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Spread of Pathogens from Marine Cage Aquaculture – A Potential Threat for Wild Fish Assemblages Under Protection Regimes?

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1. Introduction

The spread and the control of pathogens represent a serious problem in aquaculture, implying important environmental and economic issues (Huntington, 2006; Sapkota et al., 2008).

With the rapid development of aquaculture in recent decades (Food and Agriculture Organization [FAO], 2008), an increasing number of pathogens involved in fish disease, and the range of susceptible fish species have been identified and described. The increasing use of molecular tools has helped improve our knowledge in this area.

Few studies have been performed to evaluate the interactions host/pathogen in the natural environment (Hedrick, 1998), or to investigate the mechanisms of infection and interaction between farmed and wild specimens, nor the ecological role assumed by infected wild hosts has been explored yet.

In view of the current decline of fish stocks, which has recently been attributed not only to habitat loss and overfishing, but also even to the spread of pathogens (Daszak, 2000; Gozlan et al., 2005), understanding these mechanisms is essential for a proper conservation and management of marine and fisheries resources.

The effect of parasites and other pathogens on fish populations under protection regimes has received virtually no attention (McCallum et al., 2005). Marine Protected Areas (MPAs) can provide unique protection for critical areas and spatial escape for overexploited species and are regarded as essential tools for the conservation of marine environments (Lubchenco et al., 2003; Pauly, 2005; Roberts et al., 2005). However, the effectiveness of MPAs is greatly limited by several anthropogenic and natural impacts acting at scales much larger than that encompassing by the reserve boundaries. Examples of such impacts toward which MPAs cannot offer any direct protection are represented by climate change (Graham et al., 2008; McClanahan, 2000), with all the problems related to it (Keller et al., 2009), the spread of pollutants (Jarman et al., 1992; Loganathan & Kannan, 1994; Terlizzi et al., 2004), invasive

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species (Carlton et al., 1990; Katsanevakis et al. 2010; Trowbridge, 1995), and pathogens (Lessios, 1988; Littler & Littler, 1995; Rasmussen, 1977; Steinbeck et al., 1992).

In this chapter we briefly review the potential threats to wild population represented by the spread of pathogens from marine cage fish aquaculture. As an emblematic example we focus particularly on the effects of a serious disease, namely the *Viral Nervous Necrosis* (VNN), affecting several reared fish species worldwide.

In order to warn about how the spread of pathogens could represent a risk for natural populations, even under protection regimes, we report on the results of a preliminary survey for the detection of viral particles responsible for VNN in the gonads and brain homogenates in some wild specimens collected in two no take-no access Marine Protected Areas in the Southern Italy (South Adriatic and North Ionian Sea).

2. The spread of pathogens from marine cage aquaculture

There are several ways by which new pathogens can attain natural populations.

For example, a benign organism can undergo a genetic change that may increase its degree of pathogenicity (Bull, 1994; Cunningham, 2002). A pathogen may also be introduced through the expansion of the range of wild species in new regions as a result of global warming, the removal of barriers, or the transport of animals for commercial and recreational purposes (Gozlan et al., 2006).

It is now known that the activities of aquaculture farms, particularly those of offshore floating cages, are a source of various types of environmental (Pusceddu et al., 2007) and biological (Terlizzi et al., 2010) impact, including the facilitation of the spread of potential pathogens (Krkošek et al., 2006). The high density to which organisms are reared and the unnatural and stressful conditions to which they are subjected may in fact facilitate the emergence and spread of diseases, which can potentially extend to wild organisms (Naylor et al., 2005).

The use of net pen or cages systems involves a higher risk for disease transmission because there is no impermeable barrier between the farm and the aquatic environment (Huntington et al., 2006). There is therefore a strong potential for contamination between reared organisms and the wildlife of the surrounding environment.

Many species of wild fish are attracted by the presence of the cages, which provide them food and shelter (Diamant et al., 2000), often succeeding to get into cages, passing through the mesh. Farmed species may be able to escape in the environment too. This phenomenon may have important biological implications (e.g. in terms of genetic contamination) on wild populations. In addition, escaped individuals can carry infections they acquired at the culture systems (Conseil International pour L'Exploration Scientifique de la Mer Médetirranée [CIESM], 2007).

Farmed fish stocks may prove receptive to pathogens from the surrounding environment too. The exchange of pathogens between farmed and wild fish has been repeatedly observed in different geographical regions (Diamant et al., 2000; Kent, 2000; Mc Vicar, 1997; Nowak et al., 2004; Paperna, 1998; Sepulveda et al., 2004). Bacterial infections by *Mycobacterium marinum* and *Streptococcus iniae*, which are common among farmed fish stocks, have been described to cause mortality even in wild fish (Colorni et al., 2002).

