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Distinct differences in photoacclimation potential between prokaryotic and eukaryotic oceanic phytoplankton

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ABSTRACT

Six (pico)phytoplankton strains typical for the open oligotrophic oceans were acclimated to different irradiance regimes mimicking stable (ranging from 10 to 125 μmol photons m^{-2} s^{-1}) and dynamic (averaging at 50 and 125 μmol photons m^{-2} s^{-1}) water column conditions. Photoacclimation potential of the different phytoplankton species was assessed by analysis of specific growth rates, pigment composition, pigment absorption, elemental composition, and photosynthetic characteristics. Results showed distinct differences between the studied prokaryotic species, *Prochlorococcus marinus* and *Synechococcus* sp. (two strains), and the eukaryotic species, *Ostreococcus* sp., *Emiliania huxleyi*, and *Thalassiosira oceanica*. Based on growth and photosynthetic characteristics, the photoacclimation potential of the eukaryotic species was significantly higher compared to that of the prokaryotic species under high and dynamic irradiance conditions. Likewise, the eukaryotic species performed better than the prokaryotic species after photoacclimation to low irradiance conditions. No consistent differences between constant and dynamic irradiance treatments were found. Differences in pigment composition, for example the presence of a xanthophyll cycle, may have played an important role in the success of photoacclimation of the studied phytoplankton species. These results imply that the high photoacclimation potential of eukaryotic oceanic phytoplankton offers a selective advantage over prokaryotic phytoplankton, in both the upper mixed layer and the deep chlorophyll maximum. Thus, factors other than photoacclimation potential, for example low nutrient availability, are likely to explain the high abundance of prokaryotic picophytoplankton in the open oligotrophic oceans.

1. Introduction

The phytoplankton community of open oligotrophic oceans is typically dominated by prokaryotic *Prochlorococcus* spp., *Synechococcus* spp., and eukaryotic pico- and nanophytoplankton (Olson et al., 1990; Lindell and Post, 1995; reviewed by Veldhuis et al., 2005). Both *Prochlorococcus* and *Synechococcus* are described as the most abundant phytoplankton genera (Li, 1994; DuRand et al., 2001; Johnson et al., 2006), whereas eukaryotic phytoplankton species are regarded as less abundant (Campbell and Vaulot, 1993; Veldhuis et al., 2005). The success of the prokaryotic phytoplankton species is often ascribed to the occurrence of several ecotypes of a single species throughout the water column (Moore et al., 1998; Partensky et al., 1999; Fuller et al., 2003). These ecotypes are genetically and (photo) physiologically distinct and well adapted to specific water column conditions, such as the intensity and spectral composition of irradiance at the deep chlorophyll maximum. To date, three *Prochlorococcus* and eight marine *Synechococcus* ecotypes have been identified, each having differences in pigmentation, absorption, and photosynthetic characteristics (Moore et al., 1998; Fuller et al., 2003; Partensky and Garczarek, 2010). Consequently, the coexistence of ecotypes could allow for competitive growth over a broader range of conditions than could be achieved by a genetically homogeneous population (Moore et al., 1998; Fuller et al., 2006). The occurrence of different ecotypes within a single species is however not unique to prokaryotic phytoplankton. Recent studies indicate that the eukaryotic picophytoplankton species *Ostreococcus* shows this type of genetic and physiological diversification as well (Rodriguez et al., 2005). Like *Prochlorococcus* and *Synechococcus*, the four ecotypes of *Ostreococcus* show distinct differences in pigment composition and photosynthetic characteristics. In contrast to abundance, primary production rates of eukaryotic phytoplankton can potentially be significantly higher compared to prokaryotic phytoplankton (Li, 1994; Worden et al., 2004). It is however thought that the actual contribution of eukaryotic picophytoplankton to primary production in the open ocean is light limited due to their occurrence in the deep chlorophyll maximum (Veldhuis et al., 2005).

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The competitive success of a specific species depends on different factors, but primarily on the response to the (dynamic) irradiance and nutrient conditions encountered in the water column. The degree of stratification of the water column determines the depth of the wind-induced transport of phytoplankton (vertical mixing) (Brainerd and Gregg, 1993), and consequently the irradiance climate phytoplankton experience. In permanently stratified regions, phytoplankton can be trapped in the shallow upper mixed layer (UML), thereby enhancing exposure to (dynamic) photosynthetically active radiation (PAR, 400–700 nm), or can experience limiting irradiance conditions at the deep chlorophyll maximum (DCM). In seasonally stratified regions, the period of stratification is interchanged with periods of deep convective mixing that can reach below the euphotic zone. In addition to vertical mixing, cloud cover can cause fluctuations in the underwater light field. The dynamic changes between low and high light require regulation and acclimation of light harvesting and photoprotective pigments (Falkowski and La Roche, 1991) and other photosynthetically important cell components. The extent of pigment adjustment in response to varying irradiance conditions is species specific, with certain species having a more dynamic range in photoacclimation potential than others. These differences in photoacclimation potential may partly explain why certain species prefer on average high (UML) or low (DCM) irradiance conditions during stratification, while others have a competitive advantage during (deep) vertical mixing in non-stratified waters (Arrigo et al., 1999; Strzepek and Harrison, 2004; Van Leeuwe et al., 2005).

The photophysiology of oceanic (pico)phytoplankton species is often addressed separately. Both *Prochlorococcus* (Moore et al., 1998; reviewed by Partensky et al., 1999) and *Synechococcus* (Kana and Glibert, 1987; Six et al., 2004) are studied extensively in the laboratory and the field. Comparisons between the two prokaryotic species show differences in pigment composition, and consequently differences in absorption and fluorescence characteristics (Morel et al., 1993; Moore et al., 1995). Accordingly, *Prochlorococcus* and *Synechococcus* both have growth optima at different irradiance intensities and spectral compositions, with *Prochlorococcus* having an advantage over *Synechococcus* at the bottom of the euphotic zone (Morel et al., 1993; Moore et al., 1995). Direct comparisons of photoacclimation potential between key prokaryotic and eukaryotic phytoplankton species are however rare. Only a few comparative studies exist on the photophysiology of specific eukaryotic picophytoplankton species (Timmermans et al., 2005; Six et al., 2008; Dimier et al., 2009). Moreover, studies on the response of picophytoplankton to fluctuating irradiances, as experienced in the UML, are minimal (Wagner et al., 2006; Dimier et al., 2009). Thus far, these studies indicate that eukaryotic picophytoplankton species are able to maintain high growth rates under natural irradiance and nutrient conditions. Only severe light and nutrient limitation are found to affect growth and photosynthetic performance (Timmermans et al., 2005). Moreover, eukaryotic picophytoplankton seem to be able to acclimate effectively to high irradiance levels experienced in the upper water column (Dimier et al., 2007; Dimier et al., 2009). Given the relatively low abundance of eukaryotic phytoplankton in the open oligotrophic oceans, this raises the question to what extent photoacclimation potential determines *in situ* performance of the prokaryotic and eukaryotic phytoplankton species found in open ocean communities.

In the present study, a comparative analysis of the photoacclimation potential of key oceanic phytoplankton species was performed to unravel the importance of irradiance conditions in structuring the phytoplankton community in open oligotrophic oceans. Therefore, three prokaryotic and three eukaryotic phytoplankton strains were acclimated to a range of constant and dynamic irradiance regimes. Growth was assessed during photoacclimation, and in addition, pigment composition, absorption spectra, elemental composition, and photosynthetic characteristics were quantified after photoacclimation. The results are discussed in the context of ecophysiological differences between prokaryotic and eukaryotic (pico)phytoplankton species.

