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PHOTOPROTECTION AND XANTHOPHYLL-CYCLE ACTIVITY IN THREE MARINE DIATOMS[1]

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Light is one of the most important factors affecting marine phytoplankton growth, and its variability in time and space strongly influences algal performance and success. The hypothesis tested in this work is that the activity of the xanthophyll cycle and the development of nonphotochemical quenching could be considered a functional trait of algal diversity. If this hypothesis is true, a relationship must exist between fast-activated pigment variations linked to photoprotective behavior and the ecology of the species. This assumption was tested on three diatoms: *Skeletonema marinoi* Sarno et Zingone, *Thalassiosira rotula* Meunier, and *Chaetoceros socialis* Lauder. These three diatoms occupy different ecological niches. Strains of these diatoms were subjected to five changes in irradiance. Xanthophyll-cycle activity, quantum yield of fluorescence, and electron transport rate were the main parameters determined. There were marked interspecific differences in xanthophyll-cycle activity, and these differences were dependent on the light history of the cells. *Chaetoceros socialis* responded efficiently to changing irradiance, which might relate to its dominance during the spring bloom in some coastal areas. In contrast, *T. rotula* responded with a slower photoprotection activation, which seems to reflect its more offshore ecological distribution. The photoresponse of *S. marinoi* (a late-winter coastal species blooming in the Adriatic Sea) was light-history dependent, becoming photoinhibited under high light when acclimated to low light, but capable of reaching a high photoprotection level when acclimated to moderate light. Our hypothesis on the photoprotection capacity as a functional trait in microalgae seems to be validated given the results of this study.

Key index words: **diatoms; NPQ; photoacclimation; quantum yield of fluorescence; xanthophyll cycle**

Abbreviations: **Ax, antheraxanthin; β-car, β-carotene; chl *a*, chlorophyll *a*; chl *c*, chlorophyll *c*; Dd, diadinoxanthin; DES, de-epoxidation state (= Dt/[Dt + Dd] ratio); Dt, diatoxanthin; F, instantaneous fluorescence value; F_m, maximal fluorescence in dark-adapted state; F'_m, maximal fluorescence in light-adapted state; F_o, minimal fluorescence in dark-adapted cells; F'_q/F'_m, effective quantum yield of fluorescence (light-adapted cells); F_s, fluorescence intensity at steady state at given actinic level (RLC measurement); Fuco, fucoxanthin; F_v/F_m, quantum yield of fluorescence (dark-adapted cells); HL, high light; LHC, light-harvesting complex; LL, low light; ML, moderate light; NPQ, nonphotochemical quenching; PAM, pulse amplitude modulated; PFD, photon flux density; RLC, rapid light curve; SV_m, Stern–Volmer expression for NPQ calculation; Vx, violaxanthin; Zx, zeaxanthin**

The photoacclimation process in marine phytoplankton covers a large range of temporal scales and consists of many biological modifications, from molecular to morphological (Falkowski and La Roche 1991, Prézelin et al. 1991), which drive variations in the ecophysiological behavior of a species. At oversaturating irradiances, excess photons absorbed by the light-harvesting complexes (LHCs) can be dissipated (e.g., through thermal dissipation occurring as nonphotochemical quenching [NPQ]). This phenomenon involves xanthophyll-cycle activity (Ruban et al. 2004), corresponding to the reversible de-epoxidation of violaxanthin (Vx) into zeaxanthin (Zx) through antheraxanthin (Ax) for green algae and higher plants (Demmig et al. 1988). In chl *c*–containing algae, the photoprotective cycle involves the conversion of the monoepoxide diadinoxanthin (Dd) into the de-epoxidized diatoxanthin (Dt; Arsalane et al. 1994, Olaizola et al. 1994), even though the Vx, Ax, and Zx pigments are present in low quantity in this taxon (Lohr and Wilhelm 1999, 2001). Recent studies on diatoms indicate that Dt is responsible for NPQ (Jakob et al. 2001, Lavaud et al. 2002a,b,c, 2003), which can reach very high values in *Phaeodactylum tricornutum* Bohlin, much higher than the values reported for terrestrial plants (Lavaud et al. 2002b, 2004).

Comparative studies showed that the maximal value of nonphotochemical quenching (NPQ) is taxon dependent (Casper-Lindley and Björkman 1998) and can differ markedly even among different species of, for example, diatoms (Lavaud et al. 2004). However, relationships between photoprotective response diversity and algal ecological properties

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remained hypothetical. Recently, some studies revealed an adaptation of the photosynthetic properties of algae to the ecological niche in which they grow. Strzepek and Harrison (2004) showed that acclimation capacities in two diatoms depended on biochemical characteristics of their photosynthetic apparatus, which in turn are related to the ecosystem properties. In our study, we wanted to test the assumption that the capacity of short-term pigment variations, activated as a photoprotective process, depends on the ecological characteristics of the species (i.e., on the ecological niche properties). For instance, the short-term photoresponse is probably more often activated in highly mixed water columns (e.g., coastal) than in more hydrodynamically stable (e.g., oceanic) ecosystems, and this characteristic could therefore be one factor determining the competitive performance and the success of some algae. In relation to this hypothesis, the short-term photoresponse would act as a functional component of phytoplankton diversity.

Diatoms were chosen as models since they generally dominate algal communities in coastal ecosystems, accounting for ~40% of the marine primary production (Armbrust et al. 2004), and they present a high level of morphological, ecological, and molecular diversity (Medlin and Kaczmarska 2004). The short-term photoprotective capacities, mainly controlled by xanthophyll-cycle activation and development of NPQ as well as variations of fluorescence parameters, were followed during five different light-shift experiments. A comparative study of the dynamics of Vx, Ax, and Zx among the three diatoms was also undertaken to test their potential role in Dd–Dt xanthophyll-cycling intensity, since these pigments could be intermediary pigments in the photoprotective pathway activation (Lohr and Wilhelm 2001).

