Vol. 466: 43–55, 2012
doi: 10.3354/meps09898

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published October 15

Temperature-dependent growth and photophysiology of prokaryotic and eukaryotic oceanic picophytoplankton

Gemma Kulk[1,*], Pablo de Vries[1], Willem H. van de Poll[2], Ronald J. W. Visser[1], Anita G. J. Buma[1]

[1]Department of Ocean Ecosystems, Energy and Sustainability Research Institute Groningen, University of Groningen, 9747 AG Groningen, The Netherlands

[2]Department of Biological Oceanography, Royal Netherlands Institute for Sea Research, 1790 AB Den Burg, The Netherlands

ABSTRACT: It is expected that climate change will expand the open oligotrophic oceans by enhanced thermal stratification. Because temperature defines the geographic distribution of picophytoplankton in open-ocean ecosystems and regulates photophysiological responses, it is important to understand how temperature affects picophytoplankton growth and photophysiology. Two prokaryotic and 2 eukaryotic picophytoplankton strains were acclimated to 3 different temperatures, ranging from 16 to 24°C. Temperature-dependent growth and photophysiology were assessed by measurements of specific growth rates, cell size, pigment composition, absorption and electron transport rates. Growth of *Prochlorococcus marinus* (eMED4), *Prochlorococcus* sp. (eMIT9313), *Ostreococcus* sp. (clade B) and *Pelagomonas calceolata* was positively related to temperature, especially in the prokaryotic strains. Changes in photophysiology included increased light harvesting, increased electron transport and reduced photoinhibition at elevated temperatures. However, the changes related to light harvesting and electron transport could not fully explain the observed difference in growth. This suggests that other processes, such as Calvin cycle activity, are likely to limit growth at sub-optimal temperatures in these picophytoplankton strains. The overall changes in photophysiology during temperature acclimation will possibly allow photosynthesis at higher irradiance intensities, but the genetically defined low temperature tolerances and photosynthetic characteristics of the different ecotypes will likely be more important in determining picophytoplankton (depth) distribution and community composition.

KEY WORDS: *Prochlorococcus* · Eukaryotic picophytoplankton · Temperature · Growth · Pigment · Absorption · Electron transport rate

INTRODUCTION

Research related to climate change has largely focused on polar systems and organisms, although changes in other oceanic systems can also be expected. In temperate to warm-temperate oceanic regions, a rise in seawater temperature by 1.5 to 4.5°C over the next century (Houghton et al. 1995) will lead to changes in water column stratification. The subsequent modifications in mixed layer dynamics will decrease nutrient availability and increase levels of photosynthetic active radiation (PAR) and ultraviolet radiation in surface layers of the open oceans (Behrenfeld et al. 2006, Doney 2006). Elevated temperatures, as well as the indirect changes in the irradiance climate, can have pronounced effects on phytoplankton productivity and community structure (Schmittner 2005, Behrenfeld et al. 2006, Litchman et al. 2006).

*Email: g.kulk@rug.nl

The phytoplankton community of open oligotrophic oceans is dominated by *Prochlorococcus* spp., *Synechococcus* spp., and eukaryotic nano- and picophytoplankton (Olson et al. 1990, Li 1994, DuRand et al. 2001). The geographic distribution of these phytoplankton species is highly related to temperature. For example, the abundance of *Prochlorococcus* spp. is highest in warm waters with temperatures above 17°C (Partensky et al. 1999, Johnson et al. 2006). Similar trends are found for *Synechococcus* spp., with a strong positive relationship between *Synechococcus* spp. abundance and temperatures above 14°C (Agawin et al. 1998, Li 1998, Moisan et al. 2010), and for *Ostreococcus* spp., with a temperature range from 11 to 26°C (Demir-Hilton et al. 2011). The geographic ranges of picophytoplankton are thought to be species and ecotype specific, determined by genetically defined low temperature tolerances (Moore et al. 1995, Johnson et al. 2006, Zinser et al. 2007). In addition to the effect of temperature on the distribution of picophytoplankton, general trends in community production and biomass are also evident. Typically, the biomass and primary production of picophytoplankton and the contribution of picophytoplankton to the total phytoplankton production increase with temperature (Agawin et al. 2000, Feng et al. 2009, Morán et al. 2010).

Even though temperature is an important factor in picophytoplankton ecology, only a few studies have focused on the effects of temperature on growth and photophysiology of this specific phytoplankton size class (Moore et al. 1995, Fu et al. 2007, Zinser et al. 2007). Especially little information is available on the eukaryotic picophytoplankton. Like other phytoplankton, picophytoplankton show a traditional temperature-dependent growth curve (Eppley 1972, Moore et al. 1995, Zinser et al. 2007). The initial photochemical reactions are independent of temperature, but many associated aspects of photosynthesis, such as enzymatic activities, membrane fluidity and electron transport, are reduced at sub-optimal temperatures (Oquist 1983, Raven & Geider 1988). These changes are accompanied by changes in the light-harvesting complex. Typically, phytoplankton acclimated to low temperatures show a photophysiology comparable to that of high-light-acclimated phytoplankton, with low levels of cellular chlorophyll *a* (chl *a*) (Geider 1987, Maxwell et al. 1994, Stramski et al. 2002). This was also found for the prokaryotic picophytoplankton species *Prochlorococcus marinus* (eMED4) and *Synechococcus* sp. (WH7803) (Fu et al. 2007). In eukaryotic nanophytoplankton, other changes in the light-harvesting complex involved in low temperature acclimation are a reduction in photosystem II (PSII) reaction center size and abundance (Davison 1991, Wilson & Huner 2000) and an increase in photoprotective pigments relative to light-harvesting pigments (Wilson & Huner 2000, Helbling et al. 2011). As temperature increases, the constraints on photosynthesis gradually decrease with increased chl *a* synthesis and enhanced light capture (Falkowski & Raven 1997, Stramski et al. 2002). Beyond the optimal temperature, the demand in ATP and carbohydrates exceeds that of new production, and growth and photosynthesis decrease abruptly (Raven & Geider 1988, Davison 1991).

In the present study, temperature-dependent processes in oceanic picophytoplankton were studied to improve the knowledge on the direct and indirect effects of a rise in seawater temperature on picophytoplankton performance in open-ocean ecosystems. Therefore, 2 prokaryotic and 2 eukaryotic picophytoplankton strains were acclimated to 3 different temperatures. Growth and photophysiology were assessed by analysis of specific growth rates, cell size, pigment composition, absorption and electron transport rates. The results are related to the possible effect of elevated temperature on picophytoplankton photophysiology and species distribution. In addition, the use of fluorescence analysis in the assessment of picophytoplankton photophysiology is discussed.

MATERIALS AND METHODS

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- and high-light-adapted species in open-ocean ecosystems. *Ostreococcus* sp. strain RCC410 (ecotype clade B or OII) and *Pelagomonas calceolata* strain RCC879 were cultured in K medium based on natural oceanic seawater (35 PSU) as described by Keller et al. (1987). Final nutrient concentrations in K medium were 50 µM NH_4, 882 µM NO_3 and 10 µM PO_4. *Prochlorococcus marinus* strain CCMP2389 (ecotype MED4) and *Prochlorococcus* sp. strain RCC407 (ecotype MIT9313) were cultured in an adjusted version of the K medium, with a 10 times diluted concentration of trace metals minus copper (K/10–Cu; see Chisholm 1992). Final nutrient concentrations in K/10–Cu

medium were 50 µM NH_4 and 10 µM PO_4. 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.) at a diurnal cycle of 12 h:12 h light:dark at 20°C.