2. Method

2.1. Culture conditions

Cultures were obtained from the Roscoff Culture Collection (RCC) and the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP). *Synechococcus* sp. strain RCC477, *Synechococcus* sp. strain RCC543, *Ostreococcus* sp. strain RCC410, and *Emiliania huxleyi* strain CCMP2112 were cultured in K medium based on natural oceanic seawater as described by Keller et al. (1987). For *Thalassiosira oceanica* strain CCMP1616, silicate was added to K medium in a final concentration of 50.4 μmol l^{-1}. *Prochlorococcus marinus* strain CCMP2389 (ecotype MED4) was cultured in a different version of the K medium, with a ten times diluted concentration of trace metals minus copper (K/10-Cu; see Chisholm, 1992). Cultures were maintained in 100 ml glass Erlenmeyer flasks at 68 μmol photons m^{-2} s^{-1}, except for *Synechococcus* sp. strain RCC477 (9 μmol photons m^{-2} s^{-1}), in a diurnal cycle of 12:12 h light:dark at 20 °C.

2.2. Experimental design

Cultures of *P. marinus*, *Synechococcus* sp. (both strains), *Ostreococcus* sp., *E. huxleyi*, and *T. oceanica* were transferred in duplicate to 250 ml glass Erlenmeyer flasks and incubated at different irradiance regimes for typically 6 days. The incubations were prolonged up to 14 days if growth rates were low and/or the lag phase was longer than 2 days. Irradiance treatments were carried out in a U-shaped lamp setup as described by Van de Poll et al. (2007). In short, the setup consisted of 12 fluorescent lamps (six biolux and six skywhite lamps, Osram) equipped with reflectors (Doublelux) and connected to dimmers (Osram). The dimmers were computer controlled by LabVIEW software (version 8.2, National Instruments) and allowed irradiance fluctuations without changing spectral quality. In a first set of experiments, cultures were exposed to five different constant irradiance regimes, in which 10, 25, 50, 75, and 125 μmol photons m^{-2} s^{-1} PAR was provided as a square wave function with a 12:12 h light:dark cycle. For these incubations, growth and pigments analysis were performed. In a second set of experiments, the six phytoplankton strains were cultivated under two dynamic irradiance treatments, in which irradiance, mixing speed, mixing depth, and attenuation were superimposed on a diurnal cycle of 12:12 h light:dark. Two different regimes were chosen, one averaging at 50 μmol photons m^{-2} s^{-1} PAR (max. 166 μmol photons m^{-2} s^{-1}), and the other averaging at 125 μmol photons m^{-2} s^{-1} PAR (max. 400 μmol photons m^{-2} s^{-1}) during the light period (Fig. 1). For this experimental series, as well as the constant irradiance incubation of 50 and 125 μmol photons m^{-2} s^{-1}, absorption spectra, elemental composition,

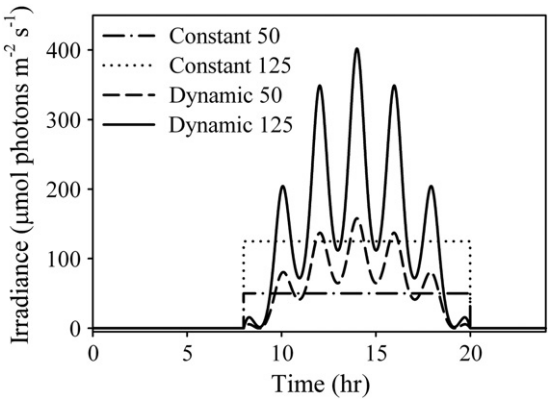

Fig. 1. Constant and dynamic irradiance regimes, averaging at 50 and 125 μmol photons $m^{-2}s^{-1}$.

and photosynthetic characteristics were measured in addition to growth and pigment analysis. In all experiments, growth was followed daily starting directly after the beginning of the irradiance treatment. In the second set of experiments, analysis of pigments, absorption spectra, elemental composition, and photosynthetic characteristics were performed simultaneously during the exponential growth phase after photoacclimation. To ensure photoacclimation during these experiments, the strains were pre-cultured up to 14 days under the experimental irradiance conditions. Measurements were performed in mid exponential phase, when fluorescence signals (flow cytometry) and maximum quantum yield of photosystem II (F_v/F_m, water—PAM fluorometry, Waltz GmbH) were stable. No additional measurements were performed in the second set of experiments if growth was not observed. Irradiance levels were frequently monitored with a QSL-100 (Biospherical Instruments).

2.3. Growth measurements

Samples (1 ml) for cell counts were obtained during the exponential and the beginning of the stationary growth phase. Cell concentrations were determined on a Coulter Epics MXL flow cytometer (Beckman Coulter). Growth rates (d^{-1}) of the exponential growth phase were calculated by linear regression of natural log-transformed cell numbers for all replicates (≥4 data points). In addition, cell sizes were estimated by calibration of the forward scatter of the flow cytometer (Flow cytometry size calibration Kit F-13838, Molecular Probes).

2.4. Pigment composition

One sample (20–60 ml) for pigment analysis was taken during the exponential growth phase for each replicate. Samples 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 Van Leeuwe et al. (2006). In short, filters were freeze-dried for 48 h and pigments were immediately extracted in 3 ml 90% acetone (v/v, 48 h, 4 °C). Detection of pigments was carried out using a HPLC (Waters 2690 separation module, 996 photodiode array detector) equipped with a C_{18} 5 μm DeltaPak column (Waters). Peaks were identified by retention time and diode array spectroscopy. Pigments were quantified using standard dilutions (DHI LAB products) of chlorophyll a_1, chlorophyll a_2, chlorophyll *b*, chlorophyll c_3, fucoxanthin, lutein, diadinoxanthin, diatoxanthin, antheraxanthin, violaxanthin, zeaxanthin, α-carotene, and β-carotene. Chlorophyll a_1 and a_2 could not be separated, however it was assumed that in *P. marinus* strain CCMP2389 only chlorophyll a_2 was present (Partensky et al., 1999) and standards for chlorophyll a_2 were used for quantification. From here on, chlorophyll *a* will refer to chlorophyll a_2 in *P. marinus* and to chlorophyll a_1 in all other strains.

2.5. Absorption spectra

Phytoplankton pigment absorption spectra were determined on a Varian Cary 3E UV–vis spectrophotometer, equipped with an integrating sphere. Spectral values of the absorption coefficient were recorded every 1 nm between 300 and 800 nm. For analysis, 20–50 ml culture was filtered onto 25 mm GF/F filters (Whatman) and the transmission and reflection of the total particulate matter was determined according to Tassan and Ferrari (1995). The filter was then extracted in sodium hypochlorite (1% chlorine) to remove phytoplankton pigments and measured again to obtain the absorption of non-pigmented material (detritus). Phytoplankton absorption was calculated and normalized to chlorophyll *a* concentrations to obtain the specific absorption coefficient by phytoplankton $a^*_{ph}(\lambda)$ (m^2 mg Chl-a^{-1}). The maximum quantum yield of photosynthesis was calculated by using the spectrally weighted mean specific absorption coefficient $\bar{a}^*$ (m^2 μg Chl-a^{-1}):

$$\overline{a}^* = \left(\frac{\sum_{700}^{400} \alpha^*_{ph}(\lambda) E(\lambda)}{\sum_{700}^{400} E(\lambda)} \right) \quad (1)$$

with E(λ) being the irradiance used in the photosynthetron during the photosynthesis versus irradiance measurements.

2.6. Elemental composition

For Particulate Organic Carbon (POC) and Nitrogen (PON) analysis, 15–30 ml culture was filtered onto 12 mm precombusted (4 h, 600 °C) GF/F filters (Whatman), snap frozen in liquid nitrogen, and stored at −80 °C until further analysis. For analysis, filters were acidified under HCl (37%) fumes for 4 h, dried overnight at 60 °C, and wrapped in tin capsules (Elemental Microanalysis Ltd.). Analysis of the samples was performed on a nitrogen and carbon analyzer type Flash EA™ 1112 (Interscience).

