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Growth and photoregulation dynamics of the picoeukaryote *Pelagomonas calceolata* in fluctuating light

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Abstract

Growth, photosynthesis, and photoacclimation properties of batch cultures of *Pelagomonas calceolata* (Pelagophyceae) were compared for 1 week under three different fluctuating light regimes with the same total daily amount of light. Treatments consisted of a sinusoidal diurnal light cycle or a high-frequency fluctuating light simulating two different regimes of vertical mixing (highly fluctuating light [HFL] or fluctuating light [FL]). Three to five samples were taken every day for analysis of pigments, absorption spectrum, variable fluorescence, non-photochemical quenching (NPQ), electron transport rate vs. light curves, and cell concentration. *Pelagomonas* achieved the same growth rate during the exponential growth phase under all three light conditions, revealing a high degree of acclimation to light and also suggesting that the daily light dose is the main factor regulating growth and division. Photophysiological adjustments occurred in the cells in response to the three light regimes. *Pelagomonas* seems to adopt the n-type photoacclimation in HFL, whereas the σ-type photoacclimation is applied in FL. The cells rapidly trigger photoprotective mechanisms such as the xanthophyll cycle and NPQ, even though these do not appear to be able to fully prevent photoinhibition. The enhanced costs for maintenance and repair associated with HFL may have limited the allocation of energy to growth, thus explaining the shorter duration of the exponential growth phase in this regime with respect to the two others.

Phytoplankton are subjected to temporal light variations ranging from seconds to hours or days that are overlaid on the diel cycle of light (Litchman and Klausmeier 2001). Light intensity variability experienced by cells can be caused by a plethora of phenomena, such as changes in incident radiation caused by cloud cover, focusing of light by surface waves, or movement through the vertical light gradient caused by cell migration, sinking, and/or the advection and turbulence associated with currents and wind mixing. These processes modify the light environment of cells and in extreme cases can transport cells between darkness and full sunlight (MacIntyre et al. 2000). Photoacclimation and photoregulation mechanisms allow cells to maximize photosynthesis under the different environmental conditions. Fast photoprotective reactions are activated when cells have to cope with high light, whereas the acclimation in response to low light is generally slower (Lavaud et al. 2007). 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. NPQ decreases the flow of excitation energy to photosystem II (PSII) reaction centers and helps to minimize the production of harmful oxygen radicals in the PSII antenna. NPQ is composed of fast quenching (q_E, ΔpH-dependent quenching), intermediate quenching (q_T, state transition), and slowly relaxing quenching (q_I, photoinhibition) components (Rascher and Nedbal 2006). q_E involves mostly the xanthophyll cycle activity (Lavaud et al. 2007), which corresponds to the reversible de-epoxidation of violaxanthin (Vx) into zeaxanthin (Zx) through antheraxanthin (Ax) for green algae and higher plants. In most chlorophyll *c* (Chl *c*)–containing phytoplankton species, the photoprotective cycle involves the conversion between diadinoxanthin (Dd) and diatoxanthin (Dt) (Lavaud et al. 2007). Diatoms are one of the groups with the greatest NPQ and xanthophyll cycle activity (Wilhelm et al. 2006; Lavaud et al. 2007). However, there are fewer studies dealing with other Dd-containing groups (Harris et al. 2005). Among these groups, the picoeukaryotic component is even less studied from an ecophysiological point of view (Timmermans et al. 2005; Six et al. 2008), even though their abundance and ubiquity is not in doubt (Guillou et al. 1999; Raven et al. 2005). Picoeukaryotes also present biological properties that make them an interesting model for ecophysiological studies. Indeed, their small size leads to a low package effect, which contributes to the light-saturated rate of photosynthesis being achieved at relatively low irradiances (Raven et al. 2005). In turn this may lead to greater susceptibility to photoinhibitory damage and higher sensitivity to ultraviolet radiation damage than is experienced by larger cells (Raven 1998). As a result of evolutionary adaptation, small cells usually have a higher diversity of photosynthetic pigments than larger phytoplankton (Raven et al. 2005). To fill the gap, this present study was undertaken to investigate the growth, photosynthesis, and photoacclimation dynamics of the picoeukaryote *Pelagomonas calceolata* under different variable conditions of light in order to provide more information on the relevance of its biological properties to its success in pelagic ecosystems. *P. calceolata* is a low-light–adapted picoplankton species that generally grows in the deep-chlorophyll maximum (DCM; Timmermans et al. 2005). Photoprotection refers to the mechanisms that dissipate excitation energy through NPQ. The

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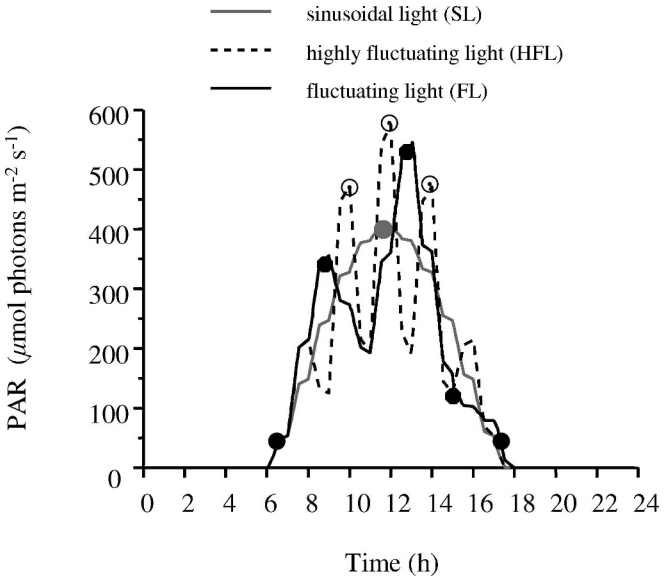

Fig. 1. Light variations applied during the experiments. Sampling times are indicated by gray circles for sinusoidal light (SL), black circles for fluctuating light (FL), and open circles for highly fluctuating light (HFL) experiments.

capacity and effectiveness of photoprotection is species-dependent (Wilhelm et al. 2006; Dimier et al. 2007*a*) and is related to the ecological niche where cells grow (Strzepek and Harrison 2004; Lavaud et al. 2007; Six et al. 2008) and to the light history (Dimier et al. 2007*b*) of the cells. Different extents or rates of light variation may stimulate or inhibit different species as a function of the capacities of their regulatory mechanisms, leading to changes in phytoplankton communities (Litchman and Klausmeier 2001). Success (or failure) in different light regimes may arise from species-specific differences in the ability to use excitation energy for photosynthesis, together with differences in the mechanisms or capacities to perform NPQ. Major physiological processes are usually affected by fluctuating light conditions (Wagner et al. 2006), and the rate of photosynthesis is enhanced (Wagner et al. 2006), depressed (Kroon et al. 1992), or possibly unaffected (Yoder and Bishop 1985) by the nature of the fluctuating light regime, depending on the species under investigation. Also, growth rate maintenance under fluctuating light appears to be species-dependent (Litchman 2000; Havelkova-Dousova et al. 2004), as does the relationship between photosynthesis and cell growth as described in different phytoplankton species and groups subjected to fluctuating light (Litchman 2000; van Leeuwe et al. 2005). Thus, as highlighted by Litchman (2000), it is necessary to gather data simultaneously on photosynthesis and growth to better understand the effect of fluctuating light on algal dynamics.

The specific questions addressed by this study are as follows: How does a shade-adapted picoeukaryote, *Pelagomonas*, regulate its photophysiology in fluctuating light? To what extent do different fluctuating light regimes affect the growth and photosynthesis of *Pelagomonas*?

Population growth and photophysiological properties of *P. calceolata* were investigated over 7 d (from the lag growth phase to the stationary growth phase) in three different fluctuating light regimes. The first condition corresponded to a sinusoidal variation of light over the daylight period (12 : 12 light : dark [LD]) and reproduced realistic values of light measured in summer at 10-m depth in the Mediterranean Sea (Brunet et al. 2007). The two other regimes superimposed on the first condition light variations simulating different vertical mixing regimes for cells in the upper layer. Pigments, variable fluorescence, NPQ, electron transport rate (ETR)–light curves, absorption spectra, and cell concentrations were measured 3–5 times per day.

Methods

Culture conditions—Strain RCC 103 of the picoeukaryote *P. calceolata* (Pelagophyceae), isolated from a low-light environment in the Red Sea, was provided by the Roscoff Culture Collection (Roscoff, France; Vaulot et al. 2004). This species was cultivated at 20°C with a photosynthetically active radiation value of 60 μmol photons m^{-2} s^{-1} (measured by a 4 π QSL-2100, Biospherical Instruments) provided by one lamp (50 W, Decostar 51, OSRAM) with a 12 : 12 LD photoperiod. Cells were grown in K medium (Keller et al. 1987) in 1-liter glass cylinders; the cultures were continuously aerated. Three weeks before the experiment, cells in 3-liter glass cylinders were transferred to a sinusoidal light (SL) regime (called hereafter "control regime") with a 12 : 12 LD cycle and a maximal photon flux density (PFD) of 400 μmol photons m^{-2} s^{-1} (Fig. 1). pH and temperature were measured daily with a HI-9214-Stick pH meter (Hanna Instruments), and cell concentration was determined by flow cytometry (*see* below). Daily and semicontinuous dilution with fresh medium (25% of the total volume) assured nutrient repletion and maintenance of cells in the exponential growth phase.