Previous photophysiological studies were generally carried out using very strong photon flux densities (PFDs; e.g., >1000 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$; Casper-Lindley and Björkman 1998, Lavaud et al. 2002b, 2003) to dissect the main traits of the photoresponse. In this study, to test the "ecological–physiological relationship" assumption, we used three diatoms representative of different ecological niches, applying PFDs ranging from 40 to 400 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$, which correspond to values often experienced by cells during their growth in coastal ecosystems. The three species used as models are *Skeletonema marinoi*, *Chaetoceros socialis*, and *Thalassiosira rotula*. The first species blooms in the coastal area of the Adriatic Sea in the late winter (Totti and Artegiani 2001, Sarno et al. 2005), while the second co-occurs with other species during the spring bloom in some coastal areas (Marshall and Ranasinghe 1989, Totti and Artegiani 2001), and the third grows in more offshore ecosystems (Syvertsen 1977). Different photoresponses were determined, depending mainly on the species but also on the growth PFD acclimation of the cells. According to the light condition and the species, the xanthophyll cycle was quickly activated (e.g., exponential synthesis of Dt), followed a slow and linear activation, or was almost inactivated when photoinhibition occurred.

MATERIALS AND METHODS

Algal cultures and experimental design. Nonaxenic strains of *S. marinoi*, *T. rotula*, and *C. socialis*, isolated from the Adriatic Sea (*S. marinoi*) or from the Tyrrhenian Sea (*T. rotula* and *C. socialis*), were provided by the Laboratory of Ecophysiology (Stazione Zoologica "A. Dohrn," Naples, Italy). Cells of *S. marinoi* are about 7–8 μm diameter, forming long chains. *Thalassiosira rotula* is a chain-forming species with cell diameter of 25 μm, while *C. socialis* forms colonies with cells of about 8–9 μm diameter.

The three species were cultured semicontinuously at 20°C in 3 L Pyrex glass bottles (Microglass, Naples, Italy) containing natural sterile seawater amended with f/2 nutrients (Guillard and Ryther 1962), with a 12:12 light:dark (L:D) photoperiod at low light (LL, 40 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$) or moderate light (ML, 100 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$). Light was provided with white fluorescent tubes (58W/840; Phillips, Paris, France), and irradiance was measured with a laboratory PAR sensor (4 π, QSL-2100; Biospherical Instruments Inc., San Diego, CA, USA). Before the beginning of the experiments, cultures were kept in exponential growth phase for at least 10 generations by repeatedly diluting them with fresh medium. Experiments were performed twice independently and consisted of the following light changes: the LL-cultured cells were shifted to 200 or 400 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$ and the ML-cultured cells were shifted to 40, 200, or 400 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$. During each experiment, one flask was kept at the same irradiance as growth conditions. Experiments lasted 3 or 4 h and samples for pigment, variable fluorescence, and flow cytometry measurements were taken at high time frequency.

Pigment content. Ten milliliters of culture was filtered through 25 mm GF/F filters (Whatman, Maidstone, UK) and immediately stored in liquid nitrogen for later pigment analysis (Casotti et al. 2005). Briefly, pigment filters were extracted in 3 mL 100% methanol, and 500 μL of 1 mol $\cdot$ L^{-1} ammonium acetate was added to the 1 mL pigment extract for 5 min before the analysis by a Hewlett Packard series 1100 HPLC (Hewlett-Packard, Wilmington, NC, USA). A 3 μm C_8 BDS column (ThermoHypersil, Runcorn, UK) was used, and the mobile phase was composed of two solvents: (A) methanol and aqueous ammonium acetate (70:30), and (B) methanol. Pigments were also measured at 440 nm using a photodiode array detector (model DAD series 1100; Hewlett Packard), which gave the 400 to 700 nm spectrum for each detected pigment. Pigments were detected by fluorometry (series 1100 fluorometer; Hewlett Packard), using a 410 nm excitation wavelength and a 665 nm emission wavelength, and quantified using standards from the VKI (Water Quality Institute, Horsholm, Denmark).

Fluorescence measurements. Variable fluorescence was measured using a Phyto-PAM (pulse-amplitude-modulated) fluorometer (Heinz Walz GmbH, Effeltrich, Germany). Quantum yield of fluorescence for the light-adapted (F'_q/F'_m, with $F'_q = F'_m - F$) and for the 15 min dark-adapted samples (F_v/F_m, with $F_v = F_m - F_o$) was determined through the measurements of the minimum fluorescence level, F (or F_o), and the maximum fluorescence level, F'_m (or F_m), respectively. The latter corresponds to the maximum fluorescence measured after a saturation pulse of bright red light (655 nm, 2400 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$) applied for 450 ms. The pulse

was saturating since the increase of its duration did not increase the fluorescence yield in any of the three species studied.

The NPQ was estimated during the rapid light curve (RLC) measurement performed three or four times per experiment, as follows: After the 15 min dark adaptation, F_v/F_m was estimated, and cells were exposed to 10 levels of increasing actinic light (from 8 to 1500 μmol photons · $m^{-2} \cdot s^{-1}$, 2 min at each PFD level). At the end of each illuminated step, the quantum yield of fluorescence was measured ($F'_q/F'_m = [F'_m - F_s]/F'_m$), and the relative electron transport rate (rETR) was estimated as follows:

$$\text{rETR} = (F'_q/F'_m) \cdot \text{PFD} \cdot 0.5 \quad (1)$$

where PFD was the incident irradiance (μmol photons · $m^{-2} \cdot s^{-1}$), and 0.5 corresponded to the assumption that half of the absorbed light is distributed to PSII. The RLCs were fitted with the equation of Eilers and Peeters (1988) to retrieve the photosynthetic parameters ${}_{rel}\alpha_{etr}$, E_k, and $rETR_{max}$. The NPQ was calculated at each light level by the Stern–Volmer expression (Krause and Weis 1991):

$$\text{NPQ} = SV_m = (F'_q/F'_m) - 1 \quad (2)$$

where SV_m is the Stern–Volmer quenching coefficient of the maximum fluorescence yield, and F'_m and F_m are the maximum fluorescence of light-adapted (after each light level) and dark-adapted samples (measured at the beginning of the RLC), respectively (Villareal 2004).

RESULTS

Acclimation of Chaetoceros socialis *to changing PFD.* The ML-growing cells of *C. socialis* (100 μmol photons · $m^{-2} \cdot s^{-1}$) had a lower F_v/F_m and photosynthetic parameter values than the LL-growing cells (Table 1) and presented a greater capacity for energy dissipation as revealed by the higher NPQ_{max} (5.60 vs. 1.12; Fig. 1, a and b). This finding was in relation to the 4-fold greater xanthophyll pool (Dd + Dt) in the ML-acclimated cells (Fig. 2, a and b). The light-limitation experienced by cells growing under 40 μmol photons · $m^{-2} \cdot s^{-1}$, as revealed by the E_k value (Table 1), might be a determinant for the increase of the chl *a* · $cell^{-1}$ content with respect to the ML condition (Table 2). The accessory pigments versus chl *a* ratio did not change between the two PFD-growing conditions, except that β-car/chl *a* doubled in the ML-growing cells (Table 2).

