Vol. 452: 11–26, 2012
doi: 10.3354/meps09606

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published April 25

OPEN ACCESS

Photoacclimation to abrupt changes in light intensity by *Phaeodactylum tricornutum* and *Emiliania huxleyi*: the role of calcification

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ABSTRACT: Phytoplankton experience strong and abrupt variations in light intensity. How cells cope with these changes influences their competitiveness in a highly dynamical environment. While a considerable amount of work has focused on photoacclimation, it is still unknown whether processes specific of phytoplankton groups (e.g. calcification and silicification) influence their response to changing light. Here we show that the diatom *Phaeodactylum tricornutum* and the coccolithophore *Emiliania huxleyi* respond to an abrupt increase in irradiance by increasing carbon fixation rates, decreasing light absorption through the decrease of light-harvesting pigments and increasing energy dissipation through the xanthophyll cycle. In addition, *E. huxleyi* rapidly increases calcium carbonate precipitation in response to elevated light intensity, thereby providing an additional sink for excess energy. Differences between the 2 species also emerge with regard to the magnitude and timing of their individual responses. While *E. huxleyi* show a pronounced decrease in chlorophyll *a* and fucoxanthin cellular contents following increased light intensity, *P. tricornutum* has a faster increase in diadinoxanthin quota, a slower decrease in F_v/F_m (ratio of variable to maximum fluorescence) and a stronger increase in organic carbon fixation rate during the first 10 min. Our findings provide further evidence of species-specific responses to abrupt changes in light intensity, which may partly depend on the phytoplankton functional groups, with coccolithophores having a supplementary path (calcification) for the rapid dissipation of excess energy produced after an abrupt increase in light intensity. These differences might influence competition between coexisting species and may therefore have consequences at the community level.

KEY WORDS: Calcification · Light · Coccolithophores · Diatoms · Phytoplankton · Pigments · Carbon fixation

INTRODUCTION

Photosynthetic organisms require light as an energy source for carbon fixation. As a result of constantly shifting cloud cover, they have to adjust to abrupt light variation in their natural environment (Falkowski 1980). Phytoplankton, unicellular photoautotrophs thriving in the sunlit surface layer of marine and freshwater systems, must deal with additional rapid light fluctuations while being passively transported through the water column or when exposed to radiation focusing and defocusing by surface waves (Dera & Stramski 1986). When brought to the surface, phytoplankton cells experience light intensities orders of magnitude higher than at greater depths. The increase in light intensity can be so high that light absorption by chlorophyll and accessory pigments exceeds the potential utilization. As a consequence, cells have developed mechanisms to dissipate excess energy.

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Chlorophyll relaxation from its excited state occurs by the following means: light emission (fluorescence), heat dissipation (non-photochemical quenching, NPQ) and photochemistry (fueling photosynthesis; based on Müller et al. 2001). These energy dissipation valves have specific contributions in photoprotection; for instance, chlorophyll fluorescence can account for 0.6 to 3% of the absorbed photons (Krause & Weis 1991). The excess energy that is not dissipated by any photoprotective mechanism might be used in the production of reactive oxygen species (Foyer & Harbinson 1999). The amount of reactive oxygen species produced may lead to pigment bleaching and cell death under extremely high light conditions. As concurrent processes, NPQ and photochemistry minimize the production of the damaging O_2 reactive by-products of photosynthesis.

In most phytoplankton (see Kirilovsky 2007 for further information concerning cyanobacteria), namely diatoms (e.g. Bertrand 2010) and coccolithophores (e.g. Llewellyn et al. 2007), NPQ is correlated with the xanthophyll cycle (Horton et al. 1996, Müller et al. 2001), which is controlled by the light-induced proton gradient across the thylakoid membrane (Lavaud & Kroth 2006). Under increasing light conditions, the deepoxidation of diadinoxanthin (Dd) into diatoxanthin (Dt) leads to an enhanced dissipation of excessively absorbed light energy in the form of heat. When light intensity decreases, Dt is converted to Dd instead (Brunet et al. 2008, Brunet & Lavaud 2010, Goss & Jakob 2010).

Photochemistry can be adjusted as cells change the quantity (Fisher et al. 1989) and activity of photosynthetic enzymes related to carbon fixation and, therefore, modify their photosynthetic and cell division rates. Cellular responses to strong light variation include changes in the cellular content of the main carboxylating enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), modulation in the ratio of Rubisco to chlorophyll or protein contents (Fisher et al. 1989) or changes in Rubisco activity (Lin & Carpenter 1997). The availability of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate hydrogenase (NADPH) for the Calvin-Benson Cycle also affects photochemical rates, but is harder to quantify. Thus, even though it is known that part of the additional light can fuel photochemistry, there are still uncertainties related to the timing and magnitude of this response.

High light intensity increases the carbon demand for carbon fixation, eventually beyond the supply. This might have implications for the carbon-concentrating mechanisms (CCMs) which increase CO_2 concentrations close to Rubisco. Hence, there might be a dual role for CCMs. On the one hand, they are vital in providing high CO_2 concentration for carbon fixation and adjusting to variable CO_2 supply (Giordano et al. 2005); on the other hand, they might play an important role in compensating for the changing CO_2 demand under variable light conditions. As CCM characteristics and activity are distinct between different phytoplankton groups (Giordano et al. 2005), this might influence the specific response to changing CO_2 demand such as under abrupt increase in light intensity.

Another potentially relevant difference between phytoplankton functional groups affecting the overall response to changing light intensity is their specific biogeochemical signature resulting from silicification or calcification, since both processes spend energy to produce either silica (diatoms) or calcite (coccolithophores) structures. Moreover, the silica frustules are produced after cell division, which is thought to occur mostly during the night or at the end of the day (Zurzolo & Bowler 2001, Ragni & D'Alcalà 2007), whereas coccolith (calcite scales) formation occurs more or less evenly throughout the day (Paasche 2001, Müller et al. 2008) when light intensity varies.

Furthermore, diatoms and coccolithophores differ in their general distribution, which correlates with their nutrient utilization strategy (Litchman 2007). The nutrient gradients found in the ocean, in turn, correspond to water column structure and light availability. Diatoms are well adapted to nutrient- (nitrate) rich turbulent waters characterized by variable light intensities, while coccolithophores predominantly occur in more stratified surface waters under conditions of moderate to low nutrient availabilities and comparably stable light intensities (Litchman 2007). Accordingly, the photoprotective capacity and regulation likely differ between these 2 phytoplankton groups, as is the case between diatoms and green algae (Wagner et al. 2006), therefore potentially influencing phytoplankton community composition. It is not known which group-specific characteristics most strongly affect the response to abrupt changes in light intensity. Studies on diatoms have correlated their distribution with photoprotective capacity (Lavaud et al. 2007), as well as with their photosynthetic architecture which is related to iron availability and demand (Strzepek & Harrison 2004). In this context, estuarine/coastal species such as *Phaeodactylum tricornutum* should have a higher photoprotective capacity than oceanic diatoms (Lavaud et al. 2007). No study has been done to date to analyse differences in the photoprotective capacity between

species of coccolithophores — for instance, the cosmopolitan *Emiliania huxleyi* versus others — or between this group and diatoms.

In summary, understanding the regulatory response of phytoplankton to light intensity variation profits from considering both the cellular contents of photoprotective pigments and carbon fixation rates. Differences in the efficiency of using the extra energy becoming available through sudden increases in light intensity for carbon fixation (organic and inorganic) could have important consequences at the community level and for species distribution. To assess the influence of abrupt increases in light intensity on phytoplankton physiology, we grew the molecularly and physiologically well-studied (e.g. Lavaud et al. 2007) diatom *Phaeodactylum tricornutum* and the most abundant coccolithophore in the modern ocean, *Emiliania huxleyi* (Paasche 2001), under controlled laboratory conditions. The combination of pigment and carbon fixation (^{14}C method) analysis of 2 species with similar cell division rates provided information about the timing of their responses in terms of both energy dissipation and utilization and offers a new perspective on the relevance of calcification in coccolithophores as an energy dissipation mechanism.