Pathological events in populations of wild abalone (Alstatt et al., 1996) suggest a potential susceptibility that makes them able to influence or be influenced by, reared individuals. Such pathogen exchange relationship seems to occur frequently between farmed and wild fish populations, as in the case of a disease known as *Infectious Hematopoietic Necrosis*, which affects mainly salmons, and which appears to be transmitted between wild and farmed specimens in both directions (Naylor et al., 2003).

Transmission of pathogens from aquaculture to wild fish can occur at different stages of the life cycle: infection can start from the earliest stages of reproduction and larval rearing (hatchery) and spread in the later stages (growing) contaminating wild stocks and causing dangerous epidemics (Naylor et al., 2005).

Krkošek et al. (2011) studied the effects of sea lice (*Lepeophtheirus salmonis*) from salmon farming facilities on populations of wild salmon (*Onchorhynchus gorbusha* and *O. keta*), noting that juveniles of these species, when parasitized, show a reduced ability to escape predators that consume selectively parasitized salmon. Therefore, the mortality of juvenile wild salmon may be higher than previously believed and this has important implications for the development of appropriate conservation policies.

The importation of feed for aquaculture can also be considered vehicle of sources of pollutants and of pathogens between fish stocks very distant from each other (Dalton, 2004).

Parasites and pathogens can therefore play an important role for fish stocks, reducing both the number and yield, increasing mortality, altering the reproductive potential, affecting the structure of the population size and/or reducing the commercial value of harvested stocks (Dobson & May, 1987; Kuris & Lafferty, 1992).

Being able to influence the survival of the host and his reproductive success, pathogens should be taken into great consideration in the design and management of MPAs (McCallum et al., 2005). Also, even if the survival or reproduction are not affected, as in the case of infections by *Anisakis simplex* and *Pseudoterranova decipiens*, pathogens may pose a problem for fisheries management-oriented reserves, because the infected hosts have a lower market value and can represent a health risk when consumed as food by humans.

3. The Viral Nervous Necrosis (VNN)

The genus *Betanodavirus*, family Nodaviridae is a widespread and well-known viral pathogen in the field of fish farming. It is the etiologic agent responsible for a severe disease known as *Viral Encephalopathy and Retinopathy* (*VER*), or *Viral Nervous Necrosis* (*VNN*). It affects more than 40 species of marine fish worldwide (Panzarin et al., 2010). VNN disease is regarded as one of the most devastating viral infections among marine fish.

Affected fish, particularly juvenile forms, show spiralling swimming, darkened colour and hyper inflated swim bladders. Internal disease signs include pale livers, empty digestive tracts and intestines filled with brownish fluid. In affected fish the virus propagates in the eye, brain and distal spinal cord causing marked vacuolations leading to encephalopathy and retinopathy (Fig. 1). Such lesions in the Central Nervous System and in the retina (nervous tissue of the eye) have always been typical of larval and juvenile stages in the sea bass (Bovo et al., 1996; Breuil et al., 1991) and in other species sensitive to nodavirosis (Glazebrook et al., 1990; Grotmol et al., 1997; Mori et al., 1991; Nguyen et al., 1996). For this

Fig. 1. Histological preparation of a sea bass larva (age 45 days) with the typical vacuolar necrosis of the brain tissue and in the retina (indicated by white and black arrows, respectively). After the collection, the sample has been readily fixed in 10% neutral buffered formalin for not more than 7 days. Thereafter it has been included in paraffin to be cut in to sections of 5 μm and then colored for histological analysis. The dye used in this histological section is hematoxylin-eosin.

reason histological analyses have often been performed in monitoring programs to evaluate the spread of the disease in the hatcheries (Sweetman et al 1996).

The virus also multiplies in the gonad, livers, kidney, stomach and intestine (Grotmol et al., 2000; Munday et al., 2002).

Experiments with *Pseudocaranx dentex* (Arimoto et al., 1992; Mushiake et al., 1994; Nishizawa et al., 1996) have shown that the virus transmission can occur either vertically, from broodstock to larvae through the eggs or sperm, either horizontally, through the contact between healthy fish and infected larvae (Arimoto et al., 1993). Experimental contamination with infected tissue homogenates (Arimoto et al., 1993; Glazebrook et al., 1990; Grotmol et al., 1999; Tanaka et al., 1998) or addition of purified virus from infected individuals (Nguyen et al., 1996) or produced in SSN-1 cells (Péducasse et al., 1999) is also able to transmit the virus. The ability of the betanodavirus to resist to a wide pH range, varying from 2 to 9 (Frerichs et al., 1996), and its resistance in sea water at 15° C for more than one year (Frerichs et al., 2000) increases the probability of horizontal transmission (Munday et al., 2002). The transmission of the betanodavirus from infected, asymptomatic specimens is also possible. Some Authors (e.g. Castric et al., 2001) have shown how specimens of *Sparus aurata* experimentally infected but apparently healthy, are able to transmit the virus to individuals of *Dicentrarchus labrax* placed in the same cage.