Photoresponses of ML-growing cells to the high PFD condition (400 μmol photons · $m^{-2} \cdot s^{-1}$) were characterized by an increase in the $rETR_{max}$ and E_k (by 40%) and a decrease in NPQ_{max} (−44%; Fig. 1a). It is noteworthy that a quenching of F_m occurred (first point of the evolution of maximum fluorescence parameter from RLC; Fig. 1c) between the start (T_o) and the end of the experiment (240 min), which could indicate dark-NPQ. A possible cause could be chlororespiration (Jakob et al. 2001) generating a transthylakoid pH gradient, allowing the maintenance of some xanthophylls in the de-epoxidized state (Villareal 2004). Indeed, the diatoxanthin pool was high since it followed a 3-fold increase mainly in the first minutes after the shift from ML to HL (Fig. 2a). This Dt increase, mainly due to de novo synthesis would be responsible for the strong quenching of F and F'_m ($P < 0.01$; Fig. 2c).

Photoresponse of LL-growing cells to high PFD was characterized by a strong decrease in PSII operating efficiency (F'_q/F'_m; Fig. 2d), which could be related to xanthophyll-cycle activity (Fig. 2b). Diatoxanthin synthesis was slower than for the ML-growing cells shifted to HL ($k = 0.37$ and 1.61 min^{-1}, respectively) and was primarily due to the conversion of preexisting Dd, followed by a de novo synthesis of Dd and Dt. A development of dark-NPQ was also revealed by the quenching of F_m between T_o and the end of experiment (180 min) from RLC measurement (Fig. 1d).

The photobiological properties of ML-growing cells shifted to low PFD remained rather similar, except for the 50% reduction in diatoxanthin and the 30% increase in chl *a* · $cell^{-1}$ (data not shown).

Acclimation of Skeletonema marinoi *to changing PFD.* The maximum PSII photochemical efficiencies, the relative photosynthetic efficiencies, and the NPQ_{max} were almost similar in ML- and LL-growing cells (≈0.65, 0.266 μmol e^- · μmol photons^{-1}, and 0.8 to 1.2, respectively; Table 1 and Fig. 3, a and b). The light-saturation of cells at 100 μmol photons · $m^{-2} \cdot s^{-1}$ with respect to their light limitation at 40 μmol photons · $m^{-2} \cdot s^{-1}$ (see E_k values, Table 1) could be a determinant for the decrease in cellular chl *a* quota and fucoxanthin (Fuco; Table 2). The latter probably induced a lowering of cell absorption capacity, which might account for the reduction of $rETR_{max}$ (Table 2), together with the 2- to 3-fold greater pool of Dd and Dt in

TABLE 1. Fluorescence and photosynthetic parameters of the three diatoms grown under low (40 μmol photons · $m^{-2} \cdot s^{-1}$) and moderate (100 μmol photons · $m^{-2} \cdot s^{-1}$) photon flux density (PFD) conditions.

	F_v/F_m	F'_q/F'_m	E_k (μmol photons · $m^{-2} \cdot s^{-1}$)	$rETR_{max}$ (μmol e^- · $m^{-2} \cdot s^{-1}$)	${}_{rel}\alpha$ (μmol e^- · μmol photons^{-1})
Chaetoceros socialis PFD: 40	0.70	0.72	98.9	28.9	0.292
C. socialis PFD: 100	0.56	0.57	77	18.7	0.243
Skeletonema marinoi PFD: 40	0.65	0.63	160.5	42.8	0.266
S. marinoi PFD: 100	0.64	0.66	118.4	31.5	0.266
Thalassiosira rotula PFD: 40	0.66	0.67	93.7	25.9	0.277
T. rotula PFD: 100	0.50	0.36	67.7	14.4	0.213

