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Xanthophyll cycling in *Phaeocystis antarctica*: changes in cellular fluorescence

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ABSTRACT: The xanthophyll cycle has been implicated as a possible photoprotective mechanism in higher plants and algae by dissipating excess excitation energy via non-photochemical quenching. To examine whether colonial *Phaeocystis antarctica* Karsten displays xanthophyll cycling, nutrient-replete cultures were initially grown under limiting (40 µmol quanta m^{-2} s^{-1}) and saturating (280 µmol quanta m^{-2} s^{-1}) irradiances for photosynthesis and their responses to irradiance transitions were examined for 1 h under 4 treatments. The *in vivo* chl-specific absorption coefficient [$a^*_{ph}(\lambda)$, m^2 (mg chl $a)^{-1}$] for the light-limited cultures was initially lower than the light-saturated cultures while chlorophyll (chl) *a*-normalized fluorescence yields were similar for both treatments. Increases in irradiance induced increases in the diatoxanthin to diadinoxanthin ratio (DT:DD, w:w) up to 9-fold whereas parallel decreases in irradiance similarly decreased the DT:DD ratio. Light-induced increases in DT concentration were reduced in cultures exposed to dithiothreitol (DTT), an inhibitor of DD to DT conversion. Short-term changes in DD and DT concentrations were attributed solely to xanthophyll cycling; no de novo synthesis of DD or DT was evident based on a constant sum of DD and DT in the 1 h experimental perturbations. It was found that DD and DT de novo synthesis required long-term acclimation; the mass ratio at steady state of (DD+DT)/chl *a* was 0.1 and 0.4 for the low and high light treatments, respectively. Pooled results from treatment and control cultures showed a linear relationship between light-induced changes in DT/chl *a* concentration and F/chl *a* (fluorescence to chl *a* ratio) and the slopes depended on the initial photoacclimated state of the culture. Cellular fluorescence changes appeared to be physiologically based; $a^*_{ph}(\lambda)$ did not change in response to abrupt irradiance changes. Xanthophyll cycling may enable *P. antarctica* to tolerate both high light environments and sudden changes in irradiance, which occur during austral spring due to shallow mixed layers and intermittent shading by ice or clouds.

KEY WORDS: Antarctic · *Phaeocystis* · Phytoplankton · Xanthophyll cycling · Fluorescence · Absorption · Diatoxanthin · Diadinoxanthin

INTRODUCTION

Phytoplankton have developed strategies to prevent reaction center over-excitation which may ultimately lead to photoinhibitory damage (Osmond 1981). In polar regions, protection from excess light may be particularly critical to the success of an organism because the light field experienced by phytoplankton can have a strong short-term gradient due to clouds, ice, waves and vertical mixing. During austral winter, phytoplankton and sea ice microalgae acclimate to low irradiances by increased chlorophyll (chl) *a* to carbon ratios, increased ratios of accessory pigments to chl *a*, increased chl-specific photosynthetic efficiency values and decreased maximal photosynthetic rates (Brightman & Smith 1989, Lizotte & Sullivan 1991, Sakshaug & Slagstad 1991, Robinson et al. 1995). Low-light adapted polar phytoplankton communities have been shown to be more susceptible to photoinhibition when abruptly exposed to increased irradiance (Platt et al. 1982, Harrison & Platt 1986, Sakshaug & Slagstad 1991). Some polar phytoplankton such as nanophytoflagellates may fail to acclimate to large and abrupt irradiance changes (Buma et al. 1993).

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Light field fluctuations of the order of seconds occur from alternate focusing and defocusing of sunlight by sea surface waves which may increase the instantaneous irradiance by 150% (Schenck 1957, Dera & Stramski 1986, Stramski & Legendre 1992). Cloud movement also alters the light field on these time scales. Several scenarios may occur during austral spring which force low-light adapted phytoplankton into an environment which is saturating or photoinhibiting for photosynthesis and changes in minutes to hours. For example, changes in the location of the marginal ice edge due to ice melt or winds will abruptly expose low-light adapted phytoplankton underneath the ice to increased irradiance on hourly time scales (Smith 1987). Increased stratification due to ice melt at the marginal ice edge can entrain phytoplankton into stable low salinity surface melt-water pools (Smith & Nelson 1985) at the ocean surface where irradiance is highest and vertical motion is reduced. Open leads and polynyas within the ice expose phytoplankton adapted to under-ice conditions to abrupt increases in irradiance. Also, water column mixing processes expose phytoplankton to a variety of irradiance histories (Smith 1987, Falkowski et al. 1994).

Phytoplankton photoacclimate on a long-term basis by changing *in vivo* chl-specific absorption, thylakoid stacking, pigmentation and photosynthetic characteristics such as size and number of photosynthetic units (Perry et al. 1981, Mitchell & Kiefer 1988, Berner et al. 1989, Nelson & Prézelin 1990, Sakshaug et al. 1991, Sosik & Mitchell 1991, Moisan & Mitchell in press). Relatively little is known about how phytoplankton acclimate to abrupt irradiance changes (Post et al. 1985, Olaizola et al. 1992, Stramski & Legendre 1993).

Xanthophyll cycling between the carotenoids diadinoxanthin (DD) and diatoxanthin (DT) responds on short time scales, enabling organisms to respond on time scales related to vertical mixing (Welschmeyer & Hoepffner 1986). It is an effective quenching mechanism, which does not affect the light harvesting efficiency (Schubert et al. 1994) and lessens the cost of synthesizing other carotenoids (Brunet et al. 1993). It has been correlated with increased thermal de-excitation in the Photosystem II (PSII) antenna which may ultimately result in protection of the PSII reaction center from excess excitation energy (Olaizola et al. 1994).

