawson 1985)

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Table 2 (*Continued*) A synthesis from the literature of the general environment, depth ranges and habitats used by syngnathid species in sub-Saharan Africa and adjacent islands

Species name	General environment	Depth range	Habitats (southern and western Africa specific)	General habitat information
		0.5–1 m (Goran & Spanier 1985)	Rock pools in Mozambique (Sindorf et al. 2015)	Flat, sandy bottom habitat with flat rocks forming crevices and some coral cover in the Red Sea (Goran & Spanier 1985)
		<30 m (Moore et al. 2014)		Associated with seagrass and hard substrata in Australia (Moore et al. 2014)
		0.5 m (Randall et al. 2010)		Reef flat habitat in Tahiti (Randall et al. 2010)
		Recorded to depths of 5 m, it is most commonly found <2 m deep (Dawson 1985)		Mangroves in New Caledonia (Thollot 1996)
				Seagrass and macroalgae in Malaysia (Lim et al. 2011)
Microphis aculeatus	Freshwater	No depth data	Recorded in *Rhizophora racemosa*-dominated estuaries in Benin (Adite et al. 2013)	Endemic to West Africa
Microphis argulus	Freshwater	No depth data	Freshwater in Madagascar and Comoros (Smith 1963)	Rivers and streams (Dawson 1985)
Microphis millepunctatus	Freshwater	No depth data	Brackish estuaries and lower reaches of freshwater streams in Réunion (Fricke et al. 2009)	No global habitat data found
			Sheltered waters and estuaries (Smith 1963)	
			Inland water in Benin (Adite et al. 2013)	
Microphis fluviatilis	Freshwater	No depth data	Found in quiet water among vegetation, where they apparently adopt a head-down orientation to conceal themselves among the fronds. Can also be found in the vicinity of logs at river edge (Okeyo 1998)	Rivers (Dawson 1985)

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Table 2 (*Continued*) A synthesis from the literature of the general environment, depth ranges and habitats used by syngnathid species in sub-Saharan Africa and adjacent islands

Species name	General environment	Depth range	Habitats (southern and western Africa specific)	General habitat information
			Fresh and brackish water in East Africa (Smith 1963)	
			Enters lower reaches of rivers (Seegers et al. 2003)	
Microphis leiaspis	Freshwater	No depth data	Rivers and estuaries in Madagascar (Smith 1963)	Rivers in Japan (Ishihara & Tachihara 2009)
				Found as drifting larvae in a river in Japan (Maeda & Tachihara 2010)
				Rivers and streams, although juveniles have been observed in estuaries (Dawson 1985)
Nannocampus elegans	Coastal	5 m range (Pattrick & Strydom 2008)	Tide pools in East Africa (Smith 1963)	Rock pools and shallow reefs (Dawson 1985)
		27 m (Brian Sellick pers. comm.)	Rock pool with the vertical sides covered by a short algal turf of corallines and *Hypnea spicigera* in South Africa (Christensen & Winterbottom 1981)	Tide pools and shallow reefs (Kuiter 2009)
		< 3 m depth (Christensen & Winterbottom 1981)		
Nerophis lumbriciformis	Coastal	Intertidal (Monteiro et al. 2002, 2005, 2006)	No habitat data found for sub-Saharan Africa and adjacent islands	Intertidal (Dawson 1986)
				Rocky shores (Monteiro et al. 2005, 2006)
				Intertidal seaweeds (Monteiro et al. 2002)
				Uses rocky shore boulders as refuge during low tide period (Monteiro et al. 2002)
Nannocampus pictus	Coastal	<9 m (Dawson 1985)	Tide pool in South Africa (Brian Sellick pers. comm.)	Algal beds, reefs and coral reefs (Dawson 1985)
		1 m (Brian Sellick pers. comm.)		

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Table 2 (*Continued*) A synthesis from the literature of the general environment, depth ranges and habitats used by syngnathid species in sub-Saharan Africa and adjacent islands

Species name	General environment	Depth range	Habitats (southern and western Africa specific)	General habitat information
Penetopteryx taeniocephalus	Coastal	<1 m (Dawson 1985)	No habitat data found for sub-Saharan Africa and adjacent islands	Sampled in surface plankton samples in Indonesia (Dawson 1985)
				Gravel and coral rubble habitats (Dawson 1985)
Phoxocampus belcheri	Coastal	1–15 m (Dawson 1985)	Intertidal habitats in Mauritius (Arndt & Fricke 2019)	Tide pools and reefs (Dawson 1985)
			Intertidally associated with coral rubble and weeds in East Africa (Smith 1963)	Shallow rock pools in Japan (Murase 2015)
				Shallow coastal waters in Malaysia (Lim et al. 2011)
Siokunichthys breviceps	Coastal	< 2 m and specimens collected from surface samples were collected over 21 m (Dawson 1983)	Coral rubble in Mozambique (Dawson 1983)	Coral and coral rubble habitats – have also been found in surface samples (Dawson 1985)
		<10 m (Dawson 1985)	Coral rubble in Mozambique (Smith 1963)	
Syngnathoides biaculeatus	Coastal and estuarine	<5 m depth (Louw Claassens unpublished data)	Seagrass beds in Vilankulo, Mozambique (Louw Claassens unpublished data)	Coastal shallows. Juveniles have been recorded in offshore surface samples (Dawson 1985)
		Average depth of 3 m (Lugendo 2007)	Mangrove creeks and channels (*Rhizophora mucronata*) with a muddy substratum with prop roots as well as in *Enhalus acoroides* interrupted by small patches of *Thalassodendron ciliatum* and the calcareous algae *Halimeda* spp. in Kenya (Lugendo 2007)	Mangrove habitat in New Caledonia (Thollot 1996)

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Table 2 (*Continued*) A synthesis from the literature of the general environment, depth ranges and habitats used by syngnathid species in sub-Saharan Africa and adjacent islands

Species name	General environment	Depth range	Habitats (southern and western Africa specific)	General habitat information
		<1 m (Mirriam 2010)	Mangrove creek (*Rhizophora mucronata* and *Avicennia marina*) in Kenya (Mirriam 2010)	Shallow coastal seagrass habitats. Juveniles can occur in offshore surface waters (Dawson 1985)
		4–7 m depths (Gajdzik et al. 2014)	Mangrove and seagrass habitats in Kenya (Van der Velde et al. 1995)	*Enhalus acoroides*-dominated seagrass bed in Japan (Nakamura et al. 2003)
		3–14 m (Berkström et al. 2012)	*Sonneratia alba* mangroves in Gazi Bay, Kenya. Juveniles found in estuaries; adults found further upstream (Crona & Rönnbäck 2007)	*Zostera capricorni*, with interspersed *Halophila ovalis* and *H. spinulosa*, seagrass beds from the east coast of Australia (Takahashi et al. 2003)
		5–6 m depths (Almeida et al. 2001)	Seagrass beds (*Thalassodendron ciliatum*/*Cymodocea serrulata*) in Mozambique (Almeida et al. 2001)	*Thalassia hemprichii*-dominated seagrass beds (within close proximity to mangroves) in Papua New Guinea (Barrows et al. 2009)
		2–5 m (Gell & Whittington 2002)	Mangroves (*Rhizophora mucronata* and *Ceriops tagal*) in Kenya (Gajdzik et al. 2014)	Associated with *Zostera muelleri* and *Caulerpa taxifolia* in Australia (Burfeind et al. 2009)
		20 m (Sanaye et al. 2016)	Seagrass beds dominated by *Enhalus acoroides*, *Thalassodendron ciliatum* and *Cymodocea serrulata* in Mozambique (Gell & Whittington 2002)	
		0.5–2 m (Nakamura et al. 2003)	Seagrass in Zanzibar, but also recorded in coral reefs (Berkström et al. 2012)	
		0.5–1 m (Takahashi et al. 2003)	Mainly found in weeds in East Africa (Smith 1963)	
Syngnathus acus	Coastal	<1 m (Gurkan et al. 2009)	No habitat data found for sub-Saharan Africa and adjacent islands	Found within *Zostera marina* eelgrass in Sweden (Goncalves et al. 2011)

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Table 2 (*Continued*) A synthesis from the literature of the general environment, depth ranges and habitats used by syngnathid species in sub-Saharan Africa and adjacent islands

Species name	General environment	Depth range	Habitats (southern and western Africa specific)	General habitat information
		10–90 m (Yildiz et al. 2015)		Found within eelgrass beds (*Zostera marina* and *Z. noltii*) in Portugal (Costa et al. 1994)
Syngnathus temminckii	Coastal and estuarine	<1 m (Harrison 1999)	*Zostera capensis* in estuaries in South Africa (Bekcley 1984, Hanekom & Baird 1984, Ter Morshuizen & Whitfield 1994, Becker et al. 2012, Ntshudisane et al. 2021) *Spartina maritima* in South Africa (Nel et al. 2018) *Cladophora* sp. in the Kleinemonde estuary and *Ruppia* sp., *Potamogeton* sp., Chara sp. and Cladophora sp. in the Bot estuary, South Africa (Bennett 1989) Surf zone of nearby South African estuaries (Strydom 2003) *Zostera capensis* and Codium spp. in the Bushmans and Kariega estuaries, South Africa (Paterson & Whitfield 2000, Claassens et al. 2021)	Endemic to southern Africa
Syngnathus watermeyeri	Estuarine	1–2 m (Cowley & Whitfield 2001)	Associated with predominantly open estuaries (Harrison & Whitfield 2006) *Ruppia cirrhosa* in Kleinemonde East estuary, South Africa (Cowley & Whitfield 2001)	Endemic to South Africa

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Table 2 (*Continued*) A synthesis from the literature of the general environment, depth ranges and habitats used by syngnathid species in sub-Saharan Africa and adjacent islands

Species name	General environment	Depth range	Habitats (southern and western Africa specific)	General habitat information
			Codium spp. in the Bushmans and Kariega estuaries in South Africa (Claassens et al. 2021)	
			Zostera capensis habitats in estuaries in South Africa (Vorwerk et al. 2007, Whitfield et al. 2017, Ntshudisane et al. 2021, Claassens et al. 2021)	
Trachyrhamphus bicoarctatus	Coastal	2–42 m (Dawson 1985)	Reefs in Madagascar (Fordyce 2016)	Sand, rubble, reef and grass habitats (Dawson 1985)
		Shallows to 40 m (Ali et al. 2020)	Seagrass beds in Vilankulo, Mozambique (Louw Claassens unpublished data)	Muddy and sandy estuaries and bays in Yemen (Ali et al. 2020)
		15–22 m (Alain Rassat pers. comm.)	Sandy habitats in Madagascar (Alain Rassat pers. comm.)	
			Sandy habitat among sea pens in Mozambique (Georgina Jones pers. comm.)	
Trachyrhamphus longirostris	Coastal	16–91 m (Dawson 1985)	No habitat data found for sub-Saharan Africa and adjacent islands	Only recorded from deep, offshore habitats (Dawson 1985)

General habitat information for species that occur outside of Africa is also provided.

submerged vegetation is essential for their occurrence. For example, *Syngnathus watermeyeri* is dependent on available submerged vegetation habitats, such as *Ruppia cirrhosa* (Sheppard et al. 2011).

Only two species, *Hippocampus capensis* and *Syngnathus watermeyeri*, are found exclusively in estuaries along the south coast of South Africa (Bell et al. 2003, Lockyear et al. 2006, Vorwerk et al. 2007, Whitfield et al. 2017). Estuarine species can withstand high variability of environmental conditions, specifically salinity. For example, *Hippocampus capensis* can tolerate salinities of 1–59 on the Practical Salinity Scale (Riley 1986). At least six estuarine species occur in mangroves along the east coast and adjacent islands of Africa (*H. histrix*, *H. kuda*, *Choeroichthys sculptus*, *C. amplexus*, *Hippichthys spicifer* and *Microphis aculeatus*) (Van Der Velde et al. 1995, De Boer et al. 2001, Crona & Rönnbäck 2007, Lugendo 2007, Huxham et al. 2008, Mwandya et al. 2009, Weis et al. 2009, Mirriam 2010, Berkström et al. 2012, Mwandya 2019).

Several species, such as *Enneacampus* spp., *Microphis* spp. and *Hippichthys* spp., move up into rivers from estuaries (Skelton et al. 1989, Okeyo 1998, Weerts & Cyrus 2002, Seegers et al. 2003, Harrison & Whitfield 2006, Crona & Rönnbäck 2007, Fricke et al. 2009). *Hippichthys cyanospilos*, *H. spicifer* and *Microphis leiaspis* are amphidromous, and adults of these species are usually associated with freshwater streams and estuaries across the Indo-Pacific (Milton 2009). The migratory seasons for these species are not known, but migration is likely to be timed with wet and dry seasons (Milton 2009). Members of the genera *Enneacampus* and *Microphis* are truly freshwater species and occur in inland river systems in Kenya, Benin, Republic of the Congo, Lower Guinea and Angola (Okeyo 1998, Seegers et al. 2003, Adite et al. 2013, Walsh et al. 2014, Skelton 2019).

Many syngnathids, particularly seahorses, have been found globally to use artificial structures (Harasti et al. 2010, Gristina et al. 2015, Correia et al. 2015b, Otero-Ferrer et al. 2015, Claassens 2016, Gristina et al. 2016, Claassens & Hodgson 2018b). In some instances, artificial structures have been used for the conservation of syngnathid species (Hellyer et al. 2011, Correia et al. 2013, Simpson et al. 2020), and two species of seahorses (*Hippocampus capensis* and *H. whitei*) have been found to actively choose artificial habitat (seahorse hotels and Reno Mattresses) over natural seagrass habitats (Claassens et al. 2018, Simpson et al. 2019). In Mozambique, *Doryrhamphus bicarinatus* inhabits concrete blocks deployed as artificial reefs in seagrass habitats (Louw Claassens unpublished data), and both *Dunckerocampus boylei* and *D. multiannulatus* were found on rigid buoys used as FAD structures in Mauritius (Forget et al. 2020), as well as on the legs of oil rigs in the Red Sea (Rilov & Benayahu 1998).

Population parameters

Limited data are available on syngnathid populations in sub-Saharan Africa and adjacent islands (Table 3). Since most ichthyological studies have not been specifically designed to focus on syngnathids, sampling approaches and equipment utilised may not have been effective in detecting them. Abundance and density data for syngnathids in the region are therefore probably underestimates owing to the non-targeted nature of most of the research. Approaches used in the studies that recorded syngnathids include various netting methods (seine nets, fyke nets, trawl nets and plankton nets) (Whitfield 1989, Harris et al. 1995, 1999, Almeida et al. 2001, Lugendo 2007, Vorwerk et al. 2007, Pattrick & Strydom 2008, O'Brien et al. 2009, Mirriam 2010, Mwaluma et al. 2010, Ntshudisane et al. 2021), light trapping (Jaonalison et al. 2016) and underwater visual surveys (Van Der Velde et al. 1995, Bell et al. 2003, Lockyear et al. 2006, Ory 2008, Pinault et al. 2013, Claassens & Hodgson 2018b, Daly et al. 2018, Forget et al. 2020) (Table 3).

Local population information exists for 37% (22 species) of African syngnathid species with seine netting being the most frequently used survey method. The most available population abundance data are for *Syngnathus temminckii* with a total of 20 studies, followed by *Hippocampus*

Table 3 Available quantitative population data on syngnathids that occur in sub-Saharan Africa and adjacent islands

Species	Country	Location	Sample approach/ gear type	Total abundance	Density	Other measures	Reference
Choeroichthys sculptus	Madagascar	Nosy Ve island and Great Reef of Toliara	Light trap	13			Jaonalison et al. (2016)
Corythoichthys amplexus	Kenya	Mombasa and Watamu	Plankton net		1.1; 0.2; 29.4 fish/100 m²		Mwaluma et al. (2010)
	Kenya	Gazi Bay	Stake net	1			Huxham et al. (2008)
Corythoichthys flavofasciatus	Madagascar	Nosy Ve island and Great Reef of Toliara	Light trap	6			Jaonalison et al. (2016)
Corythoichthys intestinalis	Madagascar	Ankilibe Bay	Underwater visual census	14	Mean (SE) density of 0.9 (0.6) per transect		Ory (2008)
Doryrhamphus bicarinatus	Réunion	Piton de la Fournaise	Underwater visual census			0–0.08 occurrence (number of observations per total number of stations)	Pinault et al. (2013)
Dunckerocampus boylei	Mauritius	Not given	Underwater visual census			10% Frequency of occurrence	Forget et al. (2020)
	Seychelles	Not given	Underwater visual census	1			Daly et al. (2018)
Dunckerocampus multiannulatus	Réunion	Saint-Leu	Photographic survey	1			Tea et al. (2020)
	Mauritius	Not given	Underwater visual census			10% Frequency of occurrence	Forget et al. (2020)
Hippichthys cyanospilos	Kenya	Gazi Bay	Net pen	13			Crona & Rönnbäck (2007)
Hippichthys heptagonus	South Africa	St Lucia	Plankton net	11	0.05 mean density/100 m³		Harris et al. (1999)
	South Africa	Kosi Bay	Plankton net	1			Harris et al. (1995)
Hippichthys spicifer	Kenya	Gazi Bay	Fyke net, underwater visual census, trawl net	1			Van der Velde et al. (1995)

(*Continued*)

Table 3 (*Continued*) Available quantitative population data on syngnathids that occur in sub-Saharan Africa and adjacent islands

Species	Country	Location	Sample approach/ gear type	Total abundance	Density	Other measures	Reference
	South Africa	Mngazi and Mngazana estuaries	Seine net			Large seine net: 0.3 catch per unit effort; Fry seine net: 0.1 catch per unit effort	Mbande (2003)
	Zanzibar	Chwaka Bay	Seine net		0.4 pipefish/km^2		Lugendo (2007)
	Kenya	Tutor	Seine net	2			Mirriam (2010)
	South Africa	Umvoti estuary	Seine net, fyke net, gill net, cast net, electrofishing	1			O'Brien et al. (2009)
Hippocampus algiricus	Senegal and The Gambia	Not given	Surveys	205			Cisneros-Montemayor et al. (2016)
	Senegal	Not given	Surveys	35			West (2012)
Hippocampus camelopardalis	Mozambique	Inhaca Island	Beam trawl	10			Almeida et al. (2001)
Hippocampus capensis	South Africa	Swartvlei Estuary	Hand collected	3000 dead seahorses			Russell (1994)
	South Africa	Swartvlei Estuary	Plankton net	4061 juveniles exiting the estuary, 205 juveniles entering the estuary			Whitfield (1989)
	South Africa	Knysna Estuary	Scoop net		0.33 (± 0.03) and 0.23 (± 0.03) seahorses per kg of *C. tenue*		De Villiers et al. (2019)
	South Africa	Swartvlei Estuary	Seine net and hand collected	78 live specimens, 371 dead specimens			Arendse & Russell (2020)
	South Africa	Knysna Estuary	Underwater visual census	44	0–0.25 seahorses/m^2; mean=0.0089 m^2		Bell et al. (2003)
	South Africa	Knysna Estuary	Underwater visual census		Reno mattress 0·26±0·02 seahorses/m^2 Vegetation: 0·01±0·002 to 0·06±0·01 seahorses/m^2		Claassens & Hodgson (2018b)

(*Continued*)

Table 3 (*Continued*) Available quantitative population data on syngnathids that occur in sub-Saharan Africa and adjacent islands

Species	Country	Location	Sample approach/ gear type	Total abundance	Density	Other measures	Reference
	South Africa	Knysna Estuary	Underwater visual census	279 in Knysna, 71 in Swartvlei, 102 in Keurbooms			Lockyear et al. (2006)
	South Africa	Knysna Estuary	Underwater visual census and scoop net	*Z. capensis* habitat: 23; Reno mattress habitat: 100–182; *Codium tenue* habitat: 82–68			Claassens (2016)
	South Africa	Knysna Estuary	Underwater visual survey	135–75			Claassens & Harasti (2020)
Hippocampus hippocampus	Senegal and The Gambia	Not given	Surveys	14			Cisneros-Montemayor et al. (2016)
	Senegal	Not given	Surveys	2			West (2012)
Hippocampus histrix	Mozambique	Inhaca Island	Beam trawl	1			Almeida et al. (2001)
	Kenya	Gazi Bay	Fyke net, underwater visual census, trawl net	2			Van der Velde et al. (1995)
	Zanzibar	Chwaka Bay	Seine net		0.1 seahorses/km^2		Lugendo (2007)
Hippocampus kuda	Mozambique	Inhaca Island	Beam trawl	1			Almeida et al. (2001)
Micrognathus andersonii	Kenya	Watamu	Quadrats	1			Sindorf et al. (2015)
Microphis aculeatus	Benin	Not given	Seine net, gill net	5			Adite et al. (2013)
	Nigeria	Not given	Survey			200 fish in a box; \$1 unit price	Ukaonu et al. (2011)
	Côte d'Ivoire	Dodo River		1			Kamelan et al. (2013)
Nannocampus elegans	South Africa	Algoa Bay	Plankton net	1	0.01 (range 0.0–0.06)		Pattrick & Strydom (2008)
	South Africa	Port Alfred	Rotenone			2% of the total sample	Bennett (1987)

(*Continued*)

Table 3 (*Continued*) Available quantitative population data on syngnathids that occur in sub-Saharan Africa and adjacent islands

Species	Country	Location	Sample approach/ gear type	Total abundance	Density	Other measures	Reference
	South Africa	Kleinemonde East	Rotenone	1			Christensen & Winterbottom (1981)
Syngnathoides biaculeatus	Mozambique	Inhaca Island	Beam trawl	35			Almeida et al. (2001)
	Kenya	Mida Creek	Fyke net	2			Gajdzik et al. (2014)
	Kenya	Gazi Bay	Fyke net, underwater visual census, trawl net	20			Van der Velde et al. (1995)
	Kenya	Gazi Bay	Net pen (Stake net?)	13			Crona & Rönnbäck (2007)
	Zanzibar	Chwaka Bay	Seine net		0.5; 02; 0.4 pipefish/km²		Lugendo (2007)
	Kenya	Tutor	Seine net	1			Mirriam (2010)
Syngnathus temminckii	South Africa	Swartvlei Estuary	Plankton net	71,000 juveniles exiting the estuary			Whitfield (1989)
	South Africa	St Lucia	Plankton net	11			Harris et al. (1999)
	South Africa	Algoa Bay	Plankton net	3	0.02 (range 0.0–0.07)		Pattrick & Strydom (2008)
	South Africa	Bushmans Estuary	Seine net	5			Ntshudisane et al. (2021)
	South Africa	Bot Estuary	Seine net		10–1 pipefish/seine net haul		Bennett (1989)
	South Africa	Bot Estuary	Seine net		0.008 pipefish/m^2		Bennett & Branch (1990)
	South Africa	Kleinemonde Estuary	Seine net		0.2 pipefish/seine net haul		Bennett (1989)
	South Africa	False Bay	Seine net	2			Clark et al. (1996)
	South Africa	Bot Estuary	Seine net	17			Harrison (1999)
	South Africa	Klein Estuary	Seine net	42			Harrison 1999
	South Africa	Kariega Estuary	Seine net		Average of 0.05 pipefish/10 m^2		Paterson & Whitfield (2000)
	South Africa	Mngazi Estuary	Plankton net	2			Pattrick et al. (2007)
	South Africa	Kariega Estuary	Otter trawl	6			Richardson et al. (2006)

(*Continued*)

Table 3 (*Continued*) Available quantitative population data on syngnathids that occur in sub-Saharan Africa and adjacent islands

Species	Country	Location	Sample approach/ gear type	Total abundance	Density	Other measures	Reference
	South Africa	Gamtoos Estuary	Plankton net	11			Strydom & Wooldridge (2005)
	South Africa	Keurbooms Estuary	Seine net	3			James & Harrison (2010a)
	South Africa	Kariega Estuary	Seine net	4			James & Harrison (2010b)
	South Africa	Gamtoos Estuary	Plankton net	11			Strydom & Wooldridge (2005)
	South Africa	Touw Estuary, Eilandvlei, Rondevlei	Seine net		Relative abundance: Touw Estuary 0.1/36 seine nets, Eilandsvlei 0.02/24 seine nets Rondevlei 0.02 /13 seine nets		Olds et al. (2016)
	South Africa	Various estuaries	Plankton net		Cool temperate estuaries: 23.03 pipefish/100 m^3, warm temperate estuaries: 6.65 pipefish/100 m^3, transition zone estuaries: 7.75 pipefish/100 m^3		Strydom (2015)
	South Africa	Kromme estuary	Seine net	22			Hanekom & Baird (1984)
	South Africa	Nxaxo-Ngqusi Estuary	Plankton net		Average density 2.5 (range 0–32.3) pipefish/100 m^3		Wasserman et al. (2010)
	South Africa	Kabeljous Estuary	Seine net	1			Strydom (2003)
	South Africa	Kariega Estuary	Seine net		0.01–0.07 pipefish/m^2		Ter Morshuizen & Whitfield (1994)
Syngnathus watermeyeri	South Africa	Bushmans Estuary	Seine net	5			Ntshudisane et al. (2021)
	South Africa	Kleinemonde East	Seine net	43			Cowley & Whitfield (2001)
	South Africa	Kariega Estuary	Seine net	1 in Kariega in 2013; 55 in Kleinemonde East			Whitfield et al. (2017)
	South Africa	Kariega Estuary	Seine net	20			Vorwerk et al. (2007)

capensis with nine studies. Studies on *Syngnathus temminckii* were, however, mostly part of general fish surveys, conducted once off and using seine nets. In contrast, the population studies on *Hippocampus capensis* were conducted using underwater visual surveys (Bell et al. 2003, Lockyear et al. 2006, Claassens & Hodgson 2018b) and on a monthly basis (Claassens 2016, Claassens & Hodgson 2018b, Claassens & Harasti 2020). A comparison of population data across studies, even for the same species, is difficult owing to the different approaches, methods and the sampling effort used during sampling (Table 3).

Most syngnathids have small home-ranges (Vincent & Giles 2003, Harasti et al. 2014). For example, in the only research investigating home-ranges for syngnathids in sub-Saharan Africa and adjacent islands, individuals of *H. capensis* were found to move an average of only 5 m over a 13-month period (Claassens & Harasti 2020). Small home-ranges and a limited ability to disperse can increase the vulnerability of syngnathid populations. For example, a major flood event in the Kleinemonde East estuary, South Africa, in 2003, resulted in the local extinction of *Syngnathus watermeyeri* in this estuary (Cowley & Whitfield 2001, Sheppard et al. 2011, Whitfield et al. 2017).

Syngnathids usually disperse as juveniles, with the duration of the juvenile stage dependent on the species (Kendrick & Hyndes 2003, Bertola et al. 2020). Whitfield (1989) observed high numbers of juvenile *Hippocampus capensis* and *Syngnathus temminckii* wash in and out of the Swartvlei estuary in South Africa, most likely as a means of dispersal. Another mode of dispersion is by attaching to drifting algae (Howard & Koehn 1985, Teske et al. 2005, Kuiter 2009). *Choeroichthys sculptus*, *Hippichthys cyanospilos*, *H. spicifer*, *Micrognathus andersonii*, *Hippocampus kuda*, *Syngnathoides biaculeatus*, *Trachyrhamphus bicoarctatus* and *T. longirostris* have all been associated with drifting algae (including *Sargassum* spp.) in Japan (Ohta & Tachihara 2004, Nishida et al. 2008), and it is possible that these species also use drifting algae as a means to disperse in African waters.

Life History

There is a dearth of data on the reproductive ecology of syngnathids within African waters. Most information is from studies in South Africa that have focused on the breeding ecology and behaviour of *Hippocampus capensis* (Grange & Cretchley 1995, Lockyear et al. 1997), *Syngnathus temminckii* and *S. watermeyeri* (Mwale et al. 2014, Whitfield et al. 2017). *Syngnathus watermeyeri* has low fecundity with small brood sizes (about 44 embryos per male; Whitfield 1995b), whereas *S. temminckii* has high fecundity and larger brood sizes (200–500 eggs in a brood pouch; Branch 1966, Mwale et al. 2014, Whitfield et al. 2017). These differences in reproduction are probably one of the reasons for the differences in vulnerability between these two species (Whitfield et al. 2017). The number of offspring produced by *Hippocampus capensis* is highly variable and was found to range from 25 to 60 (Grange & Cretchley 1995) and from 7 to 95 (Lockyear et al. 1997). Larger seahorses have greater reproductive potential (Foster & Vincent 2004), which has also been found for *Syngnathus temminckii*, which has an adult size range of 10–13 cm, and in which larger pipefish produce more embryos (Mwale et al. 2014).

Many syngnathid species form pair bonds (Rosenqvist & Berglund 2011, Brandl & Bellwood 2014) and some species are monogamous within at least a single breeding season (Vincent 1995, MasonJones & Lewis 1996), whilst *Hippocampus whitei* has been found to display long-term monogamy (Harasti et al. 2012). Monogamy has not been established for syngnathid species in African waters. However, Mwale et al. (2014) found that in *Syngnathus temmincki*, the number of eggs produced by a female was not statistically different from the number of embryos brooded by the male. This suggests that a male only mates with one female. Whether this applies to other southern African pipefishes is unknown.

Sex ratios in syngnathids are usually equal (Perante et al. 2002, Moreau & Vincent 2004, Smith et al. 2012). Female-biased sex ratios have, however, been noted for *Hippocampus hippocampus* in the Macaronesia Islands, specifically in artificial habitats (Otero-Ferrer et al. 2015); in *H. erectus* in Chesapeake Bay (Teixeira & Musick 2001); and Kvarnemo et al. (2007) found a wild population of *H. subelongatus* to be female biased with stronger sexual selection on females, a contradiction to the normal male sexual selection found in monogamous species (Vincent 1994a, b, Naud et al. 2009). Information on sex ratios for *H. capensis* varies. In 2000, a transect survey indicated male bias, but equal numbers of seahorses were recorded using a focal grid method (Bell et al. 2003). In 2001, Lockyear et al. (2006) also recorded a 1:1 sex ratio from transect surveys. During surveys between 2014 and 2017, the sex ratio varied across habitats and seasons and changed from being equal to female biased (Claassens 2016, Claassens & Hodgson 2018b). In *Codium tenue* habitats, the sex ratio for *Hippocampus capensis* remained equal over an 18-month period (De Villiers et al. 2019). Both *Syngnathus temminckii* and *S. watermeyeri* were found to have female-biased sex ratios, but only significantly so in *S. temminckii* (Mwale et al. 2014).

Extravagant courting behaviour is a common phenomenon in syngnathids (Vincent 1995). One of the most well-known courting behaviours are morning greetings, mostly considered as a means to confirm pair bonds (Vincent et al. 2005), which has been shown for *Hippocampus capensis* (Claassens & Hodgson 2018a). In Japan, the monogamous pipefish *Corythoichthys haematopterus* recognised its specific partner, with morning greetings only done with existing partners (Sogabe 2011). In addition, morning greetings were observed throughout the non-breeding season as well, which suggests that this pipefish maintains its pair bonds (Sogabe & Yanagisawa 2008).

Breeding seasons vary for different syngnathid species and are linked with seasons and weather patterns (Kendrick & Hyndes 2003). The breeding season for *Syngnathus temminckii* was thought to be limited to spring and summer (Bennett 1989), but breeding was found throughout the year except during April and May (Mwale et al. 2014). *Syngnathus temminckii* also exhibits lekking behaviour similar to the worm pipefish *Nerophis lumbriciformis* (Monteiro et al. 2017), whereby female individuals gather in temporary groups at a particular area to display their ornamentation to attract males for the sole purpose of mate choice (Georgina Jones pers. comm.; Figure 3). *Syngnathus watermeyeri* breeds in all seasons, except winter (Mwale et al. 2014). *Hippocampus capensis* breeds during spring and summer and courtship behaviour and mating occurs throughout the breeding season and the average gestation period for this species is 34 days (Lockyear et al. 1997). This means

Figure 3 *Syngnathus temminckii* aggregation in False Bay, South Africa, as part of lekking behaviour (photo: Georgina Jones).

that males can produce more than one brood during the breeding season. *Microphis aculeatus* has a gestation period that ranges between one to three weeks and gives birth to juveniles that are 1.5 cm long (Snoeks & Vreven 2008).

Feeding, prey and predators

Syngnathids have a long snout, fused jaws, lack teeth and use suction feeding to consume their prey whole in a few milliseconds (Leysen et al. 2010). Suction feeding has required the evolution of musculoskeletal specializations of the head and snout. To date, the anatomy of the feeding apparatus of species from Africa has been briefly described by Branch (1966) for *Syngnathus temminckii* (as *S. acus*) and in more detail for *Hippocampus capensis* (Leysen et al. 2010). Like other syngnathids, the snouts are composed of neurocranial and suspensorial bones in which suction feeding is accomplished by hyoid retraction followed by powerful neurocranial elevation (Leysen et al. 2010, Manning et al. 2019).

Even though there are almost no data available on the feeding behaviour of syngnathids in sub-Saharan Africa and adjacent islands, general information from a recent global review of syngnathid feeding and predation is applicable to African species (Manning et al. 2019). Syngnathids are regarded as ambush predators (Manning et al. 2019), although they will actively swim to seek prey (James & Heck 1994). Such hunting behaviour in the wild has been recorded for *Hippocampus capensis* using small action cameras (Claassens & Hodgson 2018a). Syngnathids mostly feed on small crustaceans, such as amphipods, copepods and isopods (Tipton & Bell 1988, Teixeira & Musick 2001, Woods 2002, Kendrick & Hyndes 2005, Castro et al. 2008, Yip et al. 2015). In addition, some species feed on nematodes (Castro et al. 2008) and fish larvae (Didenko et al. 2018). According to Kendrick & Hyndes (2005), syngnathids with longer snouts tend to feed on more mobile prey, whilst those with shorter snouts feed on slower moving, benthic prey. Copepods (specifically *Pseudodiaptomus hessei*) and amphipods were found to be dominant in the gut content analyses of *Syngnathus temminckii* in the Bot estuary, South Africa (Bennett 1989, Bennett & Branch 1990) and only macrurans were found in the gut of *S. temminckii* in the Kromme estuary, South Africa (Hanekom & Baird 1984). According to Bennett & Branch (1990), *S. temminckii* was the most specialised feeder of all resident fish species assessed in the Bot estuary. In addition, ontogenetic changes in prey were evident, with smaller juveniles mostly feeding on copepods and larger adults on amphipods (Bennett 1989). *Hippocampus capensis*, which has a short snout, was found to hunt actively and feed primarily on mobile epibenthos (Claassens & Hodgson 2018a). A recent study using faecal eDNA to determine the prey composition of *Syngnathus watermeyeri* and *S. temminckii* (Ntshudisane et al. 2021) found a distinct difference in the types of prey these two species feed on. The long-snouted *S. temminckii* feeds mostly on gastropod and decapod crustacean larvae, whilst the short-snouted *S. watermeyeri* mostly feeds on copepods. One reason for this difference could be the difference in gape size of these two species (*S. temminckii* has a larger body, with a wider gape size and thus an ability to feed on larger prey) (Whitfield et al. 2017, Ntshudisane et al. 2021).

There is very little information on predators of syngnathids, and it is suggested that predation is mostly opportunistic (Kleiber et al. 2011). Cape cormorant *Phalacrocorax capensis* and the grey heron *Ardea cinera* are known to prey on *Hippocampus capensis* (https://www.youtube.com/watch?v=UyCW36HRgN4; https://www.youtube.com/watch?v=pORa8DKGcgk) (Figure 4A). Twenty *H. capensis* were found within the stomach of a spotted grunter *Pomadasys commersonnii* in the Keurbooms estuary (Figure 4B), and according to Smith (1963), *Hippocampus capensis* is eaten by some other fishes. *Syngnathus temminckii* has been recorded in the diet of the African penguin *Spheniscus demersus* in Algoa Bay (Randall & Randall 1986), bluefish *Pomatomus saltatrix* (Bennett 1989) and the Cape cormorant (Duffy et al. 1987). *Syngnathus temminckii* is preyed on by the klipvis *Clinus superciliosus* in False Bay (Georgina Jones pers. comm.; Figure 4C).

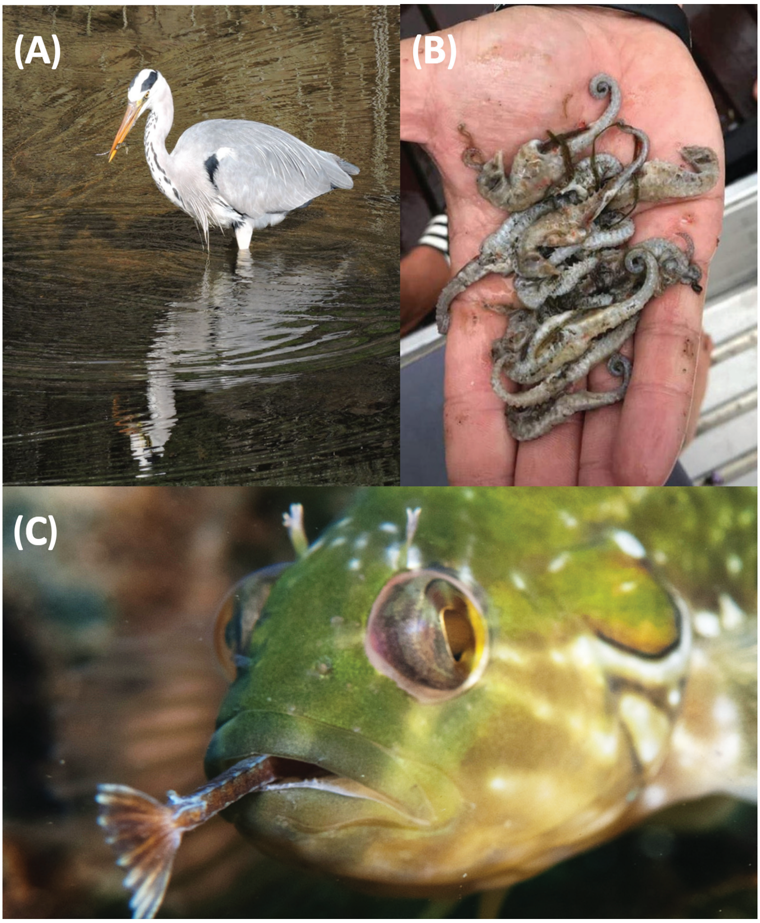

Figure 4 Examples of predation: (A) *Hippocampus capensis* in the bill of a grey heron; (B) *H. capensis* from the gut of a spotted grunter, *Pomadasys commersonnii*; (C) *Syngnathus temminckii* in the mouth of a klipvis, *Clinus superciliosus*.

Behaviour

Globally, only a handful of studies exist on the behaviour of syngnathids in the wild (MasonJones & Lewis 1996, Naud et al. 2009, Freret-Meurer et al. 2012, Harasti & Gladstone 2013), and there has been only one behavioural study in Africa, in which *Hippocampus capensis* was observed in the wild using small waterproof action cameras (i.e. GoPros) (Claassens & Hodgson 2018a). That study found that *H. capensis* was more active during the morning than midday or late afternoon and spent >80% of the active period hunting (Claassens & Hodgson 2018a). In addition, a decrease in seahorse activity during the holiday season was linked to an increase in boat noise (Claassens & Hodgson 2018a). Impacts of anthropogenic noise on seahorse behaviour have also been found in *Hippocampus erectus* (Anderson et al. 2011) and *H. guttulatus* (Palma et al. 2019).

An unusual form of behaviour is seen in *Microphis fluviatilis* in which a head-down vertical orientation is adopted in quiet water among vegetation (Okeyo 1998), which could be a means of camouflage.

Syngnathids are known for their extremely cryptic behaviour and ability to blend in with their surrounding environment (Kuiter 2009), which limits their detection by divers or researchers. For example, *Nannocampus elegans* was not detected in a visual census survey of a rock pool in

South Africa, but was found when the poison rotenone was used in the same pool (Christensen & Winterbottom 1981).

Threats and conservation

Of the 63 syngnathid species that occur in Africa, 41 are listed as Least Concern, four as Vulnerable (*Hippocampus kelloggi*, *H. kuda*, *H. histrix* and *H. algiricus*), one as Endangered (*H. capensis*) and one as Critically Endangered (*Syngnathus watermeyeri*) on the IUCN Red List (IUCN 2021). Almost 20% (12 species) are listed as Data Deficient, and four species (*Hippocampus nalu*, *Syngnathus temminckii*, *Cylix* sp. and *Hippocampus borboniensis*) have not been assessed. Limited current and regional data were used for most of the assessments, owing to the dearth of available syngnathid-focused research in Africa.

Threats

It is important to correctly identify threats that adversely affect a species (Figure 5). If a clear understanding of what threatens a species is not known or threats are misidentified, effective conservation

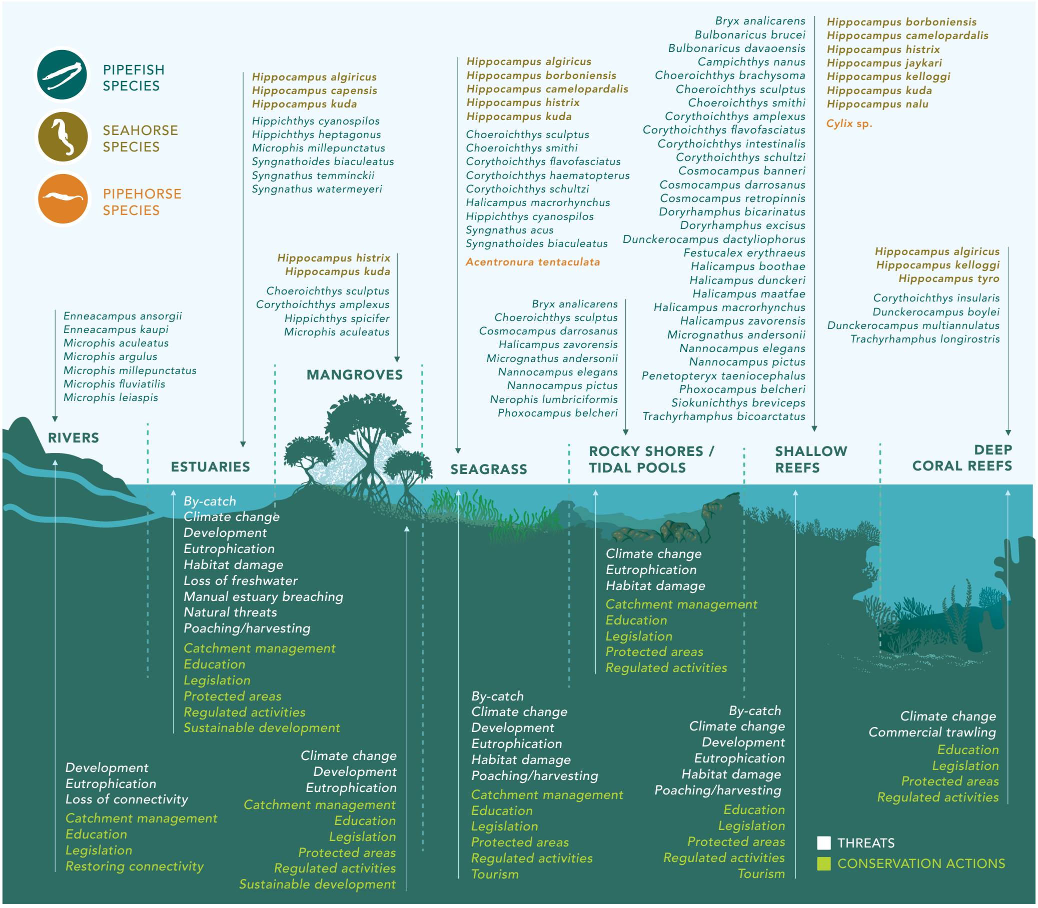

Figure 5 The distribution of syngnathid species found in sub-Saharan African and adjacent islands across their habitat ranges with a summary of habitat-specific threats and conservation actions.

actions are unlikely. Vincent et al. (2011) identified three types of threats to seahorses: (1) targeted fishing, (2) incidental capture (by-catch) and (3) habitat loss and alteration. These threats, which apply to all syngnathids, are reviewed here in the context of species that occur in Africa. The particular types of threats vary by habitat (Figure 5). For example, *Microphis* spp. are euryhaline and range from estuaries to the upper catchment of rivers (Seegers et al. 2003, Fricke et al. 2009, Weerts et al. 2014, Cutler et al. 2020). The loss of connectivity in these systems owing to dam or weir construction thus poses a direct threat to these species and their movement patterns, possibly impeding their migration upstream and downstream between rivers and the ocean (Cyrus 2001, Weerts et al. 2014, Cutler et al. 2020). Species that occur in seagrass beds face different threats than those found in deeper reef habitats. Species commonly found in seagrass beds are likely to be more vulnerable to by-catch in artisanal fisheries, impacts from recreational boating, and poaching, whereas species on deeper reefs are more vulnerable to commercial fishing (Louw Claassens pers. obs.).

Targeted fishing and trade

Syngnathids, specifically seahorses, are targeted and used for traditional Chinese medicine, the aquarium trade and curios (McPherson & Vincent 2004, Martin-Smith & Vincent 2006, Vincent et al. 2011, Stocks et al. 2017). Between 2004 and 2011, CITES reported an estimated 5.6 million seahorses were taken annually and traded internationally (Foster et al. 2016). The majority of CITES-reported seahorse trade consisted of dried specimens (98%), with a limited number (11,600 seahorses) traded live for aquaria (Foster et al. 2016). Interestingly, most seahorses in the dried trade originate from wild populations, whilst live seahorses are mostly sourced from captive breeding facilities (Foster et al. 2016). According to CITES trade data, between 2004 and 2011, *Hippocampus algiricus* made up 5.6% of total annual trade and was only sourced from wild populations along the west coast of Africa. *Hippocampus camelopardalis* and *H. capensis* made up less than 1% of annual catches, were only found in the live seahorse trade and were sourced from captive populations (Foster et al. 2016). This information is, however, questionable, because *H. capensis* is a protected species in South Africa under national legislation (see *Legislation, global agreements and regulations* section below), and all captive breeding of this species is heavily regulated and permitted in only two aquariums (Two Oceans Aquarium in Cape Town, uShaka Aquarium in Durban) and at the Garden Route National Park in Knysna. *Hippocampus histrix* and *H. kuda* made up 2.7% and 6.2%, respectively, and were used in both the dried and live trade (Foster et al. 2016).

In a recent assessment of the seahorse trade from Africa to Asia between 2008 and 2018, major discrepancies were found between total import (15,772,838) and export (11,259,098 individuals) figures (Louw & Burgener 2020). From 2004 to 2011, Senegal and Guinea were in the top three countries globally to export seahorses (Foster et al. 2016). Three African countries (Togo, Guinea and Senegal) reported seahorse exports between 2008 and 2018, and Senegal was the major exporter of seahorses during this time (Louw & Burgener 2020). However, recorded exports from these countries are less than reported imports in receiving countries, and these discrepancies highlight the limitations of CITES data to account for all trade that is taking place (Louw & Burgener 2020), especially in instances when trade is done illegally. For example, dried specimens of *H. camelopardalis,* illegally poached, probably for use in Chinese traditional medicine, have been confiscated in Mozambique en route to Asia in 2018 and 2021 (Louw Claassens unpublished data).

Between 2008 and 2018, Hong Kong was the sole importer of African seahorses, according to import records, though export records show that seahorses were also exported to mainland China and Taiwan (Louw & Burgener 2020). *Hippocampus algiricus* was the main seahorse species recorded in all African trade and was exclusively sourced from wild populations along the west coast of Africa (Louw & Burgener 2020). According to Cisneros-Montemayor et al. (2016), *H. algiricus* is one of the most traded seahorse species in the world, with an estimated annual export of 700,000 animals. It is important to note here that CITES data do not account for any animals that are captured and traded domestically.

Not much information is available for capture and trade of southern and western African pipefish and pipehorses. *Syngnathoides biaculeatus* is considered the most heavily exploited pipefish globally and is commonly used in traditional Chinese medicine (Vincent 1996, Martin-Smith & Vincent 2006, Barrows et al. 2009), probably because they are not listed under CITES. Imports of dried pipefish into Taiwan were 7500–21,300 kg per annum between 1983 and 1993 (Vincent 1996) and imports into Hong Kong in 1998–2002 were 1600–16,500 kg annually (Martin-Smith & Vincent 2006).

Seahorses caught as by-catch can be traded either in the dried seahorse trade, or as curios for tourists. In countries where seahorses and pipefish are protected species and trade only occurs illegally, the availability of accurate capture and trade data (as reported to CITES) and the extent of impacts are limited. In recent years, illegal fishing for seahorses has been recorded in a small fishing village in Vilankulo, Mozambique. A haul of 1782 dried seahorses (predominantly *Hippocampus camelopardalis* with *H. kuda*) was confiscated from local fishermen in 2018 and the fishermen were jailed. Recently, 9 kg of dried seahorses has been confiscated from a Chinese buyer in the same village (Louw Claassens unpublished data). This type of relatively small-scale unlawful harvesting and trade is not usually tracked and can have far-reaching impacts on local populations (Lawson et al. 2017). In addition, seahorses in the above two examples were targeted catch, with poachers collecting seahorses by hand from shallow seagrass beds by snorkelling or walking during low tide. In South Africa, *Syngnathoides biaculeatus* has also been confiscated together with seahorses (Louw Claassens unpublished data). In Nigeria, *Microphis aculeatus* is sold in the ornamental fish trade; a box of 200 fish can be bought for as little as 381.15 Nigerian Naira, which is equivalent to US$1 (Ukaonu et al. 2011).

By-catch and trade

As much as 95% of seahorses used in trade come from shrimp trawl by-catch (Vincent et al. 2011). Seahorse by-catch can either be used to generate a secondary income, in low-grade fishery by-products such as fishmeal, or discarded. Seahorse by-catch per vessel is generally low (Meeuwig et al. 2006), but the cumulative impact can be devastating, with 2.2 million animals caught per annum in Vietnam in the late 1990s (Giles et al. 2005). In a recent review, Lawson et al. (2017) estimated an annual seahorse by-catch of 37 million animals from the 21 countries assessed. It is especially subsistence fishermen in developing countries that turn to the sale of seahorses to make a living (McPherson & Vincent 2004). There are numerous impacts from fishing, over and above immediate reduction in population size: disruption of monogamous seahorse pairs, which can lead to reduced reproduction, alterations of population structure and habitat destruction (see Vincent et al. 2011 for a review).

Whist there are limited empirical data on syngnathid by-catch in sub-Saharan Africa and adjacent islands, there are some by-catch records for species from elsewhere in their ranges. *Corythoichthys schultzi* has been recorded as by-catch in the Nigerian *Nematopalaemon* shrimp fishery (Ambrose et al. 2016). This fishery is dominated by artisanal fishers that use conical trap nets called 'Anyima' to catch shrimps (Ambrose et al. 2016). In Sulawesi, Indonesia, *Choeroichthys sculptus* is caught as by-catch during fishing by local communities for small baitfish. The pipefish is, however, not used as bait by locals as it is considered to be poisonous (Pet et al. 2006). In addition, *Hippocampus kuda*, *H. kelloggi*, *H. histrix* and *Syngnathoides biaculeatus* have been recorded in by-catch in India, Vietnam and Malaysia (Nguyen & Do 1996, Choo & Liew 2003, Sambandamoorthy et al. 2015).

An emerging threat in East Africa is the use of mosquito nets (provided as malaria prophylaxis) by artisanal fishers (Bush et al. 2017). Mosquito nets are used to catch all edible fish in the very fine mesh and are extremely indiscriminate in the species caught, resulting in a high amount of by-catch. Unidentified species of pipefish have been recorded in mosquito net by-catch in Palma, Mozambique (Jones & Unsworth 2020). Mosquito nets are generally used in a range of shallow, coastal habitats including mangroves, estuaries, seagrass beds, intertidal mud and sand flats, rocky areas and reef platforms (Bush et al. 2017). By-catch from mosquito net fishing should thus be considered a major threat to African syngnathids, as many species occur in these habitats. This threat is, however, not limited to Africa: mosquito net fishing has also been noted in Papua New Guinea (Short et al. 2018), for example.

Habitat loss and alteration

Most habitats used by syngnathids are globally under threat and susceptible to anthropogenic impacts. In a recent study, Phair et al. (2019) found that *Zostera capensis*, the dominant seagrass species occurring in South Africa (Adams 2016), shows high clonality and low genomic diversity and concluded that this species will have limited ability to re-establish in estuarine systems once lost. The vulnerability of *Z. capensis* owing to low genetic diversity makes it a priority for protection in the context of syngnathid conservation. Unfortunately, seagrasses in Africa are threatened by growing coastal human populations and related activities such as fishing, pollution, eutrophication and sedimentation (Gullstrom et al. 2002). In addition, activities such as boating or bait digging can lead to habitat loss, especially in shallow coastal environments and estuaries (Claassens et al. 2020). First, whilst boating or bait digging is taking place, syngnathids can be disturbed within their habitats, not only by trampling or direct disturbance from a boat moving through seagrass, but noise from boats has also been found to negatively affect seahorses (Claassens & Hodgson 2018a, Palma et al. 2019). Second, seagrass can be damaged by the removal of vegetation during bait digging, anchoring or by boat propellers (Claassens et al. 2020). In areas with a high number of moored boats, damage from permanent moorings has also been found to significantly impact seagrass habitats (Glasby & West 2018).

Coral reefs are also vulnerable to various anthropogenic impacts ranging from destructive fishing to climate change, ocean acidification and disease (Lindén et al. 2002, Hoegh-Guldberg et al. 2017, Hughes et al. 2018). Mangroves in East Africa are threatened by activities such as logging for fuel and house building, removal to make room for urban expansion and salt and shrimp production (Godoy & De Lacerda 2015).

Development is another major threat to coastal and estuarine habitats and can result in infilling of aquatic habitats or dredging to develop and maintain artificial environments such as harbours and marinas (Claassens 2018). In the most recent South African National Biodiversity Assessment, it was found that 29% of South African estuaries have been subject to severe habitat modification owing to development and related land-use pressures (van Niekerk et al. 2019b). In Zanzibar, mangroves cleared to make space for solar power generation and fish farms have adverse effects on the trophic structure of fish communities (Mwandya 2019).

Habitat alteration and loss in rivers can be caused by the construction of dams and other structures (Cutler et al. 2020), which also results in the loss of connectivity (Weerts et al. 2014). Another related impact from increased damming and water abstraction is the loss of freshwater inflow into estuarine systems, which can lead to the loss of a salinity gradient and even hypersaline conditions (Grange et al. 2000, Van Niekerk et al. 2019b). In addition, many estuarine species depend on regular freshwater pulses for breeding and food, and the loss of regular pulses can have an adverse effect on the entire ecosystem (Ter Morshuizen & Whitfield 1994, Strydom et al. 2002, Vorwerk et al. 2008). Specifically, *Syngnathus watermeyeri* is threatened by the loss of freshwater inflow into the Kariega and Bushmans estuaries, South Africa, the only two estuaries in which this species is currently found (Whitfield et al. 2017, Claassens et al. 2021). This is because reduced freshwater inflow results in a decrease in important prey species such as copepods (Wooldridge 2010). The loss of freshwater in estuaries can, however, have a positive impact on seagrass and other macroalgal habitats and can result in the upstream expansion of these habitats (Adams 2016) and the subsequent expansion of available habitat to these estuarine species (Claassens et al. 2021).

Wastewater run-off and the resultant increase in nutrients can have adverse effects on coastal environments such as estuaries (Claassens et al. 2020) and mangrove forests (Machiwa & Hallberg 1995, Cannicci et al. 2009). Unnaturally high increases in nutrients within these systems commonly result in nuisance algal blooms and increases in turbidity, which have been found to displace seagrass (Human et al. 2016). Impacts from eutrophication can impact the behaviour of syngnathids. For example, in a choice experiment, *Nerophis ophidion* avoided *Zostera marina* overgrown with filamentous algae compared to seagrass without any algal growth (Sundin et al. 2011). Increased turbidity can also affect mate choice, either by decreasing visibility and the ability to choose a mate when using visual cues only (Sundin et al. 2010), or by enhancing sexual selection and reproductive success using alternative cues to select a mate (Sundin et al. 2017). Eutrophication has been identified as a major threat to the Knysna seahorse in the Knysna estuary owing to the displacement of eelgrass habitats by nuisance macroalgal blooms dominated by *Ulva lactuca* (Claassens et al. 2020).

Climate change is a global phenomenon which can have far-reaching effects on local and regional ecosystems. In the first instance, marine heat waves can lead to coral bleaching, which directly damages coral habitats. Extensive damage from coral bleaching has been recorded in the Seychelles, Mozambique, Tanzania and Kenya (Lindén et al. 2002). In addition, coral bleaching could also lead to a decrease in the productivity and diversity of a reef, which can result in a decrease in available prey for syngnathids. Climate change and related warming have also been found to negatively affect seagrass and macroalgae (Duarte et al. 2018) and mangroves (Gilman et al. 2008); however, the direct effects of increased water temperature on syngnathids in the wild require investigation.

Sometimes, syngnathids are impacted by natural threats such as floods or storms. Climate change is causing an increase in the frequency and severity of storms, which can directly damage coral reefs (Cheal et al. 2017) and mangroves (Godoy & De Lacerda 2015). Most estuaries in South Africa are temporarily open/closed systems, where the connection to the ocean varies over time (Whitfield 1992). Natural estuarine breaching events, where the connection between the estuary and ocean is re-established, in these types of estuaries can cause a substantial drop in water level, leaving syngnathids stranded; this happened in the Swartvlei estuary, South Africa (Russell 1994). The impacts from breaching can, however, be exacerbated when sand bars enclosing estuary mouths are deliberately breached by management authorities, usually to prevent flooding of properties (Arendse & Russell 2020). Artificial breaching can occur more frequently than natural breaching.

The deleterious impacts of microplastics on aquatic environments and a wide range of taxa have become increasingly recognised in recent years (Rochman et al. 2016, Avio et al. 2017). Even though the impacts from microplastics on syngnathids are not well known, preliminary research in the Knysna estuary found that microplastics occur in the gut of *Syngnathus temminckii* along with prey animals (Naidoo 2021). Similarly, a study conducted in Spain found that *Hippocampus reidi* ingest microplastics through trophic transfer from their prey (Dominguez 2020). The direct effects of microplastic ingestion by syngnathids is unknown, but could potentially cause harm through toxin absorption and impact on the ability to feed and digest prey.

Conservation

Successful conservation depends on the identification and implementation of actions to improve the conservation status of a species. Baseline monitoring is required to determine if specific conservation actions are necessary, and if so, to identify which actions to implement. Ongoing monitoring is required to ascertain if conservation actions are in fact effective, and if not, to determine an alternative approach. The IUCN Red List assessment provides important information on the extinction risk of species (IUCN 2012, 2021), and ecological data used to inform this assessment can be monitored over time to ascertain conservation success (Rodrigues et al. 2006). A recently developed approach, the Green Status of species, assesses the effectiveness of conservation actions (Grace et al. 2021).

This approach assesses past conservation actions for a species, estimates what the status of a species would be under different conservation scenarios and provides much-needed insight into the efficacy of conservation actions.

Legislation, global agreements and regulations

International and regional agreements are important components of successful species conservation (Figure 5). In 2004, the entire genus *Hippocampus* was one of the first groups of marine fishes to be covered by CITES trade regulations since 1976, when they were included in Appendix II (Vincent et al. 2013). Under Appendix II, all signatories to CITES must ensure that trade in seahorses does not harm or adversely affect natural populations and is done legally, and that all international trade is reported (Foster et al. 2016). Difficulty in the identification of seahorses being traded, a mismatch between export and import data, and the increasing threat from poaching and illegal trade, which are not reported, are problematic limitations of the implementation of CITES in respect of seahorses (Foster et al. 2016). Regardless of these limitations, CITES provides an integral legal platform to hold signatories to account by applying international pressure to comply with regulations. Most countries in Africa, with the exception of South Sudan, Republic of the Congo, and The Kingdom of Eswatini, are signatories of CITES. In addition to CITES, member organisations of the IUCN undertake to abide by the Motions and the resultant Resolutions and Recommendations adopted by the IUCN, which are used to guide policy and influence third parties. The latest motion specifically focused on syngnathids (Motion 111) was adopted by the IUCN and its member organisations in December 2020 and provides a key strategy for the conservation of syngnathids that can be used by member organisations (244 of which are in Africa) in the promotion of syngnathid-specific conservation actions. Regionally, on the east African coast, the Nairobi Convention (2021) is a partnership between governments, civil society and the private sector with the aim of building a prosperous western Indian Ocean region with healthy rivers, coasts and oceans. On the west coast, the Abidjan Convention (2021) was developed to focus on the cooperation, management and development of the marine and coastal environment of the Atlantic coast of West, Central and southern Africa.

All syngnathids are protected in South Africa under the National Environmental Biodiversity Act No 10 of 2004. According to this act, members of the family Syngnathidae are not allowed to be captured, collected or disturbed in any way (Government Notice No. 476 of 2017). Instances of seahorse exports from South Africa are thus concerning, especially if these animals originate from South Africa. A single *Hippocampus capensis* specimen was sampled from a Taiwanese traditional Chinese medicine market (Chang et al. 2013). Seahorses confiscated in 2017 in South Africa appear to be *H. kuda*, which are commonly found in Mozambican waters and are known to be captured for the dried seahorse trade by local communities. It is thus likely that seahorse and other syngnathid exports from South Africa originate instead from neighbouring countries, such as Mozambique, although seahorses are a protected species in that country as well. CITES as well as the IUCN Red List are used to inform the setting of the protection status of species in many countries and in Mozambique, all seahorse species are protected because they are listed under CITES.

In addition to legislation and global agreements, regulated activities can be used as a locally significant conservation tool. For example, to prevent adverse impacts on seagrass habitats in the Knysna estuary, the use of a shovel or rake to dig for bait is not permitted (Claassens et al. 2020), although these activities are still done illegally, highlighting the difficulty in enforcing regulations. Utilisation of coastal and estuarine areas can be regulated through zonation or community agreements. For example, fishing is not allowed on Sundays in Vilankulo, Mozambique (Louw Claassens unpublished data).

Marine protected areas and community conservation

Protected areas are one of the most effective ways to protect species and habitats (Halpern 2003), and marine protected areas (MPAs) are used globally to protect marine ecosystems and resources

(Kelleher & Kenchington 1992). According to the Protected Planet database (UNEP-WCMC 2021), only 12.3% of African marine and coastal areas are formally protected (UNEP-WCMC 2021). South Africa increased the total area within MPAs in 2019, with the addition of 20 new sites, which increased the extent of marine protected areas to 5% of its territorial waters (https://www.marine-protectedareas.org.za/) (Sink 2016). This network includes 17 offshore and deep-sea MPAs and 23 coastal MPAs. The coverage of MPAs for many other African countries is, however, very little, with some countries such as Angola, Benin, Liberia and Somalia having no MPAs and 14 other countries with less than 1% coverage (UNEP-WCMC, accessed 18 February 2021). The Seychelles, in contrast, has 32.8% MPA coverage, and Gabon 28.8% (UNEP-WCMC 2021). Successful protection is, however, not guaranteed with protected area demarcation. For example, despite being part of the Garden Route National Park and a protected area, the environmental health of the Knysna estuary in South Africa is deteriorating (Claassens et al. 2020). The level of protection within MPAs also varies, and not many MPAs are fully protected from extractive activities, which has been found to limit the effectiveness of an MPA (Edgar et al. 2014). In addition, consultation with and cooperation from local communities is integral in the development and implementation of MPAs (Burgoyne et al. 2017). In particular, adverse impacts on local livelihoods from MPA development should be avoided and benefits from protection should be experienced by all stakeholders (Levine 2006, Sunde & Isaacs 2008, Burgoyne et al. 2017).

Tourism

The charismatic nature of syngnathids makes them effective tourist attractions (Ternes et al. 2016, Giglio et al. 2019), which can generate significant revenue (De Brauwer et al. 2017). In some instances, seahorse tourism is being used as an alternative income generator to seahorse fishing and poaching (Ternes et al. 2016). In an attempt to deter seahorse poaching in Vilankulo, Mozambique, a seahorse tourism initiative was developed by a local NGO and community members (ParCo 2021). Through this initiative, former seahorse poachers turned seahorse tour guides take tourists on seahorse tours on a traditional boat (dhow). The programme is managed by the community fishing council, and funds generated are shared within the community (Louw Claassens unpublished data). Some organisations also use voluntourism, a form of tourism in which tourists participate in voluntary work, to promote the conservation of seahorses and conduct ongoing monitoring (Goffredo et al. 2004, Roques et al. 2018). Tourism activities face various risks, the most recent being the COVID-19 pandemic, which resulted in a significant reduction in tourism globally. It is thus important to develop robust conservation programmes that can withstand such unforeseen events.

It is, however, also important to limit adverse impacts from tourist activities, such as habitat damage or disturbance of seahorses (Giglio et al. 2019). For example, tour operators in Brazil collect *Hippocampus reidi* in glass containers for tourists to observe (Ternes et al. 2016), which can result in increased stress to the animals and adverse impacts to populations. The key aspect to prevent negative effects through tourism activities is to avoid any direct contact with the animal (De Brauwer et al. 2019, Giglio et al. 2019). With the discovery of *H. nalu* in Sodwana Bay (Short et al. 2020), the first pygmy seahorse to be found in Africa, an increase in interest from scuba divers can be expected and it will be important for local dive operators to implement sustainable practices such as a code of conduct for diving with pygmy seahorses (Smith 2021).

Catchment management and sustainable development

Good management of river catchments is an important requirement for healthy coastal and estuarine environments. In particular, effective stormwater management is needed to prevent high sediment loads and polluted run-off entering coastal areas. Wastewater is commonly discharged into coastal and estuarine environments, and this has necessitated the development of water quality standards for discharged effluent (Beher et al. 2016). Compliance with these standards is, however, lacking in many areas (Claassens et al. 2020). Connectivity across rivers and between rivers and the coastal environment is important for animal movement. In instances where connectivity has been compromised, fish ladders are used to allow for fish movement (Weerts et al. 2014), although the usefulness of such remediation for syngnathids is unknown. The reduction or loss of freshwater inflow into estuaries owing to abstraction and damming upstream can be remediated by scheduled freshwater releases from dams and minimum flow requirements (Van Niekerk et al. 2019b). In South Africa, provision has been made for an ecological reserve, which refers to the quantity of freshwater needed to sustain the environment (Adams et al. 2016).

Sustainable development is another important conservation tool that can be used to limit habitat loss and alteration within coastal environments. In South Africa, the National Environmental Management: Integrated Coastal Management Act 24 of 2008 regulates all urban and industrial development along the coast. Specifically, this act aims to: "ensure that development and the use of natural resources within the coastal zone is socially and economically justifiable and ecologically sustainable". Through this Act, a development setback line must be set by all coastal municipalities with the objective of preventing development encroaching on sensitive coastal ecosystems as well as to protect communities from risks such as flooding and shoreline erosion (Desportes & Colenbrander 2016). Despite this regulation, development continues almost randomly along shorelines, with piecemeal and unplanned management intervention resulting in wide-scale habitat fragmentation (Celliers et al. 2015, Jewitt et al. 2015).

Education

Effective education and outreach are important conservation tools and have become integral to achieving effective conservation. Citizen science is one approach, used to educate and enhance community conservation engagement and conservation (Kelly et al. 2020), and has also been used to promote syngnathid conservation globally. Novel technologies, such as facial recognition, are being used to gather data on seadragons in Australia as part of SeadragonSearch (2021). iSeahorse (Project Seahorse 2021) is a global citizen science programme which collates seahorse observations. Citizen science initiatives can be effective in assessing species distributions, changes in population abundance and habitat quality. According to a recent review of citizen science in marine conservation (Kelly et al. 2020), citizen science programmes in Africa are lacking and there is a need to develop and expand such networks both as a means to increase conservation engagement of local communities and to achieve effective species conservation. In addition, habitats such as seagrass meadows and mangrove forests, key habitats for syngnathids in Africa, are also underrepresented in citizen science programmes (Earp & Liconti 2020) and there is scope to develop additional programmes in these habitats. It is important to develop locally significant educational resources and outreach programmes, to involve local communities and to nurture local custodianship. For example, species-specific educational resources are available for the Knysna seahorse (IUCN SSC Seahorse, Pipefish and Seadragon Specialist Group 2021), and local communities in Vilankulo, Mozambique, are part of ongoing education initiatives about seahorses found along their coast (ParCo 2021).

Research priorities, conservation opportunities and conclusions

This synthesis of available information on African syngnathids provides key insights into future research, management and conservation needs for this group in Africa. Based on the gaps identified in this review, we outline the most pertinent syngnathid research priorities.

1. There is a dearth of species-specific ecological and basic biological data for most African syngnathids, and future focus should be placed on those species listed as Data Deficient on the IUCN Red List as well as those that have not yet been assessed. For example, data on reproduction, growth, movement, habitat use and other biological aspects are needed. There is a need to develop regionally and locally significant ecological research for African syngnathids that can be used to guide conservation efforts predominantly at a local level as well as contributing to conservation initiatives at national, regional and international levels.
2. Owing to the limited focus on syngnathid diversity research, it is important to investigate and confirm syngnathid species diversity across Africa. Specifically, synonymised species, such as *Hippocampus borboniensis*, should be reassessed. New research approaches such as environmental DNA and population genomics should be incorporated into future research. It is also important to develop targeted syngnathid research to ensure that survey approaches are suitable to detect these cryptic fishes. A concerted effort should be taken to find ‘lost species’ such as *Bulbonaricus brucei* and *Campichthys nanus* (https://www.inaturalist.org/posts/16539-lost-species). It was as recent as 2018 that *Hippocampus nalu*, the first pygmy seahorse to be found in Africa, was discovered in South Africa’s most popular dive location. This discovery highlights the potential of new species discovery in Africa, even in areas that are well known.
3. The conservation of syngnathids in Africa should be highlighted and prioritized through the incorporation of syngnathid protection in national and regional legislation, regulations and policies. To aid in conservation efforts of threatened habitats such as mangroves, estuaries, seagrass and coral reefs, the use of syngnathids as ‘flagship’ species should be developed further, and locally significant education and outreach initiatives should be prioritized, working with citizen scientists, local dive operators and divers, and in-country partners and fishers. Endemic, threatened and range-restricted species should receive specific focus. For example, the Knysna estuary has been identified as the most important system for the long-term conservation of the endangered seahorse *Hippocampus capensis* (Mkare et al. 2017) and the successful protection of this estuary and seahorse habitats should be prioritized (Claassens et al. 2020). The tourism value of seahorses and other syngnathids should be investigated further, and ethical and sustainable tourism practices should be developed and become a requirement for any future syngnathid tourism initiatives.
4. The prevention of unsustainable fishing and poaching of syngnathids should be prioritized, and focus should be placed on compliance with CITES regulations regarding seahorse trade (and improved reporting), as well as assessing the extent of syngnathid exploitation across Africa. Management and enforcement agencies should be enabled to identify syngnathids correctly when exported or confiscated, as well as be able to determine the likely origin. This can be done by providing relevant and locally significant resources to these agencies and making use of genetic tools to verify species. Aquaculture can be a sustainable alternative source to wild-caught syngnathids, and research on the efficacy and value of syngnathid aquaculture in Africa is required.
5. Successful syngnathid research and conservation in Africa will require capacity development and training. To ensure that conservation actions are sustainable, Africa will need to develop its own in-country syngnathid experts. This will require support from global syngnathid experts, and the development of research and conservation collaborations with a focus on training and capacity development of local partners. Training should include different research fields that range from syngnathid taxonomy to trade.

Concluding remarks

This review provides the first synthesis of information for syngnathids in sub-Saharan Africa and adjacent Indian Ocean islands. Available information on syngnathids in this region are limited to a handful of species with a distinct geographical bias to South Africa. Most of the available information on this group originates from *ad hoc* and general coastal research, which limits species-specific information on ecology and conservation. In addition, piecemeal research limits the comparability of data and the application of findings.

The comprehensive literature search provides a summary of current knowledge on syngnathids in sub-Saharan Africa that can be used by researchers, managers and conservationists to assist with decision-making. The identified research priorities and conservation opportunities align future syngnathid research and conservation towards the common goal of conserving this unique group of flagship fishes and their aquatic habitats.

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Oceanography and Marine Biology: An Annual Review, 2022, **60**, 243-300
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HOTSPOTS OF CENOZOIC TROPICAL MARINE BIODIVERSITY

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Abstract Hotspots of tropical marine biodiversity are areas that harbour disproportionately large numbers of species compared to surrounding regions. The richness and location of these hotspots have changed throughout the Cenozoic. Here, we review the global dynamics of Cenozoic tropical marine biodiversity hotspots, including the four major hotspots of the Indo-Australian Archipelago (IAA), western Tethys (present Mediterranean), Arabian Sea and Caribbean Sea. Our review supports the 'Hopping Hotspots' model, which proposes that the locations of peak biodiversity are related to Tethyan faunal elements and track broad-scale shallow-marine habitats and high coastal complexity created by the collision of tectonic plates. A null hypothesis is the 'Whack-A-Mole' model, which proposes that hotspots occur in habitats suitable for high diversity regardless of taxonomic identity or faunal elements. Earlier 'Centre-of' theories (e.g. centres of origin with diversity decreasing with distance from supposed areas of exceptionally high rates of speciation, for which easy connection to adjacent regions to the east and west is important) were based on the analysis of recent biotas with no palaeontological foundation, and may better explain diversity dynamics within a hotspot rather than those between hotspots. More recently, however, human disturbance is massively disrupting these natural patterns.

Keywords: Global patterns, species diversity gradients, tropics, Paleogene, Neogene, fossil records, paleobiology

Introduction

Tropical oceans support several fold more biodiversity than any other marine region (Reaka-Kudla 1997, Bouchet et al. 2002, Plaisance et al. 2011), including the ~30% of all marine species found in coral reef ecosystems alone (Costello et al. 2015, Fisher et al. 2015). Biodiversity hotspots are areas that contain disproportionately large numbers of species (species richness) compared to surrounding regions (Jefferson & Costello 2020, Manes et al. 2021). For example, within the tropics and subtropics, the Indo-Australian Archipelago (IAA) is characterised by the highest marine species richness and endemicity for numerous taxonomic groups, such as corals, molluscs, crustaceans and fishes (Bouchet et al. 2002, Hoeksema 2007, Tittensor et al. 2010, Plaisance et al. 2011, Bellwood et al. 2012, Asaad et al. 2018a, b). However, the richness and geographical location of biodiversity hotspots have changed over the Cenozoic (last 66 Myrs [million years], the latest geologic era that has much better fossil records than the, older, Mesozoic and Paleozoic eras) (O'Dea et al. 2007, Renema et al. 2008, Yasuhara et al. 2017a, Di Martino et al. 2018).

Palaeontological studies of biodiversity hotspots have been conducted in the western Tethys, Arabian, Caribbean and Indo-Pacific regions. The western Tethys has a long history of palaeontological research and accumulation of fossil data (McKenzie 1982, 1991a,b, Popov et al. 2001, Harzhauser et al. 2002, Popov et al. 2002, 2004, Harzhauser et al. 2007). The Panama Palaeontology Project and subsequent studies have described Neogene (23.0–2.6 Ma [million years ago]) biodiversity dynamics of the Caribbean in detail (Coates & Collins 1999, Jackson & Johnson 2000, O'Dea et al. 2007, Johnson et al. 2008, Di Martino et al. 2018). The IAA has been studied less, despite being the location with the highest modern-day diversity (Renema et al. 2008, Di Martino et al. 2015, 2019, Johnson et al. 2015b, Yasuhara et al. 2017a, Harzhauser et al. 2018), although pioneering studies and re-investigations of their fossil collections and localities exist (e.g. Martin 1919, Gerth 1923, van der Vlerk & Umbgrove 1927, Gerth 1933, Kingma 1948, Keij 1966, Beets 1986, Leloux & Wesselingh 2009). Further palaeontological studies have been conducted recently by international collaborations, including the Throughflow Project (Reich et al. 2014, Di Martino et al. 2015, Johnson et al. 2015a, Kusworo et al. 2015, Santodomingo et al. 2015, 2016, Yasuhara et al. 2017a, Shin et al. 2019). In addition to palaeontological studies (Wilson & Rosen 1998, Renema et al. 2008), molecular studies have improved our understanding of the evolutionary processes and mechanisms behind the development of IAA hotspot (Williams & Duda Jr 2008, Bellwood et al. 2017). However, extinction in the ancient Caribbean Sea, western Tethys and Arabia (Johnson et al. 2008, Renema et al. 2008) has made it difficult to study the macroevolutionary dynamics of these ancient hotspots using molecular techniques alone. In addition, ongoing anthropogenic ecosystem degradation is making biodiversity studies increasingly difficult in the Anthropocene (Waters et al. 2016) by obscuring or even erasing the natural baseline condition (Jackson 1997, Jackson et al. 2001, Pandolfi et al. 2003, Lotze et al. 2006, Halpern et al. 2008, 2015, 2019).

Renema et al. (2008) proposed the Hopping Hotspots model to explain the Cenozoic history of multiple biodiversity hotspots, arguing that the tropical biodiversity hotspot has migrated from the western Tethys via the Arabian Peninsula to the IAA during the Cenozoic (Renema et al. 2008).

The Caribbean region was not included in this model, although strong biogeographic ties between the Caribbean and Mediterranean existed until the end of the Oligocene (Cheetham 1968, Budd et al. 2011). Cenozoic biogeographic relationships (i.e. similarities and differences of faunal compositions) between these four (western Tethys, Arabian, IAA and Caribbean) hotspots have never been synthesized fully or updated since work in the 1960–1990s, which often had a stratigraphic rather than a biogeographic perspective (Adams 1967, McKenzie 1967, 1982, Adams et al. 1983, Adams 1987, McKenzie 1991a,b). These earlier studies also focused almost entirely on foraminifera and ostracods to the exclusion of other informative taxa. The extent to which human activities have degraded natural biodiversity patterns is also poorly established, complicating comparison of recent and fossil biogeographic patterns.

In short, a global synthesis of the Cenozoic history of tropical marine biodiversity and biogeography is still lacking, preventing a holistic understanding of the dynamics and mechanisms behind the regional waxing and waning of tropical biodiversity. Here we review the Cenozoic history of tropical biodiversity hotspots from a palaeontological perspective. Our main aims are to: (1) describe present-day total marine biodiversity patterns, including all coastal taxa, and assess their consistency with marine biodiversity patterns of groups with high fossilisation potential and good fossil records (such as bivalves and corals); (2) provide an in-depth and up-to-date review of the Cenozoic history of marine biodiversity and biogeography in the western Tethys, Arabian Sea, Indo-Australian Archipelago and Caribbean Sea; (3) synthesize the ecological and evolutionary dynamics of Cenozoic hotspots globally and their likely mechanisms and drivers; (4) summarise available evidence of human-induced degradation in the IAA and Caribbean regions; and (5) identify research opportunities and future directions for uncovering the underlying mechanisms driving the locations of hotpots through time. We mainly focus on Cenozoic marine patterns in the microfossil and macrofossil records such as ostracods, foraminifera, molluscs, corals and bryozoans. Here we firmly establish that palaeontological data are essential for understanding the current as well as ancient geographic distribution of biodiversity.

Present-day global biodiversity patterns

Global patterns and hotspot regions

Broadly speaking, marine species richness increases from high to low latitudes, referred to as the latitudinal biodiversity gradient (Tittensor et al. 2010, Chaudhary et al. 2016, 2017, Reygondeau 2019, Gagné et al. 2020, Rogers et al. 2020, Yasuhara et al. 2020b) (Figure 1). However, species richness is longitudinally heterogeneous within the relatively diverse tropics. The highest marine species richness is found in the IAA, with more diversity than in other tropical regions such as the Caribbean Sea and western Indian Ocean (Tittensor et al. 2010, Plaisance et al. 2011, Reygondeau 2019, Gagné et al. 2020, Rogers et al. 2020) (Figure 1). Species richness in the Caribbean is higher than in other areas in the Atlantic, but much lower than in the IAA hotspot (Tittensor et al. 2010, Rogers et al. 2020) (Figure 1). The Mediterranean Sea, which in the past represented the western Tethys hotspot, is no longer in the tropics (Leprieur et al. 2016). The Mediterranean does not currently have significant warm-water coral reef systems, but has more species than the adjacent Atlantic ocean (Costello et al. 2010) (Figure 1). The present-day Arabian region, including the Red Sea, Gulf of Aden and Persian Gulf, has lower biodiversity than the IAA (Figure 1) but higher biodiversity than the Caribbean for some taxa (e.g. Scleractinia; Veron et al. 2009) and comparable or somewhat lower biodiversity for other groups (e.g. fishes; Miloslavich et al. 2010, Sonnewald & El-Sherbiny 2017) (Figure 1). These trends, however, may be related to comparatively low sampling effort and high levels of endemism in the Arabian region (Sonnewald & El-Sherbiny 2017).

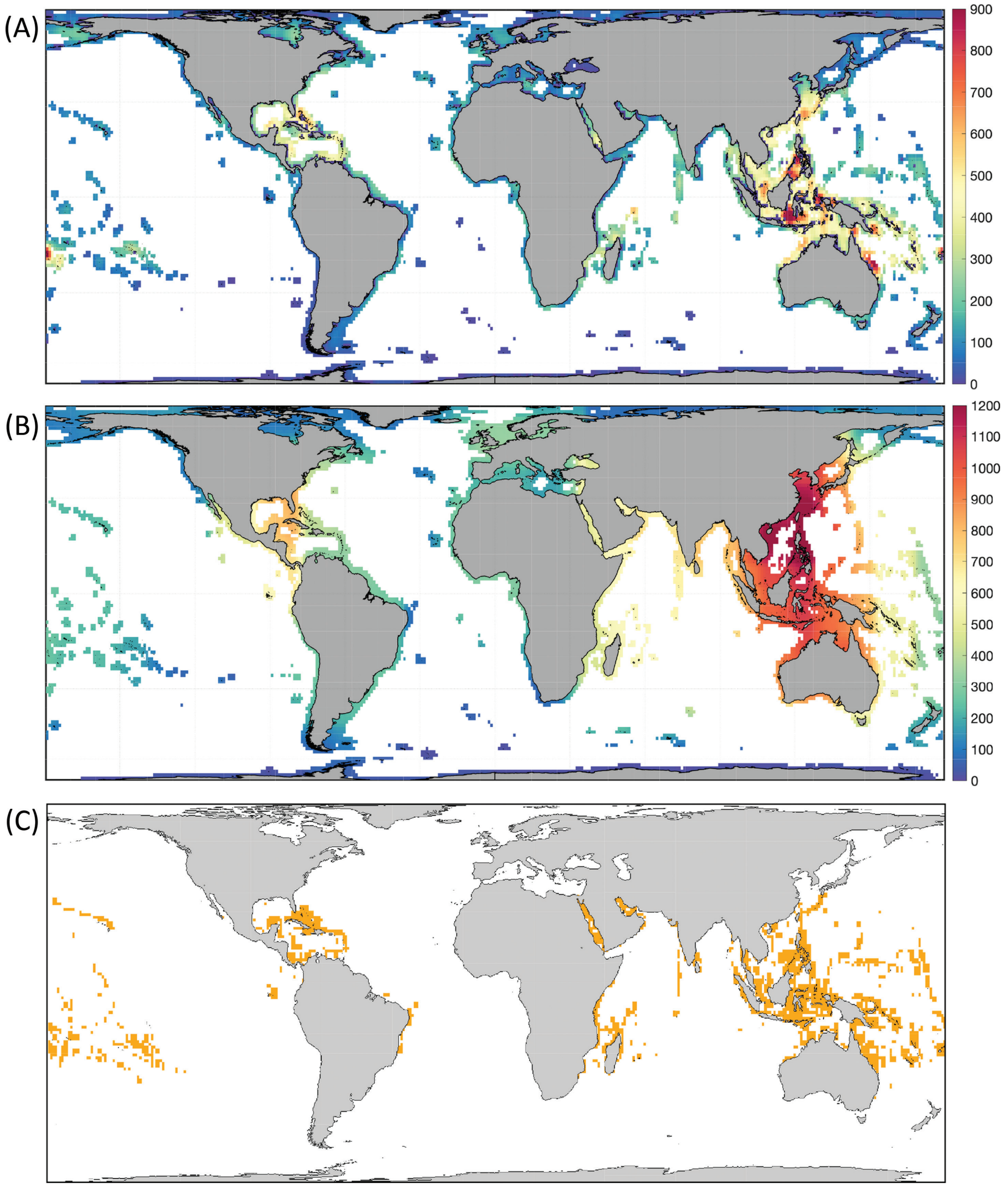

Figure 1 Present-day global biodiversity patterns. Species richness heat maps of (A) all coastal taxa (Reygondeau 2019, Reygondeau & Dunn 2019, Gagné et al. 2020, Reygondeau et al. 2020, Rogers et al. 2020) and (B) bivalves (Jablonski et al. 2013). (C) Coral reef distribution (orange) is shown to visualise habitat availability for tropical shallow-marine biodiversity (Zhao et al. 2020).

Fossilisation potential of global patterns

Among taxonomic groups with well-preserved fossil records, the most comprehensive synthesis of present-day biodiversity data is available from bivalves (Belanger et al. 2012, Jablonski et al. 2013), followed by reef corals (Hughes et al. 2002, Keith et al. 2013, Veron et al. 2015, Kusumoto et al. 2020). Bivalve and coral diversity show a distinct IAA hotspot and

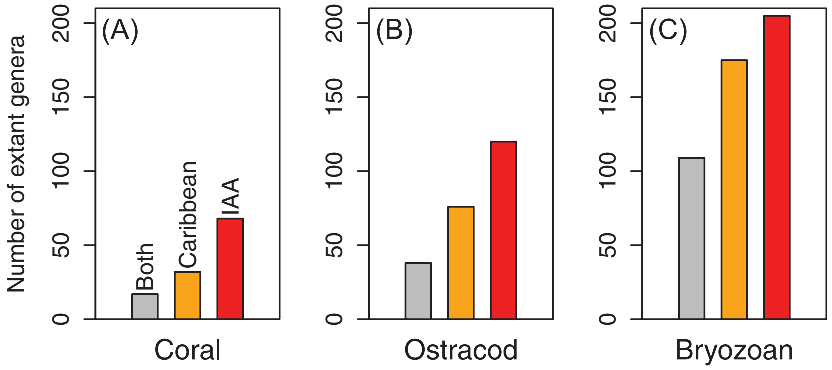

Figure 2 Comparison of IAA and Caribbean generic diversity for corals (Kusumoto et al. 2020) (A), ostracods (Yasuhara et al. 2017a and references therein, Chiu et al. 2020) (B), and bryozoans (data compiled in April 2020 from the website Recent and Fossil Bryozoa of Philip Bock, available at www.bryozoa.net) (C), illustrating richness of extant genera distributed in the Caribbean Sea (orange) and the IAA (red) as well as that of shared genera between the Caribbean Sea and IAA (grey). These data for taxonomic groups with high fossilisation potential consistently show higher extant diversity in the IAA than in the Caribbean Sea.

less diverse Caribbean Sea and western Indian Ocean (Veron et al. 2009, Jablonski et al. 2013) (Figure 1), patterns consistent with those of total marine and coastal biodiversity (Tittensor et al. 2010). Indeed, many additional taxonomic groups with a good fossil record (larger benthic foraminifera [LBF], ostracods and bryozoans) show higher diversity in the IAA than in the Caribbean (Figure 2), mirroring the pattern of total marine biodiversity. Taxonomic groups with a good fossil record are also known to show consistent biodiversity responses with other taxonomic groups (e.g. Jackson & Johnson 2000, Renema et al. 2008, Di Martino et al. 2018, Chiu et al. 2020). Thus, their fossil records may be reliable proxies for total tropical marine biodiversity trends prior to the timescale of modern observations.

History of major biodiversity hotspots

How has present-day tropical diversity emerged? To address this question, we review the regional Cenozoic history of one present-day (IAA), one waning (Caribbean) and two vanished (western Tethys and Arabian) biodiversity hotspots. Figure 3 shows the Cenozoic timescale and the names and ages of geological periods, epochs and stages, as well as names of palaeogeographical and palaeobiogeographical regions mentioned in this paper. A global summary of the Cenozoic events and biodiversity patterns are shown in Figures 4–7 and Table 1.

Vanished hotspots of the Tethys

Introduction

The Tethys Ocean included two Cenozoic biodiversity hotspots, the western Tethys and Arabia. Diversity in these regions is not high today, but they were the global centre of marine diversity during the early Cenozoic. These two hotspots have a dynamic Cenozoic history. Note that while the IAA and Caribbean regions were a part of the Tethys Ocean during the Paleogene (66–23 Ma), we treat these hotspots in the next sections separately, because these regions were hotspots primarily in the Neogene after the disappearance of the Tethys Ocean.

The Tethys was originally defined as an equatorial ocean that was present prior to the uplift of the Alpine-Himalayan mountain ranges (Suess 1893, Sengor 1985). The Tethys originated at the

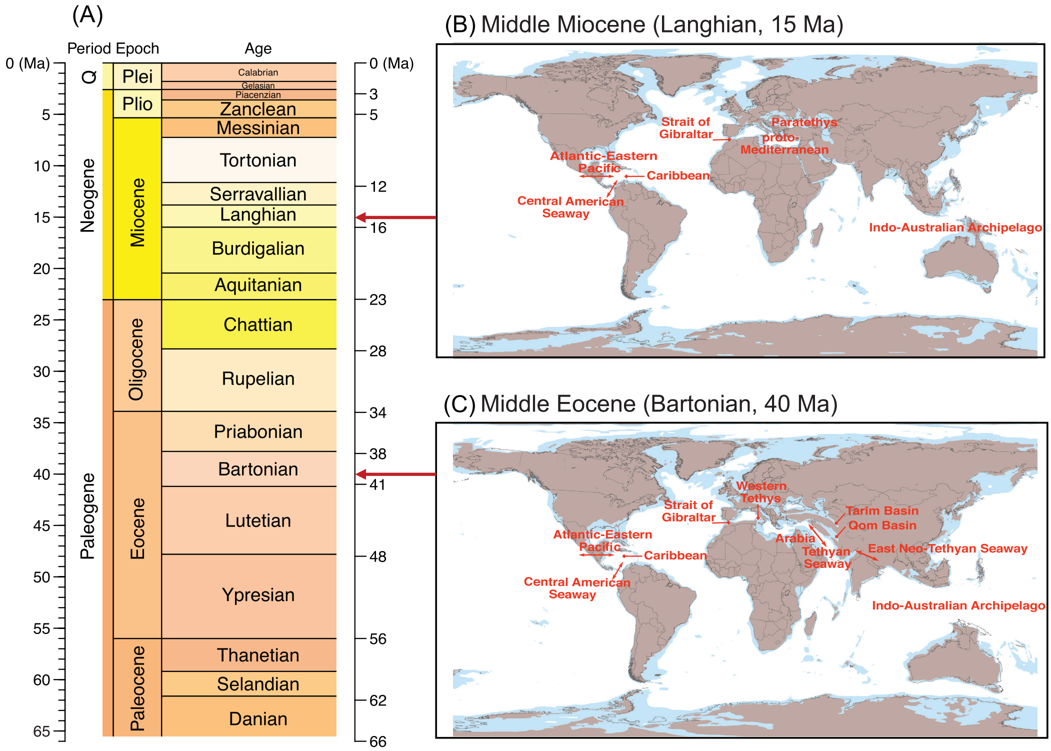

Figure 3 Geological timescale and (palaeo)geographical regions. (A) Cenozoic timescale (Walker et al. 2018, Gradstein et al. 2020). Overview of palaeogeographical and palaeobiogeographical regions on middle Miocene (B) and middle Eocene maps (C). Palaeogeographical maps (light blue denotes shallow-marine areas) based on Kocsis & Scotese (2021). Note that the east Neo-Tethyan seaway is not represented in the middle Eocene palaeogeographical map of Kocsis & Scotese (2021), but should have existed, because the Indian subcontinent had not yet collided with the Eurasian continent at that time (Harzhauser et al. 2002, Wang et al. 2020).

eastern edge of the Pangea supercontinent in the Permian with the drifting of the Cimmerian Plates away from Gondwana (Golonka 2002). Pangea broke apart during the Triassic and Jurassic, separating into Laurasia (North America and Eurasia) to the north and Gondwana (Antarctica, India, South America, Australia, Africa) to the south. This process opened a circumtropical marine connection between the Pacific and Atlantic Ocean basins across the Tethys.

The northward movement of the African, Indian and Australian plates resulted in a continuous narrowing of the vast Tethys Ocean since the Late Cretaceous (~80 Ma). This narrowing allowed water mass exchange at low latitudes between the Atlantic, Indian and Pacific oceans until the beginning of the Neogene (Harzhauser et al. 2007, Bialik et al. 2019). The western Tethys remained biogeographically connected to the Caribbean (west-central Atlantic Ocean) to the west and the east Tethys region (Indo and western Pacific) to the east until the end of the Paleogene (Cheetham 1968, Budd et al. 2011). Together, these regions constituted a broad Tethyan biogeographic realm that continued south as far as Southeast Asia and Madagascar during the Paleogene (Cowman & Bellwood 2013a, Hou & Li 2018, Yasuhara et al. 2019a) (Figure 3).

The Caribbean (western Atlantic or tropical American) region has been considered as its own distinct biogeographic unit (e.g. Harzhauser et al. 2002, Renema 2002, 2007). However, some Caribbean species had broad distributions from the east Tethys through the western Tethys to the Caribbean during the Eocene (e.g. Cheetham 1968, Givens 1989, Ivany et al. 1990, Matsumaru

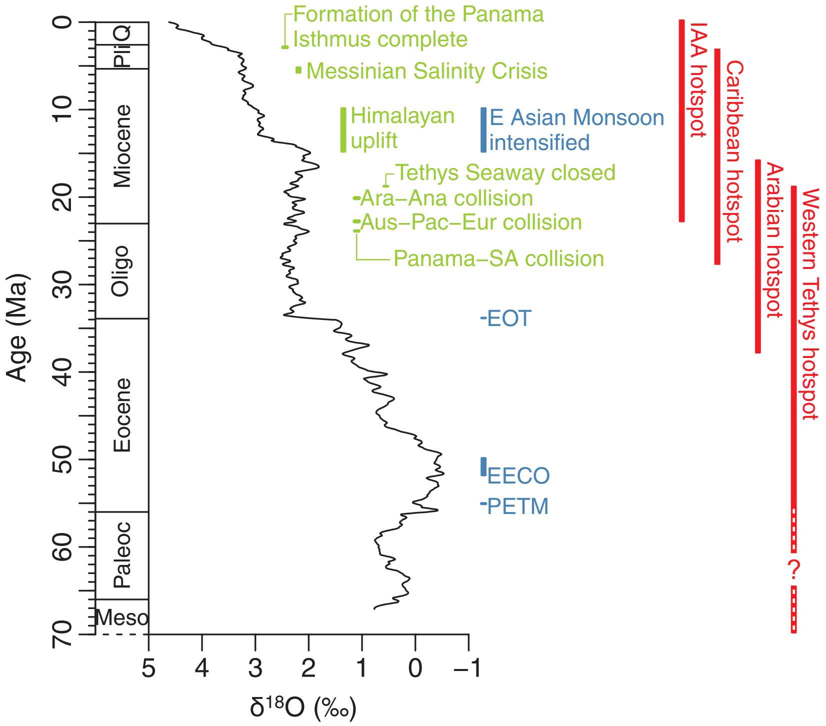

Figure 4 Cenozoic global changes and major events associated with biodiversity hotspots. Global deep-sea oxygen isotope records are from Westerhold et al. (2020). The figure is modified after Yasuhara et al. (2020a). Major tectonic-related events (green) include the Panama-South America (SA) collision (~24 Ma) (Farris et al. 2011), Australia-Pacific-Eurasia (Aus-Pac-Eur) collision (~23 Ma) (Renema et al. 2008), Arabia-Anatolia (Ara-Ana) collision (~20 Ma) (Renema et al. 2008), Tethys Seaway closure (~19 Ma) (Harzhauser et al. 2007, Yasuhara et al. 2019b), Himalayan uplift latest phase (15–10 Ma) (Tada et al. 2016), Messinian Salinity Crisis (5.96–5.33 Ma) (Krijgsman et al. 1999) and completion of the Formation of the Panama Isthmus (~3 Ma) (O'Dea et al. 2016, Jaramillo 2018). Major climatic events (blue) include Paleocene–Eocene thermal maximum (PETM, 55.5 Ma) (Cronin 2009), early Eocene climatic optimum (EECO, 52–50 Ma) (Cronin 2009), Eocene–Oligocene transition (EOT, ~34 Ma) (Cronin 2009) and late Miocene East Asian Monsoon intensification (15–10 Ma) (Tada et al. 2016). Biodiversity hotspot durations (red) include those of the western Tethys, Arabian, Caribbean and IAA hotspots (Figure 7, Table 1).

1996, Harzhauser et al. 2002, Shahin 2005, Renema 2007, Yamaguchi & Kamiya 2009, Budd et al. 2011, Yasuhara et al. 2019a). This broad circumtropical Tethys faunal element in the Eocene was characterised by a warm-water, tropical to subtropical fauna and flora that included taxa with broad geographic distributions (e.g. Harzhauser et al. 2002, 2007, Renema et al. 2008, Cowman & Bellwood 2013a, Hou & Li 2018, Yasuhara et al. 2019a). The faunal similarity between the western Tethys and the Caribbean in the Eocene varies among taxonomic groups. For example, similarity is substantial in gastropods (Harzhauser et al. 2002) but more limited in LBF (Renema 2002, 2007).

Western Tethys hotspot: Paleogene biogeography and biodiversity

Global tropical marine biodiversity peaked in the western Tethys (present Mediterranean) during the Eocene, at least when considering LBF (Renema 2007) (Figures 4–7). High diversity is also recorded for molluscan and ostracod faunas during the Eocene (Oppenheim 1894, 1896, 1901, 1909, 1912, Keij 1957, Ducasse et al. 1985, Guernet et al. 2012), but up-to-date and quantitative syntheses are lacking. Close to the Eocene/Oligocene boundary (~34 Ma), LBF suffered a large extinction

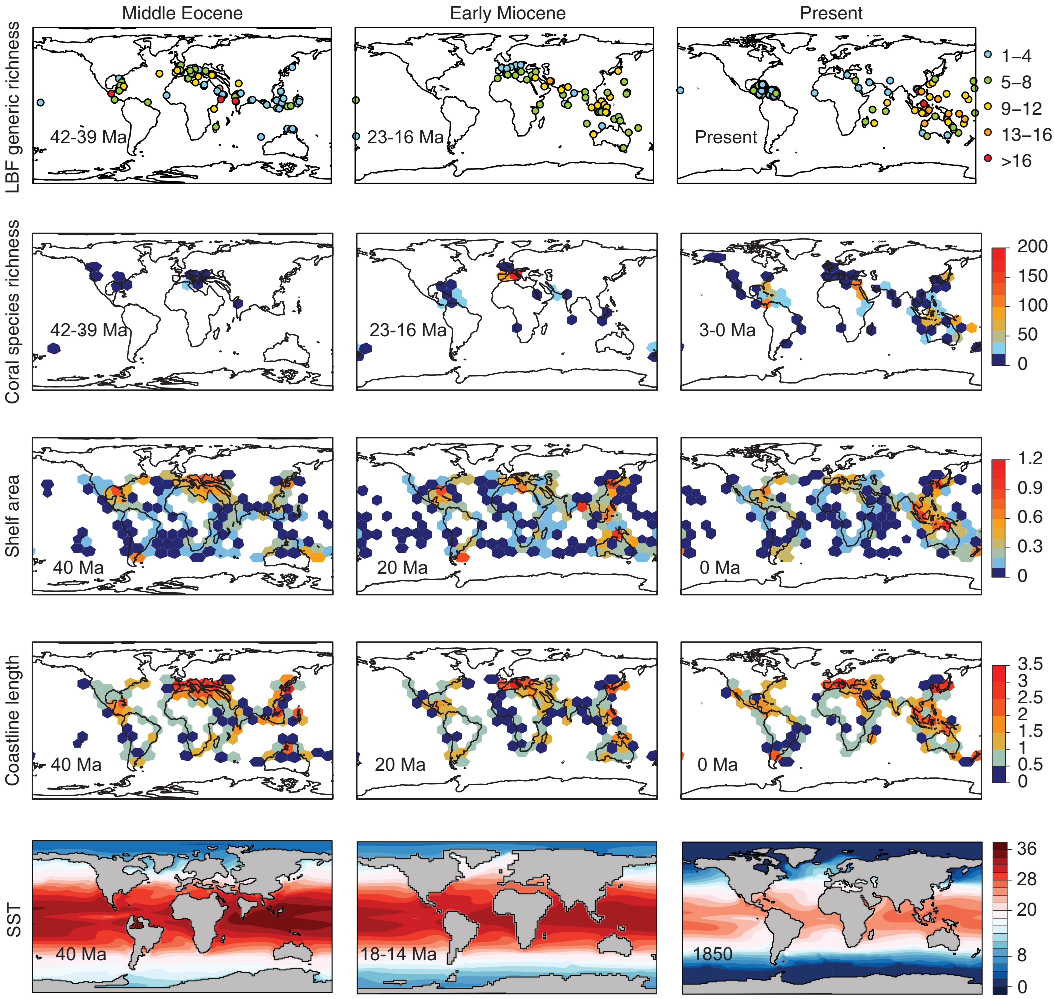

Figure 5 LBF diversity, coral diversity, shelf area, coastline length and sea surface temperature (SST) time slices. LBF data are based on Renema et al. (2008) with our own additional compilation: present (Culver and Buzas 1982 and references therein, also Buzas et al. 1977, De Araújo & De Jesus Machado 2008, Baker et al. 2009); Miocene and Eocene (Robinson 1974, de Mello e Sousa et al. 2003, Robinson 2003, Bowen Powell 2010, Baumgartner-Mora & Baumgartner 2016, Serra-Kiel et al. 2016, Cotton et al. 2018, 2019, Torres-Silva et al. 2019, Cotton Unpublished). Coral data were downloaded from Palaeobiology Database on 28 January 2021 (requested taxa: Scleractinia; identification resolution: species; filters: certain genus and species identification only, regular taxa only). See Section 'Role of shallow-marine habitat size and complexity' for the details of shelf area and coastline length reconstructions. Sea surface temperature reconstructions are from Hutchinson et al. (2018), Feng (2019) and Danabasoglu et al. (2020).

(Adams et al. 1986, Cotton & Pearson 2011) that marks the initial decrease in western Tethys diversity (Renema 2007) (Figures 6 and 7). LBF diversity continued to decrease through the Oligocene to early Miocene at ~19 Ma, leading to the final disappearance of the western Tethys hotspot (Figures 4, 6 and 7, see Section 'End of the Tethys').

Changing patterns in the generic composition of the western Tethys reef coral fauna suggest a gradual decrease in faunal exchange with the western Atlantic-Caribbean near the end of the

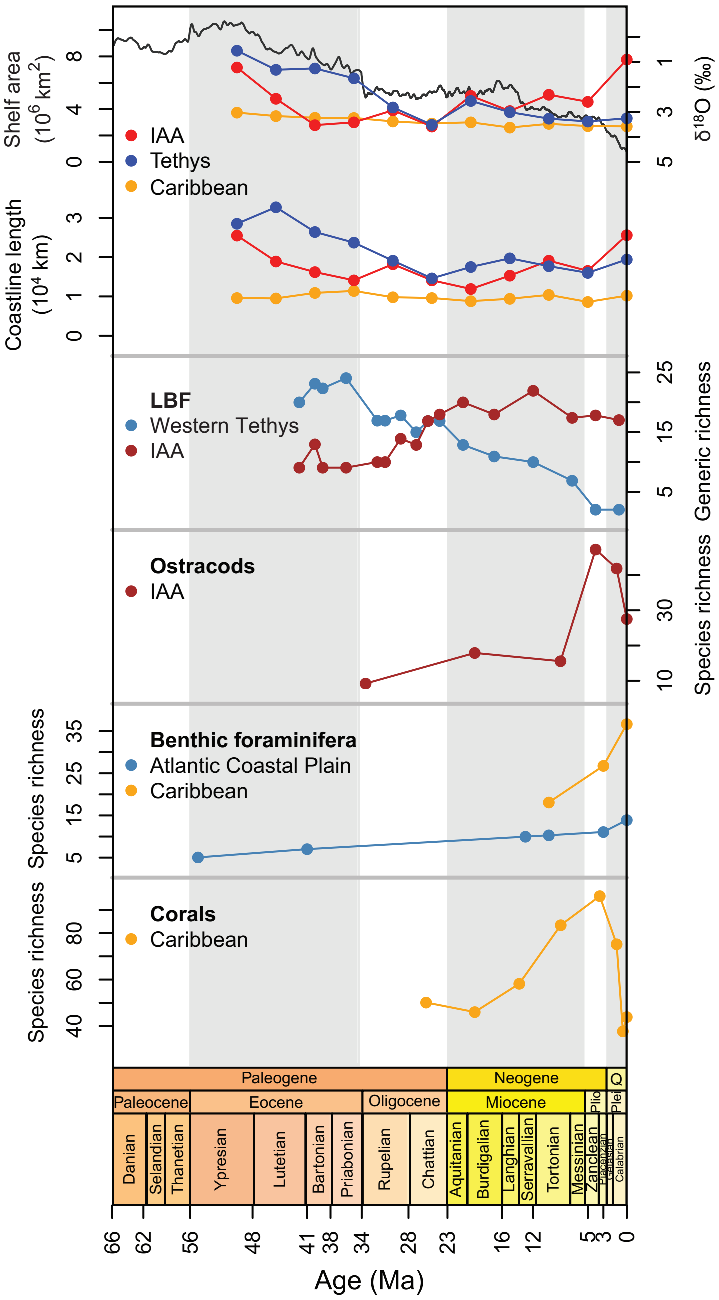

Figure 6 Diversity trends during the Cenozoic. Plots show (from top to bottom): shelf area (see Figure 10 and Section 'Role of shallow-marine habitat size and complexity' for details) and a global climate curve (Westerhold et al. 2020); coastline length (see Figure 10 and Section 'Role of shallow-marine habitat size and complexity' for details); and diversities of LBF (western Tethys and IAA, Renema 2007), ostracods (IAA, Yasuhara et al. 2017), benthic foraminifera (Fisher's alpha used for the species richness proxy; Caribbean with temperate north-western Atlantic as a reference; Buzas et al. 2002), and corals (Caribbean; Johnson et al. 2008, Chao et al. 2014). We followed Chao et al.'s (2014) method to calculate Hill numbers (q=0, minimum coverage) for the coral biodiversity estimates, using abundance data from Johnson et al. (2008).

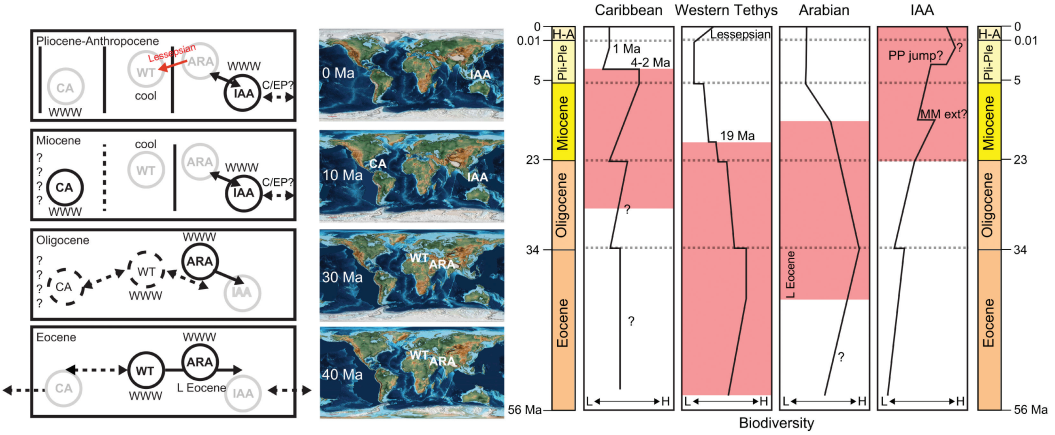

Figure 7 Hypothetical and schematic summary of global hotspots dynamics. (Left) Schematic diagrams summarising hotspots dynamics. 'WWW' indicates major continent-continent collision. Solid and dotted arrows: solid and limited connectivity/dispersal, respectively. Vertical solid and dotted bars: solid and limited separation, respectively. Questions mark indicate uncertainty. Solid circles: hotspot presence (dotted circle indicates limited diversity or uncertainty). Grey circle: hotspot absence. CA, Caribbean hotspot; WT, western Tethys hotspot; ARA, Arabian hotspot; IAA, Indo-Australian Archipelago hotspot; C/EP, central and/or eastern Pacific. (Middle) Palaeogeographical maps (Scotese 2016). (Right) Hypothetical summary of diversity trajectories of four biodiversity hotspot regions (mainly based on the taxonomic groups with good quantitative fossil records, i.e. LBF and ostracods, but also referred to other major taxonomic groups such as molluscs, corals and bryozoans). Biodiversity is shown in a relative sense (H, high; L, low). Red indicates a hotspot period. Question marks indicate uncertainty. Pli-Ple, Pliocene–Pleistocene; H-A, Holocene–Anthropocene. The Caribbean region is characterised by relatively low diversity in the Eocene, Eocene–Oligocene extinction, (uncertain) Oligocene diversification, Oligocene–Miocene coral loss (extinction event?), Miocene–Pliocene diversification (likely by increased habitat heterogeneity by diminished interoceanic seaways), major extinction event at ~4–2 Ma that basically eliminated this hotspot (likely via productivity collapse related to the establishment of the Panama Isthmus), rapid diversification since the extinction event (by oligotrophication and coral reef development; magnitude uncertain) and stable diversity for the last one Myrs. The Western Tethys region is characterised by high Eocene diversity, Eocene–Oligocene extinction, gradual diversity decline in the Oligocene (via suitable habitat decline; the hotspot shifts to Arabia in the late Eocene–Oligocene), Oligocene–Miocene extinction, final disappearance of the hotspot at ~19 Ma (by the final closure of the Tethys Seaway), further diversity decline in the middle and late Miocene (by global and regional cooling), Miocene–Pliocene (Messinian) extinction and diversity increase by Lessepsian migration from the Indian Ocean. The Arabian region is characterised by Eocene diversification, high diversity in the late Eocene, high but declining Oligocene diversity, and Miocene diversity decline with final hotspot disappearance in the middle Miocene (by habitat loss due to the final closure of the Tethys Seaway). The IAA is characterised by low (but increasing) Eocene diversity, Eocene–Oligocene extinction, Oligocene diversification, hotspot establishment at the early Miocene (~23 Ma; by increased suitable habitats by the collision), strong Miocene diversification punctuated by the middle Miocene extinction (MM ext; at least in LBF), Pliocene–Pleistocene diversity jump (the PP jump, related to cyclic glacial-interglacial sea-level changes; exact timing uncertain), and (uncertain) recent diversity decrease (by Anthropocene human-induced degradation or large late Pleistocene glacial-interglacial sea-level changes, at least in ostracods). See main text and Table 1 for further details.

Oligocene (Perrin 2002, Perrin & Bosellini 2012). This decrease is considered to reflect a major oceanographical change in the Central Atlantic, which led to the breakup of pan-tropical ocean circulation (Perrin 2002, Perrin & Bosellini 2012). Consistent with the corals, the fossil record of LBF shows the last Caribbean-western Tethys faunal exchange during the Oligocene at ~32 Ma (BouDagher-Fadel & Price 2010). The same is true of bryozoans (Cheetham 1968). Molecular phylogenies of scleractinian corals previously classified in the families Faviidae and Mussidae, however,

Table 1 Summary of the Cenozoic tropical biodiversity hotspots

	Western Tethys	Arabian	IAA	Caribbean	Remarks
Eocene	Hotspot. But Arabian diversity became higher in the late Eocene. Marginal basins such as the Tarim Basin and east Neo-Tethyan seaway had rich marine faunas, but with relatively low diversity.	Hotspot. Especially in the late Eocene, diversity was higher than the western Tethys. Faunal connection with the western Tethys.	Non-hotspot. Temperature too high?	Non-hotspot? Temperature too high?	Certain faunal elements show circumtropical distribution, indicating certain connection of all of these hotspots in the Eocene. But note that degree of pandemism/endemism depends on taxonomic groups and LBF shows high endemism and large difference between western Tethys and Caribbean faunas.
Oligocene	Extinction at E/O. Transition to non-hotspot. Paratethys Sea appeared at around the E-O boundary; enhancement of fragmentation of broad Tethys region.	Hotspot. But molluscan data indicate unstable/hypersaline environments in the late Oligocene. Decreased faunal connection with the western Tethys in the late Oligocene.	Non-hotspot. Extinction at E/O.	Extinction at E/O. Initiation of suitable habitat and so hotspot. Reef loss at O/M.	The faunal connection between the western Tethys and Caribbean is more limited compared to the Eocene, but still exists by the end of the Oligocene, even in LBF that show higher endemism than other organisms.
Miocene	Closure of the Tethyan Seaway (19 Ma) finally eliminated this hotspot. Cooling and perhaps Messinian Salinity Crisis (5.96–5.33 Ma) further decreased the diversity of Proto-Mediterranean fauna at the end of the Miocene.	Hotspot sustained by the middle Miocene. Faunal connection with the IAA. Then hotspot gradually disappeared by the middle-late Miocene due to habitat loss by the Arabia-Eurasia collision. Increasingly unstable/hypersaline environment in the early Miocene.	Hotspot initiation at ~23 Ma due to the collision of Australia with Pacific arcs and the Southeast Asian margin. End mid-Miocene (~11.6 Ma) extinction (in LBF).	Hotspot. High productivity. Mio-Pliocene diversification.	In the early Miocene, the Indian Ocean showed strong provincialism with 4 different provinces, in contrast to the homogenous fauna throughout the Indian Ocean for the last 10 million years from the late Miocene to the present. Basic biogeographic distributions have been similar for the last 10 million years.
Plio-Pleistocene	Non-hotspot. Non-tropical and likely too cold.	Non-hotspot.	Hotspot. Diversity jump via biological pump; successive cycles of sea-level change caused repeated habitat disconnection and reconnection. Indonesian Throughflow restriction from 10 to 4.4 or 1.6 Ma may have also allowed diversification.	Hotspot finally eliminated by the extinction event at ~4–2 Ma; productivity collapse and delayed extinction. Final closure of shallow-marine connection of the Central American Seaway (3 Ma). Reef proliferation and diversity increase since 2 Ma.	Exact timing of Plio-Pleistocene diversity jump remains elusive.
Holocene/ Anthropocene/ present	Non-hotspot. Non-tropical. Too cold. Lessepsian migration.	Non-hotspot.	Hotspot. Modern diversity and distribution likely affected by Anthropogenic forces (aka reef ecosystem degradation), but data deficient.	Reef proliferation and diversity increase since 2 Ma. Modern diversity and distribution affected by Anthropogenic forces (aka reef ecosystem degradation). Data deficient to quantify the anthropogenic diversity loss.	A subordinate centre of coral reef biodiversity in the northern Mozambique Channel between East Africa and Madagascar in the Indian Ocean.

indicate a deeper divergence between the dominant Atlantic and Indo-West Pacific lineages before the end of the Eocene (= before ~34 Ma) that had been obscured by conventional (only based on macromorphological characters) coral taxonomy (Fukami et al. 2004, Budd et al. 2012, 2019).

Marginal basins of the Tethys

Marginal basins of the Tethys show lower biodiversity compared to the core western Tethys hotspot in general (Renema et al. 2008). The Paratethys Sea (Figure 3) originated around the Eocene/Oligocene boundary (~34 Ma) as a northern satellite basin of the Tethys Ocean due to the uplift of the Alpine mountain chains and disintegrated during the Pliocene. The sea reached maximum extent during the early Miocene and stretched from the Rhône Basin in France towards Inner Asia (Rögl 1998) (Figure 3). During the Oligocene–Miocene, convergence of the Afro-Arabian and Eurasian plates initiated a complex pattern of changing seaways and land bridges between the eastern and western Tethys Ocean and the Paratethys Sea. These palaeogeographical changes were amplified by sea-level fluctuations in the range of ~50–60 m associated with Antarctic ice-sheet dynamics at the time (Harzhauser et al. 2007, Reuter et al. 2009, Miller et al. 2020). The Paratethys Sea is characterised by moderate biodiversity, outside of the core Tethyan hotspots of the western Tethys and Arabia, at least in LBF (Renema et al. 2008). However, the Paratethys Sea has a rich fossil record (Walkiewicz 1977, Radwańska 1984, Stolarski 1991) and harboured high biodiversity, for example, of gastropods (Baluk 1975, Bałuk 1995, 2006, Scarponi et al. 2015), as documented for recently revised taxonomic groups (Harzhauser et al. 2002, Harzhauser & Landau 2017, 2019, 2021a, b).

The Tarim Basin (Figure 3; in the western part of Xinjiang province, north-western China) was a semi-enclosed sea and a north-western branch of the Tethys seaway in the Paleogene before the establishment of the Paratethys Sea (Xi et al. 2016). The Tarim Basin fostered a rich marine invertebrate and microfossil fauna in the Paleogene but disappeared due to marine regression by the end of the Eocene (Bosboom et al. 2014, Cao et al. 2018, Jiang et al. 2018). The biodiversity of this sea is not well quantified but does not seem high judging from published data (Bosboom et al. 2014, Cao et al. 2018, Jiang et al. 2018).

The eastern Neo-Tethyan seaway (Figure 3) is the shallow-marine basin between the Eurasian continent and Indian sub-continent before the India-Asia collision (Figure 3). This seaway is characterised by relatively low LBF and ostracod biodiversity during the Eocene (Renema et al. 2008, Wang et al. 2020) and remained opened until at least ~36 Ma (Wang et al. 2020).

Arabian hotspot: late Eocene–Miocene

Renema et al. (2008) considered the Arabian hotspot to be distinct from the western Tethys hotspot from the late Eocene into the Miocene. During the late Eocene, LBF biodiversity was highest in the Arabian region (Renema et al. 2008, Serra-Kiel et al. 2016) (Figure 7). Indeed, even in the late middle Eocene (42–39 Ma), some Arabian sites record higher LBF diversity than those of the western Tethys based on our new data compilation (Figure 5). Arabian biodiversity remained highest during the middle Miocene, while western Tethys biodiversity had already diminished by that time (Renema et al. 2008) (Figures 4 and 7). The Arabian hotspot shares faunal elements with the western Tethys hotspot in the late Eocene and with the IAA hotspot in the Miocene (Renema et al. 2008) but also exhibits endemism in the Eocene (Lunt 2003), Oligocene and Miocene (Harzhauser et al. 2007, Ozcan & Less 2009).

Restriction and closure of the Tethyan Seaway: the demise of the Arabian hotspot

Marine connections through the Tethyan Seaway became gradually restricted from the late Oligocene to the early Miocene as detailed below. In the late Oligocene (Chattian, 28–23 Ma), gastropod faunas from the Kutch Basin (northwestern India, northern coast of the eastern Tethys) indicate a moderate, but still traceable, faunal exchange via the Tethyan Seaway with the western Tethys (Harzhauser et al. 2009). However, comparison of molluscan faunas from the late Oligocene

(Chattian) Warak and earliest Miocene (Aquitanian) Ghubbarrah formations in south-eastern Oman with coeval faunas from north-western India and Pakistan reveals the initial blockage of dispersal pathways for the shallow-marine biota along the Afro-Arabian margin (Harzhauser 2007, Harzhauser et al. 2009).

Along with this biogeographic development, extensive parts of the Arabian shelf sea became increasingly restricted in the south-eastern Oman region during the late Oligocene and finally emerged in the earliest Miocene (early Aquitanian, ~22.5 Ma), linked to rifting cessation and commencement of seafloor spreading in the Gulf of Aden at that time (Reuter et al. 2008). The dynamic interplay between tectonics and sea-level caused the stepwise interruption of the marine connection (Tethyan Seaway) between the western and eastern Tethys in the region that later become the Zagros Mountains and Mesopotamia (Arabia) during the early to middle Miocene (Harzhauser et al. 2007). Frequent changes from normal marine to hypersaline facies are documented in the late Oligocene–early Miocene Asmari Formation, which was deposited on a carbonate platform in the foreland of the Zagros orogenic belt (Iran), representing the inner part of the Tethyan Seaway (Mossadegh et al. 2009). Following the general shallowing-upward trend in the Asmari Formation, a change from normal marine to hypersaline conditions occurred during the late Oligocene (late Chattian) (Mossadegh et al. 2009). Hypersaline conditions with episodes of even higher salinity followed during most of the early Miocene (Aquitanian) (Mossadegh et al. 2009). Such a dynamic environment is unlikely to sustain high-level biodiversity. Larger benthic foraminiferal, gastropod and bivalve evidence indicates that the Arabian biodiversity hotspot finally collapsed in the middle or late Miocene as a result of habitat loss largely caused by regional uplift of the Arabia-Eurasia collision and the closure of Tethyan Seaway (Kay 1996, Harzhauser et al. 2007, Renema et al. 2008).

End of the trans-Tethyan marine connection

In the north-eastern sector of the Tethyan Seaway (Eurasian margin), the marine gateways to the Qom Basin (central Iran; Figure 3), which was connected to the western Tethys and Tethyan Seaway, gradually ceased during the early Miocene (Aquitanian and early Burdigalian) due to the compressive tectonic regime (Reuter et al. 2009). This favoured deposition of evaporites in the basin during the third-order sea-level lowstands at 21.4 and 20.4 Ma (Reuter et al. 2009). It is therefore assumed that the total breakdown of western Tethys connectivity in Aquitanian gastropod assemblages from SE Oman (Harzhauser 2007) and north-western India (Kutch Basin; Harzhauser et al. 2009) and in the Aquitanian–Burdigalian echinoid fauna of southern Iran (Hormuz Strait; Kroh et al. 2011) was related to an excessively saline environment throughout the Tethyan Seaway (Reuter et al. 2009). This saline environment interrupted east–west dispersal of shallow-marine biota such as molluscs and corals (Reuter et al. 2009). A shift back to normal marine conditions took place during the latest Aquitanian in the Zagros Basin and was followed by frequent changes from normal marine to slightly hypersaline in the early Burdigalian (Mossadegh et al. 2009). Although this phase coincided with the re-appearance of western Tethys gastropod taxa in northwestern India (Kutch Basin; Harzhauser et al. 2009), the Burdigalian gastropod faunas of southern Iran (Makran) and of southwestern India (Kerala Basin) exhibit no faunal affinity to the western Tethys (Harzhauser et al. 2007, Harzhauser 2014) (Figure 8). In addition, the Mediterranean and Indo-Pacific reef coral faunas shared no species after the Aquitanian (McCall et al. 1994, Schuster & Wielandt 1999, Perrin & Bosellini 2012). This lack of faunal relations point to considerable dispersal limitation via temporary and rather ineffective dispersal pathways from the western to the eastern Tethys during the Burdigalian. In line with this biogeographic pattern, the trans-Tethyan marine connection was finally interrupted during the Burdigalian third-order sea-level low stand at 19.2 Ma (Reuter et al. 2009) (Figures 4 and 7). The progressive narrowing of the Tethyan Seaway culminated in the extensive distribution of terrestrial and hypersaline sedimentary facies in the Iranian Qom, Esfahan-Sirjan and Zagros basins at the end of the Burdigalian (Reuter et al. 2009). Development of these facies documents the

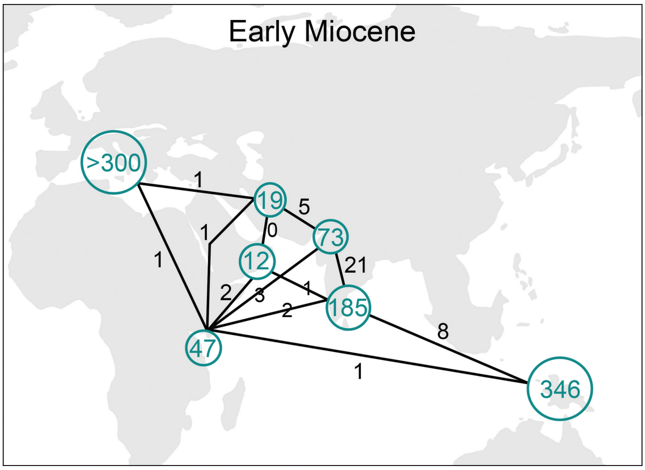

Figure 8 Diversity distribution of Early Miocene gastropod faunas. The numbers in the circles indicate the numbers of species described. Numbers over the lines indicate the number of shared species between regions. The pattern suggests a strong provincialism. Data from Harzhauser (2007, 2009, 2014), Harzhauser et al. (2009, 2017) and Leloux and Wesslingh (2009). Palaeogeographical map for 20 Ma and from Kocsis and Scotese (2021).

emergence of the *Gomphotherium* Landbridge at ~19 Ma, which allowed for the first exchange of terrestrial biota between Eurasia and Africa, but formed a biogeographic barrier for marine organisms (Harzhauser et al. 2007, 2009, Reuter et al. 2009). Although eustatic sea-level rise related to the middle Miocene climatic optimum (MMCO), a global warming event at ~17.0–14.5 Ma (Figure 4), might have re-opened the seaway for a short time via the Mesopotamian Trough (Rögl 1998, 1999), biogeographic separation between Mediterranean and Indo-West Pacific shallow-marine faunas persisted until the opening of the Suez Canal and the Lessepsian migration (Por 1978, Harzhauser et al. 2007, Rilov & Galil 2009, Perrin and Bosellini 2012, Albano et al. 2021).

End of the Tethys

With the final closure of the Tethyan Seaway during the early Miocene (~19 Ma; Harzhauser et al. 2007), the western Tethys vanished and gave rise to the proto-Mediterranean Sea (Harzhauser et al. 2002) (Figures 4–7). Some original western Tethys biota such as tridacnine bivalves (Harzhauser et al. 2008), strombid gastropods (Harzhauser et al. 2007), *Parascolymia* corals (Reuter et al. 2015) and Platinistidae dolphins (Barnes 2002) migrated into the eastern Tethys prior to the closure of the Tethyan Seaway and contribute to the enormous present-day biodiversity of the Indo-West Pacific (Figure 9). Generic richness of zooxanthellate corals markedly declined in the Mediterranean reef coral province during the Miocene. The reason for the decline in reef coral diversity was likely regional climate cooling from tectonically-induced northward shift of the Mediterranean region and global climatic change (Bosellini & Perrin 2008, 2010, Perrin & Bosellini 2012, 2013). This gradual cooling in the Miocene caused the disappearance of almost all zooxanthellate colonial corals (except for *Cladocora caespitosa*) from the Mediterranean Sea and the end of the (sub)tropical Mediterranean coral reef province until the onset of the Pliocene (Perrin & Bosellini 2013, Vertino et al. 2014). The geologically short-term (5.96–5.33 Ma) Messinian Salinity Crisis (MSC: Figures 4 and 7) additionally caused a massive disruption to marine life in the Mediterranean Sea, but small *Porites* reefs were still present in the Messinian post-evaporitic Terminal Complex in the western Mediterranean Sea (Vertino et al. 2014).

With the closure of the Tethyan Seaway, the Indian Ocean became a geographic entity in the Miocene. The data on Oligocene and early Miocene shallow-marine gastropods from central (Qom

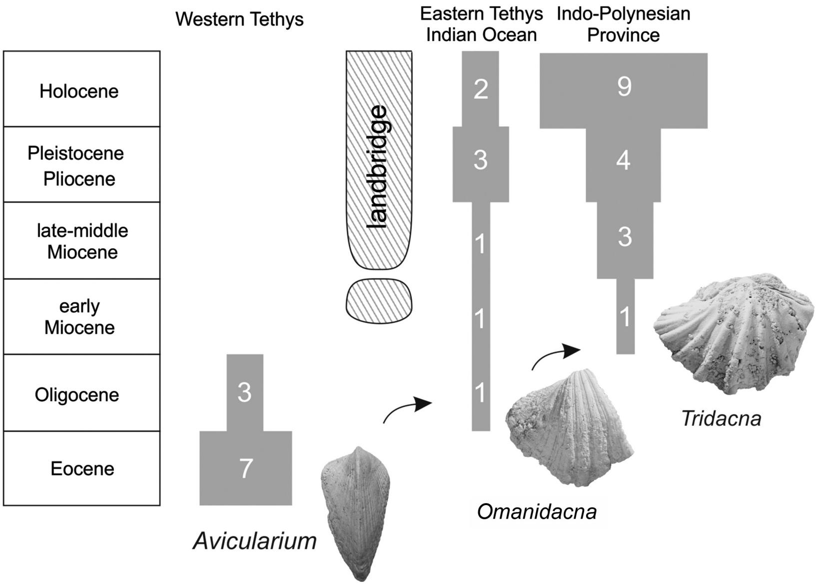

Figure 9 Evolutionary history of tridacnine bivalves. They evolved during the Eocene in the north eastern Atlantic and the western Tethys and spread to the eastern Tethys during the Oligocene. Numbers indicate species richness. Data from Harzhauser et al. (2007).

Basin) and southern (Makran) Iran, Oman, Tanzania and northwestern (Kutch Basin) and south western (Kerala Basin) India document a complex pattern of faunal relations among these areas with high rates of endemism (Harzhauser 2007, 2009, Harzhauser et al. 2009, Harzhauser 2014, Harzhauser et al. 2017) (Figure 8). Thus, a distinct early Miocene bioprovincialism can be postulated during the switch from the eastern Tethys to the Indian Ocean with a Central East African Province, an east African-Arabian Province, a western Indian Province and a Proto-Indo-Polynesian Province in the east (Harzhauser 2007, 2009, 2014). Correspondingly, Oligocene and early Miocene reef coral faunas from eastern Africa (Somalia) show a low similarity with southern and central Iran and no relation to Indonesia at species level (Reuter et al. 2019). This early Miocene palaeobiogeographical pattern differs fundamentally from the pattern of the Indian Ocean as a rather homogeneous single province that persisted during the last 10 Myrs (late Miocene–present; Costello & Chaudhary 2017, Kocsis et al. 2018).

Present-day Indian Ocean

Today, reef coral diversity peaks in the IAA, but a subordinate centre of coral reef biodiversity (across diverse taxa such as corals, mantis shrimps and LBF) occurs in the northern Mozambique Channel between east Africa and Madagascar (Reaka et al. 2008, Obura 2012, Veron et al. 2015, Förderer et al. 2018, Kusumoto et al. 2020) (Figure 1). This subordinate centre is maintained by the inflow of the South Equatorial Current, which brings coral larvae from the IAA (Obura 2012). The high compositional similarity at species level between a late Miocene (~7–6 Ma, Messinian) reef coral fauna from southern Tanzania (Rovuma Basin) and the living coral fauna of eastern Africa shows that the community structure of reef corals remained largeley unchanged in this area since the late Miocene (Reuter et al. 2019). The different biogeographic affinities of Oligocene–early Miocene and late Miocene–recent east African coral faunas indicate that this regional centre of reef coral biodiversity in the western Indian Ocean did not exist in its present form during the early Miocene. Its high diversity may have developed by the faunal connection of the region to the IAA during the middle to late Miocene (Reuter et al. 2019). The end of bioprovincialism corresponds with the onset and intensification of the Miocene Indian Ocean Equatorial Jet between 14 and 9 Ma in response to the narrowing of the Indonesian Gateway (Gourlan et al. 2008). This precursor of the South Equatorial Current is thought to have caused high biogeographic connectivity across the Indian Ocean (Reuter et al. 2019) and, thus, facilitated the formation of the east African hotspot of reef coral diversity as an offshoot of the Coral Triangle (Reuter et al. 2019).

Strengths, weaknesses and opportunities

Our knowledge of the western Tethys and Arabian hotspots is high for certain taxonomic groups such as molluscs, but there is no synthetic quantification of biodiversity trends in space and time. Renema et al. (2008) used LBF as a proxy, but the resolution of their palaeobiogeographical maps (regarding number of time slices and data points) was not sufficient to show details throughout the broad Tethys region. Consequently, we do not know when and how peak biodiversity shifted from the western Tethys to Arabia. Details for marginal regions such as the Tarim Basin, Qom Basins, and Paratethys Sea are also not well resolved. There is a pressing need for rigorous standardised assessments for other taxonomic groups, including molluscs, corals, bryozoans and ostracods.

Another major problem in understanding these hotspots is a dearth of information about Paleocene biogeography and biodiversity that preceded the above transitions. Some migration events are known during the Paleocene–Eocene transition (Speijer et al. 2012, Beasley et al. 2021). For example, the ostracod genus *Alocopocythere* is known to disperse from west Africa to the western Tethys region, and poleward shift is known in several taxa such as planktonic foraminifera (e.g. *Acarinina sibaiyaensis*) and the dinoflagellate cyst genus *Apectodinium*. However, aside from these events, we lack synthesis of Paleocene Tethys palaeobiogeography, with studies focusing mostly on taxonomy of individual taxa (e.g. in ostracods: Al-Furaih 1980).

Indo-Australian Archipelago (IAA)

Miocene origin

The Indo-Australian Archipelago region began to host substantial biodiversity (hotspot initiation) from the early Miocene (~23 Ma ago), following relatively low diversity in the Paleogene (Renema et al. 2008, Bellwood et al. 2012, Yasuhara et al. 2017a, Prazeres & Renema 2019) (Figures 4–7). With senescence of the western Tethys hotspot, narrowing of the Tethyan Seaway and contraction of the tropics to lower latitudes after the Eocene (Renema et al. 2008, Bribiesca-Contreras et al. 2019), Tethyan relicts of corals and reef fishes are believed to have shifted their distribution to the IAA during the Oligocene–Miocene. Both groups exhibited rapid lineage diversification in the IAA (Bellwood et al. 2017), resulting in the formation of the modern IAA faunal province (Titterton & Whatley 1988, Keith et al. 2013, Costello et al. 2017, Kocsis et al. 2018). After extinction of a number of widespread and long-ranging taxa at the Eocene/Oligocene boundary (Renema 2007, Cotton et al. 2014, Prazeres & Renema 2019), the diversity of LBF increased in the Oligocene and plateaued from the early Miocene to the present (Adams 1965, Adams et al. 1986, Renema 2007, Prazeres & Renema 2019) (Figures 6 and 7), with a change in faunal characteristics in the early Miocene (rise of the modern fauna) and highest diversity in the middle Miocene, where both the 'old faunal' and an already upcoming modern fauna merged (Renema et al. 2015, Prazeres & Renema 2019). After, LBF experienced a substantial extinction during the middle Miocene (~11.6 Ma) (Renema 2007) (Figures 6 and 7). Fossil corals and ostracods show substantial Miocene diversification (Renema et al. 2008, Johnson et al. 2015b, Renema et al. 2016, Yasuhara et al. 2017a) (Figures 6 and 7). Based on molecular data, the Miocene origin of the vast majority of modern fish genera has been believed to have played an important role in the development of the IAA hotspot (Bellwood et al. 2017). These lines of evidence consistently support an early Miocene initiation of the IAA hotspot. However, IAA diversity may have been lower than Caribbean diversity at that time (Di Martino et al. 2018), and Oligocene–early Miocene fossil data remain sparse in the IAA region (McMonagle et al. 2011, Reich et al. 2014, Yasuhara et al. 2017a), which means taphonomic or sampling biases may affect observed diversity patterns. Indeed, Paleogene IAA diversity was already high in LBF that have more complete fossil records compared to other taxonomic groups (Renema 2007). Although numerous molluscs have been described from the Miocene of the IAA (e.g. Indonesia, Leloux & Wesselingh 2009, see Harzhauser et al. 2018 for a literature survey), collecting is still fragmentary and biogeographic analyses and critical revisions are largely lacking.

The apparent Oligocene–Miocene increase in IAA shallow-marine biodiversity coincided with tectonic activity that formed the complex island archipelagos of the IAA region, with a dynamic mosaic of oceanic arc and microcontinental fragments (Renema et al. 2008, Leprieur et al. 2016, Hall 2017). In particular, the collision of the south-east Eurasia margin with the Australian and Pacific plates at ~23 Ma was critical in developing this vast area of complex habitat (Kuhnt et al. 2004, Renema et al. 2008, Hall 2011). Thus, an extensive array of shallow seas provided suitable habitats for corals on shallow carbonate platforms, and the geographic complexity of this region provided barriers for vicariance events, which may have together facilitated the colonisation of Tethyan relicts and diversification of new lineages as suggested by molecular studies (Pandolfi 1992, Dornburg et al. 2015, Bellwood et al. 2017, Bribiesca-Contreras et al. 2019). An example of a vicariance event within the IAA is the biogeographic separation of the late Miocene gastropod fauna from the South China Sea from those of the Java Sea, the Makassar Strait and the Celebes Sea (Harzhauser et al. 2018). This biogeographic pattern was triggered by a landmass stretching from the Asian mainland across Borneo from the Oligocene to the Pliocene (Lohman et al. 2011). With the formation of the IAA hotspot, the basic framework of modern biodiversity and biogeographic patterns in tropical oceans emerged in the Miocene (Titterton & Whatley 1988, Renema et al. 2008, Keith et al. 2013, Bellwood et al. 2017) (Figures 5 and 7).

Pliocene–Pleistocene biodiversity jump

Another important period of significant diversity increase in the IAA hotspot occurred during the Pliocene–Pleistocene, known as the Pliocene–Pleistocene biodiversity jump (Hoeksema 2007, Renema et al. 2008, Renema 2010, Bellwood et al. 2012, Yasuhara et al. 2017a, Shin et al. 2019) (Figures 6 and 7). In terms of corals and reef fishes, both fossil and phylogenetic evidence indicates rapid species-level diversification during this interval (Bellwood et al. 2017). Molecular evidence further suggests that coral reef biodiversity approximately doubled in the last 5 Ma (Bellwood et al. 2017). In addition, microfossil groups, including ostracods and LBF, show a consistent Pliocene–Pleistocene diversity increase (Renema 2007, Yasuhara et al. 2017, Shin et al. 2019). During the Pliocene and Pleistocene, the IAA hotspot played a more important role as the macroevolutionary source from which shallow-marine lineages diversified and dispersed into the Indian Ocean and Central Pacific regions (Cowman & Bellwood 2013b, Cowman et al. 2017). It is widely recognised that Pliocene–Pleistocene eustatic sea-level fluctuations are the most important mechanism of diversity increases (Bellwood et al. 2005, Briggs 2005, Hoeksema 2007, Bellwood et al. 2012, Yasuhara et al. 2017a). Successive cycles of sea-level change caused repeated disconnection and reconnection of small marine basins during lowstands and highstands, which in turn influenced habitat area and affected ocean currents within the geographically complex IAA region through a relatively short geological time (Pandolfi 1992, Hoeksema 2007, Bellwood et al. 2012, Yasuhara et al. 2017). Due to these dynamic eustatic and oceanographic processes, isolation of populations may have promoted speciation, but also increased the risk of extinction. Subsequent reconnection may have then facilitated the accumulation of peripheral species in the centre of biodiversity (Renema et al. 2008, Bellwood & Meyer 2009, Bellwood et al. 2012). Movements and/or expansion of the geographic distributions of shallow-marine species may have also led to an overlap zone in their geographic ranges, resulting in high species richness (Bellwood et al. 2012). However, it is uncertain if glacially-forced isolation was long enough to prompt speciation. In addition, diversification (Renema 2010, Yasuhara et al. 2017, Prazeres & Renema 2019, Shin et al. 2019) may predate the onset of the large glacial-interglacial sea-level variations at ~2.6 Ma (Lisiecki & Raymo 2005, Yasuhara et al. 2020b).

Switching the Indonesian Throughflow (ITF: warm ocean current running from the Pacific to Indian Ocean) on and off may have also driven Pliocene–Pleistocene diversification in the IAA. Gallagher et al. (2009) presented evidence for Indonesian Throughflow restriction between 10 and 4.4 Ma, and between 4 and 1.6 Ma. Either of these periods of restriction, which would have disrupted north-east to south-west ITF current flows from the Pacific to Indian Ocean, may have facilitated a west-to-east faunal shift from the Indian to Pacific Ocean, across the Timor and Arafura Seas, via a South Java Current flow uninhibited by the influence of the ITF. This may have resulted in the coexistence of species from the Indian Ocean and the Pacific Ocean in shallow-marine environments on the east side of a subsequently re-established Indonesian Throughflow. For example, the first records of the ostracod genera *Alocopocythere* and *Stigmatocythere* indicate that these taxa originated in the Cretaceous or early Paleogene African-Arabian-Indian region (Siddiqui 1971, Al-Furaih 1980, El Sogher et al. 1996) and went on to become widely distributed in the Eocene to Miocene of the Indian Ocean (Siddiqui 1983). The earliest records of these genera in Southeast Asia are in the Pliocene (although pre-Pliocene fossil records in this region are poor) (Hou & Gou 2007). The earliest records of *Alocopocythere* in northern Australia (east of the ITF) are in the 'Anthropocene' (Warne et al. 2006), although, again, earlier fossil faunas in this region are poorly known. Despite this patchy fossil record, if these genera migrated into the Pacific during a period of the Indonesian Throughflow restriction, migration would have occurred between 10 and 4.4 Ma or more likely between 4 and 1.6 Ma. The Indonesian Throughflow restriction may have facilitated faunal dispersal from the Indian to Pacific Ocean, subsequently resulting in the coexistence of species, or speciation in new environments after re-establishment of the Indonesian Throughflow.

Due to the incompletely known late Cenozoic ostracod fossil record of the IAA, we cannot exclude an alternate possible hitch-hiker dispersal event via recent shipping or earlier human maritime travel (Teeter 1973). Although some Tethyan ostracod genera such as *Schizocythere* are known to be widely distributed in the Asian Pacific in the Eocene (Yamaguchi and Kamiya 2009), it may be that certain Tethyan ostracod genera such as *Alocopocythere* and *Stigmatocythere* could not disperse into the Pacific until the Pliocene–Pleistocene because of the Indonesian Throughflow. Consistent with this explanation, benthic foraminifera show faunal separation between the Indian and Pacific Oceans until 4.4 Ma (Gallagher et al. 2009). These 'out of Indian Ocean' shifts might have enhanced IAA biodiversity.

In summary, incorporation of fossil and molecular data with patterns of modern species richness provide valuable insight into the origin and development of the IAA hotspot from the Miocene to the Pleistocene and the nature of biodiversity hotspots more generally. As the western Tethys hotspot senesced after the Eocene, the IAA may have inherited ancient Tethyan lineages and gradually become the key biogeographic region. The Miocene and Pliocene–Pleistocene were two critical epochs during which diversity increased, leading to the modern, bullseye-like pattern of IAA biodiversity, likely aided by the lack of substantial extinction in the region, in contrast to the Caribbean Sea as discussed below (Di Martino et al. 2018).

Strengths, weaknesses and opportunities

IAA biodiversity trends are comparatively well summarised in palaeontological and molecular data for several taxonomic groups (Renema et al. 2008, Williams & Duda 2008, Bellwood et al. 2012, Johnson et al. 2015b, Leprieur et al. 2016, Yasuhara et al. 2017). In contrast, detailed palaeobiogeographical changes in the Cenozoic IAA, and their relationship to the western Tethys, are not well understood for many taxonomic groups. With a few exceptions (Renema et al. 2008, Bromfield & Pandolfi 2012, Santodomingo et al. 2015, 2016, Mihaljević et al. 2017, Yasuhara et al. 2017a, Di Martino et al. 2018), palaeontological data and their syntheses are scanty in the IAA when compared to other hotspots. This obvious deficiency in bryozoans, corals, molluscs and ostracods will be improved with the Throughflow (Johnson et al. 2015a) and 4D-REEF (https://www.4d-reef.eu/) project samples in the near future. Given rich Pliocene–Pleistocene fossiliferous sediments in the region compared to Paleogene sediments (e.g. Yasuhara et al. 2017, Shin et al. 2019), the Pliocene–Pleistocene biodiversity jump should be better quantified.

Tropical America and the Caribbean

Continental rifting broke up Pangea, forming the proto-Atlantic Ocean as North America drifted to the north-west and opened the Central American Seaway (CAS) during the Early Jurassic (Pliensbachian) (Smith & Tipper 1986). The CAS persisted in various sizes until the Pliocene–Pleistocene. The dynamics of the resident biota in Tropical America were defined principally by regional tectonic activities leading, at first, to an ocean expansion and emergence of shallow-marine habitats. Later, general trend across all major groups shows a pattern of ever-increasing isolation and rapid *in situ* diversification until massive extinction at the beginning of the Pleistocene. Today, Tropical American biodiversity is generally low compared to the IAA, but considerably higher than in the eastern Pacific, west Africa and Brazil (Figure 1). Here we review the known geological, environmental and biotic changes that resulted in the modern-day biodiversity of Tropical America, with particular focus on the Caribbean.

Early Cenozoic tropical America

Throughout the Eocene, the eastern Pacific and western Atlantic remained connected through the CAS. Tectonic reconfigurations, uplift and emergence led to a complex arrangement of platforms. Florida formed a broad carbonate platform, with abundant LBF, echinoderms and molluscs, and isolated platforms existed across the Caribbean. However, shared faunal assemblages existed across Mexico, Florida and Cuba, characterised by the LBF lepidocyclinids and nummulitids (Frost 1974, Bowen Powell 2010, Cotton et al. 2018, Torres-Silva et al. 2019). Diverse corals are also found on the Mexican platform. Further south, LBF assemblages are found in Jamaica and the Nicaragua rise, showing higher species richness than the more northerly platforms, including multiple endemic species (Robinson 1995, Robinson et al. 2003). However, samples from Costa Rica show curiously low diversity (Baumgartner-Mora & Baumgartner 2016). Robinson (2004) suggested that the more restricted marine connections between the Gulf of Mexico and Florida (following reconstructions by Pindell 1994) would have inhibited lower to higher latitude movement from the tropical Nicaragua Rise to marginally tropical Florida and Gulf coast, and may account for the varying taxa and diversity. New data compilation of late mid-Eocene (42–39 Ma) LBF diversity in the present study shows that, while diversity was generally lower compared to the western Tethys at that time, there are a few high diversity sites (Figure 5).

The Eocene LBF assemblages of the Caribbean differ considerably to elsewhere at this time. Although the genus *Nummulites* is found in all three biogeographic provinces (i.e. western Tethys, Arabian and IAA), only two species of the genus *Nummulites* are present in the Americas, while several hundred species are present in the Tethys within the same interval (Schaub 1981, Torres-Silva et al. 2019). The lepidocyclinids evolved in the middle Eocene in the Caribbean province and became widespread in the region, but are not seen until the Oligocene in the Tethyan region and until the late early Oligocene in the IAA (BouDagher-Fadel & Price 2010). Their dispersal is thought to have been facilitated by fluctuating sea-level in this interval (BouDagher-Fadel & Price 2010).

Few Oligocene and Miocene LBF records from the Caribbean have been published. However, as in other regions, the Eocene/Oligocene boundary is followed by a decrease in LBF diversity, which occurs with the extinction of orthophragmines and several other long ranging LBF taxa (Figure 7). Shallow water mollusc records also show a general decrease in diversity towards and in the Oligocene, particularly in warm water taxa (e.g. Hansen 1987). In the Gulf of Mexico, over 90% of taxa went extinct between the latest Eocene and Oligocene (Hansen et al. 2004). Irregular echinoids also show a large diversity decrease from the Eocene to Oligocene in Florida, while regular taxa remain relatively constant (Oyen & Portell 2001). Both the cooling and sea-level fall would likely have been detrimental to tropical shelf taxa, although studies of the timing from elsewhere suggest extinctions within the LBF were not caused directly by the decrease in shelf space (Cotton & Pearson 2011).

In the Oligocene, the previous dominance of LBF across the Caribbean declined and conditions became more favourable for corals, resulting in the region-wide formation of thick sequences of coral reefs (Johnson et al. 2008, 2009). However, these coral reefs declined at the Oligocene–Miocene transition when 50% of corals species disappeared and the rate of reef growth reduced sharply (Johnson et al. 2008, 2009) (Figures 6 and 7). The cause of this biotic turnover remains to be determined. Steinthorsdottir et al. (2021) speculated that narrowing of the Tethyan Seaway reorganised ocean currents such that cool waters entered from the Pacific through the CAS into the Caribbean, causing extinction of thermophilic reef taxa.

Increasing isolation of tropical America

In the Oligocene, the Caribbean remained marginally connected to the Tethys. However, the collision of the Afro-Arabian plates with Eurasia in the early to middle Miocene brought this circum-global tropical seaway to an end and cut the Mediterranean from the IAA and isolated the Caribbean from the rapidly growing hotspot of the IAA (Figure 7, Renema et al. 2008). Indeed, a number of major clades of marine organisms that are today shared between the modern day Atlantic and Pacific hotspots ceased to disperse and began to split around this time (e.g. Duda Jr & Kohn 2005). Moreover, many genera of reef corals formerly believed to be circumtropical are now classified in different families, with many clades restricted to the Caribbean and broader tropical western Atlantic (Fukami et al. 2004, 2008).

As global continental reconfigurations continued, the Caribbean became ever more isolated from other warm-water biogeographic realms. The Atlantic Ocean continued to spread and widen, making the crossing from west Africa and the Mediterranean Sea less likely. At the same time, the tropical western Atlantic was isolated from the eastern edge of the IAA by the largest ocean barrier in the world: the eastern Pacific Barrier (EPB) that today includes around 5000 km of almost entirely deep water that continues to act as a strong dispersal filter between the west and east Pacific (Darwin 1872, Ekman 1967, Vermeij 1987, Lessios & Robertson 2006).

The rise and fall of the productive Atlantic-Eastern Pacific biogeographic realm

The continued isolation of Tropical America from both the east and the west resulted in the formation of a unique faunal province called the Atlantic-Eastern Pacific (AEP) biogeographic realm (sometimes termed the Gatunian Faunal province) (Figure 3). The Miocene AEP extended across most of the tropical western Atlantic, including Florida to the north, Brazil to the south and Panama to the west. It continued through the CAS(s) into the Tropical eastern Pacific up to California and as far south as Ecuador (Woodring 1974, Landau et al. 2008, 2009, Aguilera Socorro et al. 2011, Pimiento et al. 2013, Aguilera et al. 2014, Leigh et al. 2014). Both taxa and ecological characteristics were shared across this large range, with apparently long-food chain trophic systems (Pimiento et al. 2010, Alberti & Reich 2018, González-Castillo et al. 2020), abundance of filter-feeding benthic (O'Dea et al. 2007, 2016, 2018, Nebelsick et al. 2020) and pelagic (González-Castillo et al. 2020) organisms supported by strong upwelling of nutrient-rich waters (Jones & Allmon 1995, O'Dea et al. 2007, Anderson et al. 2017, Grossman et al. 2019) – a configuration that today is relegated to regions of the tropical eastern Pacific.

The productive AEP realm persisted through to the late Miocene, but even by the middle Miocene tectonic reconfigurations had initiated the transformation of the Caribbean towards the basin we know today. First, Pacific subduction uplifted the Andes and diverted the outflow of the enormous body of fresh water in South American from the Caribbean (Urumaco in northern South America) east to the Atlantic in the late Miocene (Hoorn et al. 2010). This ended the transport of land-based nutrients into the Southern Caribbean whose impacts probably reached to a ~1000 km radius (Subramaniam et al. 2008). Second, starting ~25 Ma, South America collided with the semi-emergent Panama volcanic arc causing it to fracture (Farris et al. 2011) and uplift – a process that continues today (O'Dea et al. 2016). Uplift and volcanic 'infilling' (Buchs et al. 2019) caused the interoceanic straits crossing the Panama volcanic arc to become increasingly narrow and shallow through the Miocene, as observed by evidence of (1) the shallowing of suites of sedimentary basins from Colombia to Costa Rica (Coates et al. 2004, 2005), (2) initiation of genetic, morphological and life-history divergences in marine sister taxa in the Pacific and Caribbean (Lessios 2008, O'Dea et al. 2016), (3) separations in Pacific-Caribbean deep-water oxygen isotope ratios (Sepulchre et al. 2014, Kirillova et al. 2019) and (4) an increase in the probability that continental mammals, birds, insects and reptiles could disperse over the narrowing seaways between the two American continents (O'Dea et al. 2016, Jaramillo et al. 2017).