The finding, in Norway (Aspehaug et al., 1999), of adults of Atlantic halibut (*Hippoglossus hippoglossus*) positive for betanodavirus suggests a possible danger of using wild animals as breeding stock.

There is obviously also the possible risk of transmission occurring in the opposite direction, namely that the virus originating from aquaculture facilities can infect wild healthy individuals. Supports to this hypothesis, in addition to the above mentioned evidences, which show that betanodavirus can spread both horizontally and vertically within a system, are provided by the detection of the virus in several wild marine species, although the infection in the examined subjects was asymptomatic (Barker et al., 2002; Gagné et al., 2004; Gomez et al., 2004; Gomez et al., 2008; Thiéry et al., 2004).

Recent studies carried out along the Italian coast have identified the etiologic agent responsible for the disease in wild organisms belonging to a wide panel of species. From a virological examination conducted by Maltese et al. (2005) on samples from the northern Adriatic and the Strait of Sicily, the presence of viruses has been detected in the sea bass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*), white grouper (*Epinephelus aeneus*), dusky grouper (*Epinephelus marginatus*), red mullet (*Mullus barbatus*), axillary seabream (*Pagellus acarne*), poor cod (*Trisopterus minutus*) and in the black goby (*Gobius niger*). The study highlighted a possible widespread of the virus in nature, the role of *S. aurata* as an asymptomatic carrier and the high sensitivity of the genus *Epinephelus*, for which episodes of mortality in other geographical areas have been reported. The study also confirmed the considerable sensitivity of *D. labrax* to the betanodavirus, which had already been proven in the context of intensive farming.

4. VNN and protected populations: A limited effectiveness of MPAs?

The two areas considered for sampling are the MPA of Porto Cesareo, located along the southwestern coast of Apulia (Ionian Sea, 40°15' N - 17°52' E) and the MPA of Torre Guaceto, located along the east coast of Apulia (Adriatic Sea, 40°43' N - 17°48' E). Both areas are characterized by the past presence of extensive aquaculture activities within or close to the reserve boundaries. The survey focused mainly on specimens of *D. labrax* but, whenever possible, considered, as a preliminary screening, some other species of commercial interest for which positive serology for the betanodavirus has been already reported in literature. For all specimens, samples of brain and gonadal tissue, the main target organs of the virus were taken in double aliquot for DNA extraction and biomolecular analysis. Virological analyses were performed by the laboratories of the Istituto Zooprofilattico Sperimentale delle Venezie (Padua, Italy). Samples were analysed through real-time TaqMan PCR. This technique proved to be highly sensitive, is suitable for a wide range of organic matrices and is able to detect the four *betanodavirus* genotypes currently recognized. The experimental protocol followed the procedure described in Panzarin et al. (2010). More particularly, samples were processed for RNA extraction using the NucleoSpin® RNA II (Macherey-Nagel GmbH & C., Düren, Germany). Real-time PCR was performed using the LightCycler 2.0 system and carried out in 20 µl with ''LightCycler® TaqMan® Master'' (Roche Diagnostics GmbH, Mannheim, Germany), 0.9 µM of each primer, 0.75 µM of probe and 5 µl of cDNA template. The thermal profile consisted of a 10-min incubation at 95°C followed by 45 cycles of 10 s denaturation at 95°C, 35 s annealing at 58°C and 1 s elongation at 72°C, followed by an additional 30-s cooling step at 40°C.

The analysis involved the collection of 28 specimens, 9 of which coming from the MPA of Torre Guaceto and 19 from the MPA of Porto Cesareo. Results are summarised in Table 1.

Several specimens resulted positive to the VNN test. The individuals showing positivity to the molecular analyses were mostly represented by the sea bass (*Dicentrarchus labrax*) but the presence of the virus was also detected in the scorpion fish (*Scorpaena scrofa*), the gray mullet (*Mugil* sp.), the goby (*Gobius* sp.) and the comber (*Serranus cabrilla*). All positive individuals appeared asymptomatic.

Fish species	No. of examined fish	No. of positive samples
	Torre Guaceto MPA	
Dicentrarchus labrax	4	3
Diplodus sargus	5	0
	Porto Cesareo MPA	
Dicentrarchus labrax	3	3
Scorpaena scrofa	3	1
Mugil sp.	3	1
Gobius sp.	2	1
Serranus cabrilla	2	1
Synodus saurus	2	0
Serranus scriba	1	0
Sarpa salpa	2	0
Oblada melanura	1	0

Table 1. Summary of VNN tests performed in the two Marine Protected Areas (MPAs)

5. Discussion

The lack of clinical signs in infected fish specimens observed in this study is consistent with the results obtained in other recent studies of wild marine fauna (Baeck et al., 2007; Gomez et al., 2004), thus confirming how rare are the observations of clinical forms in wild fish populations (but see Gomez et al., 2009).