MATERIALS AND METHODS

Experimental setup

Monospecific cultures of the coccolithophore *Emiliania huxleyi* (strain PML B92/11A) and the diatom *Phaeodactylum tricornutum* (CCMP632) were grown in modified f/2 media using 0.2 µm sterile filtered North Sea water (5 µmol l^{-1} phosphate, 40 µmol l^{-1} nitrate and 40 µmol l^{-1} silicate in the case of the diatom) at 17°C, a photon flux density of 50 µmol m^{-2} s^{-1} (supplied from Daylight 12-950 lamps, Philips TLD 18W) and a 13.5/10.5 h light/dark cycle, for a minimum of 14 generations. Silicate addition allowed the construction of frustules in the weakly silicified diatom *P. tricornutum,* a species which does not depend on silicate for growth (Brzezinski et al. 1990). This addition ensured that the cells followed the same cell cycle as any other diatom. Cultures were grown at low cell abundance to avoid changes in sea water carbonate chemistry. Initial cell abundances, ranging between 150 (regime: light change) and 1250 (regime: baseline) cells ml^{-1}, were chosen with the aim of reaching about 20 000 or 30 000 cells ml^{-1} on the days of the experiments. Both the pre-cultures and experimental cultures were mixed twice (9:00 and 16:00 h) every day, avoiding significant sedimentation and associated self-shading during the light period and, therefore, differences in light supply. Each experiment (regimes: baseline and light change) was performed twice with duplicate bottles (Fig. 1). Therefore, all parameters have 4 data points, with the exception of pigment concentrations for which there are only 2 data points (1 experiment with duplicate bottles). In the baseline experiment, cells were maintained under the conditions to which they were acclimated in the pre-cultures (50 µmol m^{-2} s^{-1}; Fig. 1a). In the next experiment (on the following day, regime: light change), light intensity was abruptly raised to 800 µmol m^{-2} s^{-1} 7 h after the beginning of the light phase (14:00 h; Fig. 1b). Sampling occurred at the same time for all parameters and throughout the light phase, starting at the beginning of the light phase (07:00 h, t0) in both the baseline and the light change regime.

Carbon fixation (^{14}C)

On the days prior to the experiments, 2 h before the dark phase, cultures were transferred to incubation flasks (65 ml) in order to reduce prolonged handling stress for the cells on the day of the experiment. On that day, both light and dark (wrapped

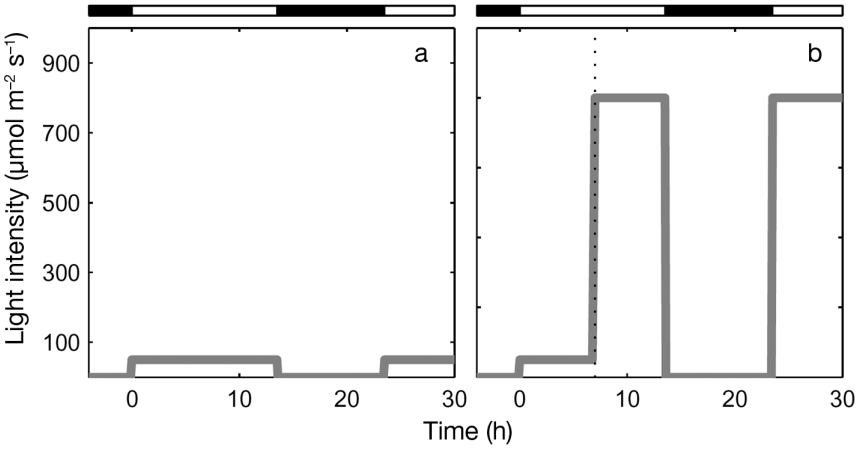

Fig. 1. Light intensity through time. (a) Baseline: light intensity constant at 50 µmol m^{-2} s^{-1}; (b) light change: light intensity increased abruptly from 50 to 800 µmol m^{-2} s^{-1} 7 h after the start of the light phase (14:00 h). The white/black bar represents the light/dark diel cycle. The vertical dotted line marks the hour of light intensity increase

several times in aluminium foil) samples were spiked with 100 µl of a 1.85×10^{12} Bq $H^{14}CO_3^-$ solution and were incubated in the same climate chamber as the cultures for a period of 1.75 to 13.25 h. Flasks were positioned to ensure equal light intensities for all vials. *Phaeodactylum tricornutum* and *Emiliania huxleyi* samples for carbon fixation determination (plus the replicates which were incubated in the dark for the same amount of time) were filtered onto cellulose acetate filters (0.45 µm) under low pressure (200 mbar). In the case of *E. huxleyi,* the filters for particulate organic carbon were rinsed with 1 ml HCl (0.01 M) for 1 to 2 s and then soaked again with 1 ml of HCl (0.01 M) for 30 s, ensuring the dissolution of all calcium carbonate (coccoliths). This protocol was optimized from Müller et al. (2008) by comparing the change in filter colour from yellowish to green of acidified *E. huxleyi* filters with those of *P. tricornutum*. The data were consistent with previous work in terms of particulate inorganic carbon (PIC) to organic carbon (POC) ratios (in this study, mean ± SE of PIC/POC was 0.98 ± 0.09), suggesting complete removal of inorganic carbon. Furthermore, an additional filter was taken without any acid treatment for total carbon. All filters were rinsed with 0.2 µm filtered seawater, removing excess radioactive dissolved inorganic carbon (DIC) and then placed in scintillation vials. Lumagel Plus (Universal LSC cocktail) was added to the filters, and the radioactive decay was measured in a liquid scintillation analyser (Tri-Carb 2900TR, Packard) after 12 h in the dark. PIC fixation (coccoliths, produced by calcification) was calculated as the difference between total carbon (non acidified filters) and organic carbon (acidified filters) fixation, assuming that the acid-labile carbon fraction consists of coccoliths only. For calculating overall carbon fixation rates from ^{14}C incorporation, DIC concentrations were measured photometrically (Stoll et al. 2001) using an automated segmented-flow analyser (Quaatro) equipped with an auto-sampler.

Cell pigments and maximum photochemical quantum yield of photosystem II (F_v/F_m)

Pigment samples were filtered onto GF/F filters and analysed by high performance liquid chromatography (HPLC; column-Microsorb-MV 100-3C8, 100 × 4.6 mm × ¼ inch, Waters) according to Barlow et al. (1997). Maximum photochemical quantum yield of photosystem II (F_v/F_m) was determined with a PhytoPAM (Phyto-ED Walz, PPAA0138), 20 min (dark incubation) after sampling, by determining the ratio between the maximum variable (F_v) and maximum (F_m) Chl *a* fluorescence yield in dark adapted cells.

Cell numbers and statistical analysis

Cell abundance and equivalent spherical diameter (ESD) were determined shortly after each sampling by using a Coulter Counter Z series (Beckmann Coulter). As *Phaeodactylum tricornutum* used here was fusiform, the actual diameter of the central area is probably slightly smaller than the measured ESD. In the case of the spherical *Emiliania huxleyi*, ESD and actual cell diameter are the same. Cell division rate was calculated as µ according to:

$$\mu = (\ln C_e - \ln C_i) / \Delta t \quad (1)$$

where C_e and C_i refer to end and initial cell concentrations, respectively, and Δt to the duration of the incubation period in days. The periods of time (see Fig. 7) for the calculation of the mean cell division rates of each species and light condition were 0 to 7 h, 0 to 10 h, 0 to 12 h and 7 to 12 h after the beginning of the light phase. Cell division rates were also calculated from 0 to 24 h, thereby including the dark phase. The data were analysed by calculating Pearson's linear correlation coefficient (R), using the program MATLAB®.

