The effect of temperature on the photophysiology of four picophytoplankton species

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Rising seawater temperatures and increased stratification that are associated with global warming are expected to cause changes in the picophytoplankton community. In this study, the picophytoplankton species *P. marinus*, *Synechococcus sp.*, *Ostreococcus sp.*, and *P. calceolata* were cultured at 16, 20, and 24 °C to study the effect of temperature on their photophysiology. Photosynthetic parameters were assessed by measurements of growth, pigmentation, absorption spectra, electron transport rates, and ^{14}C incorporation. In addition, the recovery of photosystem II (PSII) after exposure to high photosynthetic active radiation (PAR) and ultraviolet radiation (UVR) was assessed. Results showed that, maximum growth rates were generally found at higher temperatures. The activity of the xanthophyll pigment cycle was most effective at 24 °C. Temperature did not have an effect on the photosynthetic parameters of *Ostreococcus sp.*. For both *P. marinus* and *P. calceolata*, photosynthetic parameters were highest at 16 °C. In addition, *P. calceolata* showed photoinhibition at all three temperatures. *Ostreococcus sp.* showed the lowest sensitivity to UVR, whereas *P. calceolata* was most sensitive. *P. marinus* suffered from PSII damage caused by UVR, but not beyond repair. Overall, rising seawater temperatures will have a positive effect on the growth rates, xanthophyll pigment cycle activity and the electron transport rate in picophytoplankton, but will have little effect on D1 photorepair From this study, it can be concluded that all four species benefit from rising temperatures. However, this benefit could be nullified: stronger thermoclines and upper layer mixing due to rising sea water temperatures cause more frequent exposure to excessive (UV) irradiances. Furthermore, temperature induced stratification eventually causes nutrient depletion. This can cause a shift to smaller species, and eventually changes in carbon fixation and ocean species communities.

Keywords: climate change, picophytoplankton, non photochemical quenching, photoprotection, xanthophyll pigment cycle, P-E, ETR.

Introduction

The current climate change has great consequences for the oligotrophic oceans: sea water temperatures are rising, wind fields are changing (Sarmiento et al. 1998), and changes in stratification and vertical mixing influence the irradiance and the nutrient availability. These changes are likely to affect the picophytoplankton species, which are the dominant organisms inhabiting these oceans. Dominance of picophytoplankton (algae and photo-autotrophic bacteria < 2µm) in oligotrophic waters is based on their small cell size associated with small diffusion boundary layers and a large surface area per unit volume (Raven, 1986). The small cell size of picophytoplankton leads to a greater capacity to acquire nutrients and a more efficient use of nutrients for growth and maintenance (Raven 1998). Another reason for their success is the co-existing of several ecotypes throughout the water column (Moore et al., 1998; Partensky et al., 1999; Fuller et al., 2003; Rodriquez et al. 2005), all adapted to a certain water temperature and light intensity.

The open oligotrophic oceans are typically dominated by cyanobacteria and eukaryotic pico- and nanophytoplankton (Olson et al., 1990; Lindell and Post, 1995) of which the cyanobacteria *Prochlorococcus* spp. and *Synechococcus* spp. are the most abundant phytoplankton genera (Li, 1994; DuRand et al., 2001). The picophytoplankton, autotrophs contribute at least 10% to total global aquatic net primary productivity (Raven 1998).

Therefore, it is important to understand the effect of water column conditions, i.e. temperature and high irradiance, on their growth and photosynthetic characteristics to achieve a better insight on their role and abundance in the oligotrophic oceans in the future.

An aspect of global warming is the enhanced ultraviolet radiation (UVR) reaching the Earth's surface. In phytoplankton, UVR is known to cause photoinhibition, i.e. the reduction of photosynthetic rates by the damage of photosynthetic components, specifically the damage of the D1 protein of photosystem II (PSII) (Halac et al. 2010). To maintain an optimum photosynthetic activity, phytoplankton must circumvent the conflict of effective light

absorption on one hand, and a fast photoprotective response to photoinhibitory light intensities on the other hand (Goss & Jakob 2010).
When cells encounter sudden strong (UV) irradiances, fast photoprotective reactions are activated to reduce photosystem damage and avoid photoinhibition. Non photochemical quenching (NPQ) allows the thermal dissipation of the excess of energy and is one of the faster photoprotective processes activated by algal cells. It decreases the flow of excitation energy to PSII reaction centers and helps to minimize the production of harmful oxygen radicals in the PSII antenna (Dimier et al. 2009). These photoprotective processes are carried out by pigments, which are found in the form of a mere protective pigment in prokaryotic phytoplankton species (zeaxanthin) and as a cycle of pigments (xanthophyll pigment cycle) in eukaryotic phytoplankton species.
Two types of xanthophyll cycles found in eukaryotic picophytoplankton species are the Violaxanthin-Antheraxanthin-Zeaxanthin (VAZ) cycle (found in vascular plants, green, and brown algae) and the diadinoxanthin-diatoxanthin (Dd-Dt) cycle (mainly found in diatoms).The VAZ cycle consists of two de-epoxidation steps. In the first step, the light harvesting pigment violaxanthin is de-epoxydized to the photoprotective antheraxanthin. If more quenching is necessary, antheraxanthin will be de-epoxydized to zeaxanthin (fig. 1). Low light or darkness stimulates the backward reaction of the violaxanthin cycle (Goss & Jakob 2010), where zeaxanthin is epoxydized to violaxanthin. The Dd-Dt cycle can be found in the algal classes Bacillariobhyceae, Xanthophyceae, Haptophyceae, and Dinophyceae (Stransky and Hager 1970; Hager 1980; Demers et al. 1991). In this cycle, there is only one de-epoxidation step from diadinoxanthin to diatoxanthin (Fig. 1).

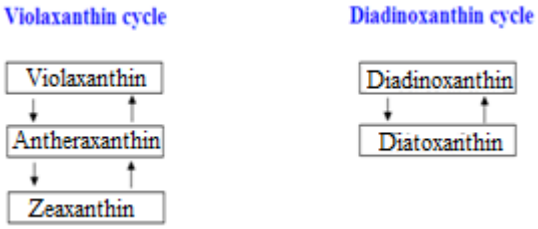

Figure 1: the violaxanthin-antheraxanthin-zeaxanthin cycle (VAZ) and the diadinoxanthin-diatoxanthin cycle (Dd-Dt)

Although photoprotective pigments dissipate a fraction of the excess energy, damage to the photosystems still occurs. These damaged photosystems cause photoinhibition and repair is important to maintain photosynthesis. In the recovery process the precursor of the D1 protein is synthesized *de novo*, incorporated into the PSII complex, and then processed to yield the active D1 protein, with resultant generation of the active PSII complex (Andersson *et al*. 1992). The extent of the photoinhibition depends on the balance between the inactivation of the PSII complex and the recovery of the complex from the inactivated state (Gombos *et al*. 1994).

Because temperature plays a key factor in enzymatic activity, elevated seawater temperatures could be beneficial for processes that imply enzymatic activity like recovery from UVR-induced damage (Bouchard et al. 2006) and growth (Fu et al. 2007; Eppley, 1972). Although the photochemical reactions (El-Sabaawi & Harrison, 2006) and the efficiency of photon-exciton conversion (Baumert et al, 2008) are temperature independent, temperature dependent cell structures like the lipid thylakoid membrane might not benefit from rising sea water temperatures. Temperatures above an optimum could loosen the thylakoid membrane and indirectly affect the embedded photosystems and photochemical reactions (El-Sabaawi & Harrison, 2006). Despite this possible negative effect on the thylakoid membrane, several studies show a positive feedback between elevated temperatures and photosynthesis (Hancke et al. 2008; Schofield et al. 1998; Davison et al. 1991) and a diminished rate of photoinhibition in the diatoms *Chaetoceros gracilis* and *Thalassiosira weissflogii* (Halac et al. 2010).

In this study, the effect of temperature on the photoprotective reactions and photosynthetic parameters were studied for four picophytoplankton species: *P. marinus, Synechococcus sp.* (cyanobacteria), *Ostreococcus sp.*, and *P. calceolata* (eukaryotes). The species were cultured at three different temperatures (16, 20, and 24°C) where after their photophysiology was assessed. In addition, the recovery of PSII after high PAR and PAR+UVR was measured.
The following questions will be answered: what is the effect of thermo-acclimation of these four species to the given temperatures on growth, pigmentation, photosynthetic ability, and PSII recovery after UVR exposure? Will the combination of rising sea water temperatures, changing mixing patterns, and increased UVB radiation, introduced by global warming, have consequences for the growth, survival, and distribution of these four picophytoplankters?

Materials and Methods

Cultures conditions
P. marinus (CCMP2389) was cultured in K/10-Cu medium (Chisholm 1992), *Synechococcus sp.* (RCC543), *Ostreococcus sp.* (RCC410), and *P. calceolata* (RCC879) were cultured in K-Si medium (Keller et al. 1987). Both media were prepared using filtered (using a Sartopore 0.2 µm filter) and sterilized natural seawater. Salinity was brought to 35 PSU. All species were pre-cultured in climate chambers to adapt to the experimental temperature conditions.

Experimental design
For the experiments, triplicate cultures of *P. marinus*, *Ostreococcus sp.*, and *P. calceolata* were grown in 500 ml glass Erlenmeyer flasks at three different temperatures, namely 16, 20, and 24 °C. Irradiance was provided at a light intensity of 50 µmol photons m^{-2} s^{-1} in a 12:12 h light/dark regime. Growth and the maximum quantum yield of photosystem II (F_v/F_m) were followed daily. Additional measurements were performed when the cultures were in mid-exponential growth phase, including non-photochemical quenching (NPQ), Electron Transport Rate (ETR), pigmentation, and absorption spectra. In addition, duplicate cultures of *P. marinus*, *Synechococcus sp.*, *Ostreococcus sp.*, and *P. calceolata* were grown at 24 °C to determine Photosynthesis-Irradiance (P-E) characteristics by a C-14 incorporation technique. Light intensities for the experimental set-up, ETR, and P-E measurements were measured using a biospherical light measuring device (QSL-100, Biospherical Instruments).

Measurements

Growth analysis
Cells were counted daily using a flowcytometer (Beckman Coulter Epics XL.MCL) to indicate the exponential and the stationary growth phase. Growth (μ d^{-1}) was calculated by linear regression of the log-transformed data.

Pigment analysis
Pigment samples were taken on 25 mm GF/F filters (Whatman), snap frozen using liquid nitrogen, folded in aluminum foil and freeze-dried at -80°C for 48 hours. *Ostreococcus sp.* pigments (chlorophyll a and b, prasinoxanthin, neoxanthin, violaxanthin, antheraxanthin, zeaxanthin, lutein and β-carotene) were quantified using High Performance Liquid Chromatography (HPLC). Pigments were quantified using standard dilutions (DHI LAB products).

Pigment absorption spectra
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 pigments and measured again to obtain the absorption of non-pigmented material (detritus). The absorption was calculated and normalized to chlorophyll a concentrations to obtain the specific absorption coefficient $\bar{a}^*$ (Kulk et al, 2011) (See ETR: Photosynthesis-Irradiance (P-I) curves for formulas).

Fluorescence measurements
F_v/F_m: The fluorescence of photosystem II (PSII) was measured daily by Pulse Amplified Modulation (PAM) fluorometry, (waterPAM, WALZ). Before measuring, ±4 ml culture was dark-adapted for 20 minutes to close all photosystems and obtain the maximum yield of PSII.

Experiments

NPQ (Non photochemical quenching) experiments
Cultures were exposed to two excessive light sources for 10 minutes: one containing only Photosynthetic Active Radiation (PAR, 400-700: PAR 1.72E+02 W/m^2, UVA 8.69 W/m^2, UVB 4.85E-02 W/m^2) and one containing PAR and ultraviolet radiation (UVR) (305-700 nm: PAR 1.57E+02 W/m^2, UVA 15.3 W/m^2, UVB 1.79 W/m^2). For both treatments, the light intensity was brought to 500 µmol photons m^{-2} s^{-1} using a neutral density screen. A cryostat (RK8 KS LAUDA) was used to control the temperature in the experimental set-up. The quantum yield of PSII (Φ_{PSII}) was measured at 0, 5, 10, 15, 20, 25, 30, 40, 50, 70, and 90 min after exposure using PAM fluorometry. Three HPLC samples were collected during the recovery period, at t=0, 10, and 40 min.