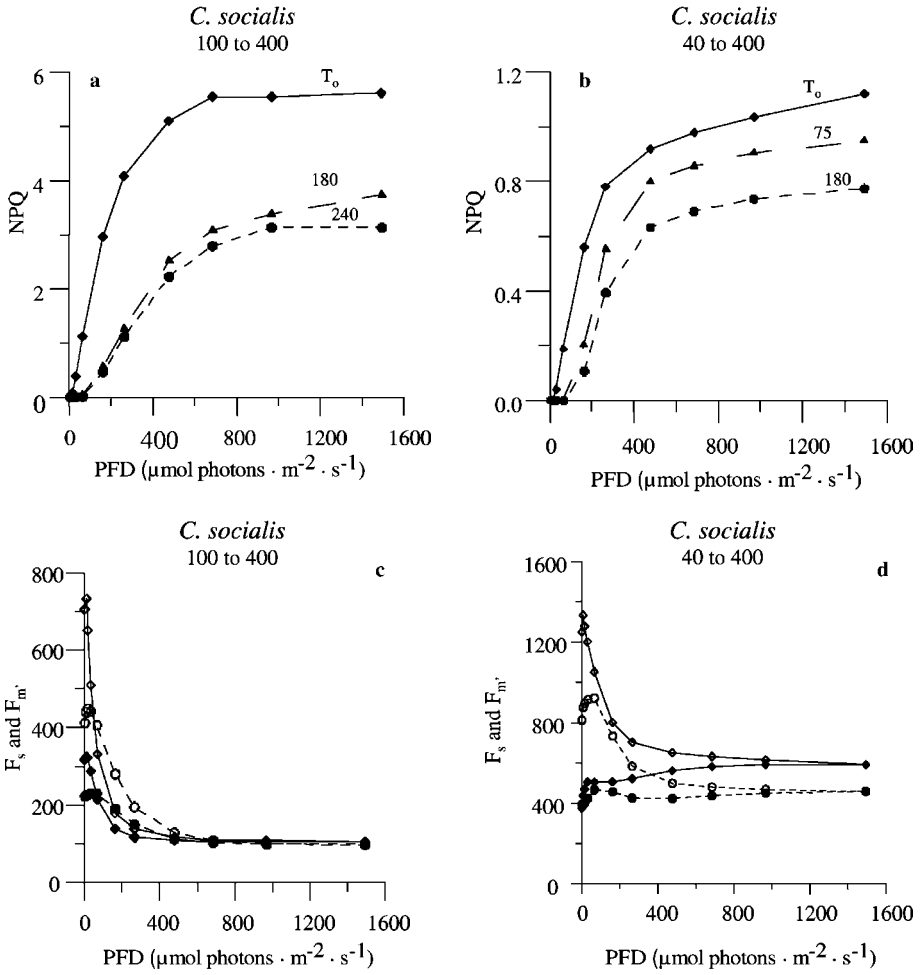

FIG. 1. Evolution of NPQ (a, b) and F_s and F'_m (c, d) from an upward shift in irradiance, from rapid light curve measurements performed on *Chaetoceros socialis*. Cells were shifted to 400 µmol photons · m^{-2} · s^{-1} from growth in medium light (100 µmol photons · m^{-2} · s^{-1}; a, c) and low light (40 µmol photons · m^{-2} · s^{-1}; b, d). All the data are means of two values. Numbers on the graph represent the time (min) after the light shift.

ML-growing cells compared to LL-growing cells (Fig. 4, a and b).

The shift in ML-growing cells to the high PFD condition (400 µmol photons · m^{-2} · s^{-1}) induced a strong accumulation of Dt (Fig. 4a) correlated to a reduction of PSII operating efficiency ($P < 0.05$; Fig. 4c). The lowering of NPQ_{max} with time (Fig. 3a) might be related to the strong decrease of F_m (first point of the RLC, Fig. 3c). This absence of F_m recovery could indicate the development of dark-NPQ. Photoinhibition could be another hypothesis, but the minimal fluorescence F did not increase (Fig. 4c), as generally observed in such cases (Müller et al. 2001). On the contrary, the LL-growing cells shifted to the high PFD condition showed a low Dt synthesis while Dd accumulated (Fig. 4b). This pigment response might account for the small decrease in the PSII operating efficiency (Fig. 4d). The increase in the minimal level of fluorescence (F_o [+14%], data not shown) might indicate the closure of the reaction centers related to photoinhibition (Krause et al. 1990). The RLC measurement revealed an increase in NPQ_{max} (Fig. 3c) and the absence of dark-NPQ (Fig. 3d).

The photobiological properties of ML-growing cells shifted to low PFD remained rather similar, except for a 42% increase of chl a · $cell^{-1}$ (data not shown).

Acclimation of Thalassiosira rotula *to changing PFD.* The ML-growing cells of *T. rotula* (100 µmol photons · m^{-2} · s^{-1}) were light-saturated (E_k = 67.7 µmol photons · m^{-2} · s^{-1}) in contrast to cells growing at low PFD (E_k = 93.7 µmol photons · m^{-2} · s^{-1}). This feature was responsible for the greater xanthophyll pigment pool (Fig. 6, a and b), as well as the low PSII quantum yield of fluorescence (Table 1) and the higher NPQ_{max} (Fig. 5, a and b) compared to the low PFD condition. The ML-growing cells appeared to be already photoinhibited. Therefore, when shifted to high PFD (400 µmol

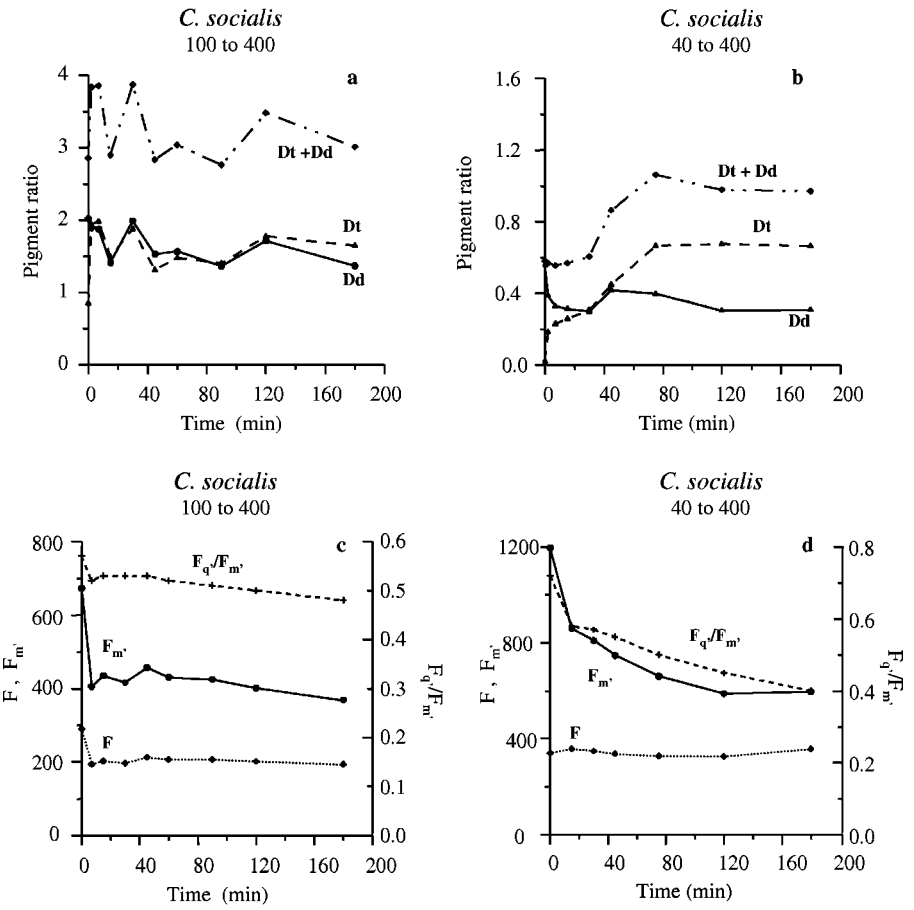

FIG. 2. Evolution of xanthophyll ratios (a, b) and F, F_m', and F_q'/F_m' (c, d) from an upward shift in irradiance on *Chaetoceros socialis*. Cells were shifted to 400 µmol photons · m^{-2} · s^{-1} from growth in medium light (100 µmol photons · m^{-2} · s^{-1}; a, c) and low light (40 µmol photons · m^{-2} · s^{-1}; b, d). Pigment ratios in mol · 10 mol chl a^{-1}. All the data are means of two values.