Experimental design

Cultures of *Prochlorococcus marinus*, *Prochlorococcus* sp., *Ostreococcus* sp. and *Pelagomonas calceolata* were transferred to 500 ml glass Erlenmeyer flasks and incubated in triplicate at 16, 20 and 24°C. Experiments were carried out in a temperature-controlled U-shaped lamp setup as described by Van de Poll et al. (2007). The temperature in the setup was maintained at 16, 20 and 24°C by a thermostat (RK 8 KS, edition 2000, Lauda Dr. R. Wobser & Co.) and deviated by 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 h:12 h light:dark cycle (monitored with a QSL-100, Biospherical Instruments). Prior to the experiments, the strains were kept in the exponential growth phase and were acclimated to the experimental irradiance and temperature conditions for at least 3 wk. Growth and maximum quantum yield of PSII were followed daily starting directly after the beginning of the incubation. In the mid-exponential growth phase, the photophysiology of the cultures was assessed by analysis of pigments, absorption spectra and electron transport rates. Cell densities during these measurements ranged from 6×10^6 cells ml^{-1} in *P. marinus* and *Prochlorococcus* sp. to 17×10^6 cells ml^{-1} in *Ostreococcus* sp. and 2×10^6 cells ml^{-1} in *P. calceolata*. Culturing of *Prochlorococcus* sp. RCC407 (eMIT9313) 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 additional measurements were performed for *Prochlorococcus* sp. under these conditions.

Growth measurements

Samples (1 ml) for cell counts were obtained daily during the exponential growth phase. Cell concentrations were determined on a Coulter Epics MXL flow cytometer (Beckman Coulter). Growth rates (µ; d^{-1}) of the exponential growth phase were calculated by linear regression of natural log-transformed cell numbers for all replicates (≥4 data points per replicate). The temperature quotient (Q_{10}) for growth was calculated as $(\mu_{T_2}/\mu_{T_1})^{10/(T_2-T_1)}$, where T_1 and T_2 are 16 and 24°C, respectively. 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).

Pigment composition

Samples (25 to 30 ml) for pigment analysis were collected during the exponential growth phase for each replicate culture. 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 Hooker et al. (2009). 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 an HPLC (Waters 2695 separation module, 996 photodiode array detector) equipped with a Zorbax Eclipse XDB-C_8 3.5 µm column (Agilent Technologies). Peaks were identified by retention time and diode array spectroscopy. Pigments were quantified using standards (DHI LAB products) of chl a_1, chl a_2, chl *b*, chl c_2, chl c_3, 19-butanoyloxyfucoxanthin, 19-hexanoyloxyfucoxanthin, fucoxanthin, neoxanthin, prasinoxanthin, lutein, diadinoxanthin, diatoxanthin, antheraxanthin, violaxanthin, zeaxanthin, α-carotene and β-carotene. From here on, chl *a* will refer to chl a_2 in *Prochlorococcus marinus* and *Prochlorococcus* sp. and to chl a_1 in *Ostreococcus* sp. and *Pelagomonas calceolata*.

Absorption spectra

Samples for phytoplankton pigment absorption spectra were taken during the exponential growth phase for each replicate culture. 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 350 and 800 nm. For analysis, 25 to 30 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 & 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 absorp-

tion was calculated (β was set to 2) and normalized to chl *a* concentrations to obtain the specific absorption coefficient by phytoplankton $a^*_{ph}(\lambda)$ (m^2 mg^{-1} chl *a*). The spectrally weighted mean specific absorption coefficient $\bar{a}^*$ (m^2 mg^{-1} chl *a*) was calculated by:

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

where $E(\lambda)$ is the irradiance used in the incubator during the electron transport rate measurements. The blue:red ratio was calculated by dividing the maximum $a^*_{ph}(\lambda)$ between 350 and 600 nm by the maximum $a^*_{ph}(\lambda)$ between 650 and 700 nm.

PSII fluorescence

PSII fluorescence analyses were performed on a WATER-PAM chlorophyll fluorometer (Waltz) equipped with a WATER-FT flow-through emitter-detector unit (blue LED) and analyzed using WinControl software (version 2.08, Waltz) according to Maxwell & Johnson (2000; and references therein). For daily analysis, the maximum quantum yield of PSII (F_v/F_m) was measured for each replicate culture. In addition, the electron transport rate (ETR) was recorded for cultures of *Prochlorococcus marinus*, *Prochlorococcus* sp., *Ostreococcus* sp. and *Pelagomonas calceolata* growing in exponential growth phase at 16 (except *Prochlorococcus* sp.), 20 and 24°C.

Maximum quantum yield of PSII. For daily analysis, 5 to 15 ml culture samples were dark-adapted for at least 20 min at 16, 20 or 24°C. For measurements, the measuring light (frequency 3) was turned on and the minimal fluorescence F_0 was recorded. During a saturating light flash (0.6 s, ±1100 µmol photons m^{-2} s^{-1}), the maximum fluorescence in the dark-adapted state, $F_m°$, was then recorded. F_v/F_m was calculated as $(F_m° - F_0)/F_m°$.

Electron transport rates. ETR was determined by exposing separate culture samples of 3.8 ml to 10 different irradiance levels ranging from 19.9 to 630.9 µmol photons m^{-2} s^{-1} (250 W MHN-TD lamp, Philips) in a temperature-controlled incubator. After 20 min of exposure, the quantum yield of PSII (Φ_{PSII}) was determined by measuring the steady-state fluorescence prior to the saturating light flash, F_t, and the maximum fluorescence in the light, F_m'. Φ_{PSII} was calculated as $(F_m'-F_t)/F_m'$. The absolute ETR (aETR; mol e^- μg^{-1} chl *a* h^{-1}) for each irradiance level was calculated by:

$$aETR = \Phi_{PSII} \cdot E \cdot \bar{a}^* \cdot 0.5 \quad (2)$$

where E (µmol photons m^{-2} s^{-1}) is the irradiance level of the incubator, $\bar{a}^*$ (m^2 mg^{-1} chl *a*) is the spectrally weighted mean specific absorption coefficient and 0.5 is a factor accounting for the partitioning of energy between photosystem I (PSI) and PSII. aETR versus irradiance curves were fitted to the empirical model described by Platt et al. (1980) using LABFit software (version 7.2.45, Wilton and Cleide P. Silva) to estimate the maximum aETR (ETR_{max}), the initial aETR (α_{ETR}), the photoacclimation index ($E\kappa$) and photoinhibition (β_{ETR}). If no photoinhibition was present, the aETR versus irradiance curves were fitted to the model of Webb et al. (1974) to estimate ETR_{max}, α_{ETR} and $E\kappa$. The Q_{10} for ETR_{max} was calculated as $(ETR_{max,T2}/ETR_{max,T1})^{10/(T2-T1)}$.

Statistical analysis

Differences between the 3 temperature conditions and differences between species were statistically tested by ANOVA using STATISTICA software (version 8.0 and 10.0, StatSoft). Before analysis, data were tested for normality and homogeneity of variances. Differences were considered significant when $p < 0.05$.

RESULTS

Growth

Temperature had a positive effect on the growth rates of the 4 oceanic picophytoplankton strains (Fig. 1). Growth rates increased significantly with increasing temperature in the prokaryotic species *Prochlorococcus marinus* (from $\mu_{16°C}$ = 0.23 ± 0.02 d^{-1} to $\mu_{24°C}$ = 0.63 ± 0.05 d^{-1}) and *Prochlorococcus* sp. (from $\mu_{20°C}$ = 0.17 ± 0.02 d^{-1} to $\mu_{24°C}$ = 0.30 ± 0.02 d^{-1}; $p < 0.005$). No growth was observed in *Prochlorococcus* sp. at 16°C, incubated at an irradiance intensity of 50 µmol photons m^{-2} s^{-1}. Several attempts showed that this *Prochlorococcus* strain was able to grow at 16°C, but only at lower irradiance intensities (25 µmol photons m^{-2} s^{-1}, µ = 0.12 ± 0.03 d^{-1}). An increasing trend in growth rate with temperature was also found in the eukaryotic species. In *Ostreococcus* sp., the lowest growth rate was found at the lowest temperature ($\mu_{16°C}$ = 0.69 ± 0.01 d^{-1}; $p < 0.0005$), but cultures grown at 20 and 24°C showed similar growth rates (µ = 1.09 ± 0.03 and 1.05 ± 0.05 d^{-1}, respectively). The highest