As the narrow interoceanic seaway(s) diminished through the Miocene–Pliocene, Caribbean faunal richness accelerated (Figures 6 and 7), potentially driven by increasing habitat heterogeneity (Jackson et al. 1993, Budd et al. 1994, Johnson et al. 2007, Klaus et al. 2011, Jagadeeshan & O'Dea 2012, Grossman et al. 2019) resulting in *in situ* diversification (Vermeij 2005, Leigh et al. 2014) and providing appropriate habitat for immigrants (e.g. Siqueira et al. 2019). Evidence from the cheilostomate bryozoans – the only group whose fossil records have been compared between the Caribbean and IAA in detail – suggests that the increasing diversity observed in the Caribbean at this time paralleled that of the IAA hotspot (Di Martino et al. 2018) (Figure 7). The same may be true for reef corals that appear to exhibit only moderate diversity in the IAA during the Miocene (Santodomingo et al. 2016). In contrast, molecular analysis suggests that parrotfish diversity in the IAA was caused by *in situ* diversification, whereas immigration played a much more prominent role in the less spectacular growth of parrotfish diversity in the Caribbean (Siqueira et al. 2019).

The Miocene–Pliocene accumulation of Caribbean marine diversity is observed in the fossil records of a wide array of animal groups including corals (Johnson et al. 2008, Klaus et al. 2011), gastropods (Kohn 1990, Duda Jr & Kohn 2005, Todd & Johnson 2013), bivalves (Leonard-Pingel et al. 2012), bryozoans (Jackson & Cheetham 1994, Cheetham et al. 1998, O'Dea & Jackson 2009, Di Martino et al. 2018), decapods (Schweitzer et al. 2006) and fish (González-Castillo et al. 2020), among others (Leigh et al. 2014) (Figures 6 and 7). However, much of this diversity was soon to face extinction associated with the closure of the shallow-marine connection of the CAS between the Atlantic and Pacific Oceans approximately 3 Ma (O'Dea et al. 2016). Between 4 and 2 Ma, extinction rates across all groups peaked, resulting in the loss of 30% up to 100% of species (depending on group) by the early Pleistocene (Stanley 1986, Allmon 2001, Todd et al. 2002, O'Dea et al. 2007, Smith & Jackson 2009, Leonard-Pingel et al. 2012, Todd & Johnson 2013, Di Martino et al. 2018) (Figures 6 and 7). This regional mass extinction event was highly selective against modes of life suited to highly productive settings, such as non-zooxanthellate-assisted filter feeding, clonal reproduction and planktotrophic larvae (Johnson et al. 1995, 1996, Allmon 2001, O'Dea & Jackson 2009). This extinction pattern implicates the previously-described oceanographic declines in planktonic productivity due to the restriction of the shallow-marine connection of the Central American Seaway as the root cause. However, several extinct bryozoan species (e.g. species of Cupuladriidae) persisted 1–2 Myrs after the diminishment of Caribbean's source of upwelling waters at ~4 Ma (O'Dea et al. 2007, O'Dea & Jackson 2009, Grossman et al. 2019), although they appear to have done so in small, isolated populations (O'Dea & Jackson 2009). The cause of this mismatch between environmental 'cause' and macroevolutionary 'effect' has yet to be resolved. Several hypotheses have been proposed, such as the random decline of metapopulations as conditions became less favourable (O'Dea et al. 2007, Smith & Jackson 2009) based on the phenomenon of extinction debt (Nee & May 1992, Tilman et al. 1997). Alternatives include the persistence of isolated pockets of nutrient-rich coastal waters (O'Dea & Jackson 2009, Grossman et al. 2019) although there is as yet no evidence of consistent environmental differences among locations to support this.

Birth of the modern Caribbean

The decline in productivity across the Caribbean also enabled coral reefs to proliferate to an extent not seen since the late Oligocene (O'Dea et al. 2007, Johnson et al. 2008, Klaus et al. 2012). The extent and rate of reef building increased sharply in the early Pleistocene, reaching rates seen in the Oligocene (Johnson & Pérez 2006, Johnson et al. 2008). This rapid reef building was, to some extent, driven by the arrival and ecological dominance of the coral genus *Acropora* in shallow Caribbean habitats around 2 Ma (Jackson 1994, Klaus et al. 2012, Renema et al. 2016). *Acropora* is characterised by rapid growth with colony extension rates many times higher than other branching coral genera and was able to thrive during the intervals of rapid sea-level change that initiated 1.8 Ma and has since intensified towards the recent.

The environmental conditions (oligotrophic and aseasonal), ecological states (fast-growing large reef tracts) and assemblages of taxa resilient to the rapid and extreme sea-level fluctuations that characterised the Pleistocene of the modern Caribbean were therefore established only within the last two million years. Since then, a few clades have experienced intense *in situ* diversifications within the Caribbean (e.g. *Polystira*; Todd & Johnson 2013, *Stylopoma*; Jackson & Cheetham 1994), but most groups appear to remain a subset of their earlier pre-extinction diversity (DiMartino et al. 2018). The last million years have seen remarkable stability in those groups whose fossil record is sufficiently well studied despite intense fluctuations in sea-level and shelf areas (Jackson 1992, Pandolfi et al. 2001) (Figure 7).

Tropical eastern Pacific: an overlooked geologically recent hotspot?

The general paradigm has been that the tropical eastern Pacific is not a biodiversity hotspot – an idea that probably stemmed from the perception that coral reefs are rare and, when they are present, they are species poor. It is true that reef building coral species are of especially low diversity in the tropical eastern Pacific, and their abundance is low when compared to the reef tracts of the Caribbean. Yet, richness of other groups (e.g. molluscs) is often comparable to the Caribbean and often exceeds it (Leigh et al. 2014) (but note that the tropical eastern Pacific is much more abundant in molluscs and so molluscan diversity could be overestimated by sampling artefact [i.e. easy to collect more specimens] [see Jackson et al. 1993, Vermeij 1993, Smith & Jackson 2009]). Ongoing work in the region is revealing high levels of previously unknown diversity, much of which is endemic, that may elevate this region to a global biodiversity hotspot (Cortés et al. 2017, Jefferson & Costello 2020). The available evidence shows that the origins of this fauna is a complex mix of high-productivity-adapted remnants of the AEP biogeographic realm (Leigh et al. 2014), descendants of lucky dispersals across the eastern Pacific barrier, and local endemics that remain poorly studied (Cortés et al. 2017).

Strengths, weaknesses and opportunities

Palaeontological studies, including those of the Panama Palaeontology Project, have documented biological patterns of diversity and ecological and evolutionary change (Jackson & Johnson 2000, O'Dea et al. 2007, Johnson et al. 2008). Dynamics in earlier periods (e.g. Paleogene to early Miocene) are less well studied (but see Iturralde-Vinent 1998) because of a general lack of replicated sedimentary rock sequences or poor preservation in the sequences that exist. In addition, the Pleistocene records of non-reef taxa are poorly known, which leaves the precise timing of the Caribbean extinction unresolved. Indeed, smaller benthic foraminifera appear to sidestep the extinction event (Smith et al. 2013) and show strong and continuous Miocene–Present diversification (Figure 6). Filling these gaps will improve our understanding of the history of the Caribbean hotspot and allow for a more detailed comparison with other hotspots.

Summary of major Cenozoic biodiversity hotspots

The post-Mesozoic Tethys Ocean belonged to a large tropical biogeographic realm connecting all of the western Tethys, Arabian, IAA and Caribbean regions to varying degrees, but the Tethys no longer exists and these four regions are now separated by land masses and/or deep ocean (Figure 7). The hotspots associated with these four regions all have dynamic ecosystem histories. As described above and clearly shown by LBF, corals and other taxonomic groups (Figures 5 and 6), the western Tethys hotspot thrived in the Eocene, diminished in the Oligocene, and vanished in the Miocene at ~19 Ma, mainly due to isolation and regional and global cooling. The Arabian hotspot emerged in the late Eocene showing higher diversity than the western Tethys hotspot and was sustained until the middle Miocene. The IAA hotspot originated in the Miocene at ~23 Ma, further diversified in the Pliocene–Pleistocene, and, mainly because of tectonics and associated oceanography, continues to host the highest biodiversity in the oceans today. The Caribbean hotspot emerged in the Oligocene, temporarily diminished during the Oligocene–Miocene transition, then rapidly diversified in the Miocene–Pliocene and collapsed by the Pliocene–Pleistocene extinction event (Figures 4–6; see summary in Table 1 and Figure 7).

Hotspot dynamics and drivers: global perspectives

Although plausible drivers of diversity changes in each hotspot have been discussed above, the explanations tended to be descriptive. Comparing differences and similarities in their histories may help to better constrain the common driver(s) and testable hypotheses of tropical biodiversity. Thus, here we try to contextualise these hotspots globally.

Hopping Hotspots or Whack-A-Mole?

The Hopping Hotspots model (Renema et al. 2008) (Table 2) is the first integrative hypothesis to explain the spatiotemporal dynamic of multiple biodiversity hotspots. This hypothesis proposes that marine biodiversity hotspots have 'hopped' or shifted across almost half of the globe throughout the Cenozoic: from the western Tethys (present Mediterranean) during the Eocene, to the Arabian region during late Eocene–early Miocene, and eventually to the IAA region from the early Miocene onwards to track suitable shallow-marine habitats created by major tectonic movements (Renema et al. 2008). Each hotspot has an initiation and, in the case of the western Tethys and Arabian hotspots, a demise.

During the Eocene, the large western Tethys was connected to the Indian Ocean. The Indian subcontinent in the middle of the Indian Ocean had not yet collided with the Eurasian continent (Harzhauser et al. 2002, Wang et al. 2020). The western Tethys, a tectonically active and geographically complex ocean surrounded by the European, African, Arabian and Indian plates, fostered high biodiversity at that time (i.e. western Tethys hotspot) (Renema et al. 2008). After, the Tethys Ocean gradually narrowed by the collision of the African with the Eurasian continent, and the Tethys Seaway to the Indian Ocean was closed in a stepwise fashion by the Miocene at ~19 Ma

Table 2 Comparison of hypotheses and how to test their relative importance

	Hopping Hotspots	Whack-A-Mole
In short	Certain taxa with high diversity or high diversification potential hopped from the western Tethys, through Arabia to the IAA (Indo-Australian Archipelago) to the east, and to the Caribbean to the west, and prospered in each region.	Moles (=biodiversity hotspots) rise and fall individually, without moving from hole to hole. High diversity regions have occurred in suitable habitats capable of many species or speciation events.
Cause of hotspot dynamics	Suitable habitat created in tectonically active, collision regions (large habitat size/shelf area, high habitat/coastline complexity); pumping out Tethyan elements from a senescent hotspot to newborn hotspots.	Suitable habitat created in tectonically active, collision regions (large habitat size/shelf area, high habitat/coastline complexity).
Assumption	Certain Tethyan taxa (e.g. genera) are responsible for hotspots and their high diversity.	Certain Tethyan elements (e.g. genera, families) are unrelated to hotspots and their high diversity.
How to test? Eocene biogeography	Key taxa might not yet extend to the IAA and Caribbean regions. Or, key taxa may have reached these regions but not yet diversified due to lack of suitable habitat size and complexity.	Whack-A-Mole will be supported if the endemism is extremely low among the western Tethys, Arabia, IAA and the Caribbean, although high endemism itself will not reject this hypothesis.
How to test? Key elements	Key taxa are responsible for hotspots' high diversity.	Absence of common key taxa among hotspots.
How to test? Oligocene–Miocene biogeography	There should be enhanced migration of key taxa from the western Tethys and Arabia to the IAA and perhaps Caribbean.	There may be less migration in general compared to the Eocene, when the western Tethys and Arabia had more connection to the IAA and Caribbean.
How to test? Pre-extinction Caribbean fauna	The key taxa in the pre-extinction Caribbean should exhibit phylogenetic relationships with those of the Eocene western Tethys.	The key elements of pre-extinction Caribbean biodiversity should have functional/ecological similarity to those of the Eocene western Tethys.
How to test? Modern IAA fauna	The key taxa of the modern IAA should exhibit phylogenetic relationships with those of the Eocene western Tethys.	The key elements of modern IAA biodiversity should have functional/ecological similarity to those of the Eocene western Tethys.
Did Caribbean senescence cause hopping?	Taxa that went extinct during the Plio-Pleistocene Caribbean extinction event should have been pumped out to the west and east to some degree.	NA

(Harzhauser et al. 2007). The biodiversity centre moved eastwards, with a diversity decrease in the western Tethys and a diversity increase in the Arabian region (i.e. Arabian hotspot) during the late Eocene–early Miocene, coinciding with the collision of the Arabian and Eurasian plates. The Arabian hotspot ceased during the middle Miocene as the continental collision proceeded, and the vast shallow-marine carbonate platforms on the Arabian Shelf and the central Iranian basins became increasingly restricted and finally subaerially exposed (i.e. the final closure of the Tethyan Seaway at ~19 Ma, Renema et al. 2008, 2009, Bellwood et al. 2012). Indo-Pacific biodiversity started to increase during the Miocene at ~23 Ma (i.e. IAA hotspot) when the collision of Australia with the Pacific arcs and the south-east Asian margin formed a complex archipelago (Renema et al. 2008, Bellwood et al. 2012), connecting shallow-marine habitats. With eastern shifts in peak biodiversity from the Arabian to IAA region, IAA biodiversity has remained persistently high from the Miocene to today. By correlating the formation and senescence of each of the three replicate hotspots with successive tectonic collisions along the Eurasian plate from west to east, the Hopping Hotspots model indicates strong abiotic control (i.e. plate tectonics) on evolutionary-scale biodiversity patterns (Renema et al. 2008).

However, the long-term impacts of plate tectonics must manifest through mechanisms that underpin the origination and accumulation of individual species to foster high biodiversity (Beaugrand et al. 2018). In the case of IAA, a complex mosaic of continental seas, island arcs and microcontinental fragments formed within the convergence zone between tectonic plates (Hall 2002, 2012). This geological complexity is of great ecological importance as it may disrupt population connectivity, increase the area and heterogeneity of shallow habitats, accumulate species from peripheral regions, and form an overlap zone of species-distribution ranges (Hoeksema 2007, Bellwood et al. 2012). All these processes may contribute to high biodiversity through speciation of marine taxa.

The Hopping Hotspots model is supported by fossil and molecular evidence. The fossil record of multiple shallow-marine taxa (e.g. large benthic foraminifera, reef corals, mangroves, bivalves, gastropods) consistently suggests eastern shifts of maximal alpha diversity following biodiversity hotspots from the Eocene Tethys to Arabia and eventually to the late Miocene–Recent IAA (Renema 2007, 2008). Larger benthic foraminiferan palaeobiogeographical data tend to show similar taxonomic composition among the hotspots, indicating spatial and temporal connectivity of component taxa from the Tethys through Arabia to the Indo-Pacific (Renema 2007, Renema et al. 2008). In addition, recent molecular and phylogenetic studies, together with first occurrence data in fossil records, demonstrate the early origination of many extant IAA taxa, ranging from the Eocene to late Miocene (Williams & Duda 2008, Bellwood et al. 2017). In some case studies of taxonomic groups with an exceptionally good fossil record (e.g. LBF), palaeobiogeographical distributions indicate that higher taxa (genera, families) originated in the western Tethys, radiated east following the Tethyan hotspot senescence and reached Indonesia as the IAA hotspot emerged (Renema 2007, Dornburg et al. 2015). As these taxa moved with the Hopping Hotspots to the IAA region, many species seem to have arisen from them thereafter (Bellwood et al. 2012). With growing recognition of the importance of historical processes in shaping modern biodiversity, the Hopping Hotspots model emphasises the spatiotemporal shifts of particular shallow-marine taxa (‘Tethyan elements’) to tectonically active (or continental collision) regions to form new biodiversity hotspots. We now understand the IAA hotspot as the latest manifestation of a biodiversity pattern analogous to that of the Eocene western Tethys (Renema et al. 2008).

However, the degree to which biodiversity hotspots really ‘hopped’ sideways or arose and fell independently through time has not been tested quantitatively. Shifts in geographic ranges from the Eocene western Tethys to the Miocene IAA have been elucidated for only a few key taxa (Bellwood et al. 2012). Limited fossil evidence in the literature is often not sufficient or robust to validate the palaeobiogeographical affinities between historical hotspots. Older literature often needs taxonomic updates and lacks stratigraphic and palaeoecological information. Palaeontological databases, while useful, often adopt the data without further critical evaluation. These factors are limiting for comparison of fossil assemblages from different chronostratigraphic stages and regions. In addition to abiotic controls (i.e. tectonic events and climate changes) over large-scale biodiversity patterns, the Hopping Hotspots model implies that biotic controls (i.e. shifts of higher taxa/Tethyan elements) are also important by proposing a correlation between palaeobiogeographical similarities and biodiversity dynamics among the hotspots (Renema et al. 2008). However, it remains unclear if the shifts of Tethyan taxa are causally linked to development of high biodiversity given that major tectonic collisions control the area and variability of suitable shallow-marine habitats. Testing the validity of the causal link between biogeography and biodiversity, and consequently the Hopping Hotspots model, may increase our understanding of the interplay between biotic and abiotic factors in shaping regional to global biodiversity patterns over evolutionary timescales. If a causal link is detected (e.g. high biodiversity is characterised by certain higher taxa, instead of miscellaneous rare species, Table 2), it may be feasible to identify a representative range of taxa that are indicative of high biodiversity. These indicator taxa will be useful to monitor biodiversity for conservation and management purposes and to reconstruct past biodiversity hotspots, without full biodiversity census data that is time-consuming to obtain.

As discussed above, the Hopping Hotspots model suggests that biogeographic shifts of certain taxa fostered biodiversity through biotic interactions in suitable habitats set by plate tectonic movements. On the other hand, Cenozoic tropical biodiversity dynamics may also be explained simply by a rise and fall of diversity in each region, regardless of biogeographic shifts of certain taxa between hotspots (=hopping). We call this null hypothesis 'Whack-A-Mole', in which moles (=biodiversity hotspots) rise and fall individually, without moving from hole to hole (Table 2). This hypothesis suggests that abiotic factors such as plate tectonics, climate and oceanography control the 'capacity' for number of species in an area (Benton 2009, Tittensor et al. 2010) regardless of 'Tethyan elements'. Shifts of taxa in this case, if occurring at all, may be regarded as random dispersal events out of the senescing hotspot where tectonic subsidence and collision caused habitat loss. As coral phylogeny indicates, a hotspot may provide suitable habitats for many species and may have nothing to do with macroevolutionary mechanisms (Pandolfi 1992). Noticeably, there is not a dichotomy between the Hopping and Whack-A-Mole hypotheses, and we need to understand their relative importance (i.e. more biotic or abiotic controls over biodiversity?) by testing the putative effects of palaeobiogeographical shifts on biodiversification (Table 2).

Global Hopping Hotspots model: Caribbean–Tethys relationship

While the original Hopping Hotspots hypothesis does not include the Americas, the tropical Americas were a part of the Tethys during the Mesozoic–Paleogene greenhouse world when the Atlantic Ocean was narrower. For example, there are ostracod species in common between the Caribbean and the western Tethys in the Eocene (McKenzie 1967, 1987, Yasuhara et al. 2019a). Even in the Miocene, some 'westward' dispersal is known in certain groups of bivalves and gastropods (Harzhauser et al. 2007) and bryozoans (Cheetham 1968). Thus, the Caribbean hotspot can be incorporated into the global Hopping Hotspots model (Figure 7, Table 2). Hotspots could hop not only to the east, but also to the west, with the Eocene–Miocene connection between the Caribbean and western Tethys and the subsequent Miocene–Pliocene diversification in the complex shallow-marine habitats created by tectonics/collision in the Caribbean. This global Hopping Hotspots process is clearly depicted in the LBF data as well as coral and other taxonomic group data (Figures 5 and 6).

'Centre-of' hypotheses and geographical barriers

A set of 'Centre-of' hypotheses have been proposed to explain the diversity centre in the IAA, focused on the centre of speciation (species originate in the centre and successively disperse to surrounding areas), centre of accumulation (species arise in the peripheral locations and subsequently move to the centre), centre of survival (species originate anywhere and better survive in the centre), centre of overlap (species geographical ranges overlap in the centre) and/or other varieties of these hypotheses (see review by Bellwood et al. 2012) (also see Pandolfi 1992, Bellwood et al. 2005, Briggs 2005, Cowman & Bellwood 2013a, Cowman 2014, Huang et al. 2018). These 'Centre-of' hypotheses can be applied to all Cenozoic diversity hotspots. The 'Centre-of' mechanisms can operate only when a tropical hotspot is situated in 'the centre' with connection to adjacent regions both to the east and west. In this regard, the death of a hotspot has occurred when a barrier is formed to block out its eastern or western connection. For instance, the closures of the Tethyan Seaway and Central American Seaway changed the Paleogene western Tethys and Miocene–Pliocene Caribbean hotspots to enclosed marginal seas, respectively. This east-west connection may be an important condition for the existence of a biodiversity hotspot (Figure 7). Although little consensus has been reached regarding the patterns and modes of macroevolution, most of these 'Centre-of' hypotheses invoke tectonic, eustatic, climatic, oceanographic and geomorphological processes (TECOG) as the underpinning mechanisms of the bullseye pattern of IAA diversity (Rosen 1984, Bellwood et al. 2012, Cowman & Bellwood 2013a) that might have also operated in the extinct western Tethys and Caribbean hotspots.

Role of shallow-marine habitat size and complexity

Habitat area and complexity are considered the main diversity drivers in the Hopping Hotspots model (Renema et al. 2008) and Phanerozoic global marine diversity (Valentine & Moores 1970, Zaffos et al. 2017), and some of drivers of the present-day coastal diversity (Tittensor et al. 2010). For example, see the similarity between coastal species richness and coral reef distributions in Figure 1. We tried to quantify habitat size and complexity by using palaeogeographical reconstructions of palaeo-coastlines and flooded continental shelf distributions for the last 50 Myr (Kocsis & Scotese 2021). Habitat size was expressed for every hotspot area in every 5 Myr bin using the total shelf area between the reconstructed maximum transgression coastline and the continental margin (–1400 m isobath, Kocsis & Scotese 2021, Figures 5, 6 and 10). We approximated habitat complexity in the coastlines by calculating the lengths of the coastlines in the same areas. These coastline length calculations were executed using the ‘yardstick’ method (O’Sullivan & Unwin 2010), with a yardstick length of 100 km. Hotspot sizes were inferred to be almost constant, but latitudinal and longitudinal extents changed over time along with changing palaeogeography (Figures 6 and 10). Thus, we operationally defined the hotspot areas as follows: The IAA ranged between 70°E–160°E and 10°S–25°N in 50–0 Ma. The Tethys (note that this is in a broad sense, including both the western Tethys and Arabian hotspots, because it is difficult to define the western Tethys and Arabian regions separately on maps) was expressed as the union of a spherical rectangle covering the Mediterranean (10°W–20°E and 19°N–45°N) and another covering the western basin of the Indian Ocean (20°E–60°E and 0°N–50°N in 50–35 Ma, 20°E–60°E and 10°S–40°N in 35–0 Ma). The Caribbean ranged between 90°W–60°W and 0°N–40°N in 50–45 Ma, 95°W–65°W and 0°N–40°N in 40 Ma, 95°W–60°W and 0°N–35°N in 35–25 Ma, 100°W–65°W and 0°N–35°N in 20–10 Ma, 100°W–62°W and 0°N–32°N in 5–0 Ma. Both habitat size and coastline length were calculated using a similar method with penta-hexagonal grids (Kocsis 2020) with a mean grid cell edge length of 6.66° (Figure 5).

Shallow-marine habitat area and complexity trend show substantial similarity to those of regional diversities (Figures 1, 5 and 6). IAA diversity increased with an increase in shelf area and coast line length, and Tethyan diversity decreased with a decrease in shelf area and coast line length, respectively. Larger habitat areas and hotspot locations are well matched on present day and palaeo maps (Figures 1, 5 and 6). This quantitative evidence supports the view that suitable habitat (size and complexity) created by active tectonics is important for the development and maintenance of biodiversity hotspots, and, thus, both the Hopping Hotspots and Whack-A-Mole models.

Role of temperature

There is increasing consensus that present-day and Quaternary large-scale marine biodiversity patterns are mainly temperature-driven, and that higher temperature areas (e.g. tropics) or times (e.g. interglacials) retain more species (Yasuhara et al. 2009, Tittensor et al. 2010, Yasuhara et al. 2012b, Beaugrand et al. 2013, Costello & Chaudhary 2017, Reygondeau 2019, Gagné et al. 2020, Rogers et al. 2020, Yasuhara et al. 2020b). In addition, global Cenozoic diversity curves tend to bear considerable similarity with temperature trends (Condamine et al. 2019, Yasuhara et al. 2020b). Indeed, the IAA is now sitting in the warmest spot in the world (Figure 5). However, the IAA has been the warmest region throughout the Cenozoic, even in the Eocene when peak biodiversity was situated

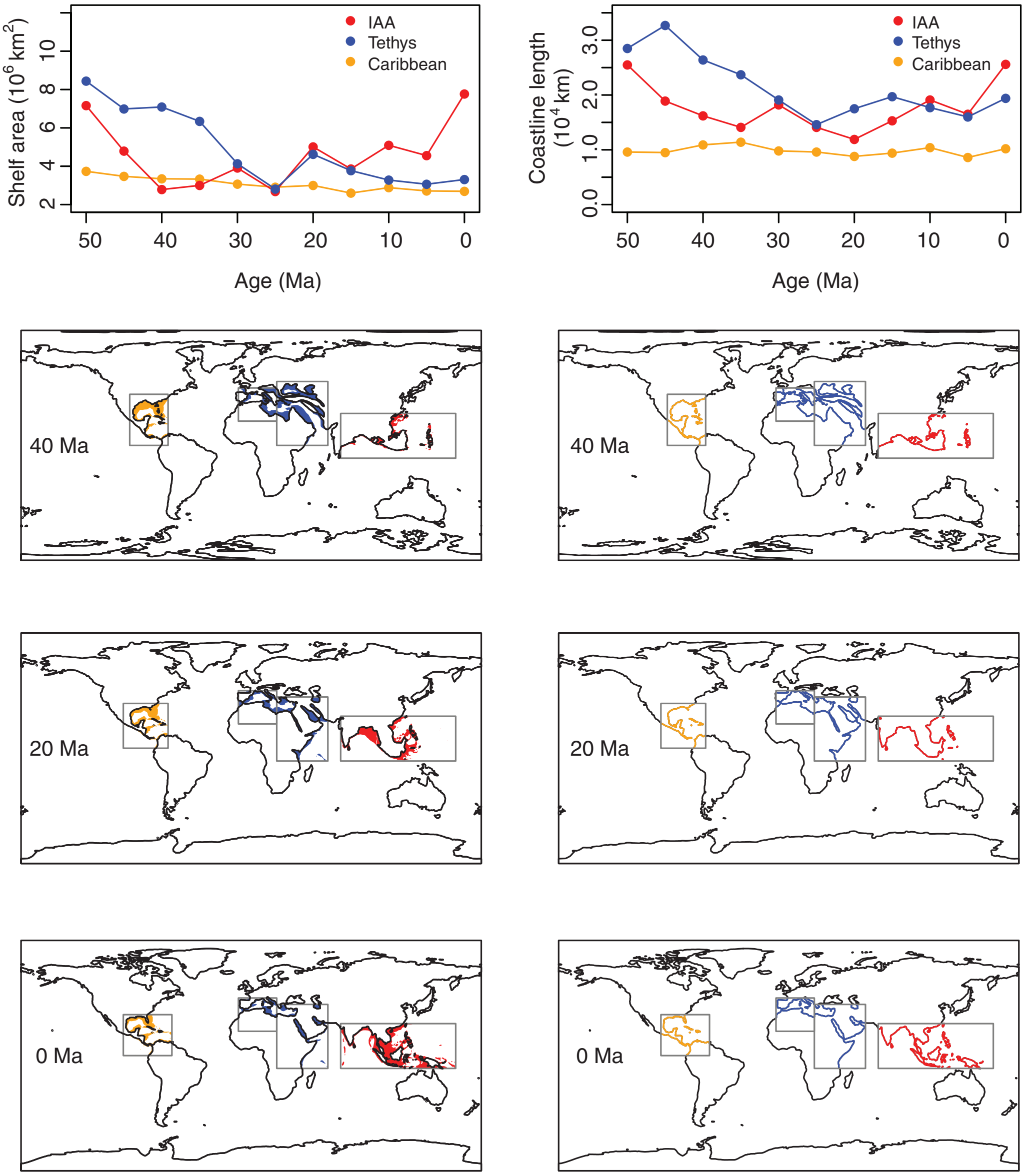

Figure 10 Shelf areas (left column) and coastline lengths (right column) in the IAA, Tethys (western Tethys+ Arabian) and Caribbean areas. The top panels show values every 5 million years from 50 to 0 Ma (see Figure 6 and Section 'Role of shallow-marine habitat size and complexity'). The maps in the second to fourth rows illustrate the calculations in the 40, 20 and 0 Ma time slices, respectively.

in the western Tethys (Figure 5). The western Tethys was not the warmest region in the Eocene, yet hosted the highest diversity at that time (Figure 5). From a temperature perspective, this inconsistency may mean that Eocene equatorial regions might have been too hot to host high levels of biodiversity.

We now know that equatorial temperatures can be too hot for marine organisms. Latitudinal diversity gradients become bimodal with an equatorial dip in both the past and present in concert with warming oceans (Kiessling & Simpson 2011, Chaudhary et al. 2016, 2017, Yasuhara et al. 2020b, Yasuhara & Deutsch 2022). Many pelagic and benthic marine species have shifted their distribution away from the equator into mid-latitudes since the 1950s as predicted by climate change models (Chaudhary et al. 2021). Major marine taxonomic groups show decreases in species richness above 20°C–25°C (Chaudhary et al. 2021). This pattern points to a role for temperature in the past development of tropical biodiversity hotspots. Hopping Hotspots from the western Tethys to IAA ranged from moderately low latitude to equatorial through time. Other than tectonics, this latitudinal shift may, in part, be due to cooling, and most equatorial oceans, including the IAA, may have been too hot in the greenhouse Paleogene (Worm & Tittensor 2018).

For the Eocene super-greenhouse world, little is known with respect to the distribution, structure and biodiversity of coral reefs in low latitudes, except for framework reefs being rather uncommon and small (Perrin 2002). In this respect, we draw a parallel with the last interglacial where coral diversity seemed to have been low near the equator and many coral taxa displayed poleward range shifts (Kiessling et al. 2012). These same shifts are occurring now in planktonic foraminifera (Yasuhara et al. 2012b, 2020) and many other groups (Poloczanska et al. 2013, Pecl et al. 2017). Tropical sea surface temperatures during the Eocene were ~5°C higher than present (Evans et al. 2018, Hollis et al. 2019, Lunt et al. 2021) (Figure 5) and therefore likely to have caused a zone depauperate in coral reefs within the equatorial warm pools, e.g. Southeast Asia (Wilson & Rosen 1998, Scheibner & Speijer 2008). In addition to temperature stress to reef corals, ocean acidification also contributed to the reef crisis during the Paleocene–Eocene thermal maximum (PETM) (Kiessling & Simpson 2011) and a possible reef gap during the early Eocene Climate Optimum (Perrin & Kiessling 2011, Norris et al. 2013). Notably, a recent molluscan study showed that the early Eocene latitudinal diversity gradient was U-shaped with minimum diversity at the tropics (Boag et al. 2021). But note that Eocene planktonic foraminifera do not show a strong equatorial dip in diversity (Fenton et al. 2016). LBF also experience a turnover event around the Paleocene–Eocene transition, but an increase in diversity through the early Eocene (Whidden & Jones 2012). Scheibner and Speijer (2008) also show a global shift across the PETM from coral-dominated to LBF-dominated platform systems. So, although corals and LBF show similar patterns of diversity relating to tectonics, perhaps temperature/ocean chemistry events differentiate between their responses. Yasuhara et al. (2012b) further showed the extinction of warm-adapted tropical planktonic foraminifera species via global cooling since the Pliocene. There may have been some taxa adapted to super high temperature in the equatorial Paleogene before the Neogene cooling caused their extinction.

During the early Eocene, sea surface temperature was >34°C at the tropics where the IAA and Caribbean regions were situated, whereas the western Tethys hotspot temperature was ~28°C, similar to the present-day IAA temperature (Figure 5). At this temperature of >34°C, oxygen will be less than 6 mg/L when fully saturated, and thus low latitude ocean habitat would have been significantly oxygen limited (Shi et al. 2021). In the middle Miocene, the equatorial IAA and Caribbean remained >30°C, when biodiversity in these hotspots was only moderately high (Figures 5 and 7). By the mid-Pliocene, the Mediterranean Sea (western Tethys region) became too cold to house a tropical fauna (Figure 5). Recent studies indicate that temperatures >25°C are too hot for many organisms and may result in diminished diversity (Yasuhara et al. 2020b, Boag et al. 2021, Chaudhary et al. 2021). Thus, Eocene and perhaps Miocene equatorial temperature was too high to generate and maintain high levels of biodiversity comparable to the present-day IAA. Even the present-day IAA temperature of ~28°C is too hot, as we see equatorial diversity declines (Yasuhara et al. 2020, Chaudhary et al. 2021). Thus, the higher present-day temperature in the IAA compared to the Caribbean probably does not explain higher diversity in the IAA. In sum, temperature may be an important constraint (i.e. hotspots cannot be fully developed in places that are too cold or too hot), but does not explain the entire history of tropical biodiversity hotspots.

Human impacts on biodiversity hotspots

A major question is how the biodiversity that we see today has been degraded by humans, decreasing diversity levels from a 'pre-anthropogenic disturbance' baseline. A substantial diversity decline from the Pleistocene to the present is known from ostracods in the IAA (Yasuhara et al. 2017a), potentially due to human-induced ecosystem degradation. Although extant tropical biodiversity of both the IAA hotspot and the Caribbean is often described with human activities evidenced by coral bleaching, IUCN endangered species and outbreaks of harmful taxa such as crown-of-thorns starfish (De'ath et al. 2012), these constitute only a few aspects of human-induced ecosystem degradation. Our understanding of the true human impact on the local (alpha) and regional (gamma) biodiversity of major taxonomic groups in these areas remains qualitative or insufficient (Rogers et al. 2020).

Caribbean Sea

In the Caribbean region, exhaustively compiled observational data have shown that coral cover declined from >30% in the 1970s to less than 20% in the 2000s, while macroalgal cover increased from ~10% to 25% in the same time period (Jackson et al. 2014). This dramatic shift in benthic dominance represents the alteration of the regional ecosystem by human activities, primarily through increased development, water pollution, anthropogenic species invasion and overfishing, and by recent climatic warming via coral bleaching (Glynn 1993, Hughes 1994, Jackson et al. 2014, Hughes et al. 2018). However, it is known that degradation began well before the start of biological observations around the 1970s (Hughes 1994, Gardner et al. 2003, Jackson et al. 2014) as it is well documented in historical and palaeontological archives (Jackson 1997, Jackson et al. 2001, Pandolfi et al. 2003, Pandolfi & Jackson 2006, Cramer et al. 2012, 2017, Cramer et al. 2020a,b).

Human perturbations likely began to permanently alter the composition of Caribbean ecosystems with the arrival of European settlers and colonisation (Jackson 1997). One of the most notable documented degradations is the complete collapse of the branching *Acropora* forests that characterised Caribbean reefs since the early Pleistocene (Jackson 1992, Pandolfi & Jackson 2006). This collapse was caused by rapid human population growth and likely agricultural impacts (Cramer et al. 2020b), white-band disease (Aronson & Precht 2001, Knowlton 2001, Jackson 2008) and bleaching (Aronson et al. 2000). It has caused a 'flattening of the Caribbean reefs', or a decrease in reef complexity, leading to further biodiversity loss and altering ecosystem function (Alvarez-Filip et al. 2009, O'Dea et al. 2020). Similar human-induced collapse of *Acropora*, the dominant reef builder for the last 1.8 Myrs, is known regionally and globally (Renema et al. 2016, Cybulski et al. 2020). Comprehensive palaeoecological works have been conducted in the Bocas del Toro region of Panama where bivalve, gastropod and foraminiferal assemblages suffered a deterioration of reef ecosystem conditions by land-based agricultural activities and resulting eutrophication in the mid-twentieth century or earlier (Cramer et al. 2015, 2020b, Figuerola et al. 2021). Declines in the accretion rates of Panamanian coral reefs were linked to the historical overfishing and decrease in parrotfish since the mid-eighteenth–mid-nineteenth century (Cramer et al. 2017). Fossil echinoid spines showed that the lack of recovery of the sea urchin *Diadema antillarum* after its mass mortality event in the 1980s (Hay 1984, Hughes 1994, Jackson 1997, 2008, Cramer et al. 2018) may have been due to the long-term overfishing of piscivorous fishes that released damselfishes, an effective competitor of *Diadema*, from predation pressure (Cramer et al. 2017, 2018). This imbalance of *Diadema* in the reef ecosystem is particularly striking when palaeorecords indicate that they were the most abundant sea urchin on reefs for the last 125,000 years (Gordon & Donovan 1992, Jackson 1997). Parrotfish and *Diadema* are both important herbivores (Hay 1984), and the loss of these populations in the region has contributed to a phase shift in the reefs from coral- to algal-dominated ecosystems (Jackson 1997). Recent invasion of predatory lionfish (*Pterois volitans*) may have also been a factor, because their prey includes juvenile parrotfishes and other small herbivorous fishes (Johnston & Purkis 2011, Albins & Hixon 2013, Côté & Smith 2018).

Historical resource overexploitation, poaching, habitat alteration, environmental pollution, European trade, and other human activities in the Caribbean has permanently changed marine faunal composition through the removal of large sharks and large trophy fish, and reduced populations of manatees, sea turtles and humpback whales (Mignucci-Giannoni et al. 2000, Reeves et al. 2001, Romero et al. 2002, Smith & Reeves 2003, McClenachan 2009, McClenachan et al. 2010, Ward-Paige et al. 2010, Lotze & McClenachan 2014, Dillon et al. 2021). It also led to the complete extinction of the Caribbean Monk Seal due to intensive human hunting (McClenachan & Cooper 2008). Perhaps the best proxy for larger animal and higher trophic level decimation in the Caribbean is seen with the collapse of sea turtle populations. Before colonial hunting, it is estimated that green and hawksbill turtle populations numbered over 6.5 million and are now reduced to around 300,000, a 95% decline (McClenachan et al. 2006). By using these larger taxa as a proxy for other marine life needed to support the food chain, it can be calculated that Caribbean fish and invertebrate biomass has seen as much as an 80% reduction since historical times (McClenachan & Cooper 2008).

IAA

Although it is the type example of a modern-day marine biodiversity hotspot that harbours much higher biodiversity than the Caribbean, much less is known for the IAA despite recent increased scientific interest. Top ranked areas for global marine biodiversity, the Philippines and Sunda Islands, are associated with very high 'threat score' from human impacts (Roberts et al. 2002). The IAA is also a region with some of the highest levels of intrinsic risk globally – or risk of extinction for modern taxa based on palaeontological data – mainly due to climate and human stressors (Finnegan et al. 2015).

Very little research has been done on the extent of human impacts on IAA ecosystems. In Papua New Guinea, a comparison of fossil and modern assemblages showed greater coral biodiversity in the past and decreased modern reef variation due to anthropogenic degradation (Edinger et al. 2001). In general, the limited historical ecology research in Papua New Guinea agrees with general biodiversity degradation due to anthropogenic forces, showing that numerous taxa (herbivores, carnivores, suspension-feeders and corals) have all been exploited by human populations since indigenous times and are being pushed closer to ecological extinction (Berzunza-Sanchez et al. 2013). Microfossils (foraminifera and ostracods) in surface sediments show ecosystem degradation in the IAA region (Delinom et al. 2009, Renema 2010, Fauzielly et al. 2013a, Nurruhwati et al. 2020). However, there are no palaeoecological studies investigating long-term changes, except an ostracod study in Jakarta Bay showing a faunal shift at ~1950 related to the increased population of Jakarta City and resulting eutrophication (Fauzielly et al. 2013b).

Nearby areas to the IAA can be used to draw general biodiversity conclusions for the western Pacific, such as in the Great Barrier Reef and the South China Sea. The Great Barrier Reef, south of the IAA, is one example of a marine region with documented biodiversity changes over the Anthropocene. Although some low diversity reefs have remained unaffected for the last 1,200 years despite high sedimentation and nutrient loads due to European colonisation (Perry et al. 2008), other turbid reefs experienced similar collapses of previously dominant *Acropora* in the 1920s–1960s from water quality degradation after European settlement and again in the 1980s–1990s due to cyclones, thermal stress and flood events (Roff et al. 2013, Clark et al. 2017).

The South China Sea, adjacent to the IAA and similar in terms of high marine biodiversity, though classified as a high-latitude marginal reef system, has become an area where recent palaeobiological reconstructions have built a strong understanding of biodiversity change through the Anthropocene. Hong Kong represents a case study for extreme levels of human development juxtaposed with high marine biodiversity that could be applied to much of the South China Sea region

(Duprey et al. 2017, Ng et al. 2017). Cybulski et al. (2020) investigated the previously unknown history of fossil coral communities in sediment cores there. Similar to the Caribbean (Cramer et al. 2020a), Hong Kong has experienced a 40% range decline in important reef building *Acropora*, which has led to a decrease in reef habitat complexity and functionality for the area (Cybulski et al. 2020). The modern assemblages in areas that are located closest to sources of poor water quality show lower genus diversities compared to those of the natural-baseline fossil assemblages. They concluded that increased nutrients and perhaps sedimentation, associated with extreme development in the region, is the leading stressor impacting modern coral communities – a threat common to most marine life throughout Southeast Asia and the IAA (Todd et al. 2010). Overall degradation of reef ecosystems and a decline in biodiversity resulting from modern Anthropogenic impacts characterise many marine communities within the South China Sea (Chen et al. 2009, Zhao et al. 2012, Li et al. 2013, Clark et al. 2019, Yan et al. 2019, Cybulski et al. 2020).

In summary, multiple lines of observational, historical and palaeobiological evidence reveal that coastal development associated with post-European settlement and overexploitation has degraded Caribbean reef ecosystems for over two centuries (Jackson 1997, Pandolfi et al. 2003, Cramer et al. 2020a). Similar threats are obvious in the IAA as seen in fossil data from adjacent areas such as the Great Barrier Reef and the South China Sea, discussed above, but much less long-term evidence is available for the IAA itself. The similarity between the degradation that occurred in the Caribbean and the degradation we are currently seeing in a region of maximum biodiversity is alarming, particularly as we do not currently have an understanding of the scale or temporal origins of these impacts. In addition, even for the better studied Caribbean region, it remains challenging to quantify how much biodiversity has been lost since the natural Pleistocene baseline estimated by fossil records (Figure 7), especially given the fact that ecosystem compositional change does not directly mean extinction.

There is increasing evidence that anthropogenic-induced climate changes leading to ocean warming, acidification and deoxygenation are degrading the world's marine tropical ecosystems (Yasuhara et al. 2012a, Anthony 2016, Altieri et al. 2017, Breitburg et al. 2018, Yasuhara et al. 2019c, 2020b, Yasuhara & Deutsch 2022). As predicted by some palaeoecological studies, the tropics may become less habitable in the near future (Kiessling et al. 2012, Yasuhara et al. 2020b, Yasuhara & Deutsch 2022). The negative impact of exceedingly high temperatures on tropical diversity is increasingly better recognised, as seen by the equatorial diversity dip for various marine organisms (Chaudhary et al. 2016, Saeedi et al. 2017, Rivadeneira & Poore 2020, Yasuhara et al. 2020b, Boag et al. 2021, Chaudhary et al. 2021, Yasuhara & Deutsch 2022). However, the detrimental impact of warming on tropical alpha diversity was not exclusively anthropogenic, because the start of tropical diversity decline predates the Anthropocene and industrialisation and did not involve any extinction but rather distributional shifts (Yasuhara et al. 2020b). Direct human impacts such as overpopulation, overexploitation, habitat degradation and loss, and pollution, have been, up until recently, more damaging (Jackson et al. 2001, Pandolfi et al. 2005, Lotze & McClenachan 2014) than those as a result of anthropogenic greenhouse gas emission.

Future directions: leading questions and testable hypotheses

Our review of all major hotspots simultaneously allowed us to identify data deficiency and areas of future research, as listed below:

1. The western Tethys and Arabian hotspots have more detailed palaeobiogeographical information, but tend to lack diversity syntheses. The IAA has better syntheses of the history of bio diversity from both palaeontological and molecular data, but palaeobiogeographical distributions are poorly known and palaeontological data remain limited compared to the

western Tethys. Although Caribbean food web and functional groups are well studied in regard to productivity and other oceanic conditions, such information is virtually absent in other hotspots. Complementary studies that focus on these missing pieces will bring a more holistic understanding of diversity dynamics across the global tropics. In general, synthetic and quantitative palaeontological biodiversity data are extremely scarce. This is the major next step.

2. Similarly, comparisons of historical patterns from palaeontological data among different hotspots are needed. The Caribbean hotspot has, for example, seldom been compared with other hotspots. Consequently, the following important questions remain unresolved.

   Is extinction in the Caribbean the primary reason for the differences in IAA and Caribbean biodiversity today? If so, was Caribbean biodiversity as high as that of the IAA in the Miocene before the Pliocene–Pleistocene Caribbean extinction event? Caribbean diversity dropped in the Pliocene–Pleistocene, while IAA fauna avoided similar levels of extinction (as far as we know), with diversity continuing to increase towards the Recent. Indeed, bryozoan data indicate that the Caribbean extinction event was a major reason for lower present-day Caribbean diversity compared to the IAA, and that Miocene Caribbean diversity was at a similar level to Miocene IAA diversity (Di Martino et al. 2018) (Figure 7). However, these patterns have been evaluated only for one well-sampled group, bryozoans, and their universality has yet to be established. This leads to the questions: Was origination, extinction or immigration more important to explain present-day tropical diversity and its heterogeneity than the others? Was diversification in the Miocene–Pliocene (i.e. pre-Caribbean extinction) in both the IAA and Caribbean driven by the same mechanism? Origination, immigration and/or extinction rates may well have been different for the two regions, e.g., based upon their relative connectivity to other regions. To resolve these questions, comparisons of diversity patterns in well-sampled geological sections need to be made, ideally with an understanding of the relative importance of in situ diversification vs immigration as well as estimates of the rates of extinction and origination across different ecological groups.
3. Although the Hopping Hotspots and Whack-A-Mole models explain high diversity as the result of habitat area and complexity provided by tectonic collision, this has neither been tested rigorously nor quantitatively. Our first preliminary attempt (Figures 5 and 6) reveals the importance of quantifying habitat size and complexity in the Cenozoic, which supports both the Hopping Hotspots and Whack-A-Mole models. Alternative drivers, such as temperature, are also important to consider, preferably through the use of regression models and a model selection framework (e.g. see Yasuhara et al. 2009, 2014, 2017b). In addition, the relationship between diversity and environmental factors may not be linear. Therefore, non-linear causality analyses, such as convergent cross mapping, could be more appropriate when attempting to identify drivers of diversity patterns (e.g. Doi et al. 2021).
4. Did the Hopping Hotspots mechanism operate in deeper times? This is a reasonable question, because continental configuration and fragmentation are argued as the main controlling factor of global Phanerozoic marine biodiversity (Valentine & Moores 1970, Zaffos et al. 2017), which is a similar argument to the Hopping Hotspots model with emphasis on habitat size and complexity (nearly equal to continental configuration and fragmentation). Thus, the global hypothesis (Valentine & Moores 1970) may be reasonably scaled down to the regional hypothesis of the Hopping Hotspots (Renema et al. 2008). For example, the Mesozoic (especially Triassic and Jurassic) is a time known for lower continental fragmentation compared to the Cenozoic (Zaffos et al. 2017). Given this, do we expect not to see a biodiversity hotspots in the Triassic and Jurassic? Or, did the Hopping Hotspots mechanism still operate in the Triassic and Jurassic in some other way?

Even in the Cenozoic, we do not have sufficient diversity data to include the Paleocene and early Eocene in our review. Shallow-marine biotic responses to the PETM are known to be complicated (McInerney & Wing 2011, Speijer et al. 2012, Self-Trail et al. 2017, Tian et al. 2021). The Cretaceous–Paleogene (K-Pg) extinction is known to have wiped out the majority of marine species (D'Hondt 2005). However, it is not certain if these events affected the distribution of tropical diversity hotspots. Mannion (2020) suggested similar flat latitudinal diversity gradients both in the Late Cretaceous and the early Eocene. However, Boag et al. (2021) showed substantially different latitudinal diversity patterns between the Late Cretaceous and late Paleocene. Thus, important questions remain: Were tropical hotspot distributions in the Paleocene and early Eocene similar to those of the middle Eocene (in other words, did western Tethys diversity remain highest at that time)? Did the PETM and K-Pg events affect biodiversity hotspots, and if so, how and how much? Did the Hopping Hotspots mechanism operate in the Mesozoic before the K-Pg extinction?

5. The western Tethys, Arabian, Caribbean and IAA regions were once a single pan-tropical Tethyan realm before the Oligocene–Miocene transition, which, then, separated into different biogeographic realms with distinct faunal elements and compositions (e.g. McKenzie 1991b, Harzhauser et al. 2007, Perrin & Bosellini 2012, Yasuhara et al. 2019a). This view may be too simplistic, since a taxonomic group that has a better fossil record than most other groups, the LBF, shows substantial provincialism already in the Eocene (Renema 2007). Accordingly, modern syntheses and updates quantifying and delineating global Eocene tropical biogeography are needed to better understand cosmopolitanism and provincialism in the Cenozoic, and the tectonic contribution to global beta and gamma diversity.

   Similarly, did the western Tethys hotspot have disproportionately high biodiversity compared to adjacent regions in the middle Eocene? In our new LBF compilation of late middle Eocene diversity (Figure 5), western Tethys diversity appears higher regionally on average, but there are a few higher diversity sites found in the Arabian and Caribbean regions (red dots in Figure 5). Thus, the important questions are: Was the western Tethys the single hotspot in the middle Eocene before the Arabian hotspot achieved higher diversity in the late Eocene? Otherwise, did the pan-tropical Tethyan realm (at least western Tethys, Arabian and Caribbean regions) have similarly high diversity during the middle Eocene and before it?

6. Did the closure of the Tethyan Seaway at ~19 Ma cause global biotic change? Oligocene–Miocene Caribbean biotic turnover may be associated with this closure as discussed in Section 'Early Cenozoic Tropical America'. A recent study discovered a global shark extinction event at ~19 Ma (Sibert & Rubin 2021a), although the validity of this event has been actively debated (Feichtinger et al. 2021, Naylor et al. 2021, Sibert & Rubin 2021b, c) and a global analysis using a comprehensive fossil dataset found a Cenozoic shark extinction event at the Eocene–Oligocene transition (~34 Ma) instead of at ~19 Ma (Condamine et al. 2019). Thus, the possibility that the Tethyan Seaway closure may have affected not only adjacent regions of the western Tethys, Arabian and IAA, but also the Caribbean and even globally requires further investigation. The breakdown of the vast and single pan-tropical ocean may have affected marine biodiversity substantially and globally.

   In addition, unidentified extinction events merit investigations. For example, the Eocene–Oligocene extinction is known in the Caribbean (Hansen 1987, Hansen et al. 2004), western Tethys (Renema 2007) and IAA regions (Renema 2007), but not in the Arabian hotspot (Figure 7). An extinction event is documented in the Oligocene–Miocene transition at ~23 Ma in the Caribbean (Johnson et al. 2008, 2009) and western Tethys regions (Renema 2007), but not in the Arabian and IAA (Figure 7). The LBF record indicates a middle–late

Miocene extinction event just after their diversity peak in the middle Miocene in the IAA (Renema 2007) (Figure 7). So far, this extinction event has not been identified in any other regions and organisms. Further efforts to obtain more original data and harmonise data from other regions and organisms may allow us to understand the nature of these extinctions (region- and/or taxon-specific or global) and, more generally speaking, to identify still unknown regional and global extinction events in different groups.

7. Quantifying the Anthropocene defaunation. Human-induced degradation of tropical biodiversity hotspots is obvious, as we reviewed here. However, accurate quantification of local (alpha) and regional (gamma) diversity loss is more difficult. Study of recent fossil records (Kidwell 2015) can help to quantify what has been lost and when. Down-core analyses of microfossils in short cores covering the Anthropocene or a few hundred years are a powerful tool to reconstruct past human-induced impacts on marine biodiversity (e.g. Yasuhara et al. 2007, Tsujimoto et al. 2008, Yasuhara et al. 2012a, Roff et al. 2013, Narayan et al. 2015), but this is seldom applied in tropical hotspot regions. The other approach is live-dead assemblage comparison (Kidwell 2007). Death assemblages can provide quantification of natural, pre-industrial baseline biodiversity (Clark et al. 2014, Tomašových & Kidwell 2017, Yasuhara et al. 2020b). Conservation palaeoecology using young (Holocene) fossils is increasingly important to understand pre-historical baselines and anthropogenic impacts on biodiversity in decadal and centennial scales beyond the time of biological monitoring (Kidwell 2015) that remains seldom applied in tropical regions (but see Pandolfi & Minchin 1996, Greenstein & Pandolfi 1997, Pandolfi & Greenstein 1997, Greenstein et al. 1998, Roff et al. 2013, Hong et al. 2021, 2022). These approaches are also applicable to investigate how invasive species have changed or affected regional biodiversity (Yasuhara et al. 2012a, Albano et al. 2018), which can be substantial as seen in the Lessepsian migration (Por 1978, Rilov & Galil 2009, Albano et al. 2021, Costello et al. 2021) and Caribbean lionfish (Johnston & Purkis 2011, Albins & Hixon 2013, Johnston & Purkis 2015, Côté & Smith 2018).
8. Some studies use alpha diversity (local diversity: number of species per sample) (e.g. Renema et al. 2008, Yasuhara et al. 2017a) and others use gamma diversity (regional diversity: total number of species in an entire hotspot region in a time bin) (e.g. Jackson & Johnson 2000, Renema 2007). Few studies quantify beta diversity (differences in faunal composition between samples/sites). It is important to understand alpha, beta and gamma diversity to fully characterise macroevolutionary (origination and extinction) and macroecological processes (coexistence and dispersal) that operate(d) on biodiversity. In addition, consistent use of a standard diversity measure will maximise the comparability of biodiversity across studies. Recently, there has been increasing consensus in ecology that Hill numbers (Hill 1973) should be the species diversity measure of choice (Ellison 2010, Chao et al. 2014, 2020). The use of Hill numbers has recently become more common in palaeontology (Yasuhara et al. 2016, Allen et al. 2020, Trubovitz et al. 2020, Yasuhara et al. 2020b, Strotz & Lieberman 2021) and is recommended.

Conclusions

The analysis of biodiversity hotspots through time provides a synthetic view on the Cenozoic history of tropical marine biodiversity (Figures 4 and 7, Tables 1 and 2). All of the biodiversity hotspots of the Eocene Tethys, Eocene–Miocene Arabian, Miocene–Pliocene Caribbean and Miocene–Modern IAA developed in a similar tectonic setting that is an ocean with connections both to the east and to the west and with complex coastlines and numerous islands derived from the collision of continental plates (Harzhauser et al. 2002, 2007, Renema et al. 2008, Coates & Stallard 2013, O'Dea et al. 2016, Hou & Li 2018, Jaramillo 2018). This common ground strongly supports the roles of Hopping Hotspots and 'Centre-of' mechanisms in generating species diversity. The global Hopping Hotspots model likely explains the Cenozoic diversity among hotspots (Figure 7, Table 2): (1) After the Eocene biodiversity peak, the western Tethys hotspot began declining from the Oligocene to the Miocene due to the narrowing connection to the Indian Ocean and cooling. (2) Related to the decrease in suitable habitats in the western Tethys at that time, Tethyan taxa pumped out to the east towards the Arabian hotspot and then to the IAA and west towards the Caribbean. (3) In the Miocene, tectonics created suitable habitats and preconditioned the IAA and Caribbean hotspots. (4) Thus, the IAA and Caribbean hotspots commenced from the Miocene (the Caribbean hotspot may have commenced a bit earlier, in the Oligocene). In contrast, the 'Centre-of' hypotheses may better explain diversity dynamics within a hotspot. Indeed, the IAA hotspot with connection to adjacent regions both to the east and to the west further diversified into the present day, aided by a Pliocene–Pleistocene biological pump with TECOG processes. The Caribbean hotspot collapsed due to a Pliocene–Pleistocene extinction event caused by the closure of its western connection (but note that the Caribbean showed substantial diversification again after the extinction and seaway closure).

Temperature may have been an important precondition in the western Tethys becoming an Eocene biodiversity hotspot, i.e., the temperature was not too hot but still sufficiently high to sustain high biodiversity compared to 'too hot' equatorial regions at that time. Human activities have subsequently degraded the IAA and Caribbean hotspots, yet a quantitative estimate and timeline of biodiversity loss (either locally with reduced coexistence in an area or regionally with extinctions) since the natural Pleistocene baseline is lacking. Aside from the iconic extinction and collapse of charismatic megafauna such as the Caribbean monk seal and sea turtles, little is known about Anthropocene marine extinctions of fishes and invertebrates.

Our review highlights recent progress in understanding Cenozoic tropical biodiversity hotspots. However, many aspects of past tropical biodiversity remain unknown. By tackling the above-mentioned questions in the last section, we will advance the understanding of tropical biodiversity in the past, present and future. Quantitative deep-time synthesis of global marine tropical biodiversity will require substantial effort.

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PAST, PRESENT AND FUTURE OF SRI LANKAN COASTAL MACROPHYTE-DOMINATED ECOSYSTEMS: BLUE CARBON, CONSERVATION, RESTORATION AND POLICY

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Abstract Mangroves, salt marshes and seagrasses are conspicuous and important features of the Sri Lankan coastline, playing an important role in delivering multiple ecosystem services including carbon sequestration (the so-called blue carbon ecosystems). Together, they support coastal biodiversity, provide habitat and nutrition for numerous plant and animal species, protect vulnerable coastlines and support millions of human beings. Sri Lanka is now placing itself at the forefront of marine conservation by becoming the first nation to legislatively protect all its remaining mangrove forests. The current area of mangrove forest in Sri Lanka (19,500 ha) is less than that of salt marsh (33,573 ha) and seagrass meadows (23,819 ha). There is, however, considerable uncertainty in these estimates, particularly in some regions, which hampers policy development and conservation goals. While knowledge of the value of ecosystem services provided by mangrove forests is improving (e.g. value to fisheries, national estimates of carbon stocks and sequestration rates), comparable studies on saltmarsh and seagrass ecosystems are rare and this is reflected in their governance and legal protection. Further, while there are considerable efforts to restore lost mangrove forests (i.e. more than 1200 ha restored, with ambitious plans for 10,000 ha), restoration of seagrasses and salt marshes remains largely unattempted. The current disparity in the state of knowledge of blue carbon ecosystems must be addressed through application of robust science that extends descriptive data and theoretical assumptions to generate deeper knowledge on the structure, function and socio-economic value of each of these important ecosystems. Overall, this review seeks to summarise the state of these important higher-plant-dominated coastal ecosystems in Sri Lanka. We seek to help set a research agenda to ensure the nation becomes a regional leader with respect the protection, conservation and restoration of its coastal zone, thereby ensuring continued delivery of important ecosystem services, especially blue carbon sequestration.

Keywords: Blue Carbon, Mangrove, Seagrass, Salt marsh, Restoration, Sri Lanka

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Introduction

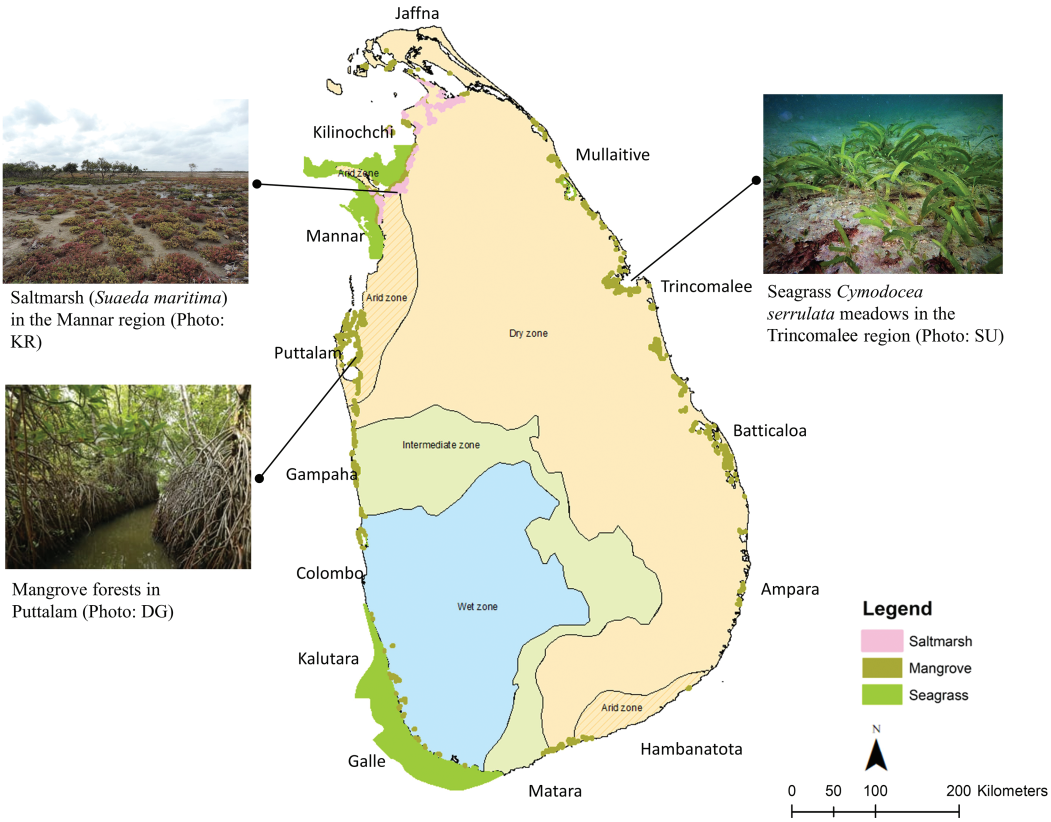
The island nation of Sri Lanka has a land area of approximately 65,000 km^2 and is home to more than 21 million people, many of whom have a deep physical and spiritual connection to the coast. The country has faced challenges, including a decades-long civil war (1983–2009) and periods of economic instability and natural disasters (i.e. most notably the 2004 Indian Ocean tsunami), but it entered into a period of relative prosperity during the early 2000s that permitted advancement in the areas of science and technology (supplementary material, Figure S1A). Research outputs mirrored the country's economic performance (i.e. GDP growing from $16 billion USD in 2000 to more than $84 billion USD in 2019; Central Bank of Sri Lanka 2019), with the number of scientific articles growing to 1348 in 2018. The number of studies focusing on coastal wetlands and seagrass meadows as 'blue carbon ecosystems' followed this trajectory, with more than 200 peer-reviewed articles published since 1979 (supplementary material, Figure S1B). The term 'blue carbon' refers to the carbon stored in the biomass (e.g. leaves, trunks, branches and roots) and soils of coastal vegetated ecosystems, including mangrove forests, salt marshes and seagrass meadows, which can remain trapped for centuries to millennia (Geraldi et al. 2019).

While the total area of macrophyte-dominated ecosystems may be less than that of other countries in the Indian Ocean (i.e. it ranks 15th in terms of mangrove distribution and 9th for seagrass; Vanderklift et al. 2019), their relative importance is profound given that the coastal zone is home to 34% of the nation's population (NARESA 1991), with over half a million relying (directly and indirectly) upon coastal fisheries production (International Labour Organization 2020). Unfortunately, the economic growth period during the early twenty-first century saw the wholesale clearing and degradation of large areas of coastal vegetation, impacting biodiversity and ecosystem functioning and the delivery of a host of important ecosystems services including carbon sequestration. Sri Lanka is believed to have lost up to three-quarters of its mangrove forests (Hansen et al. 2013), half of its salt marshes (Mcowen et al. 2017) and a large but unknown area of seagrass (estimates in some areas exceeding 96% over a 7-year period; Udagedara & Dahanayaka 2020) since the late 1970s. Together, this has resulted in a decline in fish stocks (Gunasekara et al. 2019), has created coastlines that are more vulnerable to flooding and erosion (Weerakkody 1996) and has resulted in numerous socio-economic challenges impacting the livelihoods of the coastal population (Kallesøe et al. 2008).

The history of blue carbon science in Sri Lanka can be seen through the lens of the 'ecosystem concept', popularised following the Second World War (Odum 1953). This idea adopts aspects of scientific reasoning that are applied to environmental management, policy and the popular movement of environmentalism (Golley 1991). Within the discipline of marine ecology, there was an early recognition that mangrove ecosystems are important traps for organic carbon (Golley et al. 1962). The concept outlines how early knowledge based on descriptive data and/or theoretical assumptions is gradually replaced by robustly planned experiments and deeper understanding (Odum 1969) which naturally leads to improvements in conservation and the promotion of ecosystem services under the broader concept of nature-based solutions (Cohen-Shacham et al. 2016). In Sri Lanka, these two stages have been happening simultaneously: with research and governance relating to seagrasses and salt marshes in its infancy (i.e. spatial distribution and species; Pahalawattaarachchi & Dahanayaka 2018, Ranawana et al. 2020, Udagedara & Dahanayaka 2020), while studies of mangrove ecosystems have advanced to include their ecological functioning (Cooray et al. 2021), biogeochemistry (Phillips et al. 2017), socio-environmental value (Gunawardena & Rowan 2005) and ecological restoration (Kodikara et al. 2017).

The 2004 Indian Ocean Tsunami was a watershed moment for Sri Lanka, reinforcing the inextricable link between the status of coastal ecosystems and the vulnerability of coastal inhabitants to natural disasters (Dahdouh-Guebas et al. 2021). Coastal areas where mangrove forests had been largely cleared or degraded suffered the greatest destruction and heaviest loss of life (Mulligan & Shaw 2007). Acknowledging this, successive governments have pivoted towards policy that protects and conserves coastal environments and promotes the sustainable use of natural resources (Wickramasinghe 2017). Indeed, the country's constitution states that it is the government's responsibility to protect, preserve and improve the environment for the benefit of the community, a task that requires detailed knowledge of the past and present status of ecosystems (e.g. their spatial distribution, ecology, ecosystem services and benefits to society and the national economy). While detailed information is available for certain lagoons and coastal areas along the west coast in proximity to the capital Colombo (e.g. Negombo and Puttalam lagoons; Figure 1), those in the north of the country that were closed during the civil war have received considerably less scientific attention. Despite the commendable efforts of individual researchers, fundamental knowledge gaps remain and only recently has there been a coordinated attempt to collate and synthesize available data at the national scale.

The aim of the current review was to harmonise knowledge on three distinct coastal ecosystems important for blue carbon policy informing climate mitigation, to improve current understanding of their past, present and future in Sri Lanka in a way digestible to researchers, policy makers, local communities and the public. In turn, we consider: (1) the distribution and diversity of mangrove forests, salt marshes and seagrass meadows across the country, (2) their historical losses and present status, (3) productivity, carbon stocks and potential for sequestration, (4) the provision of ecosystem



Saltmarsh (*Suaeda maritima*) in the Mannar region (Photo: KR)

Mangrove forests in Puttalam (Photo: DG)

Seagrass *Cymodocea serrulata* meadows in the Trincomalee region (Photo: SU)

Figure 1 Map of Sri Lanka showing the known distribution of three blue carbon ecosystems (mangrove forests, salt marshes and seagrass meadows), the three main climatic regions and the sites at which notable scientific investigations have been focused. Data derived from the models of Hutchison et al. (2014) (mangroves); Short (2017) (seagrasses), available through the United Nations Environment Programme World Conservation Monitoring Centre (UNEP-WCMC) (www.unep-wcmc.org); and the atlas products presented by Ranawana et al. 2020 (salt marshes).

services and employment, (5) the national policy and legal frameworks and (6) ecological restoration and future perspectives, before (7) identifying knowledge gaps and suggesting priority research areas. We present the consensus view from the scientific community (e.g. nationwide estimates of mangrove distribution), but where this was inconsistent or absent, data from different sources and/or regions were synthesized (using meta-analysis) to provide new data along with measures of associated error (e.g. estimates of carbon stocks for different climate zones). In evaluating the status and ongoing threats to ecosystems (e.g. historical and ongoing drivers of loss; Table 2), the strength of the scientific literature (in terms of number and impact of publications) and level of government action was used to quantify the potential risk facing different blue carbon ecosystems. Lastly, with respect to knowledge gaps and priorities, we incorporated the conclusions of relevant scientific literature, the goals of current government policies such as the *Mangrove Action Plan* (Ministry of Environment 2020), and the outcomes of workshops and meetings such as the Australia-Sri Lanka blue carbon symposium held in 2019, of which this review is one output.

Influence of biogeography on the distribution and diversity of blue carbon ecosystems

Climate, oceanography and geomorphology

Macrophyte ecosystems fringe much of Sri Lanka's 1705 km of coastline, with their distribution and diversity varying with climatic, oceanographic and geomorphological features (Figure 1). Three prominent climate zones divide the country: the 'wet zone' (mean annual rainfall over 2500 mm without pronounced dry periods) that encompasses the south-western region and central highlands; the 'dry zone' (mean annual rainfall of less than 1750 mm with a distinct dry season from May to September) dominating the northern and eastern parts of the country; and an 'intermediate zone' (mean annual rainfall between 1750 and 2500 mm with a short and less prominent dry season) that divides the two extremes along the coast in the south and central west (Punyawardena 2020). Under some classification schemes, there is also an 'arid zone' (Figure 1), although this has more relevance to terrestrial systems than it does to the marine and coastal environment. There is some evidence that average annual rainfall in Sri Lanka is declining, with an observed decrease of 144 mm between the years 1961 and 1990 in comparison with the previous 30 years (1931–1960) (Jayatillake et al. 2005). This may have consequences for the current spatial limits of climate zones (i.e. the Köppen-Geiger climate classification system Cui et al. 2021), with far-reaching impacts on ecological systems including the distribution of forests and other vegetated communities in Sri Lanka (Somaratne & Dhanapala 1996).

Sri Lanka is a climatically diverse country with a year-round hot climate, but one that ranges from wet Equatorial (Köppen climate classification Af) in the south-west to drier Tropical Savanna (Aw) across much of northern half and the south-eastern parts of the island. April and May are the hottest months, with average high temperatures in the 32°C–35°C range. The average low temperatures in January range between a mild 18°C and a warm 24°C. Average temperature rise in Sri Lanka is projected to reach approximately 3.2°C by the 2090s according to the CCKP model ensemble under emissions pathway RCP8.5 (World Bank 2021), and this is likely to have disproportionately greater consequences for the country's drier northern regions (De Silva 2009). Sri Lanka's position in the equatorial Indian Ocean, having the Arabian Sea on its western side and the Bay of Bengal on its eastern side, means that it experiences biannually reversing monsoon winds (De Vos et al. 2014). A recirculation feature that occurs along the east coast during the south-west monsoon (the Sri Lanka Dome; Anutaliya et al. 2017) drives longshore winds and eddy currents that direct seawater flow to the south. On the west coast, the island deflects the eastward-flowing Southwest Monsoon Current so that currents also flow to the south. The resulting convergence of these water bodies along the southern coast and their subsequent divergence result in a major upwelling region along the southern coast which leads to higher surface chlorophyll concentrations (De Vos et al. 2014). Sri Lanka regularly faces destructive weather conditions during the North Indian cyclone season, with a study by the Disaster Management Centre (DMC) of Sri Lanka and UNDP, showing that of the 22 cyclones that crossed the coasts of Sri Lanka between 1881 and 2001, 16 (approximately 73%) occurred during the months of November and December (Srisangeerthanan et al. 2015). This same study revealed that 17 of these cyclones affected the east coast, three affected the west coast and one each affected the north and south coasts of the country.

While the devastating Indian Ocean Tsunami of 2004 remains stamped in the minds of people in Sri Lanka (and the world), there is strong evidence for past major tsunamis affecting the region with a recurrence interval of 434 years (Jackson et al. 2014). At the other end of the temporal scale, semi-diurnal tides impose constant influence on the structure of blue carbon ecosystems because their amplitude governs the landward penetration of sea water into tidal creeks and lagoons and thus defines the limit of mangroves and salt marshes (depending on the region). The mean spring and neap tidal ranges in Colombo are 0.56 and 0.13 m, respectively, with tidal range declining towards the north (Wijeratne 2003). Consequently, the coastal belt inundated by seawater is quite narrow and the overall regime is considered microtidal. Despite having such a small tidal range, there is some evidence that sea level rise will drive shifts in the zonation and composition of coastal vegetation and lead to the expansion of salt-tolerant species such as mangroves and salt marsh (Mathiventhan et al. 2022). Together, the distinct climatic, oceanographic and biogeophysical patterns across the country mean that there is considerable variation in the structure of blue carbon ecosystems, with some occurring only in particular areas, while others display vastly different characteristics within contrasting regions (Table 1).

Table 1 Mean (±SD) estimates of the distribution of blue carbon ecosystem area (hectares) from regions of different climates in Sri Lanka

		Mangrove forest		Seagrass meadow		Salt marsh	
Climate zone	Region	Mean (Ha)	Std	Mean (Ha)	Std	Mean (Ha)	Std
Dry	Puttalam	2755	643	ND		3461	–
	Mannar	1351	–	13,349	–	5179	–
	Kilinochchi	1328	788	509	–	4975	–
	Jaffna	2427	131	21,225	–	4963	–
	Mullaitive	734.5	433	2054	–	517	–
	Trincomalee	2146	217	ND		1401	–
	Batticaloa	1687	543	ND		2196	–
	Ampara	359	366	ND		127	–
	Hambantota	634	82	ND		318	–
Total		13,422		37,137		23,137	
Intermediate	Gampaha	474	227	684		497	–
	Matara	23	23	ND		ND	
Total		497		684		497	
Wet	Galle	322	119	ND		185	–
	Kalutara	43.5	45	ND		ND	
	Colombo	39	–	ND		ND	
Total		405		ND		185	
Grand total		14,324		37,821		23,819	

Notes: Mean estimates of ecosystem distribution for mangrove forests (taken from Edirisinghe et al. 2012, Jayatissa et al. 2002), seagrass meadows (SLCZCRMP 2018) and salt marshes (National Atlas of Sri Lanka 2013).

'ND', no data available; '-' , no estimate of standard error.

For more information related to climate zone, refer Figure 1.

Distribution and diversity

Mangrove forests and salt marshes

Mangrove forests are found all around the island and span the three climate zones, having a long-established total area of 15,700 ha (Edirisinghe et al. 2012). This figure has recently been upgraded to 19,500 ha according to the Forest Department (Mombauer 2020) due in part to improved mapping products derived from earth observation. Expansive forests occur in the relatively dry climate regions of Puttalam, Jaffna, Trincomalee and Batticaloa (Figure 1, Table 1). Smaller expanses occur in the south and south-west (Kalutara, Galle and Matara regions), but these are generally restricted to small lagoons and river mouths and are mostly absent along exposed shorelines. Given some of the inconsistencies in distribution estimates nationally, this review compiled data for individual regions (Table 1) to identify the main sources of uncertainty and reconcile the influence of temporal change. Pooling data for all regions gave a nationwide estimate of 13,929 ha, which is less than the current consensus estimate, but is likely to reflect the evolution of methodologies (e.g. aerial photography being replaced by satellite imagery), as well as actual changes in cover over time (i.e. estimates span 2012–2020). Concerning, however, were the high levels of uncertainty at the regional level (e.g. a standard deviation of 643 ha for the well-studied Puttalam Lagoon). To reconcile such inconsistencies and improve confidence in estimates, there has been a recent intensification of mapping activities based on high-resolution remote sensing approaches (Dahanayaka et al. 2015, Dahanayaka 2017, Pathmanandakumar 2019, Athukorala et al. 2021). These studies are proving useful for long-term assessments, both retrospective and predictive, and can help understand the drivers of regional through to national land cover change to aid in policy development.

The ecological aspects of Sri Lankan mangrove forests, such as their diversity and physical structure (i.e. age, height) have comparatively been well studied by government institutions, university researchers and several NGOs. At least twenty-one true mangrove species, comprising woody trees and shrubs, are believed to exist, as well as 18 mangrove-associated species which are mostly non-woody or herbaceous (Jayatissa et al. 2002, Jayatissa 2012), although there is some ambiguity, with other authors suggesting up to 24 species (Amarasinghe & Perera 2017). The species that are considered ‘most common’ include *Avicennia marina* (Forssk.) Vierh., *Rhizophora mucronata* (Lamk, 1804), *Lumnitzera racemosa* (Willd.), *Rhizophora apiculata* (Blume), *Ceriops tagal* (Perr.) C.B.Rob., and *Bruguiera gymnorhiza* (L.) Savigny (Amarasinghe & Perera 2017). Species composition varies according to salinity differences, nutrient concentrations and soil types, moderated by seasonal variations in rainfall (Mathiventhan & Jayasingam 2010). This is important because the salinity of certain estuaries and lagoons can fluctuate from almost zero to more than that of seawater depending on freshwater discharge, tidal amplitude, topography and rates of evaporation (De Silva & De Silva 2002). Indeed, some studies have shown that vegetation complexity, tree basal area, total biomass, leaf area and gross primary productivity display an inverse relationship with soil salinity (Amarasinghe & Balasubramaniam 1992, Chandrasekara et al. 2016), and more specifically that sites located far from the estuary mouth, where soil salinity is low, exhibit the highest species richness and diversity (Perera et al. 2013). Several true mangrove species are at risk with two considered critically endangered (*Ceriops decandra* (Griff.) W. Theob. and *Lumnitzera littorea* (Jack) Voigt.), three endangered (*Bruguiera cylindrica* (L.) Blume, *Sonneratia alba* J. Smith and *Xylocarpus granatum* J. König) and five vulnerable (IUCN 2021).

The geographical distribution, ecology and status of salt marshes in Sri Lanka have received arguably the least attention of all three blue carbon ecosystems considered in this review, likely because they are not deemed a priority by many sections of government (Ranawana et al. 2020) and because they tend to occur in areas of the arid north-west in which fighting was particularly fierce during the civil war (Pemadasa et al. 1979). The largest areas of undisturbed salt marsh occur in the Mannar, Kilinochchi and Jaffna regions (Figure 1). One widely quoted estimate for the nation's total saltmarsh distribution is 33,573 ha (Gunatilleke et al. 2008), which exceeds the recognised total area of mangroves. More recent estimates given in the Coastal Zone and Coastal Resource Management Plan (CZCRMP) put the figure at only 27,520 ha, although this could reflect recent habitat loss. Synthesizing all available data by region suggests that the true value may be somewhere between these two estimates (28,696 ha, Table 1). Much of this uncertainty is driven by inconsistencies when delineating salt marshes along a tidal gradient, which has led to different classification systems. Specifically, the National Strategic Action Plan of 2009 published guidance that all previous research done on salt marshes should be referred to as 'tidal flats' (IUCN 2009). While salt marshes are often depicted in maps of individual studies, they have not been given a separate category in the coastal vegetation maps prepared by the Forest Department (Ranawana et al. 2020). Attempts are being made to reconcile the classification of salt marshes, with both the previously mentioned report by the CZCRMP 2018 and the National Biodiversity Strategic Action Plan (NBSAP) 2016–2022 referring to the presence of salt marshes in Sri Lanka.

Recent studies on the saltmarsh communities of Sri Lanka suggest that there are seven true species (i.e. salt-tolerant halophytes, succulents, small herbs and shrubs) and 18 associates including small shrubs, grasses, reeds and sedges (Patro et al. 2017, Ranawana et al. 2020). The most common true species are *Suaeda maritima* (L.) Dumort., *Suaeda vermiculata* Forssk. ex. J.F. Gmel., *Sesuvium portulacastrum* (L.) L., *Tecticornia indica* (Willd.) Paul G.Wilson and *Salicornia brachiata* Roxb., all of which can form thick ground covers that grow to 2 m in height. Typically, however, cover is sparser (~50%) and they tend to occur as intermixed assemblages with associates that include *Cyperus stoloniferus* Retz., *Indigofera oblongifolia* Forssk. and *Zoysia matrella* (L.) Merr., with species exhibiting marked zonation according to their tolerance to submersion or exposure (Soysa 1993). Ongoing studies of seven dominant species in the Mannar region are showing that both elevation and salinity have a direct effect on plant distribution (Ahalya & Suresh 2020).

Seagrass meadows

Knowledge on the distribution and ecology of seagrass in Sri Lanka is scant, especially along the north, north-west and north-east coasts (again because of a lack of scientific surveys during the civil war). While official records acknowledge at least 23,819 ha of seagrass meadows across the country (Gunatilleke et al. 2008), a recent evaluation of the *World Conservation Monitoring Centre* global seagrass distribution dataset (UNEP-WCMC and Short 2021) suggests there could be more than 290,000 ha. There have not been any attempts to estimate the proportion of seagrass meadows that occupy the intertidal versus those found in shallow subtidal or deeper waters. The distribution is not uniform along the coastline (Figure 1), but rather occurs as dense meadows in warmer northern regions such as Trincomalee, Mannar and Puttalam and in southern regions such as Batticaloa and Galle where they occur within shallow and relatively sheltered bays and lagoons (Arulnanam & Abeywickrama 1991, Baldwin 1991, Mathiventhan & Jayasingam 2004, Naveenan et al. 2015). Most studies investigating the distribution of seagrasses in Sri Lanka have also relied on remote sensing approaches (Dahanayaka et al. 2010). Dahanayaka & Pahalawattaarachchi (2017) incorporated field observations and high-resolution WorldView-2 (WV-2) imagery to provide an assessment of shallow coastal marine seagrass meadows in Palk Bay, north-western coast of Sri Lanka. Seagrass mapping in Sri Lanka remains a challenge given the issues of detecting seagrasses in optically deeper waters (which limits the applicability of remote sensing approaches) and the difficulties and costs of conducting subtidal ground-truthing surveys in deeper more turbid areas, a trait which is common to other subtidal ecosystems (e.g. kelp forests along temperate coastlines; Gorman et al. 2013).

Several *in situ* field surveys have been done in the Gulf of Mannar, Puttalam and Negombo lagoons (Pahalawattaarachchi et al. 2005, Pahalawattaarachchi & Dahanayaka 2018, Udagedara

& Kumara 2013), along the country's east coast (Mathiventhan & Jayasingam 2004, Udagedara et al. 2017) and at several locations in the south (Faazil et al. 2019). Historical records for seagrass species stretch back to the eighteenth century, with a herbarium collection of *Thalassia hemprichii* (Ehrenberg) Ascherson 1871 from Puttalam Lagoon dating back to 1826 (Dassanayake et al. 1995). Since then, 15 true seagrass species (i.e. those having a developed root system) have been identified across the country, which represents 60% of the total number found in the Indo-Pacific bio-region (Short et al. 2011, Udagedara & Dahanayaka 2020). Some of the most widespread species include *Enhalus acoroides* (Linnaeus f.) Royle 1839, *Thalassia hemprichii*, *Cymodocea rotundata* Ascherson & Schweinfurth 1870, *Cymodocea serrulata* (R.Brown) Ascherson & Magnus 1870, *Halophila ovalis* (R. Brown) J.D. Hooker 1858 and *Halodule pinifolia* (Miki) Hartog 1964. Meadows often occur in association with coral reef ecosystems or estuaries and lagoons. Data suggest that the most expansive meadows are frequently associated with calcareous deposits and show seasonal differences in their distribution and abundance (e.g. covers of *Thalassia hemprichii* in Trincomalee, ranging from 73% in February [winter] to 30% in August [summer]; Mathiventhan & Jayasingam 2004).

Historical losses and present status

Blue carbon ecosystems in Sri Lanka have undergone significant losses from both natural (e.g. tsunamis, storms and flooding; Dahdouh-Guebas 2001, Mathiventhan & Jayasingam 2011) as well as anthropogenic impacts (Joseph 2004). A more in-depth look at the anthropogenic drivers shows the importance of land conversion, direct harvesting, grazing, urban and industrial development and pollution, all of which continue to affect blue carbon ecosystems to varying degrees (Table 2). Arguably, the most pronounced driver of mangrove (but also salt marsh and seagrass) loss has been the conversion of coastal land for shrimp farming (primarily the black tiger shrimp, *Penaeus monodon* Fabricius, 1798), especially along the north-western coastline, Puttalam district and the Chilaw and Negombo lagoons. Shrimp farming was heralded as a means of diversifying and securing livelihoods in rural Sri Lanka and rose to be the country's second most valuable export fishery in 2007 (generating approx. 25 million USD; NARA 2007). Operations expanded from less than 40 ha in 1992, to well over 1100 ha in 2012, before many farms were abandoned because of disease outbreaks including the Monodon Baculovirus and white spot syndrome virus (Arthur 1998). Although productivity has declined since the peaks of the early 2000s, recent estimates confirm that shrimp exports remain a valuable foreign exchange earner (i.e. exceeding 1.8 million US$ in 2019; National Aquaculture Development Authority (NAQDA) 2020). Despite legislative protection of blue carbon ecosystems in Sri Lanka (e.g. prevention of further mangrove clearance – see policy section), continued loss driven by shrimp farming activity is widely acknowledged to be occurring through illegal clearing and release of pollutants (e.g. in the Pambala–Chilaw, Puttalam and Negombo Lagoon systems; Dahdouh-Guebas et al. 2002, Katupotha 2014, Hsieh et al. 2021).

Mangrove forests and salt marshes

It is estimated that Sri Lanka has lost between 50% and 76% of its original mangrove forest cover over the past 30 years (Hansen et al. 2013, Nagenahiru Foundation 2021). Clearly, the biggest cause was the clearing of coastal land for the construction of ponds for the shrimp aquaculture industry (Bournazel et al. 2015). Reconstructing the changes that occurred during this period has become a national priority and has received considerable scientific attention (Dahdouh-Guebas 2001, Dahdouh-Guebas et al. 2002, Gunawardena & Rowan 2005, Mathiventhan & Jayasingam 2011, Bournazel et al. 2015, Weragodatenna & Gunaratne 2015). The study by Weragodatenna & Gunaratne (2015) used satellite imagery to track the decline in mangrove cover of Puttalam Lagoon caused by aquaculture expansion between 1977 and 2015 and highlighted a 62% loss over

Table 2 Degradation drivers, impacts and mitigation approaches for three blue carbon ecosystems in Sri Lanka

Degradation driver	Mangroves	Seagrass	Salt marsh
1. Conversion of land for shrimp pond aquaculture	☆ Entire trees are removed, and soil carbon stocks are disturbed during pond excavations. Conversions are permanent and do not allow regrowth of mangroves except in abandoned in which natural recruitment can occur to a limited scale (given restricted tidal flow and propagule supply). Quantitative data (such as mangrove area converted) for calculating carbon loss are scanty (but see Bournazel et al. 2015).	☆☆ Release of briny water, chemicals, waste products and antibiotics negatively affects seagrass meadows (Senarath & Visvanathan 2001).	☆☆ Given the historical perceived low value of salt marshes (vs. mangroves) and strict laws against mangrove clearing, marsh vegetation is seen as an alternative for shrimp pond construction (especially in the north).
2. Clearance of vegetation for temporary needs	☆☆ Above-ground biomass of some mangroves of north and east were cleared during the civil war for security purposes. In other regions, trees have been removed with minimal disturbance to soil which permits regrowth depending on propagule and secondary disturbances. Quantitative data for calculating carbon loss are scanty (but see Edirisinghe et al. 2015).	☆☆ Meadows can be damaged during harmful fishing activities that involve the use of gill nets, push nets and the illegal use of dynamite to catch fish and take Dugongs (Nanayakkara 2013).	☆☆☆ Extensive grazing of saltmarsh by cattle can exacerbate the spread of invasive species such as Seemai Karuvelam trees (*Prosopis juliflora*), also known as Kalapu-Andara in Sinhala, and Karuvelam in Tamil (Ranawana et al. 2020).
3. Selective harvesting for firewood, wood or fisheries purposes	☆ Removal of selected trees could change the mangrove diversity. Harvesting of large adult trees can affect the reproductive capacity of the forest. The gaps created by harvesting trees would be colonised by other trees. Quantitative data for calculating carbon loss are scanty (except Dayarathne & Kumara 2015).	☆ Detrimental fishing practices and overharvesting of polychaete worms and brood stock from prawn hatcheries (Kallesøe et al. 2008).	☆ Selective harvesting of some species such as *Suaeda maritima* for food-based products (Ranawana et al. 2020).

(*Continued*)

Table 2 (*Continued*) Degradation drivers, impacts and mitigation approaches for three blue carbon ecosystems in Sri Lanka

Degradation driver	Mangroves	Seagrass	Salt marsh
4. Browsing by macro-herbivores such as cattle	☆ Grazing can reduce the growth of trees and removal of seedlings with overall negative impacts on carbon storage and sequestration.	There is no evidence for overgrazing of seagrass meadows by dugongs or turtles, and indeed, ‘cropping’ is believed to have beneficial effects on tropical seagrass nutrient concentration (Aragones et al. 2006).	☆☆☆ Cattle grazing is a major issue given that the ecological value of marsh systems is not well recognised by local farmers, and mapping efforts have not kept pace with other systems (no consistent classification).
5. Conversion of for industrial purposes, infrastructure and roads	☆ While clearing of mangroves is now strictly regulated, illegal clearances for agriculture and buildings still occur. Quantitative data (such as mangrove area converted) for calculating carbon loss are scanty for such conversions (but see Bournazel et al. 2015).	☆☆☆ Coastal infrastructure development can negatively affect meadows through terrestrial sediment inputs (Ayesha et al. 2015), eutrophication from land-based inputs (Pahalawattaarachchi and Bjork 2004) and changes to coast hydrodynamics.	☆☆☆ Improper construction of roads and culverts can change marsh hydrodynamics and prevent tidal inundation. Salt marsh is also regularly cleared for urban settlements and salternss (e.g. Gunathilaka 2019).
6. Pollution	☆☆ Terrestrial inputs of pollutants such as nitrogen fertilisers and heavy metals can reduce growth and sequestration. Details on the pollutant types released to mangroves and their quantitative impacts on tree growth and carbon are scanty.	☆☆☆ Seagrass meadows are susceptible to terrestrial inputs of nutrients from agricultural production and aquaculture operations (Pahalawattaarachchi & Siriwardena 2003).	☆☆ Given that they are still widely viewed as wastelands, marshes are exposed to waste disposal and effluent discharge.

Stars (☆) indicate the degree of importance of each factor and system.

this period, which equates to an average annual loss of 1.6% (Figure 2). Another study done in the smaller Negombo Estuary using multi-temporal satellite data describes three decades of land use change and the resulting impacts on the mangrove distribution and nutrient dynamics (i.e. with rates of decline reaching as high as 2.3% per year; Dahanayaka 2012). Multi-spectral Landsat satellite imagery has also been used to monitor changes in the cover of mangroves due to human activities in the Pambala–Chilaw Lagoon (Dahdouh-Guebas et al. 2002), the north-west of the country (Travaglia et al. 1999) and in the Trincomalee district along the east coast (Pathmanandakumar 2019); with all of these studies demonstrating that mangroves continue to be illegally reclaimed for aquaculture or other land uses.

Other human impacts are more subtle and involve fragmentation rather than permanent clearing and loss, examples being the exploitation for temporary needs (e.g. water withdrawal; Mathiventhan et al. 2018 and the harvesting of firewood; Ransara et al. 2012,). Harvesting of large adult trees for construction timber is another driver of forest degradation in some regions and can be rather insidious, given that the gaps created can be colonised by invasive species (Dayarathne & Kumara 2015).

Figure 2 Changes in the distribution of mangroves, shrimp farms and salterns of Puttalam Lagoon over the past four decades classified using Landsat TM and ETM+ imageries. Adopted from Weragodatenna & Gunaratne (2015).

Other pressures include browsing by cattle (Mathanraj & Kaleel 2015) and the use of mangrove branches to construct ‘brush parks’ (or ‘kraals’) that are used in certain fishing activities (Costa & Wijeyaratne 1994, Dayaratne et al. 1995, Dayarathne & Kumara 2015). Other reasons for historical deforestation have included conversion to salterns (Kaleel & Nijamir 2017), agricultural cultivation (Mathanraj & Kaleel 2015, Dalpathadu & Haputhantri 2020) and the construction of roads and other infrastructure (Silva et al. 2013, Jonnson 2017). The effects of pollutants such as nitrogen fertilisers and heavy metals can also reduce the growth and carbon sequestration capacity of mangroves (Katupotha 2016). For example, disturbance of acid-sulphate soils in the Dutch Canal Mundel lagoon system in the north of the country led to the discharge of highly acidic waters (pH 3.5) with high concentrations of heavy metals (Corea et al. 1998), known to have marked consequences for mangrove growth and soil microbial activity (Yim & Tam 1999). Finally, Sri Lanka faces an ongoing pollution crisis caused by the tons of plastic debris (especially microplastic pellets) and persistent organic pollutants (POPs) that continue to impact the beaches and lagoons of the countries east coast as a result of the 'MV X-Press Pearl' container ship sinking on the 2 June 2021. The incident has been described as the worst marine ecological disaster in Sri Lankan history (NDTV 2021) and the true scale of the ecological damage to coastal ecosystems, the fish that inhabit them and the local communities that rely on fishing is yet to be fully realised.

Sri Lankan salt marshes face a similar suite of threats to those of mangroves (Table 2), yet they may be under greater risk of future degradation given the level of government inaction, pervasive view that they are wastelands, and because of the relocation of impacts from mangrove forests which are now protected from further clearing by rangers and local communities (e.g. see article on local communities taking the lead on mangrove conservation; Perera 2021). It is believed that Sri Lanka has lost over 50% of its original area of saltmarsh habitat (Mcowen et al. 2017). Ongoing pressure comes in the form of the widespread practice of villagers encouraging their livestock (mainly cattle) to graze on saltmarsh vegetation. During the *maha* cultivation season (October – January) there is often a shortage of palatable grass for cattle on paddy fields and herders take their animals to browse the salt marsh. Soil disturbance resulting from this and other agricultural activities has also spread invasive species such as Seemai Karuvelam trees (*Prosopis juliflora*) (Ranawana et al. 2020). Saltmarshes in the countries north continue to be degraded, because of poorly designed road infrastructure and culverts that alter hydrodynamics and prevent tidal inundation (see example of the Thondamanaru lagoon ecosystem; Piratheepa et al. 2016). Lastly, there are the ubiquitous impacts caused by rapidly expanding urban populations, the construction of salterns, illegal disposal of wastes and effluent discharge (Gunathilaka 2019).

Seagrass meadows

Seagrass meadows are some of the most globally threatened coastal ecosystems (Orth et al. 2006) with some 14% of all species considered to be at elevated risk of extinction (Short et al. 2011). This is similarly true in Sri Lanka, where a large but unknown area of seagrass is believed to have been lost over the past few decades (Udagedara & Dahanayaka 2020). Meadows continue to face a range of threats (Table 2) that include pollution from discharged shrimp farm effluent (Pahalawattaarachchi & Siriwardena 2003), harmful fishing practices such as the use of bottom trawl or drag nets (Kallesøe et al. 2008), sand mining and conversion of coastal land for tourism development (e.g. see the case study from the Hikkaduwa region known to be the countries most developed beach resort area; Sullivan et al. 1995). While there are no universal estimates of exactly how much seagrass has been lost, data from the relatively well-studied Negombo lagoon document declines in biomass that range from 80% (Pahalawattaarachchi et al. 2005) to more than 96% within just a 6-year period (i.e. 1998–2004 Udagedara & Dahanayaka 2020).

Recently, coastal development and land reclamation have increased due to urbanisation and several mega-projects (e.g. the US$1.2 billion expansion of the Port of Colombo). Indeed, there are concerns that the large sand mining footprint associated with the current Port of Colombo expansion is affecting adjacent seagrass meadows as well as reefs and lagoons, to impact on fishing grounds and the livelihoods of more than 30,000 fishers (Iddamalgoda 2016). Further north in the Gulf of Mannar, seagrass meadows are becoming degraded because of elevated phosphate concentrations driven by agricultural activities and discharge from industries, tourist resorts and aquaculture operations (SACEP 2014), which create anoxic conditions that negatively affect photosynthesis and growth (Pahalawattaarachchi & Bjork 2004). Along the southern coastline, recent studies have focused on understanding how the sedimentary dynamics that characterise urban development can influence the species composition, coverage and overall survival of seagrass (Ayesha et al. 2015). It was found that *Thalassia hemprichii* preferred muddy substrate, *Syringodium isoetifolium* preferred fine sandy sediment, and *Halodule uninervis* was able to tolerate sandy sediments with relatively high nitrate and phosphate concentrations (Ayesha et al. 2015). Such species-specific research can provide practical knowledge on the sensitivity and resilience of certain species to land modification and coastal erosion and may help to better manage vulnerable species such as *Halophila beccarii* Ascherson, 1871 which are at risk because of their specific habitat requirements and fragmented populations (Short et al. 2010).

Productivity, carbon stocks and sequestration

Research on the carbon stocks and sequestration rates of blue carbon ecosystems in Sri Lanka is gaining momentum, but remains heavily biased towards mangroves (Clarivate - Web of Science results as of June 2022): eleven studies on mangrove forests, two studies for seagrasses and one for salt marsh. The task is made more challenging because of the diversity of coastal geomorphic settings and climatic zones and their profound influence on ecosystem diversity and structure. Typically, available measurements include 'above-ground' (AG) and 'below-ground' (BG) biomass, with fewer estimates of soil organic carbon (SOC). AG biomass includes all living above-ground material, such as tree trunks, branches, leaves and pneumatophores for mangroves, and branches and leaves for salt marshes and seagrass, and also includes epiphytic organisms such as encrusting algae on seagrass leaves. BG biomass includes living roots and rhizomes. SOC refers to the below-ground carbon comprising of dead plant tissues and soil organic matter, which can be both autochthonous (generated *in situ*) and allochthonous (coming from elsewhere) and can be presented as total carbon (C_{total}) or more usefully as total organic carbon (C_{org}). The scarcity of information on SOC represents a major limitation to our complete understanding of blue carbon stocks in Sri Lanka, given that the proportion of carbon stored in wetland soils is known to be far greater than that stored in living biomass (McLeod et al. 2011). While the application of remote sensing approaches can help to upscale estimates of carbon stocks in certain locations (where the drivers of variability are reasonably well understood), in areas where there are presently limited to no available data, there is little alternative but to increase the scale of *in situ* field measurements. This will become crucial when it comes to gaining knowledge of the carbon dynamics of seagrass meadows and salt marshes necessary to understand the cumulative role of coastal vegetated ecosystems in sequestering and storing atmospheric carbon dioxide (Macreadie et al. 2019).

Mangrove forests and salt marshes

The standing carbon stocks of mangrove forests have been quantified at various sites across the three climatic zones (Jonsson & Hedman 2019). One study from multiple locations in the south and east of Sri Lanka suggest C_{org} values that range from 316 Mg/ha in the Batticaloa lagoon (dry zone) to 580.8 Mg ha in the Rekawa lagoon (intermediate zone; Perera & Amarasinghe 2019). Another study, done in the Negombo estuary (wet zone), estimated total organic carbon retention by mangroves to be 500 Mg/ ha, of which 84% was contained in mangrove soils, while only 16% comprised of living biomass (Perera et al. 2018). A more recent study that integrated both climatic and intertidal zonation showed that total carbon stocks can range from 1455 Mg/ha in intermediate zones (interestingly, some of the highest values recorded globally) to 735 Mg/ha in the narrow fringing forests of dry climate zones (Cooray et al. 2021). A synthesis of all available data (AG+BG+SOC definitions in section above) done as part of this review reaffirms the strong influence of climate (Figure 3A) and suggests that intermediate forests have the greatest overall carbon stocks (i.e. AG+BG+Corg=1257 Mg/ha), followed by wet forests (899 Mg/ha), and the lowest estimates from dry and or arid forests (770 Mg/ha).

At the time of writing, there is only one published study on the total organic carbon stocks of salt marshes in Sri Lanka. This paper reports the total organic carbon stocks (i.e. BG+SOC) within

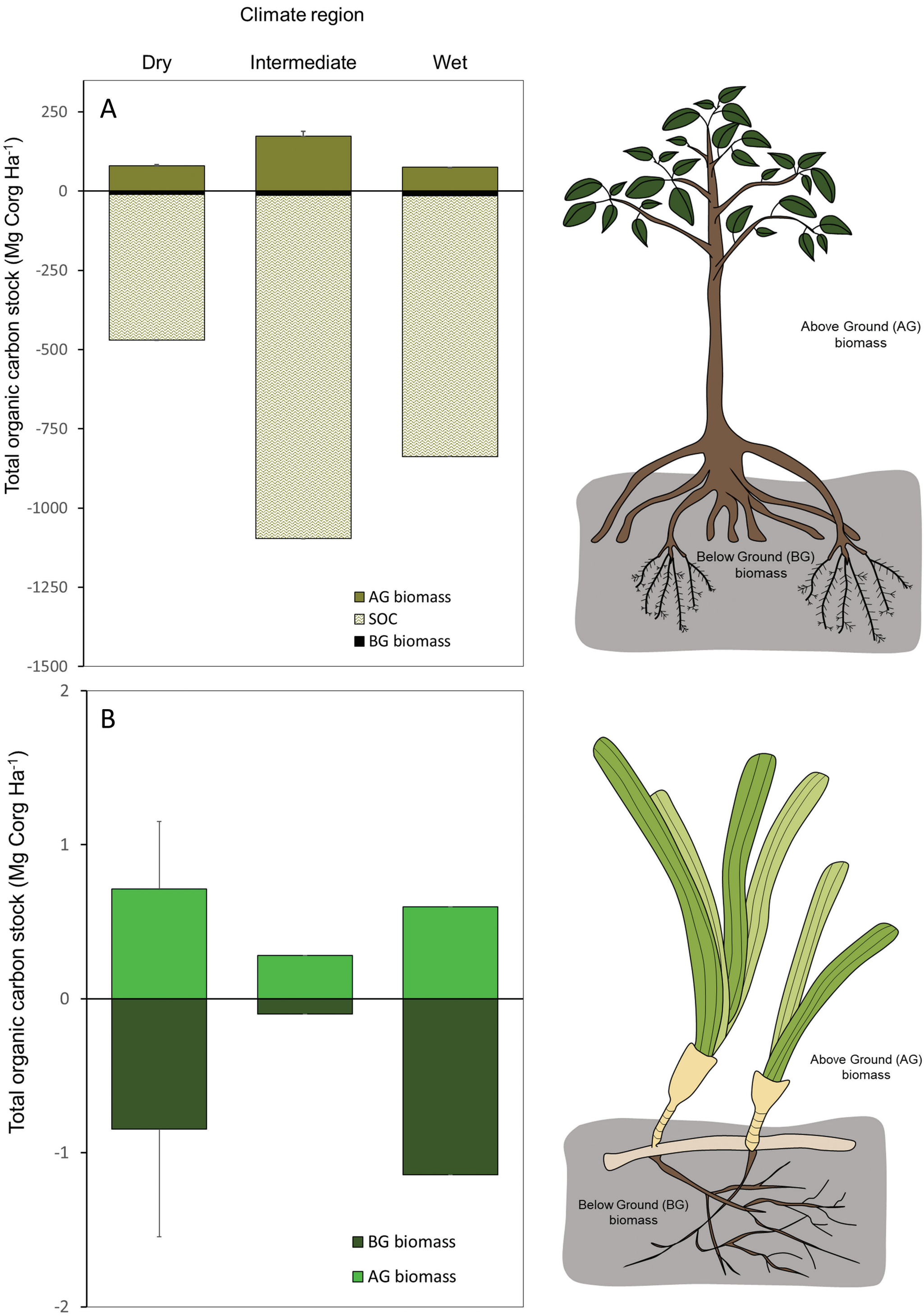


Figure 3 Estimates of total organic carbon (Corg, mean ± SEM) stocks from two blue carbon ecosystems located within the three principal climatic zones; (A) mangroves. Derived from Cooray et al. (2021), Edirisinghe et al. (2012). (B) Seagrass ecosystems. Derived from Faazil et al. (2019), NARA (2013), Pahalawattaarachchi & Dahanayaka (2018), Pahalawattaarachchi et al. (2005).

the Wedithalathive Nature Reserve in the countries north-west, to range from 48.2 to 108.1 Mg/ha across replicate sites (Perera et al. 2022). This study also showed a general decline in SOC with increasing depth and comparatively greater C_{org} concentrations in surface soil layers than deeper layers up to 50 cm. Another related conference paper highlights the variability in carbon stocks among different saltmarsh species (i.e. *Suaeda vermiculata* greater than *Salicornia brachiata* and *Halosarcia indica*) in the Vidataltivu nature reserve (Perera et al. 2021). Despite these new studies, the general lack of data describing saltmarsh carbon stocks is concerning given that their overall distribution is greater than that of mangroves and that they are arguably under sustained threat from continued loss.

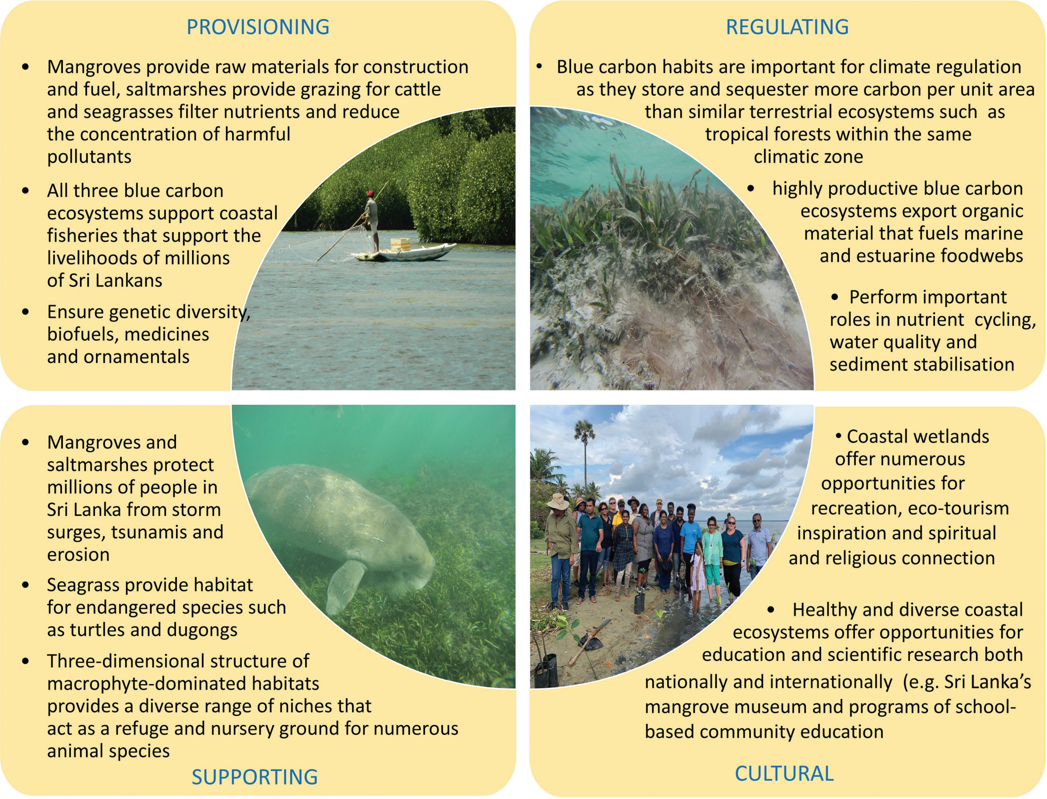
Seagrass meadows

There are few studies on the productivity, carbon stocks or sequestration rates of Sri Lankan seagrass meadows. Early estimates suggested that productivity was on a par with other tropical regions, ranging from 574 to 1518 g/m^2/year depending on epiphytic growth, water turbidity and seasonal nutrient dynamics (Johnson & Johnstone 1995). This reflects the range in total standing stock biomass (AG+BG), which can vary from 3006 Mg/ha (wet climate) to less than 1.4 Mg/ha in the temperate southern region of Rekawa (Faazil et al. 2019). When we consider other studies from dry regions such as Mannar and Kilinochchi (i.e. Palk Straight and Palk Bay, respectively), in which mixed species *Thalassia hemprichii* and *Enhalus acoroides* meadows yield values for total biomass of approximately 2.4 Mg/ha (Pahalawattaarachchi & Dahanayaka 2018), it is clear that meadow structure must be dynamic. Indeed, contrasting the low carbon stocks of dry regions in the south with high values of dry regions in the north suggests that seagrasses demonstrate a more general south-north trend related to surface circulation and upwelling patterns (De Vos et al. 2014) than mangroves, which tend globally to be more closely linked to climatic patterns related to seasonal minimum air temperature (Borges et al. 2019).

Our synthesis of available data for this review suggests that there is considerable variation in selected carbon pools of seagrass meadows occurring across Sri Lanka (Figure 4B), whereby the wet climate average (AG+BG=1.7 Mg/ha) was marginally greater than the dry climate (0.4 Mg/ha), and both were substantially greater than the intermediate climate (0.4 Mg/ha). It is important to note that these estimates don't include SOC from any climate region, or for BG biomass from intermediate regions. This means that a comparatively large component of the total carbon stocks of seagrasses in Sri Lanka is missing. Consequently, the available data across the country (2.5 Mg/ha) appear to be lower than similar estimates of seagrass carbon stocks for other Indian Ocean regions, e.g. an average of 7.3 Mg/ha for AG+BG in Gazi Bay, Kenya, which represents only ~4% of total carbon stocks (Juma et al. 2020). It is also evident that the ratio between AG and BG is more uniform across climate zones than it is for mangrove forests. This is likely because the ratio of root to rhizome volumes vary more among seagrass species, with low ratios for small-leaved species such as *Halophila* spp. and larger ratios for larger species such as *Enhalus acoroides* (Duarte & Chiscano 1999). This means that previous attempts to measure temporal change in the health and productivity of seagrass meadows are limited to comparisons of living biomass (AG+BG). One example being the studies done on several smaller and more ephemeral seagrass species (i.e. *Halophila ovalis*, *Halodule pinifolia* and *Ruppia maritima*) in the Negombo lagoon which suggested declines in average biomass from 2.3 Mg ha in 1998 to less than 0.3 Mg ha in 2004 (Pahalawattaarachchi et al. 2005). Despite these preliminary indicators, it is clear that greater effort is needed to obtain accurate and robust estimates of SOC across the different climate zones and among the larger and more perennial species such as *Thalassia hemprichii* and *Enhalus acoroides*.

Ecosystem services and livelihoods

Blue carbon ecosystems underpin the function of many of the world's coastlines and provide life-sustaining services to much of humankind (Himes-Cornell et al. 2018). In Sri Lanka, mangroves, salt marshes and seagrasses support coastal fisheries, provide a long-term store of carbon, contribute to shoreline protection and provide habitat for many threatened and endangered animals (Figure 4). These ecosystem goods and services (EGS) can be broadly separated into provisioning, regulatory, supportive and cultural services (World Bank 2017), all of which are important for humans and the long-term resilience of Sri Lanka's coastline. However, managers, planners and economists often struggle to include the social, cultural and economic values derived from nature into economic decision-making, and hence, the true value of nature's enormous wealth is often left unaccounted for or undervalued (Daily et al. 2000). Despite efforts to place an economic value on mangroves and other wetlands in Sri Lanka (e.g. Emerton & Kekulandala 2002, Gunawardena & Rowan 2005), this has not yet been widely done for salt marshes or seagrass meadows (but see; Jones 2022). There is a clear need to better understand and evaluate the services provided by each ecosystem (in both ecological and economic terms), and what is at stake if they are lost or degraded (Fisher et al. 2009). The following section presents some of the most tangible EGS provided by these three blue carbon ecosystems in Sri Lanka.



- Mangroves provide raw materials for construction and fuel, saltmarshes provide grazing for cattle and seagrasses filter nutrients and reduce the concentration of harmful pollutants
- All three blue carbon ecosystems support coastal fisheries that support the livelihoods of millions of Sri Lankans
- Ensure genetic diversity, biofuels, medicines and ornamentals

- Blue carbon habits are important for climate regulation as they store and sequester more carbon per unit area than similar terrestrial ecosystems such as tropical forests within the same climatic zone
- highly productive blue carbon ecosystems export organic material that fuels marine and estuarine foodwebs
- Perform important roles in nutrient cycling, water quality and sediment stabilisation

- Mangroves and saltmarshes protect millions of people in Sri Lanka from storm surges, tsunamis and erosion
- Seagrass provide habitat for endangered species such as turtles and dugongs
- Three-dimensional structure of macrophyte-dominated habitats provides a diverse range of niches that act as a refuge and nursery ground for numerous animal species

- Coastal wetlands offer numerous opportunities for recreation, eco-tourism inspiration and spiritual and religious connection
- Healthy and diverse coastal ecosystems offer opportunities for education and scientific research both nationally and internationally (e.g. Sri Lanka's mangrove museum and programs of school-based community education

Figure 4 Provisioning, regulating, supporting and cultural ecosystem goods and services provided by three blue carbon ecosystems in Sri Lanka. Photos by Susantha Udagedara and Daniel Gorman.

Mangrove forests and salt marshes

Mangrove forests provide a wide range of EGS including food production, raw materials, medicines, microclimate regulation, disease prevention, water purification and opportunities for recreation and spiritual connection (Millennium Ecosystem Assessment 2003, Vo et al. 2012). In Sri Lanka, most studies evaluating such services have focused on their importance as a habitat and nursery ground for fish and other marine species (Sarathchandra et al. 2018). The three-dimensional structure of mangrove roots can provide refuge from predators and enhance many ecosystem processes (Primavera 1997, Vorsatz et al. 2021). The nutrients and carbon exported in the form of detritus and dissolved organic material fuel the productivity of marine microorganisms, which form the base of many estuarine and marine food webs (Fleming et al. 1990, Nagelkerken et al. 2008). Sri Lanka's mangroves host 53 species of fish and 13 species of crustaceans, many of which are commercially important such as mud crabs, lobsters and several species of shrimps and prawns (De Silva 1985, De Silva & De Silva 2002). Fishery resources depend on healthy mangrove forests (Figure 4A), especially in estuaries and lagoons, as mangroves support both brackish and marine species that are significant for the island's fishing industry (Sarathchandra et al. 2018). The forests also support other species including 100 species of birds, 9 species of amphibians, 33 species of reptiles and 26 species of mammals (Joseph 2004) including elephants and panthers (De Silva and De Silva 2002) listed as endangered and vulnerable, respectively, by the IUCN.

Mangrove forests are among the most productive ecosystems globally and while occupying only 0.5% of the world's coastal land, they contribute 10%–15% (24 Tg C/y) to coastal sediment carbon storage and export 10%–11% of the particulate terrestrial carbon to the ocean (Alongi 2014). Their tremendous potential to sequester atmospheric carbon dioxide is encouraging conservation and restoration to help address and mitigate the effects of climate change (Figure 4B). Sri Lanka's mangrove forests are some of the most productive globally, with estimates of net primary productivity from the Negombo lagoon of 2282 $g \cdot m^{-2}/y$ (Perera & Amarasinghe 2016). This means that the average amount of sequestered carbon is nearly 500 t ha, which is twice that of tropical forests occupying a similar climatic zone (Perera & Amarasinghe 2019). This carbon accumulation capacity, which equates to approximately 12 t ha/y, is equivalent to the amount of carbon emitted as CO_2 through the combustion of approximately 19,000 L of diesel or 22,000 L of gasoline in motor vehicles (Perera & Amarasinghe 2016).

The complex three-dimensional structure of mangrove forest also protects the coastline from storms and tsunamis (Guannel et al. 2016). The Indian Ocean Tsunami of 26 December 2004 was one of the worst natural disasters in modern history, responsible for immense destruction and loss of life and livelihoods (Dahdouh-Guebas et al. 2021). In Sri Lanka, the waves impacted a 1700 km long coastal strip, reaching heights of up to 6.8 m and travelling as far as 3 km inland at some locations (Wijetunge 2006). The waves caused widespread devastation across the southern and eastern parts of the country, with a reported death toll of 23,558 that was concentrated in towns adjacent to lagoons and estuaries (Dahdouh-Guebas et al. 2005). The wave damage was influenced by a range of factors including land elevation, coastal convexity and the absence or presence of mangrove forests that reduce wave inundation distance and height and thus lead to lower human fatalities (Dahdouh-Guebas et al. 2005). The benefits of mangroves are apparent not only when it comes to reducing the risks of large-scale infrequent events such as tsunamis, but also seasonal storm surges and flooding. Indeed, while 63 tsunami events were recorded in the Indian Ocean between 1750 and 2004, there were more than three tropical cyclones per year over roughly the same period (total of 1530 within the North Indian Ocean Basin). Available literature suggests there is still considerable work to do with respect to evaluating the protective capacity of mangroves as a function of forest health, species composition and connection with other ecosystem types (e.g. seagrasses, salt marshes and coral reefs; Venkatachalam et al. 2012).

Salt marshes also provide a wide range of EGS including food, carbon sequestration and wave attenuation (Friess et al. 2020). Unlike mangroves, however, negative perceptions of salt marshes persist in Sri Lanka, driven by several of their perceived attributes (e.g. odour, wasteland and their real or perceived role in vector and disease transmission; Friess et al. 2020). Because of this, evaluating the EGS provided by salt marshes has not been an active area of research, with only a few studies examining their importance to resident and migratory bird species that use the Central Asian Flyway (Ranawana et al. 2020) and their importance for cattle grazing and habitat provision to supply juvenile fish and shellfish for pond aquaculture (Kallesøe et al. 2008). Therefore, there remain many gaps in our comprehension of salt marshes' EGS provision in Sri Lanka. These gaps will be increasingly important to address given the multitude of risks these ecosystems face.

Seagrass meadows

Seagrass meadows provide a wide range of EGS that directly or indirectly benefit humans (Cullen-Unsworth et al. 2014, Campagne et al. 2015, Nordlund et al. 2018), including habitat provision for important marine species that support livelihoods, sediment trapping and water filtration as well as carbon sequestration. The importance of Sri Lanka's seagrass meadows for endangered species such as turtles and dugongs (*Dugong dugon*, Figure 4D) have been broadly acknowledged (Ilangakoon et al. 2008). According to a study by the United Nations Environmental Program (UNEP), the global population of dugongs have decreased by 20% in the last 90 years. In Sri Lanka, these animals have disappeared from the western coast and are now only found in the north, along the coast of the Palk Strait and the Gulf of Mannar (Ilangakoon et al. 2008).

Seagrass meadows are thought to support more than 50% of the country's near-shore fishery production (Pernetta 1993), which employs more than half a million people (directly and indirectly), contributes 70% of animal protein (Amarasinghe & Amarasinghe 1991) and contributes about 2% of the country's GDP (Ministry of Fisheries 2020). Indeed, the seagrass meadows of the Negombo lagoon are thought to be the single most important habitat type within the region, supporting exceptionally high fish production that exceeds 150 kg ha/y (Jayakody 1994). Maintaining healthy seagrass meadows is therefore crucial, especially given the large coastal population and the disproportionate contribution of fishing to the national GDP (Sarathchandra et al. 2018). Unfortunately, coastal development across the country has accelerated the rates of seagrass loss to more than 1% per year, which is leading to reduced fish breeding and feeding habitat and is driving a decline in several demersal fish stocks (Gunasekara et al. 2019). This has created a range of socio-economic challenges (including human migration to other regions in search of employment, see; Thilakarathna & Amaralal 2019) that have recently been exacerbated by the SARS-CoV-2 pandemic, which has decimated Sri Lanka's tourism industry and resulted in the loss of more than 400,000 jobs (Arachchi & Gnanapala 2020).

Policy and governance

National policies and legislation

The Sri Lankan government has enacted a range of national policies and legislation to protect, conserve and restore coastal ecosystems such as coral reefs, beach dunes and blue carbon ecosystems (summarised in, Joseph 2004). Indeed, the Environmental Performance Index (EPI – a metric that measures how well countries are preserving, protecting and enhancing ecosystems and the services they provide) gave Sri Lanka a ranking of 1 for Wetland loss in 2020 (indicating virtually no habitat loss) and, moreover, was praised for its low carbon dioxide emissions when compared to other similar-sized economies (Baba 2010). Protection for coastal wetlands lies under the country's constitution which has driven successive governments to introduce policies to manage the broader environment, the species therein and the EGS they render (see previous section). The policies outlined in the following section have the broad aim of conserving biodiversity, protecting resource use, maintaining cultural values, promoting sustainable activities and regulating those known to be harmful to coastal macrophyte-dominated ecosystems.

Mangrove forests

Sri Lanka is one of the few countries to be proactive in terms of ensuring policy and regulatory frameworks for the conservation and sustainable utilisation of mangroves (Kinver 2015). In May 2015, a joint programme between the government of Sri Lanka and several non-profit organisations was announced by President Maithripala Sirisena to grant full protection to all the country's remaining mangrove forests irrespective of tenure. In 2019, a memorandum was adopted by the Cabinet of Ministers that permitted lands previously leased from government (for prawn farming or saltern construction) to be returned to the Department of Forest and Wildlife for the purpose of restoration if commercial activity had ceased for over 1 year. This decision allowed for the declaration of new conservation areas and the establishment of the National Mangrove Task Force charged with accelerating restoration programmes beginning in 2019. The latest and most direct policy *The National Policy on Conservation and Sustainable Utilization of Mangrove Ecosystem in Sri Lanka 2020* is intended to provide consistent guidance to the government, the ministry of environment and all other stakeholders with regard to the management of mangroves.

Other legal protections are found under *The Fauna and Flora Protection Ordinance 1937* which provides protection for mammals, reptiles, birds, amphibians, invertebrates and plants, and the *Fisheries and Aquatic Resources Act 1996* which can be used to declare fish breeding areas as reserves and to enforce spatial closures and special protection to endangered resources within lagoons and estuaries where mangroves are present. The *Fisheries and Aquatic Resources Act 1996* also contains provisions to promote the regeneration of aquatic life ('restoration'), preserve water quality, enhance natural beauty and promote scientific study or research in mangrove habitats where fisheries interests are also important. Although not used to date, the *National Heritage Wilderness Areas Act 1988* can also be used to declare any mangrove forest with significant heritage as a National Heritage Wilderness Area, while *The Coast Conservation and Coastal Resources Management Act 1981* can declare areas of mangroves as "Beach Parks" for the preservation of scenic beauty and biodiversity. There are additional provisions to declare "Conservation Areas" where there is a need to take special measures to conserve the coastal and aquatic ecosystems. This diverse suite of legal protections for mangroves in Sri Lanka are having the desired effect with around 8815 ha of forest being protected across the nation.

More broadly under the UN Convention on Biological Diversity, Sri Lanka has prepared a National Biodiversity Strategic Action Plan (NBSAP), which spans 2016–2022. This plan identifies the importance of mangrove restoration in the context of enhancing the capacity of ecosystems to provide services, including mitigating the risks associated with hazards such as waves and flooding associated with storms and tsunamis. In addition, Sri Lanka aims to improve knowledge of its coastal environment, by developing an accurate sea level rise forecasting system (including a Digital Elevation Model for the entire country), by undertaking a long-term data collection programme that includes measurements of waves, sediment, inundation prone areas and coastal habitats (including blue carbon ecosystems), by identifying suitable sites for conservation, rehabilitation and restoration, and by conducting pilot projects at highly prioritised sites.

Salt marshes and seagrass meadows

Policy and legal protection of seagrasses and salt marshes are not well defined and instead are usually linked to the protection of threatened species inhabiting them, such as dugongs in seagrass meadows (e.g. under the *Fauna and Flora Protection Ordinance 1993*) and migratory birds in salt marshes (e.g. under the *Ramsar Convention*). Such lack of protection is especially problematic for salt marshes, which continue to be reclaimed and used for cattle grazing (Rajkumar et al. 2019). The absence of detailed maps of saltmarsh distribution (as well as inconsistent classification) means that there is no specific conservation plan despite the multitude of risks these ecosystems face (Ranawana et al. 2020). In addition to the specific policies aimed at protecting coastal wetlands (mentioned in the previous section), several other policies of broader scope provide some protection to both salt marshes and seagrass meadows, notably *National Forest Policy 1995*, *National Wildlife Policy 2000*, *National Environmental Policy 2003*, *National Sand Policy 2005*, *National Policy on Wetlands 2006*, *National Fisheries Policy 2006*, *National Soil and Mineral Policy 2007*, *National Policy on Solid Waste Management 2007*, *National Policy on Climate Change 2012* and *National Coastal Resource Management Plan 2016*.

International commitments

Sri Lanka is a signatory to several international conventions that are relevant for blue carbon ecosystems, most importantly the United Nations Framework Convention on Climate Change (UNFCCC) and the Convention on Biological Diversity (CBD). As a signatory to the Paris Agreement, agreed at the 21st session of the UNFCCC Conference of the Parties (COP), Sri Lanka submitted its Nationally Determined Contributions (NDC) in 2016 (Ministry of Mahaweli Development and Environment 2016). Recognising that Sri Lanka is especially vulnerable to climate change, and that protection and restoration of blue carbon ecosystems is a grand challenge to humanity (Duarte et al. 2020), the NDC highlighted that climate mitigation and adaptation should include restoration, conservation and management of seagrasses and mangroves (as well as other ecosystems). Indeed, Sri Lanka's NDC has been notable for being one of few such commitments globally to mention the potential for seagrass ecosystems to store carbon and provide opportunities for carbon abatement.

Compliance and restoration

Although Sri Lanka's protected area network is impressive for the nation's size (i.e. marine protected areas totalling 100,966 ha; IUCN 2018), and despite the large number of policies and regulations, the lack of resources for detecting and monitoring offences have hampered management efforts (Krishan et al. 2020). For instance, despite formal protection, mangroves continue to be illegally cleared in some of Sri Lanka's largest and most important wetlands including the Pambala-Chilaw, Puttalam and Negombo lagoons because of the failure or inability to enforce these legal protections (Dahdouh-Guebas et al. 2002, Katupotha 2014, Hsieh et al. 2021). In other regions, there are serious concerns that existing nature reserves may be de-gazetted, to encourage a new wave of aquaculture (see case study – 'Vidattaltivu under threat'; Hettiarachchi & Deane 2020). These examples remind us that legal protection and enforcement alone will not be enough to achieve the desired goals of conserving and protecting marine and coastal resources, but rather that education and community participation will be critical to effective coastal zone management (Sanborn & Jung 2021).

Promoting the value of blue carbon ecosystems to local communities and children is key to greater appreciation and consequently enhanced protection and conservation (Figure 5). Sri Lanka has identified this as an important goal, with several unique and innovative programmes that engage with the wider community. One of these is the Seacology-Sudeesa Mangrove Museum, which is a component of the Sri Lanka Mangrove Conservation Project. The facility is the first of its kind to provide an avenue to educate locals and visiting ecotourists about the ecological and economic importance of mangroves, while introducing guests to the conservation and restoration of mangrove ecosystems in Sri Lanka through its impressive mangrove seedling nursery. The museum opened on International Mangrove Day (26 July 2016) in a ceremony attended by the Sri Lankan President Maithripala Sirisena and the leaders of Seacology and Sudeesa.

Figure 5 Sri Lanka has ambitious plans for mangrove restoration and for community education on the importance of coastal blue carbon ecosystems. Activities include (A) ongoing research into seagrass ecosystems, (B) education and outreach, (C) support for the establishment of mangrove nurseries and (D) community lead restoration. Photos by Susantha Udagedara, Daniel Gorman and Kasun Nuwarapaksha.

There is also a great deal of activity being headed by non-profit organisations (e.g. SLYCAN Trust, Sri Lankan Nagenahiru Foundation, Wildlife and Nature Protection Society) as well as the private sector (e.g. Dilmah Tea) who together are engaging in mangrove conservation and restoration in collaboration with government entities such as the Department of Forest, the Department of Wildlife Conservation, the Marine Environmental Protection Authority and the Coast Conservation Department. Additionally, and unlike most nations around the world, the Sri Lanka Navy has been at the forefront of mangrove restoration on their lands as well as supporting other government and community initiatives for several years. An example of this was the recent planting of 2,500 mangrove seedlings by the Navy and local communities in the Jaffna Peninsula, coordinated by the Northern Naval Command from December 2019 to January 2020. Heeding recent calls to “Pause before you Plant” (Huxham et al. 2020), many of the current conservation and restoration projects being undertaken in Sri Lanka are apparently yielding success because they are specifically considering both the environmental (i.e. site suitability) and ecological (i.e. biological diversity) factors, as well as employing a more wholistic approach that relies on local knowledge and sustainable cooperation with coastal communities (Mombauer 2019).

An exciting aspect of current blue carbon research in Sri Lanka is the tremendous activity relating to marine coastal restoration. This mirrors the realisation globally that rebuilding marine life will be a grand challenge for humanity, and an ethical obligation to achieve a sustainable future (Duarte et al. 2020). It also reflects a shift in government and industry thinking from one that was rather pessimistic because of previous failures, to one that focuses more on bright spots and technological fixes that can help to achieve these goals (Saunders et al. 2020).

Mangrove forests and salt marshes

Sri Lanka initiated numerous mangrove restoration projects following the 2004 Indian Ocean Tsunami, ranging from locally funded small-scale activities to island-wide projects that were facilitated by funding from external donor agencies (Ekaratne & Mallawatantri 2015). The outcomes have been mixed, with a recent review suggesting that 54% of planting attempts resulted in complete failure and roughly 40% of the sites chosen for planting had limited success, because they were done in inappropriate areas (Kodikara et al. 2017). Of 14 sites that reported some degree of success, 50% had survival rates of less than 10%. Many of these projects were relatively small (Figure 6) and not at a scale sufficient to address historical losses, nor advance the 10,000 ha commitments made in the 2019 memorandum adopted by the Cabinet of Ministers. Thus, there is a clear need to review the factors that led to early failures, and to focus on ensuring future sites are chosen based on the appropriate hydrological setting and the correct combination of target species (e.g. Gorman et al. 2022). This includes consideration of factors such as topography, soil parameters, incursions by cattle, algal accumulation and insect attack, as well as local community engagement that can ensure post-care of planted seedlings (Kodikara et al. 2017).

The National Action Plan and Guidelines for Mangrove Restoration are soon to be introduced by the Biodiversity Secretariat of Ministry of Environment and are expected to support scientific restoration, coordinate efforts and help to govern data collection that will be important as a part of Sri Lanka's commitment to the Mangrove Ecosystems and Livelihood Action Group (MELAG)

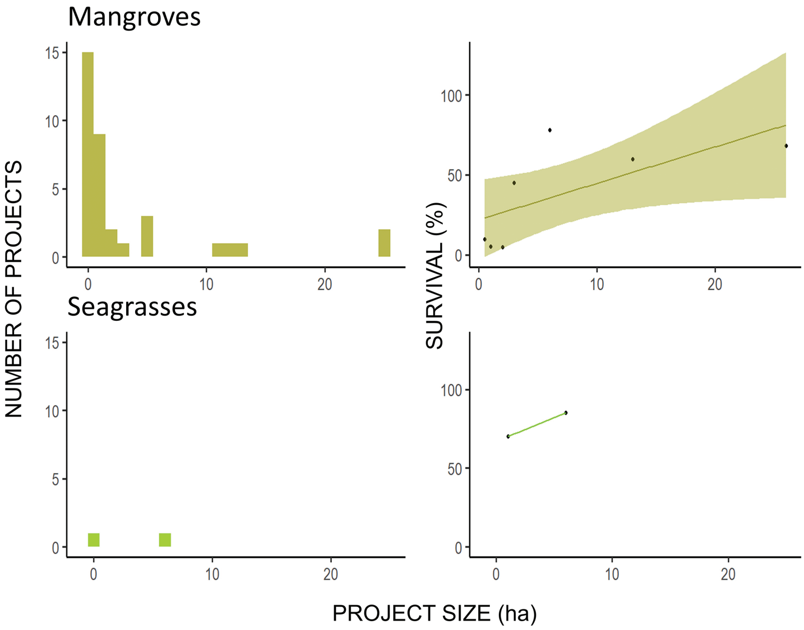


Figure 6 Histograms of active restoration project size (in hectares: ha) for mangroves and seagrasses in Sri Lanka along with relationship between project size and mean survival (%) of plants within each hectare classification. There are currently no studies on salt marshes. Data derived from Gorman et al. (Unpub. Data) and Kodikara et al. (2017).

under the Commonwealth Blue Charter. Recent restoration efforts are focusing more on these scientific and socio-economic factors, often using a broad framework that considers pre-plantation (drivers of loss, land tenure, social support and finance instruments), plantation (site selection, what species to use, employment) and post-plantation stages (monitoring, policy development and identification of challenges) (Mukherjee et al. 2009). This involves paying specific attention to environmental factors such as existing hydrology to improve site selection and hydrological modification which can dramatically improve tidal inundation (including novel approaches such as herring bone structures; Figure 7). There remains a need for robust studies that consider the feasibility of passive versus assisted interventions (see Atkinson & Bonser 2020) with respect to restoration in the Sri Lankan context. While active replanting may be necessary in areas that have been heavily degraded (and where propagule supply is low), a more feasible approach might be to restore the hydrological conditions of abandoned shrimp farms (e.g. by simply opening sluice gates or tearing down bund walls) to facilitate natural recovery. There also remains the challenge of financing such projects, with a recent report suggesting that the return on investment of restoring financially viable mangrove forests in Sri Lanka is around US$ 239,000 per year (Zeng et al. 2021). Thus, carbon financing could both provide a means of funding and implementing these interventions and help to encourage greater international interest in mangrove and other blue carbon ecosystems restoration projects.

Previous challenges related to land tenure and finding potential sites for restoration have been mitigated with the adoption of the Cabinet memorandum to return abandoned shrimp farms to the Department of Forest and Wildlife for the purpose of restoration and reforestation (see policy

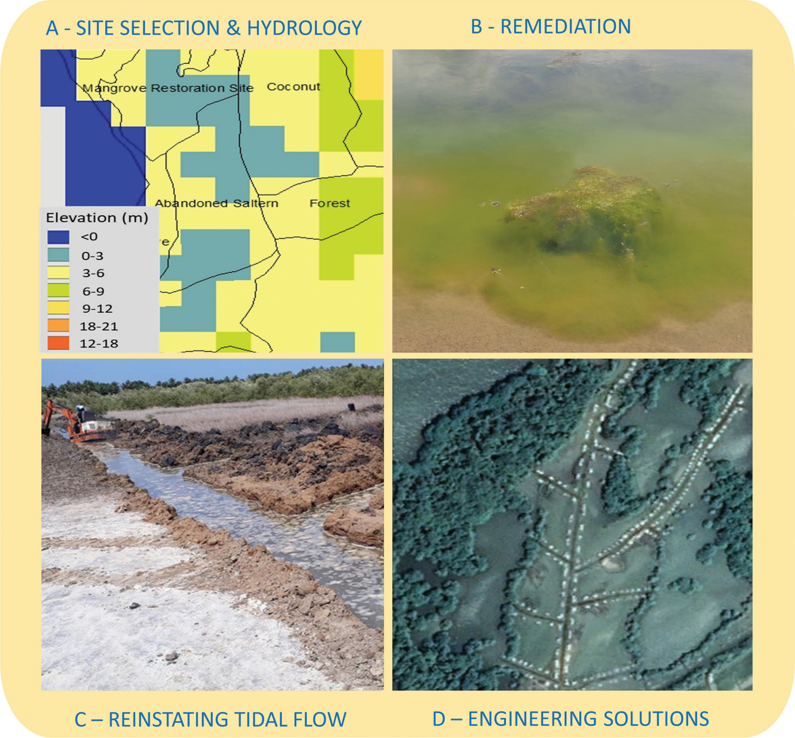


Figure 7 Scientifically informed restoration projects in Sri Lanka are now focusing on: (A) evaluating existing hydrology to improve site selection – example of elevation map from Forestry Department site, (B) dealing with issues of contamination, changes to soil characteristics and algal blooms; (C and D) hydrological modification using approaches such as the construction of ‘herring bone’ structures that facilitate tidal inundation. Photos: Forest Department of Sri Lanka.

section). It is also key to have community support that minimises conflict over land use and can provide direct benefits through employment. New partnerships such as those established through the Sri Lanka Mangrove Conservation Project are giving impoverished Sri Lankans alternative ways to earn a living by participating in restoration rather than continuing with activities that entail the cutting down of mangroves. To date, this has included sustainable livelihoods training for more than 7900 young people and women (many of whom were widowed during the civil war) and the provision of 2893 microloans in exchange for leadership in community mangrove conservation cooperatives (UNCC 2021). Positive outcomes can be achieved by encouraging community efforts in these nature-based solutions by emphasising natural heritage and also by ensuring sustained logistical and financial support by government and/or external sources (Wickramasinghe 2017).

In contrast to the efforts directed at mangrove restoration, there have been no attempts to restore salt marshes in Sri Lanka, which is surprising given both their ecological importance (e.g. for resident and migratory birds; Ahalya & Suresh 2020) and the fact that recent advances in the restoration of these communities could be readily adopted in Sri Lanka (see the use of propagules; Vanderklift et al. 2020). Unlike the considerable backlash given to proposed and realised environmental damages to freshwater wetlands (e.g. the 1777 ha Muthurajawela Sanctuary adjacent to Negombo lagoon; Emerton & Kekulandala 2002, Athukorala et al. 2021), there is still little political and/or community will to conserve salt marshes, and thus, future losses are almost a foregone conclusion (Ranawana et al. 2020).

Seagrass meadows

Seagrass restoration in Sri Lanka is in its infancy, but progress is being made (Figure 6). One ongoing project has set the goal of restoring an area of 800 m^2 in the Mannar region with a mixed community of *Halodule uninervis*, *Halophila ovalis* and *Cymodocea rotundata* (The Rufford Foundation, 2017). Another larger-scale project done in the Gulf of Mannar and Palk Bay region (i.e. a region connecting Sri Lanka with India) claims to have restored 14 acres of degraded seagrass between 2011 and 2020 with a success rate of 85%–90% (Edward et al. 2019). This project further highlights the cost-effectiveness of seagrass restoration done within this part of the Indian Ocean, with the total cost of manual transplantation and ongoing monitoring stated to range between Rs. 800,000 and 1,000,000 (approx. US$ 11,000–13,000) per acre. This is substantially less than the median cost of seagrass restoration globally, which has been estimated at approximately US$ 43,000 per acre (i.e. reported as US$ 106,782 per hectare; Bayraktarov et al. 2016). A number of reports have identified the need to understand how replanting seagrass might help to combat climate change and represent a ‘soft defence mechanism’ to aid in coastal protection (e.g. MERE 2014).

Knowledge gaps and research needs

Research challenges remain to fully understand and appreciate blue carbon ecosystems in Sri Lanka and best plan for their future. The following section outlines some of the major knowledge gaps and proposes research needs that should be prioritised.

a. *Uncertainties in geographical distribution*. There remain uncertainties with respect to the geographical distribution in Sri Lanka of all three blue carbon ecosystems considered in this review. While there is now consensus on the current distribution of mangrove forests (19,500 ha), it is necessary to reconcile the role that different methodologies have had in the variation of estimates at the regional level and how this may have influenced broader assessments of historical change. For salt marshes, the challenges of understanding geographical distribution are further complicated across a tidal gradient, because of inconsistent classification (by government agencies, and policy documents) and the general lack of studies into the salt marsh – terrestrial ecotone necessary to effectively map and manage these highly threatened ecosystems (Ranawana et al. 2020). Finally, the major challenge of mapping seagrass will be to overcome the inherent logistical and financial constraints of intertidal/subtidal surveys, as well as the limits to the application of remote sensing approaches in optically deep waters (Krause-Jensen et al. 2004). After overcoming these hurdles, it may be possible to rapidly scale up the current mapping and modelling capacity, establish carbon stock assessments of interconnected ecosystems (i.e. seagrass-sand dune-mangrove-marsh; Rupasinghe & Perera 2006) and identify potential restoration sites.

b. *Productivity and carbon dynamics.* There is a clear need to expand the scale of research focused on understanding the productivity and carbon stocks of blue carbon ecosystems. The currently scant data on carbon stocks of seagrass (two studies) and salt marshes (one) hinder the development of a complete carbon stock inventory for coastal wetlands and delays the implementation of commitments made under Sri Lanka's NDCs. Finally, there is a pressing need to better understand the productivity dynamics of all three ecosystems (i.e. sequestration rates and carbon storage) in the face of climate change and understand the consequences of inaction or further degradation that may turn these stores of carbon into sources of carbon dioxide and other greenhouse gasses such as methane and nitrous oxides into the atmosphere (Adame et al. 2021).
c. *Evaluation of ecosystem goods and services.* Scientific knowledge of the full range of EGS that are provided by mangroves in Sri Lanka is growing, but remains poor for seagrass meadows and salt marshes. Research needs to be focused on quantifying the value of these ecosystems in terms of supporting coastal biodiversity, fishery production and coastline protection (among other EGS). More valuation studies are needed across the diversity of coastal settings and social contexts present in Sri Lanka (i.e. from urbanised areas through to impoverished fishing communities).
d. *Optimising marine coastal restoration.* With respect to the current focus on mangrove restoration and lessons learnt about historical failures (Kodikara et al. 2017), there is a major need for robust studies that consider restoration approaches (i.e. passive vs. assisted interventions), soil and hydrological restoration (e.g. acid sulphate, pollution, hydrological reconstruction) and the re-establishment of EGS (through restoration of ecosystem functioning). Increased focus on salt marshes and seagrass meadows also represents a priority. Indeed, while seagrass areas are being conserved for the protection of endangered species (e.g. dugongs in the newly declared 4839 ha Vankalai Sanctuary in the Mannar region), there remains no sound management plan for salt marshes. Several challenges remain to scale up blue carbon restoration in Sri Lanka, including understanding the current distribution as well as historical losses, managing or mitigating the drivers of loss (e.g. land-based pollutants) and identifying the most feasible species, transplantation methods and avenues for financing.

Conclusions

The blue carbon ecosystems considered in this review are vitally important for the continued prosperity and health of the island nation of Sri Lanka and its people. Despite significant challenges, research into the biology, ecology and social value of mangrove forests, seagrass meadows and salt marshes has progressed over the past few decades, albeit at different pace. The country exhibits a two-tiered focus with respect to the accumulation of scientific knowledge: while studies of mangrove ecosystems have advanced from early descriptive stages to deeper understanding and mature legal protection, the science underpinning seagrasses and saltmarshes remains in its infancy. The goal of this review was to summarise the current state of knowledge, the opportunities for ecological restoration and the socio-economic value of these three blue carbon ecosystems. This information is crucial to allow decision-makers to balance the relative costs and benefits of various socio-economic and policy decisions. This type of robust, accessible and defensible knowledge is vital to ensure that Sri Lanka continues efforts aimed at the protection, conservation and restoration of these ecosystems that comprise a large and important part of the countries coastline and contribute towards the well-being of its people.

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KELP GAMETOPHYTES IN CHANGING OCEANS

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Abstract Kelp forests are ecologically diverse habitats that provide vast ecosystem goods and services, but are threatened by climate and anthropogenic stressors. Laminarian kelps have an alternating biphasic life cycle, and while there is a growing understanding of climate impacts on the macroscopic diploid sporophyte, impacts on the microscopic haploid gametophyte stage are just emerging. There exists a strong history of gametophyte literature on single species and environmental factors, but only recently studies have increasingly examined multiple climate stressors, species and populations. We synthesize studies on kelp gametophytes, building upon our understanding of their responses to environmental conditions and subsequent vulnerability in changing oceans. Kelp gametophytes have a broad tolerance to environmental conditions predicted to change with climate change, including temperature and irradiance, but large variation exists among species and populations. Key processes such as gametogenesis and early sporophyte development consistently appear more sensitive to environmental conditions than vegetative growth and may present bottlenecks to ongoing kelp persistence. Indirect effects of climate change negatively affect kelp gametophytes through competition, grazing and sedimentation. Modern genomic techniques are paving the way to transition research into field settings that include both sporophyte and gametophyte stages. Unravelling the response of gametophytes to changing environmental conditions is beginning to provide an understanding of overall kelp forest persistence and enables proactive conservation and management initiatives in changing oceans.

Keywords: Climate Change; Competition; Gametophytes; Grazing; Laminariales; Ocean Acidification; Resilience; Sedimentation; Temperature; Thermal Tolerance; UV Radiation

Introduction

Kelp forests, defined here as communities structured by macroalgae in the order Laminariales, are the dominant biogenic habitat of shallow temperate rocky reefs and underpin some of the most productive ecosystems globally (e.g. Kain 1979, Dayton 1985, Schiel & Foster 1986, Steneck et al. 2002, Graham et al. 2007, Smale et al. 2013, Wernberg et al. 2019a, Wernberg & Filbee-Dexter 2019).

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Kelp forests provide habitat for many ecologically and economically important species (Bertocci et al. 2015) and are often the main primary producers in many temperate rocky reef systems (Mann 1973). They are the third most productive system globally (Filbee-Dexter & Wernberg 2018) and store and cycle large amounts of CO_2 (Krause-Jensen & Duarte 2016, Duarte 2017, Filbee-Dexter & Wernberg 2020). Through direct exploitation by harvesting (Buschmann et al. 2017) and indirect support of economically valuable industries such as fisheries and tourism, the value of kelp forests is estimated at $US 684 billion annually worldwide (Eger et al. 2021).

Despite their importance, the cover of kelp forests is declining in many regions due to local anthropogenic impacts such as pollution, urban run-off and industrial effluents (Reed et al. 1994, Tegner et al. 1995, Coleman et al. 2008, Strain et al. 2014), overgrazing by urchins (Jones & Kain 1967, Chapman 1981, Johnson & Mann 1988, Keats et al. 1990, Filbee-Dexter & Scheibling 2014, Ling et al. 2015) and increased sedimentation (Foster & Schiel 2010, Alestra et al. 2014). Decline is also occurring due to global stressors such as ocean warming (Moy & Christie 2012, Vergés et al. 2014, Krumhansl et al. 2016, Filbee-Dexter et al. 2016, Filbee-Dexter & Wernberg 2018, Wernberg et al. 2019b, Berry et al. 2021, Coleman et al. 2022), marine heatwaves (Dayton et al. 1999, Wernberg et al. 2013, Arafeh-Dalmau et al. 2019, Smale et al. 2019) and increased frequency and intensity of storms (Dayton et al. 1992, Filbee-Dexter & Scheibling 2012, Davis et al. 2022). The decline of kelps can be due to direct impacts (Dayton et al. 1999, Smale & Wernberg 2013, Wernberg et al. 2016), or driven by indirect effects such as climate-induced range shifts of tropical grazers into temperate habitats (Johnson et al. 2011, Vergés et al. 2014, Wernberg et al. 2016) and suppressed recruitment due to algal turfs that thrive under urbanized or acidified conditions (Connell & Russell 2010, Connell et al. 2018, Filbee-Dexter & Wernberg 2018). These stressors on the marine environment have altered the ecological structure (Harley et al. 2012, Filbee-Dexter & Wernberg 2018, Vergés et al. 2019), adaptive capacity (Gurgel et al. 2020, Coleman et al. 2020a) and economic and social values derived from kelp forests (Bennett et al. 2016). In contrast to these general trends, localized increases in kelp cover have also occurred in some regions (Krumhansl et al. 2016, Krause-Jensen et al. 2020) and understanding what confers these positive responses is key for informing conservation, restoration and management of kelp forests globally (Coleman & Goold 2019, Layton et al. 2020b, Coleman et al. 2020b, Eger et al. 2020b).

Kelps are characterized by a haplo-diplontic life history with macroscopic diploid sporophytes alternating with microscopic haploid gametophytes. While we have a growing understanding of the functioning and resilience of kelp forests at their macroscopic sporophyte stage, our understanding of the microscopic gametophyte stage is considerably less (Schiel & Foster 2006). For example, a literature search using the keywords “gametophytes”, “microscopic stages”, “kelp” and “Laminariales” conducted on 5-12-2020 in Web of Science resulted in a final set of 192 papers (Figure 1), whereas a search on “sporophyte”, “kelp” and “Laminariales” in the same search engine resulted in over 5000 publications. As such, several excellent reviews exist on the effects of climate change on the ecology and persistence of kelp forests (e.g. Steneck et al. 2002, Harley et al. 2012, Wernberg et al. 2019b), the reproductive biology of kelps (Santelices 1990, Amsler et al. 1992, Schiel & Foster 2006, Liu et al. 2017) and the potential of gametophytes to persist as a bank of microscopic forms (Hoffmann & Santelices 1991, Barrento et al. 2016, Wade et al. 2020, Schoenrock et al. 2020). Moreover, there are several reviews on the biology and ecology of individual kelp species that compare both gametophyte and sporophyte life stages (e.g. Kain 1979, Graham et al. 2007, Bartsch et al. 2008, Wernberg et al. 2019a) as well as a long history of gametophyte knowledge and studies that focus on aquaculture (Valero et al. 2017).

Despite this body of literature, studies on kelp gametophytes are largely biased towards single factor experiments on individual species, with multi-factor studies in the context of climate change only gaining traction in recent years (16% of the total number of studies identified above; Figure 1). Moreover, studies examining the impact of environmental factors predicted to be altered under

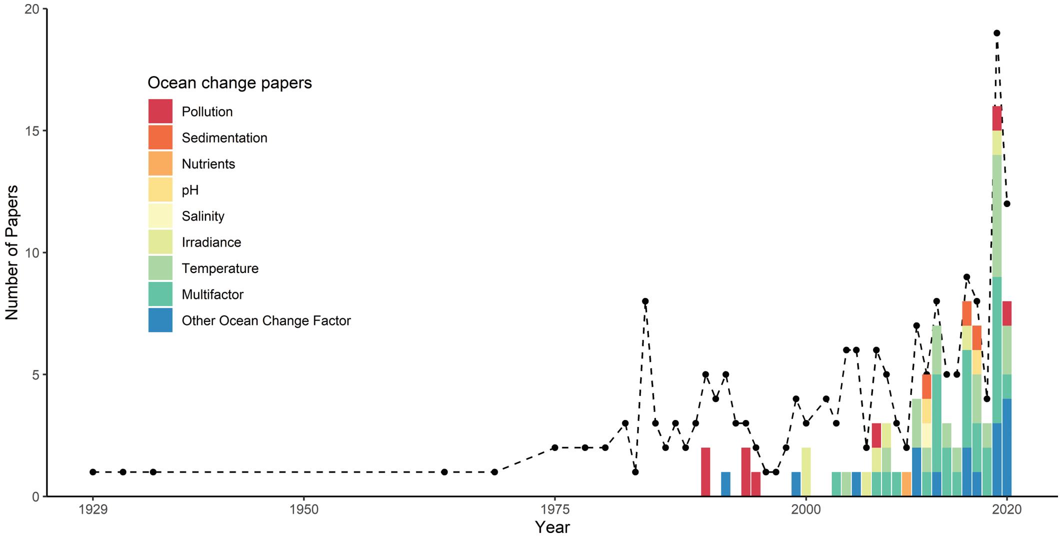


Figure 1 Database of papers collated through a Web of Science search with search terms "microscopic stages", "gametophytes", "kelp" and "Laminariales" on 5-12-2020. A total of 327 studies were found, which were then examined to concern solely laminarian kelps and gametophytes. The final database consisted of 192 papers and was sorted according to ocean change theme. The dotted line represents all papers throughout time, with the coloured bars representing the proportion of total papers that concern ocean change, sorted by theme.

anthropogenically driven climate change (e.g. marine heatwaves, acidification) on gametophytes account for less than half (45%) of papers and have only emerged in the past two decades with acceleration in the appearance of such studies in recent years (Figure 1).