TABLE 2. Mean (n = 6) and standard deviation (in brackets) of chl a · $cell^{-1}$ (mol chl a · $cell^{-1}$) and pigment ratios (mol pigment · 10 mol chl a^{-1}) for the three species after 10 generations of acclimation at 40 (LL) or 100 (ML) µmol photons · m^{-2} · s^{-1}.

	Chl a · $cell^{-1}$	β-car/chl a	(Vx + Ax + Zx)/chl a	Fuco/chl a
Chaetoceros socialis PFD: 40	8.86×10^{-16} (0.9×10^{-16})	0.356 (0.025)	0.173 (0.095)	5.33 (0.48)
C. socialis PFD: 100	7.26×10^{-16} (0.8×10^{-16})	0.602 (0.080)	0.183 (0.179)	5.94 (0.75)
Skeletonema marinoi PFD: 40	6.49×10^{-16} (0.7×10^{-16})	0.477 (0.053)	0.338 (0.061)	6.78 (0.648)
S. marinoi PFD: 100	4.23×10^{-16} (0.7×10^{-16})	0.657 (0.244)	0.708 (0.206)	8.84 (3.77)
Thalassiosira rotula PFD: 40	3.65×10^{-14} (1.8×10^{-14})	0.227 (0.027)	0.156 (0.016)	4.18 (0.45)
T. rotula PFD: 100	4.04×10^{-14} (0.9×10^{-14})	0.253 (0.017)	0.135 (0.043)	4.27 (0.21)

β-car, β-carotene; Vx, violaxanthin; Zx, zeaxanthin; Ax, antheraxanthin; Fuco, fucoxanthin; LL, low light; ML, moderate light.

photons · m^{-2} · s^{-1}), they did not accumulate a large amount of Dt (Fig. 6a), which in turn implied a slight decrease of F_q'/F_m' (Fig. 6c). The NPQ_{max} was strongly reduced with time (Fig. 5a), which might be related to development of dark-NPQ as suggested by the strong decrease of F_m (first point of the RLC, Fig. 5c). On the contrary, the LL-growing cells shifted to the high PFD condition slowly synthesized Dt (Fig. 6b), which was not correlated to the rapid decrease of F_m' ($P > 0.05$; Fig. 6d). The low NPQ_{max} was reduced with time (Fig. 5b) due to dark-NPQ and/or photoinhibition (see Fig. 5d), the latter being revealed by the increase of F_s along the RLC (+56%).

The ML-growing cells shifted to low PFD revealed a quick relaxation of PSII. The quenching of F_m and the dark-NPQ disappeared after 7 min, while Dt/chl a was reduced by 50% (data not shown).

DISCUSSION

Our results highlight a strong variability of photoresponses developed by these three centric diatoms. The high photophysiological diversity among the

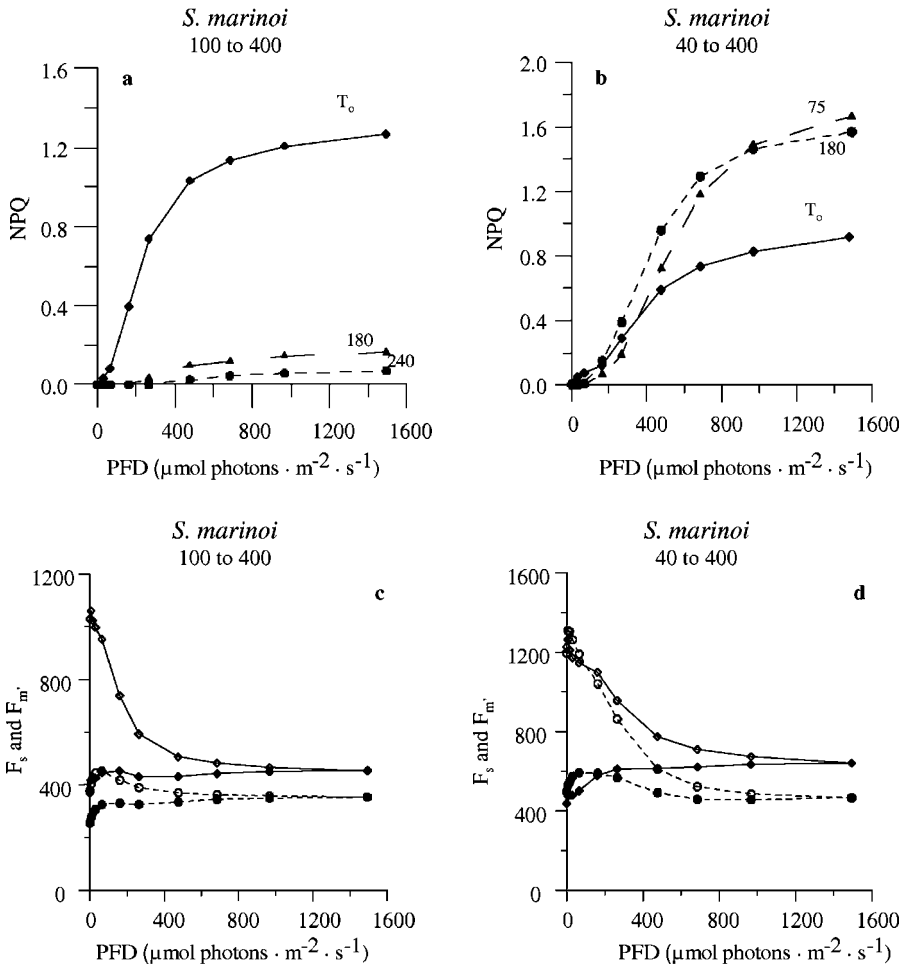

FIG. 3. Evolution of NPQ (a, b) and F_s and F'_m (c, d) from an upward shift in irradiance, from rapid light curve measurements performed on *Skeletonema marinoi*. Cells were shifted to 400 µmol photons · m^{-2} · s^{-1} from growth in medium light (100 µmol photons · m^{-2} · s^{-1}; a, c) and low light (40 µmol photons · m^{-2} · s^{-1}; b, d). All the data are means of two values. Numbers on the graph represent the time (min) after the light shift.

three species does not match their phylogenetic relationships (Medlin and Kaczmarska 2004), since *S. marinoi* and *T. rotula* are closely related, whereas *C. socialis* is more distant. The ecological properties of the species could represent one of the factors responsible for photophysiological differences. Responses varied from a quick and efficient photoacclimation to photoinhibition, and the physiological processes activated by cells concern xanthophyll cycling and NPQ as well as dark-NPQ related to the formation of a transthylakoidal Δ-pH in the dark, for instance by chlororespiration (Nixon 2000, Villareal 2004). Intraspecific photoresponse variability mainly depends on the light history of the species (i.e., on the growth PFD condition) as already observed for other species (Casper-Lindley and Björkman 1998). The higher content of diatoxanthin in ML-growing cells strongly increases the further development of NPQ as observed in *C. socialis* and *T. rotula*, in which the NPQ_{max} increased 5 and 10 times, respectively, compared to the LL-growing cells.