The carotenoids DD and DT are found in chromophyte algae such as diatoms, dinoflagellates and prymnesiophytes (Liaaen-Jensen 1978), but no evidence of xanthophyll cycling has been found for cyanobacteria, red algae, cryptophytes or prochlorophytes (Falkowski & Raven 1996). However, it should be noted that zeaxanthin in cyanobacteria may serve a photoprotective role under conditions of high light (Bidigare et al. 1989). Conversions between DD and DT are mediated by a reversible light epoxidizing enzyme in the thylakoid chloroplast membrane which utilizes a pH gradient across the lumen membrane (Gilmore & Yamamoto 1993). Under low to high light transitions, DD is de-epoxidized into DT and DT accumulates during high light conditions. In contrast, DT is epoxidized to DD during high to low light transitions and DD accumulates under low light conditions. In higher plants, analogous xanthophyll pigments such as violaxanthin, antheraxanthin, and zeaxanthin are considered to be the most important pigments that protect the photosynthetic apparatus from damage under conditions of excess light (Demmig-Adams 1990, Arsalane et al. 1994).

In this study, we tested whether *Phaeocystis antarctica* Karsten demonstrates xanthophyll cycling. It is an ideal organism to investigate this physiological process because it is a numerically and structurally important organism in the Antarctic food web and biogeochemical cycle (Fryxell & Kendrick 1983, Weisse et al. 1994). *Phaeocystis* single cells may at times be numerically abundant during austral winter (Ashworth et al. 1989). It has been shown that *Phaeocystis* acclimates to low irradiance by approaching the maximal quantum yield for growth (Cota et al. 1994, Moisan & Mitchell in press). Photoacclimation has been suggested to occur on relatively short time scales. Palmisano et al. (1986) showed that when *Phaeocystis* was advected underneath the annual sea ice, a 2- to 4-fold increase in α^B was observed in response to a 2 orders of magnitude decrease in irradiance.

MATERIALS AND METHODS

Cultures of *Phaeocystis antarctica* (CCMP 1374), obtained from the Provasoli-Guillard Center for Culture of Marine Phytoplankton, Bigelow Laboratory for Marine Sciences, Maine, USA, were grown semi-continuously at 3°C in f/2 medium (Guillard & Ryther 1962) under limiting (40 µmol quanta m^{-2} s^{-1}) and saturating (280 µmol quanta m^{-2} s^{-1}) irradiances for photosynthesis in continuous blue light. Limiting and saturating irradiances for photosynthesis were based on preliminary photosynthesis versus irradiance responses of the culture (data not shown).

Irradiance was determined with a Biospherical Instruments QSL-100 irradiance sensor (400 to 700 nm) inside a culture vessel filled with distilled water. Details regarding the spectral distribution of light can be found in Moisan & Mitchell (in press). Cultures were diluted with fresh medium to ensure they were nutrient replete and 'optically thin' to minimize light attenuation by phytoplankton. Specific growth rate (μ)

was calculated from the slope of daily estimates of the natural log of *in vivo* fluorescence of cell suspensions measured in a Turner Designs Model 10 fluorometer.

After steady-state acclimation of the cultures for ~2 wk, growth irradiance was changed with neutral density perforated nickel screens at time 0 and after 30 min under 4 different treatments (Table 1). Samples for pigments, absorption, and fluorescence were collected at time zero, 5, 15, 30, 35, 45, and 60 min. All samples for pigment and absorption measurements were stored in liquid nitrogen in less than 3 min after collection.

As a control, 100 µM of dithiothreitol (DTT) was added to parallel cultures for Treatments A, B, and C for 10 min prior to the initial irradiance change. Otherwise, the experimental treatment was the same as described in Table 1 (Treatments A to C). DTT was not added to Treatment D because it does not inhibit the conversion of DT to DD. The t_0 samples were collected before the culture was split and were assumed to be identical for both treatment and control.

Pigment concentration. Samples (n = 2) were collected at the different time points by filtering either 40 or 50 ml of culture onto Whatman GF/F filters. Filters were immediately placed in liquid nitrogen and stored for <1 mo. For analysis, frozen filters were placed in 1.5 ml of cold acetone and canthaxanthin was used as an internal standard. Filters were thawed in acetone for 30 to 60 min, ground with a teflon tipped tissue grinder and extracts were centrifuged to eliminate cell and filter debris from the supernatant.

Pigment separation was accomplished with high performance liquid chromatography (HPLC) with an Alltech Adsorbosphere C8 column (Goericke & Repeta 1993). Specifically, an autoinjector (Shimadzu SIL-10A) added water to the pigment extract (30%, v:v) before injection (300 µl total volume) to increase resolution of early peaks. We used a binary gradient (Waters 510 pumps) with a flow rate of 1.5 ml min^{-1}. Solvent A consisted of a 75:25 (v:v) methanol:aqueous ammonium acetate mixture. Solvent B was 100% methanol. The proportion of Solvent B increased with time during elution. Pigments were detected by absorption (440 nm, Shimadzu SPD-10AV) and peak areas were used for quantification using pure pigment standards obtained from batch mono-cultures. Purity was confirmed with HPLC. Individual pigments were identified by their retention time and the absorption spectrum of each peak was monitored with an on-line photodiode array detector (Waters 991). Errors for duplicate samples were ≤1%.

Table 1. Experimental conditions for cultures of *Phaeocystis antarctica* grown at 3°C under nutrient-replete conditions. Cultures were acclimated to a steady-state irradiance. Irradiance shifts started at t_0 for a 30 min duration and returned to initial conditions for 30 min. Cultures were either transferred to or from limiting (40 µmol quanta m^{-2} s^{-1}), saturating (280 µmol quanta m^{-2} s^{-1}) or photoinhibiting (540 µmol quanta m^{-2} s^{-1}) conditions for photosynthesis

Treatment	Irradiance (µmol quanta m^{-2} s^{-1})		
	Initial conditions	t_0–t_{30} min	t_{30}–t_{60} min
A	40	540	40
B	40	280	40
C	280	540	280
D	280	40	280

Estimation of time rate of change of DT:DD. The rate of change of DD:DT over time was estimated for the changes in DT:DD in the initial 5 min after each irradiance change. This time period was chosen because most of the change occurred within the initial 5 min period.

