

Photoprotection capacity differs among diatoms: Possible consequences on the spatial distribution of diatoms related to fluctuations in the underwater light climate

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Abstract

In this study, we show a fundamental difference between diatom species from different marine habitats in their ability to cope with changes in irradiance. Estuarine species show a higher and more flexible capacity for photoprotection than oceanic and coastal species, and when exposed to excess light, the impairment of their photosynthetic capacity because of photoinhibition was reduced. This resulted in maintenance of growth in a fluctuating light regime, conferring the estuarine species an adaptive advantage. The ability of diatoms, and to a larger extent other phytoplankton, to occupy a wide range of ecological niches depends critically on their capacity to exploit the differences in underwater light climate. These results might explain how diatoms adapt to the challenge of maintaining optimal photosynthetic production in turbulent waters, in which the rate of light change is high.

Phytoplankton are passively transported within the water column by turbulent mixing and must cope with highly variable light. Unlike nutrient availability and temperature, fluctuations in light intensity can show very high frequency (few seconds) coupled with high amplitude (from darkness to full sunlight) (Lewis et al. 1984; MacIntyre et al. 2000). Turbulence and light fluctuations have been identified as key parameters in the structure of phytoplankton communities and have been integrated in recent ecological modeling for species dynamics and bloom development (Huisman et al. 1999; Lichtman and Klausmeier 2001; Huisman et al. 2004).

Fluctuation in the underwater light climate strongly influences the photosynthetic productivity of phytoplankton (Lewis et al. 1984; Cullen and Lewis 1988). Fluctuating light in combination with excessive light is potentially harmful for photosynthesis because it can cause a decrease in productivity and fitness (Long et al. 1994; Külheim et al. 2002). Photosynthetic organisms attenuate these undesirable effects by safely dissipating excess energy (Niyogi

2000). The extent and regulation of this photoprotection vary among phytoplankton groups (Wagner et al. 2006 and citations within), diatoms being generally considered to have a better ability to cope with fluctuating light (Mitrovic et al. 2003; Wagner et al. 2006).

Diatoms contribute highly to Earth's primary production (25%, Sarthou et al. 2005). Their dominance in turbulent waters, in which vertical mixing exposes them to large fluctuations in irradiance (Huisman et al. 2004; Tozzi et al. 2004), suggests an unusual photosynthetic flexibility. Here we report a habitat-related difference among diatom species in their ability to cope with light. Estuarine species show a higher and more flexible capacity for photoprotection than oceanic and coastal species, conferring an adaptive advantage in a turbulent environment. These results provide an ecophysiological explanation for the spatial distribution of planktonic diatoms as a function of the underwater light climate.

Materials and methods

Culturing—The diatom strains used were *Phaeodactylum tricorutum* (clone CCAP1052, isolated near Plymouth, England), *Skeletonema costatum* (clone 'Banyuls'—Laboratoire Arago Collection, Banyuls-sur-Mer, Mediterranean Sea, France), *Thalassiosira weissflogii* (CCMP1336, estuary Long Island, New York, USA), *Thalassiosira pseudonana* (CCMP1335, Moriches Bay, Long Island), and *Thalassiosira oceanica* (CCMP1003, Sargasso Sea). *P. tricorutum* and *S. costatum* were grown photoautotrophically at 18°C

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in continuously flushed airlifts of 300-mL sterile natural seawater F/2 medium. The *Thalassiosira* species were grown in Aquil medium. The light conditions were 45 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ on a 16:8 h light:dark (LD) cycle. For the growth rate experiments on a 1:1 h LD 24-h cycle (45 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), the cells were maintained in this regime for at least 2 weeks and then transferred to 450 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (same LD cycle). All measurements were made during the exponential growth phase during the first week of growth to catch the immediate response of the cells to this switch in light regime. Specific growth rates, μ (d^{-1}), were calculated from regression of the natural logarithm of culture chlorophyll *a* (Chl *a*) concentration or fluorescence during the exponential growth phase of acclimated cultures.

