Chapter 8

Temperature-Dependent Photoregulation in Oceanic Picophytoplankton During Excessive Irradiance Exposure

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1. Introduction

The phytoplankton community of open oligotrophic oceans is dominated by prokaryotic *Prochlorococcus* spp., *Synechococcus* spp., and eukaryotic pico- and nanophytoplankton [1-3]. The competitive success of these phytoplankton species depends on different factors, including the response to the (dynamic) irradiance conditions encountered in the water column. With the occurrence of different ecotypes, picophytoplankton species such as *Prochlorococcus* spp., *Synechococcus* spp., and *Ostreococcus* spp. can grow over a broad range of irradiance conditions [4-7]. For example, the low light adapted ecotypes of *Prochlorococcus* are well adapted to the irradiance intensity and spectral composition of the deep chlorophyll maximum with high chlorophyll *b*/*a* ratios and low optimal growth irradiances [4,5,8]. In contrast, the high light adapted ecotypes of *Prochlorococcus* spp. can competitively grow in the (upper) mixed layer with low chlorophyll *b*/*a* ratios and higher optimal growth irradiances [4,5,8]. Similar differences in pigmentation, absorption, and photosynthetic characteristics have been found in ecotypes of marine *Synechococcus* spp. [9-11] and *Ostreococcus* spp. [7,12,13]. In addition to the genetically defined (photo)physiology of the different ecotypes, the photoacclimation potential of specific (pico)phytoplankton species may play an important role in the response to (dynamic) irradiance conditions [11].

Phytoplankton irradiance exposure is strongly influenced by physical processes in the ocean [14]. During stratification, phytoplankton can be trapped in a shallow upper mixed layer, thereby enhancing exposure to photosynthetically active radiation (PAR, 400-700 nm) and ultraviolet radiation (UVR, 280-400 nm), or can experience limiting irradiance conditions at

the deep chlorophyll maximum. In seasonally stratified regions, the period of stratification is interchanged with periods of deep convective mixing that can reach below the euphotic zone. This causes a strong reduction in the daily experienced irradiance, with occasional interruptions of excessive irradiance exposure. Consequently, phytoplankton irradiance exposure in open ocean ecosystems can vary by several orders of magnitude on a time scale ranging from seconds to days. Moreover, short wavelength solar radiation (UVB, 280-315 nm) can penetrate to significant depths in clear oligotrophic waters [15,16].

High irradiance exposure may have considerable effects on photosynthesis and viability in oceanic picophytoplankton species such as *Prochlorococcus* spp., *Synechococcus* spp., and *Ostreococcus* spp. [17-19]. When residing near the surface, picophytoplankton can experience irradiance intensities that exceed photosynthetic requirements. Exposure to excessive PAR and UVR causes photoinhibition, a process in which an over-reduction of the photosynthetic electron transport chain reduces photosynthetic efficiency by a decrease in functional photosystem II (PSII) reaction centers [20]. Moreover, prolonged exposure to excessive irradiance can lead to the uncontrolled formation of reactive oxygen species and viability loss [21,22]. To prevent photoinhibition and viability loss during excessive irradiance exposure, phytoplankton regulate light harvesting and other photosynthetically important processes. In prokaryotic species, the utilization of light harvesting energy can be regulated by state transitions, in which the light harvesting antenna of the phycobillisome (PBS) is redistributed between the reaction centers of photosystem I (PSI) and PSII [23,24]. In addition, light harvesting energy can be regulated by the thermal dissipation of excess energy. This photoprotective process can occur within seconds after irradiance changes in both prokaryotic and eukaryotic phytoplankton species, but the underlying mechanisms are considerably different. In eukaryotic species, the thermal dissipation of excess energy involves the xanthophyll pigment cycle. Epoxidized xanthophyll cycle pigments assist in light harvesting, whereas de-epoxidized equivalents dissipate excess energy in the form of heat [25]. In PBS containing cyanobacteria, the thermal dissipation of excess energy involves the orange carotenoid protein [24,26]. In *Prochlorococcus* spp., these proteins are not observed and the underlying mechanism remains unknown [24,27]. In addition to the regulation of light harvesting, photoinhibition and viability loss may be avoided by the increase of photochemical quenching by enhancing alternative electron transport and (non-)enzymatic scavenging of reactive oxygen species [28,29]. Simultaneously, phytoplankton can counteract the effects of photoinhibition by photorepair, a process in which damaged D1 proteins are removed from PSII and replaced by newly synthesized D1 proteins [20].

Although it has previously been reported that temperature may have a positive effect on the survival of picophytoplankton under high irradiance conditions [30], no direct assessment of the temperature-dependency of photoregulation during high PAR and UVR exposure is available for this specific phytoplankton group. A recent study showed that both prokaryotic and eukaryotic picophytoplankton may be less susceptible to the negative effects of high irradiance intensities at elevated temperatures [31]. In the prokaryotic species *Prochlorococcus* spp. (eMED4 and eMIT9313) and the eukaryotic species *Ostreococcus* sp. (clade B) and

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Pelagomonas calceolata, acclimation to elevated temperatures enhanced photoacclimation to higher irradiance intensities and reduced photoinhibition [31]. This has also been found in larger phytoplankton species, such as the diatom species *Chaetoceros gracilis*, *Thalassiosira pseudonana*, and *Thalassiosira weissflogii* [32-34]. In cyanobacteria and eukaryotic nanophytoplankton, reduced levels of photoinhibition at elevated temperatures may be associated with enhanced rates of state transitions [24], enhanced enzymatic conversions of the xanthophyll pigment cycle [35], enhanced D1 repair [36], and the potential enhancement of Rubisco activity [34]. However, the potential role of these photoregulating mechanisms at elevated temperatures remains unknown in oceanic picophytoplankton.

In the present study, a comparative analysis of the high irradiance sensitivity of oceanic picophytoplankton was performed to study the combined effect of elevated temperatures and irradiance levels near the surface of open oligotrophic oceans. To this end, two prokaryotic and two eukaryotic strains were acclimated to 16 °C, 20 °C, and 24 °C, after which they were exposed to a single high PAR dose, with and without UVR. The response to and the recovery after high irradiance exposure was assessed by analysis of PSII fluorescence and pigmentation in order to investigate immediate photoinhibition and photoprotective processes. The results are discussed in the context of differences between oceanic picophytoplankton species and are used to unravel the importance of photoinhibition in structuring the phytoplankton community in open oligotrophic oceans.