From the obtained data, total NPQ (1), fast NPQ or the xanthophyll cycle activity (NPQ_F) (2), and slow NPQ or the repair of damaged D1 proteins (NPQ_S) (3) was calculated according to Maxwell and Johnson (2000):

$$(1)\quad NPQ = (F^0_m - F_m')/F_m'$$

Where F^0_m is the maximum fluorescence level and Fm' is the fluorescence maximum in the light.

$$(2)\quad NPQ_F = (F^0_m/F_{m'}) - (F^0_m/F^r_m)$$

$$(3)\quad NPQ_S = (F^0_m - F^r_m)/ F^r_m$$

In which F^r_m is the fluorescence maximum that would have been attained if only slowly relaxing quenching had been present in the light.

ETR (electron transport rate) experiments
To measure the Φ_{PSII} (using PAM fluorometry), 10 glass vials were filled with 4 ml culture and placed in 10 separate slots. Each slot contained a different light intensity (19.9 - 630.9 µmol photons m^{-2} s^{-1}) created by neutral density screens (table 1). A cryostat (RK8 KS LAUDA) was used to control the temperature during the incubation. 4 ml culture was dark adapted for 20 minutes at the beginning of the experiment. After 20 minutes of incubation, the light was switched off and the Φ_{PSII} was measured consecutively (starting at vial 1) using PAM fluorometry.

The rETR data were normalized by the mean specific absorption coefficient $\bar{a}^*$ (m^2 mg Chl-a^{-1}) calculated by the following formula (4):

$$(4)\quad \bar{a}^* = \left(\frac{\sum_{700}^{400} \alpha^* ph(\lambda) E(\lambda)}{\sum_{700}^{400} E(\lambda)}\right)$$

In which $E(\lambda)$ is the irradiance (µmol photons m^{-2} s^{-1}) used in the photosynthetron during the P-I measurements and $\alpha^* ph(\lambda)$ (m^2 mg Chl-a^{-1}) is the specific absorption coefficient, obtained by calculating and normalizing the phytoplankton absorption to chlorophyll a concentrations.

	Light intensity (µmol photons m^{-2} s^{-1})	
Vial #	**ETR**	**P-E (C14)**
1	19.9	10.8
2	66.4	42.0
3	25.7	18.8
4	109.6	92.0
5	68.1	63.1
6	240.7	274.7
7	348.7	391.0
8	630.9	781.0
9	124.5	142.2
10	207.5	163.0

Table 1: The light intensities for each slot for the ETR experiment (left) and P-E experiment (right).

The $\bar{a}^*$ obtained was used to calculate the absETR using the following formula (5), where 0.5 is a factor that accounts for the partitioning of energy between PSII and PSI (Maxwell and Johnson, 2000)

$$(5)\quad {}_{abs}ETR = (F_v/F_m) * rETR * \bar{a}^* * 0.5$$

The data were fitted to the empirical model (6) described by Platt et al. (1980) using LABfit software (version 7.2.45, Wilton and Cleide P. Silva).

$$(6)\quad P = P_s\left(1 - e^{-\alpha\left(\frac{x}{P_s}\right)}\right)\left(e^{-\beta\left(\frac{x}{P_s}\right)}\right) - P_0$$

From these results, the maximum electron rate $absETR_{max}$ (mol e^-µg Chl-a^{-1} h^{-1}) (7) and the photoacclimation index Ek (µmol photons m^{-2} s^{-1}) (8) were calculated using the following equations:

$$(7)\quad \text{absETRmax} = P_s\left(\frac{\alpha}{\alpha+\beta}\right)\left(\frac{\beta}{\alpha+\beta}\right)^{\left(\frac{\alpha}{\beta}\right)}$$

$$(8)\quad E_k = \text{absETR}_{max}/\alpha_{ETR}$$

In which α_{ETR} is the initial slope of ETR.

Photosynthetic versus Irradiance (P-E) curves (incorporation of ^{14}C labeled bicarbonate):

The incorporation of ^{14}C labeled bicarbonate was used to measure primary production in all four phytoplankton species grown at 24°C. For the P-E curves, 7 µl ^{14}C-bicarbonate was added to 27 ml culture sample (total activity is 0.52 MBq). 2 ml culture sample was added to 13 prepared scintillation vials, labeled from 1-10 and three vials labeled with 't=0'. The vials (1-10) were placed in the ten different slots of the photosynthetron (table 1) and were incubated for 60 min at 24 °C. HPLC, A*ph and carbon samples were taken during incubation. For *Synechococcus sp.*, an additional pigment sample was taken.

For the time zero activity, three times 2 ml ^{14}C culture was acidified with 100 µl 6M HCl. The t=0 vials were placed directly in a flow box where excess ^{14}C-carbonate is removed. For the total activity, 100 µl ^{14}C culture sample was added to three vials containing 500 µl seawater and 50 µl ethanolamine. 5 ml scintillation cocktail was directly added to the totals.

After incubation, 100 µl 6M HCL was added to the vials (1-10). All ten vials were placed in the flow box with the t=0 samples. The pump was left on for 4 h to remove all excess ^{14}C-bicarbonate and samples were left overnight. 100 µl 6M NaOH was added the next day to neutralize the samples. 10 ml scintillation cocktail was added to each incubated sample (vials 1-10 and the t=0 labeled vials). All 16 vials were counted by liquid scintillation spectrometry (Tri-carb 2000 CA scintillation counter, Packard).

Results from the scintillation counter were transferred to Excel and C-uptake was calculated by :

$$\frac{\left(DPM - DPM(t0)\right) * 1.05 * DIC * volume\ totals}{Incb.vol. * incb.t.hr * [Chl].ug.l. * DPM}$$

With DPM being the disintegrations per minute and DIC the dissolved inorganic carbon. Data was then fitted according to the empirical model described by Platt et al. (1980) using LABFit software (see equation 8-10).

Statistical analysis

Data were statistically analyzed by one-way ANOVA (analysis of variance) using STATISTICA software (version 7.0, StatSoft Inc.). Three effects were tested:

1) Effect of treatment (NPQ experiment only)
2) Effect of temperature
3) Differences between the species

RESULTS

Growth

The highest growth rate was found for *Ostreococcus sp.* (1.12±0.04 d^{-1}) and the lowest for *P. marinus* and *P. calceolata* (0.25±0.04 and 0.26±0.03 d^{-1}, respectively). Maximum growth rates for both *Ostreococcus sp.* (1.12±0.04 d^{-1}) and *P. marinus* (0.49±0.01 d^{-1}) were reached at 20°C, as for *P. calceolata* the maximum growth rate was reached at 24°C (0.37±0.05 μ d^{-1}). (Table. 3, appendix)

Effect of temperature

The highest growth rates for *Ostreococcus sp.* were found at 20°C, significantly higher than 16°C (P<0.05) and 24°C (P<0.05). *P. marinus* also showed significantly higher growth rates at 20°C compared to 16°C (P<0.05) and 24°C (P<0.05). In addition, growth speeds were significantly higher at 24°C compared to 16°C (P<0.05). *P. calceolata* grew fastest at 24°C, significantly faster than at 16°C (P<0.05) and 20°C (P<0.05). No significant differences were found between 16°C and 24°C (fig. 2)

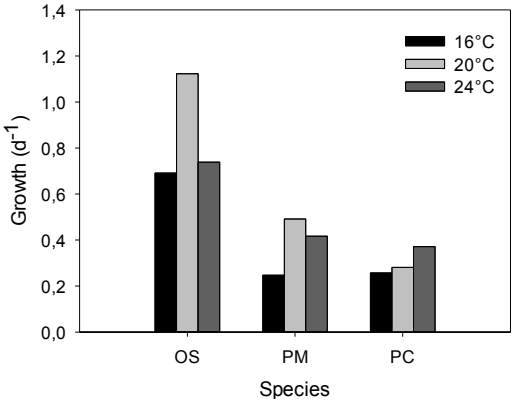

Figure 2: Average growth rates (μ, d^{-1}) ± st.dev. (*n*=3) for *Ostreococcus sp.* (OS), *P. marinus* (PM) and *P. calceolata* (PC) at 16, 20, and 24°C.

Between species

At 16 and 24°C, *Ostreococcus sp.* showed a significantly higher growth rate than *P. marinus* and *P. calceolata* (P<0.001). No differences were found between *P. marinus* and *P. calceolata* at these temperatures. At 20°C, all species showed significant differences in their growth rate: *Ostreococcus sp.* > *P. marinus* > *P. calceolata* (P<0.001).

F_v/F_m

Both highest and lowest F_v/F_m values were found at 24°C, with *P. marinus* reached the highest (0.64±0.02) value and *P. calceolata* the lowest (0.52±0.01). *Ostreococcus sp.* reached the highest values of F_v/F_m at 20°C (0.62 ± 0.02[1]) (data not shown).

Effect of temperature

Variations in F_v/F_m (fig. 3) between the different temperatures for each species were found for *Ostreococcus sp.* and *P. calceolata*, but not for *P. marinus*. For *Ostreococcus sp.*, F_v/F_m was significantly higher at 20°C than at 16°C (P<0.01) and 24°C (P<0.05). *P. calceolata* grown at 16°C gained the highest F_v/F_m, significantly higher than both 20°C (P<0.05) and 24°C (P<0.001).

Between species

Distinct differences between the species were found at 16°C, 20°C and 24°C (fig. 3). At 16°C, *P. marinus* showed a significantly higher value of Fv/Fm than both *P. calceolata* (P<0.001) and *Ostreococcus sp.* (P<0.01).

At 20°C, *P. marinus* showed a significantly higher F_v/F_m than *P. calceolata* (P<0.05). No significant differences between the other species were found.
At 24°C, *P. marinus* showed the highest F_v/F_m, significantly higher than *Ostreococcus sp.* (P<0.01) and *P. calceolata* (P<0.001). Also, *Ostreococcus sp.* showed a distinctly higher Fv/Fm than *P. calceolata* at this temperature (P<0.01)

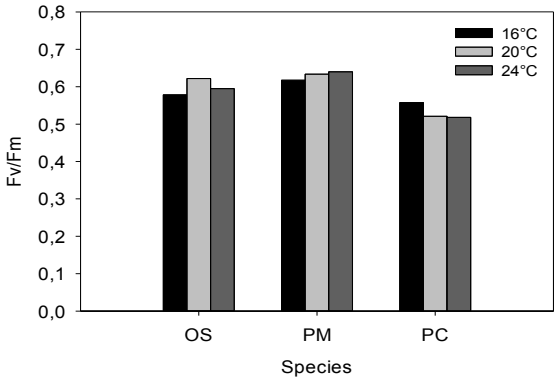

Figure 3: Maximum quantum yield of PSII (F_v/F_m) ± st.dev (*n*=3) for *Ostreococcus sp.* (OS), *P. marinus* (PM), and *P. calceolata (*PC) at 16, 20, and 24°C

Absorption spectra

Effect of temperature

Studying the effect of temperature for each species, the only significant difference was found for *P. calceolata*, where the ā* at 16°C showed a significant lower value compared to 20°C (P<0.001) and 24°C (P<0.001). The other species showed no effect of temperature for ā* (data not shown).

Between species

Differences in ā* between the species were found for all three temperatures. At 16, 20, and 24°C, *Ostreococcus sp.* showed the highest value of ā*, significantly higher than both *P. marinus* and *P. calceolata* ($P<0.001$, $P<0.01$, $P<0.05$ and $P<0.001$, $P<0.001$, $P<0.001$). Overall the following trend in ā* was shown: *Ostreococcus* sp > *P. marinus* > *P. calceolata* (data not shown).

NPQ

Data for total NPQ, NPQf and NPQs can be found in table 2.

Total NPQ

Effect of treatment

The two treatments did not have an effect on the total NPQ of *Ostreococcus sp.*. Only for *P. marinus* the treatments showed distinct effects. At 16 and 24°C, *P. marinus* produced significantly more total NPQ during the PAR+UV treatment compared to the PAR treatment ($P < 0.05$). The total NPQ of *P. calceolata* showed no effect of treatment, but *P. calceolata* did not recover after exposure to UVR radiation.

Effect of temperature

Only *P. calceolata* (PAR and PAR+UVR) and *P. marinus* (PAR) showed a difference in response to excessive irradiance. After the PAR treatment, *P. calceolata* showed significantly more total NPQ at 16°C than at 20°C ($P<0.05$) and 24°C ($P<0.01$) *P. marinus* showed significantly more total NPQ at 20°C than at 24°C($P<0.05$) after the PAR treatment. No significant differences in total NPQ were found after the PAR+UVR treatment. Temperature did not affect *Ostreococcus sp.*, no significant differences between total NPQ were found after both treatments.