Photosynthetic architecture and pigment analyses—Steady state O_2 yield per flash (Y_{SS}) and the concentration of P_{700} per Chl *a* were used as proxies for photosystems II and I (PSII and PSI), respectively, and measured as described before (Lavaud et al. 2002a). P_{700} was determined with an Aminco DW-2 spectrophotometer (American Instrument). The O_2 yield per flash was measured with a homemade flash O_2 electrode (Lavaud et al. 2002b). PSII antenna size ($1/I_{1/2}$ of Y_{SS} , the reciprocal of the half-saturating flash intensity of flash O_2 evolution saturation curves) was measured the same way while applying a set of neutral filters to vary the intensity of the flashes (Lavaud et al. 2002a). Xanthophyll analyses were performed by high-performance liquid chromatography as described (Arsalane et al. 1994). The xanthophyll de-epoxidation state (%) was calculated as $(\text{DT}/\text{DD} + \text{DT})100$, where DD is diadinoxanthin, the epoxidized form and DT is diatoxanthin, the de-epoxidized form.

Photosynthetic measurements—Flash O_2 measurements (Y_{SS} , number of active PSII), photosynthesis versus irradiance (P/E) curves, and fluorescence yield measurements were performed on dark-acclimated samples (15 min). Cells were brought to a final concentration of 10 $\mu\text{g Chl a mL}^{-1}$ following gentle centrifugation (5 min, 5,000 rpm). Cells were resuspended in F/2 medium and allowed to recover for 1 h in ambient light. P/E curves were determined at 18°C with a calibrated Clark O_2 electrode (model DW1, Hansatech) by measuring O_2 evolution normalized to Chl *a* as a function of light intensity. White light of adjustable intensity was provided by a KL-1500 quartz iodine lamp (Schott). A new sample was used for each irradiance. P_{max} (maximum photosynthetic rate) and E_k (light intensity for saturation of photosynthesis) were derived from P/E curves. Chl *a* fluorescence yield measurements were performed with a PAM-101/103 fluorometer (for *P. tricornutum* and *S. costatum*) and a PhytoPAM with an emitter-detector cuvette assembly (ED101 [for the *Thalassiosira* species], Heinz Walz). The maximum photosynthetic efficiency of PSII (F_v/F_m) was determined by inducing a transient closure of the PSII reaction centers with a saturating light pulse (600 ms, 4,000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). The capacity for energy dissipation was calculated as $\text{NPQ} = F_m/F'_m - 1$. For a comparison with the

potential for photochemistry $\text{qP} = (F_m - F_s)/(F'_m - F'_0)$ it was calculated as $\text{qN} = (F_m - F'_m)/(F_m - F'_0)$ (see Ruban et al. 2004). Photoinhibition kinetics were performed by exposing the cells to different irradiances (5 min illumination duration, a new sample was used for each irradiance step) or illumination times (at 2,000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) directly in the DW1- O_2 electrode chamber and measuring the decrease in the number of active PSII reaction centers (Y_{SS}) by regular sampling. With application of the same protocol, the extent of the PSII electron cycle was determined by measuring the O_2 deficit at the beginning of an O_2 flash sequence (see Lavaud et al. 2002b). The O_2 deficit per PSII reaction center in a 20-flash series was quantified by comparison of the O_2 deficit in illuminated (subscript L) and nonilluminated (subscript D) samples: $\text{O}_2 \text{ deficit} = (\{[20 \times Y_{\text{SSL}}] - [\Sigma(Y_{1...20})_L]\} - \{[20 \times Y_{\text{SSD}}] - [\Sigma(Y_{1...20})_D]\})/Y_{\text{SSD}}$.