Experiments were carried out in triplicate on 4-liter cultures grown in 5-liter glass cylinders with a cell concentration of 10^6 cells mL^{-1}, which corresponded to the end of the lag growth phase (data not shown). Cultures were continuously mixed with a magnetic stir bar and bubbled with filtered air.

Experimental design and light regimes—For each light condition, triplicate cultures were illuminated individually by lamps (50 W, Decostar 51, OSRAM) mounted on a prototype device allowing gradual changes of PFD on the culture flasks (Dimier et al. 2007*a*). The biological and physiological properties of the cells were investigated and compared in the three light conditions (Fig. 1) characterized by the same daily light dose (~9.67 mol photons m^{-2}). The mean PFD over the 12 h was ~225 μmol photons m^{-2} s^{-1}. The first light regime corresponded to the control regime previously described and simulated no-mixing conditions (SL, Fig. 1) with the PFD reproducing the values measured in the Strait of Sicily (Mediterranean Sea) at 10-m depth in August 1997 (Brunet et al. 2007). The two other light regimes superimposed high-frequency light variability simulating vertical mixing and transport of cells

between 20- and 5-m depths on the control regime. The PFDs at 5- and 20-m depths were measured on the same water column where the 10-m depth values were obtained. The fluctuating light regime (FL, Fig. 1) simulated a cyclical transport of the cells in the upper layer (5–20 m depth) with a period of 4 h (velocity of mixing [V] = 0.20 cm s^{-1}) with a maximal PFD of 550 μmol photons m^{-2} s^{-1}. The highly fluctuating light regime (HFL, Fig. 1) simulated a vertical mixing with a period of 2 h (V = 0.40 cm s^{-1}) with a maximal PFD of 570 μmol photons m^{-2} s^{-1}.

Sampling strategy and parameters—Experiments were conducted in triplicate and cultures were sampled 3–5 times per day according to the light regime (dots in Fig. 1). Twenty-five to fifty milliliters of culture was rapidly taken with a syringe at each sampling time, for determination of variable fluorescence, NPQ, photosynthetic ETR, and ETR–light curves and for further analysis of pigments, absorption spectrum, and cell concentrations (by flow cytometry).

The growth rate was estimated daily from cell abundance measurements using the following equation:

$$\mu = \ln[N_{t2} : N_{t1}] : [t_2 - t_1] \quad (1)$$

where μ is the growth rate (d^{-1}) and N_t is the mean cell concentration at time t, where t_1 and t_2 correspond to the first sampling times (40 μmol photons m^{-2} s^{-1}) of the 24-h period of days 1 and 2, respectively. From the growth rate, the number of cell divisions (n) per day was estimated with the following equation:

$$n = \mu : [\ln(2)] \quad (2)$$

where n is the number of cell divisions per day and μ is the growth rate.

Pigment analysis—One aliquot of algal culture (10 mL) was filtered onto GF/F glass-fiber filters (Whatman) and immediately stored in liquid nitrogen until analysis. During the first sampling time of the day, three replicates from each flask were taken. Analysis was the same as in Dimier et al. (2007*a*): the pigment extract was injected in a Hewlett Packard series 1100 HPLC (Hewlett-Packard) with a C_8 BDS 3 μm Hypersil, IP column (Thermo Electron). The mobile phase was composed of two solvent mixtures: methanol, and aqueous ammonium acetate (70 : 30) and methanol. Pigments were detected spectrophotometrically at 440 nm using a Model DAD, Series 1100 Hewlett Packard photodiode array detector. Fluorescent pigments were detected in a Hewlett Packard standard FLD cell series 1100 with excitation and emission wavelengths set at 407 and 665 nm, respectively. Determination and quantification of pigments used pigment standards from DHI Water & Environment.

Cell concentration and optical parameters—At each sampling point duplicate samples of algal culture (1 mL) were fixed with 10% paraformaldehyde with a final concentration of 1% and immediately stored in liquid nitrogen. On each day for the first sampling point, fresh samples were also analyzed and used for the estimation of the growth rates. A FACScalibur (Becton Dickinson) flow cytometer was used with 10-μm beads (Flow Check, Coulter) as an internal standard. The method and apparatus were as in Casotti et al. (2005). Forward angle light scatter (FALS), right angle light scatter (RALS), and red fluorescence from chlorophyll *a* (Chl *a*) were measured and the values expressed as relative units (r.u.) to beads.

Absorption spectrum—Ten-milliliter samples were filtered onto GF/F filters (Whatman, 25 mm) and immediately frozen. Measurements were carried out at the end of the experiment following the procedure described in Dimier et al. (2007*a*). Absorption was measured between 400 and 800 nm with 1-nm increments on a spectrophotometer (Hewlett Packard HP-8453E) equipped with an integrating sphere (Labsphere RSA-HP-53).

Variable fluorescence measurements—Photochemical efficiency of PSII was estimated with a Phyto-PAM fluorometer (Heinz Walz GmbH). Triplicate measurements of variable fluorescence were performed on both light- and 15-min dark-acclimated samples. The operating photochemical efficiency (Fq′ : Fm′, with Fq′ = Fm′ − F′) was measured on a light-acclimated sample under a PFD corresponding to the incident light in the culture at the time that the measurement was made. The maximum photochemical efficiency (Fv : Fm, with Fv = Fm − Fo) was measured on a 15-min dark-acclimated sample. The effective NPQ of fluorescence was quantified by the Stern-Volmer expression:

$$_{\text{eff}}\text{NPQ} = (\text{Fm} : \text{Fm}') - 1 \quad (3)$$

where Fm and Fm′ are the maximum fluorescence values from dark- and light-exposed samples, respectively. Fm and Fm′ were measured after a saturating pulse of red light (2400 μmol photons m^{-2} s^{-1} for 450 ms) that caused a complete reduction of the PSII acceptor pool.

The ETR–light curves were determined on 15-min dark-exposed samples by applying 10 increasing actinic irradiances (from 1 to 1500 μmol photons m^{-2} s^{-1}, for 2 min each). The absolute electron transport rate (expressed in e^- Chl a^{-1} h^{-1}) was calculated as

$$_{\text{abs}}\text{ETR} = (\text{Fq}' : \text{Fm}') \times \text{I} \times \left(a^*_{\text{ph}} : 2\right) \quad (4)$$

where I is the incident irradiance (in μmol photons m^{-2} s^{-1}). The mean absorption value (a^*_{ph}) of phytoplankton was normalized by Chl *a* (m^2 mg Chl a^{-1}) and divided by two assuming that half of the absorbed light is distributed to PSII. The ETR–light curves were fitted with the equation of Eilers and Peeters (1988) to estimate the photosynthetic parameters α_{etr}, Ek, and $_{abs}ETR_{max}$.

During determination of the curve the operating photochemical efficiency decreases as NPQ develops (Villareal 2004). NPQ capacity was calculated after each actinic irradiance level according to Eq. 3, where Fm was the maximum fluorescence measured after the 15-min dark

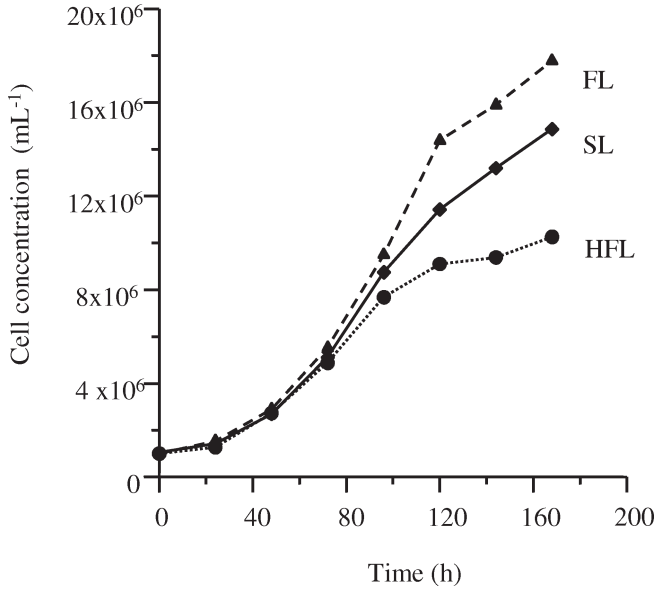

Fig. 2. Growth curves of *P. calceolata* exposed to the different light regimes: sinusoidal light (SL), fluctuating light (FL), and highly fluctuating light (HFL). Values are means of three measurements from fresh samples. Time is in hours after the start of the experiment.

acclimation and Fm′ was the maximum fluorescence measured after each actinic light level. The maximum NPQ ($_{max}$NPQ) was determined as the highest value of NPQ reached on the NPQ capacity vs. PFD curve.