This review presents a contemporary synthesis of how kelp gametophytes are influenced by variation in environmental conditions relevant to anthropogenic climatic change, indirect effects of climate change such as change of ecological interactions and other anthropogenic disturbances such as pollution and eutrophication (together termed ocean change hereafter) and the implications for kelp persistence. We build on earlier reviews to consider kelp gametophyte biology, ecology and physiology and integrate emerging studies on ocean change (Figure 1). We review the literature on kelp gametophytes across taxa and development stages to examine responses to environmental conditions (Figure 1) and highlight the implications for kelp persistence in changing oceans. Where possible, we compare differences in response to environmental conditions between gametophyte and sporophyte stages and summarize current knowledge on ecological interactions in the microenvironment that gametophytes inhabit. Finally, we identify knowledge gaps and key future research directions that will increase understanding of the role of gametophytes and provide pathways for conservation and management of kelp in a changing ocean.

The alternation of generations life cycle of kelp

Kelps are characterized by a haplo-diplontic life history with macroscopic diploid sporophytes alternating with microscopic haploid gametophytes (Figure 2). The haploid life stage begins following spore release from the mature adult sporophyte (as reviewed by Santelices 1990). Spores then develop a germ tube through which the first gametophyte cell grows (germination) and develops into male and female gametophytes that either undergo gametogenesis (i.e. production of gametes) or persist in a vegetative state, depending on environmental conditions (Harries 1932, Kain & Jones

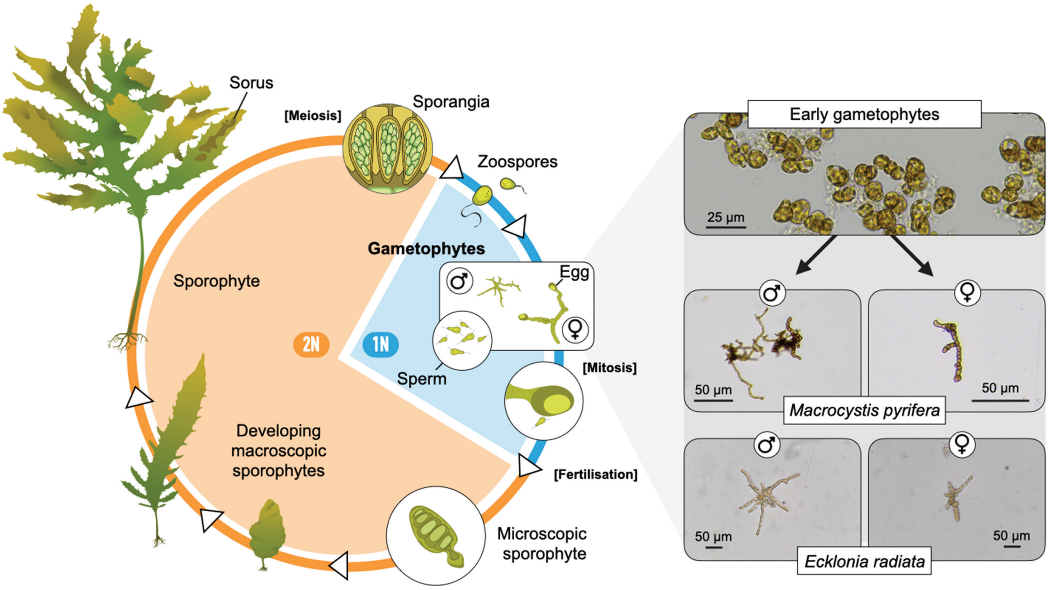


Figure 2 Haplo-diplontic life cycle of laminarian kelp. Diploid sporophytes produce spores by meiosis which develop into male and female haploid gametophytes. Fertilization can take place after the production of gametes, which results in recruitment of juvenile sporophytes.

1969, Lüning & Neushul 1978, tom Dieck 1993; Figure 2). Male gametes (sperm) are attracted to female gametes (oogonia) by pheromones released by females (Marner et al. 1984), resulting in fertilization and the subsequent development of a microscopic sporophyte and completion of the life cycle (Figure 2). While this review deals with factors influencing gametophytes rather than sporophytes, below we briefly summarise how environmental conditions and ocean change may alter sporophyte phenology as this has implications for the environment in which gametophytes grow.

Climate change has already facilitated shifts in phenology for many species (e.g. Scranton & Amarasekare 2017, Piao et al. 2019) including seaweeds (e.g. Tala et al. 2004, Coleman & Brawley 2005, Bartsch et al. 2013, de Bettignies et al. 2018), and because many aspects of sporophyte reproduction are tightly linked to environmental conditions (e.g. Lüning & tom Dieck 1989, Santelices 1990), sporophyte phenology is likely to change under predicted future climatic conditions. Reproductive phenology of kelp can be characterised as the production of sporangia in sori (fertility) and the timing of spore release and these processes, as well as the biology of spores generally, have been relatively well studied and reviewed (Santelices 1990, Amsler et al. 1992, Clayton 1992, Fletcher & Callow 1992, Schiel & Foster 2006, Liu et al. 2017, de Bettignies et al. 2018).

Kelp reproductive phenology can be strictly seasonal, or vary intra-annually depending on environmental conditions (Reed et al. 1996). Reproductive phenology may also be linked to sporophyte growth which is governed by circannual rhythms, or can be influenced directly by changing environmental factors such as temperature (tom Dieck 1991). Recent studies have shown that sori production may be the life-history phase with the narrowest environmental window (Bartsch et al. 2013, de Bettignies et al. 2018) highlighting the likely sensitivity of sporogenesis to changing environmental conditions. The dispersal of spores and their settlement are also important determinants of subsequent gametophyte development. Dispersal distance and settlement density are likely species-specific and vary seasonally (Reed et al. 1988, 1992) depending on several factors such as water motion and currents (Gaylord et al. 2002, 2006, Coleman et al. 2011). These factors can in turn be modified by kelp forest density (Graham 2003) and episodic events such as storms

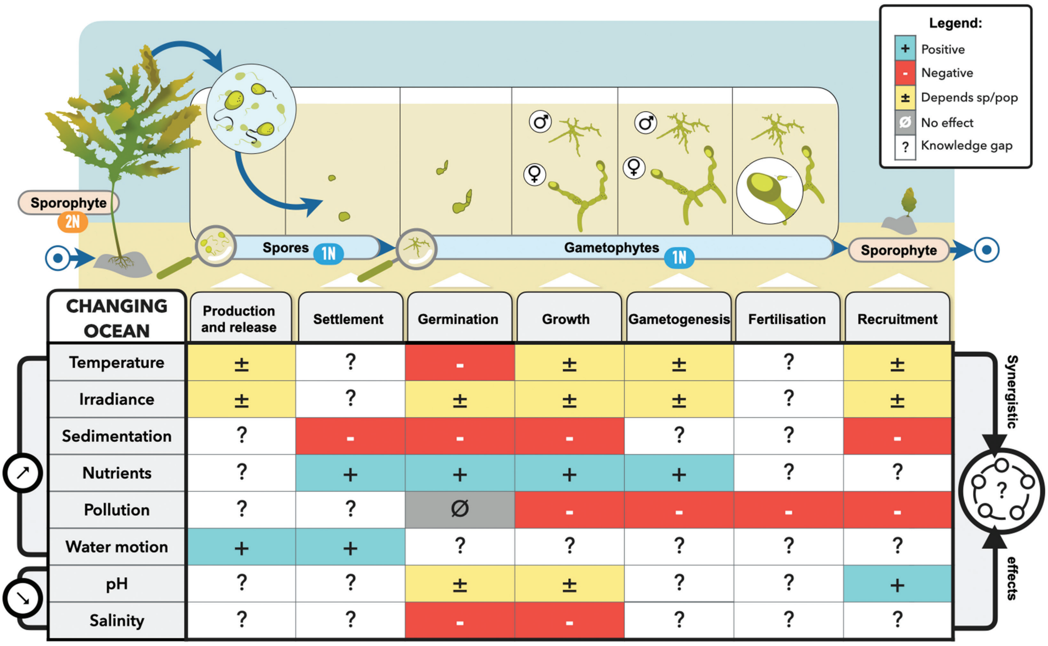
(Reed et al. 1988). As spore dispersal is integral to population structure and connectivity (Graham 2003, Coleman et al. 2011, Oppliger et al. 2014), and is predicted to be altered under climate change (Coleman et al. 2017), research into reproductive phenology and its ties to environmental factors are at the base of the overall resilience of gametophytes to ocean change.

The impact of ocean change on gametophytes

Climate change and other anthropogenic stressors are fundamentally altering environmental conditions in the world's oceans and coastal waters. Ocean warming, acidification, increasing intensity and frequency of storms and extreme events in conjunction with increasing urbanisation and pollution presently threaten kelp forests globally (Filbee-Dexter & Wernberg 2018). This section reviews research on how environmental factors expected to change in future oceans (temperature, light, pH, sedimentation, nutrients, pollution and salinity) will impact the survival, growth and physiology of the gametophyte stage and its key developmental stage transitions (e.g. gametogenesis and sporophyte production; summarized in Figure 3). Where studies exist that specifically examine such environmental factors in an ocean change context, we highlight the response of gametophytes and implications for persistence in future oceans.

Effects of change in temperature on gametophytes

Temperature is among the most important drivers in the large-scale distribution of marine species, including kelps (Bartsch et al. 2012, Harley et al. 2012, Martínez et al. 2018, Smale 2020, Krause-Jensen et al. 2020). The impact of temperature on gametophytes is the most documented environmental factor, with 55% of papers identified in our search addressing impacts of temperature



CHANGING OCEAN	Production and release	Settlement	Germination	Growth	Gametogenesis	Fertilisation	Recruitment
Temperature	±	?	-	±	±	?	±
Irradiance	±	?	±	±	±	?	±
Sedimentation	?	-	-	-	?	?	-
Nutrients	?	+	+	+	+	?	?
Pollution	?	?	Ø	-	-	-	-
Water motion	+	+	?	?	?	?	?
pH	?	?	±	±	?	?	+
Salinity	?	?	-	-	?	?	?

Figure 3 The ocean change factors influencing different microscopic life stages, including spores and juvenile gametophytes. Based on the available literature, variable, negative and positive impacts (up to a threshold) of ocean change factors are indicated in the table.

(Figure 1), and more than half (68%) of those papers specifically studying temperature in the context of ocean warming. Early research on gametophyte temperature tolerance was part of a larger effort to characterize the ecophysiology of kelps and identified a broad thermal range in which kelp gametophytes can survive (Ueda 1929, Kain & Jones 1964, Lüning & Neushul 1978, Novaczek 1984b, Bolton & Levitt 1985, tom Dieck 1993). Independent of location, most kelp gametophytes have a lower survival threshold of 0°C and their upper thermal limit ranges between 20°C and 30°C (tom Dieck 1993) which is hypothesised to have played an important evolutionary role in kelp dispersal across the equator for some species (Peters & Breeman 1992). Apparent differences in temperature tolerances between gametophytes and sporophytes, as well as the fact that many gametophytes exhibit higher temperature tolerances than conditions experienced by natural populations (e.g. Bolton & Lüning 1982), have led to the notion that gametophytes can persist even when environmental temperatures exceed the upper thermal thresholds of sporophytes (Ladah et al. 1999, Murua et al. 2013, Mohring et al. 2014, Komazawa et al. 2015, Park et al. 2017, Augyte et al. 2019, Rodriguez et al. 2019, Hollarsmith et al. 2020). This broad thermal tolerance of gametophytes relative to sporophytes is particularly important in the context of ocean warming (Ladah et al. 1999, Carney et al. 2013) and the predicted increase in extreme temperature anomalies such as marine heatwaves (Frölicher et al. 2018, Oliver et al. 2018, Smale et al. 2019).

The response of kelp gametophytes to change in temperature appears to be both species- and population-specific (Lüning & Neushul 1978, Bolton & Anderson 1987, Oppliger et al. 2012, Mohring et al. 2014, Lind & Konar 2017, Muth et al. 2019; Figure 3). Elevated temperature can negatively affect germination, particularly in colder adapted species (Lee & Brinkhuis 1988, Fredersdorf et al. 2009, Shukla & Edwards 2017, Gonzalez et al. 2018), but appears to have less impact on cosmopolitan species that occupy a broader thermal niche (Muth et al. 2019). In some instances, elevated temperatures have been found to positively influence germination (Izquierdo et al. 2002). The negative effects of increased temperature on kelps can have a cumulative impact on the transition from spore to gametophyte (Muth et al. 2019) and may depend on the temperature range over which they occur. The role of cumulative effects highlights the need for multi-factor, multi-species experiments across multiple life stages and over broad geographical ranges to fully understand the effect of changing oceans on all life-history stages (Muth et al. 2019).

Some general differences in requirements for gametogenesis are apparent between cold and warm temperate species. However, it is important to note that due to high variability in responses, these differences are not universal across locations, suggesting local adaptation. Laboratory studies have shown that species occurring in cold temperate waters (i.e. monthly means below 10°C in winter and below 15°C in summer) appear to have gametophytes that grow slower under decreased experimental temperatures and induce gametogenesis and sporophyte production at relatively low (5°C–18°C) temperatures (e.g. Lüning 1980, Lee & Brinkhuis 1988, Izquierdo et al. 2002, Nelson 2005, Martins et al. 2017, Augyte et al. 2019, Rodriguez et al. 2019). Species distributed in warm temperate (monthly means above 15°C in summer) waters generally appear to have higher thermal tolerance of the gametophyte stage, where gametophytes can survive experimental temperatures that are higher than the thermal maximum experienced by the source population and can undergo gametogenesis across a wide (10°C–25°C) temperature range (e.g. Novaczek 1984b, Deysher & Dean 1986b, Bolton & Anderson 1987, Thornber et al. 2004, Oppliger et al. 2012, Mabin et al. 2013, Hollarsmith et al. 2020). This indicates gametophytes may persist while conditions for sporophyte production are unfavourable and can become fertile and produce gametes when the environment matches their reproductive window, potentially acting as a seedbank (Hoffmann & Santelices 1991, Santelices et al. 2002). However, the reproductive window for gametophytes does not always match ideal environmental conditions for sporophyte growth, which demonstrates the importance of local environmental drivers and highlights the reproductive versatility that is often found among kelps (e.g. Muth et al. 2019, Liesner et al. 2020, Martins et al. 2020, Camus et al. 2021). Moreover, most studies have been done in laboratory settings and the ecological relevance of temperature tolerance in gametophytes, as well as their ability to persist vegetatively for periods until their 'reproductive window' occurs, remains to be validated in natural settings (Deysher & Dean 1986a, Reed et al. 1997, McConnico & Foster 2005).

Another important factor that makes the study of temperature responses more complex is the within-species variation between geographically distinct populations and genotypes, particularly those growing along latitudinal thermal gradients. For instance, geographically distinct populations are often genetically differentiated due to a combination of isolation by distance, which is common among kelps (Valero et al. 2011, Durrant et al. 2014), and local adaptations (Wood et al. 2021, Vranken et al. 2021). Such differences can drive disparity in gametophyte thermal tolerance (Oppliger et al. 2012, Mohring et al. 2014) and fertility (Bolton & Levitt 1985, Camus et al. 2021) potentially due to selection and underlying genetic variation among individuals (Alsuwaiyan et al. 2021, Vranken et al. 2021). Populations that grow in cooler waters often produce more gametes and sporophytes at lower temperatures, whereas survival of gametophytes is higher with increased temperatures in warm-adapted populations within the same species (Bolton & Levitt 1985, Oppliger et al. 2012, Mabin et al. 2013, Mohring et al. 2014, Muth et al. 2019, Rodriguez et al. 2019, Hollarsmith et al. 2020, Camus et al. 2021). Gametophytes from populations at the warmer edge of their distribution can be more tolerant of high thermal stress (Oppliger et al. 2012, Muth et al. 2019, Hollarsmith et al. 2020), while populations with more narrow distributions often produce gametophytes that are more susceptible to temperature change (Lind & Konar 2017, Augyte et al. 2019, Choi et al. 2019, Gao et al. 2019). Even different individuals (genotypes) from within the same population can display very different responses and tolerance to temperature stress (Alsuwaiyan et al. 2021).

The temperature experienced by parent sporophytes can also impact gametophytes. Spores collected at different times from the same population can produce gametophytes with different thermal tolerances, with two laboratory studies showing that spring and summer spores produce relatively heat-tolerant gametophytes with higher growth rates (Mohring et al. 2013a, Murua et al. 2013). In turn, higher growth rates of gametophytes can enhance the production of sporophytes (e.g. Mabin et al. 2013, Martins et al. 2020). Conversely, *in situ* temperature during spore release and early growth of gametophytes has recently been confirmed to impact subsequent sporophytes in *Laminaria digitata,* which had higher growth rates across a range of temperatures when gametophytes were grown in cooler temperatures (Liesner et al. 2020). Based on these studies, it is likely that gametophyte growth and performance are highly dependent on temperature conditions experienced during spore production and increased temperatures in future oceans may severely affect kelp gametophytes (Martins et al. 2017, Muth et al. 2019) and subsequent sporophyte production (but see Layton & Johnson 2021). However, time lags between the production of spores and recruitment of sporophytes may indicate persistence of gametophytes (or microscopic sporophytes; Kinlan et al. 2003) in a dormant state until temperatures become suitable for recruitment (McConnico & Foster 2005). Similarly, different temperature optima for spore production and gametogenesis (Mabin et al. 2013, Mohring et al. 2013b) could indicate the persistence of gametophytes between pulses of spore release and recruitment.

Marine heatwaves, defined as discrete periods of anomalously warm water that exceed historical norms of ocean temperature (Hobday et al. 2016) are superimposed on a background of ocean warming. Marine heatwaves are increasing in frequency and duration globally (Oliver et al. 2018) with devastating consequences for kelp forests (Dayton et al. 1999, Wernberg et al. 2013, Arafeh-Dalmau et al. 2019, Smale et al. 2019). Despite this, only 2% of studies examined the impact of heatwaves on gametophytes. Alsuwaiyan et al. (2021) simulated different types of marine heatwaves and found that all heatwaves negatively impacted *Ecklonia radiata* gametophyte performance and delayed gametogenesis and sporophyte recruitment. However, the response to heatwaves was also highly dependent on genotype, with some genotypes (from the same population) performing much better than others (Alsuwaiyan et al. 2021). Furthermore, heatwaves have been shown to decrease photosynthetic activity of gametophytes beyond a threshold of 20°C in some cold temperature species (Delebecq et al. 2016), and indeed, a simulated marine heatwave caused high mortality (80%–100%) in juvenile sporophytes of *Macrocystis pyrifera* across different populations (Camus et al. 2021). Post-heatwave recovery may be possible, though population dependent, as gametophytes from warm-adapted populations of *L. digitata* had a higher recovery rate after simulated heatwave treatments (Martins et al. 2020).

Effects of change in light on gametophytes

Climate change is predicted to increase the frequency of storms (Coumou & Rahmstorf 2012, Collins et al. 2019) that may impact light provision to kelp forests and gametophytes in several ways. Storms can remove large proportions of the adult canopy and change light levels within kelp forests. Moreover, increases in storm events and run-off may change turbidity and light in nearshore coastal systems. This may affect gametophytes given that light availability is an important determinant of the distribution of kelp (Swanson & Druehl 2000, Roleda et al. 2005). In the total database of papers, 47% studied the influence of light on gametophytes, but less than half of those papers (46%) considered the impact of changes to light in the context of climate change. Similar to the effects of temperature, the transition from one life stage to another is highly sensitive to light cues, but gametophytes can grow in a wide range of light conditions (Lüning & Neushul 1978, Lüning 1980, Novaczek 1984a, Bolton & Levitt 1985, Ebbing et al. 2020), and can even persist in darkness for extended periods (Kain & Jones 1969, tom Dieck 1993). Low light concurrent with adult canopy shading generally increases germination of spores once settlement has occurred (Augyte et al. 2019) and high light stress can decrease successful germination in *Lessonia, Pterygophora* and *Undaria* (Veliz et al. 2006, Cie & Edwards 2008, Morelissen et al. 2013), potentially affecting gametophyte density in open canopy patches created by storms.

Low light conditions generally induce growth in gametophytes (Kain & Jones 1964, Lüning & Neushul 1978, Bolton & Levitt 1985, Kinlan et al. 2003, Nelson 2005, Xu et al. 2015a, Augyte et al. 2019) and a change of light intensity and colour can be a cue for the onset of gametogenesis (Lüning & Dring 1975). The use of blue light to promote gametogenesis (Lüning & Dring 1975) has been used for decades in aquaculture settings, whereas cultures are often grown in red light to suppress gametogenesis. To cue gametogenesis, some species require increased light (Deysher & Dean 1984, Novaczek 1984a, Nelson 2005, Choi et al. 2005, Morelissen et al. 2013, Tatsumi & Wright 2016), while others require decreased light (Izquierdo et al. 2002, Pang et al. 2008, Roleda 2016). The ability to induce gametogenesis in high light conditions allows gametophytes to rapidly recruit when the canopy of adult kelps is removed after storms or other pulse disturbances (Dayton 1985, Schiel & Foster 1986), which has been documented among *Lessonia* and *Ecklonia* species growing in the southern hemisphere and the invasive *Undaria pinnatifida* (Novaczek 1984a, Nelson 2005, Choi et al. 2005, Morelissen et al. 2013, Tatsumi & Wright 2016). However, gametogenesis can also be prevented when irradiance is too high (Augyte et al. 2019) which may have implications for low latitude populations within western boundary currents where warm, nutrient poor and thus clearer waters are more frequently encroaching into temperate kelp habitats (Cetina-Heredia et al. 2014). Suppressed recruitment under light stress may have consequences for future kelp persistence, especially in view of competition with other algae that might be better adapted to changing light circumstances, such as invasive species (Morelissen et al. 2013) or in competition for light with understory algae (Tatsumi & Wright 2016).

Day length is a factor less studied when measuring gametophyte performance, but appears to be as important as light intensity (Martins et al. 2017) and may influence kelps as they shift distribution or their timing of reproduction with climate change. Cycles of day length are strongly correlated with seasonal changes in natural kelp populations, which are governed by circannual rhythms

(Lüning 1991, Lüning 1994), and can determine growth and gametogenesis in gametophytes (Deysher & Dean 1984, Mohring et al. 2013a, Martins et al. 2017). Increasing daylight hours has been found to promote both gametophyte growth and gametogenesis in several species (Mohring et al. 2013a, Martins et al. 2017, Choi et al. 2019), which is concurrent with summer/spring photoperiods. Under field conditions photoperiod, light intensity and temperature all increase concurrently meaning that teasing apart the individual influence of these factors is difficult. Some studies have tried to test the influence of these covarying factors. For instance, it has been shown for *M. pyrifera* that total irradiance received determines gametogenesis, irrespective of photoperiod and light intensity (Deysher & Dean 1986b). Conversely, short day lengths induce gametogenesis in *U. pinnatifida* rather than total light intensity (Choi et al. 2005), which allows recolonization after winter storms when day length is short, giving this species a potential competitive advantage over native perennial kelps (e.g. *L. digitata and Lessonia variegata*) where gametogenesis is induced under long day lengths (Nelson 2005, Martins et al. 2017).

UV radiation is recognized as a stressor throughout shallow coastal ecosystems and is expected to increase under climate change (Williamson et al. 2014, Barnes et al. 2019), which might alter the capacity of gametophytes to persist especially in places of high UV stress such as the Arctic (Müller et al. 2012). Both UVA and UVB radiation play a role in gametophyte development. Gametophytes are relatively more resilient to UV radiation than sporophytes, but exposure can still result in damage to the growing gametophyte (Müller et al. 2012). UVA is found to be less damaging than UVB to gametophyte growth, and in some cases even promotes germination (Tala et al. 2007, Müller et al. 2008). However, UVB can be severely damaging to gametophytes, with exposure resulting in up to 50% decreased germination (Huovinen et al. 2000) and the complete failure to recruit in some species (Tala et al. 2007, Roleda et al. 2007). Yet when the increase in UV radiation is gradual, some gametophytes have been found to survive and produce sporophytes (Tala et al. 2007). Some species can recover from the negative effects of UV radiation, and sporophyte recruitment has been observed when gametophytes are placed back under low light conditions (Roleda et al. 2007). This indicates that kelps can survive UV radiation as gametophytes, and recruitment can resume when conditions improve (Müller et al. 2012). The ability to cope with UV radiation and light stress is often observed in species growing in the intertidal and shallow subtidal waters (Augyte et al. 2019), and the ability to adapt to UV radiation could be a factor that determines the distribution of kelps in future oceans (Wood 1987, Tala et al. 2007, Müller et al. 2008, Fejtek et al. 2011).

A small number of studies have experimentally tested the interactive effects of light with other environmental factors and have shown that effects of light intensity and day length on growth and gametogenesis can interact with several other abiotic factors. Interactions of light and temperature often create a species-specific 'reproductive window' (Lüning & Neushul 1978, Lüning 1980, Deysher & Dean 1986b, Izquierdo et al. 2002, Müller et al. 2008, 2012, Martins et al. 2017, Augyte et al. 2019), and it is hypothesized that opposite regimes of light and temperature (i.e. low light, high temperature or high light, low temperature) induce fertility in gametophytes (Ebbing et al. 2021). This is important to consider in an ocean change context as the disparity between temperature and light might increase recruitment pulses, though this increased disparity may also alter the reproductive window with unknown ecological consequences. The influence of increased nutrient pulses can stimulate growth in light-limited gametophytes (Kinlan et al. 2003, Morelissen et al. 2013), which can be relevant for populations growing in upwelling sites and potentially beneficial to kelp species competing for light with understory algae. Day length, irradiance and ocean temperature covary over seasonal timescales, and gametophytes are likely to have evolved interactive responses to these variables. To obtain more accurate and comparable results in the future, it will be necessary to refine experimental methods to realistically reflect multi-stressor field conditions related to ocean change, as well as consider not only the quantity, but also the quality of light used in studies.

Effects of change in pH on gametophytes

The effect of lowered pH caused by increasing atmospheric CO_2 concentrations, known as ocean acidification, on marine organisms is a well-researched topic, but most studies have focused on negative effects on calcifying species (Hofmann et al. 2010, Connell et al. 2013). In contrast, fleshy algae such as kelps are expected to be largely unaffected by a decrease in ocean pH as their photosynthesis is saturated at current oceanic carbon concentrations (Hurd et al. 2019). When atmospheric CO_2 comes in contact with seawater it dissolves and subsequent reactions with water convert the majority of dissolved CO_2 into HCO_{3^-} (bicarbonate) (Raven et al. 2005). Aqueous CO_2 requires less energy for most aquatic plants to take up into their cells as it can diffuse passively. Bicarbonate, in contrast, requires energy to take up using active carbon concentrating mechanisms (Hepburn et al. 2011). As many laminarian species are not able to downregulate their use of active carbon uptake, the added CO_2 for passive uptake may not pose a large benefit for kelps (Roleda & Hurd 2012).

Due to the active carbon uptake mechanisms of kelp, decreased pH can have either positive or neutral effects on growth and photosynthesis of sporophytes (Roleda & Hurd 2012), but few studies (5%) have researched the effect of lowered pH on gametophyte growth and gametogenesis (Figure 1). From these limited studies, however, it seems that an increase in CO_2 concentration can either increase growth (Roleda et al. 2012, Leal et al. 2016, 2017) or have no effect (Shukla & Edwards 2017, Gonzalez et al. 2018) on gametophytes. In contrast, germination can be decreased by a reduced pH consistent with future ocean acidification (Roleda et al. 2012, Xu et al. 2015a), although increased germination has also been observed (Leal et al. 2016). Differential responses to the interaction of ocean warming and ocean acidification suggest gametophytes' response to these dual stressors might be related to local environmental conditions. For example, *M. pyrifera* has been found to grow faster and larger under ocean warming and ocean acidification independently (Leal et al. 2016), while ocean warming and ocean acidification have been found to interact to impair growth and germination in the same species (Gaitán-Espitia et al. 2014, Shukla & Edwards 2017). This varying response to ocean warming and acidification within the same species might be the result of adaptation to local temperature and pH conditions. It has been shown that geographically and genetically separated populations of *M. pyrifera* can react differently to reduced pH, where populations more often exposed to low pH had a higher egg production in reduced pH compared to populations that do not experience pH fluctuations (Hollarsmith et al. 2020). In a study spanning populations of a broad latitudinal temperature range, Hollarsmith et al. (2020) showed high resistance of gametophytes to increased temperature and reduced pH concurrent with ENSO events and upwelling, but reproduction of gametophytes presented a bottleneck where fertility was most successful in varying populations when treatment pH and temperatures were concurrent with local temperatures and acidity. This increased fertility under environmental conditions matching that of the source population shows that gametophytes can be well adapted to their local environments, and an increase in extreme events such as marine heatwaves and increased ENSO events can constrain reproduction at the gametophyte stage (Hollarsmith et al. 2020). However, if such conditions are of short nature, gametophytes could persist in their resilient vegetative stage (Hollarsmith et al. 2020).

Gametophytes that survive acidification can be more resistant to a subsequent decrease in pH suggesting acclimation (Xu et al. 2015a). Indeed, kelp forests can themselves display large diel and seasonal fluctuations in pH driven by photosynthesis and respiration which can be larger than the projected reduction in ocean pH under climate change (Delille et al. 2009, Hofmann et al. 2011, Cornwall et al. 2013), which must be considered in laboratory studies to accurately predict the response of gametophytes to ocean acidification. The natural variation in pH often found in kelp forests may influence (and potentially increase) the tolerance of kelps, including gametophytes, to future acidification as they already experience reduced pH levels concurrent with projected ocean acidification (Hofmann et al. 2011). Interestingly, due to high fluctuations in pH levels kelp farms are increasingly recognised as an efficient local buffer to decreased pH, as well as providing an environment that can facilitate adaptation for other marine organisms, suggesting that kelp farms may serve as local refugia from ocean acidification (Xiao et al. 2021).

Even if there are minimal direct effects of ocean acidification, kelps may be more likely to be indirectly affected under future acidified conditions. It has been suggested that kelp could become outcompeted by turf-forming algae, which are positively affected by the increased availability of CO_2 under ocean acidification (Connell et al. 2013, Provost et al. 2017, Connell et al. 2018). Through high turn-over rates (Copertino et al. 2005, Miller et al. 2009, Layton et al. 2019a) filamentous algal turfs can utilize the additional available carbon to increase growth rate and cover when temperatures rise (Connell & Russell 2010). It is thus possible that future ocean acidification could exacerbate declines in kelp forests associated with turf-forming algae (Filbee-Dexter & Wernberg 2018) by hindering the survival of gametophytes to reproduction and subsequent sporophyte recruitment (Connell et al. 2018, Layton et al. 2019a). The effect of turfing algae on gametophytes has not yet been studied, and so manipulative experiments of gametophytes in co-occurrence with turfs and under future ocean conditions are required to test these hypotheses.

Effects of change in nutrients, pollution and sedimentation on gametophytes

Climate change and other anthropogenic activities are also fundamentally changing levels of nutrients, pollution and sedimentation in coastal ecosystems (Brierley & Kingsford 2009). Ocean warming and changing ocean currents are decreasing nutrient availability for some temperate kelps (Behrenfeld et al. 2006, Keeling et al. 2010), while changes in storm frequency and terrestrial run-off may create local pulses of high nutrient loads and introduce pollution (Russell et al. 2009). Consequently, understanding the influence of nutrients, sediments and pollution on the survival and reproduction of gametophytes is essential for predicting future persistence of kelp.

The positive effect of nutrients on sporophyte growth has been well studied and nutrient (such as nitrate and phosphate) enrichment of culture media in a laboratory setting has a positive effect on growth rates of gametophytes and increases the proportion of gametophytes undergoing gametogenesis (Harries 1932, Carter 1935, Hoffmann & Santelices 1982, Hoffmann et al. 1984, Morelissen et al. 2013, Nielsen et al. 2016, Gao et al. 2019). However, it should be noted that nutrient addition in still cultures is necessary to mimic the replenishment of nutrients that occurs by water movement in natural settings, and it is unknown if the same effects are seen in the field. The addition of nutrients to cultures often has a positive effect on the later developmental stages of gametophytes (i.e. gametogenesis and fertilisation), as well as an increased positive impact on the growth rate of juvenile sporophytes (Harries 1932, Morelissen et al. 2013, but see Muth et al. 2019). In contrast, earlier stages (spores) do not require as many external nutrients due to lipid storage (Brzezinski et al. 1993). In field conditions, a lack of sporophyte recruitment under nutrient depletion suggests that gametogenesis is nutrient-limited in natural populations (Dayton et al. 1992), which is corroborated by increased recruitment and growth of sporophytes through nutrient addition in the field (Deysher & Dean 1986a). Some evidence exists that nutrient supply in the early stages of gametophyte development also affects the ability to recruit sporophytes. For example, *M. pyrifera* gametophytes failed to produce sporophytes in a laboratory setting after being exposed to low nutrient levels (Kinlan et al. 2003, Ladah & Zertuche-Gonzalez 2007), but showed increased production of sporophytes after a period of arrested development under low nutrient conditions (Carney & Edwards 2010). The observed difference is thought to be the result of the nutrient history in which the gametophytes were grown. Inducing spore release and germination in non-limiting conditions often results in poor performance when nutrient supply is subsequently lowered, whereas growing spores in limiting conditions from the point of spore release means gametophytes are capable of gametogenesis once nutrient limitations are lifted, often producing smaller sporophytes, but at a higher rate than gametophytes grown under continuously high nutrients (Muñoz et al. 2004, Carney & Edwards

2010, Lewis et al. 2013). Note that most of the nutrient addition studies discussed here were carried out in laboratories (and under still culture conditions), and results may vary in natural field conditions where nutrients are likely to be replenished through water motion and interact with multiple additional factors (e.g. temperature and light).

Species-specific responses to nutrients have been observed. For example, *Eisenia bicyclis* is known to require higher nutrient concentrations to induce gametogenesis than the more common *Ecklonia cava* (Choi et al. 2019). Similarly, variable effects of nutrient addition have been identified among geographically distinct populations of *M. pyrifera*, with different concentrations of nutrients required to induce gametogenesis (Lewis et al. 2013). Furthermore, nutrient enrichment interacts with increased light intensity (Morelissen et al. 2013), daylight cycles (Martins et al. 2017, Choi et al. 2019) and decreased temperature (Martins et al. 2017) to induce gametogenesis in other species. Increased gametogenesis in high nutrients and decreased temperature is concurrent with annual upwelling events; however, in future oceans warm, eutrophic waters can negatively impact gametophyte fertility (Martins et al. 2017). Even so, temperature seems more important than nutrient supply in determining the survival of gametophytes in future oceans (Muth et al. 2019). More recent multifactor experiments testing interactive effects of factors such as temperature, day length, sedimentation, nutrients, pH and salinity have demonstrated that such interactions affect stage transitions in gametophytes, which will be key for understanding kelp persistence in changing multi-stressor marine environments (e.g. Zacher et al. 2016, Martins et al. 2017, Muth et al. 2019, Rodriguez et al. 2019, Hollarsmith et al. 2020, Augyte et al. 2020; Figure 3).

Increasing urbanisation results in high wastewater output and pollution which can have a negative effect on kelp populations (Coleman et al. 2008, Connell et al. 2008, Jara-Yáñez et al. 2021). The negative effects of some pollutants can exceed that of future ocean warming and acidification (Leal et al. 2018). Copper is a well-researched metal pollutant that can negatively impact gametophyte development in several species (Garman et al. 1994a, Bidwell et al. 1998, Contreras et al. 2007, Leal et al. 2018). Copper and other metals, such as cadmium, zinc and arsenic, pollute nearshore waters via biofouling agents, wastewater from oil production and mining sites, and cadmium pollution has been observed near nuclear power plants (James et al. 1990, Reed et al. 1994, Garman et al. 1994a, Wang et al. 2019). Most studies demonstrate that these metals affect gametophyte development in successive life stages. For example, germination of spores is generally not affected, whereas gametophyte growth is increasingly negatively affected, and gametogenesis is completely inhibited in *E. radiata, Lessonia nigrescens, Lessonia spicata, M. pyrifera* and *U. pinnatifida* (Anderson et al. 1990, Bidwell et al. 1998, Contreras et al. 2007, Leal et al. 2018, Espinoza-González et al. 2021). The inhibition of gametogenesis naturally results in a loss of successive sporophyte populations. Importantly, although in high concentrations of metal pollutants (i.e. >10 mg/mL or higher) regeneration does not take place (Bidwell et al. 1998), in low concentrations (i.e. <100 µg/mL) the effects of metal pollution may be reversible (Wang et al. 2019) and long-term exposure can result in adaptation to high copper concentrations (Roncarati et al. 2015). Determining lethal thresholds and concentrations at which recovery or adaptation can be achieved for local kelp populations can inform conservation and restoration efforts through managing levels of pollution in urbanized coastal systems.

The mechanisms underlying the observed negative effects of pollutants, especially in gametogenesis, are still not fully resolved. It is likely, however, that pollutants interfere with cell division and DNA replication (Garman et al. 1994b) and possibly pheromone signalling between male and female gametophytes (Reed et al. 1994). Copper and other toxic metals may interfere with the cellular calcium pathway which makes membranes more permeable, perhaps interfering with adhesive qualities (Contreras et al. 2007, Leal et al. 2018). Alternatively, metal ions may potentially compete for space with ions in photosynthesis, interfere with signalling molecules necessary for cell determination and differentiation, and increase the number of reactive oxygen species in gametophyte cells, ultimately leading to cell degradation (Leal et al. 2018, Wang et al. 2020).

In addition to pollution, run-off from the land can increase sedimentation rates which negatively impact gametophyte germination, survival, reproduction and recruitment, likely due to the combined effects of decreased attachment surface (hard substrate) and interference with light and nutrient supply (Watanabe et al. 2016, Zacher et al. 2016, Traiger & Konar 2017) (Schiel et al. 2006, Layton et al. 2019b, 2020a, Watanabe et al. 2016, Zacher et al. 2016, Traiger & Konar 2017). Additionally, sedimentation rate can change the outcome of competition between gametophytes of different species. For example, *Nereocystis luetkeana* has a higher survival when settled first in the absence of sediment, but *Saccharina latissima* has a higher survival when settled first in the presence of sediment (Traiger & Konar 2017). Understanding multi-stressor scenarios caused by human disturbance such as pollution and increased sedimentation and their interactions with global climate stressors will be essential in assessing long-term health and resilience of kelp populations in urbanized environments (Russell et al. 2012, Zacher et al. 2016).

Effects of change in salinity on gametophytes

Salinity may decrease in some parts of future oceans, with influxes of fresh water more likely in Arctic areas due to glacial melt and nearshore areas experiencing greater run-off and freshwater input from storms (Collins et al. 2019). To date, gametophyte growth under varying salinities has been mostly assessed in Arctic and sub-Antarctic species, which are regions experiencing changes in salinity due to run-off from glacial melt. Indeed, while only 2% of studies researching change in environmental conditions on gametophytes focused on salinity (Figure 1), they were all on Arctic kelps. In all studies, a significant interaction of salinity with temperature was found, where higher temperatures interacted with lowered salinity to decrease germination and growth (Fredersdorf et al. 2009, Lind & Konar 2017, Rodriguez et al. 2019). However, temperatures needed to achieve these negative effects were often higher than these populations are expected to experience under near- to mid-term climate change (Fredersdorf et al. 2009). Additionally, populations of the same species living in different environments show maximum gametophyte growth and germination under varying salinities that match their local conditions, which indicates local adaptation to geographic change in salinity (Rodriguez et al. 2019). The inability to grow at very low salinity and high temperatures is thought to be the effect of decreased physiological functioning of cold-adapted species under higher temperatures (Fredersdorf et al. 2009). However, as some individuals were able to survive even under high temperatures and low salinities, gametophytes of these species may be resilient to reduced salinities (Lind & Konar 2017, Davis et al. 2022). Additionally, gametophytes can be more resilient to reduced salinities than sporophytes (Peteiro & Sanchez 2012), indicating microscopic stages can survive fluxes in salinity and be a source of population regrowth (Davis et al. 2022).

Sexual variation and sporophyte production in changing oceans

Factors affecting sex bias and reproductive strategies of gametophytes

The mode of reproduction and the rate and manner of sex determination can be influenced by environmental factors that are predicted to change in future oceans. While the development of gametophytes into males or females is genetically determined (Ouyang et al. 2009, Liu et al. 2009, Shan & Pang 2010, Lipinska et al. 2015, Zhang et al. 2019) environmental factors can influence sex ratios, most likely by post-germination mortality of either males or females (Oppliger et al. 2011). For example, increasing temperature has been found to increase the number of males in *M. pyrifera* (Rodriguez et al. 2019) and females in *L. variegata* (Nelson 2005). Variation in sex ratio is geographically dependent in some species, where more males of *M. pyrifera* are observed under high temperatures in poleward populations (Rodriguez et al. 2019), but more females of *L. nigrescens* are found in lower latitude populations under temperature stress (Oppliger et al. 2011). High light intensity has been shown to skew the sex ratio to a higher proportion of females in *S. japonica* (Xu et al. 2015a). While some studies have shown an influence of environmental factors on sex ratios, others have found no effect and ascribed an observed skewed sex ratio to either a genetic influence or unmeasured environmental factors (Leal et al. 2016, Shukla & Edwards 2017, Gonzalez et al. 2018). There are no studies that examine gametophyte sex ratios under field conditions, though other temperate seaweeds can show a sex bias towards males in warm, low latitude populations (Wood et al. 2021) and male bias under warm temperatures is common among dioecious land plants (e.g. Munné-Bosch 2015, Petry et al. 2016). This suggests that skewed sex ratios in warming oceans could indeed hinder fertilisation and sporophyte production in kelps because the abundance and fertility of females will determine overall sporophyte recruitment. Indeed, gametogenesis in females (production of oogonia) has been shown to decrease, while antheridia production increased under experimental high temperature stress in several species (Müller et al. 2008, Choi et al. 2019, Martins et al. 2020, Hollarsmith et al. 2020). Decreased egg production by female gametophytes as observed in laboratory studies and subsequent recruitment failure under temperature stress may potentially explain why many kelp forests often fail to rapidly recover *in situ* following thermal stress such as heatwaves, while direct stress on adult sporophytes is most likely responsible for initial loss. For example, an increased number of oogonia has been observed in *M. pyrifera* in central populations, while the warmer marginal populations had a smaller number of oogonia and a lower fertility rate (Camus et al. 2021). Conversely, *E. radiata* increases egg production under temperature stress in warm edge populations (Bolton & Levitt 1985). There is clearly much versatility and compensatory mechanisms in reproductive strategies that allow kelp gametophytes to persist under a range of conditions (Bolton & Levitt 1985, Coleman & Veenhof 2021).

As well as physiological differences between male and female gametophytes, gene expression is shown to be sex-biased (Pearson et al. 2019). Higher gene expression activity was found in female gametophytes of *S. latissima* under increased experimental temperatures, indicating a higher sensitivity to heat stress (Monteiro et al. 2019). Functional expression patterns of female gametophytes are related to protein degradation and signalling, whereas male expression links more to cell division, growth and flagella production (Ouyang et al. 2009, Pearson et al. 2019). The occurrence of signalling molecules in females and cell division and growth expression in males is consistent with females producing pheromones, mainly lamoxirene (Marner et al. 1984) to attract male gametes (Müller et al. 1985, Maier & Müller 1986, Boland 1995, Gordon & Brawley 2004), and corroborates the male reproductive strategy of continuously producing reproductive cells (Destombe & Oppliger 2011).

Gametophytes can also produce sporophytes without fertilization through parthenogenesis and apomixis which are viable in culture conditions (Oppliger et al. 2007, Li et al. 2017), and this mode of reproduction has also been observed in warm-edge wild populations (Oppliger et al. 2014). It is interesting to speculate whether this reproductive mechanism is an adaptation to ensure persistence in marginal habitats where gametogenesis may fail. However, asexual reproduction potentially has negative consequences for the genetic diversity of wild populations (Oppliger et al. 2014) and reduced genetic diversity may compromise the persistence and adaptive capacity of kelps under climate and anthropogenic change (Wernberg et al. 2018). There is mixed evidence to support this idea. For example, studies have shown that reduced genetic diversity of gametophytes inhibited sporophyte production and growth (Raimondi et al. 2004) while others have shown no negative effect or only a slight disadvantage of gametophyte selfing and inbreeding on the subsequent sporophyte generation following thermal stress (Camus et al. 2021, Layton & Johnson 2021). Certainly, the ability to asexually reproduce is also hypothesized to benefit populations by fixing beneficial genes into a population (Coleman & Wernberg 2018, Müller et al. 2019). While it is still unclear what effect apomixis may have on natural populations and how this influences climate change resilience, the use of apomixis in maintaining stock cultures of gametophytes to preserve declining wild populations is currently being explored (Müller et al. 2019).

Gametophytes can also be selfed if males and females from the same parent plant fertilise, or inbred if related individuals mate, potentially leading to inbreeding depression. Inbreeding can be enhanced where genetic diversity is limited, such as in warm, marginal populations (Wernberg et al. 2018), highly fragmented populations (Reynes et al. 2021) or where there have been population bottlenecks following stress-induced mortality (Gurgel et al. 2020). Despite this, a recent study by Camus et al. (2021) found that the degree of selfing and inbreeding in gametophytes did not affect the resilience of the F1 sporophyte generation to a marine heatwave. Even though fertility decreased with inbreeding, female gametophytes may be able to detect the degree of kinship in potential mates and increase fecundity (i.e. production of eggs) accordingly (Camus et al. 2021). Interestingly, this kinship effect was less pronounced in warm-edge populations which may represent an adaptation to having fewer available mates in marginal populations (Camus et al. 2021). These results suggest that the underlying mechanisms of fertilization in gametophytes begs much further research, and their flexibility in mating strategies can prove an important avenue for climate change resilience (Coleman & Veenhof 2021), which is especially relevant in warm-edge populations.

Transition from gametophyte to sporophyte: comparing performance

Comparison of the relative performance of both gametophyte and sporophyte stages can hint at how changing oceans may impact kelp persistence as the transition between these stages is a key bottleneck in the kelp life history. Our review identified that 30% of studies compare both gametophytes and sporophytes that grow beyond their microscopic stages. Overall, photosynthetic rates of gametophytes are less affected by changing light, temperature and salinity than the sporophyte generation (Gerard 1990, Fredersdorf et al. 2009, Borlongan et al. 2019), although some studies have recorded similar optimum temperatures for photosynthesis for both life stages (Watanabe et al. 2014, Borlongan et al. 2018). This indicates that gametophytes may have a greater ability to grow and persist under a wider range of environmental conditions. Gametophytes are also repeatedly defined as shade adapted (Lüning & Neushul 1978), with low light requirements to achieve net photosynthesis and displaying a lower light saturation point than the sporophyte generation (Fain & Murray 1982, Altamirano et al. 2003, Xu et al. 2015b, Roleda 2016, Borlongan et al. 2018, Borlongan et al. 2019, Gao et al. 2019). This reflects the circumstances under which gametophytes grow in the field, as they will usually grow under the shade of the adult canopy and/or understory algae (Schiel & Foster 2006).

The juvenile sporophyte can be more sensitive to light stress and photodamage than the gametophyte stage in several species (Kinlan et al. 2003, Altamirano et al. 2004, Roleda et al. 2007, Augyte et al. 2019). Lower sensitivity to light may allow gametophytes to survive under a range of conditions in the field and initiate gametogenesis in response to environmental conditions that are also favourable for juvenile sporophyte growth (Izquierdo et al. 2002). The adverse effects of photodamage can also be enhanced under low temperatures, which is an effect that can be more pronounced in the sporophyte generation (Borlongan et al. 2019). Gametophytes are capable of controlling photodamage by upregulating accessory pigments (Delebecq et al. 2016) and the capability of gametophytes to recover from UV damage will be of increasing relevance in safeguarding future kelp populations as increased storms (Coumou & Rahmstorf 2012, Collins et al. 2019) will create a patchier distribution (Layton et al. 2019b) which will lead to local increases in UV radiation.

There is recent interest in uncovering which genes are expressed under stressful conditions in both gametophytes and sporophytes, particularly as a line of defence against ocean change (Henkel & Hofmann 2008, Martins et al. 2019). Differential expression patterns between sporophyte and gametophyte generations indicate different cellular pathways between these life-history phases (Crepineau et al. 2000, Shan et al. 2015). For instance, gametophytes of *L. digitata* and *S. japonica* show a higher expression of metabolic genes in comparison with sporophytes (Crepineau et al. 2000, Liu et al. 2019). Gametophytes are more thermally tolerant than sporophytes in some species and are thus presumed to have a metabolic pathway in place to attenuate heat stress, such as heat shock proteins (Henkel & Hofmann 2008). These proteins have, however, not been found until recently, partly because only two kelps have their genomes sequenced (*S. japonica*; Ye et al. 2015, *U. pinnatifida*; Shan et al. 2020) and information on transcriptomics is still in its early stages (Shan et al. 2020). Heat shock proteins have been identified in *U. pinnatifida* gametophytes, which use them to attenuate thermal stress, as well as differential expression patterns between sporophytes and gametophytes under thermal stress (Henkel & Hofmann 2008, Shan et al. 2015). This differential expression was also observed in *S. japonica*, where the sporophyte stress response included expression of proteins pertaining to cell cycle control and cytoskeleton adaption, and the gametophyte heat stress response pertained to metabolism and coenzyme transport (Liu et al. 2019). Similarly, genome-wide sequencing has recently shown that loci under selection in populations of kelp sporophytes can show balancing selection suggesting that gametophytes and sporophytes may be under selection for different environmental conditions (Vranken et al. 2021).

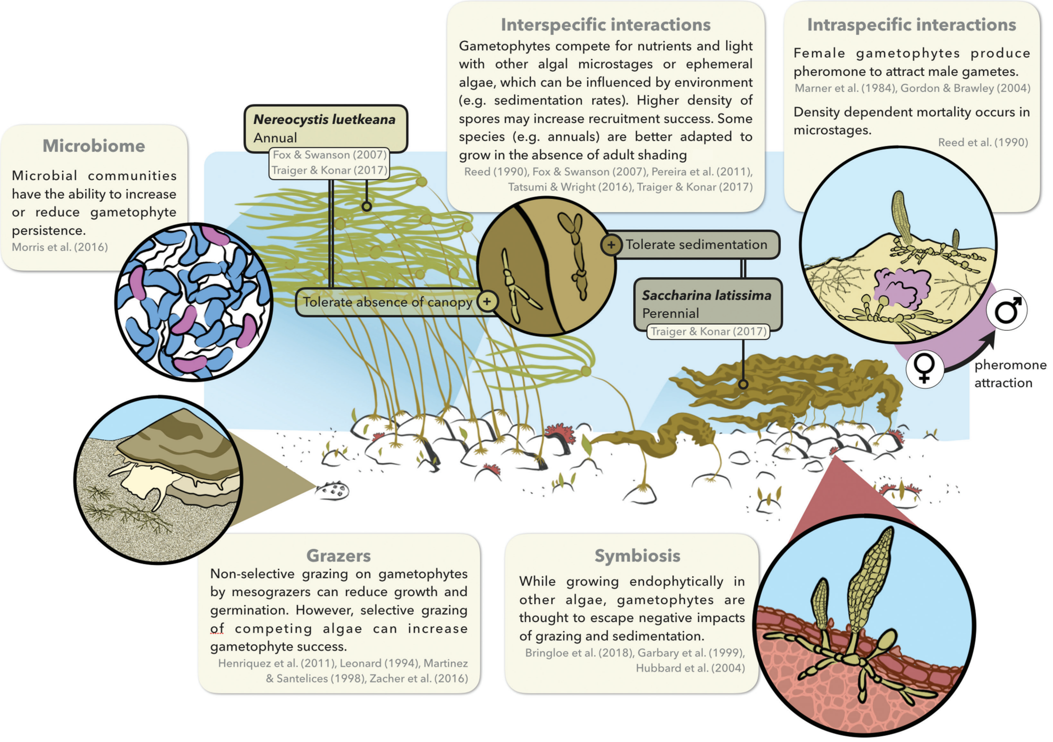
Ecological interactions of gametophytes in changing oceans

Climate mediated ecological interactions such as competition and grazing are well documented drivers of change in the sporophyte generation of kelp (e.g. Tegner & Dayton 2000, Steneck et al. 2002, Vergés et al. 2014, Wernberg et al. 2016, Filbee-Dexter & Wernberg 2018), but are less understood in the gametophyte stage. This is particularly relevant to address given that climate and anthropogenic change will impact the gametophytes themselves as well as their grazers and competitors, which can interact to either exacerbate or diminish the effects of climate change.

Interspecific interactions: competition & symbiosis

Interspecific competition between gametophytes within and among species forms an integral part of their overall survival. While gametophytes possess inherent mechanisms to combat competition such as chemical deterrents (Amsler et al. 1992) and can potentially trigger premature gamete release of competitors (Maier et al. 2001), other factors influencing interspecific competition may change in future oceans. For example, the outcome of interspecific competition can be determined by initial settlement density (Reed 1990), which may change as spore release is tied to factors predicted to change in future oceans, such as water motion and temperature (see previous sections). Increased disturbance will also create a patchier distribution in kelp forests (Layton et al. 2020a), and annual kelps and other opportunistic species that colonize rapidly can potentially be competitors of perennial kelps (Reed & Foster 1984, Pereira et al. 2011). For example, gametophytes of *Saccorhiza polyschides* perform relatively better under increased temperature stress than the perennial kelp *Laminaria ochroleuca*, necessitated by the increased reliance of annual populations on the persistence of the gametophyte stage (Pereira et al. 2011). In competition with the perennial *S. latissima,* the annual kelp *N. luetkeana* exhibits better survival success when settled first, whereas *S. latissima* is better able to cope with increased sedimentation, which is often detrimental to kelp sporophyte production (Traiger & Konar 2017; Figure 4). The relative advantage of *N. luetkeana* over *S. latissima* depending on timing of spore settlement indicates the importance of reproductive phenology noted earlier in this review.

The use of novel genetic techniques such as nested PCR with species-specific primers has allowed the occurrence of gametophytes of different kelp species, and potential interactions, to be quantified *in situ*. For example, the dominant canopy forming species *N. luetkeana* had a more widespread occurrence of their microscopic stages than *Hedophyllum sessile* (Fox & Swanson 2007) which may indicate that the gametophyte generation of *N. luetkeana* has some competitive



Microbiome

Microbial communities have the ability to increase or reduce gametophyte persistence.
Morris et al. (2016)

Interspecific interactions

Gametophytes compete for nutrients and light with other algal microstages or ephemeral algae, which can be influenced by environment (e.g. sedimentation rates). Higher density of spores may increase recruitment success. Some species (e.g. annuals) are better adapted to grow in the absence of adult shading
Reed (1990), Fox & Swanson (2007), Pereira et al. (2011), Tatsumi & Wright (2016), Traiger & Konar (2017)

Intraspecific interactions

Female gametophytes produce pheromone to attract male gametes.
Marner et al. (1984), Gordon & Brawley (2004)

Density dependent mortality occurs in microstages.
Reed et al. (1990)

Grazers

Non-selective grazing on gametophytes by mesograzers can reduce growth and germination. However, selective grazing of competing algae can increase gametophyte success.
Henriquez et al. (2011), Leonard (1994), Martinez & Santelices (1998), Zacher et al. (2016)

Symbiosis

While growing endophytically in other algae, gametophytes are thought to escape negative impacts of grazing and sedimentation.
Bringloe et al. (2018), Garbary et al. (1999), Hubbard et al. (2004)

Figure 4 Examples of interactions that take place on a microscale inside and outside of the sporophyte canopy. Studies on these subjects are few, and the field conditions in which gametophytes live and survive are an important future avenue of research.

advantage over *H. sessile* relating to dispersal potential or colonization of substrates. However, even when spore dispersal and presence of microscopic forms are high, recruitment might not take place. A recent study shows kelp recruitment in post-disturbance patches may be limited due to competition with faster-growing turfing algae for light (Layton et al. 2020a). Indeed, areas composed mostly of small understory algae are devoid of kelp gametophytes (Akita et al. 2019) and the species composition of microscopic stages can be very different from the sporophyte distribution in that same area (Robuchon et al. 2014). This disparity in distribution between gametophytes and sporophytes could be due to a seasonal effect, but can also be an indication that kelp gametophytes are losing in competition for space with understory and turf-forming algae, in which light requirements of germinating gametophytes might play a role (Tatsumi & Wright 2016).

Understanding interspecific competition and ecological interactions on microscales may benefit from the development of metabarcoding for detecting the presence of gametophytes and other species within the environment (e.g. Fox & Swanson 2007, Robuchon et al. 2014). Co-culturing different species of gametophytes or other taxa will also aid in determining the effects of competition, but to date, only a few studies have attempted this (e.g. Reed 1990, Traiger & Konar 2017). Further convergence of several avenues of research (e.g. co-culturing, *in situ* use of metabarcoding, multifactorial mesocosm ecological experiments) may help understand ecological interactions among species, as well as 'winners and losers' in a changing ocean within the microenvironment that gametophytes occupy.

Gametophytes can also live as endo- or epiphytes on red algae (Garbary et al. 1999, Hubbard et al. 2004, Bringloe et al. 2018) and other kelp (Lane & Saunders 2005; Figure 4), but the significance of this in natural settings is unknown. This symbiosis is hypothesized to attenuate the negative effects of sedimentation and grazing by growing inside the protective layer of other algal cell walls. For example, oogonia have been found protruding from red algal cell walls, which indicates that gametophytes are reproductive when living epiphytically (Garbary et al. 1999). As sedimentation and grazing are primary drivers of kelp loss (Strain et al. 2014, Ling et al. 2015), living epiphytically may prove advantageous in future oceans, though this needs to be corroborated in field settings.

Interactions of kelp gametophytes with microbes

Beneficial relationships between kelp sporophytes and associated microbes (termed the holobiont) are well established (Egan et al. 2013, Marzinelli et al. 2015, Pfister et al. 2019) and microbes may play a role in sporophyte resilience to ocean change (e.g. Qiu et al. 2019), but research establishing the role of microbes on gametophytes is still in its infancy. Microbial communities obtained from urbanised nearshore waters negatively affected *M. pyrifera* gametophyte abundance and size, whereas microbes from more pristine environments enhanced growth when present in intermediate abundance (Morris et al. 2016; Figure 4). A positive association with bacteria has also been shown when associated microbiota negate the toxic effects of polluting metals (Wang et al. 2020), but negative effects may occur when fast growing filamentous bacteria compete for space with gametophytes in polluted environments (Reed et al. 1994). Evidently, interactions with microbes may either increase or decrease the resilience of gametophytes to ocean change factors such as pollution and urbanization, and through their manipulation might prove a functional method of increasing resilience (Egan et al. 2013).

Effects of grazing on gametophytes

Increased grazing by herbivores on adult sporophytes is recognized as a pressing threat to kelp forest persistence under climate change (Santelices & Ojeda 1984, Vergés et al. 2014, Ling et al. 2015) due to either increases in rates of herbivory from co-occurring grazers (Provost et al. 2017, Rich et al. 2018, Miranda et al. 2019) or climate-driven poleward extensions of herbivores into temperate systems (Vergés et al. 2014, Ling et al. 2015, Vergés et al. 2016). Less is known, however, about how grazing affects the gametophyte stage. It has been shown in laboratory settings that grazing by echinoderms and gastropods can negatively affect gametophyte growth and recruitment (Leonard 1994, Martinez & Santelices 1998, Zacher et al. 2016, Veenhof et al. 2022; Figure 4) and it is hypothesized that amphipods, urchins and fish can also graze on gametophytes (Dayton et al. 1984, Amsler et al. 1992, Sala & Graham 2002, Franco et al. 2017). Although grazing is seen as a negative impact on successful kelp recruitment, herbivores can enhance gametophyte survival and subsequent sporophyte recruitment if herbivores graze selectively on competing understory algae (Henriquez et al. 2011, Zacher et al. 2016). In a multifactor design, Zacher et al. (2016) showed a species-specific response where temperature, sedimentation and grazing pressure can either decrease or stimulate gametophyte growth of *Alaria esculenta*, *L. digitata* and *S. latissima*. Specifically, the direction of singular effects was similar for all species, where increased temperature benefited germination and grazing increased sporophyte density, but sedimentation decreased recruitment of sporophytes. However, the interactive effects between grazing and abiotic factors were species-specific and could reinforce the positive effects of increased temperature and decreased disturbance of sedimentation (Zacher et al. 2016). As these are all factors that are influenced by ocean change, their study showed that climate change may alter kelp composition in a complex and hard to predict manner.

Grazing by herbivores appears to have a greater direct negative impact on gametophytes than on juvenile sporophytes (Martinez & Santelices 1998), but can have an indirect positive influence on gametophyte survival through removal of sediment (Zacher et al. 2016) and competing algae (Henriquez et al. 2011) as well as enhancing fertilization and sporophyte recruitment following grazer ingestion (Veenhof et al. 2022). Sustained urchin barrens indicate recruitment of adult sporophytes is prevented by grazing; however, there is an indication that algal diversity is still present on a microscale (Akita et al. 2019, Coleman & Kennelly 2019, Shum et al. 2019). Additionally, spores of *L. nigrescens* can survive digestion by sea urchins, and other seaweed species can be recruited from fertile material after digestion by molluscs (Santelices et al. 1983, Santelices & Correa 1985, Veenhof et al. 2022). The sparse amount of research on grazer and gametophyte interactions makes these conclusions preliminary, but the complexity of interactions between herbivores and microscopic stages of kelps and the paucity of knowledge of the biotic microenvironment gametophytes inhabit will mean that filling this knowledge gap will be central in understanding kelp persistence under increasing ocean change.

Future opportunities & knowledge gaps

Climate change is one of the most pressing issues faced by marine ecosystems globally (Henson et al. 2017). Predicted increases in temperature, acidification, UV radiation and non-climate related human impacts will affect ocean physics and chemistry (Collins et al. 2019), which in turn affects species physiology, distributions and interactions (Harley et al. 2012, Field et al. 2014). Negative impacts on kelp forests such as loss of biodiversity and change in ecological structure are already apparent (e.g. Wernberg et al. 2013, Filbee-Dexter et al. 2016, Berry et al. 2021) and climate projections suggest further loss in the future (e.g. Assis et al. 2017, Davis et al. 2021b). The response of gametophytes to environmental factors predicted to change in future oceans gives both concern and hope for the long-term persistence of kelp forests.

This review has revealed that gametophytes appear to be relatively tolerant to a wide range of ocean temperatures and light (Müller et al. 2012, Mabin et al. 2013, Hollarsmith et al. 2020), albeit with responses dependent on location (Oppliger et al. 2012, Mohring et al. 2014, Muth et al. 2019, Martins et al. 2020) and thermal history (Liesner et al. 2020). Temperature increases may have the largest impacts on gametophytes of temperate and warm adapted kelps (e.g. Gonzalez et al. 2018, Augyte et al. 2019, Hollarsmith et al. 2020), and could partly underpin recruitment failure and large sporophyte declines in populations around the world (Muth et al. 2019, Wernberg et al. 2019b), particularly at warm range edges (Vergés et al. 2016, Smale et al. 2019). Summer temperatures and/or temperature anomalies such as marine heatwaves are increasingly exceeding the temperature thresholds for stage transitions in gametophytes in the south-east Pacific Ocean (Shukla & Edwards 2017, Gonzalez et al. 2018, Muth et al. 2019) and the North Sea (Müller et al. 2008, Bartsch et al. 2013). As future kelp persistence and distribution may largely be driven by thermal tolerance (Martínez et al. 2018), understanding thermal plasticity in the gametophyte stage should be a prime direction of future research. Some advances have been made in understanding the genetic basis for thermal tolerance (Liu et al. 2019), how these genetic patterns manifest under naturally occurring temperature gradients in the field (Vranken et al. 2021) as well as the potential for adaptive capacity of gametophytes under increased heat stress (Mabin et al. 2019, Vranken et al. 2021), but more research is required to demonstrate causation and link these patterns to conditions characteristic of field settings (but see Liesner et al. 2020). Efforts to map and preserve thermal and adaptive genetic variation more generally will also inform the capacity of gametophytes to face the challenge ocean warming poses (Wade et al. 2020, Coleman et al. 2020b, Wood et al. 2021).

Even though gametophytes can be resilient to a variety of temperature and light regimes, their phase changes (i.e. gametogenesis, recruitment of sporophytes) are tightly linked to specific environmental cues which may be disrupted and alter population dynamics in the future (de Bettignies et al. 2018, Capdevila et al. 2019, Muth et al. 2019). The reproductive phenology of many kelps is likely to change with changes in seasonal temperatures and duration, and this may result in gametophytes growing under different environmental conditions than in the past (Martins et al. 2017). Kelp life cycles respond to circannual rhythms (tom Dieck 1991, Lüning 1991), with irradiance and temperature providing important cues in the onset of gametogenesis (Lüning 1980, Roleda 2016).

This might cause a mismatch between environment (e.g. optimum temperature for growth of gametophytes) and the developmental transition from gametophytes to sporophytes (de Bettignies et al. 2018), as optimum conditions for growth can differ from those measured for survival (Mohring et al. 2013a) or fertility (Bolton & Levitt 1985, Martins et al. 2017). In addition, thermal conditions during early stages can have repercussions on performance in successive life stages (Liesner et al. 2020). However, the ability of gametophytes to remain in a dormant state when conditions are unfavourable for weeks to several months (Hoffmann & Santelices 1991, Barradas et al. 2011, Carney et al. 2013; Figure 5) could mitigate this problem, and potentially facilitate a shift in growing season and persistence through harsh conditions.

The potential of kelp gametophytes to temporarily persist through harsh conditions as a microscopic seedbank (Hoffmann & Santelices 1991, Santelices et al. 2002, Carney et al. 2013, Schoenrock et al. 2020) is a current and relevant topic of discussion (Schoenrock et al. 2020; Figure 5). In laboratories, gametophyte cultures can be preserved for decades (e.g. tom Dieck 1993). In field studies, however, tracking gametophyte growth and recruitment on out-planted microscope slides indicates recruitment occurs over a short period, suggesting multiple short ‘reproductive windows’ rather than long-term persistence of a bank of microscopic forms (Deysher & Dean 1986a, Reed et al. 1994, 1997). Field-based experiments have also tracked the regrowth of kelp beds following canopy removal as an indication of the persistence of microscopic gametophytes in the field in a dormant form (Barradas et al. 2011, Hewitt et al. 2005, McConnico & Foster 2005) or in the form of microscopic sporophytes (Kinlan et al. 2003), and found recruitment with no apparent source of spores, indicating the presence of dormant microscopic stages (Schoenrock et al. 2020). Additionally, several studies using genetic markers have established a widespread presence of gametophytes in the field (i.e. Bringloe et al. 2018, Fox & Swanson 2007, Robuchon et al. 2014) and genetic parentage analyses of recruits suggest that a bank of gametophytes of mixed age and origin may exist (Carney et al. 2013). The benefits of a bank of microscopic forms with a mixed genetic make-up may lie in the ability to preserve genetic variability that supports the adaptive capability to changing conditions (Carney et al. 2013,

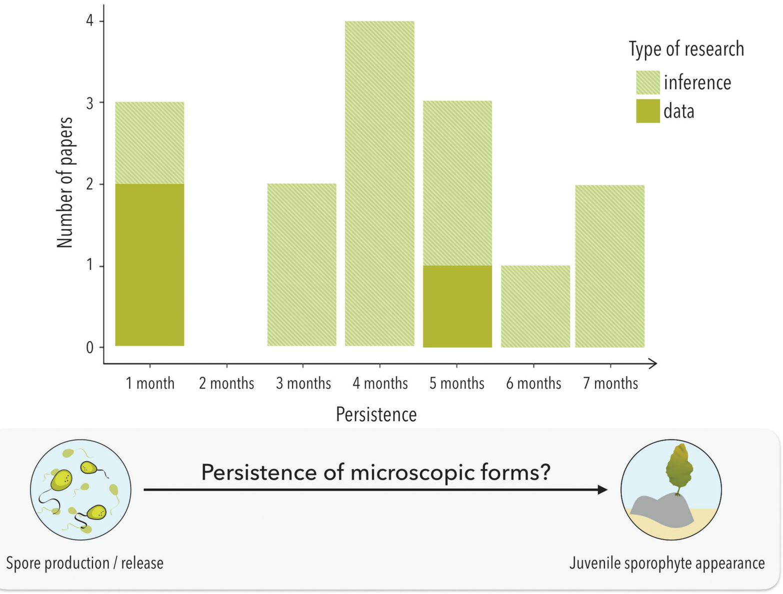


Figure 5 A collation of studies that research the possibility of microscopic forms of kelps persisting for different periods. Studies are categorized by whether the study was based on data from gametophytes tracked in the field (“Data”), or by tracking recruitment after canopy removal (“Inference”).

Coleman & Goold 2019, Coleman & Wernberg 2020, Wade et al. 2020). Persisting through harsher conditions and recruiting when environmental conditions are suitable may indeed be of great benefit for overall kelp forest health and recovery.

The existence of banks of microscopic forms would be unlikely to present a pathway for persistence through extreme events, such as marine heatwaves, that exceed the physiological thresholds of gametophytes. Indeed, failure of kelp to recover following loss of sporophytes after extreme events (e.g. Wernberg et al. 2013, Filbee-Dexter et al. 2016, Arafeh-Dalmau et al. 2019) suggests that gametophytes cannot survive, or their stage transitions are hindered, during such extreme conditions, and recovery may be dependent on dispersal of spores from surviving sporophyte populations. Indeed, refugia from grazing pressure in cryptic habitats (Zarco-Perello et al. 2021) and temperature and reduced salinity in deep kelp forests (Davis et al. 2021a, 2022) are potential sources of replenishment. Field studies suggest that the majority of sporophytes that recruit in natural populations are thought to have originated from recently settled spores (Reed et al. 1997). Certainly, there is little empirical evidence that gametophytes persist vegetatively for more than ~7 months (Figure 5). Whether and for how long gametophytes can persist as a dormant bank of microscopic forms through warming or other impacts remains a critical knowledge gap that needs to be filled to determine the future of kelp forests, by developing creative solutions to link field research with understanding from laboratory studies.

The development of germplasm or culture banks may help secure the future of kelps and their adaptive capacity (Wade et al. 2020). Germplasm banks hold the potential to preserve threatened or lost genetic and adaptive diversity and facilitate reseeding and restorative initiatives (Coleman et al. 2020b, Wade et al. 2020). Indeed, algal culture libraries that were established decades ago are still used for current experiments (e.g. Martins et al. 2019) and are commercially available (e.g. https://ku-macc.nbrp.jp/strain/list). Building on these historical culture collections, *M. pyrifera* gametophytes have been successfully preserved in germplasm banks for up to 5 years, representing genetically distinct gametophyte cultures to preserve naturally occurring genetic diversity (Barrento et al. 2016). Indeed, loss of kelp populations is already being addressed through restoration (Bekkby et al. 2020, Coleman et al. 2020b, Eger et al. 2020a,b, Layton et al. 2020b, Vergés et al. 2020) and the existence of algal culture collections can play a role in this process (Filbee-Dexter et al. 2022). Recently, gametophytes have been harnessed to develop a novel kelp restoration tool, "green gravel", whereby gravel is seeded with gametophytes in the laboratory before being out-planted into the field as juvenile sporophytes and gametophytes (Fredriksen et al. 2020). This method is now being applied globally (www.greengravel.org). Successful restoration of kelp forests has been achieved in the past by out-planting juvenile sporophytes (Wilson & North 1983, Hernández-Carmona et al. 2000, Eger et al. 2020a), but green gravel overcomes the challenges of such techniques by eliminating the need to employ divers or use engineered structures and allows control over what genotypes are seeded (Fredriksen et al. 2020). This technique provides an ideal platform to 'future-proof' kelp forests via utilising culture banks to seed gravel and restore reefs with either enhanced genetic diversity or specific adaptive traits (Coleman et al. 2020b, Coleman & Wernberg 2021) or primed individuals with increased thermal tolerance (Jueterbock et al. 2021).

Ecological interactions are also set to change in future oceans and their role in the gametophyte stage remains a critical knowledge gap. Specifically, competition of kelp gametophytes with species that perform better under higher sedimentation rates, nutrients and acidification such as turfing algae warrants more research, as this form of competition will likely become increasingly apparent under future climates (Tatsumi & Wright 2016, Filbee-Dexter & Wernberg 2018, O'Brien & Scheibling 2018). Similarly, elucidating the role of positive interactions among kelp and other algal species, such as the role of microbiomes, may present novel pathways for enhancing resilience in a restoration context (Eger et al. 2020a).

The application of novel genomic techniques has great potential to advance the understanding of the persistence of gametophytes in changing oceans. Specifically, genomics should be used to identify the presence of gametophytes in the field (e.g. Robuchon et al. 2014, Bringloe et al. 2018, Akita et al. 2019, Shum et al. 2019, Nagasato et al. 2020, Schoenrock et al. 2020) and link presence to changing environmental conditions, including persistence through extreme events. The differences in the response of gametophytes versus sporophytes to environmental change can be understood through examining patterns of selection through genomic data (Vranken et al. 2021) as well as transcriptomics (Liu et al. 2019). Further linking loci to annotated kelp genomes may provide insights into the functions that mediate such patterns (Vranken et al. 2021) and more investment into sequencing kelp genomes is needed. Identification of genes and variants that perform better under future climatic conditions could even pave the way for the development of climate-resilient transgenic gametophytes through genetic engineering or synthetic biology (Coleman & Goold 2019, Coleman et al. 2020b) transforming the way we conserve and manage kelp in future oceans.

Clearly, gametophytes are an essential part of kelp forest persistence and research investigating their responses to climate and anthropogenic impacts is beginning to reveal their role in predicted future ocean ecosystems. The ability of gametophytes to persist in a dormant state through harsh environmental conditions may be facilitated by their broader tolerance to environmental stress relative to sporophytes, but where, when and for how long this may occur remain overarching knowledge gaps. With present kelp loss in many parts of the world, it is apparent that proactive interventions are required to reverse and prevent ongoing degradation of kelp forests (Coleman et al. 2020b). Through their relative tolerance to a wide range of conditions, experimental and genetic tractability, and capability to be cultured and stored, gametophytes may hold the key to designing and implementing proactive management and conservation strategies to secure the future of kelp forests.

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PROGRESS AND DIRECTION IN THE USE OF STABLE ISOTOPES TO UNDERSTAND COMPLEX CORAL REEF ECOSYSTEMS: A REVIEW

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Abstract Coral reef ecosystems are exceptionally complex with a myriad of trophic pathways and consumer relationships. The application of stable isotopes (SIs) offers numerous advantages over traditional methods towards understanding these intricate systems. We summarise current knowledge derived from the rapidly increasing SI literature base and identify potential gaps and future directions for the use of SIs in coral reef ecosystem studies. Using topic modelling, a form of text mining, on 236 identified published works, we determined that SI research on coral reefs broadly falls into five major topics. (1) Organic matter dynamics: SI analyses (SIA) have quantified substantial variability in autochthonous (internal) and allochthonous (external) fluxes across coral reefs. (2) Holobiont metabolism: Coral nutrient acquisition, translocation and partitioning, and coral responses to various endogenous and exogenous factors have been explored through SIA. (3) Trophic niches: SIA has indicated that considerable variation in resource use facilitates co-occurrence of high densities of consumers, emphasising that many trophic categorisations on reefs are often too simplistic. (4) Fish diet variation and habitat connectivity: SIA has revealed how ontogenetic, larval and mobile predator movements link adjacent ecosystems. (5) Environmental drivers (both natural and anthropogenic): SIA can track anthropogenic nutrient inputs, revealing impacts of human-derived pollutants on reef systems. There are a number of important knowledge gaps, however. Few studies compare feeding strategies across guilds, and the literature is biased towards reef fish and hard corals. Furthermore, few studies examine multiple taxonomic groups in situ or consider multiple environmental drivers. Studies also tend to ignore the underlying, but potentially substantial, spatiotemporal variation in SI baselines as demonstrated from 741 mean SI values extracted from the literature, making inferences based on small variations in SI values problematic. Given that coral reefs face global decline, knowledge gaps need to be addressed while acknowledging the limitations of SIA; careful application of SIs can enhance the understanding of processes driving environmental change in these iconic marine ecosystems.

Keywords: stable isotope analysis; food webs; organic matter; holobiont metabolism; trophic niches; fish diet; habitat connectivity; environmental drivers

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Introduction

Knowledge of the energy fluxes and networks of consumer relationships that constitute whole ecosystems has long been considered crucial to understanding their structure (e.g. Lindeman 1942, Teal 1962), functioning (e.g. Patten 1959, Slobodkin 1959, Ulanowicz 1972, Bellwood et al. 2019) and sustainable use (e.g. Ryther 1969, Friedland et al. 2012, Link & Watson 2019). This is particularly challenging where the range of sources, diversity of consumers, and number of trophic levels are large (Link 2002). The high species diversity of coral reefs, therefore, presents a huge obstacle to any attempt to understand the ecosystem, especially as the ecologies of the majority of species are known only from short bursts of research activity at particular locations and points in time (e.g. Odum & Odum 1955, Hiatt & Strasburg 1960, Randall 1967, Vivien 1973, Hobson 1974, Harmelin-Vivien 1981, Sano et al. 1984). This creates great uncertainty in understanding coral reef functionality.

Ecosystem function relies on the movement and storage of energy and nutrients. As such, the exceptional productivity and biodiversity of coral reef ecosystems in mostly oligotrophic tropical surface waters has long been considered a paradox (Darwin 1842). The array of potential pathways sustaining these iconic ecosystems are only now being explored in the detail that their present plight demands – there is a plethora of threats to coral reefs, including multiple consequences of climate change (Hughes et al. 2003, Graham et al. 2011, Hoegh-Guldberg et al. 2017, Hughes et al. 2017) and impacts of fishery exploitation (Newton et al. 2007, MacNeil et al. 2015, Bozec et al. 2016).

Coral reef primary **production sources** (all bold words appear in the glossary, Table 1) can include algae, phytoplankton, various sponges and cnidarians which are partially photosynthetic or chemosynthetic, and suspended and sedimentary particulate and dissolved organic materials derived from these. The nature of these sources is complex, so characterising consumer relationships is non-trivial. For example, the macroalgal matrix that nominally herbivorous fish feed on may also include microbes, detritus and animal material (Wilson & Bellwood 1997). Many herbivores may also feed opportunistically on the faeces of zooplanktivorous fishes (Robertson 1982). Corallivores and spongivores, which include nominal herbivores (e.g. Burkepile et al. 2019), actually ingest material of **mixotrophic** origin. This is because the coral or sponge **holobiont** is composed of not only the host animal tissue, but also symbiotic dinoflagellates (often from the clade Symbiodiniaceae) and other potential prokaryotic symbionts, hereafter referred to as 'endosymbionts'. Marine symbioses such as these are particularly common in nutrient-poor environments such as coral reefs (Ferrier-Pagès & Leal 2019) and present another layer of complexity in understanding these ecosystems. Zooplankton have long been considered an important resource for reefs, but assessing their role is complicated by the fact that pelagic plankton are continuously advected over reefs, in conjunction with distinct reef plankton living amongst the reef substrata, with some only emerging at night (Hobson 1974). Free-living bacteria also represent an important resource in coral reefs. They experience high rates of growth and production through feeding on abundant dissolved organic matter, providing a food source to higher trophic level consumers including zooplankton and corals, thereby transferring energy and contributing to reef productivity (Sorokin 1973a,b, Sorokin et al. 1985, Ferrier-Pagès & Gattuso 1998). While detritus constitutes an important flux (e.g. Crossman et al. 2005), the origins and lability of the materials involved are diverse and little studied.

Even at this low level, understanding these diverse and complex relationships is hedged with uncertainty. Traditionally, tools used to tackle these knowledge gaps have included in situ behavioural and stomach contents analyses, mass-balanced modelling, genetics, and 'omics' methods (i.e. proteomics and metabolomics). Feeding observations offer considerable resolution in some aspects of a consumer's diet, but, without costly extension, provide data over only small temporal and spatial scales.

Table 1 Glossary of the key terms used throughout the review. Terms appear in bold in the main text.

Term	Notes/explanation
Allochthonous/external sources	Production sources that originate from places other than where they are found.
Autochthonous/internal sources	Production sources that originate from where they are found.
Autotrophy	The process through which an organism can synthesize its own food from inorganic substances (e.g. photosynthesis).
Baseline	The isotope baseline refers to the isotope ratios at the base of the food web, such as in nutrients (e.g. nitrate, dissolved inorganic carbon) and fixed organic matter (e.g. detritus, primary producers) sources, which can vary sustainably with space and time over reefs.
Bulk stable isotope analysis (SIA)	Measuring the SI values in a whole tissue (e.g. liver or muscle) or body (e.g. very small organisms), see Text box 1.
Compound-specific SIA (CSIA)	Measuring the SI values of individual biochemical compounds (e.g. fatty acids or amino acids) within a tissue type, see Text box 1.
Connectivity	The degree of movement and trophic interactions between different food web biota or between different food web compartments, whether within or between ecosystems.
Diet-tissue discrimination factor	Hereafter 'trophic discrimination factor' (TDF): change in relative abundances of the stable isotopes of an element between diet and a consumer's tissue, due to the kinetics of the various metabolic and physiological processes that occur during digestion and assimilation. Other synonyms may include fractionation factor (FF) or trophic enrichment factor (TEF).
Heterotrophy	The process through which an organism acquires external organic substances for nutrition.
Holobiont	The assemblage of a host and the organisms living within or around it in symbiosis (e.g. hard coral and Symbiodiniaceae).
Isotopic niche	An area or volume (in δ-space) with isotopic values (δ-values) as coordinates (Newsome et al. 2007).
Mixotrophy	When an organism uses a mix of newly fixed in situ and secondary production source types for nutrition (e.g. may have photosynthetic symbionts, but also captures particles).
Production source	The basal organic inputs sustaining a food web, formed from either primary (photo- or chemosynthetic) fixation of inorganic nutrients or secondary fixation of recycled, organic materials.
Trophic niche	The various ecological aspects relating to energetic acquisition of the full array of food items consumed by an organism and the strategies used to acquire them.
Trophic position (TP)	The vertical position of an organism in the food web, akin to trophic level with the exception that it need not be an integer value due to complex linkages within a food web.

They also generally do not allow quantification of some hard to observe, but significant dietary components such as microbes and plankton, and they may provide only a modest measure of what is assimilated into consumer tissues. Additionally, for interactions among marine symbioses, it is not possible to track nutrient exchanges visually. For modelling approaches, the scope is vast but severely constrained by the accuracy of parameterisation and key assumptions, such as the rate of primary production and trophic transfer efficiencies (e.g. Polovina 1984, Polunin & Klumpp 1990, Arias-González et al. 1997). While 'omics' methods are important for disentangling metabolic relationships in marine symbioses, they cannot trace nutrient exchanges or identify original sources well (Ferrier-Pagès & Leal 2019).

Although coral reef ecosystem functioning has been considered since the early 1800s (Darwin 1842), stable isotope (SI) approaches (see Text box 1) are a relatively recent addition to this endeavour, having only been applied readily since the early 1980s (e.g. Fry et al. 1982), although there are some examples of earlier works (Stephens 1960, Sorokin 1973a, Goreau 1977). These approaches fall into two categories: (1) measuring natural abundances of stable isotopes in a sample and (2) tracing the artificial addition of heavier isotopes through a system of interest, known as isotope labelling. SI approaches have many strengths (Text box 1; Fry 2006). SI compositions are time-integrated, so potentially represent material assimilation by consumers over timescales of days to months, whereas behavioural and gut contents analyses capture at most hours to days. SI ratios in a consumer's tissues also contain information about what has been assimilated from the diet, not merely ingested. This means the nutritional role of a particular source can be clarified rather than having to be assumed. This extends to quantifying the importance of materials such as gelatinous plankton and dissolved organic matter, which may be estimated poorly, or not at all, via traditional gut contents analyses. This also extends to primary producers, with tissue SIs relating to the nutrients taken up for fixation along with the fixation pathway itself. SI analyses (SIA) offer ways of tracking distinct **production sources** and testing ideas about how the importance of different pathways may vary in space and time, or in relation to other factors such as body size. SI ratios can provide a chemical proxy of **trophic position** (**TP**) and a means of estimating **trophic niche** widths and volumes, providing an opportunity to test ideas about overlaps in these parameters. Because of their snapshot character, gut contents analyses tend to require much larger sample sizes than those needed for SIA, while SI data can be derived from non-lethal sampling. Inherent in each of these strengths are important constraints, however. This includes those of the isotope ratio mass spectrometry technology involved (e.g. Mill et al. 2008), the fact that SI ratios can only be used to track sources and trophic pathways that are isotopically distinct, and that derived metrics such as **TP** rely on assumptions regarding isotopic discrimination which can vary across space, time, and trophic levels (Hussey et al. 2014).

TEXT BOX 1

Stable isotopes are variations of the same element that differ in the number of neutrons in their nuclei, and therefore their mass. Unlike radioisotopes, stable isotopes are energetically stable and therefore persist in nature indefinitely. As they have the same number of protons and electrons, isotopes of the same element have the same chemical properties. But, because isotopes differ in their masses, different isotopes of the same element have different kinetic behaviours. There are many physical processes in nature such as evaporation or the movement of molecules, which result in the separation of isotopes due to differences in kinetics. This results in variation in their natural abundances. It is this combination of persistence and kinetic variability that makes stable isotopes so useful in ecological studies.

Isotopic composition of a material is measured using mass spectrometers, which provide the ratio of the abundance of one isotope of an element relative to another, e.g. the ratio of ^{13}C to ^{12}C atoms. As heavier isotopes are typically scarce compared to their lighter counterparts, absolute isotope ratios are very small, making them impractical to work with. By convention, isotopic composition is expressed relative to the internationally agreed standard for that element (in delta, δ, parts per mil, ‰, notation), according to the equation:

$$\delta X = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 (‰)$$

where R_{sample} and R_{standard} are the respective ratios of heavy to light isotopes in the sample and international standard, with higher values (δX) equating to a composition enriched in the heavier isotope.

Bulk stable isotope analyses, which are the most common form of analysis, refer to the measurement of the isotopic composition of an element within the entire sample. This is typically in the form of a tissue or tissue fraction (e.g. separated endosymbionts or the organic fraction of a fish otolith). This can either be to measure the natural abundance of the stable isotope found in the sample, or in the case of isotope labelling pulse-chase experiments, to trace the artificial addition of heavier isotopes through a system of interest based on their excess abundance. The amount of material required for these analyses is generally small, on the order of a few milligrams or less, but depends upon the relative concentration of the element of interest within the tissue type of interest. Technological advances mean that it is now readily possible to measure multiple stable isotopes from a single sample and, coupled with the relatively low cost of sample runs, has resulted in bulk stable isotope analyses becoming a regular feature within the ecological toolbox. Commonly used stable isotopes in marine ecology are carbon ($\delta^{13}C$), for discriminating production sources, nitrogen ($\delta^{15}N$) for estimating nitrogen sources and **trophic position**, and oxygen ($\delta^{18}O$) which relates to environmental information such as prevailing temperatures. Sulfur ($\delta^{34}S$) is also now being used more frequently to further distinguish production sources and infer habitat use. In terrestrial and freshwater systems, hydrogen ($\delta^{2}H$ or δD) and strontium ($\delta^{87}Sr$) can be used to separate geographic regions based on differences in hydrology/elevation and underlying geology, respectively.

More recently, **compound-specific stable isotope analyses** have been developed to address some of the shortcomings associated with **bulk** approaches. That is, by derivatising and subsequently separating specific compounds of interest, the stable isotope composition of molecules can be determined within a sample. To date, this generally relates to the measurement of stable isotope ratios within amino or fatty acids. However, the complex methodologies involved result in longer processing times and greater expense, which is reflected in the reduced number of samples that are generally analysed in such studies.

For a more comprehensive introduction to stable isotope ecology, see Fry (2006). For other comprehensive literature reviews, please see, among others, Boecklen et al. 2011, McMahon et al. 2013, Vander Zanden et al. 2015, Pethybridge et al. 2018, McCormack et al. 2019, Whiteman et al. 2019, Shipley & Matich 2020, and Tsui et al. 2020.

There are still many opportunities to add value to SI studies, including through: (1) the use of elements other than carbon and nitrogen (such as sulfur); (2) the use of fast and slow turnover tissues to represent feeding over different timescales; (3) the use of archived materials to test ideas about past changes; (4) improved parameterisation of niche topology (Jackson et al. 2011); (5) development of SI mixing models allowing better differentiation of different **production sources** and trophic pathways (e.g. Parnell et al. 2013); (6) estimation of key processes such as food chain transfer efficiency from relationships between **TP** and body size (Jennings et al. 2002); and (7) accurate characterisation of enrichment factors between consumers and their diet, especially for **holobionts**. The ability to gain SI information on specific compounds rather than just **bulk** tissue homogenate has further expanded our ability to understand coral reef ecosystems by better resolving different sources and pathways (e.g. McMahon et al. 2016, Fox et al. 2019, Skinner et al. 2021). While important for contextual understanding, the purpose of this review is not to summarise or explain recent advances in SI methodologies and techniques (for that, we point readers to Text box 1 and, among others, e.g. Boecklen et al. 2011, McMahon et al. 2013, Vander Zanden et al. 2015, Pethybridge et al. 2018, Ferrier-Pagès & Leal 2019, McCormack et al. 2019, Whiteman et al. 2019, Shipley and Matich 2020, Tsui et al. 2020). Instead, in light of the expanding, but often disparate research occurring on coral reefs that utilises SIs and the lack of a comprehensive synthesis of general findings, we sought to summarise the current knowledge of coral reefs that has been advanced through the application of SI approaches.

Undertaking a more systematic approach to guide this review, we conducted an extensive literature search for studies utilising SI approaches in coral reef ecosystems and organisms. We screened and extracted key information pertaining to each study, including the focal taxa, when and where the study was conducted, along with SI measurements of **baseline** sources. We then analysed the text in article abstracts using topic modelling (a form of text mining which identifies clusters of words, e.g. topics; Griffiths & Steyvers 2004, Grün & Hornik 2011). This approach facilitates the objective identification of recurring themes in the articles within the extensive literature (see Supplements S1 for detailed methods). Specifically, here we (1) characterise the biological, geographical, and methodological foci of SI applications in published works, (2) quantify the isotopic variability observed among **production sources** across coral reef systems globally, and (3) identify the principal topics to which SI data have been applied. We summarise the extent to which current knowledge of coral reef ecosystems has been advanced through SIA, highlight the main areas for growth, emphasise considerations that are essential when interpreting SI data from coral reef ecosystems, and identify future challenges for obtaining fuller understanding of this complex system.

Key patterns and topics in SI approaches to coral reefs

By combining a traditional literature search with mining of pertinent information from identified articles, and topic modelling of their abstracts, we were able to identify patterns and recurring themes in the SI coral reef studies published to date (1980–2019 inclusive). Of 341 articles identified through Web of Science and 964 identified through Google Scholar, a total of 238 were retained after careful screening (Table S1). Of those, 236 had abstracts and were used in the subsequent topic modelling analysis (see Supplements S1 for detailed methods). We also extracted key information from all 238 articles (Table S1), including basic article information, study-specific information (e.g. spatial and temporal information; Table S2 for definitions), sample information (e.g. single or multi-tissue, tissue type, isotopes, and focal taxa; see Table S3 for definitions) and, when reported, SI values of **baseline** sources (Table S6). It is worth noting here that although we combined extensive literature searches across two different databases, the final 238 articles are likely not an exhaustive list of published SI studies on coral reefs. However, our literature search generated an extensive, comprehensive, and representative suite of coral reef SI studies using an objective approach.

Topic modelling identified five topics, which were named based on the highest weighted words occurring within each (Table 2). Generally, similar numbers of articles were assigned to each topic, with Topic 2 (**Holobiont** metabolism) having the highest number of articles (n=55) and Topic 1 (Organic matter dynamics) the lowest number of articles (n=41). Although topic popularity has fluctuated over time (Figure 1), the distribution of topics across regions is fairly equitable (Figure 2). These five topics

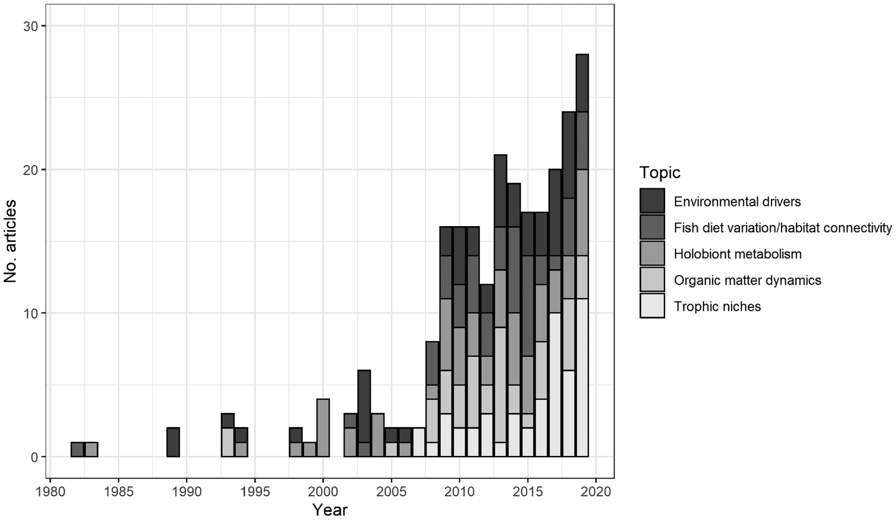

Figure 1 Number of articles published in each of the five identified topics over time (1980–2019).

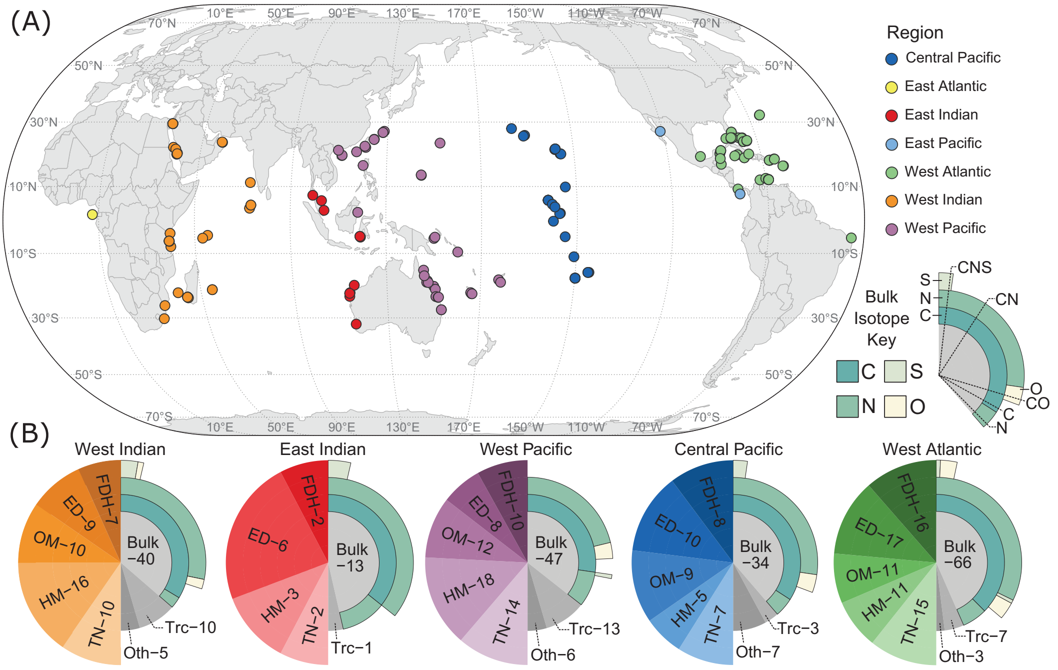

Figure 2 (A) Map of the locations of identified studies from the literature search using stable isotope approaches on coral reefs in the field, partitioned into the seven major biogeographic regions across the globe (1980–2019). (B) Plots showing the breakdown of different topics and stable isotope approaches utilised across five regions (East Atlantic and East Pacific excluded due to the small number of studies in each region, $n=1$ and $n=2$, respectively). The left-hand side represents the assignment distribution of studies to the five identified topics within each region: FDH, fish diet variation/habitat connectivity; ED, environmental drivers; OM, organic matter dynamics; HM, holobiont metabolism; TN, trophic niches. The right-hand side displays the breakdown of isotope methodology used for each region. Grey inner segments show the number of studies using natural bulk stable isotopes (Bulk), isotopic tracer (Trc) or compound-specific and other (Oth) approaches. For bulk studies, overlaid is a hierarchy of elemental identity of studies using carbon (C: δ^{13}C), nitrogen (N: δ^{15}N), sulfur (S: δ^{34}S), or oxygen (O: δ^{18}O), and combinations of these (as demonstrated by the dashed lines on the bulk isotope key which show combinations of different analyses, e.g. blue and green = CN, δ^{13}C and δ^{15}N; blue and yellow = CO, δ^{13}C and δ^{18}O). Other elements measured are recorded in Other. Note that as some studies use multiple methodologies, the sum of the right-hand side of the plots is greater than the number of studies in each region (sum of left-hand side).

were used as a guide to construct the remainder of this review. The topics appear in a logical order which reflects the structure of the coral reef ecosystem, starting from the basal **production sources** (Topic 1: Organic matter dynamics), moving onto key primary producers and consumers (Topic 2: **Holobiont** metabolism), food web and community structure (Topic 3: **Trophic niches**), higher-level consumers and the energetic linkages they construct (Topic 4: Fish diet variation/Habitat **connectivity**), and finally considering external drivers of coral reef systems (Topic 5: Environmental drivers) (Figure 3). It should be acknowledged, however, that there is some inherent crossover between topics.

In terms of SI approaches used, natural abundances of SIs of **bulk tissue** (i.e. measuring a whole tissue type such as muscle or liver) dominate the literature (84% of articles; Figure 2B), with dual analysis of carbon and nitrogen (i.e. δ^{13}C and δ^{15}N) the most popular approach (54% of articles; Figure 2B), followed by nitrogen on its own (12% of articles; Figure 2B). While SI studies using **compound-specific stable isotope analysis** (**CSIA**, i.e. profiling individual compounds such as amino acids or fatty acids) are less frequent, they are increasing as this methodology becomes more accessible (6%; Figure 2B). Analyses on organism muscle tissue ($n=99$), **holobiont** host soft tissue

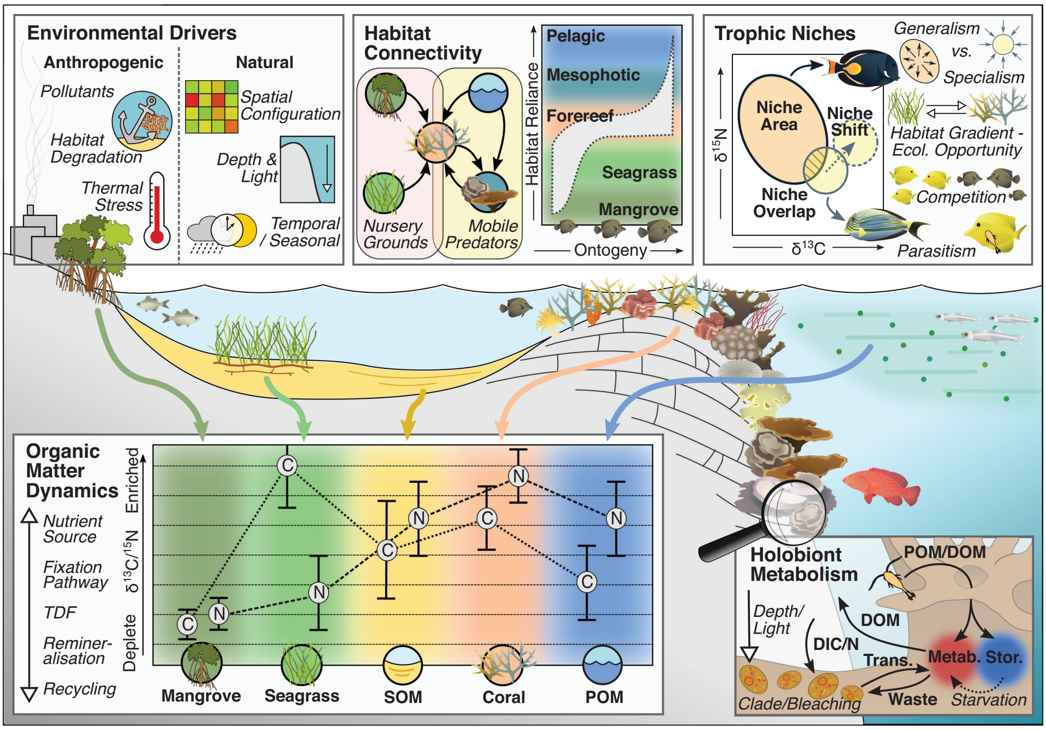

Figure 3 A conceptual diagram of the mangrove–seagrass–coral reef–pelagic continuum surrounded by panels showcasing the five major research foci of coral reef stable isotope (SI) studies to date, identified through topic modelling of identified published literature (1980–2019 inclusive, $n=236$). (1) Organic matter dynamics: relative $\delta^{13}C$ and $\delta^{15}N$ means and standard deviations of five common reef sources are depicted (not to scale, see Table 3). The arrows on the left side of the plot highlight factors which may result in more enriched or depleted values. (2) Holobiont metabolism: SIs help trace the complex cycling of nutrients between holobiont hosts and their endosymbionts. Various factors (depth, light) may influence these relationships (Trans. = translocation, Metab. = metabolism, Stor. = storage). (3) Trophic niches: SI biplots (often $\delta^{13}C$ and $\delta^{15}N$) generate an isotopic ellipse for an animal that is used to infer their trophic niche area. Niche overlaps suggest competition for resources, while shifts suggest a change in resource use. The various factors that may influence these metrics are depicted on the right side of the biplot (Ecol. = ecological). (4) Fish diet variation and habitat connectivity: SIs reveal how ontogenetic, larval, and mobile predator movements link adjacent ecosystems. (5) Environmental drivers: SIs reveal both anthropogenic and natural drivers. They can track nutrient inputs and provide evidence of thermal stress. Natural drivers such as seasonality, depth, and light can also be elucidated.

($n=79$) and their endosymbionts ($n=46$), and algal tissues ($n=66$), constitute the majority of studies, but there is a huge diversity of tissues that have been analysed. In addition to various organs (liver $n=7$; gonad $n=3$; skin $n=3$; digestive tract $n=2$; heart $n=2$), analyses have been conducted on fish scales ($n=2$) and gills ($n=2$), faeces ($n=2$), bone ($n=2$) and sea cucumber respiratory trees ($n=1$). Most studies are field based (75%), with few relying on laboratory experiments (22%), and fewer still combining both field and lab approaches (3%). While studies have been carried out around the world, there is substantial disparity between regions (Figure 2): the eastern Atlantic and eastern Pacific remain poorly represented ($n=1$ and $n=2$, respectively), likely due to a lack of expansive coral reef systems in these regions. Instead, western Atlantic studies dominate (28%), followed by

Table 2 Assigned name, number of articles, and top 15 weighted stemmed words for the five topics identified through the model. Stemmed words have been reduced to their root.

Topic number	No. articles	Top 15 words (stemmed) in weighted order	Assigned topic name
1	41	sourc, food, organ, product, benthic, sea, abund, matter, pattern, contribut, base, spatial, higher, examin, island	Organic matter dynamics
2	55	carbon, nitrogen, tissu, nutrient, host, enrich, rate, alga, coloni, algal, light, symbiont, metabol, heterotroph, assimil	Holobiont metabolism
3	48	speci, trophic, diet, feed, nich, ecolog, prey, resourc, predat, content, function, group, forag, divers, dietari	Trophic niches
4	44	fish, habitat, differ, size, popul, seagrass, individu, juvenil, shift, ecosystem, area, bodi, adult, muscl, marin	Fish diet variation/habitat connectivity
5	48	increas, chang, water, depth, high, site, communiti, rang, variat, shallow, ocean, environment, decreas, environ, effect	Environmental drivers

the western Pacific (21%) and the western Indian Ocean (20%), with notable foci localities in the Caribbean and Florida Keys, the Great Barrier Reef, and the Red Sea, respectively.

Across the literature, a variety of sources have been sampled in the field, ranging from water nutrients and organic matter through to macrophytes, zooplankton, and **holobionts**, covering both the pelagic and benthic and spanning the mangrove–seagrass–reef–offshore habitat continuum (Table 3). Of the 741 extracted values, macroalgae were the most repeatedly measured for both δ^{13}C and δ^{15}N ($n=145$ and 203, respectively). Particulate organic matter (POM), zooplankton, algal turfs, sediment organic matter (SOM), and coral (both homogenate and separated fractions) have also been well characterised across studies ($n>45$). The global variability observed in δ^{13}C and δ^{15}N across coral reef sources is substantial (Figure 4), spanning from approximately –30‰ to –4‰ for δ^{13}C and –5‰ to 15‰ for δ^{15}N, with macroalgae specifically exhibiting considerable diversity in expressed SI compositions.

Sampling regimes of SI studies are dominated by limited temporal windows (single point, period, or season, approximately 50% of screened articles), with only ~13% of studies designed to observe changes, if any, between distinct seasons or years (multi-season, monthly, annually, and interannual studies) (Figure 5A). Of considerable concern is that 17% of studies screened failed to provide enough information to discern their sampling regime, with examples found across all major regions of study. The focal taxa of studies are heavily, but unsurprisingly, skewed towards reef fish and hard corals, which combined comprise over 60% of all SI studies globally. This bias is particularly notable in the Western Indian region, whereas studies based in the Western Atlantic appear to be more evenly dispersed across focal taxa groups (Figure 5B). Barring hard coral, there is limited focus on other coral reef sources (although they are often measured within other studies, Table 3, and Figure 4). Encouragingly however, studies that explore SIs in multiple components of the ecosystem, thereby employing a more holistic approach to understanding whole coral reef systems, consist of over 5% of the observed literature.

Organic matter dynamics

Background

The dynamics of organic matter and remineralised constituents over and within coral reef ecosystems, and hence the food and dissolved nutrient resources available to reef consumers, have long been enigmatic. Odum & Odum (1955) suggested that the “changes in dissolved organic matter (DOM) in the vast flow of water crossing [a] reef” posed a central question in reef productivity.

Table 3 Summary of interstudy mean stable isotope values and their standard deviations for basal resources, extracted from across the 238 identified studies. Where studies were investigating pollution or anthropogenic nutrient inputs, no values were taken from the impacted sites. Individual values, corresponding references, and specific details on extraction are provided in the supplements.

	$\delta^{13}C$			$\delta^{15}N$			$\delta^{34}S$		
Sources	Mean	SD	***n***	Mean	SD	***n***	Mean	SD	***n***
Pelagic									
DIC/DIN[a]	−3.12 (8.59)	0.57 (0.34)	6, 6	1.30 (2.40)	1.48 (0.15)	2, 2			
DOM[b]				3.15 (0.07)	0.60 (0.42)	2, 2			
POM[c]	−20.95 (2.74)	1.22 (1.03)	49, 39	4.69 (2.53)	1.15 (0.98)	50, 40			
Phytoplankton[c]	−20.81 (2.14)	0.63 (0.45)	10, 5	3.78 (2.79)	1.04 (0.59)	8, 5	20.80		1
Zooplankton[c]	−19.21 (1.58)	1.00 (0.79)	54, 44	6.27 (2.67)	0.79 (0.60)	56, 47	18.65 (2.33)	1.33 (1.15)	2, 2
Macrophytes									
Mangrove[d]	−28.24 (0.49)	2.25 (2.28)	5, 4	1.65 (0.30)	0.54 (0.36)	4, 4	12.90	0.53	1, 1
Seagrass[d]	−9.69 (2.98)	1.24 (0.87)	12, 10	2.78 (2.72)	0.73 (1.17)	32, 30	17.67 (2.32)	1.70 (0.99)	3, 2
Macroalgae[e]	−15.68 (4.87)	1.27 (1.23)	145, 120	3.93 (3.45)	0.76 (1.12)	203, 179	20.10 (0.71)		2
Benthic mix									
Algal turf[f]	−15.63 (3.48)	1.49 (0.90)	46, 43	2.44 (1.61)	0.64 (0.51)	46, 43			
SOM[g]	−16.27 (3.26)	1.34 (1.22)	46, 44	4.69 (2.80)	0.65 (45)	47, 45			
Holobionts									
Coral[h,i]									
Holobiont	−13.80 (2.19)	0.99 (0.59)	50, 45	5.27 (1.53)	0.51 (0.39)	50, 46			
Tissue	−15.47 (1.63)	1.16 (0.69)	71, 51	6.59 (3.67)	0.74 (0.48)	55, 35			
Endosymbiont	−14.64 (2.67)	1.22 (0.63)	54, 54	5.02 (3.42)	0.74 (0.42)	39, 39			

(*Continued*)

Table 3 (*Continued*) Summary of interstudy mean stable isotope values and their standard deviations for basal resources, extracted from across the 238 identified studies. Where studies were investigating pollution or anthropogenic nutrient inputs, no values were taken from the impacted sites. Individual values, corresponding references, and specific details on extraction are provided in the supplements.

	δ^{13}C			δ^{15}N			δ^{34}S		
Sources	Mean	SD	***n***	Mean	SD	***n***	Mean	SD	***n***
Sponge[h]									
Endosymbiont	−17.25 (1.48)		2						
Soft coral[j]	−16.31 (1.30)	0.70 (0.55)	54, 44	5.23 (2.86)	0.47 (0.51)	61, 49			
Chemoautotroph[k]	−24.50	1.38	1, 1	0.40	1.96	1, 1	−17.90	5.29	1, 1

[a] Dissolved inorganic carbon/nitrogen (DIC/N) quantified as seawater bicarbonate, nitrate, and/or ammonium.

[b] Dissolved organic matter (DOM) estimated as total dissolved nitrogen.

[c] Seawater filtrate of various size fractions defined as either particulate organic matter (POM), phytoplankton, or zooplankton by the authors.

[d] Leaf tissue only.

[e] All species of macroalgae, including epiphytic and calcifying species barring turf.

[f] Includes a matrix of various organic matter sources and is therefore kept separate from macroalgae. Studies perform varying degrees of matrix separation, but typically limited to excluding macroinvertebrates.

[g] Sediment organic matter (SOM) covering various methods of sampling and fractions, including total organic matter; microphytobenthos; sedimentary bacteria/bacterial mats, coral mucus, and detritus as defined by the authors.

[h] Measured either combined host/endosymbiont homogenate (holobiont) or measured separately as host (tissue) and endosymbionts, typically via centrifugation of homogenate prior to stable isotope analysis. Asymbiotic species included in tissue.

[i] Excludes non-standard tissue types (skeletal material, gametes, and larvae) and bleached tissues.

[j] Includes both symbiotic homogenates and asymbiotic species.

[k] A single study with measurements from the lucinid bivalve, *Codakia orbicularis,* which host chemoautotrophic (sulfur) bacterial symbionts in their gill tissues.

Values are given as mean plus standard deviation in parentheses. n (x_1,x_2) denotes the number of means (x_1) and standard deviations (x_2) per source-stable isotope combination across single, dual, and tri-isotope analyses.

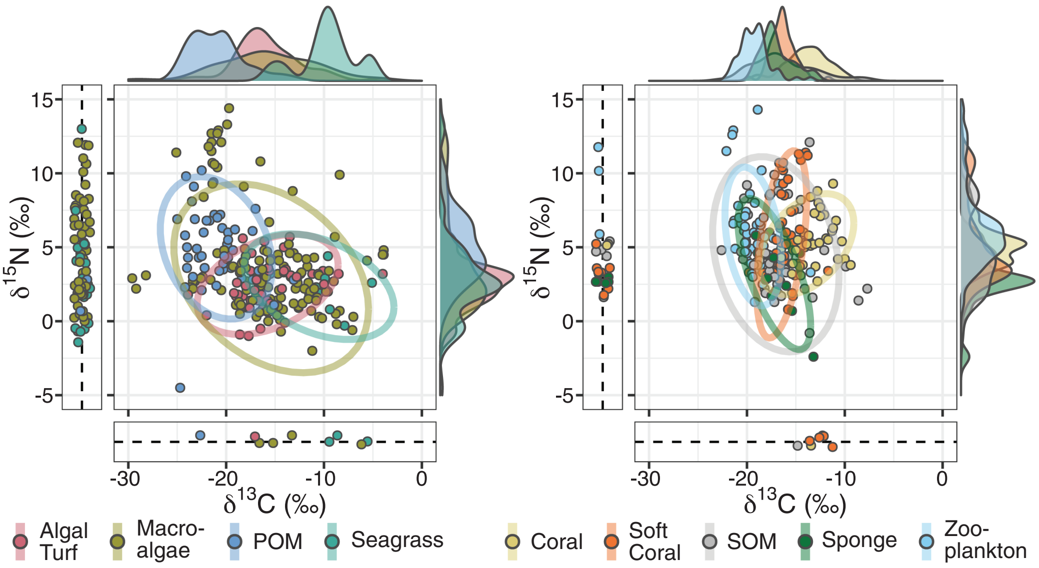

Figure 4 Mean δ^{13}C and δ^{15}N values for nine commonly measured sources in coral reef systems, separated into two panels for clarity (macrophytes and algae left, other sources right). A total of 741 single and dual stable isotope observations were extracted from the literature (see Table 3 for breakdown by group and definitions, only coral and sponge holobiont homogenate are plotted). Where studies were investigating pollution or anthropogenic nutrient inputs, no values were taken from the impacted sites. Isotope measurements are plotted as biplots with 95% ellipses. Single isotope measurements are plotted below (δ^{13}C) or to the left of (δ^{15}N) on the corresponding biplot axis. Single isotope observation data are jittered for clarity. Kernel densities of total isotope measurements by source are plotted above (δ^{13}C) and to the right of (δ^{15}N) on the biplot. Individual values are given in Table S6.

Quantifying variability in material fluxes into and within reefs was recognised in early studies as essential to understanding how reefs function in oligotrophic oceanic settings (Sargent & Austin 1954, Odum & Odum 1955, Sorokin 1973b). However, reef food webs possess a large range of potential sources, both **autochthonous** (hereafter '**internal**') and **allochthonous** (hereafter '**external**'), and these are likely to vary spatially along with hydrodynamics and zonation of community structure (Figures 3 and 4; Table 3). SI studies investigating these fluxes have predominantly been conducted in the western ($n = 12$) and central ($n = 9$) Pacific, western Atlantic ($n = 11$), and western Indian Ocean ($n = 10$), with particular foci on Japan ($n = 7$), the Caribbean ($n = 7$), and the Red Sea ($n = 5$), respectively (Figure 2). The eastern Pacific, eastern Indian, and eastern Atlantic are poorly represented with few, if any, studies conducted ($n = 0$ of the papers assigned to this topic from our literature search).

High reef productivity despite low ambient nutrient concentrations has been attributed to both internal and external processes: high rates of internal recycling or atmospheric nitrogen fixation, and therefore nutrient retention (e.g. Odum & Odum 1955, Johannes et al. 1972, Webb et al. 1975), versus flow-driven inputs of **external** oceanic nutrients (Odum & Odum 1955, Andrews & Gentien 1982, Atkinson 1992, 2011, Wyatt et al. 2012a). The high rate of respiration, with net productivity often close to zero, indicates effective recycling within reef systems (Kinsey 1985, Crossland et al. 1991, Tribble et al. 1994). However, the fluxes of materials over reefs (both **internal** and **external**) and their uses by consumers have rarely been examined at ecosystem scales. This makes it difficult to make accurate predictions regarding the future function of reefs subject to change. Indeed, non-isotope studies, mostly in mesocosms or incubations, have indicated that DOM may be a significant resource (Tanaka et al. 2009, Nakajima et al. 2010, Naumann et al. 2010a, 2012, Tanaka et al. 2011a). However, these are generally

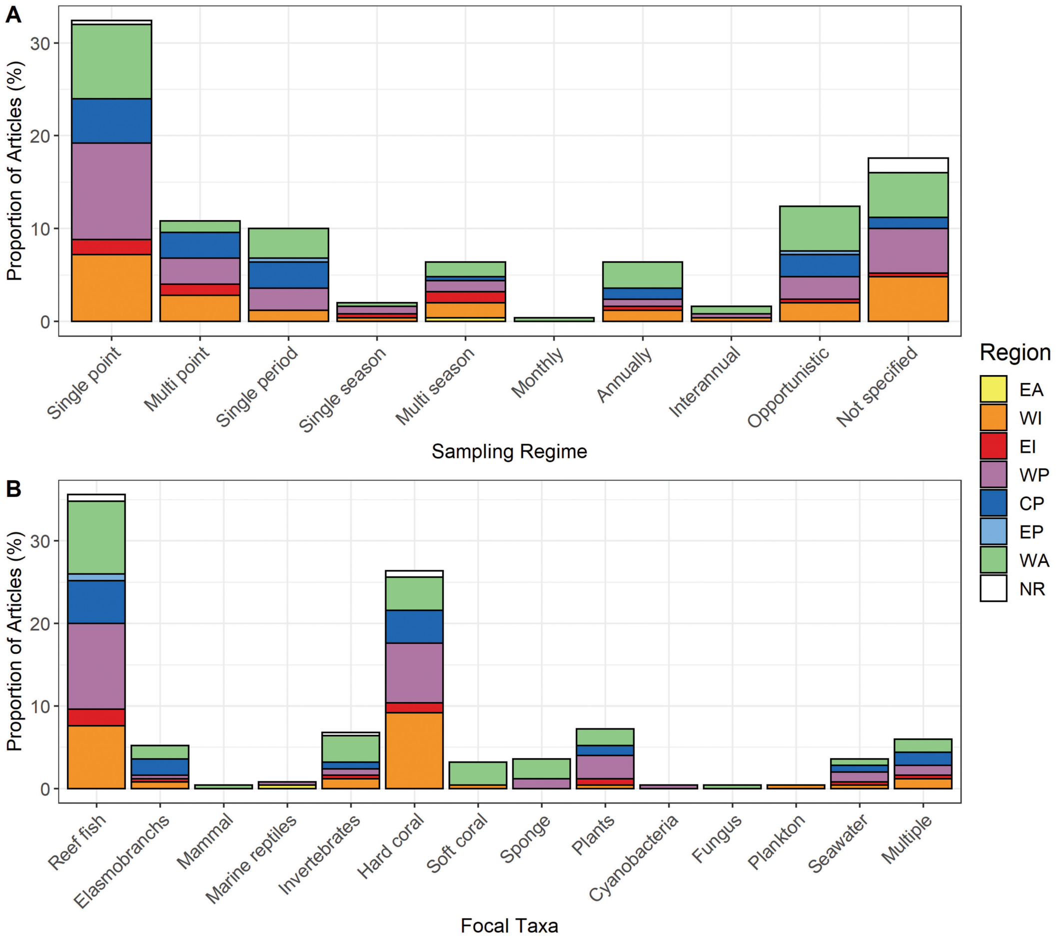

Figure 5 (A) Temporal sampling regime and (B) focal taxa of all 238 articles identified through the literature search (1982–2019). (A) Single point=all samples collected over one/several days; multi-point=all samples collected at ≥2 distinct temporal points; single period=all samples collected over a period of several weeks or more (>1 month, for logistical reasons), but no repetition of groups sampled. See Table S2 for all definitions and notes on the different sampling regimes. EA, East Atlantic; WI, West Indian; EI, East Indian; WP, West Pacific; CP, Central Pacific; EP, East Pacific; WA, West Atlantic; NR, No Region Specified.

hampered by a lack of understanding of hydrodynamic influences on DOM fluxes over natural reef systems (but see Wild et al. 2012, Thibodeau et al. 2013). SI data offer great potential to identify organic matter sources over natural reefs and measure the gross fluxes of this material, providing insights into reef functioning that are essential for predictions for the future. To date, the focal taxa of studies investigating these fluxes (n=41) are typically reef fish (n=13), other invertebrates (n=6), and hard corals (n=5). While seawater is also frequently sampled (n=9), there has been little focus on benthic algae (macroalgae n=4; turf algae n=0) regarding organic matter dynamics, despite their generally large surface area, important role in nutrient uptake, and as a resource for many reef consumers (Figure 5B).

Internal and external fluxes of DOM and POM

Organic matter in the ocean is dominated by dissolved organic matter (DOM), concentrations of which are far greater on average (1–2 orders of magnitude) than particulate organic matter (POM) (e.g. Libes 2009, Barrón & Duarte 2015). In contrast to the refractory nature and low availability of oceanic DOM to reef consumers, much of the DOM produced by reef communities is labile and rapidly remineralised (e.g. 1 month for ~80% of total organic carbon in coral mucus to be mineralised; Tanaka et al. 2011b). SI data have demonstrated that corals can release significant amounts of DOM; background concentrations of dissolved organic carbon (DOC) and nitrogen (DON) rose from 100 and 15 µM to 300–1700 and 120 µM, respectively, above coral colonies (Ferrier-Pagès et al. 1998b); as well as POM (see below). Isotope labelling is often used to trace DOM fluxes; highly isotopically enriched nutrients are supplied and subsequently traced through the various metabolic pathways within the **holobiont**. ^{13}C-labelling of reef-building corals *Porites cylindrica* and *Acropora pulchra* suggested that the DOC they release over reefs is derived from stored (>90% of total) rather than from newly synthesized organic C (<10%) (Tanaka et al. 2008). One of the few ecosystem-scale investigations of reef DON cycling used spatial patterns in δ^{15}N to demonstrate that localised release of DON (potentially supported by N_2 fixation in pristine benthic habitats) is an important means of recycling N within reef communities (Thibodeau et al. 2013). The spatial arrangement (see 'Spatial and temporal variations' below) of reef communities is therefore important in determining rates of nutrient exchange across whole coral reef systems (Smith & Marsh 1973, Steven & Atkinson 2003, Miyajima et al. 2007, Wyatt et al. 2012a, 2013).

While concentrations of DOM are greater, POM is more bioavailable (Lønborg et al. 2018), and therefore, POM dynamics are more readily studied on coral reefs. Several studies, including non-isotope approaches, have identified significant inputs of **external** POM from the ocean by analysing gross fluxes of isolated components of the **external** POM pool (particularly the smallest phyto- and bacterioplankton) (Genin et al. 2009, Wyatt et al. 2010b, Patten et al. 2011, Akhand et al. 2021). While this may suggest a less prominent role for tight recycling within reefs, rates of **internal** POM production have not been adequately quantified across reef systems. Therefore, robust generalisations regarding the overall relative importance of **external** versus **internal** POM inputs are lacking. Isotope labelling indicates that release of POM from corals may be of a similar order or higher than that of DOM (average ratios of 0.6 and 0.5 for *P. cylindrica* and *A. pulchra*, respectively) (Tanaka et al. 2008). Macroalgae can also produce large amounts of POM; small incubation chambers over intact reef habitats in Moorea (French Polynesia) have shown a dominance of algal material within POM, with increased POM δ^{13}C (–16.9‰ to –11.2‰) above ambient (–20.6‰) indicative of the contribution of algal exudates relative to ^{13}C-depleted plankton (Haas et al. 2010). More enriched nearshore δ^{13}C values of POM samples (–18.3‰) in the Florida Keys were similarly taken to indicate the degradation of seagrass detritus, while more depleted δ^{13}C of the POM on the outer reef (–21.4‰) suggested it was dominated by plankton (Lamb & Swart 2008), *cf.* Figure 4. Few studies have quantified rates of POM production over a natural reef. However, modelling of δ^{13}C and δ^{15}N data from Ningaloo Reef in Western Australia supported the premise that **external** POM inputs were balanced by correspondingly high rates of gross **internal** POM release into water flowing over the reef crest and flat (Wyatt et al. 2013). Measuring net concentration changes of, for example **bulk** POM, may therefore obscure the dynamics of organic matter uptake and release over reefs, making SI evidence indispensable for disentangling **internal** from **external** organic matter fluxes.

Organic mucus produced in abundance by corals may play a role in both **internal** cycling and **external** inputs to reefs. Studies have used δ^{13}C analyses to demonstrate that coral mucus can be both exported from reefs, but also re-enter reef food webs due to uptake by reef consumers (Naumann et al. 2010b, Wyatt et al. 2013). Mucus itself can be a significant source of organic matter, especially in inner reef habitats with decreased oceanic exposure (Wyatt et al. 2013). For example, it may enhance the flux of **external** particles by trapping oceanic plankton in mucus aggregates, augmenting sedimentation rates onto reef habitats (Naumann et al. 2009). Moreover, **internal** sources within reefs are not limited to the corals. For instance, ^{15}N-labelling has shown significant rates of POM release from upside-down jellyfish *Cassiopea* sp. (which can be abundant in some reef systems) and subsequently assimilated by zooplankton (Niggl et al. 2010). Furthermore, non-isotope work has highlighted the important role of fish communities in supplying (excretion) and storing (biomass) nutrients on coral reefs and adjacent habitats (Allgeier et al. 2014), suggesting nutrient cycling is prevalent even at higher trophic levels. To date, there is limited SI work exploring this aspect of organic matter dynamics on reefs (but see 'Habitat connectivity').

Isotope labelling has been used to demonstrate a potentially major role for sponges in organic matter uptake and retention within coral reefs. The combination of isotopic labelling (^{13}C and ^{15}N) and nanoscale secondary ion mass spectrometry (NANOSIMS) has shown that sponge host tissues are capable of directly utilising DOM in the water column through filter feeding (Achlatis et al. 2019). Indeed, some sponges are more **heterotrophic**, as indicated by their **bulk** δ^{15}N values (Figure 4) (Weisz et al. 2007). For example, **bulk** δ^{13}C and δ^{15}N indicate that coral cavity sponges typically feed on coral-derived materials (van Duyl et al. 2011, Slattery et al. 2013). Unlike other marine organisms, a few sponges show differential assimilation mechanisms for different DOM sources, as revealed by stable isotope pulse-chase experiments (^{13}C and ^{15}N); for example, algal DOM is mainly used by symbiotic bacteria, while coral DOM is assimilated by sponge cells (Rix et al. 2017). de Goeij et al. (2008) used ^{13}C-labelling to provide evidence that, in addition to POM, a coral reef sponge, *Halisarca caerulea*, was able to incorporate DOM, thereby accessing an abundant source of organic matter in reef waters. Later, using ^{13}C- and ^{15}N-labelled DOM, de Goeij et al. (2013) demonstrated both in aquaria and in situ that DOM incorporation by sponges may facilitate DOM transfer to higher trophic levels through detritus (POM) production. Using ^{13}C- and ^{15}N-labelling in the laboratory, DOM uptake and POM production by sponges were further demonstrated to facilitate the transfer of coral-mucus-derived DOM to detritivores on both warm- and cold-water reefs (e.g. ophiuroids and polychaetes) (Rix et al. 2016, 2018). These isotope studies on reef sponges have revealed an additional mechanism by which organic matter produced on reefs may be internally retained within the system.

Spatial and temporal variations

Organic matter fluxes are likely to have strong spatial and temporal dynamics (Figure 4). These will be influenced by the hydrodynamic conditions and zonation of benthic communities over small spatial scales of hundreds of metres or less (Haas et al. 2011, Kolasinski et al. 2011, Wyatt et al. 2012a), and larger-scale stochastic events such as upwelling, storm disturbances, and coral bleaching events (Leichter et al. 2007, Wild et al. 2008, Kolasinski et al. 2011, Radice et al. 2021). Further, large-scale urbanisation-eutrophication gradients can be reflected through reef food webs, particularly as elevated δ^{15}N (Kürten et al. 2014, Duprey et al. 2020; and see 'Environmental drivers'). Broad-scale SI data or 'isoscapes', i.e. a spatial pattern of isotopic values across a land or seascape (West et al. 2008, Bowen 2010), can reveal geographic patterns in organic matter and nutrient dynamics over reefs. For instance, in the Red Sea, where 11% of all coral reef SI studies have been conducted (Figure 2), low organic matter δ^{15}N values in the north (zooplankton δ^{15}N: 1.3‰) reflect the importance of N_2 fixation, while the higher δ^{15}N of organic matter in the south (zooplankton δ^{15}N: 5.8‰) reflects N inputs from the Indian Ocean (Kürten et al. 2014). Spatial and temporal variability of organic matter over reefs is poorly understood, yet there is strong SI evidence of habitat-linked variations in fluxes of DOM (Thibodeau et al. 2013), POM (Wyatt et al. 2013), and dissolved nutrients (Leichter et al. 2007). While these oceanographic drivers mean that **external** resource supply can be highly variable in space and time (Wyatt et al. 2012a, 2013, Kürten et al. 2014), most SI reef studies are based on just a single time point or period of sampling (44%), with few conducting repeat samplings across multiple time points (19%) (Figure 5A).

Mass coral spawning can periodically increase POM concentrations several fold on many reefs, and SI data have proven useful in examining the biogeochemical impact of these events. A lasting influence of spawning on POM δ^{15}N was seen on the Great Barrier Reef over a period of about

10 days (increases of ~5 ‰, from 0.7‰ to 4.8‰–5.7‰), suggesting spawning-derived organic matter is rapidly transferred to higher trophic levels (Wild et al. 2008). Similarly, in Kane'ohe Bay, Hawai'i, POM δ^{15}N remained slightly elevated (2‰–3‰) after spawning (>10 days), indicating incorporation of spawning-derived organic matter into higher trophic levels, but spawning impacts on water-column POM δ^{13}C were short-lived (2–4 days post-spawning returned to pre-spawning values) (Briggs et al. 2013). Briggs et al. (2013) also found that the δ^{13}C of tissues of the coral *Montipora capitata* increased by ~ 1‰ (−13.3‰ to −12.2‰) over the course of the spawning season, with eggs having lower δ^{13}C than host tissues (−14.5‰). This temporal change could reflect the spawning physiology; the concentration of ^{13}C-light wax esters and overall carbon content is higher in *M. capitata* eggs compared to adults (Padilla-Gamiño et al. 2013), but it may also reflect changes in feeding or relative rates of **autotrophy** and **heterotrophy.** This underscores the importance of having a good understanding of **baseline** isotope variations when assessing the impacts of stochastic events like spawning using tissue SI data.

The high spatiotemporal variability in resources means that adequately quantifying the isotopic composition of material supplied to reefs can be a non-trivial undertaking (Figure 4; Tables 3 and S6). For instance, variability in the isotopic composition of dissolved inorganic N (e.g. nitrate) can be high across the water column (3.5‰–5.5‰ between 50 and 242 m) just due to small-scale patchiness in N cycling (Leichter et al. 2007). **Internal** production can also be highly variable, with organic carbon released by benthic algae on reefs at Moorea demonstrating distinct seasonality, including δ^{13}C variation of ~ 5‰ (Haas et al. 2010). This variability is likely why few studies to date have used isoscapes on coral reefs, or tropical waters in general (but see MacKenzie et al. 2019); those in marine settings have mostly been conducted in temperate shelf regions (MacKenzie et al. 2014, Kurle & McWhorter 2017, St. John Glew et al. 2019). However, isoscapes can provide spatial (and potentially temporal) context to more general questions about community ecology, animal migration, and nutrient cycling (Hobson et al. 2010, McMahon et al. 2013, Cheesman & Cernusak 2016). In one of the few studies employing an isoscape approach on coral reefs, δ^{15}N values of long-lived benthic bivalves were used to create a nitrogen isoscape in New Caledonia that highlighted regions of eutrophication (δ^{15}N of 11.7‰ compared to 4.3‰ in lagoon), characterising the anthropogenic nitrogen footprint of the area (Thibault et al. 2020). Thus, isoscapes of coral reef ecosystems that account for sufficient natural variation could offer insight into the complex processes that might influence coral reef trophodynamics and SI values across various locations (Figures 3 and 4; Table 3). Given that environmental conditions are fluctuating due to climate change, isoscape studies would further benefit from the inclusion of SI data across both space and time where possible (McMahon et al. 2013).

Isotopic insights into the role of detritus

Detritus (i.e. non-living organic matter) is an abundant and potentially significant food resource over reefs, but its variable lability makes it challenging to characterise (Figure 4; Tables 3 and S6). Isotopes offer an opportunity to better trace and quantify fluxes of this material. Indeed, there is substantial isotopic evidence of a prominent role for detritus in reef food webs. While detrital pools are higher (1.6 times) in low-energy back reef habitats where benthic material accumulates (evidenced by enriched mean δ^{13}C −16.83‰), input rates are higher (1.7–2.9 times) over dynamic fore reefs due to oceanic detritus supply (evidenced by depleted mean δ^{13}C −19.84‰) (Max et al. 2013). Thus, while detritus can be a nutritious food source, its availability fluctuates with hydrodynamics (Max et al. 2013). Isotope labelling (^{13}C and ^{15}N) has demonstrated that detritivores, such as ophiuroid brittle stars, can play an important role in the recycling of nutritionally poor detritus to higher trophic levels (Rix et al. 2018). There is also increasing evidence of a high degree of detritivory in reef fishes previously assumed to be largely herbivorous. For instance, parrotfish such as *Chlorurus sordidus* are often classified as herbivores. Stomach and feeding observations have suggested that this species may be better described as a detritivore (Choat et al. 2002), and **bulk** and **CSIA** data confirm it to be principally detritivorous across both oceanic and inshore reefs (Wyatt et al. 2012b, McMahon et al. 2016). In agreement with observational and morphological studies (Choat et al. 2002, 2004), **CSIA** data identified that the surgeonfish *Ctenochaetus striatus* is also predominantly detritivorous (73%; McMahon et al. 2016). Dietary plasticity at the individual level (McMahon et al. 2016), or spatial changes in the importance of detritivory (Wyatt et al. 2012b), require more detailed investigation. However, it appears likely that the importance of detritus, and therefore microbial reworking, in reef food webs has been underestimated (McMahon et al. 2016). As described in detail in 'Internal and external fluxes of DOM and POM', sponges also play a key role in reworking detritus on coral reefs through the 'sponge loop': coral mucus carbon and nitrogen are transferred into sponge tissues and subsequently released as detritus (de Goeij et al. 2013, Rix et al. 2016).

Holobiont metabolism

Background

Hermatypic (reef-building) scleractinian corals play a foundational role in providing essential habitat for reef organisms (Coker et al. 2014), so it is only natural that considerable efforts have focused on their ecology and the metabolic dependency between them and their symbiotic dinoflagellates in the family Symbiodiniaceae (Figure 3). **Holobiont** metabolism had the largest number of studies principally assigned to it ($n=55$) among SI coral reef topics. Likewise, of all 238 identified SI studies, 26% ($n=62$) have had coral as their focal taxa, second only to fishes (36%, $n=85$) (Figure 5B). Studies focusing on this topic contributed a major proportion of the earliest SI works on coral reefs (Figure 1). As SIs act as natural (or artificial) tracers for elucidating energetic pathways, they are ideally placed to resolve questions regarding nutrient acquisition, translocation, and utilisation within and between **holobionts** and their symbiotic microbiomes (Figure 3). This is highlighted by the fact that most isotope labelling studies were assigned to the topic of '**Holobiont** metabolism' (28 of 37 total). Geographically, there has been a particular focus on **holobiont** metabolism regionally in the western Indian Ocean (notably experimental work in the Red Sea) and the western Pacific (Figure 2B and 5B). Key aspects revolve around the nature of nutrients that are taken up by **holobionts**; how they are proportioned internally between hosts and symbionts; and drivers of variation in the relative utilisation of photo- or chemosynthetically fixed material (**autotrophy**) and secondarily sourced production (**heterotrophy**), known as **mixotrophy**. Here we expand upon these ideas to demonstrate the understanding achieved using SIs, before considering studies whose focus is on the symbiotic relationships found in organisms other than those of hermatypic corals.

External nutrient acquisition

As highlighted in the previous section, there is a wealth of **external** nutrient sources that are potentially available to corals and other **holobionts** to underpin their metabolism, as well as those of their endosymbionts to form photosynthates. This is despite coral reef ecosystems traditionally being viewed as restricted to oligotrophic tropical surface waters (Darwin 1842). Early work by Risk et al. (1994) suggested increasing reliance on terrigenous carbon sources by corals and their endosymbionts after observing increasing $\delta^{13}C$ tissue values (from –16‰ to –11‰) with increasing distance from shore on the Great Barrier Reef. Spatially varying nutrient acquisition has also been observed with reef zonation, with varying coral tissue $\delta^{15}N$ values along an offshore to mid-shelf to inshore barrier reef transect attributed to cold-water upwellings, algae-based nitrogen fixation, and terrigenous sources, respectively (Sammarco et al. 1999, Erler et al. 2014). Elsewhere, across a reef atoll system in the Maldives, nitrates from deep ocean water that are enriched in ^{15}N have been shown to be incorporated into coral tissues via seasonal upwelling (Radice et al. 2019). In the Cook Islands in the South Pacific Subtropical Gyre, nitrogen inputs to corals also vary seasonally, with $\delta^{15}N$ in coral skeletons suggesting nitrogen sources originate from groundwater during wet seasons and from N_2 fixation during dry seasons (Erler et al. 2019). These studies highlight the environmental and temporal context dependency of patterns in coral nutrient uptake, but it is worth noting that increases in coral and endosymbiont $\delta^{13}C$ values may also be related to spatial gradients in, for example, turbidity affecting coral photosynthesis rates or endosymbiont genera.

Direct nutrient uptake from the water column is likely influenced by its form; nitrogen is primarily available as nitrate or ammonium, where ammonium appears to be principally taken up by the endosymbiotic algae. Eight-week-long ^{15}N-labelled ammonium enrichment experiments revealed a tenfold increase in ammonium uptake rates by hosted dinoflagellates compared to coral tissue (Grover et al. 2002). However, overall ammonium uptake is reduced in fed versus starved hosts (Grover et al. 2002), which may suggest secondary reliance on ammonium as a nitrogen source compared to **heterotrophic** feeding. Similar patterns were found for nitrate using ^{15}N-labelled nitrate and ammonium; nitrate is principally taken up by the endosymbionts, with uptake rate independent of prior nutrient acclimatisation, but significantly lower under high ammonium regimes (Grover et al. 2003). The metabolic response of dinoflagellate endosymbionts to **external** nutrient enrichment is rapid; significant uptake occurs within an hour of exposure to either ammonium or nitrate ^{15}N-enriched seawater (Pernice et al. 2012, Kopp et al. 2013). This suggests direct uptake and fixation of ammonium by endosymbionts from seawater filling the coelenteron rather than nutrient transfer from hosts to algae (Pernice et al. 2012, Kopp et al. 2013). Such a rapid response is facilitated by temporary nitrogen storage in intracellular uric acid crystals that can then be remobilised in the following hours (Kopp et al. 2013). Similar temporary intracellular storage structures have also been identified for carbon using pulse-chase isotopic labelling of ^{13}C-bicarbonate, whereby seawater bicarbonate is rapidly (~15 minutes) taken up by dinoflagellates and fixed into lipid droplets and starch granules (Kopp et al. 2015).

The uptake of water column nutrients can be further facilitated by recycling pathways, as has been revealed by isotope tracer experiments using artificially ^{15}N-labelled bacteria to track their incorporation into coral larvae (Ceh et al. 2013). Isotopic labelling (^{15}N) has further shown that nitrogen-fixing diazotrophic bacteria may also be taken up directly into the epidermal layers of coral larvae (Lema et al. 2016), potentially helping to meet their nitrogen demands. Nitrogen fixation can also occur within the endolithic diazotrophs found between coral tissues and their carbonate skeleton, as demonstrated with ^{15}N-labelling (Grover et al. 2014, Yang et al. 2019). However, the importance of this pathway varies according to coral metabolic status (i.e. whether they are more **auto**- or **hetero**trophic) and with depth (Bednarz et al. 2017). **Bulk** SI work ($\delta^{13}C$ and $\delta^{15}N$) has, however, shown a lack of nutrient exchange between coral polyps and adjoining epilithic algal turfs, demonstrating that at least some potential nutrient pathways are not utilised (Titlyanov et al. 2008).

Heterotrophic feeding by coral polyps further expands the potential nutrient sources available for these **holobionts**, with a diversity of trophic interactions being observed (see Houlbrèque & Ferrier-Pagès 2009 for a detailed review on coral **heterotrophy**). Lai et al. (2013) used ^{15}N-labelled seagrass to experimentally show that corals can directly consume seagrass material, in both particulate and dissolved form. This highlights a direct nutritional link between corals and adjacent seagrass meadows, which can export large quantities of fresh and detrital material. Other experimental **bulk** SI work ($\delta^{13}C$ and $\delta^{18}O$) has highlighted how grazing on zooplankton can lead to substantial increases in various measures of coral fitness, such as tissue chlorophyll concentrations, compared to when **heterotrophic** feeding is restricted (e.g. Reynaud et al. 2002). Furthermore, grazing experiments using ^{3}H-labelled bacteria and ciliates showed that although coral grazing rates decrease as light intensity increases, **heterotrophy** still contributes to coral nutrition, suggesting it complements **autotrophy** even under high light conditions in *Stylophora* sp. (Ferrier-Pagès et al. 1998a). More recent SI fingerprinting techniques using $\delta^{13}C$ of essential amino acids (**CSIA**) demonstrate the significant contribution of **heterotrophic** feeding to coral hosts, with an average of 41% contribution to assimilated material determined for the widespread Indo-Pacific scleractinian coral *Pocillopora meandrina* (Fox et al. 2019). Similar proportions of **heterotrophic** nutrient uptake have also been verified experimentally using ^{15}N-labelled rotifers to quantify nitrogen incorporation rates in another common Indo-Pacific coral *Porites lutea* (Rangel et al. 2019). However, the degree of **heterotrophic** feeding may be a species-specific trait; paired **bulk** (δ^{13}C and δ^{15}N) and amino acid **CSIA** ($\delta^{13}C_{AA}$, $\delta^{15}N_{AA}$) revealed that *Montipora capitata* did not change their nutritional strategies under different experimental nutrition regimes (Wall et al. 2021), suggesting a lack of trophic plasticity.

Internal nutrient translocation and partitioning

The fate of nutrients once assimilated into host tissues underpins the symbiotic relationship between coral and Symbiodiniaceae – basal products need to be supplied to algal cells for **autotrophy** and likewise photosynthates shuttled elsewhere for energetic demands of the host (Figure 3). Isotopic labelling experiments tracing the various metabolic pathways within the **holobiont** constitute some of the earliest uses of SIs on coral reefs. For example, feeding experiments with ^{14}C revealed that six species of coral were able to feed on DOM and planktonic bacteria, actively consuming organic phosphorus bound in the cells of the latter (Sorokin 1973a). Other studies have attempted to understand carbon translocation and turnover within coral colonies. Crossland et al. (1980) saw a 50%–60% loss of photosynthetically fixed ^{14}C from isotopically labelled and replanted coral colonies during their first 40 hours back on the reef. However, rather than the coral colonies translocating fixed carbon from outer branches to basal regions, the authors suggested that the coral tissues had released mucus and dissolved organic carbon into the environment (Crossland et al. 1980). Similarly, Rinkevich & Loya (1983) used ^{14}C sodium bicarbonate in the field to demonstrate limited translocation of photosynthetic products across coral colonies over the course of a month (from bases to branch tips), despite lower incorporation rates in the growing tip regions.

Under more controlled laboratory settings, high precision tracing can be conducted to elucidate internal translocation of metabolites over short timescales, further revealing the complexities of this symbiotic relationship. Following ^{13}C-labelled bicarbonate enriched seawater incubation, Tremblay et al. (2012) calculated that 60% of carbon fixed by endosymbionts in the scleractinian *Stylophora pistillata* is translocated to host tissue within 15 minutes, with approximately 50% of fixed carbon being respired by the **holobiont** as a whole. Whole nitrogen budgets have also been determined through ^{15}N enrichment, showing that a majority (50%–83%) of nitrogen utilised by endosymbionts is derived from coral hosts, with host species-level differences attributed to different N-biomasses per unit surface area of coral host species (Tanaka et al. 2015, 2018). One of the few studies to combine both $\delta^{13}C_{AA}$ and $\delta^{15}N_{AA}$ to explore the nutritional exchanges between coral hosts and their endosymbionts confirmed that endosymbionts do benefit from host **heterotrophy** (Ferrier-Pagès et al. 2021). Furthermore, this relationship is not one way but tightly coupled. Combining ^{13}C- and ^{15}N-labelling showed that the coral host derived 99% of its total nitrogen from the endosymbiont, suggesting the host ‘farms’ the endosymbionts to efficiently exploit both C and N (Tanaka et al. 2018).

Interestingly, the fate of acquired nutrients appears to depend upon the source of the material. Isotope labelling (^{13}C and ^{15}N) reveals that **heterotrophic** sources exhibit considerable internal exchange and retention within the coral-algae symbiosis, whereas inorganic nutrients (that are photosynthetically fixed by endosymbionts) are rapidly used and respired to meet more immediate metabolic demands (Hughes et al. 2010, Tremblay et al. 2015). The release of DOM by corals can constitute a considerable loss of fixed material, corresponding to approximately 5% of net photosynthetic production of endosymbionts as shown by ^{13}C tracer accumulation experiments (Tanaka et al. 2009). This has been corroborated elsewhere; similar work has estimated coral carbon losses due to DOM release is equivalent of 28% of gross carbon fixation (Tremblay et al. 2012).

With the advent of ever more sophisticated technology, exploring the spatial structuring of metabolic processes within coral tissues is now possible. Notably, the combination of isotopic labelling and nanoscale secondary ion mass spectrometry (i.e. NANOSIMS) has revealed the variation in net carbon fixation rates between individual cells of *Symbiodiniaceae*, with an average sixfold decrease between upper and lower tissue layers within individual polyps (Wangpraseurt et al. 2016). These combined technologies have also helped disentangle the rapid nutrient uptake dynamics within endosymbionts (Kopp et al. 2013, 2015). Such approaches can be further expanded with, for example, simultaneous immunofluorescent microscopy to correlate the presence of isotopically labelled labile nutrients with associated proteins and enzymes to further elucidate metabolic pathways (Loussert-Fonta et al. 2020). These technological advances have the potential to greatly expand the current understanding of the molecular-level underpinning of coral-algae symbioses.

Drivers of mixotrophy

The mechanisms that influence the strength of coral-algae symbiosis are key to the wider coral reef ecosystem. Therefore, understanding how coral **mixotrophy** changes with different factors provides insight into how they may be best managed and conserved in a changing world. SI studies that explore potential drivers of **mixotrophy** in corals can generally be categorised by the nature of the driver(s) of interest: whether **mixotrophy** is impacted by the external environment (exogenous) or influenced by traits that are particular to the studied host or symbiont (endogenous).

Exogenous factors

Given the photosynthetic underpinning of coral-algae symbiosis, depth represents a strong natural abiotic gradient which can impact **autotrophic** efficiency due to, among other things, diminishing ambient light levels (see 'Environmental drivers: natural drivers'). The nutritional history of host corals (fed versus starved), which is driven by the temporal dynamics of prey availability, may also play a role in **mixotrophy**. In isolation, ^{15}N-labelling indicates that nutritional history does not appear to affect the assimilation efficiency of **heterotrophic** feeding by corals (Piniak & Lipschultz 2004). However, δ^{13}C of fatty acids reveals that recent starvation, when compounded with thermal stress, leads to reductions in chlorophyll and maximal photosynthetic efficiency in coral tissues, resulting in respiration of storage fatty acids in order to maintain coral metabolism (Tolosa et al. 2011). This highlights the compounding nature of environmental factors (see 'Environmental drivers: natural drivers').

Bleaching events, which are induced by a variety of external stresses, are projected to continue to increase with ongoing climate change (Hughes et al. 2017). Given the loss of the **autotrophic** symbionts during such episodes, the impact on host energetics is likely to be significant. Early SI work suggested, however, that bleaching does not alter the ratio of **heterotrophic** to **autotrophic** dependency. This was inferred from similar δ^{13}C values in two corals (*Porites compressa* and *Montipora verrucosa*) and their endosymbionts taken from paired samples of bleached and non-bleached tissue in the field in Hawai'i (Grottoli et al. 2004). This was in contrast to pulse-chase experiments (^{13}C) on the same species (*P. compressa*) and a congener (*Montipora verrucosa* cf. *M. capitata*) that showed reduced assimilation of autotrophically derived carbon in bleached corals, but with assimilation of **heterotrophic** sources remaining similar in bleached versus non-bleached corals (Hughes et al. 2010). More recent work using **bulk** SI data (δ^{13}C and δ^{15}N) on the same two species also did not indicate increased **heterotrophic** nutrition post-bleaching (Wall et al. 2019). The δ^{13}C data also suggest corals may undergo biomass compositional changes when bleached and directly after (Wall et al. 2019), possibly as they catabolise their stores of ^{13}C-enriched lipids (Grottoli and Rodrigues 2011). **Heterotrophic** carbon sources are likely important for coral recovery as they are predominantly used to replenish coral lipids (Baumann et al. 2014), which are used to maintain metabolism during thermal stresses (Tolosa et al. 2011). For longer-term responses of corals to bleaching, see 'Environmental drivers: natural drivers'.

Endogenous factors

The diversity in form observed across Scleractinia, and even within species across, for example, depth gradients (Einbinder et al. 2009), suggests ecological trade-offs associated with different host morphologies. Previous non-SI work exploring coral morphology-feeding relationships suggested that coral surface-to-volume ratio (S/V) and polyp size might determine the importance of light or zooplankton capture. Branching corals have maximum S/V ratios and small polyps and are best suited for light capture, while corals with lower S/V ratios generally have larger polyps suiting them to zooplankton capture (Porter 1974, 1976). Subsequent non-SI work by Wellington (1982) corroborated that tentacle size was important for determining the degree of **hetero**- or **autotrophy** (i.e. corals with larger tentacles use more zooplankton), but could not confirm the coral morphology-feeding relationships. Some SI data have now supported this hypothesis. $\delta^{13}C$ values in coral tissues and endosymbionts from 14 Red Sea coral species indicated increased relative rates of **autotrophy** in branching corals with smaller polyps compared to massive species with larger polyps, attributed to reductions in carbon limitation associated with increasing surface area (Levy et al. 2006). Such a trend with host morphology has been further corroborated by Xu et al. (2020) using $\delta^{13}C$. However, in contrast, a non-isotope feeding study found no relationship between coral feeding rates and polyp size (Palardy et al. 2005), while Hoogenboom et al. (2015) using **bulk** $\delta^{13}C$ and $\delta^{15}N$ found that feeding rates were instead highest in branching corals with smaller polyp sizes. This suggests more research is needed to disentangle coral morphology-feeding relationships. More recently, corals that were more **autotrophic**, implied by similarity in host tissue and symbiont **bulk** $\delta^{13}C$ and $\delta^{15}N$, had a negative relationship with polyp size, but also bleaching resistance, suggesting they may be more susceptible to increasing water temperatures (Conti-Jerpe et al. 2020).

While less conspicuous, the different genera within Symbiodiniaceae that form symbioses with corals can also influence host metabolism due to their differing functional responses to environmental conditions. For example, isotope labelling (^{13}C and ^{15}N) revealed that **holobionts** hosting former clade C (genus *Cladocopium*) have increased uptake rates of inorganic nitrogen under non-thermal stress conditions compared to **holobionts** with former clade D (genus *Durusdinium*) (Baker et al. 2013a). This can lead to the competitive exclusion of clade D under normal conditions despite this clade resulting in increased carbon acquisition during periods of thermal stress (Baker et al. 2013a). Similarly, Ezzat et al. (2017) demonstrated increased carbon acquisition by **holobionts** with clade C symbionts compared to clade A under low irradiance levels using isotope labelling (^{13}C and ^{15}N), but this appeared to be due to increased **heterotrophic** capacity by corals hosting clade C endosymbionts. However, other non-isotope studies did not find an influence of symbiont genus on host metabolism (Matthews et al. 2020). Endosymbiont communities do influence coral SI values though. Coral colonies dominated by clade D have lower $\delta^{13}C$ in both host and symbiont tissues compared to colonies dominated by clade C (clade C host and endosymbiont $\delta^{13}C$ 1.6‰ and 1.5‰ higher than clade D in summer), but these differences were inferred to be driven by light availability rather than coral feeding (Wall et al. 2020).