Results and discussion

The key to our findings came from the measurement of nonphotochemical Chl *a* fluorescence quenching (NPQ), a parameter to evaluate photoprotection. The NPQ process allows the PSII light-harvesting complex (LHC) antenna to safely dissipate light excitation energy absorbed in excess of photosynthetic capacity during a sudden increase in irradiance (Holt et al. 2004). NPQ is one of the most important mechanisms for rapid (seconds to minutes) regulation of photochemistry. Consequently, it is one of the first lines of defense that diatoms (Arsalane et al. 1994; Lavaud et al. 2004) use to attenuate photoinhibitory oxidative damage generated by light stress (Niyogi 2000). Five plankton diatom strains were chosen as representative of marine habitats (estuary, bay, coast, and open ocean; see *Materials and methods* for details) characterized by different rates of water mixing and hence underwater light climates. A light gradient exists in the marine environment that ranges from dynamic and turbid estuarine waters, characterized by light fluctuations with high amplitude and frequency (partly because of an active tidal hydrodynamism), to calm and clear oceanic waters characterized by a more stable average irradiance (illustrated by a seasonal stratification); coastal/bay waters being intermediary (MacIntyre et al. 2000). To test whether the photoprotective capacity of the diatoms reflected their original habitat, NPQ was measured in laboratory isolates exposed to irradiances ranging from darkness to full sunlight (Fig. 1). The estuarine species showed a higher NPQ (up to three- to fivefold) than coastal and oceanic species. Within the same genus (*Thalassiosira*), photoprotective potential clearly reflected the geographical origin of the isolates: NPQ was highest in the estuarine species and lowest in the oceanic species, whereas *T. pseudonana*, originating from a bay, showed intermediary NPQ induction. Measurements of NPQ in the field suggest that this process is essential for maintaining the photosynthetic activity of diatoms in a variable light climate (Kashino et al. 2002; Fujiki et al. 2003) and that it might explain the spatial and temporal distribution of diatom species (Meyer et al. 2000; Serodio et al. 2005). We therefore examined why an estuarine species

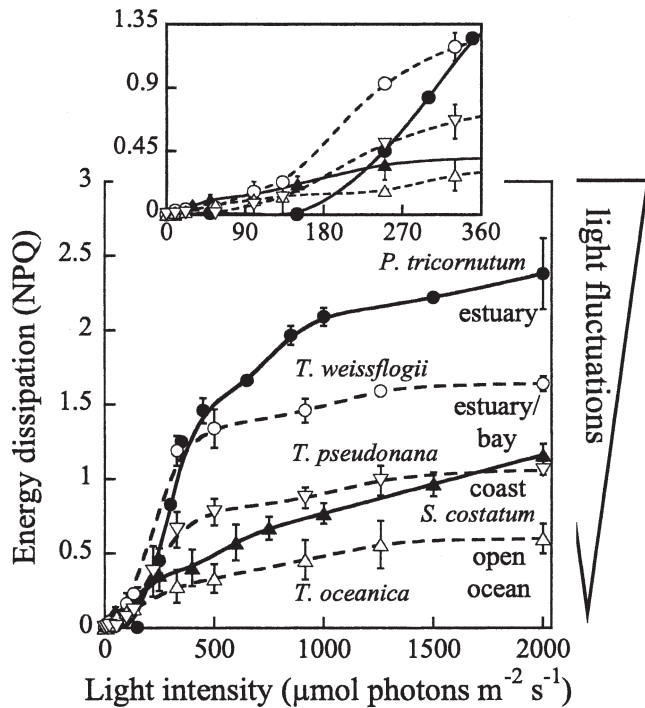


Fig. 1. Capacity for the dissipation of the light excitation energy (NPQ) versus irradiance in diatoms isolated from different ecosystems. Full sunlight in nature corresponds to $2,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Long et al. 1994). Data (\pm SD) are the average of three to four independent measurements.

is better suited to fluctuating light and what consequences this ability might have on the maintenance of photosynthetic activity and growth.