Between species

For the PAR treatment, *P. marinus* showed distinctly less total NPQ than both *Ostreococcus* sp ($P<0.05$). and *P. marinus* ($P<0.05$) at 16°C. At 20°C, *P. marinus* only showed distinctly less total NPQ than *Ostreococcus sp.* ($P<0.01$). For the PAR+UVR treatment, *Ostreococcus sp.* showed significantly more total NPQ than both *P. marinus* ($P<0.05$) and *P. calceolata* ($P<0.01$) at 24°C. No significant differences were found for the other temperatures.

NPQ_S

Effect of treatment

Exposing *Ostreococcus sp.* to UV radiation did not have an effect on the NPQ_S. The other two species did show an effect of treatment. *P. marinus* showed significantly more NPQ_S during the PAR+UV treatment at 16°C ($P<0.001$) and *P. calceolata* showed significantly more NPQ_S after PAR+UV treatment at 16°C, 20°C and 24°C ($P<0.001$, $P<0.001$, $P<0.05$).

Effect of temperature

During the PAR treatment, the effect of temperature was found for *Ostreococcus sp.* and *P. calceolata*. *Ostreococcus sp.* showed significantly more NPQs at 24°C compared to 16°C ($P<0.05$), while *P. calceolata* showed the opposite result with more NPQ_S at 16°C than at 24°C ($P<0.01$). During the PAR+UV treatment, *Ostreococcus sp.* showed significantly more NPQs at 24°C compared to 16°C ($P<0.05$). *P. calceolata* showed distinct differences between all temperatures: 16°C > 20°C ($P<0.05$), 16°C > 24°C ($P<0.01$) and 20°C > 24°C ($P<0.01$). No significant differences in NPQ_S were found for *P. marinus* in both treatments.

Between species

Distinct differences between all species were found for the NPQs at 16°C. For the PAR and PAR+UVR treatment, *Ostreococcus sp.* showed significantly less NPQs than *P. marinus* ($P<0.01$) and *P. calceolata* ($P<0.01$). In addition, *P. marinus* showed significantly less NPQs than *P. calceolata* ($P<0.05$). In addition, *P. marinus* showed distinctly less NPQs at 20°C than *P. calceolata* ($P<0.05$).

NPQ_F

Effect of treatment

No NPQf was found for *P. calceolata* (*P. calceolata* did not recover after the PAR+UV treatment). No significant differences were found for *Ostreococcus sp.*, but a trend was shown (PAR+UV > PAR). *P. marinus*, a prokaryote that is lacking a xanthophyll cycle and therefore is expected not to show NPQf. The NPQf found for the PAR treatment (probably due to measurement fluctuations) will not be analyzed.

Effect of temperature

During the PAR treatment, temperature did not have an effect on the NPQ_F of *P. calceolata*. One distinct difference between temperatures was found for *Ostreococcus sp.*, where the NPQ_F at 20°C was significantly higher than at 24°C ($P<0.05$). During the PAR+UV treatment, no NPQ_F was found for *P. calceolata* at all three temperatures. *Ostreococcus sp.* did show NPQ_F for this treatment, but no distinct differences between the temperatures were found.

Between species

For the PAR treatment, differences in NPQ_F between the species were found for 16°C and 20°C. For both temperatures, *Ostreococcus sp.* showed the highest NPQ_F, significantly higher than *P. calceolata* ($P<0.01$). *Ostreococcus sp.* was the only species to show NPQf when being exposed to PAR+UVR, therefore no comparisons can be made for this treatment.

	***Ostreococcus* sp.**		
	16°C	**20°C**	**24°C**
Total NPQ	1.215±0.206 *1.513±0.450*	1.724±0.550 *1.803±0.484*	1.361±0.273 *1.695±0.291*
NPQf	0.721±0.150 *0.975±0.337*	1.060±0.230 *0.948±0.306*	0.314±0.255 *0.560±0.266*
NPQs	0.494±0.088 *0.718±0.125*	0.664±0.318 *0.855±0.199*	1.047±0.039 *1.096±0.038*
	P. marinus		
	16°C	**20°C**	**24°C**
Total NPQ	0.867±0.043 *1.304±0.133*	0.985±0.137 *1.296±0.222*	0.661±0.074 *1.070±0.110*
NPQf	0.077±0.027 *-0.290±0.035*	0.042±0.136 *-0.185±0.252*	-0,129±0.247 *-0.160±0.127*
NPQs	0.789±0.064 *1.594±0.146*	0.943±0.184 *1.482±0.340*	0.789±0.320 *1.230±0.201*
	P. calceolata		
	16°C	**20°C**	**24°C**
Total NPQ	1.276±0.037 *1.218±0.047*	1.118±0.658 *1.105±0.029*	0.989±0.061 *0.992±0.032*
NPQf	0.265±0.018 *-0.041±0.066*	0.020±0.049 *-0.056±0.034*	0.021±0.036 *-0.048±0.007*
NPQs	1.011±0.019 *1.260±0.029*	0.914±0.024 *1.160±0.019*	0.784±0.094 *1.040±0.037*

Table 2: The total NPQ, NPQf and NPQs for *Ostreococcus sp.*, *P. marinus* and *P. calceolata* (± st.dev., n=3). PAR (normal) and PAR+UVR (italic) results for each temperature.

ETR

See table 4 (appendix) for the $absETR_{max}$, Ek_{ETR} and α_{ETR} data and fig. 1 (appendix) for absETR curves.

$absETR_{max}$

Effect of temperature

For *P. marinus*, a significantly higher $absETR_{max}$ was found at 24°C compared to 16°C ($P<0.05$). For *P. calceolata*, both 20°C and 24°C showed a significantly higher $absETR_{max}$ compared to 16°C ($P<0.001$ and $P<0.05$). No significant differences between the temperatures were found for *Ostreococcus sp.* (fig. 4).

Between species

At 16°C, *P. calceolata* showed the lowest $absETR_{max}$, significantly lower than *Ostreococcus sp.* ($P<0.01$) and *P. marinus* ($P<0.05$). The same trend was found for 20°C ($P<0.01$). At 24°C, no significant differences between the $absETR_{max}$ of *Ostreococcus sp.* and *P. calceolata* were found, but the $absETR_{max}$ of *P. calceolata* was found to be significantly lower than *P. marinus* ($P<0.01$) (fig. 4).

$E\kappa_{ETR}$

Effect of temperature

No significant effect of temperature was found for *Ostreococcus sp.* and *P. calceolata*. One significant difference between the temperatures was found for *P. marinus*, where 16°C showed a distinctly lower $E\kappa_{ETR}$ than 24°C ($P<0.01$) (fig. 4).

Between species

At 16°C, *P. calceolata* showed a lower $E\kappa_{ETR}$ than both *Ostreococcus sp.* ($P<0.01$) and *P. marinus* ($P<0.01$). At 20°C, *P. calceolata* only showed a significant lower $E\kappa_{ETR}$ compared to *P. marinus* ($P<0.05$). At 24°C, the highest $E\kappa_{ETR}$ was found for *P. marinus*, significantly higher than both *P. calceolata* ($P<0.001$) and *Ostreococcus sp.* ($P<0.001$) (fig. 4).

α_{ETR}

Effect of temperature

The only significant differences between the three temperatures were found for *P. calceolata*, where 20°C showed the highest value of α_{ETR} compared to 16°C ($P<0.01$) and 24°C ($P<0.05$) (fig. 4).

Between species

Comparing the species, distinct differences were found. At 16°C, α_{ETR} of *Ostreococcus sp.* was significantly higher than the α_{ETR} found for *P. calceolata* (P<0.05). At 20°C, *Ostreococcus sp.* showed a significantly higher value of α_{ETR} than *P. marinus* (P<0.05). At 24°C, the α_{ETR} found for *Ostreococcus sp.* was significantly higher than both *P. marinus* (P<0.05) and *P. calceolata* (P<0.01). Overall, *Ostreococcus sp.* showed the highest values of $\alpha_{ETR,}$, with a maximum of 0.0092 ± 0.0007 mol e^- µ Chl-a^{-1} h^{-1} [µmol photons m^{-2} $s^{-1}]^{-1}$ at 24°C (fig. 4).

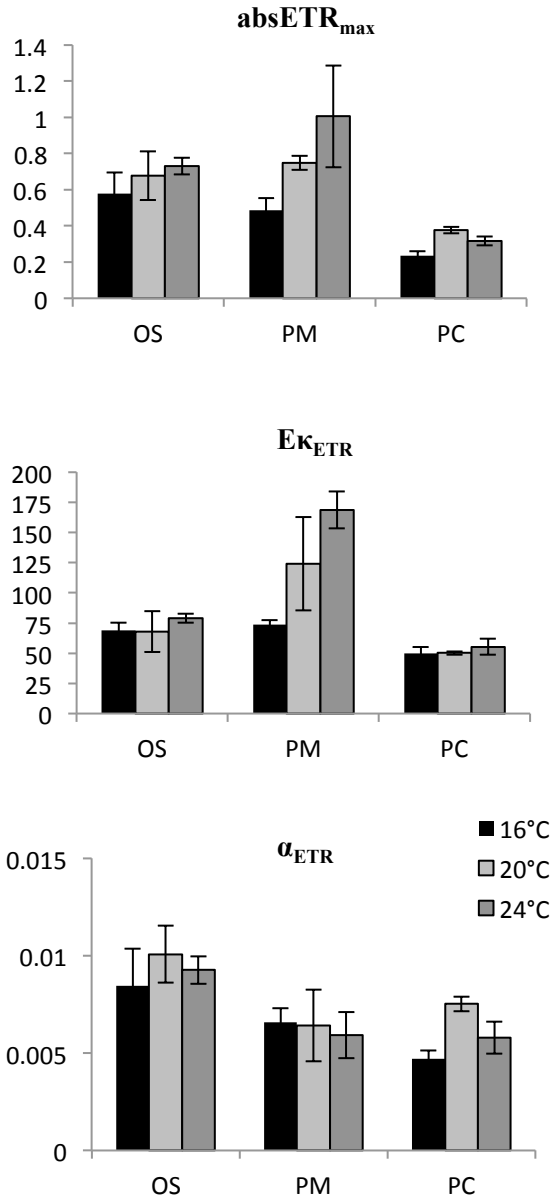

***Fig. 4*:** The **absETR$_{max}$** (mol e^-µg Chl-a^{-1} h^{-1}), **Ek$_{ETR}$** (µmol photons m^{-2} s^{-1}) and **α_{ETR}** (mol e^-µg Chl-a^{-1} h^{-1} [µmol photons m^{-2} s^{-1}]) for *Ostreococcus sp.* (OS), *P. marinus* (PM) and *P. calceolata* (PC) for each temperature (±st.dev., *n*=3).

P-E curves: incorporation of 14-C (between species)

See table 4 (appendix) for the P_{max}, E*k* and α data and fig. 2 (appendix) for carbon uptake curves.

P_{max}

P. marinus showed a significant lower P_{max} (2.80 ± 0.48 µg C µ Chl-a^{-1} h^{-1}) than *P. calceolata* (P<0.01), *Ostreococcus sp.* (P<0.05) and *Synechococcus sp.* (P<0.05) (table 4, appendix). *P. calceolata* showed the highest P_{max} (6.67 ± 0.30 µg C µ Chl-a^{-1} h^{-1}), but no significant differences between this species and the other species were found.

E_k

The lowest **E_k** was found for *P. calceolata* (18.8 ± 2.3 µmol photons m^{-2} s^{-1}) and the highest for *Synechococcus sp.* (25.8 ± 1.8 µmol photons m^{-2} s^{-1}). No significant differences between the species were found.

α

he lowest α was found for *P. marinus* (0.12 ± 0.01 µg C µ Chl-a^{-1} h^{-1} [µmol photons m^{-2} $s^{-1}]^{-1}$), significantly lower than both *Ostreococcus sp.* (P<0.05) and *P. calceolata* (P<0.01). No distinct differences between the two prokaryotes (*P. marinus* and *Synechococcus sp.*) were found. The highest α was found for *P. calceolata* (0.36 ± 0.03 µg C µ Chl-a^{-1} h^{-1} [µmol photons m^{-2} $s^{-1}]^{-1}$), which was significantly higher than *Ostreococcus sp.* (P<0.05), *P. marinus* (P<0.01) and *Synechococcus sp.* (P<0.01).