2. Method

2.1. Culture conditions

Cultures were obtained from the Roscoff Culture Collection (RCC) and the Provasoli-Guillard National Center for Marine Algae and Microbiota (NCMA). The strains were all isolated from oligotrophic regions and are representative for low light (LL) and high light (HL) adapted species in open ocean ecosystems. *Prochlorococcus marinus* strain CCMP2389 (ecotype MED4, HL) and *Prochlorococcus* sp. strain RCC407 (ecotype MIT9313, LL) were cultured in K/10-Cu medium based on natural oceanic seawater as described by [37]. *Ostreococcus* sp. strain RCC410 (clade B, LL) and *Pelagomonas calceolata* strain RCC879 (LL) were cultured in K medium as described by [38]. Cultures were maintained in 100 ml glass Erlenmeyer flasks at 9 μmol photons m^{-2} s^{-1} (*Prochlorococcus* sp. and *P. calceolata*) and 68 μmol photons m^{-2} s^{-1} (*P. marinus* and *Ostreococcus* sp.) in a diurnal cycle of 12:12 h light:dark at 20 °C.

2.2. Experimental design

Cultures of *P. marinus*, *Prochlorococcus* sp., *Ostreococcus* sp., and *P. calceolata* were transferred to 500 ml glass Erlenmeyer flasks and incubated in triplicate at 16 °C, 20 °C, and 24 °C. Experiments were carried out in a temperature controlled U-shaped lamp setup as described by [39]. The temperature in the setup was maintained at 16 °C, 20 °C, and 24 °C by a thermostat (RK 8 KS, edition 2000, Lauda Dr. R. Wobser & Co.) and deviated less than ± 0.5 °C. During the experiments, 50 μmol photons m^{-2} s^{-1} PAR (Biolux and Skywhite lamps,

Osram) was provided as a square wave function with a 12:12 h light:dark cycle (monitored with a QSL-100, Biospherical Instruments). Prior to the experiments, the picophytoplankton strains were kept in exponential growth phase and acclimated to the experimental irradiance and temperature conditions for at least three weeks. In mid-exponential growth phase, the response to high photosynthetically active radiation (PAR, 400-700 nm), with and without ultraviolet radiation (UVR, 290-400 nm), was assessed at growth temperature by pigment and PSII chlorophyll fluorescence analysis. To this end, 200 ml of each replicate culture was exposed to high PAR and PAR+UVR for 10 min in a temperature controlled (RTE-211, Neslab Instruments Inc.) irradiance set-up at 16 °C, 20 °C, or 24 °C. The irradiance set-up provided ± 500 μmol photons m^{-2} s^{-1} by a 250 W MHN-TD lamp (Philips) and two 20 W TL/12 UVB fluorescent lamps (Philips), in which the PAR and PAR +UVR conditions were obtained by using the long pass filters GG395 and WG305 (Schott AG, Mainz), respectively (Table 1). Prior to exposure (t = 0), samples for the analysis of pigmentation and the maximum quantum yield of PSII (F_v/F_m) were collected and measured as described below. After exposure, treated culture samples were transferred to dim light conditions at growth temperature (16 °C, 20 °C, or 24 °C). Subsequently, samples for the analysis of pigmentation were taken at t = 10, 20, and 40 min and recovery of the quantum yield of PSII (Φ_{PSII}) was determined at t = 10, 15, 20, 25, 30, 35, 40, 60, 80, and 100 min for both PAR and PAR+UVR treated cultures. Culturing of *Prochlorococcus* sp. at 16 °C and 50 μmol photons m^{-2} s^{-1} was attempted several times, but this condition exceeded the limit for growth of this individual strain. No measurements were performed for *Prochlorococcus* sp. under these conditions.

	PAR	PAR+UVR
PAR	172	157
UVA	8.69	15.3
UVB	0.05	1.79

Table 1. Doses (W m^{-2}) for photosynthetically active radiation (PAR, 400-700 nm) and ultraviolet radiation A (UVA, 315-400 nm) and B (UVB, 290-315 nm) are given for the PAR and PAR+UVR treatments during the experiments. Total irradiance intensity was ± 500 μmol photons m^{-2} s^{-1} in both treatments.

2.3. Photosystem II chlorophyll fluorescence characteristics

PSII fluorescence analyses were performed on a WATER-PAM chlorophyll fluorometer (Waltz GmbH) equipped with a WATER-FT flow-through emitter-detector unit and analyzed using WinControl software (version 2.08, Waltz GmbH) according to [40] and references therein. Prior to exposure to PAR and PAR+UVR (t = 0), 5-15 ml culture samples were dark-adapted for 20 min at 16 °C, 20 °C, or 24 °C. For analysis, the measuring light was turned on and F_0 was recorded as the minimal fluorescence. During a saturating light flash, $F_m°$ was then recorded as the maximum fluorescence in the dark-adapted state. The maximum quantum yield of PSII (F_v/F_m) was calculated as ($F_m° - F_0$) / $F_m°$. After exposure (t = 10-100), the quantum yield of PSII (Φ_{PSII}) was determined by measuring F_t as the steady state fluorescence prior to the saturating light flash and F_m' as the maximum fluorescence

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in the light. Φ_{PSII} was calculated as ($F_m' - F_t$) / F_m'. From the F_v/F_m measurements at t = 0 and the Φ_{PSII} measurements at t = 10, total non photochemical quenching (NPQ) was calculated as ($F_m° - F_m'$) / F_m'. Relaxation analysis was performed to estimate the contribution of slowly and rapidly relaxing non photochemical quenching. Relaxation of NPQ on a time scale of minutes is associated with photoprotective processes such as state transitions, relaxation of the xanthophyll pigment cycle or other forms of thermal dissipation [35, 40,41]. Processes that relax over a longer period of time (hours) are referred to as photoinhibition, i.e. damage to the reaction centers of PSII [40,42]. To estimate photoprotection and photoinhibition, the recorded F_m' was corrected for baseline quenching by subtracting F_0 and was log transformed for further analysis. Transformed F_m' values of the final 60 min of the Φ_{PSII} recovery curve were extrapolated to calculate the value of F_m' that would had been attained if only slowly relaxing quenching was present in the light (F_m^r). Slow relaxing non photochemical quenching (NPQ_S) was then calculated as ($F_m° - F_m^r$) / F_m^r and fast relaxing non photochemical quenching (NPQ_F) as ($F_m° / F_m'$) - ($F_m° - F_m^r$). In addition, the contribution of UVR to the decrease in quantum yield of PSII during irradiance exposure was calculated as ($\Phi_{PSII,PAR} - \Phi_{PSII,PAR+UVR}$) / $\Phi_{PSII,PAR} * 100$ [43].