Some authors argue that most infections in wildlife are presumably latent and they do not evolve into a pathological condition until the fish becomes stressed (Barker et al., 2002).

Barker et al. (2002) firstly reported piscine nodavirus in wild winter flouder *Pleuronectes americanus* in Passamaquoddy Bay (New Brunswick, Canada) considering the possibility that the virus could be transferred between wild winter flounder and sea-caged halibut or cod.

Baeck et al. (2007) detected nodavirus in 21 different apparently healthy wild marine fish collected in coastal areas of Korea, close to aquaculture facilities and Gomez et al. (2004) reported high prevalence for betanodavirus in apparently healthy cultured and wild marine fish near mariculture areas in Japan. In both studies, sampling areas were located near aquaculture facilities and the authors support the hypothesis of an horizontal transmission of the virus.

The results here reported, although preliminary, make us suppose that the distribution of the betanodavirus is currently extending and not confined to marine cage aquaculture. The genetic characterization of isolated viral strains is currently under investigation and will likely help determining more precisely the range of dispersion and residence time of these possible sources of contamination, thus improving our understanding about the epidemiological mechanisms of the disease and consequently our ability to control it.

Our findings also confirm that MPAs are not able to cope with this particular form of impact, which clearly acts at a scale larger than that covered by the measures of protection (Allison et al., 1998).

The specimens considered, although asymptomatic, might still be reservoirs of infection, by acting as virus carriers, and further favoring the spread of the pathogen. The ecological role of these asymptomatic carriers in nature remains however unclear and the possible consequences on natural population still remain to be clarified. This is not an easy task, however, as disease disorders are difficult to observe in the wild because affected individuals are rapidly removed from the population by predators (Gozlan et al., 2006). These consequences are nevertheless conceivable since in some reared species such as the sea bass, the virus causes, in addition to the above-described damages to the nervous system, failure in reproduction (often sterilization), reduced hatching rate and high larval mortality.

In the view of the impact caused by the over-exploitation of marine resources and given the current trend of decline of commercially important fish stocks, increased mortalities and failure in reproduction due to diseases should be carefully considered and adequately addressed, especially in sound quantification of the effectiveness of MPAs.

Attempts to protect biodiversity through MPAs and/or restore ecosystem functioning (e.g., through the protection of over-exploited species that generate positive community-wide effect) might be frustrated and/or biased in their quantification by external pressures.

Given the wide spread of this virus and its possible consequences in asymptomatic forms it should be important to include the issue of pathogens in disease surveillance program and monitoring of biological health of each MPA as well as any area that has or has had a significant number of intensive aquaculture plants.

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7. References

Allison, G. W.; Lubchenco, J. & Carr, M. H. (1998). Marine reserves are necessary but not sufficient for marine conservation. *Ecological applications*, Vol.8, pp. S79–S92.

Alstatt, J. M.; Ambrose, R. F.; Engle, J. M.; Haaker, P. L.; Lafferty, K. D. & Raimondi, P. T. (1996). Recent declines of black abalone *Haliotis cracherodii* on the mainland coast of central California. *Marine Ecology Progress Series,* Vol.142, pp 185-192.

Arimoto, M.; Mori, K.; Nakai, T.; Muroga, K. & Furusawa, I. (1993). Pathogenicity of the causative agent of viral nervous necrosis disease in striped jack, *Pseudocaranx dentex* (Block & Schneider). *Journal of Fish Diseases,* Vol.16, pp. 461–469.

Arimoto, M.; Mushiake, K.; Mizuta, Y.; Nakai, T.; Muroga, K. & Furusawa, I. (1992). Detection of Striped Jack Nervous Necrosis Virus (JSNNV) by enzyme-linked immunosorbent assay (ELISA). *Gyobyo Kenkyu,* Vol.27, pp. 191–195.

Aspehaug, V.; Devold, M. & Nylund, A. (1999). The phylogenetic relationship of nervous necrosis virus from halibut (*Hippoglossus hippoglossus*). *Bulletin of European Association of Fish Pathologists,* Vol.19, pp. 196-202.

Baeck, G.W.; Gomez, D.K.; Oh, K.S.; Kim, J. H.; Choresca, C. H. Jr & Park, S. C. (2007). Detection of piscine nodaviruses from apparently healthy wild marine fish in Korea. *Bulletin of the European Association of Fish Pathologists,* Vol.27, pp. 116–122.

Barker, D. E.; MacKinnon, A. M.; Boston, L.; Burt, M. D. B.; Cone, D. K.; Speare, D. J.; Griffiths, S.; Cook, M.; Ritchie, R. & Olivier, G. (2002). First report of piscine nodavirus infecting wild winter flounder *Pleuronectes americanus* in Passamaquoddy Bay, New Brunswick, Canada. *Diseases of Aquatic Organisms,* Vol.49, pp. 99-105.