RESULTS

Shortly after the 16-fold (1500%) light intensity increase, organic carbon fixation (mostly photosynthesis considering that a C_4-like mechanism may exist in diatoms; for further information concerning C_4-like and C_4 mechanisms see e.g. Morris et al. 1978, Reinfelder et al. 2004, Kroth et al. 2008) of both *Phaeodactylum tricornutum* and *Emiliania huxleyi* increased above the values found for the baseline (Fig. 2a). In fact, within 10 min, there was an approximate 880% increase in organic carbon fixation rate (calculated from the slopes) in *P. tricornutum* and a 430% increase in *E. huxleyi* (Fig. 2a). If the first 30 min were considered instead, changes in organic carbon fixation rates were lower, 380 and 340%, respectively. When the whole light period was integrated, the percentage increase in organic carbon fixation rates was even lower, especially in *P. tricornutum* (70%) in comparison to *E. huxleyi* (170%). The slower increase in organic carbon fixation rate within the first 10 min in *E. huxleyi* was associated

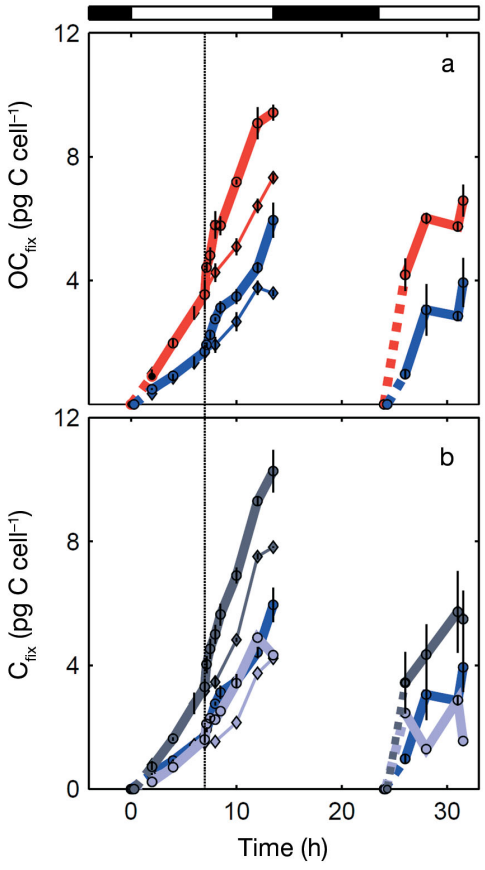

Fig. 2. *Phaeodactylum tricornutum* and *Emiliania huxleyi.* Cumulative carbon fixation of *P. tricornutum* (Pt) and *E. huxleyi* (Ehux) through time. (a) Organic carbon fixation per cell of both species (OC_{fix}); (b) carbon fixation per cell of Ehux (C_{fix}: total, organic and inorganic carbon fixation per cell). Thin and thick lines indicate values under the baseline and the light change regimes, respectively: red, Pt; blue, Ehux organic carbon fixation; light purple, Ehux inorganic carbon fixation; grey, Ehux total carbon fixation. Each light intensity profile has 4 independent replicates for each species. Mean values ± SE (error bars) are shown. The white/black bar represents the light/dark diel cycle. The vertical dotted line marks the hour of light intensity increase. The baseline values for Pt were multiplied by 0.85 for optical reasons, having no implications for the calculations

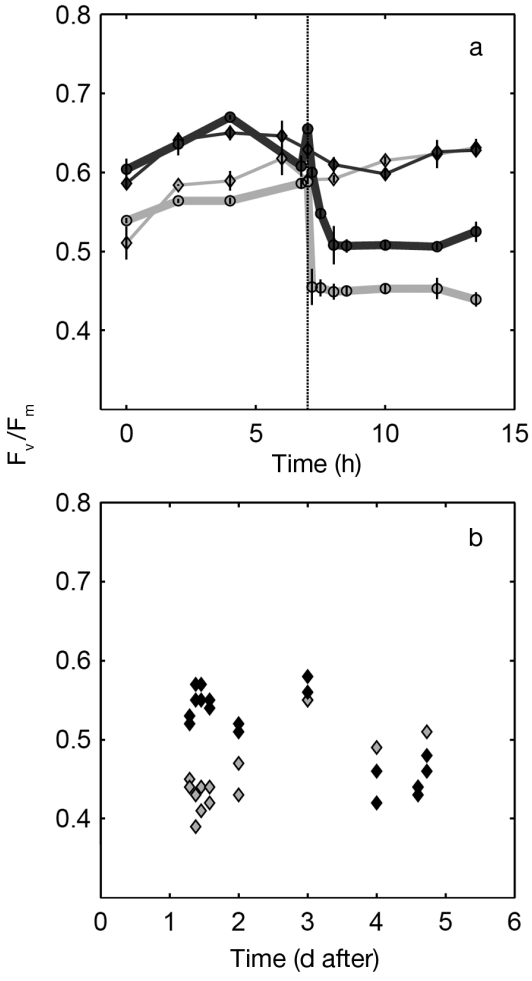

Fig. 3. *Phaeodactylum tricornutum* and *Emiliania huxleyi.* Maximum photochemical quantum yield of photosystem II (F_v/F_m) of *P. tricornutum* (Pt) and *E. huxleyi* (Ehux) through time, after a 20 min dark incubation. F_v/F_m (a) within 15 h and (b) until 5 d after the light intensity was raised and maintained at 800 µmol m^{-2} s^{-1} with a 13.5/10.5 h light/dark cycle. Thin and thick lines indicate values under the baseline and the light change regimes, respectively: black, Pt; grey, Ehux. Panel b shows Pt (black diamonds) and Ehux (grey diamonds) values on the days following the light intensity increase. Means ± SE (error bars) are given. Other details as in Fig. 2

with a pronounced increase (990%) in inorganic carbon fixation (calcification) rate combining to a 720% increase in total carbon fixation rate (Fig. 2b).

After an abrupt and strong light intensity increase, more energy is absorbed than can be utilized to fix organic compounds/carbon, thereby jamming the electron chain. Indeed, immediately after the increase in light intensity (10 min), F_v/F_m decreased 8% ($p = 0.09$) in *Phaeodactylum tricornutum* and 23% ($p = 0.04$) in *Emiliania huxleyi* and did not recover during the rest of the light phase (Fig. 3a). By the end of the light phase, the overall decrease in F_v/F_m was similar in both species: 20% ($R = -0.94$, $p < 0.001$) in *P. tricornutum* and 25% ($R = -0.96$, $p < 0.001$) in *E. huxleyi.* After 2 d under high light intensity (800 µmol m^{-2} s^{-1} with a 13.5/10.5 light/dark cycle), F_v/F_m of *P. tricornutum* remained higher than that of *E. huxleyi,* but on the third day, *P. tricornutum* values tended to decrease while *E. huxleyi* values tended to slightly increase (Fig. 3b).

Experiments done for determining the baseline and those with the light intensity increase showed

increasing cellular carbon quotas with time (Fig. 2), as well as increases in other cellular contents (see below) throughout the light period. In the following light phase (still under 800 µmol m^{-2} s^{-1}), organic carbon fixation increased to values higher than those from the previous day at the same time, with *Phaeodactylum tricornutum* having a quicker response than *Emiliania huxleyi*. No evident trend was seen in the inorganic carbon quota.

While a considerable amount of the additional energy was channelled to carbon fixation, other mechanisms and pathways were triggered as well. For example, cellular chlorophyll *a* (chl *a*) (Fig. 4a), fucoxanthin (Fig. 4b) and chl *c* (Fig. 4c) concentrations decreased relative to those in cells kept under the baseline conditions (50 µmol m^{-2} s^{-1}). Approximately 1 h after the irradiance increase, cellular chl *a* content started decreasing in relation to the baseline and, at the end of the light phase, was 16% lower ($R = -0.99$ and $p = 0.01$) in *Phaeodactylum tricornutum* and 23% lower ($R = -0.99$ and $p = 0.11$) in *Emiliania huxleyi*. Within the first hour of incubation at higher light intensities, cellular concentrations of fucoxanthin decreased 5% ($R = -0.77$ and $p = 0.23$) in *P. tricornutum* and 15% ($R = -0.78$ and $p = 0.22$) in *E. huxleyi*, and this trend became even more pronounced at the end of the light phase with a 24% ($R = -0.99$ and $p = 0.005$) decrease in *P. tricornutum* and 56% ($R = -0.90$ and $p = 0.10$) in *E. huxleyi*. Also after 1 h, the 19′hexanoyloxyfucoxanthin quota of *E. huxleyi* seemed to slightly increase with rising light intensity in comparison to the baseline (data not shown). Chl *c* decreased, but it was difficult to determine the timing of this response to elevated irradiance (Fig. 4c).