Chl-specific absorption coefficient $a^*_{ph}(\lambda)$. Samples were concentrated onto GF/F filters and immediately stored in liquid nitrogen until analysis a week later. Duplicate samples were collected at t_0 and single samples were collected at each subsequent time interval. For raw optical density estimates, each filter was rotated 4 times randomly and the mean value is presented in order to account for any non-homogeneous distribution on the filter pad. Samples were analyzed at a 4 nm slit width with a Varian Cary 1 UV-Vis spectrophotometer. The phytoplankton spectral absorption coefficient, $a_{ph}(\lambda)$, was estimated using the method of Mitchell (1990) after subtraction of a blank and normalization to zero at 800 nm. Absorption was negligible (<12% and <1% of total absorption at 436 and 676 nm, respectively) after methanol extraction of filters (cf. Kishino et al. 1985).

***In vivo* fluorescence estimates.** Samples (n = 3) were determined immediately after collection in a Turner Designs Model 10 fluorometer. Each sample was measured 3 times. Average fluorescence yield values are reported here as the relative fluorescence, F divided by the mean a_{ph} (400–700 nm). Average coefficient of variation of 9 samples is 17.9%.

Statistics. The analytical error for duplicate HPLC estimates as ≤1%. Experimental DT and DD errors (with respect to chl *a*) were derived by calculating a mean error for each treatment when DD and DT are summed (with respect to chl *a*) since the slope of the sum of DD and DT over time did not significantly differ from 0.

RESULTS

Average chl-specific absorption [$\bar{a}^*_{ph}$ (400 to 800 nm)] was lower for the culture that was initially grown at 40 µmol quanta m^{-2} s^{-1} compared to the culture grown at

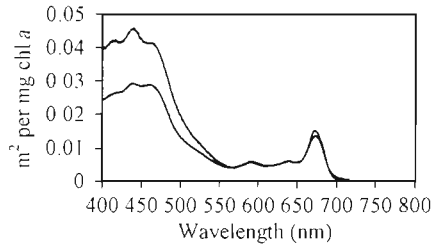

Fig. 1 Chl-specific absorption of *Phaeocystis* cultures acclimated to 40 (bottom curve) and 280 µmol quanta m^{-2} s^{-1} (top curve). Spectra represent the mean of 4 raw absorbance estimates on each of 2 replicate samples

280 µmol quanta m^{-2} s^{-1} (Fig. 1). Differences in $a^*_{ph}(\lambda)$ at the 2 light levels are due to differences in pigment packaging and the relative proportion of photoprotective pigments. The blue (436 nm) to red (676 nm) absorption ratio of the light-saturated culture was 1.3 times higher than the light-limited culture (Table 2). The *in vivo* fluorescence to chl *a* ratio (*F*/chl *a*) was statistically similar in the light-limited and light-saturated cultures and the *in vivo* fluorescence yield ($F/\bar{a}_{ph}$) was similar in the 2 cultures (Table 2). The pool of DT+DD was roughly 5 times higher in the light-saturated culture compared to the light-limited culture (Table 2).

In each of the treatments outlined in Table 1, *Phaeocystis antarctica* demonstrated active and rapid xanthophyll cycling between DD and DT and no net change in the sum of DD and DT over time. Observed changes in DD and DT cellular concentrations were related to stoichiometric changes in the respective xanthophyll components (Fig. 2) which significantly altered the cellular DT to DD ratio (DT:DD, w:w; Fig. 3). When cultures were transferred to a higher intensity, there was a stoichiometric increase in DT at the expense of the DD pool. Similarly, there was an increase in DD at the expense of the DT pool in response to a decreased irradiance.

In all treatments, the constant sum of DD and DT, within experimental error, suggested no de novo synthesis of the xanthophyll pigments during the 1 h incubation. Synthesis of DD and DT apparently requires a much longer time for acclimation as shown by the differences between the light-limited and light-saturated cultures grown at steady state (Table 2). There was no statistically significant change in the sum of the xanthophyll components during the experiments; the slope of the sum of the xanthophyll components (normalized to chl *a*) over time was not significantly different from 0 (*t*-test, $p > 0.05$). Also, the observed changes in xanthophyll pigments were unrelated to the

Table 2. Specific growth rate (μ), DT and DD concentration (normalized to chl *a*), chl-specific absorption (m^2 mg^{-1} chl *a*), *F*/chl *a*, and fluorescence yield of *Phaeocystis antarctica* cultures initially acclimated to 40 and 280 µmol quanta m^{-2} s^{-1}

	Irradiance conditions (µmol quanta m^{-2} s^{-1})	
	40	280
μ (d^{-1})	0.13	0.24
Diatoxanthin content	0.006	0.08
Diadinoxanthin content	0.08	0.36
a^*_{ph} (436 nm)	0.031	0.044
a^*_{ph} (676 nm)	0.013	0.014
a_{ph} (436:676)	2.38	3.14
F/chl *a* (rel. units)	34.7	37.2
$F/\bar{a}_{ph}$ (rel. units)	12.4	10.0

culture concentration; the slope of chl *a* concentrations during the experiment was not significantly different from 0 (*t*-test, $p > 0.05$). Constant chl *a* concentrations are supported by the constant values of a_{ph} at 676 nm where absorption is dominated by chl *a* (Table 3). Given the low growth rates at 3°C, a significant increase in photosynthetic pigments is not expected during the experimental period (Table 2).