Bovo, G.; Borghesan, F.; Mutinelli, F.; Montesi, F. & Comuzzi, M. (1996). Viral Encephalo-retinopathy of reared sea bass: first detection in Italy. *Bollettino della Società Italiana di Patologia Ittica*, Vol.19, pp. 52-64.

Breuil, G.; Bonami, J. R.; Pepin, J. F. & Pichot, Y. (1991). Viral infection (picorna-like) associated with mass mortalities in hatchery-reared sea bass *Dicentrarchus labrax* larvae and juveniles. *Aquaculture* Vol.97, pp. 109–116.

Bull, J. J. (1994). Perspective e virulence. *Evolution,* Vol.48, pp. 1423-1437.

Carlton, J. T.; Thompson, J. K.; Schemel, L. E. & Nichols, F. H. (1990). Remarkable invasion of San Francisco Bay (California, USA) by the Asian clam *Potamocorbula amurensis*. I. Introduction and dispersal. *Marine Ecology Progress Series,* Vol.66, pp. 81-94.

Castric, J.; Thiery, R.; Jeffroy, J.; de Kinkelin, P. & Raymond, J. C. (2001). Sea bream *Sparus aurata*, an asymptomatic contagious fish host for nodavirus. *Diseases of Aquatic Organisms,* Vol.47, pp. 33-38.

Colorni, A.; Diamant, A.; Eldar, A.; Kvitt, H. & Zlotkin, A. (2002). *Streptococcus iniae* infections in Red Sea cage-cultured and wild fishes. *Diseases of Aquatic Organisms,* Vol.49, pp. 165-170.

Conseil International pour L'Exploration Scientifique de la Mer Médetirranée [CIESM] (2007). Impact of mariculture on coastal ecosystems. Ciesm Workshop Monographs n°32, Monaco www.ciesm.org/online/monographs/lisboa07.pdf

Cunningham, C. O. (2002). Molecular diagnosis of fish and shellfish diseases: present status and potential use in disease control. *Aquaculture,* Vol.206, pp. 19-55.

Dalton, R. (2004). Fishing for trouble. *Nature,* Vol.431, pp. 502–504.

Daszak, P. (2000). Emerging infectious diseases of wildlife - threats to biodiversity and human health. *Science,* Vol.287, pp. 443-449.

Diamant, A.; Banet, A.; Ucko, M.; Colorni, A.; Knibb, W. & Kvitt, H. (2000). Mycobacteriosis in wild rabbitfish *Siganus rivulatus* associated with cage farming in the Gulf of Eilat, Red Sea. *Diseases of Aquatic Organisms,* Vol.39, pp. 211–219.

Dobson, A. P. & May, R. M. (1987). The effects of parasites on fish populations: theoretical aspects. *International Journal for Parasitology,* Vol.17, pp. 363–370.

Food and Agriculture Organization [FAO] (2008). State of World Fisheries and Aquaculture (SOFIA), Food and Agricultural Organization of the United Nations, Rome.

Frerichs, G. N.; Rodger, H. D. & Peric, Z. (1996). Cell culture isolation of piscine neuropathy nodavirus from juvenile sea bass, *Dicentrarchus labrax*. *Journal of General Virology,* Vol.77, pp. 2067–2071.

Frerichs, G. N.; Tweedie, A.; Starkey, W. G. & Richards, R. H. (2000). Temperature, pH and electrolyte sensitivity, and heat, UV and disinfection inactivation of sea bass (*Dicentrarchus labrax*) neuropathy virus. *Aquaculture,* Vol.185, pp. 13–24.

Gagné, N.; Johnson, S. C.; Cook-Versloot, M.; MacKinnon, A. M. & Olivier, G. (2004). Molecular detection and characterization of nodavirus in several marine fish species from the northeastern Atlantic. *Diseases of Aquatic Organisms,* Vol.62, pp. 181-189.

Glazebrook, J. S.; Heasman, M. P. & de Beer, S. W. (1990). Picorna-like viral particles associated with mass mortalities in larval barramundi, *Lates calcarifer* Bloch. *Journal of Fish Diseases,* Vol.13, pp. 245–249.

Gomez, D. K.; Baeck, G. W.; Kim, J. H.; Choresca, C. H. Jr & Park, S. C. (2008). Molecular detection of betanodaviruses from apparently healthy wild marine invertebrates. *Journal of Invertebrate Pathology,* Vol.97, pp. 197–202.

Gomez, D. K.; Sato, J.; Mushiake, K.; Isshiki, T.; Okinaka, Y. & Nakai, T. (2004). PCR-based detection of betanodaviruses from cultured and wild marine fish with no clinical signs. *Journal of Fish Diseases,* Vol.27, pp. 603-608.