Light harvesting and photoprotective pigments in *Ostreococcus sp.*

See table 2 (appendix) for data.

Before exposure

Highest concentrations of Chl *a* (9.00 ±1.23 fg $cell^{-1}$) and Chl *b* (6.73±1.01 fg $cell^{-1}$) were found at 24°C. No significant differences were found between the temperatures.

For the xanthophyll cycle pigments, highest amounts of violaxanthin were also found at 24°C (0.39±0.05 fg $cell^{-1}$). For both antheraxanthin and zeaxanthin, highest amounts per cell were found for 16°C (0.11±0.07 fg $cell^{-1}$ and 0.07±0.03 fg $cell^{-1}$, respectively). No significant differences between the temperatures were found for all three xanthophyll pigments (fig. 5).

The highest de-epoxidation state of violaxanthin was found at 20°C (0.84±0.02), significantly higher than at 16°C ($P<0.01$). For the VAZ/Chl *a* ratio, the highest value was found at 16°C (0.08±0.01), but not significantly different compared to the other temperatures. For lutein (not shown in graph), a significantly higher amount was found at 16 °C (0.12 ± 0.05 fg cell^{-1}) compared to 20°C ($P<0.05$) (fig. 5).

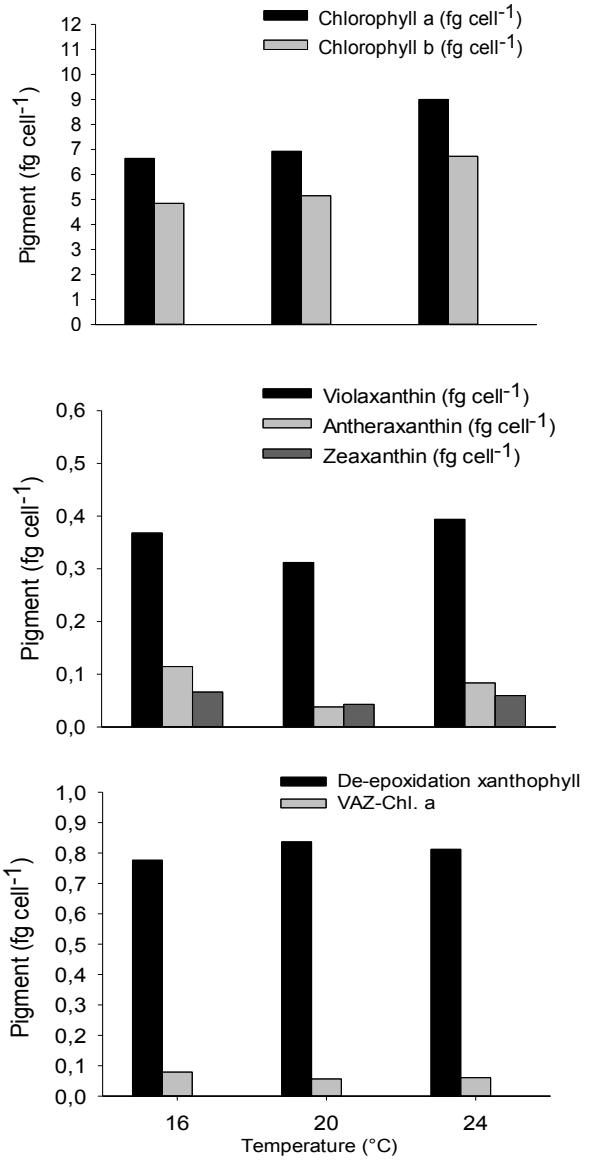

Fig. 5: The amounts of pigments (fg cell^{-1}) ±st.dev. **before exposure** in *Ostreococcus sp.*: The main light harvesting pigments Chl. *a* and Chl. *b*, the xanthophyll pigments violaxanthin, antheraxanthin and zeaxanthin (VAZ), the de-epoxidation state of violaxanthin and xanthophylls pigments (VAZ) per Chl. *a* for all three temperatures (±stdev, *n*=3).

Changes in pigments quantities (fg cell^{-1})

See table 2 (appendix) for data.

Chlorophyll *a* and *b*

For each temperature, no change in the amount of Chl. *a* and *b* was found during and after excessive irradiance exposure for both PAR and PAR+UVR treatments. No significant differences between the temperatures were found.

Violaxanthin, antheraxanthin, zeaxanthin and lutein

16°C

No significant changes the three xanthophyll cycle pigments or lutein were found during the PAR treatment.

In the PAR+UVR treatment, significantly more antheraxanthin was found at t=10 than before exposure ($P<0.05$). No other significant changes in pigment quantities were found at this temperature (fig. 6).

20°C

No significant changes over time were found for the PAR treatment.

For the PAR+UV treatment, antheraxanthin concentrations increased significantly compared to before exposure ($P<0.05$ for t=0, $P<0.01$ for t=10 and $P<0.01$ for t=40). Zeaxanthin also changed over time, with significantly more zeaxanthin at t=10 than before exposure ($P<0.05$). No changes in violaxanthin and lutein were found in both treatments.

24°C

For the PAR treatment, the amounts of violaxanthin dropped slowly after exposure. Significantly less violaxanthin at t=40 than before exposure($P<0.05$) and t=0 ($P<0.01$) was found.

For the PAR+UVR treatment, violaxanthin dropped significantly at directly after exposure (t=0) ($P<0.01$). At t=10, violaxanthin levels significantly increased compared to t=0 ($P<0.05$) and slightly dropped at t=40. The amount of violaxanthin found at t=40 were significantly lower than before exposure ($P<0.01$).

For the PAR treatment, the amounts of antheraxanthin significantly increased directly after exposure and stayed distinctly higher than before exposure ($P<0.001$ for t=0, $P<0.05$ for t=10 and t=40). Nonetheless, antheraxanthin levels dropped significantly after t=0 ($P<0.05$).

The PAR+UVR treatment showed a different change in antheraxanthin levels. The highest amounts of antheraxanthin were found at t=10, with a value significantly higher than before exposure ($P<0.001$), at t=0 ($P<0.01$) and t=40 ($P<0.05$). Lowest amounts

were found before exposure, significantly lower than at t=0 (P<0.05), t=10 (P<0.001) and t=40 (P<0.01)

No significant change in zeaxanthin was found for the PAR treatment, but for the PAR+UV treatment a distinct increase in zeaxanthin was found at t=10 compared to the other sampling times (P<0.01 for before exposure, P<0.05 at t=0 and t=40). No

The PAR+UVR treatment showed the same trend (P<0.001 for t=0, t=10 and t=40). No changes in VAZ/Chl *a* were found for both treatments.

24°C

No significant changes in de-epoxidation state of violaxanthin were found for the PAR treatment.
The PAR+UVR treatment showed the same trend as for 20°C (P<0.001 for t=0, t=10 and t=40 compared

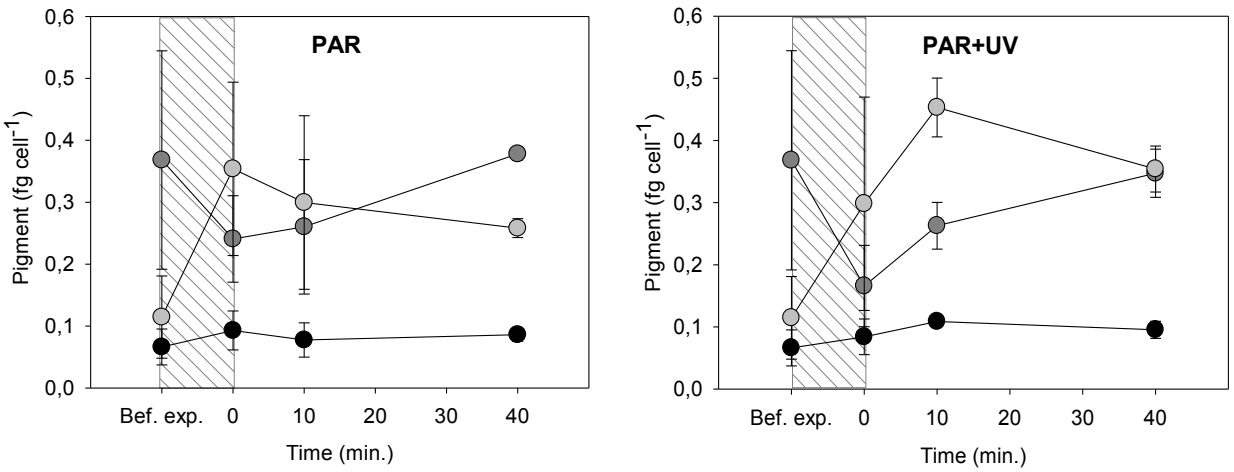

Fig. 6: The change in violaxanthin (dark gray), antheraxanthin (light gray) and zeaxanthin (black) concentrations for *Ostreococcus sp.* at 16°C (±stdev., n=3) for both treatments.

De-epoxidation state of violaxanthin and VAZ/Chl. *a*

16°C

For the PAR treatment, the de-epoxidation state of violaxanthin significantly dropped after exposure and stayed below before exposure levels (P<0.001 for t=0 and t=10; P<0.01 for t=40). After t=0, levels increased and significantly higher levels were found at t=40 (P<0.001) compared to t=0. In addition, significantly higher levels were found at t=40 compared to t=10 (P<0.01).
The same drop in the de-epoxidation state of violaxanthin was found for the PAR+UVR treatment (P<0.001 for t=0, t=10 and =40 compared to before exposure). Like the PAR treatment, levels increased after t=0 and significantly higher levels were found at t=40 (P<0.001). In addition, significantly higher levels were found at t=40 compared to t=10 (P<0.01).No changes in VAZ/Chl. *a* were found.

20°C

For the PAR treatment, the de-epoxidation state of violaxanthin significantly dropped at t=0 (P<0.01) and stayed significantly lower than before exposure (P<0.01 for t=10 and P<0.05 for t=40). No other significant differences were found.

significant lower values were found before exposure (P<0.05) and at t=0 (P<0.05) compared to t=10.

Light harvesting and photoprotective pigments in *Ostreococcus sp.*, *P. marinus*, *P. calceolata* and *Synechococcus sp.* at 24°C

See table 2 (appendix) for data.

The following pigments were found:
For *Synechococcus sp.* Chl *a,* zeaxanthin, α-carotene, and β-carotene; for *P. marinus*, Divinyl *a,* zeaxanthin, and α-carotene; for *Ostreococcus sp.*, Chl *a,* Chl *b*, neoxanthin, prasinoxanthin, violaxanthin, antheraxanthin, zeaxanthin (VAZ), lutein, and β-carotene; for *P. calceolata*, Chl. *a*, 19-butanoyloxyfucoxanthin, fucoxanthin, violaxanthin, diadinoxanthin, diatoxanthin, lutein, α-carotene, and β-carotene.
Of all species, *Synechococcus sp.* showed the highest Chl. *a* (fg cell^{-1}) levels at 24 °C. The lowest levels of Chl. *a* were found for *P. marinus* and *Ostreococcus sp.*, both lower than *Synechococcus sp.*. *Ostreococcus sp.* showed low amounts of Chl. *a* when comparing to *P. marinus* and *P. calceolata.*
Synechococcus sp. showed the highest amounts of photoprotective pigments, followed by *P. marinus*, *P. calceolata*, and *Ostreococcus sp.* Despite the high

amounts of photoprotective pigments and Chl *a* per cell, *Synechococcus sp.* did not contain the most photoprotective pigments per Chl *a*. *P. marinus* showed the highest amounts of photoprotective pigments per Chl *a,* higher than the other species (figure 7), followed by *Synechococcus sp.*, *Ostreococcus sp.*, and *P. calceolata*..

Conclusions and Discussion

Growth.

Differences in growth rates were found at different temperature in all four species. The response in growth rate can possibly be explained by changes in molecular mechanisms (Jumars et al, 1993). Temperature dependent molecular mechanisms are enzyme activity, protein synthesis, membrane permeability, and active uptake mechanisms. For example, increasing temperature causes an increase in the substrate–saturated reaction of RUBISCO, which causes an increased growth rate (if not limited by inorganic carbon or other factors) (Beardall and Raven, 2004).