Phaeodactylum tricornutum and *S. costatum* were chosen as model species adapted to high and low rates of light fluctuation, respectively. *P. tricornutum* is primarily both tychopelagic and estuarine, whereas *S. costatum* is often pelagic and coastal. The rate of light change is higher in estuary than in coastal waters because of the higher vertical diffusivity and the higher light attenuation (MacIntyre et

al. 2000). This choice was justified because the species had comparable photosynthetic capacity (Table 1) in our growth conditions. This similarity allowed us to rule out an adaptive response of the architecture of the photosynthetic apparatus to iron availability, as has been observed in oceanic species (Strzepek and Harrison 2004). Even though we did not perform in-depth analysis for the three *Thalassiosira* species, we observed that in the light conditions we used to grow the cells ($45 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, 16:8 h LD), the three *Thalassiosira* species showed a similar F_v/F_m , rate for linear electron transport ($= 11 \pm 3 \mu\text{mol m}^{-2} \text{s}^{-1}$ for all the species) and growth rate as *P. tricornutum* and *S. costatum*. Additionally, a compilation of several growth and photosynthetic parameters from four of the species used here (*P. tricornutum*, *S. costatum*, *T. weissflogii*, and *T. pseudonana*) showed that there is no clear evidence for systematic differences, especially as a result of size/shape and pigment absorption, when they were grown under similar light and nutrient conditions (MacIntyre et al. 2002). Hence, the similar global photosynthetic capacity for *P. tricornutum* and *S. costatum* on one side and some also similar essential photosynthetic features for the three *Thalassiosira* species on the other side are of first importance for the comparison of NPQ capacity (Fig. 1) and for deeper characterization of photoprotection ability under different light conditions. It is at least reasonable to argue that the physiological state versus growth irradiance was similar for all the species under the conditions used here and that this allows a reliable comparison of their ability to cope with changes in light intensity. The differences in the response of the species are then likely to be more adaptive (i.e., genetic) than acclimative (i.e., phenotypic), even if the acclimative aspect cannot be totally ruled out.

The reaction of *P. tricornutum* and *S. costatum* to a gradual increase in incident light was dramatically different. NPQ was induced in *S. costatum* at low light intensities (typically just over the growth intensity), whereas in *P. tricornutum*, NPQ was induced only when light intensities were five- to about threefold higher than the growth light intensity (inset Fig. 1). The most striking

Table 1. Photosynthetic properties of the two model diatom species (*Phaeodactylum tricornutum* and *Skeletonema costatum*) adapted to high and low rates of light fluctuation, respectively.

Measure	<i>P. tricornutum</i>	<i>S. costatum</i>
PSII : PSI	1.7 : 1	2.1 : 1
$1/I_{1/2}$ of Y_{SS}	0.32	0.33
F_v/F_m	0.74 ± 0.02	0.73 ± 0.04
P_{max} ($\mu\text{mol O}_2 [\text{mg Chl } a]^{-1} \text{ h}^{-1}$)	122.4 ± 13.0	155.6 ± 21.1
E_k	132 ± 10	125 ± 10
XDep (%)	2.4 ± 2.4	20 ± 3.0
	$2,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$	40.2 ± 2.8
(d^{-1}), 1 : 1 h LD	$45 \mu\text{mol photons m}^{-2} \text{s}^{-1}$	$+1.03 \pm 0.08$
	$450 \mu\text{mol photons m}^{-2} \text{s}^{-1}$	$+0.48 \pm 0.04$
		-0.06 ± 0.02

Data (\pm SD) are the average of three to four independent measurements. All measurements were performed on cultures growing at $45 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, 16:8 h LD except for μ . $1/I_{1/2}$ of Y_{SS} is a measurement of the PSII light-harvesting antenna size (the pigment, especially xanthophyll, content of the species is very similar to that in Lavaud et al. 2004). F_v/F_m is the maximum photosynthetic efficiency of PSII, P_{max} is the maximum photosynthetic rate, E_k is the light intensity for saturation of photosynthesis, XDep is the level of xanthophyll de-epoxidation, and μ is the growth rate; $2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ is equivalent to full sunlight (Long et al. 1994).