Gomez, D.K.; Matsuoka, S.; Mori, K.; Okinaka, Y.; Park, S.C. & Nakai, T. (2009). Genetic analysis and pathogenicity of betanodavirus isolated from wild redspotted grouper *Epinephelus akaara* with clinical signs. *Archives of Virology*, Vol.154, pp. 343-346.

Gozlan, R. E.; St-Hilaire, S.; Feist, S. W.; Longshaw, M. & Peeler, E. J. (2006). The effect of microbial pathogens on the diversity of aquatic populations, notably in Europe. *Microbes and Infection,* Vol.8, pp. 1358–1364.

Gozlan, R. E.; St-Hilaire, S.; Feist, S. W.; Martin, P. & Kent, M. L. (2005). Biodiversity: disease threat to European fish. *Nature,* Vol.435, p. 1046.

Graham, N. A. J.; McClanahan, T. R.; MacNeil, M. A.; Wilson, S. K.; Polunin, N. V. C.; Jennings, S.; Chabanet, P.; Clark, S.; Spalding, M. D.; Letourneur, Y.; Bigot, L.; Galzin, R.; Ohman, M. C.; Garpe, K. C.; Edwards, A. J. & Sheppard, C. R. C. (2008). Climate Warming, Marine Protected Areas and the Ocean-Scale Integrity of Coral Reef Ecosystems. *PLoS ONE*, Vol.3, pp. e3039. doi:10.1371/journal.pone.0003039

Grotmol, S.; Bergh, O. & Totland, G. K. (1999). Transmission of viral encephalopathy and retinopathy (VER) to yolk-sac larvae of the Atlantic halibut *Hippoglossus hippoglossus*: occurrence of nodavirus in various organs and a possible route of infection. *Diseases of Aquatic Organisms,* Vol.36, pp. 95–106.

Grotmol, S.; Nerland, A. H.; Biering, E.; Totland, G. K. & Nishizawa, T. (2000). Characterization of the capsid protein gene from a nodavirus strain affecting the Atlantic halibut *Hippoglossus hippoglossus* and design of an optimal reverse-transcriptase polymerase chain reaction (RT-PCR) detection assay. *Diseases of Aquatic Organisms,* Vol.39, pp. 79–88.

Grotmol, S.; Totland G. K.; Thorud, K. & Hjeltnes, B. K. (1997). Vacuolating encephalopathy and retinopathy associated with a nodavirus-like agent: a probable cause of mass mortality of cultured larval and juvenile Atlantic halibut *Hippoglossus hippoglossus*. *Diseases of Aquatic Organisms*, Vol.29, pp. 85-97.

Hedrick, R. P. (1998). Relationships of the host, pathogen and Environment: implications for disease of cultured and wild fish populations. *Journal of Aquatic Animal Health*, Vol.10, pp. 107-111.

Huntington, T. C.; Roberts, H.; Cousins, N.; Pitta, V.; Marchesi, N.; Sanmamed, A.; Hunter-Rowe, T.; Fernandes, T. F.; Tett, P.; McCue, J. & Brockie, N. (2006). Some Aspects of the Environmental Impact of Aquaculture in Sensitive Areas. Report to the DG Fish and Maritime Affairs of the European Commission. Rep. No. 221-EC/R/02/B. Poseidon Aquatic Resource Management Ltd, Windrush, Warborne Lane, Portmore, Lymington, Hampshire SO41 5RJ, UK.

Jarman, W. M.; Simon, M.; Norstrom, R. J.; Burns, S. A.; Bacon, C. A.; Slmonelt, B. R. T. & Risebrough, R. W. (1992). Global distribution of tris(4-chlorophenyl) methanol in high trophic level birds and mammals. *Environmental Science and Technology,* Vol.26, pp. 1770–1774.

Katsanevakis, S.; Issaris, Y.; Poursanidis, D. & Thessalou-Legaki, M. (2010). Vulnerability of marine habitats to the invasive green alga *Caulerpa racemosa* var. *cylindracea* within a marine protected area. *Marine Environmental Research,* Vol.70, pp. 210-218.

Keller, B. D.; Gleason, D. F.; McLeod, E.; Woodley, C. M.; Airamé, S.; Causey, B. D.; Friedlander, A. M.; Grober-Dunsmore, R.; Johnson, J. E.; Miller, S. L. & Steneck, R. S. (2009). Climate Change, Coral Reef Ecosystems, and Management Options for Marine Protected Areas. *Environmental Management*, Vol.44, pp. 1069-1088.

Kent, M. L. (2000). Marine net-pen farming leads to infections with some unusual parasites. *International Journal for Parasitology, Vol.*30, pp. 321-326.

Krkošek, M.; Connors, B. M.; Ford, H.; Peacock, S.; Mages, P.; Ford, J. S.; Morton, A.; Volpe, J. P.; Hilborn, R.; Dill, L. M. & Lewis, M. A. (2011). Fish farms, parasites, and predators: implications for salmon population dynamics. *Ecological Applications,* Vol.21, pp. 897–914.