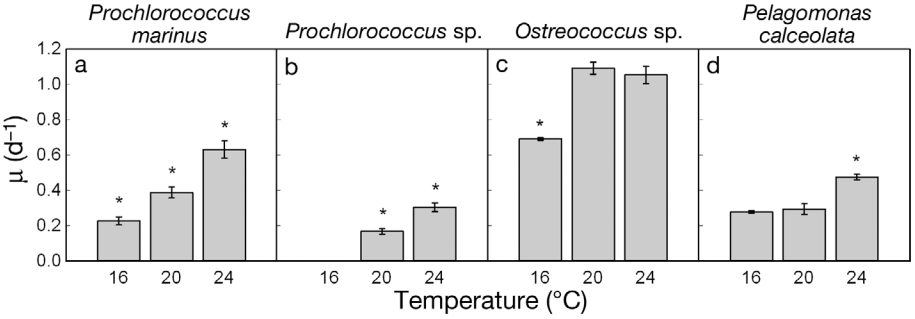

Fig. 1. Mean (±SD, n = 3) growth rates (μ) for (a) *Prochlorococcus marinus* eMED4, (b) *Prochlorococcus* sp. eMIT9313, (c) *Ostreococcus* sp. clade B and (d) *Pelagomonas calceolata* at 16, 20 and 24°C. No growth was observed for *Prochlorococcus* sp. at 16°C and 50 μmol photons m^{-2} s^{-1}. Asterisks indicate significant effects ($p < 0.05$) of temperature on the growth rate within each species

growth rates in *Pelagomonas calceolata* were found at 24°C (μ = 0.47 ± 0.02 d^{-1}) ($p < 0.0005$), but no difference was found between the growth rates at 16 and 20°C (μ = 0.28 ± 0.01 and 0.30 ± 0.03 d^{-1}, respectively). Overall, *Ostreococcus* sp. showed the highest growth rates, followed by *P. marinus*, *P. calceolata* and *Prochlorococcus* sp., respectively ($p < 0.005$). Q_{10} values for growth were 3.59 for *P. marinus*, 4.41 for *Prochlorococcus* sp., 1.69 for *Ostreococcus* sp. and 1.96 for *P. calceolata*.

Temperature affected the cell size of the picophytoplankton, but this effect was small and not uniform among the different strains (Table 1). In *Prochlorococcus marinus* and *Ostreococcus* sp., cell size decreased with increasing temperature by −1.4% ($p < 0.05$, not significant between 20 and 24°C) and −5.6% ($p < 0.05$, not significant between 16 and 20°C), respectively. In contrast, in *Prochlorococcus* sp. and *Pelagomonas calceolata*, cell size increased with increasing temperature by +1.6% ($p < 0.05$) and +10.1% ($p < 0.05$, not significant for 20°C), respectively. Overall, *P. calceolata* had the largest cell size, followed by the eukaryotic species *Ostreococcus* sp. and the prokaryotic species *Prochlorococcus* sp. and *P. marinus*, respectively ($p < 0.05$). No significant relationship was found between temperature-induced changes in cell size and cellular chl *a* concentration, total cellular pigment concentration (data not shown) or the spectrally weighted mean specific absorption coefficient.

Table 1. Mean (±SD, n = 3) cell size (diameter in μm) of *Prochlorococcus marinus* eMED4, *Prochlorococcus* sp. eMIT9313, *Ostreococcus* sp. clade B and *Pelagomonas calceolata* grown at 16, 20 and 24°C. Different superscript letters indicate significant effects of the temperature treatment within each species ($p < 0.05$). n/a: data not available, growth was not observed under the used conditions and no additional measurements were performed

	Prochlorococcus marinus	*Prochlorococcus* sp.	*Ostreococcus* sp.	*Pelagomonas calceolata*
16°C	0.767 ± 0.001^{a,b}	n/a	1.033 ± 0.005^{d}	2.294 ± 0.047^{f}
20°C	0.759 ± 0.002^{a}	0.848 ± 0.006^{c}	1.035 ± 0.032^{e}	2.471 ± 0.017
24°C	0.756 ± 0.004^{b}	0.862 ± 0.002^{c}	0.975 ± 0.011^{d,e}	2.553 ± 0.144^{f}

Pigment composition

Changes in the pigment composition with temperature were most evident in the prokaryotic species. In *Prochlorococcus marinus* and *Prochlorococcus* sp., the pigments chl a_2 and *b*, zeaxanthin and α-carotene were identified. The effect of temperature on the pigmentation was similar in both *Prochlorococcus* strains, although differences in *Prochlorococcus* sp. were not significant. The light-harvesting pigments, here indicated by the cellular chl *a* levels and the chl *b*/*a* ratio, increased (by 3 to 29%) with increasing temperature ($p < 0.05$ for *P. marinus*, not significant between 20 and 24°C; Fig. 2a,b,d,e). The photoprotective pigmentation decreased with increasing temperature ($p < 0.001$ for *P. marinus*; Fig. 2c,f). This was especially evident in *P. marinus*, in which the zeaxanthin to chl *a* ratio decreased by 33%.

In the eukaryotic species, the effects of temperature on pigmentation were evident, but the observed differences were often not significantly different. In *Ostreococcus* sp., the pigments chl *a* and *b*, prasinoxanthin, neoxanthin, violaxanthin, antheraxanthin, zeaxanthin, lutein and β-carotene were identified. All cellular light-harvesting pigment concentrations, including chl *a* (26%; Fig. 2g), increased with temperature in *Ostreococcus* sp., but the ratios per chl *a* generally remained unaffected (Fig. 2h). The xanthophyll cycle pigment to chl *a* ratio (Fig. 2i) and the de-epoxidation of the xanthophyll pigment cycle (18 to 32%, data not shown) showed a decreasing trend with increasing temperature. The other eukaryotic species, *Pelagomonas calceolata*, contained the pigments chl *a*, c_2 and c_3, 19-butanoyloxyfucoxanthin, 19-hexanoyloxyfucoxanthin, fucoxanthin, diadinoxanthin, diatoxanthin and β-carotene. The cellular concentrations of these pigments increased with increasing temperatures (chl *a* by 23%; Fig. 2j), except for 19-hexanoyloxyfucoxanthin and β-carotene. The main light-harvesting