2.4. Pigment composition

Samples (25-30 ml) for untreated (t = 0), PAR treated (t = 10, 20, 40), and PAR+UVR (t = 10, 20, 40) treated cultures were filtered onto 25 mm GF/F filters (Whatman), snap frozen in liquid nitrogen, and stored at -80 °C until further analysis. Pigments were quantified using High Performance Liquid Chromatography (HPLC) as described by [44]. In short, filters were freeze-dried for 48 h and pigments were extracted in 3 ml 90% acetone (v/v, 48 h, 4 °C). Detection of pigments was carried out using a HPLC (Waters 2695 separation module, 996 photodiode array detector) equipped with a Zorbax Eclipse XDB-C_8 3.5 μm column (Agilent Technologies, Inc.). Peaks were identified by retention time and diode array spectroscopy. Pigments were quantified using standards (DHI LAB products) of chlorophyll a_1, chlorophyll a_2, diadinoxanthin (Dd), diatoxanthin (Dt), violaxanthin (Vio), antheraxanthin (Ant), and zeaxanthin (Zea). From here on, chlorophyll *a* (Chl-a) will refer to chlorophyll a_2 in *P. marinus* and *Prochlorococcus* sp. and to chlorophyll a_1 in *Ostreococcus* sp. and *P. calceolata*. The de-epoxidation state (DPS) of the xanthophyll pigment cycle was calculated as (Ant + Zea) / (Vio + Ant + Zea) for *Ostreococcus* sp. and as Dt / (Dd + Dt) for *P. calceolata*. In addition to the DPS, the rate of de-epoxidation of the xanthophyll pigment cycle (k_{DPS} in min^{-1}) was estimated as the increase in DPS during exposure to high PAR and PAR+UVR [45].

2.5. Statistical analysis

All measurements were performed for triplicate cultures (n = 3) at each temperature. Differences between the three temperature conditions, differences between irradiance treatments, and differences between species were statistically tested by analysis of variance (ANOVA) using STATISTICA software (version 8.0 and 10.0, StatSoft Inc.). Before analysis, data were tested for normality and homogeneity of variances. Differences were considered significant when $p < 0.05$.

3. Results

3.1. Non photochemical quenching and photosystem II recovery

P. marinus, *Prochlorococcus* sp., *Ostreococcus* sp., and *P. calceolata* all showed non photochemical quenching (NPQ) upon exposure to high photosynthetically active radiation (PAR), with and without ultraviolet radiation (UVR) (Figure 1). The effect of temperature on NPQ was most pronounced in the prokaryotic strains *P. marinus* and *Prochlorococcus* sp. (Figure 1). Although total NPQ did not change with temperature in *P. marinus*, the proportion of slow and fast non photochemical quenching changed significantly. Slow relaxing non photochemical quenching (NPQ_S) decreased with increasing temperature ($p < 0.05$, not significant between 20 °C and 24 °C), whereas fast relaxing non photochemical quenching (NPQ_F) increased significantly with increasing temperature ($p < 0.05$). In *Prochlorococcus* sp., total NPQ increased from 20 °C to 24 °C (Figure 1). The proportion of NPQ_S and NPQ_F was also affected by temperature in *Prochlorococcus* sp., with a significant increase in NPQ_F with increasing temperature ($p < 0.05$) and unchanged levels of NPQ_S. In the eukaryotic species *Ostreococcus* sp., temperature had no effect on NPQ (Figure 1). In *P. calceolata*, total NPQ decreased with increasing temperature ($p < 0.05$, not significant between 20 °C and 24 °C). This was associated with a decrease in NPQ_S with increasing temperature ($p < 0.05$, not significant between 16 °C and 20 °C), whereas NPQ_F remained unaffected by temperature.

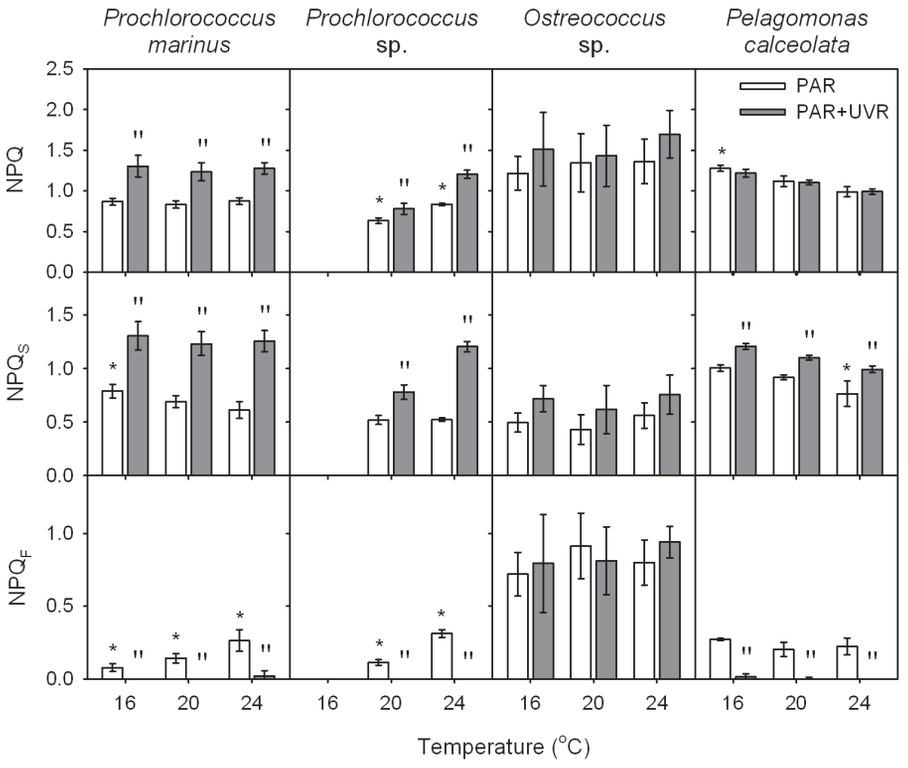

Figure 1. Non photochemical quenching. Mean (± standard deviation, $n = 3$) total non photochemical quenching (NPQ), slow relaxing NPQ (NPQ_S), and fast relaxing NPQ (NPQ_F) are given for *Prochlorococcus marinus* eMED4, *Pro-*