Krkošek, M.; Lewis, M. A.; Morton, A.; Neil Frazer, L. & Volpe, J. P. (2006). Epizootics of wild fish induced by farm fish. *Proceedings of the National Academy of Sciences*, Vol.103, pp. 15506–15510.

Kuris, A. M. & Lafferty, K. D. (1992). Modeling crustacean fisheries – effects of parasites on management strategies. *Canadian Journal of Fisheries and Aquatic Sciences,* Vol.49, pp. 327-336.

Lessios, H. A. (1988). Mass mortality of *Diadema antillarum* in the Caribbean: what have we learned? *Annual Review of Ecology and Systematics,* Vol.19, pp. 371-393.

Littler, M. M. & Littler, D. S. (1995). Impact of CLOD pathogen on Pacific coral reefs. *Science,* Vol.267, pp. 1356-1360.

Loganathan, B. G. & Kannan, K. (1994). Global organochlorine contamination trends: an overview. *Ambio,* Vol.23, 187-191.

Lubchenco, J.; Palumbi, S. R.; Gaines, S. D. & Andelman, S. (2003). Plugging a hole in the ocean: The emerging science of marine reserves. *Ecological Applications,* Vol.13, pp. S3–S7.

Maltese, C.; Antonetti, P.; Quartesan, R.; Ormelli, S.; Borghesan, F.; Manfrin, A.; Selli, L.; Castiglione, F.; Ferrantelli, V.; Guercio, A. & Bovo, G. (2005). Isolation of viral encephalopathy and retinopathy virus (VERV) from wild marine fish species in the Mediterranean sea. In: 12th International Conference "Diseases of Fish and

Shellfish", European Association of Fish Pathologists, Copenhagen (Denmark), 11-16 September 2005: Poster 8.19.

McCallum, H.; Gerber, L. & Jani, A. (2005). Does Infectious Disease Influence the Efficacy of Marine Protected Areas? A Theoretical Framework. *Journal of Applied Ecology,* Vol.42, pp. 688-698.

McClanahan, T. R. (2000). Bleaching Damage and Recovery Potential of Maldivian Coral Reefs. *Marine Pollution Bulletin,* Vol.40, pp. 587–597.

McVicar, A. H. (1997). Interaction of pathogens in aquaculture with wild fish populations. *Bulletin of European Association of Fish Pathologists,* Vol.17, pp. 197-200.

Mori, K.; Nakai, T.; Nagahara, M.; Muroga, K.; Mekuchi, T. & Kanno, T. (1991). A viral disease in hatchery-reared larvae and juveniles of redspotted grouper. *Fish Pathology*, Vol.26, pp. 209-210.

Munday, B. L.; Kwang, J. & Moody, N. (2002). Betanodavirus infections of teleost fish: a review. *Journal of Fish Diseases,* Vol.25, pp.127–142.

Mushiake, K.; Nishizawa, T.; Nakai, T.; Furusawa, I.; & Muroga, K. (1994). Control of VNN in striped jack: selection of spawners based on the detection of SJNNV gene by polymerase chain reaction (PCR). *Fish Pathology,* Vol.29, pp. 177–182.

Naylor, R.; Eagle, J. & Smith, W. (2003). Salmon aquaculture in the Pacific North-west: a global industry with local impacts. *Environment,* Vol.45, pp. 18–39.

Naylor, R.; Hindar, K.; Fleming, I. A.; Goldburg, R.; Williams, S.; Volpe, J.; Whoriskey, F.; Eagle, J.; Kelso, D. & Mangel, M. (2005). Fugitive salmon: assessing the risks of escaped fish from net-pen aquaculture. *BioScience,* Vol.55, pp. 427–37.

Nguyen, H. D.; Nakai, T. & Muroga, K. (1996). Progression of striped jack nervous necrosis virus (SJNNV) infection in naturally and experimentally infected striped jack *Pseudocaranx dentex* larvae. *Diseases of Aquatic Organisms*, Vol.24, pp. 99-105.

Nishizawa, T.; Muroga, K. & Arimoto, M. (1996). Failure of the polymerase chain reaction (PCR) method to detect striped jack nervous necrosis virus (SJNNV) in striped jack *Pseudocaranx dentex* selected as spawners. *Journal of Aquatic Animal Health,* Vol.8, pp. 332–334.

Nowak, B. F.; Dawson, D.; Basson, L.; Deveney, M. & Powell, M. D. (2004). Gill histopathology of wild marine fish in Tasmania: potential interactions with gill health of cultured Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases,* Vol.27, pp. 709-717.

Panzarin, V.; Patarnello, P.; Mori, A.; Rampazzo, E.; Cappellozza, E.; Bovo, G. & Cattoli, G. (2010). Development and validation of a real-time TaqMan PCR assay for the detection of betanodavirus in clinical specimens. *Archives of Virology,* Vol.155, pp. 1193-1203.