Results

Growth and cell parameters—The mean growth rates in the exponential phase were similar for the three light regimes (Fig. 2; SL, $\mu = 0.61 \pm 0.06$ d^{-1}; FL, $\mu = 0.61 \pm 0.06$ d^{-1}; HFL, $\mu = 0.60 \pm 0.16$ d^{-1}) representing 0.87 ± 0.13 divisions d^{-1}. In HFL, cells entered into the stationary growth phase earlier than in the two other regimes (Fig. 2), and therefore a lower cell concentration was measured at the end of the experiment (18×10^6, 15×10^6, and 10×10^6 cells mL^{-1} in the FL, SL, and HFL regimes, respectively). FALS (measured by flow cytometry) was similar in the three conditions (mean 0.018 ± 0.004 r.u., 0.020 ± 0.004 r.u., and 0.020 ± 0.005 r.u. in the SL, FL, and HFL regimes, respectively), revealing that cell size was quite similar in the three light regimes. The variation of FALS, which can be used as an indicator of cell growth and division, indicated that the division of *P. calceolata* was synchronized to the LD cycle, with minimal values of FALS recorded at dawn and maximal values near dusk (data not shown). The significant correlation among FALS, RALS, and red Chl *a* autofluorescence ($p < 0.001$, $n = 20$ in SL or 35 in FL and HFL) shows that cell growth and division mainly drove the cell variability in our experiments. The red fluorescence : FALS ratio slightly increased with increasing light variation frequency (1.01 ± 0.09, 1.06 ± 0.11, and 1.16 ± 0.06 in the SL, FL, and HFL regimes, respectively), reflecting an increase of the intracellular pool of Chl *a*.

Photosynthetic pigments—The average cellular Chl *a* content was significantly higher in HFL than in SL and FL (for the exponential phase, $4.13 \times 10^{-17} \pm 0.98 \times 10^{-17}$ mol Chl *a* cell^{-1} vs. $2.30 \times 10^{-17} \pm 0.54 \times 10^{-17}$ and $2.58 \times 10^{-17} \pm 0.57 \times 10^{-17}$ mol Chl *a* cell^{-1}, respectively; Table 1). As observed for the red Chl *a* autofluorescence, the Chl *a* cell^{-1} showed a trend with 24-h periodicity, increasing in the light period, reflecting cell growth, and decreasing during night, reflecting division (Fig. 3). The less significant correlation between Chl *a* cell^{-1} and RALS and FALS in HFL ($p < 0.005$, $n = 35$) relative to the SL and FL regimes ($p < 0.001$, $n = 20$ and 35 for SL and FL, respectively) could be linked to a little alteration during the diel cycle of the Chl *a* cell^{-1} because of the stronger light variability.

Concerning the accessory photosynthetic pigments, the main changes occurred in HFL with increased Chl c_2 : Chl *a* and decreased Chl c_3 : Chl *a* ratios. In SL and FL (Table 1) these ratios were constant with time and similar under the SL and FL regimes (Table 1). The fucoxanthin (Fuco) : Chl *a* and 19-butanoyloxyfucoxanthin (19′BF) : Chl *a* ratios strongly decreased (by 25%) during the exponential phase in HFL, whereas they were constant over time in the SL and FL regimes (data not shown). The mean 19′BF : Chl *a* ratio decreased with the greater frequency of light fluctuation from 0.24 (SL) to 0.15 (HFL) mol 19′BF mol Chl a^{-1}, although the Fuco : Chl *a* ratio was higher under FL (0.60 mol Fuco mol Chl a^{-1}) than in SL (0.50 mol Fuco mol Chl a^{-1}) or HFL (0.45 mol Fuco mol Chl a^{-1}; Table 1). Significant relationship between these two pigments was found under fluctuating light conditions ($p < 0.001$ and $p < 0.05$ under FL and HFL, respectively) and

Table 1. Pigment content in *P. calceolata* grown under sinusoidal light (SL), fluctuating light (FL), and highly fluctuating light (HFL) regimes. Chl *a*, chlorophyll *a*; Chl c_2, chlorophyll c_2; Chl c_3, chlorophyll c_3; Fuco, fucoxanthin; 19′BF, 19′-butanoyloxyfucoxanthin; Dd, diadinoxanthin; Dt, diatoxanthin. Chl *a* content is expressed in mol Chl *a* cell^{-1}. Pigment ratios are expressed in mol pigment mol Chl a^{-1}. Values are means±SD calculated over the exponential growth phase (between 24 and 96 h) for each light condition.

Light regime	Chl *a* cell^{-1}	Chl c_2 : Chl *a*	Chl c_3 : Chl *a*	Fuco : Chl *a*	19′BF : Chl *a*	(Dd + Dt) : Chl *a*
SL	$2.30\times10^{-17}\pm0.54\times10^{-17}$	0.86±0.03	0.18±0.01	0.50±0.02	0.24±0.02	0.08±0.03
FL	$2.58\times10^{-17}\pm0.55\times10^{-17}$	0.92±0.06	0.20±0.02	0.60±0.02	0.21±0.01	0.10±0.04
HFL	$4.13\times10^{-17}\pm0.98\times10^{-17}$	0.99±0.05	0.13±0.03	0.45±0.05	0.15±0.02	0.07±0.02

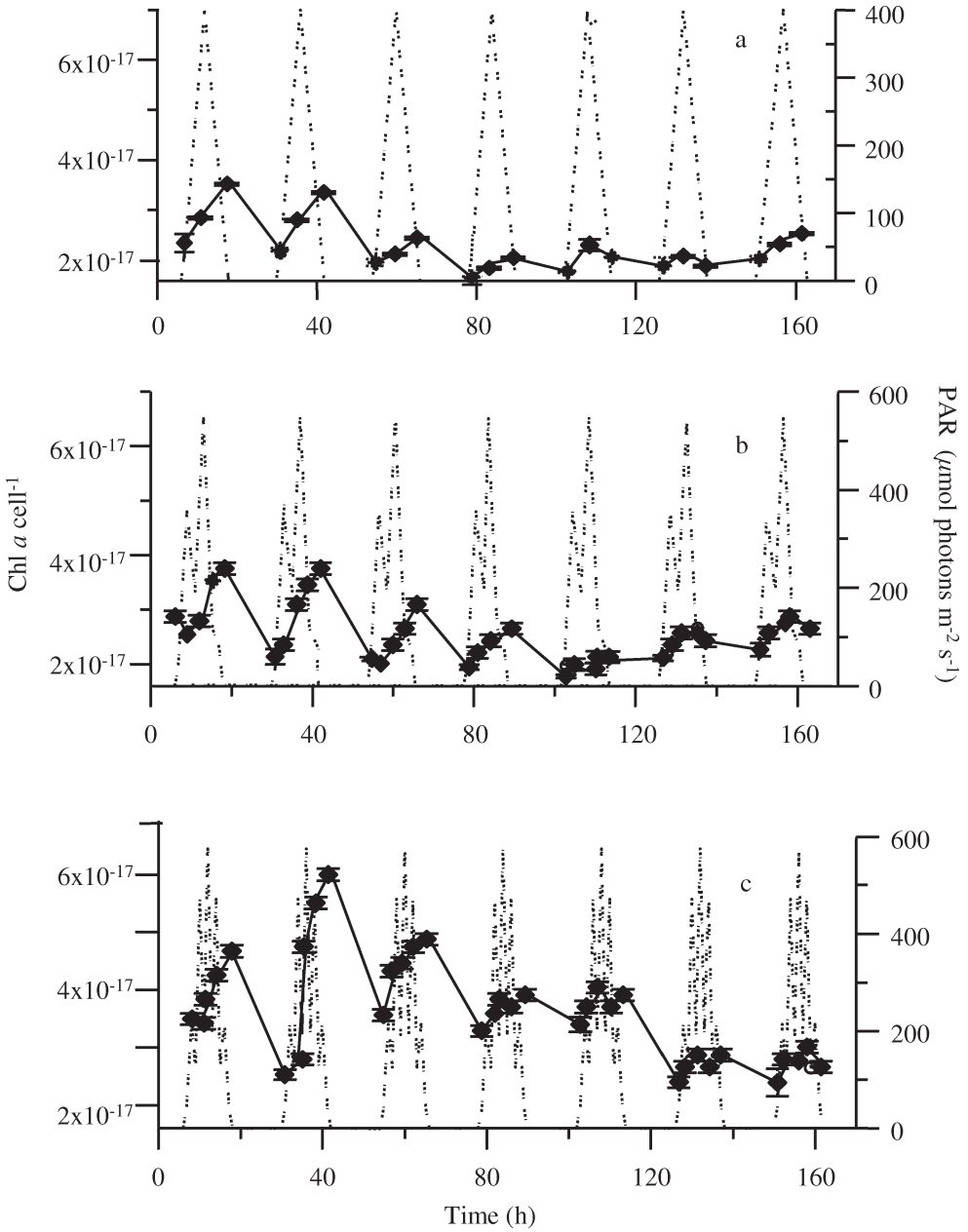

Fig. 3. Variations of intracellular pool of Chl *a* (in mol Chl *a* cell^{-1}) in (a) SL, (b) FL, and (c) HFL regimes. Time is in hours after the start of the experiment. The dashed line indicates light intensity (in μmol photons m^{-2} s^{-1}). Data are the average of three measurements. Error bars are SD.

not under SL, revealing that light variability favors covariation of these pigments.