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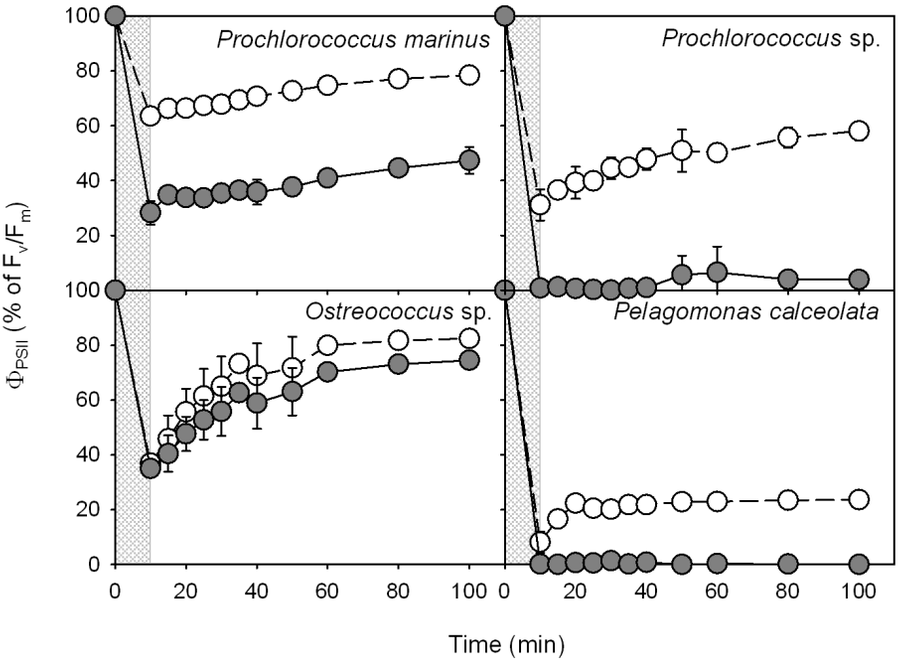

Figure 2. Recovery of PSII after high irradiance exposure. Mean (± standard deviation, n = 3) quantum yield of PSII (Φ_{PSII} in % of F_v/F_m) during and after exposure to high irradiance for *Prochlorococcus marinus* eMED4, *Prochlorococcus* sp. eMIT9313, *Ostreococcus* sp. clade B, and *Pelagomonas calceolata* acclimated to 20 °C. The picophytoplankton strains were exposed to high photosynthetically active radiation (PAR, white circles) and high PAR with ultraviolet radiation (PAR+UVR, dark grey circles) for 10 minutes (light grey area).

chlorococcus sp. eMIT9313, *Ostreococcus* sp. clade B, and *Pelagomonas calceolata* at 16 °C, 20 °C and 24 °C. The picophytoplankton strains were exposed to high photosynthetically active radiation (PAR, white bars) and high PAR with ultraviolet radiation (PAR+UVR, grey bars) for 10 minutes. Significant effects ($p < 0.05$) of the growth temperature (*) and the spectral composition of the irradiance treatment (") are indicated.

The spectral composition of the irradiance treatment influenced non photochemical quenching and the recovery of the quantum yield of PSII (Φ_{PSII}) considerably in the prokaryotic strains (Figure 1, Figure 2). In both *P. marinus* and *Prochlorococcus* sp., total NPQ and NPQ_S were significantly higher during exposure to PAR+UVR compared with PAR, whereas NPQ_F decreased significantly during exposure to UVR ($p < 0.05$) (Figure 1). In *Prochlorococcus* sp., this was associated with almost no recovery of Φ_{PSII} after exposure to PAR+UVR (Figure 2). In the eukaryotic species *Ostreococcus* sp., the spectral composition of the irradiance treatment did not have a significant effect on NPQ (Figure 1). However, recovery of Φ_{PSII} in *Ostreococcus* sp. was lower after exposure to PAR+UVR compared with PAR (significant for t = 60-100, $p < 0.05$, Figure 2). In *P. calceolata*, exposure to PAR+UVR significantly increased NPQ_S and decreased NPQ_F ($p < 0.05$), but total NPQ remained unaffected by the spectral composition of the irradiance treatment (Figure 1). *P. calceolata* showed no recovery of Φ_{PSII} after exposure to PAR +UVR (Figure 2).

Comparison of NPQ between the different picophytoplankton strains demonstrated significantly lower total NPQ in the prokaryotic species *P. marinus* and *Prochlorococcus* sp. compared with the eukaryotic species *Ostreococcus* sp. and *P. calceolata* ($p < 0.05$) (Figure 1). In *P. calceolata*, NPQ_S was significantly higher compared with the other species ($p < 0.05$, not significant

at 24 °C). *P. marinus* and *Prochlorococcus* sp. showed intermediate levels of NPQ_S, whereas *Ostreococcus* sp. showed significantly lowest NPQ_S ($p < 0.05$, not significant at 24 °C). The relative low levels of NPQ_S in *Ostreococcus* sp. were accompanied by significantly higher NPQ_F compared with the other species ($p < 0.005$). No differences in NPQ_F were found between *P. marinus*, *Prochlorococcus* sp., and *P. calceolata*.