Photoprotective behavior as a functional trait in diatoms. In *T. rotula*, the low capacity for short-term photoprotective acclimation prohibits this species from coping with rapidly increasing irradiance when grown under low PFD. Its very low capacity of NPQ (NPQ_{max} = 0.17) probably determines photoinhibition of cells under high PFD, as revealed by the increase in F_o (+154%), reflecting the closure of reaction centers (Müller et al. 2001, Voronova et al. 2002). This species when acclimated under low- or moderate-PFD conditions exhibits the slowest Dt synthesis under high PFD, which might reflect a slow formation of a transthylakoidal ΔpH. A low cytochrome b_6f content could be hypothesized to be responsible for this feature, as found in another oceanic *Thalassiosira* species (*T. oceanica* Hasle, Strzepek and Harrison 2004). The presence of NPQ in ML-growing cells of *T. rotula* and the stability of

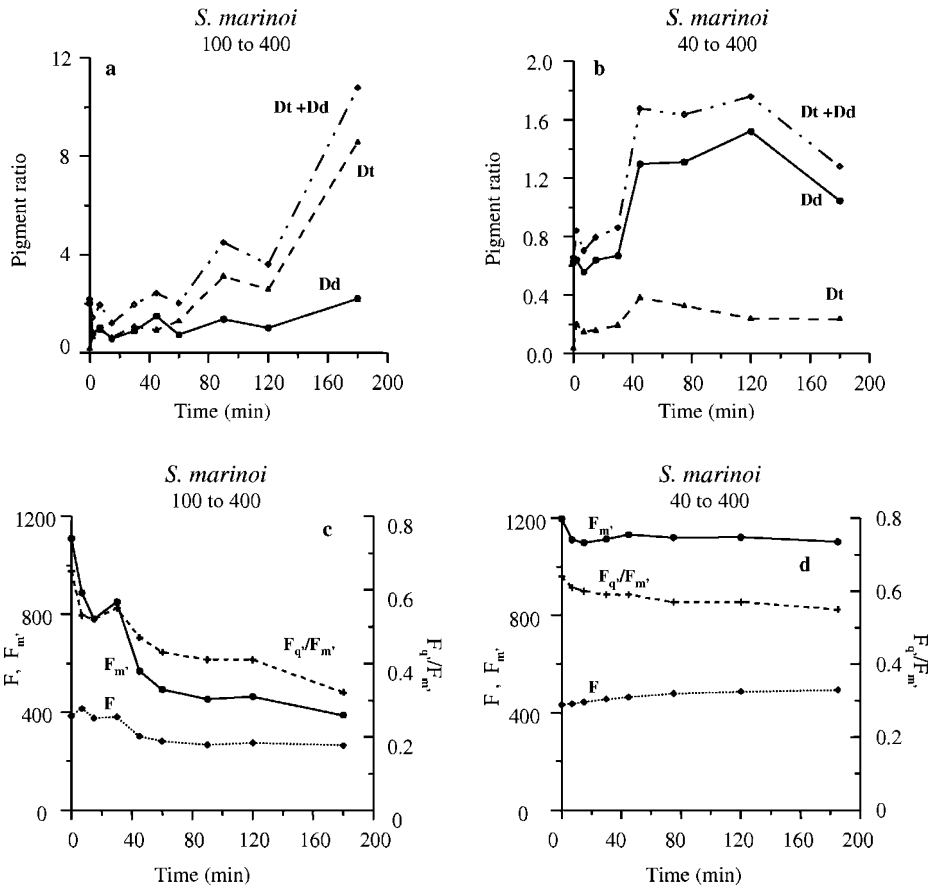

FIG. 4. Evolution of xanthophyll ratios (a, b) and F, F'_m, and F'_q/F'_m (c, d) from an upward shift in irradiance on *Skeletonema marinoi*. Cells were shifted to 400 μmol photons · m^{-2} · s^{-1} from growth in medium light (100 μmol photons · m^{-2} · s^{-1}; a, c) and low light (40 μmol photons · m^{-2} · s^{-1}; b, d). Pigment ratios in mol · 10 mol chl a^{-1}. All the data are means of two values.

Dt/chl *a* during transfer to 400 μmol photons · m^{-2} · s^{-1} suggest that these processes act on a longer-term scale, as already seen in studies carried out on different *Thalassiosira* species (Meyer et al. 2000, Lavaud et al. 2004, Strzepek and Harrison 2004). *Thalassiosira rotula* is also peculiar in that it was the only one of the three species studied that does not appear to have a significant change in the most common long-term acclimation processes, such a decrease in chl *a* content or an increase in the accessory pigments linked to photoprotection (Dd, β-car, Vx, Ax, or Zx; see below). This species appears, therefore, to be able to cope with "limited" increasing light by activating slow physiological responses. This physiological property fits well with the low physical motions present in the offshore, seasonally stratified ecosystems. The very fast recovery to the low-light state of PSII is in agreement with this hypothesis since these very large cells may also sink to escape from the very high-light level of the surface in such stable water columns.