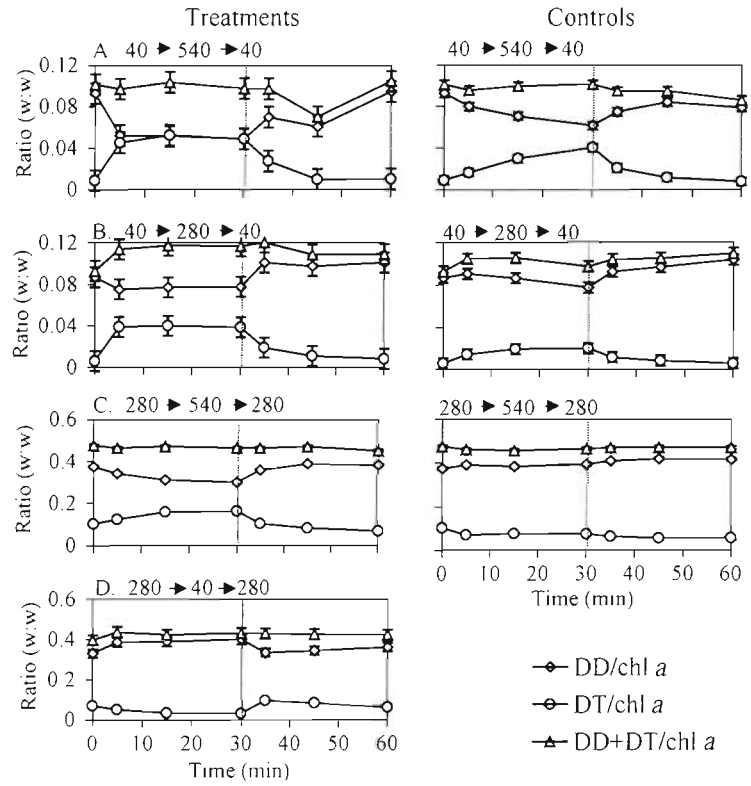

Fig. 2. Average (±SE) diadinoxanthin (DD) and diatoxanthin (DT) (with respect to chl *a*). Numbers at the top of the graphs refer to the irradiance change at initial acclimation, t_0 and $t_{30\ min}$, respectively. No control for Treatment D (cf. Table 1). Vertical dotted line indicates where second light switch occurred

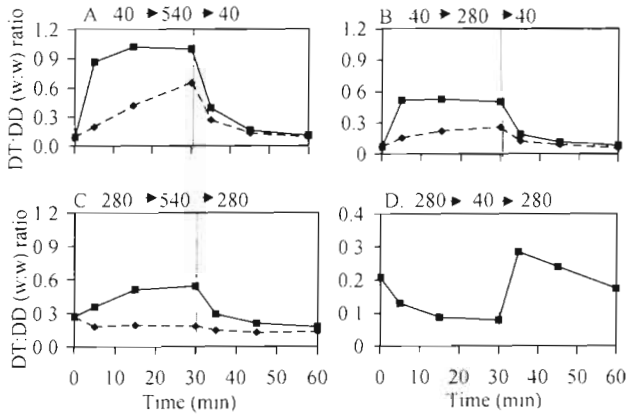

Fig. 3. DT:DD (w:w) over time for treatment (solid line) and control (dashed line) experiments. No control for Treatment D. Vertical dotted line indicates where second light switch occurred. Values are mean ± SD (n = 2)

Changes in DT:DD were rapid in all treatments and occurred within 5 min of the initial irradiance shift. The time rate of change of DT:DD appeared to be related to the state of photoacclimation (Table 4). The most rapid time rate of change in DT:DD, as measured by the initial time rate of change of DT:DD, was observed when cultures were shifted from a limiting to a photoinhibiting condition for photosynthesis (Fig. 3A, Table 4). DT:DD approached steady state within 15 min and returned to the initial value at $t_{60\ min}$. When irradiance was shifted from limiting to saturating conditions for photosynthesis, the initial time rate of change of DT:DD was more rapid compared to the irradiance shift from saturating to photoinhibiting conditions (Fig. 3B, C). DT:DD decreased when the growth irradiance was down-shifted from saturating to limiting conditions for photosynthesis (Fig. 3D) and recovery back to initial DT:DD ratios was as fast as at saturating light levels (Table 4). DT:DD was higher at $t_{35\ min}$ compared to t_0 levels but returned to initial DT:DD levels after an hour. The initial conversion of DT to DD was comparable to that of DD to DT when cultures were transferred from saturating to photoinhibiting conditions (Table 4).

In controls where DTT was added, changes in the DT:DD were observed in the culture initially acclimated to a limiting irradiance (Fig. 3A, B). However, it is in these experiments that the largest net change in DT:DD mass ratios occurred compared to when cultures were acclimated to a saturating irradiance (Fig. 3C). No control experiments were conducted on the experiment where the irradiance was down-shifted (Fig. 3D). To our knowledge, an inhibitor for the conversion of DT to DD is not available.

Despite changes in DT:DD, major pigment concentrations did not change during the course of the treatments. Examples are shown for chl *a*-normalized values of 19'hexanoyloxyfucoxanthin and chl ($c_1 + c_2 + c_3$) for treatment and control experiments (Fig. 4). Minor carotenoids such as 19'-butanoyloxyfucoxanthin, fucoxanthin and β-carotene also did not change significantly over the course of any treatment (Table 5).

In all experiments, the differences between $a^*_{ph}(\lambda)$ values at all time points were found to be statistically insignificant (*t*-test, $p > 0.05$, data not shown). Mean changes in a_{ph} values are given in Table 3. No changes were expected in the DD and DT absorption region because the pigment-specific absorption coefficients

Table 3. Mean a_{ph} (SD) at 676 nm for time 0, 30 min and 60 min. Differences between each time interval for each treatment were not significantly different (*t*-test, $p > 0.05$) except for t_0 for Treatment A. The t_0 samples were obtained prior to the culture split. Controls are indicated by the addition of dithiothreitol (DTT). ND: data not available

Treatment	t_0	$t_{30\ min}$	$t_{60\ min}$
A -DTT	0.31 (0.01)	0.29 (0.02)	ND
A +DTT		0.33 (0.01)	0.33 (0.008)
B -DTT	0.27 (0.008)	0.28 (0.019)	0.26 (0.008)
B +DTT		0.28 (0.007)	0.26 (0.007)
C -DTT	0.07 (0.006)	0.06 (0.006)	0.06 (0.005)
C +DTT		0.06 (0.006)	0.07 (0.005)
D -DTT	0.05 (0.003)	0.07 (0.004)	0.07 (0.005)