3.2. Inhibition of photosystem II by ultraviolet radiation

The inhibition of Φ_{PSII} due to UVR was affected by temperature in *P. marinus*, *Ostreococcus* sp., and *P. calceolata* (Table 2). In *P. marinus*, UVR inhibition decreased significantly with increasing temperature ($p < 0.01$ for 16 °C compared with 24 °C). In the eukaryotic species *Ostreococcus* sp. (not between 20 °C and 24 °C) and *P. calceolata*, UVR inhibition of Φ_{PSII} also decreased with increasing temperature, but not significantly. In *Prochlorococcus* sp., no effect of temperature was found on the UVR inhibition of Φ_{PSII}. Comparison of the different picophytoplankton strains showed that *Ostreococcus* sp. was least inhibited by UVR ($p < 0.001$) (Figure 2, Table 2). *P. marinus* showed intermediate levels of UVR inhibition, whereas Φ_{PSII} was most inhibited by UVR in *Prochlorococcus* sp. and *P. calceolata* ($p < 0.001$).

	Prochlorococcus marinus	***Prochlorococcus*** sp.	***Ostreococcus* sp.**	***Pelagomonas calceolata***
16 °C	66.4 ± 3.9[a]	n/a	24.5 ± 18.1	100.0 ± 0.0
20 °C	55.6 ± 4.9	97.9 ± 3.6	5.3 ± 5.4	97.3 ± 4.7
24 °C	49.5 ± 4.8[a]	97.3 ± 3.8	13.0 ± 5.5	77.0 ± 20.7

Table 2. Mean (± standard deviations, $n = 3$) inhibition by ultraviolet radiation (% of photosynthetically active radiation treatment) after 10 min high irradiance exposure in *Prochlorococcus marinus* eMED4, *Prochlorococcus* sp. eMIT9313, *Ostreococcus* sp. clade B, and *Pelagomonas calceolata* acclimated to 16 °C, 20 °C, and 24 °C. *abc* indicate significant effects of the temperature treatment within each species. n/a: data not available, growth was not observed under the used conditions and no additional measurements were performed.

3.3. Photoprotective pigmentation

Temperature acclimation affected the initial photoprotective pigment pool in *P. marinus* (t = 0, Table 3), with higher zeaxanthin per chlorophyll *a* levels at lower temperatures ($p < 0.001$). In *Prochlorococcus* sp., no significant effect of temperature acclimation was observed in the initial zeaxanthin per chlorophyll *a* level. In both prokaryotic strains, exposure to high irradiance did not influence photoprotective pigmentation, as the zeaxanthin per chlorophyll *a* levels remained similar during and after high irradiance exposure (Table 3). In *Ostreococcus* sp., acclimation to higher temperatures increased the initial xanthophyll cycle pigment pool (30-40%), but not significantly (t = 0, Table 3). In response to high irradiance exposure, large fluctuations in the sum of violaxanthin, antheraxanthin, and zeaxanthin per chlorophyll *a* were observed and no significant effect of temperature acclimation on the photoprotective pigment pool was found (Table 3). In *P. calceolata*, the initial photoprotective pigments per chlorophyll *a* ratio was highest at 24 °C (19 %, not significant). Temperature had no effect on the total xanthophyll cycle pigment pool in response to high irradiance in *P. calceolata* as the sum of

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diadinoxanthin and diatoxanthin per chlorophyll *a* remained unchanged during and after exposure to high irradiance (Table 3). No significant effect of the spectral composition of the irradiance treatment was observed in the photoprotective pigments pools of *P. marinus*, *Prochlorococcus* sp., *Ostreococcus* sp., and *P. calceolata* (Table 3).

	PAR			PAR+UVR		
	16 °C	**20 °C**	**24 °C**	**16 °C**	**20 °C**	**24 °C**
Prochlorococcus marinus						
t = 0	0.647±0.060[a]	0.499±0.004[a]	0.431±0.007[a]	0.647±0.060[b]	0.499±0.004[b]	0.431±0.007[b]
t = 10	0.644 ± 0.081	0.488 ± 0.019	0.434 ± 0.017	0.649 ± 0.057	0.488 ± 0.013	0.426 ± 0.017
t = 20	0.657 ± 0.066	0.493 ± 0.006	0.426 ± 0.031	0.655 ± 0.071	0.494 ± 0.002	0.421 ± 0.031
t = 40	0.661 ± 0.067	0.498 ± 0.009	0.424 ± 0.014	0.663 ± 0.053	0.492 ± 0.013	0.437 ± 0.014
Prochlorococcus sp.						
t = 0	n/a	1.062 ± 0.034	1.025 ± 0.023	n/a	1.062 ± 0.034	1.025 ± 0.023
t = 10	n/a	1.206 ± 0.076	0.976 ± 0.009	n/a	1.198 ± 0.039	0.946 ± 0.039
t = 20	n/a	1.209 ± 0.093	0.966 ± 0.036	n/a	1.189 ± 0.059	0.936 ± 0.032
t = 40	n/a	1.226 ± 0.088	0.996 ± 0.041	n/a	1.192 ± 0.044	0.974 ± 0.039
Ostreococcus sp.						
t = 0	0.079 ± 0.030	0.057 ± 0.024	0.061 ± 0.014	0.079 ± 0.030	0.057 ± 0.024	0.061 ± 0.014
t = 10	0.109 ± 0.017	0.062 ± 0.026	0.084 ± 0.032	0.058 ± 0.004	0.053 ± 0.012	0.060 ± 0.013
t = 20	0.091 ± 0.036	0.052 ± 0.020	0.060 ± 0.009	0.109 ± 0.003	0.082 ± 0.002	0.105 ± 0.008
t = 40	0.106 ± 0.005	0.078 ± 0.006	0.074 ± 0.031	0.109 ± 0.003	0.079 ± 0.014	0.077 ± 0.020
Pelagomonas calceolata						
t = 0	0.089 ± 0.008	0.089 ± 0.005	0.106 ± 0.012	0.089 ± 0.008	0.089 ± 0.005	0.106 ± 0.012
t = 10	0.096 ± 0.009	0.095 ± 0.002	0.106 ± 0.013	0.092 ± 0.010	0.094 ± 0.004	0.103 ± 0.014
t = 20	0.093 ± 0.005	0.096 ± 0.007	0.107 ± 0.014	0.080 ± 0.031	0.093 ± 0.005	0.105 ± 0.014
t = 40	0.093 ± 0.009	0.094 ± 0.006	0.109 ± 0.013	0.090 ± 0.008	0.092 ± 0.005	0.104 ± 0.012