There is marked interspecific diversity in the capacity of NPQ development, as already shown for species belonging to different groups (Casper-Lindley and Björkman 1998) and among diatoms (Lavaud et al. 2004). The highest value reported for *C. socialis* (NPQ_{max} = 5.6) is in the range of the highest values reported by other authors on diatoms and other microalgal groups (Casper-Lindley and Björkman 1998, Lavaud et al. 2004, Ruban et al. 2004). The quick activation of the xanthophyll cycle and the strong accumulation of Dt in cells exposed to high PFD are responsible for this high NPQ and probably contribute to the dark-NPQ measured in this species. *Chaetoceros socialis* had the greatest physiological plasticity of the three species studied, being able to efficiently acclimate to high PFD when grown under either low or moderate PFD. We interpret this plasticity as a functional trait that contributes to its ecological success in the turbulent environment where it grows. This species dominates the algal community during the spring bloom in the northern Adriatic Sea (Totti and Artegiani 2001) and in coastal waters of the northwest Atlantic (Marshall and Ranasinghe 1989). *Chaetoceros* as a genus might be seen as having high photophysiological plasticity since other species have the same acclimation behavior with respect to light variations

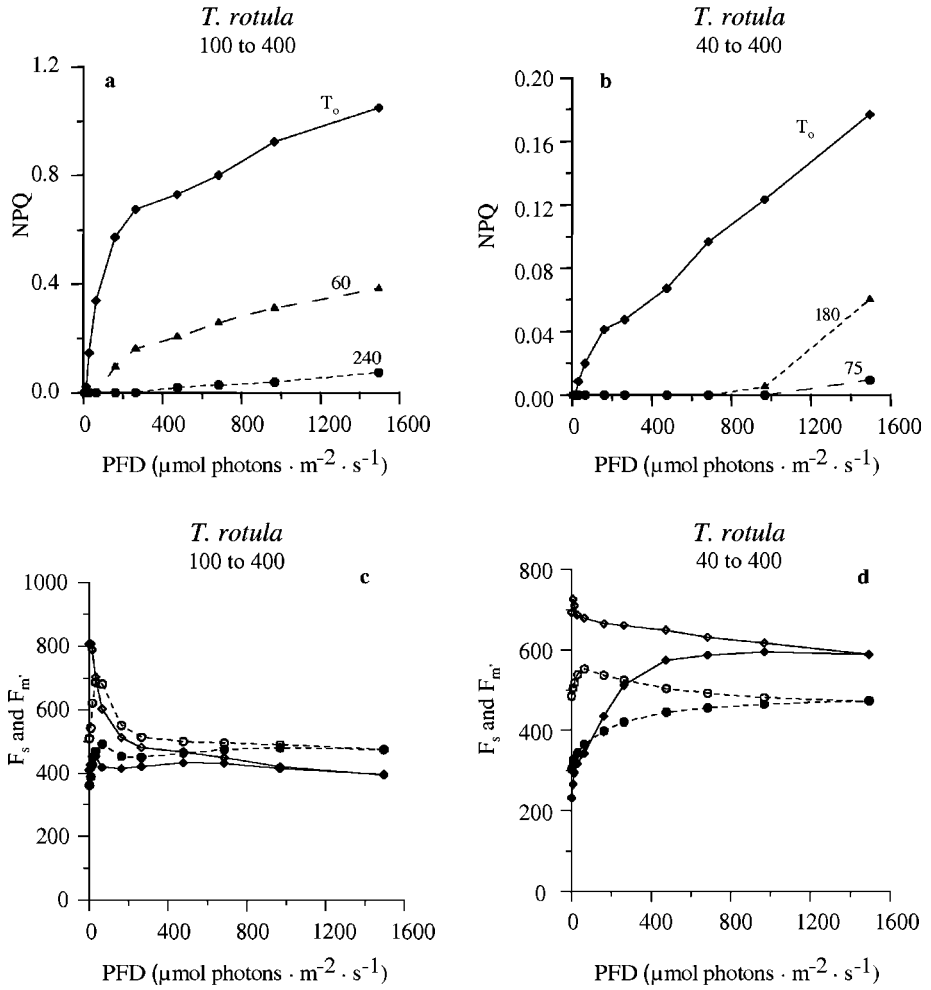

FIG. 5. Evolution of NPQ (a, b) and F_s and F'_m (c, d) from an upward shift in irradiance, from rapid light curve measurements performed on *Thalassiosira rotula*. Cells were shifted to 400 µmol photons · m^{-2} · s^{-1} from growth in moderate light (100 µmol photons · m^{-2} · s^{-1}; a, c) and low light (40 µmol photons · m^{-2} · s^{-1}; b, d). All the data are means of two values. Numbers on the graph represent the time (min) after the light shift.

(*C. muelleri* Lemmerm., Olaizola and Yamamoto 1994; *C. gracilis* Pant., Kashino and Kudoh 2003).

In contrast, *S. marinoi* does not show high capacity for energy dissipation, which is also strongly dependent on the light history of the cells. The LL-growing cells are not capable of accumulating high amounts of Dt when transferred under high light, probably leading to photoinhibition. In the LL-growing cells, the lack or the delay of Dt synthesis could be due to the low content of Dd (~0.065 mol Dd · mol chl a^{-1}) most probably strongly attached to FCPs and therefore not really convertible. A low thylakoid lumen pH due to the delay of photoprotection might inactivate the de-epoxidase (Olaizola et al. 1994) because of conformational change of the enzyme (Emanuelsson et al. 2003). When the new synthesis of Dd occurs (i.e., after 30 min), efficient photoprotection is thus prevented. On the other hand, the ML-growing cells activate a strong synthesis of Dt, reaching 0.80 mol · mol chl a^{-1} under high PFD. This response is relatively slow due to the delay of about 30 min before the Dd de novo synthesis (Fig. 4a), which is needed when this pigment reaches the minimal value of 0.065 mol · mol chl a^{-1}. This value, identical for both LL- and ML-growing cells of *S. marinoi*, could correspond to a nonconvertible Dd pool. A lag of 30 min would be the time needed to activate the photoprotective biosynthetic pathway, leading to the synthesis of new Dd molecules. The high Dt content could be responsible for the development of dark-NPQ and the nonreversibility of F'_m in the dark (see Fig. 3c). This dark-NPQ is a bias for the interpretation of NPQ measurement based on F_m, the maximal fluorescence of dark-adapted cells. The maintenance of a ΔpH in the dark could be an advantage under fluctuating irradiance, allowing the rapid development of energy dissipation upon exposure to oversaturating PFD (Lavaud et al. 2002c). Over long timescales, in ML-growing cells, the strong increase in Dd and Dt is accompanied by an