The photoprotective response could also be detected through changes in the xanthophyll cycle. While cellular content of Dd increased 40% within 1 h ($R = 0.95$ and $p = 0.05$) in *Phaeodactylum tricornutum*, *Emiliania huxleyi* quotas did not change much on this time scale. After 3 h, Dd quotas of *E. huxleyi* increased 62% ($R = 0.98$ and $p = 0.02$), already similar to the 57% increase ($R = 0.96$ and $p = 0.04$) of *P. tricornutum* (Fig. 5a). However, considering the entire light period, both had the same percentage of increase. Dt increased in both species immediately after the increase in light intensity. In less than 30 min, Dt increased 148% ($R = 0.96$ and $p = 0.04$) from the baseline values in *E. huxleyi* (Fig. 5b). In *P. tricornutum*, it was not possible to estimate the relative increase since Dt was undetectable under baseline light conditions.

After the dark period, cellular concentrations of all pigments considered here were lower than values measured at the beginning of the previous light phase, in both the baseline and the light change regime. Moreover, most of the pigment concentrations at 800 µmol m^{-2} s^{-1} were also lower than the cellular quotas under 50 µmol m^{-2} s^{-1} at the same time of the day. The only exception was Dt cellular content of *Phaeodactylum tricornutum*, which could only be measured after the abrupt increase in the light intensity and could still be measured after the dark period.

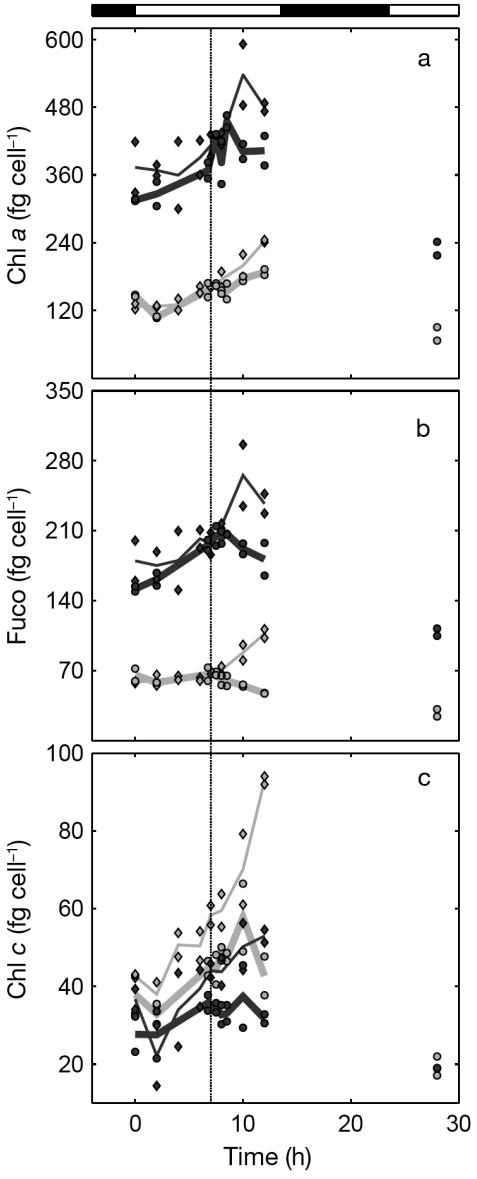

Fig. 4. *Phaeodactylum tricornutum* and *Emiliania huxleyi*. Cellular pigment content of *P. tricornutum* and *E. huxleyi* through time. (a) Chlorophyll *a* (chl *a*) per cell, (b) fucoxanthin (Fuco) per cell and (c) chlorophyll *c* (chl *c*) per cell. Each light intensity profile has 2 independent replicates for each species. Lines correspond to a tendency line based on the average of both data points. Other details as in Figs. 2 & 3

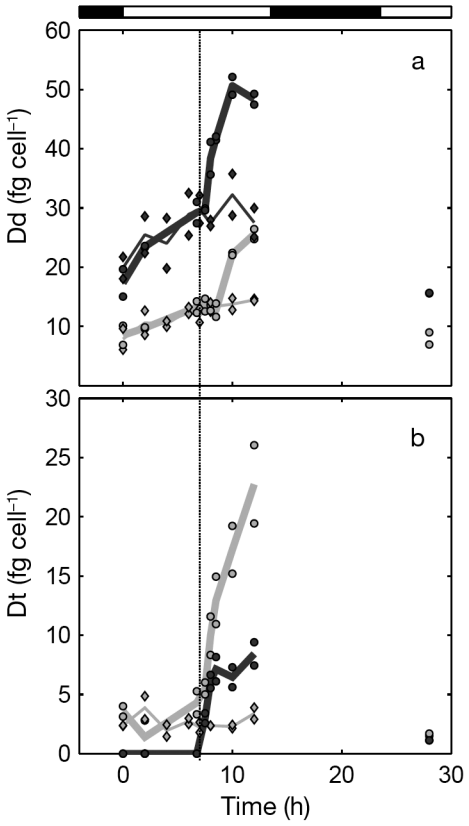

Fig. 5. *Phaeodactylum tricornutum* and *Emiliania huxleyi.* Cellular pigment contents of *P. tricornutum* and *E. huxleyi* through time. (a) Diadinoxanthin (Dd) per cell and (b) diatoxanthin (Dt) per cell. Each light intensity profile has 2 independent replicates for each species. Lines correspond to a tendency line based on an average of both data points. Other details as in Figs. 2 & 3

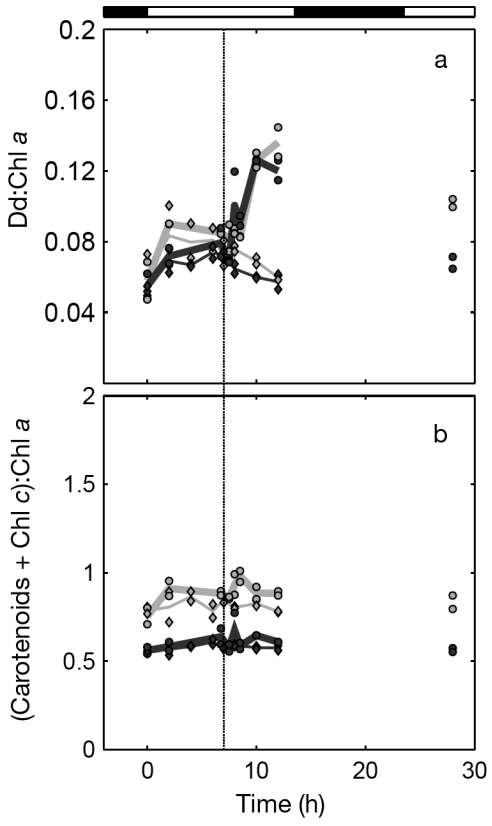

Fig. 6. *Phaeodactylum tricornutum* and *Emiliania huxleyi.* Pigment ratios of *P. tricornutum* and *E. huxleyi* through time. (a) Diadinoxanthin (Dd):chlorophyll *a* (chl *a*) and (b) total carotenoids plus chl *c*:chl *a*. Each light intensity profile has duplicate measurements for each species. Lines correspond to a tendency line based on an average of both data points. Other details as in Figs. 2 & 3

Even though the species considered have different cellular pigment concentrations, the ratio between Dd and chl *a* changed similarly in both of them (Fig. 6a). The sum of all carotenoids (Dd, Dt, fucoxanthin, β-carotene) and chl *c* normalized to chl *a* did not change with the light intensity increase in either species, but had higher values in *Emiliania huxleyi* than *Phaeodactylum tricornutum* (Fig. 6b).

The cell division rate during the light period decreased (Fig. 7) from approximately 0.80 (± 0.13, SE) in the baseline regime to 0.62 (± 0.17) for *Phaeodactylum tricornutum* and from 0.41 (± 0.09) to 0.24 (± 0.06) for *Emiliania huxleyi* from 0 to 12 h after the light was turned on. The reduction in cell division rate was also seen in both species between 0 to 10 and 7 to 12 h. Cell division rate from 0 to 7 h of both species was similar between the 2 light regimes. When the dark phase was included, the cell division rate of *E. huxleyi* stayed lower than under 50 µmol m^{-2} s^{-1} while *P. tricornutum* reached rates comparable to those of the baseline. The increase in carbon fixation together with a decrease in cell division rates led to slightly increased cell diameters in *E. huxleyi* (data not shown).