Table 3. Mean (± standard deviations, $n = 3$) photoprotective pigments per chlorophyll *a* ratio in *Prochlorococcus marinus* eMED4 (zeaxanthin), *Prochlorococcus* sp. eMIT9313 (zeaxanthin), *Ostreococcus* sp. clade B (violaxanthin, antheraxanthin, and zeaxanthin), and *Pelagomonas calceolata* (diadinoxanthin and diatoxanthin) acclimated to 16 °C, 20 °C, and 24 °C. Pigment ratios were obtained before (t = 0) and after (t = 10, 20, 40) exposure to high photosynthetically active radiation (PAR), with and without ultraviolet radiation (UVR). *abc* indicate significant effects of the temperature treatment within each species. n/a: data not available, growth was not observed under the used conditions and no additional measurements were performed.

3.4. De-epoxidation of the xanthophyll cycle

In both *Ostreococcus* sp. and *P. calceolata,* the de-epoxidation state (DPS) of the xanthophyll pigment cycle increased significantly during exposure to high irradiance ($p < 0.001$) (Figure 3). In both strains, the DPS of the xanthophyll pigment cycle decreased over time, but the DPS did not return to initial values after 30 min of recovery in low light conditions (t= 40, Figure 3). In *Ostreococcus* sp., the de-epoxidation of the xanthophyll pigment cycle mainly included the de-epoxidation of violaxanthin to antheraxanthin, whereas the de-epoxidation of antheraxanthin to zeaxanthin was small. Temperature had an effect on the DPS of the xanthophyll

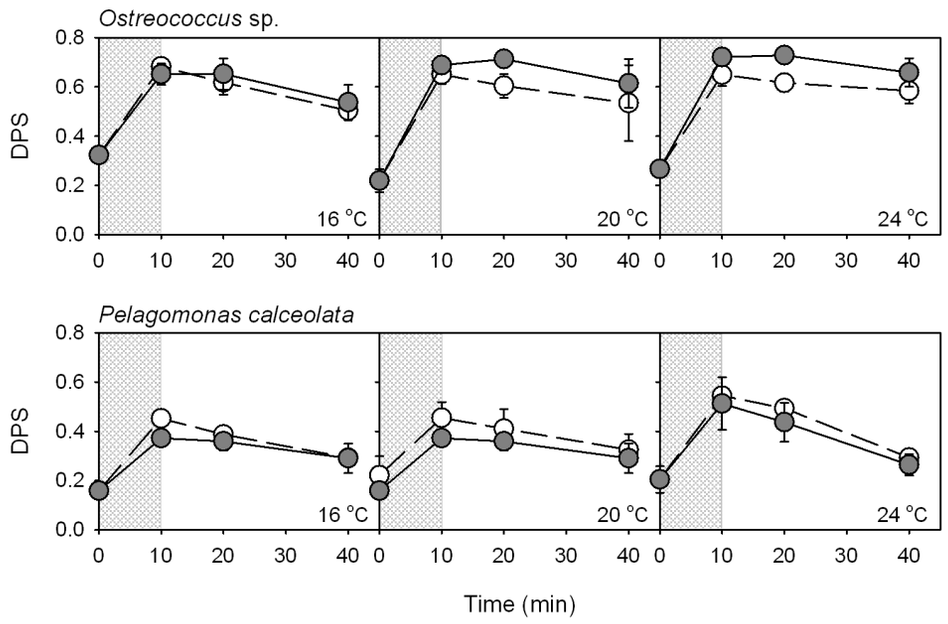

Figure 3. De-epoxidation of the xanthophyll pigment cycle. Mean (± standard deviation, *n* = 3) de-epoxidation state (DPS) of the xanthophyll pigment cycle in *Ostreococcus* sp. clade B and *Pelagomonas calceolata* are given during and after 10 minutes of exposure to high photophotosynthetically active radiation (PAR, white circles) and high PAR with ultraviolet radiation (PAR+UVR, grey circles) at 16 °C, 20 °C, and 24 °C.

pigment cycle in *Ostreococcus* sp. (Figure 3, Table 4), but differences were mostly not significant. The initial DPS of the xanthophyll pigment cycle (t = 0) in *Ostreococcus* sp. was 21-47% higher at 16 °C compared with 20 °C and 24 °C. During exposure to high PAR and PAR+UVR, the increase in the DPS was fastest at 20 °C (Table 4), as was the epoxidation of the xanthophyll pigment cycle after exposure to high irradiance (Figure 3). In *P. calceolata*, the initial DPS of the xanthophyll pigment cycle was 22-28% lower at 16 °C compared with the higher temperatures (not significant) (Figure 3). During irradiance exposure, the rate of de-epoxidation of the xanthophyll pigment cycle increased with increasing temperature in *P. calceolata* (not significant) (Figure 3, Table 4). In accordance with the rate of de-epoxidation, the epoxidation of the xanthophyll pigment cycle was fastest at 24 °C ($p < 0.05$).

The effect of the spectral composition of the irradiance treatment on the de-epoxidation of the xanthophyll pigment cycle was most evident in *Ostreococcus* sp. (Figure 3). During irradiance exposure (t = 0-10), the DPS in *Ostreococcus* sp. did not differ significantly between the PAR and PAR+UVR treatment (Figure 3, Table 4). However, in the PAR treatment, epoxidation of the xanthophyll pigment cycle started directly after exposure (t = 10), whereas the epoxidation was delayed in the PAR+UVR treatment and started after 10 minutes of recovery in low light (t = 20). After 30 minutes of recovery (t = 40), the DPS in *Ostreococcus* sp. was similar in both PAR and PAR+UVR treatments (Figure 3). In *P. calceolata*, no significant effect of the spectral composition of the irradiance treatment was found, but it seemed that exposure to UVR limited the de-epoxidation of the xanthophyll pigment cycle, especially at lower temperatures (Figure 3).