The strong focus on adult coral colonies can ignore the importance of vulnerable larval stages that support the maintenance of coral reefs through successful recruitment and ontogenetic development. In an effort to better understand planktonic coral larvae metabolism, Alamaru et al. (2009b) conducted feeding experiments with various potential food sources – phytoplankton, zooplankton, and bacteria – but failed to observe active feeding or **heterotrophic** uptake via $\delta^{13}C$ and $\delta^{15}N$ SI data. This is despite planulae larvae having an oral opening. They therefore inferred that coral larvae rely wholly on lipid reserves or photosynthates from symbionts that are already present in the endoderm. This was further elaborated by pulse-chase experiments using labelled ^{13}C-bicarbonate and ^{15}N-nitrate conducted by Kopp et al. (2016), who determined that there is minimal contribution by symbionts to host metabolism, demonstrating that coral larvae are essentially lecithotrophic pre-settlement.

Metabolism in non-hard coral symbioses

A significant number of SI studies have explored the metabolic underpinning of other non-coral symbiotic groups, revealing complex physiologies. Sponges possess an intricate symbiotic system due to the diversity of the microbiomes they can host, including photosymbionts (Davy et al. 2002, Weisz et al. 2010). However, the strength of the symbiotic dependency appears to vary between host sponge species, with both tight and weak metabolic couplings being observed (Freeman et al. 2015). Isotopically enriched seawater with ^{13}C and ^{15}N tracers has been used to demonstrate nutrient transfer between microbes and host sponges, the rate of which appears dependent on symbiont identity and irradiance rather than overall symbiont abundance (Freeman et al. 2013). Interestingly, labelled ^{15}N-ammonium and ^{13}C-bicarbonate revealed that coral-excavating sponges often host both Symbiodiniaceae and prokaryotic symbionts, with the former undertaking significant inorganic nutrient fixation and transfer to host bioeroding sponges, but the latter not contributing to nutrient assimilation (Achlatis et al. 2018). Conversely, ^{13}C- and ^{15}N-enriched DOM show that sponge hosts can directly take up and utilise DOM and subsequently transfer significant dissolved organic waste products to symbionts (Achlatis et al. 2019). This can constitute the entire nitrogen budget of hosted algae (Davy et al. 2002), in a similar fashion to coral hosts. Pulse-chase experiments with isotopically labelled ^{13}C- and ^{15}N-enriched coral- and algal-derived DOM show that various sources of DOM are utilised by sponges, but algal-derived sources are predominantly transferred to the microbiome while coral-derived DOM is used directly by the sponge host (Rix et al. 2017). Interestingly, **bulk** SI (δ^{13}C and δ^{15}N) and isotope labelling (^{13}C and ^{15}N) revealed that the encrusting sponge, *Terpios hoshinota*, which kills corals by overgrowing them, does so for space, not for food; in the new sponge tissues, only 9.5% of the C and 16.9% of the N was derived from the corals underneath (Syue et al. 2021). In contrast, contact association with macroalgae appears to competitively inhibit sponges, with SI enrichment experiments (^{13}C and ^{15}N) demonstrating nitrogen transfer from sponge to macroalgae (Easson et al. 2014). As with coral **holobionts**, changes in the surrounding environment can alter the dependency of sponge hosts on photoautotrophic symbiont production. Recent **bulk** and amino acid **CSIA** work in the Caribbean demonstrated increasing **heterotrophy** with depth for sponges, but highlighted species-specific trends in host utilisation of POM and DOM, and internal translocation of these (Macartney et al. 2020).

The host-symbiont relationship in gorgonians has been the focus of more recent interest in **holobiont** metabolism utilising SIs, likely due to their increasing presence in some coral reef systems (Rossi et al. 2020). While overall **autotrophic** reliance varies seasonally and with species, endosymbionts can deliver the majority of energetic demands (>95%), with heterotrophically acquired carbon sources contributing less than 5% year-round for gorgonian species examined in the Caribbean (Rossi et al. 2020). Conversely, gorgonians examined in Taiwan appear to be highly dependent on **heterotrophic** inputs based on host and symbiont δ^{13}C and δ^{15}N values, with relatively little energetic benefit garnered from their endosymbionts (Hsu et al. 2020). Similarly, recent work on Antipatharian soft corals (black corals) using isotope labelling (^{13}C and ^{15}N) has demonstrated that they can use a range of different food sources, likely allowing them to exploit seasonal fluctuations in prey concentrations (Rakka et al. 2020). These studies highlight the diversity in energy acquisition within this group. While environmental sensitivities to gorgonian symbioses have yet to be thoroughly explored, depth appears to have little impact on gorgonian tissue δ^{15}N values (change in only 1.4‰ over 20 m), implying limiting physiological effects associated with ambient light (Baker et al. 2011). Further, short-term (seven day) nutrient enrichment does not appear to significantly impair the symbiosis between gorgonians and their dinoflagellate endosymbionts, but can result in increased chlorophyll content and algal densities within hosts (McCauley & Goulet 2019).

Other forms of symbiotic relationships, while lesser studied, are likely commonplace on coral reefs and constitute important components to ecosystem functioning (Pinnegar & Polunin 2006). Anemones may also host *Symbiodinium* as a source of **autotrophic** nutrition; however, SI labelling

(^{13}C and ^{15}N) highlights the competitive nature of resource acquisition by both host and endosymbiont, implying a less stable symbiotic association compared to true coral **holobionts** (Radecker et al. 2018). Carbon and nitrogen SI data from both lab and field isotope labelling feeding experiments have been used to demonstrate nutrient transfer from anemonefishes to both fish-hosting anemones and their endosymbionts forming a tripartite symbiosis (Cleveland et al. 2011). Parmentier & Das (2004) examined relationships between four species of apparently parasitic carapid fishes and their echinoderm hosts using **bulk** SIs, but only found evidence for feeding on host tissues for one species (*Echeliophis gracilis*), suggesting commensal associations for the other three fishes. Similarly, while shallow-water black corals host a variety of macrosymbionts, **bulk** δ^{13}C and δ^{15}N reveal that they do not use their host as a main food source, but instead use the coral's structure to access nutrition from the water column (Terrana et al. 2019). True parasitic-host relationships have also been explored using **bulk** SIs in reef fish and gnathiid ectoparasites (Demopoulos & Sikkel 2015). Finally, upscaling from individual metabolic processes to whole community metabolic functioning can be facilitated through SI approaches. Spatial differences in isotopic discrimination of overlying seawater DIC are attributable to the varying ratio of calcification:primary production of the underlying ecological community, e.g. ~5‰ higher $\delta^{13}C_{DIC}$ discrimination for portions of the reef with more non-calcifiers compared to portions of the reef with more calcifiers (Koweek et al. 2019).

Trophic niches

Background

Ecological niches are multidimensional spaces defined by environmental conditions and resource utilisations that are occupied by an organism (or population) where their survival curves are optimised (Hutchinson 1957). The **trophic niche** relates to the array of food items consumed by an organism, which constitutes a subset of its overall ecological niche. Understanding an animal's resource use, and how this varies within and among species and guilds, helps determine its trophic function within an ecosystem and how this might respond to environmental change. Increasingly, studies are using SIs to estimate an animal's **trophic niche**. Isotope data are typically presented on a biplot using the isotope values (δ-values) as coordinates (Figure 3) (however, note that tri-isotope plots are now being used; Skinner et al. 2019a). The area (δ-space) of these coordinates is determined to be the animal's **isotopic niche**, providing an understanding of its trophic ecology by reflecting some aspects of their **trophic niche** (Figure 3) (Newsome et al. 2007). For example, the size of the **isotopic niche** and position of the individual coordinates indicate intraspecific variation in resource use, known as the niche width (Bearhop et al. 2004). SI approaches to **trophic niche** determination are the newest of our defined topics to emerge in SIs on coral reefs (first paper published in 2007), and it has expanded rapidly since then (n=48; Figure 1). This is likely due to the instrumental Layman et al. (2007) and Jackson et al. (2011) papers which have brought community SIA into the foreground. However, although related, **trophic niches** and **isotopic niches** are not interchangeable, and care should be taken when using these terms (see Reddin et al. 2018, Hette-Tronquart 2019). In some cases, variation in SI values may be independent of diet, e.g. where habitat-derived isotopic **baselines** differ (Figures 3 and 4). Organism foraging behaviour and habitat use must also be considered before converting an **isotopic niche** to an ecological or **trophic niche** (Flaherty & Ben-David 2010).

Isotopic niches

To date, coral reef **isotopic niche** studies have predominantly explored resource partitioning within guilds, likely as the high densities of species with seemingly similar functional roles raises questions as to the mechanisms of their coexistence. This can be elucidated by SI data, revealing dietary variation and intricacies which were previously overlooked. For example, while traditional techniques suggest that herbivorous surgeonfish and parrotfish consume similar **production sources** on reefs, **bulk** δ^{13}C and δ^{13}N SI data reveal complex trophic ecologies, indicating a high level of functional diversity (Plass-Johnson et al. 2013, Dromard et al. 2015). Similarly, **isotopic niche** overlap (see Figure 3) among sympatric spotted (*Panulirus guttatus*) and Caribbean (*P. argus*) spiny lobsters is minimal, with each utilising different food sources and occupying unique **TPs** (evidenced by δ^{15}N) in the food web (Segura-García et al. 2016). Higher up the food chain, coral reefs have high biomasses of predators with seemingly similar traits and trophic ecologies. Yet, δ^{13}C and δ^{13}N reveal that sympatric species of coral trout *Plectropomus laevis* and *P. leopardus* have resource uses that differ (Matley et al. 2017, 2018). They likewise also vary from other predatory teleosts (*Lethrinus miniatus* and *Lutjanus carponotatus*), implying degrees of trophic specialisation in coral reef mesopredators (Frisch et al. 2014). Indeed, in the Maldives, the resource uses of seven sympatric teleost reef predators across multiple families vary both within and among species and spatially, with δ^{34}S acting as a useful third isotope further differentiating individual feeding behaviours (Skinner et al. 2019a). Furthermore, on Bahamian reefs, while isotope biplot data (δ^{13}C and δ^{15}N) of the invasive lionfish (*Pterois* spp.) and a native snapper *Lutjanus griseus* appeared to overlap considerably, their core **isotopic niches** did not, suggesting competition was not as high as initially perceived (Layman & Allgeier 2012). These predators appear to occupy the same functional roles within their guilds, but by partitioning trophic resources both spatially and temporally, inter- and intraspecific competition is likely reduced, thereby facilitating their coexistence and altering previously assumed ecological roles (Dale et al. 2011, Gallagher et al. 2017, Matich et al. 2017, Curnick et al. 2019, Skinner et al. 2019a).

Individual specialisation within a population is a mechanism through which sympatric individuals within an age or size class may reduce competition for resources by focusing on a narrower set of resources than that of the population as a whole (Bolnick et al. 2003, Araújo et al. 2007, 2011). Occurrences of individual specialisation are expected to be greater where resource diversity is high, as there is increased ecological opportunity (Semmens et al. 2009, Araújo et al. 2011). Coral reefs, with their high rates of biodiversity, should therefore be prime locations for occurrences of individual specialisation. This is compounded for populations with access to two or more adjacent resource pools (e.g. benthic and pelagic) or habitats (e.g. reef and seagrass), as there is a greater array of potential resources (Araújo et al. 2011, Matich et al. 2019). SIs are a powerful tool which can quantify these specialisations and are less costly and labour-intensive than long-term dietary records (Newsome et al. 2009). To date, studies investigating individual specialisation using SI on coral reefs have concentrated on elasmobranchs and large predatory teleosts (Shipley et al. 2018, Shiffman et al. 2019, Skinner et al. 2019a, Wyatt et al. 2019). The degree of specialisation across lower trophic levels is little explored. However, 34% of SI coral reef studies involve a single species, which suggests there is sufficient opportunity to investigate individual specialisation more generally on coral reefs. Indeed, there is some evidence of such specialisation at lower trophic levels; **bulk** δ^{13}C and δ^{13}N SI show that damselfish, *Dascyllus aruanus*, are more specialised when colonies are larger, suggesting local abundances drive intra-group competition, which is then modulated by individual specialisation (Frédérich et al. 2010). Groups of damselfish with narrower **trophic niches** (evidenced by their **isotopic niches** using δ^{13}C, δ^{13}N, and δ^{34}S) also have lower genetic diversity, highlighting potential links between population and trophic ecology (Gajdzik et al. 2018). Exceptional variation in feeding strategies from **heterotrophy** to **autotrophy** at scales of metres to kilometres among colonies of the hard coral *Pocillopora meandrina* has also been identified using δ^{13}C of essential amino acids, showing no relationship with site or depth (Fox et al. 2019). Although consumer dietary specialisation does not occur in all systems or between all species (Gallagher et al. 2017), it warrants further study, particularly in systems where resources are fluctuating. If consumers partition resources, their ecological roles may be vastly different, which could be masked by traditional species- or guild-level categorisations.

Attempts by ecologists to categorise species functional traits to better understand ecosystem function conflicts with the natural variability inherent in complex systems such as coral reefs. SI data have successfully been used to refute some strict dietary classifications of reef organisms derived from traditional ecological studies using gut contents data and in situ observations. For example, in Papua New Guinea, of seven damselfish species sampled, their δ^{13}C and δ^{15}N SI values and corresponding **isotopic niches**, indicated that none were strict herbivores, despite traditionally being classified as such (Eurich et al. 2019). In fact, using δ^{13}C and δ^{13}N damselfish have relatively distinct **isotopic niches,** reflecting varying degrees of planktonic to benthic reliance (Frédérich et al. 2009, Lepoint et al. 2016, Olivier et al. 2019). Similarly, cardinalfishes, thought to be generalist carnivores based on stomach contents data, are sustained by **production sources** from across a benthic-planktonic gradient, and δ^{13}C and δ^{13}N indicate distinct species-specific **isotopic niches** (Frederich et al. 2017). Indeed, community-wide δ^{13}C and δ^{15}N data show that many strictly categorised reef fish rely on **production sources** outside their putative diet source and this spans multiple trophic levels (Zhu et al. 2019), highlighting how narrow functional group categorisations should be applied with caution. However, care should be taken when interpreting isotopic spread as solely due to diet variation; spatiotemporal differences in **production source** isotopic **baselines** and a myriad of other drivers may also cause such variations (Figures 3 and 4; and see 'Environmental drivers').

In some cases, there may be greater redundancy among different guilds rather than within them; δ^{13}C and δ^{15}N SI values of muscle tissue from reef sharks and large teleost mesopredators indicated that they occupy the same **isotopic niche** on the Great Barrier Reef, suggesting they perform similar functional roles (Frisch et al. 2016). Similarly, in the Hawaiian archipelago, there was considerable overlap in the δ^{13}C and δ^{15}N values of reef sharks and larger teleosts, highlighting a degree of functional redundancy which could contribute to ecosystem stability (Hilting et al. 2013, Roff et al. 2016). However, a study further north on the Great Barrier Reef using multi-tissue δ^{13}C and δ^{13}N data (muscle, blood, and plasma) to compare resource use of reef sharks and large predatory teleosts identified significant trophic separation (Espinoza et al. 2019). While Frisch et al. (2016) call for apex predator reef sharks to be reassigned as high-level mesopredators, Espinoza et al. (2019) suggested there is substantial diversity in their trophic ecologies and likely wider functional roles. This suggests that different localities may have populations with different behaviours. Beyond these studies of reef mesopredators, few studies compare **isotopic niches** among differing guilds on coral reefs, likely because they are expected to have different dietary strategies. For example, overall, the structure of the reef fish assemblage remains fairly consistent, with herbivores occupying lower trophic levels (reflected by low δ^{15}N), while carnivores and piscivores feed across a broader range of trophic levels and resources (Carassou et al. 2008, Yang et al. 2012). Most studies comparing niches and categorisations do so across similar species groups; of the 47 articles assigned to the **Trophic Niche** topic, 64% focus on reef fish and 15% on elasmobranchs. Few, if any, compare resource use across broader groupings (e.g. some invertebrates may feed on the same prey as some reef fish; Zapata-Hernández et al. 2021), so wider competitive feeding relationships may be missed.

Multi-tissue SI data can reveal detailed feeding strategies and how these fluctuate over different timescales, particularly regarding individual specialisation within a population (Bond et al. 2016). For example, a multi-tissue (plasma, cartilage and faeces), multi-isotope approach identified consistent individual specialisation on either oceanic or coastal prey in whale sharks, *Rhincodon typus* (Wyatt et al. 2019). Yet, multi-tissue approaches are infrequently utilised on coral reefs. Of the 29% of identified SI reef studies employing a multi-tissue approach, only 9% measure multiple whole tissue types, e.g. muscle and liver, of the same individual consumer to elucidate short- and longer-term fluctuations in resource use. Instead, 20% of multi-tissue studies focus on exploring the **trophic niches** of **mixotrophic holobionts**, e.g. host soft tissue and endosymbionts. For example, the degree of overlap in the **isotopic niches** (determined using **bulk** δ^{13}C and δ^{15}N) of seven different genera of coral hosts and their endosymbionts was linked to coral trophic strategy; higher overlap indicated resource sharing between host and endosymbionts (i.e. greater host **autotrophy**), while lower overlap indicated less resource sharing (i.e. greater host **heterotrophy**) (Conti-Jerpe et al. 2020). While multi-tissue analyses may offer additional insight, comparing **isotopic niches** among guilds helps in the understanding of how resource use and partitioning are structured within the wider community. One of the few studies using SIA to investigate trophic interactions at the community level confirmed previous hypotheses that the sea cucumber *Stichopus herrmanni* has a top-down influence (consistently higher δ^{15}N across seasons) on its meiobenthic prey in a lagoon system on the Great Barrier Reef (Wolfe et al. 2021).

For inferences to be made about resource use and **trophic positions** with confidence, knowledge on **diet-tissue discrimination factors** (hereafter 'trophic discrimination factor' (**TDF**), usual notation is Δ) between a consumer and their diet are necessary (see reviews referenced in 'Introduction'; Boecklen et al. 2011, McCormack et al. 2019, Whiteman et al. 2019). The standard **TDFs** that are typically applied to **bulk** carbon (~0.5) and nitrogen (~3.4) are averages drawn from broader syntheses of laboratory experiments, but are known to vary among species, by life stage, and season, among other factors (Wyatt et al. 2010a, 2019). For example, the estimated Δ^{15}N between the hard coral *Porites lutea* and its food source is only 1‰ (Rangel et al. 2019), three times lower than the commonly applied value. Average **TDF** values may be appropriate for studies at the ecosystem level, but greater resolution specificity is required for individual species. To date, very few studies ($n \approx 5$) have empirically determined species-specific **TDF** for coral reef organisms, despite a call for more lab studies to do so over a decade ago (Wyatt et al. 2010a). Evidence suggests that typically employed **TDFs** may not be wholly applicable to coral reef ecosystems: feeding observations and modelling indicated that three herbivorous reef fish have consistently higher Δ^{15}N (model estimates ranged from 4.30‰ to 5.68‰) than the accepted value of 3.4‰ (Mill et al. 2007). Furthermore, a comprehensive study using **CSIA** of >200 samples from 47 species of marine teleosts including many reef fish found that the estimated **TDF**$_{AA}$ was significantly lower (2‰) than previously accepted values for **CSIA** (Bradley et al. 2015). Given the huge diversity of organisms on coral reefs, a greater understanding of how these values vary among species and compounds, and with other factors such as seasonality, is required (Whiteman et al. 2019) to improve the understanding of coral reef trophic interactions. One such avenue might involve parasites (an understudied group on coral reefs), which often reflect their host's feeding ecology and **TP** (indicated by their δ^{13}C and δ^{15}N values) (Jenkins et al. 2018). This suggests future research could explore this relationship to better understand the complex trophic interactions and feeding strategies that could affect reef organism's **TDF** values, but SI data between fish hosts and their parasites are likely to be complicated (Pinnegar et al. 2001).

As time-integrated values of the major **source** reliance and **TP** of an organism in the food web, SI data cannot generally be used to identify specific prey. For example, while **bulk** δ^{13}C and δ^{15}N values were similar across four species of mesopredatory teleosts, stomach contents data revealed substantial differences in the types of prey taxa consumed (Ashworth et al. 2014). Similarly, among 21 species of butterflyfish, gut contents data and in situ feeding observations were required to resolve finer-scale differences in prey items (Nagelkerken et al. 2009). SIs are therefore not necessarily suitable at differentiating ecological niche differences that vary over smaller scales, especially when isotopic differences between sources are small. SIs are also unable to distinguish between behavioural differences in feeding: two sympatric species of fish that have approximately the same diet, but with one feeding nocturnally while the other diurnally, would occupy the same position in isotopic space (given the same **TDF**s). It is therefore often useful to combine multiple methodologies to obtain a comprehensive overview of the complexities of an organism's feeding strategies across different temporal and spatial scales on coral reefs. As researchers become more accustomed to using SI data to understand food webs, the number of **isotopic niche** studies on coral reefs is increasing, but other data, including traditional techniques, often complement and add to the inferences deduced from SI data alone (e.g. inclusion of fatty acids; see Dethier et al. 2013).

Drivers of isotopic niche variation

Isotopic niches may vary spatially, particularly in diverse systems such as coral reefs, helping to explain how species partition resources (Figure 3). This variation can occur across relatively large scales: across the Southern Line Islands, **isotopic niche** widths (from $\delta^{13}C$ and $\delta^{15}N$) of reef fish populations change in relation to available primary production (Miller et al. 2019). They may also change at much smaller scales: **bulk** $\delta^{13}C$ and $\delta^{15}N$ data show that several reef shark species have different patterns of resource use among coastal areas of Florida (Shiffman et al. 2019) and dolphins have varying levels of coastal vs offshore resource use in Panama (Barragan-Barrera et al. 2019). While **isotopic niche** variation can be driven by factors other than dietary preference (Figure 3), understanding the relationships between SI values and these drivers is non-trivial. For example, predator biomass influences the dietary diversity (represented by the $\delta^{13}C$ and $\delta^{15}N$ **isotopic niche** area) of two herbivorous reef fish in the Florida Keys, likely by causing a change in their group foraging activities and perceived predation risk (Catano et al. 2014). Other biotic influences on **isotopic niches** include the presence of parasites: ectoparasitic isopods affect the resource use (inferred through $\delta^{13}C$ and $\delta^{15}N$) of grunts, likely causing them to feed in different localities to uninfected fish, and changing their condition (Welicky et al. 2017). On reefs in New Caledonia, partitioning of resources indicated by **bulk** $\delta^{13}C$ and $\delta^{15}N$ data by two species of sea krait indicated habitat-based dietary divergence (Brischoux et al. 2010). Environmental conditions may also influence **isotopic niches**: various flow conditions led to differing physiological adaptations of two sympatric bonefish species based on $\delta^{13}C$ and $\delta^{18}O$ data (Haak et al. 2018). These studies highlight how a range of factors may influence an organism's **isotopic niche** at any one time and underline the inherent difficulty in extrapolating findings from one location to another. Other factors which may influence **isotopic niches** include growth rate, metabolism, and diet quality; however, to our knowledge, there are no studies investigating these relationships on coral reefs to date. With few studies investigating the effect of multiple variables or drivers, or the indirect relationships occurring between them, the ability to generalise such patterns across coral reef ecosystems is limited.

Anthropogenic impacts can influence **isotopic niches** and resource use, as habitats degrade or prey groups fluctuate, but such responses vary among species. For example, corallivores had smaller $\delta^{13}C$ and $\delta^{15}N$ **isotopic niches** at a degraded reef site compared to a healthy reef site, likely reflecting a narrower pool of available resources from a loss of live coral cover (Letourneur et al. 2017). In contrast, invertivores had larger **isotopic niches**, and herbivores and zooplanktivores displayed no difference (Letourneur et al. 2017). Similarly, $\delta^{15}N$ in spiny lobster muscle tissue did not differ between reefs of different levels of degradation, with no apparent trend with the loss of habitat structural complexity (Lozano-Álvarez et al. 2017). The impact of reef habitat degradation on organic matter sources and the trophic ecology of reefs in the Caribbean (where 14% of SI reef studies have been conducted; Figure 2) was quantified by Morillo-Velarde et al. (2018), on the basis of switches or widening in carbon sources as evidenced by reef fish $\delta^{13}C$ in degraded habitats. This indicates that there is a degree of trophic plasticity, with some groups adapting to degradation while others do not, but there may be a cost to this adaptation. As the biomass of planktonic damselfish decreased on a degraded reef, $\delta^{13}C$ and $\delta^{15}N$ of the spotted coral grouper, *Plectropomus maculatus*, indicated it switched to foraging on herbivorous damselfish. This switch was linked to long-term declines in the groupers' growth rate, fecundity and survivorship (Hempson et al. 2017, 2018), highlighting potential negative, long-term consequences of anthropogenic impacts across the wider coral reef food web.

Fish diet variation and habitat connectivity

Background

In coral reef SI studies, reef fish are the dominant focal taxa (36% of all articles; Figure 5B) and a major area of research involves using SIs to determine how their habitat and resource use varies spatially and throughout their life cycle ($n=44$ identified studies; Figure 3). Although this topic includes one of the earliest known papers using SIs on coral reefs (Fry 1982), much of the **bulk** of the work on fish trophodynamics was conducted from the late 2000s onwards, with a focus on the western

Atlantic ($n=16$) (Figure 2B). While there are thousands of fish species living on coral reefs, only a few have been the focus of multiple SI studies, namely the whitetail damselfish *Dascyllus aruanus* ($n=5$), leopard coral grouper *Plectropomus leopardus* ($n=6$), blackspot snapper *Lutjanus ehrenbergii* ($n=5$), and the red lionfish *Pterois volitans* ($n=5$). Similarly, coral reef SI studies tend to focus on common groups (e.g. damselfish, surgeonfish, groupers, snappers), with few to none investigating the trophodynamics of poorly understood and often cryptic groups such as blennies and gobies.

Ontogenetic habitat shifts

Ontogenetic habitat shifts are common in marine organisms as they strive to maximise their fitness strategies (Schmitt & Holbrook 1985, Holbrook & Schmitt 1988, Dahlgren & Eggleston 2000). In coral reef ecosystems, it is common for juvenile reef fishes to spend time in a nursery habitat, such as mangroves or seagrass meadows, prior to migrating to the reef (Figure 3). SIA can be a cost-effective tool to pinpoint when and how these changes occur by analysing the SI values of juveniles through to adults across the nursery and reef habitats. This allows the timing of the ontogenetic habitat and diet shifts to be estimated (Cocheret de la Morinière et al. 2003, Frédérich et al. 2012, McMahon et al. 2012, Berkström et al. 2013). For example, the δ^{13}C of juvenile snappers in mangroves reflect mangrove habitat (–23‰ to –17‰), while individuals on the reef shift to a reef δ^{13}C signature with increasing body size (–16‰ to –8‰), indicating that the smaller individuals within reefs have migrated there from the mangroves (Nakamura et al. 2008). Acoustic telemetry can be used to further support inferences from SI data; δ^{13}C of fin tissue and acoustic tracking of the schoolmaster snapper, *Lutjanus apodus*, revealed that they move from bays to coral reefs as they get larger (i.e. smaller fish mean δ^{13}C –16.7‰, larger fish –12.2‰) (Huijbers et al. 2015).

Fish otoliths, as an incrementally grown, metabolically inert biological structure, provide the ability to determine when ontogenetic shifts occur within a single individual, and have been utilised across several coral reef SI studies ($n=13$). As surface otolith material is continuously deposited with age, a time series of SI data can be derived by sampling progressive segments from the core (reflecting larval life stages) to the edge (age of the fish just prior to otolith sampling). Changes in SI values will reflect potential changes in habitat, diet, or physiology. In the Red Sea, otolith δ^{13}C essential amino acid values of the blackspot snapper, *Lutjanus ehrenbergii*, across a gradient from coral reefs to seagrass were influenced by the habitats the fish resided in, offering an opportunity to track their movements across the isoscape (McMahon et al. 2011). In addition to δ^{13}C, some studies (6%; Figure 2B) use oxygen isotopes (δ^{18}O) in otolith segments (Blamart et al. 2002), as they vary with temperature and salinity, thus providing information on the external environment. Both δ^{13}C and δ^{18}O in otolith core and edge segments of the trumpet emperor, *Lethrinus miniatus*, on the Great Barrier Reef revealed contrasting movements of juveniles across different latitudes and corresponding isotopic environments (Currey et al. 2014). Similarly, δ^{13}C and δ^{18}O of grey, *Lutjanus griseus*, and yellowtail, *Ocyurus chrysurus*, snapper sub-adult otoliths in Florida determined the distinct nursery areas in the Florida Bay that the fish had migrated to the reef from (Gerard et al. 2015).

Otolith SI values can be incorporated into spatial simulation models, revealing that the geographic distribution of the nursery areas plays an important role in driving the spatial distribution of the adults on the reefs (Hujibers et al. 2013). Recently, fish eye lenses have also been advocated as an alternate, metabolically stable, incrementally grown structure with which to track resource and habitat use shifts with SIs across different life stages (Quaeck-Davies et al. 2018, Curtis et al. 2020, Vecchio and Peebles 2020). Clearly, SI data can be used to delineate fish movement patterns, tracking animal movements in the absence of electronic tagging, and identifying nursery habitats that should be prioritised for management; habitat impacts may disproportionately affect certain life stages thus influencing populations elsewhere, e.g. recruitment of juveniles to reefs. However, if the animals move through the habitat before an isotopic signature can be recorded, or if the habitats are isotopically indistinguishable, then movement information may be missed (McMahon et al. 2013). As such, the use of traditional approaches in conjunction such as tagging (e.g. Huijbers et al. 2015), spatial simulation (e.g. Hujibers et al. 2013), and fish size distribution modelling (e.g. Mumby et al. 2004) should be considered.

Body size

Multiple studies indicate that variation in resource use may occur as a function of body size (e.g. Layman et al. 2005, Romanuk et al. 2011). Indeed, many predatory fishes across various biomes tend to feed at higher **TP** (evidenced by higher δ^{15}N) as body size increases, a pattern that also holds true for coral reef systems (Layman et al. 2005, Greenwood et al. 2010). For example, in Moorea, French Polynesia, plasma of blacktip reef sharks, *Carcharhinus melanopterus*, was enriched in δ^{15}N with increasing body size, but there was no change in δ^{13}C, suggesting they were feeding on higher trophic-level prey, but reliant on the same basal **production sources** (Matich et al. 2019). Such trends are, however, far from ubiquitous in coral reefs. For blacktip reef sharks at Palmyra Atoll, there was a positive relationship between body size and δ^{15}N in one lagoon, but no relationship with body size at all in another, suggesting that the two shark populations have different trophic ecologies despite their proximity to one another (Papastamatiou et al. 2010). Similarly, in the Caribbean reef shark *Carcharhinus perezii*, there was no relationship between δ^{15}N and body size, but a significant positive relationship with δ^{13}C, implying larger individuals relied more on lagoonal food sources with increasing size rather than feeding at higher trophic levels (Bond et al. 2018). Clearly, relationships between predator **TP** and body size are not always positive or absolute (Gallagher et al. 2017, Matley et al. 2017, Shipley et al. 2018, Skinner et al. 2019a, Eddy et al. 2020). This is demonstrated by the invasive lionfish in the Caribbean, which are smaller than native Nassau groupers, yet occupy the highest **TP** in the study region (represented isotopically by higher δ^{15}N) (O'Farrell et al. 2014). These studies highlight how trophic ecology of fishes can vary with body size, indicating that an organism's ecological role may be complex and life stage- as well as species-specific. However, for most herbivorous reef fish, **TP** (represented by δ^{15}N) remains relatively unchanged as body size increases (Cocheret de la Morinière et al. 2003, Greenwood et al. 2010, Plass-Johnson et al. 2013, 2015a). This is likely as, although they may access different resources with increasing body size, the δ^{15}N of these basal **production sources** remains fairly similar.

Despite being lesser studied, similar trends with body size have also been observed in invertebrates. The fireworm, *Hermodice carunculata*, a facultative corallivore, is enriched in δ^{15}N and has fluctuating δ^{13}C with increasing body size, potentially reflecting feeding at higher trophic levels while diversifying resource use across ontogeny (Wolf et al. 2014). It should be noted, however, that body size relationships with SIs assume there is minimal change in isotopic **baselines** and growth/metabolic influences on isotopes independent of diet (e.g. constant **TDF**s across ontogeny). Intraspecific and life-stage feeding specialisations, such as these, may help promote population resilience to environmental change, as individuals and populations are reliant on a wider range of resources across ontogeny. However, given that many of the anthropogenic impacts on reef fishes are size- and species-selective, with many targeted fish often larger and functionally important (Benoît & Swain 2008, Lokrantz et al. 2009, Plass-Johnson et al. 2015a), this may have serious consequences for the trophodynamics of the coral reef food web.

Residency and population connectivity

SIA can reveal an organism's residency within a habitat at various life stages, representing a more cost-effective approach than tagging or tracking. This can be useful for the management of certain species when assessing the efficacy of protected areas. In Australia, δ^{13}C and δ^{15}N values of reef fish liver and muscle revealed three species were resident in the area, while others had migrated from coastal riverine habitats (Davis et al. 2015). In western Australia, $\delta^{13}C$ and $\delta^{15}N$ of muscle tissue of several reef sharks in coastal habitats confirmed residency was high across four species (Speed et al. 2012). Examination of $\delta^{13}C$ and $\delta^{15}N$ in lionfish, *Pterois volitans*, muscle tissue showed they did not move between mangroves and reefs; in fact, there was no overlap in habitat or resource use of lionfish between habitats, confirming them as site-attached opportunistic foragers (Pimiento et al. 2015). SI data can also help assess the importance of different habitats to more mobile consumers that have larger home ranges; for example, one of the few studies to use $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ to examine habitat residency determined that the reef manta ray, *Manta alfredi*, was heavily dependent on lagoonal resources, suggesting long periods of residency in the lagoon (McCauley et al. 2014). Combining SI data with fatty acids further revealed that *M. alfredi* are secondary consumers that rely on both epipelagic and demersal zooplankton, reflecting their ability to access disparate resources through vertical and horizontal movements (Couterier et al. 2013). While SI data can provide much needed insight into movement patterns and habitat usage, it should be noted that **connectivity** ultimately depends on the spatial configuration of the seascape (Nagelkerken et al. 2008, Rooker et al. 2018).

Larval population dynamics are inherently more difficult to study; larvae are hard to follow due to their high natural mortality and rapid dispersal by ocean currents (Cowen & Sponaugle 2009). Nevertheless, SI approaches provide an opportunity to track larval dispersal and understand population **connectivity** more easily. Transgenerational isotope labelling involves injecting adult female fish with labelled isotopes (^{137}Ba) and results in consistently and permanently marked larvae throughout a reproductive season. This method has minimal impact on the fish, their eggs and larvae as they develop, or on those that consume them (Williamson et al. 2009a,b, Roy et al. 2012, Cuif et al. 2014). By analysing the SI values of the otolith cores of the new cohorts, the degree of **connectivity** and self-recruitment within a population can be determined. In New Caledonia, this approach revealed that self-recruitment of damselfish *Dascyllus aruanus* varied significantly between months and years, but was independent of the proportion of self-recruits within the population (Cuif et al. 2015). This suggests that self-recruitment can successfully indicate population openness, but may not relate to population persistence (Cuif et al. 2015).

Habitat connectivity

There are important exchanges of organisms and energetic material between coral reefs and other adjacent habitats that can be difficult to measure (Polis & Strong 1996, Huxel & McCann 1998). SI data offer distinct opportunities to identify these cross-system linkages and quantify fluxes across habitat boundaries; the flow of nutrients into and across shallow coral reef ecosystems is increasingly being documented (Figure 3). In shallow-water reef habitats in the Caribbean, $\delta^{13}C$ suggests that benthic algae and seagrass contribute 48%–76% of carbon to reef fish (Fry et al. 1982), but in Moreton Bay, in eastern Australia, dietary proportions vary with distance to adjacent mangrove and seagrass habitats (Davis et al. 2014). Such patterns are not necessarily surprising; however, they highlight that contributions of various exogenous materials to reefs are likely to be site-dependent due to varying seascapes (Briand et al. 2015). Nevertheless, looking at the wider spatial context can reveal less intuitive habitat linkages. In the Chagos Archipelago, rats interrupt nutrient flows between pelagic, coral reef, and island ecosystems. Rat-free islands had significantly greater densities of seabirds and therefore larger deposits of nitrogen and subsequent runoff, leading to higher $\delta^{15}N$ in the soil, macroalgae, turf algae and reef fish compared to rat-infested islands where seabird densities were lower (Graham et al. 2018). Sulfur isotopes ($\delta^{34}S$) have rarely been applied to infer habitat **connectivity**, but they may be well placed to distinguish and identify sources produced under anaerobic conditions, e.g. decomposition of mangrove organic matter, due to the strong fractionations that occur during such processes (Okada & Sasaki 1998, Granek et al. 2009). More widely, mangrove, microalgae, macroalgae, and seagrass exhibited greater separation in $\delta^{34}S$ compared to $\delta^{13}C$ in a study conducted in Bocas del Toro (Panama) (Granek et al. 2009). Used together, $\delta^{34}S$ and $\delta^{13}C$ suggested that mangrove-coral reef nutrients contributed up to 57% to the biomass of sessile reef invertebrates (Granek et al. 2009). SI data thus point to mangroves as an important source of nutrients for adjacent reef consumers (Carreón-Palau et al. 2013, Briand et al. 2015) despite their apparent low labile organic matter content and nutritional quality (Granek et al. 2009).

While common in the deep sea, chemosynthesis has rarely been explored as a potential source of nutrient exchange for shallow water marine food webs (Table 3), yet SI data point to chemosynthesis being important for some consumer species on coral reefs. $\delta^{34}S$, together with $\delta^{13}C$ and $\delta^{15}N$, provides evidence that chemosynthesis in lucinid clams supports 20% of the diet of Caribbean spiny lobster (Higgs et al. 2016). They therefore play an important part in transferring the chemosynthetically fixed carbon into reef food webs (Higgs et al. 2016). While there are undoubtedly many energetic connections between coral reefs and adjacent habitats, the intricacies of many have yet to be fully identified. This should be addressed going forward given that such subsidies are expected to contribute to wider ecosystem resilience and stability (Bascompte et al. 2005).

Predators, typically being more mobile, often have greater opportunity to feed across ecosystem boundaries, playing an important ecological role in connecting distinct food webs (Figure 3). While tracking studies can identify these predator movements (e.g. Papastamatiou et al. 2009, Heupel & Simpfendorfer 2015), they cannot in isolation define the role of predators in nutrient cycling. This is in contrast to SI data that offer an opportunity to track these energetic linkages. In Palmyra atoll, $\delta^{13}C$ and $\delta^{15}N$ from muscle tissue of blacktip reef sharks, *Carcharhinus melanopterus*, grey reef sharks, *C. amblyrhynchos*, and red snapper, *Lutjanus bohar*, indicated they relied on pelagic **production sources** from outside their primary fore reef habitats, playing a key role in providing ecological coupling as cross-system foragers (McCauley et al. 2012). The reef manta ray, *Manta alfredi*, also constructs an important link between adjacent pelagic and reef/lagoonal systems (evidenced by $\delta^{13}C$ and $\delta^{15}N$) by feeding on pelagic zooplankton, which is then excreted over shallow reefs (Peel et al. 2019). In fact, many reef predators are similarly subsidised by pelagic inputs (evidenced by $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$), likely arising through their feeding on reef-based planktivorous fish, suggesting they play an important role in reef-pelagic **connectivity** (Frisch et al. 2014, 2016, Matley et al. 2018, Skinner et al. 2019b).

Energetic connections across depth ranges have also been identified using $\delta^{13}C$, $\delta^{15}N$ and $\delta^{15}N_{AA}$ in combination with acoustic telemetry. Galapagos sharks, *Carcharhinus galapagensis*, and giant trevally, *Caranx ignobilis*, forage in both shallow- and deep-water mesophotic reef habitats, transporting nutrients between them (Papastamatiou et al. 2015). In some cases, these energetic linking movements may be nocturnal. $\delta^{13}C$ and $\delta^{15}N$ data combined with acoustic telemetry revealed that the grunt, *Haemulon plumierii*, in the Caribbean is predominantly sustained by organic matter from habitats they visit at night, likely to minimise encounters with barracuda (Rooker et al. 2018). These studies highlight the potential for SIs to complement and enhance inferences from acoustic telemetry or tracking data, by providing detailed information on energy fluxes which may help elucidate organism movements. Although SI studies documenting cross-system linkages are increasing, only 13% of the studies in this topic have focused on sharks, despite their acknowledged important role in nutrient transfer (Williams et al. 2018b). There is therefore much that remains to be understood about energy subsidies to and from coral reefs, particularly the generality of the role that these mobile organisms play in establishing them.

Production source use in coral reef consumers can be difficult to define compared to other, simpler systems with fewer energy pathways. A significant overlap between the myriad of reef-associated sources (Figure 4; Table 3; and see ‘Organic matter dynamics’) often makes it impossible to clearly identify, for example, carbon sources over a reef using **bulk** $\delta^{13}C$ data. As such, isotope modelling to confidently assign consumers to energy sources in such ‘underdetermined’ systems becomes impractical (Fry 2013). The use of **CSIA** of amino acids (**CSIA**-AA), particularly essential amino acid carbon isotopes ($\delta^{13}C_{EAA}$), which exhibit minimal discrimination between resources and consumers across the food web, has great potential to help resolve carbon resources over reefs due to the increased dimensionality of the data. This technique effectively separates benthic versus planktonic pathways, further separating the latter into distinct nearshore reef-associated plankton and offshore pelagic plankton groups (Skinner et al. 2021). Analysis of consumer tissue $\delta^{13}C_{EAA}$ allows the source of primary productivity supporting consumers to be identified with more precision than is possible with **bulk** $\delta^{13}C$. Less consideration of variations in isotopic **baselines** and **TDFs** is required when interpreting $\delta^{13}C_{EAA}$ data, which can often confound inferences from **bulk** SI data. Surprisingly, $\delta^{13}C_{EAA}$ data suggest that even highly mobile top predators may rely predominantly on a single carbon source at the base of the food web. For instance, in the Red Sea, $\delta^{13}C_{EAA}$ data suggested that the snapper, *Lutjanus ehrenbergii*, and giant moray, *Gymnothorax javanicus*, may receive >70% of their C from a single end-member, phytoplankton, indicating quite tightly linked food chains supporting these predators (McMahon et al. 2016). Similarly, in the Maldives, several species of grouper were primarily supported by offshore pelagic plankton across an oceanic atoll (73%–86%; Skinner et al. 2021). Contrasting $\delta^{13}C_{EAA}$ within more wide-ranging apex predators like the tiger shark, *Galeocerdo cuvier*, could provide important information on the role of mobility in integrating across ocean and reef food webs (Hilting et al. 2013, Frisch et al. 2016).

Environmental drivers

Background

It is intuitive following the identification of spatial and temporal patterns in data for scientists to try and elucidate the underlying processes and mechanisms that give rise to them. Despite the complexity of coral reefs, SI-based studies focusing on particular components of these ecosystems often infer the key drivers at play that underpin observed trends. This is evident in all of the preceding topics, where studies attribute the importance of inherent environmental and biological factors (see, for example, ‘Organic matter dynamics: spatial and temporal variations’ and ‘Holobiont metabolism: drivers of mixotrophy’). However, there are a collective of SI studies that specifically investigate the general role spatial and temporal forcings play on coral reefs, providing context for other studies utilising SI approaches ($n=48$ identified topics). These environmental drivers can be broadly separated into two categories: natural drivers and anthropogenic drivers (Figure 3). The first category investigates how the isotopic composition of various components of coral reef ecosystems responds to a range of natural variables such as depth, resource availability, and salinity. Hard coral ($n=17$) and reef fish ($n=5$) are the dominant focal taxa in this regard, with most studies conducted in the Caribbean ($n=10$), the Red Sea ($n=6$), or the Central Pacific ($n=6$) (Figure 2B). The second category almost exclusively focuses on measuring SIs in structure forming organisms, that is marine plants ($n=9$), soft corals ($n=6$), and hard corals ($n=6$), to explore anthropogenic nutrient inputs to coral reef communities, but also considers the impacts of thermal stress. These are conducted mostly in the Caribbean ($n=7$) or the western Pacific ($n=6$) (Figure 2B).

Natural drivers

Environmental conditions and nutrient availability fluctuate across both fine and broad spatial and temporal scales. Much like those of the **holobionts** (see ‘Holobiont metabolism’), primary producer and higher consumer SI values may reflect this variability (Figure 4; Tables 3 and S6), as organisms alter their resource use accordingly and/or integrate isotopic differences in production **baselines**. For example, gradients in resource availability exist across coral reef habitats depending on their proximity to open ocean resources (Wyatt et al. 2013). Primary producers have higher $\delta^{15}N$ **baseline** values on outer reefs compared to lagoon regions (Page et al. 2013), and many reef fish are increasingly reliant on oceanic nutrients with proximity to the open ocean (Wyatt et al. 2012b, Gajdzik et al. 2016, McMahon et al. 2016). In New Caledonia, $\delta^{13}C$ provided clear evidence of spatial changes in the primary sources of carbon across a reef habitat, with increased oceanic inputs on the outer slopes (grouped teleosts mean $\delta^{13}C$ –17.9‰) compared to increased **internal** subsidies in the lagoon (grouped teleosts mean –14.8‰) (Le Bourg et al. 2017). Interestingly, $\delta^{15}N$ data did not demonstrate any differences in food chain length or trophic level, supporting the idea of consistent food web structure across the reef scale despite differences in **production sources** supporting that structure (Le Bourg et al. 2017). At a regional spatial scale, Zgliczynski et al. (2019) similarly did not find strong isotopic evidence of shifts in foraging patterns in a range of species and functional groups across the central Pacific. Isotopic changes were attributed to **baseline** changes across the ~1000 km scale oceanographic gradient studied and taken as indicative of regional consistency in foraging (Zgliczynski et al. 2019). This demonstrates that coral reef food webs will often reflect fluctuations in resource availability and illustrates how SI data can be used to test ideas about spatial and temporal variation in key trophodynamic processes at different scales, and the factors likely to be driving these.

If assessed in the context of well quantified temporal and spatial isotope **baselines**, SI data can demonstrate spatial changes in organic resource use that have significant implications for reef food web structure (Glass et al. 2020). Such changes can be exceedingly difficult to observe with traditional techniques such as feeding studies. As an example, **bulk** $\delta^{13}C$ and $\delta^{15}N$ data suggested a switch between planktivory and herbivory between fore and back reef environments for both nominally planktivorous and herbivorous species (Ho et al. 2009, Wyatt et al. 2012b). At Ningaloo Reef (western Australia), $\delta^{13}C$ and $\delta^{15}N$ modelling suggested that nominally herbivorous *Stegastes* spp. derived over half their diet from the plankton in more oceanic habitats while planktivores increased herbivory in the back reef (Wyatt et al. 2012b). On a reef flat in Okinawa (Japan), $\delta^{13}C$, $\delta^{15}N$, and fatty acid data showed that the algal gardening damselfish *Stegastes nigricans* supplemented its diet with unexpectedly large amounts of animal material for a herbivore (Hata & Umezawa 2011). This suggests that notions of resource reliance in reef consumers may need to be more flexible. Using $\delta^{13}C_{EAA}$, McMahon et al. (2016) showed that while *S. nigricans* gained the majority (75%) of their dietary C from macroalgae, in more oceanic habitats 13%, and up to 30% for some individuals, came from phytoplankton. Importantly, the error on the estimates from the $\delta^{13}C_{EAA}$ data was much lower than from **bulk** SIA observations. It is not clear whether such changes reflect real spatial differences in actual food web structure, however (McMahon et al. 2016), as SI data cannot distinguish between active consumption or incidental ingestion of particular sources that can be influenced by exposure rates and therefore spatial proximity and foraging strategies.

Interactions between the complex topography of reefs and hydrodynamic processes that alter physical and biogeochemical conditions such as internal waves and upwelling can lead to highly variable isotope ratios in reef organisms across depth ranges at the same location. As light availability decreases with depth so, generally, does coral **autotrophy;** coral host tissue $\delta^{13}C$ values become closer to oceanic carbon values through feeding (Muscatine et al. 1989, Gattuso et al. 1993, Lesser et al. 2010, Crandall et al. 2016). Coral skeletal $\delta^{13}C$ values also reflect fluctuations in light and **heterotrophy** because they are influenced by metabolic fractionation; decreasing light and zooplankton levels resulted in significant decreases in coral skeleton $\delta^{13}C$ (Grottoli & Wellington 1999, Grottoli 2000). Furthermore, both host and endosymbionts are depleted in ^{15}N with depth, with impacts on endosymbiont growth rates (Muscatine & Kaplan 1994).

This pattern of trophic zonation (i.e. where coral **heterotrophy** increases with depth) is not always consistent, however. Some evidence suggests that increasing depth does not necessarily result in increased **heterotrophy** as is typically expected (Einbinder et al. 2009), with observed changes in $\delta^{13}C$ along depth axes potentially driven by internal carbon cycling processes once other biological traits are accounted for. Controlled feeding experiments of Reynaud et al. (2009) found no effect of ambient light levels on internal nutrient metabolism. They did, however, identify increased nitrogen cycling of host metabolic waste products to endosymbionts when **heterotrophic** feeding was limited, by tracing **bulk** $\delta^{15}N$ through different tissues. This is in contrast to carbon metabolism, which appears to change with irradiance levels when **heterotrophic** feeding occurs, with increasing rates of photosynthesis and carbon translocation from endosymbionts to coral host under high light conditions (Tremblay et al. 2014). At Palmyra Atoll, $\delta^{13}C$ and $\delta^{15}N$ data suggested that increases in coral **heterotrophy** with depth were absent at sites where resources were assumed to be readily available from extensive water mixing (Williams et al. 2018a). Similarly, in one of the few studies to use both $\delta^{13}C_{AA}$ and $\delta^{15}N_{AA}$, while **autotrophy** was the dominant source of carbon to the hard coral *Stylophora pistillata*, **heterotrophic** energy contributions were equal across shallow (5 m) and deep (60 m) reefs (Martinez et al. 2020). Furthermore, SI data reveal that some species are heterotrophs throughout their depth ranges (Alamaru et al. 2009a, Crandall et al. 2016, Radice et al. 2019) or vary in their degree of **heterotrophy** across depths based on sampling region (Santos et al. 2021). Even within the same genus and at the same location, SI values ($\delta^{13}C$ and $\delta^{18}O$) show that three species of *Madracis* differ in their depth adaptations and ecological plasticity (Maier et al. 2003), indicating that this phenomenon could depend on prey encounter, resource availability, and resource acquisition (Maier et al. 2010, Plass-Johnson et al. 2015b, Fox et al. 2018, Santos et al. 2021). Indeed, Leichter et al. (2007) suggested that depth gradients in reef macroalgal SI values can reflect gradients of exposure to offshore nutrient sources, such as increased use of deep-water nitrate by macroalgae exposed to high-frequency upwelling. Conversely, variability in SI values ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$) in two abundant macroalgal species over scales of 10s of metres were perhaps independent of depth and instead reflected large amounts of spatial heterogeneity (Stokes et al. 2011). Clearly, there are limited universal trends with depth that can be identified thus far on coral reefs, which suggests that biological traits and their plasticity may play a significant role in buffering effects of physical drivers over a species' observed distribution.

CSIA can be especially powerful in examining variability in resource use across environmental gradients that could influence tissue SI signatures independent of diet changes. While primary producer **bulk** SI values vary across regions and seasons (Figure 4; Tables 3 and S6), which can confound interpretation of dietary changes, **CSIA** measures individual compounds which are shaped by the biochemical processes of the primary producers and are thought not to vary spatially, temporally, or with growth rates (Whiteman et al. 2019). Using **CSIA**, Papastamatiou et al. (2015) were able to discount the isotope **baseline** as a source of variation in the **bulk** SI data of giant trevally (*Caranx ignobilis*) from deep-water reefs on a Pacific atoll; differences were due to individual trophic flexibility, with **trophic positions** determined with **CSIA**-AA ranging from 3.5 to 4.6. Acoustic tracking demonstrated individual variability in diel migration and feeding behaviour leading to a range of **trophic positions**, perhaps reflecting individual foraging preferences and intraspecific competition (Papastamatiou et al. 2015). By demonstrating changes in $\delta^{15}N_{AA}$ derived **TP** of spiny and slipper lobsters across large spatial scales in the north-western Hawaiian Islands, which were thus independent of **baseline** isotope variation, O'Malley et al. (2012) robustly demonstrated that spatial variability in growth was due to different responses between the two species to limited prey availability. Resource availability across spatial gradients thus may be a driver of consumer SI values. Of two obligate corallivore butterflyfish that both preferentially feed on *Acropora* coral, $\delta^{13}C_{AA}$ reveal that the specialist *Chaetodon baronessa* is more selective with depth and continually seeks out *Acropora* despite decreased availability, while the generalist *Chaetodon octofasciatus* becomes more flexible with depth (MacDonald et al. 2019). Furthermore, while planktivores have a consistent feeding strategy across shallow to mesophotic reefs, benthic invertivores and omnivores have significantly broader niches (with benthic invertivores also occupying a higher **TP**) (Bradley et al. 2016). These studies highlight the trophic versatility of many reef organisms along spatial gradients.

Anthropogenic drivers

Land and seascapes are under increasing pressure from human activities worldwide, and shallow coral reef ecosystems are particularly vulnerable to these threats (Figure 3); coral bleaching events are now occurring every six years or less (Hughes et al. 2017). **Bulk** $\delta^{18}O$ SI values can reveal when a coral has been subjected to extreme thermal stress by directly relating the $\delta^{18}O$ values to in situ temperature (Porter et al. 1989, Mayal et al. 2009). SIs can also identify how the coral animal host and endosymbionts alter their trophic strategies and resource use in response to bleaching events (see 'Holobiont metabolism: exogenous factors'). Over the longer term, pulse-chase experiments using ^{13}C-labelled bicarbonate revealed that some corals may increase their use of **heterotrophic** carbon for up to a year after bleaching, but it is not known whether this is a sign of resilience or prolonged stress (Hughes & Grottoli 2013). Generally, it seems that different coral species have different bleaching responses, with some maintaining energy reserves or **heterotrophic** capacity, but most recovering within a year if the bleaching is a mild and isolated event (Grottoli et al. 2017, Levas et al. 2018). There is currently little understanding of how coral and endosymbionts trophic strategies will change through successive bleaching events, despite predictions that these will occur ever more frequently. Furthermore, studies assessing thermal stress effects using SIs have focused on corals, with few, if any, investigating these impacts on other **holobionts.** For non-symbiotic organisms, Vaughan et al. (2021) suggested increases in $\delta^{15}N$ (~1 ‰) in natural and transplanted macroalgae, *Sargassum mangarevense*, were linked to the release of coral-derived nutrients post-bleaching. While a better understanding of SI changes in reef biogeochemical cycles post-bleaching is required (but see Radice et al. 2021), it is crucial to understand and measure the underlying natural variation in SI source data when making inferences, i.e. by measuring the underlying $\delta^{15}N$ **baseline**.

Global bleaching events and declines in live coral cover have been linked to declines in structural complexity, coral biodiversity, and the abundance and diversity of reef-associated fishes (Jones et al. 2004, Carpenter et al. 2008, Pratchett et al. 2018). Overfishing also contributes to the latter, while simultaneously reducing fish-mediated storage and supply of nutrients by up to 50% (Allgeier et al. 2016). These drivers may be reflected in consumer SI values. For example, as the amount of rubble increases along a habitat disturbance gradient, there are significant differences in the $\delta^{13}C$ and $\delta^{15}N$ ranges and the **isotopic niche** area of the parrotfish *Chlorurus bleekeri*, the foraging of which varies according to the surrounding habitat (Plass-Johnson et al. 2018). Similarly, as prey densities fluctuate in response to declines in structural complexity, the peacock grouper *Cephalopholis argus* maintains their **TP** (as indicated by their $\delta^{15}N$) by switching foraging modes from ambush to widely active foraging (Karkarey et al. 2017). Loss of habitat structural complexity is predicted to cause a threefold reduction in fishery productivity (Rogers et al. 2014). However, some species such as the peacock grouper may be more resilient to habitat loss than previously thought, as their foraging plasticity may enable them to adapt to coral degradation (Karkarey et al. 2017). In addition to habitat loss, ocean acidification (i.e. reductions in oceanic pH through elevations in partial pressure of seawater CO_2 (pCO_2) due to increasing global carbon emissions) may negatively impact calcifying marine organisms. By studying coral skeleton $\delta^{13}C$ and $\delta^{18}O$ values, Zhou et al. (2016) determined that *Acropora gemmifera* photosynthesis and calcification were only impaired at the highest pCO_2 treatment, with their microbial communities remaining stable. More recently, benthic communities have been exposed to increased pCO_2 and warming in mesocosms to determine how trophic architecture (represented by organism $\delta^{13}C$ and $\delta^{15}N$) would respond under future scenarios (Nagelkerken et al. 2020). While trophic pyramids and community structure (i.e. biomass and productivity) shifted, the food web architecture remained inflexible and stabilising processes were absent, suggesting a lack of adaptive capacity in the ecosystem (Nagelkerken et al. 2020). SIs therefore offer a unique opportunity to study potential impacts of changing environmental conditions on organism and whole food web trophic ecology (Plass-Johnson et al. 2018).

SI data offer great potential for exploring other anthropogenic impacts on coral reef food webs, notably the role of human-derived pollutants in reef systems. By measuring SI values of various reef organisms, these nutrient inputs can be identified to determine the extent of their impact on the local reef communities (Yamamuro et al. 2003). Elevated δ^{15}N values in sampled organisms have suggested artificial nutrient inputs to reef communities pertaining to aquaculture (Herbeck et al. 2014), shoreline sewage (Todd et al. 2009, Baker et al. 2017, Abaya et al. 2018, Lachs et al. 2019), stormwater discharge (Lapointe and Bedford 2011), seepage water (Mwaura et al. 2017), river plume pollution (Risk et al. 2014), and even wood pulp effluent (Schleyer et al. 2006). This highlights how spatially explicit context is required to determine the role of pollution in nutrient cycling of nearshore reefs and flow on effects for other taxa like coral (Umezawa et al. 2002, Huang et al. 2013, Adam et al. 2021). While most of these studies use macroalgae as bio-indicators of pollution, seagrass δ^{15}N has also been proposed as a tool to monitor time-integrated changes over coral reef habitats; there are fewer seagrass species than the diverse macroalgae that are found across reefs, facilitating both identification and standardisation (Yamamuro et al. 2003). Indirect incorporation of anthropogenic inputs can result from **connectivity** to the pelagic food chain and reef planktivore grazing. Lower δ^{13}C and higher δ^{15}N in two damselfishes demonstrated how POM released from nearby fish farms in southern Taiwan can enter coral reef food webs (Jan et al. 2014). One of the few studies to employ sulfur isotopes to examine anthropogenic inputs attributed decreasing δ^{34}S in coral skeletons off Yoron Island to rain-driven inputs of low δ^{34}S terrestrial material from sugar cane-dominated farmland (Otani & Nakanishi 2019). Oxygen isotopes may also be useful in anthropogenic impact monitoring, with shifts in δ^{13}C and δ^{18}O in coral skeleton correlating with local oil spills (Xu et al. 2018). However, studies need to be cautious when using taxon-specific variations in tissue isotopes to infer nutrient fluxes based on SI data, especially across small ranges that might be explained by metabolic or hydrodynamic variations (see 'Natural drivers'), which is especially the case for coral skeleton. Relationships between anthropogenic nutrient inputs and SI data, and subsequent ecological effects may be more nuanced. For example, a long-term experiment involving δ^{13}C and δ^{15}N in the hard corals *Acropora palmata* and *Porites porites* showed that moderate doses of anthropogenic nutrients had no additional effects and the corals continued growing (Allgeier et al. 2020). However, models revealed that nutrient and carbon flows were dominated by the symbiont, leading to algal dominance in the **holobiont** and greater algal demand on coral resources, likely increasing the corals' future vulnerability to bleaching due to stress (Allgeier et al. 2020).

It should be emphasised that elevated δ^{15}N values alone are insufficient evidence of anthropogenic impacts over reef ecosystems. There is an extensive range in natural isotope abundances in the ocean in the absence of anthropogenic inputs; isotope effects across the marine nitrogen cycle span approximately −0.5‰ to +38‰ (Sigman et al. 2009). Increased macroalgal δ^{15}N with depth can be indicative of upwelling increasing dissolved nitrogen availability, rather than a sewage impact (Huang et al. 2013). Instead of anthropogenic sources (e.g. Lapointe 1997, Lapointe et al. 2005), POM isotopic variations may reflect mineralisation of organic material and nitrification along with inputs of DIN from upwelling, run-off, sediments, and the atmosphere (Lamb & Swart 2008). Natural N cycling and resultant isotopic variation could account for δ^{15}N variations in benthic components of the reef. For instance, Lapointe et al. (2005) reported algal δ^{15}N elevated by +2‰ as indicative of land-based pollution; however, the range in POM δ^{15}N across the Florida Keys varies over a 20‰ range with a standard deviation of ±3.6 ‰, apparently independent of human influences (Lamb & Swart 2008). This underscores the importance of understanding spatial and temporal variations in potential source isotopes (Figure 4; Table 3), due to, for example, water column sources and upwelling (Leichter et al. 2007), before invoking anthropogenic perturbation. This is especially important where the variations are small (e.g. a few per mil or less) relative to a potential natural range of SI data variations.

Due to their incremental growth, corals, and in particular gorgonians, provide an opportunity to investigate long-term trends in anthropogenic nutrient inputs. The biochemistry of skeletal banding reflects the ambient nutrient levels and can therefore be used to infer long-term pollution trends (Ward-Paige et al. 2005). Indeed, gorgonian $\delta^{15}N$ values accurately reflect tourism levels over multiple years, with declines in $\delta^{15}N$ linked to declines in tourism and increases in $\delta^{15}N$ linked to its recovery (Baker et al. 2013b). Hard coral skeletons can track anthropogenic nitrogen fluctuations over decades with ^{15}N enrichment linked to increasing sewage levels and population density (Duprey et al. 2017, 2020). Archived museum samples may provide another means to monitor human pollution levels over longer time spans. Gorgonian samples from a 143-year time span had $\delta^{13}C$ and $\delta^{15}N$ values which reflected increasing atmospheric CO_2 and use of agricultural fertilisers, respectively (Baker et al. 2010), while samples in Bermuda spanning 50 years reflected changes in management policies which were effective in reducing local pollution levels (Baker et al. 2017). Despite the wealth of information they can convey on prior isotopic **baselines**, the use of archival samples is exceedingly low across SI reef studies ($n \approx 3$).

Knowledge gaps, caveats, limitations, and future directions

Throughout this review, we have summarised the key findings of studies that have used SIs to understand coral reef ecosystems. In doing so, we have highlighted that there are still areas where research, knowledge, and understanding are lacking. This should be taken as an opportunity to focus future research. Below, we have included a numbered list of identified gaps and opportunities for future SI studies. Given the logistical ease for researchers to now obtain and analyse SI data, we also emphasise why care must be taken not to misuse or overinterpret SI data. The *Knowledge gaps and opportunities* section is followed by important *Caveats and limitations* which must be considered. We hope that these points serve as a roadmap to direct future research involving SIs on coral reefs.

Knowledge gaps and opportunities

1. *Improve characterisation of basal sources.* Corals have been the focus of much SI research due to their foundational importance, but the living proportion of whole reefs that they cover is generally low. Conversely, 'turf algae' cover a greater surface area and are intensively grazed, but there is sparse understanding of the fate of turf-derived fluxes in the ecosystem. Similarly, substantial fluxes of detritus are derived from benthic reef sources both directly (e.g. exudates) and secondarily (e.g. grazer defecation), but the detrital pool, its origins and fluxes are little resolved. SI data, both **bulk** and **CSIA**, have a lot to offer well-focused investigations which can advance our understanding of these lesser studied fluxes on reefs.
2. *Extend sampling over longer time frames.* Resource availability on coral reefs fluctuates through time and consumer resource use may vary accordingly. However, to date, many coral reef SI studies sample during a single time point or limited temporal window (Figure 5A). Future studies exploring the dynamics and stability of coral reef ecosystems should consider longer time periods over which inferences can be made. Multi-tissue approaches are one technique which may reveal dietary variations across a range of timescales from a single individual, yet currently, only 9% of studies measured multiple tissues within the same individual organism. Employing a multi-tissue approach by sampling faster turnover tissues, e.g. organs and blood, in addition to slower turnover tissues, e.g. muscle tissue, could better discriminate dietary consistency or the lack thereof (Wyatt et al. 2019).
3. *Employ an isoscape approach.* To date, and likely due to their inherent complexity, there are few studies employing an isoscape approach on coral reefs. However, isoscapes of coral reef ecosystems could offer valuable insight into the complex processes that might influence coral reef trophodynamics and SI values across various locations (Figure 4; Table 3). For example, spatial variations in the relative importance of different **production sources** to reef food webs are evident. Given that environmental conditions are fluctuating due to climate

change, there is a need for studies examining the drivers of these likely differences in coral reef ecosystems, which could be facilitated by a broadening of the spatial scale across which studies are conducted. Isoscapes provide a solid isotopic foundation of a region, providing the context upon which other avenues of research can be conducted (McMahon et al. 2013).

4. *Explore trophodynamics across the wider community.* SI data have helped to highlight that categorising reef consumers into simple trophic groups masks dietary specialisations and the complexities of this dynamic system. However, few studies compare niche dynamics and resource partitioning outside of species groups or guilds. For example, most studies that focus on reef fish do not consider how invertebrates occupying the same **TP** may share similar resources (but see Zapata-Hernández et al. 2021). A better understanding of trophic interactions across the wider reef community is required. This will help understand the importance of potential competitiveness among disparate taxa on coral reefs. Given increased feasibility, this body of knowledge is expected to grow, and it will be important for greater resolution to be assimilated into whole ecosystem models.
5. *Quantify species-specific TDF and tissue turnover rates.* To date, few studies have accurately quantified species-specific **TDF** and isotopic turnover rates (i.e. the time it takes for a given consumer tissue to reflect the isotopic composition of its diet) across different tissue types for coral reef consumers. While this will be challenging to determine for species with longer turnover times (e.g. hard coral; Tanaka et al. 2008), it is highly recommended that more laboratory feeding studies be conducted. Where environment- and species-specific **TDF** are not available, analyses must rely on **TDF** across a range of values to account for uncertainty surrounding the inherent variability (e.g. likely ≥1‰ SD for $\Delta^{15}N$).
6. *Consider parasites.* Parasites may be involved in >50% of food web links, but they are rarely considered in food web science (Dunne et al. 2013). Indeed, on coral reefs, there are estimated to be ten times more parasite species than fish species (Justine et al. 2012). In addition to their huge biodiversity, parasites can affect food web structure indirectly by modifying their host's behaviour and subsequent resource use (Welicky et al. 2017). Despite their important ecological role in shaping trophic interactions in coral reef ecosystems, parasite trophodynamics on reefs are poorly understood. While several more recent studies are beginning to address this knowledge gap (e.g. Jenkins et al. 2018, 2020, Riekenberg et al. 2021), few have considered parasite life-stage dietary shifts.
7. *Measure other isotopes.* Despite limited application on coral reefs to date, **bulk** $\delta^{34}S$ shows promise for distinguishing among reef **production sources** and revealing consumer habitat use (Connolly et al. 2004, Granek et al. 2009, Skinner et al. 2019a). Moreover, recent advances in SI technology mean that $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ can now be measured from the same sample aliquot with a high level of precision (Fourel et al. 2015). Similarly, to our knowledge, there are currently no coral reef SI studies that utilise mercury isotopes ($\delta^{202}Hg$), which can characterise resource partitioning and identify foraging depth separation among predators in marine environments (Besnard et al. 2021). Given the myriad of co-occurring predators on coral reefs, $\delta^{202}Hg$ may offer a useful tool to understand resource partitioning across depths. Finally, copper ($\delta^{65}Cu$) and zinc ($\delta^{66}Zn$) isotopes have recently been suggested as a proxy for coral stress, as both increase in coral host and symbiont tissue at higher temperatures (Ferrier-Pagès et al. 2018). Given the difficulty in disentangling the **production sources** and consumer resource use on reefs, studies incorporating such additional isotopes are strongly encouraged.
8. *Apply compound-specific SIA (CSIA).* The number of studies employing **CSIA** on coral reefs is rapidly increasing (of coral reef SI studies >5 in 2020 alone, compared to 15 between 1982 and 2019). This advance will certainly offer new insights into the complexities of the coral reef ecosystem. Although multiple isotope approaches show significant potential for better elucidation of organic matter fluxes and resource use in this complex ecosystem, few

studies combine **CSIA** of both carbon and nitrogen in the compounds of interest. Given the depth of information they can provide on **production sources** and trophic interactions, studies combining both are likely to provide a more holistic understanding of the specific systems of interest.

9. *Incorporate and explore relationships with environmental drivers.* Laboratory studies generally focus on manipulating one aspect of the environment, while field studies use SIs as indicators rather than testing what drives changes in their values. Few studies consider the effect of multiple environmental drivers, whether natural or anthropogenic, or the interactions occurring between them. Measuring multiple environmental variables (e.g. nutrients, pH, temperature, and salinity) and linking those with SI values is a multi-method approach which could better explain spatial gradients (of human impacts) on reef ecosystems (Teichberg et al. 2018).
10. *Utilise archival samples.* Analysis of archival samples, held in museums and other institutions across the globe, can provide additional information on prior isotopic **baselines** and how they have shifted over time. Soft and hard corals are common, slow-growing, benthic organisms, which may comprise important **baselines** against which current pollution levels can be monitored. This gives the historical context against which the significance of contemporary isotope values can be compared. Archival specimen preservation is a considerable cause for concern; however, improved mass spectrometer technology means that very little material is physically required to obtain SI data. Where possible, we encourage researchers to incorporate archival or historical **baseline** samples. Such approaches are particularly powerful when drawing conclusions about current anthropogenic impacts.
11. *Conduct multiple analyses on the same sample material.* There are considerable logistical efforts involved with collecting samples on coral reefs and conservation concerns involved with intensive sampling, especially of threatened species. As such, we advocate for multiple analyses on the same sample material. Not only would this facilitate more collaboration between different regions (which at present tend to specialise on particular fields of research), but it would maximise the information derived from each individual sample.
12. *Adopt a multi-regional, aggregative research approach.* There are now many SI studies (and therefore data) conducted on coral reefs, but almost all are isolated to a single area. Further still, there are regional disparities in research focus. Here, it is worth acknowledging that collecting SI data often requires extensive sampling to which there may be substantial limitations, particularly in coral reef regions (i.e. lack of access, funding, infrastructure, requiring specialist vessels or equipment, sampling during extreme conditions to assess seasonality). Regardless, generality at the regional to global scale is currently lacking. Moreover, inferences from different studies can be contradictory (e.g. see 'Trophic niches: isotopic niches'). Given the number of studies now published, a more aggregative research approach (i.e. meta-analyses) may help discern ubiquity in patterns and drivers of these. Complementary to this, we advocate for larger, multi-region studies to detect, if any, general trends in SIs (e.g. variability in **production sources** *cf.* Figure 4) on coral reefs to act as ecological **baselines.** This could help generate mechanistic theory of overall 'typical' coral reef SI functioning. Deviations from this may point to interesting, but currently not-yet-realised avenues of further study.

Caveats and limitations

1. *Provide enough detail for SI data to be useful to others.* Researchers should strive to clearly report their sampling design and SI data so that they can be used by others. Over 15% of identified articles did not report when or over what period samples were taken (Figure 5A),

despite the high spatiotemporal variability of SI data on coral reefs. Furthermore, when extracting SI baseline data (i.e. Table 3), we found that many articles only published data in Figures and/or did not provide sample sizes that are required to understand data spread and error. More rigorous reporting will facilitate point 12 above, allowing researchers to employ an aggregative approach to future coral reef SI studies.

2. *Interpret SI data within the context of underlying SI baselines.* Variation in SI **baselines** (values at the base of the food web) must be considered when assessing variation in resource and nutrient use using isotopes. There is a tendency to extrapolate from taxon-specific variations in tissue SI data to infer biogeochemical rates or anthropogenic impacts, which is especially concerning across small ranges in isotope values. These variations may be within ranges of a few per mille or less, which alone can be explained by discrimination and metabolic variations, or fluctuations in isotopic **baselines**. This presents a high risk of overinterpretation, especially given the dynamic fluxes present on coral reefs. Understanding natural variation in SI values is also needed when analysing the influence of stochastic events (e.g. coral spawning, coral bleaching) with SIs, especially when SI perturbations are small relative to the magnitude of the natural variance in SI **baselines** (see Figure 4). We urge researchers to take careful consideration when interpreting spatial and temporal heterogeneity in reef organism SI values and to measure isotopic **baselines** wherever possible. This might involve comprehensive source sampling for **bulk** SIA or the application of more source-specific **CSIA**. Complementary data, e.g. acoustic telemetry or nutrient concentrations, and data simulations prior to SIA (to estimate the likely impact, for example, **TDF** variability could have on observed data) could also be hugely beneficial for considering SI variation in context. In some cases, it may not be possible to measure **baseline** source values in situ. As a first estimate, researchers should consider the source SI values presented herein (Figure 4 and Table 3 for interstudy means; Table S6 for study-specific values). The presented values integrate differences attributable to spatiotemporal dynamics, biodiversity, and inter-laboratory differences, highlighting the variability that has been observed across studies. For example, macroalgae SI values show high variance (mean ± S.D. for $\delta^{13}C = -15.68 \pm 4.87$ and $\delta^{15}N = 3.93 \pm 3.45$; Table 3), especially between species, and, as such, authors should consider some form of abundance weighting by species.
3. *Isotopic niche ≠ trophic niche.* While variability in SI data within a population may indicate there are individuals with consistent differences in trophic ecology, low variability in SI data in a population does not necessarily indicate a narrow **trophic niche**. In the latter case, because of the time integrating character of the SI signatures, there are two possibilities: (1) individuals could be constantly feeding on one **production source** or (2) they could be feeding on different sources in proportions or combinations that happen to integrate to the same SI value. Specifically, how equivalent is the **isotopic niche** to the **trophic niche**? What is the magnitude of isotopic variation expected from processes independent of feeding variation (e.g. growth rates, diet quality)? Given the diversity of **production sources** available on coral reefs, these questions and the discrepancy between the **trophic niche** and the **isotopic niche** may be more of a problem in this ecosystem compared to others with less diversity in available sources for consumers. Consequently, care must be taken when making inferences regarding **trophic niches** based on **isotopic niches** on coral reefs.

Conclusions

SIA are an important tool that have elucidated many of the diverse and complex processes and relationships occurring on coral reef ecosystems. By combining a traditional literature search of databases with topic modelling of article abstracts, we identified recurring patterns and themes in the SI coral reef studies published to date. Summarising how SIs have advanced our understanding of coral reefs is challenging due to the inherent crossover between studies, but the topic modelling approach partitioned the article text data to generate non-biased categories, providing a clear guide on the most logical structure for the review.

One of the fundamental components of SI advances for coral reef ecology, and also one of the challenges, involves identifying the available energy fluxes to reef food webs and how these vary across different scales. SIA have been used to successfully identify and quantify inputs to reefs, and releases of material from reef organisms, showing that both are closely linked to the spatial arrangement of organisms and hydrodynamics across reefs. Fluctuations in available resources, related to the structure and layout of the seascape, are reflected in reef primary producer and consumer SI values. This is highlighted by the global variability in reported SI **baselines** across the literature (Table 3, Figure 4), emphasising the importance of considering variations in these when studying trophodynamics across coral reef food webs.

Corals represent one of the most studied organisms on coral reefs due to their instrumental role as ecosystem engineers. SI studies have revealed just how complex their nutrient uptake and feeding strategies are, often with stark contrasts between species and little consistency in their reliance on **auto**- or **hetero-trophic** resources spatially and temporally. SI studies have also highlighted the importance of previously underappreciated **holobiont** groups to the overall coral reef ecosystem, notably sponges that underpin diverse pathways of in situ DOM and POM recycling. The myriad of interactions occurring between the vast numbers of reef primary producers and consumers is challenging to explore, but SI data have begun to disentangle some of these. For example, many **isotopic niche** studies have shown that traditional dietary classifications mask individual-level variations in resource use. They suggest that many reef organisms have more varying and flexible functional roles than those inferred from traditional techniques. Such insight also points to the fact that a better understanding of wider feeding relationships across guilds, not only within guilds, is required, particularly among invertebrates.

Coral reef ecosystems do not persist in isolation, and SI studies demonstrate energetic linkages with adjacent habitats. These linkages are formed not only from ontogenetic shifts in habitat use of reef fishes, from their nursery grounds in mangroves and seagrass beds to the reef, but also through the movement patterns of larger, mobile predators. SIs also reveal vertical movement patterns across depth gradients, highlighting the extent of **connectivity** between shallow and deeper reefs. **Connectivity** is not only determined by energy flows, however, with SIs providing a methodological means of transgenerational tagging to follow larval distributions, highlighting the diversity of research questions that can be addressed using SI techniques.

SIA have become an increasingly important tool for exploring anthropogenic impacts on coral reef food webs; they can highlight nutrient inputs, ocean acidification effects, and thermal stress, although care is needed not to conflate such effects with natural SI **baseline** variations. Climate change is increasing global water temperatures, resulting in catastrophic bleaching of coral reefs worldwide. Such a loss of live coral can impact associated food webs by reducing biodiversity and degrading habitats, resulting in lower trophic complexity across the community (Gabara et al. 2021), as well as reduced ecological stability due to loss of functional redundancy. SIA represents a crucial tool to increase our understanding of the complex trophic interactions occurring on coral reefs that are modulated by environmental drivers and their associated dynamics, including human-induced climate change.

The number of SI reef studies has been increasing rapidly in recent years. By objectively drawing on the published literature, we have synthesized the current knowledge and understanding acquired through the application of SIA on coral reefs into five broad bodies of research focus. In doing so, we have highlighted potential research avenues that warrant further exploration, including increasing the scale at which SI studies are conducted (both through time and across space) in the hope of identifying more general patterns and processes that underpin coral reef structure and functionality. While acknowledging the considerations that need to be made when utilising SIs, we hope this review acts as a useful synthesis and will serve to bolster the expanding literature on ecological applications of SI approaches to coral reef ecosystems.

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LARVAL SETTLEMENT IN ECHINODERMS: A REVIEW OF PROCESSES AND PATTERNS

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Abstract Echinoderms are a common component of benthic marine ecosystems, with many being ecologically and/or economically important. Like many marine organisms, most echinoderms have a bipartite life history with a planktonic larval phase and a benthic adult phase. The transition between these phases (i.e. settlement) is complex and comprises a cascade of events including the location, exploration and selection of suitable benthic habitat, and metamorphosis to adapt from a pelagic to a benthic lifestyle. This review provides a comprehensive synthesis of the various processes involved in the settlement phase across all five extant classes of echinoderms. Central to the review is a detailed assessment of settlement behaviour and the diverse mechanisms of settlement induction. Most echinoderms, including keystone sea urchins, starfishes and sea cucumbers, do not settle indiscriminately; specific environmental conditions or cues are often necessary for settlement to occur, resulting in marked spatial and temporal variability in settlement rates. Fluctuations in settlement, in turn, lead to major changes in the local abundance of echinoderms and often have profound ecological consequences, due to the pivotal role that many echinoderms play in ecosystem functioning. Given important knowledge gaps persist, this review also explores opportunities for future research to advance our understanding of this critical early life-history phase.

Keywords: Marine Ecology; Environmental Cues; Settlement Induction; Larval Behaviour; Metamorphosis; Recruitment; Marine Benthic Invertebrates

Introduction

Echinoderms (phylum Echinodermata) are commonly observed across all benthic marine habitats, ranging from exposed rocky shores and tropical coral reefs to the sandy floor of deep-sea habitats (Byrne 1990, Keesing et al. 1993, Sumida et al. 2000). There are approximately 7000 extant species (Pawson 2007) across five classes (Byrne & O'Hara 2017): Echinoidea (sea urchins, sand dollars and heart urchins), Asteroidea (starfishes or seastars and sea daisies), Ophiuroidea (brittle stars, basket stars and snake stars), Holothuroidea (sea cucumbers) and Crinoidea (feather stars and sea lilies). Echinoderms are economically important with extensive commercial fisheries for sea cucumbers (Holothuroidea) and sea urchins (Echinoidea), which are increasingly supplemented by aquaculture (Brown & Eddy 2015). Echinoderms are also ecologically important. Sea cucumbers, for example, make important contributions to nutrient cycling and energy transfer (Purcell et al. 2016). Other

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echinoderms have been shown to be important ecosystem engineers (Lessios et al. 1984, Carpenter 1985) and/or keystone species (Paine 1969, Hughes et al. 1985, Ling et al. 2015, Byrne et al. 2016, Menge et al. 2016).

Large fluctuations in population density are a salient characteristic of many echinoderms (Uthicke et al. 2009) and can have important consequences for ecosystem structure and function (Chesher 1969, Birkeland 1989, Estes et al. 2011, Ling et al. 2015). For example, the proliferation of sea urchins and subsequent overgrazing in many temperate reef systems has contributed to loss of kelp forest and macroalgal beds (Ling et al. 2015). Conversely, population collapse in keystone predator starfish species across the Northeast Pacific (Menge et al. 2016, Schultz et al. 2016, Harvell et al. 2019, Kay et al. 2019) has triggered trophic cascades and changed shallow water seascapes. On tropical reefs in the Caribbean, population collapse of the sea urchin *Diadema* contributed to shifts from coral- to algal-dominated habitats (Lessios et al. 1984, Carpenter 1985, Hughes et al. 1985). Periodic population eruptions of coral-feeding crown-of-thorns starfishes (*Acanthaster* spp.) have also been a major contributor to sustained coral loss and reef degradation on many Indo-Pacific reefs (Bruno & Selig 2007, Kayal et al. 2012, Mellin et al. 2019). Ecological studies of echinoderms are, therefore, often focused on understanding causes of population fluctuations (Ling et al. 2019, Glockner-Fagetti & Phillips 2020, Caballes et al. 2021), which are inextricably linked to life-history processes that affect larval ecology and patterns of settlement or recruitment (e.g. Ebert 1983, Uthicke et al. 2009, Metaxas 2013).

Echinoderms exhibit considerable life-history diversity (Byrne et al. 1999, McEdward & Miner 2001, 2007, Byrne & O'Hara 2017, Ebert 2021a), but are generally gonochoric, and reproduce sexually by broadcast-spawning gametes that are fertilised externally. Accordingly, most echinoderms have a planktonic larval phase, which lasts from days to months (Strathmann 1987, Hadfield et al. 2001). Larval development is characterised by a series of distinct developmental stages (Byrne 2013, Byrne & O'Hara 2017). Many echinoderms have lecithotrophic larvae (McEdward & Miner 2001, 2007, Uthicke et al. 2009), meaning that they do not or cannot feed, and larval duration is constrained by initial energy reserves. Most of the best known and extensively studied echinoderm species (e.g. *Acanthaster* spp., *Diadema* spp.) are, however, planktotrophic (Uthicke et al. 2009), and this capacity to feed means that larvae may remain in the plankton for much longer (*cf.* lecithotrophic larvae) and potentially disperse much further (Emlet 1995). More importantly, egg size and energy content (= maternal provisioning) are significantly higher in species with lecithotrophic larvae (McEdward & Chia 1991, Falkner et al. 2015), which consequently constrains fecundity. Echinoderms with planktotrophic larvae have potentially much higher reproductive capacity (Uthicke et al. 2009), but the extent to which the reproductive capacity is realised depends on exogenous food availability for larvae.

Despite the wide range of biotic (e.g. predation and starvation) and abiotic factors (e.g. dispersal to unfavourable habitats) that may constrain larval development and survival (Rumrill 1990, Lamare & Barker 1999, Cowan et al. 2016a, 2020), population replenishment and dynamics of echinoderms (like all marine species with a dispersive larval phase) is also conditional on settlement success and early post-settlement survival (Balch and Scheibling 2001). After completing larval development, echinoderms undergo metamorphosis, wherein they rapidly develop morphologies that are adapted to their benthic adult habit (*sensu* Hadfield et al. 2001). This planktonic-benthic transition, hereafter referred to as settlement (*sensu* Scheltema 1974), comprises a cascade of events that often occur rapidly, but can have far-reaching consequences for the population dynamics and distribution of echinoderms, and the ecosystems in which they play important roles. To assess these potential consequences, a comprehensive understanding of the environmental conditions and cues necessary for echinoderm larvae to settle, and how they translate to concomitant settlement patterns in the field is needed.

Past reviews of settlement induction and metamorphosis in marine invertebrate larvae have largely focused on ascidians, cnidarians, corals, hydroids, molluscs and/or polychaetes (e.g. Burke 1983a, 1986, Pawlik 1992, Rodríguez et al. 1993, Wieczorek & Todd 1998, Hadfield & Paul 2001, Hadfield 2011). Despite extensive primary research on settlement in echinoderms (e.g. Birkeland et al. 1971, Cameron & Hinegardner 1974, Highsmith 1982), there has been limited synthesis or review of this work. Strathmann (1978) and Pearce (1997) reviewed settlement induction in echinoderm larvae. Meanwhile, Balch & Scheibling (2001) provided a comprehensive synthesis of patterns of echinoderm recruitment, building on the initial review of recruitment patterns by Ebert (1983). General patterns of settlement are also briefly summarised in more recent syntheses focusing on the larval ecology of echinoids (Metaxas 2020) and asteroids (Metaxas 2013). However, a considerable body of research from the past two decades has substantially advanced our understanding of the processes and patterns of settlement in echinoderms. It is therefore timely to provide a comprehensive, phylum-wide review covering all aspects of this critical and complex early life-history phase.

The aim of this review is to synthesise foundational research and recent advances in the study of the various processes and patterns in echinoderm settlement. Importantly, our review provides a detailed overview of published work on larval settlement across a wide range of marine habitats and environments. We start with a summary of the complex mechanisms and processes taking place during this planktonic-benthic transition. Central to our review is a detailed synthesis of settlement behaviour and the various mechanisms of settlement induction, including both natural and artificial chemical cues. We then critically assess predominant sampling techniques used to measure settlement *in situ*, followed by a review of spatial and temporal settlement patterns, which allows for comparisons among echinoderm classes and species across various marine habitats, locations and climate zones. The final section of this review highlights persistent and critical information gaps and outlines future directions for research pertaining to this important early life-history phase.

Larval settlement: the planktonic-benthic transition

Terminology

While often used interchangeably to describe planktonic-benthic transitions, settlement and metamorphosis are distinct processes for echinoderms. Scheltema (1974) defined settlement as a behavioural response of planktonic larvae resulting in the termination of their pelagic larval stage and assumption of a benthic life. As such, settlement typically refers to the descent from the water column and contact with the substratum. However, such substratum contact is reversible (Burke 1983a, Crisp 1984, Pawlik 1992, Hadfield & Paul 2001), whereby larvae may abort their benthic habit and resume swimming to seek out alternative settlement substrates or habitats (Figure 1). By contrast, metamorphosis is considered an irreversible morphogenic event, which includes the loss of larva-specific and emergence of juvenile-specific structures (Figures 2 and 3, Hadfield & Paul 2001). Moreover, metamorphosis may occur before or after the contact with the substratum, depending on the species (Figure 1).

For the purpose of this review, settlement will be used to refer to the more general definition of the transition from the planktonic larval to the benthic, post-metamorphic stage, as used in many other marine taxa (Hadfield 1986, Pawlik 1992, Rodríguez et al. 1993). In many echinoderms, this settlement phase may include various behavioural components (*sensu* Crisp 1984). We only use the morphogenic term ‘metamorphosis’ in specific reference to the physiological and morphological changes involved in this event, which appears necessary given some echinoderms metamorphose in

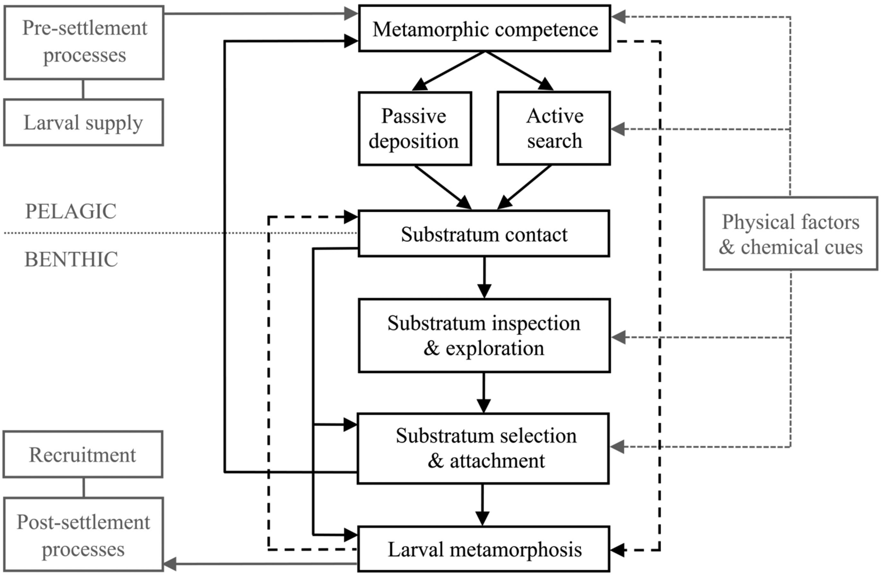

Figure 1 Key stages and processes in the settlement of echinoderms with planktonic larvae. Some echinoderms metamorphose in the plankton prior to contacting the substratum (dashed black arrows).

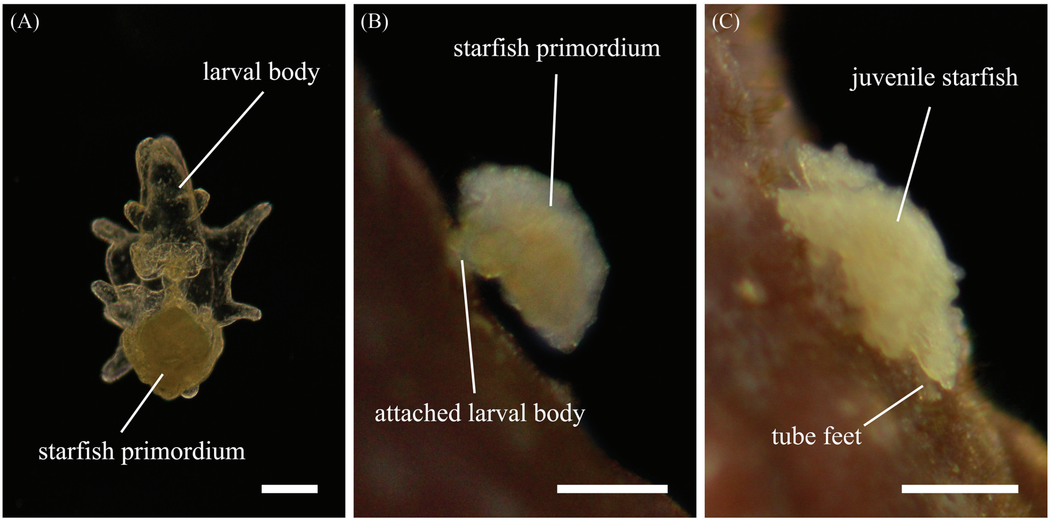

Figure 2 Larval development through metamorphosis in the Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*), an ecologically important asteroid with a pelagic planktotrophic larval stage and complex larval morphogenesis: (A) brachiolaria larva; (B) metamorphosing larva absorbing the larval body; (C) post-metamorphic juvenile. Scale = 0.25 mm. Photographs by C.F. Caballes.

the plankton prior to contacting any substratum (Domanski 1984, Hendler 1991, McEdward & Miner 2001, Selvakumaraswamy & Byrne 2004), and numerous studies specifically report metamorphic responses of echinoderm larvae (e.g. Pearce & Scheibling 1991, Yazaki & Harashima 1994, Naidenko 1996, Matsuura et al. 2009).

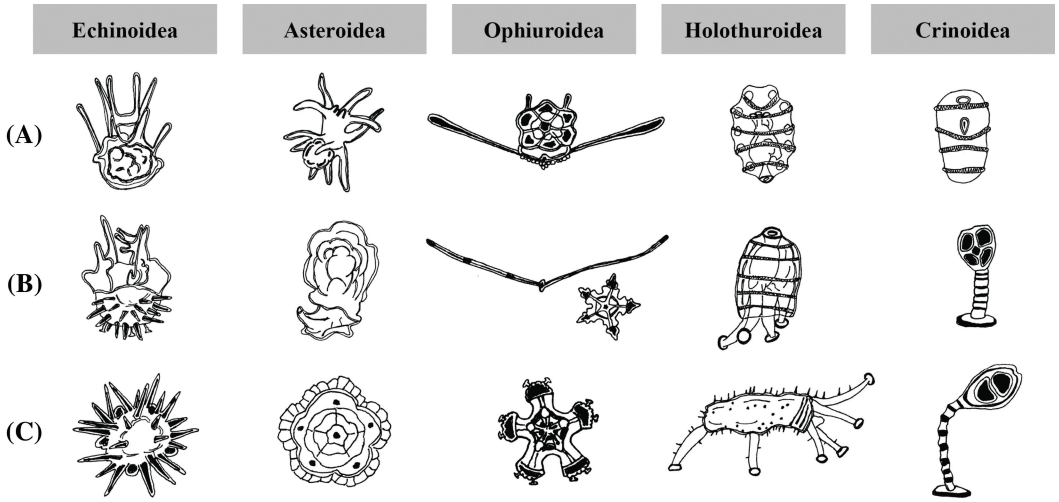

Figure 3 Echinoderm larval development through metamorphosis, as exemplified by an echinoid (*Tripneustes gratilla*), asteroid (*Acanthaster* cf. *solaris*), ophiuroid (*Ophiothrix exigua*), holothuroid (*Holothuria scabra*) and crinoid (*Antedon serrata*): (A) late-stage larva; (B) settlement and metamorphosis; (C) post-metamorphic juvenile. Illustrations by C.F. Caballes; based on Caballes & Pratchett (2014), Kitazawa et al. (2015), Toha et al. (2017), Yamakawa et al. (2020) and Nontunha et al. (2021).

Pre-settlement processes

A range of abiotic and biotic factors may influence echinoderm larvae prior to settlement. Crucially, mortality is thought to be very high during the sometimes highly prolonged planktonic larval stage (Cameron et al. 1985, Bosch et al. 1987, Pratchett et al. 2017, Ebert 2021b). With a few exceptions (Rumrill 1987, López et al. 1998, Lamare & Barker 1999), most estimates of larval mortality in echinoderms come from controlled laboratory studies, such that larval mortality may be even higher in natural environments, due to increased environmental fluctuations, limited prey availability and potentially high rates of predation (Pearse & Cameron 1991, Ebert 2021b).

The principal abiotic (environmental) factors affecting echinoderm larvae during their planktonic life stage are thought to be advection, temperature and salinity (reviewed by Balch & Scheibling 2001). Most notably, the dispersal and distribution of planktonic larvae depends on advective transport, which in turn is driven by large-scale oceanographic features and currents, and local hydrodynamics (Ebert 1983, Pearse & Cameron 1991). Given their generally limited swimming capacity, larval dispersal models often assume that echinoderm larvae are passive particles (Montgomery et al. 2017), although swimming is critical in determining larval distribution and settlement patterns at relatively small spatial scales. The important role of hydrodynamic forces in determining large-scale (e.g. inter-reef) patterns of species' distributions and population connectivity has been demonstrated for the Pacific crown-of-thorns starfish *Acanthaster* cf. *solaris* (Black & Moran 1991; Hock et al. 2014) and the echinoid *Centrostephanus rodgersii* (Banks et al. 2007). However, detailed biophysical models which incorporate species-specific knowledge of larval biology and behaviour (e.g. Bode et al. 2019) are yet to be developed for any echinoderm species.

Changes in key physical variables such as temperature and salinity may also influence larval development (Byrne et al. 2009, Li et al. 2011a, Privitera et al. 2011, Kamya et al. 2014), survival (Lucas 1973) and planktonic larval duration (Thorson 1950, Agatsuma et al. 1998). For example, larvae of the echinoid *Dendraster excentricus* take three times as long to reach the six-armed larval stage at 12°C compared to 22°C (McEdward 1984). Numerous studies attribute fluctuations in reproductive behaviour and settlement rates of echinoderms to changes in temperature (Balch et al. 1999, Hernández et al. 2010, Sotelo-Casas et al. 2016, Glockner-Fagetti & Phillips 2020, Okamoto et al. 2020, Caballes et al. 2021), although these patterns may be conflated by simultaneous changes in a range of environmental conditions. In some echinoids, temperature changes have explicitly been linked to population irruptions and range extensions (Hart & Scheibling 1988, Ling et al. 2008, 2009).

In terms of biotic factors, predation and starvation in the pelagic developmental habitat have long been recognised as key factors contributing to high levels of mortality among planktotrophic larvae across a range of marine taxa (Thorson 1950, Birkeland 1982, Young & Chia 1987). For echinoderms, laboratory experiments have demonstrated the importance of predators in regulating larval densities (Rumrill & Chia 1985, Cowan et al. 2016a, 2020). At the same time, food availability and potential starvation inherently constrain the condition, size and survival of planktotrophic echinoderm larvae that are reliant on exogenous nutrition to complete development and facilitate settlement (Basch & Pearse 1995). Larval starvation has been viewed as less important than predation in directly limiting larval supply (Pearse & Cameron 1991); however, for several echinoderm species, there is experimental evidence that larval nutrition influences the quality and quantity of planktotrophic larvae (Fenaux et al. 1994, Fabricius et al. 2010). Spatiotemporal variation in settlement rates of echinoderms has been attributed to differences in phytoplankton availability in the weeks or months preceding settlement (Hernández et al. 2010, García-Sanz et al. 2014, Glockner-Fagetti & Phillips 2020, Okamoto et al. 2020), suggesting that food availability may exert significant effects on planktotrophic echinoderm larvae. The qualitative effect of starvation on the condition of echinoderm larvae prior to and during settlement was confirmed by Basch & Pearse (1995), who demonstrated a strong effect on larval size, development, swimming and substrate searching behaviour of the asteroid *Asterina miniata*. Likewise, Pratchett et al. (2017) reported delays in peak settlement of *Acanthaster* cf. *solaris* at low food levels, which are likely reflective of delays in larval development. Thus, even if direct mortality due to starvation is unlikely under natural conditions, prolonged planktonic existence may increase the risk of predation and thus lead to higher total mortality, or advection to less suitable settlement sites (Thorson 1950). Conversely, a shortened planktonic phase, due to early metamorphosis of less developed echinoderm larvae in response to settlement cues, may also increase total mortality within a cohort of echinoderms (Mos & Dworjanyn 2016).

While a range of abiotic and biotic factors can influence the abundance and distribution of larval echinoderms, and thus the temporal and spatial variation in the supply of competent larvae, the individual roles and combined effect of these processes on larval settlement remain unclear. Further adding complexity to this puzzle, larval cloning has been described for some echinoderms (Balser 1998, Allen et al. 2019, Hart et al. 2021). If supported by environmental conditions, cloning has the potential to influence dispersal distance of planktonic larval stages and increase the number of larvae reaching metamorphic competence (Allen et al. 2019), which, in turn, may influence rates of settlement and recruitment (Hart et al. 2021).

Species with complex, planktonic, feeding larvae are common across all echinoderm classes, with the exception of crinoids (McEdward & Miner 2001, Uthicke et al. 2009). However, all five classes also possess lecithotrophic larval types in both planktonic and benthic developmental habitats (McEdward & Miner 2001, Raff & Byrne 2006, Byrne & O'Hara 2017), with benthic development and brooding documented in some echinoids (Emlet et al. 1987), crinoids (Lahaye & Jangoux 1985), holothuroids (McEuen 1986) and ophiuroids (Ebert 1983). Importantly, the dispersal, condition and survival of planktonic echinoderm larvae appears to be governed by a suite of abiotic and biotic variables, which will, in turn, affect spatiotemporal patterns of settlement (Basch & Pearse 1995). When analysing the drivers of settlement patterns, however, the capability for long-distance dispersal also needs to be considered, especially for species with prolonged planktonic phases, as observed in asteroids of the genus *Luidia* which are known to spend >1 year in the water column as exceptionally large post-larvae (Wilson 1978, Domanski 1984).

Metamorphic competence and substratum search

Metamorphic competence is the developmental capacity of larvae to undergo complete morphogenesis that is necessary for settlement including metamorphosis (Hadfield et al. 2001). Such competence is conditional upon complete larval development, which requires a minimum prescribed, but species-specific, pre-competency period (Sutherby et al. 2012). Larval developmental rates and the minimum pre-competency period are, however, influenced by nutritional resources and temperature (Lucas 1982, Hadfield et al. 2001). In general, most planktotrophic larvae in the pelagic habitat attain competence after a few weeks to several months. For example, planktotrophic larvae of the echinoid *Strongylocentrotus purpuratus* acquire metamorphic competence after 4–6 weeks post-fertilisation (Strathmann 1987). Lecithotrophic echinoderm larvae may acquire competence within days (Pawlik 1992, Hadfield et al. 2001). Another echinoid, *Heliocidaris erythrogramma*, for example, has a highly reduced larva, which develops from large eggs and attains metamorphic competence within 3–6 days (Byrne et al. 2001).

While metamorphic (or settlement) competence is conditional upon larval development, metamorphosis itself is often induced by specific environmental conditions or cues (as discussed later). Importantly, echinoderm larvae may attain competence long before they actually settle, deferring metamorphosis until settlement opportunities present (Birkeland et al. 1971, Hadfield et al. 2001, Pratchett et al. 2017). Extended competency periods are particularly pronounced for planktotrophic larvae (Hadfield et al. 2001), with larvae remaining in the water column until the presence of suitable habitat is signalled by abiotic or biotic cues, as opposed to immediate metamorphosis in a potentially unsuitable habitat. For example, short-term exposure to turbulence signalling proximity to reefs can cause echinoid larvae to immediately transition from a pre-competent to a competent state (Gaylord et al. 2013, Hodin et al. 2015, Ferner et al. 2019), which allows them to respond to chemical cues before and after they contact the substratum (Hodin et al. 2020). Similarly, histamine, a compound associated with various marine algae, has been shown to modulate metamorphic competence in *Strongylocentrotus purpuratus* (Sutherby et al. 2012). At the same time, this organic compound is hypothesised to maintain the developmental priming of competence once larvae are morphologically competent by inhibiting settlement in the absence of suitable cues (Sutherby et al. 2012). More specifically, histamine achieves the maintenance of competency in echinoid larvae by inhibiting caspase-mediated apoptosis (Sutherby et al. 2012), and thus suppressing the destruction of larval-specific structures necessary to settle and metamorphose until a suitable settlement site is found.

Throughout this competency period, larvae continue to live a functional planktonic life until it culminates in either passive deposition onto the substratum, or active selection of a suitable habitat/substratum (Figure 1). Likewise, larvae are thought to delay settlement in response to cues indicative of an unsuitable settlement site (Hadfield & Paul 2001), although inhibitory cues have rarely been explored in echinoderm studies. All ophiuroids and some groups of asteroids and holothuroids metamorphose in the plankton and then often settle indiscriminately (Wilson 1978, Domanski 1984, Hendler 1991, Komatsu et al. 2000, McEdward & Miner 2001, Selvakumaraswamy & Byrne 2004, Morgan & Jangoux 2005). However, other echinoderm larvae with a planktonic larval phase generally require physical or chemical settlement cues and subsequent contact with the substratum in order to metamorphose (Hadfield & Paul 2001). Once a larva has the ability to discriminate cues, substratum choice appears to be primarily limited by the availability of suitable substrata, and the availability and concentration of pertinent cues in the water column. Waterborne chemical cues originating from conspecifics (Pearce & Scheibling 1990a, Dworjanyn & Pirozzi 2008) and various algal substrata (Williamson et al. 2000, Swanson et al. 2004) have been demonstrated to initiate settlement and subsequent metamorphosis in echinoderm taxa. Such cues can be highly specific (Table 1) and are mostly detected in relatively close proximity to their source organisms.

Passive transport is generally thought to be the major determinant in the dispersal of echinoderm larvae over broad spatial scales. Echinoid larvae are hypothesised to also use environmental signposts such as turbulence to narrow down potential areas of settlement at relatively localised scales (Gaylord et al. 2013, Hodin et al. 2020). Short-term behavioural responses of echinoid larvae to such signposts include the cessation of swimming, which facilitates sinking and contact with the substratum (Ferner et al. 2019). Although direct evidence of active vertical movement is sparse, the extremely fast sinking and substratum attachment in response to inductive settlement cues (Tables 1 and 2) suggests that most competent echinoderm larvae do not randomly or passively sink to the bottom. Rapid sinking may also be a response to the high densities of planktivorous invertebrates and fish that associate with the substratum (Tegner & Dayton 1981, Balch & Scheibling 2001).

Other key physical variables with the potential to substantially alter settlement patterns are temperature and pH (Mos et al. 2011, Li et al. 2011b). Settlement pulses in echinoids, holothuroids and ophiuroids have been linked to temperature peaks and fluctuations (Balch et al. 1999, García-Sanz et al. 2014, Sotelo-Casas et al. 2016, Glockner-Fagetti & Phillips 2020). For example, larval settlement of the echinoid *Strongylocentrotus purpuratus* in southern California was orders of magnitude lower during warm, El Niño conditions (Okamoto et al. 2020), while settlement of *Diadema africanum* was found to be high during warm years in the Canary Islands (Hernández et al. 2010). However, these links could be driven by other factors, such as spawning induced by temperature change (Caballes & Pratchett 2017, Caballes et al. 2021), and need to be explicitly tested in field and laboratory experiments. In a laboratory study, settlement of the echinoid *Tripneustes gratilla* was unaffected by temperatures between 24 and 30°C, but was substantially reduced at 33°C (Mos et al. 2011). Likewise, warmer temperatures reduced settlement rates in the holothuroid *Apostichopus japonicus* (Li et al. 2011b) and negatively affected development in settlement-stage individuals of the echinoid *Heliocidaris erythrogramma* (Byrne et al. 2011). Moreover, reduced settlement rates at lowered pH have been demonstrated in some echinoids (García et al. 2015), asteroids (e.g. Uthicke et al. 2013) and holothuroids (e.g. Li et al. 2011b). Low pH negatively affected morphological traits, but not settlement rates in the echinoid *Centrostephanus rodgersii*; however, high pH reduced settlement rates considerably (Mos et al. 2020). Conversely, the echinoids *Evechinus chloroticus* (Espinel-Velasco et al. 2020) and *Pseudechinus huttoni* (Houlihan et al. 2020) appeared unaffected by changes in pH. Settlement was delayed at reduced pH in the echinoid *Paracentrotus lividus* (García et al. 2015), but not in *Arbacia lixula* (Wangensteen et al. 2013) and *Heliocidaris erythrogramma* (Byrne et al. 2011). Espinel-Velasco et al. (2018) and Nelson et al. (2020) argued that decreases in seawater pH may affect microbial communities and thus also indirectly alter the settlement of echinoderms that respond to cues associated with biofilms. On balance, both passive processes and active vertical migration in response to environmental cues appear to play significant, but highly variable roles between the attainment of competence and the first contact with the substratum in planktonic echinoderm larvae.

Substratum contact, exploration and selection

Once a competent larva has found its way to the substratum, contact is established and attachment of the larva to the surface of the substratum occurs (Yamaguchi 1973, Hamel & Mercier 1996, Gosselin & Jangoux 1998). For many sessile marine invertebrates, attachment to a substratum may already represent the initiation of metamorphosis into their juvenile form (Hadfield & Paul 2001). Conversely, many motile species, including echinoderms, have the ability to detach and reattach to substrata while delaying metamorphosis and exploring the suitability of various settlement

Table 1 Summary of natural chemical cues known to induce larval settlement and metamorphosis in echinoderm classes and species

Chemical stimulus	Class	Species	Reference
Gregarious settlement cues			
Peptide	Echinoidea	*Dendraster excentricus*	Highsmith (1982), Burke (1984), Highsmith & Emlet (1986)
Not characterised	Echinoidea	*Echinarachnius parma*, *Scaphechinus mirabilis*, *Tripneustes gratilla*	Highsmith & Emlet (1986), Pearce & Scheibling (1990a), Takeda (2008), Dworjanyn & Pirozzi (2008), Mos et al. (2011)
	Ophiuroidea	*Ophiothrix fragilis*	Warner (1971), Morgan & Jangoux (2004), Morgan & Jangoux (2005)
	Holothuroidea	*Molpadia intermedia*, *Psolus chitonoides*	Young & Chia (1982), McEuen & Chia (1985)
	Crinoidea	*Antedon bifida*, *Florometra serratissima*, *Dorometra sesokonis*	Mladenov & Chia (1983), Lahaye & Jangoux (1985), Obuchi et al. (2010)
Associative settlement cues			
Seagrass			
Enhalus acoroides	Holothuroidea	*Holothuria scabra*	Mercier et al. (2000), Agudo (2007)
Halophila ovalis	Echinoidea	*Holopneustes purpurascens, H. inflatus*	Swanson et al. (2012)
Posidonia australis	Echinoidea	*Holopneustes purpurascens, H. inflatus*	Swanson et al. (2012)
Posidonia oceanica	Echinoidea	*Paracentrotus lividus*	Privitera et al. (2011)
Thalassia hemprichii	Holothuroidea	*Holothuria scabra*	Mercier et al. (2000), Agudo (2007)
Zostera capricorni	Echinoidea	*Holopneustes purpurascens, H. inflatus*	Swanson et al. (2012)
Red algae			
Chondrus crispus	Echinoidea	*Strongylocentrotus droebachiensis*	Pearce & Scheibling (1991)
Delisea pulchra	Echinoidea	*Holopneustes purpurascens*	Williamson et al. (2000), Swanson et al. (2004, 2006)
Carbohydrate		*Holopneustes purpurascens*	Williamson et al. (2000)
Floridoside-isethionic acid			
Histamine		*Holopneustes purpurascens, H. inflatus*, *Heliocidaris erythrogramma, Centrostephanus rodgersii*	Swanson et al. (2004, 2006, 2012)
Laurencia rigida	Echinoidea	*Holopneustes purpurascens*	Williamson et al. (2000)
Laurencia obtusa, L. rigada, *Laurencia* sp.	Echinoidea	*Centrostephanus rodgersii*, *Tripneustes gratilla*	Dworjanyn & Pirozzi (2008), Swanson et al. (2012)
Mastocarpus stellatus	Echinoidea	*Strongylocentrotus droebachiensis*	Pearce & Scheibling (1991)
Palmaria palmata	Echinoidea	*Strongylocentrotus droebachiensis*	Pearce & Scheibling (1991)
Polysiphonia harveyi, *P. lanosa*	Echinoidea	*Strongylocentrotus droebachiensis*	Pearce & Scheibling (1991)
Solieria robusta	Echinoidea	*Holopneustes purpurascens*	Williamson et al. (2000)

(*Continued*)

Table 1 (*Continued*) Summary of natural chemical cues known to induce larval settlement and metamorphosis in echinoderm classes and species

Chemical stimulus	Class	Species	Reference
Red algal turf	Echinoidea	*Strongylocentrotus purpuratus, Paracentrotus lividus*	Rowley (1989), Privitera et al. (2011)
Coralline red algae			
Amphiroa anceps	Echinoidea	*Centrostephanus rodgersii, Holopneustes purpurascens, H. inflatus, Heliocidaris erythrogramma, Tripneustes gratilla*	Williamson et al. (2000), Huggett et al. (2006) Swanson et al. (2006, 2012), Dworjanyn & Pirozzi (2008)
Corallina elongata	Echinoidea	*Arbacia lixula, Echinocardium cordatum, Paracentrotus lividus*	Nunes & Jangoux (2008), Privitera et al. (2011)
Corallina pilulifera	Echinoidea	*Pseudocentrotus depressus, Anthocidaris crassispina*	Kitamura et al. (1992, 1993, 1994)
Polyunsaturated fatty acids			Kitamura et al. (1993, 1994)
Eicosapentaenoic acid			Kitamura et al. (1993)
Arachidonic acid			Kitamura et al. (1993)
Dihomo-γ-linolenic acid			Kitamura et al. (1994)
Eicosatrienoic acid			Kitamura et al. (1994)
Corallina officinalis	Echinoidea	*Centrostephanus rodgersii, Heliocidaris erythrogramma, Holopneustes purpurascens, Strongylocentrotus droebachiensis, Tripneustes gratilla*	Pearce & Scheibling (1990b, 1991), Williamson et al. (2000), Huggett et al. (2006), Dworjanyn & Pirozzi (2008), Mos et al. (2011), Swanson et al. (2012)
Corallina sp.	Echinoidea	*Evechinus chloroticus*	Lamare & Barker (2001)
Lithothamnion californicum	Echinoidea	*Strongylocentrotus purpuratus*	Rowley (1989)
Lithothamnion glaciale	Echinoidea	*Strongylocentrotus droebachiensis*	Pearce & Scheibling (1990b, 1991)
Lithothamnium sp. (likely *Lithothamnion proliferum*)	Asteroidea	*Acanthaster* cf. *solaris*	Johnson et al. (1991), Johnson & Sutton (1994), Uthicke et al. (2018)
Lithophyllum incrustans	Echinoidea	*Arbacia lixula, Paracentrotus lividus*	Privitera et al. (2011)
Lithophyllum yessoense	Echinoidea	*Strongylocentrotus nudus*	Taniguchi et al. (1994)
Dibromomethane			Taniguchi et al. (1994)
Mesophyllum insigne	Asteroidea	*Stichaster australis*	Barker (1977)
Neogoniolithon fosliei	Asteroidea	*Acanthaster* cf. *solaris*	Johnson et al. (1991)
Phymatolithon laevigatum, R. rugulosum	Echinoidea	*Strongylocentrotus droebachiensis*	Pearce & Scheibling (1990b)
Coralline red algae spp.	Echinoidea	*Echinometra mathaei, E. oblonga, E.* spp., *Strongylocentrotus droebachiensis, Tripneustes gratilla*	Rahman & Uehara (2001), Dworjanyn & Pirozzi (2008), Scheibling & Robinson (2008)
Non-coralline crustose red algae			
Hildenbrandia rubra	Echinoidea	*Strongylocentrotus droebachiensis*	Pearce & Scheibling (1991)

(*Continued*)

Table 1 (*Continued*) Summary of natural chemical cues known to induce larval settlement and metamorphosis in echinoderm classes and species

Chemical stimulus	Class	Species	Reference
Brown algae			
Dictyota dichotoma	Echinoidea	*Centrostephanus rodgersii*, *Tripneustes gratilla*	Dworjanyn & Pirozzi (2008) Swanson et al. (2012)
Dilophus marginatus	Echinoidea	*Centrostephanus rodgersii*, *Tripneustes gratilla*	Dworjanyn & Pirozzi (2008), Swanson et al. (2012)
Ecklonia radiata	Echinoidea	*Heliocidaris erythrogramma*, *Holopneustes purpurascens*, *Tripneustes gratilla*	Huggett et al. (2006), Swanson et al. (2006), Dworjanyn & Pirozzi (2008)
Fucus distichus	Echinoidea	*Strongylocentrotus droebachiensis*	Pearce & Scheibling (1991)
Homeostrichus olsenii	Echinoidea	*Holopneustes purpurascens*, *Tripneustes gratilla*	Swanson et al. (2006), Dworjanyn & Pirozzi (2008)
Laminaria digitata & *L. longicruris*	Echinoidea	*Strongylocentrotus droebachiensis*	Pearce & Scheibling (1991)
Pylaiella littoralis	Echinoidea	*Strongylocentrotus droebachiensis*	Pearce & Scheibling (1991)
Sargassum linearifolium	Echinoidea	*Centrostephanus rodgersii*, *Heliocidaris erythrogramma*, *Tripneustes gratilla*	Huggett et al. (2006), Dworjanyn & Pirozzi (2008), Mos et al. (2011), Swanson et al. (2012)
Sargassum vestitum	Echinoidea	*Heliocidaris erythrogramma*	Huggett et al. (2006)
Sargassum sp.	Echinoidea	*Tripneustes gratilla*	Juinio-Meñez & Bangi (2010)
	Holothuroidea	*Holothuria scabra*	Agudo (2007)
Stypocaulon scoparium	Echinoidea	*Paracentrotus lividus*	Privitera et al. (2011)
Zonaria angustata	Echinoidea	*Tripneustes gratilla*	Dworjanyn & Pirozzi (2008)
Green algae			
Codium fragile	Echinoidea	*Heliocidaris erythrogramma*	Huggett et al. (2006)
Enteromorpha linza	Echinoidea	*Paracentrotus lividus*	Gosselin & Jangoux (1996)
Enteromorpha sp.	Echinoidea	*Strongylocentrotus droebachiensis*	Pearce & Scheibling (1991)
Spongomorpha aeruginosa	Echinoidea	*Strongylocentrotus droebachiensis*	Pearce & Scheibling (1991)
Ulva lactuca	Echinoidea	*Tripneustes gratilla*	Dworjanyn & Pirozzi (2008)
Endophytic microalgae			
Ulvella lens	Echinoidea	*Apostichopus japonicas*, *Strongylocentrotus nudus*	Taniguchi et al. (1994), Takahashi et al. (2002), Matsuura et al. (2009)
Dibromomethane	Echinoidea	*Strongylocentrotus nudus*	Taniguchi et al. (1994)
Glycoglycerolipids	Echinoidea	*Strongylocentrotus intermedius*, *S. nudus*	Takahashi et al. (2002)
Polychaete			
Phyllochaetopterus prolifica	Asteroidea	*Mediaster aequalis*	Birkeland et al. (1971), Bryan (2004)
Biofilm-associated cues			
Bacteria			
Photobacterium phosphoreum	Echinoidea	*Holopneustes purpurascens*	Swanson et al. (2006)
Photobacterium spp.	Echinoidea	*Heliocidaris erythrogramma*	Huggett et al. (2006)
Pseudoalteromonas luteoviolacea, other *Pseudoalteromonas* spp.	Echinoidea	*Heliocidaris erythrogramma*	Huggett et al. (2006)
Shewanella spp.	Echinoidea	*Heliocidaris erythrogramma*	Huggett et al. (2006)

(*Continued*)

Table 1 (*Continued*) Summary of natural chemical cues known to induce larval settlement and metamorphosis in echinoderm classes and species

Chemical stimulus	Class	Species	Reference
Thalassomonas viridans	Echinoidea	*Holopneustes purpurascens*	Swanson et al. (2006)
Vibrio spp.	Echinoidea	*Heliocidaris erythrogramma*	Huggett et al. (2006)
Other/unspecified bacteria	Echinoidea	*Arachnoides placenta*, *Arbacia punctulata*, *Heliocidaris erythrogramma*, *Lytechinus pictus*, *Tripneustes gratilla*	Cameron & Hinegardner (1974), Huggett et al. (2006), Dworjanyn & Pirozzi (2008), Mos et al. (2011)
	Asteroidea	*Acanthaster* cf. *solaris*	Johnson et al. (1991), Johnson & Sutton (1994)
Periphytic diatoms			
Achnanthes sp.	Holothuroidea	*Apostichopus japonicus*	Ito & Kitamura (1997)
Amphora sp.	Holothuroidea	*Apostichopus japonicus*	Ito & Kitamura (1997)
Navicula sp.	Holothuroidea	*Holothuria scabra*, *Apostichopus japonicus*	Ito & Kitamura (1997), Agudo (2007)
Nitzschia spp.	Echinoidea	*Tripneustes gratilla*	Mos et al. (2011)
	Holothuroidea	*Holothuria scabra*, *Apostichopus japonicus*	Ito & Kitamura (1997), Agudo (2007)
Platymonas sp.	Holothuroidea	*Holothuria scabra*	Agudo (2007)
Multiple species (predominantly *Navicula*, *Amphora*, *Achnanthes* & *Nitzschia* spp.)	Echinoidea	*Anthocidaris crassispina*, *Pseudocentrotus depressus*	Rahim & Kitamura (2004)
Unspecified biofilm	Echinoidea	*Anthocidaris crassispina*, *Evechinus chloroticus*, *Pseudocentrotus depressus*, *Tripneustes gratilla*	Lamare & Barker (2001), Rahim & Kitamura (2004), Mos et al. (2011)
	Asteroidea	*Stichaster australis*, *Coscinasterias calamaria*	Barker (1977)

sites (Crisp 1984, Hadfield & Paul 2001, Scheibling & Robinson 2008, Vellutini & Migotto 2010). Competent larvae of the crown-of-thorns starfish *Acanthaster* cf. *solaris* (Figure 2) have been observed to drift downwards and flex the anterior body dorsally to orient their brachiolar arms towards the substratum in an attempt to test the suitability of substratum surfaces for settlement (Yamaguchi 1973). Similarly, the asteroid *Patiriella regularis* swims along the substratum surface and exhibits searching behaviour using its median brachium (Byrne & Barker 1991). Echinoid larvae (*Paracentrotus lividus*) interrupt the beatings of their epaulette cilia to reach the substratum, which is then tested by primary podia (Gosselin & Jangoux 1998, Flammang et al. 1998). Without appropriate cues, the larvae fold back their podia and ascend back into the water column via ciliary beating of the epaulettes (Gosselin & Jangoux 1998). In crinoids, the adhesive pit serves to attach the larval body to the substratum by developing the attachment disc of a juvenile (McEdward & Miner 2001). Upon first substratum contact with its tentacles, the holothuroid *Cucumaria frondosa* spent up to 40 hours searching the substratum surface for a suitable settlement site using its ambulacral podia (Hamel & Mercier 1996).

In most cases, specificity in echinoderm settlement is mediated by chemical cues associated with the biogenic substrate (Pawlik 1992, Hadfield & Paul 2001, Dworjanyn & Pirozzi 2008). Evidence of the inductive capacity of cues associated with conspecifics (e.g. Pearce & Scheibling 1990a), prey (e.g. Birkeland et al. 1971), algae (e.g. Williamson et al. 2020) and biofilms (Hugget et al. 2006) is highly abundant for echinoderms (Table 1). When such cues signal the suitability of

Table 2 Summary of 'artificial' chemical cues (i.e. neurotransmitters and ions) known to induce larval settlement and metamorphosis in echinoderm classes and species

Compound	Class	Species	Reference
Amino acid derivatives			
Glutamine	Echinoidea	*Scaphechinus mirabilis*, *Strongylocentrotus intermedius*, *Pseudocentrotus depressus*	Yazaki & Harashima (1994), Naidenko (1996)
γ-Amino butyric acid (GABA)	Echinoidea	*Echinometra mathaei*, *E. oblonga*, *E.* spp., *Strongylocentrotus droebachiensis*	Pearce & Scheibling (1990a), Rahman & Uehara (2001)
	Asteroidea	*Mediaster aequalis*	Bryan (2004)
	Holothuroidea	*Apostichopus japonicus*	Sun et al. (2014)
		Holothuria scabra	Nontunha et al. (2021)
Glutamic acid	Echinoidea	*Scaphechinus mirabilis*, *Strongylocentrotus intermedius*	Naidenko (1996)
Monosodium glutamate	Holothuroidea	*Holothuria scabra*	Nontunha et al. (2021)
Tyrosine derivatives			
Dopamine	Echinoidea	*Dendraster excentricus*	Burke (1983b)
	Holothuroidea	*Apostichopus japonicus*, *Holothuria scabra*	Matsuura et al. (2009), Sun et al. (2014), Nontunha et al. (2021)
L-3,4-Dihydroxyphenylalanine (L-DOPA)	Echinoidea	*Dendraster excentricus*	Burke (1983b)
	Holothuroidea	*Apostichopus japonicus*	Matsuura et al. (2009), Sun et al. (2014)
		Holothuria scabra	Nontunha et al. (2021)
Epinephrine	Holothuroidea	*Apostichopus japonicus*	Matsuura et al. (2009)
Norepinephrine	Holothuroidea	*Apostichopus japonicus*	Matsuura et al. (2009) Sun et al. (2014)
Choline derivative			
Acetylcholine	Holothuroidea	*Apostichopus japonicus*	Sun et al. (2014)
Ions			
K^+	Echinoidea	*Clypeaster rosaceus*, *Colobocentrotus atratus*, *Dendraster excentricus*, *Diadema antillarum*, *Echinarachnius parma*, *Echinometra lucunter*, *E. viridis*, *Heterocentrotus mamallatus*, *Leodia sexiesperforata*, *Lytechinus* variegatus, *Mellita tenuis*, *Mesocentrotus franciscanus*, *Strongylocentrotus droebachiensis*, *S. fragilis*, *S. pallidus*, *S. purpuratus*	Cameron et al. (1989), Pearce & Scheibling (1994), Carpizo-Ituarte et al. (2002), Heyland et al. (2004, 2006), Heyland & Hodin (2004), Gaylord et al. (2013), Hodin et al. (2015, 2019)
	Holothuroidea	*Apostichopus japonicus*	Sun et al. (2014)
Ca^{2+}	Echinoidea	*Lytechinus variegatus*	Cameron et al. (1989)
NH_4^+	Holothuroidea	*Apostichopus japonicus*	Sun et al. (2014)

a site, the cascade of settlement events, comprising initial adhesion (Crisp 1984) and metamorphic morphogenesis of echinoderm larvae, can occur rapidly (Juinio-Meñez & Bangi 2010, Swanson et al. 2012). If, however, no inductive cue is present, the explorative period may be prolonged (Hamel & Mercier 1996), and competent larvae may swim off in a broad-scale search for other surfaces. As a result, a larva may risk being exposed to benthic and sometimes planktonic predators during this period (Tegner & Dayton 1981, Cowan et al. 2016a,b), but the trade-off is an increased chance of settlement and post-metamorphic success.

Although their importance appears to be secondary to chemical cues, physical factors, such as light intensity and the physical properties associated with the substratum, may also play a significant role in settlement induction once a competent larva has contacted the substratum surface (Young & Chia 1984, Privitera et al. 2011). More specifically, sedimentation, the contour, microtopography, texture, exposure and the thermal capacity of a substratum may affect where competent larvae choose to settle, depending on taxon-specific habitat requirements and cue responsiveness (Young & Chia 1984, Walters & Wethey 1991, Hamel & Mercier 1996). For example, fine sediments have been identified as a potential inhibitor of settlement for *Evechinus chloroticus* (Phillips & Shima 2006, Walker 2007), while larvae of the crown-of-thorns starfish *Acanthaster* cf. *solaris* are thought to favour particular substrata because their rough texture and depressions suit their metamorphosing larvae (Lucas 1974, Ormond & Campbell 1974). Other asteroids (Barker 1977, Barker & Nichols 1983, Metaxas et al. 2008) and holothuroids (Hamel & Mercier 1996) predominantly settle on the underside of rocks and rubble. This pattern of substratum selectivity may be linked to light exposure, as settlers of the holothuroid *Cucumaria frondosa* not only showed strong selectivity for the undersides of the physical substratum, but were highly sensitive to light (Hamel & Mercier 1996). When exposed to light, settlement of *C. frondosa* larvae was highest on rocky substrates with crevices, relatively high on gravel and smooth rocky substrates and substantially lower on mud, sand and glass (Hamel & Mercier 1996). Largely photopositive during their exploration and substratum selection, these larvae were observed to rapidly change their initial site of substratum contact and move to light sheltered areas on the substratum (Hamel & Mercier 1996). Similarly, *Patiriella regularis* asteroids settle on the undersides of substrata and appear to be light sensitive during the settlement phase (Byrne & Barker 1991). Given the generally high prevalence of light sensitivity across this phylum (Byrne & O'Hara 2017), light availability and intensity are likely also to play an underappreciated role in the substratum search and selection by other echinoderm groups.

Larval metamorphosis

Upon reception of metamorphic cues, larval metamorphosis, a complex network of cellular processes which involve the transformation and reorganisation of most tissues and cells, is induced (Figure 3, Pearse & Cameron 1991, Morgan & Jangoux 2005). All ophiuroids, and some asteroids, holothurians and echinoids are able to metamorphose in the water column and sink to the substratum in their juvenile form (Wilson 1978, Domanski 1984, Hendler 1991, McEdward & Miner 2001, Morgan & Jangoux 2005, Selvakumaraswamy & Byrne 2006). However, crinoids and most asteroid, echinoid and holothuroid groups, including the ecologically and economically important taxa discussed in this review, generally attach to a substratum prior to metamorphosis (Pearse & Cameron 1991, McEdward & Miner 2001). Metamorphosis in echinoderms begins with a larva initiating the degeneration of larva-specific characters and ends once all juvenile characters have emerged and the juvenile is fully functioning in its (usually benthic) habitat (*sensu* Hadfield et al. 2001). In general, this includes a transformation of the fundamental bilateral symmetry of echinoderm larvae into a juvenile form with a radial symmetry (Figures 2 and 3, McEdward & Miner 2001). The numerous morphogenic events occurring during this period have been documented for some species, including echinoids (e.g. Mazur & Miller 1971), asteroids (e.g. Caballes & Pratchett 2014) and ophiuroids (e.g. Morgan & Jangoux 2005), and have been reviewed in detail for Echinoidea (e.g. Burke

1983a, Pearse & Cameron 1991). For example, competent (late brachiolaria) larvae of the asteroid *Acanthaster* cf. *solaris* attach to the substratum surface and metamorphose into their juvenile form by absorbing the larval body into the asteroid (Figure 2). Similarly, most echinoids must undergo substantial physiological changes concomitant with the transition from a pelagic and planktotrophic larva to a benthic and herbivorous juvenile (Figure 3).

Neural mechanisms for the induction of echinoderm metamorphosis have been supported by multiple lines of evidence (Sutherby et al. 2012, Caballes and Pratchett 2014). Specifically, neurons in the apical organ of competent echinoid larvae are suggested to detect exogenous metamorphic cues and translate that information into behavioural and physiological responses (Burke 1979, 1983b, Brandhorst et al. 2001). However, little is known about the causal relationships among the various cellular processes occurring during metamorphic transformation. In echinoids, inductive cues are likely transduced by epithelial cells via Ca^{2+}-dependent K^{+} conductance, triggering the involution of the larval epithelium (Emlet 1988, Pearse & Cameron 1991). Few superficial organs have been characterised in echinoid larvae; however, cues for the nerve cells may be received through sensory cells on the surface of the podia of the echinoid rudiment (Burke 1980, Smith et al. 2008, Ullrich-Lüter et al. 2011, Valero-Garcia et al. 2016, Marconi et al. 2019). At the same time, the eversion of the urchin rudiment causes the musculoepithelial cells to contract, which alters its shape and everts the rudiment (Pearse & Cameron 1991, Vellutini & Migotto 2010). Once the larval epithelium is fully collapsed, the adult epithelium fuses on the aboral surface (Pearse & Cameron 1991, Vellutini & Migotto 2010). The enclosed larval cells then lose their attachments and degenerate (Cameron 1975, Chia & Burke 1978). Some tissues documented to break down soon after the start of metamorphosis include the epidermis, oesophagus, epaulettes, larval mouth and the ciliary bands, while the skeletal mesenchyme and larval gut are transformed into adult tissues (Chia & Burke 1978, Burke 1983a). Similar transformations occur in other echinoderm larvae, including many asteroids, echinoids and ophiuroids, which see adult rudiments unfolded and the larval body involuted into what will become the aboral surface of the juvenile body (Figure 3, Burke 1993a, Hadfield et al. 2001). In crinoids, the vestibule and associated ventral structures rotate towards the posterior end of the larvae; however, metamorphic transformations do not include a distinct adult rudiment oriented orthogonally to the larval plane of symmetry (Figure 3, McEdward & Miner 2001). Altogether, the complex network of these metamorphic processes, which are variable among taxa, transforms competent echinoderm larvae into fully functioning juveniles, which, in most cases, are able to live, move and feed in their benthic habitat.

Post-settlement processes

Newly settled echinoderms are generally subject to very high mortality within the first weeks to months of their benthic life stage (Gosselin & Qian 1997, Hunt & Scheibling 1997, Balch & Scheibling 2001). As such, our understanding of post-settlement processes is critical to determine how patterns established at settlement are altered, and the consequent effects on adult populations (Underwood & Fairweather 1989). Recruitment to juvenile and adult populations is highly variable in echinoderms, both temporally and spatially (reviewed by Ebert 1983, Balch & Scheibling 2001, Ebert 2021b), and plays a vital role in the dynamics of echinoderm populations and, in the case of ecologically important taxa, may substantially alter marine ecosystems (McClanahan 1988, Bonaviri et al. 2012, Pratchett et al. 2014). Settlement rates do not always explain patterns of distribution and abundance of conspecific juveniles and adults (Loosanoff 1964, Rowley 1989, Vermeij et al. 2010, Williams et al. 2010, 2011, Rogers & Lorenzen 2016), and adult population dynamics may be driven by early post-settlement processes, including high mortality rates soon after settlement. In the case of *Diadema antillarum* in the southern Caribbean Sea, for example, unknown sources of high post-settlement mortality may have prevented a recovery of the local adult population following the 1983 die-off, despite a substantial recovery of settlement rates (Vermeij et al. 2010).

The vast majority of early post-settlement mortality in echinoderms has been attributed to various invertebrate and fish predators (Sala & Zabala 1996, Sala et al. 1998, Hereu et al. 2005, Pederson & Johnson 2006, Clemente et al. 2009, Bonaviri et al. 2012, Cowan et al. 2017, Keesing et al. 2018). Small, recently emerged size classes are often most vulnerable to predation (McClanahan & Muthiga 1989, Pederson & Johnson 2006, Clemente et al. 2007, Ling & Johnson 2009), such as the size-dependent predation by rock lobsters on the kelp overgrazing echinoids *Heliocidaris erythrogramma* and *Centrostephanus rodgersii* (Pederson & Johnson 2006, Ling et al. 2009). Other factors that may contribute to the loss of post-settlement echinoderms from populations include starvation (Keesing and Halford 1992), disease (Hughes et al. 1985, Lessios 1988), abiotic disturbances (Cameron & Fankboner 1989), space competition (Hamel & Mercier 1996) and migration (Young & Chia 1982, Hamel & Mercier 1996), although movement over large distances is rare for small post-settlement juveniles (Rowley 1989). Of all post-settlement processes, mortality is generally viewed as having the greatest effect on recruitment to juvenile populations, but determining the relative contributions of these processes in the field has proven to be challenging (Andrew & Choat 1985, Balch & Scheibling 2001). Few studies have assessed post-settlement mortality of echinoderms under field conditions (but see Andrew & Choat 1985, Rowley 1990, Sewell & Watson 1993, Ling & Johnson 2012). Keesing et al. (2018) demonstrated the effect of predation on small post-settlement juveniles of *Acanthaster* cf. *solaris* in a caging experiment and developed a model of size- and age-dependent mortality to calculate a settlement rate threshold that leads to destructive population irruptions of this corallivorous asteroid. Quantitative estimates of both settlement rates and early post-settlement mortality may not only improve our understanding of the supply-side ecology of echinoderms (*sensu* Lewin 1986), but also help resolve priority research questions pertaining to these processes in ecologically or economically important species.

Settlement behaviour

Many echinoderm larvae that are competent to settle and metamorphose respond to chemical cues indicating the suitability of a substratum and environment for their benthic life-history stage. Chemical settlement cues originate from a wide range of sources, and their characterisation appears critical to our understanding of spatiotemporal settlement patterns, and thus the supply-side ecology of echinoderm taxa (Hadfield & Paul 2001). Moreover, identifying mechanisms of induction has applicability in the successful and efficient settlement of echinoderms in aquaculture settings (reviewed by Hodin et al. 2019). Chemical cues identified to induce echinoderm settlement generally fall under one of four categories: gregarious cues, associative cues (e.g. prey), biofilm-associated cues and neurotransmitter or ion-associated cues (Tables 1 and 2). These cues, associated with various biotic sources, can be surface bound or waterborne cues and may thus act before the substratum is physically contacted by the larva or once this contact has been established (Figure 1). Initially, only surface-bound, non-polar settlement cues were considered capable of inducing larval settlement (Pawlik 1992), because the inductive effectiveness of waterborne cues was assumed to be hindered by rapid dilution and dispersion in the surrounding seawater, inhibiting larvae from reaching the appropriate substratum (Chia et al. 1984). More recently, numerous studies have shown that water-soluble cues associated with conspecifics or live substrata can function as *in situ* inducers of larval settlement and metamorphosis in echinoderms (e.g. Williamson et al. 2000, Swanson et al. 2004, Dworjanyn & Pirozzi 2008). In most echinoderms, a single inducer is expected to trigger the entire settlement process, including the initial substratum attachment and larval metamorphosis (e.g. Burke 1984, Pearce & Scheibling 1990b). However, in cases where the inductive compound has not been fully characterised, or only conditioned water treatments have been demonstrated to induce both settlement and metamorphosis, multiple chemical compounds may be present and affecting the two processes (Hadfield & Paul 2001).

Echinoid larvae settle readily in response to a wide range of natural chemical cues, including conspecifics, seagrasses, fleshy and coralline algae, and biofilms (Table 1). Echinoid larvae often respond to a suite of cues as opposed to a single chemical signal. With the exception of a few species known to respond to cues associated with conspecifics (Highsmith 1982, Highsmith & Emlet 1986), chemical cue specificity is relatively low in echinoids. Likewise, chemical cues appear to be required for the induction of settlement and metamorphosis in asteroids, although this has only been demonstrated for few asteroid species of ecological importance (Birkeland et al. 1971, Barker 1977, Johnson et al. 1991, Johnson & Sutton 1994). In these studies, asteroids exhibited associative settlement in response to cues associated with polychaetes (Birkeland et al. 1971, Bryan 2004) and coralline algae with bacterial films (Johnson et al. 1991, Johnson & Sutton 1994). Few studies have examined possible settlement and metamorphic cues in holothuroids, ophiuroids and crinoids; however, some species of these echinoderm classes appear to exhibit gregarious settlement (Table 1). Holothuroids will settle without stimulation, but the presence of periphytic diatoms (Ito & Kitamura 1997, Agudo 2007), seagrass (Mercier et al. 2000) and macroalgal surfaces (Agudo 2007) can increase settlement and metamorphic success. More so than other echinoderms, competent ophiuroid larvae metamorphose spontaneously in the plankton and settle naturally in the absence of specific exogenous cues (Hendler 1991, Selvakumaraswamy & Byrne 2006, Hodin et al. 2019).

Gregarious settlement

Gregarious settlement, the settlement of larvae in response to cues from conspecific individuals, has been well documented in many benthic marine invertebrates (reviewed by Burke 1986). Sessile marine benthic invertebrates demonstrating chemically specific gregarious settlement have been studied extensively, and many of the chemical cues they respond to have been fully or partially characterised (Pawlik 1992, Hadfield & Paul 2001). Although the vast majority of echinoderms are motile, their locomotory capacities are often limited to relatively small spatial scales and some species are even considered effectively sessile (Young & Chia 1982). Gregarious settlement can thus be of high ecological importance. The presence of conspecifics likely indicates species-specific habitat suitability, and may support post-settlement growth and survival due to prey availability, and suitable environmental conditions (Slattery 1997). In addition, population aggregations can potentially reduce mortality rates via predator swamping (Highsmith 1982, McCallum 1987) and enhance fertilisation rates and reproductive success (Crisp 1979, Babcock et al. 1994). At the same time, conspecific aggregations could increase competition for resources, especially for species with narrow ecological niches (Crisp 1979, Slattery 1997). Settlement and metamorphosis in response to conspecifics has been demonstrated in all classes of echinoderms except asteroids (Table 1). In contrast to many sessile marine invertebrate taxa, the specific chemical stimuli triggering these responses in echinoderms have not been characterised, with the exception of one irregular echinoid (Highsmith 1982, Burke 1984).

Larvae of the sand dollar *Dendraster excentricus* preferentially settled and metamorphosed on sand from beds of adult conspecifics (Highsmith 1982, Burke 1984, Highsmith & Emlet 1986). The chemical cue produced by adults, which was extracted from sand that has been isolated for 7 weeks, effectively induced metamorphosis in *D. excentricus* (Highsmith 1982). Larval settlement responses to sand treated with proteolytic enzymes and sand in dialysis tubing indicated that the responsible chemical cue is a small peptide with low molecular mass (Highsmith 1982). This characterisation was confirmed by Burke (1984), who isolated a purified 980 Da peptide that induced metamorphosis from sand extracts using both gel permeation and liquid chromatography. However, even for purified inducers like this peptide, we do not have any information about its amino acid composition and structure, and thus the reason for its specificity in settlement response is unclear. Peptide pheromone signalling may be driving the settlement in response to substrata or water conditioned with conspecifics in other irregular (Pearce & Scheibling 1990a, Takeda 2008) or regular echinoids (e.g. Dworjanyn & Pirozzi 2008), but their chemical inducers have yet to be characterised. The sand dollar *Scaphechinus mirabilis* metamorphosed faster in adult-conditioned sand (Takeda 2008). Likewise, larvae of the sand dollar *Echinarachnius parma* showed a comparably high responsiveness to a water-soluble cue associated with sand conditioned with adult conspecifics in aquaria (Highsmith & Emlet 1986, Pearce & Scheibling 1990a). In the field, such specialised responses likely result in strongly preferential settlement of larvae in or near established sand dollar beds, demonstrating that settlement can occur onto populations of motile echinoderms that live in dense aggregations (Burke 1984). Small proportions of larvae of *E. parma* and *Dendraster excentricus* settled on non-conditioned substrata (Highsmith 1982, Burke 1984, Pearce & Scheibling 1990a) and newly settled *D. excentricus* have been observed outside of adult sand dollar beds in the field (Cameron and Rumrill 1982). Unlike in some sessile species (Nelson 1979), adult aggregations of these species can therefore not entirely be attributed to gregarious settlement of their larvae. However, the gregarious settlement of *D. excentricus*, *Scaphechinus mirabilis* and *Echinarachnius parma* is generally assumed to be contributing to an aggregated pattern in the distribution of adult populations.

In general, cue specificity is not high in echinoid settlement (Chia et al. 1984), and the sand dollar species mentioned above are assumed to be an exception to this rule. Accordingly, no evidence of gregarious settlement was found for the echinoid *Heliocidaris erythrogramma* (Huggett et al. 2006). Likewise, adult urchins, adult-conditioned seawater, their faecal matter and conspecific settlers did not induce larval metamorphosis in the echinoid *Strongylocentrotus droebachiensis* (Pearce & Scheibling 1991). A regular echinoid, *Tripneustes gratilla*, has been induced to settle in response to conspecific chemical cues (Dworjanyn & Pirozzi 2008). Larvae settled at high rates in the presence of conspecific juveniles and their faeces. Settlement of this echinoid, however, was also readily induced by cues associated with numerous types of seagrass, algae, coralline algae and, specifically, bacterial biofilms (Dworjanyn & Pirozzi 2008). These results were corroborated in a second study, which reported settlement induction of *T. gratilla* larvae by adult conspecifics, however, at significantly lower rates than in response to macroalgal cues (Mos et al. 2011). This suggests that even echinoid species found to be highly responsive to conspecific cues probably settle in response to a wide range of biotic chemical cues in nature.

Evidence of gregarious settlement in holothuroids, crinoids, ophiuroids and asteroids is highly fragmented. Competent larvae of the holothuroid *Psolus chitonoides* preferred to settle in response to conspecifics in experimental studies (Young & Chia 1982). Field experiments reporting high settlement either directly on top or around the sole of conspecific adults substantiated the hypothesis of gregarious settlement in this holothuroid (Young & Chia 1982). Likewise, larval crinoids settling gregariously in culture (Mladenov & Chia 1983, Obuchi et al. 2010) are supported by reports of crinoid settlers found on top or associated with the cirri of conspecific adults (Mladenov & Chia 1983), suggesting that gregarious settlement in crinoids may be a common and overlooked phenomenon. Preferential settlement in the proximity of conspecific adults has also been reported in the ophiuroid *Ophiothrix fragilis* (Warner 1971, Morgan & Jangoux 2004, 2005). Larval metamorphosis in ophiuroids begins spontaneously in the plankton; however, the presence of adult conspecifics clearly enhances the loss of two posterolateral rods and larval settlement (Morgan & Jangoux 2005). The loss of their posterolateral rods near a brittle star bed increases the likelihood of larvae settling in a suitable habitat (Morgan & Jangoux 2005), and hooked spines on settlers without their posterolateral rods may help them cling directly onto adults (Morgan & Jangoux 2004). However, in cases where the habitat or dietary requirements of settlers and conspecific adults differ, larval echinoderms may instead settle in response to chemical cues associated with prey or substratum they require during their early post-settlement stage. In the case of motile echinoderms, these juveniles may later migrate into habitat utilised by conspecific adults. For example, larvae of the asteroid *Mediaster aequalis* settled in response to tubes of a polychaete worm that juveniles were frequently observed on, while the presence of juvenile *M. aequalis* failed to trigger a settlement response (Birkeland et al. 1971). Spines and tube feet of conspecifics did not affect settlement rates in larvae of *Acanthaster* cf.

solaris (Henderson & Lucas 1971), although analyses of settlement preferences based on static choice chambers suggest that larvae may be attracted to adult conspecifics (Cowan et al. 2016b).

Associative settlement

Associative settlement, or settlement onto heterospecific plant or animal species (Crisp 1974), is seemingly widespread among echinoderms. Associative settlement can be broadly categorised based on predatory, herbivorous, parasitic and other non-parasitic associations between species (Pawlik 1992). In the case of echinoderms, this phenomenon most commonly refers to species responding to chemical cues associated with suitable habitat (Williamson et al. 2000, Ling et al. 2019) and potential prey species of herbivorous juveniles or adults (Rowley 1989, Pearce & Scheibling 1990b, 1991, Johnson et al. 1991).

Numerous species of echinoids, asteroids and holothurians have been found to settle and metamorphose in response to seagrass, coralline red algae, and red, green and brown fleshy algae (Table 1). However, the relative capacity of these substrata to induce settlement in echinoderm larvae varies greatly. For example, studies testing for differential settlement rates of echinoids in response to these substratum types reported that species of coralline red algae and red macroalgae generally induced the highest percentage of larval settlement, while the inductive capacity of brown algae, green algae and seagrasses was relatively low (Pearce & Scheibling 1991, Dworjanyn & Pirozzi 2008, Swanson et al. 2012). Moreover, chemical cue specificity is highly variable within and among echinoderm classes (Table 1). The echinoid *Tripneustes gratilla* was induced to settle by all 11 macroalgae tested including 6 brown algae, 1 green algae, 2 red algae and 3 coralline algae (Dworjanyn & Pirozzi 2008). In contrast, settlement in the asteroid *Mediaster aequalis* is induced predominantly by a specific chemical cue associated with the polychaete *Phyllochaetopterus prolifica* (Birkeland et al. 1971, Bryan 2004). In many cases, differences in the inductive capacity of natural substrata, as well as cue specificity of settlers, are likely linked to the presence or absence of specific chemical compounds in these substrata or animal prey. Purified compounds that induce associative settlement in echinoids have been isolated from red algae (Williamson et al. 2000, Swanson et al. 2004, 2006, 2012), coralline red algae (Kitamura et al. 1993, 1994) and endophytic microalgae (Taniguchi et al. 1994, Takahashi et al. 2002).

Naturally occurring settlement cues associated with red algae have been demonstrated for several ecologically important echinoid species (Rowley 1989, Pearce & Scheibling 1991, Williamson et al. 2000, Dworjanyn & Pirozzi 2008, Privitera et al. 2011), and chemical constituents of red algae that induce settlement and metamorphosis have recently been identified (Swanson et al. 2004, 2006, 2012). Newly settled echinoids of the species *Holopneustes purpurascens* are predominantly found on the red algae *Delisea pulchra* (Williamson et al. 2000, Swanson et al. 2006), although adult densities in the canopy of *D. pulchra* and the brown algae *Ecklonia radiata* are similar (Williamson et al. 2000). This pattern is hypothesised to be a response to a chemical cue produced by *Delisea pulchra*, as competent *Holopneustes purpurascens* larvae metamorphosed to cues associated with *Delisea pulchra*, but not the brown algae *Ecklonia radiata* (Williamson et al. 2000). The metamorphic cue was isolated and characterised by nuclear magnetic resonance spectroscopy as a water-soluble floridoside-isethionic acid complex (Williamson et al. 2000). Swanson et al. (2004) corrected this finding and identified the chemical identity of this cue produced by *Delisea pulchra* as histamine, by isolating the cue from the polar extract via cation-exchange chromatography. Corroborating this characterisation, the histamine content found in *D. pulchra* was reported to be one or more orders of magnitude higher than in the brown algae *Ecklonia radiata* and four other common species of fleshy and coralline algae (Swanson et al. 2004). Histamine exposure also appears to modulate metamorphic competence in other echinoids (Sutherby et al. 2012). With the first quantitative *in situ* measurements of a natural settlement cue in the habitat of a marine organism, Swanson et al. (2006) showed that seawater surrounding *Delisea pulchra* contained far higher concentrations of histamine than for all other fleshy and coralline algae tested. The effectiveness of histamine as a metamorphic cue was demonstrated further for the lecithotrophic larvae of the echinoids *Holopneustes inflatus* and *Heliocidaris erythrogramma*, and the planktotrophic larvae of *Centrostephanus rodgersii* (Swanson et al. 2012). Conversely, histamine had a weak or no inductive effect on larvae of *Heliocidaris tuberculata* and *Tripneustes gratilla* (Swanson et al. 2012). A lower specificity in the metamorphic cues of these two habitat-generalist herbivores may be due to a relatively low restriction in their habitat and prey selection as juveniles and adults (Swanson et al. 2012). In contrast, dissolved histamine that originates from red algae on other plants likely serves as an exogenous cue for competent larvae of more habitat-specialised species such as *Holopneustes purpurascens* and *H. inflatus*, signalling the presence of a suitable habitat in which to settle (Swanson et al. 2012). Cues associated with histamine certainly have a role in settlement and metamorphic processes of echinoderms, which may extend past this group of ecologically important echinoids.

Settlement and metamorphic responses to natural chemical cues in other echinoids and asteroids have been described for numerous species of coralline red algae (Table 1). Both articulate and encrusting assemblages of coralline red algae may be comparably or even more inductive to some larvae than fleshy red macroalgae (Dworjanyn et al. 2008, Swanson et al. 2012). For example, encrusting coralline species of the genus *Lithothamnion* induce settlement and metamorphosis in competent *Acanthaster* cf. *solaris* larvae (Johnson et al. 1991, Johnson & Sutton 1994), and the echinoids *Strongylocentrotus purpuratus* (Rowley 1989) and *S. droebachiensis* (Pearce & Scheibling 1990b, 1991). Similarly, many echinoid species settle and metamorphose in response to cues associated with the articulated corallines *Amphiroa anceps* (Williamson et al. 2000, Swanson et al. 2006, 2012, Dworjanyn & Pirozzi 2008) and *Corallina* spp. (Lamare & Barker 2001, Pearce & Scheibling 1990b, 1991, Kitamura et al. 1992, Mos et al. 2011). Purified compounds responsible for the induction of settlement and metamorphosis in two commercially important echinoid species, *Pseudocentrotus depressus* and *Anthocidaris crassispina*, have previously been characterised from lipophilic extracts of the coralline red alga *Corallina pilulifera* (Kitamura et al. 1992, 1993, 1994). Silica gel column chromatography demonstrated that simple lipids including free fatty acids were the inducers of this settlement response (Kitamura et al. 1992). Further chemical analyses of a mixture of free fatty acids detected in *C. pilulifera* revealed that larvae of both echinoids only underwent settlement and metamorphosis in response to eicosapentaenoic acid and arachidonic acid (Kitamura et al. 1993). Responses of both echinoid larvae to a wide range of saturated and unsaturated fatty acids were highly specific and suggested that only highly unsaturated fatty acids (dihomo-γ-linolenic acid and eicosatrienoic acid) triggered strong metamorphic larval responses (Kitamura et al. 1994), which appears to be a similar induction mechanism for other motile marine invertebrates (Pawlik & Faulkner 1986).