Photoprotective pigments—Dt was synthesized during the light period and decreased substantially under low light and darkness (Fig. 4). During the exponential phase, the mean (Dt + Dd) : Chl *a* ratio was almost the same under the three conditions (Table 1), suggesting that this ratio was related to total photon dose over the day rather than to variability in PFD during the day.

The mean de-epoxidation state (DPS = Dt : [Dd + Dt] ratio) at peak light during the exponential phase was higher in FL and HFL (71% ± 5% and 71% ± 9%, respectively) than in SL (60% ± 9%, Fig. 4). This is probably related to the higher maximal light intensity in FL and HFL (~560 vs. 400 μmol photons m^{-2} s^{-1}) and/or to the greater induction of the xanthophyll cycle under fluctuating light. This increase in Dt was mainly because of the de-epoxidation of Dd with a limited new synthesis of the latter except during the latency phase (Fig. 5). The

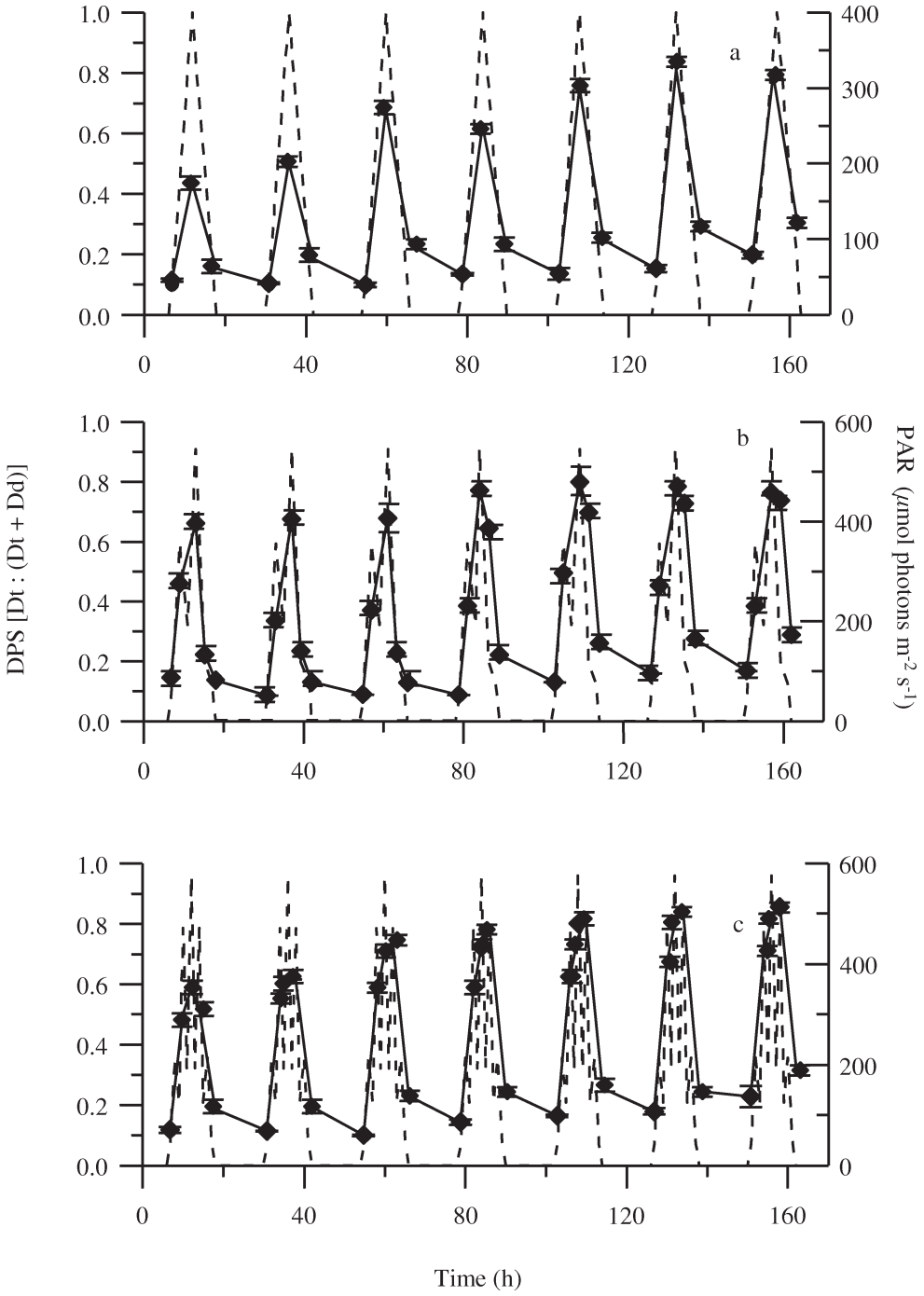

Fig. 4. Variations of de-epoxidation state (DPS) in (a) SL, (b) FL, and (c) HFL regimes. Time is in hours after the start of the experiment. The dashed line indicates PFD (in μmol photons m^{-2} s^{-1}). Data are the average of three measurements. Error bars are SD.

synthesis of Dt was higher when the culture entered the stationary phase (Fig. 5), which suggested an increased need for photoprotection in mature senescent cells. Significant linear regressions between Dt : Chl *a* and Dd : Chl *a* ratios in the three light conditions reveal that the Dd pool was reduced in favor of Dt with increasing light fluctuations (Fig. 6). Moreover, it appeared that Dd and Dt were lower during the exponential phase than in the stationary phase in HFL, and that the rate of de-epoxidation—as indicated by the slope of the regression—was almost similar in the exponential phase to that in the stationary phase (Fig. 6).

Only two (Vx and Ax) of the three-xanthophyll pigments (Vx, Ax, and Zx) of the other xanthophyll cycle were detected. Vx was constant and present in a very low amount ($\sim$0.0013 $\pm$ 0.0002 mol Vx mol Chl a^{-1}, Fig. 7).