Table 4. Rate of change (min^{-1}) in the diadinoxanthin to diatoxanthin ratio (DT:DD) for the different treatments (cf. Table 1). The rate of change is calculated for the initial 5 min after the irradiance change

Treatment	$t_{0-5\ min}$	$t_{30-35\ min}$
A	0.155	−0.121
B	0.090	−0.062
C	0.018	−0.051
D	−0.016	0.041

Table 5. Average ± SD (n = 7) of minor carotenoids (normalized to chl *a*). Together, minor carotenoids were ≤9% of total pigments (w:w). DTT: dithiothreitol

Treatment	19'-Butanoyloxy-fucoxanthin	β-Carotene	Fucoxanthin
A -DTT	0.016 ± 0.001	0.010 ± 0.001	0.082 ± 0.007
A +DTT	0.017 ± 0.001	0.010 ± 0.001	0.081 ± 0.007
B -DTT	0.017 ± 0.001	0.010 ± 0.001	0.059 ± 0.002
B +DTT	0.017 ± 0.001	0.009 ± 0.001	0.063 ± 0.002
C -DTT	0.017 ± 0.001	0.020 ± 0.001	0.034 ± 0.001
C +DTT	0.018 ± 0.002	0.019 ± 0.002	0.036 ± 0.002
D -DTT	0.017 ± 0.002	0.018 ± 0.001	0.037 ± 0.003

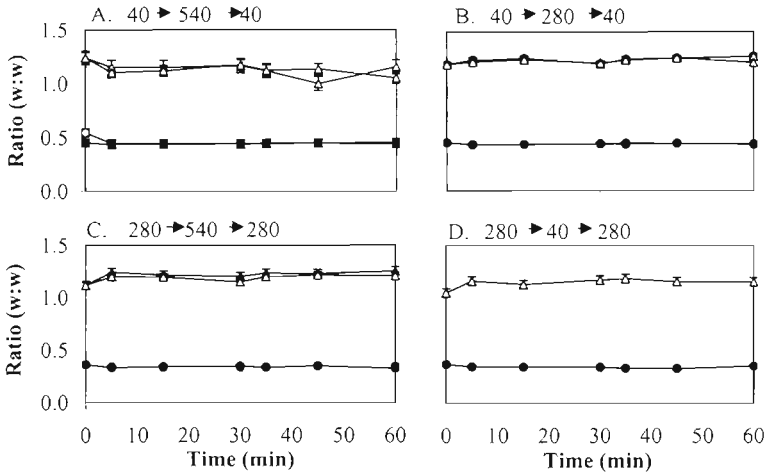

Fig. 4. 19'-Hexanoyloxyfucoxanthin (triangles) and chl *c* ($c_1 + c_2 + c_3$, circles) normalized to chl *a* in treatment (filled) and control experiments (unfilled). Vertical dotted line indicates where second light switch occurred. Values are mean ± SD (n = 2)

are similar (Bidigare et al. 1990) and the sum of DD and DT did not change over the course of the experiment.

Changes in fluorescence appeared to be related to the physiology rather than the optics of the cell because the chl-specific absorption cross section of *Phaeocystis antarctica* cells did not change significantly over the course of the experiments. In each of the treatments, *F*/chl *a* changed in response to changes in irradiance. Changes in *F*/chl *a* were reversible. In all treatments, the response of *F*/chl *a* was slower when transitions were made from a higher to a lower irradiance and were more rapid when transitions were made from a lower to a higher irradiance. When cultures were transferred to a higher irradiance at t_0, a rapid decrease in *F*/chl *a* resulted. The response in *F*/chl *a* was much slower when the cultures were returned to the lower irradiance at $t_{30\,min}$ (Fig. 5A). Conversely, there was little change in *F*/chl *a* when the culture was acclimated to a higher irradiance and transferred to a lower irradiance. The initial rate of change of *F*/chl *a* was greater when the culture was returned to the initial environmental conditions at $t_{30\,min}$ (Fig. 5B).

Pooled results including treatments and controls showed that the chl-normalized *in vivo* fluorescence was linearly related to the chl-normalized DT concentration. The slope of the relationship depended on whether the culture was initially limited or saturated for photosynthesis (Fig. 6). The slope of this relationship for the low light-limited cells (–442 ± 48.9 SE) was roughly 4 times higher than for cells that were initially acclimated to the saturating irradiance (–106 ± 21.5 SE).

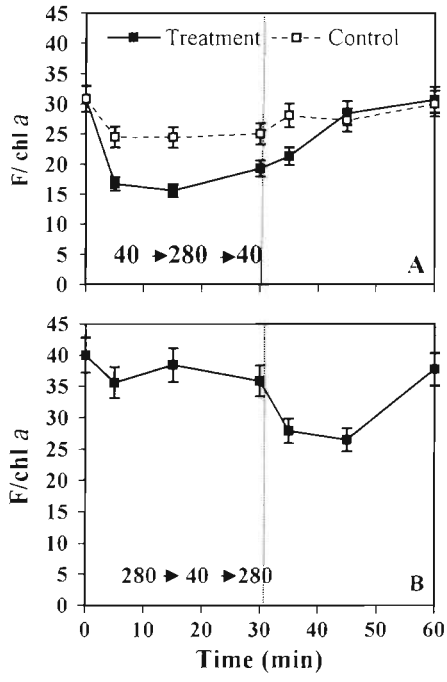

Fig. 5. (A) Relative fluorescence to chl *a* ratios (*F*/chl *a*) over time for Treatment B. (B) *F*/chl *a* over time for Treatment D. No control was performed for Treatment D. Values are mean ±SE (n = 9). Vertical dotted line indicates where second light switch occurred