Different chemical compounds isolated from extracts of the endophytic green microalgae *Ulvella lens* have been identified to induce larval settlement and metamorphosis in the echinoids *Strongylocentrotus nudus* (Taniguchi et al. 1994, Takahashi et al. 2002) and *S. intermedius* (Takahashi et al. 2002). The compound dibromomethane, which was found in seawater in which *Ulvella lens* and three coralline algae species were cultured, is the principal inducer of settlement and metamorphic responses in *Strongylocentrotus nudus* (Taniguchi et al. 1994). Further investigating chemical cues associated with *Ulvella lens*, Takahashi et al. (2002) reported that settlement and metamorphic responses of *Strongylocentrotus nudus* and *S. intermedius* were only triggered by relatively high concentrations of dibromomethane, and a polyunsaturated fatty acid (eicosapentaenoic acid) did not induce settlement at all. Instead, several active compounds isolated from *Ulvella lens* extracts were identified as glycoglycerolipids (Takahashi et al. 2002), which are a class of cell wall-associated compounds abundant in marine algae (Tebben et al. 2015). These findings underline the importance of assessing taxa-specific inducers to gain an accurate understanding of the chemical cues driving the mechanisms and patterns of larval settlement in echinoderms.

Biofilm-induced settlement

Biofilms are complex and dense consortia of microorganisms, which accumulate on the surfaces of most marine substrata (Dobretsov 2010). Marine biofilms usually consist of various species of bacteria, *Archaea* and unicellular organisms such as diatoms (Rahim & Kitamura 2004, Dobretsov 2010) and commonly include secreted extracellular substances in which the cells of these component organisms are situated (Hadfield 2011). The reasons why competent larvae choose to settle on biofilms are not always clear. Biofilms may be a possible food source for some newly settled echinoderms (de la Uz et al. 2013), while in some cases, settlement is thought to occur in response to cues simply signalling the presence of a nontoxic surface, which accumulated a biofilm and microorganisms over time (Hadfield 2011). Most research on biofilm-induced settlement in echinoderms has focused on cues associated with biofilm bacteria (Table 1); however, echinoderm larvae have also been induced to settle by various diatoms (Ito & Kitamura 1997, Rahim & Kitamura 2004, Agudo 2007, Mos et al. 2011), which are single-celled algae commonly found in marine biofilm assemblages (Dobretsov 2010). Among echinoderm classes, the inductive capacities of marine biofilm consortia have been demonstrated in echinoids (e.g. Lamare & Barker 2001, Rahim & Kitamura 2004), asteroids (e.g. Barker 1977, Johnson & Sutton 1994) and holothuroids (e.g. Ito & Kitamura 1997).

Early reports of settlement induction in echinoderms by marine biofilms (e.g. Cameron & Hinegardner 1974, Barker 1977) have raised the question whether many of the natural chemical cues of echinoderm settlement attributed to marine algae and other live substrata may, in fact, be produced by marine bacteria and unicellular eukaryotes on the surfaces of these substrata. Differential inductive capacity of naturally occurring algal substrata with and without biofilms, as well as isolated cues from the biofilms and their compounds, has been investigated for echinoderm larvae (Pearce & Scheibling 1990b, Johnson et al. 1991, Johnson & Sutton 1994, Swanson et al. 2006, Dworjanyn & Pirozzi 2008, Mos et al. 2011). It appears that in some cases, only the biofilm is required to induce settlement (Swanson et al. 2006, Dworjanyn & Pirozzi 2008), while others demonstrated that the algal substratum, but not the biofilm (Pearce & Scheibling 1990b), or both (Johnson & Sutton 1994) were essential to produce the inductive response.

Antibacterial treatment of the coralline red algae *Amphiroa anceps* substantially reduced larval settlement rates of the echinoid *Holopneustes purpurascens* (Swanson et al. 2006). Here, a settlement cue from the coralline algae is proposed to be bacteria-derived histamine, which is released into the surrounding seawater. Two isolated bacterial strains, *Photobacterium phosphoreum* and *Thalassomonas viridans*, cultured from the surfaces of the corallines induced settlement and contained histamine, while no histamine was detected in coralline extracts (Swanson et al. 2006). Further supporting the hypothesis of Steinberg et al. (2001) that generalist herbivorous echinoids metamorphose in response to biofilms, Dworjanyn & Pirozzi (2008) demonstrated the necessity of bacterial film growing on algal surfaces in producing an inductive compound. The removal of bacteria from the surface of three macroalgae significantly inhibited settlement of *Tripneustes gratilla* larvae in response to these macroalgae (Dworjanyn & Pirozzi 2008), which demonstrates the larvae did not respond to cues originating from the algae per se. The echinoid *T. gratilla* appears to have very little specificity in its settlement cues and likely settles on a wide range of surfaces with bacterial films present. Conversely, larvae of the same echinoid species were induced to settle at substantially higher rates by the addition of macroalgae-conditioned seawater to biofilm treatments (Mos et al. 2011), which indicates that compounds associated with the alga itself may at least contribute to settlement induction. Bacteria-derived settlement cues as inducers of echinoid settlement were also not supported by Pearce & Scheibling (1990b). Here, antibiotic treatment of the coralline red algae *Lithothamnion glaciale* and subsequent reduction of surface bacteria by two orders of magnitude did not affect the capacity of *L. glaciale* to induce metamorphosis in the echinoid *Strongylocentrotus droebachiensis* (Pearce & Scheibling 1990b).

Substratum selectivity by crown-of-thorns starfish (*Acanthaster* cf. *solaris*) also appears to be mediated by chemical cues produced by epiphytic bacteria (Johnson et al. 1991, Johnson & Sutton 1994). Larval settlement and metamorphosis of this asteroid is inhibited in the absence of bacteria on highly inductive shards of the coralline alga *Lithothamnium pseudosorum* (likely *Lithothamnion proliferum*), with settlement rates reduced to low levels when the coralline alga was treated with antibiotics (Johnson et al. 1991). Likewise, Yamaguchi (1973) reported that larval settlement of *Acanthaster* cf. *solaris* was not induced by bleached coralline algae. Moreover, settlers of this asteroid were only observed in sections of the coralline algal substratum with high densities of epiphytic bacteria, but not on the sections with little or no bacterial film cover (Johnson et al. 1991, Johnson & Sutton 1994). However, when isolated from soluble algal compounds, surface bacteria failed to induce settlement in *Acanthaster* cf. *solaris* larvae (Johnson & Sutton 1994). This demonstrates that either chemical compounds from both the coralline algae and the bacteria are essential to induce settlement and metamorphosis, or, more likely, the bacteria require the substratum from the alga to produce the inductive compound (Johnson & Sutton 1994).

Among a range of other marine bacteria identified to induce echinoderm settlement (Table 1), the bacterial genus *Pseudoalteromonas*, and especially the bacterium *P. luteoviolacea*, has been recognised as strongly inductive and important to biofilm-associated settlement (Hadfield 2011). Huggett et al. (2006) isolated 250 bacterial strains from coralline algae and tested their capacity to induce settlement in the echinoid *Heliocidaris erythrogramma*. Many strains of bacteria induced substantially higher settlement rates than the corallines, and *Pseudoalteromonas* dominated highly inductive strains (Huggett et al. 2006). This culture-based result was supported by an assessment of larval responses to characterised biofilms in the field, which reported the highest metamorphic rates for the highly inductive *P. luteoviolacea* (Huggett et al. 2006). Other bacterial genera represented in highly inductive strains were *Photobacterium*, *Shewanella* and *Vibrio*; however, the same genera were also abundant in low inducers (Huggett et al. 2006). This indicates that the inductive capacity of marine bacteria is species-specific, and the settlement response of echinoderm larvae to bacterial films is likely determined by its specific bacterial composition. Altogether, this body of research suggests that the role of biofilms as cues in the settlement behaviour and metamorphosis of echinoderm larvae may be much more complex and widespread than previously suspected and certainly warrants further research.

Neurotransmitter and ion-induced settlement

Aside from the natural chemical cues associated with conspecifics, prey species and biofilms, various ‘artificial’ compounds have been identified to trigger larval settlement in echinoderms (Table 2). These inducers mimic natural chemical compounds and include numerous neurotransmitters (e.g. dopamine), neurotransmitter precursors (e.g. L-DOPA) and ions (e.g. potassium), which corroborates the role of neuronal receptors in initiating a settlement response (Rodriguez et al. 1993). Although these neuroactive compounds and ions may not always act as predominant cues of settlement in nature, their study furthers our understanding of chemoreception, neural control and the mechanisms driving larval response (Slattery 1997). Investigating and understanding their effects is of considerable interest, particularly their potential to improve settlement success and survival rates in echinoderm aquaculture (Slattery 1997, Nontunha et al. 2021).

Amino acid derivatives

Several amino acid derivatives have been shown to effectively induce settlement and metamorphosis in echinoderms, including glutamine (Yazaki & Harashima 1994, Naidenko 1996), gamma aminobutyric acid (GABA; Pearce & Scheibling 1991, Rahman & Uehara 2001, Bryan 2004, Sun et al. 2014, Nontunha et al. 2021), glutamic acid (Naidenko 1996) and monosodium glutamate (Nontunha et al. 2021). Glutamine is an essential amino acid and the precursor of GABA, an inhibitory neurotransmitter. Levels of GABA are enhanced by glutamic acid, which, in turn, is often used in the form of its sodium salt, monosodium glutamate (Nontunha et al. 2021). Induction of metamorphosis by glutamine has been observed in the echinoid species *Pseudocentrotus depressus* (Yazaki & Harashima 1994), *Scaphechinus mirabilis* and *Strongylocentrotus intermedius* (Naidenko 1996). Glutamic acid was also shown to induce metamorphosis in some echinoid species (*Scaphechinus mirabilis* and *Strongylocentrotus intermedius*; Naidenko 1996), but not all (*Pseudocentrotus depressus*; Yazaki & Harashima 1994), and had a toxic effect on *Scaphechinus mirabilis* and *Strongylocentrotus intermedius*, which was possibly caused by a decrease in seawater pH from 8.4 to 7.5 at a glutamic acid concentration of 100 µg/mL (Naidenko 1996). Monosodium glutamate (MSG), a glutamic acid derivative, induced larvae of the holothuroid *Holothuria scabra* to settle at high rates, and MSG was also shown to be the least toxic in comparison with other neurotransmitters tested (Nontunha et al. 2021). Another neuroactive compound, serotonin, which is a derivative of tryptophan, failed to trigger any settlement and metamorphosis response in the Japanese holothuroid *Apostichopus japonicus* (Sun et al. 2014, Matsuura et al. 2009) and the Pacific sand dollar *Dendraster excentricus* (Burke 1983b).

GABA is an inhibitory neurotransmitter, which can produce depolarisation of cells capable of activating metamorphosis (Baloun & Morse 1984). Induction by this endogenous chemical cue is known to occur in a wide range of benthic marine invertebrates (Slattery 1997) and has thus been extensively tested for numerous echinoids, asteroids and holothuroids (Table 2), although results are variable both among and within echinoderm classes. Five echinoid species, including *Echinometra mathaei*, *E. oblonga* and *Strongylocentrotus droebachiensis*, have been found to metamorphose in response to GABA (Pearce & Scheibling 1990, Rahman & Uehara 2001). On the other hand, no effect at all was observed in *Dendraster excentricus* (Burke 1983b) and the echinoid *Pseudocentrotus depressus* (Yazaki & Harashima 1994). Likewise, bioassays with GABA induced settlement and metamorphosis in larvae of the asteroid *Mediaster aequalis* (Bryan 2004), but not the crown-of-thorns starfish, *Acanthaster* cf. *solaris* (Johnson et al. 1991). Holothurians have successfully been induced by a wide range of neurotransmitters (Table 2), including GABA (Sun et al. 2014, Nontunha et al. 2021). GABA induced high rates of settlement in *Holothuria scabra* larvae, while a pair of studies found this compound to trigger larval settlement behaviour (Sun et al. 2014), but not metamorphosis (Yazaki & Harashima 1994) in *Apostichopus japonicus*. The highly variable results encountered in the response of echinoderm larvae to GABA may explain why this neurotransmitter has not been generally used as an inducer of settlement and metamorphosis in the aquaculture of commercially important echinoderm taxa.

Tyrosine derivatives

Catecholamines (dopamine, epinephrine and norepinephrine) and their precursor levodopa (L-DOPA) are tyrosine derivatives with numerous biological functions, such as structural proteins, hormones and neurotransmitters. As in many other marine benthic invertebrates, these tyrosine derivatives are reliable inducers of larval settlement in echinoderms (Table 2). Epinephrine and norepinephrine are similar tyrosine derivatives that act as neurotransmitters and hormones. Epinephrine has been shown to initiate metamorphosis, but not settlement, in the Japanese holothuroid *Apostichopus japonicus*, while norepinephrine initiates both settlement behaviour and metamorphosis (Matsuura et al. 2009, Sun et al. 2014). Conversely, neither compound was found to be an effective inducer of larval metamorphosis in the echinoid *Dendraster excentricus* (Burke 1983b). Dopamine and L-DOPA, which increases dopamine neurotransmission, have been identified as the most efficient chemical cues to induce settlement of *Apostichopus japonicus* (Matsuura et al. 2009, Sun et al. 2014), *Holothuria scabra* (Nontunha et al. 2021) and the sand dollar *Dendraster excentricus* (Burke 1983b). These findings suggest that dopamine acts as the chemical transmitter during settlement induction in various echinoderm taxa. This endogenous chemical cue is thought to either directly stimulate larval tissues containing the effectors of metamorphosis, or indirectly trigger it by inducing the release of substances stimulating these tissues (Burke 1983b). The interference and inhibition of metamorphosis by reserpine (Burke 1983b), a compound known to deplete catecholamines, further supports this conclusion.

Choline derivatives

The neurotransmitter acetylcholine is the sole choline derivative documented to induce effective larval settlement in an echinoderm, the holothuroid *Apostichopus japonicus* (Sun et al. 2014). This neuroactive compound, however, failed to induce larval metamorphosis in the sand dollar *Dendraster excentricus* (Burke 1983b). Choline, a precursor to acetylcholine, is a bound constitute of membranes surrounding cells. In contrast to acetylcholine, this compound had no significant inductive effect on *Apostichopus japonicus* larvae (Sun et al. 2014); however, it has been suggested to have an indirect effect on holothuroid settlement by participating as precursors in acetylcholine biosynthesis rather than by acting directly on the cholinergic receptors (Rodriguez et al. 1993). Similar to some catecholamine derivatives, choline derivatives are thus generally unlikely to directly induce echinoderm settlement and metamorphosis in nature by acting directly on cholinergic receptors, but instead more likely act as precursors in the synthesis and release of neurotransmitters (Hirata & Hadfield 1986, Slattery 1997).

Ions

In addition to neurotransmitters and their precursors, some ions can stimulate larval settlement in echinoderms (Table 2). The sensory bases of induction indicate that the nervous system plays a significant role in the cascade of events comprising benthic marine invertebrate settlement and metamorphosis (Burke 1983b). The successful transmission of neural signals relies on the electrical potential across cell membranes, which, in turn, is affected by the permeability of these membranes to ions (Rodriguez et al. 1993). Hence, any ions or compounds that affect ionic transport through cell membranes can trigger inductive responses, and their potential effects on echinoderm larvae should be assessed.

The highly inductive effects of potassium ions (K^+) on echinoderm settlement have been documented, particularly for echinoids (Table 2). At least 15 echinoid species from seven families have been induced to settle and metamorphose at variable potassium chloride concentrations within 1 hour of exposure (Hodin et al. 2019). More broadly, the inductive effects of potassium have also been observed in the holothuroid *Apostichopus japonicus* (Sun et al. 2014). Increased exogenous K^+ likely activates settlement responses through a depolarisation of externally accessible cells, which are directly involved in the recognition of natural inducers (Yool et al. 1986). Tetraethyl ammonium, a compound that selectively occludes potassium channels, inhibits metamorphosis (Rodriguez et al. 1993), which suggests that potassium could act through this channel in echinoderm larvae. Conversely, no settlement was induced by high concentrations of potassium in larvae of the crown-of-thorns starfish *Acanthaster* cf. *solaris* (Johnson et al. 1991).

Other ions that may trigger settlement and metamorphosis in echinoderms are calcium (Ca^{2+}), ammonium (NH_4^+) and magnesium (Mg^{2+}). Calcium has been implicated in the transmission or modulation of signals in the echinoid *Lytechinus variegatus* (Cameron et al. 1989) and other marine invertebrate larvae (Yool et al. 1986, Yu et al. 2008). Sun et al. (2014) reported inductive effects of Ca^{2+} on the settlement behaviour of *Apostichopus japonicus* larvae; however, no obvious effect on settlement success was found. Larvae of this holothuroid have also been induced to settle in response to ammonium ions (NH_4^+), although ammonium was concluded to be a relatively ineffective inducer in comparison with potassium (Sun et al. 2014). Magnesium ions appear to have no effect on *A. japonicus* larvae (Sun et al. 2014), which corroborates previous conclusions in other benthic marine invertebrates (Baloun & Morse 1984, Yool et al. 1986). Conversely, an inhibitory effect of Mg^{2+} was documented in the echinoid *Lytechinus variegatus*

(Cameron et al. 1989). Here, excess magnesium interferes with echinoid metamorphosis in the presence of inductive biofilms by competing with Ca^{2+} for binding molecules involved with the release of neurotransmitters (Cameron et al. 1989). Similar inhibitory effects of magnesium may be found in *Apostichopus japonicus* or other echinoderm larvae if tested in the presence of metamorphically active biofilms.

Multiple lines of evidence support the notion that ionic fluxes play a role in the induction of larval settlement and metamorphosis (Cameron et al. 1989). Because of their inductive capacity, some of these ions, especially potassium, are more commonly utilised as agents in the cultivation of numerous benthic marine invertebrates, replacing the use of neurotransmitters such as GABA or L-DOPA (Rodriguez et al. 1993).

Sampling methods for the study of settlement patterns

A variety of sampling methods have been trialled and used to study echinoderm settlement patterns (Figure 4); however, there are no standard or established sampling techniques used among or even within echinoderm classes (Tables 3–6). The extremely small size and often cryptic nature of recently settled echinoderms has greatly constrained progress in the study of this important life-history stage in their natural environments. In the past, inter-annual settlement patterns of some species have commonly been inferred based on the distribution and abundance of juveniles or adults

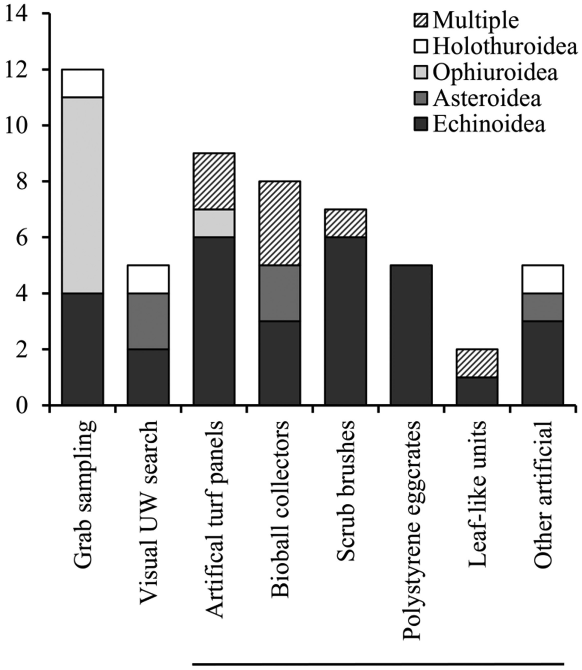

Figure 4 Frequency distribution of sampling methods used in 53 studies that assessed spatial and/or temporal patterns of larval settlement in one or multiple classes of echinoderms. The horizontal line indicates the sampling methods utilising artificial settlement collectors.

Table 3 Summary of studies assessing spatial and temporal settlement patterns in echinoids

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Arbacia lixula	Canary Islands, Spain (subtropical)	Four subtidal habitats: seagrass meadows, urchin barrens, macroalgal beds and sandy patches; 5–10 m	Settlement rates were relatively low and varied among seasons, as all newly settled echinoids were recorded between January and May.	Monthly; Feb 2009–Feb 2010	Plastic cushion-shaped leaf-like units	García-Sanz et al. (2014)
Arbacia lixula	Canary Islands, and Costa Brava, Spain (subtropical)	Gently sloping rocky barren bottom; 5–10 m	The plastic bioball collectors reported the highest settler densities. No settlers were found at the Mediterranean site (Costa Brava). At the eastern Atlantic site (Canary Is.), a considerably higher number of settlers was recorded in the collectors deployed for 28 days than in those deployed for 14 days.	Deployments in Feb (Canary Is.) and Jun 2012 (Costa Brava); two retrievals from both locations 14 and 28 days post-deployment	Plastic bioballs in nylon nets; vertical scrub brushes with vegetal bristles; horizontal triangular mats of coconut fibre	Balsalobre et al. (2016)
Dendraster excentricus	Monterey Bay, the USA (subtropical)	Inshore sand dollar bed; 8–15 m	Settlement occurred at all sampling times during the summer months of 1980, and settler densities were relatively constant throughout this time period.	Fortnightly; Jul–Oct 1980	Eckman grab samples and cylindrical cores	Cameron & Rumrill (1982)
Diadema africanum	Canary Islands, Spain (subtropical)	Rocky subtidal zone; 5–6 m	Substantial intra-annual variation in settlement observed at both sites. The primary peak between August and October may be related to spawning intensity (April–June), while a secondary peak in January/February suggests that factors other than spawning intensity may also be driving local settlement patterns.	Monthly (with exceptions); Apr 2002–Apr 2003	Plastic bioballs inside net bags	Hernández et al. (2006)
Diadema africanum	Canary Islands, Spain (subtropical)	Rocky subtidal zone; 5–10 m	Settlement was observed at all sites within three survey locations (MPA; fished; and heavily fished). The number of settlers found was similar between sites and locations, which suggests larval supply is unlikely to be limiting recruitment in any of the three locations.	Single deployment; Sep–Oct 2006	Plastic bioballs inside net bags	Clemente et al. (2009)

(*Continued*)

Table 3 (*Continued*) Summary of studies assessing spatial and temporal settlement patterns in echinoids

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Diadema africanum	Canary Islands, Spain (subtropical)	Urchin barrens, and macroalgal beds; 6–10 m	Single annual peak in settlement during August to October, which was consistent among years and locations; however, inter-annual variability in settlement was high at the Abades and Boca Cangrejo locations. Higher densities of settlers were found in the barren areas than in the macroalgal beds. Strong spatiotemporal settlement variability likely driven by temperature; however, settlement rates were also negatively correlated with food availability 2 months pre-settlement.	Monthly; Apr 2002–Apr 2008 (Abades), May 2002–Apr 2003 (Masca), Dec 2003–Apr 2008 (Boca Cangrejo) and Oct 2006 (El Hierro)	Plastic bioballs inside net bags	Hernández et al. (2010)
Diadema africanum	Canary Islands, Spain (subtropical)	Four subtidal habitats: seagrass meadows, urchin barrens, macroalgal beds and sandy patches; 5–10 m	Settlement followed a clear unimodal pattern with a main peak occurring in September and October and few newly settled echinoids observed in the months following. Densities were greater in urchin barrens and sandy patches than in the other habitats.	Monthly; Feb 2009–Feb 2010	Plastic cushion-shaped leaf-like units	García-Sanz et al. (2014)
Diadema antillarum	Curaçao, southern Caribbean Sea (tropical)	Coral reef; 8 m	Settlement occurred continuously and peaked in the spring and autumn months. Settlement rates were relatively similar among years, but differed between sites.	Fortnightly with exceptions; 1982–1984	Settlement plates consisting of two polystyrene egg crates and plexiglass in-between	Bak (1985)
Diadema antillarum	Barbados, eastern Caribbean Sea (tropical)	Coral reef	Low settlement was detected between June and August and peaked in July. Settlers were always found within the front third of the reef (offshore) on rugose substrata and aggregated with adults.	Monthly; Oct 1984–Dec 1985	Visual UW searches for settlers	Hunte & Younglao (1988)
Diadema antillarum	Florida Keys, the USA (subtropical); Puerto Rico (tropical)	Variable habitats (fore reef, back reef, drowned reef and bank reef) and sampling depths; 3–19 m	While settlers were detected in low numbers at all locations, only one of the Puerto Rico sites recorded a substantial settlement pulse. These results corroborate the hypothesis of low larval supply (and settlement) limiting the recovery of this species in the Florida Keys.	Monthly; Aug 2005–Jul 2006 (Florida Keys), and Jun 2006–Oct 2006 (Puerto Rico)	Settlement plates consisting of two polystyrene egg crates and plexiglass in-between	Miller et al. (2009)

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Table 3 (*Continued*) Summary of studies assessing spatial and temporal settlement patterns in echinoids

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Diadema antillarum	La Parguera, Puerto Rico (tropical)	Coral reef; 19–6 m (shelf-edge), 10–5 m (mid-shelf) and 2–3 m (inshore)	Substantially higher settlement at shelf-edge reef (275 recruits) compared to mid-shelf (two recruits) and inshore reefs (0 recruits). Unimodal settlement peak in July, with low settler densities from October to June.	Monthly; Sep 2005–Sep 2006	Artificial turf on horizontal and vertical plates	Williams et al. (2010)
Diadema antillarum	Curaçao, southern Caribbean Sea (tropical)	Coral reef; 3–5 m	Settlement peaked in early June, similar to peaks in 1982 and 1983 (historic data comparison). Mean settlement rates in 2005 were > two times lower compared to pre-*D. antillarum* die-off numbers (1982–83), but substantially higher than those measured after the die-off (1984). No spatial variation among sites around the island.	Fortnightly; Mar–Jul 2005	Settlement plates consisting of two polystyrene egg crates and Formica sheets in-between	Vermeij et al. (2010)
Diadema antillarum	La Parguera, Puerto Rico (tropical)	Coral reef; 3–17 m (depending on water depth)	Highest settlement at 9 m, intermediate at 6 and 12 m, and lowest at 3 and 15 m. Substantially higher settlement at two shelf-edge reefs (217 of 220 settlers), with little and no settlement detections at mid- and inner-shelf reefs. Settlement peaked in September, but was also relatively high in July.	Monthly; Feb–Apr and Jul–Sep 2008	Artificial turf on vertical plates	Williams et al. (2011)
Diadema antillarum	Curaçao, southern Caribbean Sea (tropical)	Variable habitats (fore reef, sheltered lagoon or back reef)	Settlement was variable in space. Significantly higher settlement rates were observed in sheltered habitats (lagoon and back reef) than in fore reef habitats.	Monthly; May/ Jun–Aug 2010	Settlement plates consisting of two polystyrene egg crates and plastic sheets in-between	Rogers & Lorenzen (2016)
Diadema antillarum	Xcalak, Mexico (tropical)	Coral reef lagoon and fore reef zone; <1, 2.5, 3 and 7 m	Sampled a total of five settlers during the 11-month period, all of which settled in the fore reef habitat at 7 m (4 in August and 1 in December).	Monthly; May 2014–Apr 2015	Settlement plates consisting of polystyrene egg crates and acrylic sheets in-between	Maldonado-Sánchez et al. (2019)

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Table 3 (*Continued*) Summary of studies assessing spatial and temporal settlement patterns in echinoids

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Evechinus chloroticus	Hauraki Gulf, New Zealand (subtropical)	Urchin barrens & shallow mixed-algal assemblages; 4–5 m	No settlers were found at the sheltered, high-sediment location (Little Manly), while spatiotemporal variability in settlement was evident among sites and sampling months at the wave-exposed, low-sediment location (Leigh).	Nov 2003–Mar 2004	Underwater suction sampling and sieving	Walker (2007)
Evechinus chloroticus	Wellington region, New Zealand (temperate)	Rocky reef; 4–7 m	Highly variable settlement among sampling years and locations. Settlers were most abundant in 2019 at Shelly Bay, and smaller pulses of settlement occurred at Beaker Bay (2018, 2019) and Kau Bay (2018). Overall echinoderm settlement pulses were positively correlated with shell cover, seawater temperature and chlorophyll.	Annually; Nov 2016–Apr 2017; Nov 2017–Apr 2018; Dec 2018–Feb 2019	Plastic bioballs inside nylon nets	Glockner-Fagetti & Phillips (2020)
Mesocentrotus franciscanus	Northern & southern California, the USA (subtropical)	Not stated, but likely variable	Marked seasonality in settlement as most settlement occurred during one or multiple settlement pulses between February and July each year. Settlement was highly variable among years, and spatial settlement clusters did not form contiguous geographic subunits.	Weekly; Feb/Mar 1990–Jul 1993 at most sites, with shorter periods at others	Scrub brushes with nylon bristles	Ebert et al. (1994)
Mesocentrotus franciscanus	Southern Oregon, the USA (temperate)	18 m (Port Oxford) & 22 m (Gregory Point)	Settlement occurred from April to August at both locations. Settler densities were variable between locations and sampling years, but were most consistently high in June. Settlement pulses were stronger in 1995 and generally associated with warm water events characterised by a mixed water column and northward wind stress.	Deployments ranging from 9 to 35 days; Feb to Aug in 1994 and 1995	AstroTurf inside PVC pipe	Miller & Emlet (1997)
Paracentrotus lividus	Tossa de Mar, Spain (temperate)	Vertical wall occupied by seaweeds; 3, 6 and 9 m	Settler densities (<2 mm) varied substantially within and among years. Unimodal peaks in settlement were observed during the each of the four recruitment seasons (May–August), but peak densities were much higher in 1992 and 1995, compared to 1993 and 1994.	Fortnightly; Jun 1992–Jul 1993, Jun 1994–Dec 1995	Substrate collection using 20 × 20 cm^2	López et al. (1998)

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Table 3 (*Continued*) Summary of studies assessing spatial and temporal settlement patterns in echinoids

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Paracentrotus lividus	Medes Islands Marine Reserve, Spain (temperate)	Rocky bottoms colonised by a rich algal assemblage; 5, 10, 15 and 20 m	Reported single annual settlement peak within 3 weeks (May–June) and substantial inter-annual differences in settlement by more than two orders of magnitude. Preferential settlement at shallow depths (5 and 10 m). Spatial variation was found at all scales, and settlement was related to wave exposure and currents, but not to substratum type, level of protection and adult densities, suggesting physical processes may be driving these variable settlement patters.	Weekly (spring–late summer) or every other week (rest of the year); Mar 1998–Oct 2000	Scrub brushes with vegetal bristles	Hereu et al. (2004)
Paracentrotus lividus	Medes Islands Marine Reserve, Spain (temperate)	Seagrass meadow, and vertical rock wall; 3, 5 (and 10 m at seagrass meadow only) in Apr 2000–July 2001; then 5 m only	Major settlement peak in April/May and minor peak in October/November. Inter-annual variability in settlement was high (~one order of magnitude). Settlement was generally higher in the vertical rock wall habitat than in the seagrass meadow.	Weekly or fortnightly (Apr 2000–Jul 2001); additional sampling in autumn–winter 2002 and early summer 2003	Scrub brushes with vegetal bristles	Tomas et al. (2004)
Paracentrotus lividus	Canary Islands, Spain (subtropical)	Four subtidal habitats: seagrass meadows, urchin barrens, macroalgal beds and sandy patches; 5–10 m	Densities of newly settled echinoids differed substantially among the four habitats surveyed, with collectors deployed in sandy patches reporting greater numbers than in the other habitats.	Single deployment for 4 weeks from early Aug to early Sep 2008	(A) Plastic leaf-like units; (B) cushion-shaped, leaf-like units; and (C) cushion-shaped units	García-Sanz et al. (2012)
Paracentrotus lividus	Canary Islands, Spain (subtropical)	Four subtidal habitats: seagrass meadows, urchin barrens, macroalgal beds and sandy patches; 5–10 m	Almost all newly settled echinoids were recorded during a main peak between February and April. During this peak, abundances of settlers differed substantially among the four habitats surveyed, with soft substrata (sandy patches and seagrass) reporting greater densities than hard substrata (barrens and macroalgal beds).	Monthly; Feb 2009–Feb 2010	Plastic cushion-shaped, leaf-like units	García-Sanz et al. (2014)

(*Continued*)

Table 3 (*Continued*) Summary of studies assessing spatial and temporal settlement patterns in echinoids

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Strongylocentrotus droebachiensis	Isles of Shoals, the USA (temperate)	Kelp beds and barren grounds; 0–30 m	Annual settlement pulses were substantial and settlement decreased with depth below 9 m.	Single deployments in Jun–Jul 1990, 1992 and 1993	Plastic turf	Harris et al. (1994)
Strongylocentrotus droebachiensis	Gulf of Maine, the USA; Bay of Fundy and Nova Scotia, Canada (temperate)	Kelp beds, silt and barren grounds; 5–30 m	Settlement in the Gulf of Maine was three orders of magnitude higher than in the Bay of Fundy and one order of magnitude higher than on Nova Scotia's Atlantic Coast. Spatial differences in settlement among sites within these regions were within the same order of magnitude. Settler densities were higher in barren grounds than in adjacent kelp beds.	Fortnightly to bi-monthly sampling intervals; May–Sep 1994	Plastic turf and scrub brushes	Balch et al. (1998)
Strongylocentrotus droebachiensis	Nova Scotia, Canada (temperate)	Kelp beds and urchin barren grounds; 5–10 m	Nearly all settlement occurred in July each year, but few settlers were reported up to October. Settlement was very low in 1992 and 1993, and the only major settlement occurred in early July 1994. Settlement rates were quite similar between kelp and barren sites.	Fortnightly; Jun–Nov 1992, May–Nov 1993 and Jun–Oct 1994	AstroTurf inside PVC pipe	Balch & Scheibling (2000)
Strongylocentrotus droebachiensis	Gulf of Maine, the USA (temperate)	7 m	Settlement peaked in mid-June, relatively high throughout June and early July, and low to absent from mid-July to early September. No differential settlement was reported during the peak in settlement among six substrate types deployed; however, settlers preferentially settled on coralline algae encrusted substratum during the remainder of the sampling period.	Weekly; 48-hour deployments in Jun–mid-Jul; 1-week deployments in late Jul–early Sep	AstroTurf plexiglass panels	Lambert & Harris (2000)
Strongylocentrotus droebachiensis	Bocabec Cove, Canada (temperate)	Rocky subtidal zone	Highly variable settlement among years, intra-annual sampling periods and sites. Highest density of settlers in mid-July 2005 at the site closest to the larger body of water, Passamaquoddy Bay. If transported to the area by currents, larvae would likely reach this site first and, if competent, settle.	Fortnightly; Jun–Oct 2004; Jun–Oct 2005	AstroTurf inside PVC pipe	Jennings & Hunt (2010)

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Table 3 (*Continued*) Summary of studies assessing spatial and temporal settlement patterns in echinoids

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Strongylocentrotus purpuratus	Northern and southern California, the USA (subtropical)	Not stated, but likely variable	Settlement pulses were strongly seasonal (spring); however, there was a strong spatiotemporal variability in the number of settlers. Sites in northern, central and southern California were shown to form geographic subunits with respect to coherence in settlement pulses.	Weekly; Feb/Mar 1990–Jul 1993 with exceptions	Scrub brushes with nylon bristles	Ebert et al. (1994)
Strongylocentrotus purpuratus	Southern Oregon, the USA (temperate)	18 m (Port Oxford) & 22 m (Gregory Point)	Settlement occurred, but was generally low from April to August at both locations. Settler densities peaked at Gregory Point in June 1995, while settlement at Port Oxford was highest in late July–early August 1994 and March–April 1995. Settlement pulses were generally associated with warm-water events characterised by a mixed water column and northward wind stress.	Deployments ranging from 9 to 35 days; Feb–Aug in 1994 and 1996	AstroTurf inside PVC pipe	Miller & Emlet (1997)
Strongylocentrotus purpuratus	Southern & northern California, the USA (subtropical)	Shallow subtidal and intertidal rocky habitats	Settlement varied greatly, both inter- and intra-annually, at all three locations over 27 years. Following a seasonal pattern with peak settlement during April–May, settlement fluctuations were highly synchronous among sites within Southern California locations. Large spatiotemporal settlement patterns were related to differential responses to fluctuations in ocean temperature and climate.	Weekly (1990–2003) and fortnightly (2003–2016)	Scrub brushes with nylon bristles	Okamoto et al. (2020)
Strongylocentrotus purpuratus & *Mesocentrotus franciscanus*	Santa Barbara, the USA (subtropical)	Urchin barren grounds and kelp beds	Newly settled echinoids were found in low numbers from April to July in 1984 and 1985, and in April 1986. In early May 1986, many settlers of both species were present in high numbers on both foliose red algal turf and crustose coralline algae substrata.	Irregular sampling frequency; 1984–1986, mostly during summer	Collection of reef shale pieces	Rowley (1989)

(*Continued*)

Table 3 (*Continued*) Summary of studies assessing spatial and temporal settlement patterns in echinoids

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Strongylocentrotus purpuratus & *Mesocentrotus franciscanus*	Monterey Bay, the USA (subtropical)	Kelp forest; 10 m	Two distinct peaks in settlement occurred from December through February and April through July.	Monthly; Jul 1988–Jul 1989	Plastic matrix or articulated coralline algae inside a PVC pipe	Harrold et al. (1991)
Strongylocentrotus purpuratus & *Mesocentrotus franciscanus*	Northern California, the USA (subtropical)	Rocky habitat; 10–12 m	The total seasonal settlement rate varied substantially among the locations sampled. While very few settlers were recorded at Duxbury Reef, Bodega Head and Salt Point, while settlement was markedly higher at Point Reyes.	Weekly; Apr–early Sep 1993	Scrub brushes with polypropylene bristles	Wing et al. (1995a)
Strongylocentrotus purpuratus & *Mesocentrotus franciscanus*	Northern California, the USA (subtropical)	Rocky habitat; 5–20 m	Settlement occurred at low levels from late April to late July, and synchronous peaks were reported at both locations in mid-July.	Weekly; Apr–early Sep 1992	Scrub brushes with polypropylene bristles	Wing et al. (1995b)
Strongylocentrotus purpuratus & *Mesocentrotus franciscanus*	Southern California, the USA (subtropical)	Kelp forest, inshore and offshore habitats; 12–15 m	Settlement was generally low (0–10 settlers per collector) across locations in both years, and there was no evidence of lower settlement rates of either species at sites situated under the kelp canopy as opposed to offshore sites.	Weekly (5–20 days); Mar–May 1991, Jan 1992 and Apr–Jul 1992	Scrub brushes with nylon bristles	Schroeter et al. (1996)
Strongylocentrotus nudus	Hokkaido, Japan (temperate)	Rocky substratum; 1–9 m	Settlement annually occurred at generally low densities between 1980 and 1995, with the exception of a strong settlement pulse recorded in 1984. Relatively high densities of newly settled echinoids were also found in 1990–1992 and 1995. Settler densities varied between the two sampling locations Okushiri Island and Shimamaki.	Annually; Aug or Sep from 1980 to 1995, excluding 1981 and 1983	Underwater visual quadrat survey	Agatsuma et al. (1998)
Echinoid spp. (i.a. *Mespilia globulus* and *Echinometra* sp.)	Central Great Barrier Reef, Australia (tropical)	Coral reef; 15 m	Numbers of echinoid settlers were generally high at all sites, although settlement was significantly greater at the front reef sites than on the back reef sites.	Single deployment for 2 months from Nov 1991 to Jan 1992	Plastic bioballs inside net bags	Keesing et al. (1993)

Table 4 Summary of studies assessing spatial and temporal settlement patterns in asteroids

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Acanthaster cf. *solaris*	Suva Barrier Reef, Fiji (tropical)	Coral reef; shallow depth (crest)	A major settlement pulse only occurred in one (1984) of the nine sampling years (1979–1987). The low number of newly settled asteroids recorded in most years is likely the 'normal' amount of settlement success, while unusually high settlement of larvae in some years may give rise to population irruptions.	Monthly to annually; 1979–1987	Quadrat searches for newly settled individuals	Zann et al. (1987)
Acanthaster cf. *solaris*	Central Great Barrier Reef, Australia (tropical)	Coral reef; 15 m	Low numbers of settlers were found in the artificial collectors during the summer sampling period (late November to late February). Settlement was only observed at two front reef sites, while no settlers were recorded at any of the back reef sites.	Single deployment for 2 months from Nov 1991 to Jan 1992	Plastic bioballs inside net bags	Keesing et al. (1993)
Acanthaster cf. *solaris*	Moore Reef, Australia (tropical)	Coral reef; 2–9 m	Settlement occurred between October 2016 and February 2017. The settlement pulse was stronger between December and March than between October and February.	Oct 2016–Feb 2017, Dec 2016–Mar 2017	Plastic bioballs inside leaf baskets	Uthicke et al. (2019)
Acanthaster cf. *solaris*	Central Great Barrier Reef, Australia (tropical)	Coral reef; 1–18 m	Densities of settlement-stage individuals were highest at intermediate depths (8–14 m), and increased and decreased with the availability of rubble and coral substrata, respectively. At the reef scale, settler density was greatest in south-western and northern fore reef habitats.	May and Jun 2017	Underwater visual quadrat survey	Wilmes et al. (2020)
Acanthaster cf. *solaris*	Northern and central Great Barrier Reef, Australia (tropical)	Coral reef; 4–12 m	Settlement varied considerably among sampling periods and peaked between October 2016 and January 2017. Reports substantial latitudinal variation as collectors deployed in the central GBR were > ten times more likely to record settlers than in the northern GBR. No preferential settlement was detected among depths, suggesting these larvae readily settle in shallow reef environments.	Four sampling periods: Oct 2016–Jan 2017; Dec 2016–Mar 2017; Nov 2017–Feb 2018; and Nov 2019–Feb 2020	Plastic bioballs inside leaf baskets	Doll et al. (2021)
Asterias forbesi	Long Island Sound, the USA (temperate)	3–9 m	Settlement mostly occurred between early July and early September for an average period of 52 days, but the timing and length of the settlement period varied between years. Settlement was also highly variable among the three areas and ten nested sites; however, settler densities were remarkably similar among the three depths studied (3, 6 and 9 m) at any given year.	Twice per week; 1937–1961	Shells inside collectors	Loosanoff (1964)
Asterias forbesi & *A. vulgaris*	Nova Scotia, Canada (temperate)	Kelp bed and urchin barren ground; 5–10 m	Settlement was generally high, but variable among years and occurred from late July to early October. Higher densities of settlers were observed in and above kelp beds than in the urchin barrens.	Fortnightly; Jun–Nov 1992, May–Nov 1993 and Jun–Oct 1994	AstroTurf inside PVC pipe	Balch & Scheibling (2000)

(*Continued*)

Table 4 (*Continued*) Summary of studies assessing spatial and temporal settlement patterns in asteroids

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
A. forbesi & *A. rubens*	Bocabec Cove, Canada (temperate)	Rocky subtidal zone	Highly variable settlement among years, intra-annual sampling periods and sites. Settlement peaked from early August to early September 2005. Settler densities were lower in 2004 and peaked later. Highest densities for both years were recorded at the site closest to the larger body of water, Passamaquoddy Bay. If transported to the area by currents, larvae would likely reach this site first and, if competent, settle.	Fortnightly; Jun–Oct 2004, Jun–Oct 2005	AstroTurf inside PVC pipe	Jennings & Hunt (2010)
Choriaster granulatus	Central Great Barrier Reef, Australia (tropical)	Coral reef; 15 m	Low numbers of settlers were recorded at front and back reef sites during the summer sampling period (late November to late February).	Single deployment for 2 months from Nov 1991 to Jan 1993	Plastic bioballs inside net bags	Keesing et al. (1993)
Coscinasterias muricata	Wellington region, New Zealand (temperate)	Rocky reef; 4–7 m	Highly variable settlement among sampling years and locations. Settlers were highly abundant in 2019 at two locations (Kau Bay & Shelly Bay), and a smaller pulse of settlement occurred in 2017 at the same sites. Overall echinoderm settlement pulses were positively correlated with seawater temperature, chlorophyll and shell cover (exclusively found at these Harbour sites).	Annually; Nov 2016–Apr 2017, Nov 2017–Apr 2018, Dec 2018–Feb 2019	Plastic bioballs inside nylon net	Glockner-Fagetti & Phillips (2020)
Culcita novaeguineae	Central Great Barrier Reef, Australia (tropical)	Coral reef; 15 m	Low numbers of settlers were found in the artificial collectors during the summer sampling period (late November to late February). Settlement was only observed at one back reef site and two front reef sites.	Single deployment for 2 months from Nov 1991 to Jan 1994	Plastic bioballs inside net bags	Keesing et al. (1993)
Asteroid sp.	Northern California, the USA (subtropical)	Rocky habitat; 5–20 m	Settlement occurred at low levels from early April to late July and peaked in mid-July at the Bodega Marine Laboratory location. At Salt Point, a strong, synchronous settlement pulse was recorded in mid-late July, but settlement was absent before and after.	Weekly; Apr–early Sep 1992	Scrub brushes with polypropylene bristles	Wing et al. (1995b)
Asteroid sp. A	Wellington region, New Zealand (temperate)	Rocky reef; 4–7 m	Inter-annual and spatial variation in settlement observed. Settlement occurred in 2018 and 2019 at Princess Bay and Breaker Bay. Overall echinoderm settlement pulses were positively correlated with shell cover, seawater temperature and chlorophyll.	Annually; Nov 2016–Apr 2017, Nov 2017–Apr 2018, Dec 2018–Feb 2020	Plastic bioballs inside nylon net	Glockner-Fagetti & Phillips (2020)
Asteroid sp. B	Wellington region, New Zealand (temperate)	Rocky reef; 4–7 m	Little settlement occurred in 2019 at Princess Bay. Overall echinoderm settlement pulses were positively correlated with shell cover, seawater temperature and chlorophyll.	Annually; Nov 2016–Apr 2017, Nov 2017–Apr 2018, Dec 2018–Feb 2021	Plastic bioballs inside nylon net	Glockner-Fagetti & Phillips (2020)

Table 5 Summary of studies assessing spatial and temporal settlement patterns in ophiuroids

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Amphiura chiajei	Øresund, Denmark (temperate)	Muddy sand; 27 m	Settlers were observed in relatively small numbers, and the settlement period was found to start in early November in 1964, which was 3 months later than in *Amphiura filiformis*.	Fortnightly; Oct 1963–Oct 1966	Mouse-trap sampler	Muus (1981)
Amphiura filiformis	Øresund, Denmark (temperate)	Muddy sand; 27 m	Settlement occurred annually between August and December and peaked during a 2- to 6-week period in September to November.	Fortnightly; Oct 1963–Oct 1965	Mouse-trap sampler	Muus 1981
Amphiura filiformis	Galway Bay, Ireland (temperate)	Silty sand; 20 m	Little settlement over the 8-year period as only approximately 5% of the population were newly settled individuals (<4 mm). Settlement likely peaked in the autumn months from September to November.	3–10 monthly samples; 1974–1976	Van Veen grab and diver-operated suction sampling	O'Connor et al. (1983)
Amphiura filiformis	German Bight & North Sea (temperate)	Muddy sand; 38 and 54 m	Suggests the annual settlement season begins in July to August and the timing of settlement likely progresses from SW to NE during the summer and autumn months.	Every Mar/Apr in 1983, 1984 and 1986 to 1988; also in Jun, Jul/Aug, and Nov in 1983 and 1987, and in Dec 1987	Van Veen grab and Reineck box sampling	Künitzer (1989)
Ophiocten gracilis	Rockall Trough, NE Atlantic (temperate)	Silty sand; 600–1200, 2200 and 2900 m	Highest densities of settlers were recorded during sampling at depths of approximately 2900 m in May and July 1975, June 1978 and May 1980.	Sampling every 2–7 months; May 1975–Sep 1980	Epibenthic sled sampling	Gage & Tyler (1981a)
Ophiocten gracilis	Rockall Trough, NE Atlantic (temperate)	1000 m and 1400 m	Settlement occurred in both traps (1000 m; 1400 m) during mid-May.	May–Jul 1996	Sediment traps	Sumida et al. (2000)
Ophioderma longicaudum	Canary Islands, Spain (subtropical)	Four subtidal habitats: seagrass meadows, urchin barrens, macroalgal beds and sandy patches; 5–10 m	Densities of newly settled ophiuroids differed substantially among the four habitats surveyed, with collectors deployed in macroalgal beds reporting greater numbers than in the other habitats.	Single deployment for 4 weeks from early Aug to early Sep 2008	(A) plastic leaf-like units; (B) cushion-shaped, leaf-like units; and (C) cushion-shaped units	García-Sanz et al. (2012)

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Table 5 (*Continued*) Summary of studies assessing spatial and temporal settlement patterns in ophiuroids

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Ophionereis reticulata	Baleeiro Isthmus, Brazil (subtropical)	Intertidal rock boulder habitat	Analysis of recruitment patterns suggests intense settlement pulses in February and from September to November.	Jan 2001–Dec 2002	-	Yokoyama & Amaral (2011)
Ophiopholis aculeata and *Ophiura* spp.	Nova Scotia, Canada (temperate)	Kelp bed and urchin barren ground; 5–10 m	Within the annual 2-week settlement period, a major settlement pulse occurred over a 3-day interval, and settlement declined over the following 6 days. This settlement pulse was preceded by rapid temperature and salinity fluctuations and related to a change in current direction.	Three-day intervals during 2-week settlement period in Jul/Aug 1993	AstroTurf inside PVC pipe	Balch et al. (1999)
Ophiopholis aculeata and *Ophiura* spp.	Nova Scotia, Canada (temperate)	Kelp bed and urchin barren ground; 5–10 m	Settlement was observed from July to early August each year. Spatial differences in settlement between sites were likely related to variable larval supply due to local differences in hydrodynamic conditions. Settlement rates were higher in the barrens than in kelp beds, and substantially more settlers were collected in the high (2.3 m off bottom) than in the low (0.2 m) collectors.	Fortnightly; Jun–Nov 1992, May–Nov 1993 and Jun–Oct 1994	AstroTurf inside PVC pipe	Balch & Scheibling (2000)
Ophiothrix fragilis	Blanes, Spain (temperate)	Subvertical rocky walls & sandy bottom; 8–12 m	Compared the pattern found after settlement had ceased (July 1997 survey) with that found while settlement was still under way (June 1999 survey). Settlement occurred in multiple batches and peaked in June.	July 1997 and June 1999	Grab sampling of paired sponge-turf samples	Turon et al. (2000)
Ophiura ljungmani	Rockall Trough, NE Atlantic (temperate)	Silty sand; 2260–2925 m	Settlement occurs annually in summer, but the timing and magnitude of settlement likely varies among years.	Irregular sampling frequency; Jan 1979–Sept 1980	Epibenthic sled sampling	Gage & Tyler (1981b)
Ophiuroid species	Central Great Barrier Reef, Australia (tropical)	Coral reef; 15 m	High numbers of ophiuroid settlers were sampled at both the front reef and back reef locations. While no difference in settlement rates was observed between these two locations, settlement was patchy, with variable rates at the scale of hundreds of metres.	Single deployment for two months from Nov 1991 to Jan 1992	Plastic bioballs inside net bags	Keesing et al. (1993)
Ophiuroid species	Wellington region, New Zealand (temperate)	Rocky reef; 4–7 m	Settlers were recorded continuously from year to year; however, significant spatial variation was recorded. Settlement occurred during all three sampling years at Princess Bay and Beaker Bay, and little settlement occurred in 2017 at Moa Point. These sites had relatively high macroalgal cover.	Annually; Nov 2016–Apr 2017; Nov 2017–Apr 2018; Dec 2018–Feb 2019	Plastic bioballs inside nylon net	Glockner-Fagetti & Phillips (2020)

Table 6 Summary of studies assessing spatial and temporal settlement patterns in holothuroids

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Australostichopus mollis	Wellington region, New Zealand (temperate)	Rocky reef; 4–7 m	Settlement occurred only in 2017 and 2019 at Shelly Bay. Overall echinoderm settlement pulses were positively correlated with shell cover, seawater temperature and chlorophyll.	Annually; Nov 2016–Apr 2017; Nov 2017–Apr 2018; Dec 2018–Feb 2019	Plastic bioballs inside nylon net	Glockner-Fagetti & Phillips (2020)
Cucumaria frondosa	St. Lawrence Estuary, Canada (temperate)	Rocky subtidal zone; 0–60 m	Newly settled individuals were predominantly found at shallow depth between 0 and 20 m, rare between 20 and 40 m and virtually absent below 40 m. The highest densities of settlers were recorded in areas with high cover of gravel, small rocks and boulders, and also high light availability.	Seasonally; at the beginning of each season from spring 1992 to winter 1994	Belt transect surveys	Hamel & Mercier (1996)
Leptosynapta clarki	Vancouver Island, Canada (temperate)	Mid-intertidal mudflat	Unimodal settlement likely occurred in both years around February to March prior to annual recruitment pulses in April to May.	Bi-monthly; May 1990–Aug 1991	Sediment coring	Sewell (1994)
Pentamera chierchia & *Neothyone gibber*	Marietas Islands, Mexico (tropical)	Coral reef with sandy and rocky bottom areas	Recruitment density presented seasonal changes, with peaks for both species in May to June, and low recruitment for the rest of the year. This peak is suggested to be due to a settlement event following the March–April spawning time. Thus, a pulse in settlement likely occurred in April or May.	Bi-monthly; Mar 2011–Feb 2012	Coral skeletal structures as settlement collectors	Sotelo-Casas et al. (2016)
Holothuroid spp. (mostly *Psolus fabricii*, and few *Cucumaria frondosa*)	Bocabec Cove, Canada (temperate)	Rocky subtidal zone	Settler densities varied substantially between and within years; however, no spatial variation in settlement was reported. Only one settler was found during 2004, while high settlement pulses were detected in July 2005 at multiple sites.	Fortnightly; Jun–Oct 2004; Jun–Oct 2005	AstroTurf inside PVC pipe	Jennings & Hunt (2010)

(e.g. Zann et al. 1987). However, as demonstrated for the asteroids *Asterias forbesi* (Loosanoff 1964) and *Acanthaster* cf. *solaris* (Keesing & Halford 1992), and the echinoid *Strongylocentrotus purpuratus* (Rowley 1989), high larval settlement rates do not always give rise to high rates of recruitment to juvenile and adult populations. Conventional field-based studies usually only detect newly settled individuals that have had time to grow (e.g. Wilmes et al. 2020), such that it is very likely that differential post-settlement survival will have already modified patterns of abundance established at settlement. Visual underwater searches within quadrats or transect belts have reported densities of settlers soon after predicted settlement for echinoids (Hunte & Younglao 1988, Agatsuma et al. 1998), asteroids (Wilmes et al. 2020) and holothuroids (Hamel & Mercier 1996). Densities of recently settled ophiuroids have mostly been studied using various grab samplers, including mouse-trap samplers, Van Veen grab samplers, sled samplers and sediment traps (Gage & Tyler 1981a,b, Muus 1981, O'Connor et al. 1983, Künitzer 1989, Sumida et al. 2000). These techniques usually limit ophiuroid studies to few and short sampling intervals (Table 5); however, the logistical difficulties of sampling such cryptic settlers, particularly in deep-sea habitats, do not allow extensive sampling designs as seen in some shallow-water echinoderm taxa (e.g. Ebert et al. 1994). Conventional underwater surveys, inferences based on adult densities or back-calculations based on size distributions (e.g. Yokoyama & Amaral 2011) almost invariably lack data on early post-settlement processes, including mortality rates, to effectively represent settlement patterns (Keesing et al. 2018). Mortality may be very high during this period due to individuals adapting to the new benthic habitat and exposure to a new suite of predators (Wilmes et al. 2018), necessitating novel approaches of sampling individuals during or soon after settlement.

To address the inherent limitations in recording settlement in natural substrates and habitats, various different artificial settlement collectors have been employed (Loosanoff 1964, Bak 1985, Harrold et al. 1991, Keesing et al. 1993, Ebert et al. 1994, Lamare & Barker 2001, Uthicke et al. 2019). It is not clear how rates of settlement and early post-settlement survival differ between these artificial collectors and natural substrata, such that the collectors only allow relative comparisons of settlement potential, as opposed to absolute rates of larval settlement (Keesing et al. 1993). Early deployment, prior to anticipated settlement, to allow for extensive pre-conditioning and colonisation of the collectors could mitigate, but not resolve this limitation. If absolute rates of settlement had to be obtained (e.g. for stock-recruitment modelling), experimental assays comparing settlement rates and early post-settlement mortality on natural and conditioned artificial substrata could provide an indication of differential inductive capacity and survival. Potential differences could be then applied when calculating settlement rates for a reef area based on settler densities found in artificial collectors. In any case, these artificial collectors facilitate separation of settlement from post-settlement processes to produce a more accurate census of settlers and have thus been widely used in recent decades. More than two-thirds of the 53 studies on echinoderm settlement patterns summarised here used some form of artificial settlement collector (Figure 4), and some of these studies compared the relative efficiency of multiple collector designs for quantitative assessments of echinoderm settlement rates (Lamare & Barker 2001, Hereu et al. 2004, García-Sanz et al. 2012, Balsalobre et al. 2016). In general, these artificial samplers are designed to maximise microhabitat complexity and surface area, which is favourable for settlement and metamorphosis (Hunte & Younglao 1988, Harrold et al. 1991, Keesing et al. 1993). Moreover, these methods minimise disturbance to the benthos and can be easily deployed and retrieved for variable sampling durations and intervals.

Artificial turf panels, scrubbing brushes and some other similar collector designs are commonly used to assess spatiotemporal settlement patterns in echinoids (Figure 4), but have also been shown to facilitate settlement of ophiuroids, asteroids and holothuroids in the field (Wing et al. 1995b, Balch et al. 1999, Balch & Scheibling 2000, Jennings & Hunt 2010). Both types of sampling devices are usually suspended vertically in the water column, either from overhanging ledges from shore, or anchored to the substratum. Harrold et al. (1991) examined *Strongylocentrotus purpuratus* settlement using pieces of PVC pipe, which were filled with either high surface area plastic matrix or articulated coralline algae and suspended 1 m above the substratum. Higher densities of settlers were recorded in the designs using the plastic matrix, although this may have been due to a higher number of predators in the coralline algal collectors (Harrold et al. 1991). This design was later modified by Miller & Emlet (1997) and Balch et al. (1998) who lined PVC pipes with plastic turf, which is an effective settlement surface for echinoids (Harris et al. 1994) and has frequently been used in subsequent studies (Balch & Scheibling 2000, Lambert & Harris 2000, Jennings & Hunt 2010). Balch & Scheibling (2000) demonstrated the utility of suspending collectors in the water column for mitigating the potential effects of early post-settlement mortality due to predation, as substantially more ophiuroid settlers were collected in the high (2.3 m off bottom) than in the low (0.2 m) collectors.

Scrubbing brushes with nylon bristles were first used by Ebert et al. (1994) to document settlement rates of the echinoids *Mesocentrotus franciscanus* and *Strongylocentrotus purpuratus*. Due to their ease of use and cost-effectiveness, scrubbing brushes are still widely used (e.g. Schroeter et al. 1996, Okamoto et al. 2020). Studying *Evechinus chloroticus* settlement in New Zealand, Lamare & Barker (2001) compared scrubbing brush samplers (Ebert et al. 1994), AstroTurf panels (Ebert et al. 1991) and PVC pipes filled with plastic matrix (Harrold et al. 1991) and found the unstandardised mean number of settlers recovered from the PVC pipe design to be three- to fivefold higher than from the other two sampler designs. Conversely, wooden scrubbing brushes with vegetal bristles recorded considerably higher numbers of settlers than oyster shells, artificial grass and plastic matrix in trials measuring *Paracentrotus lividus* settlement in the Western Mediterranean Sea (Hereu et al. 2004). These comparative assessments reveal that it is probably not viable to compare settlement rates measured using contrasting methods, although most methods in and of themselves will provide insights into settlement patterns.

The settlement collectors designed by Keesing et al. (1993) consisted of a net bag containing 100 plastic aquarium filter bioballs, which provide an extremely high surface area, and successfully sampled settlers of numerous species of asteroids, ophiuroids, crinoids and echinoids on Australia's Great Barrier Reef. Laboratory trials demonstrated that competent larvae of crown-of-thorns starfish (*Acanthaster* cf. *solaris*) readily settle on plastic surfaces, which had been pre-conditioned in seawater aquaria (Keesing et al. 1993). Aquarium filter bioballs have since been incorporated in various modified versions of these collectors to efficiently sample settlement in a diverse range of habitats and different echinoderm species (Hernández et al. 2006, 2010, Clemente et al. 2009, Uthicke et al. 2019, Glockner-Fagetti & Phillips 2020, Doll et al. 2021). The suitability of this method to sample settlement rates in a wide range of echinoderms was corroborated by quantitative comparisons of multiple collector designs (Balsalobre et al. 2016); nylon nets containing plastic bioballs showed higher reproducibility and efficiency in sampling settlement than vertically oriented scrubbing brushes with vegetal bristles, or horizontal triangular mats of coconut fibre. Other artificial settlement collectors shown to effectively sample echinoid and ophiuroid species include settlement plates consisting of polystyrene egg crates and plexiglass (Bak 1985, Miller et al. 2009, Vermeij et al. 2010, Roger & Lorenzen 2016), and various designs of leaf-like units consisting of plastic mesh frames and plastic raffia (García-Sanz et al. 2012, 2014a).

The biggest constraint to using artificial settlement collector methods is the post-collection process time required to remove, sort and identify newly settled echinoderms, which are often microscopic (Keesing et al. 1993). Moreover, reliable visual identification of newly settled individuals may not be possible, especially if large numbers of sympatric species exist within sampling regions (Keesing et al. 1993). Innovative genetic analyses (e.g. eDNA) have emerged as a promising tool to address these constraints and further the utility of artificial settlement collectors to advance the understanding of settlement patterns (Uthicke et al. 2019, Doll et al. 2021). Uthicke et al. (2019) successfully detected newly settled *Acanthaster* cf. *solaris* in settlement collectors using taxon-specific mtDNA primers and standard polymerase chain reaction (PCR) (Uthicke et al. 2019). Further tests by Doll et al. (2021) validated the utility of genetic analyses using digital droplet PCR in assessing spatiotemporal settlement patterns. If developed as an accurate and quantitative tool, this method would be of particular utility for large-scale, high replicate studies of settlement for ecologically important taxa, and echinoderm groups for which reliable visual identification is not possible.

Although artificial settlement collectors cannot be regarded as providing absolute numbers of echinoderm settlers, they certainly provide comparable estimates of spatial and temporal variation in settlement and can complement *in situ* sampling of older juveniles in natural habitats. Simultaneous sampling using both these approaches will allow for improved discrimination between settlement and post-settlement processes (Harrold et al. 1991). Overall, artificial settlement collectors provide a relatively non-destructive sampling method and standardised index to study spatial and temporal settlement patterns for most echinoderm groups. Genetic methods are also likely to reduce processing time and greatly enhance capacity to assess spatiotemporal variability in settlement.

Spatial and temporal patterns of settlement

Echinoidea

Marked seasonality in settlement has been documented in the vast majority of echinoid taxa, and this pattern appears to be consistent among different habitat types and climate zones (Table 3). Species of the genera *Strongylocentrotus* and *Mesocentrotus*, commonly studied along the California coast (Ebert et al. 1994, Wing et al. 1995b, Miller & Emlet 1997, Okamoto et al. 2020) and in the western Atlantic (Balch & Scheibling 2000, Lambert & Harris 2000, Jennings & Hunt 2010), predominantly settle between April and July each year. Along the California coast, unimodal settlement peaks or primary settlement pulses occurred in June (Miller & Emler 1997, Okamoto et al. 2020) and July (Wing et al. 1995b). This timing coincides with warmer (or increasing) water temperatures, suggesting that temperature-induced spawning, as observed in other echinoderms (e.g. Caballes et al. 2021), may be governing this pattern. In contrast, Harrold et al. (1991) reported two distinct peaks in the settlement of *Mesocentrotus franciscanus* and *Strongylocentrotus purpuratus* in Monterey Bay, occurring from April through July and December through February, respectively. Ebert et al. (1994) recorded multiple annual settlement pulses, however, all between February and July each year. As part of a 27-year study, Okamoto et al. (2020) demonstrated annual settlement peaks during April and May and strong seasonal settlement fluctuations, which were highly synchronous among sites within Southern California locations. Here, temporal patterns of larval settlement were linked to fluctuations in ocean temperature and climate, which may impact pre-settlement processes and larval supply in *S. purpuratus* (Okamoto et al. 2020). Strong intra-annual variability in settlement rates was further reported for *Evechinus chloroticus* in New Zealand (Lamare & Barker 2001, Walker 2007, Glockner-Fagetti & Phillips 2020). Likewise, major settlement peaks of the echinoid *Paracentrotus lividus* only occurred between February and April on the Canary Islands (García-Sanz et al. 2014b) and between April and June in the Western Mediterranean Sea (Hereu et al. 2004, Tomas et al. 2004). On tropical and subtropical reefs, the echinoid *Diadema antillarum* settles continuously, but in low numbers throughout the year (Bak 1985, Miller et al. 2009, Williams et al. 2010), with substantial settlement pulses detected in the spring and autumn months in Curaçao (Bak 1985, Vermeij et al. 2010), and in July (Williams et al. 2010) or August (Millet et al. 2009) in Puerto Rico. Settlement of *D. africanum* follows a clear unimodal pattern, with a main settlement peak between August and October (Hernández et al. 2006, 2010, García-Sanz et al. 2014), which is probably linked to highly seasonal spawning events in the months prior (Hernández et al. 2006). This strong seasonal settlement pattern is likely directly or indirectly driven by temperature; however, settlement rates may also be negatively affected by reduced food availability in the months prior to settlement (Hernández et al. 2010).

While the timing of annual peaks in the settlement of most echinoids is relatively consistent among years, inter-annual variability in the magnitude of these pulses is generally high (Table 3). Settlement of *Strongylocentrotus nudus* occurred annually at relatively low densities off the northwest coast of Japan, with the exception of a strong settlement pulse in 1984 and some smaller pulses in 1990–1992 and 1995 (Agatsuma et al. 1998). Highly variable settlement among years was also documented for *Mesocentrotus franciscanus and Strongylocentrotus purpuratus* off the California coast (Miller & Emler 1997, Okamoto et al. 2020), and *S. droebachiensis* in Nova Scotia (Balch & Scheibling 2000) and Bocabec Cove, Canada (Jennings & Hunt 2010). Similarly, Tomas et al. (2004) and Hereu et al. (2004) reported substantial inter-annual differences in settlement of *Paracentrotus lividus* in the Mediterranean Sea by one and more than two orders of magnitude, respectively. Conversely, settlement rates of *Diadema antillarum* were relatively similar among years in the southern Caribbean Sea, although rates were only monitored over three consecutive years (Bak 1985). High inter-annual variability in *D. africanum* settlement at Canary Islands locations between 2002 and 2008 was likely driven by temperature or food availability during the planktonic larval phase (Hernández et al. 2010). Likewise, Glockner-Fagetti & Phillips (2020) suggested that settlement pulses, which varied considerably among years, were correlated with seawater temperature and chlorophyll concentration. These field studies did not explicitly demonstrate the causative nature of these links; however, the potential effects of starvation (Byrne et al. 2008) and temperature (McEdward 1984, Privitera et al. 2011) on the condition of echinoderm larvae prior and during settlement indicate that these processes may, in fact, be driving temporal settlement patterns.

A range of abiotic factors, including hydrodynamic forces, appear to be driving spatial differences in settlement rates of echinoids, particularly at large scales. Jennings & Hunt (2010) described highly variable settlement of *Strongylocentrotus droebachiensis* among sampling locations. As highest settler densities occurred at the site closest to the larger body of water, larvae transported to the area by currents would reach this site first and settle if competent and stimulated by an inductive cue (Jennings & Hunt 2010). The geographical position in relation to hydrodynamic processes may be an important factor in explaining larger-scale spatial variation in the settlement of other *Strongylocentrotus* species (Wing et al. 1995a, Agatsuma et al. 1998), and echinoids more broadly (Lamare & Barker 2001, Balsalobre et al. 2016). For example, the settlement of *S. droebachiensis* in the Gulf of Maine was one and three orders of magnitude higher than on Nova Scotia's Atlantic Coast and the Bay of Fundy, respectively, indicating substantial differences in larval supply to these locations (Balch et al. 1998). Synchrony in settlement pulses of *S. purpuratus* among geographical subunits off the California coast may be governed by physical processes affecting larval supply at regional scales (Ebert et al. 1994, Okamoto et al. 2020), although *Mesocentrotus franciscanus* did not form such geographic subunits with respect to coherence in settlement pulses (Ebert et al. 1994). At a smaller spatial scale, Clemente et al. (2009) found similar numbers of *Diadema africanum* settlers among sampling locations and sites in the Canary Islands and concluded that larval supply is unlikely to be limiting *D. africanum* settlement at any of the sampled locations. Settler densities of echinoid species, including *Mespilia globulus* and *Echinometra* sp., were generally high across ten sites at Davies Reef on Australia's Great Barrier Reef, although settlement was considerably greater at the front reef sites than on the back reef sites (Keesing et al. 1993). In contrast, settlement rates of *Diadema antillarum* in the southern Caribbean Sea were higher in sheltered lagoons and back reefs than in fore reef habitats (Rogers & Lorenzen 2016). Comparing settlement rates of *Evechinus chloroticus* in the Hauraki Gulf, New Zealand, Walker (2007) found no settlers at a sheltered, high-sediment location, while settler densities were high, although spatiotemporally variable, at a wave-exposed, low-sediment location.

Larval supply and the exposure to physical factors thus appear to also play a role in determining settlement patterns at smaller spatial scales. However, with decreasing spatial scale, taxon-specific chemical cues have been shown to also commonly play an important role in the processes surrounding larval settlement and thus relative rates of settlement at varying sites, habitats and depths.

Hereu et al. (2004) found settlement rates of *Paracentrotus lividus* to relate to wave exposure and currents, but not substratum type or adult densities, suggesting settlement patterns were not driven by chemical stimuli. Conversely, settlement of *P. lividus* was found to be generally higher on vertical rock wall habitats than in seagrass meadows at the same location in the Western Mediterranean Sea (Tomas 2004). García-Sanz et al. (2014) reported the same species to preferentially settle on soft substrata including seagrass, as opposed to hard substrata such as barrens and macroalgal beds, in the Canary Islands. Competent *P. lividus* larvae readily settle in response to chemical cues associated with various substrata (Table 1), including seagrass, brown macroalgae and coralline red algae (Privitera et al. 2011). Thus, the presence of chemical stimuli associated with inductive substrata may be driving these contrasting patterns at different locations.

Settler densities of *Strongylocentrotus droebachiensis* were higher in barren grounds than in adjacent seagrass beds (Balch et al. 1998). Larvae of this species respond to numerous types of coralline algae and red, brown and green fleshy algal species (Rowley 1989, Pearce & Scheibling 1990b, 1991). The main food source of *Strongylocentrotus* spp. is brown macroalgae, with occasional omnivory (Tegner 2001, Scheibling & Hatcher 2007). Competent *S. droebachiensis* larvae are likely induced to settle by a chemical cue associated with their preferred prey or conspecifics present in the urchin barrens, but not the adjacent seagrass beds. Similarly, the relatively high densities of *Diadema africanum* in urchin barren habitats, compared to macroalgal beds (Hernández et al. 2010, García-Sanz et al. 2014) and/or seagrass meadows (García-Sanz et al. 2014a), may be driven by gregarious settlement cues, which have been demonstrated in other echinoid species (Highsmith 1982, Burke 1984, Highsmith & Emlet 1986, Pearce & Scheibling 1990a, Dworjanyn & Pirozzi 2008, Mos et al. 2011, Ling et al. 2019). Differential settlement of echinoids along depth gradients has only been assessed in two studies. Larvae of both *Paracentrotus lividus* and *Strongylocentrotus droebachiensis* preferentially settled at shallow depths, with decreased settlement rates reported below 10 m (Harris et al. 1994, Hereu et al. 2004). In the case of *P. lividus*, this pattern is likely governed by physical processes or cues affecting larval movement or behaviour, as the results of this study suggest that the rocky substratum, although colonised by a rich algal assemblage, is not a cue for its settlement (Hereu et al. 2004).

Asteroidea

Available evidence suggests the settlement of asteroids is also highly variable within and among years (Table 4), although much of the research to date has focused on two genera, the corallivorous crown-of-thorns starfish (*Acanthaster* spp.) on coral reefs (Zann et al. 1987, Keesing et al. 1993, Uthicke et al. 2019, Wilmes et al. 2020, Doll et al. 2021), and asteroids of the genus *Asterias* in North America (Loosanoff 1964, Balch & Scheibling 2000, Jennings & Hunt 2010). Loosanoff (1964) monitored settlement rates of the temperate asteroid *Asterias forbesi* using shells inside collectors at Long Island Sound for bi-weekly intervals from 1937 to 1961. Settlement of *A. forbesi* predominantly occurred between early July and early September for a mean duration of 7 weeks; however, both the annual timing and the duration of this settlement period varied among years (Loosanoff 1964). This high inter-annual variability of settlement in this asteroid was further confirmed by Jennings & Hunt (2010) off Canada's Atlantic Coast. Here, settlement of *A. forbesi* and *A. rubens* peaked around the same time in 2005, from early August to early September, but settler densities were considerably lower in the last year (Jennings & Hunt 2010). Likewise, Balch & Scheibling (2000) reported generally high, but variable settlement among years for *A. forbesi* and *A. vulgaris* in the same region. Temporal trends, such as the highly variable settlement among sampling years of *Coscinasterias muricata* in New Zealand, are likely governed by the timing of pre-settlement processes and variable larval supply, as settlement pulses of *C. muricata* were positively correlated with larval food availability and seawater temperature (Glockner-Fagetti & Phillips 2020). Similar biotic and abiotic factors may be driving inter-annual differences in settlement observed in other asteroid species, such as *Stichaster australis* in New Zealand (Barker 1979). In Fiji, Zann et al. (1987) observed a major settlement pulse of *Acanthaster* cf. *solaris* in only one of the nine sampling years from 1979 to 1987. The low number of settlers recorded in most years was suggested to represent 'normal' settlement events, while spikes in the abundance of settlers in some years may give rise irruptive population growth (Zann et al. 1987).

Temporal settlement variability within (Uthicke et al. 2019) and among years (Doll et al. 2021) has only recently been explicitly demonstrated for crown-of-thorns starfish. However, the marked temporal patterns and propensity to undergo major population irruptions displayed by *Acanthaster* cf. *solaris* (Pratchett et al. 2014) are most likely linked to differential rates of larval supply (Birkeland 1982, Brodie et al. 2005), settlement and subsequent early post-settlement processes. It is unclear whether strong pulses and inter-annual differences in the settlement of both *A.* cf. *solaris* and *Asterias* spp. are driven by adult population density fluctuations of these species (Uthicke et al. 2009), or whether it is, in fact, settlement patterns that drive these adult population fluctuations in the first place.

Settlement patterns of both *Acanthaster* cf. *solaris* and *Asterias* spp. are also highly variable among regions and locations throughout their geographical ranges (Loosanoff 1964, Jennings & Hunt 2010, Doll et al. 2021). In the case of *Acanthaster* cf. *solaris*, spatial patterns of larval settlement on the Great Barrier Reef likely depend on spatiotemporal availability of larvae, driven by the latitudinal progression of their population irruptions, with regional estimates of settlement coinciding with densities of adult asteroids (Doll et al. 2021). Settlement of asteroid species is also highly variable at smaller spatial scales. Settlement of *Coscinasterias muricata* was high at two sites inside the Wellington Harbour, New Zealand, but completely absent at the three sites on the southern coast and the mouth of the harbour (Glockner-Fagetti & Phillips 2020). At this localised scale, such contrast in settlement success may be driven by substratum-related cues, as shell cover was exclusively found in the harbour and correlated with echinoderm settlement pulses overall (Glockner-Fagetti & Phillips 2020).

Similarly, physical or chemical cues acting at the site or habitat scale may also play a role in the smaller-scale settlement patterns displayed by these asteroids (Keesing et al. 1993, Balch & Scheibling 2000, Wilmes et al. 2020). For example, densities of juvenile *Acanthaster* cf. *solaris* (approx. 6 months post-settlement) were positively related to the cover of rubble, and more commonly observed in fore reef as opposed to back reef habitats (Keesing et al. 1993, Wilmes et al. 2020). Settlement of competent *A.* cf. *solaris* larvae in response to cues from some crustose coralline algae species (preferred settlement substratum and juvenile prey; Wilmes et al. 2020) and associated biofilms are well established (Johnson et al. 1991, Johnson & Sutton 1994). Thus, the availability of rubble beds encrusted with certain species of crustose coralline algae appears to govern the settlement patterns of this species within reefs. The high occurrence of coral rubble supporting epilithic crustose coralline algae at the base of reef slopes in deep water has led to the hypothesis that *A.* cf. *solaris* larvae predominantly settle on these deep-water habitats (Johnson et al. 1991). However, both their preferred substratum and settlers are also commonly found in shallow water environments in other studies (Wilmes et al. 2020, Doll et al. 2021). Doll et al. (2021) found no difference in settlement among three depth ranges sampled between 4 and 12 m, and records of newly settled *A.* cf. *solaris* across a wide range of depths (1–18 m) (Wilmes et al. 2020) support the conclusion that *A.* cf. *solaris* readily settle in shallow water environments. Similarly, settlement rates of *Asterias forbesi* were remarkably similar among three shallow water depths (3–9 m) assessed at any given year (Loosanoff 1964). These studies suggest that it is the presence of particular reef substrata and associated chemical cues, rather than depth, that is driving reef-scale settlement patterns of *Acanthaster* cf. *solaris* and other asteroids showing strong substratum selectivity.

Ophiuroidea

Compared to other echinoderm classes, the magnitude and timing of ophiuroid settlement appears to be relatively consistent (Table 5). Studies on *Ophiocten gracilis* (Gage & Tyler 1981a, Sumida et al. 2000) and *Ophiura ljungmani* (Gage & Tyler 1981b) indicate that settlement in these deep-sea ophiuroids occurs annually at the Rockall Trough in the East Atlantic. Highest densities are generally recorded during early summer in May and June, across a wide range of deep-sea sampling depths (Gage & Tyler 1981a, Sumida et al. 2000). Spatial settlement patterns remain largely unexplored; however, a range of physical processes affecting larval supply and advection in deep-sea environments may generally be driving recruitment in these species (Ebert 1983). Ophiuroid larvae can randomly metamorphose in the plankton and then settle indiscriminately (Hendler 1991, McEdward & Miner 2001, Morgan & Jangoux 2005, Selvakumaraswamy & Byrne 2006), which suggests that chemical cues associated with the substratum are unlikely to play an important role, especially given that such deep-sea habitats are characterised by relatively uniform soft substrata (Gage & Tyler 1981a,b).

Consistent annual settlement seasons have also been observed in ophiuroid species at relatively shallow depths in the North Sea and North-East Atlantic Ocean (Muus 1981, O'Connor et al. 1983, Künitzer 1989). Settlement of *Amphiura filiformis* in Øresund, Denmark, occurred annually between August and December and peaked during a short 2- to 6-week window in September to November (Muus 1981). The seasonal settlement pattern displayed by *A. filiformis* was consistent among temperate locations, as settlement in the North Sea, German Bight and off the Irish coast occurred and peaked during the same months (O'Connor et al. 1983, Künitzer 1989). Similarly, larval settlement of *Ophiopholis aculeata* and *Ophiura* spp. was observed in Nova Scotia, Canada, from July to early August each year (Balch & Scheibling 2000). Here, spatial differences in settler densities between sites appear to be driven by variable larval supply due to differences in hydrodynamic conditions, although settlement rates were also higher in urchin barren grounds than in seagrass beds (Balch & Scheibling 2000). Importantly, spatiotemporal settlement patterns and recruitment may also be influenced by cloning of ophiuroid larvae, if environmental conditions support planktonic existence and dispersal (Balser 1998, Hart et al. 2021). Settlement of *Ophiothrix fragilis* in the Western Mediterranean Sea took place in several patches and peaked in June (Turon et al. 2000). This ophiuroid species is thought to settle gregariously at times (Warner 1971, Morgan & Jangoux 2004, Morgan & Jangoux 2005), which may enhance the chance of settlement by competent larvae in suitable habitats; however, this link has not been demonstrated in field-based studies.

Holothuroidea

While there has been extensive research on settlement induction (and cues) for sea cucumbers (discussed above), as necessary for aquaculture, there have been few field-based studies that examine settlement patterns or early life-history processes (Table 6). Settlement of *Australostichopus mollis* was only detected at one of five sites in the Wellington region in New Zealand during two sampling years (Glockner-Fagetti & Phillips 2020). Settler densities of holothuroids in Bocabec Cove, Canada, including *Psolus fabricii* and *Cucumaria frondosa*, varied considerably between and within sampling years (Jennings & Hunt 2010). However, settlement patterns did not vary spatially, as high pulses occurred in July 2005 at multiple sites, likely driven by currents differentially affecting larval supply at different points in time (Jennings & Hunt 2010). Newly settled *Cucumaria frondosa* were predominantly found at shallow depths (0–20 m) along a rocky subtidal depth gradient, while settlers were rare and absent in intermediate (20–40 m) and deeper waters (40–60 m), respectively (Hamel & Mercier 1996). The highest number of newly settled individuals was recorded in areas characterised by high cover of gravel, small rocks and boulders, and also high light availability (Hamel & Mercier 1996). In complimentary experimental studies, Hamel & Mercier (1996) demonstrated strong selectivity for the undersurfaces of rocks and rubble, which may be linked to the high light sensitivity displayed by *C. frondosa* during the settlement phase. Largely photopositive during their substratum search and selection, competent larvae likely react to not only physical cues associated with the substratum, but also light intensity. In laboratory studies, other holothuroid species have also been induced to metamorphose in response to cues associated with seagrass (Mercier et al. 2000, Agudo 2007), brown algae (Agudo 2007), periphytic diatoms associated with biofilms (Ito & Kitamura 1997, Agudo 2007) and conspecifics (Young & Chia 1982); however, the relevance of these results has yet to be tested in their natural environments.

Crinoidea

In contrast to the other echinoderm classes, many groups of crinoid species do not have complex planktotrophic larvae, with lecithotrophic larval types in both pelagic and benthic developmental habitats being more common (Lahaye & Jangoux 1985, McEdward & Miner 2001). Keesing (1993) recorded low numbers of crinoid settlers at Davies Reef on Australia's Great Barrier Reef between November 1991 and February 1992. A dozen crinoid larvae settled at the front reef sites, while only three settlers were found at the back reef sites (Keesing et al. 1993). *Antedon bifida* doliolaria appear to settle selectively and gregariously in aquaria and the field, with larvae most commonly observed near or within adult colonies (Lahaye & Jangoux 1985). Larvae of the planktotrophic crinoid *Florometra serratissima* and the external brooder *Dorometra sesokonis* have also previously been shown to metamorphose in response to conspecific cues (Mladenov & Chia 1983, Obuchi et al. 2010). While there have been no observations of gregarious settlement by these two species in the field, the strong gregarious settlement responses of *Florometra serratissima* in culture suggest that gregarious settlement may play an important role in the formation and maintenance of adult aggregations of *F. serratissima* and many other crinoid species with a planktonic larval stage (Mladenov & Chia 1983).

Future directions in research

There is a suite of environmental stimuli that induce settlement of echinoderm larvae, although the specificity and relative importance of these cues vary greatly among species and classes. Environmental cues are thought to become progressively more important at smaller spatial scales and play a critical role in settlement processes once a planktonic larva has attained metamorphic competence. Physical factors have been demonstrated to induce, inhibit or affect the processes occurring during this phase; however, chemical cues in particular have received considerable attention in echinoderm studies. Despite decades of extensive research effort, specific chemical compounds from environmental inducers of echinoderm settlement have not been explicitly identified, with a few exceptions (e.g. four polyunsaturated fatty acids in Kitamura et al. 1993, 1994; histamine in Swanson et al. 2004). In contrast to some other marine invertebrate taxa (reviewed by Hadfield & Paul 2001), there have only been very few attempts to fully or partially characterise the various chemical cues proven to induce echinoderm settlement. As echinoderm larvae have the ability to settle in response to low molecular-weight, water-soluble chemical cues, these cues need to not only be fully characterised, but also quantified (i.e. relevant concentrations) in the habitat of the species. The challenges in carrying out such purifications and characterisations are large, and often complicated by the polarity of molecules and low concentrations of compounds (Hadfield & Paul 2001), but can be overcome using new methodologies. By isolating the chemical cue produced by an alga from the polar extract by cation-exchange chromatography, Swanson et al. (2004) presented evidence that histamine, an invertebrate neurotransmitter, is an important natural inducer of echinoid settlement, correcting the characterisation in a previous study (Williamson et al. 2000). Larvae perceived low concentrations of histamine and responded rapidly to the inducer, and the characterisation was further corroborated by analyses of histamine concentrations in various algae and in the seawater surrounding the most inductive alga (Swanson et al. 2004, 2006, 2012). Similarly, the physiology and settlement ecology of many other echinoderms could be linked, if chemical cues are fully characterised.

The roles played by biofilms as cues in echinoderm settlement are much more widespread and complex than formerly thought. When tested in isolation from the substratum, many studies have identified biofilms, and associated bacteria or diatoms, as the 'true' inducer of settlement in echinoderms (e.g. Huggett et al. 2006). Previous settlement assays that did not differentiate biofilms from the underlying substrata may thus be of limited use in drawing conclusions about echinoderm settlement behaviour in the field. At the same time, the specific chemical components of biofilms, which serve as cues for settlement in larvae, are largely unknown. Portions of complex protein-lipopolysaccharides, which make up much of the extracellular polymeric substances secreted by microorganisms, have been suggested as a cue (Hadfield 2011); however, such evidence is generally weak, especially for water-soluble cues. Many past studies on the effects of biofilms tested single-taxon cultures of bacteria or other microorganisms in their assays, which does not allow generalisations about larval responses under natural conditions, but nevertheless progresses the identification of specific chemical components that may be important inducers or inhibitors of echinoderm settlement. The development of new *omic* techniques certainly presents an opportunity to characterise microbes in biofilms and generally enhance our understanding of specific receptors and genes responsible for settlement processes in echinoderms (reviewed by Dobretsov & Rittschof 2020). To gain a greater understanding of the ecological and developmental processes surrounding settlement, it is critical not only to test how widespread and relatively important biofilm-induced and bacteria-mediated settlement is among echinoderm taxa, but also to identify the specific chemical components of biofilms which serve as cues. More generally, research on chemosensory-mediated settlement behaviour could also facilitate our ability to address ecological issues, such as population irruptions of the corallivorous crown-of-thorns starfish (Pratchett et al. 2014). While there is likely a multitude of semiochemicals detectable by echinoderm larvae, the characterisation of the few that regulate their behaviour, such as the movement towards or away from the source, could inform the management of such a complex issue (Hall et al. 2017, Motti et al. 2018). Different echinoderm species are likely to respond to different suites of distinct chemical or biochemical cues, but early work has demonstrated that there are many common features to inductive and inhibitory factors.

Further descriptive studies of larval responses to single physical or chemical cues in nature are unlikely to improve our general understanding of the mechanisms driving these processes. There have been numerous studies on the role of physical factors in settlement behaviour (e.g. turbulence), and even more on the induction of settlement by chemical cues, commonly associated with substrata or conspecifics. A considerable shortcoming of these studies has been the highly unnatural conditions in which these effects were tested, as echinoderm larvae do not encounter these environmental cues in isolation in the field. The relevance and potential applications of these cues in the natural environment thus remain largely unknown. Specifically, most studies tested these isolated cues in small containers and in the absence of flow. Larval settlement in echinoids is enhanced by turbulence through a competence shift and short-term behavioural responses facilitating substratum contact (Gaylord et al. 2013, Ferner et al. 2019). Hydrodynamic processes may play an underappreciated role in the induction of echinoderm settlement at the habitat scale and should thus be integrated into settlement assays. Chemical cues associated with substrata and conspecifics could be tested under more realistic flow regimes, and additionally, other physical factors such as light intensity could be considered. Concomitant efforts towards advancing our ability to track larval movements in the field would complement this research. Testing a suite of environmental cues is a non-trivial challenge, but natural conditions must be replicated as closely as possible when studying settlement in the laboratory. This endeavour is worth the effort for a more realistic and accurate understanding of the complex processes in larval settlement of echinoderms.

The ability to link information on settlement processes and induction to patterns of settlement and recruitment observed in the field has been constrained by challenges to effective and accurate sampling of echinoderm settlers, due to their extremely small size and cryptic nature. To address the limitations of conventional sampling techniques, various artificial settlement collector designs have been developed and successfully used in recent decades. The number of echinoderm studies documenting spatial and temporal settlement patterns in the field has thus markedly increased, although the majority of studies are focused on a few echinoid, asteroid and holothuroid species of ecological or economic importance. In theory, artificial collectors facilitate separation of settlement from post-settlement processes. However, this separation depends on how frequently collectors are deployed and retrieved, and in the case of unimodal annual settlement pulses, the timing of retrieval with respect to the settlement event. Because echinoderm settlers are subject to various early post-settlement processes including high mortality during the first days and weeks of their benthic life stage (Hunt & Scheibling 1997, Balch & Scheibling 2001), field studies with relatively long deployment periods are unlikely to accurately describe settlement rates, but rather relative rates of recruitment. Nevertheless, further assessments of spatiotemporal settlement patterns at appropriate scales appear critical to our understanding of the supply-side ecology of echinoderms, especially if such assessments occur in conjunction with quantitative descriptions of abiotic (e.g. temperature and hydrodynamics) and biotic factors (e.g. chlorophyll, availability of settlement substrata or conspecific aggregations) acting at the same scales. Some echinoderm studies have reported correlation of spatiotemporal settlement patterns with environmental variables, including temperature, chlorophyll concentrations and currents. However, most of these studies have failed to establish causative links with appropriate field or laboratory experiments. To explain the roles of settlement cues and processes in the natural environment, future studies on settlement patterns should thus not only aim to quantify pertinent environmental variables, but also attempt to resolve critical information gaps surrounding the links between these factors and settlement patterns observed in the field.

In brief, we argue that to meaningfully advance our understanding of echinoderm settlement, the following four directions in research should be prioritised in future studies: (1) detailed characterisation of specific chemical compounds inducing echinoderm settlement; (2) examination of biofilm-induced and bacteria-mediated settlement; (3) settlement assays testing suites of abiotic and biotic cues, replicating natural conditions as closely as possible; and (4) *in situ* quantification of settlement rates and pertinent environmental variables, with complementary experimental studies to establish causative links.

Concluding remarks

Our review of larval settlement in echinoderms has highlighted considerable spatial and temporal variability in settlement patterns across all classes (see also Balch & Scheibling 2001), although variability is much less apparent for ophiuroids. It has long been known that echinoderm larvae do not settle randomly in space and time (Ebert 1983), yet the processes and mechanisms that influence settlement patterns are highly complex and poorly understood. Settlement patterns, and the factors that influence them, are nonetheless fundamental to the population dynamics, distribution and abundance of echinoderms. Our review highlights the wide range of abiotic and biotic factors involved in the movement (locomotion and dispersal) and distribution of echinoderm larvae prior to settlement, and complex interactions between larvae and their environment throughout the settlement process. Important settlement and metamorphic cues are often associated with particular substrata or organisms that occur in benthic habitats. However, the specificity and the relative importance of settlement cues vary among classes, whereby echinoids appear to have much more generalised settlement cues, at least compared to asteroids. Further research is clearly warranted to better understand settlement in echinoderms, especially for species that are ecologically or economically important. Critically, marked fluctuations in settlement success, and thereby local abundance, of ecologically important echinoderms can have broad-scale impacts on the structure and functioning of marine ecosystems.

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THE INTIMATE RELATIONSHIP BETWEEN BOXER CRABS AND SEA ANEMONES: WHAT IS KNOWN AND WHAT IS NOT

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Abstract Crabs of the xanthid subfamily Polydectinae (boxer crabs) have the remarkable habit of holding another animal, usually a sea anemone, in each of their claws. Some boxer crab species hold nudibranchs and holothurians. Boxer crabs are the only known crab species to have effectively lost all ability to use their claws in typical fashion, having formed what appears to be an obligate dependence on the animal held in its claws. Several, although not all, of the associated anemone species are known to occur free-living. Due to the anemones being held in the 'hands' of the crab, it is easy to envision how the stinging anemones are used for defence and food gathering. Boxer crabs have been mentioned often in this regard in popular culture and are commonly kept by marine aquarists. However, since their first appearance in the literature over 200 years ago, very few systematic studies have been conducted into the particulars of this symbiosis. Recent laboratory studies have expanded on various aspects of the natural history of boxer crabs, as well as the discovery of additional species in this subfamily. This review covers the literature on the boxer crab–anemone association, using the more extensively studied hermit crab–anemone association as a point of comparison. The review covers many aspects of the symbiosis, including the cost and benefits to each of the partners, the defensive value of the anemones to the crab, how the crabs locate their anemones, their respective morphological adaptations, anemone splitting and theft, as well as distribution and phylogeny. Due to their small size, most of the experimental work conducted to date has been limited to the laboratory. Recent advances in video recording as well as other tracking methods may allow for a closer look at this association in the wild, laying out the path to answering the many questions in this fascinating partnership.

Keywords: boxer crab, sea anemone, symbiosis, asexual reproduction, tool use, defense, Lybia, Alicia

Introduction

Members of the xanthid crab subfamily Polydectinae are the only group of crabs whose claws have lost the ability to fulfil typical functions such as food gathering, burrowing and grooming, since they have become specialized for holding anemones (Figure 1D). Other brachyuran crabs that carry anemones do so by placing them on their carapace or appendages. They often hold them with their fourth and or fifth walking legs without loss of claw function (see reviews by Guinot et al. 1995,

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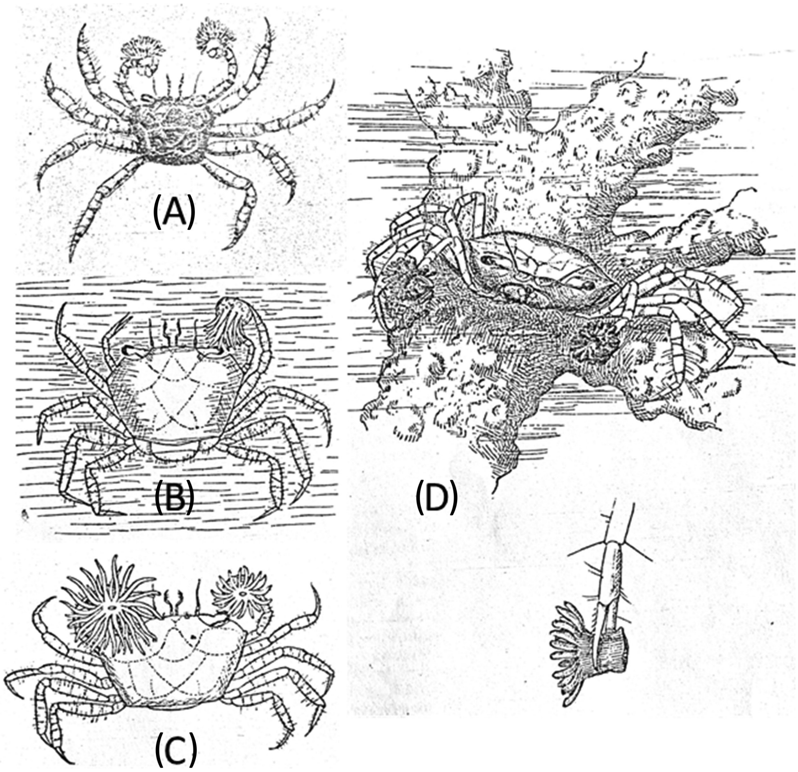

Figure 1 Early descriptions of polydectine crabs associated with anemones. (A) *Lybia tessellata* holding an actinian in each claw. After Richter (1880). (B) *Lybia edmondsoni* detaching a fixed actinian with its first walking leg (Duerden 1905). (C) *Lybia edmondsoni* holding an expanded actinian in each claw (Duerden 1905). (D) *Lybia tessellata* on a live coral, bearing in each claw an anemone, and an enlarged claw holding an anemone (Borradaile 1902).

Guinot and Wicksten 2015). The dependence of boxer crabs on their associated invertebrates to replace their claws renders these partnerships so interrelated and unique.

The first description of the association between a polydectine, *Lybia tessellata* (initially designated as *Melia tessellate*; see Appendix 1 for further details on polydectine crabs) and anemones was published at the end of the nineteenth century, as a small footnote by Mobius to a taxonomic study of the Crustacea of Mauritius and the Seychelles Islands (Richter 1880). *Lybia tessellata* was described as holding a living anemone *Actinia prehensa* (Moebius), in each of its claws by means of delicate hooks, slightly embedded in the anemone column (Figure 1A). It was suggested that the anemone's stinging cells assisted the crab in securing food. A detailed study of this association was carried out in Hawaii at the beginning of the twentieth century (Duerden 1905) (Figure 1B and C). Even though this study was based on only three specimens of boxer crabs (two in the genus *Lybia* and one *Polydectus cupulifer*), it contributed much to our understanding of the crab–anemone relationship. Duerden (1905) dealt with crab–anemone partner specificity, the mechanism of anemone acquisition, defensive movements carried out by the crab with its anemones, feeding behaviour of the crab and anemone, as well as the cost and benefit of the association for both crab and anemone. Due to their peculiar behaviour of holding anemones, crabs of the genus *Lybia* have received since then much attention from the public, receiving such popular names as 'boxer crabs', 'pom-pom crabs' and 'cheer leaders'. Boxer crabs despite their small size are very photogenic, often featured in popular books on coral reefs and annual calendars. These crabs, and particularly *Lybia tessellata,* are also popular in the ornamental crustacean trade (Calado et al. 2003; Calado, 2020). In contrast to the interest in boxer crabs from the non-scientific community, little systematic research has focused on these partnerships. Ross (1983) in his review on symbiosis in crustaceans emphasized that since the pioneering study by Duerden (1905) very little research addressed the behaviour of these creatures and more research is needed on the relationship between boxer crabs and their anemones. Several studies have recently been published on these partnerships (e.g. Karplus et al. 1998, Mendoza and Ng 2011, Schnytzer et al. 2013, 2017).

This review examines all the information available on Polydectine crabs and their associated anemones and other invertebrates, emphasizing what is known and what should be investigated in the future. We review the first accounts of boxer crabs and the studies that followed, covering what is known about their taxonomy, morphology, distribution, partner specificity and associated behaviours, as well as phylogeny and evolution. Throughout this review, the relationship of hermit crabs and other crustaceans with their associated anemones is contrasted with that of boxer crabs to better understand the peculiarity of the latter. This association is unique since the crabs cause temporary morphological changes in their claw-held associates, as well as inducing their asexual reproduction, at least in the case of the more commonly held anemones, raising interesting questions about the ultimate costs and benefits to each partner.

Taxonomy and distribution of boxer crabs and associated anemones

The Polydectinae is a small subfamily of the Xanthidae (124 genera and 639 species) with only 11 species and 3 genera (Figure 2). Within the subfamily, *Lybia* has eight species; *Tunebia* has two species; and *Polydectus* is monotypic (Table 1; Appendix 1). The morphology and taxonomy of the Polydectinae have been extensively reviewed by Guinot (1976) and Serène (1984). Results from Lai et al. (2011) using molecular data and developmental comparisons of first stage zoea are consistent with the adult morphological parameters which remain diagnostic. Guinot (1976), in her study of the Polydectinae, summarized the major morphological traits to include form of carapace, morphology and position of the antennas, mouth appendages, structure of the pincers and the ability to grasp anemones (Figure 2). Guinot (1976) recognized three species groups within *Lybia* based on the structures of the carapace, anterolateral border, the endopod of the first and third maxillipeds, male first pleopod and chelipeds. The first group consists of *Lybia tessellata* (Figure 2A) and *Lybia edmondsoni* (Figure 2D). Both these crabs are conspicuously coloured (Figure 3A and C). *Lybia tessellata* has a wide Indo-West Pacific distribution and *Lybia edmondsoni* is apparently endemic to Hawaii. The second group consists of *Lybia plumosa* (Figure 2F) and *Lybia leptochelis* (Figure 2E); both are cryptically coloured and occur in the Indian Ocean. *Lybia denticulata* was left by itself, regarded as intermediary between the genera *Polydectus* (Figure 2I and J) and *Lybia*. The status of *Lybia australiensis* (Figure 3F), *Lybia caestifera* and *Tunebia hatagumoana* (Figures 2G and 3E) was left undecided. *Lybia tutelina* was subsequently defined from New Caledonia by Tan and Ng (1994; Figure 2H). Mendoza and Ng (2011) erected *Tunebia* for *Tunebia hatagumoana* and *Tunebia tutelina*. Both species appear to have a relatively limited distribution, *Tunebia hatagumoana* occurring in Japan and the Philippines (Mendoza and Ng 2011) and *T. tutelina* in New Caledonia. To date, both species have only been found in relatively deep water of not less than 70 m.

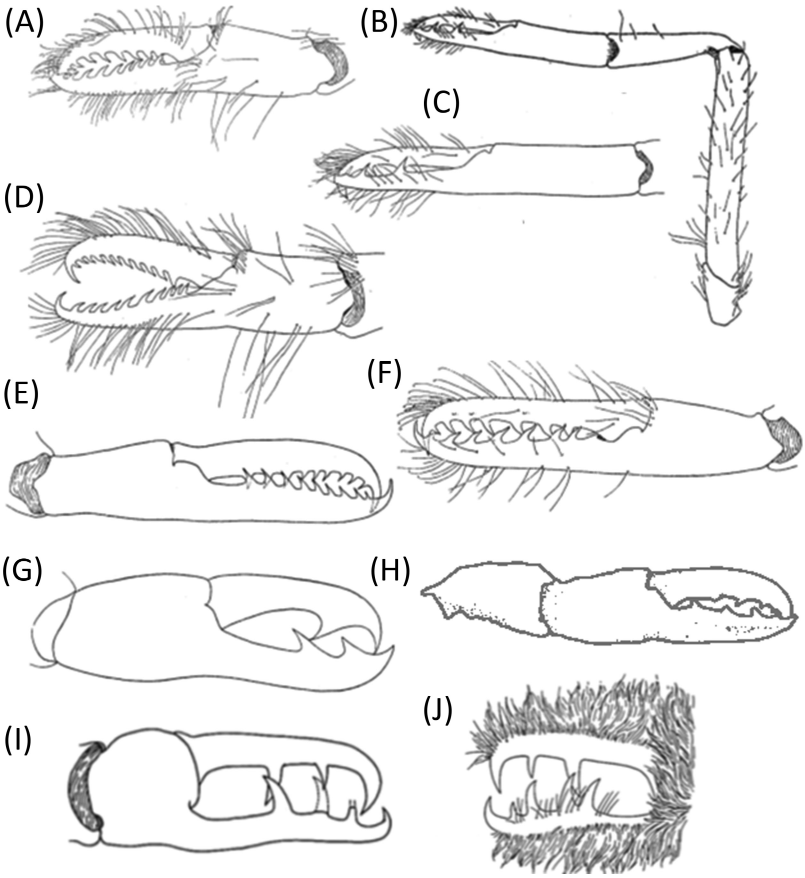

Figure 2 Chelipeds of Polydectine crabs. (A) *Lybia tessellata*. (B and C) *Lybia denticulata*. (D) *Lybia edmondsoni*. (E) *Lybia leptochelis*. (F) *Lybia plumosa*. (G) *Tunebia hatagumoana*. (H) *Tunebia tutelina*. (I and J) *Polydectus cupulifer*. (A–F) After Guinot (1976); (G) after Guinot & Wicksten (2015); (H) after Tan & Ng (1994); (I and J) after Guinot & Wicksten (2015).

Thus far, there have been no studies that focused on the ecology of boxer crab–anemone associations. These partnerships were only briefly mentioned in overviews of non-scleractinian anthozoans of the shallow Red Sea (Fishelson 1970), the shallow benthic fauna of the Red Sea (Fishelson 1971) and the fauna of the coral reefs of northern Mozambique (Kalk 1959). Their habitat and depth distribution has been often briefly mentioned in taxonomic studies (Table 1). In most cases, boxer crabs live in the shallow littoral zone, under rocks, basalt slabs and coral rubble during the day, leaving their shelters at night for foraging.

The taxonomy of the anemones carried by Polydectine crabs has not been well studied. In 6 out of 11 recognized species of boxer crabs the identity of their symbiotic anemones is unknown (Table 1). According to Crowther (2013), the difficulty in the identification of these symbiotic anemones is due to their small size and changes in morphology (e.g. lack of body outgrowths) which are different from that of the fully grown non-associated anemones and important for their classification. Cutress (1977) suggested that several misidentifications of symbiotic anemones associated with boxer crabs may have arisen due to the altered morphology of held anemones.

The anemones of the family Aliciidae are typically involved in symbiosis with zooxanthellae. They typically possess branched outgrowths from the column which harbour these intracellular

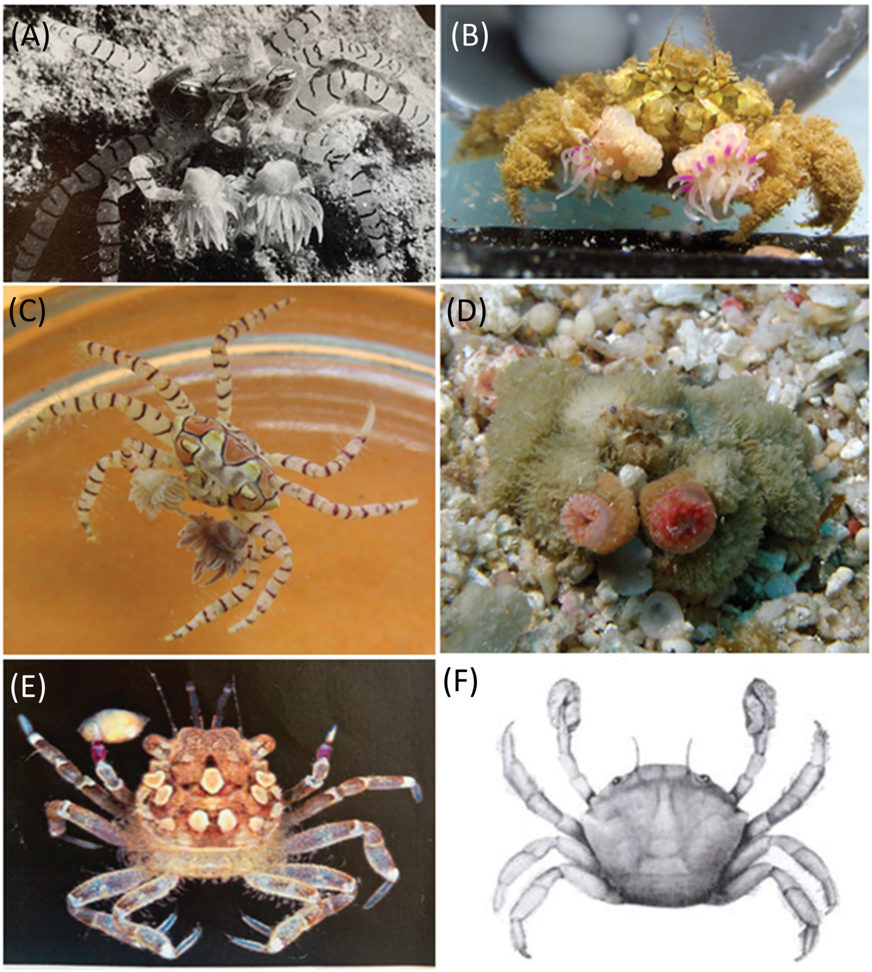

Figure 3 Polydectine crabs and associated invertebrates. (A) *Lybia edmondsoni* holding in each claw a *T. producta* (Karplus et al. 1998). (B) *Lybia leptochelis* holding in each claw an *Alicia* sp. (C) *Lybia tessellata* holding in each claw a *Triactis producta* anemone: the right claw anemone is bleached, and the left claw anemone is dark due to the presence of symbiotic zooxanthellae. (D) *Polydectus cupulifer* holding unidentified anemones. Taken by Kaoru Imagawa. (E) *Tunebia hatagumoana* holding a nudibranch in one claw (Lai et al. 2011). (F) *Lybia australiensis* holding unidentified anemones (Ward 1933).

photosynthetic algae and spherical defence structures that contain nematocysts (Crowther 2013). This family consists of four genera, *Lebrunia*, *Triactis, Phyllodiscus* and *Alicia*, and a total of nine species (Crowther 2013). Two of these species are associated with boxer crabs, *Triactis producta* and *Alicia* sp. The former is highly venomous (Levi et al. 1970) and has a wide Indo-West Pacific distribution. This species is involved in more partnerships with boxer crabs than any other anemone, occurring in four out of the five associations with boxer crabs whose anemones were positively identified. *Alicia* sp. has been found in the Red Sea only in association with *Lybia leptochelis*. A further six species of anemones have also been identified in association with boxer crabs, albeit to a lesser extent; *Boloceractis prehensa* (Fam. Boloceroididae), *Actinia prehensa* (Fam. Actiniidae), *Bunodeopsis* sp. (Fam. Boloceroididae) *Sagartia pugnax* (Fam. Sagartiadae), *Phelia* sp. and *Telmatactis decora* (Fam. Isophellidae; see Table 1). Members of the families Isophellidae

Table 1 Boxer crabs and associated anemones

Species	Distribution	Habitat & depth	Associated anemones	References
Lybia australiensis (Ward 1933)	New South Wales Australia	Under large rock	Unknown	Guinot (1976)
Lybia caestifera (Alcock 1898)	Western Indian and eastern Pacific Ocean	Shallow water	Unknown	Lee et al. (2008)
Lybia denticulata (Nobili 1906)	Red Sea	Unknown	Unknown	Guinot (1976)
Lybia edmondsoni (Takeda & Miyake 1970)	Hawaii[a]	Under basalt & coral rocks 6–20 m[a]	*Triactis producta*[a]	Karplus et al. (1998)[a], Takeda & Miyake (1970)[b]
Lybia leptochelis (Zehntner 1894)	Indian Ocean[b]	Under rocks in the shallow sublittoral[b]	*Triactis producta*[a] *Alicia* sp.[b]	Fishelson (1970)[a] Schnytzer et al. (2013)[b]
Lybia plumosa (Barnard 1947)	Indian Ocean[a]	Coral pools Shallow water[b]	*Triactis producta*[c]	Guinot (1976)[a] Kalk (1959)[b] Giman (2008)[c]
Lybia pugil (Alcock 1896)	Indian Ocean[a]	Unknown	Unknown	Guinot (1976) Mendoza & Ng (2011)
Lybia tessellata (Latreille 1812)	Indian Ocean & Western Pacific[f]	Living coral branches,[g] under dead coral and stones,[i] intertidal,[h] 4 m[b]	*Triactis producta*[a] *Boloceractis prehensa*[b] *Actinia prehensa*[c] *Bunodeopsis* sp.[d] *Sagartia pugnax*[e]	Giman (2008)[a] Jeng (1994)[b] Richter (1880)[c] Duerden (1905)[d] Verrill (1928)[e] Guinot (1976)[f] Borradaile (1902)[g] Serène (1984)[h] Tweedie (1950)[i]
Tunebia hatagumoana (Sakai 1961)	Japan[a] The Philippines[d]	Unknown 60[a]–100 m[b]	*Gymnodoris* sp. (nudibranch) Unknown anemone[c]	Sakai (1961)[a] Miyake (1983)[b] Baba & Noda (1993)[c] Mendoza & Ng (2011)[d]
Tunebia tutelina (Tan & Ng 1994)	New Caledonia	Unknown 74–76 m	Unknown	Tan & Ng (1994)
P. cupulifer (Latreille 1812)	Indo-Pacific[d]	Under coral & stone blocks,[a] intertidal & several m[d]	*Phelia* sp.[a] *Sagartia pugnax*[b] *Telmatactis decora*[c] Small holothurians[d]	Duerden (1905)[a] Edmondson (1946)[b] Schmitt (1965)[c] Guinot (1976)[d]

and Sagartiadae possess acontia or stinging threads, which are expelled by the anemones in defence against predators. Some of the species in the list may actually be synonyms and misidentifications of *Triactis producta* (Cutress 1977, 1979, Crowther 2013). According to Cutress (1979), the *Bunodeopsis* sp. identified by Duerden (1905) as the anemone carried by crab number one in his study, is almost certainly a misidentification of *Triactis producta*. Forty specimens of *Lybia edmondsoni* examined in Hawaii all carried *Triactis producta* (Cutress 1977). As aforementioned, the small specimens and fragments carried in the chelae of *Lybia* are often not of typical morphological form (lacking, outgrowths, changes in size, etc.) and are not readily identified. The 'sagartiids' carried by Duerden's (1905) second crab are likely young *T. decora*, the species usually carried in the chelae of *Polydectus cupulifer* (Figure 3D).

In addition to anemones, boxer crabs occasionally collect and hold in their claws other small invertebrates. Most notably, *Tunebia hatagumoana* appear to almost always hold small nudibranchs of the genus *Gymnodoris,* possibly *Gymnodoris citrina* (Sakai 1961, Baba & Noda 1993), although

Sakai (1961) reported one specimen found holding an unidentified anemone. *Tunebia hatagumoana* have been observed holding nudibranchs in both Japan and the Philippines (Mendoza & Ng 2011); however, a majority of the studied specimens are preserved, impeding on the identification of small soft tissue animals held by the crabs. *Polydectus cupulifer* usually hold sea anemones but has also been retrieved holding small unidentified holothurians in each claw (Guinot 1976). Little is known about the nature of the association of these crabs with nudibranchs or holothurians. The notion that they hold animals such as nudibranchs which are typically far more mobile than anemones is intriguing.

Schnytzer (2008; see Appendix 2) documented a novel relationship between *Lybia leptochelis*, who always hold a pair of *Alicia* sp., and another anemone, typically twice the size or more of the claw-held

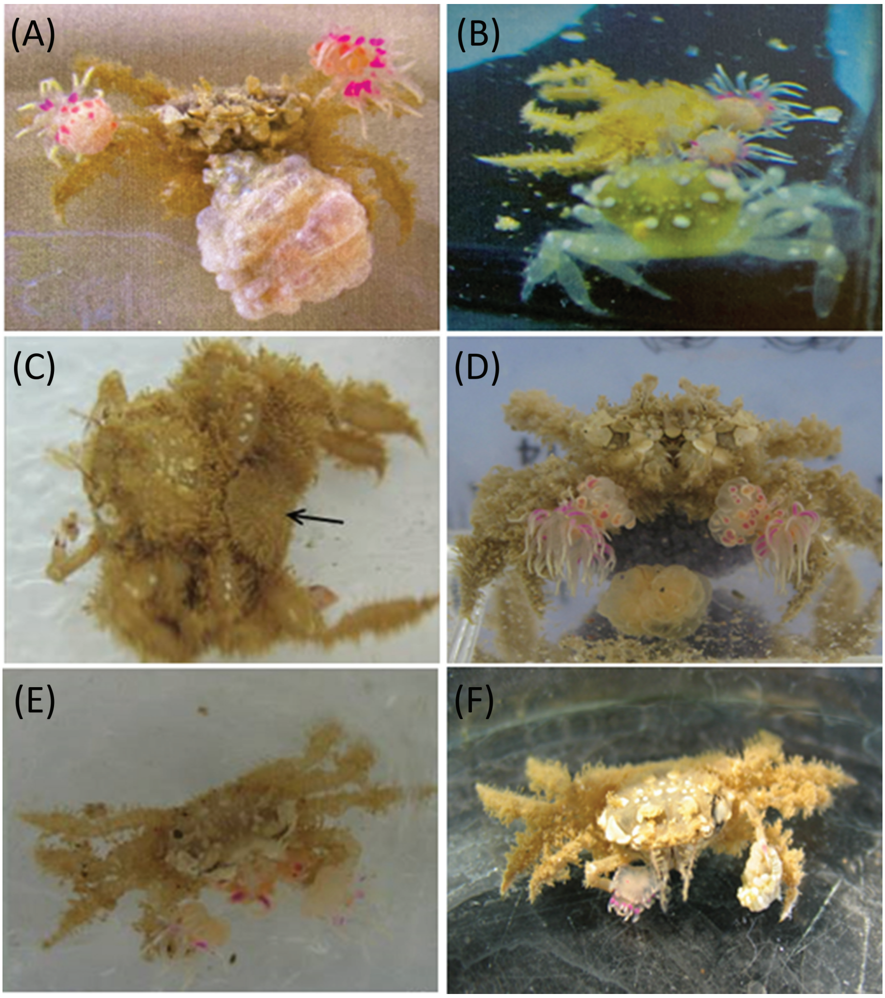

Figure 4 *Lybia leptochelis* and associated anemones. After Schnytzer (2008). (A) The crab is holding an *Alicia* sp. in each of its claws and retaining an *Anthopleura elatensis* with the aid of its walking legs. (B) Newly moulted crab in the front, behind is the moult with both anemones still secured in the claws. (C) Mating *Lybia leptochelis*. The black arrow points at the female abdomen. (D) Female crab with attached purple eggs holding two large anemones with a large *Anthopleura elatensis* in front of the crab. (E) A crab with four *Alicia* sp., two held in the claws and two retained with the walking legs. (F) *Lybia leptochelis* holding a typical *Alicia* sp. anemone in the right claw, and what appears to be a fragmented *Anthopleura elatensis* in the left claw.

Supplemental Video 1 *Lybia leptochelis* with a large *Anthopleura elatensis* kept between its walking legs. The crab is holding its *Alicia* sp. anemones at the sides, distanced from the *Anthopleura elatensis,* a behaviour typically observed when crabs are put together, presumably to prevent their theft or damage (Schnytzer 2008).

anemones (Figure 4A; Supplemental Video 1). About a quarter of all collected *Lybia* crabs were found retaining an additional anemone *Anthopleura elatensis* with the aid of their walking leg(s). Although the fourth walking leg was usually used to hold the anemone, occasionally, further walking legs were used, depending on the size of the anemone. The larger the anemone the more legs used to hold it. In the laboratory, this anemone was retained for several weeks at least. The most common 'additional anemone' was *Anthopleura elatensis,* but occasionally a crab was found retaining *Boloceroides mcmurrichii* and once a *Paractis paldella*. *Anthopleura elatensis* appeared also to be the most commonly found anemone in the crab's habitat occurring in clonal groups of four to six under rocks that are similar to those under which the crabs are found. The function of retaining these relatively large anemones by boxer crabs is unknown. Possible functions include chemical or physical camouflage, 'active' defence or perhaps they are holding a food reserve (Schnytzer 2008). Identifying anemones held by crabs using classical morphological techniques is both time-consuming and uncertain. There is a very large gap in our knowledge regarding the taxonomy of anemones associated with boxer crabs. Such a gap could be closed by the application of molecular techniques. Contrasting the DNA of a small symbiotic unknown anemone to that of a large, fully developed and identified non-associated anemone should provide the answer. This procedure was successfully applied by Giman (2008; see Appendix 3) and Crowther (2013) for the identification of *Triactis producta* held by several species of crabs.

Morphological adaptations for holding and wielding anemones

The claws of polydectine crabs are structured to efficiently hold live anemones, sea slugs or small holothurians. The chelipeds exhibit no sexual dimorphism. These chelipeds are slender with long fingers armed with spiniform recurved hooks on their cutting margins (Figure 2). The hooks are directed obliquely inwards and gradually increase in size from those near the tip of the finger to the proximal ones. The tips of both fingers are strongly curved downwards and upwards, respectively. The anemones are held tightly by the middle of the column below the tentacles, with the sharp hooks of the fingers dug into the anemone tissue. The anemones are usually held with the pedal disc facing up and the tentacles facing down. The shape of the chela and fingers and the number and shape of the hooks along the cutting margins are species specific for polydectine crabs (Figure 3) (Guinot 1976, Guinot & Wicksten 2015, Davie et al. 2015).

The adaptive and comparative morphology of boxer crabs has been studied extensively using light microscopy (Guinot 1976). The use of SEM (Scanning Electron Microscope) has never been employed to this end. Since the claws are occupied with holding anemones, boxer crabs must use their walking legs to perform behaviours such as food gathering (Duerden 1905), food theft (Schnytzer et al. 2013), intraspecific fighting (Karplus et al. 1998), anemone detachment (Duerden 1905) and even the carrying of additional anemones (Schnytzer 2008). These observations led to the hypothesis that the crabs may have sensory adaptations enabling them to feel if, when, how and where they are holding their symbiont and/or other anemones, as well as the collection or theft of food particles. A SEM study using three representatives of *Lybia*, male and female specimens of *Lybia leptochelis, Lybia tessellata* and *Lybia plumosa*, revealed that the claws, particularly on the inward-facing 'hooks' used for retaining anemones, and tips of the walking legs (Figure 5), particularly the first, have numerous pores on them. Two main types of pores were observed on the legs,

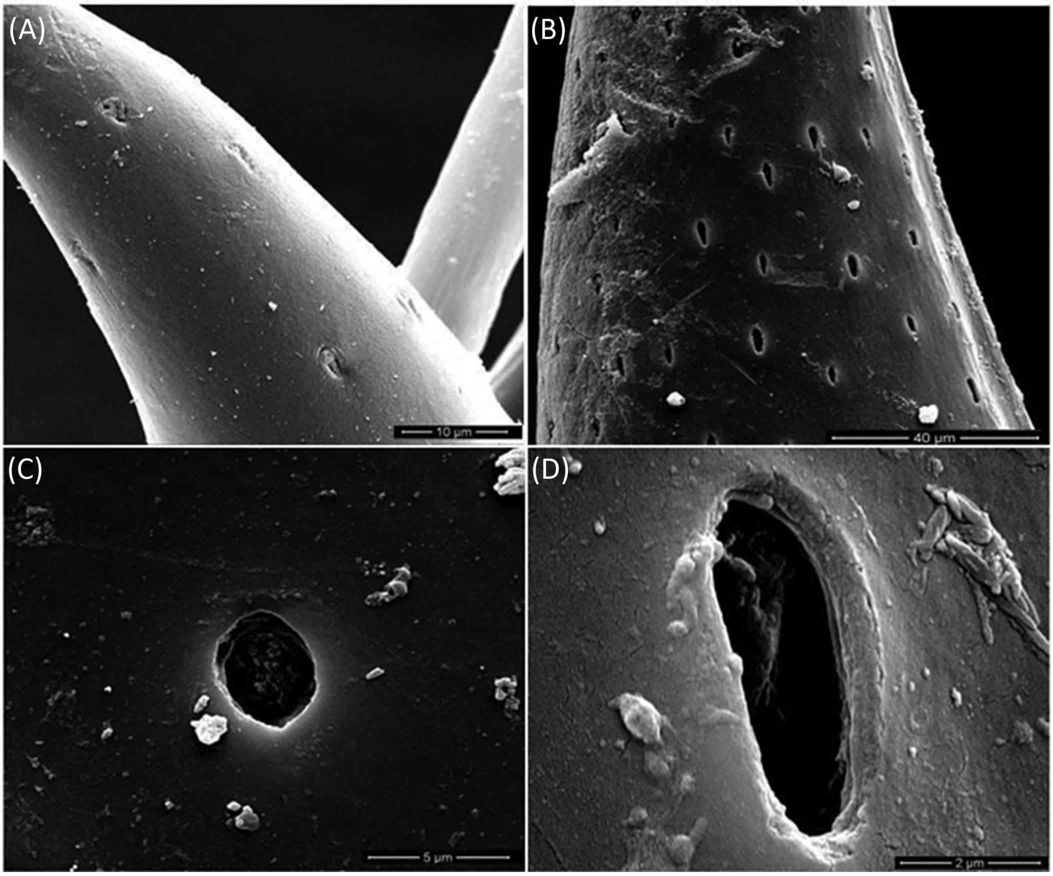

Figure 5 SEM images of possible *Lybia tessellata* sensory pores. (A) Tip of claw. (B) Tip of first walking leg. (C) Close-up of round-type pore. (D) Close-up of oblong-type pore. All images were taken from *Lybia tessellata*.

one rounder and one more oblong (Figure 5C and D), and one type of pore was observed on the claws. Similar structures, such as hair peg and campaniform organs, are known to have mechanosensory and chemosensory functions in other brachyuran crabs (Davie et al. 2015). Some variances in the number and distribution of pores were observed between the *Lybia* species tested. Although further work is required, *Lybia* crabs provide a promising model for studying the functioning of putative crustacean sensory organs in the performance of their unique behaviours (Schnytzer 2008).

The range of motion of each of the segments of crustacean chelipeds is usually restricted to a single plane. Each plane is typically positioned so that it is perpendicular to that of its neighbour (Davie et al. 2015). However, the propodal-carpal joint in polydectine crabs is different, allowing much more freedom of movement and precision (Guinot 1976). This capacity allows boxer crabs to precisely wield their anemones to target a source of disturbance, as described by Duerden (1905).

Outside the Polydectinae, *Diogenes edwardsii* is the only hermit crab which places an anemone, *Sagartia paguri,* on top of its claw, but without impending the grasping ability of its chelae. All other hermit crabs place their anemones atop of their shells. When *Diogenes edwardsii* withdraws into its shell, the anemone is pulled back into the aperture facing the outside. As the crab emerges from its shell the anemone comes out first, usually fully expanded (Ross 1975).

Measurements of field-collected *Lybia leptochelis* revealed a size range of maximal carapace width of adults between 4 and 13 mm (Schnytzer et al. 2017) and that of *Lybia edmondsoni* ranged between 10.5 and 14 mm (Karplus et al. 1998). *Alicia* sp. pedal disc diameters range between 0.5 and 3 mm. Within this range, there is a positive correlation between *Lybia leptochelis* carapace width and the mean pedal disc diameter of the associated anemone (Figure 6A) (Schnytzer et al. 2017). There appear to be no signs of handedness (Figure 6B). There seems to be an optimal anemone size for the crab to carry. The correlation between carapace width and anemone diameter is unlikely to be the result of acquiring the right size of anemone, but probably due to the crab regulating the size of anemone (Schnytzer et al. 2017). Overly large anemones may cause an excessive strain on the delicate propodal-carpal joint. *Polydectus cupulifer* is the only boxer crab which can handle anemones near its own size (e.g. *Teliopsis* sp.; Guinot 1976). According to Guinot (1976), the propodal-carpal joint of *Polydectus cupulifer* is different from that of other boxer crabs and has a more robust structure.

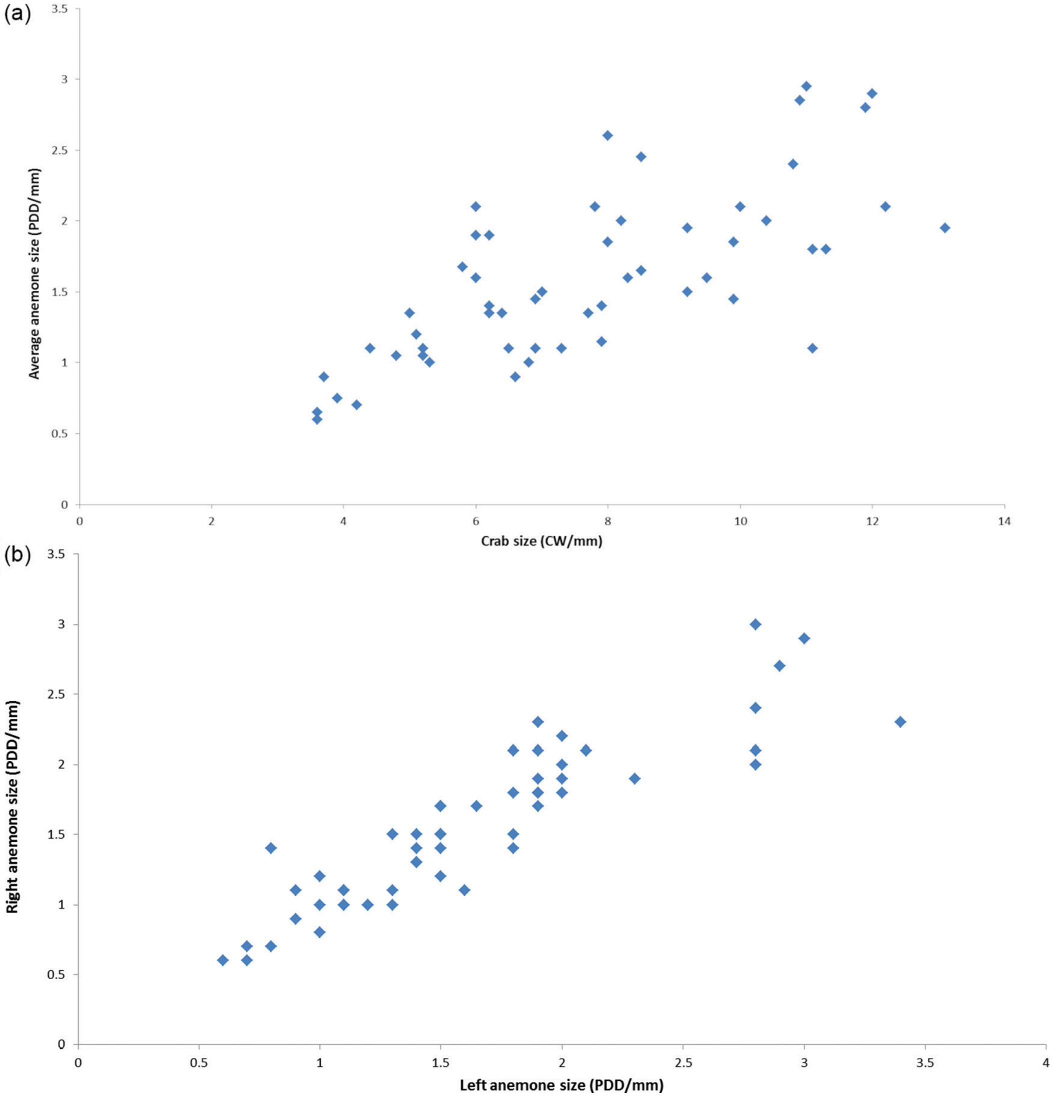

Figure 6 *Lybia leptochelis* and *Alicia* sp. size relationships. (a) Correlation between pedal disc diameter of held anemones (i.e. average of left and right anemones) and crab carapace width, as observed in nature. (b) Correlation between pedal disc diameter of left- and right-held anemones as observed in nature. CW– carapace width, PDD– pedal disc diameter. After Schnytzer et al. (2017).

Alterations in associated anemones

Anemones held by boxer crabs undergo with time alterations in size, structure and colouration in comparison with free-living specimens. As will be discussed in the following sections, unique behaviours such as food restriction, splitting (induction of asexual reproduction) and intraspecific theft of whole and fragmented anemones, all presumably contribute to these alterations and changes. *Triactis producta* held by *Lybia tessellata* also differ from free-living specimens by lacking column outgrowths (Figure 7). Anemones with outgrowths would render their handling difficult, thus the crabs either choose anemones that lack column outgrowths, or the crabs impede their formation (Crowther 2013). A further possibility is that the anemones held by the crab are too small for outgrowth development. *Triactis producta* held by *Lybia edmondsoni* became bleached

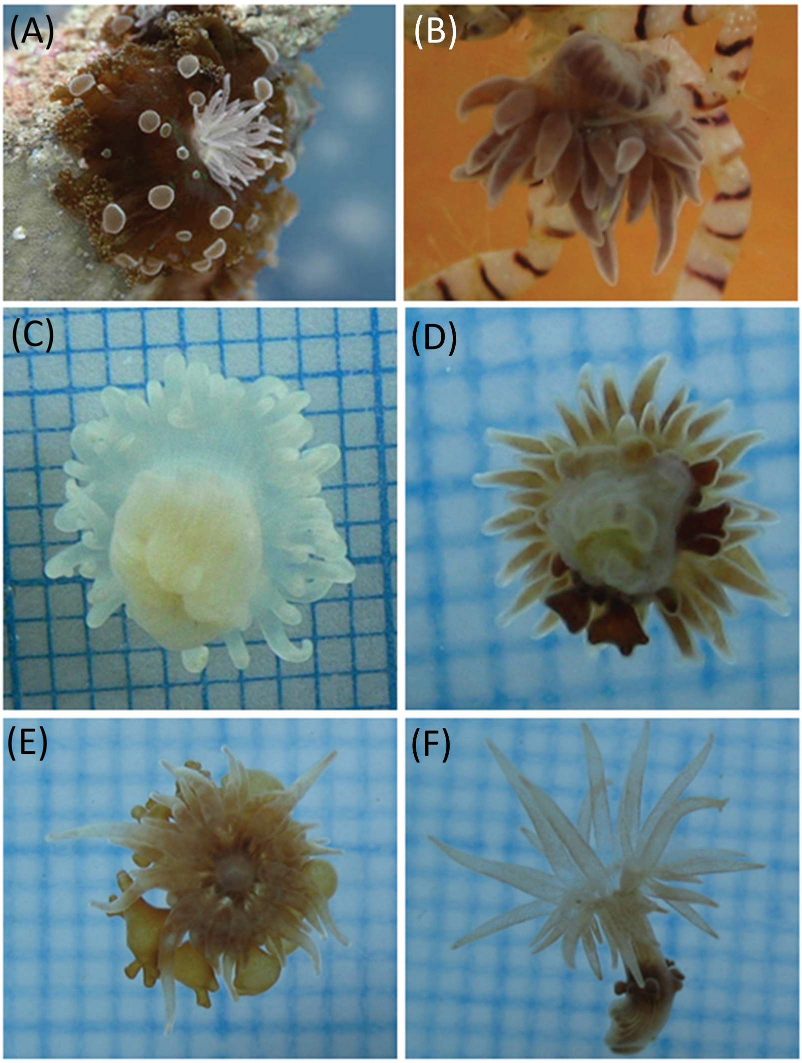

Figure 7 *Triactis producta* wild vs. crab held morphology. (A) Free-living *Triactis producta* in nature. (B) Crab held, with photosymbiotic zooxanthellae. (C) Bleached anemone, immediately after removal from crab. (D) Two weeks post-removal from crab, kept under LD lighting conditions. (E) Two months post-removal from crab – top view. (F) Two months post-removal from crab – side view.

(Figure 7C) due to loss of zooxanthellae because of the sheltering of the crab in the dark during the day. In comparison, free-living anemones are dark brown, except for their frosty white tips, due to thriving zooxanthellae exposed to sunlight (Figure 7A; Cutress 1977). Experimentally, a small whitish coloured *Triactis producta* lacking any outgrowths from the column was removed from *Lybia tessellata*. When fed and exposed to light, gradually this anemone increased in size, became darker and developed outgrowths of its column (Figure 7D-F; Yanagi and Iwao 2012). Furthermore, *Lybia edmondsoni* have been observed mouthing their held anemones, whereby the crabs will bring the anemones in contact with their mouth appendages. It was suggested that this activity affects the anemone structure, giving its pedal disc a cone shape, making it easier to handle (Karplus et al. 1998). Boxer crabs may also control the morphology of other invertebrates held in their claws. Nudibranchs (*Gymnodoris* sp.) held by *Tunebia hatagumoana* are small and have no visible gills or oral tentacles compared with free-ranging gymnodorids (Baba & Noda 1993). Baba & Noda (1993) note that this species of *Gymnodoris* most closely resembles *Gymnodoris citrina*; however, they were unable to confirm this due to the structural changes possibly due to being held by the crab.

Crab and anemone activity rhythms

The activity rhythm of boxer crabs in the field has been briefly described based on limited observations. *Lybia* crabs presumably spend the days hidden in crevices and under stones in shallow tropical reefs, whereas at night they emerge from their shelters to scavenge (Crowther 2011). *Lybia edmondsoni* was observed during night dives by Debelius (1984) in the stony infra littoral of Hawaii. The crabs were active moving on top of rocks as long as they were not targeted by the underwater flashlight causing them to freeze.

Triactis producta, a common associate of *Lybia tessellata and Lybia edmondsoni,* has a diurnal activity rhythm. This anemone is associated with photosynthetic zooxanthellae and gains most of its nutrition from its symbionts. *Triactis producta* is attached to firm substrate such as dead or live scleractinian corals (particularly *Porites* and *Acropora*) and rocks at depths of 1–15 m in areas of strong light penetration (Levi et al. 1970).

The activity rhythm of *Lybia leptochelis* associated with *Alicia* sp. was studied in the laboratory under controlled light conditions (Schnytzer 2008). *Lybia leptochelis,* with and without anemones, were maintained in isolation in small aquaria, each equipped with a shelter (see Schnytzer et al. 2013). The crabs displayed a distinct nocturnal activity rhythm. They spent about 80% of the time inside the shelter during the day and about 20% at night. Time spent in locomotion was relatively high at night and reduced while inside the shelter during the day. Minor grooming (i.e. grooming with the third maxilliped) occurred both inside and outside the shelter whereas major grooming (i.e. grooming with the chelipeds, involving the temporary removal of an anemone from the claw) occurred only inside the shelter. Major and minor grooming occurred during both day and night. The same activity patterns were displayed by crabs with anemones and after the anemones had been removed. In the case of *Lybia* crabs associated with *Triactis producta* (e.g. *Lybia edmondsoni* and *Lybia tessellata*), the presumed nocturnal activity of the crabs probably enforces its own activity rhythm on that of the anemone, causing it to bleach due to loss of zooxanthellae. That said, previous work on bleached *Entacmaea quadricolor*, a common host of symbiotic clown fish, has shown that they do not lose their ability to deploy toxic nematocysts when bleached (Hoepner et al. 2019).

Boxer crab and anemone feeding and growth

Feeding in anemones is diverse, ranging from complete metabolic dependence on symbiotic zooxanthellae to predation of benthic and planktonic organisms, suspension feeding and a combination of some of the above (Schick 1991). *Triactis producta* and *Alicia* sp. are both associated with boxer crabs but differ in their feeding habits. Aquarium studies revealed that *Triactis producta* largely depends on photosynthetic algae embedded in its tissues. *Daphnia* and *Artemia* feed is usually refused in the aquarium. Glutathione has been found to produce a feeding response in most anemones but fails to do so in *Triactis producta* (Levi et al. 1970).

Alicia sp. lacking zooxanthellae, as revealed by Pulse Amplitude Modulation (PAM) fluorometry measurements, epifluorescent microscope observations and histological work, depend largely on predation and scavenging (Schnytzer 2008, Schnytzer et al. 2013).

Boxer crabs use their associated anemones in three different strategies to facilitate feeding:

A. *Stunning of prey. Lybia tessellata* and *Lybia leptochelis* crabs in aquaria attacked live small fishes and amphipods using their anemones to stun and immobilize them. Elusive prey could then be captured and consumed (Schmitt 1965). By contrast, *Lybia leptochelis* holding *Alicia* sp. in the laboratory did not use its anemones for stunning large prey (Supplemental Video 3; Schnytzer et al. 2013). That being said, when presented with live brine shrimp, however, the shrimp will adhere to the *Alicia* sp. tentacles and cease to move shortly thereafter. Indeed, free-living *Triactis producta* colonies are known to cause serious stings even in humans (Levi et al. 1970), whereas *Alicia* sp. that were kept in the lab and held on many occasions caused no apparent discomfort (Schnytzer et al. 2013).

B. *Anemones used to collect food*. Duerden (1905) reported in his classical study on the crab–anemone feeding reactions that *Lybia edmondsoni* robbed food from their held anemones *Bunodeopsis* sp. and *Sagartia* sp. He observed that the crabs perform a 'mopping' action with the anemones using them as tools for gathering food. The crabs were described removing food particles adhering to the walls of the polyp (Supplemental Video 2). Pieces of meat attached to the anemone oral disc were removed by the crab with their first pair of walking legs. In cases that the food fragments have already been swallowed by the polyp, the crab extracted the food from the stomodeum of the anemone. The crab might have detected the presence of food on the anemone by movements of the anemone during ingestion and or due to stimulation by meat juices emanating from the food particles.

C. *Anemones distanced from food*. *Lybia leptochelis* associated with *Alicia* sp. attempts to reduce the amount of food ingested by the anemones by distancing the held anemones from the food and removing any food captured by the anemones (see the following section for further details; Supplemental Video 3; Schnytzer et al. 2013). This is of course in contrast to the more active use of anemones exhibited by *Lybia tessellata* and *Lybia edmondsoni*.

Supplemental Video 2 *Lybia tessellata* holding out its *Triactis producta* to capture food (dead *Artemia*), then proceeds to remove particles from anemone tentacles (Schnytzer 2008).

Supplemental Video 3 *Lybia leptochelis* holding its *Alicia sp.* anemones away from presented food (dead *Artemia*). Any food particles that are caught by the anemone are then removed by the crab for its consumption (Schnytzer et al. 2013).

Only one study has systematically assessed the impact of lone and crab-held anemone feeding and growth (Schnytzer et al. 2013). This study included four experimental groups. The first group included fed *Lybia leptochelis* holding *Alicia* sp. anemones which shrunk significantly over the course of the experiment. The crabs reduced the amount of food ingested by the anemones by distancing them and removing food captured by the anemone. The successful removal of food by the crab was due to their much more rapid response to the food compared with the response of the anemones. The second experimental group consisted of fed crabs without anemones and made almost no use of their vacant claws. If a crab would try to use its modified chelae to grasp and lift an *Artemia* they failed in their attempts. However, they were successful, as were the anemone-bearing animals, in pushing and shoving presented food into their mouths with the first pair of walking legs. No growth was recorded for the crabs since none of them moulted during the study.

Experimental group three consisted of anemones that were ‘freed’ from the crabs ingested up to four *Artemia* per feeding session (Figure 8A; Supplemental Video 4) and ate about 8 times more than the crab-held anemones. These anemones underwent remarkable changes in morphology, colour and size, with a 250% expansion in pedal disc diameter. Experimental group four consisted of ‘free’ starved anemones shrunk in size until they disappeared altogether, probably due to lack of zooxanthellae, being dependent on predation for survival (Figure 8B). The relationship between *Lybia leptochelis* and *Alicia* sp. is an extreme example of a kleptoparasitic interaction. *Lybia* preserves the minute size of its associated anemones by food reduction much as gardeners produce ‘Bonsai’ trees (Figure 8C). The boxer crab thus maintains a ‘Bonsai’ symbiont that is conveniently carried around as a tool to trap its food and provide protection.

Boxer crab reproduction

No information is available on the breeding and larval culture of boxer crabs (Calado et al. 2003, Calado 2020). The demand for boxer crabs and particularly *Lybia tessellata*, *Lybia edmondsoni* and *Polydectus cupulifer* in the ornamental crustacean trade is supplied exclusively by capture of crabs in the field, damaging and depleting natural populations.

Our limited knowledge of the reproductive biology of *Lybia leptochelis* has been accumulated partly by chance as a by-product of other projects. While collecting boxer crabs, we noticed that during the winter months only a single crab was found under a rock, whereas, during the summer on many occasions two, and sometimes even more crabs, were found under the same rock. On two such occasions both crabs were collected and found to be a male and a female. On one such occasion the pair proceeded to mate shortly after being brought to the laboratory. As they were to be used for a separate study, the anemones were removed from the crabs, leaving only small fragments in their claws. Within several minutes the crabs encountered one another. Both crabs held their claws away from the opposing crab, while their bodies and appendages were in close contact. Several minutes later the male lifted its pleon from his cephalothorax, whereas about 20 minutes later the female lifted her broad and round abdomen exposing the pleopods. The crabs remained copulated for about two and a half hours (Figure 4C) and subsequently disengaged. Approximately 2 weeks following the mating, the female laid a grey egg clutch of several hundred eggs which gradually turned purple over the course of about 2 weeks. *Lybia leptochelis* females hold significantly larger anemones than males (Schnytzer et al. 2017). Possibly the larger size of their anemones contributes additional protection to the eggs attached to the female pleopods (Figure 4D). Hatching of zoea larvae was observed several times in the laboratory. The larval hatching is assisted by the female leading to larval dispersal. The morphology of zoea 1 of *Lybia plumosa* was described by Clark and Paula (2003), which also predicted this species has four different zoeal stages, typical for other related xanthids. There seems not to be a strict ‘high season’ of settling since ovigerous females were seen all year round at the collection sites except in the middle of the winter, with small individuals found throughout the year (unpublished data).

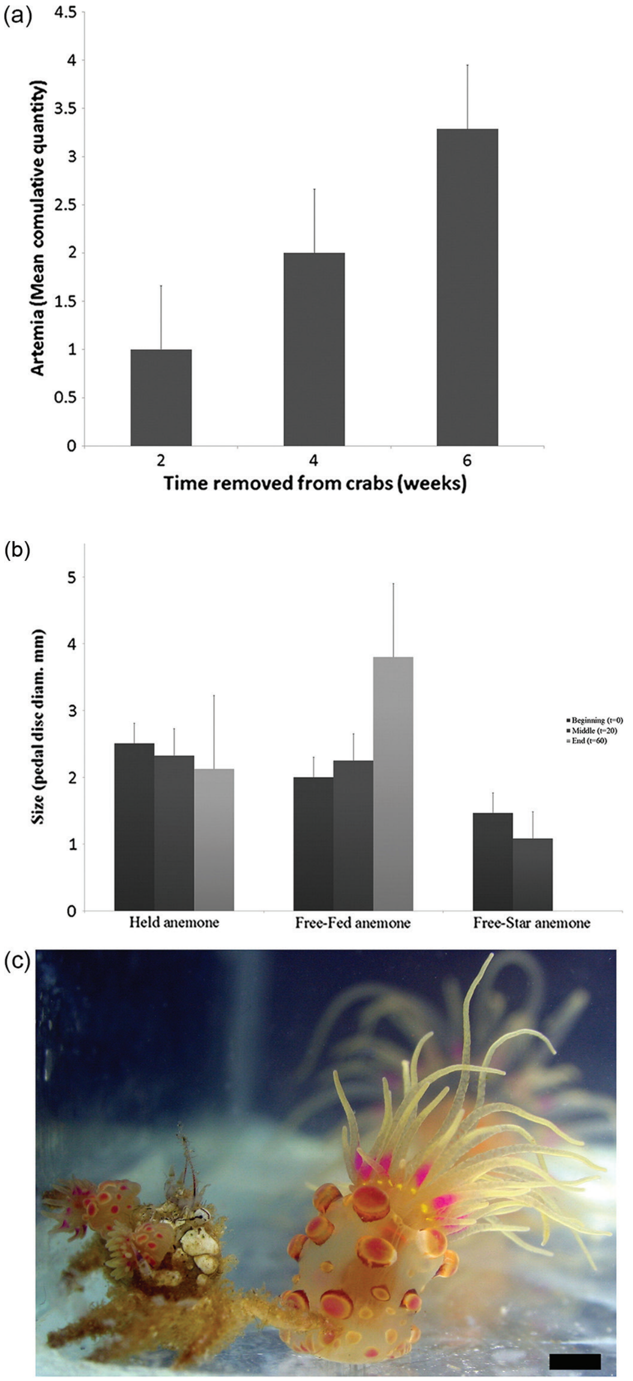

Figure 8 Growth of *Alicia* sp. while being held or removed from *Lybia leptochelis*. (a) The quantity of *Artemia* consumed by free *Alicia* sp. anemones per feeding trial following 2, 4 and 6 weeks after removal from the crab. (b) Anemone pedal disc diameter before (0 days), during (20 days) and after (60 days) the feeding experiment in anemones held by crabs, free anemones fed to satiation and starved anemones. (c) *Lybia leptochelis* holding a small *Alicia* sp. anemone in each of its claws (left side) and a large *Alicia* sp. grown free from the crabs for 3 months (right side). Scale bar = 2 mm.

Supplemental Video 4 Lone *Alicia sp.* presented with dead *Artemia* and feeding (Schnytzer 2008).

Anemone location by boxer crabs

According to Duerden (1905), *Lybia edmondsoni* is not able to locate its associated anemones from a distance. He observed the crabs apparently moving aimlessly in the proximity of the anemone (i.e. *Bunodeopsis* sp. and *Sagartia* sp.) and only after coming in physical contact with the anemone did the crab seize it.

In contrast to Duerden (1905), in a recent study (Schnytzer 2008) *Lybia leptochelis* was found both visually and chemically attracted to its associated anemone *Alicia* sp. (Supplemental Video 5). In a tank in which anemones were in a cell behind a transparent partition, both crabs with and without anemones were attracted to the anemones, spending significantly more time close to a cell containing sea anemones, in comparison with the cell without anemones.

Chemical attraction of the crabs to the anemones was tested in an elongated aquarium that contained two permeable black nylon bags, one with anemones and one left empty, that were placed at either end. A significant majority of the tested crabs, both with and without anemones, were attached to the sacs that contained the anemones. Some crabs were even found to have torn the bag open and were holding two anemones. A similar experiment was carried out with *Anthopleura elatensis*, the anemone that is often carried by *Lybia leptochelis*. Once again, the crabs with and without anemones were chemically attracted to this anemone. Many of the questions concerning the chemical attraction of *Lybia leptochelis* such as the chemical nature of the anemone compound that attracts the crab and the specificity of the chemical attraction are still unknown. The differences in the positive findings of Schnytzer (2008) on the optical and chemical attraction in *Lybia leptochelis* to the lack of such attraction in *Lybia edmondsoni* as described by Duerden (1905) may be due to differences in the tested species or differences in the testing procedures, being more quantitative in the former and of a general descriptive nature in the latter.

A comparison with the study on the chemical attraction of the hermit crab *Dardanus venosus* to its associated anemone *Calliactis tricolor* by Brooks (1991) is of interest. In contrast to *Lybia*, hermit crabs were only attracted to anemones if they had no anemone on their shell. It was suggested that when carrying an anemone possibly all their chemoreceptors were saturated with specific molecules resulting in random movement of the crab with regard to the anemone. The chemical attraction of *Lybia leptochelis* was the same irrespective whether it held anemones. In preliminary observations, we found that *Lybia leptochelis* and *Lybia tessellata* will replace a small anemone with a larger one when available, perhaps explaining their attraction to anemones even when holding them.

Initiation of the partnership

How young boxer crabs initially find anemones is largely unknown. No one has documented acquisition in the wild by any polydectine. Our group as well as others have observed in the laboratory the hatching of *Lybia* crabs and vertical transfer, i.e. anemones passing from parent to larvae, does not appear to occur following the observation of many hatching events in the lab. Furthermore, the zoea lack the claws or other appendages to hold the anemones during their initial stages post-hatching (Clark & Paula 2003, Schnytzer 2008). It is perhaps easier to understand the initiation of the partnerships between *Triactis producta* and its associated boxer crabs such as *Lybia tessellata* and *Lybia edmondsoni* as they have been observed in close proximity in the wild. This anemone is by far the most common associate of boxer crabs, has a wide Indo-West Pacific distribution and occurs in well illuminated shallow water often in rock crevices or vertices of branching scleractinian corals

(Fishelson 1970). Large specimens of *Triactis producta* are often surrounded by smaller individuals, possibly the product of asexual reproduction (Crowther 2013). *Lybia tessellata* megalopa larvae may be chemically attracted to the large colonies of *Triactis producta*. A newly settled crab could presumably remove the small anemones located at the base of the large anemones with their maxillipeds and first walking legs. Subsequently the crabs with their newly acquired anemones would move into shallow water. It is important to note that various *Lybia* take part in intraspecific anemone theft and anemone splitting, the relative importance of these behaviours in *Lybia* anemone acquisition is unknown.