2.7. Photosynthetic characteristics

A ^{14}C-bicarbonate method was used to determine photosynthetic versus irradiance (P–I) characteristics as described by Lewis and Smith (1983). First, 10 μl ^{14}C-bicarbonate (0.74 MBq) was added to 42 ml of culture, after which 20 times 2 ml was dispensed in scintillation vials. 17 vials were then incubated for 60 min at 20 °C in a photosynthetron consisting of a temperature controlled aluminum block illuminated by a 250 W lamp (MHN-TD power tone, Philips) with irradiance levels ranging from 4 to 897 μmol photons m^{-2} s^{-1}. After incubation the samples were acidified with 100 μl 6 N HCl to remove excess ^{14}C-bicarbonate and left overnight under active air filtration. The next day, samples were neutralized with 100 μl 6 N NaOH and 10 ml scintillation cocktail (Ultima Gold XR, PerkinElmer) was added. To obtain time zero activity, 3 times 2 ml ^{14}C culture sample was immediately acidified with 100 μl 6 N HCl and thereafter treated equal to the other samples. For total activity, 100 μl ^{14}C culture sample was added to 500 μl 0.2 μm filtered seawater and 50 μl ethanolamine in three prepared vials, where after 10 ml scintillation cocktail was directly added. After at least 24 h, radioactivity in all samples was measured by liquid scintillation spectrometry (Tri-Carb 2000 CA scintillation counter, Packard).

All data from the P–I curves were normalized to chlorophyll *a* derived from HPLC measurements, and the carbon uptake measured during the incubation was plotted against the irradiance levels of the photosynthetron. The data were then fitted to the empirical model described by Platt et al. (1980):

$$P = P_S\left(1-e^{-\alpha\left(\frac{x}{P_S}\right)}\right)\left(e^{-\beta\left(\frac{x}{P_S}\right)}\right) - P_0 \quad (2)$$

using LABFit software (version 7.2.45, Wilton and Cleide P. Silva) to perform the nonlinear least-squares regression, in which P is the chlorophyll *a* specific CO_2 fixation rate (μg C μg Chl-a^{-1} h^{-1}) at irradiance E (μmol photons m^{-2} s^{-1}), P_S is the theoretical maximum for photosynthesis in the absence of photoinhibition (μg C μg Chl-a^{-1} h^{-1}), α is the initial rate of photosynthesis (μg C μg Chl-a^{-1} h^{-1} [μmol photons m^{-2} s^{-1}]$^{-1}$), β is a measure of photoinhibition (μg C μg Chl-a^{-1} h^{-1} [μmol photons m^{-2} s^{-1}]$^{-1}$), and P_0 was used to indicate respiration or dark carbon fixation at zero irradiance. The parameters obtained from Eq. (2) were used to calculate the maximum photosynthetic rate P_{max} (μg C μg Chl-a^{-1} h^{-1}) and the

photoadaptation index Eκ (μmol photons m^{-2} s^{-1}) by the following equations:

$$P_{max} = P_S\left(\frac{\alpha}{\alpha+\beta}\right)\left(\frac{\beta}{\alpha+\beta}\right)^{\left(\frac{\alpha}{\beta}\right)} \quad (3)$$

$$E_\kappa = P_{max}/\alpha \quad (4)$$

The maximum quantum yield of photosynthesis ϕ_{max} (μmol C μmol photons absorbed^{-1}) was calculated according to Geider and Osborne (1992):

$$\phi_{max} = \frac{\alpha}{43.2\overline{a^*}E} \quad (5)$$

with 43.2 as a unit conversion factor and E as the irradiance intensity (μmol photons m^{-2} s^{-1}).

In addition, the P–I data were normalized to POC to exclude a possible effect of cellular chlorophyll *a* variability (MacIntyre et al. 2002). The carbon uptake measured in the P–I curves was multiplied by the chlorophyll *a* to carbon ratio and refitted by Eq. (2). Carbon specific maximum photosynthetic rate P^C_{max} (μg C μg C^{-1} h^{-1}) and initial rate of photosynthesis α^C (μg C μg C^{-1} h^{-1} [μmol photons m^{-2} s^{-1}]$^{-1}$) were calculated using Eqs. (3) and (2), respectively.

2.8. Statistical analysis

Data were statistically analyzed by analysis of variance (ANOVA) and regression analysis using STATISTICA software (version 8.0, StatSoft Inc.).

3. Results

3.1. Growth

Under constant irradiance conditions (first experimental series) growth rates varied widely between the different species (Fig. 2). Highest rates were found for the diatom species *T. oceanica* (μ ranging from 0.29 ± 0.27 to 1.24 ± 0.06 d^{-1}), whereas lowest values were found for *Synechococcus* sp. RCC543 (μ ranging from 0.22 ± 0.01 to 0.29 ± 0.01 d^{-1}). Distinct differences in growth rates and response to irradiance treatment were found between the prokaryotic strains *P. marinus*, *Synechococcus* sp. RCC477, and RCC543 and the eukaryotic strains *Ostreococcus* sp., *E. huxleyi*, and *T. oceanica* (Fig. 2). In general, the prokaryotic species showed lower growth rates at all irradiance regimes compared to the eukaryotic species. Moreover, the maximum growth rates of *P. marinus* (μ_{max} = 0.39 ± 0.03 d^{-1}), *Synechococcus sp.* RCC477 (μ_{max} = 0.40 ± 0.03 d^{-1}), and *Synechococcus* sp. RCC543 (μ_{max} = 0.29 ± 0.01 d^{-1}) were found during treatments with the lower irradiance regimes, at 50, 50, and 25 μmol photons m^{-2} s^{-1}, respectively. Noteworthy was the absence of growth in both *Synechococcus* sp. strains at the highest light intensity (125 μmol photons m^{-2} s^{-1}). On the other hand, *Ostreococcus* sp. (μ_{max} = 0.85 ± 0.08 d^{-1}), *E. huxleyi* (μ_{max} = 0.90 ± 0.05 d^{-1}), and *T. oceanica* (μ_{max} − 1.24 ± 0.06 d^{-1}) showed maximum growth at irradiances of 75, 125, and 125 μmol photons m^{-2} s^{-1}, respectively.

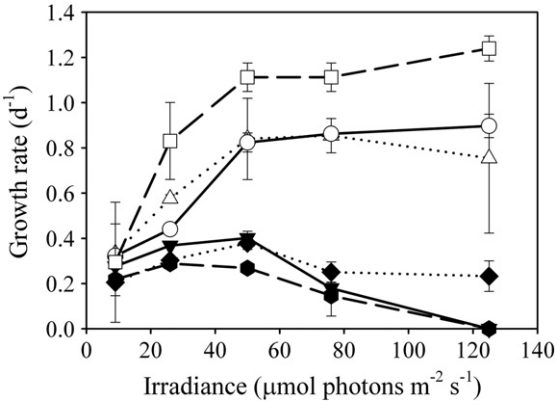

Fig. 2. Average growth rates (± standard deviation, $n \geq 2$) for *Prochlorococcus marinus* (filled diamonds), *Synechococcus* sp. RCC477 (filled triangles), *Synechococcus* sp. RCC543 (filled octagons), *Ostreococcus* sp. (open triangles), *Emiliania huxleyi* (open circles), and *Thalassiosira oceanica* (open squares). Negative values in *Synechococcus* sp. (both strains) at 125 μmol photons m^{-2} s^{-1} were set to zero.

When growth rates of the dynamic and constant irradiance treatments were compared (second experimental series, Table 1), different trends were visible for the low and high irradiance treatments. At 50 μmol photons m^{-2} s^{-1}, all species showed reduced growth under dynamic irradiance exposure ($p<0.05$, not significant for *Synechococcus* sp. RCC477 and *Ostreococcus* sp.). In contrast, at 125 μmol photons m^{-2} s^{-1}, the prokaryotic strains *P. marinus*, *Synechococcus* sp. RCC477, and RCC543 failed to grow when exposed to dynamic irradiance, whereas the eukaryotic strains grew at similar rates (*Ostreococcus* sp. and *E. huxleyi*) or slightly faster (*T. oceanica*, $p<0.01$) at dynamic compared to constant irradiance.

Cell sizes (data not shown) were on average 0.79 ± 0.004 μm for *P. marinus*, 0.98 ± 0.02 μm for *Synechococcus* sp. RCC477, 0.90 ± 0.04 μm for *Synechococcus* sp. RCC543, 1.05 ± 0.12 μm for *Ostreococcus* sp., 5.07 ± 0.49 μm for *E. huxleyi*, and 6.37 ± 0.54 μm for *T. oceanica*. No trend with irradiance treatment was found.