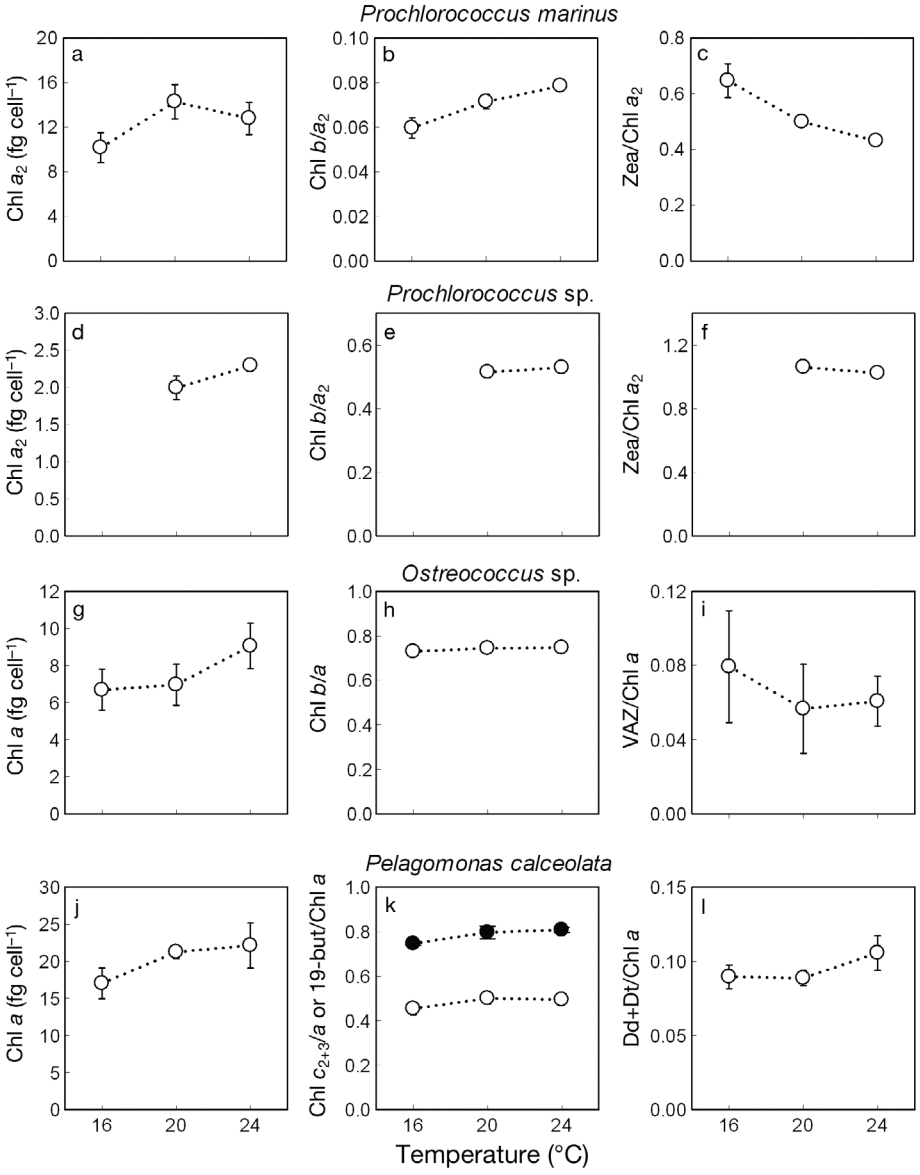

Fig. 2. Overview of the main light-harvesting and photoprotective pigments. Mean (±SD, n = 3) cellular chl *a* concentrations and the ratios of the main accessory and photoprotective pigments per chl *a* are given for (a–c) *Prochlorococcus marinus* eMED4, (d–f) *Prochlorococcus* sp. eMIT9313, (g–i) *Ostreococcus* sp. clade B and (j–l) *Pelagomonas calceolata* grown at 16, 20 and 24°C. For *P. calceolata*, the accessory pigment ratios for chl c_2 and c_3 are indicated by open circles and the pigment ratios for 19-butanoyloxyfucoxanthin by filled circles. 19-but: 19-butanoyloxyfucoxanthin; Dd: diadinoxanthin; Dt: diatoxanthin; VAZ: violaxanthin, antheraxanthin and zeaxanthin; Zea: zeaxanthin. Note that the scales of the pigment concentrations and ratios are different between the panels

pigments, chl c_2, chl c_3 and 19-butanoyloxyfucoxanthin, increased with increasing temperature per chl *a* ($p < 0.05$, not significant between 20 and 24°C; Fig. 2k). An increasing trend with increasing temperature was also found for the xanthophyll cycle pigment pool (16%, not significant; Fig. 2l) and the de-epoxidation state of the xanthophyll pigment cycle (22 to 24%, data not shown).

The pigment compositions of the different phytoplankton species were considerably different, and are therefore difficult to compare. However, it was clear that the prokaryotic species contained the highest levels of photoprotective pigments relative to chl *a* ($p < 0.005$; Fig. 2, note the differences in scale).

Absorption spectra

No significant effect of temperature was found on the spectrally weighted mean specific absorption

Table 2. Mean (±SD, n = 3) spectrally weighted mean specific absorption coefficient $\bar{a}^*$ (m^2 mg^{-1} chl *a*) and the blue:red ratio of the absorption spectra of *Prochlorococcus marinus* eMED4, *Prochlorococcus* sp. eMIT9313, *Ostreococcus* sp. clade B and *Pelagomonas calceolata* grown at 16, 20 and 24°C. Different superscripted letters indicate significant effects of the temperature treatment within each species ($p < 0.05$). n/a: data not available, growth was not observed under the used conditions and no additional measurements were performed

	Prochlorococcus marinus	*Prochlorococcus* sp.	*Ostreococcus* sp.	*Pelagomonas calceolata*
$\bar{a}^*$				
16°C	$0.015 \pm 1.38 \times 10^{-3}$	n/a	$0.019 \pm 2.35 \times 10^{-3}$	$0.017 \pm 0.87 \times 10^{-3}$
20°C	$0.013 \pm 1.68 \times 10^{-3}$	$0.033 \pm 0.31 \times 10^{-3}$	$0.018 \pm 1.86 \times 10^{-3}$	$0.018 \pm 0.94 \times 10^{-3}$
24°C	$0.013 \pm 0.75 \times 10^{-3}$	$0.033 \pm 5.52 \times 10^{-3}$	$0.017 \pm 0.74 \times 10^{-3}$	$0.019 \pm 1.61 \times 10^{-3}$
Blue:red				
16°C	2.49 ± 0.39	n/a	2.03 ± 0.09	2.17 ± 0.13[a]
20°C	2.41 ± 0.06	3.60 ± 0.18	1.93 ± 0.03	1.92 ± 0.05[a]
24°C	2.40 ± 0.05	3.59 ± 0.24	1.88 ± 0.01	2.11 ± 0.06

coefficient ($\bar{a}^*$) (Table 2). When the different strains were compared, *Prochlorococcus* sp. had the highest $\bar{a}^*$ ($p < 0.001$), whereas *Prochlorococcus marinus* had the lowest $\bar{a}^*$ ($p < 0.05$, except at 16°C). No significant differences in absorption were found between the 2 eukaryotic species *Ostreococcus* sp. and *Pelagomonas calceolata*, and levels of $\bar{a}^*$ of the eukaryotic strains were in between those of *P. marinus* and *Prochlorococcus* sp.

The blue:red ratio remained similar (*Prochlorococcus* sp.) or decreased somewhat with increasing temperature (other strains), but differences were not significant (except for *Pelagomonas calceolata* between 16 and 20°C, $p < 0.05$; Table 2). Overall, *Prochlorococcus* sp. had the highest blue:red ratios ($p < 0.001$). *Prochlorococcus marinus*, *Ostreococcus* sp. and *P. calceolata* showed similar ratios at 16°C, whereas the blue:red ratio was significantly higher in *P. marinus* at higher temperatures ($p < 0.005$). The blue:red ratio did not differ between the 2 eukaryotic species *Ostreococcus* sp. and *P. calceolata*.

Table 3. Mean (±SD, n = 3) maximum quantum yield of photosystem II (F_v/F_m) of *Prochlorococcus marinus* eMED4, *Prochlorococcus* sp. eMIT9313, *Ostreococcus* sp. clade B and *Pelagomonas calceolata* during exponential growth at 16, 20 and 24°C. Different superscript letters indicate significant effects of the temperature treatment within each species ($p < 0.05$). n/a: data not available, growth was not observed under the used conditions and no additional measurements were performed

	Prochlorococcus marinus	*Prochlorococcus* sp.	*Ostreococcus* sp.	*Pelagomonas calceolata*
16°C	0.599 ± 0.015[a]	n/a	0.559 ± 0.003[c,d]	0.527 ± 0.009
20°C	0.626 ± 0.010[a]	0.520 ± 0.012[b]	0.607 ± 0.010[c]	0.524 ± 0.002
24°C	0.622 ± 0.009	0.559 ± 0.003[b]	0.597 ± 0.005[d]	0.523 ± 0.003

Maximum quantum yield of PSII

In general, an increase in temperature had a positive, but small (4 to 8%), effect on the maximum quantum yield of PSII (F_v/F_m) (Table 3). In *Prochlorococcus marinus*, F_v/F_m increased from 16 to 20°C ($p < 0.05$), but no other significant differences were observed. In the other prokaryotic strain, *Prochlorococcus* sp., F_v/F_m increased with increasing temperature ($p < 0.005$). Similar to the results on growth rates, F_v/F_m in *Ostreococcus* sp. was significantly lower at 16°C compared with 20 and 24°C ($p < 0.005$), but similar between 20 and 24°C. In contrast, F_v/F_m of the other eukaryotic species, *Pelagomonas calceolata*, was not affected by temperature. Overall, *P. marinus* showed the highest F_v/F_m values, followed by *Ostreococcus* sp., *Prochlorococcus* sp., and *P. calceolata*, respectively ($p < 0.05$, not significant at 20°C).