With regard to *Lybia leptochelis*, the most pressing question is whether the crab-held *Alicia sp.* also occur free-living or they are perhaps obligate symbionts of the crab only occurring in association. A limited histological study on the morphology of *Alicia* sp. taken from *Lybia leptochelis* indicated that they may be an undescribed species of *Alicia* (Fautin & Crowther pers. comm.). Furthermore, very small crabs (i.e. 2–3 mm c.w.) already possess two tiny anemones which indicates that the association between boxer crabs and anemones is formed very early in the crab's life cycle. We have searched for free-living *Alicia* sp. in the vicinity of the habitat of *Lybia leptochelis* without success. *Alicia* sp. is hard to miss since it is very conspicuous, having bright violet spots forming a ring at the base of the tentacles, a vibrant orange coloured mouth and orange-red coloured outgrowths around its column and base. However, anemones living in fissures and crevices are hard to detect, more so when such predatory anemones, such as *Alicia* sp., are nocturnal (Kruzic et al. 2002, Katsanevakis & Thessalou-Legaki 2007) and are presumably contracted during the day. We may cautiously assume that anemone splitting, and theft are part of the crab–anemone acquisition mechanism in nature. This is supported by the genetic identity of anemone pairs held by individual crabs collected at sea, as well as the size similarity of left- and right-held anemones (Schnytzer et al. 2017). However, it is reasonable to assume that splitting and theft are not the full picture of the acquisition mechanism. The discovery of free-living *Alicia* sp. will allow to investigate their genetic structure and the role they play in the initiation of these partnerships.

Mechanism of anemone acquisition and temporary release

Removal of sea anemones from substrate

The removal of symbiotic anemones attached with their pedal disc to the substrate has been described in detail in two species of boxer crabs, *Lybia edmondsoni* (Duerden 1905) and *Lybia leptochelis* (Supplemental Video 5; Schnytzer 2008). In both species, the anemone removal progressed in three phases: probing, detachment and readjustment. In the probing stage, the crab touched the anemone stalk and pedal disc with its first walking legs and third maxillipeds, administrating tactile stimulation to the anemone which leads to its relaxation. In the detachment stage the crab introduces its first walking legs under the pedal disc, loosening the anemone attachment (Figure 1B). In the event the crab encounters a large anemone it may make use of additional walking legs to aid in the detachment and acquisition of the anemone. During the probing and detachment stages, the crab claws, whether empty or holding anemones, were not involved. Only after detachment was completed did the crab use its chelae to grasp the anemone. The chelae seized the anemone in any position initially. However, to achieve what we perceive as the 'optimal holding position' the anemone was temporary

Supplemental Video 5 *Lybia leptochelis* without anemones (previously removed) grabbing newly presented *Alicia sp.* anemone (Schnytzer 2008).

held with the third maxillipeds and first walking legs. Finally, the anemone was re-grasped by the chela at the middle of the stalk with the oral disc facing down.

The process of anemone removal from the substrate and its placement on a hermit crab shell has been studied in great detail. Special attention was given to the active vs. passive role filled by the crab and anemone, respectively, and the particular mechanism employed by different pairs of associated hermit crab–anemone species in the initiation phase of the association (Ross & Sutton 1961, 1968, Ross 1967, 1974). The comparison between boxer and hermit crabs in relationship with symbiotic anemones during the probing and detachment stages is of interest. Unlike hermit crabs that make use of the claws and walking legs during the probing and detachment stages, boxer crabs do not use their claws but only their first walking legs and maxillipeds even when they are not holding anemones and could potentially make use of their claws. The tactile stimulation by hermit crabs has been experimentally replaced by tactile stimulation of the anemones with rods as well as by an electric current (Ross & Sutton 1968).

Theft of anemones

The first record of anemone theft by boxer crabs was reported by Karplus et al. (1998) observing *Lybia edmondsoni* holding *Triactis producta*. Schnytzer et al. (2017) demonstrated that anemone theft was a frequently practiced behaviour among *Lybia leptochelis* associated with *Alicia* sp. The interactions between pairs of crabs matched in size and sex, each consisting of one individual with two anemones and the other without anemones were tested. In 73% of the encounters intense fighting occurred culminating in anemone theft. Crabs of both genders, with or without anemones, were equally likely to initiate a fight. The time from encountering one another until the start of fighting was not affected by crab gender or whether the initiator was deprived or possessed an anemone. Fight duration was variable and was not related to gender or contest outcome (i.e. removal of an entire anemone or of a fragment).

In a typical contest, the individuals first approached one another. The crab with the anemones held its anemones at a distance away from the other (Figure 9A). Next, the initiator gently touched the other crab with the tip of its first walking leg for about a minute (Supplemental Video 6; Figure 7B). Following this gentle leg contact the two crabs typically proceeded to a back-to-back configuration (Figure 9C). Following this, the crabs rapidly locked their walking legs and commenced a close physical struggle (Figure 9D), grasping one another with their legs forming a tight ball (Figure 9E). During these phases, both crabs distanced their claws (either holding anemones or vacant) as far as possible from the other (Figure 9E). Next, the crab without anemones strived to move into a dominant position, typically on top of the crab holding anemones. The crab without anemones then tried to hold one of the opposing crab's claws and to lock it with the aid of its walking legs. No use was made of its unoccupied delicate claws. Upon achieving a claw lock of the opposing crab, the crab without anemones tried to remove the anemone held by the other crab using its first walking leg to pry at the claw holding the sea anemone. After it has been pried open sufficiently, the attacking crab for the first time used its vacant claw to take hold of the anemone (Figure 9G). Sometimes, an entire anemone was taken and sometimes only a fragment was torn off. Never was a contest witnessed with two whole anemones removed. Typically, after a whole anemone or a fragment was stolen, the contest was over (Figure 9H). No crabs were injured or killed in fights. In about a half of the contests an entire anemone was stolen, in a third an anemone fragment was taken, occasionally the crab stole two fragments. Splitting (see the following section) occurred in all cases that a single anemone or fragment were stolen. Presumably splitting is not induced when both claws are occupied. Contests were staged between very small juveniles deprived of their anemones and fully grown crabs with anemones. In all cases it was the small crab which initiated the fight and, in all instances, managed to come away with a fragment or a full anemone.

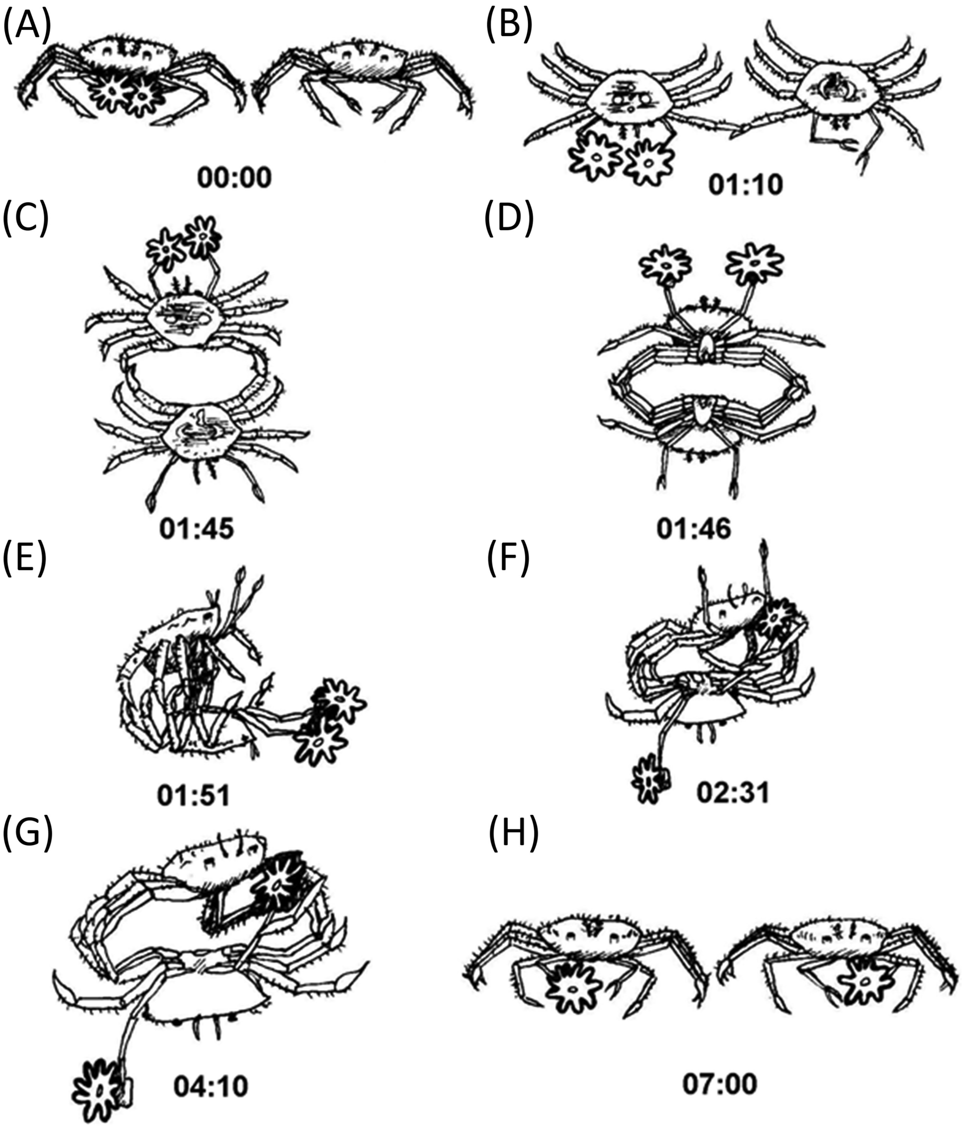

Figure 9 Sequence of typical anemone theft by *Lybia leptochelis*. Line drawings from video. (A) The crab with anemones holds them at a distance away from the other crab. (B) The initiator gently touches the other crab with the tip of its first walking leg for about a minute. (C) The crabs then typically proceeded to move into a back-to-back configuration. (D) The crabs then rapidly lock their walking legs and commence a close struggle grasping one another with their legs forming a tight ball. Note that during these phases both crabs distanced their claws (holding anemones or vacant) as far as possible from the other. (E) The crab without anemones strives to move into a dominant position, typically on top of the crab holding anemones. (F) The crab without anemones then tries to hold one of the opposing crab's claws and lock it with the aid of its walking legs. No use was made of its unoccupied delicate claws. (G) Once a claw lock of the opposing crab without anemones is acheived, it proceeds to remove the anemone held by the other crab. At first, it made use of its first walking leg to pry at the claw holding the anemone. After it has been pried open sufficiently, the attacking crab for the first time used its vacant claw to take hold of the anemone. (H) The crabs post contest holding a whole or fragmented anemone. Time presented in mm:ss. See text for elaboration of theft sequence. After Schnytzer et al. (2017).

Supplemental Video 6 *Lybia leptochelis* anemone theft. Two equally sized crabs are placed together, one with and one without anemones. The crab without anemones then proceeds to steal a large fragment (Schnytzer et al. 2017).

Supplemental Video 7 *Lybia leptochelis* anemone theft. Disproportionate matchup between small crab without anemones and a large crab with anemones. The small crab proceeds to steal an anemone fragment (sped up X2; Schnytzer et al. 2017).

In contrast to boxer crabs, very little intraspecific stealing of anemones was demonstrated for three species of Mediterranean hermit crabs, *Dardanus arrosor, Paguristes oculatus* and *Pagurus alatus* (Ross 1979). The rare intraspecific stealing was not immediate, almost never seen and was suggested to be due to some inhibiting influence deterring stealing from conspecifics. In contrast, stealing of anemones from conspecifics was very common among boxer crab, in line with the crucial role of the anemone in the boxer crab life. Moreover, in Giraud's (2011) study, only large *Dardanus pedunculatus* removed the anemone *Calliactis tricolor* from small conspecifics, whereas the reverse never occurred. In contrast, small boxer crabs removed anemones from conspecifics irrespective of the existing size difference, again indicating the importance of the anemone to the boxer crab (Supplemental Video 7).

Splitting

Duerden (1905) and Karplus et al. (1998) reported that *Lybia edmondsoni* may split its associated anemone *Triactis producta* into two fragments that later regenerate. Splitting was further studied in *Lybia leptochelis* associated with *Alicia* sp. demonstrating that splitting is common in this species and has implications for both the initiation of the anemone–boxer crab partnership as well as the genetic structure of associated anemone populations (Schnytzer et al. 2017). *Lybia leptochelis* of both genders that had either their left or right anemone removed were monitored. Splitting was a highly significant response performed by the crabs in 77% of the trials. The torn anemone fragments typically had less than a 10% size difference. The complete splitting process was observed several times (Figure 10; Supplemental Video 8). Splitting started with the crab holding the anemone with its claw, across the column, with the pedal disc facing upwards. The crab then took hold of the anemone with its free claw, thus holding the anemone in the aforementioned conformation between both claws. Next, the crab slowly began stretching the anemone between both claws in an outward motion, utilizing its front walking legs to surgically tear the anemone in half. Occasionally, the crab momentarily ceased the stretching to re-grasp the anemone in a central position as far as possible, so that the final splitting will produce two equal parts. Once the anemone was split into two, the remaining strands of connecting tissue were cut by the front walking legs. All split anemones fully regenerated within 4–7 days.

To establish whether splitting is a common occurrence in the wild, the genetic relatedness of anemone pairs taken from wild-caught crabs was tested. Anemones removed from freshly collected crabs were used for DNA analysis employing AFLP (Amplified Fragments Length Polymorphism; Amar et al. 2008, Douek et al. 2011, Brazeau et al. 2013). Pairs of anemones from a given crab were genetically identical. Furthermore, there was genetic identity between most pairs of anemones held by different crabs, with only three genotypes showing slight genetic differences. The resemblance of

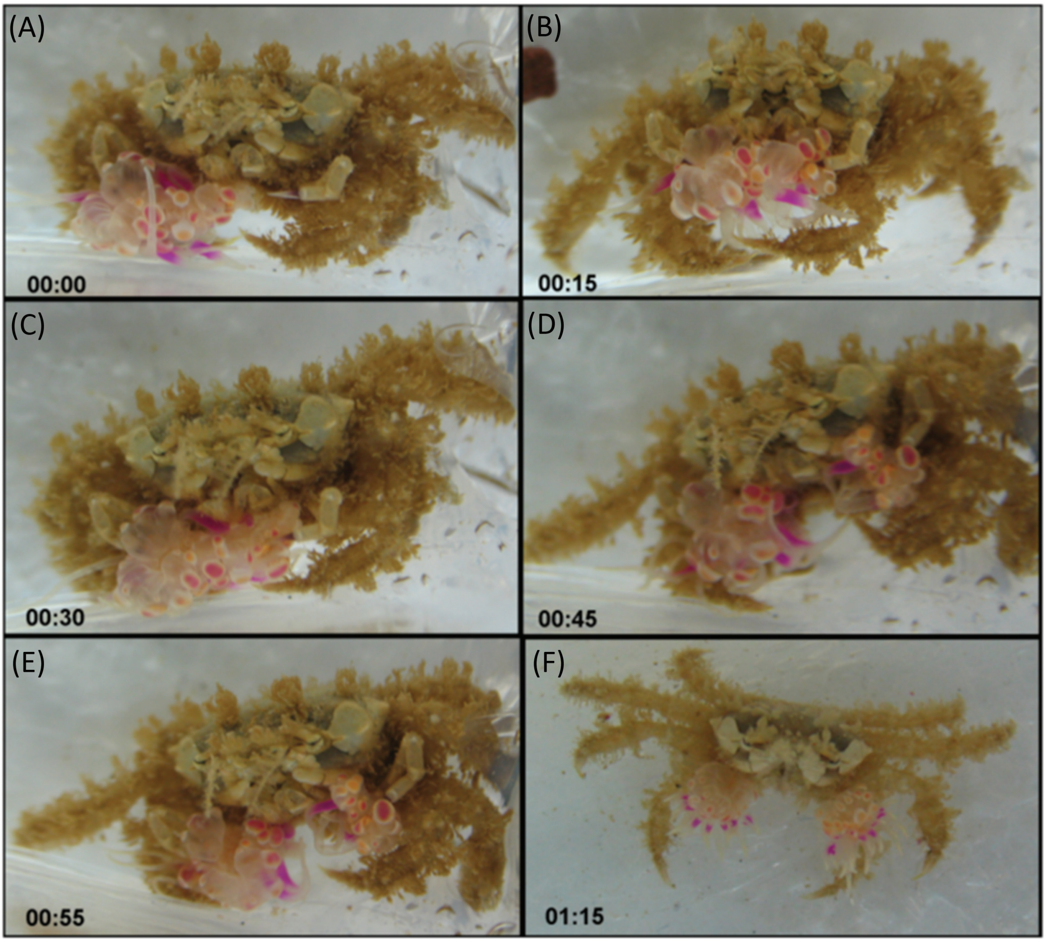

Figure 10 Sequence of anemone splitting by *Lybia leptochelis*. Time presented in hh:mm format. (A) The crab holding an anemone in one claw; the second is vacant. (B) Typical anemone splitting conformation with pedal disc up and oral disc down. (C) Stretching of anemone between both claws and use of front walking legs to tear it down the middle. (D) Tearing of anemone into two. (E) Final strand of anemone tissue are cut with front walking legs. (F) The crab holding two identical clones of the original anemone. After Schnytzer et al. (2017).

Supplemental Video 8 *Lybia leptochelis* splitting *Alicia* sp. (sped up X16; Schnytzer et al. 2017).

the DNA profiles between the three anemone groups is probably the result of asexual reproduction not involving the crab (Giman 2008). Measurement of the pedal disc diameter of the anemones held in the left and right claw of a crab revealed a highly significant size correlation (Figure 6B). This size correlation between anemone pairs from wild-caught crabs further support the notion of widespread splitting in natural populations of *Alicia* sp. anemones. This is a unique case in which one animal induces asexual reproduction of another, consequently also affecting its genetic diversity.

The phenomenon of splitting anemones appears to be unique to boxer crabs as it has not been documented among hermit crabs or any other crustacean associated with anemones.

Temporary release of anemones – moulting

Verril (1928), in his study of Hawaiian shallow water anthozoans, stated that when boxer crabs shed their shells they must be 'intelligent' enough to remove and transplant the actinian to their new claw, but he doubts that this operation has been seen. Jeng (1994) suggested that *Lybia tessellata* when moulting deliberately release the anemones, setting them aside until the new shell hardens, then retaking them. A different sequence of events has been observed in *Lybia leptochelis*. While keeping this species in captivity over several years, we frequently witnessed moulting in *Lybia leptochelis* (Supplemental Video 9; Schnytzer 2008). Sexually mature adult crabs moult about once every 2–3 months, during both day and night. The crabs withdrew from their old exoskeleton leaving the anemones held in the claws of the exuvia (Figure 4B). After a short period of time, an hour at most, from when the crab had fully moulted it would move rapidly towards the moult and within an hour take back its anemones one at a time. Great care was taken by the crab to remove the anemones complete and unharmed, each to its original claw. For approximately 30 minutes after retaking its anemones the crab would exhibit acclimatization signs in the form of claw 'twitching' (see section on 'Boxer crab immunity'). In contrast to boxer crabs which retake their original anemones after moulting, the spider crab *Inachus phalangium* (Inachidae) switches to a new host following each moult. This crab leaves its relatively large host *Anemonia sulcata* prior to moulting and following its night-time moulting will move to another nearby anemone (Wirtz & Diesel 1983, Landmann et al. 2016).

Temporary release of anemones – grooming

Many decapod crustaceans spend considerable amounts of time grooming, with their claws in many cases playing an important role (Bauer 1981). Highly conserved grooming behaviours were observed to take place in *Lybia leptochelis* and *Lybia tessellata* and have two distinct forms of grooming, defined as major and minor (Schnytzer 2008). Major grooming was only performed inside the shelter (Supplemental Video 10). During which the crab held one of their anemones in a raised position, facing the entrance of the shelter. The second anemone on the inner side of the shelter was held down on the substrate, and in a highly conserved sequence, the anemone was released from the claw and held tightly by the first walking leg on the same side as the claw. The tip of the leg was pressed against the pedal disc of the anemone and the mouth and tentacular region were folded between the joints of the leg. The crab then proceeded to move the free claw towards the mouth appendages and used the vacant claw to brush over its antennules, antennae, eyes and

Supplemental Video 9 *Lybia leptochelis* moulting and retaking *Alicia* sp. anemones (video shortened for convenience; Schnytzer et al. 2017).

Supplemental Video 10 *Lybia leptochelis* performing major grooming. *Lybia leptochelis* in shelter, releasing one anemone, holding it with walking legs and then proceeding to clean claw and facial appendages before retaking the anemone (Schnytzer 2008).

general frontal region. It repeated this process several times. The crab then retakes the anemone in its claws. The retaking of the anemone can take some time, the crab would rotate it vertically and horizontally until it was in the correct conformation. The anemones were almost always held across the middle of the column with the pedal disc facing up and the mouth region facing down. On some occasions, the crab moved to the other side of the shelter and proceed to remove the other anemone and implement the same sequence of actions. The unique aspect of major grooming is that the claw function of holding the anemones was temporarily abandoned in favour of grooming. A transition from holding to any other activity (e.g. feeding, fighting or anemone detachment) was not observed in boxer crabs, including minor grooming which only involves grooming with the third maxilliped without anemone removal.

Anemone swapping

The swapping of anemones has been described for two species of boxer crabs, *Lybia edmondsoni* (see Duerden 1905) and *Lybia leptochelis* (see Schnytzer 2008). Duerden (1905) twice observed a crab holding in one claw an intact *Bundeopsis* sp. and a fragment in the other, replacing the fragment with an intact anemone. The fragment was once replaced by an intact *Bunodeopsis* and once by an intact *Sagartia*. After dropping the fragment, the crab cleaned its free chela before grabbing the intact anemone. *Lybia leptochelis* holding two intact *Alicia* sp. in their claws were exposed to a single *Alicia* sp. larger as well as smaller than those held in their claws. Anemones larger than those carried by the crab were swapped following their examination by the crab with its first walking legs and maxillipeds. Anemones smaller than those held in their claws were invariably not swapped. A crab holding an *Aiptasia* sp. would swap it in favour of an *Alicia* sp.; however, the reverse never occurred.

Boxer crab immunity

The studies of crustacean immunity from cnidarians is mainly focused on palaemonid shrimps associated with giant anemones. Palaemonids that were isolated for several days from their anemone, or whose integument was wiped, undergo a process of acclimatization when returning to the anemone. The shrimp gradually approach the anemone, first retreating after being stung, but gradually being less and less stung until being able to move freely over the entire anemone without releasing any response (Levine & Blanchard 1980, Crawford 1992, Karplus 2014). However, the anemone shrimp *Periclimenes brevicarpalis,* after isolation from the anemone *Entacmaea quadricolor*, did not require any acclimation behaviour before entering its host without being stung (Fautin et al. 1995). Three mechanisms have been suggested for preventing the discharge of nematocytes while the shrimp are in close contact with anemones: (1) The shrimp covers its body with the anemone secretions, becoming chemically camouflaged (Levine & Blanchard 1980, Giese et al. 1996). (2) The shrimp, upon contact with an anemone, may secrete a chemical that acts on its own and inhibits nematocyte discharge by binding to a receptor site of the nematocyte (Crawford 1992). (3) Similar to the previous hypothesis except that the chemical secreted by the shrimp combines with an anemone-derived substance to inhibit nematocyte discharge (Crawford 1992).

Unlike the shrimp dynamic acclimation process, boxer crabs undergo acclimation to their anemones while continuously holding them. For approximately 30 minutes after retaking its *Alicia* sp. a freshly moulted *Lybia leptochelis* would exhibit acclimation signs in the form of claw ‘twitches’. ‘Twitches’ were produced at a rate of 1–2 times a minute. These movements were analogous to what might be expected when a person is stung by an anemone. ‘Twitching’ was never observed a day following anemone retake. It is not known which of the three mechanisms suggested to prevent nematocyte discharge operates in *Lybia leptochelis*. However, the second suggested mechanism is highly unlikely since shrimp that produce their own secretion to inhibit nematocyte discharge are not affected by ecdysis in the retake of their anemones.

Boxer crab–anemone partner specificity

The earliest observations related to partner specificity in the boxer crab–anemone associations were carried out in Hawaii by Duerden (1905). In this pioneering study, one of two captured specimens (presumably *Lybia edmondsoni*) was holding a pair of *Bunodeopsis* sp. anemones, and the other specimen was holding a pair of *Sagartia* sp. anemones. The two species of anemones were found to be interchangeable, in that following removal of their anemones and exposure to anemones previously held by the other specimen, the crabs readily accepted the other species of anemone. In another experiment an additional crab–anemone combination, namely crabs that held in each claw a different species of anemone, was formed. Duerden (1905) concluded that in all his experiments the crabs appeared to seize one or the other species with equal readiness. A lack of partner specificity was thus demonstrated among the two species of carried anemones.

The evaluation of the level of partner specificity of boxer crab–anemone associations is speculative since many of the anemones have not been identified and few crabs from specific species were collected (Table 1). However, looking at Table 1, there are at least some crab species such as *Lybia tessellata* and *Polydectus cupulifer* which seem to be host generalists, found in association with five species of anemones in the former and three species of anemones and a holothurian in the latter. Nonetheless, in a specific locality, crabs often seem to hold anemones of only a single species. One hundred examined *Lybia leptochelis* from the northern Red Sea invariably held *Alicia* sp. in their claws, except for one case of a crab that held a fragment of an unidentified anemone, clearly not *Alicia sp.*, presumably *Anthopleura elatensis* (Schnytzer et al. 2013; Figure 4F).

Some insight concerning partner specificity may be gained by attempting to form in the laboratory associations between boxer crabs and anemones which do not occur in their natural habitat. Guinot (1976) reported that *Polydectus cupulifer* in the absence of *Sagartia pugnax* agreed to hold in aquaria an anemone of the genus *Teliopsis* sp., almost as large as its own size. *Lybia leptochelis*, which typically hold *Alicia* sp., had their anemones removed and were presented with *Aiptasia* sp. Crabs without anemones treat *Aiptasia* sp. in captivity in a similar way to *Alicia* sp., including holding and splitting. However, several crabs that were holding *Aiptasia* sp. were found to lose them after one to several months. This occurred possibly due to the lack of intake of photosynthetic metabolites due to bleaching when carried by the night-active crabs or due to feeding on these anemones which was observed on several occasions (Schnytzer 2008). It is noteworthy that *Aiptasia* sp. occurs in the Red Sea but not in the same habitat as *Lybia leptochelis* and has not been observed associated with boxer crabs in the wild.

Boxer crab intraspecific fighting

The involvement of anemones in boxer crab intraspecific fighting was studied in *Lybia edmondsoni* associated with *Triactis producta* (Karplus et al. 1998). Contests were staged between pairs of crabs matched to size and gender. Five out of the 15 recorded acts were performed with anemones (Figure 11). Three of these acts – 'twitch' (i.e. a rapid and short (1–3mm) lateral extension of the propodus), 'wave' (i.e. slow movement of chela or chelae in the horizontal plane) and 'extend' (extension of chelipeds with anemones) – comprised nearly 50% of all acts performed by the crabs. Both males and females avoided proximity and contact with their adversaries. Even when extending one claw at close range crabs invariably did so with the claw farthest away from the opponent. Actual contact between anemones and an opponent was extremely rare and seemed to occur due to an accidental movement of one of the crabs. Short contacts between legs during agonistic interactions (e.g. 'single-leg contact' and 'full grapple' (Figure 11A)) seem to have replaced the usual ritualized intertwining of the chelae reported in several other brachyurans (Schone 1968, Warner 1970, Lindberg 1980) and forcefully grasping (Huber 1987). There was no sexual dimorphism in claw dimensions since this structure is involved in anemone holding in both genders. Likewise, there was a marked similarity

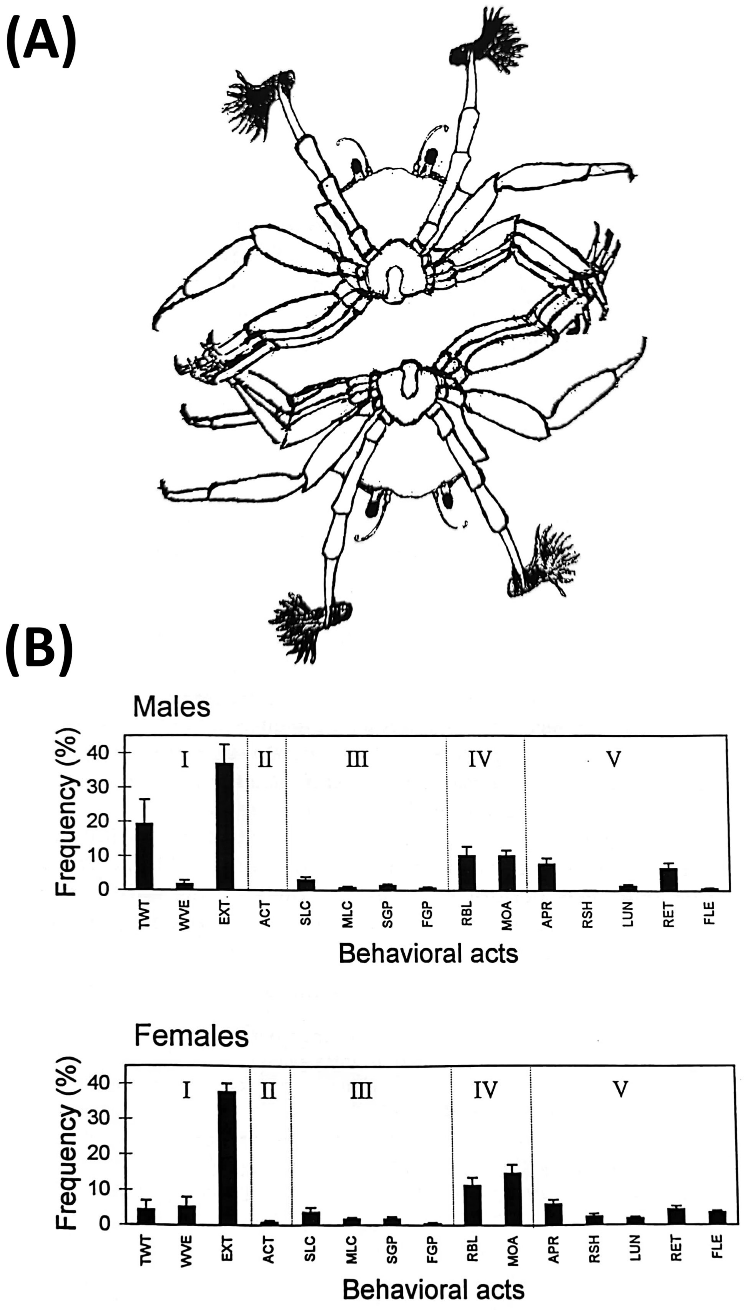

Figure 11 Intraspecific fighting behaviour of *Lybia edmondsoni*. (A) Two fighting males in the full grapple position (FGP). Walking legs are interlocked while the anemones *Triactis producta* are extended in opposite directions. (B) Mean frequency with standard error of 15 different acts during intraspecific aggressive encounters presented separately for males and females. I. Non-contact acts carried out with anemones (i.e. TWT – twitch; WVE – wave; EXT – extend). II. Contact acts carried out with anemone (i.e. act-anemone contact). III. Other acts with physical contact between crabs (i.e. SLC – single-leg contact; MLC– multiple-leg contact; SGP – semi-grapple; FGP – full grapple). IV. Maintenance activities (i.e. RBL – rub legs; MOA – mouth anemone). V. Acts increasing and decreasing distance between crabs (i.e. APR – approach; RSH – rush; LUN – lunge; RET – retreat; FLE – flee). After Karplus et al. (1998).

in the fighting behaviour of male and female crabs during contests. Several hypotheses were raised for the lack of use of anemones as a contact weapon during intraspecific contests:

1. *Triactis producta is a valuable resource to Lybia edmondsoni*. The anemones are not used in combat to prevent their damage or removal by the opponent.
2. *Triactis producta is highly toxic to L. edmondsoni.* Crabs avoid their use in fighting due to their high potential of severely harming the winner and loser of fights (Maynard-Smith & Price 1973), similar to fighting poisonous snakes (Shaw 1948, Thomas, 1961, Carpenter et al. 1976).
3. *Triactis producta are non-toxic to L. edmondsoni*. The anemones constitute an inefficient weapon and therefore are not used in combat.

So far, it is unclear which of the three hypotheses, or a combination of them, underlies the lack of anemone use in intraspecific fighting.

Boxer crab anti-predator defence

The involvement of anemones in boxer crab defence by deterring predators has been suggested several times (Borradaile 1902, Duerden, 1905, Jeng, 1994, Karplus et al. 1998, Davie et al. 2015). According to Verril (1928) the anemones serve as a protective living shield against fishes and perhaps other predators which fear the venomous stings of the actinian tentacles. When threatened, *Lybia* crabs perform a waving display by waving both chelipeds from side to side for a few seconds while either holding anemones (Jeng 1994) or nudibranchs (Baba and Noda 1993), bringing their defensive partners closer to the threatening object (Duerden 1905, Baba and Noda 1993). However, the claims concerning the anemone function to deter predators has so far not been supported by systematic experimentation. Preliminary observations on interactions of *Lybia leptochelis* associated with *Alicia* sp. with two species of fishes and a crab were carried out (Schnytzer 2008). A juvenile sciaenid *Argyrosomus regius*, a predator of small crustaceans, was placed in an aquarium with *Lybia leptochelis*. The fish, several times the size of the crab, very soon swam towards the boxer crab and came very close to it. The crab in response raised its anemones and struck the fish near the eye (Figure 12B). The fish appeared to 'shiver' for a brief moment and then retreated, not coming close to the crab again. Furthermore, *Lybia leptochelis* was introduced into an aquarium that contained a juvenile puffer fish, several times the size of the crab. The puffer fish several times slowly approached *Lybia leptochelis*. In one of these occasions the boxer crab struck at the puffer fish that retreated (Supplemental Video 11). An average sized *Lybia leptochelis* (c.w. 6–10 mm) was placed in the same aquarium with an Indo-Pacific crab, *Pilodius areolatus* several times its size. The crab approached the boxer crab which subsequently touched it with one of its anemones. The crab did not appear to be affected but did stop harassing the crab. In a single observation a *Lybia tessellata* (presumably holding *Triactis producta*) was approached by a juvenile octopus, about twice the size of the crab. The crab pointed its anemones at the octopus and touched it, causing the octopus to retreat (Figure 12A; Vaninni, pers. comm.). The crab's use of its anemones in anti-predatory defence is completely different from its avoidance of contact during intraspecific fighting.

Further experiments with boxer crabs with and without anemones exposed to different predators are needed to establish the protective role of the anemones. Similar experiments were carried out with hermit crabs with anemones attached to their shells and hermit crabs without anemones, exposed to predators (Ross 1971, McLean & Mariscal 1973, Brooks 1988). These studies clearly demonstrated the role of the anemones in helping to protect hermit crabs from octopuses and crabs.

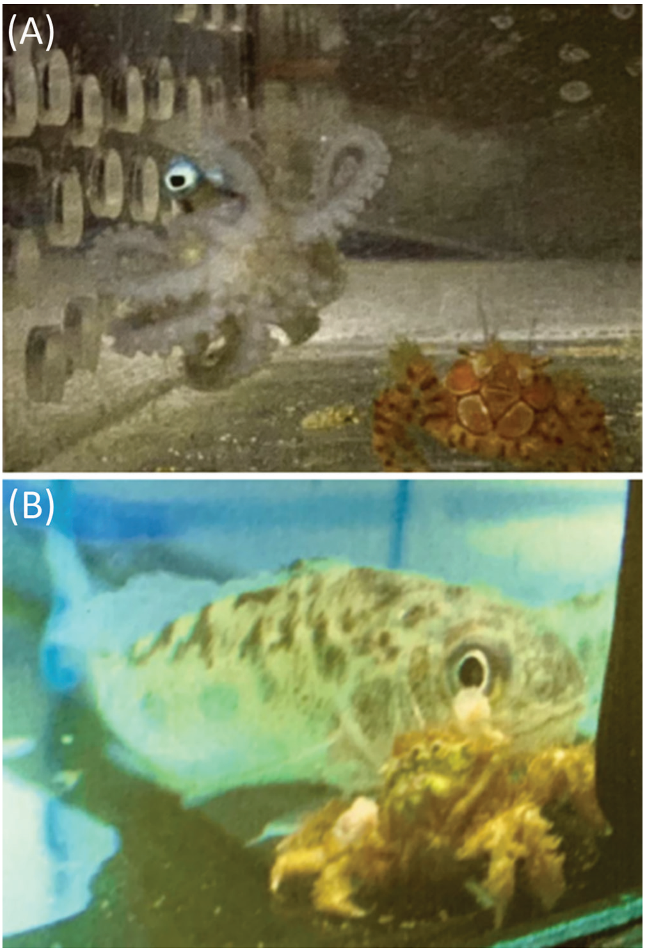

Figure 12 Boxer crabs deterring predators. (A) *Lybia tessellata* probably holding *Triactis producta* induced a retreat in a juvenile octopus by touching it (Vannini pers. com.). (B) *Lybia leptochelis* extending its left anemone towards the eye of an approaching sciaenid *A. regius*. After Schnytzer (2008).

Obligatory vs. facultative partnerships

The partnership between boxer crabs and anemones appears to be obligatory, as there is only one reference of several cases pertaining to the finding of several boxer crabs in the wild without symbiotic anemones (Borradaile 1902). In other references, each covering large numbers of at least 50 examined specimens (Richter 1880, Fishelson 1970, Karplus et al. 1998, Schnytzer et al. 2013),

Supplemental Video 11 *Lybia leptochelis* deterring Pufferfish. PBS 2018. https://www.youtube.com/watch?v=BUdpqFQYeZA; BBC 2018. https://www.bbc.co.uk/programmes/p06fnfmj/player.

crabs invariably held anemones in their claws. Furthermore, almost all specimens of these crabs in museums were preserved with their anemones (Guinot 1976). *Lybia* spp. were reared in captivity for several months without anemones (Karplus et al. 1998, Schnytzer et al. 2013). It is not surprising that they managed to survive under laboratory conditions considering the lack of predators and availability of food. However, in the wild, boxer crabs are not expected to survive given the lack of protection from predators and the lack of efficient means of collecting food.

Several *Lybia leptochelis* kept up to 1 year in captivity never lost their *Alicia* sp. anemones (Schnytzer 2008). This long adherence to their preferred anemone is a further indication of the obligatory boxer crab–anemone relationship. In contrast, hermit crabs which are facultatively associated with anemones, loose their anemones, frequently damaging and eating them following several months of captivity in the absence of predators. Chemical effluent from a tank containing an octopus reactivated the hermit crab habit of transferring anemones to its shell (Balasch & Mengual 1973, Ross & Von Boletzky 1979, Brooks & Mariscal 1986).

Anemones are probably facultatively associated with boxer crabs, given the fact that several of them such as *Triactis producta* (Fishelson 1970), *Phelia* sp. (Duerden 1905) and *Sagartia pugnax* (Verril 1928) have been observed free-living. Although *Alicia* sp. has so far not been observed free-living (Schnytzer et al. 2017), it will be quite surprising if it is not.

Costs and benefits from the partnership

Boxer crabs benefit from their association with anemones in three main ways: (1) Predator defence (Schnytzer 2008); (2) Stunning prey for feeding (Schmitt 1965) and food collection (Duerden 1905; Schnytzer et al. 2013); (3) Intraspecific ritualized fighting (Karplus et al. 1998). The presence of the anemones held by the crab claws emphasize and render more conspicuous the ritualized movements of the boxer crabs (e.g. behaviours such as wave and twitch), which would presumably be an unimpressive display by the crabs vacant, thin and feeble claws.

Anemones may also be involved in crab camouflage and conspicuousness. *Lybia tessellata* has derived its name from the special tessellated pattern of its carapace consisting of red and white polygons bordered with black lines. The crab legs are white with red to black rings (Figure 3C). The very conspicuous colour and patterns on *Lybia tessellata* (Figure 1D), as well as *Lybia edmondsoni*, may help them to blend into their colourful natural background as a form of disruptive camouflage (Ng pers. comm.). Alternatively, this colouration may be involved in aposematism, advertising its symbiotic anemone venom to deter potential predators. So far, no systematic work has confirmed aposematism in decapods (Caro 2018). In contrast, some of the polydectine crabs are cryptically coloured and indeed typically occur in rocky and muddy areas more suited to their colouration. *Polydectus cupulifer* possess a very thick covering of soft setae all over its external surfaces and has therefore been called ‘teddy bear crab’ (Figure 3D). The sediment that adheres to these setae conceal the form of the crab making it look like a lump of mud (Verril 1928, Chen and Hsueh 2007). The anemones carried by this crab possibly also add to its concealment.

Tool use by boxer crabs, related to benefits from the partnership, was already addressed close to the discovery of the association (Borradaile 1902, Duerden 1905). Since then, boxer crabs have been listed among tool users. Recently, they have been grouped together with elephants and lemurs as tool waving animals (Shumaker et al. 2011). Among invertebrates, only ants, crabs and octopus are known to construct tools (Shumaker et al. 2011), which raises the question: Do the modifications seen in anemones and nudibranchs held by Polydectine crabs constitute as tool construction? Broader invertebrate tool use is more common; however, the foregoing of the ‘hands’ or ‘claws’ which are permanently replaced by another animal are unknown outside of Polydectine crabs. There is, however, a comparable and interesting, yet little studied association between *Tremoctopus violaceus*, the blanket Octopus, which has special dorsal arm suckers to which they attach *Physalia physalis* (Portuguese man o' war) tentacles, obviously providing them with a formidable weapon (Jones 1963, Norman et al. 2002). It is important to note that there they make use of tentacle strands and not the whole animal. The main problem, which is out of the scope of this study to discuss, is the issue of invertebrate cognition and to what extent and where the definition 'tool use' is applicable (Hansell & Ruxton 2008). In a recent review of tool use in animals (Amodio et al. 2018), boxer crabs were classified as stereotyped in contrast to flexible tool users. In stereotyped tool use the actions are absent prolonged individual practice and social influences showing little variation within species and genera. However, the use of a single tool (i.e. anemone) for different purposes in boxer crabs is exceptional among stereotyped tool users. The question as to the possible ontogenetic changes in the crab, such as individual practice and experience while handling anemones, has still to be resolved.

The major cost of the partnership to the crab is the loss of the common grasping function of its claws, which are highly adapted for the sole purpose of anemone holding. These functions are replaced or compensated for by the crabs' walking legs and maxillipeds. A further potential cost is the utter dependence of the crabs on their held anemones and consequently the availability and abundance of their anemones which dictates the distribution of each particular boxer crab species.

Benefits to anemones from their association with boxer crabs, have been suggested to relate to changes in the crabs' position, with increased oxygen supply and greater proximity to potential food items, though not necessarily ingesting them (Duerden 1905). Anemones are also dispersed by the crabs and occasionally forced to reproduce asexually. Other than this, the anemone pays a heavy toll for being associated with the crab, namely, growth suppression due to food deprivation and prevention of sexual reproduction (Schnytzer et al. 2017). Furthermore, it appears that in cases where *Lybia* hold anemones in symbiosis with zooxanthellae, they bleach over time due to the nocturnal activity of the crabs, resulting in a loss of one of their primary methods of nutrition (i.e. photosynthesis). As already stated by Duerden (1905), the advantage for the actinian appears largely negative. Many, but not all, of the anemones associated with Polydectine crabs, such as *Lybia tessellata*, *Lybia edmondsoni* and *Polydectus cupulifer* are found free-living. It would appear that the crab is essentially a parasite of the anemone as the anemones are also found commonly free-living and thus has little or nothing to benefit from being held by the crabs which limit their food intake and suppress growth (Yanagi & Iwao 2012, Schnytzer et al. 2017). It is compelling to suggest that the association is of a parasitic nature. In contrast, *Alicia sp.* associated with *Lybia leptochelis*, has not been found free-living to date and so we must be cautious about defining the nature of that particular association. In the case of *Tunebia Hatagumoana*, typically found holding gymnodorid nudibranchs, it is all the less clear what gain could come to a mobile predatory slug. Overall, the nature of this association appears to be of a parasitic nature, with very little if any gain to the crabs 'partners'. Such is the case with many other crab–anemone associations that have been documented, in which there is a highly intimate association, such as *Dorippoides facchino* and the anemone *Carcinactis* (Holthuis & Manning 1990) which are known to collect small anemones and grow with them simultaneously. Yet, it appears that none of those studies have experimentally assessed the cost and benefit to each of the associates and so we must be cautious with such comparisons.

Evolution of the boxer crab–anemone partnership

The reconstruction of the evolution of a complex behavioural relationship is always complicated and speculative. According to Duerden (1905), there were no simpler or intermediate stages of the boxer crab–anemone partnership which could suggest the lines along which the evolution has taken place. A change from predation to association has been suggested by Imafuku et al. (2000) for hermit crabs and could also be applied to the boxer crab–anemone partnership evolution. Hermit crabs, according to Imafuku et al. (2000), occasionally feed in captivity on their symbiotic anemones particularly during starvation. These crabs also eat non-symbiotic anemones. The complex behaviour of removing firmly attached anemones likely shifted from detachment for feeding to detachment for placement on the shell for protection. Boxer crabs also feed occasionally in captivity on non-symbiotic as well as symbiotic anemones (Schnytzer 2008). In the case of the symbiotic *Alicia sp.* this was observed to happen when the crabs were already holding a pair of anemones and were provided with a surplus of anemones. The partnership between *Lybia leptochelis* and *Anthopleura elatensis* carried by its walking legs, may have originated as a food reserve, perhaps representing an early phase in the boxer crab–anemone partnership. In addition, a very small *Lybia leptochelis* of about 3 mm c.w. was collected holding in its claws two *Alicia* sp. as well as two additional similar sized anemones of the same species held in the fold of the two first walking legs (Figure 4E). The carrying of *Alicia* sp. by the legs as well as the claws, may provide a clue as to the evolutionary pathway of associated anemone holding mechanism.

Molecular analysis of the Xanthidae using three concatenated mitochondrial 12S rRNA, 16s rRNA and cytochrome oxidase I (COI), and the nuclear marker histone H3 was carried out on a large number of xanthid crabs, containing five species of the subfamily Polydectinae (Lai et al. 2011). The four *Lybia* species included in the study were split into two sister clades, each with two species. One clade included *Lybia edmondsoni* and *Lybia tessellata* as sister species and the other *Lybia hatagumoana* and *Lybia leptochelis*, with *Polydectus cupulifer* basal to them. The subfamily Polydectinae appears to be monophyletic, indicating that the partnership between boxer crabs and anemones evolved once among the sampled boxer crabs nested among non-associated xanthid crabs.

An additional molecular phylogeny was carried out by Giman (2008) on the subfamily Polydectinae using the three mitochondrial genes used by Lai et al. (2011) and Giman (2008), and two species of *Lybia*, *Lybia plumosa* and *Lybia caestifera*, that were not included in Lai et al. (2011). Included in this study were also *Lybia tessellata* from four different localities (i.e. Japan, Indonesia, Kenya and Somalia). In this study, based on the combined trees, the subfamily Polydectinae was resolved as a monophyletic group within the Xanthidae (Figure 13). The molecular analysis supports separation of *Lybia* into two clades, the setose carapace clade (i.e. *Lybia caestifera, Lybia plumosa, Lybia leptochelis*) with *Polydectus cupulifer* forming a sister group to this clade. The smooth crabs, *Lybia edmondsoni* and *Lybia tessellata*, forming a separate clade. The most intriguing result was that of *Lybia tessellata* from Somalia. The genetic distance found in the specimens from Somalia is enough to doubt its inclusion within the species *Lybia tessellata* (Figure 13). According to Guinot (pers. comm.) *Lybia tessellata* from Somalia looks different from the other *Lybia tessellata* and may be a different species. The three setose species (i.e. *Lybia caestifera, Lybia plumosa, Lybia leptochelis*) are closely related, sharing almost identical sequences (Giman 2008).

The evolution of the claws for only holding anemones is hard to comprehend according to Duerden (1905) since while holding anemones by the claws, the same claws have to be used for seizing and conveying food to the mouth as in most other decapods. The solution to this dilemma is in the plasticity of the periopod function. In decapod crustaceans the walking legs may change their function and replace that of lost appendages reflecting possible convergent evolution. For example, in the giant freshwater prawn, *Macrobrachium rosenbergii*, the amputation of the second pair of large claws resulted in the involvement of the small first pair of claws in intraspecific fighting behaviour. The small claws usually mainly involved in food acquisition and grooming were used in the absence of the large claws in the performance of aggressive acts (Karplus et al. 1992). Similarly, with the evolution of the boxer crab claws for holding anemones, the walking legs, particularly the first pair, took over the functions of feeding, anemone detachment and intraspecific fighting. The capacity for such a compensatory mechanism enabled the benefits of using the sea anemones to outweigh the costs of neutralizing the claws and thereby the evolution of boxer crab – anemone partnership.

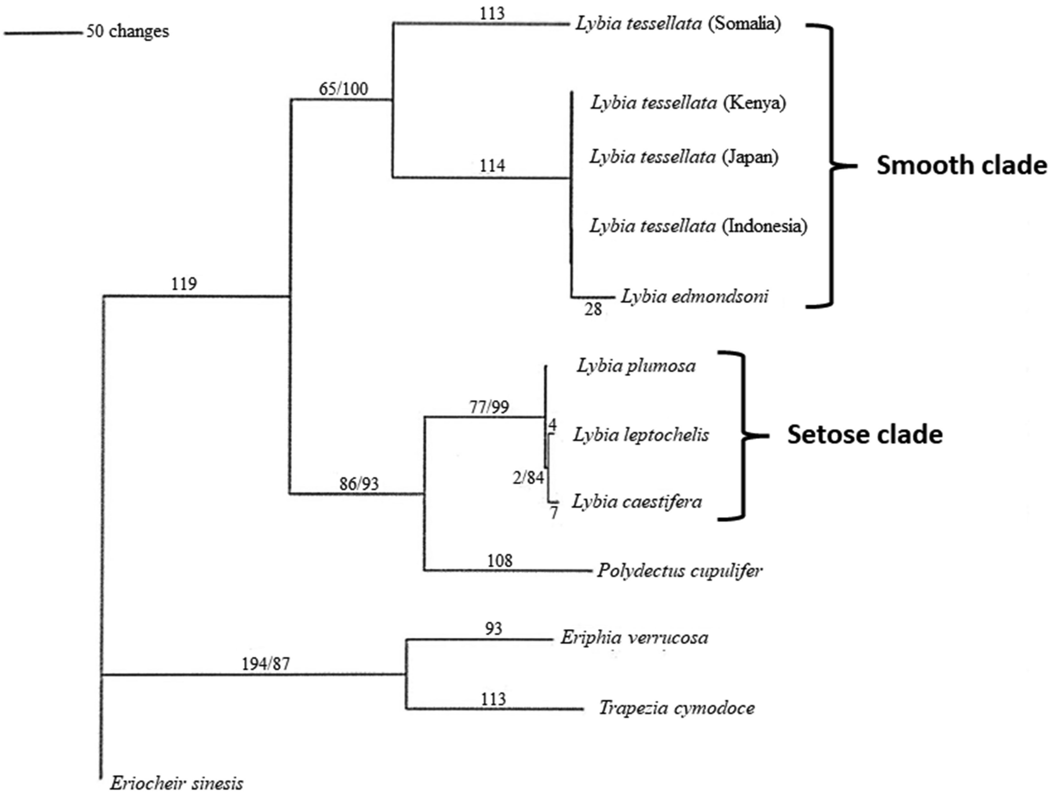

Figure 13 Phylogeny of polydectine crabs based on three combined mitochondrial genes (12S + 16S + COI). The tree was built according to the ML method using GARLI software. Left numbers are branch length, and right numbers are bootstrap values based on 100 replications. After Giman (2008).

Recommendations for future research

Several recent studies have contributed significantly to the limited knowledge to date on this association, followed by increased attention in the popular literature to boxer crabs. Nevertheless, important knowledge gaps remain, including the following:

a. There are substantial gaps in the ecology of boxer crabs and their associates. Some species, such as *Lybia leptochelis*, are known to occur at disparate locations, with little information of their distribution in between. There are some indications, both morphological and molecular, that *Lybia tessellata* from different locations may not be the same species. For other species such as *Lybia australiensis* and *Lybia denticulata*, *Lybia pugil* and *Tunebia tutelina*, the entire body of knowledge is based on one or two preserved museum samples. Others, such as *Tunebia hatagumoana*, have only been recorded on several occasions and always from relatively deep (60–80 m) trawls, their specific macro- and microhabitat/s are unknown. However, there is some anecdotal evidence of their occurrence in shallower water, like other boxer crabs. Due to their small size, cryptic behaviour and camouflage, it is difficult to find boxer crabs, certainly most of which are not as colourful as *Lybia tessellata* and *Lybia edmondsoni*.

b. The occurrence and ecology of some boxer crab associates is well known (e.g. *Triactis producta*). However, due to the external changes in the held associates, it is often unclear which species is being held. In the case of *Alicia* sp. held by Red Sea *Lybia leptochelis*, the form and size of the crab-held anemones is nothing at all like that of lab-grown and lab-fed anemones. Indeed, they have never been found free-living. So too for the gymnodorids held by *Tunebia hatagumoana*, nothing is known about the exact species held and where or if they occur free-living.

c. The benefits gained by the crabs holding anemones are clear. The cost and benefit for the anemones (and other invertebrates) is less evident. Although there appears to be no long-term morphological damage to the anemones, no studies have been conducted on their reproduction abilities, especially sexual, in and out of association. Furthermore, for those species harbouring photosynthetic zooxanthellae (thus making it a triple symbiosis), the possible effects of being held by the reclusive crabs are even less clear. So too in the case of nudibranchs and holothurians, typically found free-living. Further growth as well as histological studies are required to better understand this.

d. Boxer crabs are often mentioned regarding the aquarium trade and hobby. They are a popular species (especially the 'common' and colourful *Lybia tessellata*) and are widely available from pet store suppliers worldwide. All the animals sold to date are wild-caught. Further studies on the crab's reproduction and aquaculture methods may be quite beneficial to the sustainability of boxer crabs.

e. Although some work has been done on the visual and chemosensory abilities of boxer crabs, particularly for adult *Lybia leptochelis*, the mechanisms involved in the initiation of the association, especially at the post-larval stage, as the crabs metamorphose into megalopa and settle, are unknown. It is reasonable to assume that they may navigate to the general area of settlement and then home in on their associates rapidly and precisely. Further lab and field experiments are required to understand this. Current advances in video recording as well as tagging methods may finally enable these studies to be carried out, which were always impeded by the crabs' small size.

f. The idea of holding an anemone for defensive purposes is apparently obvious. However, little experimental, and no field work, has shown this. Further studies are required to fully establish the protective value of holding anemones. Regarding the nudibranchs and holothurians that some *Lybia* have been found holding, the utility is even more mysterious, as at least in the case of the gymnodorids held by *Tunebia hatagumoana*, as it is unclear what sort of defensive mechanisms they have. Nothing is known about the species of holothurians held by boxer crabs.

g. There is evidence from Japan (Yanagi pers. comm.) that Okinawan *Lybia caestifera* are found holding several species of anemones, including an *Alicia sp.* very similar to the one held by Red Sea *Lybia leptochelis*. In addition, *Lybia caestifera* have been observed to hold with their legs an *Anthopleura sp.*, again very similar to the one held by Red Sea *Lybia leptochelis*. These similarities, in such disparate regions, and in different species, warrant further research.

h. From a molecular perspective, most of the research on this association has focused on the identity and phylogeny of the partners in the association, with no functional work conducted. This association could prove to be a promising model for understanding the underlying molecular mechanisms governing and regulating such symbioses. For example, one can envision various transcriptomic and proteomic studies, testing both the crabs and anemones, in and out of association, experimentally parted, and wild-caught for those animals that occur free-living, in order to discover which genes and proteins are involved in the symbiosis.

i. Past morphological studies have focused primarily on the species-specific claw differences, as well as other macro-level markers. Little work has been conducted using electron microscopy. The finding of putative sensory pores in abundance on both the tips of legs and claw ‘teeth’, which are typically embedded in the held associates, warrants further functional studies.

Conclusions

Since their first recording over two centuries ago, boxer crabs have fascinated scientists and enthusiasts alike, presumably due to their easily anthropomorphized form, holding in both claws’ ‘little tools’. It is something about their unique appearance that induces this curiosity. However, so few studies have been conducted, practically none in nature. The current body of knowledge has established, especially in one or two representative species, the fundamentals of the association; however, further comparative work is much required. Due to the particular nature of the association, such apparent morphological changes, both short and long term, in both associates, are uncommon in other ‘macro’-scale associations, making this an important model for further research.

The crab’s habit to steal and split their anemones raises many questions regarding costs and benefits as well as long-term effects on associated anemone distribution and longevity, a topic which has received much recent attention. Boxer crabs and their associates may prove to be a flagship model for intensive aquaculture research and development, in limiting the continued collection and depletion of wild populations. Preliminary lab work and observations indicate that this is quite possible. They could follow the giant footsteps of the ever-popular panda in promoting conservation.

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AMOUNTS, SOURCES, FATES AND ECOLOGICAL IMPACTS OF MARINE LITTER AND MICROPLASTICS IN THE WESTERN INDIAN OCEAN REGION: A REVIEW AND RECOMMENDATIONS FOR ACTIONS

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Abstract The Western Indian Ocean (WIO) is a region where biodiversity is increasingly being impacted by anthropogenic marine debris, but information is scattered widely in the literature. Herein, we reviewed 147 marine litter studies from the WIO region covering the time period from 1973 (first study published in the region) to the end of 2021, in order to (1) synthesize available information on the quantities and composition of marine litter and microplastics in the main ecosystem compartments, (2) identify the main sources and pathways of litter and (3) summarize which organisms have suffered from interactions with marine litter. This information was evaluated to (4) determine important knowledge gaps and (5) develop recommendations to address the issue in the region. The oceanography of the WIO is dominated by the western boundary currents (WBC), which are characterized by high current velocities and frequent onshore flows, potentially influencing the dynamics of marine litter in the region. The literature review confirms that higher macrolitter densities (mostly plastic packaging) on the seashore were generally found on urban, recreational beaches close to point sources (urban run-off, beachgoers). Identification of litter types and local concentrations indicate that most litter does not disperse far from the source, suggesting that shorelines in the WIO region are important sinks for litter. Some buoyant litter also reaches WIO countries after extended oceanic journeys and onshore transport in the WBCs, mainly from Southeast Asia, but most foreign PET bottles are dumped illegally from ships. Many studies (37%) reported interactions between organisms and marine litter; ingestion (103 species) and entanglement (58 species) were documented most frequently, and all four species of sea turtles studied had ingested plastic debris. Major knowledge gaps identified were related to the studied compartments and countries (most studies have been conducted on sandy beaches and in South Africa), to the quantification of litter from land-based versus offshore sources and to the determination of litter impacts on organisms, at both the individual and population level. Reducing current levels of plastic pollution requires reusable materials and better waste management, as well as international efforts to reduce the input of litter from shipping and fisheries. Despite local particularities (e.g. socioeconomic systems, hydrology, geomorphology), the general patterns and processes in the WIO region (high local retention, some input from offshore sources) likely apply to marine litter dynamics in other WBCs, requiring similar solutions.

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Keywords: Anthropogenic Marine Debris; Entanglement; Ingestion; Land-Based Sources; Prevention; Reusable Products; Sea-Based Sources; Western Boundary Currents

Introduction

General background

Marine anthropogenic litter is a pervasive and growing problem. Of particular concern are waste plastics, of which at least 710 million metric tons is projected to be released into the environment by 2040 (Lau et al. 2020). These plastics can persist for long periods and tend to accumulate in aquatic systems, especially in the sea. Marine plastics can have many impacts, including being ingested by and entangling marine organisms (Kühn et al. 2015), smothering the seabed (Gregory 2009, Green et al. 2015) and facilitating the spread of invasive species and diseases (Barnes 2002, Lamb et al. 2018, Rasool et al. 2021). As a result, plastics pose a threat to marine biodiversity (Moore 2008, Deudero & Alomar 2015) and it is therefore important that effective mitigation measures are implemented to greatly reduce the release of waste plastics into marine environments (Coe & Rogers 1997, Alpizar et al. 2020). To do this, up-to-date information on the quantities, distribution and fate of plastic pollution, as well as its main sources, is urgently needed.

After entering the oceans, the fate and impacts of marine litter depend not only on the characteristics of the litter itself (e.g. buoyancy, size, durability), but also on abiotic (e.g. currents, temperature, solar radiation) and biotic environmental conditions (e.g. ingestion, entanglement, overgrowth). Currents move litter through the oceans, leading to small- or large-scale accumulations in particular areas (van Sebille et al. 2020). Along continental margins, where most litter enters the sea, major coastal current systems play a key role in the fate of marine litter. For example, in the highly productive eastern boundary current (EBC) systems, coastal upwelling leads to frequent offshore transport of surface waters (e.g. Marín & Delgado 2007), which may lead to rapid export of floating litter entrained in the slow-moving coastal currents. In contrast, in the fast-flowing western boundary currents (Lutjeharms 2006, Gupta et al. 2021) with frequent onshore transport of surface waters (e.g. Hood et al. 2017), floating litter may be rapidly transported onshore. It could thus be expected that most land-based litter entering the sea is retained locally, and that some (unknown) proportion of litter washing ashore originates from distant sources, arriving with the rapid western boundary currents (WBC). Whether these conditions and dynamics in the WBCs also affect the impacts on local biota is unknown, but if most litter is retained on local shores, then densities of floating litter (large and small) in coastal waters might be low, reducing the risk of interactions with surface-feeding organisms. An ideal system to examine these questions is the Western Indian Ocean (WIO), where several studies are suggesting large amounts and/or proportions of mismanaged waste (Jambeck et al. 2015, 2018, IUCN-EA-QUANTIS 2020a,b,c,d) and high densities of marine litter (e.g. Kerubo et al. 2020, Ryan et al. 2020a, Okuku et al. 2021a). Furthermore, recent reports indeed confirm the arrival of litter from offshore sources (Ryan 2020a, Ryan et al. 2021). The large-scale oceanography in the WIO region is dominated by the northward-flowing East African Coastal Current and the southward-flowing Agulhas Current, which, along with the upstream Mozambique Current and large anticyclonic eddies that propagate southwards in the Mozambique Channel, form one of the world's largest western boundary current systems (Beal & Elipot 2016, Halo & Raj 2020). These principal currents are hypothesized to drive the transport and retention of marine litter in the WIO region.

The Western Indian Ocean

The Western Indian Ocean (WIO) region comprises five continental countries (South Africa, Mozambique, Tanzania, Kenya and Somalia) and five island states or territories (La Réunion, Mauritius, Madagascar, Comoros including the French territory of Mayotte, and Seychelles). These

Table 1 Demographic and socioeconomic backgrounds of the ten countries and territories that comprise the Western Indian Ocean (WIO) region

	Population size (World Bank 2020a)	GDP per capita (current US$; World Bank 2020b)	HDI (UNDP 2020)	HDI Rank (UNDP 2020)
South Africa	59,308,690	5090.7	0.709	High
Mozambique	31,255,440	448.6	0.456	Low
Tanzania	59,734,210	1076.5	0.529	Low
Kenya	53,771,300	1838.2	0.601	Medium
Somalia	15,893,220	309.4	NA	NA
La Réunion	895,310[a]	NA	NA	NA
Mauritius	1,265,740	8622.7	0.804	Very high
Madagascar	27,691,020	495.5	0.528	Low
Comoros (and Mayotte)	869,600 (272,810)[a]	1402.6	0.554 (NA)	Medium (NA)
Seychelles	98,460	11425.1	0.796	High

[a] Population sizes of La Réunion and Mayotte are not provided by the World Bank, and thus, the source for the 2020 estimates provided here was the website https://www.worldometers.info, which is also fed by UN data.

GDP, gross domestic product; HDI, Human Development Index; NA, data not available.

countries present widely varying population sizes and socioeconomic statuses, based on their GDP (gross domestic product), HDI (Human Development Index) and HDI Rank (Table 1).

In Africa, waste management (including waste collection, treatment, transportation, storage and final disposal) is a major problem (Bello et al. 2016), and the WIO countries are no exception to this situation. For instance, in most continental WIO countries, plastic waste collection rates (i.e. the proportion of waste that is properly collected over the total waste generated) are very low, ranging from 27% and 30% in Kenya and Mozambique to 40% in Tanzania, while a large fraction of those percentages (i.e. 19%, 29% and 36%, respectively) corresponds to improperly disposed waste in, for example, unsanitary and illegal landfills (IUCN-EA-QUANTIS 2020a,b,d). Furthermore, landfilling (legal and illegal), which is the least preferred alternative for waste management (Smith et al. 2001), is usually the cheaper and the only option in continental countries (e.g. in Kenya, Njoroge et al. 2014), as well as in island states such as the Seychelles (Lai et al. 2016) and Mauritius (Beerachee 2012, Mohee et al. 2015). These characteristics combined inevitably lead to large waste mismanagement rates (i.e. the proportion of mismanaged waste over the total waste generated; see below). However, it should be noted that the capacity to manage waste has been linked with a country's national income and HDI Rank, as Mauritius, Seychelles and South Africa (Table 1) have fairly adequate waste management capacities, in contrast to the limited capacity in Comoros, Kenya, Madagascar, Mozambique and Tanzania (UNEP & WIOMSA 2008).

The WIO hosts a high marine biodiversity, with many threatened species to which plastics and other forms of marine litter pose a serious threat (Ryan et al. 2016a, Abreo et al. 2019, Cartraud et al. 2019). The region is also well known for its beaches, the beauty and value of which are threatened by increasing amounts of stranded litter (Ballance et al. 2000, Dunlop et al. 2020), and the cost of cleaning these beaches can be extreme (Burt et al. 2020, Rodríguez et al. 2020). Moreover, marine anthropogenic debris could negatively affect tourists' experiences and discourage them from returning or recommending these areas to fellow travellers (Jang et al. 2014, Krelling et al. 2017), potentially affecting the income of many WIO coastal communities.

The last review about marine litter across the region was published in 2008 (UNEP & WIOMSA 2008) and was based on consultant reports prepared by national experts on marine litter. It focused on eight of the ten countries within the WIO region (South Africa, Mozambique, Tanzania, Kenya, Mauritius, Madagascar, Comoros and Seychelles), most of which have a large coastal population generating large amounts of marine litter (Figure 1A and B). According

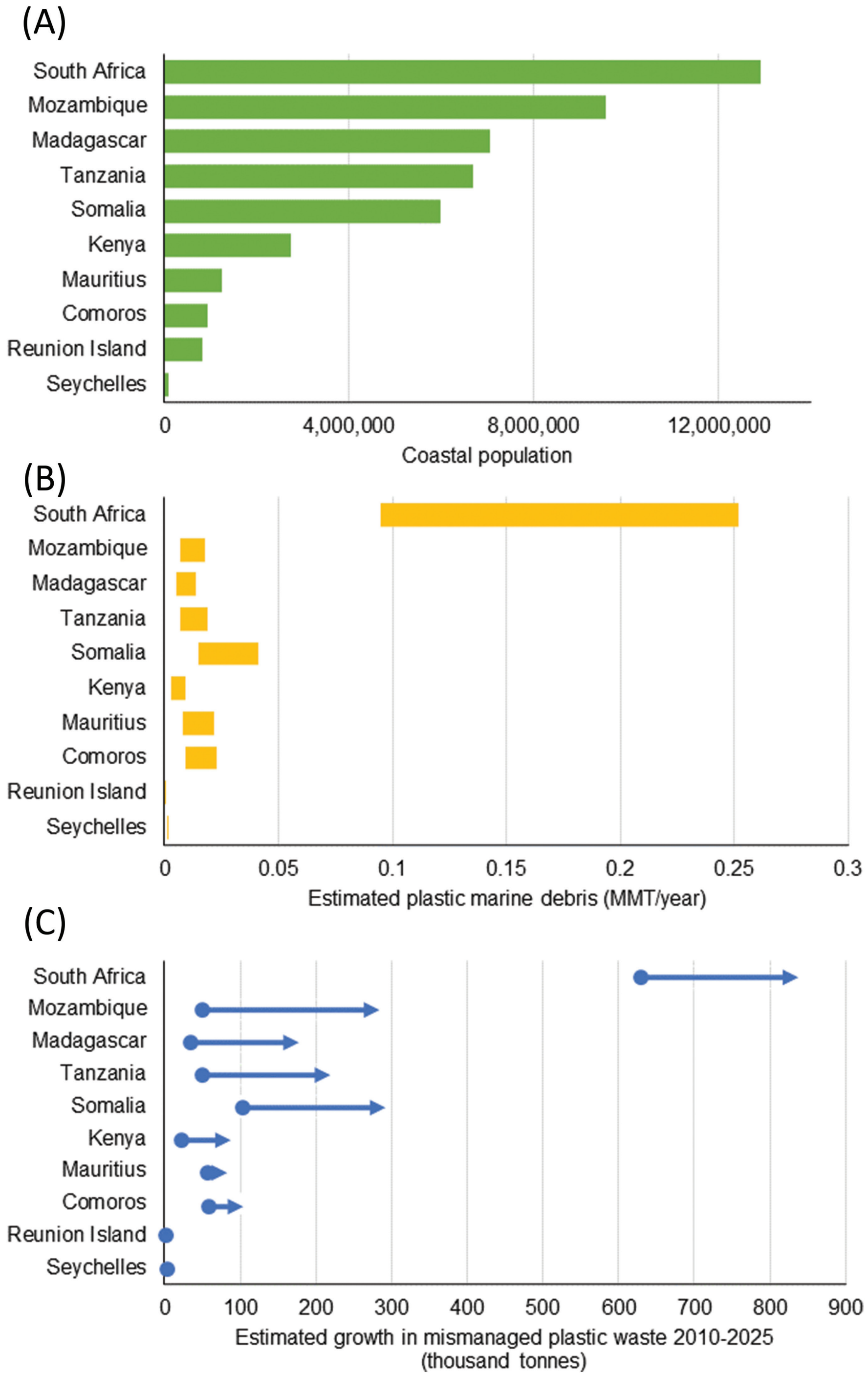


Figure 1 (A) The number of people living within 50 km of the coast in each of the WIO countries, (B) the amount of plastic litter estimated to have been released into the sea in 2010 and (C) the expected growth in mismanaged plastic waste between 2010 and 2025 (the dots indicate the amount of mismanaged plastic waste in 2010 and the tips of the arrows show the estimated amount in 2025). All data are from Jambeck et al. (2015).

to that report, most of the litter found in the region likely originates on land, although some was attributed to at-sea activities such as illegal dumping from ships and fishing activities. Mismanaged plastic waste has been identified as a main issue in the continental countries of the WIO, with mismanagement rates (i.e. proportion of the mismanaged waste over the total waste generated) ranging from 58% in South Africa to 99% in Mozambique (IUCN-EA-QUANTIS 2020a,b,c,d), and the amount of mismanaged plastic waste being predicted to increase in the coming years (Figure 1C; Jambeck et al. 2015, 2018), which could result in even more litter entering the WIO.

The region of the Western Indian Ocean is characterized by a system of poleward-flowing currents. The South Equatorial Current reaches the coast of northern Madagascar between 17.5°S and 19°S, where it divides into the Northeast Madagascar Current (NEMC) and the Southeast Madagascar Current (SEMC, Figure 2) (Chen et al. 2014). The NEMC connects to the persistent East African Coastal Current (EACC), which can reach velocities of 1–2 $m{\cdot}s^{-1}$ (Painter 2020). Near the Equator, the EACC meets the Somali Coastal Current (SC); during the SW monsoon (June–October), the EACC continues to flow northwards along the Somali coast, whereas during the NE

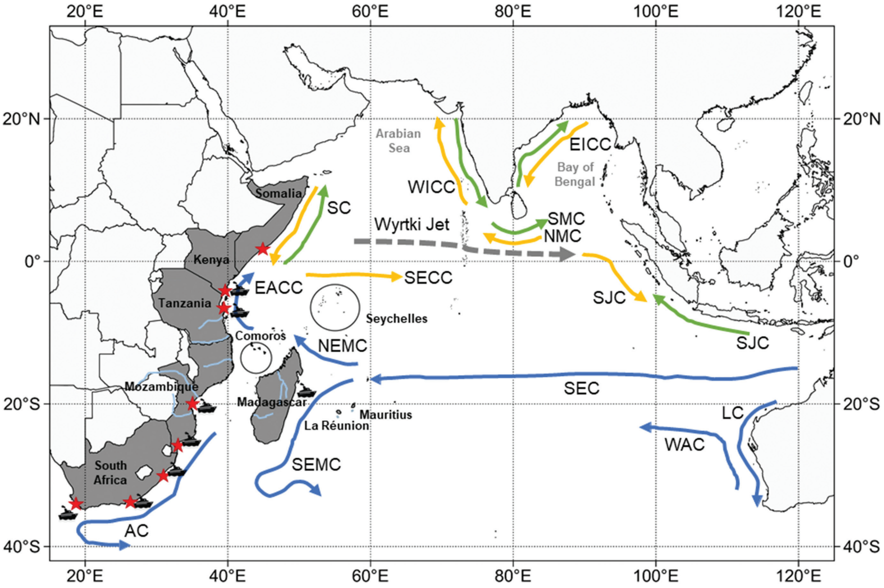


Figure 2 Map showing the major ocean currents of the Indian Ocean during the NE (yellow arrows) and SW (green arrows) monsoon. Annual currents not affected by the monsoon are shown by the blue arrows, and the semi-annual, inter-monsoon Wyrtki Jet is shown by the dashed grey line. Currents are the following: AC, Agulhas Current; SEMC, Southeast Madagascar Current; NEMC, Northeast Madagascar Current; EACC, East African Coastal Current; SC, Somali Current; WICC, West India Coastal Current; SMC, Southwest Monsoon Current; NMC, Northeast Monsoon Current; EICC, East Indian Coastal Current; SJC, South Java Current; SEC, South Equatorial Current; SECC, South Equatorial Counter Current; WAC, West Australian Current; and LC, Leeuwin Current. Western Indian Ocean countries included in this review are shown in dark grey, and ocean currents are based on Wijeratne et al. (2018), Rahaman et al. (2020) and van der Mheen et al. (2020). Potential land-based sources of marine litter into the WIO are also shown, such as the largest and most populated coastal cities (red stars), the major ports (black ships), which usually coincide with the largest cities, and the biggest rivers (light blue lines). Cities from north-east to south-west: Mogadishu, Mombasa, Dar Es Salaam, Beira, Maputo, Durban, Port Elizabeth and Cape Town.

monsoon (January–March), the SC flows southwards, joining the EACC around the equator to veer offshore forming the South Equatorial Counter Current (SECC). This likely has seasonal effects on the litter transport and stranding dynamics in this region.

The SEMC reconnects with moderate poleward transport through the Mozambique Channel, giving rise to the Agulhas Current (AC), which reaches maximum velocities of 2–2.5 $\mathrm{m{\cdot}s^{-1}}$ (Lutjeharms 2006). Consistent onshore drift within the AC proper (e.g. Shannon & Chapman 1983, Steinke & Ward 2003, Collins & Hermes 2019) will likely lead to local retention of floating litter, including objects coming from more distant sources (Ryan 2020a, Ryan et al. 2020a, 2021).

In addition to the large-scale ocean currents and processes described above, several small- and meso-scale coastal processes, such as internal tides, windage, Langmuir circulation, vertical mixing, river plumes, coastal fronts, coastal currents and surface waves, can also influence the transport and dynamics of marine litter in nearshore waters (van Sebille et al. 2020) and deposition on the shoreline (Chenillat et al. 2021). These processes can then contribute to, for example, the transport of litter offshore, its retention or accumulation nearshore, or even its beaching (van Sebille et al. 2020), and it is likely that these forces influence the dynamics of litter in the WIO region as well. For instance, models suggest that most microplastic particles released from coastal urban and industrialized centres along the South African coastline tend to beach and to accumulate close to their entry point, whereas a minor fraction is transported offshore to the Atlantic or to the Indian Ocean, but these dynamics partly depend on the presence or absence of nearshore coastal processes at or near the site of release (Collins & Hermes 2019).

Furthermore, the WIO region lies downstream from Southeast Asia, one of the world's largest sources of land-based plastics (Jambeck et al. 2015). Marine litter may therefore also be transported across the Indian Ocean to the region via the South Equatorial Current (Ryan 2020a, Ryan et al. 2021), and from there throughout the WIO region via the Somali, East African Coastal, Mozambique and Agulhas currents (van der Mheen et al. 2020), underscoring the need for regional solutions that complement national strategies.

The 2008 UNEP/WIOMSA report highlighted large knowledge gaps in all countries in the region except South Africa, but substantially more research on marine litter has subsequently been published, providing a much better understanding of the characteristics, sources, distribution and sinks of marine plastics in the WIO. Moreover, four WIO continental countries have recently conducted comprehensive national hotspotting assessments about plastic waste, which provide up-to-date information on the sources, density and distribution of plastic waste, as well as recommended interventions to curb its generation and release to the environment (IUCN-EA-QUANTIS 2020a,b,c,d). However, regardless of these advances, no recent region-wide review of the densities, distribution, sources, fate and threats of waste plastics has been conducted. Further, the countries from East Africa (i.e. the WIO region) are least well known with respect to the abundance, distribution and impacts of aquatic litter (Akindele & Alimba 2021).

Consequently, there are important knowledge gaps that remain unattended since the 2008 UNEP/WIOMSA report and the most adequate actions to tackle plastic waste at the regional level. The main goal of this review is thus to identify the principal sources of marine litter in the WIO countries, which is essential knowledge for effective prevention and management measures. We assess the status of marine plastics knowledge in the WIO region by conducting a comprehensive review of the literature on the amounts, sources and fate of marine litter and microplastics in the region, and their resultant ecological impacts. The focus is on publications reporting litter, its abundance, distribution and fate in order to identify sources, pathways and sinks of the marine litter in the WIO region. Furthermore, we have synthesized all studies reporting interactions with organisms, but we do not explore direct impacts on human health or on local economy because this would have required a more extensive and substantially different analysis. The knowledge gained with this review is applied to (1) identify important research gaps and (2) recommend local and regional strategies to reduce marine litter in the large marine ecosystems (LME) of the WIO region, namely the Somali Coastal Current system and the Agulhas Current system.

Analysis of literature

Methodology

For this review, we analysed the literature available in Google, Google Scholar and the Web of Science. Given that research on marine litter in the Western Indian Ocean region is relatively recent and much of it has not been yet published in peer-reviewed journals, as was informed to us by local stakeholders, we decided to include all types of scientific reports, including peer-reviewed papers, book chapters and grey literature. For the same reason (unpublished reports not captured by the principal reference databases), we used a multi-pronged approach, including back-tracking of key references, stakeholder interviews, own reference records and database searches. In order to keep the literature search as broad as possible and increase the chances of covering all available literature, we used the following general keywords: *litter*, *debris*, *microplastics*, *mesoplastics*, *plastic*, in combination with *WIO*, *Western Indian Ocean*, *South Africa*, *Tanzania*, *Mozambique*, *Kenya*, *Somalia*, *Madagascar*, *Seychelles*, *Mauritius*, *Comoros*, *Mayotte* or *Reunion*.

A study was included in the review database if it complied with the following criteria: (1) it corresponded to a field study, a modelling study or a review, (2) it dealt with any of the topics of interest, i.e. amounts, distribution, characteristics, sources, fate, transport or ecological impacts of marine litter and/or microplastics, (3) it reported data from at least one marine compartment/habitat in the WIO region, i.e. seashore, sea surface, water column, seafloor or rivers/estuaries/drainage systems, or in biota, and (4) it was published at any given time before the end of 2021.

Specifically, for the scanning and selection of the studies to be included in the review database, a stepwise process was followed: (1) our own reference records were compiled, (2) literature was searched using the selected keywords in the mentioned search engines, (3) titles and abstracts were scanned to determine whether the studies complied with the selection criteria, and, in case of doubt, the full-texts were skimmed as well, (4) once a study was selected and reviewed, all the references cited therein were examined and searched on the web if their titles suggested compliance with the selection criteria, and (5) Steps 3 and 4 were reiterated multiple times with new references until no new titles were located. In addition, we requested researchers working in WIO countries to check the list of references found for their country and to share with us any study that we might have missed. With these, Steps 3 through 5 were conducted as well.

Importantly, regarding selection criterion 3, there were two exceptions to the rule. First, since Weideman et al. (2020c) demonstrated limited long-distance transport of plastic litter in a major river in South Africa, studies conducted or data collected at inland sites of rivers were not included. Second, given that important water masses from the Agulhas Current keep intermittently flowing westwards around the south-western cape of South Africa under certain conditions mainly governed by the westerly winds (van Sebille et al. 2009, Morris et al. 2017), thus influencing the transport and abundance of litter in that area (Ryan 1988), we decided to also include studies reporting macrolitter and microplastic data from the west and south-west coasts of South Africa. Moreover, we consider that those data are particularly relevant also because similar socioeconomic conditions pertain throughout the country, and therefore, trends reported for the south-west coast of South Africa are likely to be representative of trends along the south and east coasts of the country. Nevertheless, to avoid generalizing on the basis of west and south-west coast data, we indicate where results correspond to or where inferences are drawn based on those data.

Following up on the aforementioned selection criteria, studies on other aspects of marine litter were not included in the reviewed database, such as experimental studies to, for example, determine the effects of marine litter and/or microplastics, studies that quantified or analysed litter or microplastics in wastewater treatment plants (WWTPs), the evaluation of the impacts of active fishing gear on organisms and the impacts of marine litter on human health (including persistent organic pollutants or POPs), as well as studies on the production/treatment/management/policy of waste or plastics and on socioeconomic/sociological aspects of marine litter.

Types of reviewed literature

The resultant database included 147 studies on marine litter and microplastics in the WIO region (listed in the Electronic Supplement). Of these, 81% were articles published in peer-reviewed scientific journals, 18% were grey literature (student theses, NGO, government, internship and consultancy reports, a meeting document, a conference output, a colloquium presentation and a paper published in an undergraduate journal), and two studies (1%) were published as book chapters (Figure 3).

Geographic coverage and marine compartments

Most studies reported data from South Africa (53%), followed by Kenya (9%), Mozambique and Seychelles (4% each), and smaller proportions from Madagascar, Comoros, Tanzania,

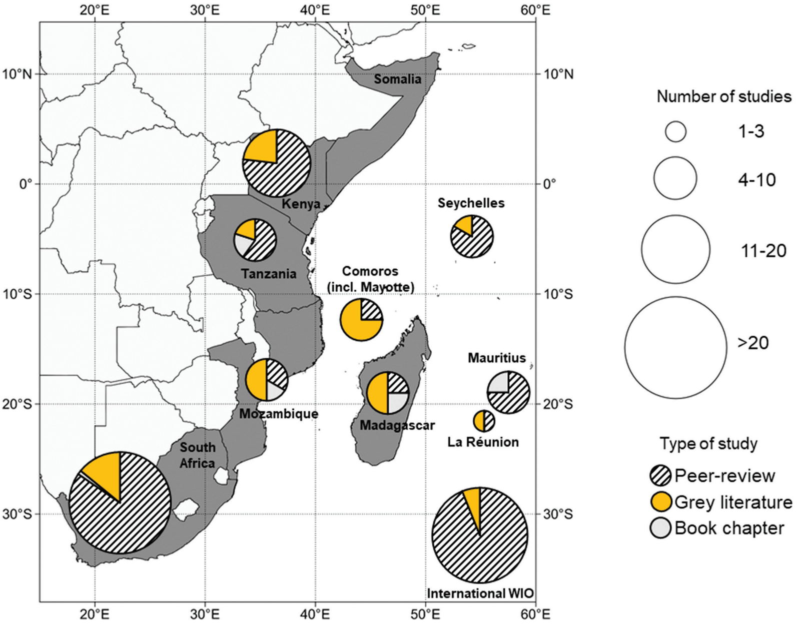


Figure 3 Map of the Western Indian Ocean (WIO) region where the size of the pie charts indicates the number of studies on marine litter or microplastics from each of the WIO countries ($n = 147$ across entire region) and size of the slices showing the proportion of studies that are grey literature (NGO and government reports, student theses, etc.), book chapters and papers published in peer-reviewed scientific journals. Western Indian Ocean countries included in this review are shown in dark grey, where Comoros also includes Mayotte. No studies were available for Somalia. An additional 33 studies were grouped as 'international WIO' (refer to main text for details).

Mauritius and La Réunion; no studies were found from Somalia (Figure 3). We classified 33 studies (22%) as 'international' if they included data (1) on more than one WIO country without distinguishing between the countries, (2) from areas within the WIO region not relating to a specific country (e.g. international waters), (3) from areas outside the WIO region, but relevant to it, for example ingestion of plastics by seabirds that forage widely across the Indian and Atlantic Oceans, or (4) from the entire Indian Ocean without identifying the data specific to the WIO region.

The best-documented habitat or compartment was the seashore (Figure 4), especially sandy beaches for which there were data available for all countries except La Réunion and Somalia (Figure 4, see also Tables in Electronic Supplement). Other types of shorelines, such as rocky shores, estuaries and mangroves, were seldom studied. The next best-documented compartment was the sea surface followed by the seafloor, whereas only one study collected samples in the water column (Figure 4). Interactions of litter with marine biota were reported from all WIO countries (except Somalia), typically comprising >25% of the studies in a country (Figure 4).

Size classes of litter

The studies were classified according to the size of the litter reported following the classification by GESAMP (2019): macro- (>25 mm), meso- (5–25 mm) and microplastics (<5 mm). However, given that many studies did not clearly distinguish between meso- and microplastics, the studies that sampled for mesoplastics using sieves, cores or nets were pooled with microplastic studies, whereas those in which mesoplastics were sampled without sieving or filtering (e.g. as part of beach transects) were included in macrolitter.

Among the studies reporting amounts and distribution of litter in the marine environment, most (47 out of 73) reported on macrolitter (>25 mm), although our understanding of the distribution

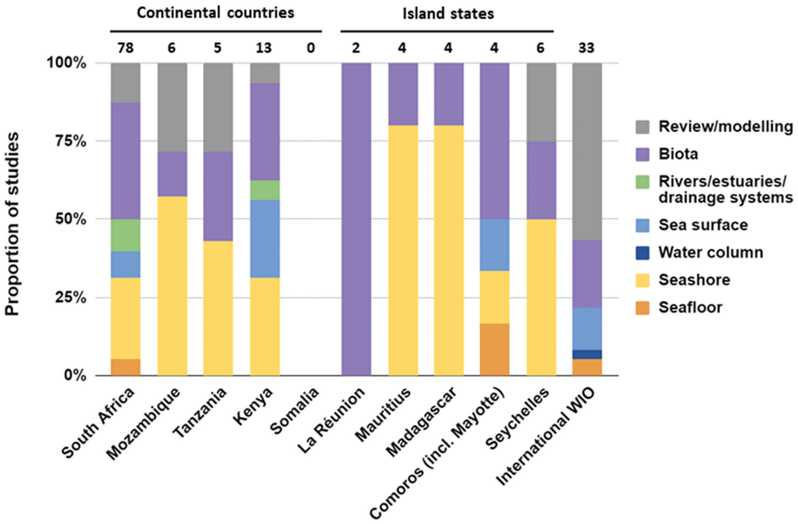
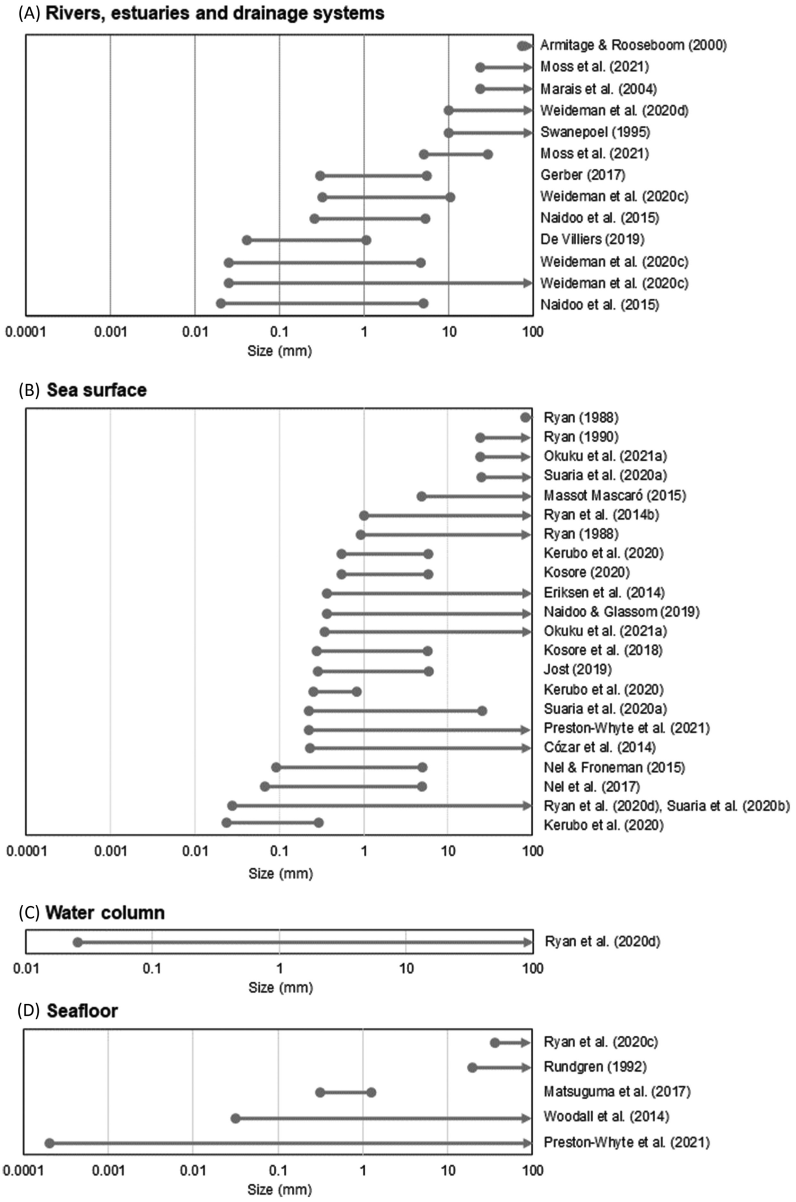


Figure 4 The proportion of studies from each of the WIO countries ($n = 147$ for entire region) focusing on different aspects of marine litter or microplastics and different compartments/habitats. Numbers above the bars indicate studies reporting data on corresponding country; they do not add up to 147, because six studies reported data on more than one country. Also, several studies reported data on more than one compartment and thus are included in more than one category.

of microplastics on the seashore has greatly improved since the 2008 UNEP/WIOMSA report (Figures 5 and 6). Most studies conducted on the seashore sampled for macrolitter, either by conducting standing stock or by accumulation studies, whereas most of the studies on the sea surface focused on microplastics (Figure 6).



(A) **Rivers, estuaries and drainage systems**

(B) **Sea surface**

(C) **Water column**

(D) **Seafloor**

Figure 5 Size ranges of macro- (>25 mm), meso- (5–25 mm) and microlitter (<5 mm) sampled in (A) rivers, estuaries and drainage systems, (B) on the sea surface, (C) in the water column (5 m deep), (D) on the seafloor (studies ranged from the subtidal zone to 1000 m depth) and (E) on the seashore of the WIO region. The circles show the minimum and maximum sizes sampled, while the lines ending in an arrow indicate studies which sampled for all litter larger than the minimum size (i.e. there was no maximum size mentioned). There were studies that reported sizes in terms of surface area (i.e. 1 cm^2, 5 cm^2), but these have not been included here (see Tables S1–S9 for details).

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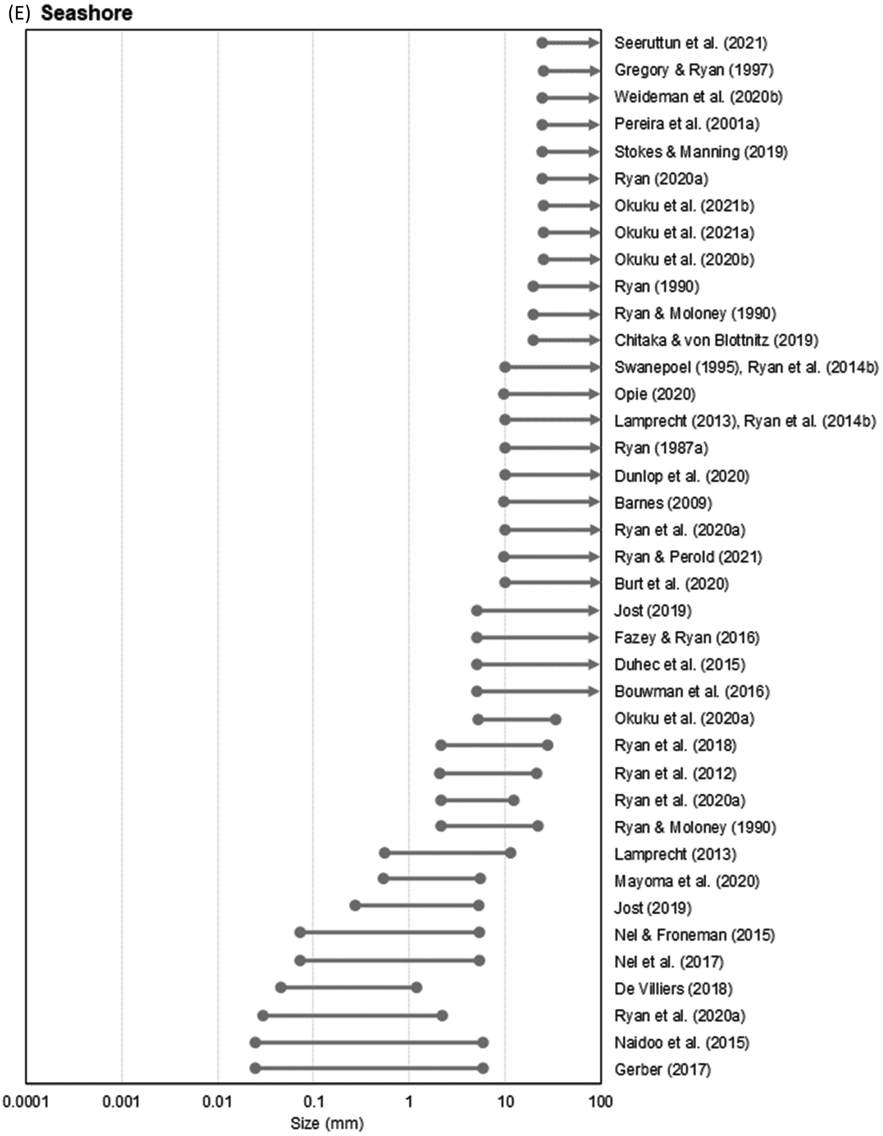

Figure 5 (*CONTINUED*) Size ranges of macro- (>25 mm), meso- (5–25 mm) and microlitter (<5 mm) sampled in (A) rivers, estuaries and drainage systems, (B) on the sea surface, (C) in the water column (5 m deep), (D) on the seafloor (studies ranged from the subtidal zone to 1000 m depth) and (E) on the seashore of the WIO region. The circles show the minimum and maximum sizes sampled, while the lines ending in an arrow indicate studies which sampled for all litter larger than the minimum size (i.e. there was no maximum size mentioned). There were studies that reported sizes in terms of surface area (i.e. 1 cm^2, 5 cm^2), but these have not been included here (see Tables S1–S9 for details).

The greater focus on macrolitter on beaches is likely explained by its higher visibility and accessibility, the overwhelming dominance of macrolitter in terms of the mass of plastic on beaches (Ryan et al. 2020a) and that macrolitter is also more informative for the identification of litter sources (Ryan et al. 2020b, 2021). Furthermore, macrolitter can be tackled more effectively through clean-ups than microplastics, but if left *in situ* will break down into microplastics.

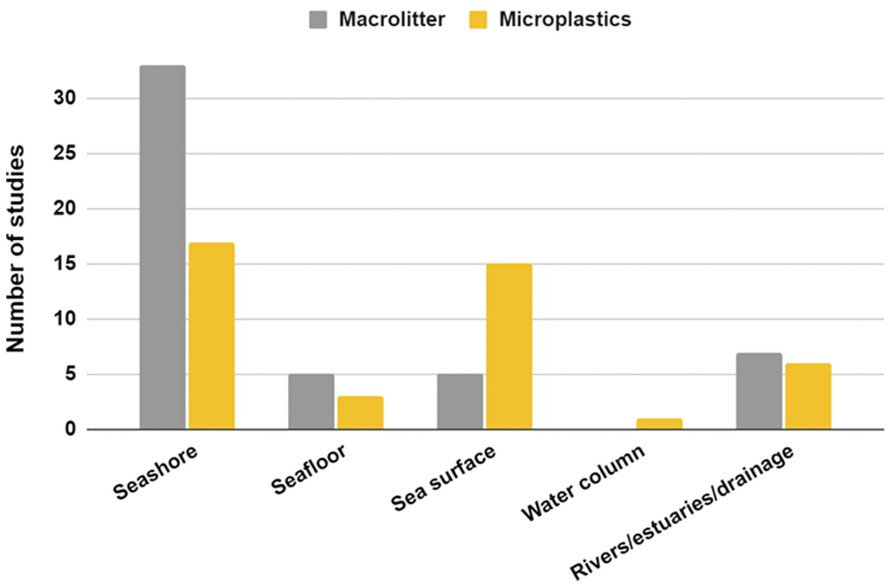

Figure 6 The number of studies on marine litter ($n=147$ for entire region) that sampled for macrolitter (>25 mm) and/or microplastics (<5 mm) in each type of marine compartment/habitat. Studies which sampled for mesoplastics (5–25 mm) with sieves, cores or nets are included in 'microplastics', whereas those where mesoplastics were sampled without sieving or filtering are included in 'macrolitter'. Some studies reported data on more than one compartment and/or more than one litter size and are thus included in all the respective categories.

Status of marine litter and microplastics knowledge in the WIO

Seashore – sandy beaches, rocky coasts, mangroves and estuaries

Macrolitter

Amounts, mass and distribution While several studies have sampled for macrolitter on seashores in the WIO region, it is difficult to compare litter densities between studies given that different sampling methods were applied; for example, most studies report litter per linear metre of shoreline (e.g. Bouwman et al. 2016, Ryan 2020b, Opie 2020), but some report densities per unit area (e.g. Pereira et al. 2001, Gjerdseth 2017, Figure 7B). It is also difficult to compare standing stock versus accumulation surveys (although both these types of sampling provide valuable information about litter densities on shorelines; Ryan et al. 2020b), and even among accumulation studies sampled at different temporal intervals (Ryan et al. 2014a). Efforts should thus be made to harmonize beach survey methods (Ryan et al. 2009, GESAMP 2019, Galgani et al. 2021), which have already been initiated by the Sustainable Seas Trust with the support of WIOMSA (Barnardo & Ribbink 2020), whose methodologies have been implemented in the region for the last 2 years.

Litter densities registered on sandy beaches of the WIO region vary between 4 and 10,060 items 100 m^{-1} and between 88,000 and 1,350,000 items km^{-2} (Figure 7B). The lowest and highest values per linear metre were recorded on different beaches of South Africa (Fazey & Ryan 2016a,b, Ryan et al. 2020a), whereas the lowest density per unit area was registered in Madagascar (Gjerdseth 2017) and the highest in Mozambique (Pereira et al. 2001). In general, these densities are fairly similar to those reported in other regions of the world (Serra-Gonçalves et al. 2019), which commonly range between 0.5 items m^{-2} (=500,000 items km^{-2}, Vlachogianni et al. 2018) and 2.0 items m^{-2} (=2,000,000 items km^{-2}, Pieper et al. 2015, Hidalgo-Ruz et al. 2018, Ríos et al. 2018).

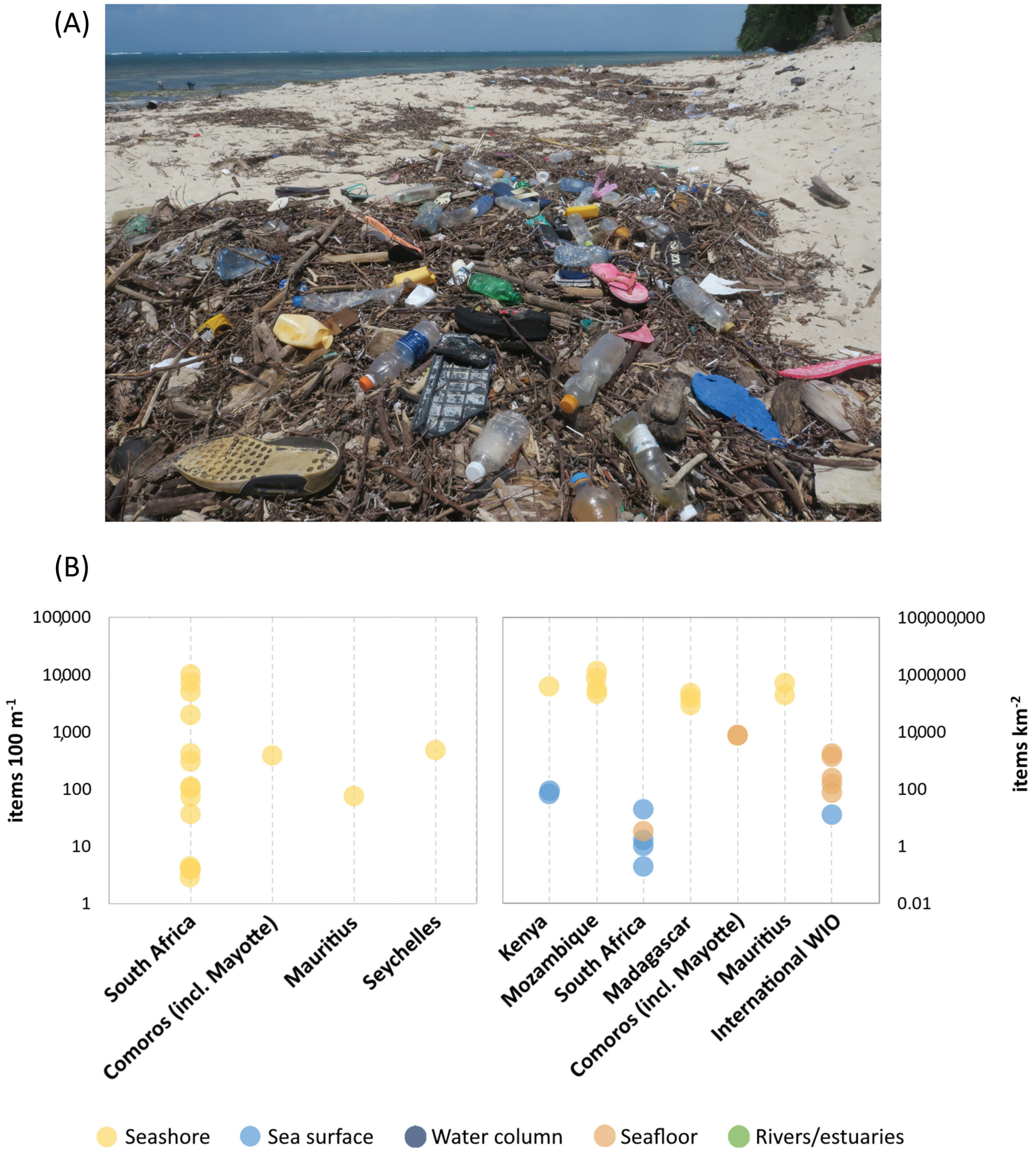

Figure 7 (A) Typical items of macrolitter accumulated on a Kenyan beach. Photo: Peter Ryan. (B) Macrolitter densities in different habitats in WIO countries with the different reporting units shown in the left and right panels (items·100 m^{-1}, items·km^{-2}; units expressed on a logarithmic scale). Panels in B include macrolitter (>25 mm) and mesoplastics (5–25 mm) collected without sieving/filtering. Only studies that reported all types of litter (i.e. not just the density of bottles or packaging) are included. Each point represents the mean density of litter at the finest scale reported in the study; for example, the mean value for each sampling site is included when studies reported at the site level, whereas the overall mean density is included when studies reported on a broader scale.

In South Africa, sandy beach surveys have been conducted since the 1980s (Ryan 1987a, Ryan 1990, Ryan & Moloney 1990), thus providing valuable baselines against which trends in litter characteristics and densities can be compared (Ryan et al. 2020b). Subsequent surveys showed that litter densities increased significantly between 1984 and 1989, and that this increase was driven by increases in locally produced plastic packaging (Ryan & Moloney 1990). However, more recent surveys have struggled to detect the rise in the amount of litter, but show that macrolitter is consistently concentrated around coastal urban centres despite greater cleaning effort on urban beaches (Ryan 2020b; Table S1). This suggests that most litter comes from local land-based sources (see below) and that litter does not disperse far from sources (see below). In Madagascar, litter densities were higher on a popular tourist beach than on more remote beaches (Gjerdseth 2017; Table S1). However, litter items on tourist beaches are often smaller and have a faster turnover rate due to increased cleaning efforts, which tend to target larger items, whereas large litter items tend to persist for longer periods on remote beaches. Therefore, while the number of items on tourist beaches may be higher, the mass of litter is normally concentrated on remote beaches, highlighting the importance of also reporting litter densities by mass (Ryan et al. 2020a).

Macrolitter standing stock surveys have also been conducted on sandy beaches in Kenya (Okuku et al. 2021a,b), Mozambique (Pereira et al. 2001), Tanzania (Maione 2021), the Seychelles (Duhec et al. 2015, Burt et al. 2020), Mayotte (Comoros Archipelago; Jost 2019) and on St Brandon's Rock, an isolated atoll 430 km north-east of Mauritius (Bouwman et al. 2016; Table S1). These studies support the findings that most marine litter is land based, most particularly in the continental countries and Mayotte (Pereira et al. 2001, Jost 2019, Maione 2021, Okuku et al. 2021b); however, in island states, there is an important input from sea-based sources as well, such as from long-distance drift (Duhec et al. 2015, Bouwman et al. 2016, Burt et al. 2020; see below). Interestingly, Okuku et al. (2021b) suggested that litter densities, composition and the relative contributions of different sources are seasonally influenced by monsoons. Litter densities on beaches at 4°S were highest during periods of high rainfall at the onset of the NE monsoon and lowest after the peak of the NE monsoon (Okuku et al. 2021b), when the confluence of the southward-flowing SC and the EACC may result in enhanced offshore flushing of floating coastal litter (see above); low proportions of litter from long-distance sources during the peak NE monsoon (Okuku et al. 2021b) further support this interpretation.

Daily accumulation studies have been conducted in South Africa (Swanepoel 1995, Lamprecht 2013, Ryan et al. 2014a, Chitaka & von Blottnitz 2019, Opie 2020, Barnardo et al. 2021), Kenya (Okuku et al. 2020b) and Cousine and Alphonse Islands in the Seychelles (Duhec et al. 2015, Dunlop et al. 2020). Accumulation rates tend to be higher on popular recreational beaches and on urban beaches closer to source areas; for example, ongoing monitoring of two beaches in South Africa since 1995 has shown that litter accumulation rates are consistently an order of magnitude higher close to central Cape Town than 30 km away (Table 2; Swanepoel 1995, Lamprecht 2013, Ryan et al. 2014a,b, Opie 2020; Table S1). While these results were obtained on the south-west coast of South Africa, accumulation rates by counts also tended to be higher on popular, easily accessible tourist beaches in Kenya, whereas the highest accumulation rate by mass was found on a less crowded, but still popular beach (Okuku et al. 2020b). Similarly, Barnardo et al. (2021) reported the same trend when comparing an urban, popular beach with open access in Port Elizabeth (south coast of South Africa) with a more remote beach (2.5 km away from Port Elizabeth) with limited access (Table 2). In both studies, the high accumulation rates by mass on these beaches were driven by the stranding of a few heavy items not found on other beaches (Okuku et al. 2020b, Barnardo et al. 2021). Litter accumulation rates are within the same ranges as those from other areas of the world, but tend to fall into the higher range (compare Table 2 in Thiel et al. 2021a).

On remote islands, accumulation surveys have been conducted on Alphonse Island (Duhec et al. 2015) and Cousine Island (Dunlop et al. 2020), both belonging to the Seychelles. Given that these studies utilized different sampling methods and frequencies, their results on accumulation rates should be compared with caution, as highlighted by Dunlop et al. (2020). Nevertheless, while it is important to consider these caveats, if we roughly calculate the daily accumulation rate of litter on the windward side of Alphonse Island (0.11 items m^{-1} day^{-1}, Duhec et al. 2015; Table 2), it was still

Table 2 Summary of litter accumulation rates on sandy beaches in the WIO region. The values for densities and mass correspond to the mean ± standard deviation, unless indicated that the latter corresponds to the standard error [SE]. Values with an asterisk (*) were calculated according to the data reported in the respective study

Country	Beach	Type of beach	Year of study	Density ($\boldsymbol{n}\cdot\mathrm{m}^{-1}\cdot\mathrm{day}^{-1}$)	Mass ($\mathrm{g}\cdot\mathrm{m}^{-1}\cdot\mathrm{day}^{-1}$)	Reference
South Africa	Paarden Eiland	Light industrial zone in Cape Town	2017	29.61 ± 23.86 [SE]	44.21 ± 33.29 [SE]	Chitaka & von Blottnitz (2019)
South Africa	Hout Bay	Harbour, popular recreational beach	2017	5.94 ± 4.94 [SE]	12.94 ± 7.32 [SE]	Chitaka & von Blottnitz (2019)
South Africa	Milnerton	Urban and popular beach in Cape Town	1995	1.57 ± 0.70	Not given	Swanepoel (1995)
South Africa	Milnerton	Urban and popular beach in Cape Town	2013	14.58 ± 2.73	Not given	Lamprecht (2013)
South Africa	Milnerton	Urban and popular beach in Cape Town	2019	3.29 ± 4.10	12.15 ± 15.21	Opie (2020)
South Africa	Milnerton	Urban and popular beach in Cape Town	2017	5.21 ± 3.64 [SE]	8.27 ± 8.71 [SE]	Chitaka & von Blottnitz (2019)
South Africa	Muizenberg	Popular recreational beach in Cape Town	2017	0.36 ± 0.28 [SE]	1.34 ± 1.34 [SE]	Chitaka & von Blottnitz (2019)
South Africa	Bluewater Bay	Urban, popular beach in Port Elizabeth, open access	2019–2020	0.56	1.63	Barnardo et al. (2021)
South Africa	Wolfgat	Coastal nature reserve in Cape Town	2017	21.97 ± 8.25 [SE]	23.40 ± 11.81 [SE]	Chitaka & von Blottnitz (2019)
Kenya	Kenyatta	Popular tourist beach, easily accessible	2019	18.2 ± 13.6	0.12 ± 0.08	Okuku et al. (2020b)
Kenya	Mkomani	Popular tourist beach, easily accessible	2019	16.5 ± 12.5	0.31 ± 0.20	Okuku et al. (2020b)
Kenya	Pirates	Popular tourist beach, easily accessible	2019	24.9 ± 19.1	0.16 ± 0.06	Okuku et al. (2020b)
Kenya	Tradewinds 1	Popular tourist beach	2019	3.8 ± 3.1	0.04 ± 0.02	Okuku et al. (2020b)
Kenya	Tradewinds 2	Popular tourist beach	2019	9.0 ± 7.8	0.08 ± 0.05	Okuku et al. (2020b)
Kenya	Baobab	Semi-populated, used by fishermen and beachgoers	2019	12.7 ± 12.5	0.04 ± 0.02	Okuku et al. (2020b)
South Africa	Cape Recife	Rural (nature reserve), 2.5 km from Port Elizabeth, limited access	2019–2020	0.46	8.38	Barnardo et al. (2021)
South Africa	Koeberg	Remote beach, 30 km from Cape Town	1995	0.35 ± 0.03	Not given	Swanepoel (1995)
South Africa	Koeberg	Remote beach, 30 km from Cape Town	2013	1.02 ± 0.43	Not given	Lamprecht (2013)
South Africa	Koeberg	Remote beach, 30 km from Cape Town	2019	1.00 ± 0.85	1.70 ± 0.93	Opie (2020)
Seychelles	Alphonse Island	Remote island	2013	0.11*	3.39*	Duhec et al. (2015)
Seychelles	Cousine Island	Remote island	2003–2019	0.008 ± 0.008	Not given	Dunlop et al. (2020)

more than one order of magnitude higher than that on the sheltered beach studied on Cousine Island (<0.01 items m^{-1} day^{-1}, Dunlop et al. 2020; Table 2). This suggests that wind direction might play a pivotal role in determining how much litter strands, which has also been reported for other islands (Debrot et al. 1999, Thiel et al. 2021a). Furthermore, models suggested that low-windage floating litter (i.e. not driven by wind) also arrived to Alphonse Island coming from India, Sri Lanka and Southeast Asia by means of surface currents, especially during the SE monsoon season (Duhec et al. 2015). These results highlight not only the importance of wind and currents as major drivers of floating litter (for other regions, see also Thiel et al. 2021a), but also the importance of litter originating from offshore sources for small island states in the WIO region.

Little is known about the vertical distribution of litter on beaches because few studies sample for buried litter (Ryan et al. 2020a). On a remote, uncleaned sandy beach in South Africa, over 80% of macrolitter items were buried below the surface, but buried items only contributed 6%–34% of the mass of plastic debris on the beach because smaller items are more easily buried than larger items (Ryan 2020b, Ryan et al. 2020a). The proportion of buried litter is higher on regularly cleaned beaches (Ryan 2020b). These findings suggest that burial is not a major factor affecting the total mass of litter on a beach, although this should be investigated in other WIO countries. However, it is important to note that buried items are exposed when storm seas erode sandy beaches, and that as sea levels rise, we are likely to witness increasing amounts of buried litter being released from beaches and perhaps even from coastal landfills if they are located close to sea (e.g. in Cape Town and Mombasa).

Much less is known about the densities, distribution and characteristics of litter on other types of shorelines. The only study conducted on a rocky shore, which was carried out in the south-west coast of South Africa, found higher litter loads at the beginning of the rainy season when litter from streets and stormwater systems was washed into False Bay, Cape Town (Weideman et al. 2020b). Among the studies conducted in mangroves, Seeruttun et al. (2021) sampled macroplastics in two mangrove forests on the east coast of Mauritius, while Stokes & Manning (2019) sampled along a stretch of sandy beach and in a mangrove close to a village in Madagascar. In Mauritius, a mangrove forest close to human settlement presented significantly more litter and also the densest litter aggregation in comparison with the more remote mangrove sampled (Seeruttun et al. 2021). Similarly, litter in the Madagascar study was significantly more abundant on the beach close to the village than along a transect farther away (Stokes & Manning 2019). However, it is important to highlight that standardized litter densities were not reported in the latter case; furthermore, the authors did not differentiate between litter in the mangrove versus that on the beach, so it is difficult to tell if densities differed between these two habitats (Stokes & Manning 2019). While no systematic surveys of macrolitter in estuaries are available for the WIO region, Ryan & Perold (2021) conducted daily beach surveys adjacent to the Zandvlei Estuary mouth in False Bay, south-west South Africa. They showed that litter densities were much more varied during the rainy season and that they peaked throughout the season 1–3 days after rain events, suggesting that most beach litter is washed out of the estuary. Furthermore, experimental releases of marked litter blocks showed that most blocks were washed into the estuary by a rising tide despite strong offshore winds; while this study was conducted in the south-west coast of South Africa, the results illustrate that exchanges of litter between estuaries, the sea and nearby beaches can be complex (Ryan & Perold 2021).

Characteristics and composition Almost all surveys show that plastics are the most common material found on sandy beaches, although denser materials such as glass, rubber and metal can contribute more to the mass of litter (but few studies report litter mass). Plastics generally make up at least >50% of litter items on beaches, and the daily accumulation rate of plastic litter on sandy beaches in Kenya was one to three orders of magnitude higher than that of other types of litter (Okuku et al. 2020b). However, plastics can contribute an even higher proportion of litter, especially on remote beaches (Madzena & Lasiak 1997, Pereira et al. 2001, Duhec et al. 2015, Bouwman et al. 2016, Gjerdseth 2017, Jost 2019, Dunlop et al. 2020; Table S1), which has also been reported elsewhere, e.g. on Easter Island and on German remote beaches along the North Sea coast (Honorato-Zimmer et al. 2019) or on Atlantic Islands (Pieper et al. 2015, Ríos et al. 2018). In a global study that assessed the composition of marine litter across seven different environments worldwide, plastic items accounted for 83% of total litter items on shorelines (Morales-Caselles et al. 2021).

Of particular concern is plastic packaging (food wrappers, packing strips, bottles, takeaway cups, etc.), which often dominates litter loads at least numerically, especially on urban sandy beaches (e.g. Figure 8; Ryan & Moloney 1990, Chitaka & von Blottnitz 2019, Okuku et al. 2020b, Opie 2020, Ryan 2020b, Barnardo et al. 2021). Most of this type of litter is either left by beachgoers, or washed onto the local beaches as part of river and urban run-off, although some is dumped from ships (Ryan 2020a, Ryan et al. 2021). This should not be unexpected, given that the packaging sector has been identified as a main source for litter in Kenya, Mozambique, South Africa and Tanzania, contributing from 50% to more than 70% of the total plastic leakage by mass into oceans and waterways, compared to other sectors (IUCN-EA-QUANTIS 2020a,b,c,d). Furthermore, takeaway consumer items (which include plastic packaging items, single-use plastic items, glass bottles and cans) have made up from 50% to 88% of the total litter items across different environments worldwide (Morales-Caselles et al. 2021).

Fish aggregating devices (FADs) are also items of concern, given that significant numbers are stranded along the east African coast, especially in Somalia and the Seychelles, being transported by the strong surface currents of the WIO region (Maufroy et al. 2015). FADs play a significant role in ghost fishing in the WIO and are regularly found entangled with dead sea turtles and corals (Balderson & Martin 2015).

Little is known about the types of litter found on other types of shorelines in the WIO region. Surveys of litter stranding on a rocky shore in False Bay, South Africa, showed that items with high specific density such as glass and rubber make up a larger proportion of litter than on nearby sandy beaches, although less dense items such as plastic bags can be trapped on the rocky shore if they become filled with sand (Weideman et al. 2020b). In Mauritius, Seeruttun et al. (2021) showed that

Figure 8 Plastic items are the most common type of litter throughout the WIO region, with litter from local land-based sources dominating on beaches close to urban source areas such as Cape Town. Photo: Peter Ryan.

plastics were the most common type of material in mangroves, in terms of both number and mass, although the proportions of other materials differed between two study areas (Table S1), highlighting how the contribution of specific materials differs locally. For instance, a few large items tend to dominate the mass of litter at certain sites and these almost always come from offshore sources (e.g. fishing gear, dunnage and other shipping waste; Ryan et al. 2020a,c), while Ryan (2020b) showed that plastics become increasingly dominant in terms of litter mass as one moves from land-based litter (e.g. street litter) to continental beaches to oceanic islands. Similar patterns of higher proportions of plastic litter with increasing distances from sources have also been reported for other oceans (e.g. Honorato-Zimmer et al. 2019). Much more research is needed to determine how the types of litter differ between different regions and how this relates to litter sources and post-disposal transport. Interestingly, however, Morales-Caselles et al. (2021) have suggested that litter composition does vary across different regions, presenting lower proportions of single-use plastics in less densely populated areas and in high-income countries (Morales-Caselles et al. 2021).

Meso- and microplastics

Amounts, mass and distribution Data on the density and distribution of meso- and microplastics on the seashore are only available for sandy beaches and estuaries, with no studies having sampled for meso- or microplastics on any other type of shoreline in the WIO region (although overall there are few data from rocky shores globally, due to the difficulty of sampling, Melvin et al. 2021). As is the case with macrolitter studies, it is difficult to compare meso- and microplastic densities among studies and sites due to the variety of different sampling methods (e.g. sieving transects, sediment cores, bulk sediment samples), as well as different size classes and reporting units (Figures 5 and 9B; Table S2). The first surveys of mesolitter on sandy beaches were conducted on 52 beaches in South Africa in the 1980s and reported an increase in the average densities from 1984 to 1989 (Ryan & Moloney 1990; Table S2). However, subsequent surveys between the 1990s and 2010s detected little change in the density of mesolitter (Ryan et al. 2018). As is the case with macrolitter, mesolitter was concentrated around coastal urban centres in South Africa (Ryan et al. 2018). In Kenya, higher densities of mesolitter were reported on beaches closer to populated areas than on semi-populated and remote beaches (Okuku et al. 2020a; Table S2). Similarly, the highest densities of mesolitter in Mauritius were found on two not-so-popular beaches among tourists and locals, but which are located in close proximity to inhabited towns and coastal villages, being possibly littered by local inhabitants (Mattan-Moorgawa et al. 2021). No studies have been published about mesolitter on the seashore in the other WIO countries.

There has been great improvement in our knowledge of the density and distribution of microplastics on the seashore of the WIO region since the 2008 UNEP/WIOMSA review, although this field of research is still in its infancy. Most research has been conducted in South Africa; for example, Nel & Froneman (2015) collected sediment cores from 21 South African beaches and showed that almost all particles are microfibres (although they did not identify the polymer types and it therefore remains unclear whether these fibres are plastic). However, they found little difference in microfibre densities between beaches, suggesting that the distribution of microfibres and microplastics on the seashore is governed by large-scale water circulation instead of population density or the proximity to land-based sources (Nel & Froneman 2015, see also Nel et al. 2017). In contrast, a more comprehensive study by de Villiers (2018) showed that microfibres on 175 sandy beaches were concentrated around coastal urban centres, as is the case with macro- and mesolitter, demonstrating the influence of population density and proximity to point sources.

Both de Villiers (2018) and Ryan et al. (2020a) found similar densities of microplastics in sediment cores on the south-west coast of South Africa, but de Villiers (2018) only sampled at the high tide mark, while Ryan et al. (2020a) sampled at set intervals across the width of the beach, from spring low to the storm high tide line. This showed that microfibres are distributed across the entire beach profile, unlike more buoyant meso- and macrolitter, which tend to accumulate on the high shore. Studies that only sample for microfibres along the high tide mark thus provide a distorted

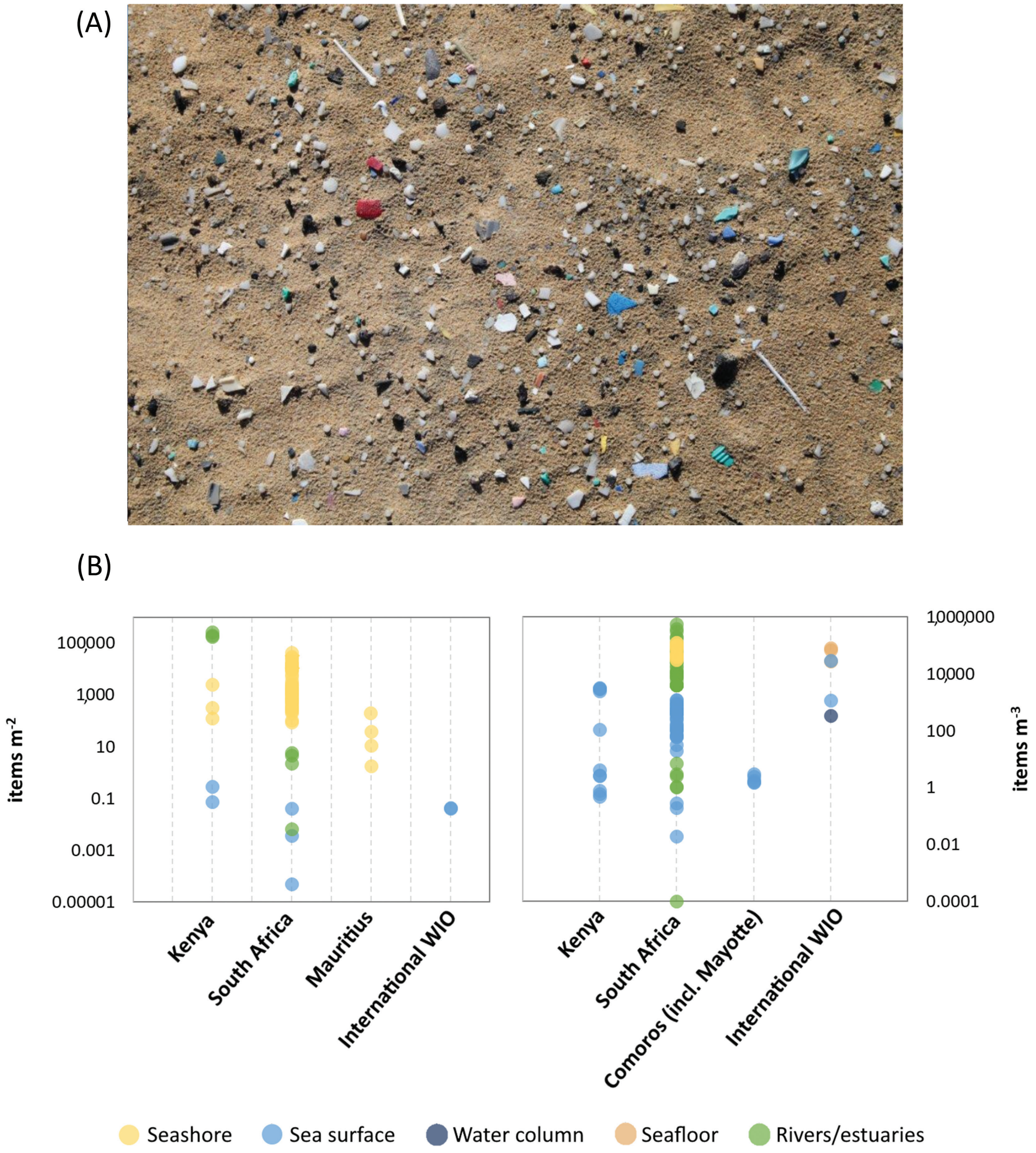

Figure 9 (A) Meso- and microplastics accumulated on a South African beach. Photo: Peter Ryan. (B) Microplastic densities in different habitats in WIO countries with the different reporting units shown in the left and right panels (items·m^{-2}, items·m^{-3}; units expressed on a logarithmic scale). Panels in B include microplastics (<5 mm) and mesoplastics (5–25 mm) collected by sieving/filtering or towing net. Each point represents the mean density of microplastics at the finest scale reported in the study; for example, the mean value for each sampling site is included when studies reported at the site level, whereas the overall mean density is included when studies reported on a broader scale.

view of microfibre densities and distribution. When extrapolated across the entire beach profile, Ryan et al. (2020a) found an average density of microfibres that completely dwarfed counts of macro- or mesolitter, but contributed <0.01% to the total mass of litter, again highlighting that larger items dominate the mass of litter on beaches (and floating in oceanic gyres, Lebreton et al. 2018).

Within urban centres, harbours may act as significant sources of microplastics into the marine environment as they often receive high levels of urban run-off via stormwater drainage systems. Nel et al. (2017) sampled at 13 sandy beaches and three harbours spanning the entire coastline of South Africa and found the highest densities of microplastics at sites near harbours. Similarly, the highest densities of microplastics on sandy beaches from Tanzania were reported near the industrial port in Dar es Salaam, being significantly higher than the densities found at 17 other sites (Mayoma et al. 2020; Table S2). In turn, within an important South African harbour, namely Richards Bay Harbour, the highest concentrations of microplastics were found near sites of major human use, such as recreational beaches and a restaurant and business area (Mehlhorn et al. 2021).

Interestingly, on the main island of Mauritius, the highest densities of microplastics on sandy beaches were recorded at windward sites compared to leeward and southern sites (Mattan-Moorgawa et al. 2021). The authors partly attributed this to the potential influence of the offshore Indian Ocean Gyre located to the east of Mauritius, and to the presence of the south-east trade winds and their associated currents and waves (Mattan-Moorgawa et al. 2021), which reinforces our aforementioned suggestion about the role of wind in the transport and accumulation dynamics of marine litter and microplastics in remote islands.

Regarding microplastics in estuaries, Gerber (2017) collected sediment cores from three estuaries in KwaZulu-Natal, South Africa, while Naidoo et al. (2015) sampled at another four estuaries in the same province (Table S2). Interestingly, both studies also measured microplastics on adjacent beaches, but while Naidoo et al. (2015) found no change in microplastic densities with distance from the estuary mouth, Gerber (2017) reported higher microplastic densities close to estuary mouths, suggesting that estuaries may act as sources of microplastics into the sea and onto adjacent beaches. In support of this, a longitudinal survey of micro-, meso- and macrolitter in the Orange-Vaal River system in South Africa showed that larger meso- and macrolitter were retained upstream near point sources, whereas microfibres were more evenly distributed along the length of the river with particularly high concentrations found near the river mouth in the dry season before seasonal rains flushed the system (Weideman et al. 2020c). This suggests that smaller items are more easily transported long distances by rivers, which has also been suggested for rivers in other regions of the world (Nizzetto et al. 2016, Honorato-Zimmer et al. 2021).

Characteristics and composition All studies surveying mesolitter and microplastics on sandy beaches indicated that plastics were the most frequent material found: they made up >99% of items by number in South Africa (e.g. Ryan et al. 2018, 2020a), 90% of items on Kenyan beaches (Okuku et al. 2020a), and between 50% and 70% of items on 12 beaches along Mauritius' main island's coast (Mattan-Moorgawa et al. 2021). However, the composition of mesolitter tends to vary according to proximity to urban areas, with a more diverse array of litter on beaches close to urban areas than on remote beaches where only plastics tend to be found (Okuku et al. 2020a, Ryan 2020b).

In South Africa, most plastics were industrial pellets (Ryan & Moloney 1990, Lamprecht 2013), most of which were either polyethylene or polypropylene (Ryan et al. 2012). However, it should be noted that the proportion of industrial pellets has decreased over time, from 80% in 1984 to 68% in 1989 (Ryan & Moloney 1990, Gregory & Ryan 1997), and Ryan et al. (2018) showed that pellets are now concentrated at a few beaches where they have probably accumulated over decades. On most beaches, pellets are now scarce (except after accidental losses of containers transporting pellets at sea, Schumann et al. 2019) and there has been a significant decrease in the proportion of pellets ingested by seabirds in the region (Ryan 2008).

Microfibres have by far been the most common kind of item found on sandy beaches in South Africa (Naidoo et al. 2015, Nel & Froneman 2015, Gerber 2017, Nel et al. 2017, Ryan et al. 2020a; see Table S2) and Mayotte (Jost 2019), and in estuary mouths in South Africa (Naidoo et al. 2015, Gerber 2017). While the composition of microlitter varied among 18 beaches along the Tanzanian coastline, fibres and fragments were found at all sites (Mayoma et al. 2020). Polyethylene and polypropylene were shown to be the main polymer types of fragments and pellets on Tanzanian shores, although it should be noted that only 15 out of a total of 14,681 microparticles found were subjected to ATR-FTIR for their polymer identification (Mayoma et al. 2020), which highlights that more extensive analyses should be conducted in the future whenever possible.

Furthermore, it is important to highlight that results and interpretations on the abundances and composition of meso- and microplastics should be treated with caution, as most of the cited studies did not identify polymer types (e.g. Naidoo et al. 2015, Nel & Froneman 2015, Gerber 2017, Nel et al. 2017, de Villiers 2018, Ryan et al. 2020a), and thus, it is difficult to ascertain the plastic nature of particles and especially fibres, considering that most microfibres in the marine environment are of natural origin (Suaria et al. 2020b). Nevertheless, while not identifying polymers, some of the studies employed other methods to determine the plastic nature of particles, such as following the guidelines for visual identification described by Hidalgo-Ruz et al. (2012) (Naidoo et al. 2015, Ryan et al. 2020a) or using visual identification guidelines in combination with the 'hot needle test' (Gerber 2017). This highlights the importance of determining polymer types in the future to validate the plastic nature of sampled items, preferably by means of spectroscopy techniques such as FTIR or Raman; however, when this is not possible due to different types of constraints, other methods can and should be used as a first approach.

Sea surface

Macrolitter

Observations for floating macrolitter have been conducted off the coasts of Kenya (Okuku et al. 2021a) and off the south-west and south coasts of South Africa (Ryan 1988, 1990, Ryan et al. 2014b), as well as along a transect stretching south-east from South Africa to Marion Island (Suaria et al. 2020a) and a transect running south-east from South Africa to Crozet and Kerguelen Islands (Connan et al. 2021; Table S3). These studies show that macrolitter is orders of magnitude more abundant in coastal waters closer to land-based sources than those farther away, although there is some evidence that litter accumulates in the Southern Indian Ocean gyre (Connan et al. 2021). For example, litter densities were particularly high in coastal waters of Kenya (Okuku et al. 2021a), and in south-west South Africa, litter densities were significantly higher 10 km offshore compared to 50 km offshore (Ryan 1988; Table S3). It is possible that meso-scale coastal processes, which seem to accumulate floating litter in nearshore waters and even push it onto the seashore (van Sebille et al. 2020), are also important drivers of the aforementioned results. In contrast, litter was scarce in the southern fraction of the Agulhas Current and its retroflection (Ryan 1990, Ryan et al. 2014b), in temperate waters south-west of Cape Town, South Africa (Ryan et al. 2014b), as well as in international temperate waters south-east of South Africa (Suaria et al. 2020a; Figure 7B; Table S3).

While the types of macrolitter floating off the coast of Kenya were not specified, >90% of macrolitter off South Africa was plastic, with the most common types being plastic packaging (Ryan et al. 2014b), and larger and more buoyant items being more abundant farther offshore than close to the coast (Ryan 1988, Fazey & Ryan 2016b). Interestingly, plastic packaging items contributed up to 65% in temperate waters and their proportion decreased to 25% in sub-Antarctic waters, whereas the share of fishing/boating items increased from 13% to 25% from temperate to sub-Antarctic waters (Ryan et al. 2014b). These findings agree with those of a global model, which revealed a higher proportion of single-use items in nearshore waters, while a higher contribution of fishing-related items was identified in oceanic waters (Morales-Caselles et al. 2021).

Meso- and microplastics

There have been significant improvements in our understanding of the densities, distribution and characteristics of floating meso- and microplastics since the 2008 UNEP/WIOMSA review. However, few studies used the same mesh sizes or reporting units, making it difficult to directly compare meso- and microplastic densities across the region (Figures 5 and 9B; Table S4), once again highlighting the need for harmonized approaches to microplastics sampling (Twiss 2016, GESAMP 2019, Provencher et al. 2020, Galgani et al. 2021). Also, similar to the case of meso- and microplastics on the seashore, several studies conducted at the sea surface also did not identify polymer types (e.g. Nel & Froneman 2015, Nel et al. 2017, Naidoo & Glassom 2019, Kosore 2020, Okuku et al. 2021a), making it difficult to determine whether the totality of the reported particles corresponded to plastics. Moreover, in some of those studies the methods for preventing contamination of the samples were not well detailed or not conducted, and thus the following results and interpretations should be treated with caution as well.

Among the studies that sampled for floating micro- and mesoplastics with nets (Table S4), the highest densities were found in coastal waters in Kenya (mesh size = 0.3 mm, Kosore 2020, Okuku et al. 2021a) and South Africa (mesh = 0.08 mm, Nel & Froneman 2015; mesh size = 0.2 mm, Suaria et al. 2020a; Table S4). All studies reported that plastics were the most common anthropogenic material caught in the nets. Interestingly, Nel & Froneman (2015) did not find a correlation between microplastic and human population densities and thus suggested that microplastic abundance is more likely governed by surface water currents than proximity to land-based sources. In contrast, Naidoo & Glassom (2019) found the highest concentrations of micro- and mesoplastics at sites close to the urban centre of Durban, thus suggesting that urban run-off is a major source of litter into the sea. Naidoo & Glassom (2019) did, however, also state that large-scale ocean currents and mesoscale coastal processes played a significant role in determining the distribution and accumulation of meso- and microplastics, particularly in winter, which is supported by earlier drift card (Shannon & Chapman 1983) and modelling (Collins & Hermes 2019) studies. In Kenya, seasonal variations (i.e. SE monsoon and NE monsoon) were found to influence microplastic abundances in surface waters, with the highest densities reported during the SE monsoon season (Kosore 2020), as reported for macrolitter on Kenyan beaches (Okuku et al. 2021b).

As for samplings of microplastics and microfibres by collecting bulk surface water samples, Nel et al. (2017) sampled at 16 coastal sites in South Africa, while Preston-Whyte et al. (2021) sampled at nine sites in Durban Harbour. The most polluted site reported by Nel et al. (2017) was Durban Harbour, whereas Preston-Whyte et al. (2021) found significantly lower concentrations in that location. This difference is likely explained by the different mesh sizes used, as Nel et al. (2017) used a 0.063 mm mesh, while Preston-Whyte et al. (2021) used a 0.2 mm mesh, illustrating the importance of considering mesh size when comparing studies (Lindeque et al. 2020), especially for fibres (Ryan et al. 2020d). Both Nel et al. (2017) and Preston-Whyte et al. (2021) found mostly microfibres, although most fibres in surface waters are not synthetic (Suaria et al. 2020b). Both studies also suggested that harbours may be important sources of microplastics and microfibres into the sea because they often receive stormwater and other run-off from adjacent urban areas, which is in agreement with several studies from other parts of the world (e.g. Ballent et al. 2016, Rose & Webber 2019, Ory et al. 2020). More research is needed to determine whether this is true for the rest of the WIO region. Furthermore, no study has examined whether there is a relationship between wastewater discharges and microplastics in coastal waters of the WIO.

Outside of South Africa, bulk water samples have been collected along the coasts of Mozambique and Tanzania (Suaria et al. 2020b), in Kenya's Exclusive Economic Zone (EEZ; Kosore et al. 2018) and within coastal creeks (Kerubo et al. 2020). Suaria et al. (2020b) used two different mesh sizes (0.7 µm and 0.025 mm) and found a median density of 1200 items·m^{-3}, although there was large variation between samples (interquartile range = 300–3000 items·m^{-3}). They showed that most particles were microfibres and that >90% were of natural materials (cellulose or animal origin) rather than synthetic. In Kenya, Kosore et al. (2018) used a 0.25 mm mesh and found an overall density of 110 items·m^{-3}. They also found that most particles were fibres, but unlike Suaria et al. (2020b), Kosore et al. (2018) only identified synthetic polymers, of which polypropylene (PP) was the most common. For the surface waters of three creeks along the Kenyan coast, Kerubo et al. (2020)

reported a considerably higher overall mean microplastics density (2898 items·m^{-3} using a mesh of 0.02 mm), of which 93% were fibres.

Water column

Macrolitter

No systematic studies have been conducted to study macrolitter in the water column in the WIO region. Some macrolitter observations off the south and south-west coasts of South Africa have detected sub-surface litter, but it is unclear what proportion of litter was missed due to poor visibility below the surface of the water (Ryan 1988, 1990, Ryan et al. 2014b), and thus, they do not provide clear information about macrolitter densities in the water column. Litter in the water column is either made of materials with a higher density than seawater that will automatically sink, or litter that has become colonized by epibionts, which reduces its buoyancy (Fazey & Ryan 2016a,b). For example, an LDPE bread bag has recently been found at a depth of 185 m off the coast of South Africa (Ryan et al. 2020c). The date stamp on the bag indicated that it was only 3 months old and, judging by the size of the goose barnacles growing on it, it had floated at sea for 20–30 days before becoming fouled enough to sink (Ryan et al. 2020c). Litter that sinks in this way could 'yo-yo' up and down the water column (see below) or become trapped on the seabed if it becomes buried in bottom sediments or further fouled by benthic organisms.

Meso- and microplastics

Only one study sampled for microlitter in the water column in the WIO region by collecting bulk water samples from the ship's underway system (5 m depth) along a transect from Cape Town to the Prince Edward Islands (Ryan et al. 2020d; Table S5). The study showed that microfibres are significantly less abundant in the water column than at the sea surface and that most microfibres are of natural origin rather than made of plastic polymers (Ryan et al. 2020d, Suaria et al. 2020b). Interestingly, a recent study conducted in the Atlantic Ocean by Pabortsava and Lampitt (2020) found considerable loads of meso- and microplastics suspended in the water column down to 200 m depth. When extrapolated to the whole ocean, these high loads could account for the estimated plastic inputs into the ocean since 1950 (Pabortsava and Lampitt 2020), thus providing a possible answer to the long-time enigma of where all the plastics that have once entered the ocean have gone. In light of the results by these authors in the Atlantic ocean, and since the only study conducted in the water column of the WIO region sampled at a shallow depth of 5 m (Ryan et al. 2020d), it would be interesting to conduct a large-scale study similar to that of Pabortsava and Lampitt (2020) in the WIO waters. Furthermore, while several other studies have sampled for microplastics in the water column in other parts of the world's oceans (e.g. Reisser et al. 2015, Kanhai et al. 2018, Choy et al. 2019, Erni-Cassola et al. 2019), this remains one of the least studied marine habitats globally and there is an urgent need for more research on the amounts, vertical distribution, movement and fate of all sizes of marine litter in the water column.

Seafloor

Macrolitter

Among the studies on seafloor macrolitter (Table S6), the highest litter densities were found using remotely operated vehicles (ROVs) at deep-sea sites (100–1500 m deep) along the Southwest Indian Ocean Ridge >1300 km south of Madagascar (Woodall et al. 2015). Most of this litter was fishing gear, suggesting that the deep seafloor off the coast of southern Africa might be a long-term sink for litter originating from fisheries. In contrast, trawl surveys conducted on the continental shelf

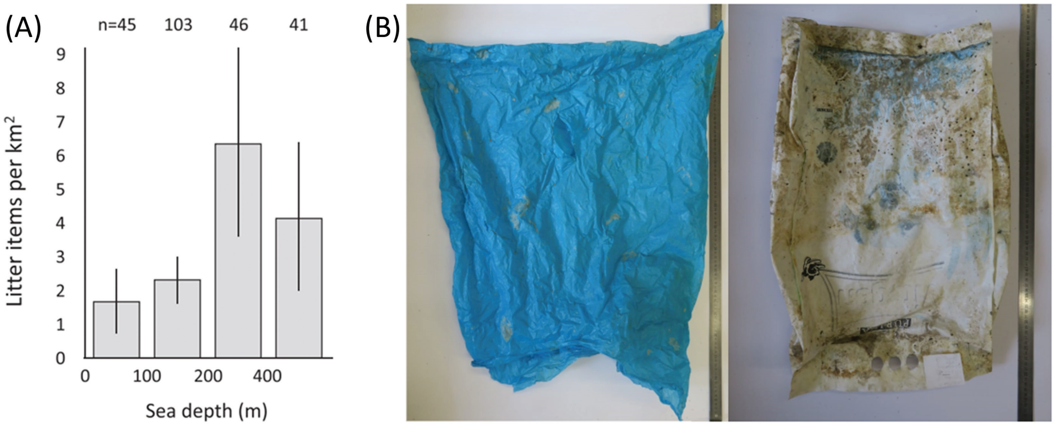

Figure 10 (A) Litter densities on the continental shelf of South Africa. From Ryan et al. (2020c). (B) Examples of the trawled-up litter samples. Photos: Peter Ryan.

(30–850 m deep) off the south and west coasts of South Africa found significantly lower densities of litter (Ryan et al. 2020c), with highest densities around the shelf break (Figure 10). Most of this litter was plastic packaging (48%) and discarded fishing gear (17%, Ryan et al. 2020c), which is in agreement with the average litter composition in nearshore seafloors worldwide (Pham et al. 2014, Morales-Caselles et al. 2021) and suggests that items littering the seafloor originate from both land-based sources and at-sea activities (Ryan et al. 2020c). The low litter densities reported by Ryan et al. (2020c) were confirmed by ROV footage taken in several habitat types on the South African continental shelf and slope, which found almost no litter (Ryan 2020b).

Mulochau et al. (2020) conducted dive transects on reefs fringing Mayotte (Comoros Archipelago) where they found similar litter densities in both winter and summer. More than 90% of litter was plastic (Table S6), with >60% fishing gear, but also numerous bags and bottles probably washed into the sea with urban and river run-off (Mulochau et al. 2020). Given that plastics have been associated with diseases in coral reefs (Lamb et al. 2018), more research is needed to determine the density, types and possible sources of marine litter on other coral reef systems in the rest of the WIO region.

Meso- and microplastics

Similar to the studies conducted in the other compartments, it is also difficult to directly compare the studies reporting microplastic densities in bottom sediments in the WIO region because they used different filter sizes (Figure 5D; Table S7). In Durban Harbour, Preston-Whyte et al. (2021) found largely variable microplastics densities among nine sites (2400–111,933 microplastics·kg^{-1} dry weight sediment), with the highest densities found close to point sources such as sewage overflows, stormwater drains and river mouths. Most items were PE (47%) although it is unclear why such a high proportion of fibres in sediment were buoyant (density of PE = 0.88–0.96 g·cm^{-3}). Interestingly, 21% of fibres were made of cellophane, which supports the findings of Suaria et al. (2020b) that a high proportion of microfibres collected from environmental samples are of natural origin. In contrast, at deep-sea sites, Woodall et al. (2014) found 1.4–4 fibres·50 mL^{-1} sediment, all of which were polyester (Woodall et al. 2014). A modelling study releasing virtual particles from the main South African harbours indicated that non-buoyant plastic particles accumulate along the continental shelf and on the western edge of the Agulhas Bank (Collins & Hermes 2019).

Sources, transport and fate of marine litter and microplastics in the WIO

Land-based sources

The published literature confirms that land-based sources account for most of the litter entering the WIO marine environment. For example, field surveys in South Africa (Gerber 2017, de Villiers 2018, Ryan et al. 2018, Ryan 2020b, Ryan & Perold 2021), Kenya (Okuku et al. 2020b, Ryan 2020a) and Tanzania (Mayoma et al. 2020) show that both micro- and macrolitter on shorelines are concentrated around large coastal urban centres or river mouths, suggesting that most litter enters the sea as part of urban and river run-off. Indeed, the national hotspotting assessments implemented in these countries and Mozambique indicate that low waste collection rates coupled with high rates of improperly disposed waste (due to a lack of official sanitary landfills and incineration facilities in these continental countries) are major drivers of the plastic waste leakage into rivers and consequently to the ocean (IUCN-EA-QUANTIS 2020a,b,c,d). This is further supported by brand audits in South Africa (Madzena & Lasiak 1997, Ryan & Perold 2021, Ryan et al. 2021) and Kenya (Okuku et al. 2020b, 2021b, Ryan 2020a), which show that most beach litter close to urban centres is locally manufactured. However, as suggested by a higher proportion of foreign brands on a Kenyan beach during the SE monsoon over the NE monsoon, some litter also arrives from the ocean and the proportion varies seasonally (Okuku et al. 2021b). On remote beaches, the proportion of foreign bottles increases to more than 50% due to inputs from shipping and long-distance drift (Ryan 2020a, Ryan et al. 2021). The most important land-based sources are described in detail below.

Illegal dumping, tourism and beachgoers

For most WIO countries, beaches are one of the main tourist attractions and beachgoers contribute significantly to local and national economies. However, tourists and beachgoers can also negatively affect local marine environments by littering, which is supported by several studies that show higher densities of beach litter on popular tourist beaches (e.g. Gjerdseth 2017, Okuku et al. 2020b), particularly during the summer, when more people presumably visit the coast (Barnardo et al. 2021). Most of the litter left by beachgoers is packaging, particularly single-use food-packaging items (e.g. Okuku et al. 2020b, Barnardo et al. 2021). Local residents can also contribute significant amounts of litter (as suggested by, for example, Mattan-Moorgawa et al. 2021), especially in areas with poor waste management and service delivery. For example, Gjerdseth (2017) noted that a common practice in northern Madagascar is for residents to leave their rubbish on the edge of their property where it can easily be blown onto the beach, resulting in high litter densities on the backshore.

Importantly, global concern has recently risen around a newer type of litter mainly left by beachgoers, namely personal protective equipment (PPE) items associated with the COVID-19 pandemic, such as face masks and plastic gloves. In the WIO region, the abundance of COVID-related litter has been studied and reported on 25 Kenyan beaches (Okuku et al. 2021a). Although the recorded densities of PPE litter were considerably higher in Kenya than on beaches in other regions (e.g. De-la-Torre et al. 2021, Haddad et al. 2021, Thiel et al. 2021b), they were still relatively low (<0.01 items m^{-2}) and accounted for only 0.43% of the total litter items, which was attributed to lockdown and the closure of beaches for recreation (Okuku et al. 2021a). Along the same vein, while touristic and recreational beaches presented the highest abundances of PPE litter in Peru and Morocco (De-la-Torre et al. 2021, Haddad et al. 2021), in Kenya the most polluted with this type of litter were remote beaches, instead of touristic and urban beaches, which the authors partly attributed to a lower compliance of rural areas with the government's regulations on closing public beaches (Okuku et al. 2021a).

Industries, sewage and wastewater effluent

Industries may also act as significant sources of litter into the environment. Beach surveys around the coast of South Africa showed that industrial pellets were concentrated around urban centres where most plastic-producing industry is located, suggesting that at least historically many pellets were released into the environment from manufacturing plants that did not dispose of their waste properly (Ryan et al. 2018). This was supported by a more recent study in Cape Town, South Africa, which found industrial pellets being released into the Salt River via urban run-off from storm drains (Weideman et al. 2020d). However, as noted earlier, the density of industrial pellets on many South African beaches (Ryan et al. 2018) and the proportion ingested by seabirds (Ryan 2008) have significantly been reduced since the implementation of Operation Clean Sweep (https://www.opcleansweep.org/), showing that proper waste management is key to reducing the amount of litter released into the environment. A recent spill of industrial pellets in Durban Harbour indicated that most pellets were retained in close vicinity to their point of origin, but that moderate winds could lead to long-distance transport of these pellets (Schumann et al. 2019). No research has been conducted on the level of contamination of treated wastewater and sewage sludge in the WIO region, thus highlighting a major knowledge gap in our understanding of the sources of microplastics and microfibres. However, given the high proportions and densities of microfibres on sandy beaches and estuaries, some studies have suggested that wastewater is likely an important source of microfibres and other microplastics (Gerber 2017, de Villiers 2018, Jost 2019).

Rivers and urban run-off

Urban run-off has been suggested as one of the main land-based sources of litter in some WIO countries, given the ‘haloes’ of litter seen around urban centres in South Africa (Ryan et al. 2018, Ryan 2020b; Figure 11), Kenya (Okuku et al. 2020b, Ryan 2020a) and Tanzania (Mayoma et al. 2020), and the high proportion of locally manufactured goods found on urban beaches (Ryan 2020a, Ryan et al. 2021). Similarly, Jost (2019) inferred that most litter seen on beaches in Mayotte is washed into the sea and onto beaches via urban run-off. Additionally, several studies in South Africa have shown that significant amounts of street litter are washed into the sea via storm drains, with the highest litter loads found in industrial and low-income residential areas with poor service delivery (Armitage & Rooseboom 2000, Marais et al. 2004, Weideman et al. 2020d; Table S8). However, no data are available on the amount of litter in stormwater and other drainage systems in any of the other WIO countries, making it difficult to quantify how much litter is released into the sea as part of urban run-off.