3.2. Pigment composition

Two different trends in cellular chlorophyll *a* levels between prokaryotic and eukaryotic species were observed after photoacclimation (data not shown). *P. marinus*, *Synechococcus* sp. RCC477, and RCC543 showed no significant trend in cellular chlorophyll *a* concentrations with irradiance. Average concentrations were 6.16 ± 2.75, 20.0 ± 3.95, and 31.7 ± 7.99 fg $cell^{-1}$ for *P. marinus*, *Synechococcus* sp. RCC477, and RCC543, respectively. In contrast, *Ostreococcus* sp., *E. huxleyi*, and *T. oceanica* showed a significant decrease in cellular chlorophyll *a* concentrations with increasing irradiance ($p<0.05$). Chlorophyll *a* concentrations in the eukaryotic species ranged from 9.08 to 5.32 ± 0.82 fg $cell^{-1}$ in *Ostreococcus* sp., from 0.35 ± 0.01 to 0.22 ± 0.03 pg $cell^{-1}$ in *E. huxleyi*, and from 0.73 ± 0.21 to 0.24 ± 0.05 pg $cell^{-1}$ in *T. oceanica* (values given for 10–125 μmol photons m^{-2} s^{-1}). The accessory pigments chlorophyll *b* in *P. marinus* and *Ostreococcus* sp. and fucoxanthin in *E. huxleyi* and *T. oceanica* also showed a decreasing trend with increasing irradiance ($p<0.01$), whereas prasinoxanthin in *Ostreococcus* sp. showed no significant trend with irradiance (data not shown).

In *P. marinus*, *Synechococcus* sp. RCC477, and RCC543 zeaxanthin was found as the main photoprotective pigment. In the eukaryotic

Table 1
Average growth rates (± standard deviation, $n \geq 2$) (d^{-1}) for all strains in the constant and dynamic (d) irradiance treatments at 50 and 125 μmol photons m^{-2} s^{-1}. * mark significant differences ($p<0.05$) in growth rates for each species between constant and dynamic irradiance treatments.

	50	50 d	125	125 d
Prochlorococcus marinus	0.38 ± 0.03*	0.28 ± 0.04	0.23 ± 0.07*	−0.62 ± 0.04
Synechococcus sp. RCC477	0.40 ± 0.03	0.39 ± 0.09	−0.18 ± 0.05*	−0.46 ± 0.01
Synechococcus sp. RCC543	0.27 ± 0.02*	0.16 ± 0.003	−0.66 ± 0.04*	−0.42 ± 0.04
Ostreococcus sp.	0.84 ± 0.18	0.76 ± 0.02	0.75 ± 0.33	1.20 ± 0.29
Emiliania huxleyi	0.82 ± 0.04*	0.66 ± 0.04	0.90 ± 0.05	0.96 ± 0.05
Thalassiosira oceanica	1.11 ± 0.08*	0.79 ± 0.05	1.24 ± 0.06*	1.40 ± 0.04

species, a xanthophyll cycle was present, consisting of violaxanthin, antheraxanthin, and zeaxanthin in *Ostreococcus* sp. and diadinoxanthin and diatoxanthin in *E. huxleyi* and *T. oceanica*. Effects of irradiance on photoprotective pigments showed similar trends for all species (Fig. 3). The ratio of photoprotective pigments to chlorophyll *a* increased with irradiance in both prokaryotic and eukaryotic species ($p<0.02$). Typically, an increase in photoprotective pigments was related to the irradiance at which growth saturated. For *P. marinus* and all eukaryotic species (Fig. 3A, B, D and F) this was around 50 μmol photons m^{-2} s^{-1} (also see Fig. 2). This trend was less clear for *Synechococcus* sp. RCC477 and RCC543, but most likely valid, as growth saturated at low irradiances and photoprotective pigments showed a strong increase from the lowest irradiance treatment upward. Noteworthy were the relatively high photoprotective pigment to chlorophyll *a* ratios in the prokaryotic species (up to 1.24 ± 0.02 in *Synechococcus* sp. RCC543), compared to the eukaryotic species (up to 0.25 ± 0.08 in *T. oceanica*).

When constant and dynamic irradiance treatments were compared, significant decreases in cellular chlorophyll *a* concentration were found under dynamic irradiance in *T. oceanica* at 50 μmol photons m^{-2} s^{-1} ($p<0.04$) and in *Ostreococcus* sp. at 125 μmol photons m^{-2} s^{-1} ($p<0.01$). In addition, a significant decrease in the photoprotective pigments per chlorophyll *a* ratio was found in the dynamic light treatment at 125 μmol photons m^{-2} s^{-1} for *Ostreococcus* sp. and *E. huxleyi* ($p<0.01$) (Fig. 3). No other significant differences in pigmentation were found between the constant and dynamic irradiance treatments.

3.3. Absorption spectra

Although the main light harvesting pigments in *Synechococcus* sp., phycobilins, were not quantified, some observations were made based on the measured absorption spectra of *Synechococcus* sp. strains RCC477 and RCC543. When specific absorption coefficients of both strains were compared during constant irradiance exposure at 50 μmol photons m^{-2} s^{-1} (Fig. 4), a difference in phycobilins absorption was found. Absorption of phycoerythrobilin (PEB) around 550 nm was much higher in *Synechococcus* sp. RCC477 compared to RCC543, whereas absorption of phycourobilin (PUB) around 495 nm was similar in both strains. This resulted in significantly different PUB:

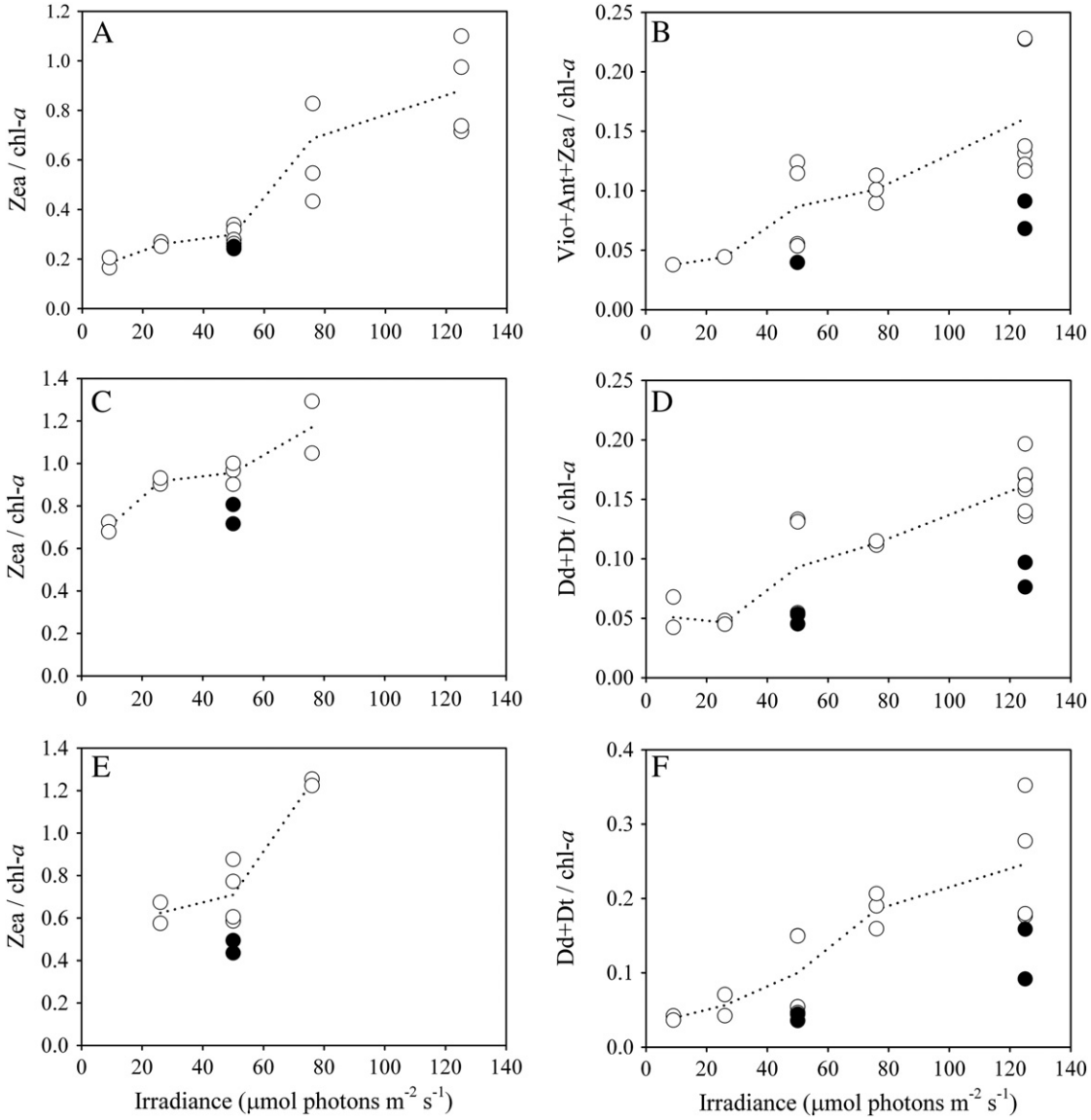

Fig. 3. Photoprotective pigments to chlorophyll *a* ratio for (A) *Prochlorococcus marinus*, (B) *Ostreococcus* sp., (C) *Synechococcus* sp. RCC477, (D) *Emiliania huxleyi*, (E) *Synechococcus* sp. RCC543, and (F) *Thalassiosira oceanica* at the different irradiances of the constant (open circles) and dynamic (filled circles) irradiance regimes. Abbreviations: Chl-*a*: chlorophyll *a*, Vio: violaxanthin, Ant: antheraxanthin, Zea: zeaxanthin, Dd: diadinoxanthin, and Dt: diatoxanthin.

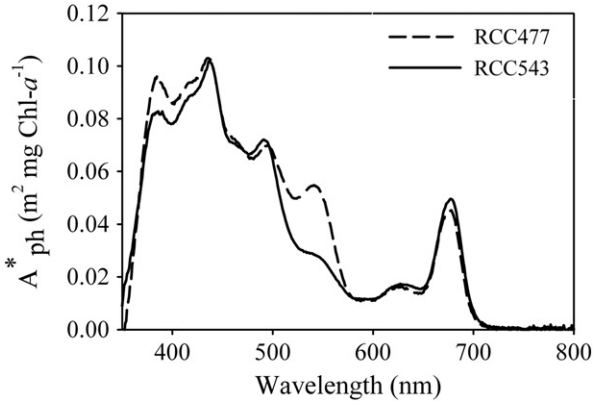

Fig. 4. Example of the specific absorption coefficient α^*_{ph} for *Synechococcus* sp. RCC477 and RCC543 at 50 μmol photons m^{-2} s^{-1}. Differences in phycobilisome pigmentation are clearly visible around 550 nm.

PEB ratios between the strains, 1.39 ± 0.16 and 2.51 ± 0.06 for strains RCC477 and RCC543, respectively (p<0.01). Moreover, the PUB:PEB ratio increased during the dynamic irradiance treatment in *Synechococcus* sp. RCC543 (to 3.21 ± 0.08, p<0.01), whereas no significant differences were found between irradiance treatments in strain RCC477.

When absorption spectra of all species were compared, no significant differences were found between the constant and dynamic irradiance treatments. Therefore, data of the constant and dynamic irradiance treatments of identical light intensity (on average 50 or 125 μmol photons m^{-2} s^{-1}) were grouped. The spectrally weighted mean specific absorption coefficients (ā*) were negatively related to cell size and cellular chlorophyll *a* concentrations (p<0.001), but there was considerable variation in this trend for the prokaryotic species (Table 2). At 50 μmol photons m^{-2} s^{-1}, ā* was significantly lower in *P. marinus* and *T. oceanica* compared to all other species (p<0.03). Highest values were found in *Synechococcus* sp. RCC477 (p<0.05). At 125 μmol photons m^{-2} s^{-1}, only *P. marinus* showed a significant increase in ā* compared to the lower irradiance treatment of 50 μmol photons m^{-2} s^{-1} (p<0.005). The blue:red absorption ratio (Table 2) was found to be significantly different between all species (p<0.02), with the exception of *Synechococcus* sp. RCC543 and *Ostreococcus* sp. at 50 μmol photons m^{-2} s^{-1}. Noteworthy was the relatively low blue:red ratio in *T. oceanica* compared to the other species. The blue:red ratio increased slightly with irradiance in *Ostreococcus* sp., *E. huxleyi*, and *T. oceanica*, but was more than one and a half times higher in *P. marinus* at 125 compared to 50 μmol photons m^{-2} s^{-1}.

3.4. Elemental composition

No clear trends were found in elemental composition between the irradiance treatments. C:N ratios (data not shown) varied from 3.50 ± 0.10 (*Synechococcus* sp. RCC477) to 6.63 ± 0.29 (*P. marinus*), and were significantly highest in *P. marinus* and *E. huxleyi* (p<0.01). Cellular nitrogen and carbon levels (data not shown) were considerably higher in *E. huxleyi* (up to 12.1 ± 0.06 pg N cell^{-1} and 1.97 ± 0.03 pg C cell^{-1}, respectively) and *T. oceanica* (up to 12.5 ± 1.81 pg N cell^{-1} and 2.84 ± 0.48 pg C cell^{-1}, respectively) compared to the other species (up to 2.51 ± 1.12 pg N cell^{-1} and 0.72 ± 0.34 pg C cell^{-1} in *Synechococcus* sp. RCC477, respectively) (p<0.001). The found differences in cellular C and N concentrations were related to cell size (p<0.001).

3.5. Photosynthetic characteristics

The photosynthetic versus irradiance curves resulted in clear differences between prokaryotic and eukaryotic phytoplankton species. However, significant differences in maximum photosynthetic rate (P_{max}), initial rate of photosynthesis (α), and photoadaptation index (E_K) were only found occasionally between the constant and dynamic irradiance treatment. The general trend, but not significant for all parameters, indicated a similar or slightly increased P_{max}, α, and E_K under dynamic compared to constant irradiance exposure at 50 μmol photons m^{-2} s^{-1}, whereas the opposite trend was observed at 125 μmol photons m^{-2} s^{-1} (Table 3). For further interpretation, data of the constant and dynamic irradiance treatment of identical light intensity (on average 50 or 125 μmol photons m^{-2} s^{-1}) were grouped.

The maximum photosynthetic rate (P_{max} both chlorophyll *a* and carbon normalized) was significantly lower in the prokaryotic strains *P. marinus*, *Synechococcus* sp. RCC477, and RCC543 compared to the eukaryotic strains *Ostreococcus* sp., *E. huxleyi*, and *T. oceanica* at all irradiances conditions (p<0.03) (Table 3, Fig. 5, Fig. 6A). This trend was remarkably strong for *T. oceanica* exposed to 125 μmol photons m^{-2} s^{-1}, which showed photosynthetic rates more than four times faster compared to the other species. The initial rate of photosynthesis (α) showed no marked differences between the species (Table 3) when normalized to chlorophyll *a*, but was lowest in *P. marinus* (p<0.05). α increased at 125 compared to 50 μmol photons m^{-2} s^{-1}, but only significantly in *T. oceanica* (p<0.03). In contrast, the initial rate of photosynthesis normalized to carbon (α^C) showed significantly lower rates under all irradiance conditions for the prokaryotic species compared to the eukaryotic species (p<0.03) (Fig. 6B). The photoadaptation index (E_K) was similar in the 'true' picophytoplankton *P. marinus*, *Synechococcus* sp. RCC477, RCC543, and *Ostreococcus* sp., around the average irradiance of the treatment, whereas the E_K of the larger species, *E. huxleyi* and *T. oceanica*, were significantly higher (p<0.001) compared to the other species (Table 3, Fig. 6C). Although E_K increased with irradiance in all species (not significant for *E. huxleyi*), most pronounced was the increase in E_K of *P. marinus* at 125 μmol photons m^{-2} s^{-1}, which again was acclimated to the average irradiance given during the irradiance treatment (Table 3, Fig. 6C). Results given for E_K related well to those found for photoinhibition (β), which was highest in *E. huxleyi* and *T. oceanica* (data not shown). The maximum quantum yield of

Table 2
The average (± standard deviation, n = 4) spectrally weighted mean specific absorption coefficient ā* (m^2 mg Chl-a^{-1}) and the blue:red ratio of the absorption spectra for all strains at 50 and 125 μmol photons m^{-2} s^{-1}. Constant and dynamic irradiance treatments did not show significant differences and are therefore grouped under the average irradiance condition (50 or 125 μmol photons m^{-2} s^{-1}). n/a: data not available, growth was not observed in cultures and additional measurements were not performed.