Increased growth rates were found at the higher temperatures, indicating that the mechanisms described above could have played a role in faster growth. Nonetheless, lower growth rates were found for both *Ostreococcus sp.* and *P. marinus* at the highest growth temperature of 24°C compared to 20°C. A comparative result was found by Fu et al. (2007), where the cell division rates of *P. marinus* (CCMP1986) cultured at a similar irradiance were found to be quite invariant between 20°C and 24°C. This suggests that the optimum growth rate of *P. marinus* can be found between 20°C and 24°C. On the other hand, *P. marinus* (MED4) cultured at 24°C, shows an optimum growth irradiance of 90 µmol photons m^{-2} s^{-1} (Moore et al. 2005). This suggests that light could have played a limiting factor in this study. These findings by Moore et al. could also apply to *Ostreococcus sp.*, which shows the same trend between the higher temperatures as *P. marinus.*

Fv/Fm.

The inversely relationship between temperature and dark-adapted values of Fv/Fm found for *P. calceolata* has also been found for the dinoflagellate *Alexandrium fundyense* (Schofield et al, 1998). This would appear to be related to the change in the fatty acid composition of thylakoid membranes with the growth temperature. These relationships could explain the dependence of growth temperature and the temperature of maximum fluorescence. This relationship was not found for *Ostreococcus sp.*, where the maximum Fv/Fm was found at 20°C. This suggests that maximum values of Fv/Fm are influenced by preferred temperatures rather than a change in fatty acid composition of the thylakoid membrane. Fv/Fm of *P. marinus* was not affected by temperature, suggesting that temperature had little to no effect on the fatty acid composition of the thylakoid membrane.

NPQ

Since temperature is a key factor that enhances enzymatic activity, an increase in its levels would, in principle, benefit species that have suffered damage in their photosynthetic apparatus.(Halac et al. 2010). In the present study, this trend was found in *P. calceolata* and to a lesser extend in *P. marinus*, where total NPQ decreased with increasing temperatures. No increase of enzyme activity was found for *Ostreococcus sp.*, in which the total NPQ was not affected by temperature.

The decrease in NPQf during the PAR treatment found for *Ostreococcus sp.* between 20°C and 24°C suggests faster xanthophyll cycle activity with increasing temperatures (fig. 3, appendix), which can be due to increased enzyme activity in the de-epoxidation of violaxanthin. However, no significant increase in the de-epoxidation of violaxanthin was found between these temperatures (table 1, appendix).

During the PAR treatment, increased temperatures did not affect the NPQf of *P. calceolata*, suggesting no increased enzyme activity at higher temperatures. The observations that *P. calceolata* strongly suffered from high irradiances (both seen for the NPQ and ETR experiments) can be explained by the shade adaption strategy of this species (Dimier et al., 2009). Nevertheless, Dimier et al. (2009) found that *P. calceolata* still maintained photoprotection and growth under fluctuating light regimes (simulating vertical mixing in a 2h period with light regimes up to 570 µmol photons m^{-2} s^{-1}), even when adapted to the deep chlorophyll maximum. Gradual changes in light regimes during vertical mixing would not cause inconveniences for this species, but this study shows that sudden exposure to excessive irradiances will, especially when UVR radiation is involved.

UVR did not affect NPQ in *Ostreococcus sp.*, suggesting that increased irradiances and sudden exposure to UVR does not affect photoprotection. Although this strain is DCM adapted, vertical mixing to upper layers will not cause significant damage and *Ostreococcus sp.* has a strong photoprotective system. This is in accordance with the specialization of *Ostreococcus* ecotypes to contrasting environments, suggesting that they may have evolved distinct capacities to cope with rapid fluctuations in light. (Six et al. 2009). This trend was also shown for *P. marinus*, although UVR did cause significant damage to the photosystems (fig. 7 appendix).

P-E (absETR)

The positive relationship between $absETR_{max}$ and increasing temperature in algae has been found for

short term exposure (Morris and Kromkamp, 2003) and thermoacclimation (Claquin et al. 2008, Necchi, 2004) due to increased enzyme activity. This trend was also found for *P. marinus* and *P. calceolata*, but not for *Ostreococcus sp.* An increase in absETR was shown for this species when comparing 16°C to 20 and 24°C, but without significant differences. The absETR of *Ostreococcus sp.* therefore seemed little affected by temperature, a result also found for NPQ.

Although initial photochemical reactions are independent of temperature (Davison, 1991), cell sizes and relative chlorophyll a concentrations may be factors influencing α due to self-shading (Taguchi, 1976). The self-shading effect could explain the distinctly higher α for *P. calceolata* at 20°C, but chlorophyll a concentrations and cell sizes at the different temperatures are needed to confirm this theory. The temperature independency of α was shown for *Ostreococcus sp.* and *P. marinus*, following the effect described by Davison (1991).

P-E (incorporation of C-14)

The results of the present study were compared to a similar research carried out for *P. marinus*, *Synechococcus sp.*, and *Ostreococcus sp.* cultured at the same light intensity, but at 20 °C (Kulk et al. 2011). The comparison showed that higher seawater temperatures do not affect the maximum photosynthesis (P_{max}) in these species, but the photoadaptation index (E_k) decreased a twofold at 24 compared to 20 °C. This suggests that P_{max} is reached at lower light intensities when sea water temperatures increase. This could have a negative effect on these species when being exposed to excessive irradiances due to vertical mixing.

Several correlations between photosynthetic characteristics and temperature were found in earlier studies. A positive correlation of temperature with Pmax and α have been shown for the dinoflagellate *Alexandrium fundyense* (Schofield et al. 1998) for α in the brown alga *Laminaria saccharina* (Davison et al. 1991), and for the P_{max}, E_k, and α in surface phytoplankton in the Black Sea (Finenko et al, 2002). In contrast, a lack of relationship between α and temperature has been found by Davison (1991), because photosynthesis is mainly restricted by light exposure and charge separation rather than electron transport. These studies show that temperature on the photosynthetic characteristics is species dependent, and a general effect of temperature cannot be drawn.

In theory, four electrons are required to reduce a single molecule of CO_2 to the level of carbohydrate (Kolber and Falkowski, 1993). Comparing the $absETR_{max}$ of *Ostreococcus sp.*, *P. marinus* and *P. calceolata* to the P_{max} of these species (24°C) found in the P-E experiment, this relationship did not exist. For both *Ostreococcus sp.* and *P. marinus*, the P_{max} showed a distinctly higher value compared to $absETR_{max}$ * 4. This lack of relationship can be explained by the findings of Cosgrove (2007): While studies on higher plants have outlined a close relationship between PSII electron transport and carbon fixation, results from studies on microalgae reveal significant variations in relationship. It was found that predicted values of primary production both underestimated and overestimated actual carbon fixation measured via radioisotope C-14, due to a non-linear relationship between carbon fixation with chl. a fluorescence measurements at higher irradiances. Moreover, Cosgrove found that this non-linear relationship was greatest for microalgae adapted to low light conditions.

Pigments

Several studies have shown that acclimation to low temperature mimics adaptation to high irradiance, with an increased content of photoprotective pigments and an decrease in chlorophyll content (Anning et al. 2001, El-Sabaawi & Harrison, 2006). This was not shown for *Ostreococcus sp.*, where the different temperatures showed no significant changes in chlorophyll a and b, nor in xanthophyll pigments.

The highest de-epoxidation state of violaxanthin was found for 20°C, significantly higher than 16°C. The de-epoxidation of violaxanthin depends on the activity of the enzyme violaxanthin de-epoxidase and membrane fluidity (Latowski et al, 2002), and is therefore temperature dependent. The significantly higher de-epoxidation of violaxanthin at 20°C compared to 16°C shows this temperature dependence. The expected increase in de-epoxidation of violaxanthin for 20°C compared to 24°C was not found. As for growth and Fv/Fm, *Ostreococcus sp.* seems to have an optimum (enzyme activity) temperature between 20°C and 24°C.

Other pigments might have played an additional role in photoprotection in *Ostreococcus sp.*. Six et al. (2008) found that significant levels of lutein were produced by *Ostreococcus* spp. growth at light intensities above 50 µmol photons m^{-2} s^{-1} and during high irradiance exposure (Six et al. 2009). In higher plants, a lutein epoxide cycle works in parallel with the violaxanthin cycle (Garcia-Plazaola et al. 2003; Matsubara et al. 2005). In this study, similar levels of lutein were found in *Ostreococcus sp.* compared to Six et al. (2008, 2009), with significantly higher levels at the lowest temperature. However, no effect of high irradiance was found for this pigment. Possibly, the duration of our treatment was too short for additional synthesis of lutein. The high amounts

of lutein present in *P. calceolata* (table 2, appendix) might suggest that this pigment, compared to the amounts of lutein found in *Ostreococcus sp.* plays a more important role in photoprotection in this species. Unfortunately, the analysis of pigments was not complete for *P. calceolata*, so implication of temperature cannot be made.
Pigment samples taken from each species incubated at 24°C showed a great investment in photoprotective pigments for *P. marinus* compared to the other prokaryotic species *Synechococcus sp.*, which invested more in light harvesting pigments. The high temperature of 24°C could be a stress factor for *P. marinus,* but since this species contains a high light adaptation strategy, this investment could be normal for this species.
P. calceolata showed low levels of xanthophyll pigments at 24°C, which might explain the photoinhibition at relatively low irradiance intensities. The little investment in xanthophyll cycle pigments could derive from the low light adapted strategy of this species. Pigment samples from lower temperatures are needed to examine if these investments are temperature related or not.

Overall
Looking at the eukaryotic species, a rise in seawater temperature will have consequences for both *Ostreococcus sp.* and *P. calceolata*. High water temperatures might negatively affect *Ostreococcus sp.*, which seems to prefer the intermediate temperature of 20 °C for both growth and PSII performance. Elevated temperatures will have a positive effect on the growth rate of *P. calceolata*. For both species the elevation of seawater temperature had a negative effect on PSII performance. Taking the effect of sudden exposure to high (UVB) irradiances into account, the advantage of higher seawater temperatures for *P. calceolata* growth will be nullified, as this species was incapable to cope with both high PAR and UVR. Since this certain strain can be found at 100 m depth, and is therefore low light adapted, mixing to upper water levels will cause great inconvenience or, when being exposed to high (UVB) irradiances, even death. The most important factor might be the duration of exposure, as for these experiments the picophytoplankton species were exposed for 10 minutes. A shorter or irregular exposure (most likely to occur during a strong storm) might cause less damaged photosystems, resulting in a higher probability to repair when being mixed to darker water layers.
Ostreococcus sp. is only little affected by sudden exposure to high irradiances. This species will still do well when being mixed to higher surface layers since it has a good ability to protect itself from both photoinhibition and photosystem damage.
For the prokaryote *P. marinus*, temperature is an important factor on growth and photosynthetic rates, but seems not so important for PSII performance. The prospect of higher seawater temperatures is not threatening for this species and might even give an advantage. Living in the surface waters and therefore being high light adapted, this strain of *P. marinus* is used to high irradiances and has probably invested highly in the photoprotective pigment zeaxanthin. Light limitation might cause a problem for this species when deep vertical mixing occurs. In the experiments, it was shown that, when being adapted to an intermediate light intensity of 50 photons m^{-2} s^{-1}, *P. marinus* does suffer from UVB radiation and even from sudden exposure to high PAR (500 photons m^{-2} s^{-1}). Still, this cyanobacteria, can recover, but only slowly.

Conclusion
A rise in seawater temperature will not cause inconveniences for all three species. Vertical mixing and stronger solar irradiances will, especially for *P. calceolata*. One problem can occur, when stronger thermoclines are caused by higher surface water temperatures. If deep water species like *P. calceolata* and *Ostreococcus sp.* become trapped in this thermocline they will be exposed to high irradiances for a long period of time. *P. calceolata* will most certainly not survive these thermocline traps, while *Ostreococcus sp.* will be able to grow and sustain under the increased (UV) irradiance conditions. The abundant *P. marinus* will be most likely become the dominant species in the surface waters (together with other small picophytoplankton species like *Synechococcus sp.*) with having the advantage of being small (thermoclines will cause rapid nutrient depletion as no exchange of nutrients from lower waters can occur) and will outcompete relatively larger species like *Ostreococcus sp.* in time. Unlike *P. calceolata* and *Ostreococcus sp.*, *P. marinus* and *Synechococcus sp.* might have a 'bright' future ahead.