DISCUSSION

Physiological plasticity of unicellular phytoplankton often allows a strong, quick (minutes to days) and reversible response to light intensity change (Falkowski 1980). This photoacclimation involves several photoprotection mechanisms (Fig. 8) which react in parallel, but not necessarily simultaneously. Here, we followed the acclimation response of *Phaeodactylum tricornutum* and *Emiliania huxleyi*, i.e. their light harvesting and dissipation capacity, rate of electron transfer and, most importantly, effective use of energy absorbed (photochemistry). Carbon fixation measurements provided unexpected data, revealing

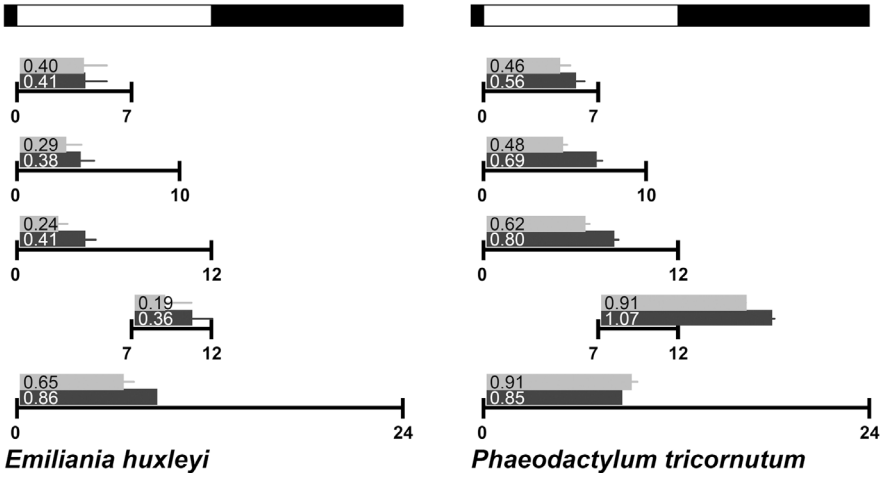

Fig. 7. *Phaeodactylum tricornutum* and *Emiliania huxleyi*. Cell division rates based on cell counts of *P. tricornutum* (right) and *E. huxleyi* (left) in relation to the light intensity regimes. Estimates under the baseline regime (dark grey bars) and under the light change regime (light grey bars) are shown. Cell division rates were calculated as the average of 4 independent replicates for the periods 0 to 7 h, 0 to 10 h, 0 to 12 h, 7 to 12 h, and 0 to 24 h after the light phase started. Horizontal error bars are + SE of the means of 4 independent replicates at the light condition considered and of each species. The white/black bar represents the light/dark diel cycle

an important role of calcification in energy dissipation especially at short time scales. We discuss consequences of these mechanisms for cell division rates and potential implications for species-specific competitive advantages in the following sections.

Photochemistry

After the light intensity increase, carbon fixation increased considerably. In fact, organic carbon fixation rate of *Phaeodactylum tricornutum* increased 9-fold when the light intensity increased 16-fold. The utilization of considerable amounts of the additional energy available in the first 10 min may be partly explained by enhanced Rubisco activation or increase of Rubisco contents, for instance through assembly of its subunits, since the inverse trend (reduction of Rubisco activity) has been observed in *P. tricornutum* under low light intensities (Beardall & Morris 1976).

In *Emiliania huxleyi*, organic carbon fixation rate did not increase as dramatically (5-fold) within the first 10 min, but calcification rate increased almost 11-fold. Therefore, calcification had a significant role in dissipating the excess energy during the first minutes, when the cell was still adjusting to the new conditions. Irrespective of whether this is a primary function of calcification, it has been seen that cell division rates of acclimated cultures of calcifying *E. huxleyi* become higher than those of a non-calcifying strain under increasing irradiance (Leonardos & Harris 2006). Interestingly, this was observed even though both calcifying and non-calcifying strains of *E. huxleyi* have a very similar response to light intensity variation in terms of changes in pigment composition (Leonardos & Harris 2006). The contribution of calcification in dissipating excess energy and therefore decreasing photoinhibition has also been seen indirectly in a study where the non-calcifying haploid phase of *E. huxleyi* already showed signs of photoinhibition above 400 µmol m^{-2} s^{-1}, while the calcifying diploid did not show this until 1000 µmol m^{-2} s^{-1} (Houdan et al. 2005). Thus, calcification might mitigate photoinhibition in organisms that are not able to rapidly increase their photosynthesis in an environment with fluctuating light, potentially by reducing oxidative stress after an abrupt light intensity increase. A previous study with *E. huxleyi* at varying light intensities and calcium (Ca^{2+}) concentrations (Trimborn et al. 2007) corroborates our results in terms of POC production. Cells acclimated to low light intensity (30 µmol m^{-2} s^{-1}) had lower organic carbon production, but cells under 800 µmol m^{-2} s^{-1} could not sustain rates higher than those under 300 µmol m^{-2} s^{-1}, similarly to our results. However, Trimborn et al. (2007) dismissed the possibility that calcification provides a way of energy dissipation, expecting that there should be photoinhibition when calcification is inhibited under very low calcium concentrations. In this context, it is important to note that the absence of coccoliths covering the cells at reduced Ca^{2+} concentrations does not necessarily imply the absence of intracellular calcification. As the growth medium was highly corrosive to $CaCO_3$ at both low Ca^{2+} treatments in Trimborn et al. (2007), coccoliths produced by the cells may have simply disappeared due to dissolution. Hence, it cannot be excluded that calcification played a role in mitigating photoinhibition even at strongly reduced Ca^{2+} concentrations.

Similarly to calcification, silicification in diatoms could potentially reduce the production of reactive O_2 species, since it could function as a concurrent sink for the excess energy produced after an abrupt