	ā*		Blue:red	
	50	125	50	125
Prochlorococcus marinus	0.018 ± 2.16 × 10^{-3}	0.031 ± 3.80 × 10^{-3}	2.22 ± 0.05	3.45 ± 0.27
Synechococcus sp. RCC477	0.047 ± 4.89 × 10^{-3}	n/a	2.41 ± 0.16	n/a
Synechococcus sp. RCC543	0.035 ± 6.42 × 10^{-3}	n/a	1.99 ± 0.05	n/a
Ostreococcus sp.	0.037 ± 4.17 × 10^{-3}	0.041 ± 9.17 × 10^{-3}	2.06 ± 0.05	2.16 ± 0.04
Emiliania huxleyi	0.030 ± 3.77 × 10^{-3}	0.030 ± 2.09 × 10^{-3}	1.85 ± 0.02	1.93 ± 0.08
Thalassiosira oceanica	0.016 ± 4.54 × 10^{-3}	0.019 ± 2.46 × 10^{-3}	1.31 ± 0.03	1.40 ± 0.05

Table 3
Photosynthesis versus irradiance characteristics (normalized to chlorophyll *a*). Averages (± standard deviations, $n=2$) of maximum photosynthetic rate P_{max} (μg C μg Chl-a^{-1} h^{-1}), initial rate of photosynthesis α (μg C μg Chl-a^{-1} h^{-1} [μmol photons $m^{-2}s^{-1}$]$^{-1}$), photoadaptiation index E_K (μmol photons m^{-2} s^{-1}), carbon to chlorophyll *a* ratio C:Chl-*a* (wt:wt), and maximum quantum yield Φ_{max} (mol C mol photons absorbed^{-1}) are given for the six phytoplankton strains. n/a: data not available, growth was not observed in cultures and additional measurements were not performed.

	Prochlorococcus marinus	*Synechococcus* sp. RCC477	*Synechococcus* sp. RCC543	*Ostreococcus* sp.	*Emiliania huxleyi*	*Thalassiosira oceanica*
P_{max}						
50	1.79 ± 0.12	4.79 ± 0.69	3.87 ± 0.35	6.15 ± 1.54	7.71 ± 0.28	11.4 ± 1.10
50d	3.12 ± 0.81	8.71 ± 4.69	4.03 ± 0.15	6.76 ± 2.45	8.48 ± 0.51	15.0 ± 1.78
125	3.90 ± 0.09	n/a	n/a	8.41 ± 0.67	9.43 ± 3.31	30.7 ± 6.33
125d	n/a	n/a	n/a	8.71 ± 0.06	5.88 ± 1.96	24.8 ± 3.58
α						
50	$0.032 \pm 2.44 \times 10^{-5}$	$0.122 \pm 3.06 \times 10^{-2}$	$0.065 \pm 4.50 \times 10^{-3}$	$0.106 \pm 1.60 \times 10^{-4}$	$0.088 \pm 2.61 \times 10^{-4}$	$0.071 \pm 5.88 \times 10^{-3}$
50d	$0.055 \pm 6.53 \times 10^{-3}$	$0.194 \pm 9.60 \times 10^{-2}$	$0.082 \pm 1.58 \times 10^{-2}$	$0.141 \pm 3.97 \times 10^{-2}$	$0.096 \pm 5.23 \times 10^{-4}$	$0.089 \pm 9.19 \times 10^{-3}$
125	$0.031 \pm 4.22 \times 10^{-4}$	n/a	n/a	$0.102 \pm 3.01 \times 10^{-4}$	$0.079 \pm 2.42 \times 10^{-2}$	$0.151 \pm 1.84 \times 10^{-2}$
125d	n/a	n/a	n/a	$0.108 \pm 1.45 \times 10^{-2}$	$0.076 \pm 2.57 \times 10^{-2}$	$0.094 \pm 2.57 \times 10^{-2}$
E_K						
50	55 ± 3.5	40 ± 4.3	60 ± 1.3	58 ± 15	88 ± 3.5	161 ± 29
50d	57 ± 8.0	44 ± 2.2	50 ± 11	47 ± 4.0	88 ± 4.8	169 ± 2.6
125	128 ± 4.6	n/a	n/a	82 ± 6.4	119 ± 5.4	202 ± 17
125d	n/a	n/a	n/a	81 ± 10	78 ± 0.4	261 ± 18
Φ_{max}						
50	$0.042 \pm 1.28 \times 10^{-3}$	$0.063 \pm 9.17 \times 10^{-3}$	0.037 ± 9.63 x 10^{-5}	$0.065 \pm 7.34 \times 10^{-3}$	$0.075 \pm 4.09 \times 10^{-4}$	$0.088 \pm 1.14 \times 10^{-2}$
50d	$0.075 \pm 6.94 \times 10^{-3}$	$0.094 \pm 5.42 \times 10^{-2}$	$0.062 \pm 6.37 \times 10^{-3}$	$0.089 \pm 1.15 \times 10^{-2}$	$0.069 \pm 7.57 \times 10^{-3}$	$0.170 \pm 8.85 \times 10^{-2}$
125	$0.023 \pm 3.19 \times 10^{-3}$	n/a	n/a	$0.063 \pm 2.46 \times 10^{-2}$	$0.047 \pm 3.16 \times 10^{-2}$	$0.174 \pm 1.75 \times 10^{-2}$
125d	n/a	n/a	n/a	$0.062 \pm 1.02 \times 10^{-2}$	$0.058 \pm 1.51 \times 10^{-2}$	$0.128 \pm 3.38 \times 10^{-2}$
C:Chl-*a*						
50	14 ± 1.27	76 ± 6.57	59 ± 1.69	39 ± 1.89	32 ± 2.22	25 ± 0.77
50d	22 ± 2.70	116 ± 0.49	58 ± 10.5	32 ± 1.65	41 ± 2.80	29 ± 5.29
125	43 ± 1.17	n/a	n/a	40 ± 0.99	57 ± 0.68	44 ± 1.33
125d	n/a	n/a	n/a	38 ± 0.68	39 ± 0.67	28 ± 0.27

photosynthesis (Φ_{max}, Table 3) was significantly higher in the eukaryotic strains *Ostreococcus* sp., *E. huxleyi*, and *T. oceanica*, compared to the prokaryotic strains *P. marinus*, *Synechococcus* sp. RCC477, and RCC543 at 50 μmol photons m^{-2} s^{-1} ($p<0.02$). At the higher irradiance treatment of 125 μmol photons m^{-2} s^{-1} this trend was less clear, but *T. oceanica* showed significant higher Φ_{max} than the other species ($p<0.002$).