Urban run-off and rivers go hand in hand because many stormwater systems drain directly into canals and rivers that can then transport litter to the sea. In the WIO region, no field studies have quantified the amount of litter washing into the sea from rivers for any countries except South Africa (Weideman et al. 2020c, Moss et al. 2021; Tables S8 and S9). These show that litter loads tend to be highest in rivers whose catchments are highly urbanized or industrialized (Swanepoel 1995, Moss et al. 2021), with significantly lower amounts of litter seen in rivers in areas with low population densities such as at the mouth of the Orange River (Weideman et al. 2020c). Further inland, Weideman et al. (2020c) found the highest densities of meso- and macrolitter in the Vaal River downstream of the Johannesburg-Pretoria conurbation, but this litter was not transported far downstream, suggesting that river sediments and riparian vegetation are significant sinks for litter (Verster & Bouwman 2020, Weideman et al. 2020c). The litter that is transported is washed into mangroves, into the sea and onto beaches by seasonal rains, as was validated by the higher litter loads on urban beaches on the south-west coast of South Africa at the beginning of the rainy season (Chitaka & von Blottnitz 2019, Weideman et al. 2020a,b). Ryan & Perold (2021) showed that the movement of litter between estuaries, the sea and beaches can be complex, with rain and

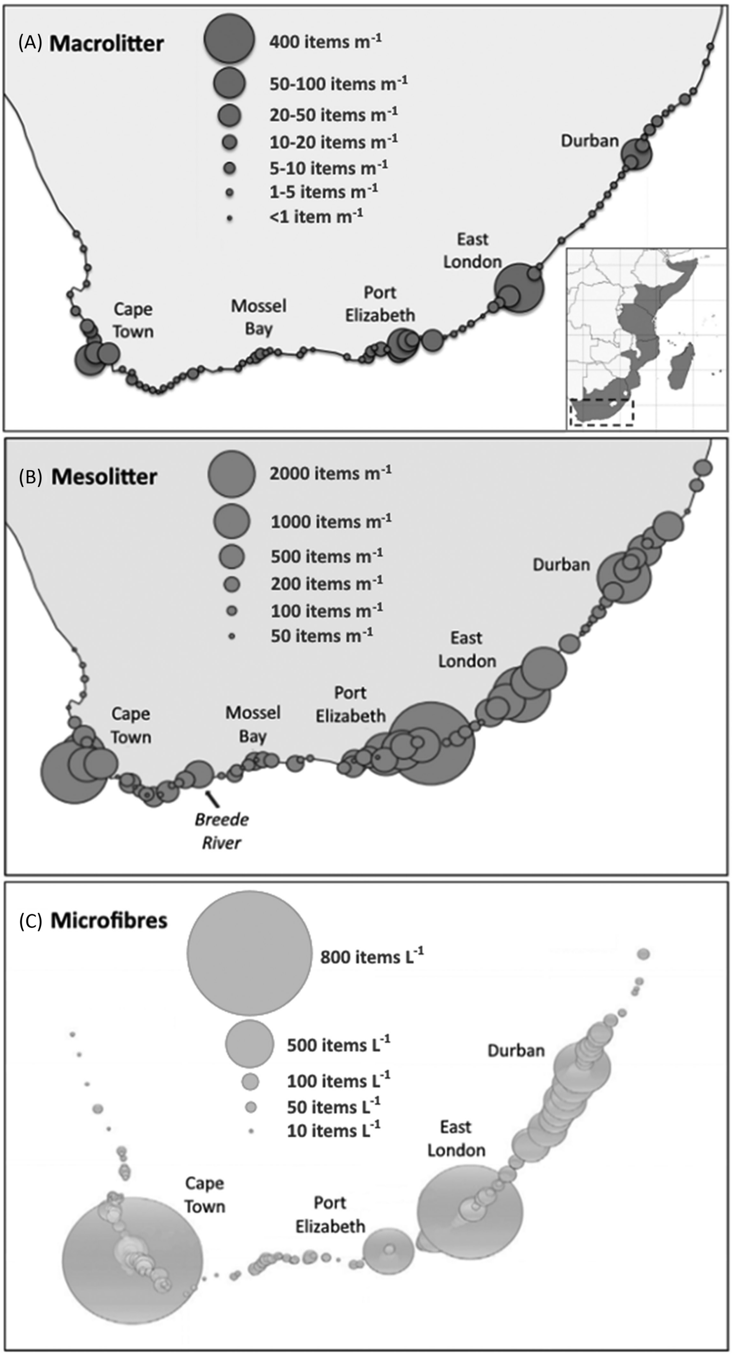

Figure 11 Maps showing how (A) macrolitter, (B) mesolitter and (C) microfibres sampled on sandy beaches are concentrated close to large coastal urban centres in South Africa (study area shown in the dashed square inside insert in A). Densities of macrolitter, mesolitter and microfibres are represented by the circles, increasing with circle size. Panels were modified after Ryan (2020b), Ryan et al. (2018) and de Villiers (2018), respectively. The scales in panels A and B were included in the original figures by the indicated authors, whereas those in panel C were approximated according to the database provided by de Villiers (2017; Creative Commons CC-BY-3.0 license).

tides playing a significant role in determining how litter is transported and where it is retained (see above). However, this has not been studied in other areas of the WIO and is an important knowledge gap, especially given that studies in other parts of the world show that litter can become trapped in estuaries for many years (e.g. Tramoy et al. 2020), or, conversely, can be rapidly flushed downstream, reaching the ocean without much retention nor accumulation, as has been suggested for rapid, dynamic mountain rivers (Honorato-Zimmer et al. 2021).

Sea-based sources

While most litter enters the sea from land-based sources, a significant amount still originates at sea, for example from fishing and shipping activities or via long-distance drift (which could originate from either land- or sea-based littering). These different sea-based sources are summarized below.

Fishing and shipping

Discarded fishing gear is commonly found across the WIO region (e.g. Burt et al. 2020, Okuku et al. 2020b), although the proportion of fishing gear versus other types of litter varies regionally and temporally. For example, fishing gear and other shipping-related gear tend to make up a larger proportion of litter mass on remote beaches (Ryan 2020b) and islands (Burt et al. 2020) than on beaches close to land-based source areas. Similarly, fishing gear has by far been the most common type of litter at deep-sea sites south of Madagascar (Woodall et al. 2015), suggesting the deep sea is a sink for debris from pelagic fisheries. In contrast, trawl surveys along the continental shelf of South Africa found a mix of fishing gear and other types of litter, suggesting that litter originates from both sea- and land-based sources (Ryan et al. 2020c). The abundance of fishing gear can also vary temporally; for example, the proportion of fishing-related litter regurgitated by seabirds on Marion Island decreased dramatically after the collapse of the Patagonian toothfish fishery around the islands (Perold et al. 2020).

Litter can also be dumped or lost from shipping activities. For example, date stamps on many of the foreign bottles that wash up along the coast of South Africa show that bottles were found within a few months of manufacture, which is too short a period for them to have drifted from their country of origin. Rather, these bottles are likely illegally dumped from ships (Ryan et al. 2019, 2021). There have also been several accidental industrial pellet spills when containers full of pellets were lost from ships off the coast of South Africa, releasing tens of tonnes of pellets into the sea (e.g. Schumann et al. 2019). Tracing a spill that occurred in 2017 in Durban Harbour, Schumann et al. (2019) found that pellets disperse across the ocean remarkably fast due to prevalent winds, waves and larger-scale ocean currents, emphasizing that ocean regions are intimately connected and thus marine litter is not a localized issue. The authors further confirmed the important role of wind for the transport of microplastics in the ocean, given that pellets tended to stay for long periods in certain sections of the coast, where they could only be removed by sustained wind events (Schumann et al. 2019).

Long-distance drift

East Africa is downstream of Southeast Asia, which is estimated to be the world's largest contributor to land-based plastic into the sea (Jambeck et al. 2015, Lebreton et al. 2017). Lagrangian drift simulations predict that ~1%–5% of litter released from Southeast Asia is transported by surface currents to the Southern Indian Ocean (see below for details), where much of it strands on WIO island states and along the east coast of Africa (van der Mheen et al. 2020), highlighting the important role of WBCs as drivers of floating litter transport and deposition in the WIO region. This is further supported by the presence of Asian-branded litter items on shorelines in

South Africa (Ryan & Perold 2021, Ryan et al. 2021), Kenya (Ryan 2020a) and in Alphonse Island in the Seychelles (Duhec et al. 2015). Most of such items stranded in Kenya and eastern South Africa corresponded to HDPE bottles manufactured in Indonesia, which were 4–6 years old, were heavily fouled by epibionts and presented bite marks; together, these features on the bottles suggest long periods of time floating at sea, thus probably coming from their country of origin (Ryan 2020a, Ryan et al. 2021). However, given that >80% of litter on Kenyan and South African beaches is locally manufactured, the contribution of litter via long-distance drift is likely moderate to low when compared to local sources, at least for continental countries on the African east coast. This highlights the need for local solutions to the marine debris problem (see below). In contrast, a high proportion of litter stranded on the remote islands of the WIO clearly originates from offshore sources (e.g. Duhec et al. 2015, Burt et al. 2020), most of which likely comes from Southeast Asia, and the cost of removing this litter is extreme (Burt et al. 2020). There is therefore also a need for stricter international regulations to be put in place to limit land-based leakage of litter and to provide potential mechanisms for severely impacted countries to seek support from polluting nations in tackling local litter issues.

Transport and fate of marine litter

Beaches are major sinks for marine litter

In the WIO region, oceanographic models predict that most buoyant litter entering the sea strands on local shorelines close to source (Collins & Hermes 2019, van der Mheen et al. 2020, Chenillat et al. 2021). While some of these models do not take into account important processes such as the variability in sinking rates and fragmentation of plastic particles, their findings are supported by the concentration of macro- (Ryan 2020b), meso- (Ryan et al. 2018) and microlitter (de Villiers 2018) around coastal urban centres in South Africa (de Villiers 2018, Ryan et al. 2018, Ryan 2020b; Figure 11), Kenya (Okuku et al. 2020b, Ryan 2020a) and Tanzania (Mayoma et al. 2020), and close to estuaries and river mouths in South Africa (Gerber 2017, Ryan & Perold 2021). This is further supported by experimental releases of marked plastic and wood blocks in South African rivers in both the east and south-west coasts, which show that at least 80% of litter strands within a few kilometres of the river mouth (Maclean et al. 2021, Ryan & Perold 2021). Strong onshore transport also drives floating litter coming from sea-based sources onto local shores of the WIO countries as shown by drift card and drifter studies (Shannon & Chapman 1983, Steinke & Ward 2003, Semba et al. 2019). Together, these studies suggest that shorelines in the WIO region are major sinks for litter, supporting this review's hypothesis that WBC systems promote stranding of floating litter on the seashore, as opposed to EBCs (Ryan 2020a).

The stranding rate and turnover of marine litter on shorelines does, however, depend on several factors. For example, items made from polymers that are more dense than seawater are more likely to be transported offshore in undertow currents (Collins & Hermes 2019), whereas less dense polymers with high windage such as expanded polystyrene or sealed empty bottles that float well above the water surface are more easily blown onto the shore (Maclean et al. 2021, Ryan 2020b). However, it is difficult to predict the fate of wind-blown marine litter: it can be blown inland and become trapped in coastal vegetation during periods of strong onshore winds, but might also be blown back into the surf zone by offshore winds (Brennan et al. 2018). This is particularly true for lightweight items such as expanded polystyrene, which have particularly high turnover rates on beaches (Ryan et al. 2014a). Once litter re-enters the surf zone, high-density items are again more likely to be carried offshore, although these can be washed back onto shorelines during upwelling events (Spencer 2020, Weideman et al. 2020a,b). Low-density items may once again be blown onto the shore where they can be buried, trapped in coastal vegetation or return into the surf zone (Ryan 2020b). These movements and dynamics are also strongly affected by the complex small- and meso-scale processes that occur in coastal waters (van Sebille et al. 2020). Stranded litter can also be picked up by beachgoers, although the impact of cleaning depends on the frequency of cleaning programmes and the number of cleaners (Ryan 2020b). Beach cleaners also often target larger items, and cleaning efforts thus have a greater impact on the mass of litter on beaches rather than on the number of items (Ryan et al. 2009, Ryan 2020b).

The type of habitat and its physical characteristics also influence the turnover rate of stranded litter. Rocky shores may act as sinks for high-density items (Weideman et al. 2020b), while the turnover rate of litter is higher on coarse or pebbly beaches with steep slopes (from which litter is easily washed back into the surf zone) than on gently sloping sandy beaches (Ryan 2020b) or beaches with back-vegetation that trap litter items (Ryan et al. 2014a, Okuku et al. 2020a). On sandy beaches, litter can become buried under the sand, but few beach surveys sample for buried litter, making it difficult to determine the turnover rate of buried litter for much of the WIO region (Ryan et al. 2020a; see above).

Dispersal of floating litter that does not strand on beaches

While much land-based litter strands on shorelines close to where it was washed into the sea, a small proportion may also be transported offshore (Duhec et al. 2015, Collins & Hermes 2019, van der Mheen et al. 2020, Chenillat et al. 2021). For example, Lagrangian drift simulations predict that ~5%–10% of litter released from east African rivers will be transported to the open ocean (van der Mheen et al. 2020), where it is predicted to either accumulate along salinity fronts (as reviewed by Pattiaratchi et al. 2021), particularly where rivers enter the sea (e.g. Acha et al. 2003, Ryan 2020b), or be retained within the Indian Ocean gyre (Cózar et al. 2014, Eriksen et al. 2014, van der Mheen et al. 2019, 2020, Chenillat et al. 2021, Connan et al. 2021). Drift card studies revealed that some floating items may get entrained into the Benguela Current, whereas others may enter the West Wind Drift, being transported towards Australia and New Zealand (Shannon & Chapman 1983, Steinke & Ward 2003). However, more at-sea surveys are needed to determine the extent of these accumulation zones in the WIO region (Connan et al. 2021, Pattiaratchi et al. 2021). It is not surprising that such a small proportion of litter is predicted to be transported offshore given that the east African coast is a WBC. Here, strong onshore currents wash debris back onto beaches (Chenillat et al. 2021), compared to EBCs where upwelling carries floating litter offshore (Ryan 2020a). In support of this, ocean circulation models predict that >90% of litter released off the east coast of South Africa strands on local beaches, compared to only 19% of litter released from Cape Town on the south-west coast (Collins & Hermes 2019). However, the models assumed all litter from Cape Town was released into Table Bay, but litter released into False Bay is much more likely to strand due to the semi-enclosed nature of the bay (Ryan 2020b). Furthermore, the models did not account for nearshore conditions and assumed all litter was released 8–10 km offshore and it is therefore unclear how accurate they are (Ryan 2020b).

Lagrangian drift simulations predict that litter entering the Northern Indian Ocean during the NE and SW monsoons will be transported back and forth between the Bay of Bengal and the Arabian Sea by seasonal surface currents (Figure 12; van der Mheen et al. 2020, Pattiaratchi et al. 2021). Almost all of this litter is predicted to beach along the Northern Indian Ocean coastline within a few years of being washed into the sea, although a small proportion may be transported south by the Somali Current where it is predicted to beach in Somalia (van der Mheen et al. 2020, Pattiaratchi et al. 2021) or move offshore into the SECC (see above). However, in the inter-monsoon period, following the SW monsoon (September–November), up to 5% of litter released into the Northern Indian Ocean is predicted to be transported eastwards along the equator by semi-annual Wyrtki Jets (dashed grey line in Figure 2) where it is picked up by the South Java Current and transported south-east across the equator (van der Mheen et al. 2020). The South Java Current then feeds into the South Equatorial Current, which transports the litter south-west into the Southern Indian Ocean. This litter can then be transported south along the east coast of Madagascar by the

Southeast Madagascar Current, north along the coasts of Tanzania, Kenya and Somalia by the Northeast Madagascar, East African Coastal and Somali Currents, or south along the coast of South Africa by the Agulhas Current (Figure 12; van der Mheen et al. 2020). All these currents frequently form cyclonic or anticyclonic eddies (Figure 12), which may temporarily enclose water masses and concentrate floating litter, as reported for Gulf Stream rings (Brach et al. 2018). How long litter is retained in these eddies is unknown, but it is likely that it is spilled where the eddies break up when hitting the continental shelf. Much of this litter is expected to beach on WIO island states and along the east African coast, and field surveys in Seychelles (Duhec et al. 2015), Mauritius (Bouwman et al. 2016), South Africa (Ryan & Perold 2021, Ryan et al. 2021) and Kenya (Okuku et al. 2020b, Ryan 2020a) have found Asian-branded packaging covered in epibionts or with fish bite marks, indicative of long periods adrift.

Litter entering the Southern Indian Ocean from the west coast of Australia can also cross the Indian Ocean and eventually beach in east Africa (Trinanes et al. 2016, Pearce et al. 2019). Several modelling studies using a variety of data predict that litter released off the Australian west coast is transported in three phases: litter first becomes entrained in the Leeuwin Current eddy system where it can be retained for up to a year; it then enters the westward-flowing South Equatorial Current, which transports it across the Southern Indian Ocean; and it is then picked up by the WBC system off southern Africa where it can be transported along the east African coast to strand on WIO shorelines (Figure 12; Trinanes et al. 2016, Pearce et al. 2019, van der Mheen et al. 2019, 2020). However, Australian bottles are rare (<1% of foreign bottles) along the coasts of Kenya (Ryan 2020a) and South Africa (Ryan et al. 2021), which is likely due to Australia's effective waste management system (Jambeck et al. 2015) or because ships docking in Australia manage their waste appropriately (given that most foreign PET bottles probably are discarded illegally from ships; Ryan 2020a, Ryan et al. 2021).

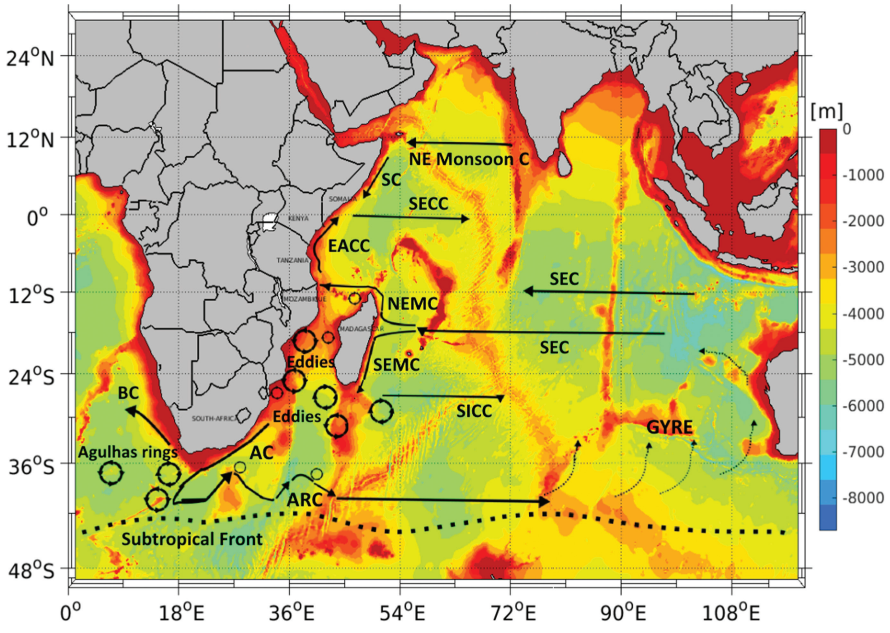

Figure 12 Indian Ocean with the main current systems in the WIO region; also shown are the areas where eddies frequently form, likely influencing the transport dynamics of marine plastic litter. Modified from Halo & Raj (2020).

Transport by marine animals

Animals may act as transport vectors of marine litter onto land. For example, birds often incorporate marine litter into their nests (Nel & Nel 1999, Witteveen et al. 2017, Perold et al. 2020, Ryan 2020c, Tavares et al. 2020; see below for details) and marine predators such as seabirds and seals may transport litter onto land when they haul out to moult, either by regurgitating or by excreting ingested litter (Ryan 2020b, Perold et al. 2020). Any ingested litter will also be retained on land when an individual dies (Nel & Nel 1999). However, despite the large populations of many of these marine predators, this behaviour probably only accounts for a very small amount of marine litter in the WIO region due to the low ingestion rates for seals (Ryan et al. 2016b) and low plastic loads for most species of seabirds (Ryan 2020b).

The seabed as a long-term sink for marine litter

Several studies have suggested that the seabed is a long-term sink for marine litter (e.g. Woodall et al. 2014), especially for materials that have a higher density than seawater or items that have become less buoyant due to fouling by marine organisms. This has been supported by surveys on South African beaches, which showed that buoyant items dominate stranded litter on beaches farther from source areas (Fazey & Ryan 2016b) and that most items collected in trawls off the coast of South Africa floated after being cleaned (Ryan et al. 2020c). However, the limited data on the characteristics and densities of litter on the seafloor of the WIO region (see above) make it difficult to ascertain to what degree the seafloor acts as the ultimate sink for marine litter.

Floating marine litter can also sink to the seafloor when it becomes colonized by epibionts, decreasing its buoyancy. For example, experiments conducted in shallow coastal waters of South Africa showed that small, tethered items with large surface area-to-volume ratios tended to sink within 2–3 weeks, while fouling rates were much slower on larger items with small surface area-to-volume ratios, which only sank after >2 months (Fazey & Ryan 2016a). However, it remains unclear whether fouling rates vary seasonally, how tethering affects fouling rates, and whether fouling rates differ between inshore versus offshore waters (Ryan 2020b). The fate of items that sink in this way also remains unclear. In shallower waters, litter probably sinks to the seafloor where it can quickly become fouled by benthic organisms and retained in sediment. In contrast, litter that sinks in deeper waters may move up and down the water column because epibionts will die and fall off as the item sinks below the photic zone (thus increasing its buoyancy), but recolonize the litter (and again decrease its buoyancy) as it moves back up to shallower waters (Ye & Andrady 1991, Kooi et al. 2017). However, if an item reaches the seafloor before surface epibionts fall off, it will likely be further fouled by benthic organisms or become weighed down by sediment and thus retained on the seafloor. While the increase in seawater density with depth may also affect the buoyancy of litter and slow down or even limit its sinking, leading litter to remain suspended at a certain depth where its density is similar to that of the surrounding water (Kowalski et al. 2016, Wang et al. 2016), this and the previous points need to be further studied to better understand the dynamics of litter in the deeper ocean in the WIO region.

The ecological impacts of marine debris

More than one-third of the 147 reviewed studies (37%) reported interactions between organisms and marine litter or microplastics. Interactions have been documented in all countries except in Comoros and Somalia (Figure 4), and the most commonly documented interactions were ingestion and entanglement (Figure 13). It should be noted, however, that we did not include entanglement in active fishing gear, although it is often hard to differentiate entanglement in ghost gear from

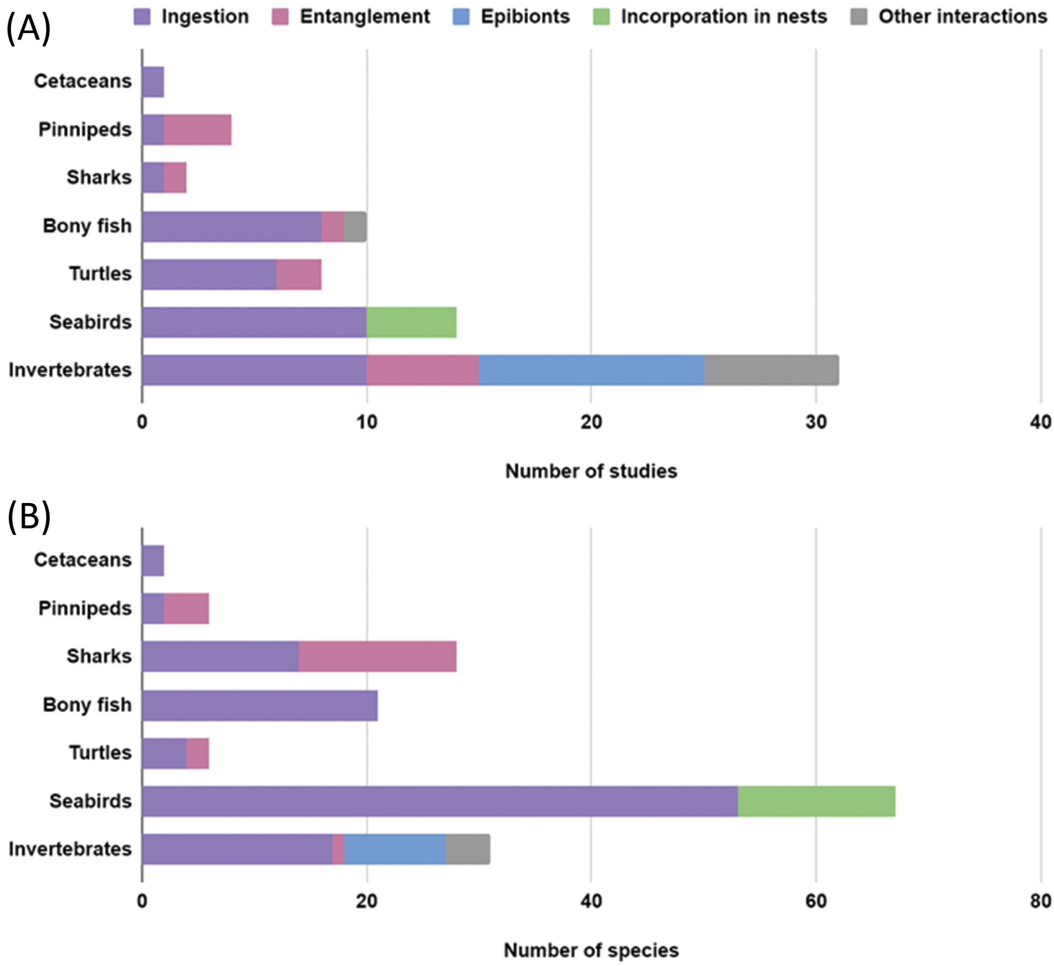

Figure 13 (A) The number of studies reporting on interactions between biota and marine litter or microplastics per studied taxon and type of interaction (total $n=55$ studies); some studies report data on more than one taxon and/or more than one type of interaction and are thus included in all the respective categories. (B) The number of studied species per taxon and type of interaction; only organisms identified to the species level were included in this figure, as well as non-identified organisms when only one individual was sampled/recorded within a taxonomic group. 'Other interactions' include the incorporation of microplastics in the tube structure of polychaetes, as well as the use of marine litter as sunshades, as camouflage or as habitat.

by-catch in active gear (Ryan 2018). Entanglement in active gear appears to be a significant problem for whales, which often become entangled in the buoy lines attached to crayfish or octopus traps (e.g. Best et al. 2001, Meÿer et al. 2011), and sharks and sea turtles, which easily become entangled in drifting FADs (Filmalter et al. 2013, 2015, Poisson et al. 2014, Balderson & Martin 2015, Forget et al. 2015, Maufroy et al. 2015, Bonnin et al. 2020).

There were also studies reporting the presence of epibionts on marine litter, although most of them did not identify the organisms to the species level, but rather reported the broader taxa (Figure 13A and B). Marine litter was also found and reported in the nests of seabirds, as well as interacting with organisms in less common ways, such as being incorporated into the tube structures of polychaetes, or being used as sunshades, camouflage and habitat (Figure 13).

Ingestion

Plastic ingestion has been studied and recorded in several groups of organisms in the WIO region, such as seabirds, sea turtles, fish, marine mammals and invertebrates, and the sum of species that were found to have ingested plastics is 103 species out of 113 species that have been sampled (i.e. ten species that were examined had no signs of plastic ingestion; Table 3).

The most well-studied group is seabirds, and 94% of all seabird species studied so far have been shown to ingest plastic litter (Table 3). Studies of seabirds ingesting marine litter have largely focused on those species that forage widely across the south-west Indian, Atlantic and Southern Oceans and breed on sub-Antarctic islands (Table S10). Thorough reviews of plastic ingestion by these seabirds include Ryan (1987b), who recorded plastic ingestion in 36 out of 60 species and showed that ingestion was most frequent in Procellariiformes, and Ryan (2008), who reported a decrease of 44%–79% in the proportion of ingested virgin pellets in five species of seabirds from the 1980s to 2006. Interestingly, Ryan et al. (2016b) analysed the ingestion of plastics >0.5 mm in seven species of albatrosses off South Africa and only found plastics in the stomachs of one species (shy albatross *Thalassarche cauta* sensu lato), highlighting the importance of also reporting negative records. Albatrosses and giant petrels breeding on Marion Island regularly regurgitate fishing gear and other plastic items at their nests, but litter loads tend to be low (Nel & Nel 1999, Perold et al. 2020), especially compared to burrow-nesting species (Ryan 1987b). On La Réunion and Juan de Nova islands, the most common types of litter ingested by seabirds were fibres and fragments, which were found in the stomach contents of nine species of seabirds, of which tropical shearwaters (*Puffinus bailloni*) and Barau's petrels (*Pterodroma baraui*) were the most affected (Cartraud et al. 2019). Interestingly, Vanstreels et al. (2020) reported that adult female African penguins (*Spheniscus demersus*) possibly supplement their consumption of calcium by ingesting seashells, and that anthropogenic litter might be accidentally ingested in the process. However, this interpretation should be treated with caution, given that the frequency of occurrence of litter ingestion by penguins was very low (Vanstreels et al. 2020).

Plastic ingestion has been studied in four species of sea turtle, with samples collected in both south-east and south-west South Africa (Hughes 1973, Ryan et al. 2016a), Mayotte (Comoros Archipelago; Claro & Hubert 2011, Chebani 2020), La Réunion (Claro & Hubert 2011), the Seychelles (Stokes et al. 2019) and in oceanic waters around La Réunion and Madagascar (Hoarau et al. 2014, Barret et al. 2018). All four species were found to have ingested plastic debris (Tables 3 and S11), with the first records dating back almost 50 years, when plastic bags, strips, sheets and beads were found in the stomach contents of loggerhead turtles (*Caretta caretta*) in South Africa (Hughes 1973).

Overall, loggerheads have been the most studied sea turtle species (Hughes 1973, Ryan et al. 2016a, Hoarau et al. 2014, Barret et al. 2018) and are also the species with the highest proportion of individuals with ingested litter (Claro & Hubert 2011). Hard-backed turtles (Cheloniidae) switch from pelagic to benthic foraging as they mature, and pelagic juveniles are therefore most likely at risk given the prevalence of plastics floating on the sea surface (Ryan et al. 2016a). Indeed, all individuals sampled around La Réunion and Madagascar were juveniles foraging on pelagic prey (Hoarau et al. 2014). In contrast, leatherback turtles (*Dermochelys coriacea*) feed on jellyfish at the sea surface and the water column and are thus at risk of ingesting marine debris throughout their lives.

In the WIO region, 21 species of bony fishes and 14 shark species have been sampled for ingested marine litter, with most of them being in South Africa (Tables 3 and S12). Microlitter (microfibres and fragments) was found in the stomachs of all species of bony fishes sampled (Tables 3 and S12; Naidoo et al. 2016, 2017, Ross 2017, Bakir et al. 2020, Chebani 2020, McGregor & Strydom 2020, Naidoo et al. 2020a, Sparks & Immelman 2020). Plastic ingestion was much less prevalent in shark species, at least in the 1980s and 1990s: Cliff et al. (2002) sampled sharks killed in shark nets off

Table 3 Number of species which have been sampled for ingested marine litter or microplastics. The percentage of species found to have ingested marine debris or microplastics is shown in brackets. No studies on debris ingestion were available for Mauritius, Mozambique and Somalia

	Forage widely across south-west Indian, Atlantic and Southern Ocns.	Deep-sea sites in international waters	Kenya[a]	Madagascar, La Réunion, Mayotte (Comoros Archipelago)	Seychelles	South Africa	Tanzania	All countries/ regions combined
Seabirds	42 (93%)	0	0	10 (100%)	0	1 (100%)	0	53 (94%)
Charadriiformes	*5 (100%)*	*0*	*0*	*4 (100%)*	*0*	*0*	*0*	*9 (100%)*
Phaethontiformes	*0*	*0*	*0*	*1 (100%)*	*0*	*0*	*0*	*1 (100%)*
Procellariiformes	*35 (91%)*	*0*	*0*	*4 (100%)*	*0*	*0*	*0*	*39 (92%)*
Sphenisciformes	*1 (100%)*	*0*	*0*	*0*	*0*	*1 (100%)*	*0*	*2 (100%)*
Suliformes	*1 (100%)*	*0*	*0*	*1 (100%)*	*0*	*0*	*0*	*2 (100%)*
Sea turtles	0	0	0	4 (100%)	1 (100%)	1 (100%)	0	4 (100%)
Bony fish	0	0	0	1 (100%)	0	20 (100%)	0	21 (100%)
Sharks	0	0	0	0	0	14 (71%)	0	14 (71%)
Pinnipeds	0	0	0	0	0	2 (0%)	0	2 (0%)
Cetaceans	0	0	0	2 (100%)	0	0	0	2 (100%)
Invertebrates	0	4 (75%)	5 (100%)	2 (100%)	0	5 (100%)	1 (100%)	17 (94%)
All species combined	42	4	5	19	1	43	1	113 (91%)

[a] Kosore et al. (2018) showed that four groups of zooplankton (Amphipoda, Chaetognatha, Copepoda and fish larvae) ingested microplastics, but did not identify the zooplankton to species level.

the coast of South Africa for 23 years and showed that 71% of species (Table 3), but only 0.4% of individuals had ingested plastic litter. However, cartilaginous fishes tend to regurgitate and even evert their stomachs to remove indigestible materials, parasites or mucus and maintain a healthy digestive tract (Sims et al. 2000, Brunnschweiler et al. 2005), which might explain the low incidence reported by Cliff et al. (2002). Bite marks, presumably caused by fish, have been found on mainly HDPE bottles stranded on Kenyan and South African beaches, which were more frequent on bottles inferred to come from foreign than local sources, due to the time spent at sea (Ryan 2020a, Ryan et al. 2021).

Very little is known about plastic ingestion by marine mammals in the WIO region (Table 3). Seals seldom ingest plastic; for example, no plastic was found in 8066 fur seal scats collected from 1989 to 2014 on Marion Island (Table S13; Ryan et al. 2016b), although plastic ingestion may be higher in seals living close to urban centres. Ingestion of macrolitter also seems to be uncommon in whales and dolphins; no litter was found in the digestive tracts of two individuals belonging to two species of cetaceans in Mayotte (Comoros Archipelago, Table S13; Chebani 2020) and of 40 smaller whales and dolphins washed up between Cape Agulhas and the Great Brak River in South Africa (Department of Forestry, Fisheries and the Environment, unpubl. data). Similarly, among eight cetacean individuals stranded in La Réunion and necropsied by the organization GLOBICE, none had ingested macrolitter (GLOBICE, unpubl. data), even though in other regions whales and dolphins have been found with ingested plastics (Unger et al. 2016, Alexiadou et al. 2019). Regardless of the scarcity of studies, however, it is inevitable that many individuals have likely ingested microplastics, especially for filter-feeding species.

Ingestion of marine litter and microplastics has also been recorded in few species of invertebrates and zooplankton (Tables 3 and S14), although most species probably ingest microplastics and microfibres given the high densities of litter at the sea surface and on the seafloor (see above). While the impact of this ingestion remains unknown for WIO species, it is likely negligible given that many of these species have evolved to ingest indigestible items and excrete them. Across all these species, 60%–100% of ingested microplastics were microfibres (Gerber 2017, Awuor 2020, Awuor et al. 2020, 2021, Chebani 2020, Mayoma et al. 2020, Sparks 2020; Table S14), which were also found to be ingested by other benthic organisms sampled at deep-sea sites off the coast of Madagascar (i.e. one hermit crab, one sea pen and one zoanthid; Taylor et al. 2016). Likewise, fibres/filaments dominated the microplastics ingested by zooplankton in the central part of Kenya's EEZ (Kosore et al. 2018). Among macroinvertebrates, filter-feeders such as oysters ingested more microfibres than deposit-feeders (three species of brachyuran crabs), presumably because they filter large volumes of water to concentrate suspended materials, including microplastics (Awuor et al. 2020). Ingestion of macrolitter has been documented in only one invertebrate species, the sandy sea anemone (*Bunodactis reynaudi*), which was studied at an urban beach in False Bay, south-west South Africa (Weideman et al. 2020a). More than 99% of the ingested litter was plastic and mostly comprised flexible plastics such as bags and food packaging (Weideman et al. 2020a). Ingested plastic was normally regurgitated within a few hours of ingestion, and it is unclear whether this had any impact on the animals (Weideman et al. 2020a).

Whether the low proportion of examined individuals with ingested plastic items is a consequence of regular egestion or regurgitation or whether individuals generally swallow fewer plastics is not known at present. Densities of floating plastics in the WIO region seem to be lower than in other regions of the world, especially compared with the densities in the central areas of the subtropical gyres, which could be caused by short residence times in coastal waters due to rapid onshore transport and stranding on shorelines. Possibly, this reduces the risk of surface-feeding organisms of being confronted with floating plastic litter. Future studies should examine interactions and also thoroughly study residence times and drift trajectories of floating litter in the WIO region.

Entanglement

In the WIO region, entanglement has been studied in 64 species of different taxa, of which 58 species have effectively been found entangled (Table 4). However, most data about entanglement in marine debris are anecdotal, given the infrequent nature of most entanglement events. Furthermore, entanglement studies and records from the WIO region are only available for South Africa and the Seychelles. Using Google Images and unpublished records, Ryan (2018) found evidence of 28 species of seabirds and 15 of freshwater and coastal birds in South Africa being entangled in debris. However, the author noted that it is difficult to differentiate entanglement in marine debris from by-catch in active fishing gear (Ryan 2018). There are no other published records of seabird entanglement from the rest of the WIO region.

Hawksbill (*Eretmochelys imbricata*) and olive ridley (*Lepidochelys olivacea*) turtles have been found entangled in FADs in the outer islands of Seychelles, but again it is unclear whether this was active or ghost fishing gear (Balderson & Martin 2015). As is the case with ingestion, some individuals of four WIO sea turtle species have likely become entangled in marine debris, but this is unlikely to affect species at a population level.

Records of sharks entangled in marine debris are only available for South Africa where the incidence rate was low (Table 4), suggesting that entanglement poses little threat to sharks at the population level (Cliff et al. 2002). Similarly, while entanglement has been found to affect four species of seals in South Africa, the proportion of entangled individuals was low at all study sites (Shaughnessy 1980, Hofmeyr et al. 2002, Hofmeyr & Bester 2002; Table S15). However, these studies of shark and seal entanglement were conducted ~20 years ago and more research is needed to quantify how often and to what extent sharks and marine mammals become entangled in marine litter in other regions of the WIO. In La Réunion, the organization GLOBICE registered 13 cases of cetaceans entangled in fishing lines between 2007 and 2019 (i.e. long-beaked dolphin, humpback whales and bottlenose dolphins of the Indo-Pacific), but this information has not yet been published (GLOBICE, unpubl. data).

Only a few records of invertebrates being entangled in marine debris are available for the WIO region. Rundgren (1992) noted the sea fan (*Lophogorgia flammea*, currently known as *Leptogorgia palma*) entangled in fishing line in False Bay, south-west South Africa, while Schleyer & Tomalin (2000) found fishing line entangled around corals and tunicates in Sodwana Bay, east South Africa, although the species were not specified. More than half of the coral colonies surveyed in the reefs fringing Mayotte (Comoros Archipelago) showed signs of breakage and abrasion associated with marine litter (Mulochau et al. 2020), similar to the impacts observed by Schleyer & Tomalin (2000).

Table 4 Number of species in which entanglement has been studied. The percentage of species which have been found entangled in debris is shown in brackets. No studies on debris entanglement were available for Comoros (including Mayotte), Kenya, Madagascar, Mauritius, Mozambique, La Réunion, Somalia and Tanzania

	Seychelles	South Africa	All countries combined
Seabirds	0	43 (100%)[a]	43 (100%)
Sea turtles	2 (100%)	0	2 (100%)
Sharks	0	14 (57%)	14 (57%)
Pinnipeds	0	4 (100%)	4 (100%)
Invertebrates	0	1 (100%)	1 (100%)
All species combined	2	62	64 (91%)

[a] Includes 28 species of seabirds and 15 of freshwater and coastal birds. As noted in the running text, the author highlighted the difficulty of differentiating entanglement in marine debris and by-catch in active fishing gear (Ryan 2018).

At deep-sea sites south of Madagascar, Woodall et al. (2014, 2015) found fibres entangled around octocorals, sponges, fish and crustaceans, but did not identify the species or note the frequency of occurrence (Table S15).

Epibionts

Among the studies that have found epibionts on marine litter (Table S16), Rundgren (1992) found encrusting coralline alga, barnacles (*Balanus* sp.), a mussel (*Choromytilus meridionalis*) and an anemone (*Bunodosoma capensis*) growing on litter collected from the seafloor of False Bay, south-west South Africa, while Woodall et al. (2015) saw corals and hydroids encrusting marine litter at deep-sea sites south of Madagascar. Ryan et al. (2020c) found that 77% of benthic litter trawled up from the continental shelf of South Africa had been colonized by epibionts, and most of this litter was buoyant once the epibionts had been removed, suggesting it has been colonized while floating at the sea surface, and perhaps retained on the seabed after being colonized by benthic epibionts.

Six species of goose barnacles *Lepas* spp. were found growing on litter collected from 22 sites along the east, south and south-west coasts of South Africa (Whitehead et al. 2011). Interestingly, on the east coast, most goose barnacles were found growing on pieces of plastic and rubber, while goose barnacles were mostly found on natural flotsam such as kelp on the south and south-west coasts. This is likely due to the absence of kelp on the east coast and also to the prevalence of rubber and plastic debris arriving on the east coast of South Africa from other east African countries via the Mozambique and Agulhas Currents (Whitehead et al. 2011) and is consistent with the higher stranding rates of litter in WBC systems (versus EBCs where upwelling carries floating litter offshore, Marín & Delgado 2007, Ryan 2020a). In support of this, epibionts were uncommon on stranded litter items collected along the south-west coast of South Africa (<1% of litter colonized in Table Bay, Swanepoel 1995), with much higher fouling rates seen along the south-east South African coast (Ryan et al. 2021), in Kenya (Ryan 2020a), in Mozambique (Barnes 2004) and in the Seychelles (Barnes et al. 2009). Furthermore, foreign bottles on beaches in Kenya and South Africa also had more bite marks and were more frequently colonized by epibionts than locally manufactured bottles, which is indicative of longer periods spent floating at sea (Ryan 2020a, Ryan et al. 2021). These high rates of colonization raise concerns about the role of floating anthropogenic litter in facilitating the spread of alien and potentially invasive species across the Indian Ocean (Barnes 2004), which has been reported and reviewed in other oceans (Póvoa et al. 2021).

Marine debris in seabird nests

Plastic and other marine debris have been recorded at or in the nests of 14 seabird species in the WIO region. This litter may either have been regurgitated by birds while sitting on the nest (e.g. Perold et al. 2020) or have been incorporated into the nest as building material (e.g. Witteveen et al. 2017; Table S17). On Marion Island, the proportion of fishing-related debris found in the nests of albatrosses and giant petrels decreased dramatically after the fishery for Patagonian toothfish around the island collapsed, and seabirds on Marion Island now ingest mostly rigid pieces of plastic such as fragments from larger items and bottle lids (Perold et al. 2020). In coastal dunes of the Western Cape, South Africa, plastic packaging and ropes/strapping were the most common types of litter found in kelp gull (*Larus dominicanus*) nests (e.g. Figure 14). Litter was more common in nests located in open areas as opposed to vegetated areas, suggesting that kelp gulls tend to use more plastic items for nest construction where natural nest construction material is scarce (Witteveen et al. 2017). Marine debris use tends to be much less common in other species of seabirds; for example, litter was found much less frequently in nests built by Hartlaub's gulls (*Chroicocephalus hartlaubii*), African penguins (*Spheniscus demersus*), great white pelicans (*Pelecanus onocrotalus*) and white-breasted cormorants (*Phalacrocorax lucidus*; Ryan 2020c, Tavares et al. 2020; Table S17).

Figure 14 Kelp gull (*Larus dominicanus*) using plastic packaging in its nest in South Africa. Photo: Peter Ryan.

However, rope and other litter is often incorporated in nests of Cape cormorants (*Phalacrocorax capensis*) that nest in harbours, where there is little other nesting materials.

Other interactions between marine debris and organisms

Some studies have also reported on other less common interactions between organisms and marine litter in the WIO. In False Bay, South Africa, Rundgren (1992) observed 43 sea urchins (*Parechinus angulosus*) with plastic fragments attached to their spines, suggesting that the species uses plastic fragments as sunshades and camouflage although it is unclear how common this behaviour is given that the author did not state how many urchins were checked for plastics. In recent years, Spencer (2020) and Weideman et al. (2020b) have reported the same behaviour in *P. angulosus* on the rocky shore of False Bay and also observed sandy anemones (*Bunodactis reynaudi*) and cask sea cucumbers (*Pentacta doliolum*) with plastic fragments adhered to their sides. At deep-sea sites south of Madagascar, Woodall et al. (2015) observed several species of benthic invertebrates (including crinoids, anemones, sea urchins and brittle stars) and fish using marine macrolitter as a habitat. Microplastics have been found and quantified in the tube structures of the Cape reef worm (*Gunnarea gaimardi*) along the west and south-east coasts of South Africa, although it remains unclear whether *G. gaimardi* deliberately incorporates microplastics into its tube structures and whether this interaction has any detrimental ecological effects (Nel & Froneman 2018). Together, these studies and observations highlight the variety of ways in which organisms interact with marine debris, and it is important that the ecological costs of these interactions are quantified, especially in WIO countries where few data are available.

Improvements in marine litter understanding and remaining knowledge gaps

In general, the findings of the present review show that there have been vast improvements in our understanding of the amounts, characteristics, sources, transport and fate of marine litter since the 2008 UNEP/WIOMSA report. First, at the time of that report's preparation there were quantitative records of marine litter available only for South Africa, whereas no marine litter amounts were known for the other WIO countries, which limited the capacity to assess the magnitude of the litter problem in the region and to determine spatial and temporal trends. Meso- and microplastics data, for their part, were almost non-existent (except for a few studies by Ryan and colleagues) and were not mentioned or individualized in the previous review (UNEP & WIOMSA 2008).

While the main sources of marine litter in the region (i.e. land-based sources such as urban and river run-off) were already apparent back in 2008, they had been inferred rather than demonstrated (UNEP & WIOMSA 2008), and the findings of the present review provide a clearer and more complete overview of the different land- and sea-based sources of marine litter and microplastics in both continental countries and island states.

Similarly, ocean currents had already been identified as potentially important drivers of marine litter transport and it was even suggested that marine litter could become a significant transboundary problem in the region if not addressed urgently (UNEP & WIOMSA 2008). The present review shows that this actually happened, as marine litter effectively travels long distances within the WIO region transported by the ocean currents, and it even comes from outside the region (e.g. Southeast Asia). This pattern is similar along the North American coasts, where the proportion of litter from sea-based sources is comparatively high on the beaches bordering the Gulf Stream, the North Atlantic WBC (Ribic et al. 2010, 2011), but substantially lower along the Pacific coasts' EBC (Ribic et al. 2012). Most island states of the WIO region receive very large proportions of sea-based litter, which is also similar to other oceanic islands, e.g. Hawaii (Ribic et al. 2012) or Easter Island (Luna-Jorquera et al. 2019) in the North and South Pacific, respectively. In the WIO region, other transport dynamics such as those driven by nearshore processes are also better known at present, which, together with the general knowledge about WBCs, contribute to a greater understanding of marine litter fate.

Lastly, the findings of this review also allow better comprehension of the different ways in which marine litter is interacting with marine organisms in the WIO region, which is fundamental information to further research about its potential impacts, whereas this aspect of the issue was not considered in depth in the previous report (UNEP & WIOMSA 2008).

However, regardless of all these important improvements in our understanding of marine litter in the WIO region since the last review, there are still large knowledge gaps that urgently need to be addressed. The main knowledge gaps for the WIO region are summarized below and in Table 5, although recent reviews for South Africa by Naidoo et al. (2020b), Ryan (2020b), Ryan et al. (2020b) and Verster & Bouwman (2020) also highlight important evidence gaps that are applicable to most other WIO countries. Most studies have been conducted on sandy beaches, while other habitats, such as mangroves, rocky shores, rivers and estuaries, the water column, coral reefs and the seafloor, are lacking important information across the region. There is also a geographic gap in marine

Table 5 Main knowledge gaps on marine litter that need to be addressed in the WIO region and their priorities

Actions to address knowledge gaps in the WIO region	Priority
Determine amounts and types of litter in habitats other than sandy beaches	High
Quantify the amounts of litter from land-based versus offshore sources	High
Determine breakdown dynamics of macroplastics under different conditions	Medium
Determine the role of eddies in temporary aggregation and transport of litter	Medium
Determine the individual-level impacts on organisms	High
Evaluate the impacts on populations of susceptible organisms	High
Improve identification of litter sinks to help focus clean-up efforts	Low
Improve understanding of littering behaviour to effectively address it	High

litter research, with most studies having been conducted in South Africa that cover the most diverse array of topics and habitats.

The most important research priorities are those focusing on improving our understanding of the main sources of litter and on testing the effectiveness of different mitigation measures. While it is clear that most litter in the WIO region comes from land-based sources, particularly in continental East Africa, more research is needed to quantify the amounts of litter being released from specific land-based sources, including rivers (Akindele & Alimba 2021). For example, no studies have quantified how effectively wastewater treatment plants in the WIO region remove microplastics from wastewater or what proportion of microplastics is retained in sewage sludge (Verster & Bouwman 2020). Furthermore, data on the quantity of litter washing out of rivers or stormwater outlets are only available for some parts of South Africa, and it remains unclear what proportion of litter is trapped in riverine sediments or riparian vegetation (Verster & Bouwman 2020, Weideman et al. 2020c). This makes it difficult to assess the accuracy of global models that attempt to predict the amounts of litter washing out from land-based sources (Jambeck et al. 2015, Lebreton et al. 2017). Field surveys in South Africa suggest that these global models are overestimating litter loads by several orders of magnitude (Ryan 2020b; Verster & Bouwman 2020; Weideman et al. 2020d), but this needs to be assessed for other WIO countries. It is also unclear how the proportion of litter originating on land versus that released from offshore, sea-based sources varies spatially and temporally, and this needs to be addressed so that effective mitigation measures can be implemented.

The lack of data on the types of litter stranding on rocky shores and in mangroves also makes it difficult to determine to what extent these habitats act as sinks for litter, and it remains unknown how much and what types of litter are found in the water column or on the seafloor for most of the WIO region. This is particularly true for microplastics, and much more research is needed to determine the sources, fates, sinks and impacts of microplastics in all habitats in the WIO region. Additionally, future studies should identify microplastic polymers using Raman or FTIR spectroscopy as this information can be used to improve our understanding of microplastic sources and transport mechanisms, as well as to determine the true nature of microparticles, in terms of whether they effectively correspond to synthetic polymers or whether they are of natural origin. Furthermore, the WIO region lies between ~12°N and 35°S, which has high incident solar radiation. This may result in high fragmentation rates of stranded macrolitter, but the conversion rate of macrolitter to microplastics has not been quantified anywhere in the WIO region.

In general, the findings of this review coincide with the results of recent modelling studies (e.g. Chenillat et al. 2021, Morales-Caselles et al. 2021), but future studies should specifically investigate litter dispersal and transport duration in the WIO region, especially in those parts affected by seasonally changing flow and transport directions. Moreover, due to a scarcity of research and to contrasting results, it is currently unclear whether (and how much) litter floating in the WIO region disperses to Antarctic waters, where the Antarctic Polar Front and associated currents have long been considered an important barrier to dispersal of marine organisms (Clarke et al. 2005, Barnes et al. 2006). For instance, Ryan et al. (2014b) suggested that, at least in the African sector of the Southern Ocean, the Subtropical Front (north of the Antarctic Polar Front) effectively acts as a barrier to the transfer of floating litter to sub-Antarctic and Antarctic waters (Ryan et al. 2014b). On the other hand, it was later demonstrated that drifting kelp can readily disperse north-south across the Antarctic Polar Front in both the Atlantic and African sectors of the Southern Ocean, which suggested that this front was not such a strong barrier as previously believed (Fraser et al. 2016). Thus, this issue is still a matter of debate and needs further research.

Importantly, all research on marine litter and microplastics in the environment should be conducted following harmonized methodologies, size classes and reporting units, to allow for comparability among studies and for determining spatial and temporal trends (GESAMP 2019; Galgani et al. 2021). In the case of studies on microplastics, it will also be fundamental that in the future all of them incorporate well-detailed and rigorously conducted protocols to minimize or to account for potential contamination of the samples.

While extensive research has been conducted on ingestion of marine litter by seabirds around South Africa, little is known about their potential impacts on most other organisms, such as bony fish, sharks, sea turtles, marine mammals and invertebrates, both in South Africa and in the rest of the WIO region. However, given the prevalence of plastic litter in most marine habitats, systematic studies will inevitably find individuals of almost all species ingesting (especially microplastics) or entangled in debris. Future studies should therefore focus on quantifying the effects of these interactions rather than just documenting them. For example, quantitative data about the impacts of litter on marine organisms at a physiological level (for example, toxicological impacts of chemicals associated with plastics, potential diseases caused by litter, increased drag and breakage) and at the population level are lacking. It has also been shown that plastics are associated with diseases on coral reefs (e.g. Lamb et al. 2018) and plastics entangled around benthic organisms, corals or kelp may increase their drag and make them more susceptible to breaking, but this has not been studied in the WIO region. In this context, it is also important to better understand the role of meso-scale eddies in concentrating floating plastics (and food organisms), which will not only affect the transport dynamics of this litter, but also the interaction with organisms of all trophic levels, including top predators seeking out these eddies during foraging trips (e.g. de Pascalis et al. 2021).

Socio-cultural and socioeconomic impacts of marine litter have been described in the WIO region and elsewhere, such as the loss of national, local or community incomes due to effects of beach litter on tourism and related productive sectors (e.g. transport and restaurants, Rangel-Buitrago et al. 2017, see also Ballance et al. 2000), the high financial cost of removing litter in the environment (Ballance et al. 2000, Burt et al. 2020) and various navigational threats caused by propeller entanglement by derelict fishing gear (Hong et al. 2017). Although these impacts can be as relevant as ecological impacts, they were not included in the current review about the WIO region; thus, we can only offer these first insights, but other studies (e.g. sociological research) will be needed to better elucidate these particular impacts and offer appropriate recommendations and solutions. Moreover, research should also focus on identifying effective mitigation efforts by, for example, testing the impact of programmes to reduce particular types of litter (such as bans on plastic bags), the retention rate of river booms and stormwater traps, and the effectiveness of beach, river and street clean-up programmes (Ryan et al. 2020d).

While there remain many knowledge gaps about marine litter in the WIO region, currently available data and information are sufficient to determine that impacts can potentially be severe on some highly susceptible organisms, which also suffer numerous other threats (e.g. most sea turtles), and thus, actions can be recommended based on existing information.

Recommendations

Our review highlights the need to address the marine litter problem by means of locally driven measures and a life cycle intervention approach. We recommend a series of actions mostly focused on prevention and management that can be implemented at the regional, national and local levels in the WIO region (Table 6). Since most marine litter comes from local, land-based sources, and most especially from the packaging sector, the most effective way to reduce plastic pollution is to prevent the generation of litter at sources. Modifying and converting the production of single-use plastics to reusable/returnable materials (which could also be reusable/returnable plastics) is recommended as an effective and feasible strategy to reduce litter loads. Promoting designs of alternative materials or processes that favour reuse will be essential (IUCN-EA-QUANTIS 2020c). Governments should approve, implement and enforce extended producer responsibility (EPR), including take-back schemes, given that EPR is a fundamental and integral policy tool covering the entire waste life

Table 6 Main recommendations to address the marine litter and microplastics problem in the WIO region, based on the information gathered in this review and the recommendations provided by the national hotspotting assessments implemented in Kenya, Mozambique, South Africa and Tanzania (IUCN-EA-QUANTIS 2020a,b,c,d)

Research and monitoring	Actions for addressing land-based sources			Actions for addressing sea-based sources
	Prevention	Management	Clean-up	
• Regional monitoring programme within all WIO countries • Harmonized protocols and reporting units • Strengthen research of understudied topics and habitats • Effective data validation and sharing	• Reduction of single-use plastics • Promote design, production and consumption of reusable/returnable alternatives • Extended producer responsibility (EPR) • Tax imported plastic products • Educational campaigns	• Adequate and more frequent waste collection • Infrastructure for proper waste disposal • Anti-littering campaigns • Improved recycling capacity • Effective waste segregation in private and public spaces • Reduce open burning of waste	• Community-based clean-ups on land • Intercept litter before it enters the sea • Strengthen beach clean-up efforts near source points (e.g. river mouths)	• Stricter international regulations and enforcement (for example, to ensure adhesion of ships to MARPOL Anx. V) • Waste audits on ships upon departure and arrival • Coordinated regional approach for litter reception facilities in ports

cycle and involves all sectors of society (Raubenheimer & McIlgorm 2018). These types of policies need to be accompanied and supported by educational campaigns directed towards citizens.

Waste management also must be improved at the municipal level, given that the national hotspotting assessments revealed low rates of collection and proper disposal of waste, which translates to high rates of mismanaged waste (IUCN-EA-QUANTIS 2020a,b,c,d). This is particularly relevant in Kenya, Mozambique and Tanzania, where 92%–99% of the generated waste is mismanaged (IUCN-EA-QUANTIS 2020a,b,d). Thus, while effective measures to prevent the generation of litter at the sources are urgently needed (see above), better waste collection and a higher capacity for proper disposal are also fundamental mitigation strategies to tackle marine litter.

Since most litter on remote beaches and small island states originates from offshore sources (e.g. fishing, shipping and long-distance drift, mostly from Southeast Asia), stricter international regulations need to be put in place and enforced. Major polluters such as Indonesia and other Southeast Asian countries must also pledge their support for small island nations by implementing local measures to curb the release of litter into the sea, which is fundamental to keeping litter out of downstream regions such as the WIO. In this regard, the Indian Ocean Rim Association (IORA) might play a key role in developing, implementing and enforcing policies and programmes aimed at reducing plastic waste in Southeast Asia, as well as supporting the involved states to comply with such measures. While IORA does not currently have a particular focus on marine litter, this is an issue of global concern that should be considered as an important part of the association's Focus Area on Blue Economy, given the impacts that marine litter can have on coastal tourism, shipping and fisheries, as briefly discussed in the previous chapter.

The regional monitoring programme that has recently been established by the Western Indian Ocean Marine Science Association (WIOMSA) in collaboration with the Sustainable Seas Trust (SST) through the African Marine Waste Network and country partners (Barnardo & Ribbink 2020) should continue and be strengthened and extended within all WIO countries, given that ongoing litter monitoring is important to (1) help identify the major sources of litter and (2) provide information to evaluate whether measures and policies aimed at reducing marine litter are effective or not (Ryan et al. 2020d). Regional monitoring activities should be coordinated and harmonized in terms of protocols and reporting units (in coordination with global programmes, such as GESAMP 2019), all data should be readily available, and the resulting information should be regularly shared with decision-makers. Importantly, a Group of Experts on Marine Litter and Microplastics already exists in the WIO region, which might contribute to the accomplishment of these recommendations.

Finally, we recommend taking advantage of other various organizations that are already operating in the region (e.g. IORA mentioned above), some of which are even working towards preventing and managing the transboundary problem of marine litter and microplastics, such as the Indian Ocean Commission which has already proposed a preliminary action plan to reduce and manage waste in the WIO island states (COI 2019).

Conclusions and outlook

The aim of this review was to collate the available information on marine debris in the WIO region with a specific focus on the sources, transport and fate of litter as well as the ecological impacts. Our knowledge has greatly improved since the last review for the region was published (UNEP & WIOMSA 2008), but there are still large knowledge gaps that need to be filled. However, while further research is needed, there is sufficient information to recommend and take actions. It is clear that most litter comes from land-based sources, especially in the case of continental countries, although this needs to be verified with more field data in countries such as Tanzania and Somalia where little research has been conducted, and where some studies suggest that some litter might leak out to offshore sinks, especially during the NE monsoon. This review confirms estimates from recent modelling studies, namely that beaches of the WIO region are important sinks for floating litter (Chenillat et al. 2021), which is also reflected in high stranding intensity of FADs in the same region (Imzilen et al. 2021).

This overview of the large marine ecosystems (LME) in the WIO region (Somali Coastal Current LME and Agulhas Current LME) shows that most marine litter is retained adjacent to local sources or within these LMEs. Some export of litter from the WIO region is only being suggested for the northern region via the SCC into the Arabian Sea or the SECC into the NW Indian Ocean, and for the southern AC via the Benguela Current into the Atlantic Ocean or the Agulhas Return Current into the West Wind Drift (Hood et al. 2017, Halo & Raj 2020). Import occurs predominantly via the SEC, which may bring floating litter via long-distance drift from foreign nations in the Eastern Indian Ocean and from sea-based sources (both from fisheries and shipping). As shown throughout this review, the WIO region functions mostly as a trap for marine litter, and particularly floating litter is pushed onto the local shores due to the particular dynamics within this WBC. These findings show that efficient solutions need to be designed and implemented within the WIO region.

In general, urgent action is needed to curb the release of plastic litter into the sea (Borrelle et al. 2020). Substantial reductions in the amounts of land-based litter can be achieved by promoting the use of reusable items such as reusable plastics, by improving waste management at the municipal level and by educating citizens about the negative effects of litter on the marine environment (Napper & Thompson 2020). Such an integral approach towards local and regional solutions is postulated as the most promising strategy to reduce the amounts of litter entering the oceans (Vince & Hardesty 2017). Since a significant proportion of WIO litter also originates offshore, which is especially relevant on remote coasts and islands that receive little or no local input of litter, effective international measures are needed to address these sources. Given that waste generation in all WIO countries is expected to increase in the future, these action plans should be made a priority by WIO governments and municipalities, and monitoring programmes should continue in order to assess the efficacy of these prevention measures. As marine litter dynamics in other WBCs are likely to be very similar to what we reported here for the WIO region, similar mitigation and prevention strategies should be effective in those systems.

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VERTICAL FAUNAL EXCHANGE IN THE OCEAN AND THE DEEP SOURCE-SINK HYPOTHESES: A REVIEW FROM THE PERSPECTIVE OF PRESSURE TOLERANCE STUDIES

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Abstract Faunal exchanges between shallow-water and deep-sea environments, throughout geological times, have resulted in the broad colonization of oceanic depths we see today. Attempts have been made to explain the current distribution of benthic animals by the submergence (shallow to deep) hypothesis or the high-latitude emergence (deep to shallow) hypothesis. More recently, discussions of bi-directional exchanges have emerged. Here we aim to summarize and explore empirical support for the various hypotheses by reviewing over 130 studies on the tolerance (survival) of adults and larvae of ~260 species following compression or decompression. Secondarily, we attempt to tease out the importance of ontogeny (comparing tolerance of adult and larval life stages), phylogeny (comparing tolerance among major phyla) and geographic location of collection (looking for latitudinal trends). Overall, empirical evidence supports the formalization of a bi-directional exchange hypothesis as the most parsimonious explanation for current bathymetric patterns of biodiversity and highlights the pressure tolerance of benthic animals occupying bathyal depths. No clear ontogenetic patterns emerged, but comparison of pressure tolerance between adult and larval stages remains tentative due to the limited number of species for which this was assessed across life stages (nearly all under decompression scenarios). Pressure tolerance varies across and within phyla, and more basal taxa do not show consistent patterns of lower mortality (or longer survival) upon pressure shifts, although organismal simplicity and protective features (e.g. calcified exoskeleton) seem to increase tolerance. Adults of deep-sea species collected at lower latitudes appear to survive to pressure in equal or higher proportions, but for much shorter durations than those collected from higher latitudes, partly supporting suggestions that bathymetric transitions may be favoured in isothermal water columns (closer to the poles). Globally, this synthesis highlights technical and conceptual gaps offering a framework for further investigation of vertical movements of marine species across depths, which will be particularly useful in predicting ecosystem shifts in response to climate change.

Keywords: Barotolerance, bathymetric distribution, biodiversity, compression, deep sea, emergence, faunal exchanges, hydrostatic pressure, submergence, vertical movements

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Introduction

Evolving views of the deep sea

A fundamental division in the world ocean involves the distinction between shallow-water and deep-sea environments (Webb et al. 2010). The transition between the two is not universally defined, but it is often considered to occur at ~200 m, which typically marks the depth of the continental shelf break (Thistle 2003, Ramirez-Llodra et al. 2011) or the lower limit of light penetration in the water column (Yancey 1991). Oceanic depths have long exerted great fascination on humans. Some believed them to be devoid of life and proposed the azoic theory (Forbes 1844), yet deep dwelling marine fauna had already been discovered, and Risso (1810, 1820) had reported that the vertical distribution of certain fishes extended 500 m below the sea surface. Eventually, there was broad recognition that fauna occurred at all ocean depths, but the deep sea continued to be depicted as a harsh environment, exhibiting lower biodiversity than shallow water (Sanders 1968). Paradigms regarding the low biodiversity and productivity of the deep sea were revised when hundreds of benthic macrofaunal species were recovered from deep-sea trawls for the first time (Hessler & Sanders 1967, Gage & Tyler 1991) and exceptional ecosystems such as those occurring at hydrothermal vents were discovered (Corliss & Ballard 1977). Over the last few decades, hotspots of biodiversity in the deep sea have been highlighted (e.g. seamounts, coral and sponge gardens, hydrothermal vents and cold seeps), which rival some of the most diversified environments on the planet. In fact, recent extrapolations suggest that deep-sea habitats could host over 10 million species (Ramirez-Llodra et al. 2011), more than the total number of species currently known to populate Earth (Mora et al. 2011).

The realization that the deep-sea fosters high levels of biodiversity, and the fact that depths >200 m cover more than 60% of the surface area of the globe, prompted contrasting hypotheses regarding the origin of extant deep-sea species. These hypotheses proposed either that deep-sea taxa evolved *in situ* with representatives subsequently colonizing shallow waters, or that deep-sea species evolved from shallow-water species following range extension into deep waters (Locket 1977, Gage & Tyler 1991). Studies focusing on deep-sea and shallow-water colonization developed during the 1960s and 1970s (Menzies & Wilson 1961, Kussakin 1973, Menzies et al. 1973, Hessler & Thistle 1975, Hessler et al. 1979), although hypotheses were postulated a few decades earlier (Dahl 1954, Wolff 1960). While deep-sea research has intensified during the past 50 years, the role of the deep ocean as a biodiversity source or sink remains obscure (Gage & Tyler 1991, Miglietta et al. 2011). Resolving this is complicated by ongoing uncertainty regarding the importance of specific depths as biodiversity sinks or sources at different geological times (Rex et al. 2005, Bik et al. 2010).

Hypotheses on ocean colonization and bathymetric ranges

Shallow to deep

The submergence hypothesis postulates that taxa from shallow waters extended their bathymetric range downwards following climate-driven extinction events in the deep sea (related to oxygen depletion), and radiated at bathyal (>200–2000 m) and abyssal depths (>2000–6000 m) (Kussakin 1973, Jablonski & Bottjer 1991, Jablonski 2005). This hypothesis relies in part on phylogenetic and taxonomic evidence that deep-sea species are derived from shallow-water species, together with ancestral state reconstructions (extrapolation back in time to common ancestors from measured characteristics of individuals or populations) based on current biogeographic distributions (Brown & Thatje 2014). Fossil evidence suggests that higher taxonomic levels predominantly originated in nearshore environments whereas lower taxonomic levels originated in offshore environments where radiation occurred subsequently (Jablonski & Bottjer 1991). Deep-sea colonization by shallow-water taxa is suggested to have occurred throughout geological history, especially during the late Mesozoic and early Cenozoic periods, when the water column was isothermal across low latitudes (Menzies et al. 1973, Jablonski et al. 1983, Wilson 1999). Jacobs and Lindberg (1998) provided support for this hypothesis, suggesting that onshore to deep-sea diversification predominated in the Mesozoic prior to the Turonian stage of the Cretaceous. Extinct deep-sea fauna identified in fossil records from these periods have been linked to extant shallow-water animals whose ancestors may have colonized the deep sea (Kussakin 1973, Cottin et al. 2012). In fact, it is argued that the colonization of the deep sea by shallow-water animals may be continuous in isothermal water columns, such as in regions of deep (cold) water formation (Wolff 1960, Tyler & Young 1998, Tyler & Dixon 2000, Oliphant et al. 2011). Smaller temperature differences between shallow water and deep sea are thought to greatly reduce the physiological barrier to bathymetric range extension (Gage & Tyler 1991, Raupach et al. 2009), but shallow-water species still must acclimatize or adapt to increasing pressure conditions to migrate downwards (Clarke et al. 1992, Hall & Thatje 2009, Brown & Thatje 2014, Gaither et al. 2016, Lemaire et al. 2018).

Shallow-water organisms that colonize the deep sea may possess sufficient physiological (and possibly developmental) plasticity to directly invade the deep sea and thrive under relatively extreme conditions, which include high hydrostatic pressure, constant low temperature, complete darkness and minimal nutrients from primary production (Hessler & Wilson 1983, Pradillon et al. 2004). Alternatively, if acclimatization to these conditions is beyond the physiological scope of invading shallow-water organisms, indirect invasion through adaptation may occur. Subsequent reproductive isolation from shallow relatives (Hessler & Wilson 1983) may lead to speciation and radiation (Brown & Thatje 2014). Adaptation to more specialized habitats such as hydrothermal vents and cold seeps is thought to happen subsequently (Kiel & Little 2006, Kiel 2016, Sun et al. 2018), although direct colonization of other chemosynthetic deep-sea environments such as whale falls has also been suggested (Distel et al. 2000). The movement of taxa from shallow to deep waters may occur in response to catastrophic events (Speijer & van Der Zwaan 1996), giving rise to the concept of the deep-sea refuge (Cavin 2001, Guinot et al. 2013). The deeper dwelling populations of taxa with broad bathymetric ranges are proposed to have allowed these species to survive surface-water mass extinction events, becoming the source populations for subsequent recolonization and repopulation of shallow waters (see examples in Cavin 2001, Friedman & Sallan 2012, Guinot et al. 2013).

Deep to shallow

The opposing emergence hypothesis proposes that modern deep-sea invertebrates evolved from ancestors that already occupied deep water (Hessler & Thistle 1975), subsequently migrating upwards and populating shallow depths at high latitudes (Hessler & Thistle 1975, Hessler et al. 1979, Wägele 1989, Wilson 1999). This hypothesis suggests that the high extant deep-sea biodiversity could not be a product of small colonization events from shallow waters (Birstein 1963, Hessler & Sanders 1967, Hessler & Thistle 1975). The vast majority of species diversity in many deep-sea families without primitive or less evolved shallow-water representatives is restricted to deep bathymetric ranges, which is proposed to challenge the possibility that these lineages migrated from shallower waters (Hessler & Wilson 1983). For example, some deep-sea isopod lineages evolved *in situ*, within the deep sea, before colonizing shallow water, based on the deep-water occurrence of the most primitive genera or species and the occurrence of shallow-water taxa that lack eyes (Hessler & Wilson 1983). This pattern of origination was reportedly reversed during periods of the Cretaceous and Cenozoic in response to changing environmental conditions, such as increasing oxygenation in the deep benthic environment, extinction of taxa associated with low-oxygen conditions, and increased oxygenation of sediments by bioturbation with the expansion of deep burrowing species into offshore environments (Jacobs & Lindberg 1998).

Bi-directional movement

Although not a formal hypothesis, recent evidence suggesting bi-directional transitions between shallow-water and deep-sea environments within some lineages provides an alternative to unidirectional shallow-deep and deep-shallow colonizations. For example, foraminiferan microfossils from shallow-water populations and deep-sea populations have been discovered to derive from a range of depth strata, evoking an active exchange of fauna (Lipps & Hickman 1982). Similarly, a combination of early-derived and late-branching lineages are found in deep-sea populations of nematodes, hinting at dynamic exchanges between fauna from different depths (Bik et al. 2010). Clade structures showing recent close relationships between deep-water and shallow-water nematode species suggest interchanges between deep-sea and intertidal zones are common in this group (Bik et al. 2010). In addition, a study of the echinoderm class Ophiuroidea revealed complex macro-evolutionary patterns of bathymetric movement (Bribiesca-Contreras et al. 2017). Notably, researchers found that no hypotheses pertaining to "uniform onshore or offshore origins" could be directly supported since there was "a long history of multiple transitions between deep and shallow water lineages" (Bribiesca-Contreras et al. 2017). These studies provide valuable insight into the origin and evolution of many species and their bathymetric distribution. However, despite gathering evidence for bi-directional exchange (see also Hall & Thatje 2009, Strugnell et al. 2011, Riehl & Kaiser 2012), strong support for this view has yet to be presented.

Exploring colonization theories through the pressure tolerance lens

The bathymetric distribution of species in the ocean is constrained by their tolerance and adaptation to many factors, on the whole dominated by the combined effects of temperature and hydrostatic pressure gradients (Menzies et al. 1973, Wilson et al. 2007, Mestre et al. 2009, Brown & Thatje 2011, Cottin et al. 2012, Kiel et al. 2012, Brown & Thatje 2014). Since all environmental factors (i.e. temperature, illumination, food supply, substrate type) except pressure are affecting other ecosystems of the globe, hydrostatic pressure is perhaps the most fundamental characteristic of the deep sea. Yet, compared to our knowledge of thermal biology and of the influence of temperature on deep-sea biodiversity (Yasuhara & Danovaro 2016), the contribution of hydrostatic pressure is understudied. This is largely because the study of pressure as an experimental factor is much more recent than the study of temperature (Rivalain et al. 2010), even though the physiological action of pressure on immersed cells, tissues and whole metazoans has been the subject of laboratory investigations since the late nineteenth century (Regnard 1884, Regnard 1885, Regnard 1891, Fontaine 1930, Cattell 1936). Incomplete knowledge of the specific role of pressure is limiting our global understanding of bathymetric adaptations, which is something that has been highlighted as a major gap in knowledge for a long time (Cattell 1936, Schlieper 1968, Macdonald 1997, Pradillon & Gaill 2007).

Fundamental characteristics and effects of pressure

The basic definition of pressure as a thermodynamic parameter is the force per unit area applied on a surface in a direction perpendicular to this surface. Hydrostatic pressure exhibits a linear gradient from the surface to the bottom of the ocean (Tyler & Young 1998, Pradillon & Gaill 2007), increasing steadily by ~1 MPa (~10 bar or ~10 atm) with every 100 m depth (Macdonald 1997, Pradillon et al. 2004). It must be specified that our knowledge of pressure effects centres largely on the outcome of compression (exposure to a higher pressure than that of the natural state or native habitat), by exposing animals or biological systems normally adapted to atmospheric (surface) pressure to increasing hydrostatic pressure. Decompression (exposure of deep-adapted life forms to surface pressure) and recompression (when deep-adapted lifeforms are brought to the surface and recompressed to the pressure of their native habitat) are not equally well understood. This is not surprising since compression studies of shallow-water taxa predate decompression studies of deep-water taxa, which have until very recently been thought to be incapable of surviving at atmospheric pressure (Pradillon & Gaill 2007).

The complex effects of hydrostatic pressure at the various levels of biological organization (genes, molecules, cells, organisms) will not be detailed here since they have previously been reviewed independently and in relation to temperature (Pradillon & Gaill 2007, Yancey 2020, Macdonald 2021). Pressure primarily produces changes in energy, volume and chemical reactivity (Rivalain et al. 2010): in water, the energy conveyed by pressure is small compared to the energy conveyed by temperature; compressibility means that volumes under high pressure are smaller than under lower pressure (with cascading effects on various processes at different organizational levels); and pressure typically improves solubility and enhances chemical reactivity.

Given these properties, changes in pressure (from outside the natural range of an organism) affects molecular, cellular, biochemical and physiological processes, which can be reflected at the level of the whole organism through loss of motor function and other sub-lethal effects, all the way to mortality (Macdonald & Teal 1975, Morris et al. 2015). Even if dysfunctions are temporary, they may jeopardize basic ecological behaviours such as foraging and escaping from predators, which are essential to survival (Oliphant et al. 2011, Munro et al. 2015, Ammendolia et al. 2018). Overall, the full range of biochemical and physiological effects of shifts in hydrostatic pressure and how they differ among marine phyla is incompletely understood (Pradillon & Gaill 2007), in part because experimental approaches face technological challenges, as discussed below.

Experimental equipment and approaches

Our ability to assess the impacts of hydrostatic pressure on marine organisms essentially revolves around the availability of suitable laboratory facilities and equipment, the choice of appropriate response variables and the selection of representative study systems/organisms.

The evolution of technologies and experimental approaches has been decisive in developing our understanding of pressure impacts (i.e. compression) on aquatic species. There are numerous types of pressure systems of different sizes (from tiny chambers to large vessels) with various corresponding fixtures (e.g. temperature controls, viewing ports, gas extractors) described in reviews by Pradillon & Gaill (2007) and by Macdonald (2021). For the most part, the equipment used for compression studies with macrobenthic animals regulates pressure through a piston and vessel design (Schlieper 1968, Childress 1976, Pradillon & Gaill 2007, Shillito et al. 2014, Macdonald 2021). Generally speaking, there are: (1) large systems used to test pressure tolerances and maintain adults organisms (e.g. Macdonald & Gilchrist 1980, Quetin & Childress 1980, Pradillon et al. 2001, 2004); (2) smaller systems to test pressure tolerances on embryonic and larval stages of organisms (e.g. Marsh et al. 2001, Pradillon et al. 2001, 2004), and; (3) isobaric systems for *in situ* collection of organisms at native pressure (e.g. Yayanos 1981, Koyama et al. 2002). While early systems were surprisingly ingenious, including glass windows and attempts at flow-through (Schlieper 1968), the experimental study of hydrostatic pressure was revolutionized by the development of a large-volume flow-through pressure system called IPOCAMP (Incubateur Pressurisé pour l'Observation et la Culture d'Animaux Marins Profonds) (Shillito et al. 2001, 2014). With built-in temperature control, this system enables the study of thermo- and barotolerance of macrofauna for durations ranging from hours to weeks. In some cases, pressure aquaria have been outfitted with specialized features to maintain chemosynthetic species (Shillito et al. 2015). However, approaches that expose animals from chemosynthetic habitats are relatively novel and have been limited by additional technical constraints in terms of volume and trial duration. Systems designed to mimic the chemically complex hydrothermal vent environment can either be maintained at surface pressure (e.g. LabHorta;

Miyake et al. 2007, Bettencourt et al. 2010) or native deep-sea pressures (e.g. AbyssBox; Sarradin et al. 2007, Shillito et al. 2015).

In the most fundamental way, the pressure tolerance of a species (i.e. the ability to survive changes in pressure) can be assessed by measuring survival (often in study-specific terms rather than standard terms such as LD_{50}) to pressures beyond the known bathymetric range, following compression or decompression (Villalobos et al. 2006, Oliphant et al. 2011, Brown & Thatje 2015, Ammendolia et al. 2018). In addition, various non-lethal responses can be measured, which may rely on shifts in behaviour (Wilcock et al. 1978, Shillito 2006, Thatje et al. 2010, Brown et al. 2017, Ammendolia et al. 2018, Pallareti et al. 2018, Brown et al. 2019), developmental anomalies (Young & Tyler 1993, Tyler & Dixon 2000, Villalobos et al. 2006, Sumida et al. 2015), physiological processes (Childress 1976, Childress & Thuesen 1993, Ravaux et al. 2009, 2013, Brown et al. 2017, Pallareti et al. 2018, Brown et al. 2019, 2020) and genetic markers (Dixon et al. 2002, Barros et al. 2015, Morris et al. 2015, Brown et al. 2017).

Another layer of complexity resides in the fact that the measurable effects of pressure may vary both across taxa (Brown & Thatje 2015) and across life stages of a given species (e.g. Tyler & Dixon 2000, Aquino-Souza 2006, Villalobos et al. 2006, Yoshiki et al. 2006, 2008, 2011, Munro et al. 2015, Brown et al. 2017). Beyond the choice of model animals (or life stages), procuring them may present additional challenges. While obtaining shallow-water organisms for experimental studies is straightforward, there are technical difficulties surrounding the collection and holding of deep-sea organisms (Shillito et al. 2001, 2015, Pallareti et al. 2018). Offshore expeditions are costly and logistically complex, thereby limiting opportunities for collecting animals at depth and potentially introducing geographic and habitat-based sampling bias. For instance, chemosynthetic environments, such as hydrothermal vents and cold seeps, have been and remain a focus of cross-disciplinary oceanographic expeditions (Gaill et al. 1997, Tyler & Dixon 2000, Marsh et al. 2001, Martinez et al. 2001, Pradillon et al. 2001, Shillito et al. 2001, Dixon et al. 2002, Lee 2003, Pruski & Dixon 2003, Shillito et al. 2004, Shillito 2006, Ravaux et al. 2009, 2013, Smith et al. 2013, Bettencourt et al. 2017). While high pressure is typical of both non-chemosynthetic and chemosynthetic environments (Ravaux et al. 2009), the latter are also characterized by high sulphide concentrations (Ravaux et al. 2003) and hydrothermal vents present high and potentially extreme temperatures (Shillito et al. 2001). Hence, pressure tolerance in deep-sea taxa collected from chemosynthetic environments may not necessarily be representative of this tolerance in species from the wider deep sea.

Experimental results are informative as long as the test subjects are well acclimated, and the factor of interest is the only applied change. Long delays associated with ascent and retrieval generate stress in deep-sea organisms during collection, and subsequent maintenance on the ship under minimally suitable conditions (for days or weeks) brings additional space-related and technical challenges. Fortuitously, most marine invertebrates do not have internal gas spaces that result in damage from gas expansion during decompression, meaning that adult animals of most marine taxa can be used for laboratory investigations if there is no cell or tissue damage (Dixon et al. 2004). However, the risk of internal and external injuries associated with sampling apparatuses (e.g. claws or suction devices of remotely operated vehicles, trawl nets, box cores) remains fairly high.

Rationale and objectives of the review

Some 95% of the marine biosphere lies below 200 m depth (Jaenicke 1983, Jannasch & Taylor 1984, Somero 1992, Danovaro et al. 2014, Brown et al. 2017), and climate-driven increases in ocean surface temperatures are predicted to drive vertical range shifts in benthic organisms over relatively short timescales (Brown & Thatje 2015, Morris et al. 2015, Brown et al. 2017, Pallareti et al. 2018). These movements of marine species across depths could potentially alter existing ecosystems, emphasizing the importance of developing our understanding of colonization theories in the face of anticipated climatic shifts.

The last synthetic contributions aiming to evaluate responses to hydrostatic pressure and tease out their role on the vertical distribution of benthic megafauna through an experimental lens were presented in the 1960s (Menzies & Wilson 1961, Rice 1964, Schlieper 1968). As outlined in these reviews, early experiments were tentative, and their methodologies were poorly described for the most part. Since then, technologies have evolved, and studies involving compression and/or decompression have bloomed, yet no recent assessment of the pressure tolerance literature has been conducted to determine how it may feed into the hypotheses of ocean colonization. The objective of the present synthesis is to fill this gap by summarizing and exploring experimental and observational evidence from the past 60 years that provide insight into the pressure tolerance of extant macrobenthic taxa. The challenges associated with pressure studies (briefly outlined in the previous section) have given rise to a diversity of experimental approaches that make results difficult to compare directly, precluding a formal meta-analysis. However, an exhaustive qualitative analysis is long overdue, which might help provide directions for future studies in this field. Our goal is to build a first comprehensive dataset and attempt to ground truth the various conceptual propositions with the empirical information currently available.

Pressure tolerance is often viewed as one of the predictors of vertical migration in marine species (Young et al. 1997, Tyler & Young 1998, Tyler et al. 2000, Aquino-Souza 2006, Villalobos et al. 2006, Smith & Thatje 2012, Sumida et al. 2015). Based on this assumption, we evaluate whether empirical data of pressure tolerance (as per cent survival and survival duration; see Appendix) provide support for shallow-deep or deep-shallow transitions, or whether bi-directional colonization may offer a more parsimonious explanation. This assessment includes general comparisons between compression and decompression studies, as well as an examination of the effect of depth zone of origin (bathyal vs. abyssal) in the latter. In addition, we attempt to tease out trends in pressure tolerance based on the most probable modulators: ontogeny (comparing tolerance of adult and larval life stages), phylogeny/taxonomy (comparing tolerance among major phyla) and geographic location of collection (looking for latitudinal trends). In line with the colonization hypotheses, this review focuses on macrobenthic species; therefore, pelagic and planktonic animals, and unicellular organisms, are not considered. Where possible, efforts are made to distinguish the responses of deep-water representatives from chemosynthetic and non-chemosynthetic environments, given that the former must additionally cope with habitat-specific gradients in temperature and water chemistry.

Review of pressure tolerance studies

Overview

We have compiled and organized the findings of over 130 studies conducted between 1961 and 2020 (see Appendix method and Table 1). Within this full dataset, some form of empirical measure of pressure tolerance exists for at least 262 species of metazoans across nine phyla. Of these, 244 (93%) have been recorded as having survived pressure shifts, regardless of type and duration of exposure and proportion (or life stage) of survivors. Globally, more species have been exposed to decompression than compression scenarios, but both types of exposure have yielded high proportions of minimal survivorship in early and/or later (adult) life stages (Figure 1).

Importantly, empirical evidence of pressure tolerance is heterogeneous in nature, ranging from acute hour-long pressurization trials of a single life stage in a selected species to long-term holding of decompressed species for months to years. Some species have only been exposed to one stable pressure level, whereas others have been tested under a suite of pressure conditions, including incremental or stable compression/decompression and recompression, and others yet have concurrently

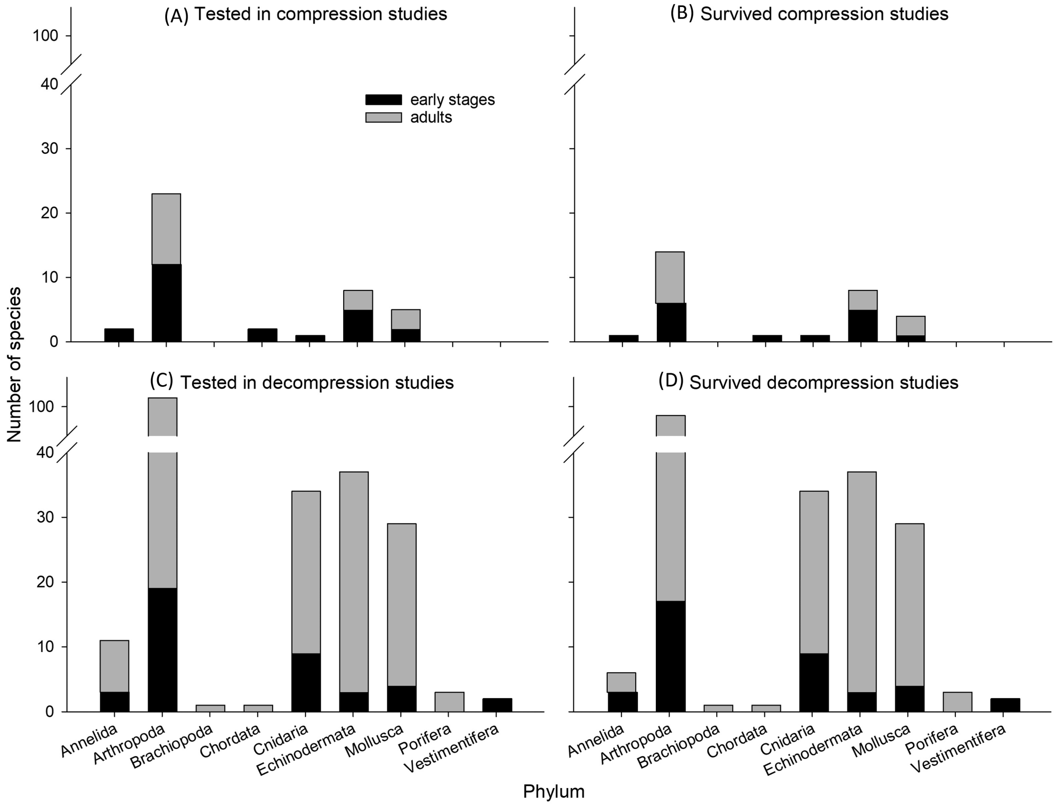

Figure 1 Overview of available empirical evidence of pressure tolerance separated into metazoan phyla, type of study (compression, decompression) and life stage (early stages or adults). (A) Number of benthic species that have been the subject compression studies, of which (B) survival was demonstrated in one way or another. (C) Number of benthic species that have been the subject decompression studies, of which (D) survival was demonstrated in one way or another.

been exposed to a range of pressures and temperatures in multifactorial designs (Appendix Table 1). Depending on the study objectives, the outcomes are also reported using various descriptive and quantitative metrics. Unfortunately, standard measures such as LD_{50} have typically not been used. The simplest, most universal means of assessing pressure tolerance involves measuring per cent survival inside a group of animals exposed to compression or decompression, with or without a complementary measure of survival duration (or inversely). Except in cases of long-term holding, the measure of momentary survival makes abstraction of the state of the survivors, which does not guarantee that fitness was maintained or that the exposure was not ultimately lethal. Therefore, teasing out the meaning of the findings shown in Figure 1, and how they can be compared is challenging. In an attempt to extract further information, we have adopted a two-stage approach: first we try to draw generalizations where possible, notwithstanding the caveats mentioned above; and second, we present case studies inside each phylum to provide a more detailed understanding of the state of knowledge.

Combining a subset of compression and decompression studies that provide clear survival rates and durations (see criteria in Appendix, and selected records in Appendix Table 2), the nine major metazoan phyla are still represented, including 45 species tested at early life stages (embryos and larvae) and 144 species tested at later life stages (juveniles and adults), some of which may be the

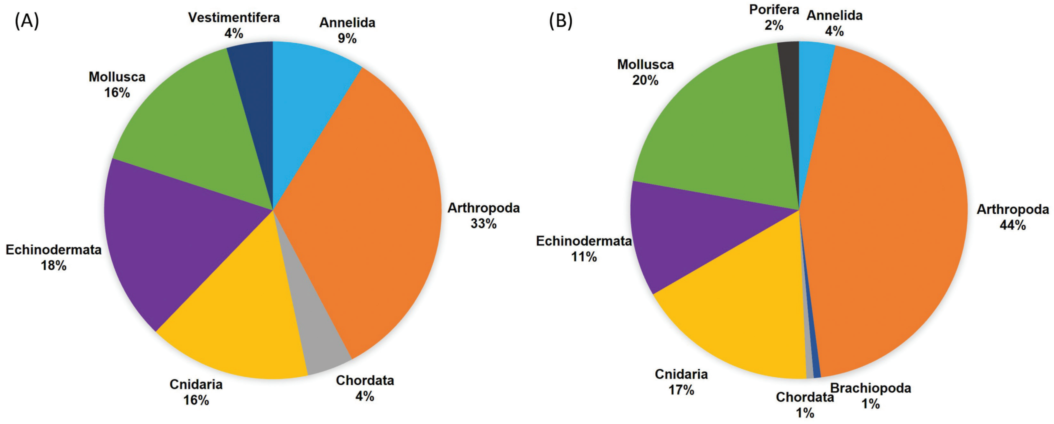

Figure 2 Taxonomic distribution of macrobenthic species used in studies of pressure tolerance (compression or decompression) that examined (A) early life stages (embryos or larvae, $N = 45$ species) and (B) late life stages (juveniles or adults, $N = 144$ species) and for which per cent survivors and duration of survival (until mortality or end of study) were both provided. Refer to Appendix for a description of studies included.

same species. In each of these two groups, most species belong to Arthropoda (33%–44%), followed by Cnidaria, Echinodermata and Mollusca (11%–20%), and the rest (Annelida, Chordata, Foraminifera, Porifera, Vestimentifera) each represent 1%–9% (Figure 2). Looking at the type of tolerance in this subset reveals that three of the most studied phyla (Arthropoda, Echinodermata and Mollusca) provide fairly balanced assessments, with both life stages having been submitted to compression and decompression scenarios (and a slight predominance of decompression studies conducted with adults). In contrast, assessments are more biased for Cnidaria, which have only been the subject of decompression studies at both life stages.

Regardless of the type of exposure (compression or decompression), most species survived exposure of ~1.5 to >26,000 hours to pressure that differed from their native pressure by at least 2 MPa (~200 m), suggesting some level of tolerance. Maximum proportion of survivors ranged broadly from as little as 2%–7% and up to 100% of exposed individuals. There was considerable variability, but average per cent survival was more commonly higher in decompression than compression studies in phyla where both metrics were available, i.e. across 9 species of Annelida, 79 species of Arthropoda, 24 species of Echinodermata and 36 species of Mollusca (Figure 3). The only exception was reported in a single invertebrate Chordata species where compression of larvae for 24 hours was highly successful.

Ontogeny

An overview of the subset shown in Figure 3A does not reveal any clear trend in per cent survival (regardless of exposure duration) between studies conducted on adults or larvae of different taxa within a phylum. The survival duration (Figure 3B) cannot be readily compared between life stages inside a phylum because studies are always of shorter duration for embryos and larvae (≤500 hours; constrained by developmental tempo). However, we found 21 species across six phyla for which survival rates were provided for the early and late life stages of the same species (Figure 4). All but one of these were tested under decompression scenarios (deep-water species exposed to atmospheric pressure) and, as stated above, duration of exposure was not necessarily equivalent for both stages. The only compression study was conducted with an arthropod and resulted in 100% survival for both life stages tested. In the decompression studies, per cent survival was the same or was similar

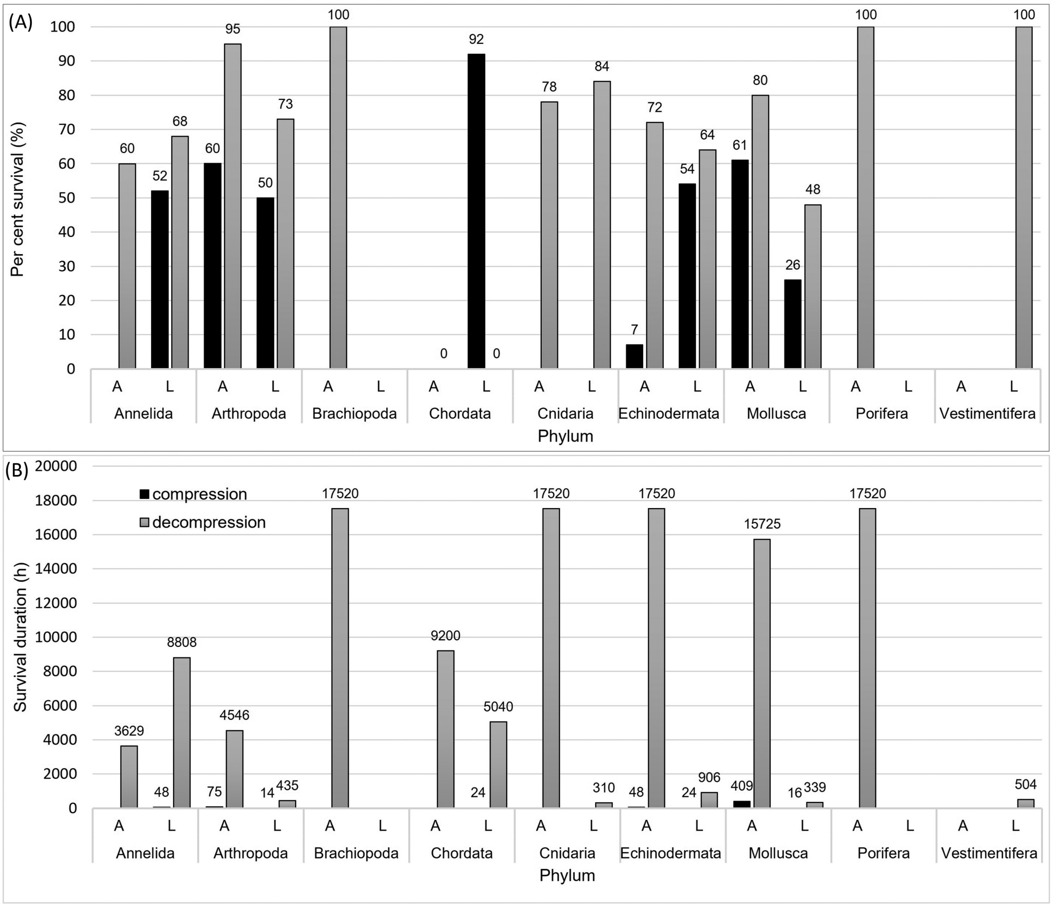

Figure 3 (A) Mean per cent survival and (B) mean survival duration (until mortality or end of study) reported following compression and decompression studies conducted with juvenile or adult stages (labelled A) and early life stages, mostly larvae (labelled L) of benthic species belonging to the major metazoan phyla (N=9 Annelida, 79 Arthropoda, 1 Brachiopoda, 1 Chordata, 32 Cnidaria, 24 Echinodermata, 36 Mollusca, 3 Porifera, 2 Vestimentifera). Bars without values are subgroups for which data are not available. See dataset in Appendix Table 2.

(within 5%) between life stages in roughly half the species (Figure 4). Survival was lower for the early life stages than for the adults in one of two annelids, two of four arthropods, the only chordate, one of seven species of cnidarians, one of three echinoderms, and in the single mollusc; conversely, it was only lower for the adults in one of the two annelids.

Taxonomy

Based on the compiled data for the main metazoan phyla, per cent survival and survival duration did not display any clear phylogenetic trend. The recorded survival of adults to pressure shifts (regardless of sample size and exposure duration) was commonly ≥50% and often as high as 100% in many taxa, and results varied markedly both across and within phyla (Figures 3 and 4). During maintenance, the longest surviving taxa were typically Cnidaria, Echinodermata, Mollusca and Porifera, while Arthropoda survived the shortest duration, but still well above 100 days (to over a year for some studies).

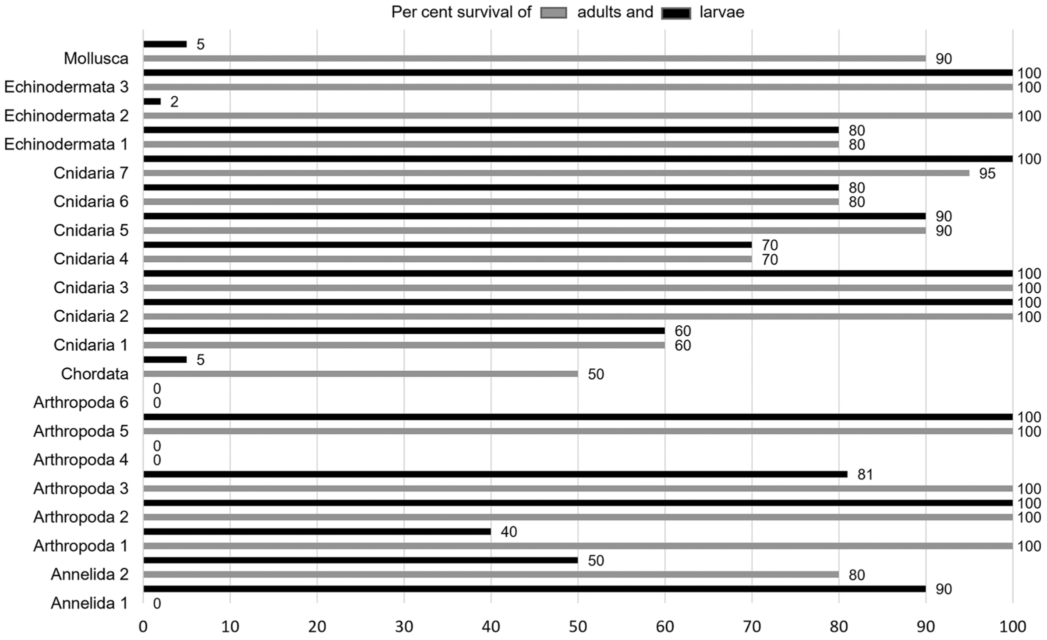

Figure 4 Exploration of per cent survival in adults and early life stages (mostly larvae) of the same deep-sea species following decompression to atmospheric pressure (grouped per phylum). Each pair of bars represents a species. See details of dataset in Appendix Table 3.

Depth zone and geographic region of origin

Based on the compiled subset, per cent survival and survival duration were highest/longest for decompressed adult individuals collected from bathyal depths, compared to both compression of taxa from shallower zones and decompression of taxa from deeper zones (Figure 5A). When considering only decompression of adults originating from the bathyal zone (minus those from chemosynthetic habitats such as vents and seeps), per cent survival was slightly higher, but survival duration much shorter for studies conducted at lower latitude (Gulf of Mexico, Mediterranean Sea, Tropical Indian and Pacific Oceans) compared to higher latitudes (Northwest/Northeast Atlantic and Pacific Oceans) (Figure 5B).

Apart from the high/low latitude trend, no pattern emerged from comparisons among geographic regions, and the influence of the habitat of origin (i.e. including or excluding chemosynthetic environments) was minimal (Figure 6). Worth mentioning is the fact that studies at higher latitudes were chiefly conducted in the Atlantic and studies at lower latitudes were chiefly conducted in the Mediterranean, creating a bias where ocean basins are not equally represented. With respect to taxonomy, the top four phyla identified at the onset still dominate.

Case studies of decompression

Adults and juveniles from deep chemosynthetic environments

Organisms from chemosynthetic habitats have been reported to survive decompression quite well (often >70%) from a substantial number of studies (Appendix Table 1) (Mickel & Childress 1982, Childress et al. 1984, 1991, Gaill et al. 1997, Shillito et al. 2001, Dixon et al. 2002, Ravaux et al. 2003, Shillito et al. 2004, Shillito 2006, Cottin et al. 2008, Kádár et al. 2008a, Boutet et al. 2009,

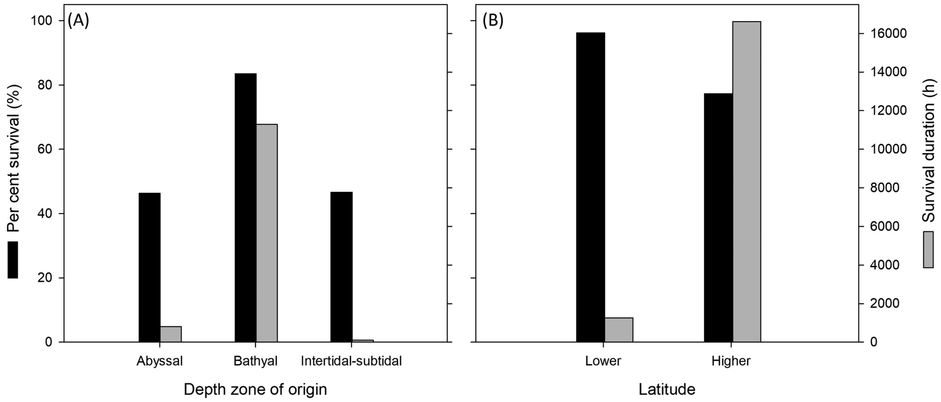

Figure 5 Exploration of per cent survival (left axis) and survival duration (right axis) from case studies of adults of the main phyla exposed to compression or decompression. (A) Comparison among depth zones of origin, regardless of habitat and exposure type ($N=142$, including 6 Annelida, 62 Arthropoda, 1 Brachiopoda, 1 Chordata, 16 Echinodermata, 25 Cnidaria, 28 Mollusca and 3 Porifera). (B) Comparison among latitudes for bathyal taxa (from non-chemosynthetic habitats) exposed to decompression ($N=104$, including 2 Annelida, 43 Arthropoda, 1 Chordata, 25 Cnidaria, 12 Echinodermata, 18 Mollusca and 3 Porifera). See details of dataset in Appendix Table 4.

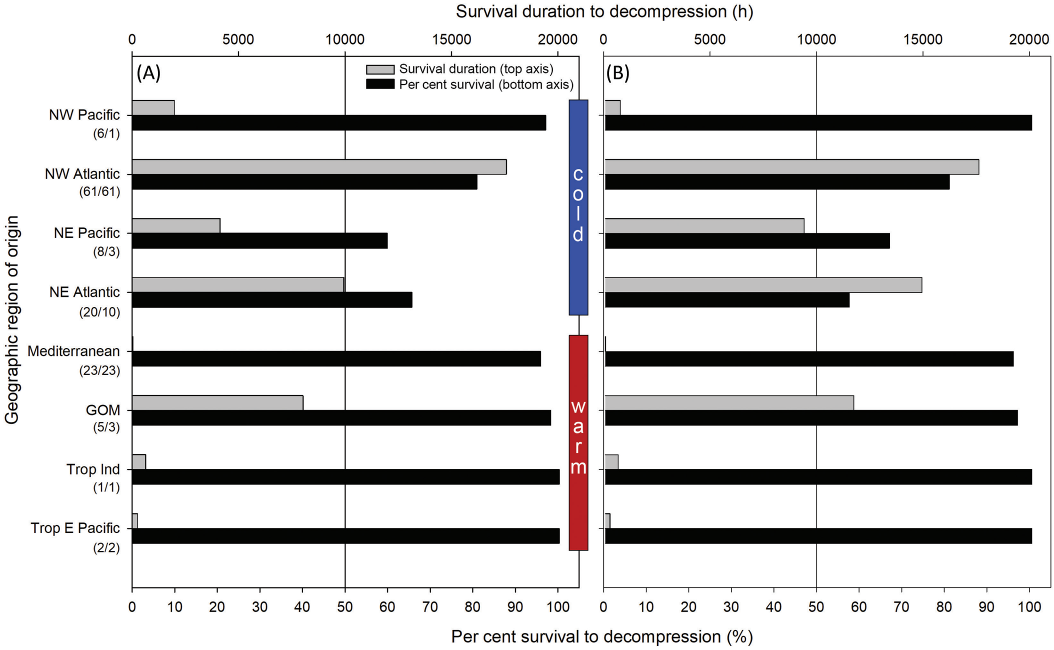

Figure 6 Exploration of per cent survival (bottom axis) and survival duration (top axis) among geographic regions from case studies of adults of the main benthic phyla collected at bathyal depths exposed to decompression. (A) Studies of chemosynthetic and non-chemosynthetic taxa; $N=126$ species (6 Annelida, 55 Arthropoda, 1 Chordata, 25 Cnidaria, 12 Echinodermata, 24 Mollusca and 3 Porifera). (B) Studies of non-chemosynthetic taxa only; $N=104$ with taxonomic distribution as in Figure 4B. The top four regions are higher latitudes (blue rectangle), and the bottom four regions are lower latitudes (red rectangle). The number of records per region is shown in parentheses (as panel A/panel B). NW, Northwest; NE, Northeast; GOM, Gulf of Mexico; Trop, Tropical; E, East. See details of dataset in Appendix Table 4.

Cottin et al. 2010, Durand et al. 2010, Ravaux et al. 2013, Shillito et al. 2015). Phylum-specific examples are outlined below.

Annelida. There are relatively few examples of chemosynthetic Annelida successfully maintained at surface pressure, but the hydrothermal vent tubeworm *Lamellibrachia luymesi* was collected and held for 1–15 months in laboratory conditions (Dattagupta 2006). It was noted that most individuals survived despite physical damage to the base of the tube sustained during the collection process. By contrast, multiple species of chemosynthetic Annelida did not survive decompression (Childress et al. 1984, Pradillon 2005). This inability to survive long exposures to decompression was also noted in unpublished work with the species *Paralvinella palmiformis* held at surface pressure (Lee, pers. comm).

Arthropoda. Survival of vent/seep arthropods at surface pressure is generally >50% (only one experiment resulted in 0% survival; Appendix Table 1), and individuals have been found to survive for sustained periods. The vent shrimp *Mirocaris fortunata* collected from 1617 m was maintained at atmospheric pressure for >12 months, although it was not made clear how much mortality occurred (Smith et al. 2013). The shrimp *M. fortunata* was also collected from the Northeast Atlantic while five species of crab and shrimp were collected from the Northwest Pacific off the coast of Japan: all were maintained for over a year at atmospheric pressure (Miyake et al. 2007, Hamasaki et al. 2010, Smith et al. 2013).

Mollusca. Molluscs collected at vents and seeps that were brought to the surface exhibited 100% survival to decompression for durations ranging from 12 hours to a year (Martinez et al. 2001, Kádár et al. 2005, 2006, Bettencourt et al. 2008, Kádár et al. 2008b, Colaço et al. 2011). The mussel *Bathymodiolus azoricus* was collected from the Northeast Atlantic at Menez Gwen and Rainbow vent fields from depths of 840, 850 and 2300 m. Individuals survived anywhere from 10 to 365 days (e.g. Pruski & Dixon 2003, Dixon et al. 2004, Kádár et al. 2008b, Bettencourt et al. 2010, Martins et al. 2014, Barros et al. 2015, Bettencourt et al. 2017). Likewise, the cold seep mussel *Bathymodiolus childressi* was collected from the Gulf of Mexico and survived decompression to surface pressure conditions for periods ranging from 15 to 365 days (Arellano & Young 2009).

Adults and juveniles from deep non-chemosynthetic environments

Macrobenthic species collected from the deep Northwest Atlantic have shown a consistent ability to survive and thrive at surface pressure for years (Appendix Table 1). Furthermore, several species have also reproduced, some persisting through multiple generations (see details below). Reports from many other regions indicate that a diversity of deep-sea taxa from non-chemosynthetic environments survive at surface pressure for days to months and beyond (Appendix Table 1). Notably, 13 species of Arthropoda and 14 species of Echinodermata were collected from 250 to 1000 m depth in the Tropical West Pacific Ocean and maintained in the laboratory for 120 days (Wilson et al. 2013). Some phylum-specific examples are provided below.

Annelida. Annelid species collected from the Northwest Atlantic survived differently when decompressed to atmospheric pressure under very similar settings (in the same laboratory). For instance, the polychaete *Neopolynoe acanellae* collected from depths of 466 to 1406 m exhibited 100% survival (Hamel et al. 2015), whereas *Ophryotrocha* sp. collected from 60 to 730 m exhibited 50% survival (Mercier et al. 2014, Verkaik et al. 2017). Despite lower survival, the latter was maintained for several generations.

Arthropoda. Generally, arthropods tolerated decompression well, but there was variability across studies. An amphipod from the Northeast Atlantic, *Stephonyx biscayensis*, survived decompression from 1528 to 1765 m depths over acute exposure periods (0.17 hour) without exhibiting any mortality, and was subsequently held for 60 days at surface pressure (Brown & Thatje 2011). Similarly, the Lophogastrida *Gnathophausia ingens* collected from 400 to 900 m tolerated acute decompression to surface pressure (for 4–6 hours), with some specimens surviving for 45 days post-experimentation (Mickel & Childress 1982). In the case of collections from deeper abyssal depths down to 3900 m in the Indian Ocean, survival rates to decompression varied among species (Treude et al. 2002). Specimens of the amphipods *Paralicella* spp. and *Abyssorchomene abyssorum* did not survive at surface pressure while *Abyssorchomene distinctus* survived for 21 days (Treude et al. 2002). In the case of *Paralicella* aff. *alberti,* individuals were collected from two different depth zones, bathyal and abyssal; the individual collected from the former survived for 26 days whereas the individual collected from the latter died shortly after being brought to surface (Treude et al. 2002). Several pycnogonids (*Nymphon hirtipes*), collected from depths of 1350 to 1450 m in the Northwest Atlantic off eastern Canada not only survived long-term holding, but laid eggs and yielded juveniles (Mercier et al. 2015). Moreover, several species of barnacles, shrimps and amphipods from the bathyal Northwest Atlantic survived for years at atmospheric pressure in mesocosms.

Cnidaria. Some 22 different species of cnidarians originating from a range of depths in the North Atlantic, down to 2500 m, were maintained for over 2 years at surface pressure with some of them spawning (sea anemones and corals) and producing offspring (e.g. Mercier & Hamel 2009a, Sun et al. 2009, Hamel et al. 2010, Sun et al. 2010, Mercier et al. 2011b, 2011d, Baillon et al. 2014, Mercier et al. 2017). One of them, the deep-sea scleractinian coral *Flabellum alabastrum*, exhibited growth between 1 and 5 mm/year over 2 years (Hamel et al. 2010). Two other scleractinian coral species collected from the Mediterranean Sea at depths of 214–218 m were held at surface pressure for 517 days to study their growth (Orejas et al. 2008). The commensal sea anemone *Allantactis parasitica* from the Northwest Atlantic, living in association with large deep-water gastropods, survived also for several years at atmospheric pressure; some individuals spawned, and interactions with the hosts were characterized (Mercier & Hamel 2008b, 2009b, Mercier et al. 2011a).

Mollusca. Under similar decompression conditions, only 50% of the bathyal mollusc species, including *Frigidoalvania brychia* (Weinberg 1990), survived for 772 days. Several species from the Northwest Atlantic, e.g. the gastropod *Buccinum scalariforme*, were seen to mate and lay egg masses that produced surviving offspring (Montgomery et al. 2017).

Porifera. Three species of demosponges from the bathyal Northwest Atlantic fed and exhibited growth over years of holding at atmospheric pressure; some individuals were used in experimental trials to estimate their filtration rates under various environmental conditions (Robertson et al. 2017).

Echinodermata. Several species of sea stars, brittle stars and sea urchins collected from deep waters off eastern Canada survived for months to years under atmospheric pressure. Among them, the brooding sea star *Henricia lisa* (Mercier & Hamel 2008a) and *Hippasteria phrygiana* fed and reproduced (Stuckless et al. 2021). Others sea stars were used in series of experimental trials over a period of several months, including *Ceramaster granularis* (Stuckless et al. 2021).

Embryos and larvae from chemosynthetic environments

Relative to the number of studies that investigated the pressure tolerance of adults, there were fewer studies of decompression in early life stages (eggs, embryos and various larval stages) of species from chemosynthetic environments (Appendix Table 1), which can be largely attributed to logistical challenges (Pradillon et al. 2001, Pradillon 2005, Cottin et al. 2008, Ravaux et al. 2009). Very few investigators obtained larvae directly from chemosynthetic environments and reported their development. Among them, Arellano et al. (2014) collected eggs and veligers of vent gastropods and Epifanio et al. (1999) collected megalopa larvae of vent crabs (see findings below). More reports exist of deep-sea species from chemosynthetic environments having spawned at surface pressure with successful larval development, as exemplified below.

Arthropoda. The deep-water barnacle *Neoverruca* sp. demonstrated greater survival in treatments that exposed larvae to lower pressures (surface pressure) than treatments that compressed larvae (born in the laboratory from decompressed genitors) to native pressure conditions (Watanabe et al. 2004). Survival at surface pressure decreased slightly from 100% to 97% over the course of

14–17 days. Many deep-sea arthropods sampled from the Nikko Seamount vents of the Northwest Pacific successfully spawned, including the vent crab *Austinograea yunhana* and vent shrimp *Opaepele* spp. (Miyake et al. 2007). However, the larvae of the latter did not continue development post-hatching and those of the former experienced early mortality, which investigators suggested was unrelated to pressure issues (Miyake et al. 2007). A similar suggestion was made when larvae of *Gandalfus yunohana* were obtained in the laboratory and successfully metamorphosed into megalopal stages, but died without moulting into juveniles after 104 days (Hamasaki et al. 2010). It was proposed that chemical cues from the natural environment were required for moulting to juvenile and that pressure was not the immediate limiting factor. Wantanabe et al. (2004) offered similar conclusions when adults of hydrothermal vent barnacles *Neoverruca* from the Northwest Pacific spawned larvae under surface pressure, and none successfully metamorphosed/settled before dying after 183 days. In a rare study of larvae obtained from the deep, Epifanio et al. (1999) collected megalopa stages of the hydrothermal vent crab *Bythograea thermydron* from 2500 m, which survived only until their third stage of development (while juveniles collected at the same time were maintained at surface pressure for a period of 201 days).

Mollusca. Generally, larvae of deep-sea vent species of molluscs (obtained following spawning of adults collected at depth) survived well (>58%) when decompressed and exposed to surface pressure (Arellano & Young 2011, Arellano et al. 2014). Arellano et al. (2014) also collected eggs of the gastropod *Bathynerita naticoidea* and veligers of *B. childressi* in the Gulf of Mexico, which were said to have successfully hatched and developed at surface pressure, but the maximum survival duration was not assessed.

Embryos and larvae from non-chemosynthetic environments

Propagules that resulted from the reproduction of deep-water adults collected from non-chemosynthetic environments generally survived well under various experimental conditions (Appendix Table 1).

Annelida. The deep-water polychaete *Ophryotrocha* sp., collected from the Northwest Atlantic, reproduced several times under mesocosm conditions at atmospheric pressure, with offspring reaching maturity and reproducing themselves over successive generations (Mercier et al. 2014). The reproductive output of this species was later used in an investigation of the impact of ocean acidification (Verkaik et al. 2017).

Arthropoda. Several individuals of the deep-sea pycnogonid *Nymphon hirtipes* collected at 700–1450 m depths in the Northwest Atlantic laid egg masses at atmospheric pressure, which underwent embryonic, larval and juvenile development until their release by the brooding males (Mercier et al. 2015). Survival rates remained 100% over 9 months of monitoring. In a series of pressure experiments on the early life stages of copepod species that reproduce in high-pressure environments (*Neocalanus cristatus, N. flemingeri* and *N. plumchrus*), which were collected at 1000–1500 m depths in the Northwest Pacific, development and hatching success were not affected by pressure (Yoshiki et al. 2011).

Cnidaria. Larvae produced by deep-sea corals, including *Drifa* sp., *Drifa glomerata, Flabellum angulare* and by the sea anemone *Urticina* sp. had high survival rates (>70%) to the planula and juvenile stages (Sun et al. 2009, 2010, Mercier et al. 2011b,c,d), but their ability to survive to later stages was not evaluated due to logistical constraints. Moreover, propagules (embryos, larvae) of the sea anemone *Allantactis parasitica* developed into juveniles that grew over several months at atmospheric pressure (Mercier & Hamel 2009a).

Echinodermata. Progeny of two deep-water sea stars (*Hippasteria phrygiana* and *Henricia lisa*) from the Northwest Atlantic were reported to develop from fertilized oocytes to gastrulae and fertilized oocytes to fully developed juveniles, respectively. In the case of *H. lisa*, the brooded offspring had a survival rate of 100% (Mercier & Hamel 2008a).

Mollusca. The deep North Atlantic gastropod *Buccinum scalariforme* developed from egg to juvenile inside egg masses laid during holding at atmospheric pressure; juveniles emerged after 120 days of development, with a survival rate of ~5% (Montgomery et al. 2017). Low survival is likely part of the reproductive strategy of this species, whereby most oocytes are used as nurse cells by a limited number of developing offspring.

Case studies of compression

Adults and juveniles

Shallow-water species submitted to compression generally did not survive as long as decompressed deep-sea species outlined above (Appendix Table 1). Nevertheless, many species demonstrated an ability to withstand pressure beyond that prevailing inside their native distributions, as shown in the phylum-specific examples below.

Arthropoda. The shallow-water shrimp *Palaemonetes varians* demonstrated 70% survival at 10 MPa after 28 days of exposure (Cottin et al. 2012). The authors of the study suggested that mortalities may have been associated with cannibalism resulting from the absence of feeding rather than from compression itself. In a series of experiments on the subtidal crab *Lithodes maja*, survival at the end of 240 hours of exposure was 100% at 7 MPa and down to 0% at 12.5 MPa (Brown et al. 2017). In another shallow-water crab, *Maja brachydactyla*, 100% of individuals survived 90 hours of exposure to 15.2 MPa (Thatje and Robinson 2011). Similarly, all (100%) of the hermit crabs *Pagurus cuanensis* survived 1-hour compression to 5 MPa (Thatje et al. 2010).

Echinodermata. The survival of sea urchins *Strongylocentrotus droebachiensis* and sea cucumbers *Cucumaria frondosa* collected from the Northwest Atlantic decreased from 100% to 0% with increasing duration (24 vs. 72 hours) of compression corresponding to depths beyond their natural range, i.e. 2–3 times the deepest depth of natural occurrence (Ammendolia et al. 2018). As for the sea star *Leptasterias polaris*, it survived compression well for 24–48 hours (83%–100%), but there was 100% mortality after 9 days of exposure at the same pressure, demonstrating decreased tolerance over prolonged experimental durations.

Mollusca. The same trend of decreasing tolerance with exposure duration was noted for the shallow-water bivalve *Mytilus edulis,* with mortality occurring after 216 hours of compression at 22 MPa (Ammendolia et al. 2018). Menzies and Wilson (1961) had also found that *M. edulis diegensis* could cope with acute compression up to 23.3 MPa for 5.8 hours, whereas no individuals were able to survive to 35 MPa for 8 hours, suggesting that barotolerance was met at depths beyond ~3500 m. In another species, *M. galloprovincialis* collected from 0 to 10 m, compression to 46.5 MPa for 144 hours resulted in 0% survival while compression to slightly lower pressures (15.5 MPa) for 1656 hours resulted in 38% survival (Galgani et al. 2005).

Embryos and larvae

A limited number of studies have tested the compression of early life stages because long-term investigations require appropriate holding facilities and continuous monitoring over more or less extended developmental periods. Globally, there were trends of decreasing survival with increasing pressure beyond the scope of natural bathymetric range in larvae of shallow-water species.

Annelida. After 48 hours of pressure exposure, survival of the larvae of the annelid *Pomatoceros lamarcki* decreased from 69.1% at 10.1 MPa to 41.4% at 20.3 MPa and 19.3% at 30.4 MPa (Vevers et al. 2010).

Arthropoda and Cnidaria. George & Marum (1974) tested the effect of experimental compression (6.1–65.5 MPa) for 1 hour on the survival of larvae of some species of shallow-water Arthropoda and Cnidaria from either the Caribbean or Northwest Atlantic, with variable survival from 0% to 100%. In short compression experiments that lasted ~4 hours, no difference in survival was found among development stages of the crab *Lithodes maja* (Munro et al. 2015).

Chordata. In the Caribbean ascidian *Polyandrocarpa zorritensis,* survival rates of tadpole larvae increased with exposure to pressure, from 62% at 5 MPa to 92% at 20.3 MPa (Sumida et al. 2015). However, survival of juveniles was not in line with this trend, suggesting there was limited capacity to adapt fully to these high-pressure conditions (Sumida et al. 2015).

Echinodermata. Species of shallow-water echinoderms (sea stars and sea urchins) collected from the Northeast Atlantic and Antarctica had high survival (mostly >80%) when compressed for 24–48 hours to pressures characteristic of depths greater than their natural occurrence (Tyler & Young 1998, Aquino-Souza 2006, Villalobos et al. 2006). Other experiments tested the larvae of sea cucumbers *Apostichopus japonicus* from the western Pacific for as little as 7 minutes and found that survival reduced as pressure increased (Ding et al. 2007). Embryos of the sea urchin *Sterechinus neumayeri,* collected from Antarctica, survived compression better than later prism and 4-arm pluteus larval stages (Tyler et al. 2000). Specifically, survival at 25.3 MPa was 91%, 18% and 4%, for the three stages, respectively. Similar trends were observed with another sea urchin (*Psammechinus miliaris*) from the Northeast Atlantic in which survival to compression diminished as development progressed, i.e. gastrulae survived more than late prism stages (Aquino-Souza et al. 2008). This was also reflected in a shallow-water sea star, *Asterias rubens,* collected from the Northeast Atlantic where the late bipinnaria generally survived more than the early bipinnaria when compressed over 48 hours (Villalobos et al. 2006).

Mollusca. Survival of the mollusc *Crepidula fornicata* exposed for 24 hours to various compression levels decreased from 100% at 20 MPa to 75% at 30 MPa and 52% at 40 MPa (Mestre et al. 2013). Interestingly, pressure tolerance varied between different larval stages, whereby late veligers of *C. fornicata* survived slightly better under elevated pressure than early veligers (Mestre et al. 2013).

Discussion

Pressure tolerance and the hypotheses of ocean colonization

Many short-term compression studies have proposed pressure tolerance (survival for a relatively brief duration) to be a predictor of deep-sea invasion by shallow-water species (Young et al. 1997, Tyler & Young 1998, Tyler et al. 2000, Aquino-Souza 2006, Villalobos et al. 2006, Smith & Thatje 2012, Sumida et al. 2015). The present review aimed to use a similar lens to take a broader look at currently available results from both compression and decompression studies, exploring how they might inform vertical colonization patterns. Evidence of tolerance to higher or lower pressures was overall quite widespread in the studies of macrobenthic species compiled here. Strictly based on the number of records demonstrating higher per cent survival and/or longer survival duration following decompression of deep-water species than compression of shallow-water species, we globally found stronger support for the deep-shallow than the shallow-deep hypothesis. Generally, the survival of adults after exposure to compression and/or decompression was higher for deep-sea than shallow-water taxa. Furthermore, adults of many deep-sea species seem to possess the capacity to withstand shallow-water pressure conditions for prolonged periods, whereas evidence of adult shallow-water organisms surviving high pressure remains limited. For instance, studies of numerous deep-sea species (from a range of phyla) have reported survival at surface pressure over months to years (e.g. Weinberg 1990, Miyake et al. 2007, Mercier et al. 2011d), both in laboratory chemosynthetic conditions (e.g. Colaço et al. 2006, Miyake et al. 2007, Colaço et al. 2011, Miyake et al. 2012, Matabos et al. 2015, Shillito et al. 2015) and non-chemosynthetic cold-water conditions (e.g. Mercier & Hamel 2008a, 2009a, Sun et al. 2009, Mercier et al. 2014, 2015). While more limited, good examples also exist of shallow-water taxa surviving long-term compression, e.g. 69 days for the mussel *Mytilus* *galloprovincialis* maintained in sea cages at depths to 1550 m (Galgani et al. 2005) and 28 days for the shrimp *Palaemonetes varians* maintained in vessels pressurized to 10 MPa, or ~1000 m (Cottin et al. 2012). However, this review found many reports of survival to pressure in shallow-water taxa decreasing both as a function of pressure level and exposure duration.

Beyond survival rates, several long-term studies observed key behaviours such as feeding, growth, spawning and larval development in deep-sea species at surface pressure, indicating that those species acclimatized successfully to surface pressure under appropriate holding conditions. The ability of deep-sea species to survive at surface pressure may not even be fully captured within the available literature because information on total survival duration beyond experimental trials was often absent. For example, Brown & Thatje (2011) assessed tolerance to compression in the deep-sea crustacean *Stephonyx biscayensis* following 60 days acclimation to decompression and surface pressure, and reported that survival at surface pressure exceeded the acclimation period.

Interestingly, the length of post-collection survival of deep-sea adults at surface pressure was generally longer for taxa collected from bathyal (>200–2000 m) than abyssal depths (>2000–6000 m). This trend was evidenced for both chemosynthetic (i.e. coming from deeper depths, but the same thermal regime) and non-chemosynthetic species, suggesting that species from bathyal depths could more easily acclimatize to surface pressures than those from abyssal depths. In fact, bathyal species also exhibited high survival at pressure greater than that experienced in their natural distribution, suggesting that their adults have the physiological capacity to acclimatize to abyssal depths too. While more limited, there is still evidence that abyssal taxa can survive decompression, which is at odds with early (and persisting) assumptions that animals collected from depths below 1500 to 2000 m would be largely incapable of surviving at surface pressure (Pruski & Dixon 2003, Dixon et al. 2004, Pradillon & Gaill 2007). It may be that the numerous cases of abyssal species showing shorter survival at surface pressure result from physical/physiological damage incurred during collection, rather than from an innate physiological barrier. For instance, animals collected deeper may undergo more severe/acute temperature and/or pressure shocks during recovery, causing damage to nervous tissue at both transcriptional and cellular levels, potentially leading to serious injuries and death (e.g. Morris et al. 2013). Hence, bathymetric thresholds in decompression tolerance may be weaker or non-existent under more isothermal regimes, as exemplified by deep-sea corals of the genus *Flabellum* surviving equally well after being collected from bathyal or abyssal zones of the Northwest Atlantic (Hamel et al. 2010, Mercier et al. 2011d). The fact that genetic and individual pressure resistance was found to be highest at the optimum temperature of a given intertidal species (Schlieper 1968) brings support to enhanced pressure tolerance (and greater potential for vertical migrations) in isothermal water columns (see also the discussion of latitudinal trends).

The tolerance of deep-sea species to decompression was confirmed when looking at the frequency of successful development, from oocytes to late larvae, settlement and survival to advanced juvenile stages. Spawning events at surface pressure leading to propagule cultures that reached metamorphosis and settlement were documented in several deep-sea species across various phyla. At least one deep-sea species (the annelid *Ophryotrocha* sp.) underwent multiple generations at surface pressure in flow-through laboratory conditions (Mercier et al. 2014). Furthermore, adult sea stars, sea anemones and octocorals collected from as deep as 2500 m produced larvae that were reared to juvenile stages at surface pressure (e.g. Mercier & Hamel 2008a, Sun et al. 2010, Mercier et al. 2015, 2017) (see discussion of ontogeny for details). Even adult tonguefish larvae developed at surface pressure, forming eyes and actively feeding at 7 days old (Miyake et al. 2007), which is intriguing because deep-sea fish do not typically survive under surface pressure conditions (Pradillon 2005). Many investigative teams have argued that the ability for deep-sea larvae to survive under laboratory conditions may be underestimated, as mentioned for adult stages in the earlier discussion. Studies do not typically sample larvae directly from the deep sea (though some did); rather, most eggs, embryos and larvae come from the reproduction of genitors collected at depth. Even where propagules are subsequently extracted or released naturally under laboratory conditions, the extent of the trauma that gametes or brooded larvae experience during sampling and recovery of adults has yet to be determined. Ultimately, collection methods, including rapid decompression or use of sampling technologies that may inflict physical damage, may negatively affect the reproductive health/fitness of animals. Other factors susceptible to undermine the survival of early life stages include the absence of natural chemical cues required for metamorphosis and settlement of deep-sea larvae (Watanabe et al. 2004, Hamasaki et al. 2010), and poor water quality (Colaço et al. 2006). For example, many deep-sea ascidian larvae (Chordata) developed until juvenile stages and demonstrated adequate feeding, but died after 7 months because of what was described as a ciliate infestation (Havenhand et al. 2006). Numerous factors are known to similarly prevent full development to juvenile in cultures of shallow-water species (Hodin et al. 2019).

While there is substantial evidence that deep-sea adults and larvae are able to tolerate shallow-water pressures (present dataset), and that deep-sea larvae may undergo extensive vertical migrations all the way to the surface (Young et al. 2018), many of these decompression-tolerant species are nevertheless absent from habitats found at shallow depths. Other pre- and post-settlement processes may affect the survival of deep-water taxa at shallow-water pressures. For example, a field study that transplanted deep-sea Antarctic acorn barnacles (*Bathylasma corolliforme*) from 400 to 25 m reported that some individuals survived at transplanted depths for over 2 years (Dayton et al. 1982). Differences in survival were said to be modulated by substrate availability, thus supporting the ability for deep-sea species to acclimatize to shallow water under otherwise suitable environmental conditions.

The submergence (shallow to deep) hypothesis has been supported mainly by fertilization trials with echinoderms and molluscs, along with survival of embryos and larvae during short-term compression (Tyler & Young 1998, Tyler & Dixon 2000, Villalobos et al. 2006). However, the present review noted a general decrease in tolerance to compression as development progressed (e.g. from early to late larvae; though at least one exception was compiled). We also found no evidence that complete development of shallow-water species from fertilization to juvenile is possible at elevated hydrostatic pressure and limited evidence (duration-wise) that adults of shallow-water species can tolerate deep-sea pressures. An important limitation is that compression studies seldom test the tolerance of larval and adult stages of the same species (e.g. Pechenik et al. 1984, Childress & Thuesen 1993, Tyler & Young 1998, Tyler & Dixon 2000, Villalobos et al. 2006, Ding et al. 2007, Aquino-Souza et al. 2008, Mestre et al. 2009, Vevers et al. 2010, Smith & Thatje 2012, Smith et al. 2013, Munro et al. 2015, Smith et al. 2015), which is important since pressure tolerances can vary ontogenetically (e.g. Munro et al. 2015, Brown et al. 2017) and all life stages and processes must be tolerant to variations in pressure to allow successful colonization. Further, experiments where shallow-water larvae survived pressures outside of their natural bathymetric ranges, and where successful metamorphosis or settlement was documented, are in contrast to the lack of adults of these species recorded at such depths in nature (Brown & Thatje 2014). While empirical evidence supporting the shallow-deep hypothesis appears weaker than that supporting the deep-shallow hypothesis at present, technological and logistical difficulties contribute to limiting the strength of compression studies on shallow-water taxa. For example, high-pressure tanks are few and it is generally difficult or impossible to feed animals for long-term holding within these pressure vessels (but see Shillito et al. 2020), let alone provide true mesocosms. Thus, the potential for shallow-water fauna to tolerate deep-sea pressure may be underestimated to the same (or greater) extent as the potential of deep-sea species to survive surface pressure discussed earlier. Furthermore, early studies found that exposure to non-lethal pressure (10–20 MPa) had a stimulating effect, i.e. caused an increase in the locomotor activity, ciliary action and heart rates of many intertidal marine species (Schlieper 1968). Similarly, increased feeding was noted in shallow-water echinoderms exposed to pressures of 5–6 MPa (Ammendolia et al. 2018). This initial metabolic stimulation may facilitate a slow stepwise descent of species towards greater depths, where they may have a chance to adapt.

Overall, the present synthesis found evidence for physiological capacity enabling transitions both from shallow water to deep sea and from deep sea to shallow water, with strongest evidence for the physiological capacity for bathyal taxa to transition to both shallow and abyssal depths.

Potential modulators of pressure tolerance

Ontogeny (trends across life stages)

The available empirical results that relate to the pressure tolerance of early (mostly larvae) versus late (mostly adult) life stages remain difficult to compare directly due to heterogeneous durations of exposure (inherently shorter for early, rapidly transitioning, life stages) and because few studies were conducted on embryos, larvae, juveniles and adults of the same species. After narrowing down to per cent survival obtained with early and late stages of the same species, this review found that decompression was tested ontogenetically in 20 deep-sea species and compression in a single shallow-water species. Most studies resulted in some degree of survival and no clear ontogenetic trends could be detected. Overall, larvae were not found to be more tolerant to decompression than adults, as might be predicted on the basis of early pelagic stages occupying broader bathymetric ranges than their benthic genitors. In the species-specific dataset, tolerance was the same or similar between life stages in half the species; in the other half, early life stages survived less than the adults (across six phyla) and the inverse was true in only one (annelid) species.

More broadly, reproduction/mating occurred, larvae developed and resulting juveniles survived for prolonged periods (years) at surface pressure in a diversity of deep-sea taxa collected from the Northwest Atlantic (Figure 7). Most of these events occurred in mesocosms where cold conditions (<10°C) were maintained by flowing seawater pumped directly from the ocean, which included natural planktonic particles; dark conditions were also generally implemented, thereby minimizing stress. Notably, adult pycnogonids *Nymphon hirtipes* collected at 700–1450 m depths successfully reproduced; egg masses were brooded by the males (Figure 7B) for 4 months until hatching and the young developed for another 5 months under the protection of the male, from walking leg-bearing stages until juvenile stages, before dispersing in the mesocosm (Mercier et al. 2015). Moreover, deep-sea annelids (*Ophryotrocha* sp.) collected between 500 and 1500 m reproduced at surface pressure after being maintained for a year (Figure 7C); juvenile stages had survival rates of 80%, with some individuals surviving to adult stages and themselves successfully reproducing, yielding a total of three generations at surface pressure (Mercier et al. 2014, Verkaik et al. 2017). Successful recruitment of species from non-chemosynthetic deep-sea environments is not restricted to taxa from the Northwest Atlantic. For example, deep-sea ascidians (Chordata) collected in the Northwest Pacific released larvae under surface pressure conditions that developed into juveniles and survived 7 months (Havenhand et al. 2006).

The compression of early life stages of shallow-water species was also assessed, showing that larvae of Chordata, Echinodermata and Mollusca are capable of surviving and developing under pressures that typically exceed the natural distribution of their adult stages (e.g. Young et al. 1997, Tyler & Young 1998, Tyler & Dixon 2000, Villalobos et al. 2006, Aquino-Souza et al. 2008, Mestre et al. 2009, Smith & Thatje 2012, Mestre et al. 2013, Brown & Thatje 2014, Sumida et al. 2015). High-pressure tolerance was often found to be greatest in early embryonic stages during cleavage, and to decrease with further development (Tyler & Dixon 2000, Pradillon & Gaill 2007, Mestre et al. 2013). Notably, fewer studies have investigated the effects of hydrostatic pressure on settlement processes and juvenile development, or even on adults in these shallow-water species (Young & Tyler 1993). In this review, compression studies across larval and adult stages were only found to exist for one arthropod species, resulting in survival for both.

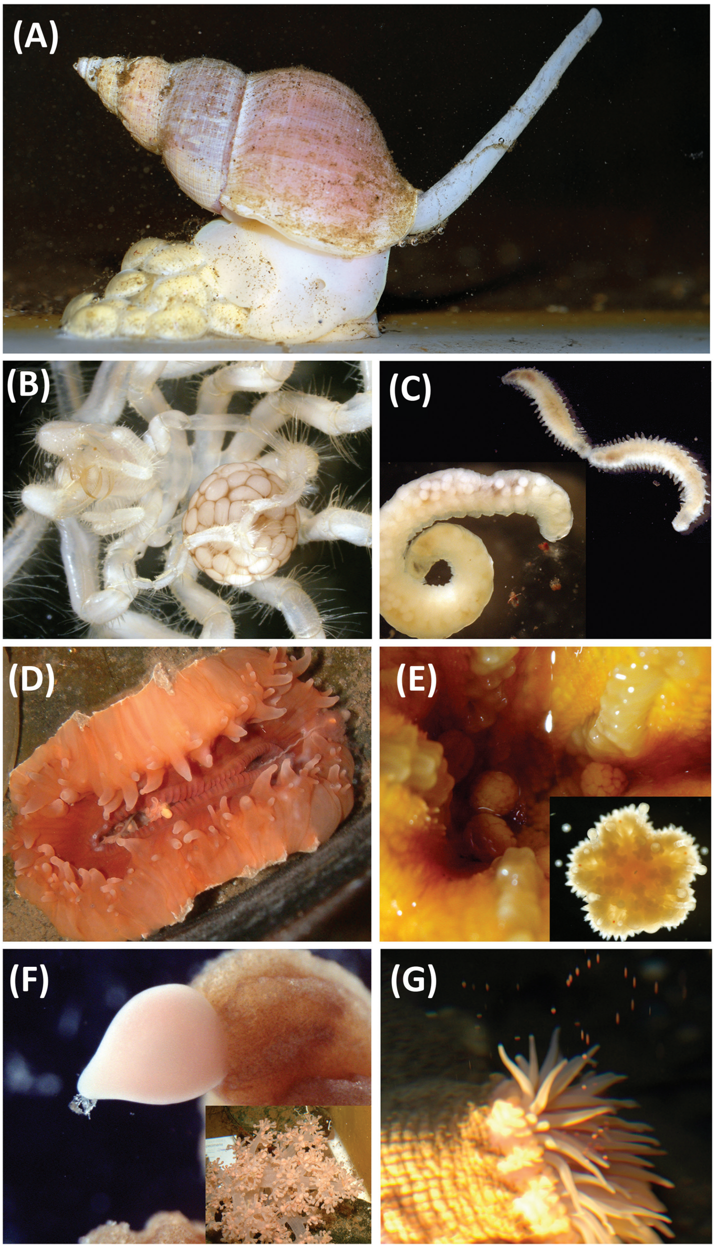

Figure 7 Examples of deep-sea benthic animals from the Northwest Atlantic, collected between 360 and 1500 m depth, held in mesocosms at atmospheric pressure and displaying reproductive behaviours. (A) The gastropod *Buccinum scalariforme* (~70 mm shell height) laying eggs (Montgomery et al. 2017). (B) A male pycnogonid *Nymphon hirtipes* (~55 mm leg span) brooding an egg mass (Mercier et al. 2015). (C) The polychaete worm *Ophryotrocha* sp. (~12 mm long) displaying mating behaviour, with insert showing mature oocytes (Mercier et al. 2014). (D) Female cup coral *Flabellum angulare* (~3.9 cm wide) releasing oocytes attached to threads (Mercier et al. 2011d). (E) Undersurface of a female sea star *Henricia lisa* (~4 cm in diameter) brooding embryos, with inset showing fully developed juvenile (1100 µm in diameter) (Mercier & Hamel 2008a). (F) Soft coral *Drifa* sp. releasing planula larvae (~300 µm in diameter), with inset showing an adult colony (~12 cm high) (Sun et al. 2009). (G) The sea anemone *Allantactis parasitica* (~4 cm in column diameter) broadcasting oocytes in the water column (Mercier & Hamel 2009a).

Taxonomy (trends across phyla)

The results of early pioneer experiments with intertidal species had proposed that taxonomic groups with the best resistance to high pressure were those with the greatest distributions in the deep sea: echinoderms, molluscs, amphipods, isopods and polychaetes (Schlieper 1968). It was also assumed that pressure resistance decreased as the level of organization increased, with protozoans being more resistant than metazoans, and the latter being decreasingly tolerant the higher their position in the taxonomic system (Cattell 1936, Schlieper 1968). However, taxonomic/phylogenetic trends were not evidenced in the present review, largely due to variability within phyla. For instance, survival in adults varied from 0% to 100% in Arthropoda and Annelida and from 60% to 100% in Cnidaria. Adult pressure tolerance had been proposed to differ among phyla in previous analyses based on more limited data (Brown & Thatje 2015). The present synthesis highlighted that generalization are difficult to make because sample sizes of species within phyla are often small, whereby repeated experiments (yielding separate records here) involve the same species. As an example, numerous studies have been conducted in the Azores on the life history, development and physiology of hydrothermal vent mussels (*Bathymodiolus azoricus*) collected from the Mid-Atlantic Ridge at ~850 m depth (Colaço et al. 2006, Kádár et al. 2006, Bettencourt et al. 2008, Kádár et al. 2008a, Bettencourt et al. 2010, Colaço et al. 2011, Martins et al. 2014, Barros et al. 2015, Bettencourt et al. 2017). Much of the Mollusca records therefore reflect the pressure tolerance of this species; investigators were able to maintain individuals for greater than 12 months under laboratory conditions at surface pressure. This mussel is protected by a hard shell and presumably sustains minimal physical damage from changes in pressure. Whether other types of molluscs would do equally well remains unclear.

The limited evidence we have suggests that the ability of a taxon to survive pressure shifts may relate to its complexity and level of protection (e.g. presence/absence of calcified exoskeleton). Mollusca, Echinodermata and Arthropoda tolerate compression and/or decompression better than invertebrates in the Chordata phylum (ascidians). These differences may reflect increasing difficulty in maintaining oxygen supply with greater organismal complexity, for example through ventilation and circulation, as suggested by Brown & Thatje (2015). Therefore, it is unlikely that vertical range extensions would involve mass movement/relocation of all taxa, but rather would be restricted to those that are more barotolerant under relatively isothermal conditions. For example, Ammendolia et al. (2018) tested the behavioural responses of three different echinoderms at pressures representing depths beyond their current bathymetric ranges. Increasingly severe responses to high pressures were demonstrated from Holothuroidea to Asteroidea and to Echinoidea (which are increasingly calcified). Establishing more definite conclusions regarding the contribution of body design complexity (morphology, calcification) and systems (circulatory and nervous) to barotolerance will require a study of the responses of multiple species from multiple classes under standardized conditions, following a comparative physiology approach (Garland et al. 2005). Physiological thresholds to pressure are also variable among highly mobile and wide-ranging vertebrate taxa. The present review did not examine barotolerance in fishes given their mobility and the additional layers of physiological complexity, which typically require studies to employ biochemical analyses (Yancey et al. 2014, Treberg & Speers-Roesch 2016). Through such work, it was generally found that fishes had more shallow physiological limits than non-chordate taxa, as demonstrated by groups such as Liparidae and Chondrichthyes limited by depths of 8500 and 4000 m, respectively (Yancey et al. 2014, Treberg & Speers-Roesch 2016).

While some phyla demonstrate greater pressure tolerance than others, taxon-specific modes of locomotion may affect potential for shifts in bathymetric distribution and range extension. Certain taxa may exhibit limited motility and be unable to successfully migrate, i.e. sessile adults of sponges and corals (anchored to a substrate). Instead, these taxa will depend entirely on dispersal of propagules for range shifts, and the capacity for broad vertical migration in some deep-sea larvae was already mentioned (Young et al. 2018). In contrast, adults of mobile taxa may have equal or greater capacity for movement within their lifespan than their brief larval stage. Consequently, more studies exploring pressure tolerances in all life-history stages of individual species are required. Further, whether taxa are able to exploit pressure tolerance and extend bathymetric range depends on ecological factors. For example, habitat availability (e.g. continental shelf and slope area for benthic species), suitability (e.g. primary productivity and food availability, seasonality) and accessibility (e.g. dispersal ability, current direction) will also limit the ability of species to migrate bathymetrically (see Barve et al. 2011 and references therein, Hamel et al. 2019). Similarly, light penetration may be particularly critical since adaptations in vision have been reported to shift with bathymetric changes in light parameters (Warrant & Locket 2004). Determining the most likely candidates for vertical range extension will therefore require further investigations to test the physiological, developmental and genomic barotolerance of multiple taxa.

Geographic location (latitudinal trends)

From the standpoint of geographic location of collection, the reviewed literature highlighted a possible difference in pressure tolerance between species occurring at lower versus higher latitudes rather than across specific regions. Bathymetric gradients in temperature are typically small at high latitudes and moderate at mid-latitudes, although there is seasonal variation at mid-latitudes as stratification builds between spring and autumn and dissipates during autumn (Gage & Tyler 1991). In contrast, bathymetric temperature gradients are typically large at low latitudes where permanent stratification occurs (Friedman & Sallan 2012). Consequently, deep-sea animals collected at high or mid-latitudes experience less severe temperature shocks during recovery from the deep sea than those collected in warmer/tropical waters (Tyler & Young 1998). Ravaux et al. (2009) highlighted the potential confounding effect of temperature stress by reporting that using insulated collection boxes on remotely operated vehicles markedly increased the survival of deep-sea species collected at abyssal depths at low latitudes. Similarly, the potential for bathymetric range extension in shallow-water taxa within tropical regions may be limited by thermotolerance. For instance, low temperatures cause physiological stress that increases mitochondrial oxygen demand in the subtropical shallow-water spider crab (*Maja squinado*), compounding challenges that high pressure presents to the maintenance of aerobic homeostasis (i.e. ventilation and circulation) (Frederich & Portner 2000, Brown & Thatje 2015). Consequently, bathymetric range extension in tropical areas may be restricted by thermal physiological bottlenecks that impose species-specific thresholds.

Perspectives

Current limitations in interpretation of findings

While the study of responses to hydrostatic pressure has progressed in leaps and bounds over the past 60 years, with major breakthroughs and the introduction of deep-water organisms to the experimental arena over the past 20 years, we are still comparing apples with oranges due to technical challenges. When it comes to the decompression of animals collected from the deep sea, some investigations have successfully maintained sufficiently adequate holding conditions to allow studies of complete development and even generational successions. For compression studies, only short-term exposure is yet possible, with very few exceptions. The situation was reversed in pioneer work up to the 2000s, whereby deep-sea animals could not be collected and held alive for more than a few hours. Hence, initial studies offered more convincing (if limited) demonstration of shallow-to-deep tolerance, whereas current studies offer more convincing (now fairly complete) demonstration of deep-to-shallow tolerance. For this reason, bi-directional movement is emerging as the most parsimonious explanation. Only further transplantation studies or technological advances allowing the maintenance of animals in compressed state for months to year will eventually confirm this.

Another short-term goal will be to refine our understanding of ontogenetic patterns, because comparing survival metrics does not paint an accurate enough picture. For example, full development of deep-sea progeny through embryonic and larval stages until the settlement of juveniles comes with natural mortality that may be unrelated to decompression (the number of surviving offspring may fundamentally decrease in a stage-wise manner) down to 50% or even 5%–10% survival. On the other hand, compression of a single chosen stage for a brief period may yield survival of 100% (e.g. a handful of pluteus or veliger larvae may all survive 24-hour exposure). Comparing these two examples will allude to better tolerance of the compressed than the decompressed larval species, when the inverse may be true since a single stage of the latter survives but only briefly, while the former successfully completes development outside its natural pressure range. Also of note is the fact that deep-sea larval stages were mostly obtained from the reproduction of decompressed genitors, rather than directly from the field.

Looking beyond lethal responses

Studies reporting sub-lethal responses to changes in hydrostatic pressure in shallow-water and/or deep-sea species are still relatively scarce. However, sub-lethal metabolic costs imposed by shifts in hydrostatic pressure may establish a narrower bathymetric range than suggested by the basic survival/mortality reviewed here. For example, the lithodid crab *Lithodes maja* (bathymetric range 4–790 m depth, approximately equivalent to 0.1–7.9 MPa hydrostatic pressure) has been reported to survive exposure to 20 MPa (Brown et al. 2017). However, heart rate decreased with increasing hydrostatic pressure, and was lower at ≥10 MPa than at 0.1 MPa. Oxygen consumption increased with increasing hydrostatic pressure to 12.5 MPa, before decreasing as hydrostatic pressure increased to 20 MPa: oxygen consumption was higher at intermediate levels of 7.5–17.5 MPa than at 0.1 MPa. Increases in expression of genes associated with neurotransmission, metabolism and stress were also observed between 7.5 and 12.5 MPa. Consequently, it has been suggested that hyperbaric tolerance in *L. maja* may be oxygen-limited by compression effects on heart rate and metabolic rate, but that its bathymetric range is limited by metabolic costs imposed by the effects of high hydrostatic pressure. Subsequently, it was proposed that hydrostatic pressure be included in a complex model of environmental tolerance, where energy-limitation constrains biogeographic range, and facilitating incorporating hydrostatic pressure into the broader metabolic framework for ecology and evolution (Brown et al. 2017).

Such an approach may be crucial for accurately projecting biogeographic responses to changing climate conditions, and for understanding the ecology and evolution of life at depth; it may also have implications for the present study. While individuals can survive exposure to hydrostatic pressure outside their normal range, individual fitness may be reduced by moderate stress imposed (Brown et al. 2017), as additional homeostatic energy costs lead to energetic trade-offs and result in reduced activity, scope for growth and/or reproductive output (Sokolova 2013). For example, increased allocation of energy to maintenance diminishes energy available for other functions such as buffering fluctuating food availability or provisioning offspring (Sokolova 2013). Consequently, analyses based on survival (even formal LD_{50} approaches) and short-duration pressure treatments may overestimate the capacity for range extension. Evidently, studies reporting persistence of a species through multiple generations at pressure outside that at which they normally live provide stronger support for potential range extension; as already discussed, they currently exist only for decompressed deep-water species.

Conclusions

Following a broad assessment of available studies, evidence suggesting that deep-sea benthic animals might have the physiological capacity to survive under surface pressure is at least as strong as evidence for shallow-water species tolerating high pressures, possibly stronger. Empirical data accumulated over the past seven decades also shed light on factors that may modulate pressure tolerance, providing insight into the potential for future vertical range extension in the ocean during changing environmental conditions. Overall, several conclusions may be drawn, and knowledge gaps identified from this review.