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When the dynamics of the xanthophyll pigment cycle of both species were compared, it was shown that *Ostreococcus* sp. had a significantly higher DPS compared with *P. calceolata* ($p < 0.05$) (Figure 3). In addition, the increase in de-epoxidation of the xanthophyll pigment cycle during high irradiance exposure was faster in *Ostreococcus* sp. ($p < 0.05$) (Table 3), whereas no differences in epoxidation rate were observed between *Ostreococcus* sp. and *P. calceolata.*

	Ostreococcus sp.	***Pelagomonas calceolata***
PAR		
16 °C	$0.036 \pm 3.75 \times 10^{-3}$	$0.029 \pm 2.76 \times 10^{-4}$
20 °C	$0.043 \pm 6.01 \times 10^{-3}$	$0.030 \pm 4.53 \times 10^{-3}$
24 °C	$0.038 \pm 6.33 \times 10^{-3}$	$0.034 \pm 5.39 \times 10^{-3}$
PAR+UVR		
16 °C	$0.033 \pm 4.79 \times 10^{-3ab}$	$0.021 \pm 6.38 \times 10^{-4}$
20 °C	$0.047 \pm 4.08 \times 10^{-3a}$	$0.023 \pm 5.33 \times 10^{-3}$
24 °C	$0.045 \pm 1.12 \times 10^{-3b}$	$0.031 \pm 7.34 \times 10^{-3}$

Table 4. Mean (± standard deviation, $n = 3$) rate of increase in the de-epoxidation state of the xanthophyll pigment cycle (k_{DPS} in min^{-1}) in *Ostreococcus* sp. clade B and *Pelagomonas calceolata* during exposure to high photosynthetically active radiation (PAR) and high PAR with ultraviolet radiation (PAR+UVR) at 16 °C, 20 °C, and 24°C. *abc* indicate significant effects of the temperature treatment within each species.

4. Discussion

Climate change is expected to mediate a rise in seawater temperature by 1.5-4.5 °C over the next century [46]. This rise in seawater temperature will lead to changes in water column stratification in open oligotrophic oceans [47,48]. The subsequent modifications in mixed layer dynamics increase the exposure of phytoplankton to high levels of photosynthetic active radiation (PAR) and ultraviolet radiation (UVR). Because temperature and irradiance conditions play an important role in the success of specific oceanic phytoplankton species [4,49,50], it is important to understand how oceanic phytoplankton will respond to elevated temperatures and whether this will affect their (photo)physiological performance. The present study focused on the temperature-dependence of photoinhibition and photoregulating processes that are essential for survival during high (dynamic) irradiance conditions.

During short periods of high irradiance exposure, both the prokaryotic picophytoplankton strains *P. marinus* and *Prochlorococcus* sp., as the eukaryotic picophytoplankton strains *Ostreococcus* sp. and *P. calceolata* were susceptible to photoinhibition. The response to high irradiances was species specific and appeared to be related to the genetically defined light adaptation of the different strains. In the prokaryotic species, the low light adapted ecotype *Prochlorococcus* sp. (eMIT9313) was highly sensitive to high PAR and UVR, whereas the high light adapted ecotype *P. marinus* (eMED4) showed lower sensitivity. Similar differen-

ces in photoinhibition during high irradiance exposure were observed for other low and high light adapted ecotypes of *Prochlorococcus* spp. during exposure to high blue irradiance [18]. The differential response to excessive irradiance intensities found in the present study related well to the occurrence of different *Prochlorococcus* ecotypes in the upper mixed layer (eMED4) and the deep chlorophyll maximum (eMIT9313) [4,49]. In the eukaryotic species, the levels of total non photochemical quenching induced by a tenfold increase in irradiance intensity were similar compared with earlier observations for *Ostreococcus* sp. and *P. calceolata* [12,51]. Although the two eukaryotic species were both isolated at 100 m depth from oceanic regions, *Ostreococcus* sp. showed considerably lower levels of photoinhibition compared with *P. calceolata*, especially during UVR exposure. It therefore seems that *Ostreococcus* sp. clade B is not specifically adapted to low light [7], but rather adapted to open ocean irradiance conditions (also see [50,52]) with a relatively low sensitivity to high irradiance intensities compared with other oceanic picophytoplankton [this study, 11,31]. The low light adapted ecotype *P. calceolata* showed highest levels of photoinhibition during exposure to high PAR compared with the other species. However, photoinhibition increased dramatically in the prokaryotic strains during exposure to UVR. This confirms the relative sensitivity of *Prochlorococcus* spp. to high levels of UVR, as has been observed in oligotrophic waters [53,54].

Temperature acclimation influenced photoinhibition and related processes during high irradiance exposure in *P. marinus*, *Prochlorococcus* sp., *Ostreococcus* sp., and *P. calceolata*. The effect was not uniform among the different strains, but temperature acclimation influenced the response to high irradiance exposure by changes in the relative contribution of photoinhibition and photoprotective mechanisms to non photochemical quenching in all strains. This general response corresponds well with the observation that both prokaryotic and eukaryotic picophytoplankton may benefit from high irradiance intensities at elevated temperatures by alterations in photophysiology and electron transport [31]. In addition, elevated temperatures had a beneficial effect on the response to high irradiance intensities by partially counteracting the UVR-induced photoinhibition in *P. marinus*, *Ostreococcus* sp., and *P. calceolata*. This was earlier observed in several diatom species and related to an increase in Rubisco activity and gene expression in *Thalassiosira weissflogii* [34], an increase in repair rates in *T. pseudonana* [32], and an increase in photoprotection by the dissipation of excess energy in *T. weissflogii* and *C. gracillis* [33]. In this study, fast relaxing non photochemical processes, i.e. photoprotection, and the influence of temperature acclimation on these processes was further investigated in the response to excessive irradiance intensities in oceanic picophytoplankton.