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References

Anning T., Harris G. and Geider R.J. 2001.
Thermal acclimation in the marine diatom Chaetoceros calcitrans . European journal of Phycology. Vol. 36(3): 233-241
Baumert H.Z. and Petzoldt T. 2008.
The role of temperature, cellular quota and nutrient concentrations for photosynthesis, growth and light–dark acclimation in phytoplankton. Limnologica –

Ecology and Management of Inland Waters. Vol. 38 (3-4): 313-326.

Beardall J. and Raven J.A. 2004. The potential effects of global climate change on microalgal photosynthesis, growth and ecology. PHYCOLOGIA Volume: 43 (1): 26-40 .

Claquin P., Probert I., Lefebvrel S., Veron B. 2008. Effects of temperature on photosynthetic parameters and TEP production in eight species of marine microalgae. Aquat. Microb. Ecol. Vol. 51: 1–11.

Cosgrove J.J. (2007) Marine phytoplankton primary production and ecophysiology using chlorophyll a fluorescence (thesis PHD).

Davison, I.R. 1991. Environmental effects on algal photosynthesis: temperature. J. Phycol. 27:2-8.

Davison I.R. , Greene R.M. and E.J. 1991. Temperature acclimation of respiration and photosynthesis in the brown alga *Laminaria saccharina* *. Marine Biology 110, 449-454

Demers S., Roy S., Gagnon R., Vignault C. 1991. Rapid light-induced changes in cell fluorescence and in xanthophyll-cycle pigments of Alexandrium excavatum (Dinophyceae) and Thalassiosira pseudonana (Bacillariophyceae): a photo-protection mechanism. Mar Ecol Prog Ser 76:185–193

Dimier C., Brunet C., Geider R., Raven J. 2009 Growth and photoregulation dynamics of the picoeukaryote Pelagomonas calceolata in fluctuating light. Limnol. Oceanogr. 54(3): 823-836.

DuRand, M.D., Olson, R.J., Chisholm, S.W., 2001 Phytoplankton population dynamics at the Bermuda Atlantic Time-series station in the Sargasso Sea. Deep-Sea Res. Pt. II 48, 1983-2003.

El-Sabaawi R. and Harrison P.J. 2006. Interactive effects of irradiance and temperature on the photosynthetic physiology of the pinnate diatom Pseudo-nitzschia granii (bacillariophyceae) from the northeast subarctic pacific. Journal of phycology. Vol 42 (4): 778-785.

Eppley W. Temperature and phytoplankton growth in the sea. 1972. Fishery bulletin: vol 70, no 4.

Finenko Z.Z., Churilova T.Y., Sosik, H.M. and Basturk O. 2002. Variability of photosynthetic parameters of the surface phytoplankton in the Black sea. OCEANOLOGY Vol. 42(1): 53-67.

Fu F., Warner M.E., Zhang Y., Feng Y., Hutchins D.A. 2007. Effects of increased temperature and CO2 on photosynthesis, growth, and elemental ratios in marine *synechococcus* and *prochlorococcus* (cyanobacteria). Delaware: J. Phycol. 43: 485-496

Fuller, N.J., Marie, D., Partensky, F., Vaulot, D., Post, A.F., Scanlan, D.J. 2003. Clade-specific 16S ribosomal DNA oligonucleotides reveal the predominance of a single marine Synechococcus clade throughout a stratified water column in the Red Sea. Appl. Environ. Microb. 69: 2430-2443.

Garcia-Plazaola J.I., Hernández A., Olano J.M. and Becerril J.M. 2003. The operation of the lutein epoxide cycle correlates with energy dissipation. *Functional Plant Biology*. Vol. 30(3): 319 – 324

Gombos Z., Wada H., Murata N. 1994 The recovery of photosynthesis from low-temperature photoinhibition is accelerated by the unsaturation of membrane-lipids – a mechanism of chilling tolerance. Proceedings of the national academy of sciences of the united states of America. Vol. 91 (19): 8787-8791

Goss R. & Jakob T. 2010. Regulation and function of xanthophylls cycle-dependent photoprotection in algae Photosynth. Res. 106:103–122

Halac S.R., Villafañe V.E., Helbling E.W. 2010. Temperature benefits the photosynthetic performance of the diatoms Chaetoceros gracilis and Thalassiosira weissflogii when exposed to UVR. Journal of Photochemistry and Photobiology B: Biology 101 (2010) 196–205

Hancke K., Hancke T.B., Olsen L.M., Johnsen G., Glud R.N. 2008. Temperature effects on microalgal photosynthesis-light responses measured by O_2 production, pulse-amplitude-modulated fluorescence and ^{14}C assimilation. Journal of Phycology. Vol. 44 (2): 501-514.

Keller, M.D., Selvin, R.C., Claus, W., Guillard, R.R.L. 1978. Media for the culture of oceanic ultraphytoplankton. J. Phycol. 23, 633-638

Kolber Z. and Falkowski P.G. 1993 Use of active fluorescence to estimate phytoplankton photosynthesis in situ Limnology and Oceanography. Vol 38(8): 646-166.

Kulk G., van de Poll W.H., Visser R.J.W., Buma A.G.J. 2011. Distinct differences in photoacclimation potential between prokaryotic and eukaryotic oceanic phytoplankton. Journal of experimental Marine Biology and Ecology. Vol. 398: 63-72.

Li, W.K.W., 1994. Primary production of prochlorophytes, cyanobacteria, and eukaryotic ultraphytoplankton – measurements from flow cytometric sorting. Limnol. Oceanogr. Vol. 39: 169-175

Lindell, D. , Post, A.F., 1995. Ultraphytoplankton succession is triggered by deep winter mixing in the Gulf of Aqaba (Eilat), Red Sea. Limnol. Oceanogr. Vol. 40: 1130-1141.

Matsubara, S. and others. 2005. Slowly reversible de-epoxidation of lutein-epoxide in deep shade leaves of a tropical tree legume may 'lock-in' lutein-based photoprotection during acclimation to strong light. Journal of Exp. Bot. 56: 461-468

Maxwell K. & Johnson G.N. 2000. Chlorophyll fluorescence – a practical guide. Journal of experimental botany, vol. 51, no. 345: 659-668.

Moore L.R., Goericke R., Chisholm S.W. 1995. Comparative physiology of Synechococcus and Prochlorococcus: influence of light and temperature on

growth, pigments, fluorescence and absorptive properties. Mar. Ecol. Prog. Ser. Vol. 116: 259-275

Moore, L.R., Rocap, G., Chisholm, S.W. 1998. Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecotypes. Nature 393: 464-467

Morris E.P. and Kromkamp J.C. 2003. Influence of temperature on the relationship between oxygen- and fluorescence-based estimates of photosynthetic parameters in a marine benthic diatom (Cylindrotheca closterium).Eur. J. Phycol. 38: 133 – 142.

Necchi O. 2004. Photosynthetic responses to temperature in tropical lotic macroalgae. Phycological Research Volume 52, Issue 2, pages 140–148

Olson, R.J., Chisholm, S.W., Zettler, E.R., Altabet, M.A., Dusenberry, J.A., 1990. Spatial and temporal distributions of prochlorophyte picoplankton n the North-Atlantic Ocean. Deep ea Res. Pt. I 37: 1033-1051.

Partensky, F., Hess, W.R., Vaulot, D. 1999. *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. Microbiol. Mol. R. 63: 106-127

Raven 1986. Physiological consequences of extremely small size for autotrophic organisms in the sea. In T. Platt and W. K. W. Li [eds.], Photosynthetic picoplankton. Can. Bull. Fish. Aquat. Sci. **214:** 1–70.

Raven, J. A. 1998. The twelfth Tansley Lecture. Small is beautiful: The picophytoplankton. Funct. Ecol. 12: 503–513.

Rodriguez F., Derelle E., Guillou L., Le Gall F., Vaulot D. and Moreau H. 2005. Ecotype diversity in the marine picoeukaryote *Ostreococcus* (Chlorophyta, Prasinophyceae). Environmental Microbiology 7 (6), 853–859

Sarmiento, J.L., Hughes, T.M.C., Stouffer, R.J., Manabe, S. 1998. Simulated response of the ocean carbon cycle to anthropogenic climate warming. Nature 393: 245-249.

Schofield O., Grzymski J., Moline M.M.A. and Jovine R.V.M. 1998. Impact of temperature acclimation on photosynthesis in the toxic red-tide dinoflagellate *Alexandrium fundyense* Journal of Plankton Research Vol.20 no.7 pp.1241-1258.

Six C., Sherrard R., Lionard M., Roy S., Campbell D.A. 2009. Photosystem II and Pigment Dynamics among Ecotypes of the Green Algae *Ostreococcus.* Plant Physiol. vol .151

Stransky H., Hager A. 1970 Das Carotinoidmuster und die Verbreitung des lichtinduzierten Xanthophyllcyclus in verschiedenen Algenklassen. Arch Microbiol 71:164–190.

Taguchi, S. 1976. Relationship between photosynthesis and cell size of marine diatoms. J. Phycol. 12:185-9

Tassan S., Ferarri G.M. 1995. Proposal for the measurement of backward and total scattering by mineral particles suspended in water. Appl. Optics 34, 8345-8353.

Appendix

		PAR			PAR+UV		
Pigment (fg cell^{-1}) **16°C**	Before exp.	t=0	t=10	t=40	t=0	t=10	t=40
Chlorophyll a	6.64 ± 1.10	7.47 ± 0.30	7.01 ± 0.48	6.90 ± 0.38	7.17 ± 0.16	7.41 ± 0.37	7.20 ± 0.52
Chlorophyll b	4.84 ± 0.82	5.44 ± 0.28	5.10 ± 0.43	5.06 ± 0.19	5.23 ± 0.24	5.43 ± 0.38	5.29 ± 0.45
Violaxanthin	0.37 ± 0.18	0.24 ± 0.07	0.26 ± 0.11	0.38 ± 0.01	0.17 ± 0.07	0.26 ± 0.04	0.35 ± 0.04
Antheraxanthin	0.11 ± 0.07	0.35 ± 0.14	0.30 ± 0.14	0.26 ± 0.02	0.30 ± 0.17	0.45 ± 0.05*	0.35 ± 0.04
Zeaxanthin	0.06 ± 0.03	0.09 ± 0.03	0.08 ± 0.03	0.09 ± 0.01	0.08 ± 0.03	0.11 ± 0.00	0.10 ± 0.01
Lutein	0.12 ± 0.05	0.13 ± 0.04	0.11 ± 0.03	0.10 ± 0.01	0.11 ± 0.02	0.14 ± 0.01	0.10 ± 0.01
de-epoxidation Violaxanthin	0.78 ± 0.01	0.61 ± 0.02*	0.64 ± 0.01*	0.70 ± 0.02*	0.58 ± 0.01*	0.59 ± 0.01*	0.66 ± 0.02*
VAZ-chlorophyll a^{-1}	0.08 ± 0.03	0.09 ± 0.03	0.09 ± 0.03	0.10 ± 0.00	0.08 ± 0.04	0.11 ± 0.01	0.11 ± 0.00
Pigment (fg cell^{-1}) **20°C**	Before exp.	t=0	t=10	t=40	t=0	t=10	t=40
Chlorophyll a	6.92 ± 1.10	7.29 ± 0.96	7.18 ± 1.06	7.53 ± 0.99	7.37 ± 1.27	7.74 ± 5.83	7.80 ± 0.10
Chlorophyll b	5.19 ± 0.83	5.44 ± 0.71	5.37 ± 0.87	5.65 ± 0.82	5.49 ± 0.99	5.83 ± 0.83	5.85 ± 0.77
Violaxanthin	0.31 ± 0.15	0.16 ± 0.06	0.16 ± 0.09	0.27 ± 0.10	0.12 ± 0.04	0.18 ± 0.04	0.24 ± 0.10
Antheraxanthin	0.04 ± 0.01	0.23 ± 0.12	0.18 ± 0.10	0.23 ± 0.07	0.21 ± 0.10*	0.35 ± 0.06*	0.28 ± 0.03*
Zeaxanthin	0.04 ± 0.02	0.06 ± 0.02	0.05 0.02	0.08 ± 0.02	0.07 ± 0.02	0.10 ± 0.02*	0.09 ± 0.01
Lutein	0.04 ± 0.01	0.04 ± 0.02	0.03 ± 0.00	0.04 ± 0.03	0.04 ± 0.02	0.05 ± 0.04*	0.04 ± 0.03
de-epoxidation Violaxanthin	0.84 ± 0.02	0.60 ± 0.03*	0.63 ± 0.04*	0.67 ± 0.09*	0.57 ± 0.02*	0.56 ± 0.03*	0.62 ± 0.07*
VAZ-chlorophyll a	0.06 ± 0.02	0.06 ± 0.03	0.05 ± 0.03	0.08 ± 0.01	0.05 ± 0.01	0.08 ± 0.00	0.08 ± 0.01
Pigment (fg cell^{-1}) **24°C**	Before exp.	t=0	t=10	t=40	t=0	t=10	t=40
Chlorophyll a	9.00 ± 1.23	9.41 ± 1.03	8.98 ± 0.99	8.73 ± 1.36	9.56 ± 1.44	10.43 ± 1.54	9.55 ± 1.20
Chlorophyll b	6.73 ± 1.02	7.04 ± 0.82	6.69 ± 0.82	6.56 ± 1.15	7.09 ± 1.16	7.83 ± 1.31	7.15 ± 0.99
Violaxanthin	0.39 ± 0.05	0.27 ± 0.11	0.21 ± 0.07	0.29 ± 0.20	0.16 ± 0.04*	0.30 ± 0.05	0.24 ± 0.01*
Antheraxanthin	0.08 ± 0.02	0.50 ± 0.07*	0.26 ± 0.05*	0.29 ± 0.12*	0.31 ± 0.09*	0.61 ± 0.04*	0.38 ± 0.10*
Zeaxanthin	0.06 ± 0.02	0.10 ± 0.07	0.07 ± 0.01	0.09 ± 0.05	0.10 ± 0.02	0.18 ± 0.02*	0.11 ± 0.03
Lutein	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.00	0.02 ± 0.00*	0.04 ± 0.01	0.06 ± 0.02	0.03 ± 0.01
de-epoxidation Violaxanthin	0.81 ± 0.01	0.70 ± 0.18	0.63 ± 0.02	0.64 ± 0.02	0.55 ± 0.01*	0.55 ± 0.02*	0.60 ± 0.04*
VAZ-chlorophyll a	0.06 ± 0.01	0.07 ± 0.05	0.06 ± 0.01	0.07 ± 0.03	0.06 ± 0.01	0.10 ± 0.01*	0.08 ± 0.02