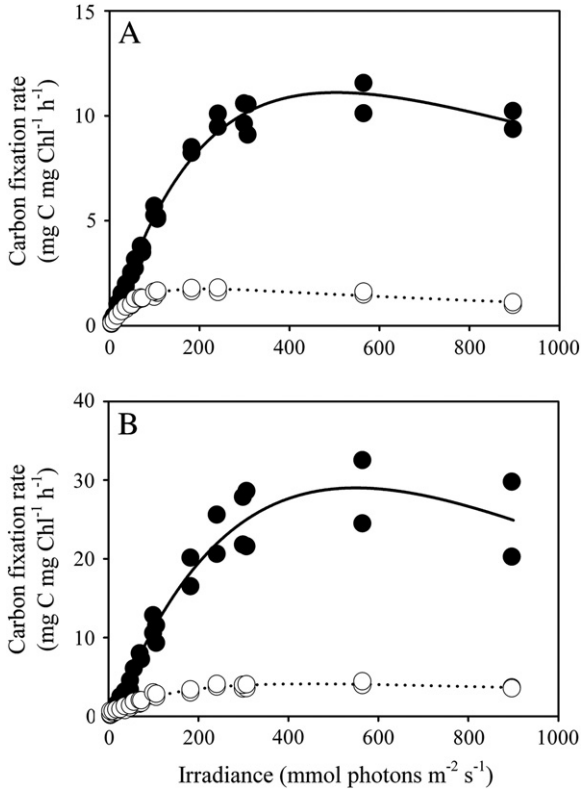

Fig. 5. Photosynthesis versus irradiance curves for *Prochlorococcus marinus* (open circles) and *Thalassiosira oceanica* (filled circles) for the (A) 50 and (B) 125 μmol photons m^{-2} s^{-1} constant irradiance treatments. The average fit is given ($n=2$). Note that the scales of the carbon fixation rates are different between the figures.

4. Discussion

A better understanding of photoacclimation mechanisms in picophytoplankton and corresponding photosynthetic performance is essential, because it is expected that climate change mediates a rise in seawater temperature as well as changes in wind fields (Sarmiento et al., 1998). Consequently, the onset and break-up of stratification in temperate and warm-temperate oceanic regions are affected, which will alter nutrient availability and the intensity, spectral composition, and dynamics of phytoplankton irradiance exposure (Boyd and Doney, 2002; Behrenfeld et al., 2006; Polovina et al., 2008). Because phytoplankton productivity provides the basis of open ocean ecosystems and a feedback for anthropogenic carbon emissions, it is important to understand how these changes affect phytoplankton performance and community composition.

Although it has previously been reported that both prokaryotic and eukaryotic species can successfully acclimate to higher irradiances (Kana and Glibert, 1987; Moore and Chisholm, 1999; Van de Poll et al., 2007), this study shows that when both groups are compared under identical experimental conditions relevant for the open ocean, eukaryotic species show a higher photoacclimation potential than prokaryotic species. The observed differences in photoacclimation to high irradiances (125 μmol photons $m^{-2}s^{-1}$) between prokaryotic and eukaryotic species are evident in both growth and photosynthetic parameters. The eukaryotic species *Ostreococcus* sp., *E. huxleyi*, and *T. oceanica* are able to maintain substantial growth under high irradiance conditions. This is in agreement with earlier studies, performed with the same (Sakshaug et al., 1987; Rodriguez et al., 2005; Leonardos and Harris, 2006) or other eukaryotic (pico)phytoplankton species, such as *Pelagomonas calceolata* (Dimier et al., 2009) and *Chlorella vulgaris* (Wagner et al., 2006). Consistent with the irradiance at which photosynthesis saturates (E_K), the observed growth rates indicate that *Ostreococcus*

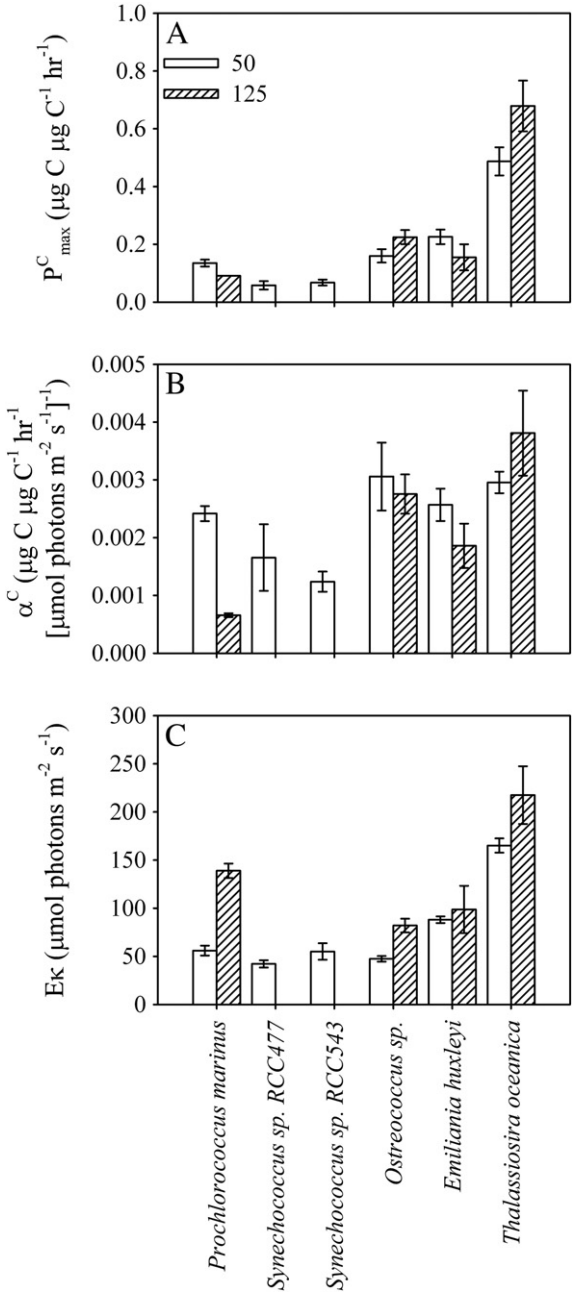

Fig. 6. Averages (±standard deviation, $n=4$) for the maximum photosynthetic rate (A), initial rate of photosynthesis (B), and photoadaptation index (C) normalized to carbon of the six phytoplankton strains at 50 and 125 µmol photons m^{-2} s^{-1}. Values for constant and dynamic irradiance treatments were grouped according to the average irradiance given during the treatment (50 or 125 µmol photons m^{-2} s^{-1}).

sp., *E. huxleyi*, and *T. oceanica* can photoacclimate relatively well to high irradiance intensities. In contrast, growth and photosynthesis in the prokaryotic species *P. marinus* and *Synechococcus* sp. (both strains) saturates at relatively low irradiances. This indicates that the *Prochlorococcus* strain used in this study, a low B/A ecotype generally assumed to be high light adapted (Partensky et al., 1993; Moore et al., 1995; Moore et al., 1998), shows a lower photoacclimation potential at high irradiance intensities compared to that of the eukaryotic species. *Synechococcus* sp. is in this study represented by two strains that have not been addressed previously in laboratory experiments. The isolation depth (120 and 10 m, respectively) and pigmentation could indicate that strains RCC477 and RCC543 are two different light adapted ecotypes of *Synechococcus*. However, both *Synechococcus* sp. strains show a low photoacclimation potential to high irradiances comparable to that of *P. marinus*. This is concurrent with earlier observations made for other *Synechococcus* strains under similar experimental conditions (Barlow and Alberte, 1985; Moore et al., 2005; Fu et al., 2007). Under higher temperature conditions however, higher growth rates and photoacclimation to higher irradiance intensities have been observed (Kana and Glibert, 1987; Morel et al., 1993; Six et al., 2004). It therefore appears that temperature plays an important role in the photosynthetic response of *Synechococcus* (see Moore et al., 1995).