1. Empirical evidence supporting the deep-shallow hypothesis is extensive, with many deep-sea species thriving for months to years in shallow-water laboratory conditions, at least one through multiple generations. Empirical evidence supporting the shallow-deep hypothesis is more limited, with fewer shallow-water species shown to survive at deep-sea pressure for tens of days only. However, the latter body of evidence may be biased by as yet limited capacity to maintain optimal environmental conditions at experimental pressure (i.e. logistical constraints).
2. Species found in the bathyal zone (intermediate ocean depths) exhibit the greatest tolerance to decompression (and compression) in laboratory studies, suggesting physiological and developmental capacity to move both shallower and deeper than their known bathymetric ranges. It also evokes the possibility that range extensions/transitions by bathyal species have been important in establishing current biodiversity patterns. A formal assessment of eurybathic species with ranges that encompasses bathyal depths would be welcome. Future studies might collect individuals of these species from different depths and assess their tolerance to pressure shifts to determine if it is independent of depth of collection (whether it is expressed at the species or population level).
3. The tolerance to decompression shown by bathyal species, combined with the fact that empirical evidence exists to support both the shallow-deep and deep-shallow hypotheses, advocates formalization of a bi-directional hypothesis as the most parsimonious explanation for current biodiversity patterns. Such a hypothesis is consistent with inferences drawn from fossil and phylogenomic studies. However, ecological factors other than hydrostatic pressure must contribute to limiting bathymetric ranges, since the latter do not appear to strictly reflect the potential of a species to tolerate pressures shifts.
4. Differences between trial conditions when testing pressure tolerance in adults and larvae, and the very limited number of species for which pressure tolerance was assessed across life stages (nearly all under decompression scenarios), mean that ontogenetic comparisons remain tentative at best. No clear trend emerged from this review. Future compression and decompression studies should strive to combine the assessment of at least two life stages of a given species.
5. The pressure tolerance of species (chiefly in adult individuals) appears to vary widely both among and within the phylum they belong to, with more derived taxa (e.g. chordates) displaying slightly greater sensitivity to pressure shifts, possibly due to greater organismal complexity. The presence of protective features (e.g. calcification of exoskeleton) also appears to contribute to pressure tolerance. Additional research is required to assess which precise morphological, physiological and/or molecular aspects might drive variation in pressure tolerance among (and within) the various taxa.
6. Geographic location of occurrence impacts pressure tolerance, with some indication that species studied at mid- to high latitudes are more tolerant to changes in pressure than those studied at low (tropical) latitudes, likely due to the synergistic effect of thermotolerance. Indeed, the effect of temperature stress during collection and recovery from the deep sea at different latitudes, resulting from contrasting vertical temperature gradients, prevents unequivocal assertion of differences in the pressure tolerance of deep-sea taxa collected at different latitudes. Nonetheless, the potential for bathymetric range extensions appears greater in periods and regions associated with small vertical temperature gradients, such as are currently established at high latitudes.

7. Key knowledge gaps remain since high-pressure vessels for experimental studies are rare commodities and technological limitations constrain the duration of pressure exposures, restricting understanding of potential for shallow-deep, bathyal-abyssal and abyssal-bathyal transitions. Logistical constraints impeding the collection and maintenance of deep-sea animals also remain, which need to be overcome to further our understanding of potential for deep-shallow transitions.

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ADAPTATIONS AND PLASTIC PHENOTYPIC RESPONSES OF MARINE ANIMALS TO THE ENVIRONMENTAL CHALLENGES OF THE HIGH INTERTIDAL ZONE

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Abstract The high intertidal zone is home to an incredible variety of marine animals, as it offers an escape from low intertidal/subtidal predation and competition, among other advantages. However, this area of the shore also comes with many tide-driven and emersion-associated environmental stressors, such as desiccation, high temperatures and freezing stress, hypoxia, salinity fluctuations, nitrogenous waste accumulation, ultraviolet (UV) radiation, wave and ice disturbance and hydrogen sulphide (H_2S) toxicity. This review explores the diversity of evolutionary adaptations and plastic phenotypic responses that high intertidal animals use to cope with these challenges. Examples are provided of behavioural, morphological, physiological and biochemical adaptations/responses, along with some of the underlying molecular mechanisms that have been elucidated to date. Adaptations of many different worms, anemones, molluscs, crustaceans and fishes are highlighted. Many adaptations and mechanisms of plasticity are universal among animal phyla, and some are multifunctional (serve more than one function) or provide tolerance to multiple stressors (i.e. 'cross-tolerance'). High intertidal animals have received considerable attention by scientists, given their accessibility and that they can provide valuable insights into the transition from a marine to a terrestrial lifestyle. Nevertheless, further research is needed to understand the adaptations/responses of these animals more thoroughly, and the future holds great promise for accomplishing this with recent advances in epigenetics, transcriptomics, protein biochemistry and other molecular tools.

Keywords: Intertidal zone, marine animals, evolutionary adaptations, phenotypic plasticity, cross-tolerance.

Introduction

Scope of review

The intertidal zone is the interface between the marine and terrestrial realms and can be described as the area on the shore that is 'in between tides', i.e. the habitat between the lowest low tide and the highest high tide (Figure 1A) (Nybakken 1993). The intertidal can be divided into low, middle and high zones, although there is no standard/universal classification in the literature to distinguish them. For the purpose of this review, the high intertidal zone (also known as the upper eulittoral zone; Raffaelli & Hawkins 1999) is defined as the area most distantly removed from the ocean (the subtidal) and is generally characterized by exposure to air (i.e. emersion) during the majority of the day. Exceptions are high-shore tidepools and the upper intertidal region of estuaries where animals may be continuously submerged in water, and which can be better distinguished by the shore height relative to the tidal amplitude (e.g. at 1.4 m above the lowest low tide level with the highest high tide at 2.1 m).

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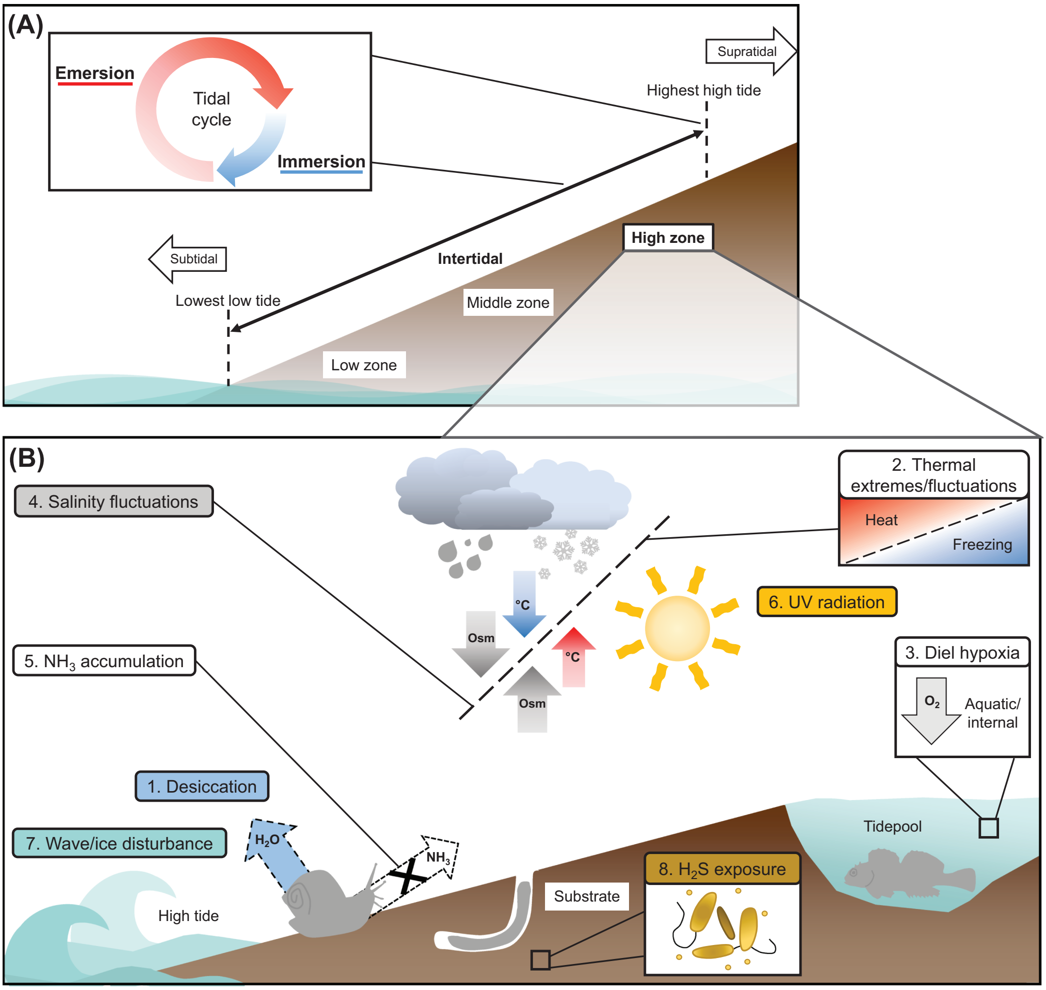

Figure 1 The location and definition of the high intertidal zone, and overview of the main abiotic challenges that marine animals are exposed to in this environment. (A) The intertidal (eulittoral) zone is the area on the shore that covers the distance between the lowest low tide and the highest high tide (Nybakken 1993), and it can usually be divided into a low, middle and high zone. The high intertidal zone is defined here as the furthest removed from the ocean/located at the highest relative shore height (e.g. at 1.4 m above the lowest low tide level with a 2.1 m tidal amplitude), and is often characterized by exposure to air (emersion) during the majority of the day (e.g. 16 hours). The intertidal zone is bordered at the lower end by the subtidal zone, which is never air-exposed during the tidal cycle, and at the upper end by the supratidal zone, which may only be wetted with seawater by wave splash. (B) Inhabitants of the high intertidal zone encounter various abiotic stressors in their environment that are often the result of the daily shift between immersion and emersion, and that are typically less severe in lower areas on the shore (Raffaelli & Hawkins 1999). Many of these stressors may occur simultaneously or at different times of the day (see Figure 2). The following abiotic challenges faced by these organisms are described in this review. (1) Emersion of considerable duration (hours) at low tide puts animals at risk of losing too much water through evaporation (i.e. desiccation stress). (2) High intertidal animals are exposed to a more thermally variable environment and greater heat stress and/or risk of freezing compared with low intertidal animals. This is largely due to the lower specific heat capacity of air as compared to that of seawater (see Table 1). (3) Aquatic hypoxia (e.g. in tidepools) can occur daily during low tide because of crowding, warming (during the day) or insufficient photosynthesis/excess respiration (at night) (Schulte 2011). Internal hypoxia may also be experienced by animals as a result of the collapse or retraction of respiratory structures during emersion, which are no longer able to take up oxygen (O_2), or due to

(Continued)

Figure 1 (*Continued*) stagnated haemolymph flow during freezing, which limits the O_2 supply to tissues. (4) Tidal movements, evaporation and freshwater input from precipitation and/or rivers can cause dramatic fluctuations in salinity (osmolarity) on the upper shore, which challenges animals to maintain their water and ionic balance. (5) Emerged animals are unable to excrete ammonium (NH_3) into the water, leading to a potential build-up of this toxic waste product in the body. (6) Because ultraviolet (UV) radiation is not filtered out by shallow seawater or during emersion, high intertidal animals may be at a greater risk of suffering UV damage (direct or indirect through the generation of reactive oxygen species) to their macromolecules (Denny & Gaines 2007). (7) Intertidal animals are subjected to wave action and other physical disturbances (e.g. from ice) at high tide, and this disturbance is more pronounced than in the subtidal zone given the ocean-to-land transition in the intertidal. (8) In intertidal habitats with a muddy or sandy substrate, hydrogen sulphide (H_2S) is produced by sulphate-reducing bacteria in the anoxic region of the sediment, and burrowing animals are exposed to high concentrations of this toxic compound.

There are many environmental challenges associated with the high intertidal zone (Figures 1B and 2), and these are often more pronounced/severe than elsewhere in the intertidal. For instance, the shift between immersion and emersion is problematic for marine animals, as seawater and air have very distinct, often opposite, characteristics (Dejours 1989) (Table 1). Compared to seawater, air is highly desiccating, has a very limited capacity to buffer temperature fluctuations, a low solubility for nitrogenous waste, and it does not filter out ultraviolet (UV) radiation (Nybakken 1993, Moyle & Cech 1996, Raffaelli & Hawkins 1999, Wright & Turko 2016). As a result, due to long emersion times, animals in the high intertidal are exposed to desiccation stress, temperature extremes and UV radiation, and face difficulties with nitrogenous waste excretion. High intertidal animals also frequently experience diel hypoxia (Richards 2011, Schulte 2011). Furthermore, large fluctuations in salinity can occur in estuaries and tidepools (Marshall 2012), and rocky intertidal zones are exposed to physical stress from waves and ice (Raffaelli & Hawkins 1999). Finally, in many intertidal environments, the sediment contains high concentrations of hydrogen sulphide (H_2S) which is toxic to most animals (Schulte 2011).

The aim of this review is to describe, and provide mechanistic bases for, the variety of adaptations and plastic (phenotypic) responses that high intertidal marine animals use to survive these various environmental challenges and flourish on this part of the shore. This topic has not been revisited since Raffaelli & Hawkins (1999), and to our knowledge, a synthesis entirely dedicated to this subject does not exist. In each of the following sections, adaptations are discussed and organized according to the environmental challenges that they protect against – although some are ‘multifunctional’ (serve multiple functions, e.g. see Hui et al. 2019) and/or allow for ‘cross-tolerance’ (tolerance against multiple stresses, e.g. see Sinclair et al. 2013); with the latter summarized at the end of this review. The high intertidal zone is an extremely diverse environment: it exists in all climates (from polar to tropical), is associated with substrates of different particle sizes (e.g. rock, gravel, sand, mud), can contain a large amount of decomposing seaweeds and debris (strandlines), terrestrial vascular plants can be present/absent (mangroves and salt marshes versus mudflats), and it can be associated with significant freshwater input (estuaries) (Nybakken 1993, Raffaelli & Hawkins 1999, Schulte 2011). Therefore, the exact nature of the local environment can greatly determine the type and severity of abiotic stressors that are encountered (Figure 3), and in turn, which adaptations and responses are required from its animal inhabitants; this will be discussed in the sections below.

In this review, the adaptations/physiological responses of a wide range of marine intertidal animals is illustrated with examples from fishes, crustaceans, molluscs, anemones and worms. This comprehensive exploration allows for the identification of adaptations that are universal among animal groups, and these are discussed in the concluding section of this article. Adaptations can be categorized as general strategies (e.g. avoidance versus tolerance) and can occur at various levels of biological organization – genetic, biochemical, physiological, morphological or complex

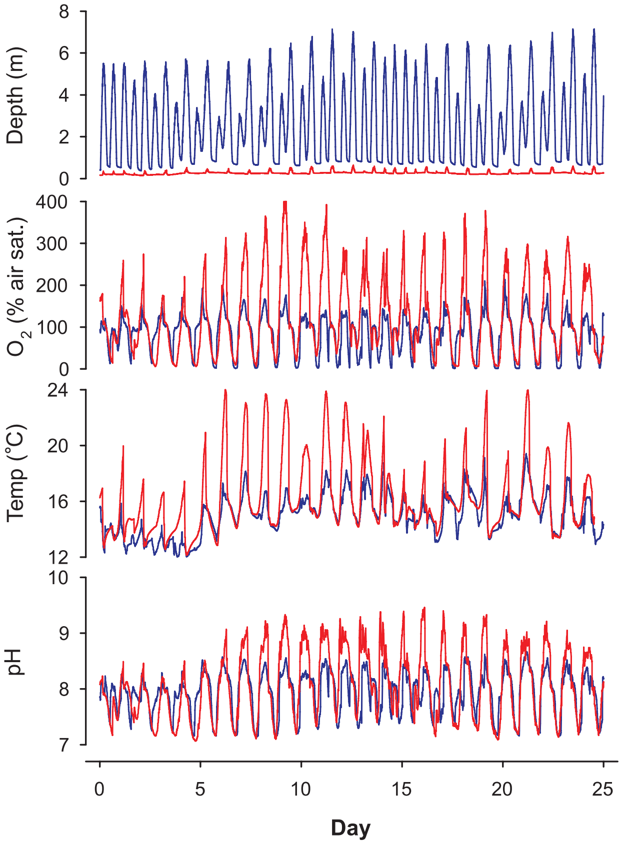

Figure 2 On-site measurements of several environmental variables (i.e. water depth, O_2 level, temperature, pH) within tidepools in the marine rocky intertidal zone at Wizard Island near the Bamfield Marine Sciences Centre, Bamfield, British Columbia, Canada. The data were collected over the course of 25 days, illustrating the type and scope of abiotic challenges that animals can experience in lower and upper parts of the shore. High tidepools (red lines) are shallower and have a very variable environment, with large fluctuations and greater extremes in O_2 level, temperature and pH. Environmental parameters in low tidepools (blue lines) are much more stable. Figure is reproduced/adapted from Richards (2011) with permission from the Journal of Experimental Biology (Company of Biologists, Cambridge, the UK).

behaviours. In some cases, it is possible to distinguish between responses that are short term and reversible within an animal's lifetime (i.e. phenotypically plastic), and those that are long-term and involve heritable genetic changes (i.e. evolutionary adaptations). We define phenotypic plasticity herein as the flexibility of animals with the same genetic background to develop different phenotypes depending on their environment (West-Eberhard 1989). The review concludes with brief perspectives on: (1) what makes the high intertidal zone an attractive habitat for marine animals, despite its harsh environmental conditions; (2) the valuable insights that can be obtained from research on high intertidal animals, especially in the current era of accelerated global climate change; and (3) important knowledge gaps and recommendations for future research on intertidal animals.

Table 1 Some important properties of seawater versus air, and the physiological consequences of emersion

Property	Seawater (35‰)	Air	Consequences of emersion
Water availability	High	Low	Desiccation[a]
Heat capacity[b]	5	1	Rapid heating/cooling
O_2 solubility	1	33	Higher O_2 availability if able to breathe air
NH_3 solubility	939[c]	1	Accumulation of NH_3
UV radiation absorption	Yes[d]	No	Higher UV radiation exposure
Density	821	1	Effect of gravity is more noticeable

Source: After Dejours (1989), Nybakken (1993), Moyle & Cech (1996) and Wright & Turko (2016).

[a] Seawater is desiccating for hypoosmotic animals due to osmotic water loss; however, air is more desiccating.

[b] Amount of heat (joules or calories) required to raise the temperature of 1 g of a substance by 1°C.

[c] In fresh water. There is no information available on the NH_3 solubility in seawater.

[d] The rate of UV absorption by seawater depends primarily on the concentration of dissolved organic matter (Tedetti & Sempéré 2006).

Values are ratios. If properties are dependent on temperature, the average is taken of the values at 0°C and 20°C.

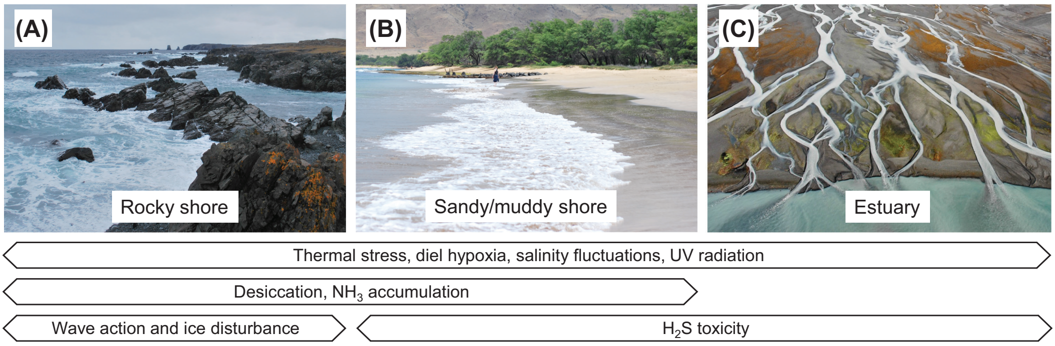

Figure 3 The abiotic stressors encountered by animals depends on the type of high intertidal habitat. For instance, the type of thermal stress (i.e. heat or freezing stress) depends on the climate of the intertidal habitat. Here, three main shore types are shown: rocky shores, sandy/muddy shores and estuaries. The occurrence of a stressor in these particular types of intertidal habitat is indicated by the horizontal bars. (A) Example of a rocky shore in Bonavista, Newfoundland, Canada. The rocky intertidal usually encounters more severe wave action than sandy/muddy shores, but generally lacks an anoxic sediment layer in which sulphate-reducing bacteria produce high levels of H_2S (Raffaelli & Hawkins 1999). (B) Example of a sandy beach in Kihei, Maui, Hawaii. Intertidal habitats with sandy/muddy shores include sand and mud flats, salt marshes and mangroves (Peterson 1991). (C) Example of an estuary in Kachemak Bay, Alaska, the USA. Because estuaries receive freshwater input from a river, estuarine animals are typically not exposed to many of the environmental challenges that are a consequence of emersion (Table 1). Estuaries are also often sheltered shores with minimal exposure to wave action. Photographs by the authors (panel A–B) and Alaska ShoreZone (panel C).

Topics not addressed

There are certainly more stressors associated with the high intertidal zone than those listed above. Large pH variations occur in tidepools and mudflats (due to fluctuations in the production and consumption of carbon dioxide, CO_2) (Figure 2) (Ganning 1971, Morris & Taylor 1983, Richards 2011). Due to frequent emersion on the upper shore, filter-feeders have limited time for feeding which can reduce the amount of energy available for growth and reproduction (Bayne et al. 1988), animals experience reduced buoyancy (Table 1), and may be unable to release sufficient quantities of metabolically produced CO_2 (Schulte 2011, Martin 2014, Wright & Turko 2016). High intertidal animals are also exposed to terrestrial pathogens and parasites, with characteristics that may differ from those in the marine environment (You et al. 2014, 2018). However, these stressors/challenges are not the focus of this review, because information on these topics is limited or only available/relevant for a specific animal group (e.g. suspension-feeders). Adaptations of high intertidal animals that allow them to avoid terrestrial predation (e.g. the use of camouflage in rockpool fish; see Stevens et al. 2014) or that are important in competitive/territorial behaviour (e.g. the role of the decapod chelipeds in agonistic interactions; see Lee 1995) are also not discussed, because this review concentrates on abiotic stressors, and similar adaptations against biotic stressors are found among animals in other marine environments. Thus, the emphasis of this work is on adaptations and phenotypic responses that are unique to animals inhabiting the high intertidal zone or driven/magnified by the tidal cycle.

This review focuses on studies that specifically compare high intertidal animals with low intertidal species of similar taxonomic classification (e.g. the high shore sculpin *Oligocottus maculosus* versus the low shore sculpin *Blepsias cirrhosus*), or with individuals of the same species that also inhabit the low intertidal zone (e.g. high shore versus low shore *Mytilus edulis* mussels), because this approach minimizes confounding phylogenetic effects. Studies on species that are abundant in the eulittoral fringe (the extreme upper end of the shore that is only wetted by waves during spring tides) are also highlighted whenever possible (e.g. the snail *Echinolittorina malaccana*, Marshall & Ng 2013). Sometimes, there are few studies of high intertidal animals related to a particular topic, and in such cases, adaptations of high intertidal animals that have not (yet) been recorded/observed among subtidal animals are also briefly discussed. Marine animals that are tidal migrants [i.e. move along with the wave splash, like the common sand crab (*Emerita analoga*); Lastra et al. 2004], or that exclusively inhabit the supratidal/splash zone (the area above the eulittoral zone/highest high tide level), are not covered by this review. The same applies to the few terrestrial animals found in the high intertidal zone that can tolerate exposure to the marine environment to some degree, such as insects, centipedes and pseudoscorpions (Raffaelli & Hawkins 1999). Shores with gravel or very coarse sand as a substrate are not discussed in this review, because they appear inhospitable to marine animals and are regarded as 'intertidal deserts' (Raffaelli & Hawkins 1999).

Almost all phylogenetic groups are represented in the high intertidal environment and covered in this review, with the exception of echinoderms which are typically restricted to the low intertidal zone. The high intertidal zone has tidal and seasonal visitors, but this review is focused on residents that inhabit this area during the majority of the year and/or their life cycle (Moyle & Cech 1996, Lam et al. 2006). Therefore, the adaptations of subtidal animals that temporarily remain in the high intertidal to build nests, deposit their eggs and/or provide parental care (e.g. plainfin midshipman, *Porichthys notatus*; and sharpnose sculpin, *Clinocottus acuticeps*) (Martin et al. 2004), are also not discussed here.

Adaptations to the environmental challenges of the high intertidal zone

Desiccation tolerance and resistance

Animals in the high intertidal zone are typically air-exposed for the majority of the time (e.g. 20 hours/day in the high intertidal snail *Littorina scutulata*, Stickle et al. 2017; and 98% of the time in some eulittoral fringe snail species as an extreme case, Monaco et al. 2017). With the exception of animals on exposed rocky shores that receive a large amount of wave splash, the most important coping mechanisms during emersion are probably those against the desiccating effects of air exposure (Table 1) (McMahon 1990, Raffaelli & Hawkins 1999). Some intertidal invertebrates (e.g. sipunculid worms and the high intertidal limpet *Lottia digitalis*) are highly tolerant of desiccation and can survive large short-term water losses (up to ~45% of their total body water content) (Oglesby 1969, Roland & Ring 1977). Likewise, there are examples of intertidal fishes (e.g. clingfish species *Tomicodon humeralis* and *Pherallodiscus funebris* from the Gulf of California) that are able to sustain extreme (as great as 60% of their total body water content) water losses (Horn & Gibson 1988). Differences in desiccation tolerance have also been reported between high versus low intertidal gastropods. In Brunei Darussalam, the high intertidal tropical snail species *Echinolittorina malaccana* only experiences low levels of mortality (<20%) when exposed to warm air for several days despite a ~13% water loss, while two local lower intertidal species *Planaxis sulcatus* and *Echinolittorina vidua* experience 100% mortality after only a ~7% water loss, and cannot withstand more than 1–2 days under these conditions (Miranda et al. 2019). Although differences in upper thermal tolerance between these tropical species is also a contributing factor, it is clear that the greater survival time during emersion (LT_{50}) and the amount of water loss until mortality (LD_{50}) both facilitate the upward extension of the intertidal distribution of *Echinolittorina malaccana*. In the South African limpet *Siphonaria oculus* from the uppermost intertidal zone, both LT_{50} and LD_{50} are also higher than reported for the local lower shore limpet *Patella granularis* (Marshall & McQuaid 1992a). Among snail species of the *Nucella* and *Littorina* genera from the northern Washington coast, LT_{50} increases similarly with shore height (Stickle et al. 2017).

However, it appears that an enhanced desiccation tolerance is not the most common strategy used by high intertidal animals when emersed. Instead, they appear to be primarily adapted to avoid, or reduce the rate of, water loss (e.g. see McMahon 1990 with regard to the decline in evaporative rates in eulittoral fringe gastropods). Further, several high shore ecotypes of the rough periwinkle *Littorina saxatilis* (from the White and North Seas) survive longer in air because they avoid water loss better than their low intertidal relatives; although the mechanisms underlying the reduced evaporation rates/improved water conservation were not identified (Sokolova et al. 2000, Sokolova & Pörtner 2001a). Ecotypes are morphological variants within a species that have undergone incomplete speciation, and they are often well studied, and can be useful models for understanding adaptations across a vertical shore gradient. *Littorina saxatilis* ecotypes and populations (also described as a 'species aggregation') have received particular attention as they display substantial phenotypic variation in response to environmental factors, while genetic divergence is limited (Panova et al. 2014). In the avoidance strategy, the first line of defence against desiccation upon emersion is a behavioural response (Table 2). High intertidal animals minimize exposed surfaces by, for instance, retracting feeding appendages (tentacles in anemones and cirri in barnacles) or tightly closing their shell (bivalves) (Shick 1991, Wolcott & Wolcott 2001, Denny & Gaines 2007). The mussel *Mytilus californianus* frequently uses this strategy, and individuals in the high intertidal zone close their shell for ~81% of the time compared to ~39% in low intertidal individuals (Gleason et al. 2017). In addition to contracting their tentacles, anemones commonly attach gravel or shells to themselves. In *Anthopleura elegantissima*, this is particularly important at high temperatures (>20°C) and wind speeds (2.2 m/s), as under these conditions such actions reduce the rate of water loss by ~64% and increase survival 2-fold (Hart & Crowe 1977). High intertidal individuals also cover themselves with more gravel than low intertidal individuals when exposed to sunlight (Dykens & Shick 1984). Finally, high intertidal *Littorina* species in Panama substantially reduce water loss during emersion by retracting inside their shell and sealing off the shell's opening with an opercula (Garrity 1984).

Mobile animals are also able to cope behaviourally by finding shelter/a favourable microhabitat. Many high intertidal animals have been reported to seek refuge in tidepools, crevices/moist places underneath rocks (e.g. snails, crabs, stichaeid fishes, clingfish) (Garrity 1984, Denny & Gaines 2007, Martin 2014, Wright & Turko 2016), in beach wrack (e.g. isopods, amphipods) (Wolcott & Wolcott 2001), inside galleried mangrove logs (e.g. the mangrove killifish) (Taylor et al. 2008), or in burrows

Table 2 Overview of the adaptations and plastic phenotypic responses of marine animals to the main environmental challenges of the high intertidal zone

Challenge	Strategy	Type of adaptation	Examples of adaptations across animal groups
Desiccation	↑ Avoidance	Behavioural	Attaching gravel (anemones); contracting/clamming up (anemones, molluscs, barnacles); seeking shelter (snails, crustaceans, fishes); retreating to low tidal area/tidepool; rolling in mud (fishes)
		Morphological	↑ Shell volume:circumference and height; ↓ aperture size (snails); ↑ body size (crabs); ↓ surface area:weight (fishes)
		Physiological/biochemical	↑ Mucus (snails, fishes); ↑ embryo/total body water content (crabs, fishes); ↓ water permeability of gills/skin/eggs/embryos; staying in water triggered by vaso- and isotocin release; ↑ water intake by activation of forebrain thirst centre (fishes)
	↑ Tolerance	Physiological/biochemical	↑ Ability to survive large water losses (worms, snails, fishes)
Heat stress	↑ Avoidance	Behavioural	Aggregate, strategic shell orientation/standing (snails); use thermal refuge in crevice/tunnelled wood (snails, fishes); sponging/emergence to ↑ evaporative cooling (crabs, fishes); cooling by wave splash (fishes)
	↑ Tolerance	Physiological/biochemical	↑ Heat stability of metabolic enzymes (molluscs); metabolic depression (snails); ↑ heat resilience of the heart; ↑ aerobic/air-breathing capacity (snails, crabs); ↑ constitutive and/or heat-induced heat shock protein levels (molluscs, fishes)
Freezing stress	↑ Avoidance	Physiological/biochemical	↓ Ice crystal growth by antifreeze proteins (worms) or viscous mucus (snails)
	↑ Tolerance	Morphological	↑ Body size to ↓ freezing rate (snails)
		Physiological/biochemical	Extracellular ice-nucleating agents; ↑ intracellular compatible organic osmolytes; ↑ anaerobiosis (molluscs); ↓ protein synthesis by RNA interference (snails)
Thermal fluctuations	↑ Avoidance	Behavioural	Thermoregulatory shuttling (crabs)
	↑ Tolerance	Physiological/biochemical	Ability to tolerate extreme temperature changes (snails, fishes); ↓ thermal sensitivity of metabolism (Q_{10}) or aerobic scope (crabs, fishes)

(*Continued*)

Table 2 (*Continued*) Overview of the adaptations and plastic phenotypic responses of marine animals to the main environmental challenges of the high intertidal zone

Challenge	Strategy	Type of adaptation	Examples of adaptations across animal groups
Diel hypoxia	↑ Aerobic capacity	Behavioural	Gaping (bivalves); aquatic surface respiration (shrimps, fishes); emergence; tidepool hopping; ferrying air into burrows (fishes)
		Morphological	Respiratory leg structures, water recirculation over gills (crabs); ↑ epithelial vascularization (crabs, fishes); ↑ gill surface area and structural support; ↓ thickness of gill secondary lamellae (fishes)
		Physiological/biochemical	↑ O_2 affinity of haemoglobin/haemocyanin or stability thereof (decapods, fishes); ↑ haemoglobin level; ↑ efficiency of oxidative phosphorylation; ↑ maximum O_2 consumption/O_2 affinity by cytochrome *c* oxidase (fishes)
	↑ Anaerobic capacity	Physiological/biochemical	↑ Fermentable energy reserves (snails, crabs, fishes); ↑ lactate dehydrogenase activity (fishes)
	↓ Metabolism	Physiological/biochemical	↓ Heart rate (mussels, fishes); ↓ metabolic enzyme activities (constitutively/temporarily) (molluscs); ↓ expression of genes involved in energy-consuming and O_2-dependent processes (fishes)
Salinity stress	↑ Avoidance	Behavioural	Contracting/clamming up (bivalves, barnacles), seeking wetter microhabitats (amphipods, crabs)
		Physiological/biochemical	↓ Respiration and heart rate (worms, snails, fishes); ↓ ion loss by ionocyte retraction; ↓ gill/skin/intestinal water permeability (fishes)
	↑ Osmoregulatory capacity	Physiological/biochemical	Adjustments in free amino acid levels by changing metabolic/biosynthetic gene expression (anemones, worms, molluscs); regulating urine tonicity (crustaceans, fishes); NaCl uptake/secretion by gill/operculum ionocytes (crabs, fishes)
NH_3 accumulation	↓ NH_3 production	Physiological/biochemical	↓ Protein and (partial) amino acid catabolism (fishes)
	↑ NH_3 excretion	Morphological	Water trapping in the gills (amphipods, fishes)
		Physiological/biochemical	↑ Nitrogenous secretion rates (snails); NH_3 volatilization (isopods, fishes); active secretion and ↑ NH_3 transporter effectivity in the gills (fishes)
	↑ NH_3 detoxification	Physiological/biochemical	Recycling through endosymbiotic algae (anemones); ↑ conversion into glutamine and/or urea (snails, crustaceans, fishes)
	↑ NH_3 tolerance	Physiological/biochemical	↑ NH_3 tolerance of brain (fishes)

(*Continued*)

Table 2 (*Continued*) Overview of the adaptations and plastic phenotypic responses of marine animals to the main environmental challenges of the high intertidal zone

Challenge	Strategy	Type of adaptation	Examples of adaptations across animal groups
UV radiation	↑ Avoidance	Behavioural	↓ Exposed external surface (anemones, molluscs); seeking shelter (fishes)
	↑ Protection	Physiological/biochemical	↑ Sunscreens (mycosporine-like amino acids) (fishes); ↑ antioxidant levels in column (anemones) and gametes (bivalves); ↑ gene expression of antioxidants and DNA repair enzymes (snails)
Wave action/ice disturbance*	↑ Avoidance	Behavioural	Restricting activity to low tide (snails)
	↑ Physical resilience	Morphological	Adhesion with byssus threads (mussels), cement (barnacles), suction (snails, fishes); ↓ wave drag by ↓ spiral shell height/weight; ↑ foot/aperture size (snails)
		Physiological/biochemical	↑ Shell strength by ↑ constitutive gene expression of shell matrix proteins (snails)
H_2S toxicity	↑ Avoidance	Behavioural	Staying at surface of sediment/water; emergence (fishes)
	↑ H_2S detoxification	Physiological/biochemical	Use of H_2S as electron donor for ATP production (chemolithoheterotrophy); symbiosis with H_2S-oxidizing bacteria (worms, bivalves); ↑ enzymatic detoxification by oxidation into thiosulphate (worms, bivalves, fishes)

* The intertidal zone is exposed to a greater amount of wave action and ice disturbance than the subtidal zone. However, this stressor is more severe in the lower than in the higher intertidal zone (see text). Adaptations that are found among intertidal animals, in general, are included in this table, whereas adaptations specific to low intertidal animals are excluded.

in the mud or sand (e.g. mudskippers) (Marshall 2012). Shelter seeking is particularly important for intertidal individuals of the mud crab *Eurypanopeus depressus*, as their desiccation tolerance is not different from the subtidal population (Grant & McDonald 1979). Finally, in addition to withdrawing to their burrows, mudskippers also retreat to other sources of water (e.g. tidepools or the low tidal zone) to drink/rehydrate (Sakamoto et al. 2015), and regularly roll onto their sides on wet substrates (e.g. the mud) to keep their skin moist (Gordon et al. 1968, Fenwick & Lam 1988).

Among intertidal snails, it has been well-documented that a specific shell morphology is often associated with greater desiccation avoidance (e.g. see Branch 1985 and references below). In limpets, for instance, water loss is reduced by having a higher volume to circumference ratio (e.g. in intertidal versus subtidal *Nacella concinna*; Weihe & Abele 2008) and a higher shell height (e.g. in high versus low shore *Siphonaria lessonii*; Nuñez et al. 2018). The latter general trend towards taller shells with increasing shore height has also been suggested to be a morphological adaptation to avoid/reduce heat stress, as it facilitates convective cooling by the wind, and the relatively smaller contact area with the substratum reduces conductive heat transfer (Harley et al. 2009). In periwinkles, a smaller aperture (i.e. the main shell opening) has also been shown to minimize desiccation (e.g. in high versus low shore *Littorina saxatilis* from Spain) (Martínez-Fernández et al. 2008). In eel-like stichaeid fishes, both desiccation tolerance and resistance are greatest in species that live high in the intertidal zone. This has been linked to a higher whole-body water content (thus, larger water losses can be sustained) and a reduced surface area to weight ratio (i.e. a more cylindrical body with a wider cross section, which allows for lower weight-specific water evaporation rates) (Horn & Riegle 1981). Total body water content is the same among sympatric hermit crab species (i.e. species that evolved in the same environment from a common ancestor) that have different vertical distributions within the intertidal. However, high intertidal species (*Clibanarius vittatus* and *C. sclopetarius*) have the largest body size, and this was associated with a slower rate of water loss upon emersion and a longer survival time (Turra & Denadai 2001).

There are also examples of anti-desiccation adaptations associated with physiology in high intertidal animals. High intertidal limpet species from the genus *Lottia* (also known as the genus *Acmaea*) have desiccation rates 1/10th of their low intertidal relatives, which is attributed to the production of a mucous barrier between the shell and the substrate (Wolcott 1973). Mudskippers and rockskippers (blennies) also secrete considerable amounts of mucus onto their gills and skin to reduce evaporation when exposed to air (Whitear & Mittal 1984, Yokoya & Tamura 1992, Lam et al. 2006). In various intertidal and estuarine fishes (e.g. Australian, Atlantic and mangrove killifish, *Austrofundulus limnaeus*, *Fundulus heteroclitus*, *Kryptolebias marmoratus*; and the giant mudskipper, *Periophthalmodon schlosseri*), water permeability of the gills, skin and eggs/embryos is very low, or can be reduced via remodelling/plasticity, and this minimizes water loss on land (Marshall 2012). *Kryptolebias marmoratus* can maintain whole-body water levels while emersed for several days, and is also able to increase cutaneous water influx during this time, which may benefit water absorption through the skin (e.g. while remaining in a moist refuge during the dry season) (LeBlanc et al. 2010, Heffell et al. 2018). These changes in water permeability could be, in part, related to a rapid (i.e. within hours) increase in the expression of aquaporin genes (e.g. aquaporin 3) (Dong et al. 2021). Finally, females of some upper intertidal porcelain crab species (e.g. *Petrolisthes laevigatus* and *P. violaceus*) provide ~20% more water to their embryos than subtidal species (such as *Allopetrolisthes punctatus*) from the same local environment, which likely improves their survival in air (Viña et al. 2018). The higher embryo water content is either accomplished during oogenesis, or during embryonic development as brooding females may behaviourally select more environmentally stable/favourable microhabitats.

Among amphibious fishes – which are able to spend time out of the water as a normal part of their life history – the mudskippers (Periophthalminae) are probably the most ‘terrestrial’ and active on land, so they face the greatest risk of desiccation (Sayer & Davenport 1991, Martin 1995). A detailed overview of the behavioural, morphological and physiological adaptations in mudskippers (and other fishes) to the tropical intertidal environment, including adaptations to lower the risk of desiccation, can be found in Lam et al. (2006) and Jaafar & Murdy (2017). The mudskipper's behaviour of seeking refuge in burrows is associated with an increase in water intake and an enhancement of water reabsorption in the gut and kidney (see review by Katayama et al. 2018). All of these responses appear to be regulated/stimulated by the release of various hormones, such as prolactin and the neurohypophysial hormones vasotocin and isotocin. During terrestrial exposure, prolactin mRNA levels in the pituitary, and prolactin levels in the blood, increase in the mudskippers *Periophthalmus modestus* (Sakamoto et al. 2005) and *P. chrysospilos* (Lee & Ip 1987), respectively. Further, when the high intertidal *Periophthalmus modestus* is kept out of water, the expression of vaso- and isotocin precursors in the brain is upregulated, and after an intracerebroventricular injection of vaso- and isotocin, there is an increased tendency to remain in the water (Sakamoto et al. 2015). Interestingly, these hormones also regulate water balance in aquatic fishes. However, mudskippers are unique in that they need to experience the sensation of thirst, and actively move to water, to rehydrate (Katayama et al. 2018). In the shuttles mudskipper (*Periophthalmus modestus*), a putative thirst centre similar to that of mammals has been identified in the forebrain (lamina terminalis), which is neuronally activated by dehydration, and that has vascular permeability (i.e. it is permeable to circulating proteins, in contrast to the blood-brain barrier), and thus, may be involved in sensing blood osmolarity (Hamasaki et al. 2016).

Tolerance to temperature-related stress

Temperature is another dominant environmental factor impacting the lives of intertidal animals. Mechanisms responsible for the heat tolerance of intertidal animals have also received a considerable amount of attention among scientists, because of their relevance in the context of global warming. Animals in the high intertidal zone, which are isolated from the ocean (i.e. in tidepools) for prolonged periods, experience greater temperature extremes and more rapid thermal fluctuations than those in the lower intertidal zone (Figure 2) (Raffaelli & Hawkins 1999, Richards 2011). Further, as seawater has a 5-fold greater heat capacity as compared to air (Table 1), these thermal challenges are magnified in those species that are emerged for long durations. Adaptations that help them to cope with heat, freezing temperatures and high temperature variability, are discussed below.

Heat tolerance

Numerous field measurements in tidepools, rocky shores and mussel beds in temperate and tropical climates have shown that animals in the high intertidal zone typically experience much higher temperatures (on a diurnal and seasonal basis) than those in the low intertidal zone (Figure 2) (Morris & Taylor 1983, Huggett & Griffiths 1986, Roberts et al. 1997, Nakano & Iwama 2002, Pulgar et al. 2005, Petes et al. 2008, Fangue et al. 2011, Richards 2011, Gleason et al. 2017, Monaco et al. 2017, Stickle et al. 2017). For example, during the summer months, the high edge of mussel beds (*Mytilus californianus*) (at ~2 m above the lowest tide level) on the north-western Pacific coast can experience average high temperatures of ~30°C, in contrast to ~15°C for the low edge beds (at ~1 m above the lowest tide level) (Petes et al. 2008). In tropical regions, high intertidal animals can be exposed to extreme heat, such as in Hong Kong, where the two high-shore snail species *Echinolittorina malaccana* and *E. radiata* are found on rock surfaces that reach temperatures of 52°C–55°C (Seuront & Ng 2016). Although heat stress can occur in all intertidal habitat types (Figure 3), extreme high temperatures in the tropics may be more common on rocky shores as compared to sandy shores or mudflats. For example, in Singapore, temperatures on the upper rocky shore often exceed 50°C, while they rarely reach 40°C on sandy/muddy substrates (Nguyen et al. 2011). This may be related to differences in physical substrate characteristics such as colour and wettability, or because of the higher occurrence of shade-providing vegetation on soft substrate shores. For instance, the maximum estimated body temperatures of tropical snails in sheltered/shaded mangroves across various geographical locations are ~20°C lower than in sun-exposed rocky shores (Marshall et al. 2015).

Some animals, such as the clingfish *Sicyases sanguineus*, are able to behaviourally avoid high temperature extremes as they remain close to the waves and are cooled by the spray (Ebeling et al. 1970). The tropical high intertidal gastropod *Nerita scabricosta* also successfully lowers its body temperature (by ~6°C) by avoiding open rock surfaces and seeking refuge in crevices, and can substantially reduce warming by aggregating with conspecifics (Garrity 1984). In contrast, the periwinkle *Echinolittorina peruviana* appears to use a thermoregulatory orientation behaviour. On warm days it faces the sun frontally, so that the small lateral side of the shell is exposed, and this reduces heat gain by as much as 7°C (Muñoz et al. 2005). These examples of thermoregulatory behaviour represent two types of responses that high intertidal ectotherms can exhibit to cope with high temperatures: the 'flight' response (to escape the most extreme heat by seeking a cooler environment, i.e. the clingfish using wave spray, and *Nerita scabricosta* hiding in crevices) and the 'fight' response (where individuals modify their own environment to minimize extreme temperatures, i.e. *N. scabricosta* aggregations, and the periwinkles' shell orientation strategy) (Ng et al. 2017). The importance of thermoregulatory behaviours was highlighted by Marshall et al. (2015) in a study of 40 tropical eulittoral snail species. This research showed that animals in rocky shore habitats can experience extreme temperatures that on average exceed their heat coma temperature (HCT) and upper lethal limit temperatures (ULT), and thus, finding sheltered microhabitats is essential to survival.

High intertidal Littorinid snails have been reported to display various flight and fight thermoregulatory behaviours (reviewed by Ng et al. 2017), and 'shell standing' is an interesting illustration of a multifunctional adaptation in this taxa. Shell standing is when aestivating snails (withdrawn into their shells during the hottest time of the day) attach their shell to the substratum with mucus in such a way that the aperture faces perpendicularly to the surface, as opposed to downwards. This posturing behaviour allows for reduced heat gain/improved cooling, but has also been suggested to play a role in mating (e.g. in the rocky-shore, eulittoral fringe species *Echinolittorina malaccana*) (Marshall & Ng 2013, Seuront & Ng 2016). Likewise, the multifunctional 'sponging' behaviour (lowering of the body onto the sediment to take up pore water by setal capillary action) of the high tropical shore crab *Scopimera intermedia* helps with cooling (reduces average body temperature by ~1.3°C), while also benefitting feeding (supplies water for flotation feeding where lighter food particles are separated from heavier sediment grains) (Hui et al. 2019). In the latter, cooling is likely facilitated through evaporation and increasing the animal's specific heat capacity. Another example of a multifunctional adaptation is seen in dartfish (*Parioglossus interruptus*) that seek refuge during low tide in tunnelled mangrove wood (Hendy et al. 2013). This behaviour's primary purpose is most likely to avoid high water temperatures, as within the wood daytime thermal maxima are 6.5°C lower as compared to adjacent tidepools. However, these cryptic niches may also allow for protection against predation and desiccation.

Although behavioural thermoregulation is an important strategy that allows high intertidal animals to stay cool during warm days, it is not always possible. The 'gaping' behaviour of mussels (intermittently opening the shell to allow for aerial O_2 uptake, see 'Tolerance to diel hypoxia' section), for instance, could theoretically also allow for evaporative cooling. Nevertheless, it was shown in *Mytilus californianus* that gaping has no significant effect on body temperature (Fitzhenry et al. 2004), probably as the shell is only occasionally opened (<20% of the time) to minimize water loss (Gleason et al. 2017). Littorinid snails restricted to the eulittoral fringe appear to rely little on evaporative cooling (or are limited in the use thereof) compared to species in lower parts of the eulittoral zone, because of the greater need to conserve water (McMahon 1990). Furthermore, slow-moving animals (e.g. limpets) are not always able to effectively utilize behavioural thermoregulation, because they may not be able to get to a favourable microhabitat in a relevant timescale (i.e. within a few hours) (Harley et al. 2009). For fish in tropical tidepools, thermal refuge options can also be limited, and they may need to stay at high temperatures until they decrease naturally (Lam et al. 2006). Even when escaping stressful thermal conditions in the water is possible, there may be important benefits to remaining in this habitat. For instance, when mangrove rivulus (*Kryptolebias marmoratus*) are exposed to hot water temperatures, they can jump out of the water to take advantage of evaporative cooling on the land (Gibson et al. 2015). However, remaining in the water allows the fish to continue to forage for aquatic prey and avoid terrestrial predators.

Because of the high temperatures encountered, high intertidal animals also typically acquire an intrinsically higher heat tolerance. For instance, a survey of 60 intertidal gastropod species representing seven superfamilies showed that thermal tolerance (measured as HCT) increases with shore height (McMahon 2001). This trend was particularly well-supported given that species included in the survey occupied various habitat types (rocky shores, mangroves and salt marshes), and were from ten geographically separate sites with different climates (temperate, subtropical and tropical). Littorinid snails have also been reported to have a 2.7°C greater mean HCT than non-littorinid snails, which may explain the worldwide dominance of this superfamily on high shores. In another study, involving 34 tropical invertebrate species, acute thermal tolerance was also found to be positively correlated with tidal height (Nguyen et al. 2011). Sometimes, this relationship is visible even among individuals of the same species. For example, tidepool sculpins (*Oligocottus maculosus*) from the high intertidal, have a slightly (0.5°C) higher critical thermal maximum (CT_{max}; the temperature at which loss of equilibrium occurs) than those from the low intertidal (Fangue et al. 2011).

There are several physiological/biochemical mechanisms that have been linked to the higher heat tolerance of high intertidal animals. In high intertidal crustaceans and molluscs, the heat resilience of the heart and the maintenance of O_2 supply/consumption play an important role. *Petrolisthes* porcelain crabs exhibit a clear correlation between thermal tolerance (measured as the LT_{50}; the temperature at which 50% mortality occurs) and intertidal vertical position (Stillman & Somero 2000). In the higher intertidal porcelain crab *Petrolisthes cinctipes*, which can be emerged for more than half of the day, the temperature of cardiac failure (Arrhenius breakpoint temperature; ABT) was 5°C higher than in its lower intertidal relative *Petrolisthes eriomerus*, which is emerged only during low spring tides (Stillman & Somero 1996). During emersion, *P. cinctipes* could also maintain a higher rate of O_2 consumption ($\dot{M}O_2$) and better avoid lactate accumulation than *P. eriomerus*. This was attributed, in part, to *P. cinctipes* having a specialized membranous structure on each walking leg that has an aerial respiratory function. Similar to porcelain crabs, there is a strong relationship between vertical distribution, thermal tolerance and ABT in several intertidal snail species from both temperate and tropical climates (Tomanek & Somero 1999, Stenseng et al. 2005, Dong & Williams 2011, Monaco et al. 2017). For instance, the ABT of the limpet *Cellana grata* which is abundant in the high zone of tropical rocky shores (at ~1.5–2 m above the lowest tide level) is ~5.6°C higher than in the local low intertidal congener *Cellana toreuma* (at ~1.25–1.5 m above the lowest tide level) (Dong & Williams 2011). Further, $\dot{M}O_2$, cardiac performance and thermal limits are linked when the upper intertidal limpet *Lottia digitalis* from California is exposed to increasing temperatures, and surprisingly, these parameters are higher under emersion than immersion (Bjelde & Todgham 2013, Bjelde et al. 2015). The fact that cardiac and metabolic performance are optimal in *L. digitalis* when aerially exposed, highlights how well adapted it is to the high intertidal environment. Interestingly, the high intertidal tropical snail *Echinolittorina malaccana* reduces crawling speed and depresses metabolic rate during heat stress, as $\dot{M}O_2$ is completely thermally insensitive between 35°C and 46°C (Marshall & McQuaid 2011, Marshall et al. 2011). Cardiac activity is also depressed in this species within this thermal range, and enzymatic and metabolomic analyses suggest that there is a switch to anaerobic metabolism up to this species' ULT (Chen et al. 2021). Thus, under heat stress, *Echinolittorina malaccana* reduces O_2 demand rather than enhancing O_2 uptake and supply. This ability to enter a state of protective metabolic depression may contribute to the extremely high thermal tolerance of this littorinid snail (i.e. its LT_{50} or ULT is ~50°C) (Lee & Lim 2009, Nguyen et al. 2011).

High intertidal molluscs also often have metabolic enzymes with a higher heat stability than their low intertidal relatives, and these differences can be found both between and among species, so they are driven by both evolution and phenotypic plasticity. In the genera *Lottia* and *Echinolittorina*, for instance, upper intertidal snail species that are more thermally tolerant than their lower intertidal congeners (Wolcott 1973, Li 2012) have cytosolic enzyme malate dehydrogenase (cMDH) isoforms/orthologues that are more resistant to heat inactivation (Dong & Somero 2009, Liao et al. 2017). Interestingly, these differences in enzyme thermal stability result from only minor amino acid substitutions (i.e. at one or two positions) that each arise from a single nucleotide polymorphism. cMDH is involved in many metabolic pathways, such as the tricarboxylic acid cycle, amino acid synthesis and gluconeogenesis (Goward & Nicholls 1994), so it is feasible that this enzyme is under strong selective pressure for thermal adaptation. Aminotransferases, which catalyse the conversion between amino acids and keto acids and are important in amino acid metabolism, also appear to have undergone selection for heat resistance; this conclusion is based on comparisons among several species of littorinids that occupy different tidal heights. For instance, in Britain, high intertidal species had higher activity levels of aspartate aminotransferase than low intertidal species (Hull et al. 1999), and this enzyme was more heat stable than alanine aminotransferase which was more active in low intertidal species. Furthermore, within one periwinkle species (*Littorina saxatilis*) that inhabits different intertidal heights, both aspartate and alanine aminotransferase were more heat tolerant in high intertidal compared to low intertidal individuals. In Singapore, the heat stability of alanine aminotransferase (also known as glutamate oxaloacetate transaminase) was the highest in the snail species living the furthest up the shore (*Echinolittorina malaccana*) (Lee & Lim 2009).

The heat shock protein (Hsp) response has been particularly well studied in intertidal animals (Hofmann 1999), and Hsp expression levels clearly mark the onset of cellular heat stress (e.g. in tidepool sculpins at 2°C below their CT_{max}) (Fangue et al. 2011). Hsps are molecular chaperones, whose expression is upregulated by heat shock transcription factors (HSF) during thermal stress (or another form of stress), and they limit the denaturation (unfolding) of proteins, aid with their repair and inhibit the induction of apoptosis (Morimoto 1998, Tomanek & Somero 2002, Beere 2004). In four *Chlorostoma* snail species (formerly the genus *Tegula*), the temperatures at which the synthesis of Hsps began, peaked and was heat-inactivated (ceased), were positively correlated with their intertidal vertical distribution and upper thermal tolerance limits (Tomanek & Somero 1999). In the rocky shore limpet *Patella vulgata*, Hsp70 levels were also higher in warmer sun-exposed microhabitats compared to cooler shaded sites (Lima et al. 2016). These data strongly suggest that these chaperone proteins play a key ecological role. In numerous intertidal molluscs found in temperate and tropical climates [e.g. in *Lottia* limpets (Sanders et al. 1991, Dong et al. 2008), *Cellana* limpets (Dong & Williams 2011) and *Mytilus californianus* mussels (Roberts et al. 1997, Halpin et al. 2004, Gracey et al. 2008)], increasing shore height, heat exposure and heat tolerance have also been associated with greater constitutive and/or heat-induced Hsp levels. Very similar trends have also been observed among sculpin species (Nakano & Iwama 2002). This indicates that in high intertidal molluscs and fishes, Hsps are thermoprotective during heat exposure, but can also be used as a 'preparative defence' against future heat stress. This latter strategy involves the maintenance of high baseline (constitutive) Hsp levels that may protect against protein damage during extreme and unpredictable high temperature events (Nakano & Iwama 2002, Dong et al. 2008). Because maintaining higher constitutive Hsp levels likely requires the allocation of energy (given that protein synthesis can be a major energy expenditure in ectotherms; Hawkins 1985, Houlihan 1991), this may not be advantageous for animals in the low intertidal, less thermally stressed, environment.

The production of Hsps in intertidal animals is highly plastic and affected by thermal history, which further helps intertidal animals to adjust to the highly variable thermal environment in which they live. This plasticity is illustrated in *Mytilus* mussels, for example, where: the induction temperature for Hsp70 production is 5°C higher in the summer than in the winter (a form of seasonal acclimatization), and similar Hsp70 induction temperatures are measured in high bed mussels as compared to low bed mussels transplanted to the former habitat (a form of acclimatization to microhabitat temperature) (Buckley et al. 2001, Halpin et al. 2004). It has also been suggested that intertidal animals induce the Hsp response more frequently than animals in thermally stable marine environments (e.g. the subtidal Antarctic, coral reefs), whereby the associated metabolic cost is likely outweighed by the benefits (Tomanek 2010). In the tidepool sculpin *Oligocottus maculosus*, the transcription and production of Hsps is not directed by an endogenous rhythm, but directly by tidepool temperature fluctuations (Todgham et al. 2006). Interestingly, in the same species, Hsp70 production following heat shock increases the tolerance to hyperosmotic and hypoxic stress exposure 8–48 hours later, which is a timeframe similar to the interval between low tides (Todgham et al. 2005). This finding suggests that the production of Hsps may allow for cross-tolerance, by helping the animal deal with multiple, and different, environmental stressors.

Freezing resistance

High intertidal animals can sometimes be exposed to extreme cold, which can occur in any environment (e.g. see Firth et al. 2011), but especially in boreal and polar climates (Raffaelli & Hawkins 1999). For example, in the Antarctic high intertidal, mean winter temperatures of –15°C are often recorded, whereas in the low intertidal/subtidal, –5°C is typically not surpassed (Waller et al. 2006, Clark et al. 2008). The duration of exposure to freezing temperatures (hours/days of temperatures below 0°C) also increases with intertidal height, as shown in several gastropod species on the Alaska and Washington coasts (Stickle et al. 2015, 2017). Temperatures close to zero can slow down the metabolism of animals to the extent that cardiac activity ceases, as seen in *Mytilus trossulus* mussels (Braby & Somero 2006). However, the freezing of body fluids is more problematic, because ice crystals cause physical damage to delicate cell structures, and ice crystal growth leads to desiccation by removing intracellular water (Denny & Gaines 2007). This section focuses on freezing, because it is the most severe form of cold stress, and resistance to its effects also involves avoidance and tolerance (Ansart & Vernon 2003, Yancey 2005, Duman 2015, Peck 2018). The body fluids of 'freezing avoiders' do not freeze, whereas 'tolerators' allow their extracellular body fluids to freeze.

Intertidal molluscs and barnacles are generally considered to be freeze tolerant, and survive with up to 65%–80% of their tissue water frozen (Roland & Ring 1977, Murphy 1983, Storey & Storey 1996, Ansart & Vernon 2003). There are a few bivalves and gastropods that also display an evolutionary trend or plasticity towards higher freezing tolerance with increasing shore height, based on comparisons among congeners or conspecifics, respectively. For instance, *Nacella concinna* sampled from the Antarctic intertidal zone in the summer have higher rates of survival following (almost) complete freezing of their body fluids as compared to individuals from the subtidal (95% versus 67% survival, respectively) (Waller et al. 2006). In the winter, high intertidal *Mytilus trossulus* from the Vancouver coast (at 3.5 m above the lowest tide level) had a 1°C–1.5°C lower freezing lethal temperature (LLT_{50}, temperature which causes 50% mortality) than low intertidal individuals (at 1 m above the lowest tide level) (Kennedy et al. 2020). A similar relationship between freezing LLT_{50} and intertidal distribution has been reported among *Nucella* and *Littorina* snail species from the Pacific Northwest (Stickle et al. 2015, 2017).

Information regarding the drivers of patterns of freezing tolerance across shore heights is still limited, compared to what is known about heat resilience-related adaptations in intertidal animals. In the periwinkle *Littorina littorea*, a larger body size provides increased freeze tolerance (i.e. a lower freezing mortality) by reducing the freezing rate, thus minimizing the length of time being frozen (Murphy & Johnson 1980). This might explain why larger individuals of this species are more common in the high intertidal zone (Gendron 1977). An important physiological adaptation allowing for the high freeze tolerance in intertidal invertebrates is the secretion of ice-nucleating proteins. The mussel *Mytilus edulis* (Aunaas 1982, Lundheim 1997, Denny & Gaines 2007) and snail *Melampus bidentatus* (Loomis 1985), for instance, deposit ice-nucleating proteins in the extracellular space that induce ice formation at a relatively high temperature, before intracellular fluids begin to freeze. As the extracellular compartment freezes, water is drawn out of the cell due to osmotic pressure, and this reduces the probability of intracellular ice formation. In the ribbed mussel *Geukensia demissa*, the gill-associated bacterium *Pseudomonas fulva* serves as an ice-nucleating agent (Loomis & Zinser 2001). Compatible organic osmolytes such as free amino acids (see the section 'Resistance to salinity fluctuations' below) may also improve freeze tolerance (i.e. serve as cryoprotectants), as they prevent excessive cellular water loss to growing extracellular ice crystals by retaining water inside the cell (Storey 1997). In the saltmarsh snail *Melampus bidentatus*, the amount of such osmolytes (glycerol and proline) was slightly elevated in the winter (Loomis 1985). Recently, using a metabolomics approach, it was shown that the concentration of several osmolytes [taurine, betaine, trimethylamine N-oxide (TMAO), glycine and alanine] was also elevated in *Mytilus trossulus* in the winter, and that this was correlated with an increase in freezing tolerance (i.e. reduced LLT_{50}) during this season in both low and high intertidal individuals (Kennedy et al. 2020). However, osmolyte concentrations did not explain the increase in freezing tolerance with shore height, so this must be facilitated by other mechanisms.

Freezing avoiders typically produce antifreeze proteins or glycoproteins, which inhibit the growth of ice crystals, or they accumulate high levels of compatible osmolytes such as glycerol to lower their freezing temperature. Although these mechanisms are used by various fishes (e.g. rainbow smelt, winter flounder, Antarctic notothenioids, Arctic gadids) and terrestrial arthropods (Yancey 2005, Duman 2015, Peck 2018), they are quite uncommon among intertidal animals (Storey & Storey 1996, Ansart & Vernon 2003). In a survey of 11 Antarctic intertidal invertebrates, only the nemertean worm *Antarctonemertes validum* was found to use antifreeze proteins, which results in 1.4°C of thermal hysteresis in its haemolymph (Waller et al. 2006). Thermal hysteresis is the delay in the growth of ice crystals in the blood/haemolymph when cooled below 0°C (i.e. the separation of the melting and freezing point of a liquid), and it is characteristic of the presence of antifreeze proteins (whereas osmolytes do not produce this phenomenon) (Peck 2018). Waller et al. (2006) also showed that the Antarctic intertidal snails *Margarella antarctica* and *Nacella concinna* have a lower freezing point than subtidal individuals of the same species, and that all intertidal species included in their study had freezing points well below that of the surrounding seawater (i.e. below −5°C). The mechanism(s) underlying freezing point depression in these animals still remain to be elucidated – although in both subtidal and intertidal *Nacella concinna*, the mucus secreted by the foot has been reported to contribute to its freezing avoidance, as the physical viscosity of this mucal film delays the proliferation of ice crystals (Hawes et al. 2010).

In molluscs, the ability to switch to anaerobiosis or to suppress metabolism, is important during hypoxia survival (see the section 'Tolerance to diel hypoxia' below), and freeze tolerance may rely on a very similar metabolic adaptation. Freezing has been shown to induce anaerobiosis in the periwinkle *Littorina littorea* (Churchill & Storey 1996) and mussel *Geukensia demissa* (Storey & Churchill 1995). During freezing, haemolymph flow is halted, which might limit O_2 supply and generate internal hypoxia, and this would require the generation of ATP though O_2-independent means to maintain energy balance. In *Littorina littorea*, freezing was also associated with the upregulation of many microRNAs and protein levels of Dicer (an enzyme that processes pre-microRNAs into mature ones) (Biggar et al. 2012). This suggests a role for 'RNA interference' in the suppression of protein synthesis (an energetically expensive process) during freezing survival in *L. littorea*, possibly as part of a metabolic depression response. As Biggar et al. (2012) argue, this would explain the lack of a decrease in overall mRNA levels. In RNA interference, mRNA transcription is unaffected, but mRNA translation into protein is suppressed through the binding of microRNAs and mRNA is subsequently degraded.

Hypoosmotic marine teleosts are particularly vulnerable to freezing compared to isosmotic invertebrates. However, research on freezing resistance in intertidal fishes is lacking. Perhaps, this is because intertidal fishes in boreal climates (e.g. the rock gunnel *Pholis gunnellus*) leave the intertidal zone during the winter months to avoid exposure to freezing temperatures (Sawyer 1967, Moring 1990). Antarctic intertidal areas are usually covered by ice in the winter (Aghmich et al. 2016), resulting in harsh conditions that may not allow for the survival of fish. Even in the summer, the Antarctic high intertidal often experiences temperatures below zero (Waller et al. 2006). Indeed, summer surveys of faunal assemblages rarely report the presence of fish (Aghmich et al. 2016), and indicate that the few species found are confined to the middle and low intertidal zones (with exception of the Antarctic spiny plunderfish *Harpagifer antarcticus* which can be found in high tidepools) (Sakurai et al. 1996). This pattern is likely driven by exposure to freezing temperatures, although it is conceivable that summer high temperatures, desiccation and other factors also make the upper tidal area in the Antarctic uninhabitable to fish.

Tolerance to high thermal variability

From polar to tropical climates, *in situ* temperature recordings show that the high intertidal zone typically experiences more rapid and larger thermal fluctuations than lower areas, on both a daily and a seasonal basis (Morris & Taylor 1983, Richards 2011, Schulte 2011, Kuklinski & Balazy 2014, Monaco et al. 2017) (Figure 2). For instance, on a single day in the tropics, the temperature can fluctuate on an emerged high shore by ~20°C versus ~15°C on an emerged low shore (Monaco et al. 2017). Further, during the summer in the Antarctic, the average temperature variability was 10°C higher at upper tidal sites as compared to lower tidal sites (Kuklinski & Balazy 2014). Temperature variability can differ substantially across intertidal environments, as it may depend on factors such as substratum type (Nguyen et al. 2011), whether the shore side receives sun exposure (Seabra et al. 2011, Amstutz et al. 2021), and the presence of vegetation that provides shading (Marshall et al. 2015). On the Atlantic coast of the Iberian Peninsula, for example, data loggers mimicking limpets (‘robolimpets’, see Lima & Wethey 2009) recorded far greater temperature variability on south-facing sun-exposed shores than on north-facing shaded shores (Seabra et al. 2011); and subtleties like these are important to consider in the study of high intertidal animals. Adaptations to cope with high thermal variability also have some overlap with those covered by the two preceding sections on heat and freezing stress, hence, the discussion of this topic is limited.

Rapid temperature fluctuations in the high intertidal zone have a considerable impact on animals given that a change in only 1°C can alter the rate of biological reactions by 10% (Lam et al. 2006). Therefore, mobile animals will often avoid exposure to the largest temperature changes using behavioural thermoregulation, i.e. by shuttling between cooler and warmer microenvironments, or between the air and water in which they may cool down/warm up more quickly. This is seen, for instance, in the middle to high intertidal crab *Hemigrapsus nudus*, which in the laboratory manages to maintain its body temperature within much narrower limits (~8°C to ~20°C) than that of the environment (~5°C to 35°C) (McGaw 2003). These results have been confirmed in the field. Intertidal animals are also typically eurythermal, and capable of tolerating large changes in temperature over short periods of time. For instance, limpets (*Lottia gigantea*) can tolerate an increase in body temperature of >20°C within a day (Denny & Harley 2006). The killifish *Fundulus heteroclitus* can live at near freezing temperatures, but also tolerates heating from 5°C to 37°C over as little as an hour (Moyle & Cech 1996, Schulte 2011).

A physiological adaptation that might improve tolerance to thermal fluctuations, is having a less thermally sensitive metabolism. Among hermit crabs, the temperature sensitivity of aerobic metabolism ($\dot{M}O_2$) in intertidal species (*Pagurus granusimanus* and *P. hirsutiusculus*) is lower than in subtidal species (*Paguristes turgidus* and *Elassochirus tenuimanus*), as indicated by lower values for the temperature coefficient Q_{10} (1.4–1.6 versus 2.1–2.4) (Burggren & McMahon 1981). An organism’s Q_{10} is the degree of change for a physiological process (in this case $\dot{M}O_2$) resulting from a 10°C change in temperature. The intertidal hermit crab species also had a smaller $\dot{M}O_2$ 'overshoot' (i.e. quick increase in O_2 consumption) in response to an abrupt 10°C increase above acclimation temperatures, and a more rapid acclimation (i.e. return of $\dot{M}O_2$ to normal resting levels), than the subtidal species. This could be advantageous by reducing the use of energy resources during the summer, as well as allowing the animal to remain active during winter. In killifish (*Fundulus heteroclitus*), the large thermal tolerance range is associated with an extremely wide thermal breath (at least 5°C–33°C) over which it can maintain a positive aerobic scope (i.e. the difference between maximum and basal/resting $\dot{M}O_2$) (Healy & Schulte 2012). Furthermore, thermal acclimation is not required to establish the majority of this breadth; and therefore, it is an inherent (fixed) characteristic of this species that its aerobic capacity suffers few negative effects from acute temperature changes.

Tolerance of diel hypoxia

Aquatic hypoxia occurs in many marine ecosystems (e.g. oxygen minimum zones and estuaries) (Breitburg et al. 2018). In the intertidal zone, this environmental challenge is characterized by short-term (acute), and often severe, decreases in available oxygen (O_2) that frequently become more extreme higher up the shore (Figure 2) (Morris & Taylor 1983, Raffaelli & Hawkins 1999, Richards 2011, Schulte 2011, Mandic & Regan 2018, McArley et al. 2019). During high tide, conditions are usually normoxic, whereas during low tide, water O_2 levels can drop to near zero in high tidepools on rocky shores and in the high tidal zone of estuaries, mangroves, salt marshes and mudflats (Figures 2 and 3) (Richards 2011, Schulte 2011). Aquatic hypoxia may occur during the day because of increased organismal respiration due to issues such as crowding (e.g. many animals are aggregated in a small tidepool) and/or overheating (which increases the metabolic demands of animals), and during the night because O_2 is not being replenished by primary production (Ganning 1971, Morris & Taylor 1983, Huggett & Griffiths 1986). Although, intertidal rockpools can also become hyperoxic (supersaturated) at low tide during the day due to photosynthetic processes (Figure 2) (Ganning 1971, Morris & Taylor 1983, Huggett & Griffiths 1986, Richards 2011, Schulte 2011, McArley et al. 2018, 2019). In addition to aquatic hypoxia, high intertidal animals can also experience 'internal hypoxia' (hypoxemia) when emerged, as a result of the retraction or collapse of respiratory structures which renders them non-functional (Raffaelli & Hawkins 1999, Wolcott & Wolcott 2001, Turko et al. 2014). Emersion-induced hypoxemia and aquatic hypoxia affect animals in a similar way (both involve constrained O_2 uptake), although there can be subtle differences in the adaptations/plastic responses against these environmental cues (e.g. see Turko et al. 2014, 2018). This section is more detailed/comprehensive than many other sections in this review because of the abundance of information on the topic, which perhaps reflects the multitude of adaptations that intertidal animals have to deal with the stress of limited O_2. These various adaptations and responses are divided into three main strategies (Table 2).

Enhanced aerobic capacity

To avoid depending on anaerobiosis during hypoxia, many high intertidal animals enhance their aerobic capacity by improving O_2 uptake from water/air, O_2 transport and/or mitochondrial O_2 utilization. For more elaborate discussions of the adaptations of intertidal fishes to hypoxia, see the recent reviews by Martin (2014), Wright & Turko (2016) and Mandic & Regan (2018). Intertidal fishes generally use two types of behaviour to enhance O_2 uptake: aquatic surface respiration (ASR), whereby the O_2-rich water is skimmed from the surface, and emergence for aerial respiration. The mangrove rivulus, *Kryptolebias marmoratus*, even has physical characteristics compatible with ASR, including an upward-facing mouth and a flattened head (Taylor 2012). Fishes such as sculpins, killifish, clingfish and mudskippers, actively leave hypoxic waters to exchange gases using their skin, gills or other epithelial surfaces, which are more vascularized to allow for better aerial O_2 uptake (Ebeling et al. 1970, Wright & Turko 2016, Mandic & Regan 2018, Livingston et al. 2018). For instance, intertidal sculpins perform ASR and emergence behaviours at a much higher frequency than subtidal relatives to escape aquatic hypoxia (Mandic et al. 2009a), and in *Oligocottus maculosus* and *O. snyderi*, O_2 uptake from the air can be as high as from the water (Yoshiyama & Cech 1994, Sloman et al. 2008). Amphibious fishes (e.g. the high intertidal mudskipper *Periophthalmodon schlosseri*) commonly have modified gills, for instance, with improved support structures, which do not collapse during emersion and can be used for effective aerial respiration (Ebeling et al. 1970, Low et al. 1988, Sayer 2005, Lam et al. 2006). However, emergence behaviour in fish might not always be for the purpose of air breathing. Emergence of the rockpool blenny *Praealticus tanegasimae* during low tide is often followed by migration to nearby tidepools ('tidepool hopping'), likely to find more favourable conditions (Kimura & Sakai 2016). Several species of intertidal burrow-inhabiting mudskippers, such as *Periophthalmodon schlosseri* (Ishimatsu et al. 1998), *Periophthalmus modestus* (Ishimatsu et al. 2007) and *Scartelaos histophorus* (Lee et al. 2005), repeatedly emerge at the surface of their burrows to take in mouthfuls of air. These are then deposited into the burrow's air chamber to avoid hypoxic exposure of the inhabitant and any eggs or embryos that it is guarding.

When aerial predation is present, high intertidal fishes may remain submerged in hypoxic water, and instead rely on physiological/biochemical strategies to maintain aerobic metabolism while exposed to limited O_2 levels (Mandic & Regan 2018). Such strategies have been described in fishes such as sculpins (Cottidae family), triplefins (Tripterygiidae family) and the mangrove rivulus (*Kryptolebias marmoratus*), and include increases in mass-specific gill surface area, haematocrit (Hct), haemoglobin levels and/or haemoglobin O_2-binding affinity (as indicated by a lower P_{50}, the O_2 level at which haemoglobin is 50% saturated with O_2) (Mandic et al. 2009b, Turko et al. 2014, 2018, Mandic & Regan 2018, McArley et al. 2019). Intertidal triplefin species also have thinner gill secondary lamellae, which results in a shorter diffusion distance for O_2 to reach the blood during ventilation (McArley et al. 2019). In addition to these adaptations at the level of O_2 uptake and transport, changes in mitochondrial respiration/the electron transport chain have been identified. In the intertidal triplefin *Bellapiscis medius*, oxidative phosphorylation in the heart is more efficient (coupled), and maximum O_2 consumption by cytochrome *c* oxidase (COX) is higher, as compared to the two subtidal species *Forsterygion varium* and *F. malcolmi* (Hilton et al. 2010). This may explain why *Bellapiscis medius* is more hypoxia tolerant (i.e. has a lower critical O_2 tension or P_{crit}; the O_2 level at which the O_2 consumption rate becomes dependent on the O_2 level) than the other triplefin species. Recently, it was also shown that the upper intertidal sculpin *Oligocottus maculosus* has a higher COX and mitochondrial O_2-binding affinity in the brain compared to lower intertidal species, which is associated with a higher hypoxia tolerance (Lau et al. 2017). *In silico* analysis of the COX3 subunit, which is the putative entry point for O_2 into the COX protein catalytic core, showed that the higher COX O_2 affinity of *Oligocottus maculosus* could be attributed to higher COX3 protein stability. This increased stability was further linked to two amino acid substitutions in the COX3 v-cleft structure (i.e. at residue positions 55 and 224, phenylalanine is replaced by leucine, and leucine by phenylalanine, respectively), which likely strengthen the high-affinity interaction with cardiolipin from the mitochondrial membrane.

Similar to fishes, intertidal crustaceans such as crabs and shrimps use ASR when exposed to hypoxia, or are capable of switching to air breathing during emersion. The temperate rockpool shrimp (*Palaemon elegans*) moves to shallow water at low O_2 levels, exploiting the higher O_2 level at the air/water interface, and this allows it to maintain a higher haemolymph O_2 content and resist increases in lactate levels (Taylor & Spicer 1988). This partial-emergence, hypoxia-induced behaviour, has also been described in the tropical burrowing ghost shrimp *Upogebia africana* (Hill 1981) and other intertidal shrimp species (Hagerman 1998). In air-exposed *Pagurus* hermit crabs, intertidal species are relatively well-adapted to aerial respiration, as they are better able to defend aerobic metabolic rate (O_2 consumption; $\dot{M}O_2$) as compared to their subtidal relatives (Burggren & McMahon 1981). The estuarine crab *Chasmagnathus granulatus* uses highly vascularized branchial chambers for air breathing (Halperin et al. 2000). Finally, some intertidal porcelain crabs (*Petrolisthes*) have an aerial gas exchange organ on each of their walking legs, which appears to be especially important in larger bodied species (Stillman 2000), and a similar structure (a 'gas window') can be found in small burrowing intertidal crabs of the genera *Scopimera* and *Dotilla* (Maitland 1986).

Some intertidal crabs have evolved other mechanisms to sustain/optimize gas exchange while in or out of the water. The high intertidal mudflat crab *Helice crassa* is able to recirculate water in the branchial chambers for re-oxygenation, whereas the mid-intertidal crab *Macrophtalmus hirtipes* is unable to do this; which might be why it is restricted to a lower area of the intertidal zone (Hawkins & Jones 1982). Intertidal crabs can also improve aerial or aquatic O_2 uptake by having haemocyanin with a higher O_2 affinity (a lower P_{50}) (DeFur 1988). Among gammaridean, hyalid and talitrid amphipods, other adaptations of respiratory pigments have been described in high intertidal species that optimize O_2 supply concordant with their semi-terrestrial/amphibious lifestyle. For instance, in *Talitrus saltator*, which inhabits sandy shores in Europe around the high tide mark, haemocyanin-O_2 affinity is relatively insensitive to organic (lactate) and inorganic ionic (Ca^{2+}, Cl^-) fluctuations in the haemolymph (Spicer et al. 1990), which would help to maintain O_2 uptake during the desiccation/acidosis that is associated with emersion. Spicer (1993) and Spicer & Taylor (1994a) confirmed this in other amphipod species, and further described a general decline in the Bohr effect in upper tidal amphipod genera as compared to aquatic amphipods. This avoids a loss of haemocyanin-O_2 affinity due to high haemolymph CO_2 levels during emersion.

In bivalves, gaping behaviour allows for aerial O_2 uptake, and is used by several intertidal species in response to emersion. This is a trade-off between shell closure to avoid desiccation, and shell opening to avoid hypoxia (Nicastro et al. 2010). Bivalves that engage in gaping activity have higher aerial O_2 uptake rates (~75% of the aquatic $\dot{M}O_2$ in *Mytilus californianus* and *Cardium edule*) than non-gaping species (~17% of the aquatic $\dot{M}O_2$ in *Mytilus edulis*) (Bayne et al. 1976, Widdows & Shick 1985, Zippay & Helmuth 2012). In addition, differences in air-breathing abilities are seen among mollusc species that inhabit both the intertidal and subtidal zone [e.g. in the mussels *Mytilus edulis* and *M. galloprovincialis* (Altieri 2006, Tagliarolo et al. 2012) and the limpet *Nacella concinna* (Weihe & Abele 2008)] and this underscores the role of phenotypic plasticity in allowing intertidal colonization. Interestingly, in *Mytilus edulis*, the difference in survival during emersion and aquatic hypoxia among inter- and subtidal individuals was almost completely reversed 7 weeks after reciprocal transplantation (Altieri 2006). Reciprocal transplantations are a useful tool to elucidate processes such as local adaptation in a species, the level of phenotypic plasticity and its reversibility, and epigenetic effects; and this specific finding highlights the flexibility of *M. edulis* to acclimate to different shore heights. Although this study does not explore the mechanisms behind this ability, it is very likely that changes in gene transcription are a key driver.

Enhanced anaerobic capacity

Bivalves from the high intertidal zone – including those that gape – also rely greatly on anaerobiosis to survive emersion, given that their air-tight shells are closed for most of the time and the water retained within the valves is depleted of O_2 within minutes (Bayne et al. 1976, Zippay & Helmuth 2012). This enhanced anaerobiosis may be reflected by a greater accumulation of anaerobic end products such as alanine, as seen in *Mytilus californianus,* when individuals from the high versus low intertidal zone are compared (Gleason et al. 2017). Estuarine oysters, such as *Ostrea chilensis*, also use anaerobiosis when 'clammed up' in response to reduced salinities (see the 'Tolerance to salinity fluctuations' section below), including embryos that are brooded by females inside the mantle cavity (Segura et al. 2015). The disadvantage of this anaerobic strategy is that an 'O_2 debt' must be repaid during re-immersion to allow for the aerobic processing of anaerobic end products, as seen in several bivalve species (Bayne et al. 1976, Widdows & Shick 1985, Zippay & Helmuth 2012). *Mytilus californianus* has a strong tide-driven metabolic cycle of aerobic metabolism during submergence and anaerobiosis during emersion, and interestingly, this intrinsic property is still visible under continuous experimental submergence (Connor & Gracey 2012, Gracey & Connor 2016). These data emphasize the importance of this metabolic cycle for *M. californianus*, which apparently does not rely on external signals from the environment that are associated with emersion.

Some high intertidal invertebrates appear to increase the amount of anaerobic fuel sources compared to their low intertidal counterparts, to help maintain energy levels under O_2 limited conditions. This is reported, for instance, in high versus low intertidal periwinkles of the species *Littorina saxatilis*, although anaerobiosis accounts only for a relatively small fraction of the ATP turnover compared to aerobic metabolism (Sokolova & Pörtner 2001a). Females of high intertidal crab species have also been reported to produce embryos with higher levels of organic matter (total protein, carbohydrates and lipids, etc.), and thus, with more available anaerobic substrates, as compared to subtidal species (Viña et al. 2018).

Although high intertidal sculpins have many adaptations to enhance aerobic capacity, they still use anaerobiosis when access to aquatic surface respiration and emergence is denied (Sloman et al. 2008), and have an enhanced capacity to do so. In several species of sculpin with differing vertical distributions on the Pacific coast, a high maximal lactate dehydrogenase (LDH) enzyme activity in the brain is linked to high hypoxia tolerance (Mandic et al. 2012). For instance, hypoxia tolerance and LDH activity were highest in the upper tidal species *Oligocottus maculosus*, and lowest in the subtidal species *Myoxocephalus scorpius* and *Blepsias cirrhosus* (i.e. P_{crit} of ~30 versus ~50 mm Hg, and enzymatic substrate conversion rate of ~170 versus ~100 µmol/min/g wet tissue, respectively). Optimizing the brain's LDH activity allows for maximum glycolytic ATP synthesis capacity, which might be particularly important in sculpins given that they elicit complex behaviours during low tide. The glycogen content in brain, liver and muscle of *Oligocottus maculosus* is also generally greater under normoxia and after hypoxic exposure, as compared to that of *Blepsias cirrhosus* (Speers-Roesch et al. 2013). Thus, *Oligocottus maculosus* appears to have larger anaerobic fuel stores in its tissues, and to deplete these stores at a slower rate. Similarly, New Zealand intertidal triplefin species have higher brain and muscle glycogen levels compared to subtidal, and less hypoxia-tolerant, congeners (McArley et al. 2019).

Metabolic depression

For the purposes of this review, metabolic depression is defined as the temporary (and reversible) reduction of metabolism below what would be considered normal at a particular temperature. Similar to anaerobiosis, metabolic depression as a means to survive hypoxia/hypoxemia during emersion is common among intertidal molluscs, and high intertidal animals appear to use this strategy to the greatest extent. For instance, in the mussel *Mytilus edulis* and cockle *Cardium edule*, both aerial O_2 uptake and heat dissipation rates were lower in intertidal compared to subtidal individuals (i.e. this reduced aerobic respiration rate was not compensated for by anaerobiosis) (Widdows & Shick 1985). Likewise, aerial O_2 consumption rates in the upper shore limpet *Siphonaria oculus* were below that of the lower shore limpet *Patella granularis*, and no 'O_2 debt' repayment was observed upon re-immersion (Marshall & McQuaid 1992a). Finally, high intertidal periwinkles (*Littorina saxatilis*) have been reported to use metabolic depression, as they accumulate less anaerobic end products during emersion while maintaining the same $\dot{M}O_2$ as individuals from the low intertidal (Sokolova & Pörtner 2001a).

In some high intertidal animals, metabolic depression as an adaptive response during emersion or aquatic hypoxia might be reflected by, or linked with, a decline in cardiac activity (though keeping in mind that heart rate and O_2 consumption are not always correlated, e.g. see Marshall & McQuaid 1992b). In the mussels *Mytilus californianus* (Connor & Gracey 2012) and *M. edulis* (Curtis et al. 2000), a greatly reduced heart rate (bradycardia), to near zero beats per min (acardia), is observed during emersion/valve closure, which would reduce O_2 requirements of the heart and of the whole organism. This bradycardic response does not change even in the absence of a tidal cycle (akin to the switch to anaerobiosis described above), and this suggests that this is an innate and crucial mechanism for survival (Gracey & Connor 2016). Similarly, exposure to hypoxia (~50 mm Hg or ~30% air saturation) induces bradycardia in the limpets *Siphonaria capensis* and *Patella granularis*, whereby *Siphonaria capensis* which occupies high shore tidepools appears to depress metabolic rate more effectively, and does not undergo the heart rate overshoot (tachycardia) upon return to normoxia as is typical for the lower intertidal species *Patella granularis* (Marshall & McQuaid 1993). Bradycardia has also been observed in the clingfish *Sicyases sanguineus* when out of the water, concordant with a reduction in $\dot{M}O_2$, a loss of respiratory movements and minimal lactate accumulation – all of which likely reflect metabolic depression (Gordon et al. 1970). However, it appears that metabolic depression in response to short-term hypoxia/emersion-related hypoxemia (i.e. hours to days) is not that common among intertidal fishes. This is possibly because it increases predation risk (i.e. the capacity for quick escape movements is likely diminished greatly). Although, it can play an important role during prolonged exposure (i.e. weeks to months) (Livingston et al. 2018, Mandic & Regan 2018, Turko et al. 2019).

Constitutively lower enzyme activities may also facilitate metabolic depression in high intertidal animals, and reduce overall energy demands. In *Littorina saxatilis* from Sweden, there are two allele variants of the metabolic enzyme alanine aminotransferase (AAT), whereby AAT^{100} and AAT^{120} homozygotes represent >70% and >80% of the population on the low and high shore, respectively (Panova & Johannesson 2004). AAT^{120} has a lower activity than AAT^{100}, which might help high shore periwinkles conserve energy during low tide. There are several more aerobic and anaerobic metabolic enzymes that have been reported to have a lower activity in high intertidal *Littorina saxatilis* from the White and North Sea coast (Sokolova & Pörtner 2001b). The mechanism(s) underlying these differences in allozyme activity (e.g. non-synonymous amino acid substitutions) have not yet been elucidated, but they appear to be under strong selection given that their vertical gradients within the intertidal zone occur in various geologically separated areas (Johannesson 2016). A temporary reduction in enzyme activity and energy-consuming processes to downregulate metabolism during hypoxia, is also seen among intertidal molluscs (Storey & Storey 1990). In *Littorina littorea* exposed to anoxia, for instance, there is a considerable decrease in the activity of two important glycolytic enzymes, phosphofructokinase (PFK) and pyruvate kinase (PK) (Greenway & Storey 2001). In the case of PK, the change in activity is the result of reversible phosphorylation, a type of covalent modification that affects the enzyme's kinetic properties, and that can switch it from an active (dephosphorylated) to an inactive (phosphorylated) form. This anoxia-induced regulatory mechanism of PK activity has also been described in the whelk *Busycotypus canaliculatus* and mussel *Mytilus edulis* (Storey & Storey 1990). Recently, it was shown that metabolic suppression during hypoxia in the hypoxia-tolerant intertidal clam *Mercenaria mercenaria* is facilitated by a drastic downregulation of protein synthesis and ion transport, as indicated by the inactivation of translation initiation factor EIF-1a, and a reduction of 26S proteasome and Na^+/K^+ ATPase activity (Ivanina et al. 2016). In contrast, this response was not observed in the hypoxia-sensitive subtidal scallop *Argopecten irradians*.

Despite the fact that intertidal fishes do not appear to use metabolic depression to the same extent as in molluscs, there is some evidence that they reduce the expression of genes involved in energetically expensive processes. In the intertidal mudskippers *Periophthalmus magnuspinnatus* and *Boleophthalmus pectinirostris*, genes involved in cell growth and proliferation were downregulated during 6 hours of air exposure, which suggests that they employ this as an energy-saving strategy (You et al. 2014). However, no comparison with subtidal mudskipper species was made in that study, leaving it uncertain as whether this strategy is a specific adaptation to the intertidal zone. In the upper tidal sculpin *Oligocottus maculosus*, on the other hand, it was reported that genes involved in O_2-dependent (e.g. fatty acid oxidation, oxidative phosphorylation) and energy-consuming (e.g. cell proliferation, protein synthesis) processes were downregulated during aquatic hypoxia, whereas this was not the case in the less hypoxia-tolerant, low intertidal sculpin *Blepsias cirrhosus* (Mandic et al. 2014). However, these transcriptional changes did not occur until after 24 hours of hypoxia exposure, which is beyond the typical duration of diel hypoxia (6–8 hours) in the intertidal zone. These authors suggested that rapid changes in gene transcription may not be used for daily hypoxia survival in *Oligocottus maculosus*, and that it instead uses translational or post-translational regulation to modulate gene expression (i.e. changes in protein synthesis or folding). Alternatively, it may maintain a 'fixed generalist phenotype' because of the metabolic costs associated with the continuous alteration of mRNA transcription during the tidal cycle. Indeed, many physiological/biochemical adaptations to diel hypoxia have already evolved in this sculpin species, and with its innate hypoxia resilience, the benefits of additional phenotypic plasticity may be limited. This theory would be somewhat consistent with the 'plastic floors and concrete ceilings' concept that was recently put forward by Sandblom et al. (2016). This concept suggests that limitations in physiological plasticity constrain the ability of fish to cope with high temperatures.

Tolerance to salinity fluctuations

All intertidal habitat types can experience tremendous fluctuations in salinity (Figure 3), which can be more extreme higher up the shore. River input or heavy precipitation (e.g. tropical monsoon rains) can reduce salinity down to that of fresh water, whereas evaporation or droughts can increase it above that of seawater (hypersaline conditions) (Ganning 1971, Morritt et al. 2007, Firth & Williams 2009, Schulte 2011, Marshall 2012). For instance, high shore rockpools may fill with seawater at high tide, but with fresh water at low tide (Marshall 2012), resulting in more pronounced salinity fluctuations than in low rockpools (Ganning 1971, Morris & Taylor 1983). In estuaries, salinity can vary between ~5‰ and ~30‰–35‰ (parts per thousand, ppt) throughout the year (e.g. as recorded on the Vancouver and Hong Kong coasts by Chan et al. 2001, Chan & Williams 2004 and Coverton & Harley 2020), and in mangroves and lagoons, evaporation can increase salinity to over 50‰–60‰ (Raffaelli & Hawkins 1999, Lam et al. 2006). Therefore, intertidal animals are typically euryhaline (able to cope with a range of salinities), and both osmoconformers [animals that allow their internal osmolarity to fluctuate along with the environmental osmolarity (predominantly invertebrates)] and osmoregulators [animals that maintain a constant internal osmotic pressure (most vertebrates and some crustaceans)] are able to withstand these fluctuations (Yancey 2005, Rivera-Ingraham & Lignot 2017). For example, among osmoconformers, the intertidal and estuarine Pacific oyster (*Crassostrea gigas*) tolerates salinities between 5‰ and 40‰ (Meng et al. 2013), and the boreo-Arctic high intertidal barnacle *Semibalanus balanoides* can tolerate/remain active at salinities from ~15‰ up to ~50‰ (Foster 1970, 1971). Among osmoregulators, the mangrove rivulus (*Kryptolebias marmoratus*), estuarine Nile tilapia (*Oreochromis niloticus*) and common killifish (*Fundulus heteroclitus*) can be acclimated to salinities ranging between 0‰ and 60‰–80‰ (King et al. 1989, Genz & Grosell 2011, Marshall 2012, Taylor 2012).

The first coping mechanisms with regard to salinity challenges are behavioural. These are particularly important for osmoconformers in the high intertidal zone (although osmoregulators use them as well), and this is probably because they are economical/do not require large physiological adjustments. Behavioural responses revolve around seeking refuge in a more favourable microhabitat, or reducing the amount of exposed surface area; that is, they are much like the behavioural defences against desiccation (Table 2). For instance, intertidal worms may move vertically in the sediment/their burrow in response to a salinity gradient (Oglesby 1969). The oyster *Ostrea chilensis* that inhabits estuaries isolates its tissues from the environment by 'clamming up' (closing the shell) when exposed to low salinity stress (Segura et al. 2015) and this is a common behavioural response among intertidal bivalves and barnacles when exposed to hypo- or hypersaline conditions (Foster 1970, Wolcott & Wolcott 2001, Chan et al. 2001). For example, the barnacle *Tetraclita japonica,* which is common on the high shore in Hong Kong (at 1.5–2 m above the lowest tide level), closes its opercular plates during acute hyposalinity stress (immersion in 10‰ seawater for up to 36 hours) and stops cirral activity/beating to prevent the influx of water into the mantle cavity (Chan et al. 2001). Although this prevents the barnacles from feeding (or reproducing, which has negative consequences in the long term), in the short term it allows them to protect their haemolymph and mantle water osmolality (by maintaining this at >500 mOsmol/kg above that of the surrounding seawater). Limpets may also use this type of 'behavioural osmoregulation' by tightly clamping to the substrate and isolating soft tissues from the environment. Such a behaviour may play a role in ionoregulation in the high intertidal *Cellana grata*, which has a limited ability to hyper-osmoregulate during monsoonal rainfall (Morritt et al. 2007, Williams et al. 2011).

An important physiological mechanism that allows intertidal osmoconformers to regulate cellular volume under salinity stress (i.e. to avoid excessive cell swelling/shrinkage), is the accumulation or catabolism of intracellular organic osmolytes, in particular free amino acids and their derivatives (Yancey 2005). In contrast to inorganic osmolytes, which at high concentrations can destabilize proteins and nucleotides, these organic osmolytes are 'compatible' as they do not interact with or damage macromolecules (Yancey et al. 1982, Yancey 2005, Schulte 2011). Therefore, their concentrations can safely fluctuate. In intertidal anemones, the amount of free amino acids (predominantly glycine, alanine and glutamate) increases along with salinity, which is not the result of the passive diffusion of water, but from their active production (Shick 1991). The high intertidal rockpool copepod *Tigriopus californicus* also appears to modulate its free fatty acid pool in response to hypo- or hyperosmotic stress (Burton & Feldman 1982). The Pacific oyster (*Crassostrea gigas*) likely uses free amino acids in a very similar way, as their concentration in the gills is positively correlated with acclimation salinity, with taurine being the most abundant free amino acid (up to 80% of total amino acids) (Hosoi et al. 2003, Meng et al. 2013, Zhang et al. 2016). Underlying the control of free amino acid levels in the gills, is the differential mRNA expression of key enzymes involved in free amino acid metabolism, such as cysteine sulphinate decarboxylase (CSAD), the main biosynthetic enzyme for taurine (Meng et al. 2013, Zhao et al. 2017). Taurine, together with glycine, betaine, alanine and trimethylamine N-oxide (TMAO), are also the most abundant organic osmolytes in intertidal mussels (Kennedy et al. 2020), polychaete worms, snails and clams (Yancey 2005). However, not all intertidal invertebrates are reported to actively modulate their intracellular osmolyte pool during salinity stress. For instance, the snail *Littorina littorea* appears to, instead, rely on a passive tolerance to large changes in cell volume (Taylor & Andrews 1988). Interestingly, in both high and low intertidal *Mytilus trossulus*, the accumulation of osmolytes in response to hypersalinity exposure is associated with a higher freezing tolerance, thus providing an example of cross-tolerance (Kennedy et al. 2020).

Osmoregulators use regulatory organs (e.g. gills, kidneys, antennal glands) to keep internal fluids at the same osmolarity (as much as possible) during salinity stress, and organic osmolytes do not play a major role (Morritt & Spicer 1998, Yancey 2005). Intertidal crustaceans vary in their capacity for osmoregulation and reliance on it as a coping strategy. For instance, the saltmarsh and estuarine gammarid amphipod *Gammarus duebeni* produces urine hypotonic to its haemolymph to conserve ions in the body during acute low salinity exposure (Lockwood 1961). The high intertidal hyalid amphipod *Hyale nilssoni* is also able to hyper-osmoregulate when exposed to dilute seawater, although it is unable to hypo-osmoregulate (Morritt 1988). Instead, this species likely adapts behaviourally rather than physiologically to hyperosmotic stress, by actively moving down the shore into wetter microhabitats (e.g. into seaweed or under gastropod shells). This appears to generally be the case for talitrid amphipods, as well as other crustaceans such as anomuran crabs, in intertidal habitats (for details, see reviews by Morritt & Spicer 1998 and Greenaway 2003). Although, the talitrid amphipod (beach flea) *Traskorchestia traskiana* that occurs at and above the high water mark, has the ability to hyper- and hypo-osmoregulate in diluted and full-strength seawater, respectively, and probably uses its gills to exchange ions accordingly (Spicer 2013). Other high intertidal crustaceans, e.g. isopods *Ligia occidentalis* and *L. pallasii* (Wilson 1970) and several crab species (e.g. *Pachygrapsus crassipes* and *Chasmagnathus granulatus*) (Gross 1964, Luquet et al. 2002), are also capable of a high degree of osmoregulation (relative to subtidal species). In the estuarine *Chasmagnathus granulatus*, which encounters salinities from <1‰ to hypersaline conditions in tidepools, the gill epithelium contains characteristics typical of a salt-transporting tissue, e.g. with numerous mitochondria and a high Na^+/K^+ ATPase activity that is involved in both NaCl uptake and secretion (Luquet et al. 2002, Genovese et al. 2004).

The osmoregulatory mechanisms/adaptations in (tropical) intertidal and estuarine fishes are reviewed by Lam et al. (2006) and Marshall (2012), and thus, will only be described here very briefly, with a focus on responses to short-term/cycling salinity stress (as this is most reflective of changes encountered in the intertidal environment). Intertidal and estuarine fish are typically able to quickly (and reversibly) adjust their gill functionality to external salinity (Schulte 2011, Marshall 2012). For instance, when the mummichog (*Fundulus heteroclitus*) is transferred from fresh- to seawater, the Na^+/K^+ ATPase in the gills is upregulated within 3 hours, allowing for a rapid increase in NaCl secretion by the ionocytes (Mancera & McCormick 2000). The chloride cell-rich opercular membrane of the mummichog also rapidly enhances NaCl secretion when exposed to a hypertonic shock (Zadunaisky et al. 1995). Hypotonic exposure of mudskippers (*Periophthalmus modestus*; Sakamoto et al. 2000) and killifish (*Fundulus heteroclitus*; Daborn et al. 2001), on the other hand, results in the retraction of gill ionocytes from the surface (i.e. the chloride cells close/contract the opening to their apical crypt). This response occurs within an hour, which provides almost immediate protection against ion loss. The contraction is facilitated by a ring of actin in the apex of chloride cells, although the signalling/triggering mechanism(s) has/have not been described. In addition to these dynamic physiological responses, intertidal fishes have a low gill, skin and intestinal permeability to water, which reduces the overall costs of osmoregulation (Schulte 2011, Marshall 2012).

High intertidal animals (both osmoconformers and -regulators) may also use a hypometabolic response (i.e. a limited form of metabolic depression, or behavioural quiescence) to help tolerate hyposalinity exposure. In seawater-acclimated upper tidal black prickleback (*Xiphister atropurpureus*), O_2 consumption ($\dot{M}O_2$) dropped by twofold and opercular beating stopped almost entirely, after transfer to a salinity of 6‰, whereas these parameters changed little in the low intertidal/subtidal penpoint gunnel (*Apodichthys flavidus*) from the same local environment and with a similar gross morphology (Haynes et al. 2009). By reducing breathing rate and water flow over the gills, the black prickleback is potentially minimizing ion loss and water gain. A decline in heart rate (bradycardia), and sometimes episodes of no cardiac activity (acardia), have also been observed in the high intertidal limpets *Cellana grata* and *Siphonaria capensis* when submerged in fresh water (Chelazzi et al. 2001), diluted seawater (20‰) (Marshall & McQuaid 1993), or exposed to simulated monsoonal rainfall (Morritt et al. 2007). This strongly suggests a degree of metabolic depression; although the cardiac response in *Cellana grata* was not seen in a later study (Williams et al. 2011) and the local low intertidal species *Cellana toreuma* and *Patella granularis* displayed bradycardia and acardia as well (Marshall & McQuaid 1993, Chelazzi et al. 2001). In the upper intertidal flatworm *Macrostomum lignano*, respiration rate decreases by ~50% during low salinity exposure (transfer from 35‰ to 5‰), and this is associated with a ~75% reduction in activity levels (Rivera-Ingraham et al. 2016). Hypersalinity exposure (transfer from 35‰ to 55‰), on the other hand, was associated with an increase in respiration and mitochondrial density, and the upregulation of antioxidant genes such as superoxide dismutase. Similarly, the mangrove rivulus (*Kryptolebias marmoratus*) increases its metabolic rate during acute hypersalinity exposure, as opposed to its hypometabolic response to hyposalinity stress (Sutton et al. 2018). These findings highlight that, in contrast to tolerance to low salinity, tolerance to high salinity comes with an energetic cost, and that it potentially induces oxidative stress which the animal may need to invoke protective measures against. The concept of salinity-induced oxidative stress and the corresponding antioxidant defences may also apply to other intertidal/estuarine animals, given that they are all frequently subject to salinity stress (Rivera-Ingraham & Lignot 2017).

Coping with nitrogenous waste accumulation

The catabolism of amino acids releases ammonia (NH_3), which has deleterious effects on many cellular processes including ion regulation (Ip & Chew 2018). Typically, marine animals are ammoniotelic and excrete NH_3 directly into the seawater due to its high solubility in this medium (Table 1). Animals with gills usually excrete NH_3 at this interface, because of its large surface area and the large volume of seawater that runs over it. However, during emersion, this mode of NH_3 excretion is interrupted (e.g. as seen in the mangrove rivulus, Livingston et al. 2018), potentially resulting in a build-up of NH_3 in the body. Nevertheless, high intertidal animals typically do not accumulate high systemic levels of NH_3, and the clingfish *Sicyases sanguineus* can even avoid NH_3 build-up while being air-exposed for over a day (Gordon et al. 1970). Because the risk of nitrogenous waste accumulation is related to air exposure, it may not be a major stressor for animals in estuaries or tidepools that remain immersed throughout the tidal cycle. Thus, this section focuses on animals in high intertidal habitats (i.e. rocky and sandy/muddy shores) that are typically emerged during low tide (Figure 3).

The adaptations that allow high intertidal (amphibious) fishes to cope with NH_3 accumulation and excretion during emersion have recently been reviewed (Chew & Ip 2014, Wright & Turko 2016, Ip & Chew 2018), and detailed information specifically for mudskippers is also available in Clayton (1993) and Jaafar & Murdy (2017); therefore, these adaptations are only briefly summarized here. Three major strategies can be identified that avoid NH_3 accumulation: (1) a reduction in NH_3 production by reducing proteolysis and amino acid catabolism; (2) active excretion of NH_3 or ammonium (NH_4^+) by the gills, skin and gut; and (3) NH_3 detoxification by conversion into glutamine and, to a lesser extent, urea (Table 2). When excreting NH_3 across a non-branchial surface, some species [e.g. the mangrove rivulus *Kryptolebias marmoratus* (Frick & Wright 2002) and common blenny *Blennius pholis* (Davenport & Sayer 1986)] are able to release NH_3 as a gas (volatilization). In *Kryptolebias marmoratus*, this can account for over 40% of total ammonia secretion, which is likely promoted/facilitated by the increase in NH_4^+ concentration and the pH (alkalinization) on its cutaneous surfaces upon emersion, and by ammonia-transporting Rhesus glycoproteins (Rhcg1 and Rhcg2) detected in the skin that are normally absent in fully aquatic fish (Litwiller et al. 2006, Cooper et al. 2013, Livingston et al. 2018). It should be noted that this mechanism of NH_3 volatilization is not limited to intertidal fish; as it has also been described in freshwater amphibious fishes (Livingston et al. 2018). Urea synthesis (ureotely) is not a major detoxifying mechanism among intertidal fishes (as compared to lungfishes), and few species possess a complete and functional ornithine-urea cycle (i.e. the enzymes needed to convert NH_3 into urea in the liver, and at sufficient levels of activity) (Chew & Ip 2014, Wright & Turko 2016, Ip & Chew 2018, Livingston et al. 2018). This is likely because it is an energetically expensive process, as a total of 5 mol of ATP are hydrolysed for each mol of urea synthesized. Indeed, tidepool sculpins (*Oligocottus maculosus*) only secrete 8%–17% of their nitrogenous waste as urea (Wright & Wood 1995). In addition to these preventative strategies against NH_3 build-up, intertidal fishes can have a high tolerance to elevated NH_3 levels in the brain, an organ that is particularly sensitive to NH_3 toxicity (Chew & Ip 2014, Ip & Chew 2018).

The high intertidal mudskipper *Periophthalmodon schlosseri* has adaptations that illustrate many of the above strategies, that are often not shared by mudskipper species inhabiting lower shore heights. During emersion, it reduces protein and amino acid catabolism (or it only partially catabolizes amino acids, forming alanine) and mitochondria-rich branchial cells actively excrete NH_3 against the concentration gradient (Wilson et al. 2000, Lim et al. 2001, Randall et al. 2004).

The modified gills of *Periophthalmodon schlosseri* have branched filaments and fused lamellae, which trap water during emersion allowing NH_3 excretion to continue, whereas the gills of the lower tidal relative *Boleophthalmus boddaerti* are comparable to those of aquatic fishes and are not able to hold water (Ip & Chew 2018). This trapped water could also reduce the risk of desiccation of the gills during air exposure, and hence, can be regarded as a multifunctional adaptation (Lam et al. 2006). Furthermore, *Periophthalmodon schlosseri* excretes more NH_3 than *Boleophthalmus boddaerti* once re-immersed (Chew et al. 2003). This may be explained, on a molecular level, by the fact that an important gill NH_3 transporter (Rhcg1) in two species of high intertidal mudskippers (*Periophthalmodon schlosseri* and *P. magnuspinnatus*) contains three more hydrophobic amino acid residues at the central pore, than in their low intertidal relative *Boleophthalmus pectinirostris* (You et al. 2014, 2018). Such an adaptation might allow for more effective NH_3 excretion. Finally, *Periophthalmodon schlosseri* can excrete 26% of its nitrogenous waste as urea (Ip et al. 2004b), and both high and low intertidal species of mudskippers (*Periophthalmodon schlosseri* and *Boleophthalmus boddaerti*) can tolerate high levels of NH_3 in the brain (>14 µmol/g whereas mammalian brains rarely tolerate >1 µmol/g) and are able to detoxify NH_3 into glutamate in this tissue (Ip et al. 2005, Lam et al. 2006).

Intertidal invertebrates vary in which mechanism(s) they utilize to avoid emersion-related nitrogenous waste accumulation, although, some mechanisms are similar to those observed in fishes. High intertidal *Anthopleura elegantissima* anemones that are exposed to air for up to 18 hours/day, do not excrete NH_3 at a higher rate during immersion than low intertidal individuals (Zamer 1986, Zamer & Shick 1987). However, they appear to use metabolic depression during emersion, which would not only reduce energy expenditure, but also NH_3 production. It is also possible that anemones recycle NH_3 by passing it to their endosymbiotic algae, reducing the requirement for exogenous nitrogen uptake by these endosymbionts (Shick 1991). The high intertidal snail *Littorina saxatilis* has a higher nitrogenous excretion rate in seawater, and excretes proportionally more nitrogen as urea as compared to its lower intertidal relative *Littorina obtusata* (Aldridge et al. 1995). Finally, the littoral fringe beachflea *Orchestia gammarellus* retains exosomatic water during emersion, which allows NH_4^+ excretion from the gills to continue unimpeded (Spicer & Taylor 1994b).

Among other intertidal crustaceans, some isopod species (*Alloniscus perconvexus*, *Tylos punctatus* and *Ligia occidentalis*) accumulate glutamine as a non-toxic intermediary nitrogen store, whereas *A. perconvexus* and *T. punctatus* can also release considerable amounts of NH_3 into the air by volatilization (Nakamura & Wright 2013). Although all three isopod species still excrete NH_3 directly into the seawater during high tide, similar to their subtidal relatives, these adaptations are key innovations that are also seen in terrestrial isopods (Wright & Pena-Peralta 2005). Glutaminase catalyses the release of NH_3 from glutamine, which is needed to create a high concentration of NH_3 for volatilization. In intertidal isopods, glutaminase activity is half that of terrestrial species, which reflects their dual mode of nitrogen excretion (Nakamura & Wright 2013). The intertidal crab *Carcinus maenas* can also detoxify NH_3 by accumulating glutamine in the muscle during air exposure (Durand & Regnault 1998, Durand et al. 1999). This allows *Carcinus maenas* to avoid high NH_3 levels in the haemolymph (below or equal to 0.1 mmol/L) for at least 72 hours of emersion. This is in contrast to the subtidal crab *Necora puber* from the same local environment, which continuously accumulated NH_3 (up to 10.4 mmol/L) during 18 hours of emersion because it is unable to detoxify NH_3 into glutamine.

Protection against UV radiation

All marine animals that inhabit shallow waters are exposed to ultraviolet (UV) radiation, so this environmental stressor is not unique to the high intertidal zone (Shick & Dunlap 2002). However, compared to the subtidal zone, the intertidal zone experiences more UV radiation – with the high intertidal zone at the extreme end (Peterson 1991). This is because the lower water depth and longer duration of air exposure result in less UV radiation being filtered out by seawater (i.e. there is less attenuation of short wavelength radiation) (Table 1 and Figure 2) (Raffaelli & Hawkins 1999, Rawlings 1999, Denny & Gaines 2007, Richards 2011); although, field measurements of the UV radiation intensity (in mW/m^2) across the vertical intertidal gradient are still lacking. Nonetheless, it is apparent from on-site data that intertidal animals need to cope with large variations in UV radiation intensity throughout the day, on a seasonal basis, and with changes in cloud cover (Carrasco-Malio et al. 2014, Pulgar et al. 2015, 2017). UV radiation is harmful because UV-B rays (~280–320 nm) are absorbed by pyrimidine bases and cause DNA mutations. Further, the photoreactivity of UV-A (~320–400 nm) leads to the formation of reactive oxygen species (ROS), which in turn damage macromolecules (Cockell & Knowland 1999, Shick & Dunlap 2002, Carrasco-Malio et al. 2014). In a few intertidal animals, the harmful effects of UV have been illustrated, in particular in vulnerable early life-stages (e.g. in mollusc embryos/larvae/juveniles when the UV-protective shell is still absent or translucent/thin). For instance, in the intertidal gastropods *Nucella emarginata* (Rawlings 1996), *Siphonaria denticulata* and *Bembicium nanum* (Przeslawski et al. 2005), UV exposure of embryos was reported to cause substantial mortality, especially in combination with high temperature and salinity conditions (in the case of the latter two species).

There are various DNA repair mechanisms, and damaged proteins/lipids can be synthesized anew (Cockell & Knowland 1999). However, this requires energy, which is observed, for example, in *Girella laevifrons*. This is a fish species that occupies tidepools in the high intertidal zone during its 2-year juvenile stage (after which it migrates to the subtidal zone for reproduction) (Pulgar et al. 2005). Exposure to UV radiation at an intensity and time period similar to that experienced in its natural habitat, was shown to increase metabolic rate ($\dot{M}O_2$) and reduce growth (weight gain) (García-Huidobro et al. 2017, Pulgar et al. 2017). Therefore, a more energetically efficient strategy is to avoid or protect against UV radiation (Table 2) (Cockell & Knowland 1999, Shick & Dunlap 2002). Intertidal animals minimize UV radiation exposure by using several behaviours. These include reducing the amount of exposed external surfaces and seeking shelter; which are the same behavioural responses used to avoid desiccation, and heat and salinity stress (discussed above). For instance, peak levels of UV irradiation cause contraction of the anemone *Anthopleura elegantissima* (with and without endosymbiotic algae) even when it is continuously immersed, and it also attaches debris to its column (Shick & Dykens 1984). The high tidepool fish *Girella laevifrons* actively seeks shelter underneath rocks when experimentally exposed to UV radiation (Pulgar et al. 2017). Embryos of the snail *Nucella emarginata* are encased in a thick egg capsule. This capsule functions as a form of shield or shelter, and blocks out a substantial amount of UV radiation (>95% of UV-B and >45% of UV-A) and this improves survival during UV exposure; although the properties of the capsule that block UV radiation remain unclear (Rawlings 1996).

Adaptations that are particularly/more unique to this type of stressor include the use of: (1) ‘sunscreens’ (UV-absorptive pigments) such as melanin and mycosporine-like amino acids (MAAs) in the skin and mucus; and (2) antioxidants such as carotenoids, ascorbic acid, superoxide dismutase (SOD) and catalase that quench and deactivate ROS (Cockell & Knowland 1999, Shick & Dunlap 2002). These adaptations are very widespread across phylogenetic groups and climates. For example, MAAs are found in temperate Pacific intertidal *Anthopleura* anemones (Shick et al. 2002, Stochaj et al. 1994) and numerous Antarctic intertidal species of annelids, molluscs and crustaceans (Karentz et al. 1991). Though, these UV-absorptive compounds are not exclusive to intertidal marine animals and are also synthesized by subtidal animals such as corals. In the intertidal *Anthopleura elegantissima*, SOD and catalase activity are disproportionally high in the external column tissue, which is exposed to the most sunlight during contraction (Dykens & Shick 1984). The endosymbiotic algae in *Anthopleura elegantissima* could also induce oxidative stress. However, there are only a few algae in the column, which suggests that the higher antioxidant activities are an adaptation to protect against oxidative stress from UV exposure rather than from photosynthesis.

The upper tidepool fish *Girella laevifrons* also actively increases catalase and SOD enzyme activities upon UV exposure (Carrasco-Malio et al. 2014).

In a few cases, high intertidal animals have been specifically shown to exhibit greater defences against UV radiation than their low intertidal relatives. Upper intertidal sculpin species have higher mucus MAA concentrations than lower intertidal species (Zamzow 2003). In *Mytilus californianus*, individuals on the high edge of the mussel bed accumulate more carotenoids in their gonadal tissues than those on the low edge of beds (Petes et al. 2008), possibly to protect their gametes or vulnerable early developmental stages against UV radiation once they are released into the water column and no longer sheltered by the parent shell. SOD activity levels in the digestive gland and gills were also higher in middle/high versus low intertidal *Mytilus edulis* (Letendre et al. 2008); although in this case, it is doubtful that this trend in oxidative stress defence is explicitly associated with UV exposure. In adult mussels, UV rays are unlikely to reach these internal tissues (thus, a different abiotic stressor may be driving this response).

Intertidal animals, in general, also appear to enhance their protection against UV radiation compared to subtidal animals. For instance, in the Antarctic limpet *Nacella concinna*, transcriptome analysis (i.e. contrasting gene expression in inter- versus subtidal individuals) showed that the intertidal ecotype upregulated genes for DNA repair enzymes (such as DNA ligase 1), antioxidants (including SOD and others) and the pentose shunt enzyme 6-phosphogluconate dehydrogenase (PGD) (Clark et al. 2018). PGD generates NADH, which can be used as an energy donor by antioxidant enzymes to combat ROS formation. Interestingly, after transplantation to the subtidal zone for 1 month, and even after 9 months of submergence in the laboratory, the intertidal limpets maintained a higher expression of most antioxidant genes. This underlines the importance of this protective mechanism in allowing limpets to inhabit the intertidal zone. DNA methylation, a form of epigenetic regulation which is often linked to reduced gene expression (Nelson & Cox 2000, Bossdorf et al. 2008), is at least partially responsible for creating the distinct, and generally permanent, gene expression profile of intertidal limpets (Clark et al. 2018). The methylation of cytosine residues (5-methylcytosine) was strongly associated with ecotype shore height, and while this pattern was largely unchanged by reciprocal transplantation, it disappeared after laboratory submergence. This phenotype reversal indicates that laboratory conditions can be very different from the environment experienced by limpets in the field.

Protection against wave action or ice disturbance

Animals in the intertidal zone experience considerably more wave action than those in the subtidal zone, because of the breaking waves that are formed at the sea-to-land transition. The severity of wave action varies greatly among intertidal habitats (Figure 3). For instance, the shores of isolated oceanic islands are usually exposed to heavy waves, as winds can build up over large distances, whereas in sheltered areas such as estuaries and fjords, wave exposure can be minimal (Raffaelli & Hawkins 1999). For the inhabitants of the high intertidal zone, wave action is not necessarily stressful and can be beneficial, as it helps with the supply of oxygen and nutrients, and in warmer climates the wave spray offers some cooling. On wave-exposed rocky shores certain predators (e.g. green crab, *Carcinus maenas*) may also be less abundant, so particular species (e.g. dog whelk, *Nucella lapillus*) may experience a lower predation risk (e.g. see Kitching et al. 1966, Hughes & Elner 1979, Gibbs 1993). However, for many sessile and slow-moving animals, being swept away from the substrate by waves has severe consequences; for example, by increasing vulnerability to subtidal predation or causing physical damage (Denny & Gaines 2007). In the rocky intertidal, wave exposure is typically the most substantial, especially when smaller rocks are ‘thrown’ against the shore by waves, and act as projectiles that can lead to mortality of barnacles and limpets (Shanks & Wright 1986). In colder climates, brash ice also mechanically disturbs the animals in the intertidal zone.

To avoid being swept away by waves, intertidal animals use several strategies to strongly adhere to the substrate: for example, by secreting cement (barnacles) or byssus threads (mussels) (Denny & Gaines 2007) or using suction (snails, clingfishes) (Table 2) (Ebeling et al. 1970, Branch 1985). The clingfish *Sicyases sanguineus*, which inhabits heavily wave-exposed high intertidal areas, attaches itself to vertical rock surfaces with a ventral sucking disc ('sucker') formed by modified pelvic fins (Ebeling et al. 1970). Among two ecotypes of the snail *Trophon geversianus* found on rocky shores in Patagonia, the intertidal ecotype has an expanded aperture and lower-spired shell compared to the subtidal ecotype. This morphology allows for better attachment to the substrate because it accommodates a larger foot and reduces wave drag (Márquez et al. 2015). Similar plastic changes in shell morphology (i.e. larger aperture and lighter shell construction) have been described in the Atlantic dogwinkle *Nucella lapillus* in response to wave action based on comparisons of individuals from wave-exposed and sheltered shores (see Kitching et al. 1966, Hughes & Elner 1979, Gibbs 1993 and references below). However, as wave exposure and predation pressure, which is also known to directly or indirectly affect shell shape and thickness in *Nucella lapillus*, can interact, it can be challenging to tease apart their individual effects (e.g. Bourdeau 2010, Pascoal et al. 2012). In Antarctica, the intertidal ecotype of the limpet *Nacella concinna* has a stronger/thicker shell compared to the subtidal ecotype, and this provides better protection against mechanical stress from ice (Clark et al. 2018). This adaptation is associated with higher constitutive expression levels of genes putatively involved in the formation of the shell matrix (thrombospondin- and proline-rich proteins) (Clark et al. 2018, Marin 2020). Interestingly, some limpet species have evolved rhythms of activity as a behavioural adaptation that works in concert with strong physical adhesion. This ensures that they are not off their 'scars' (i.e. homing site on the rock where the shell fits tightly into the substrate) when wave action can dislodge them (Branch 1985). This behaviour is seen, for instance, in the high intertidal *Siphonaria capensis* from South Africa which only feeds at low tide (Branch & Cherry 1985).

It is important to note that among intertidal animals, those in the upper zone are on average exposed to a lower frequency and force of wave action/ice disturbance than in the lower zone (e.g. see McQuaid 1981). This is simply because of the shorter contact time with the sea and the larger distance from the strongest breaking waves. Thus, this environmental stressor can result in the opposite vertical intertidal gradient in morphological and physiological adaptations as compared to the previously discussed stressors. For instance, small-sized chiton species (e.g. *Acanthopleura echinata*) have been found to be more abundant in the low intertidal than larger-sized species (e.g. *Chiton barnesi*) (Otaíza & Santelices 1985), and this pattern might be explained by the lower wave drag associated with a small size. Similarly, the wave-exposed low intertidal ecotype of the periwinkle *Littorina saxatilis* – also called the 'wave type' – has a smaller shell than the high intertidal ecotype (Johannesson 2016). Finally, the *L. saxatilis* wave type has a larger aperture along with a 1.4-fold larger foot muscle, and this helps it to avoid being dislodged by the waves (Carvajal-Rodríguez et al. 2005, Martínez-Fernández et al. 2008). Proteomic analysis of whole-animal tissue homogenates has also revealed that the wave type expresses ~3-fold higher levels of the glycolytic enzyme fructose-bisphosphate aldolase, and ~73-fold higher levels of arginine kinase which catalyses the transfer of phosphate to ADP from phosphoarginine (Martínez-Fernández et al. 2008). This allows the low intertidal ecotype to enhance ATP supply to support the energy demands of withstanding wave action.

Adaptations to H_2S toxicity

In sandy/muddy intertidal habitats such as mangroves, mudflats and salt marches, animals are exposed to the toxic gas hydrogen sulphide (H_2S), which is produced by sulphate-reducing bacteria in anoxic regions of the sediment (Figure 3) (Schulte 2011, Dufour 2018). This anoxic region is the result of oxygen consumption by bacterial decomposers in the upper sediment layer, and the subsequent use of sulphate as an alternative chemical substrate for O_2 leads to the accumulation of H_2S (Dufour 2018). Animals in muddy intertidal environments are, in particular, exposed to H_2S as O_2 diffusion into fine sediment is more limited than in coarse substrate (i.e. the anaerobic layer starts at less than 1 cm below the surface whereas in sandy shores it begins at a considerably deeper depth). H_2S is toxic to animals because it binds to the ferric haem site of the cytochrome *c* oxidase 3 subunit (COX3). This prevents O_2 from binding to COX3, inhibiting aerobic respiration and blocking the production of ATP through the electron transport chain (Ip et al. 2004a, Tobler et al. 2016, Cochrane et al. 2019). The binding is reversible, but more potent than that of cyanide, as 50% inhibition occurs at H_2S concentrations as low as 1 µmol/L. Hydrogen sulphide also binds to the haem groups of haemoglobin to form sulphhaemoglobin (SHb), which inhibits the binding and transport of O_2. Muddy/sandy intertidal zones are certainly not the only H_2S-rich marine environments (e.g. cold seeps and hydrothermal vents also exhibit high H_2S levels) (Dubilier et al. 2008, Tobler et al. 2016). However, H_2S concentrations in intertidal environments can show diel fluctuations (i.e. low during the day, high at night; Hagerman 1998, Lam et al. 2006), and the exposure of intertidal animals to H_2S can be linked to tidal movements. For instance, the intertidal mudskipper *Boleophthalmus boddaerti* is actively emerged on mudflats at low tide, but during high tide it seeks shelter in burrows in the sediment (to avoid aquatic/terrestrial predation), where it can be exposed to up to 0.3 mmol/L of H_2S (Ip et al. 2004a). Other intertidal animals, such as the mudflat worm *Urechis unicinctus*, permanently reside in burrows and are constantly exposed to high levels of H_2S (Liu et al. 2015).

Among fish, intertidal species generally exhibit higher H_2S tolerance than those which inhabit the open ocean (Lam et al. 2006) and some persist in environments with extremely high H_2S levels (>1.1 mmol/L) (Rossi et al. 2019). Because H_2S is concentrated in the sediment, fishes that live on mudflats and among mangroves avoid exposure to high H_2S levels by remaining at the top layer of the sediment or at the water surface, or by exiting the water altogether (e.g. a behaviour displayed by the mangrove rivulus *Kryptolebias marmoratus* and mangrove molly *Poecilia orri*) (Table 2) (Schulte 2011, Taylor 2012, Cochrane et al. 2019, Rossi et al. 2019). In the mangrove rivulus, the outer epithelial skin layer may also act as a barrier that limits the entry of H_2S into the body (Martin & Currie 2020). When behavioural avoidance or emersion is not possible, and harmful amounts of H_2S diffuse into internal tissues, an alternative coping strategy for intertidal animals is to switch to anaerobic respiration, which is independent of COX3 function. However, this does not appear to be a major strategy among animals that inhabit H_2S-rich environments, possibly because of the low sustainability of anaerobiosis. For instance, the mudskipper *Boleophthalmus boddaerti* does not accumulate lactate during H_2S exposure (Ip et al. 2004a), and the expression of glycolytic genes is low in the intertidal mudflat worm *Urechis unicinctus* which also inhabits H_2S-rich burrows (Liu et al. 2015). A more common adaptation is to enzymatically detoxify H_2S by oxidation. In *Urechis unicinctus* (Liu et al. 2015) and *Boleophthalmus boddaerti* (Ip et al. 2004a), the mRNA expression and activity of detoxifying mitochondrial enzymes increases upon H_2S exposure. These enzymes include sulfur transferase (ST) and sulphide-quinone oxidoreductase (SQR), which convert H_2S into thiosulphate ($S_2O_3^{2-}$) and other harmless oxidized compounds. The sand clam (*Donax serra*) primarily detoxifies H_2S into $S_2O_3^{2-}$, and this mechanism appears to be very effective during acute H_2S exposure as H_2S itself was not detected in the tissues (Laudien et al. 2002). In the saltmarsh resident *Fundulus parvipinnis*, erythrocyte mitochondrial H_2S oxidation also plays an important role, and results in lower SHb formation (Lam et al. 2006). Although, it is important to note that H_2S detoxification by oxidation can only occur in the presence of sufficient O_2. This is why the H_2S tolerance of animals can be reduced when combined with anoxia/hypoxia (e.g. in the green crab *Carcinus maenas*, see Hagerman 1998), which is relevant given that these two environmental stressors often co-occur.

In contrast to avoiding or detoxifying H_2S, many intertidal invertebrates are able to take advantage of H_2S exposure. For example, *Urechis unicinctus* is thought to use a super-complex composed of SQR and electron transport chain complexes III and IV (which are also upregulated during H_2S exposure) to catalyse the production of ATP by using H_2S as an electron donor instead of NADH and $FADH_2$ (Liu et al. 2015). The oxidation of H_2S releases electrons that can be transferred into the oxidative phosphorylation pathway (Tobler et al. 2016). A few sulphide-tolerant intertidal animals such as the ribbed mussel *Geukensia demissa* (Doeller et al. 2001) and the lugworm *Arenicola marina* (Völkel & Grieshaber 1997) indeed appear to use sulphide to produce ATP, which is a remarkable form of chemolithoheterotrophy. Unlike most animals that solely produce ATP from organic molecules such as glucose, these particular invertebrates are able to obtain energy from the oxidation of inorganic compounds (although they still require organic molecules as a carbon source). However, far more common are intertidal animals (nematodes, annelids and bivalves) with chemosymbiotic relationships, where H_2S is detoxified by ecto- or endosymbiotic H_2S-oxidizing chemosynthetic/chemolithoautotrophic bacteria. These bacterial symbionts can occupy the gut lumen (in nematodes), or are associated with the gills (in clams), for instance, and are able to convert carbon dioxide (CO_2) into organic carbon-molecules using the energy from H_2S oxidation. This type of symbiosis does not only allow for H_2S detoxification, but the bacteria are also an essential food source for the intertidal animals. See reviews by Taylor & Glover (2000), Dubilier et al. (2008), Taylor et al. (2011) and Dufour (2018) for detailed information on H_2S-oxidizing chemosymbiotic invertebrates (such as lucinid bivalves) in the intertidal zone, as well as in other marine ecosystems (e.g. the deep sea).

Concluding remarks

The high intertidal zone is a unique marine environment, because many of its animal inhabitants need to be highly adapted to living in two worlds: the marine and the terrestrial environment. As a result of the typical daily transition between immersion in seawater and emersion in air (which have very different properties, see Table 1), and given that the duration of emersion is often greater than that of immersion, these animals are exposed to severe/extreme physicochemical conditions (Figures 1 and 2). Which specific abiotic stressors are encountered depends on the type of intertidal habitat (e.g. the climate, substrate, extent of freshwater input), although high intertidal habitats have many challenges in common (Figure 3). This review has provided a comprehensive overview of the various adaptations and responses of marine animals that help them deal with the many stressors characteristic of the high intertidal zone: desiccation, thermal and osmotic stress, hypoxia, nitrogenous waste accumulation, UV exposure, wave/ice disturbance and H_2S toxicity (Table 2). For each specific stressor, these adaptations can be further categorized according to the strategy utilized to deal with them. Often, these strategies first involve behaviours used to avoid the stressor, followed by adaptations or responses that improve the physiological tolerance of the animal to the stressor. Adaptations and plastic phenotypic responses can be related to changes in animal behaviour, morphology, physiology or biochemistry, but frequently several levels of biological organization are integrated into the animal's coping mechanism(s). For instance, the release of vaso- and isotocin from the neurohypophysis in intertidal mudskippers triggers their retreat into burrows to avoid desiccation (i.e. this adaptation is behavioural with a physiological driver) (Katayama et al. 2018), and the higher constitutive gene expression for shell matrix proteins in intertidal limpets strengthens the shell as a defence against wave and ice disturbance (i.e. it is a biochemical change that underlies this morphological trait) (Clark et al. 2018). Interestingly, an adaptation/response to a particular environmental stressor can have various effects (synergistic, additive, antagonistic or potentiating) with respect to the animal's ability to respond to or tolerate another stressor, and when the stressors co-occur, animals need to find a balance between adaptive strategies. An example of a trade-off (antagonistic effect) is gaping in intertidal bivalves. This behavioural strategy allows for evaporative cooling during heat stress and aerial O_2 uptake to avoid hypoxemia during emersion; however, this comes with the risk of desiccation, and this is likely why this behaviour is only sparsely used by some high intertidal mussels (Gleason et al. 2017). The opposite situation is when adaptations allow for cross-tolerance, which appears to be more common (see the 'Adaptations allowing for cross-tolerance' section below).

Another conclusion that can be drawn from this review is that many adaptations are shared among diverse animal groups. For example, high intertidal sculpins (Speers-Roesch et al. 2013), crabs (Viña et al. 2018) and periwinkles (Sokolova & Pörtner 2001a) improve their survival during diel hypoxia (i.e. aquatic hypoxia or internal hypoxia due to air exposure) by enhancing their anaerobic capacity and by increasing fermentable energy reserves. In addition, increased (gene expression/activity) levels of antioxidants to protect against reactive oxygen species (ROS), which are generated by UV radiation, are used by high intertidal mussels (Letendre et al. 2008, Petes et al. 2008) as well as intertidal limpets (Clark et al. 2018). The examples provided in Table 2 illustrate the types of adaptations that occur in multiple taxa. It is striking that these adaptations are often universal among animals that are very different in their mobility (sessile or mobile) or phylogeny (invertebrate or vertebrate), and perhaps they can be considered as examples of convergent evolution/phenotypic convergence. Finally, this review shows that several adaptations are phenotypically plastic (i.e. gained during the animal's lifetime through exposure to the high intertidal environment), and sometimes can be reversible. Examples of phenotypic plasticity are common among higher and lower intertidal mollusc ecotypes. Plastic responses may be energetically expensive for an animal (Wright & Turko 2016), but the advantage to a species as a whole is that they provide flexibility with regard to where an individual settles across the vertical range of the intertidal zone. On the other hand, many high intertidal animals have evolved adaptations that are permanent and embedded in their genome (e.g. non-synonymous amino acid substitutions that alter the function of a protein), and these 'fixed' changes may allow them to be more optimally adapted to cope with the specific challenges of the upper tidal environment.

Adaptations allowing for cross-tolerance

In this review we have identified several adaptations that appear to confer cross-tolerance to other abiotic stressors (Sinclair et al. 2013). The following is a list of exemplar adaptations. (1) In intertidal snails (*Lottia* and *Nucella* limpets) and crabs (*Petrolisthes* species), adaptations that improve air breathing ability (i.e. capacity for O_2 uptake from the air and the maintenance of aerobic metabolism) allow for a greater tolerance to emersion-induced hypoxemia as well as high temperatures. These data strongly suggest that heat tolerance is O_2 limited in these animals. This hypothesis is consistent with the widely known, but also controversial, Oxygen and Capacity Limited Thermal Tolerance concept (see Pörtner et al. 2017, Jutfelt et al. 2018) which provides a theoretical framework that relates O_2 consumption and aerobic scope to the thermal tolerance of aquatic animals. (2) In fishes (tidepool sculpins), a heat-induced heat shock protein (Hsp) response can improve tolerance against subsequent osmotic or hypoxic stress. (3) The trend towards high-spired shells in high-shore limpets has the benefit of reducing water loss in air. However, this morphological adaptation also potentially avoids heat stress by reducing conductive heat gain and increasing convective heat loss. (4) Organic compatible osmolytes are essential for maintaining osmotic balance and avoiding excess cellular water loss/gain in intertidal osmoconforming invertebrates (anemones, worms, molluscs) during salinity fluctuations, but also appear to have an important role in freeze tolerance. (5) In some high intertidal fish (mudskippers) and crustaceans (amphipods and crabs), water trapping in the gills allows both gas exchange and NH_4^+ secretion to continue while emerged. (6) The behaviour of seeking refuge underneath some form of cover and reducing exposed surfaces during emersion is truly multifunctional, as it helps a diversity of intertidal animals to avoid exposure to various environmental stressors including desiccation, heat exposure, salinity fluctuations and UV radiation. (7) Anaerobiosis, and/or the use of varying degrees of metabolic depression, are strategies that improve hypoxia survival in all major intertidal animal groups, but have also been shown to contribute to the heat and freeze tolerance of intertidal molluscs, and to the tolerance of worms, snails and fishes to low salinity. (8) Finally, in intertidal and estuarine fishes (e.g. killifish, mudskippers), the water permeability of the skin, gills and intestines is lower, which helps protect against desiccation during air exposure, and minimizes the costs of osmoregulation during salinity stress.

More adaptations allowing for cross-tolerance are likely to exist in high intertidal animals, given that this review only covers a selection of challenges that animals need to respond and/or adapt to in their environment, and further examples of cross-tolerance may be uncovered by future research. The existence of 'multi-stressor'-related, multifunctional, adaptations is not at all surprising, as it reflects the wide range of environmental stressors that high intertidal animals are exposed to, often simultaneously (Figure 2). For instance, on rocky shores in warmer climates, desiccation stress is typically encountered along with heat stress, whereas in soft substrate environments, hypoxia is frequently associated with high H_2S levels. Although it is important for our understanding of adaptions and phenotypic plasticity that we isolate individual stressors in experimental studies, these stressors inevitably co-occur in nature. This is why it is key to also perform 'multi-stressor' studies in a laboratory setting (e.g. see Nancollas 2020 and below).

Perspectives

Benefits of life in the high intertidal zone

The inevitable question that comes up after discussing the multitude of challenges in the high intertidal zone, is why this ecological region is inhabited by such a great diversity of marine animals (why are these animals going through all the trouble?). However, the high intertidal zone has some important biotic and abiotic advantages over lower coastal areas. For some animals, the upper tidal zone allows for an escape from low intertidal/subtidal predators that are unable to survive in this environment. Although these animals may then be exposed to terrestrial predators (e.g. birds), it seems that the 'risk of being eaten' is less than in lower intertidal areas. For instance, the common killifish *Fundulus heteroclitus* has higher survival rates in the intertidal compared to the subtidal zone (Banikas & Thompson 2012), and the subtidal ecotype of the snail *Trophon geversianus* suffers higher predation pressure from crabs than the intertidal ecotype (Márquez et al. 2015). Periods of avian predation on intertidal sandy/muddy shores are also usually brief, coinciding with migration, so this may only temporarily affect intertidal animals (Peterson 1991). The high intertidal zone, specifically, offers protection to *Mytilus* mussels and acorn barnacles from low intertidal predatory *Pisaster* seastars that are unable to extend beyond the mid-intertidal (Denny & Gaines 2007, Harley 2011). In the case of the snail *Littorina sitkana* from rocky shore habitats in British Columbia, Canada, predation by the pile perch *Rhacochilus vacca* is also virtually absent at high tidal levels, but considerable at low tidal levels (McCormack 1982). In many species, juveniles find a refuge in the high intertidal, as adult counterparts dominate the low areas with more favourable conditions. For example, recruits of the sculpin *Oligocottus snyderi* use suboptimal shallow tidepools that are more common in the high intertidal zone to avoid the aggression and cannibalism by adults that dominate the more favourable deeper tidepools in the low intertidal zone (Ritter 2017).

In addition, intertidal fishes may take advantage of the relatively high O_2 level in air (Table 1) which benefits the development of their eggs (Sayer 2005, Martin 2014). In air, eggs may also be incubated at a higher temperature (which speeds up embryo development), and they are protected from aquatic predators (Martin et al. 2004). For example, the common killifish (*Fundulus heteroclitus*) purposefully lays its eggs at the upper limit of the seawater during spring tide (i.e. the highest high tide of the tidal cycle), which leaves them on the beach exposed to the air for the rest of the tidal cycle (Martin et al. 2004, Schulte 2011). There are also several subtidal fishes (e.g. plainfin midshipman, *Porichthys notatus*; sharpnose sculpin, *Clinocottus acuticeps*; white stickleback, *Gasterosteus* sp.) that enter the high intertidal zone solely to construct nests, spawn and, in some cases, provide parental care (e.g. guarder males), in order to take advantage of its several benefits (Martin et al. 2004, Bose et al. 2019).

Mudskippers that spend the majority of their life emerged, and are capable of air breathing, take full advantage of the O_2-rich air. Although there are many adjustments required to survive/thrive as this more terrestrial phenotype, it appears to have several advantages as compared to dealing with poor water conditions in their environment (such as hypoxia, high temperatures and toxic H_2S levels) (Giomi et al. 2014, Martin 2014, Wright & Turko 2016). Finally, there may be a lower energetic cost to ventilating air for O_2 uptake as compared to seawater, because of its much lower density and higher O_2 content (Table 1). These benefits have even been suggested to underlie the greater physiological performance and thermal tolerance of some intertidal invertebrates during emersion (Giomi et al. 2014, Bjelde et al. 2015).

High intertidal animals as model organisms

High intertidal marine animals have received considerable attention from biologists, which is partially due to their accessibility compared to animals found in other marine ecosystems (e.g. the deep sea) (Nybakken 1993). However, there are other reasons why high intertidal animals have garnered so much attention. These marine animals have adjusted to life that often involves little immersion in water, and this may provide a unique opportunity for gaining insights into the evolution of early terrestrial animals and the adaptations needed to make the water-to-land transition (Sayer 2005, You et al. 2014, Wright & Turko 2016, Katayama et al. 2018). For instance, paleontological studies imply that the intertidal zone is the place from where the tetrapods entered the terrestrial realm (Graham & Lee 2004, Lam et al. 2006). High intertidal animals may also reflect the selective pressures that animals experienced when moving towards a terrestrial lifestyle. For instance, aquatic hypoxia is often a trigger for modern intertidal fishes (such as sculpins and mudskippers) to actively emerge (Table 2), and it is generally thought that hypoxia was an initial driver of the development of air-breathing in vertebrates that allowed for terrestrial colonization early in the evolutionary record (Sayer & Davenport 1991, Martin 1995, Bayley et al. 2019). On the other hand, it has been proposed that heat stress in the intertidal environment triggered the development of air-breathing abilities in some marine animals (Giomi et al. 2014), as aerial respiratory structures appear to be a key adaptation with regard to enhancing the thermal tolerance of present-day intertidal crabs in the tropics (Table 2). What extant intertidal animals can reveal about the evolutionary transition to the land, and specifically whether mudskippers provide a good model for early tetrapods, is an ongoing topic of discussion (Graham & Lee 2004).

In the light of global warming and climate change, a case can be also made for the importance of research on high intertidal animals. Because they are exposed to environmental challenges from both terrestrial and marine climate regimes, these animals could serve as early warning systems for the impacts of climate change (Helmuth et al. 2006). For instance, upper shore *Mytilus californianus* are projected to undergo larger changes in body temperature compared to lower shore conspecifics with global warming, due to the additive effect of warming water and air temperatures (Gilman et al. 2006). Furthermore, compared to low intertidal animals that inhabit less thermally variable environments, high intertidal species experience more severe heat stress, have optimal performance temperatures and upper thermal limits that are generally closer to their environmental temperatures (i.e. they have a smaller ‘thermal safety margin’, TSM), and they appear to have a reduced capacity to increase their heat tolerance through phenotypic plasticity/acclimation (Stillman 2002, 2003, Berger & Emlet 2007, Tomanek 2010, Somero 2010, 2012, Nguyen et al. 2011, Bjelde & Todgham 2013). The latter finding may be counterintuitive, but is based on experimental results collected from a large variety of animals groups, such as *Balanus* barnacles, *Mytilus* mussels, *Lottia* limpets and *Petrolisthes* crabs. In other words, despite their relatively high thermal tolerance, high intertidal animals might be especially vulnerable in this era of accelerated climate change, given that the susceptibility of all animals to climate change appears to depend to a large extent on the level of exposure to environmental temperature extremes, and their physiological tolerance and acclimation potential (Williams et al. 2008, Huey et al. 2012). Although, some of the impacts of global warming may be ameliorated ('buffered') by behavioural thermoregulation, as suggested by Marshall et al. (2015), given that snail species on the tropical rocky shore already have negative TSMs (i.e. the maximum environmental temperatures exceed their lethal temperatures). On the other hand, indirect effects from ecological biotic interactions can add a layer of complexity with regards to how high intertidal animals are affected by climate change, and they can further exacerbate the direct physiological impacts of warming. For instance, *Mytilus* mussels and acorn barnacles that rely on the higher intertidal zone as a refuge from lower intertidal/subtidal predators (e.g. the *Pisaster ochraceus* seastar) are losing a large amount of this predator-free habitat due to rising shore temperatures that have gradually limited their upper range (Harley 2011).

Future research directions

Although intertidal animals have been extensively studied, research comparing high versus low intertidal relatives or conspecifics are in relatively short supply. This is especially true for adaptations related to freezing and salinity stress, and UV radiation exposure, where the effect of shore height is little explored. Such comparative studies will be instrumental to understanding the specific evolutionary adaptations or plastic phenotypic responses of high intertidal animals while minimizing phylogenetic effects. In particular, studies using species comparisons that follow the 'comparative phylogenetic method', whereby phylogeny is carefully incorporated in the statistical contrasts (Garland et al. 2005), would contribute greatly to our understanding of evolutionary trends among high intertidal animals, i.e. by ensuring that the effect of interest (shore height) is not confounded by phylogenetic affinities. A small amount of research included in this review did apply this phylogenetically independent approach (e.g. Mandic et al. 2009a,b, 2012), but it is expected that this will become a more frequently followed methodology. The use of reciprocal transplantations is also a helpful experimental tool that allows for investigations of the specific role of phenotypic plasticity in sessile or sluggish species that colonize the high intertidal – provided that appropriate controls are included (i.e. transplantations to the same vertical level on the shore) to account for the effect of disorientation that an animal may experience after transplantation (see Chapman 2000, Underwood 2000). This is now a standard practice (e.g. Halpin et al. 2004, Altieri 2006), but was less common in the older literature (e.g. McQuaid 1981, McCormack 1982).

To a large extent, research efforts so far have concentrated on the behavioural, morphological, physiological and biochemical adaptations of high intertidal animals. While this research has revealed numerous and intriguing responses/adaptations (Table 2), recent studies on the genetics, epigenetics, transcriptomics, proteomics, metabolomics and protein biochemistry of high intertidal animals have provided additional insights into the underlying mechanisms that allow them to be successful in this environment (e.g. Mandic et al. 2014, You et al. 2014, Kennedy et al. 2020, Chen et al. 2021, Dong et al. 2021). However, there are only a small number of these molecular studies, and thus, they are a promising venue for further research that may further advance this field. In comparative physiology, 'omics' approaches can, for instance, allow for a better understanding of factors involved in cross-tolerance, by looking for common transcriptional responses to different environmental stressors (Torson et al. 2020). Rapid changes (i.e. ranging from minutes to hours) in gene expression or in the proteome (e.g. post-transcriptional and post-translational modifications, respectively) that play a role in the response of high intertidal animals to the tidal cycle can also be elucidated with these methods and/or described in more detail.

There is also a need for research that attempts to better reflect the complex physicochemical conditions encountered by high intertidal animals in the wild – including experiments that specifically account for the high variability, unpredictability (stochasticity) and the combined and repeated occurrence of stressors that are characteristic of this environment (Figure 2) (Raffaelli & Hawkins

1999, Gunderson et al. 2016). Such work (e.g. multi-stressor studies) has been done with several high intertidal animals, for instance *Lottia* sp. and *Cellana grata* limpets (Williams et al. 2011, Denny & Dowd 2012, Pasparakis et al. 2016, Drake et al. 2017), porcelain and green crabs (*Petrolisthes cinctipes* and *Carcinus maenas*; Paganini et al. 2014, Nancollas 2020, Nancollas & McGaw 2021) and the tidepool sculpin (*Oligocottus maculosus*; Somo et al. 2020), but overall, this area is still little explored (e.g. the majority of multi-stressor studies appears to be focused on subtidal animals; see review by Gunderson et al. 2016). Multi-stressor experiments can evaluate the interactive effects of various abiotic factors, many of which may be exacerbated with anthropogenic climate change. For instance, along with rising temperatures, high intertidal animals may encounter greater increases in environmental salinity through evaporation, or sudden exposure to hyposalinity stress due to more frequent and extreme rainfall events (Williams et al. 2011, Rivera-Ingraham & Lignot 2017). Multi-stressor experimental regimes are also helpful when trying to determine whether there is cross-tolerance to environmental stressors (i.e. if response X to stressor A increases tolerance to subsequent exposure to stressor B) (Sinclair et al. 2013).

In the area of freezing tolerance and avoidance, there is a lack of understanding of whether, and how, high intertidal animals cope with freezing stress as compared to those in the low intertidal (even though freezing exposure is evidently more severe on the high shore) – so this area is ripe for discovery. The potential link between oxidative stress and defences, and abiotic stressors on the high shore (e.g. UV radiation and salinity fluctuations), could also be further investigated (Rivera-Ingraham & Lignot 2017). Even in areas where there is abundant literature, for example on adaptations associated with heat stress in high intertidal animals, there are knowledge gaps. While studies where high intertidal animals are acclimated to warmer conditions for a few weeks or months are relatively common (see Stillman 2002, Berger & Emlet 2007, Tomanek 2010, Somero 2010), their informative value is somewhat unclear. Transgenerational experiments that involve the acclimation of multiple generations to a particular stressor may more profoundly improve our assessment of their capacity to adapt to global warming and other climate change-related environmental challenges. This is because they also consider potential transgenerational plasticity and evolutionary shifts (through epigenetics and genetic changes). This type of research can be time-consuming, but has already been done with animals from other aquatic environments (e.g. tropical reef and freshwater fish, see Donelson et al. 2011 and Morgan et al. 2020, respectively). Further, it would be a novel approach in the field of intertidal animal biology and may be pivotal in better defining their adaptive abilities and strategies.

BOX 1. GLOSSARY

Amphibious fishes
Fishes that spend periods of time out of the water as a natural part of their life history.

Antifreeze proteins or glycoproteins
Proteins that inhibit the growth of ice crystals, which is observed as thermal hysteresis – the delayed growth of an ice crystal during cooling below 0°C.

Antioxidants
Enzymes and other bioactive compounds that protect against oxidative stress by quenching and deactivating reactive oxygen species.

Aquatic surface respiration
A behaviour by aquatic breathers where the O_2-rich layer is skimmed from the water's surface.

Bradycardia
A significant decline in heart rate, for instance in response to aquatic hypoxia.

Compatible organic osmolytes
Organic solutes, such as free amino acids, that do not interact with or damage macromolecules when their concentration fluctuates.

Cross-tolerance
When an adaptation that enhances tolerance to a specific environmental stressor also allows for greater tolerance to another, or other, stressor(s).

Ecotype
Morphological variant within a species that occupies a particular habitat and that has undergone incomplete speciation.

Gaping
Opening of the shell by bivalves during emersion to allow for aerial O_2 uptake and/or evaporative cooling.

Heat shock proteins
Molecular chaperones whose expression is upregulated during heat stress (or another form of stress), and that improve the stability and repair of other proteins in the cell.

Ice-nucleating agents
Compounds that trigger ice crystal formation at a relatively high temperature.

LT_{50}
The emersion time or temperature at which 50% mortality occurs in a group of animals, which can be used as a measure for their desiccation tolerance or upper thermal tolerance, respectively.

Metabolic depression
A temporary, and reversible, reduction in metabolism below the standard (minimal) metabolic rate.

$\dot{M}O_2$
Mass-specific O_2 consumption rate.

Multifunctional adaptation
An adaptation that serves multiple functions.

NH_3 volatilization
Release of NH_3 as a gas, instead of excreting it into the water.

Phenotypic plasticity
The flexibility that allows animals with the same genetic make-up to express multiple phenotypes depending on the environment.

Q_{10}
The fold change for a physiological process (e.g. metabolic rate) following a 10°C increase or decrease in temperature.

Reciprocal transplantations
The transplantation of animals (typically sessile or sluggish species) from one site to another, and vice versa.

RNA interference
The suppression of mRNA translation into protein through the binding of microRNAs followed by degradation of the mRNA.

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Oceanography and Marine Biology: An Annual Review, 2022, **60**, 681-682
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E. M. Marzinelli, P. J. Mumby, B. D. Russell, J. Sharples, I. P. Smith, S. E. Swearer, and P. A. Todd, Editors
Taylor & Francis

EPILOGUE: MARGARET BARNES 1919–2009: AN APPRECIATION

ROBIN N. GIBSON

The 1960s saw the appearance of two major new outlets in which marine biologists could publish their work. The review series *Oceanography and Marine Biology: an Annual Review* (*OMBAR*) was first published in 1963 and was followed four years later by the *Journal of Experimental Marine Biology and Ecology* (*JEMBE*). Both were the brainchildren of Harold Barnes who acted as their editor. Under Harold's stimulation and guidance, *JEMBE* grew from one volume of three issues at the time of his death to what will shortly reach beyond 550 Volumes in 2022. *OMBAR* was equally successful and has now reached its 60th volume. His wife Margaret was an integral, experienced and tireless other half of the editorial team who shared Harold's vision and enthusiasm; so that on his untimely death in 1978 it was natural for her to assume the editorship of both publications, and so ensure their smooth continuation.

She reluctantly retired from *JEMBE* in 1999, but continued to work as the Managing Editor of *OMBAR* until 2002, after 40 years association with the series. Margaret always had a proprietary interest in both publications and throughout her long association with them, always applying the same consistently high standards. Appreciating the fact that authors liked to have their papers processed as quickly as possible, she dealt with incoming manuscripts on the day of receipt or very shortly afterwards. She was a master of the diplomatic letter to authors, referees and publishers to ensure that the progress of manuscripts was not impeded. She insisted on high standards of English, spending many hours improving the texts, both of authors whose first language was not English and of many whose it was. Both she and Harold strongly believed in encouraging young authors to revise their papers rather than rejecting them out of hand. Her wide knowledge built up over the years meant that she could rapidly assess the general quality of a submitted paper even though she might not have been familiar with the detail. Others could deal with that aspect and her network of referees in all branches of marine biology was considerable.

She was a founder member of the European Marine Biology Symposium and in 1988 was elected for a term as president. She was intimately involved with the two Symposia that were held in her hometown of Oban in 1974 and 1989 and was instigator, organizer and Senior Editor of the Proceedings of the latter meeting.

Through her research, correspondence and extensive travels she made contact with many people the world over, with many of these contacts developing into lasting friendships. She was always encouraging to young scientists, especially young women. She was very proud of her DSc, a public acknowledgement of her contribution to science. She was an independent and determined woman largely overshadowed initially by her husband. Her true scientific and editorial abilities only became apparent after his death. She was also a gentle, modest, courteous and charming person, and a good listener with a terrific sense of humour. In her younger days she was very active and remained sprightly until her death, working in her garden throughout the year.

Robin N. Gibson
Former Colleague of Margaret Barnes and Editor in Chief of OMBAR,
volumes 40–50

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Index

Note: **Bold** page numbers refer to tables and *italic* page numbers refer to figures.