Electron transport rate

Comparable to the growth rates, the absolute electron transport rates were positively affected by temperature in all strains (Fig. 3). The maximum electron transport rate (ETR_{max}) increased with increasing temperature in *Prochlorococcus* sp. and *Ostreococcus* sp. ($p < 0.05$; Fig. 3a). This trend in ETR_{max} with temperature was also shown for *Prochlorococcus marinus* and *Pelagomonas calceolata*, but differences between the temperatures were small and not significant in these strains. Q_{10} values for ETR_{max} were 1.10 for *P. marinus*, 2.35 for *Prochlorococcus* sp., 1.62 for *Ostreococcus* sp. and 1.19 for *P. calceolata*. The initial electron transport rate (α_{ETR}) increased

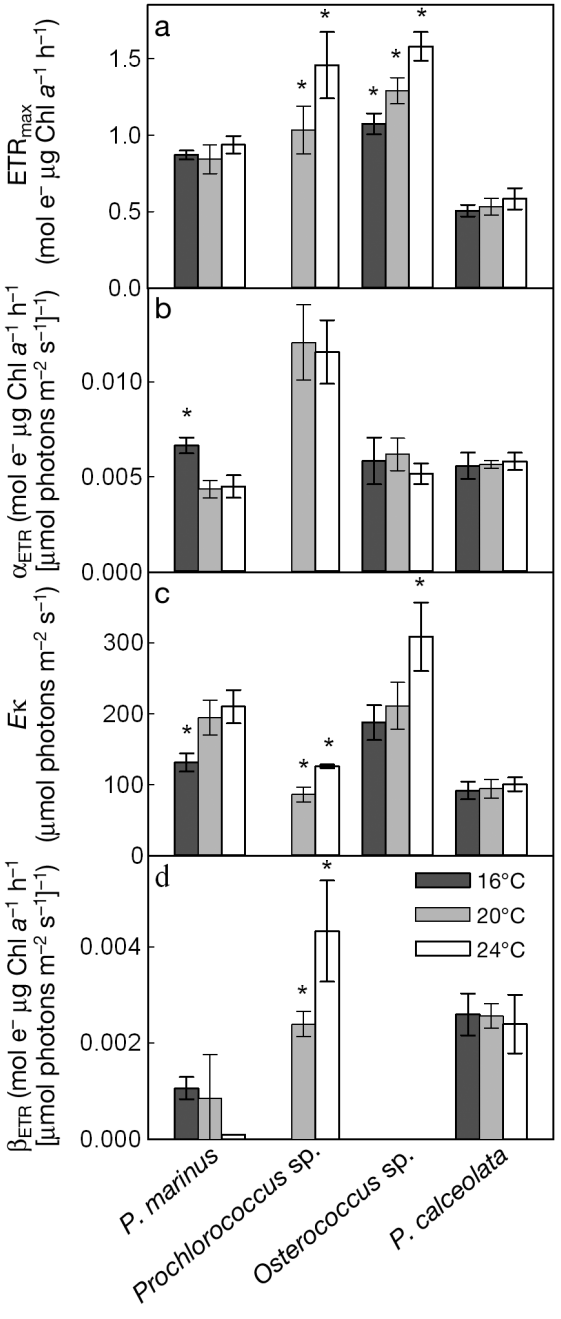

Fig. 3. Characteristics of electron transport. Means (±SD, n = 3) of (a) the maximum electron transport rate (ETR_{max}), (b) the initial electron transport rate (α_{ETR}), (c) the photoacclimation index ($E\kappa$) and (D) photoinhibition (β_{ETR}) are given for *Prochlorococcus marinus* eMED4, *Prochlorococcus* sp. eMIT9313, *Ostreococcus* sp. clade B and *Pelagomonas calceolata* grown at 16, 20 and 24°C. Asterisks indicate significant effects ($p < 0.05$) of temperature on the characteristics of electron transport within each species

significantly at 16°C compared with 20 and 24°C in *P. marinus* ($p < 0.005$), but remained similar at higher temperatures (Fig. 3b). In *Prochlorococcus* sp., *Ostreococcus* sp. and *P. calceolata*, α_{ETR} was not affected by temperature. The photoacclimation index ($E\kappa$) increased with increasing temperature in *P. marinus* ($p < 0.05$, not significant between 20 and 24°C), *Prochlorococcus* sp. ($p < 0.005$) and *Ostreococcus* sp. ($p < 0.05$, not significant between 16 and 20°C), indicating that these strains were acclimated to higher irradiance intensities at elevated temperatures (Fig. 3c). In *P. calceolata*, $E\kappa$ was not affected by temperature. Photoinhibition (β_{ETR}) showed a decreasing trend with increasing temperature in *P. marinus* (Fig. 3d). In *Prochlorococcus* sp., photoinhibition increased significantly at higher temperatures ($p < 0.05$). No photoinhibition was observed in *Ostreococcus* sp., although the aETR versus irradiance curves showed significantly lower electron transport rates at high irradiance intensities at 16°C compared with 20 and 24°C ($p < 0.05$). In *P. calceolata*, photoinhibition remained largely unaffected by changes in temperature.

The electron transport rates were markedly different between the species (Figs. 3 & 4). *Prochlorococcus* sp. and *Ostreococcus* sp. showed the highest ETR_{max}, followed by *Prochlorococcus marinus* and *Pelagomonas calceolata* ($p < 0.05$) (Figs. 3a & 4). The latter strain showed up to 63% lower electron transport rates compared with the other species. The initial electron transport rate was significantly highest in *Prochlorococcus* sp. ($p < 0.001$), but similar in *P.*

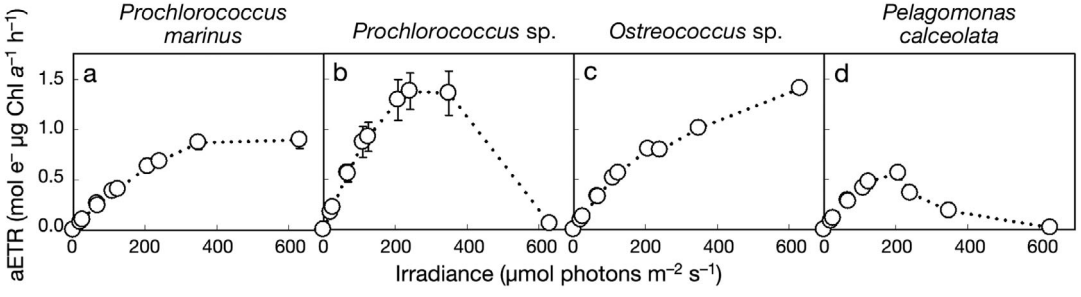

Fig. 4. Mean (±SD, n = 3) absolute electron transport rate (aETR) versus irradiance curves for (a) *Prochlorococcus marinus* eMED4, (b) *Prochlorococcus* sp. eMIT9313, (c) *Ostreococcus* sp. clade B and (d) *Pelagomonas calceolata* grown at 24°C

marinus, *Ostreococcus* sp. and *P. calceolata* (Figs. 3b & 4). *Ostreococcus* sp. was acclimated to the highest irradiance intensities ($p < 0.05$, not significantly different from *P. marinus* at 20°C). *Procholorococcus marinus* also showed a high E_K. Compared with *P. marinus* and *Ostreococcus* sp., *Prochlorococcus* sp. and *P. calceolata* were acclimated to significantly lower irradiance intensities (Figs. 3c & 4). Indicated by the acclimation to relative low irradiance intensities, *Prochlorococcus* sp. and *P. calceolata* showed the highest photoinhibition ($p < 0.05$; Figs. 3d & 4). *P. marinus* showed intermediate to low levels of photoinhibition, whereas *Ostreococcus* sp. showed no photoinhibition.

DISCUSSION

It is expected that climate change will mediate a rise in seawater temperature, thereby expanding the open oligotrophic oceans (Behrenfeld et al. 2006, Polovina et al. 2008). The changes in the onset and break-up of stratification and the mixed layer depth will alter nutrient availability and the intensity, spectral composition and dynamics of phytoplankton irradiance exposure (Behrenfeld et al. 2006, Doney 2006). Because temperature plays an important role in the geographic distribution of picophytoplankton in open-ocean ecosystems (Johnson et al. 2006, Moisan et al. 2010, Demir-Hilton et al. 2011) and might influence photophysiology (Geider 1987, Davison 1991), it is important to understand how changes in temperature affect picophytoplankton performance.