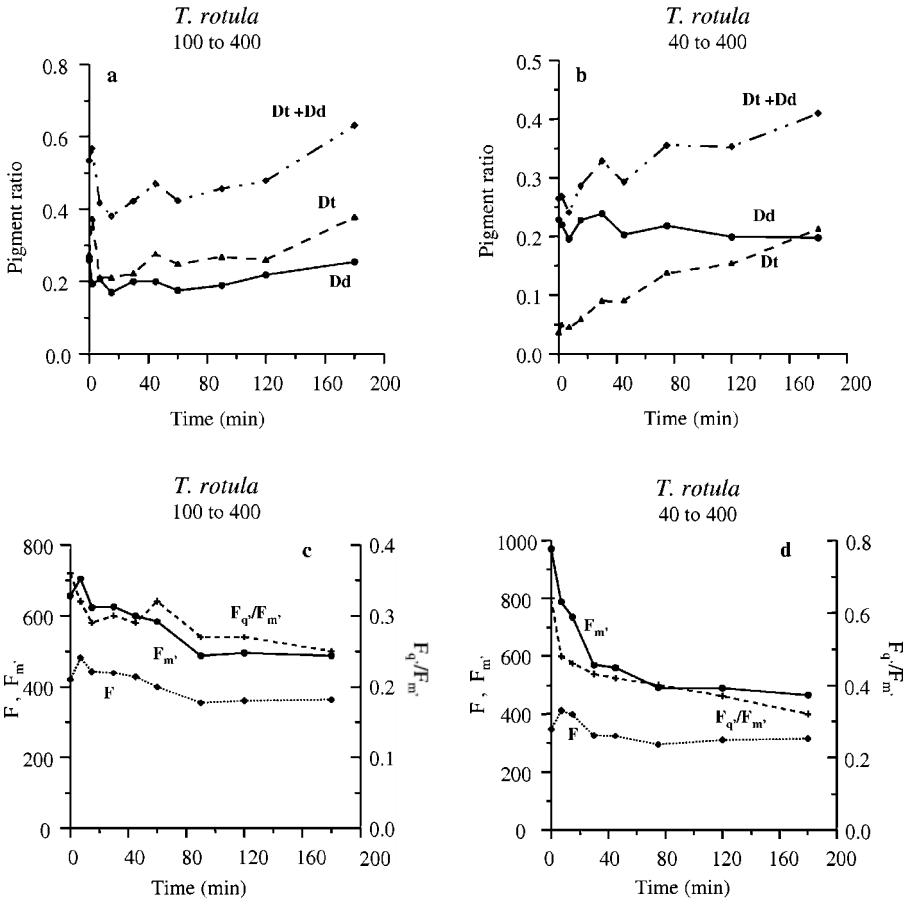

FIG. 6. Evolution of xanthophyll ratios (a, b) and F, F_m', and F_q'/F_m' (c, d) from an upward shift in irradiance on *Thalassiosira rotula*. Cells were shifted to 400 μmol photons · m^{-2} · s^{-1} from growth in medium light (100 μmol photons · m^{-2} · s^{-1}; a, c) and low light (40 μmol photons · m^{-2} · s^{-1}; b, d). Pigment ratios in mol · 10 mol chl a^{-1}. All the data are means of two values.

important reduction in chl *a* content, indicating a probable decrease in the number of thylakoids. A reduction in the antenna size is not proposed since $_{rel}\alpha$ is the same between LL- and ML-growing cells. These changes in pigment content act as a relevant acclimation process, optimizing the maximum PSII photochemical efficiency (see parameters in Table 1). The physiological plasticity of *S. marinoi* is "intermediate" between *T. rotula* and *C. socialis*. This feature is in agreement with the ecological characteristics of this species growing in the coastal area of the Adriatic Sea (Totti and Artegiani 2001). It dominates the late winter algal bloom, characterized by very low-light conditions, and can be present—without dominating—in the springtime community, when light increases.

Pool of accessory xanthophyll pigments. Low variations in the concentration of Vx, Ax, and Zx at our experimental timescale (<4 h) support findings by Lohr and Wilhelm (1999) that these pigments have no direct function in energy dissipation. At long-term scales, they might have a structural role, for instance, by decreasing the thylakoid membrane fluidity (Havaux and Gruszecki 1993). The higher

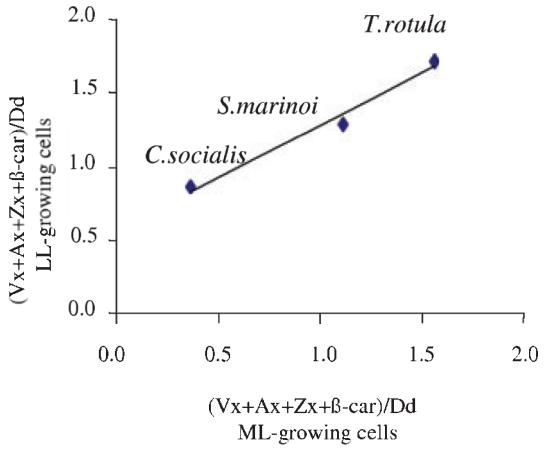

FIG. 7. Relationship between the (Vx + Ax + Zx + β-car)/Dd ratio measured for LL- and ML-acclimated cells for the three species (*Chaetoceros socialis*, *Skeletonema marinoi*, and *Thalassiosira rotula*). Data are means of two values. The regression was significant ($P < 0.05$; $r^2 = 0.98$; $y = 0.72x + 0.56$). Ax, antheraxanthin; β-car, β-carotene; Vx, violaxanthin; Zx, zeaxanthin.

content of these pigments (per mol chl *a*), together with β-car, Dd, and Dt, in the ML-growing cells with respect to LL-growing cells corresponds to their role as intermediaries in the photoprotective biosynthetic pathway (Lohr and Wilhelm 1999, 2001). The significant relationship between the (Vx + Ax + Zx + β-car)/Dd ratio estimated in the ML- and LL-growing cells (Fig. 7) reveals that the quantity of precursor pigments is linked to the quantity of Dd, and vice versa. The slope of the linear regression (Fig. 7) being <1 indicates that when this ratio increases, the differences between the LL- and ML-growing cells tend to be smaller, perhaps indicating a saturation of the loci for these pigments in the PSII. The low capacity for changing the xanthophyll content inside the PSII between the two PFD conditions could be responsible for the slow photoresponse capacity exhibited by the algae, for instance *T. rotula*. It appears that the faster the kinetics of the xanthophyll cycle, the lower the (Vx + Ax + Zx + β-car)/Dd ratio (reaching values <1, Fig. 7), indicating a trend toward net Dd accumulation. This could be a key factor in quickly activating the conversion from Dd to Dt, which is one of the main short-term processes of photoprotection of PSII.

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