Paperna, I. (1998). Mariculture versus environment: risks and impacts. *Biologia Marina Mediterranea,* Vol.5, pp. 381-389.

Pauly, D. (2005). Global trends in world fisheries: Impacts on marine ecosystems and food security. *Philosophical Transactions of the Royal Society* B, Vol.360, pp. 5–12.

Péducasse, S.; Castric, J.; Thiéry, R.; Jeffroy, J.; Le Ven, A. & Baudin Laurencin, F. (1999). Comparative study of viral encephalopathy and retinopathy in juvenile seabass *Dicentrarchus labrax* infected in different ways. *Diseases of Aquatic Organisms,* Vol.36, pp. 11–20.

Pusceddu, A.; Fraschetti, S.; Holmer, M.; Mirto, S. & Danovaro, R. (2007). Effects of intensive mariculture on sediment biochemistry. *Ecological Applications,* Vol.17, pp. 1366–1378.

Rasmussen, E. (1997). The wasting disease of eelgrass (*Zostera marina*) and its effects on environmental factors and fauna, In: *Seagrass ecosystems: a scientific perspective,* McRoy, C. P. & Helfferich, C., (Ed), pp. 1-51, Marcel Dekker, New York, USA.

Roberts, C. M.; Hawkins, J. P. & Gell, F. (2005). The role of marine reserves in achieving sustainable fisheries. *Philosophical Transactions of the Royal Society* B, Vol.360, pp. 123–132.

Sapkota, A.; Sapkota, A. R.; Kucharski, M.; Burke, J.; McKenzie, S.; Walker, P. & Lawrence, R. (2008). Aquaculture practices and potential human health risks: Current knowledge and future priorities. *Environment International,* Vol.34, pp. 1215–1226.

Sepulveda, F.; Marin, S. L. & Carvajal, J. (2004). Metazoan parasites in wild fish and farmed salmon from aquaculture sites in southern Chile. *Aquaculture,* Vol.235, pp. 89-100.

Steinbeck, J. R.; Groff, J. M.; Friedman, C. S.; McDowell, T. & Hedrick, R. P. (1992). Investigations into mortality among populations of the California black abalone, *Haliotis cracherodii*, on the central coast of California, USA, In: *Abalone of the World*, Shephard, S. A.; Tegner, M. J.; Guzmán del Próo, S. A., (Ed), pp. 203–213, Blackwell Scientific, Oxford.

Sweetman, E.; Sweetman, J.; Le Breton, A. & Grisez, L. (1996). Nodavirus: a review of the findings of the XIV/NODA/95 investigation. In: “Seabass and seabream culture: problems and prospects” European Aquaculture Society (ed.). Verona, Italy, 16-18 October 1996, pp. 87-101.

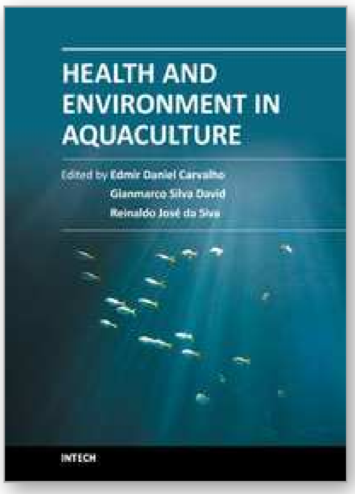
Tanaka, S.; Aoki, H. & Nakai, T. (1998). Pathogenicity of the nodavirus detected from diseased sevenband grouper *Epinephelus septemfasciatus*. *Fish Pathology,* Vol.33, pp. 31–36.

Terlizzi, A.; De Falco, G.; Felline, S.; Fiorentino, D.; Gambi, M. C. & Cancemi, G. (2010). Effects of marine cage aquaculture on macrofauna assemblages associated to *Posidonia oceanica* meadows. *Italian Journal of Zoology,* Vol.77, pp. 362–371.

Terlizzi, A.; Delos, A. L.; Garaventa, F.; Faimali, M. & Geraci, S. (2004). Limited effectiveness of marine protected areas: imposex in *Hexaplex trunculus* (Gastropoda, Muricidae) populations from Italian marine reserves. *Marine Pollution Bulletin,* Vol.48, pp. 188–192.

Thiéry, R.; Cozien, J.; De Boisséson, C.; Kerbart–Boscher, S. & Névarez, L. (2004). Genomic classification of new betanodavirus isolates by phylogenetic analysis of the coat protein gene suggests a low host-fish species specificity. *Journal of General Virology*, Vol.85, pp. 3079-3087.

Trowbridge, C. D. (1995). Establishment of the green alga *Codium fragile* spp. *Tomentosoides* on New Zealand rocky shores: current distribution and invertebrate grazers. *Journal of Ecology,* Vol.83, pp. 949-965.



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