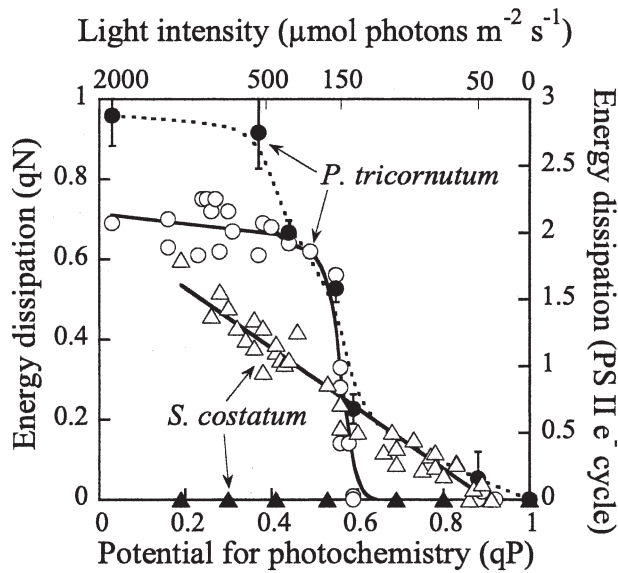


Fig. 2. Relationship between the potential for photochemistry (0-min < qP < 1-max) and the two rapid photoprotective processes for the dissipation of the excitation energy: qN (similar to NPQ; open symbols) and the PSII electron cycle (closed symbols) in *P. tricornutum* and *S. costatum*. Data were obtained by varying the light intensity at 5-min illumination intervals. Data (\pm SD) are the average of three to four independent measurements.

difference between the species was the maximal extent of NPQ, which was 2.7 times higher in *P. tricornutum* (Fig. 1). Both the delay in induction and the higher extent of NPQ in *P. tricornutum* are characteristic of high-light acclimated diatoms (J. Lavaud unpubl. data). NPQ is dependent on the xanthophyll cycle (Holt et al. 2004), an enzymatic interconversion of epoxidized to de-epoxidized xanthophylls regulated in response to changes in irradiance (Lavaud et al. 2004). However, our results suggest that the difference in NPQ was not due to a difference in the xanthophyll cycle operation because de-epoxidation levels were even higher in *S. costatum* (Table 1). The lower NPQ in *S. costatum* could be because of a different LHC antenna polypeptide composition and organization, because of fewer xanthophylls involved in the NPQ process, or because of both conditions (Lavaud et al. 2004; Ruban et al. 2004).

Compared with *S. costatum*, *P. tricornutum* was able to fine-tune the dissipation of light excitation energy (qN , similar to NPQ) relative to light-dependent photochemical activity (qP). The relationship between the two parameters was sigmoidal in *P. tricornutum*, with a rapid phase of qN development/dissipation within a very narrow range of qP (Fig. 2, open circles) that could be the result of fast activation of the xanthophyll cycle enzymes (Jakob and Wilhelm 2001; Goss et al. 2006). In *S. costatum*, the relationship between qN and qP was linear (Fig. 2, open triangles) and lacked the rapid change around E_k observed in *P. tricornutum*. This rapid change in the distribution of photosynthetic (qP) versus photoprotective (qN) allocation is of importance for an organism that is rapidly mixed through a light gradient as in estuarine ecosystems. *P.*

tricornutum outperformed *S. costatum* not only in having a higher ability for NPQ in high light but also by being able to keep NPQ low up to a high qP value of 0.6, meaning that 40% of the PSII reaction centers are in a reduced state (also highlighted in Ruban et al. 2004). Indeed, if NPQ induced near the surface is not dissipated rapidly and nearly completely when the cells are mixed back to the bottom of the water column (i.e., to low irradiances), their light-harvesting ability, and consequently their quantum yield for photosynthesis, could be reduced. Hence, in *P. tricornutum*, the sigmoidal relationship between photosynthetic versus photoprotective energy allocation is consistent with adaptation to low mean irradiance punctuated by episodic exposure to very high irradiance that characterizes the thychopelagic/estuarine niche. On the other hand, the linear relationship observed in *S. costatum* is more consistent to adaptation to a more stable average irradiance characterized by slower light fluctuations, as is encountered in pelagic/coastal ecosystems. It is noteworthy that the relationship qN/qP is also linear in *Arabidopsis thaliana* (Ruban et al. 2004).