Temperature dependency of growth in relation to geographic distribution

The studied picophytoplankton strains showed a different temperature dependency of growth, which is in accordance with their geographical distribution. Growth was positively affected by increasing temperatures in the prokaryotic species, with growth rates of *Prochlorococcus marinus* and *Prochlorococcus* sp. similar to those found in earlier studies (Moore et al. 1995, Johnson et al. 2006, Zinser et al. 2007). An optimal temperature for growth was not detected for *P. marinus* and *Prochlorococcus* sp. during the present study, but earlier studies with these strains showed that the optimal temperature for growth is 24°C for *P. marinus* and 28°C for *Prochlorococcus* sp. (Moore et al. 1995, Johnson et al. 2006, Zinser et al. 2007). The cold tolerance of *Prochlorococcus* sp. is ~16°C (present study, Zinser et al. 2007), although this specific low-light-adapted strain of *Prochlorococcus* spp. (eMIT9313) is detected at lower temperatures in the Atlantic Ocean (Zinser et al. 2007). It has been suggested that the occurrence of eMIT9313 in waters below the minimum temperature for sustained growth could be explained by the occurrence of genetic variants within the ecotype or by the detection of dying cells that are recently exposed to low temperatures (Zinser et al. 2007). However, the present study showed that growth of *Prochlorococcus* sp. is possible at 16°C, but at lower irradiance intensities (25 vs. 50 µmol photons m^{-2} s^{-1}). This suggests that temperature has a strong effect on the photoacclimation potential of *Prochlorococcus* sp. and would possibly explain the observed distribution of this strain in cold, relatively deep (i.e. low irradiance intensities) waters.

For the eukaryotic picophytoplankton species *Ostreococcus* sp. and *Pelagomonas calceolata*, the temperature dependency of growth has not been studied before. In general, growth rates of *Ostreococcus* sp. are similar to those found in earlier studies (Rodriguez et al. 2005, Kulk et al. 2011). In the present study, the optimal growth temperature of *Ostreococcus* sp. was found between 20 and 24°C. This is consistent with the observation that this ecotype of *Ostreococcus* sp. (clade B or OII) is detected in oceanic waters with a mean temperature of 22 ± 3°C, but remains undetected at higher temperatures around 26 ± 3°C (Demir-Hilton et al. 2011). Thus, it seems that *Ostreococcus* sp. is a more temperate species compared with *P. marinus* and *Prochlorococcus* sp. The growth rates of *P. calceolata* are somewhat lower compared with another strain of *P. calceolata* (Dimier et al. 2009). This difference in growth rate is most likely strain specific, as other cell characteristics, such as 19-butanoyloxyfucoxanthin concentration, are also considerably different between the 2 strains of *P. calceolata* (present study, Dimier et al. 2009). No optimal temperature for growth was observed in this study for *P. calceolata*, suggesting that the temperature optimum is above 24°C. This is in accordance with the geographic origin of this strain and other *P. calceolata* strains in the Pacific Ocean (Le Gall et al. 2008), with sea surface temperatures reaching up to 26°C. It is likely that the optimal temperature for growth of *P. calceolata* is somewhere between 24 and 26°C, as small changes above the optimal temperature would have a negative effect on survival because of the rapid decrease of growth and photosynthesis above this temperature (Eppley 1972, Li 1985, Davison 1991).

Generally, phytoplankton growth doubles with every 10°C before reaching the optimal temperature, representing a Q_{10} of ~2 (Eppley 1972, Raven & Geider 1988). Although there are reservations on the use of Q_{10} (Ahlgren 1987, Berges et al. 2002), it is a useful parameter in the comparison of the effect of temperature between different phytoplankton species. The Q_{10} values for growth were considerably different between the 4 picophytoplankton strains used in the present study. The prokaryotic species showed almost double the values found for the eukaryotic species, suggesting that the 2 *Prochlorococcus* strains benefit more from an increase in temperature below the optimal temperature for growth than *Ostreococcus* sp. and *Pelagomonas calceolata*.

Effect of temperature on picophytoplankton photophysiology

The overall photophysiology and the photoacclimation potential of *Prochlorococcus marinus*, *Prochlorococcus* sp., *Ostreococcus* sp. and *Pelagomonas calceolata* are primarily defined by the genetic differences of the specific ecotypes (Moore et al. 1998, Rodriguez et al. 2005). The strains used in the present study represent low- and high-light-adapted ecotypes of the picophytoplankton community. Based on the comparison of light-dependent growth, pigmentation and absorption properties, *Prochlorococcus* sp. and *P. calceolata* are typified as low-light-adapted (present study, Moore et al. 1998), whereas *P. marinus* and *Ostreococcus* sp. are adapted to higher irradiance intensities (Moore et al. 1995, Demir-Hilton et al. 2011, Kulk et al. 2011). In response to elevated temperatures, the 4 picophytoplankton strains extended their light-harvesting capacity by an increase in cellular chl *a* concentrations. In *P. marinus*, *Prochlorococcus* sp. and *Ostreococcus* sp. this was accompanied by a decrease in photoprotective pigmentation. These changes in the light-harvesting complex associated with temperature acclimation are comparable to those found for other phytoplankton species (Geider 1987, Davison 1991), such as *Chlorella vulgaris* (Wilson & Huner 2000) and *Thalassiosira pseudonana* (Stramski et al. 2002). The increase in cellular chl *a* concentrations is typically associated with a decrease in light absorption due to changes in pigment packaging (Geider 1987, Stramski et al. 2002, Hancke et al. 2008). However, the effect of temperature on the absorption properties of *P. marinus*, *Prochlorococcus* sp., *Ostreococcus* sp. and *P. calceolata* was small. It is possible that the changes in light absorption are restricted by the relatively small effect of pigment packaging in picophytoplankton compared with larger phytoplankton species (Bricaud et al. 1999).

The increase in electron transport at higher temperatures corresponds well to the increase in light harvesting in *Prochlorococcus marinus*, *Prochlorococcus* sp., *Ostreococcus* sp. and *Pelagomonas calceolata*. Although the idea that electron transport is sensitive to changes in temperature (caused by changes in membrane fluidity) (Geider 1987, Davison 1991) was confirmed, the increase in electron transport could not fully explain the increase in growth at high temperatures (Q_{10} of 1.10–2.35 vs. 1.69–4.41). This suggests that processes other than electron transport, such as Calvin cycle activity or DNA replication (Geider 1987), are likely to limit growth at sub-optimal temperatures in these picophytoplankton strains. The electron transport characteristics showed enhanced photoacclimation to higher irradiance intensities at elevated temperatures for *P. marinus*, *Prochlorococcus* sp., *Ostreococcus* sp. and *P. calceolata*. This was also evident from the increase in the maximum quantum yield of PSII, which related well to earlier observations (Fu et al. 2004, Six et al. 2008, Dimier et al. 2009). The enhanced photoacclimation was accompanied by a reduced photoinhibition in *P. marinus*, *Ostreococcus* sp. and *P. calceolata*, suggesting that picophytoplankton are less susceptible to the negative effects of high irradiance intensities at higher temperatures. This has also been found in larger phytoplankton species, such as the diatoms *Chaetoceros gracilis*, *Thalassiosira pseudonana*, and *T. weissflogii* (Sobrino & Neale 2007, Halac et al. 2010, Helbling et al. 2011). Reduced levels of photoinhibition may be associated with enhanced enzymatic conversions of the xanthophyll pigment cycle (Demmig-Adams & Adams 1992), enhanced D1 repair (Bouchard et al. 2006) and the potential enhancement of Rubisco activity (Helbling et al. 2011). Further study is neccessary to confirm whether these processes are also involved in temperature acclimation in *P. marinus*, *Prochlorococcus* sp., *Ostreococcus* sp. and *P. calceolata*.