Table 1: The main pigments, de-epoxidation state of violaxanthin and VAZ-chlorophyll a of *Ostreococcus* sp. for 16°C, 20°C and 24°C (±stdev., *n*=3) (NPQ experiment). Asterisks indicate significant differences compared to 'before exposure'.

	***Ostreococcus* sp.**			***P. calceolata***		
	16ºC	**20ºC**	**24ºC**	**16ºC**	**20ºC**	**24ºC**
Chl. a	6.643±1.105	6.925±1.099	9.002±1.233	n/a	n/a	25.95±1.174
Chl. b	4.844±0.821	5.149±0.0831	6.725±1.015	n/a	n/a	-
Div. Chl. a	-	-	-	n/a	n/a	-
Vio	0.368±0.176	0.312±0.147	0.394±0.052	n/a	n/a	0.159±0.077
Ant.	0.115±0.066	0.038±0.002	0.084±0.185	n/a	n/a	-
Zea.	0.066±0.029	0.043±0.020	0.059±0.016	n/a	n/a	-
De-epox.	0.776±0.011	0.837±0.020	0.812±0.010	n/a	n/a	-
Dd	-	-	-	n/a	n/a	1.805± 0.037
Dt	-	-	-	n/a	n/a	0.401± 0.025
PP/Chl. a	0.079±0.030	0.056±0.024	0.061±0.014	n/a	n/a	0.085±0.004
Lutein	0.123±0.054	0.037±0.015	0.045±0.009	n/a	n/a	0.496±0.039
	P.marinus			***Synechococcus sp.***		
	16ºC	**20ºC**	**24ºC**	**16ºC**	**20ºC**	**24ºC**
Chl. a	n/a	n/a	-	n/a	n/a	98.30±13.01
Chl. b	n/a	n/a	-	n/a	n/a	-
Div. Chl. a	n/a	n/a	9.552±0.000	n/a	n/a	-
Vio	n/a	n/a	-	n/a	n/a	-
Ant.	n/a	n/a	-	n/a	n/a	-
Zea.	n/a	n/a	5.028±0.033	n/a	n/a	17.10±1.991
De-epox.	n/a	n/a	-	n/a	n/a	-
Dd	n/a	n/a	-	n/a	n/a	-
Dt	n/a	n/a	-	n/a	n/a	-
PP/Chl. a	n/a	n/a	0.522±0.007	n/a	n/a	0.174±0.003

Table 2: Pigments of *Ostreococcus* sp. ± stdev. (n=3) for each growth temperature, *P. calceolata* ± stdev (n=2) for 24ºC, *P. marinus* ± stdev. (n=2) for 24ºC and *Synechococcus* sp. ± stdev (n=2) for 24ºC. Pigments shown are Chl. a and b, divinyl Chl. a (Div. Chl. a), violaxanthin (Vio.), antheraxanthin (Ant.), zeaxanthin (Zea.), de-epoxidation state of violaxanthin (De-epox.), diadinoxhanthin (Dd), diatoxanthin (Dt), photoprotective pigment(s) per Chl. a (PP/Chl. a) and lutein.

T (°C)	Species	Growth (μd^{-1})
16	OS	0.69±0.01
20	OS	1.12±0.04
24	OS	0.74±0.06

16	PM	0.25±0.04
20	PM	0.49±0.01
24	PM	0.42±0.02
16	PC	0.26±0.03
20	PC	0.28±0.02
24	PC	0.37±0.05

Ostreococcus sp. (OS),
*eolata (*PC) at the three

Species (16°C)	**absETR$_{max}$**	**E$_{kETR}$**	**α$_{ETR}$**
P. marinus	0.49±0.07	73.63±3.64	0.007±0.001
Ostreococcus sp.	0.58±0.11	69.08±6.04	0.008±0.002
P. calceolata	0.23±0.03	49.65±5.56	0.005±0.000
Species (20°C)	**absETR$_{max}$**	**E$_{kETR}$**	**α$_{ETR}$**
P. marinus	0.75±0.04	124.04±38.56	0.006±0.002
Ostreococcus sp.	0.68±0.13	68.01±17.01	0.01±0.001
P. calceolata	0.38±0.02	50.15±1.46	0.008±0.000
Species (24°C)	**absETR$_{max}$**	**E$_{kETR}$**	**α$_{ETR}$**
P. marinus	1.01±0.28	168.65±15.23	0.006±0.001
Ostreococcus sp.	0.73±0.05	79.01±3.91	0.009±0.001
P. calceolata	0.32±0.03	55.35±6.62	0.006±0.001

Species (24°C)	**Pmax**	**Ek**	**α**
P. marinus	2.80±0.47	22.43±2.30	0.12±0.01
Synechococcus sp.	5.22±0.54	25.84±1.79	0.20±0.01
Ostreococcus sp.	5.09±0.08	22.18±3.61	0.23±0.03
P. calceolata	6.67±0.30	18.84±2.29	0.36±0.03

Table 4: Photosynthetic characteristics of the P-E ETR experiment (upper table, *n*=3) and the P-E C-14 experiment (lower table, *n*=2) ± stdev.

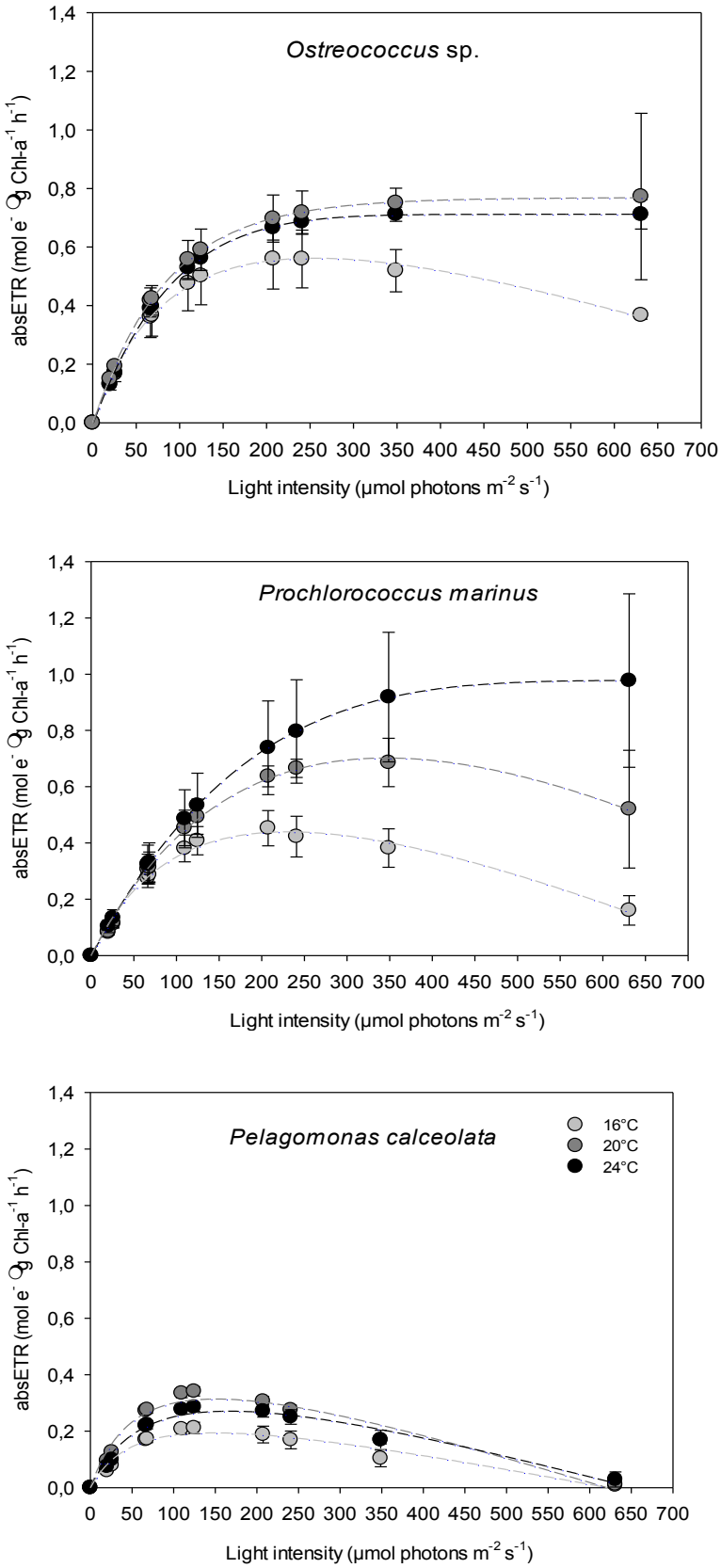

Figure 1: P-E curves (absETR) *Ostreococcus* sp., *P. marinus* and *P. calceolata* for all three temperatures (±stdev., *n*=3)