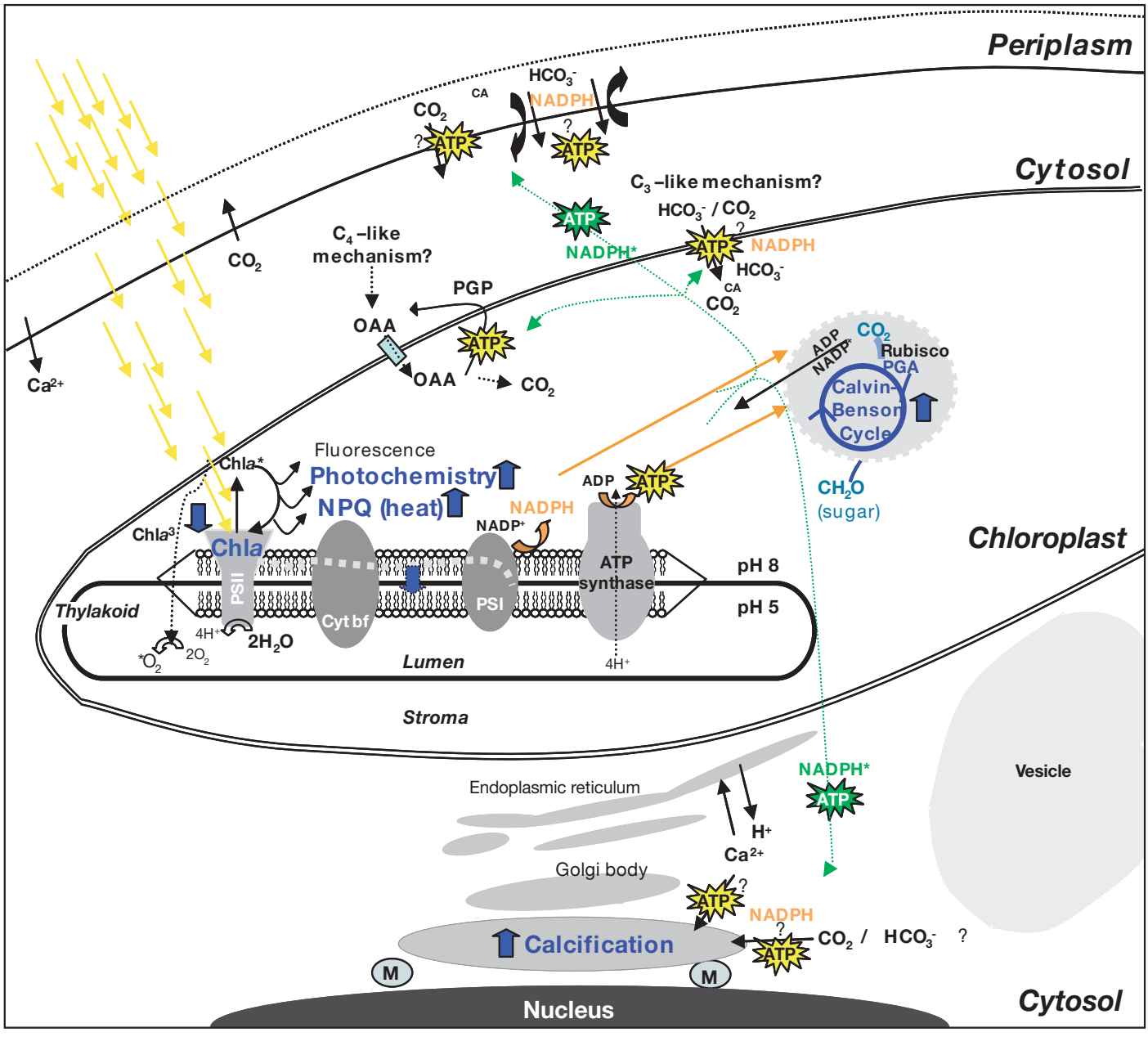

Fig. 8. Model eukaryotic cell based on *Phaeodactylum tricornutum* and *Emiliania huxleyi*. Light (arrows coming from the surrounding environment) is absorbed by chlorophyll *a* (chl *a*), turning it into its excited state (chl *a**). Chlorophyll can then relax by several mechanisms, e.g. fluorescence, photochemistry, heat (non-photochemical quenching, NPQ) and/or producing chl a^3 by intersystem crossing which can then produce $^*O_2$ (reactive oxygen species). See 'Introduction' for more detailed information. Energy dissipation through photochemistry starts with extracting electrons from water in photosystem II (PSII), transferring them to the cytochrome b_6f complex (cyt bf) and then to photosystem I (PSI), where NADP is reduced to NADPH. The dashed grey line represents the electron-transport chain. The energy and reductive power formed can then be used to fix carbon in the pyrenoid (grey circle), which is a globular structure enriched with Rubisco and encountered in the chloroplast (Dodge 1973). The chloroplast has 4 membranes both in diatoms and coccolithophores (represented with only 2 lines). Calcification is represented for *E. huxleyi*. The calcification vesicle is located next to the nucleus. Question marks note uncertainties. Blue text highlights the processes addressed in this study. Blue arrows indicate the effect found after the light intensity increase. Dashed green arrows indicate potential path of ATP and NADPH* (equivalent redactor of NADPH). C_3/C_4 with a question mark indicates the uncertainties associated with carbon fixation in these phytoplankton groups. Carbonic anhydrase (CA) may or may not be present in the periplasm depending on the species and carbon dioxide concentrations of the surrounding environment. ADP: adenosine diphosphate; M: mitochondria. General scheme based on Müller et al. (2001) and Falkowski & Raven (2007)

light intensity increase . However, contrary to calcification which occurs throughout the day, silicification occurs mostly close to the end of the light phase and thereby not during a period of great light variation. Moreover, silicification has been seen to decrease with increased light intensity (e.g. Taylor 1985), implying that it does not play a role in energy dissipation.

The difference between the 2 species in response time until the observed increase in organic carbon fixation and the difference in its magnitude may also be the consequence of a lower carbon use capacity (organic) in *Emiliania huxleyi* compared to *Phaeodactylum tricornutum*, either by lower or less flexible transport capacity into the pyrenoids (lower CCM efficiency in terms of capacity, rate or energetic efficiency), differences in organic carbon fixation (potentially C_4-like as observed in some diatoms in comparison to C_3 in coccolithophores, see Roberts et al. 2007b, Raven 2010) and/or different 'stock' concentration of substrates for the Calvin-Benson Cycle. According to previous investigations, there is evidence for the dependence of carbon fixation on the concentration of substrates available when the light intensity increases (Emerson & Arnold 1932). However, the potential increase in carbon fixation with rising light intensity might also be moderated by CCM efficiency of the species, since light intensity variation may occur in a matter of seconds, increasing the demand for carbon available for carbon fixation eventually beyond the rate of supply. Both species considered in this study have CCMs, increasing their carbon concentration potential. The stronger increase in organic carbon fixation in *P. tricornutum* than in *E. huxleyi* might indicate changes in carbon uptake and/or may be a consequence of the presence of a C_4-like mechanism in *P. tricornutum* as found in *Thalassiosira weissflogii* (Reinfelder et al. 2004). A C_4-like pathway in *P. tricornutum* could quickly supply substrates for carbon fixation after the abrupt light intensity increase due to the availability of stored carbon in the form of a C_4-carbon compound, allowing a faster response than in *E. huxleyi*, which likely has a C_3 mechanism (Tsuji et al. 2009), in analogy to the differences observed between C_4 and C_3 plants. Indeed, C_4 plants can reach higher photosynthetic rates at lower light intensity and higher maximum rates of photosynthesis than C_3 plants. The difference between the 2 species considered here becomes smaller when considering total carbon fixation (*P. tricornutum*: 880%, *E. huxleyi*: 720%) because inorganic carbon fixation of *E. huxleyi* also increased, therefore increasing carbon utilization in both species similarly. Assuming that carbon utilization corresponded to carbon acquisition would imply that *E. huxleyi* channelled less CO_2 towards Rubisco compared to *P. tricornutum*, since the carbon acquired by *E. huxleyi* was used to increase both organic and inorganic carbon fixation. Finally, CCM operation requires a considerable amount of energy as has been seen in the utilization of 43% of the ATP formed by photosynthesis in the CCM activity of *Chlamydomonas reinhardtii* grown under low CO_2 concentrations (Yokota et al. 1987). Therefore, species-specific CCM characteristics might influence the response to abrupt light intensity increase both directly by compensating for the increase in carbon demand and indirectly by spending part of the extra energy in its operation (Fig. 8). This re-distribution of energy or reductive power might, consequently, promote the observed increase in calcification. Still little attention has been given to the relationship between changes in carbon demand, CCM efficiency and competitive fitness (Tchernov et al. 1998, Rost et al. 2006). Future work should further explore the role of calcification as a light dissipating mechanism and its relationship to CCM operation and efficiency.

The abrupt initial increase in organic carbon fixation was not sustained after 30 min in either species, potentially due to (1) the readjustment of pigment concentrations, (2) a deficit of adenosine diphosphate (ADP) or reductive power for carbon acquisition, (3) limiting concentrations of Calvin-Benson Cycle substrates, or (4) a decrease in Rubisco activity. In barley leaves, the regeneration of the substrate ribulose-1,5-bisphosphate limits CO_2 assimilation and not Rubisco activity (Dujardyn & Foyer 1989). Here, a combination of the above mentioned processes is probably responsible for the observed response.

With the abrupt light intensity increase there is a concomitant increase in absorbed energy and electrons to be transported. Their passage through the electron chain depends on the redox state of the proteins involved in the electron transport, especially plastoquinone (Falkowski & Chen 2003). The increase in electron transfer, with no comparable increase in the velocity of the Calvin-Benson Cycle (possibly due to limited substrates, both inorganic carbon and 3-phosphoglyceraldehyde) would deplete $NADP^+$ and ADP (Fig. 8). This would potentially cause a 'clogging' of the electron chain, measurable as a decrease in F_v/F_m. The decrease in F_v/F_m might also be indicative of photodamage of photosystem II (PSII) in both species, since the 20 min dark incubation done previous to the measurements would enable full epoxidation of Dt in cells of *Phaeodacty-*

lum tricornutum (Lavaud et al. 2002) and *Emiliania huxleyi* (tested in this study). *E. huxleyi* had a higher percentage decrease in F_v/F_m than *P. tricornutum* with consequently lower organic carbon fixation (see above in this section). However, *E. huxleyi* seemed to slightly recover F_v/F_m after 3 d under 800 µmol m^{-2} s^{-1}, at the same time that *P. tricornutum* decreased its values. Because F_v/F_m on the days following the light intensity increase is quite variable, no assertive conclusion can be drawn.