The overall photophysiology of *Prochlorococcus marinus*, *Prochlorococcus* sp., *Ostreococcus* sp. and *Pelagomonas calceolata* moved towards the use of higher irradiance intensities to support the enhanced growth rates at higher temperatures. These changes in photophysiology during temperature acclimation will possibly broaden the (depth) distribution of these species via the positive effect of temperature on the response to high irradiance intensities. However, the

genetically defined photosynthetic characteristics of the different ecotypes (i.e. low or high light adaptation) will be more pronounced on picophytoplankton distribution and community composition.

Use of PSII fluorescence analysis

Although PSII fluorescence analysis is a readily available technique in both laboratory and field studies, it should be interpreted with care for assessing differences between species. The relationship between different phytoplankton strains based on growth or measurements of productivity by a ^{14}C incorporation technique (present study, Kulk et al. 2011) is different from the relationship based on electron transport, especially in *Prochlorococcus* sp. The comparison of electron transport rates between species is highly influenced by photosystem stoichiometry. In the calculation of the absolute electron transport rates, it is assumed that the distribution of absorbed light between PSI and PSII is equal, i.e. a PSI:PSII of 1. Assuming that *Prochlorococcus* sp. (eMIT9313) has a PSI:PSII of around 2 (Bibby et al. 2003) and *Ostreococcus* sp. has a ratio of 0.39 (Cardol et al. 2008), the electron transport rates reported here might be overestimated by 50% in *Prochlorococcus* sp. and underestimated by 44% in *Ostreococcus* sp. This would affect the maximum and initial rate of electron transport considerably, but not the photoacclimation index. When the effect of temperature within a specific species is considered, photosystem stoichiometry might be less important. Although it is known that temperature can affect PSII reaction center size and abundance (Davison 1991, Wilson & Huner 2000), the relative amounts of PSI and PSII seems insensitive to changes in temperature (Wilson & Huner 2000). In addition to photosystem stoichiometry, the absorption by non-photosynthetic pigments might overestimate electron transport (Bidigare et al. 1990, Suggett et al. 2003), especially in *Prochlorococcus* spp. because of the high levels of zeaxanthin. Moreover, the presence of alternative electron pathways can overestimate linear electron transport (for a review, see Cardol et al. 2011). In both prokaryotic and eukaryotic oceanic phytoplankton, the PTOX-based water to water cycle is an important alternative electron pathway that limits the electron flow downstream of PSII (Bailey et al. 2008, Cardol et al. 2008, Mackey et al. 2008). This may have a considerable effect on the estimation of productivity or growth from electron transport rates, but it is unknown to what extent the comparison of electron transport rates between different species is influenced.

CONCLUSIONS

The differences in the optimal temperatures for growth of *Prochlorococcus marinus*, *Prochlorococcus* sp., *Ostreococcus* sp. and *Pelagomonas calceolata* related well to the geographic distribution of these picophytoplankton strains. Although temperature and irradiance are important factors in picophytoplankton community composition and distribution in oligotrophic oceans (Johnson et al. 2006, Zinser et al. 2007, Demir-Hilton et al. 2011), these factors are difficult to distinguish in field studies. Here, it was shown that both prokaryotic and eukaryotic picophytoplankton may benefit from the altered photophysiology at elevated temperatures, with a higher light-harvesting capacity and reduced photoinhibition. It is likely that these changes in photophysiology may alter the depth distribution of the picophytoplankton to some extent, but photophysiology remains highly influenced by the specific photoadaptation of different ecotypes. In addition to temperature and irradiance, nutrient availability may play a significant role in the (photo)physiology and distribution of oceanic phytoplankton (Agawin et al. 2000, Behrenfeld et al. 2006, Zinser et al. 2007). It is therefore necessary to assess the effects of nutrient availability on picophytoplankton performance for further interpretation of the expected changes associated with climate change on picophytoplankton distribution and community composition in open-ocean ecosystems.

Acknowledgements. Remote access to the Roscoff Culture Collection of strains RCC407 and RCC879 was facilitated by ASSEMBLE grant number 227799 (to G.K.). This work was supported by the Netherlands Organization for Scientific Research (N.W.O.), grant numbers 817.01.009 (to G.K.) and 839.08.422 (to W.H.P.).

LITERATURE CITED

Agawin NSR, Duarte CM, Agustí S (1998) Growth and abundance of *Synechococcus* sp. in a Mediterranean Bay: seasonality and relationship with temperature. Mar Ecol Prog Ser 170:45–53

Agawin NSR, Duarte CM, Agustí S (2000) Nutrient and temperature control of the contribution of picophytoplankton to phytoplankton biomass and production. Limnol Oceanogr 45:591–600

Ahlgren G (1987) Temperature functions in biology and their application to algal growth constant. Oikos 49: 177–190

Bailey S, Melis A, Mackey KRM, Cardol P and others (2008) Alternative photosynthetic electron flow to oxygen in marine *Syenchococcus.* Biochim Biophys Acta 1777: 269–276

Behrenfeld MJ, O'Malley RT, Siegel DA, McClain CR and

others (2006) Climate-driven trends in contemporary ocean productivity. Nature 444:752–755

Berges JA, Varela DE, Harrison PJ (2002) Effects of temperature on growth rate, cell composition and nitrogen metabolism in the marine diatom *Thalassiosira pseudonana* (Bacillariophyceae). Mar Ecol Prog Ser 225: 139–146

Bibby TS, Mary I, Nield J, Partensky F, Barber J (2003) Low-light-adapted *Prochlorococcus* species possess specific antennae for each photosystem. Nature 424:1051–1054

Bidigare RR, Ondrusek ME, Morrow JH, Kiefer DA (1990) *In vivo* absorption properties of algal pigments. In: Spinrad RW (ed) Ocean Optics X. Proc Soc Photo-opt Instrum Eng 1302:90–302

Bouchard JN, Roy S, Campbell DA (2006) UVB Effects on the photosystem II-D1 protein of phytoplankton and natural phytoplankton communities. Photochem Photobiol 82:936–951

Bricaud A, Allali K, Morel A, Marie D, Veldhuis MJW, Partensky F, Vaulot D (1999) Divinyl chlorophyll *a*-specific absorption coefficient and absorption efficiency factors for *Prochlorococcus marinus*: kinetics of photoacclimation. Mar Ecol Prog Ser 188:21–32

Cardol P, Bailleul B, Rappaport F, Derelle E and others (2008) Original adaptation of photosynthesis in the green alga *Ostreococcus.* Proc Natl Acad Sci USA 105: 7881–7886

Cardol P, Forti G, Finazzi G (2011) Regulation of electron transport in microalgae. Biochim Biophys Acta 1807: 912–918

Chisholm SW (1992) What limits phytoplankton growth. Oceanus 35:36–46

Davison IR (1991) Environmental effects on algal photosynthesis: temperature. J Phycol 27:2–8

Demir-Hilton E, Sudek S, Cuvelier ML, Gentemann CL, Zehr JP, Worden AZ (2011) Global distribution patterns of distinct clades of photosynthetic picoeukaryote *Ostreococcus.* ISME J 5:1095–1107

Demmig-Adams B, Adams WW (1992) Photoprotection and other responses of plants to high light stress. Annu Rev Plant Physiol 43:599–626

Dimier C, Brunet C, Geider RJ, Raven J (2009) Growth and photoregulation dynamics of the picoeukaryote *Pelagomonas calceolata* in fluctuating light. Limnol Oceanogr 54:823–836

Doney SC (2006) Plankton in a warmer world. Nature 444: 695–696

DuRand MD, Olson RJ, Chisholm SW (2001) Phytoplankton population dynamics at the Bermuda Atlantic Time-series station in the Sargasso Sea. Deep-Sea Res II 48: 1983–2003

Eppley RW (1972) Temperature and phytoplankton growth in the sea. Fish Bull 70:1063–1085

Falkowski PG, Raven JA (1997) Aquatic photosynthesis. Blackwell Science, Malden, MA

Feng Y, Hare CE, Leblanc K, Rose JM and others (2009) Effects of increased pCO_2 and temperature on the North Atlantic spring bloom. I. The phytoplankton community and biogeochemical response. Mar Ecol Prog Ser 388: 13–25