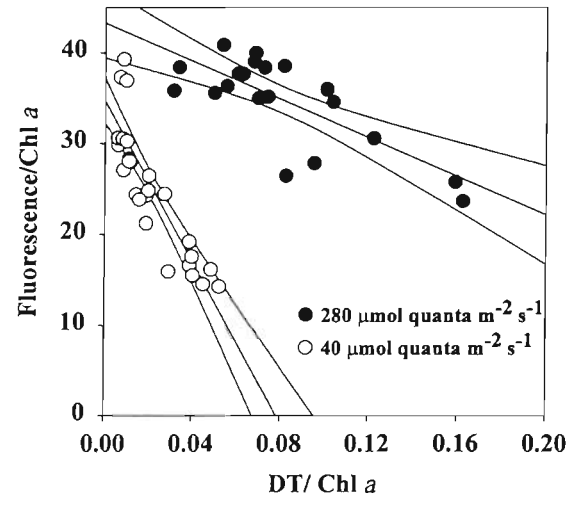

Fig. 6. Relative fluorescence (*F*/chl *a*) and xanthophyll cycle activity (DT/chl *a*) for all experimental treatments and controls after cultures were initially acclimated to either 40 µmol quanta m^{-2} s^{-1} (r^2 = 0.76) or 280 µmol quanta m^{-2} s^{-1} (r^2 = 0.56). 95% confidence intervals are shown for linear regressions

DISCUSSION

Xanthophyll cycling in *Phaeocystis antarctica*

The ability of a phytoplankton species to successfully compete depends in part on their rates and mechanisms of photoacclimation. Our results showed that xanthophyll cycling between the carotenoids, DT and DD occurs rapidly in *Phaeocystis antarctica* and was related to the light history of the cell.

We compare our xanthophyll cycling rates to previous studies, however, it should be noted that the activity has been assessed for different gradients in irradiance at different temporal resolutions. Therefore, all comparisons are made for the same time interval (initial 5 min). Our observed rates of changes in DD/chl *a* and DT/chl *a* (Fig. 2) compare well for temperate phytoplankton initially acclimated to 50 and 500 µmol quanta m^{-2} s^{-1} and transferred to 1000 µmol quanta m^{-2} s^{-1} in a series of light dark cycles (Demers et al. 1991). However, our xanthophyll cycling rates are at least 4 times higher than the temperate cultures of Demers et al. (1991) which were exposed to a fluctuating light environment at a frequency of h^{-1}. Our DT accumulation rates due to xanthophyll cycling are roughly 2 to 4 times lower than those of *Phaeodactylum tricornutum* (Olaizola et al. 1994). Because xanthophyll cycling is due to a photoprotective mechanism and DT:DD rates of change are dependent on the irradiance gradient then this observation must be accounted for in the application of DT:DD ratios as an index of vertical mixing rates. This has not been considered in earlier work (Welschmeyer & Hoepffner 1986).

As a control, DTT was added to illuminated cultures prior to the initial shift in irradiance. Results from preliminary short-term incubation photosynthesis versus irradiance experiments showed that DTT did not affect the photosynthetic performance of the cells on the experimental time scale (data not shown). DTT is a known inhibitor of violaxanthin de-epoxidase in the higher plant violaxanthin cycle (Yamamoto & Kamite 1972) and has been successfully used to block DD cycling for *Phaeodactylum tricornutum* (Olaizola et al. 1994). This is the first use of DTT as an inhibitor of the xanthophyll cycle in prymnesiophytes.

We did not observe complete blockage of the xanthophyll cycle by DTT in the treatments where cultures were initially acclimated to 40 µmol quanta m^{-2} s^{-1}. A possible explanation may be that this is caused by incomplete diffusion of DTT into the cells within the colony which are surrounded by a mucilage. However, this explanation is unlikely because the culture initially acclimated to 280 µmol quanta m^{-2} s^{-1} and transferred to 540 µmol quanta m^{-2} s^{-1} also exhibited a colonial morphology and the DTT successfully blocked the xanthophyll cycle. Also, xanthophyll cycling persisted even after 30 min which is probably ample time for diffusion of DTT into the cells. It is possible that increases in DT:DD were so strong that the amount of DTT added was insufficient to completely inhibit the reaction.

Our results showed no evidence of de novo synthesis of either DD or DT on the time scale during which xanthophyll cycling was observed. The slope of the mean DD+DT pool (normalized to chl *a*) over time was not significantly different from 0 (*t*-test, $p > 0.05$). These results differ from previous reports for temperate diatoms where de novo synthesis of these pigments was observed in response to short-term changes in irradiance. Olaizola et al. (1994) found that de novo synthesis occurred at about 30 min after the irradiance shift. In our study, DD and DT de novo synthesis required a longer period for acclimation as shown by the differences between the cultures that were initially acclimated to the 2 irradiances prior to the experiments (Table 2).

Relationship between diatoxanthin and nonphotochemical fluorescence quenching

In the photosynthetic apparatus, antennae pigments absorb light and excitation energy is either transferred to the reaction center for photosynthesis or re-emitted as fluorescence or dissipated as heat. Under all conditions, the latter 2 processes compete with photochemistry. Under conditions of low light, sufficient nutrients and optimal temperature, photochemistry is most efficient. While under high light, the dissipation of excitation energy via other pathways becomes relatively more important and leads to non-destructive thermal de-excitation of pigments through non-photochemical fluorescence quenching (Vincent et al. 1984, Demmig-Adams 1990). As a result, the *in vivo* fluorescence yield is lowered by competition with other pathways of de-excitation (Kraus & Weis 1991).

Nonphotochemical quenching in algae may be caused by 'photoinhibitory quenching' (Neale 1987), increased heat dissipation within the reaction centers (Weis & Berry 1987), and thermal dissipation in the pigment antenna (Demmig-Adams 1990, Olaizola et al. 1994).