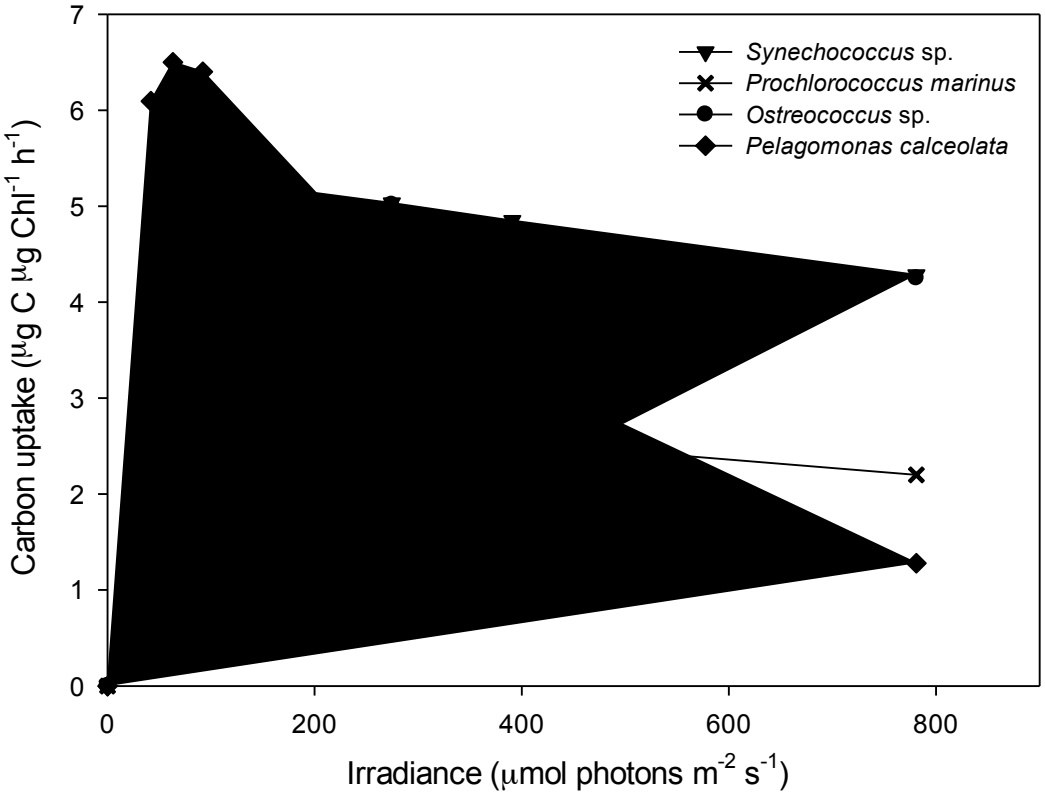

Figure 2: P-E curves (C-14) *Synechococcus* sp., *P. marinus*, *Ostreococcus* sp. and *P. calceolata* at 24°C (*n*=2)

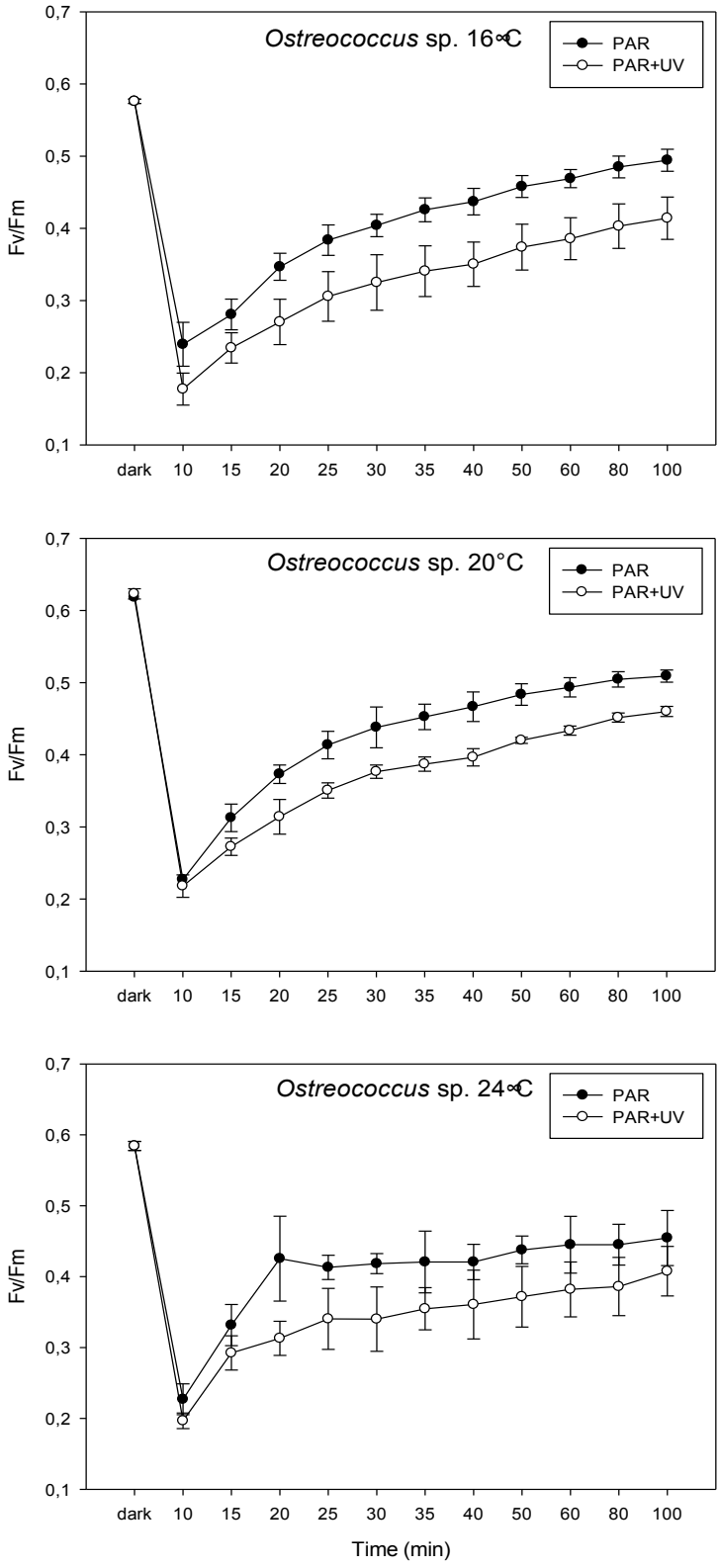

Figure 3: Fv/Fm *Ostreococcus* sp. for each temperature (PAR and PAR+UV treatment) ±stdev., *n*=3

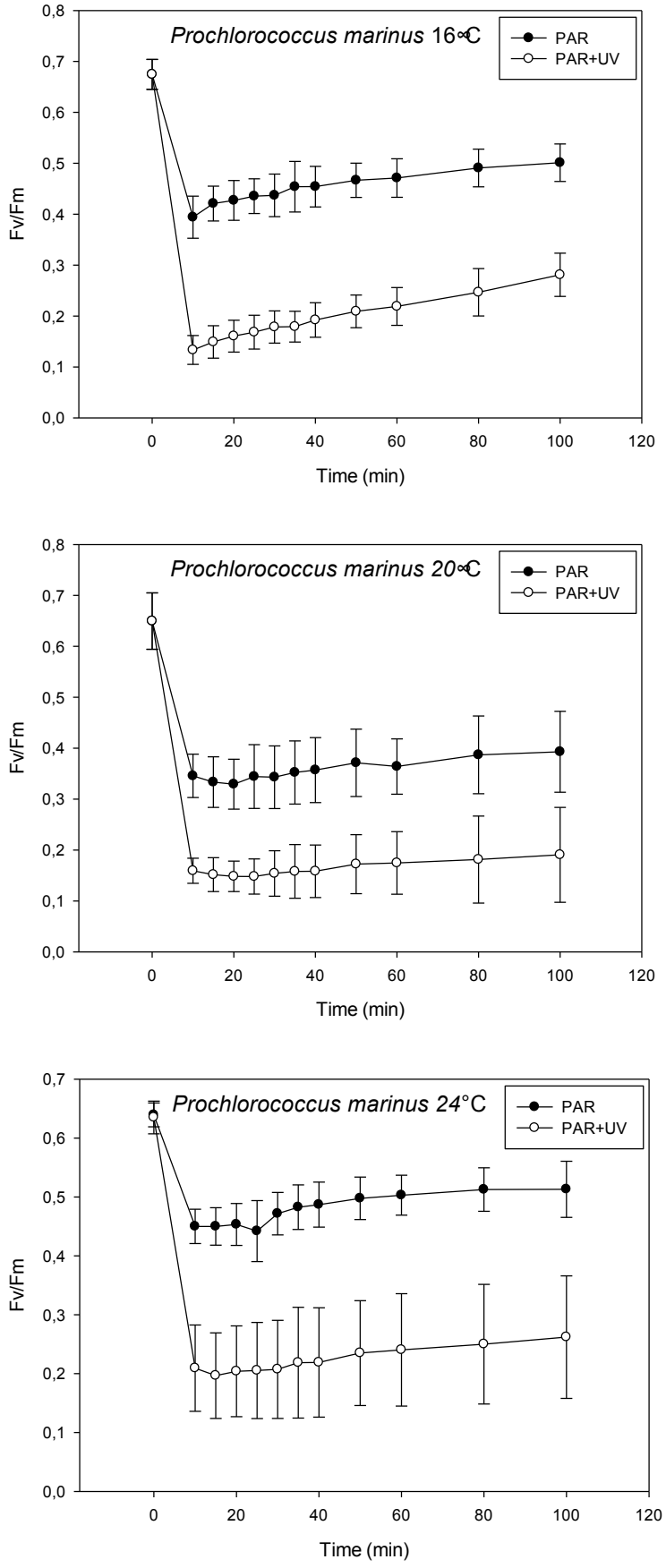

Figure 4: Fv/Fm *Prochlorococcus marinus* for each temperature (PAR and PAR+UV treatment)) ±stdev., *n*=3

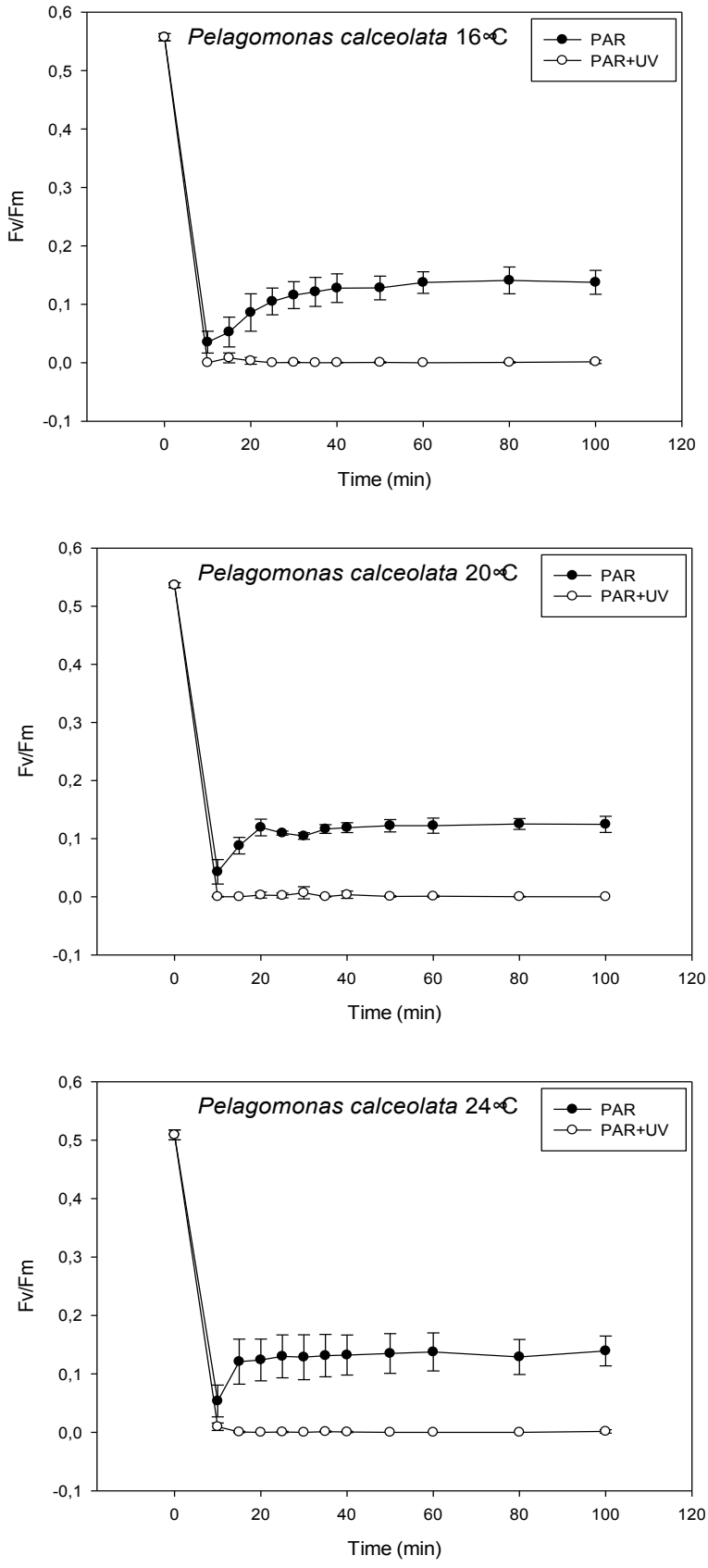

Figure 5: Fv/Fm *Pelagomonas calceolata* for each temperature (PAR and PAR+UV treatment)) ±stdev., *n*=3.