The dynamic irradiance treatments represent mixed water column conditions, and are based on measurements from the North Atlantic Ocean during stratification (Stratiphyt cruise-I, July–August 2009, unpubl.). The irradiance climate experienced by phytoplankton in the upper mixed layer of the North Atlantic Ocean varies from 0.1 to 475 µmol photons m^{-2} s^{-1} ($K_d = 0.057$ m^{-1}) (also see Kirk, 1994), consistent with the dynamic irradiance regime of this study averaging at 125 µmol photons m^{-2} s^{-1}. However, higher irradiance levels can be encountered near the surface (upper 10 m). The other dynamic irradiance regime (averaging at 50 µmol photons m^{-2} s^{-1}) could represent the irradiance climate phytoplankton experience deeper in the water column. Although dynamic irradiances have previously been found to affect photosynthetic performance in eukaryotic species (Van Leeuwe et al., 2005; Van de Poll et al., 2007; Dimier et al., 2009), the present study indicates that dynamic irradiances do not necessarily affect growth or photosynthesis at lower irradiance intensities in the eukaryotic species *Ostreococcus* sp., *E. huxleyi*, and *T. oceanica*. However, the long-term growth measurements do not directly reflect the differences found between the constant en dynamic irradiance treatments in the short-term measured photosynthetic characteristics. In the prokaryotic species *P. marinus* and *Synechococcus* sp., growth is possibly affected by photoinhibition at the maximum irradiance intensity of the dynamic irradiance regimes (166 and 400 µmol photons m^{-2} s^{-1}). In a separate experiment, *P. marinus* CCMP2389 is able to grow under high dynamic irradiance when acclimated to the irradiance regime by incrementing steps (as suggested by Moore et al., 2007). This is consistent with the observation that *Prochlorococcus* occurs in the upper layer of the water column of the seasonally stratified open oceans (Olson et al., 1990; Goericke and Welschmeyer, 1993; Partensky et al., 1999), where irradiance levels can occasionally be high. On the other hand, the rate at which *Prochlorococcus* photoacclimates raises questions on the productivity of this species during sudden exposure to high irradiances. Earlier studies on *Prochlorococcus* show that, during a light shift from low to high irradiance (from 8 to 57 µmol photons m^{-2} s^{-1}), photoacclimation is completed after 40 h in a low B/A ecotype of *Prochlorococcus* (MED4), while it may take more than three days in a high B/A ecotype (Bricaud et al. 1999). This is consistent with the observed lag-phase during growth measurements in this study (data not shown). Moreover, the uptake of inorganic carbon does not immediately result in cell growth when irradiance increases rapidly (this study; Cailliau et al., 1996). In contrast, the eukaryotic species used in this study, *Ostreococcus* sp., *E. huxleyi*, and *T. oceanica*, and other studies, such as *P. calceolata* (Dimier et al., 2009) and *Picochlorum* sp. (Dimier et al., 2007), can regulate photosynthesis and growth at much smaller timescales. For these species, maximum growth rates are established within 24 h after transfer to higher irradiance levels (this study; Dimier et al., 2007; Dimier et al., 2009).

The initial response of photoacclimation is related to the regulation of light harvesting and photoprotective pigments. Although many other processes are involved in photoacclimation (Falkowski and La Roche, 1991), the regulation of pigments is one of the fastest responses to irradiance fluctuations. One of the most pronounced differences in pigmentation between prokaryotic and eukaryotic phytoplankton species is the presence of an epoxydation/de-epoxydation (xanthophyll) cycle in eukaryotic species (Olaizola et al., 1994; Horton et al., 1996; Muller et al., 2001). Xanthophyll cycle

pigments fulfill a dual function, because the epoxidized pigments assist in light harvesting, whereas the de-epoxidized pigments dissipate excessive irradiance in the form of heat. The enzymatic conversion of xanthophyll cycle pigments is crucial in preventing photoinhibition and viability loss during excessive irradiance exposure (Moisan et al., 1998; Van de Poll et al., 2005). Apart from the flexibility in photoprotective pigment content in the eukaryotic species, the initial response of photoacclimation could be significantly faster in the eukaryotic species compared to the prokaryotic species due to the presence of a photoprotective (xanthophyll) pigment cycle. The importance of the photoprotective (xanthophyll) pigment cycle in *Ostreococcus* sp., *E. huxleyi*, and *T. oceanica* is also evident in the increased de-epoxidation state of the xanthophyll cycle pigments at higher irradiances (data not shown). In contrast, prokaryotic phytoplankton species contain the photoprotective xanthophyll pigment zeaxanthin that is not regulated by an epoxydation/de-epoxydation cycle (Bidigare et al., 1989; Goericke and Repeta, 1992; Moore et al., 1995). The photoprotective function of zeaxanthin in *Prochlorococcus* and *Synechococcus* is often questioned, since zeaxanthin is spatially separated from the photosystems in other cyanobacteria (Siefermann-Harms, 1985). Yet, based on the observation that zeaxanthin increases with irradiances in this study, it is assumed that this pigment does provide some form of photoprotection in strains *P. marinus* CCMP2389, *Synechococcus* sp. RCC477, and RCC543. This idea is supported by the high concentrations of zeaxanthin relative to chlorophyll *a* found in the field (Letelier et al., 1993; Claustre and Marty, 1995). In addition to the enhancement of photoprotection at high irradiance, the eukaryotic species *Ostreococcus* sp., *E. huxleyi*, and *T. oceanica* decrease light harvesting pigmentation to reduce the energy entering the photosystems. Based on chlorophyll *a* levels, such a trend could not be observed in the prokaryotic species *P. marinus* and *Synechococcus* sp. (both strains). When chlorophyll *b* (chl-*b*/*a*) in *P. marinus* is considered, this species does show a decrease in light harvesting pigmentation. However, the main light harvesting pigments in *Synechococcus* sp., phycobilins, are not quantified and it is therefore unsure if *Synechococcus* sp. RCC477 and RCC543 additionally decrease light harvesting at high irradiance.

It is often argued that *Prochlorococcus* and *Synechococcus* are better adapted to the irradiance climate experienced at the bottom of the euphotic zone and that eukaryotic picophytoplankton would be light limited under such conditions (Moore et al., 1995; Palenik, 2001; Veldhuis et al., 2005). However, in this study the eukaryotic species *Ostreococcus* sp., *E. huxleyi*, and *T. oceanica*, although light limited, show higher growth rates than the prokaryotic species at the lowest irradiance intensity (10 μmol photons m^{-2} s^{-1}). This shows that the eukaryotic phytoplankton species can competitively grow at irradiance intensities found in the deep chlorophyll maximum (between 0 and 15 μmol photons m^{-2} s^{-1}; Stratiphyt cruise-I, July–August 2009, unpubl.). As for the spectral composition of irradiance, the small differences found in the blue:red absorption ratios between the different species suggests that the absorption of relative blue irradiance could be as effective in eukaryotic as in prokaryotic phytoplankton species at low irradiances. This is supported by the pigment composition of the eukaryotic species used in this study. Especially *Ostreococcus* sp. shows a pigment signature suitable for the absorption of blue irradiance due to the presence of high concentrations of chlorophyll *b* (data not shown) (Rodriguez et al., 2005; Six et al., 2005). In addition, *E. huxleyi* and *T. oceanica* contain several carotenoids that reinforce blue absorption, such as fucoxanthin (Wright et al., 1991). Moreover, the absorbed energy in the blue part of the irradiance spectrum is partly dissipated as heat by photoprotective pigments and is therefore lost for photosynthesis (Bidigare et al., 1989). This dissipation of energy could be relatively high in the prokaryotic compared to the eukaryotic phytoplankton strains used in this study, since the ratio of photoprotective pigments to chlorophyll *a* is almost five times higher in *P. marinus*, *Synechococcus* sp. RCC477, and RCC543 compared to *Ostreococcus* sp., *E. huxleyi*, and *T. oceanica*. When ecotypes of *Prochlorococcus* prevailing at the bottom of the euphotic zone (high B/A ecotypes) are considered, it is however, clear that some cyanobacterial ecotypes do have a possible advantage of the spectral irradiance composition at depth (Morel et al., 1993; Partensky et al., 1993; Moore et al., 1995).

The observed species composition and community structure in open oligotrophic oceans is often explained by a combination of the response of specific phytoplankton species to certain irradiance and nutrient conditions. However, from the strains studied here, it can be stated that, when nutrients are readily available, eukaryotic phytoplankton species would outperform prokaryotic species. The ability to photoacclimate to a wide variety of both stable and dynamic irradiances regimes offers the eukaryotic species an advantage in the mixed layer, but also at the bottom of the euphotic zone. This implicates that other factors influencing production and growth of phytoplankton, such as nutrient availability, play a more important role in structuring the phytoplankton community found in the oligotrophic systems.

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