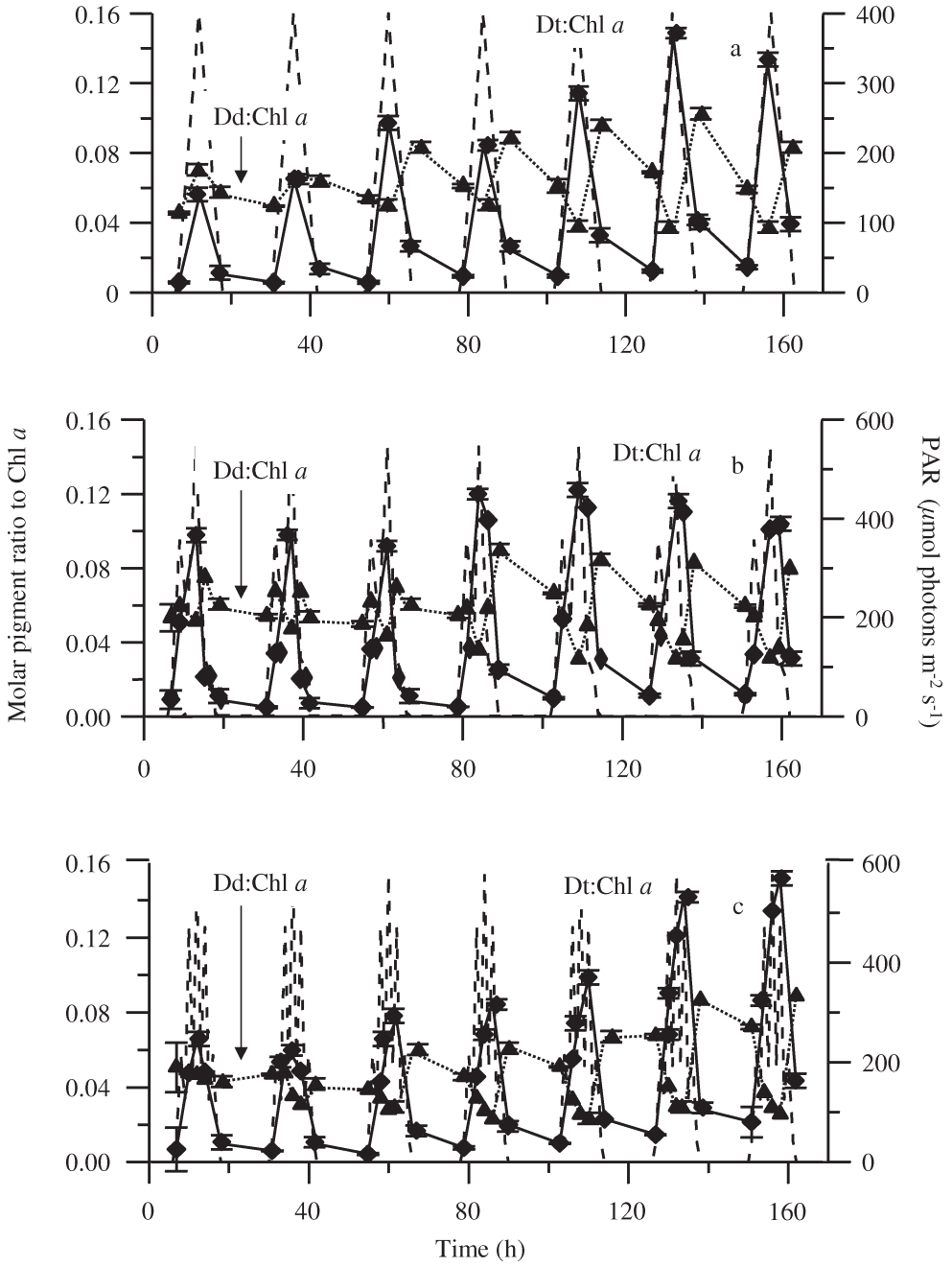

Fig. 5. Variations of Dt : Chl *a* (diamonds) and Dd : Chl *a* (triangles) ratio in (a) SL, (b) FL, and (c) HFL regimes. Time is in hours after the start of the experiment. The dashed line indicates PFD (in μmol photons m^{-2} s^{-1}). Data are the average of three measurements. Error bars are SD.

Ax was 10 times higher than Vx (~0.015 mol Ax mol Chl a^{-1}) under the three light regimes and increased with culture age (Fig. 7). Despite the lack of significant correlation between Ax : Chl *a* and Dt : Chl *a* in the pooled data ($p > 0.05$), significant relationships were observed between these variables at different times of the day (Table 2). This suggests a dependence of the relationship between these two pigments—and probably between the Vx cycle and the Dd cycle—on the dynamics in the requirement for Dt (Table 2). When data at dawn, midday, and dusk were examined separately, strong dependencies on the PDF could be inferred. At dawn, the slope of Ax : Chl *a* vs. Dt : Chl *a* was <1, revealing a biosynthetic trend mainly towards Ax. At midday, more Dt was synthesized relative to Ax under high light than at low light (slope >1). Similarly, at dusk, i.e., when these pigments decreased, the decrease seemed to be stronger for Dt that for Ax (slope >1).

Variable fluorescence and NPQ—Both Fv : Fm and Fq′ : Fm′ were significantly correlated with light, DPS,

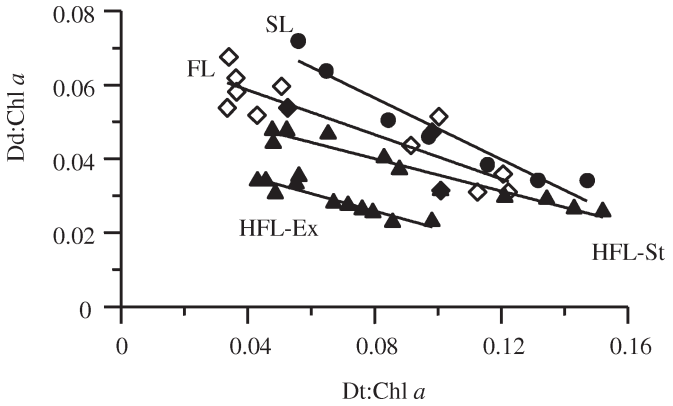

Fig. 6. Relationship between Dt : Chl *a* and Dd : Chl *a* measured at the light peaks (i.e., excluding the values measured at 40 μmol photons m^{-2} s^{-1}). SL (dots, Dd : Chl *a* = −0.42 Dt : Chl *a* + 0.090, r^2 = 0.93), FL (diamonds, Dd : Chl *a* = −0.30 Dt : Chl *a* + 0.070, r^2 = 0.77), and HFL (triangles). For HFL, stationary (St, Dd : Chl *a* = −0.24 Dt : Chl *a* + 0.045, r^2 = 0.85) and exponential (Ex, Dd : Chl *a* = −0.22 Dt : Chl *a* + 0.057, r^2 = 0.94) phases were separated.

and Dt : Chl *a* (at least $p < 0.01$; *n* ranged between 20 and 35), revealing the preponderant role of light on determining the dynamics of the maximal PSII photochemical efficiency and PSII operating efficiency and suggesting a substantial role of the xanthophyll cycle activation in photoprotection. In general, cells did not experience strong irreversible physiological stress in any of the three light regimes, as revealed by the rather similar mean values of the maximal PSII photochemical efficiency (Fv : Fm, between 0.47 and 0.65; Table 3) and PSII operating efficiency (Fq′ : Fm′, between 0.35 and 0.64; Table 3).

Fq′ : Fm′ decreased by up to 45% during light peaks. The recovery of about 70% (between 64% and 76%) of the initial value of Fq′ : Fm′ after 15-min dark exposure (which corresponds to the value of Fv : Fm) indicated the effectiveness of a rapid reversible quenching of Chl *a* fluorescence to minimize reduction of PSII operating efficiency. However, the decrease in photochemical efficiency that did not recover rapidly was probably because of photoinhibition (Harris et al. 2005). In contrast to the FL and SL, the recovery of Fq′ : Fm′ in darkness decreased during the stationary phase in HFL (data not shown). In other words, in stationary-phase HFL a significant proportion of the decrease in Fq′ : Fm′ attributable to high light was not reversible; this was probably dependent on the physiological state and not only on cell senescence, because this feature appeared only in HFL and not in the SL or FL regime.

The data from the three conditions pooled together revealed that NPQ ($_{eff}$NPQ) was significantly correlated with light ($p < 0.01$, $n > 20$), DPS, and Dt : Chl *a* (at least $p < 0.05$, $n > 20$), reflecting the involvement of the xanthophyll cycle in energy dissipation. Separating the three experimental conditions, significant correlations between NPQ and Dt : Chl *a* were found for the SL ($p < 0.01$, r^2 = 0.70) and FL ($p < 0.05$, r^2 = 0.55) regimes, but not for the HFL regime, showing that increased light variability frequency prevents the relationship's being significant and thus the linear dependence of NPQ on Dt. In the FL condition, when we did not take into account the data measured at the two added light peaks, the correlation became highly significant ($p < 0.01$, r^2 = 0.70), whereas in HFL this operation did not increase the correlation. Importantly, the slope of the regression was almost constant for the different correlations (range between 3.60 and 4.40 NPQ [mol Dt mol Chl a^{-1}]$^{-1}$).

The lack of significant correlation with increasing light variability might be related to the different temporal dynamics of NPQ development and Dt synthesis or decrease; for instance, it might be related to the slow Dt epoxidation and the much more rapid decrease of NPQ. This has been observed for this species and for other Chl *c*–containing species when light is gradually varied (C. Brunet unpubl. data), suggesting that linearity between these two features is not a general rule for short time scales and depends on the experimental conditions.

The means of $_{eff}$NPQ measured during the light peaks were quite similar under the three light regimes (range 0.32–0.35), in agreement with the small range of DPS variations, even though the maximum light values were different (400 vs. 560 μmol photons m^{-2} s^{-1}). The mean of $_{eff}$NPQ over the daylight period increased a little from SL to HFL (0.13 ± 0.18, 0.16 ± 0.14, and 0.18 ± 0.15 in SL, FL, and HFL, respectively), as related to the increase in number of light peaks during the day, confirming the role of NPQ as a short-term response and not just a diel long-term photoprotective feature.

The $_{eff}$NPQ was lower in the stationary than in the exponential phase in FL and, especially, in HFL (Fig. 8), contrasting with SL (Fig. 8a). This was probably because of an increase of photoinhibition, as also shown by Fq′ : Fm′. The incomplete recovery of Fq′ : Fm′ in darkness because of photoinhibition may have lowered the Fm value, which in turn affected the $_{max}$NPQ calculation. This feature might be the reason why $_{max}$NPQ increased during the stationary phase whereas $_{eff}$NPQ decreased, and more generally why an inverse relationship was found between $_{max}$NPQ and $_{eff}$NPQ in FL and HFL ($p < 0.01$, n = 35) and why $_{max}$NPQ and Dt : Chl *a* were not correlated.