In our study, we observed large changes in *in vivo* fluorescence due to abrupt irradiance changes which were accompanied by a change in the DT and DD cellular concentration (normalized to chl *a*) due to xanthophyll cycling (Fig. 6). When all experimental results are pooled, *F*/chl *a* is linearly related to DT/chl *a*; however, there were some residual changes in *F*/chl *a* ratio in experiments where the xanthophyll cycle was com-

pletely blocked. The rate of change for the low-light acclimated cultures was 4 times higher than for the high-light acclimated cultures. Therefore, it appears that fluorescence quenching is related to the initial conditions of acclimation which also determines the xanthophyll pool size (Table 2, Demers et al. 1991, Willemoës & Monas 1991). DT is found in the antenna bed of PSII in dinoflagellates (Johnsen et al. 1997); therefore, this process could be associated with thermal dissipation of absorbed photons in the antenna. Thus, the energy arriving at the reaction center would decrease and contribute to the observations of low photosynthetic quantum yield at high irradiances.

Our observed slopes of *F*/chl *a* and DT/chl *a* (Fig. 6) were significantly different for high-light and low-light acclimated cells. This observation contrasts with the results of Demers et al. (1991) which showed a similar slope between fluorescence and DT (normalized to chl *a*) for cultures initially grown at 50 and 500 µmol quanta m^{-2} s^{-1} and transferred to an irradiance of 1000 µmol quanta m^{-2} s^{-1}. However, they did observe a different slope for this relationship when cells were grown in a fluctuating light regime.

CONCLUSIONS

Our results demonstrate that *Phaeocystis antarctica* shows active xanthophyll cycling in response to the photoacclimated state of the cell rather than changes in the optical absorption on short time scales. Stoichiometric conversions between DD and DT are rapid and responded in minutes to shifts in irradiance. Concomitant changes in *F*/chl *a* were also observed and were related to the DT content of the cells. The relationship between changes in *F*/chl *a* and DT accumulation was related to the light history of the cell. Based on these results, it appears that xanthophyll cycling is a metabolically conservative process because it requires no de novo synthesis of costly carotenoids on hourly time scales. We hypothesize that xanthophyll cycling may help to optimize photosynthesis in fluctuating light environments which change on minute to hourly time scales and enhance the capacity of *P. antarctica* to tolerate sudden changes in irradiance.

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LITERATURE CITED

Arsalane W, Rousseau B, Duval JC (1994) Influence of the pool size of the xanthophyll cycle on the effects of light stress in a diatom: competition between photoprotection and photoinhibition. Photochem Photobiol 60:237–243

Ashworth TK, Fryxell GA, Prasad AKSK (1989) AMERIEZ 88: phytoplankton distribution across the Weddell Sea Ice Edge during austral winter. American Geophysical Union meeting, 12–16 February, New Orleans, Louisiana. AGU Publications, Washington, DC

Berner T, Wyman K, Falkowski PG (1989) Photoadaptation and the 'package effect' in *Dunaliella tertiolecta* (Chlorophyceae). J Phycol 25:70–78

Bidigare RR, Ondrusek M, Morrow J, Kiefer DA (1990) *In vivo* absorption properties of algal pigments. Ocean Optics X: SPIE 1302:290–302

Bidigare RR, Schofield O, Prézelin BB (1989) Influence of zeaxanthin on quantum yield of photosynthesis of *Synechococcus* clone WH7803. Mar Ecol Prog Ser 56:177–188

Brightman RI, Smith WO (1989) Photosynthesis-irradiance relationship of Antarctic phytoplankton during austral winter. Mar Ecol Prog Ser 53:143–151

Brunet C, Brylinski JM, Lemoine Y (1993) *In situ* variations of the xanthophylls diatoxanthin and diadinoxanthin: photoadaptation and relationships with a hydrodynamical system in the eastern English Channel. Mar Ecol Prog Ser 102:69–77

Buma AGJ, Noordeless AAM, Larsen J (1993) Strategies and kinetics of photoacclimation in three Antarctic nanophytoflagellates. J Phycol 29:407–417

Cota GA, Smith WO, Mitchell BG (1994) Photosynthesis of *Phaeocystis* in the Greenland Sea. Limnol Oceanogr 39: 948–953

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

Demmig-Adams B (1990) Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthin. Biochim Biophys Acta 1020:1–24

Dera J, Stramski D (1986) Maximum effects of sunlight focusing under a wind-disturbed sea surface. Oceanologia 23: 15–42

Falkowski PG, Greene R, Kolber Z (1994) Light utilization and photoinhibition of photosynthesis in marine phytoplankton. In: Baker NR, Bowyer JR (eds) Photoinhibition of photosynthesis: from molecular mechanisms to the field. BIOS Scientific Publishers, Oxford, p 407–432

Falkowski PG, Raven JA (1996) Aquatic photosynthesis. Blackwell Science, Oxford

Fryxell GA, Kendrick GA (1983) Austral spring microalgae across the Weddell Sea ice edge: spatial relationships found along a northward transect during AMERIEZ 83. Deep Sea Res 35:1–20

Gilmore AM, Yamamoto HY (1993) Linear models relating xanthophylls and lumen acidity to non-photochemical fluorescence quenching. Evidence that antheraxanthin explains zeaxanthin-independent quenching. Photosynth Res 35:67–78

Goericke R, Repeta DJ (1993) Chlorophylls *a* and *b* and divinyl-chlorophylls *a* and *b* in the open subtropical North Atlantic Ocean. Mar Ecol Prog Ser 101:307–313

Guillard RRL, Ryther JH (1962) Studies on marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. Can J Microbiol 8:229–239

Harrison WG, Platt T (1986) Photosynthesis-irradiance relationships in polar and temperate phytoplankton. Polar Biol 5:153–164

Johnsen G, Prézelin BB, Jovine RVM (1997) Fluorescence excitation spectra and light utilization in two red tide dinoflagellates. Limnol Oceanogr 42(5):1166–1177

Kishino M, Takahashi M, Okami N, Ichimura S (1985) Estimation of spectral absorption coefficients of phytoplankton in the sea. Bull Mar Sci 37:634–642

Kraus GH, Weis E (1991) Chlorophyll fluorescence and photosynthesis: the basics. Annu Rev Plant Physiol Plant Mol Biol 42:313–349