The divergence in photoprotective versus photochemical regulation observed in *P. tricornutum* was dependent on other rapid processes. Specifically, energy dissipation via the PSII electron cycle is an important alternative photoprotective mechanism in diatoms (Lavaud et al. 2002b). Measurements of the PSII electron cycle provided additional evidence of the ability of the estuarine species to rapidly and flexibly cope with changes in light excitation energy. PSII electron cycling varied in the same sigmoidal way with photochemistry as NPQ in *P. tricornutum* but was not detectable in *S. costatum* (Fig. 2, closed circles and triangles, respectively). These results illustrate how rapid regulatory processes work cooperatively as an adaptive response to changing light intensity. At irradiances up to threefold the growth irradiance, *P. tricornutum* only dissipates excess excitation energy via the PSII electron cycle. This feature probably explains the ability of *P. tricornutum* to keep NPQ low even if a high proportion of PSII are in a reduced state (Ruban et al. 2004). At higher irradiances, NPQ operates in concert with the PSII electron cycling. In *S. costatum* such a relationship between these mechanisms appears not to exist.

An enhanced ability to cope with excess light exposure protects the photosynthetic apparatus from photoinhibitory damage (Neale 1987; Demmig-Adams and Adams 1992; Long et al. 1994), as shown previously, specifically in diatoms (Arsalane et al. 1994; Lavaud et al. 2002a, 2004). The extent of photoinhibition was measured during short light exposures (5 min) of increasing irradiance (Fig. 3A). In both species, photoinhibition began at $E_k \times 3-4$ (see Table 1). Above 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, a difference between the two species was apparent: photoinhibition reached a maximum (of about 20%) for *P. tricornutum* (closed circles), whereas in *S. costatum* (open triangles), it increased gradually to nearly 40% at a light intensity comparable to full sunlight. After 15 min at this light intensity, *S. costatum* was more photoinhibited than *P. tricornutum* (Fig. 3B), but the difference started to decrease after 45 min of light (only 10%). Most of the photoinhi-

bitory damage took place during the first 5–10 min of light stress, and its extent was higher in *S. costatum* (by about 20%, Lavaud et al. 2004), thus highlighting the importance of rapid regulatory mechanisms in attenuating the deleterious effects of light stress on photosynthetic activity. Interestingly, the first-order reaction time constants of energy dissipation were shorter than the estimated time (up to a few seconds) needed for the irradiance to double in estuarine waters (MacIntyre et al. 2000), suggesting that the rapidity and flexibility of these mechanisms is well suited for regulating the photosynthetic activity of diatoms living in a turbulent aquatic environment. To distinguish between the energy dissipated via NPQ and the PSII electron cycle, photoinhibition was measured in cells of *P. tricornutum* exhibiting a higher capacity for NPQ (up to 9–10 obtained by specific culture conditions) but a comparable ability for the PSII electron cycle (Lavaud et al. 2002a,b). These cells were less photoinhibited during both a short light exposure (Fig. 3A) and under prolonged light stress (Fig. 3B) than cells with lower NPQ. For a short light exposure, light intensity $\geq 1,500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ were needed to observe photoinhibition. When the light stress was prolonged (45 min) under a maximal irradiance (Fig. 3B), the difference between low and high NPQ cells started to be significant (20% instead of <10%). These results provide evidence that dissipatory pathways protect the photosynthetic apparatus, not only during short variations in light intensity (a few minutes) but also during a more prolonged exposure to excess light (about 1 h), and that NPQ plays an important role in the global photoprotection in these light conditions.