Fu FX, Warner ME, Zhang YH, Feng YY, Hutchins DA (2007) Effects of increased temperature and CO_2 on photosynthesis, growth, and elemental ratios in marine *Synechococcus* and *Prochlorococcus* (Cyanobacteria). J Phycol 43:485–496

Geider RJ (1987) Light and temperature dependence of the carbon to chlorophyll *a* ratio in microalgae and cyanobacteria: implications for physiology and growth of phytoplankton. New Phytol 106:1–34

Halac SR, Villafañe VE, Helbling EW (2010) Temperature benefits the photosynthetic performance of the diatoms *Chaetoceros gracilis* and *Thalassiosira weissflogii* when exposed to UVR. J Photochem Photobiol B 101:196–205

Hancke K, Hancke TB, Olsen LM, Johnsen G (2008) Temperature effects on microalgal photosynthesis-light responses measured by O_2 production, pulse-amplitude-modulated fluorescence, and ^{14}C assimilation. J Phycol 44:501–514

Helbling EW, Buma AGJ, Boelen P, Van der Strate HJ, Giordanino MVF, Villafañe VE (2011) Increase in Rubisco activity and gene expression due to elevated temperature partially counteracts ultraviolet radiation-induced photoinhibition in the marine diatom *Thalassiosira weissflogii*. Limnol Oceanogr 56:1330–1342

Hooker SB, Van Heukelem L, Thomas CS, Claustre H and others (2009) The third SeaWiFS HPLC Analysis Round-Robin Experiment (SeaHARRE-3). NASA Tech Memo 2009-215849, NASA Goddard space flight center, Greenbelt, MD

Houghton JT, Meir Filho LG, Callander BA, Harris N, Kattenberg A, Maskell K (1995) Climate change 1995: the science of climate change. Cambridge University Press, Cambridge

Johnson ZI, Zinser ER, Coe A, McNulty NP, Malcolm E, Woodward S, Chisholm SW (2006) Niche partitioning among *Prochlorococcus* ecotypes along ocean scale environmental gradients. Science 311:1737–1740

Keller MD, Selvin RC, Claus W, Guillard RRL (1987) Media for the culture of oceanic ultraphytoplankton. J Phycol 23:633–638

Kulk G, Van de Poll WH, Visser RJW, Buma AGJ (2011) Distinct differences in photoacclimation potential between prokaryotic and eukaryotic oceanic phytoplankton. J Exp Mar Biol Ecol 398:63–72

Le Gall F, Rigaut-Jalabert F, Marie D, Garczarek L, Viprey M, Gobet A, Vaulot D (2008) Picoplankton diversity in the South-East Pacific Ocean from cultures. Biogeosciences 5:203–214

Li WKW (1985) Photosynthetic response to temperature of marine phytoplankton along a latitudinal gradient (16°N to 74°N). Deep-Sea Res Part A 32:1381–1391

Li WKW (1994) Primary production of prochlorophytes, cyanobacteria, and eukaryotic ultraphytoplankton measurements from flow cytometric sorting. Limnol Oceanogr 39:169–175

Li WKW (1998) Annual average abundance of heterotrophic bacteria and *Synechococcus* in surface ocean waters. Limnol Oceanogr 43:1746–1753

Litchman E, Klausmeier CA, Miller JR, Schofield OM, Falkowski PG (2006) Multi-nutrient, multi-group model of present and future oceanic phytoplankton communities. Biogeosciences 3:585–606

Mackey KRM, Paytan A, Grossmans AR (2008) A photosynthetic strategy for coping in a high-light, low-nutrient environment. Limnol Oceanogr 53:900–913

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence—a practical guide. J Exp Bot 51:659–668

Maxwell DP, Falk S, Trick CG, Huner NPA (1994) Growth at low temperature mimics high-light acclimation in *Chlorella vulgaris.* Plant Physiol 105:535–543

Moisan TA, Blattner KL, Makinen CP (2010) Influences of temperature and nutrients on *Synechococcus* abundance and biomass in the southern Mid-Atlantic Bight. Cont Shelf Res 30:1275–1282

Moore LR, Goericke R, Chisholm SW (1995) Comparative physiology of *Synechococcus* and *Prochlorococcus*: influence of light and temperature on growth, pigments, fluorescence and absorptive properties. Mar Ecol Prog Ser 116:259–275

Moore LR, Rocap G, Chisholm SW (1998) Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecotypes. Nature 393:464–467

Morán XAG, López-Urratia Á, Calvo-Díaz AC, Li WKW (2010) Increasing importance of small phytoplankton in a warmer ocean. Glob Change Biol 16:1137–1144

Olson RJ, Chisholm SW, Zettler ER, Altabet MA, Dusenberry JA (1990) Spatial and temporal distributions of prochlorophyte picoplankton in the North-Atlantic Ocean. Deep-Sea Res Part I 37:1033–1051

Oquist G (1983) Effects of low temperature on photosynthesis. Plant Cell Environ 6:281–300

Partensky F, Hess WR, Vaulot D (1999) *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. Microbiol Mol Biol Rev 63:106–127

Platt T, Gallegos CL, Harrison WG (1980) Photoinhibition of photosynthesis in natural assemblages of marine-phytoplankton. J Mar Res 38:687–701

Polovina JJ, Howell EA, Abecassis M (2008) Ocean's least productive waters are expanding. Geophys Res Lett 35: L03618

Raven JA, Geider RJ (1988) Temperature and algal growth. New Phytol 110:441–461

Rodriguez F, Derelle E, Guillou L, Le Gall F, Vaulot D, Moreau H (2005) Ecotype diversity in the marine picoeukaryote *Ostreococcus* (Chlorophyta, Prasinophyceae). Environ Microbiol 7:853–859

Schmittner A (2005) Decline of the marine ecosystem caused by a reduction in the Atlantic overturning circulation. Nature 434:628–633

Six C, Finkel ZV, Rodriguez F, Marie D, Partensky F, Campbell DA (2008) Contrasting photoacclimation costs in ecotypes of the marine eukaryotic picoplankter *Ostreococcus.* Limnol Oceanogr 53:255–265

Sobrino C, Neale PJ (2007) Short-term and long-term effects of temperature on photosynthesis in the diatom *Thalassiosira pseudonana* under UVR exposures. J Phycol 43: 426–436

Stramski D, Sciandra A, Claustre H (2002) Effects of temperature, nitrogen, and light limitation on the optical properties of the marine diatom *Thalassiosira pseudonana.* Limnol Oceanogr 47:392–403

Suggett DJ, Oxborough K, Baker NR, MacIntyre HL, Kana TM, Geider RJ (2003) Fast repetition rate and pulse amplitude modulation chlorophyll *a* fluorescence measurements for assessment of photosynthetic electron transport in marine phytoplankton. Eur J Phycol 38: 371–384

Tassan S, Ferrari GM (1995) Proposal for the measurement of backward and total scattering by mineral particles suspended in water. Appl Opt 34:8345–8353

Van de Poll WH, Visser RJW, Buma AGJ (2007) Acclimation to a dynamic irradiance regime changes excessive irradiance sensitivity of *Emiliania huxleyi* and *Thalassiosira weissflogii*. Limnol Oceanogr 52:1430–1438

Webb WL, Newton M, Starr D (1974) Carbon dioxide exchance of *Alnus rubra.* Oecologia 17:281–291

Wilson KE, Huner NPA (2000) The role of growth rate, redox-state of the plastoquinone pool and the trans-thylakoid ΔpH in photoacclimation of *Chlorella vulgaris* to growth irradiance and temperature. Planta 212:93–102

Zinser ER, Jonhson ZI, Coe A, Veneziano D, Chisholm SW (2007) Influence of light and temperature on *Prochlorococcus* ecotype distributions in the Atlantic Ocean. Limnol Oceanogr 52:2205–2220

Editorial responsibility: Antonio Bode, A Coruña, Spain

Submitted: March 19, 2012; Accepted: June 24, 2012
Proofs received from author(s): October 2, 2012