Photosynthetic parameters—The photosynthetic ETR was stimulated in FL, the condition giving the highest mean of absolute electron transport rate ($_{abs}ETR_{max}$ ~ 0.75 ± 0.17 mol e^- g Chl a^{-1} h^{-1}, Table 3) although HFL caused a large reduction of $_{abs}ETR_{max}$ (~0.42 ± 0.13 mol e^- g Chl a^{-1} h^{-1}). Photosynthetic efficiency under light-limited conditions (α^B, Table 3) showed the same trend, with the highest mean value in FL than in SL and in HFL (~0.023, 0.018, and 0.015 mol e^- g Chl a^{-1} h^{-1} (μmol photons m^{-2} s^{-1})$^{-1}$, respectively).

Under HFL the light saturation parameter, Ek, was lower (~28.10 ± 7.50 μmol photons m^{-2} s^{-1}) than in SL and FL (>37.00 μmol photons m^{-2} s^{-1}; Table 3), reinforcing the suggestion of the shade-acclimation strategy of the cells in HFL. The low values of Ek indicated that the cells were subjected to saturating irradiance during most of the light period in all three experimental conditions.

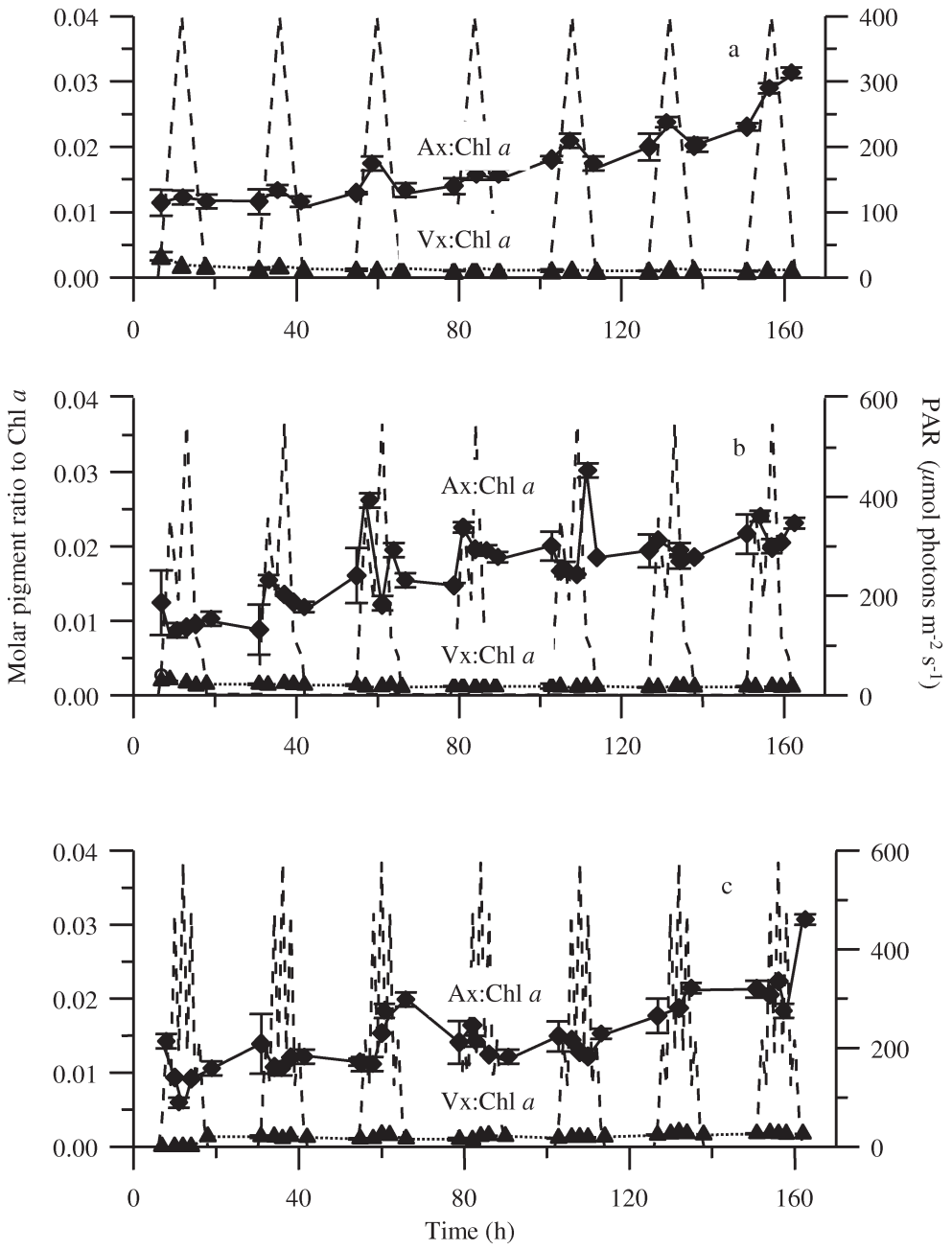

Fig. 7. Variations of xanthophyll pigments in (a) SL, (b) FL, and (c) HFL regimes. Triangles, Vx : Chl *a*; diamonds, Ax : Chl *a*. Pigment ratios are expressed in mol pigment mol Chl a^{-1}. Time is in hours after the start of the experiment. The dashed line indicates light intensity (in μmol photons m^{-2} s^{-1}). Data are the average of three measurements. Error bars are SD.

Discussion

Fluctuating light does not affect the cell cycle of *P. calceolata*, which is well synchronized with the LD cycle in all three light conditions, with cell division occurring at night in agreement with Jacquet et al. (2001). The growth rate (μ = 0.60 d^{-1}, equivalent to 0.87 divisions d^{-1}) is within the range of values found by Jacquet et al. (2001) and by Timmermans et al. (2005) on the same species and more generally on picophytoplankton (Veldhuis et al. 2005). *P. calceolata*, generally growing in the DCM (Guillou et al. 1999), is a shade-adapted species with maximum growth rate >0.35 d^{-1} for light ranging between 10 and 50 μmol photons m^{-2} s^{-1} and <0.30 d^{-1} for light > 60 μmol photons m^{-2} s^{-1} (C. Brunet unpubl. data), in agreement with Timmermans et al. (2005). The shade-adaptation strategy of *P. calceolata* can explain the observed strong down-regulation of the PSII photochemical efficiency (up to 45%) as related to NPQ (q_E + q_I) under high light. This adaptation to

Table 2. Linear regression equations between diatoxanthin (Dt) : chlorophyll *a* (Chl *a*) and antheraxanthin (Ax) : Chl *a* for the three light conditions. Pigment ratios are expressed in mol pigment mol Chl a^{-1}. Dawn, group of data corresponding to the dawn sampling; midday, group of data corresponding to the midday sampling, i.e., the highest PFD value; dusk, group of data corresponding to the dusk sampling; added peak 1, group of data corresponding to the first peak of light (before midday) in fluctuating light (FL) and highly fluctuating light (HFL); added peak 2, group of data corresponding to the third peak of light (after midday) in FL and HFL (i.e., the peak occurring after midday). The three experimental replicates were considered. For sinusoidal light (SL), FL, and HFL, $n=21$, except for dawn, where $n=63$. All together, $n=63$, except added peaks $n=42$ and dawn $n=189$. All correlations are significant at least at $p<0.05$.

	SL	FL	HFL	All together (SL, FL, HFL)
Dawn	Dt=0.70Ax−0.002 $r^2=0.84$	Dt=0.49Ax+0.0001 $r^2=0.46$	Dt=0.63Ax+0.0004 $r^2=0.56$	Dt=0.61Ax−0.0004 $r^2=0.55$
Midday	Dt=5.36Ax−0.002 $r^2=0.85$	Dt=1.65Ax+0.081 $r^2=0.34$	Dt=4.53Ax+0.021 $r^2=0.70$	Dt=3.95Ax+0.033 $r^2=0.61$
Dusk	Dt=1.22Ax+0.006 $r^2=0.57$	Dt=2.21Ax−0.016 $r^2=0.75$	Dt=1.10Ax+0.001 $r^2=0.51$	Dt=1.48Ax−0.002 $r^2=0.56$
Added peak 1	—	Dt=−0.86Ax+0.057 $r^2=0.45$	Dt=−0.87Ax+0.066 $r^2=0.31$	Dt=−1.06Ax+0.065 $r^2=0.41$
Added peak 2	—	No regression	Dt=8.05Ax−0.02 $r^2=0.79$	No regression

grow in a low-light environment also translates into the presence of numerous photosynthetic accessory pigments, which, however, does not preclude regulation of the light-harvesting apparatus, as shown by the modulation of the content in Chl c_2 and c_3, fucoxanthin, and 19′BF.