To probe the response of cells to both a change in the light regime and intensity over longer time scales (generations), we grew *P. tricornutum* and *S. costatum* under

a frequently changing light regime (1 : 1 h LD) designed to mimic the light environment of cells spending 1 h at the surface of the water column followed by 1 h below the euphotic zone and so on. When both species were grown at low irradiance, they had similar F_v/F_m (0.70 ± 0.02) and growth rates (Table 1). At a 10-fold higher growth irradiance ($450 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, just before the start of photoinhibition in both species in the light conditions of Fig. 3A), their growth rates drastically decreased (Table 1), but whereas *P. tricornutum* was able to maintain growth as well as F_v/F_m (0.70 ± 0.01), *S. costatum* failed to grow and its photosynthetic potential decreased ($F_v/F_m = 0.56 \pm 0.01$). Similar results were obtained with the *Thalassiosira* species (decrease of 20–25% in the growth rate and F_v/F_m in the high light 1 : 1 h LD regime for *T. oceanica* compared with *T. weissflogii*). Even though the light regime used was unlike the underwater light climates typically reported for estuarine ecosystems (MacIntyre et al. 2000), the difference between species in their photoadaptive response was striking and corroborated the measurements performed under shorter light exposures, particularly in light of the fact that, at the high irradiance used for this experiment, energy dissipation was higher in *P. tricornutum* than in *S. costatum* (see Fig. 2). It is then likely that the higher photosynthetic flexibility and photoprotection ability confer *P. tricornutum* with an advantage over generations when grown under a frequently changing light regime with periodic exposure to high light.

Our results suggest that the geographical and spatial distribution of diatoms might be influenced by their ability to cope with rapid fluctuations in light intensity that are dictated by the rate of mixing and light attenuation of waters. It has been previously shown that the availability of iron drives differential adaptive responses and ensures the

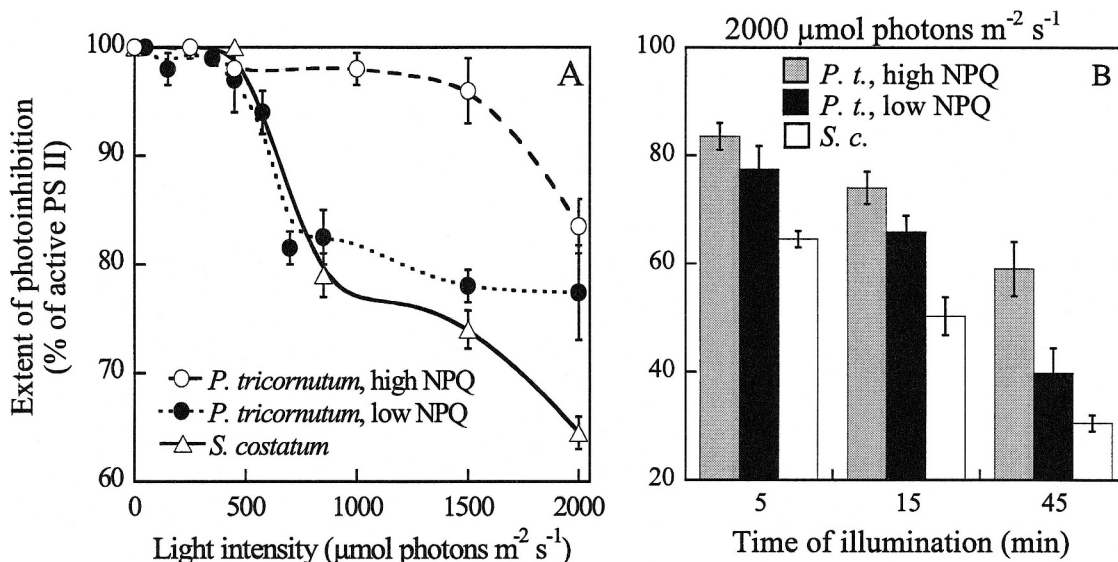


Fig. 3. (A) Extent of photoinhibition (estimated by the loss of PSII active centers as a percentage of initial PSII concentration per Chl *a*) in *P. tricornutum* and *S. costatum*. For *P. tricornutum*, “high-NPQ cells” (Lavaud et al. 2002a; Ruban et al. 2004; see the text for details) are shown. Data were obtained by varying the light intensity at 5-min illumination intervals. (B) Percentage of active PSII versus the illumination time ($2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) in *P. tricornutum* (low- and high-NPQ cells) and *S. costatum*. Data (\pm SD) are the average of three to four independent measurements.