Light harvesting

Although both species decreased their light harvesting pigment concentrations under increased irradiance (as e.g. reported by Fisher et al. 1989), absolute values of chl *a* and fucoxanthin quotas in *Emiliania huxleyi* remained lower than those in *Phaeodactylum tricornutum* during the whole day. This difference may well be size related, since even though the species considered in this work have similar maximum diameters (when considering the central area of *P. tricornutum* to be a sphere), *P. tricornutum* has an approximately 8 times higher cell volume (the cell length of *P. tricornutum* is about 16 to 17 µm, Zhuravel et al. 2009, and both the diatom and coccolithophore have a maximum cell diameter of about 4 µm). In diatoms, the chl *a*-specific light absorption coefficient has been seen to decrease with increasing cell size (Geider et al. 1986). Thus, if this can be extrapolated to other phytoplankton groups, the larger *P. tricornutum* with a potentially lower light absorption coefficient may need a higher chl *a* content than *E. huxleyi* to achieve the same light absorption. Additionally, pigment quotas might vary with light intensity, time of day (pigments accumulate towards the end of the light phase when most cell divisions occur) and species-specific characteristics. Here, both species were exposed to the same light intensities, and samples were collected simultaneously during the light phase, therefore excluding the latter possibilities. As for the group-specific characteristics, it has been hypothesized that the coccoliths (in *E. huxleyi*) could increase light scattering (e.g. Gordon et al. 2009), but their organization has also been thought to amplify the light reaching the chloroplasts in species occurring in deep waters (Young 1994) and consequently allow for lower cellular chl *a* contents.

The 2 species showed chl *a* breakdown just after the light intensity increase, but differed in the exact timing. In the case of *Emiliania huxleyi*, the rates of synthesis might also have changed, while in *Phaeodactylum tricornutum* it is hard to determine. Finally, in both species there is a breakdown of fucoxanthin, visible as a decrease in concentration after the sudden light intensity increase.

Photoprotection

Changes in light-harvesting capacity are important in photoprotection, but it is in energy dissipation that cells modify the most. Indeed, both species investigated here and in previous studies (Lavaud et al. 2003) generally had a higher relative increase in Dd and Dt contents after rising irradiance than decrease in fucoxanthin, chl *c* or chl *a.*

It has been hypothesized that diatoms might follow a pathway from violaxanthin via Dd to Dt or fucoxanthin (Lohr & Wilhelm 2001, Bertrand 2010). The increase in Dd found here, even though there is an enzymatically-controlled conversion of Dd to Dt under increasing irradiance (Brunet et al. 2008) could be misleading. The reason for this apparently paradoxical response is that the increase in Dd cellular contents is greater than the amount of Dd being converted into Dt. *Phaeodactylum tricornutum* has been previously reported to increase its Dd content after exposure to several (Lavaud et al. 2002) or a single (as tested here) irradiance increase.

After a sudden light intensity increase, there might be an increased conversion rate of Dd to Dt at the expense of the conversion of Dd to the important constituent of the light-harvesting complex, fucoxanthin. This can at least partially explain the observed trend in fucoxanthin. The replacement of some subunits of the light-harvesting complex rich in fucoxanthin by others rich in Dd in diatoms acclimated to intermittent light instead of continuous light has already been described (Lavaud et al. 2003).

The increase in the cellular concentration of Dd took 3 h in *Emiliania huxleyi* but only 1 h in *Phaeodactylum tricornutum* after the light intensity increase. Dt, on the other hand, increased practically immediately in both species. Pigment regulation occurred from less than minutes to 3 h, which agrees well with the time frame defined by Falkowski & LaRoche (1991) as photoacclimation, but is shorter than proposed by Fisher et al. (1989). The formation of Dt determines the onset of NPQ within the light-harvesting complexes (Olaizola et al. 1994, Kashino et al. 2002, Lavaud et al. 2002), which reduces the photoinhibitional damage to the antenna of PSII after exposure to increasing light intensity as seen in pre-

vious work with *P. tricornutum* (Ting & Owens 1994). Even though cells regulate the xanthophyll cycle on relatively short time scales (Lavaud et al. 2004, Dimier et al. 2007), there has not been as much emphasis on their short-term photoacclimation ability before. Yet, it is on this time scale that differences between species could lead to certain advantages in the natural environment. The slight time lag between the response of *E. huxleyi* and *P. tricornutum* may have major implications in an environment where such light intensity changes are frequent and sudden, as in coastal or estuarine areas.

Pigment ratios

The proportion of Dd to chl *a* increased with rising light intensity in both *Emiliania huxleyi* and *Phaeodactylum tricornutum*. This change in the chl *a*-specific xanthophyll pool affects the potential for dissipating excess light through NPQ (Kashino et al. 2002).

The sum of all carotenoids (β-carotene, fucoxanthin, Dd, Dt) and chl *c* per chl *a* did not change with the increase in light intensity in the 2 species considered here. This is because both chl *c* (dividend) and chl *a* (divisor) showed similar decreasing trends, cancelling each other out in the calculation. Thus, our results agree well with the finding that the sum of all carotenoids synthesized in relation to chl *a* is genetically predefined, while the cellular content of every single carotenoid might change with the light intensity (Leonardos & Harris 2006).

Emiliania huxleyi had slightly higher absolute values in the ratio between total carotenoids plus chl *c* and chl *a* than *Phaeodactylum tricornutum*. Furthermore, the Dd/chl *a* trend observed for *E. huxleyi* was prolonged into the next day and was relatively high in comparison to *P. tricornutum*. This is another indicator for an adaptation to more constant light conditions and/or a higher average light intensity of the coccolithophore *E. huxleyi* than the estuarine *P. tricornutum*.

Cell division rate

Cell division rate decreased hours after the increase in light intensity (Fig. 7). Cells acclimated to high irradiance usually have higher cell division rates than those acclimated to lower light conditions. Here, however, cells were still adjusting to the abrupt changes and not acclimated to the light conditions (compare e.g. Harris et al. 2005). *Phaeodactylum tricornutum* and *Emiliania huxleyi* cells exposed to an abrupt increase in light intensity slowed down cell division rate during the day, possibly resulting from a reallocation of cellular machinery, energy and/or reductive power for photoprotection and carbon fixation. However, *P. tricornutum* cells were able to recover by increasing cell division rates at the end of the day and during the dark phase, therefore showing a gross 24 h increase in cell division from approximately 0.85 under 50 µmol m^{-2} s^{-1} to 0.91 including the period of time after the light intensity increased to 800 µmol m^{-2} s^{-1}, while cell division rate of *E. huxleyi* decreased from approximately 0.86 to 0.65.

The increase in cell division rate of *Phaeodactylum tricornutum* only towards the end of the light phase might be tightly connected to a prolongation of the interphase in the first hours following the abrupt light increase as a result of mobilization of energy/reductive power to increase organic carbon fixation. This probably occurred at the expense of the time available for frustule construction between 2 cell divisions, potentially decreasing its silica content (Taylor 1985). Cell division rate of *Emiliania huxleyi* did not increase as strongly at the end of the light phase since in addition to the concurrent process of carbon fixation (not as high as in *P. tricornutum*), it also increased calcification, decreasing the energy/reductive power available. Moreover, irrespective of the light regime, *E. huxleyi* had similar or potentially decreasing cell division rates throughout the day and continued to divide even in the dark phase, while *P. tricornutum* increased cell division rate during the light phase, reaching a maximum hours before the onset of the dark phase. Thus, along with photoprotective capacity, the timing of cell division and calcification/silicification might be important in determining the capacity to cope with a light-changing environment.