Interestingly, *P. calceolata* is able to maintain a constant growth rate under all three light regimes over 4 d following the shift from a sinusoidal to a highly fluctuating regime. This suggests that the integrated daily light dose is more important than the light fluctuation dynamics experienced by cells in the light period. Similar growth rates under constant and fluctuating lights have been found in species such as *Picochlorum* RCC337 (C. Dimier and C. Brunet unpubl. data) and *Dunaliella tertiolecta* (Queguiner and Legendre 1986), although generally the growth rate decreases under fluctuating light, as in *Emiliania huxleyi*, *Thalassiosira weissflogii* (van de Poll et al. 2007), *Phaeodactylum tricornutum*, and *Chlorella vulgaris* (Wagner et al. 2006). Instead, high-frequency changes in irradiance enhance growth rate in *Pyramimonas* sp. and *Chaetoceros brevis* (van Leeuwe et al. 2005). For this last species and for four freshwater algal species, the frequency of light fluctuations also influences growth rate (Litchman 2000). In general, the growth rate responds to fluctuating light and is species- and irradiance-dependent (Litchman 2000), and is also related to the acclimation state of the species (Litchman and Klausmeier 2001) and to its light history (van de Poll et al. 2007). The maintenance of a constant growth rate was probably related to the high capacity for physiological regulation and acclimation of *Pelagomonas*, which might be related to its presence in, and adaptation to conditions in, the DCM, where environmental oscillations would be important and could permit its persistence (Huisman et al. 2006). As discussed by Huisman et al. (2001), the diversity of life history and physiological responses might promote the high biodiversity usually present in the plankton community. This may suggest that the variability of responses to environmental variations on a shorter time scale than would permit competitive exclusion allows the coexistence of a multitude of species. It could be interesting to test the capacity for physiological

Table 3. Photochemical efficiency and photosynthetic parameters (α^B in mol e^- g Chl a^{-1} h^{-1} [μmol photons m^{-2} s^{-1}]$^{-1}$; $_{abs}ETR_{max}$ in mol e^- g Chl a^{-1} h^{-1}; Ek in μmol photons m^{-2} s^{-1}) in *P. calceolata* grown in sinusoidal light (SL), fluctuating light (FL), or highly fluctuating light (HFL). Fv : Fm, maximal PSII photochemical efficiency, measured on 15-min dark-acclimated samples. Fq′ : Fm′, PSII operating efficiency, measured on light-acclimated samples. Values are means±SD calculated over the exponential growth phase in each light condition.

Sampling time	Experimental regime	Fq′ : Fm′	Fv : Fm	α^B	$_{abs}ETR_{max}$	Ek
Dawn	SL	0.64±0.005	0.65±0.006	0.020±0.003	0.60±0.15	33.25±2.28
	FL	0.63±0.006	0.65±0.003	0.024±0.003	0.76±0.20	32.50±1.96
	HFL	0.62±0.004	0.64±0.006	0.018±0.004	0.42±0.05	26.50±1.45
Midday	SL	0.35±0.009	0.48±0.005	0.015±0.001	0.74±0.11	48.50±6.31
	FL	0.35±0.010	0.47±0.004	0.019±0.001	0.85±0.25	45.00±5.26
	HFL	0.36±0.009	0.47±0.005	0.013±0.002	0.41±0.02	37.50±4.85
Dusk	SL	0.59±0.007	0.59±0.003	0.019±0.005	0.50±0.16	32.20±4.43
	FL	0.60±0.006	0.61±0.003	0.022±0.002	0.68±0.11	31.25±3.59
	HFL	0.59±0.004	0.59±0.003	0.015±0.003	0.42±0.03	20.25±3.36

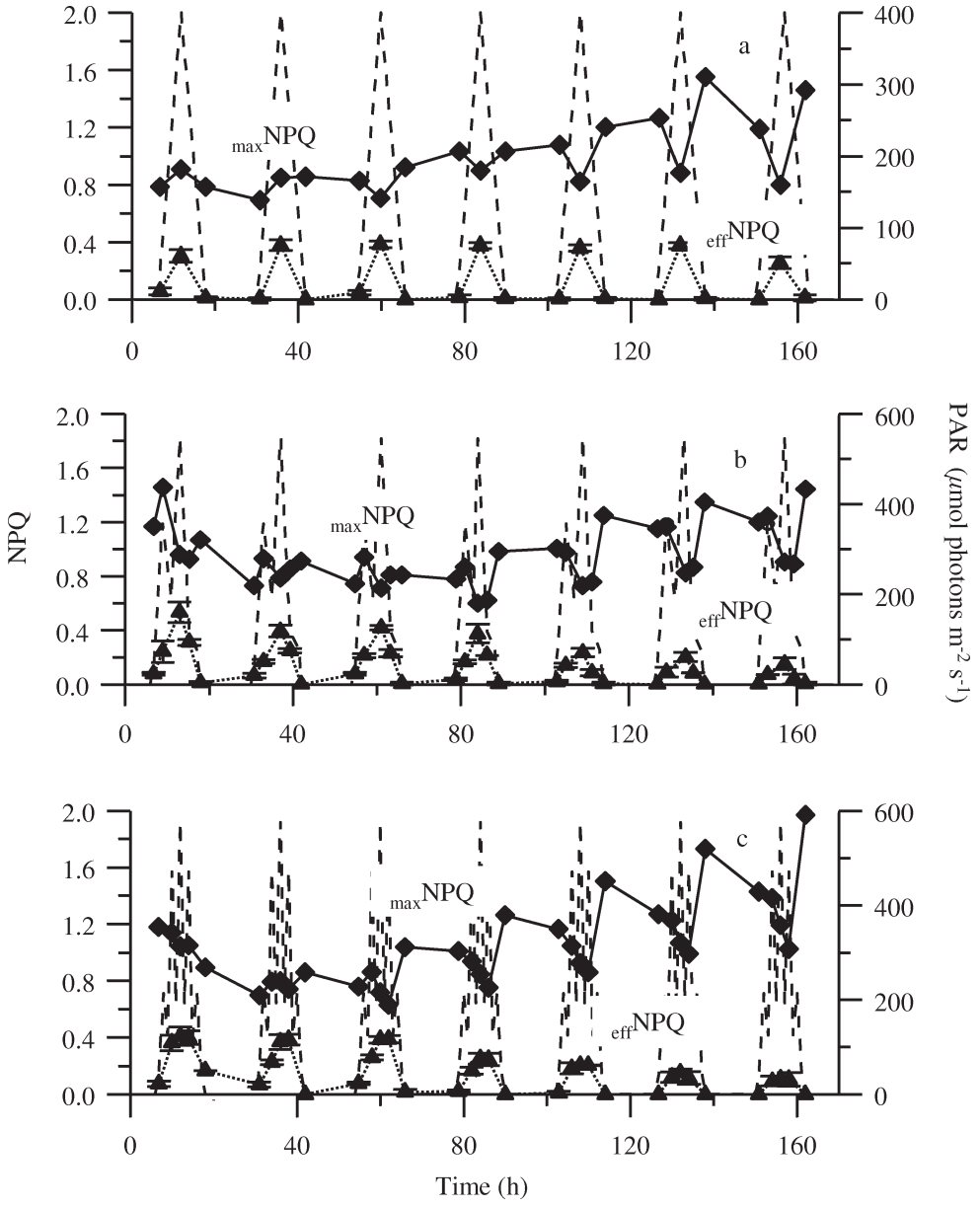

Fig. 8. Variations of non-photochemical quenching (NPQ) in (a) SL, (b) FL, and (c) HFL regimes. Diamonds, $_{max}$NPQ; triangles, $_{eff}$NPQ. Time is in hours after the start of the experiment. The dashed line indicates PFD (in μmol photons m^{-2} s^{-1}). Data are the average of three measurements. Error bars are SD.

regulation and acclimation on other DCM-adapted species, both picoeukaryotes and picocyanobacteria.

Cell division rate and $_{abs}ETR_{max}$, when expressed on a per-cell basis (i.e., multiplied by Chl *a* cell^{-1}), are similar in all three light regimes (only 25% variation for $_{abs}ETR_{max}$ on a per-cell basis), evidence of a large capacity for fine-tuning of the physiology so that photosynthetic and growth rates are kept constant. In many studies, growth and photosynthetic parameters are poorly coupled—for instance, in a diatom and a prasinophyte subjected to fluctuating irradiance (van Leeuwe et al. 2005)—reinforcing observations on the variability of the relationship between photosynthetic organic matter production and cell growth (Litchman 2000). This variability has been attributed to factors such as variations in respiration, dissolved organic matter loss from cells, and changed extent of storage of metabolites such as polysaccharides (van Leeuwe et al. 2005). It is also possible that the relationship between ETR and the production of organic matter is not linear, especially at high irradiances (Gilbert et al. 2000). As recommended by Litchman (2000), it is crucial to have both photosynthesis and growth data together to assess the effect of fluctuating light on algal dynamics.

In *Pelagomonas*, the small cell size would limit large-scale intracellular storage of energy (Raven 1998), consistent with the constancy of size-related parameters (e.g.,

FALS) under the three light regimes. This could be a reason why production and growth might be more strictly coupled in picoplankton than in larger cells, although further investigations are needed to test this hypothesis on picoplanktonic species.