Both low and high light adapted *Prochlorococcus* strains were capable of producing fast relaxing non photochemical quenching (NPQ_F). Interestingly, the level of NPQ_F in the low light adapted strain *Prochlorococcus* sp. (eMIT9313/clade LLIV) was considerably higher compared with that of another low light adapted strain of *Prochlorococcus* (strain SS120/clade LLII) [27]. It therefore seems that some low light adapted ecotypes of *Prochlorococcus* are capable of inducing high levels of NPQ_F comparable to that of high light adapted ecotypes (this study), but others are not [27]. This might possibly be related to the differential occurrence of *pcb* genes encoding the

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major chlorophyll binding and light harvesting antenna proteins in both low and high light adapted ecotypes of *Prochlorococcus* [27,55]. Although the precise underlying mechanism remains unknown, the process of NPQ_F in *P. marinus* and *Prochlorococcus* sp. was sensitive to changes in temperature. It is therefore likely that the underlying mechanisms of NPQ_F in *Prochlorococcus* spp. involves an enzymatic reaction or changes due to the improved fluidity of the thylakoid membrane at elevated temperatures [56,57]. This contrasts to earlier observations of NPQ_F in phycobillisome containing cyanobacteria [58] (for a review see [24]), which supports the notion that the underlying mechanisms are different between *Prochlorococcus* spp. and other prokaryotic species [24]. It was further shown in the present study that the mechanism of photoprotection in *P. marinus* and *Prochlorococcus* sp. was highly sensitive to UVR, possibly related to increased oxidative stress on the thylakoid membrane [59]. Fast relaxing non photochemical quenching was not related to changes in pigmentation during high irradiance exposure in *P. marinus* and *Prochlorococcus* sp. The xanthophyll pigment zeaxanthin is not regulated by an epoxydation/de-epoxidation cycle in prokaryotic species and its function is often debated [60,61]. However, the photoprotective role of zeaxanthin is not excluded, since the concentration of zeaxanthin increases relative to chlorophyll *a* in high light acclimated cells [8,11,61] and zeaxanthin is found in high concentrations in the field [62,63]. The presence of zeaxanthin might have overestimated the calculation of photoinhibition by slowly relaxing non photochemical quenching in the light-harvesting antenna of PSII (F_0 quenching) [40,64]. This was however, not observed in *P. marinus* and *Prochlorococcus* sp. (data not shown), suggesting that slowly relaxing non photochemical quenching related to damage to the reaction center of PSII in these strains.

In the eukaryotic picophytoplankton species *Ostreococcus* sp. and *P. calceolata*, fast relaxing non photochemical quenching coincided with the de-epoxidation of the xanthophyll pigment cycle. The rate of de-epoxidation of the xanthophyll pigment cycle in *Ostreococcus* sp. and *P. calceolata* was within the range reported for other eukaryotic pico- and nanophytoplankton [45], as was the relative increase in the de-epoxidation state of the xanthophyll pigment cycle upon high irradiance exposure [12,19,45,51]. For *Ostreococcus* sp. clade B it was previously shown that both the xanthophyll pigment cycle [19] and alternative electron transport [13] play an important role in the response to high irradiance, whereas photorepair is relatively slow compared with other *Ostreococcus* ecotypes [19]. This study showed that the photoprotective processes were also effective during UVR exposure, since *Ostreococcus* sp. was the only strain used in this study that showed substantial NPQ_F during UVR exposure. The influence of temperature acclimation was also most pronounced during UVR exposure, especially on the xanthophyll pigment cycle. Different effects may add to the high levels of fast relaxing non photochemical quenching observed in *Ostreococcus* sp. The xanthophyll cycle pigments may have an additional photoprotective function in *Ostreococcus* sp., including the stabilization of the thylakoid membrane by antheraxanthin and zeaxanthin, providing protection against reactive oxygen species under conditions of a highly reduced electron transport chain (for a review see [65]). In addition, the de-epoxidation of the xanthophyll pigment cycle and the consequent non photochemical quenching in *Ostreococcus* sp. may be promoted by an increase in the trans-membrane proton gradient due to the presence of chlororespiratory electron flow [13,65]. In *P. calceolata*,

the rate of de-epoxidation and the relative de-epoxidation of the xanthophyll pigment cycle increased at elevated temperature, but this was not associated with an increase in fast relaxing non photochemical quenching. It is possible that the membrane stability necessary for the dissipation of excess energy trough the xanthophyll pigment cycle was affected by oxidative stress [66,67]. This could also explain the diminished fast relaxing non photochemical levels during UVR exposure in this species. Because *P. calceolata* is a low light adapted ecotype, this species might possibly use additional photoprotective mechanisms, such as the chlororespiratory electron flow observed in *Ostreococcus* sp., to a lesser extent.

This study showed that oceanic picophytoplankton were susceptible to photoinhibition during short periods of high irradiance. The genetically defined light adaptation of *P. marinus*, *Prochlorococcus* sp., *Ostreococcus* sp., and *P. calceolata* played an important role in their PAR and UVR sensitivity, likely related to the presence of different (combinations of) photoprotective mechanisms. Temperature acclimation influenced the response to excessive irradiance exposure by changes in the relative contribution of photoinhibition and photoprotective mechanisms to non photochemical quenching. These changes were found to be species specific. Acclimation to elevated temperatures increased the dissipation of excess energy in both *P. marinus* and *Prochlorococcus* sp., indicating a strong dependence on temperature of this photoprotective mechanism. In combination with decreased photoinhibition during both PAR and UVR exposure at elevated temperature, the high light adapted ecotype *P. marinus* may benefit considerably from elevated temperatures in response to high irradiance intensities encountered in the upper mixed layer of open oligotrophic oceans. Considering exposure to UVR, the effect of elevated temperature was most pronounced in the eukaryotic strain *Ostreococcus* sp., indicating that this species can effectively regulate light harvesting in relatively warm, UVR rich waters near the surface of the open oligotrophic ocean. Even though *Prochlorococcus* sp. and *P. calceolata* are unlikely to experience high irradiance intensities in the deep chlorophyll maximum, photoinhibition in these low light adapted ecotypes is highly relevant, since damage to PSII can occur at relatively low irradiance intensities [18,31,68]. At elevated temperatures, the prokaryotic strain *Prochlorococcus* sp. benefitted by increasing dissipation of excess energy, whereas the eukaryotic strain *P. calceolata* was less susceptible to photoinhibition. Overall, the differential response to high irradiance may have considerably effect on phytoplankton species distribution and community composition in the open oligotrophic oceans, with some ecotypes and/or species being more susceptible to photoinhibition than others. Photoinhibition and/or photoprotective processes may be positively affected by the rise in seawater temperature associated with climate change, but species specific differences in (photo)physiology remain important in the performance of oceanic picophytoplankton.

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