distribution of diatoms among coastal/estuarine and oceanic ecosystems (Strzepek and Harrison 2004). It is also well known how iron as well as other nutrients (silica, nitrogen, phosphate) strongly influence the ecophysiology of diatoms, especially their photosynthetic physiology (Geider et al. 1993; Sunda and Huntsman 1997; Claquin et al. 2002; Staehr et al. 2002; Sarthou et al. 2005). All of our experiments were conducted with nutrient-replete media. Still, it is possible that for the 1:1 h LD experiment, the switch in the light regime could have changed the enzymatic uptake of nutrients that could feed back on photosynthetic capacity and ultimately growth. Nevertheless, it is rather difficult to evaluate the real effect of such a process on our measurements because most of the previous studies on the diatom species used here were conducted on nutrient-limited cultures (Kolber et al. 1988; Geider et al. 1993; McKay et al. 1997; Strzepek and Harrison 2004). In this report, we further demonstrate that fluctuations in the light underwater climate could also explain the differential spatial distribution of diatom species. It confirms and provides an explanation for the previous results on the ecological niche occupancy as a function of light regime between species (Sakshaug et al. 1987) and between ecotypes (Gallagher et al. 1984) and can be extended to other important phytoplankton groups such as the cyanobacteria and prochlorophytes that also use NPQ to acclimate to the underwater light climate (Bailey et al. 2005).

Accordingly, our estuarine model species shows a higher and more rapid capacity for safely dissipating energy when irradiance is suddenly increased, thus ensuring a higher flexibility in photosynthetic regulation, and ultimately the maintenance of photosynthesis. Even though the fluctuating light regime we used (1:1 h LD, low/high light) to probe the response of the species over generations is not very realistic, it allowed us to conclude that the estuarine species was better able to maintain its growth in these light conditions, and we suggest that this was because of its higher photosynthetic flexibility and photoprotection ability. This attribute could well explain both the ecological success of diatoms in turbulent fluctuating light environment, in which they often outcompete other phytoplankton groups (Mitrovic et al. 2003; Huisman et al. 2004), and patterns of species succession related to turbulence (Lichtman 1998; Huisman et al. 2004). Together with other recent studies (Fietz and Nicklish 2002; Wagner et al. 2006), our work also provides an ecophysiological basis for the improvement of predictive models for light competition between phytoplankton species in aquatic ecosystems (Huisman et al. 1999; Lichtman and Klausmeier 2001). Our results, as well as previous ones (Strzepek and Harrison 2004), thus provide an explanation at a physiological level for the early "Margalef's theory" (Margalef 1978) proposing that the competition amongst phytoplankton species could be related to the upper ocean turbulence and the supply of nutrients. Additionally, protection from specific grazers can also be important in determining the spatial as well as temporal distribution of diatom species (see Smetacek 1999). It has been recently debated (Falkowski et al. 2004; Tozzi et al. 2004) how modifications in

the upper ocean turbulence, through its effect on the supply of nutrients, has shaped the functional and evolutionary ecology of diatoms and currently influences their geographical distribution. We propose that this report strengthens and expands this theory by showing that the spatial distribution of diatoms might be influenced, via water turbulence, not only by nutrient availability but additionally by the underwater light climate, the ability for nutrient uptake and the photoadaptive strategy probably influencing each other (Kolber et al. 1988; Raven 1990; Sunda and Huntsman 1997; Staehr et al. 2002) and ultimately defining the success of a given species/group of organisms in a given ecosystem/niche (Strzepek and Harrison 2004).

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