Biochemical and physiological variations driven by changes in the frequency of fluctuation of light, and thus by the number of periods of decreasing light, could favor shade acclimation. α^B, when expressed per cell (instead of on a Chl *a* basis) was much higher in FL and HFL than in SL, i.e., a trend toward shade acclimation was observed. The photoacclimation strategy appears to be different under the FL and HFL regimes, suggesting a high degree of plasticity of *P. calceolata* in response to different light environments.

The cell quotas of accessory pigments increased from SL to FL to HFL. However, the ratio of accessory pigments to Chl *a* declined, suggesting an increase in the number of reaction centers without any increase in light-harvesting units. Wagner et al. (2006) suggested that increased photosynthetic unit (PSU) number with decreased PSU size allows cells to maximize photochemistry in different light regimes. Related to that is the decrease in a^*_{440} (=absorption coefficient at 440 nm vs. Chl *a*, mean value $\sim 0.037 \pm 0.004$ vs. $\sim 0.0565 \pm 0.009$ m^{-2} mg Chl a^{-1} in HFL vs. FL and SL) because of the decline in the ratio of accessory pigments to Chl *a*. The parallel large increase in Chl c_2 vs. Chl c_3 accompanying this strategy is not well explained, although it might increase light-harvesting capacity towards the lower wavelengths of the spectrum (Schofield et al. 1990).

The increase of Fuco : Chl *a* coupled with the small increase of Chl *a* in FL relative to SL and HFL suggests an increase in the relative size of the light-harvesting antenna. This strategy is often interpreted in algae as an acclimation to subsaturating irradiance (e.g., Harris et al. 2005). As pointed out by Rascher and Nedbal (2006), acclimation to fluctuating light is not the same as to low or high light, and the periodicity of light variations is as crucial as the irradiance to the outcome of acclimation. Our results suggest a threshold of light variability, in frequency and/or duration, in causing small or large physiological changes. The *n*-type photoacclimation corresponding to changes in the number of PSII reaction centers (Suggett et al. 2007) that seems to be adopted by *Pelagomonas* in HFL allows a dynamic regulation of PSII, allowing exploitation of a large range of irradiances but with a high nutrient cost for the cells (Six et al. 2008). In the water column, *Pelagomonas* growing in the nutrient-rich DCM would be able to exhibit this strategy. The highly variable light history may explain the poor physiological state of cells at the end of the exponential growth phase and the shorter duration of the exponential growth phase in HFL with respect to the other fluctuating light condition. In maintaining as rapid a growth rate as possible, and in increasing the number of PSII reaction centers, the energetic costs for rapid photoprotection and PSII repair processes needed under high light (up to 560 μmol photons m^{-2} s^{-1}) have increased. For instance, we can expect an increasing energy expenditure for the synthesis of xanthophyll-cycle pigments, or protein D1 turnover associated with a photoinhibition cycle or decreased energy acquisition because of the lower efficiency of use of excitation energy for electron transfer because of increased NPQ.

When light fluctuations are slower with fewer low-light periods (FL condition) *P. calceolata* is able to modulate the photosynthetic antenna, as in σ-type photoacclimation, which is less costly in terms of nutrients (Six et al. 2008). Surprisingly and interestingly, *Pelagomonas* seems to be able to deploy one or the other photoacclimatory strategy with respect to the light fluctuation dynamics, without any variation of the daily-integrated light dose. It will be interesting to investigate whether all pelagic algae share this capacity or whether it is more pronounced in the shade-acclimated species such as *P. calceolata*. Indeed, this could be a response to environmental pressure on growing in the euphotic pelagic environment, where changes in light fluctuation dynamics are very common as a result of wind stress, advection, or current variations, and are not always related to great changes in daily-integrated light dose.

Although the photoacclimatory strategy (either *n*- or σ-) allowed maximal photosynthesis, the potential damage to PSII was higher in FL and HFL because the peaks in irradiance were higher. In these conditions cells presumably need to allocate more energy to photoprotection and repair mechanisms than in SL. Evidence of this is seen in the higher DPS measured in FL and HFL than in SL because of the higher peak light intensity, even though the NPQ is similar. This means that the two processes are not linearly coupled, as already observed in other studies (Dimier et al. 2007*a,b*; and in the low-light strain of *Ostreococcus tauri*; Six et al. 2008). This feature is particularly evident in HFL, with DPS being highest during the third peak of light of the daylight period ($\sim$450 μmol photons m^{-2} s^{-1}), although NPQ is similar to the previous light peak (550 μmol photons m^{-2} s^{-1}).

In general, it is more difficult for the cells to cope with high irradiance under HFL than in the two other regimes, which is also reflected by the lower photochemical efficiency (Fv : Fm and Fq′ : Fm′) and by the lower fluorescence recovery seen during declining growth. This may explain why cells enter the stationary phase earlier in HFL than in FL or SL. Once cells entered the stationary phase, during which no nutrient limitation occurred (data not shown), physiological modifications appeared in all the light regimes and could not be attributed to the light history of the cells. DPS reached 80% in the stationary phase, as previously documented in senescent diatoms (Brunet et al. 1996). This is a very high value relative to the values generally observed in the exponential phase (Lavaud et al. 2007). In HFL, the combination of the higher concentration of Dd—and Dt—in the stationary phase with a similar rate of de-epoxidation to that seen in the exponential phase could have revealed slower enzymatic processes and/or a lower availability of substrates for the enzymatic reaction in these senescent and non-healthy cells than in growing cells. During the stationary phase of the same experimental condition, and to a lesser extent in SL, the large increase of Dt with the small increase of Dd was

coupled to a dramatic decrease of NPQ. This indicates that light is not the only factor responsible for the DPS increase, and suggests a potential role for Dt as an antioxidant during oxidative stress. Such a role has been suggested for the components of the xanthophyll cycle in plants, in which the synthesis of photoprotective xanthophylls has been linked to an increased activity of antioxidant enzymes (Foyer and Harbinson 1999). It is probable that this is also the case in *Pelagomonas*, in which an increase of Ax was detected together with the increase in Dt and Dd with aging of cultures. The large increase in Ax could be because of the absence of Zx from this alga, as in some prasinophytes (Goss et al. 1998). The similar trends of Dt (and Dt + Dd) and Ax might suggest a link between the two xanthophyll cycles (Wilhelm et al. 2006). The similar trend of these two pigments suggests that they could be formed together, for instance because of the parallel activities of the Vx- and Dd-de-epoxidase that transform Vx into Ax and Dd into Dt, respectively (Wilhelm et al. 2006). The role of the increase in Ax is not understood, even though it could be used for further Dt increase when necessary, in agreement with the hypothetical pathway between Ax and Dt of Wilhelm et al. (2006). This hypothesis would explain the greater increase of Ax than Dt at dawn, preparing a pool of available Ax that could be used during the diel cycle. This proposal is strengthened by the observation that in these conditions Dd does not vary so much (except when the cells reach the stationary phase) and would not be participating in the synthesis of Dt, at least predominantly. Indeed, when light is increasing very rapidly, as it is the case for the first added peak in the FL and HFL, it seems that Dt increases using Ax. This means that in the organisms missing Zx and under certain light conditions, it is not Vx that would be the main precursor of Dt, but Ax. Only a small fraction of Ax would be available for Dt synthesis; that may be because of a structural role of some molecules of Ax, which, for instance, could add rigidity to the thylakoid membrane, thereby stabilizing PSII complexes (Havaux and Gruszecki 1993).

Contrasting with the putative proposal (Wilhelm et al. 2006) and with previous studies (Anning et al. 2000; Harris et al. 2005), no inverse relationship was found between Fuco and/or 19′BF and the two photoprotective pigments Dd and/or Dt. One of the reasons could be the fact that light received by cells over the diel cycle was the same for the three experiments, and that the main difference between light conditions was the rate of light variation, which could be too fast to induce any biosynthetic reactions between Fuco and Dd or Dt. Moreover, the presence of both Fuco and 19′BF, which are probably related one to the other, could lead to more complex dynamics of interrelationship between Fuco-like pigments and Dd-cycling pigments.

In conclusion, few studies have dealt with the ecophysiological responses of algae to fluctuating light conditions (van Leeuwe et al. 2005; Rascher and Nedbal 2006), and many of those employed very large light variations (Wagner et al. 2006; van de Poll et al. 2007), allowing investigation of the photodependent processes but not readily yielding an ecological interpretation. Our results for *Pelagomonas*, together with recent observation on *Ostreococcus* by Six et al. (2008), reveal some striking ecophysiological responses in the capacity to grow and photoprotect, even though *Pelagomonas* is DCM-adapted. Picoeukaryotes are confirmed as good biological models for photophysiological studies. It would be interesting to investigate the relationship between growth and productivity in other picoeukaryote species with other adaptation strategies, such as high-light–adapted ones.

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