Liaaen-Jensen S (1978) Marine carotenoids. In: Sheuer PJ (ed) Marine natural products. Academic Press, New York, p 1–73

Lizotte MP, Sullivan CW (1991) Photosynthesis-irradiance relationships in microalgae associated with Antarctic pack ice: evidence for in situ activity. Mar Ecol Prog Ser 71: 175–184

Mitchell BG (1990) Algorithms for determining the absorption coefficient of aquatic particulates using the quantitative filter technique (QFT). In: Spinrad R (ed) Ocean Optics X. Society of Photo Optical Instrumentation Engineers, Bellingham, WA, p 137–148

Mitchell BG, Kiefer DA (1988) Chlorophyll *a* specific-absorption and fluorescence excitation spectra for light-limited phytoplankton. Deep Sea Res 35:639–663

Moisan TA, Mitchell BG (1997) Photophysiological adaptation of *Phaeocystis antarctica* Karsten under PAR Light Limitation. Limnol Oceanogr (in press)

Neale PJ (1987) Algal photoinhibition and photosynthesis in the aquatic environment. In: Kyle D, Osmond DB, Arntzen CJ (eds) Photoinhibition. Elsevier, New York, p 39–65

Nelson NB, Prézelin BB (1990) Chromatic light effects and physiological modeling of absorption properties of *Heterocapsa pygmaea* (= *Glenodinium* sp.). Mar Ecol Prog Ser 63:37–46

Olaizola M, Bienfang PK, Ziemann DA (1992) Pigment analysis of phytoplankton during a subarctic bloom: xanthophyll cycle. J Exp Mar Biol Ecol 158:59–74

Olaizola M, La Roche J, Kolber Z, Falkowski PG (1994) Non-photochemical fluorescence quenching and the diadinoxanthin cycle in a marine diatom. Photosynth Res 41: 357–370

Osmond B (1981) Photorespiration and photoinhibition. Some implications for the energetics of photosynthesis. Biochim Biophys Acta 639:77–98

Palmisano AC, SooHoo JB, SooHoo SL, Kottmeier ST, Craft LL, Sullivan CW (1986) Photoadaptation in *Phaeocystis pouchetii* advected beneath annual sea ice in McMurdo Sound, Antarctica. J Plankton Res 8:891–906

Perry MJ, Talbot MC, Alberte RS (1981) Photoadaptation in marine phytoplankton: response of the photosynthetic unit. Mar Biol 62:91–101

Platt T, Harrison WG, Irwin B, Horne EP, Gallegos CL (1982) Photosynthesis and photoadaptation of marine phytoplankton in the Arctic. Deep Sea Res 29:1159–1170

Post AF, Dubinsky Z, Wyman K, Falkowski PG (1985) Physiological responses of a marine planktonic diatom to transitions in growth irradiance. Mar Ecol Prog Ser 25:161–169

Robinson DH, Arrigo KR, Iturriaga R, Sullivan CW (1995) Microalgal light-harvesting in extreme low-light environments in McMurdo Sound, Antarctica. J Phycol 31: 508–520

Sakshaug E, Johnsen G, Andresen K, Vernet MV (1991) Modeling of light-dependent algal photosynthesis and growth: experiments with the Barents Sea diatoms *Thalassiosira nordenskioeldii* and *Chaetoceros furcellatus*. Deep Sea Res 38:415–430

Sakshaug E, Slagstad D (1991) Light and productivity of plankton in polar marine ecosystems: a physiological view. In: Sakshaug E, Hopkins CCE, Oritsland NA (eds) Proceedings of the Pro Mare Symposium on Polar Marine Ecology. Polar Res 10:69–85

Schenck H (1957) On the focusing of sunlight by ocean waves. J Opt Soc Am 60:1072–1079

Schubert H, Kroon BMA, Matthijs HCP (1994) *In vivo* manipulation of the xanthophyll cycle and the role of zeaxanthin in the protection against photodamage in the green alga *Chlorella pyrenoidosa*. J Biol Chem 269:7267–7272

Smith WO (1987) Phytoplankton dynamics in marginal ice zones. Oceanogr Mar Biol Annu Rev 25:11–38

Smith WO, Nelson DM (1985) Phytoplankton bloom produced by a receding ice edge in the Ross Sea: spatial coherence with the density field. Science 227:163–167

Sosik HM, Mitchell BG (1991) Absorption, fluorescence and quantum yield for growth in nitrogen limited *Dunaliella tertiolecta*. Limnol Oceanogr 36(5):910–921

Stramski D, Legendre L (1992) Laboratory simulation of light-focusing by water-surface waves. Mar Biol 114:341–348

Stramski D, Legendre L (1993) Photosynthetic and optical properties of the marine chlorophyte *Dunaliella tertiolecta* grown under fluctuating light caused by surface-wave focusing. Mar Biol 115:363–372

Vincent WF, Neale PJ, Richerson PJ (1984) Photoinhibition: algal responses to bright light during diel stratification and mixing in a tropical alpine lake. J Phycol 20:201–211

Weis E, Berry JT (1987) Quantum efficiency of Photosystem II in relation to 'energy'-dependent quenching of chlorophyll fluorescence. Biochim Biophys Acta 894:198–208

Weisse T, Tande K, Verity P, Hansen F, Gieskes W (1994) The trophic significance of *Phaeocystis* blooms. J Mar Sys 5: 67–79

Welschmeyer NA, Hoepffner NA (1986) Rapid xanthophyll cycling: an in situ tracer mixing in the upper ocean. EOS 67:969

Willemoës M, Monas E (1991) Relationship between growth irradiance and the xanthophyll cycle pool in the diatom *Nitzschia palea*. Physiol Plant 83:449–456

Yamamoto HY, Kamite L (1972) The effects of dithiothreitol on violaxanthin de-epoxidation and absorbance changes in the 500 nm region. Biochim Biophys Acta 267:538–543

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