Implications for functional group distribution

Phytoplankton species occurrence and biomass depend on several abiotic conditions such as nutrients, mixing, temperature and light intensity. Light intensity in the well mixed surface layer varies between long periods of low irradiance and abrupt high irradiance, reaching even supersaturation (Falkowski & Wirick 1981). Differences in the capacity to respond to these quick variations influence the distribution of phytoplankton species or functional groups through time and in different environments such as

estuarine, coastal and oceanic areas. Part of photoprotection varies in its extent and regulation according to the phytoplanktonic group (e.g. Wagner et al. 2006). Diatoms generally dominate in more turbulent coastal areas in contrast to coccolithophores which occur predominantly in more stratified water columns. Previous studies also considering the effects of sudden light intensity increase found differences between the photosynthetic apparatus of offshore and estuarine/coastal species, with *Phaeodactylum tricornutum* showing a faster and stronger capacity to dissipate excess energy than the other diatom species tested (Lavaud et al. 2007). A possible explanation is that oceanic phytoplankton, such as *Thalassiosira oceanica*, developed lower iron requirements because they evolved in oceanic iron-poor areas (Sunda et al. 1991, Sunda & Huntsman 1995). This might be related to reduced concentrations of PSI and the cytochrome b_6f complex (Strzepek & Harrison 2004), potentially affecting the pH variation in the thylakoid membrane since the cytochrome b_6f complex is involved in proton translocation, and therefore to a less efficient short-term energy dissipation through heat (Munekage et al. 2001). While this short-term capacity effectively dissipates the excess light, it is not very often needed in an environment where irradiance changes occur within hours to days (MacIntyre et al. 2000). In contrast, in a turbulent estuarine or coastal environment, phytoplankton cells need to avoid potential damage caused by irradiance variation in a matter of minutes. In fact, estuarine species are able to maintain their cell division rate under fluctuating light regimes (Lavaud et al. 2007). This was also observed here in *P. tricornutum* when the full light/dark cycle was considered, in contrast to *Emiliania huxleyi* which decreased its cell division rate (see section 'Cell division rate' above).

The tempo of activation of changes in a species' phenotypic plasticity to light variation provides acclimation advantages in the environment. *Phaeodactylum tricornutum* showed properties important in a light-fluctuating regime (estuarine/coastal); for instance, it dramatically increased carbon fixation rate in a short period of time and more gradually decreased its F_v/F_m than *Emiliania huxleyi* did. This response, however, could not be sustained. In fact, when integrated over a longer time period, the differences between the 2 species were not as pronounced, indicating that the advantageous position of *P. tricornutum* holds only on short time scales. The difference between the species considered here cannot be fully extrapolated to their functional groups, since these species are rather unusual for their groups; however, it may help in understanding that there are important group-specific characteristics which affect the cellular response to changing light intensity, such as calcification in coccolithophores.

In the future, carbon dioxide availability is expected to increase due to the increase in atmospheric and surface water CO_2 (Solomon et al. 2007). Light is also thought to change in the surface ocean due to the increase in global average temperature and consequent stronger stratification. This is thought to decrease the mixed layer depth and, therefore, trap phytoplankton closer to the surface where they will be exposed to an average higher photosynthetically active radiation as well as to more frequent high light events, potentially changing the cells' demand for CO_2. Phytoplankton groups (such as diatoms and coccolithophores) have different phenotypical plasticities (e.g. CCM) to react to abrupt environmental changes such as light. Therefore, how and how fast species respond may influence their fitness in competing for different ecological niches, with potential implications for future phytoplankton community composition.

The fate of excess energy after a sudden light intensity increase in *Emiliania huxleyi* and *Phaeodactylum tricornutum*

The potential energy/reductive power and carbon flow created by an abrupt light intensity increase in *Phaeodactylum tricornutum* and *Emiliania huxleyi* is depicted in Fig. 8. The sudden light intensity increase increased excitation of chl *a* in both species. The excited chl *a* (chl a^*) would have then relaxed by several mechanisms (see Fig. 8), such as fuelling photochemistry. This would have transferred electrons of water from PSII to PSI, reducing NADP in the process. Both NADPH and ATP formed in the electron chain can be used to fix carbon in the Calvin-Benson Cycle. However, shortly after the sudden light intensity increase, the Calvin-Benson cycle might have become limited by its substrates (phosphoglyceraldehyde, PGA; and CO_2). As a consequence, energy created in the photosynthetic electron chain might have exceeded the demand from the Calvin-Benson cycle, becoming potentially available for other mechanisms, such as carbon transport in *P. tricornutum* and *E. huxleyi*, C_4-like carbon fixation in *P. tricornutum* and calcification, namely carbon and calcium (Ca^{2+}) transport into the calcification vesicle, in *E. huxleyi* (dashed green arrows in Fig. 8).

The excess electrons could also have been partially fuelled to other sinks like the Mehler reaction, cyclic electron transport around PSI, photorespiration or even chlororespiration (see e.g. Feikema et al. 2006, Goss & Jakob 2010, Cruz et al. 2011 for further information).

Inorganic carbon acquisition characteristics of *Phaeodactylum tricornutum* and *Emiliania huxleyi* such as DIC uptake speciation, transporters or transport complexes and energy requirements are uncertain in various aspects (see e.g. Roberts et al. 2007a, Mackinder et al. 2011). Both species are known to utilize CO_2 and HCO_3^- (Matsuda et al. 2001, Schulz et al. 2007, respectively). CO_2 transport into the cells was hypothesized to take place through aquaporins (e.g. Mackinder et al. 2011), but considering that the pH in the cytosol is lower than in the surrounding seawater, CO_2 would be expected to efflux by diffusion rather than enter the cell. Other phytoplankton such as cyanobacteria (Price et al. 2008) and *Chlamydomonas* (Spalding 2008) are known to use reductive power or ATP to transport CO_2 into the chloroplast. The HCO_3^- transport in *E. huxleyi* seems to be associated with a co-transport of sodium (Na^+) or an antiport of chloride (Cl^-) (Mackinder et al. 2011), potentially at the expense of NADPH or ATP in analogy to some transporters found in cyanobacteria (Price et al. 2008).

In coccolithophores, additional energy or reductive power is necessary for carbon and Ca^{2+} transport into the calcification vesicle, but the exact requirements are unknown. In *Emiliania huxleyi*, Ca^{2+} is thought to cross the plasma membrane through channels following its electrochemical gradient, then being transported into the endoplasmic reticulum and calcification vesicle potentially by ATP-dependent pumps or ion exchangers (possibly with H^+) and Ca^{2+}-ATPase, respectively (Mackinder et al. 2011). The rapid increase in inorganic carbon fixation already after 10 min could be linked to an increased calcification rate together with faster exudation of coccoliths (*E. huxleyi* produces about 1 coccolith h^{-1}, Paasche 2001) similarly to *Coccolithus pelagicus* (Taylor et al. 2007).

Finally, carbon transported into the proximities of Rubisco in the chloroplast (C_4 or C_3, since some diatoms were found to operate C_4-like photosynthesis, Roberts et al. 2007b; and *Emiliania huxleyi* might execute C_3-like photosynthesis, Tsuji et al. 2009) had energetic costs, which potentially benefited from the extra energy produced after the abrupt increase in light intensity. Our results led us to speculate that the fast increase in organic carbon fixation rates after the sudden light intensity increase produced excess energy which might have been mostly utilized in carbon transport to the proximities of Rubisco and into the calcification vesicle in the case of in *E. huxleyi*. Thus, carbon and energy or reductive power might be shared between carbon transport into the cytoplasm, further into the chloroplast and, in the coccolithophore, into the calcification vesicle.

Acknowledgements. We thank M. Meyerhöfer for the DIC measurements and J. Franz for laboratory assistance. We also thank 3 anonymous reviewers for their useful suggestions on the previous version of this manuscript. This research was supported by the European Union Framework Program 6 project DIATOMICS (grant no. LSHG–CT–2004–512035).

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Editorial responsibility: Graham Savidge, Portaferry, UK

Submitted: November 2, 2011; Accepted: January 14, 2012
Proofs received from author(s): April 10, 2012