Photoadaptive response during the development of a coastal Antarctic diatom bloom and relationship to water column stability

Abstract—The ratio of the xanthophyll pigments diadinoxanthin and diatoxanthin to chlorophyll a [(DD + DT):Chl a] was used as a photoadaptive index during the development of a large Antarctic diatom bloom. This index was found to track fluctuations in the incident solar irradiance and the in situ light field over a 3-order-magnitude change in the water column biomass. Depth profiles of the (DD + DT):Chl a ratio show that the upper mixed layer, assessed by physical data, was in fact stable over the course of the month. Diel experiments conducted over the same period showed a delayed (5–8 h) response of the DD + DT pool to the instantaneous $Q_{uv}$ (400–700 nm) irradiance. These time-series results illustrate the potential use of xanthophyll pigments in assessing phytoplankton light histories and the degree of water column stability.

The Southern Ocean supports a rich biotic ecosystem and has been recognized as playing an important role in many global processes, such as biogeochemical cycling (Nelson and Smith 1991) and the sequestration of atmospheric CO$_2$ (Siegenthaler and Sarmiento 1993). These processes are ultimately dependent on autotrophic production by phytoplankton. Although a continued subject of debate (Chisholm and Morel 1991), the primary mechanism controlling phytoplankton production over much of the Southern Ocean is thought to be light limitation as a result of the deep vertical mixing, characteristic for the region (Mitchell et al. 1991; Nelson and Smith 1991; Sakshaug et al. 1991). This is especially true in neritic and shelf water where potentially lim-
Dharmalingam replete (de Barr et al. 1995). Assessing the ability of phytoplankton to adapt to changing light fields in these turbulent environments is therefore fundamental to improving our mechanistic understanding of the magnitude and distribution patterns of biomass and primary production in the Southern Ocean.

To date, most studies in the Southern Ocean have been conducted shipboard and have focused on describing the spatial variability of phytoplankton abundance, distribution, physiology, and productivity (El-Sayed and Weber 1982; El-Sayed et al. 1983; Smith and Nelson 1985; Bodungen et al. 1986; Wilson et al. 1986). Although spatial studies have shown photoadaptive indices in relation to instantaneous conditions, interpretation of results are either in the context of steady-state conditions or an assumed prior condition (Mitchell and Holm-Hansen 1991; Boyd et al. 1995). The ability of phytoplankton to adapt to transient conditions is a dynamic process and warrants investigation over the temporal scales of adaptation. Temporal variability in phytoplankton dynamics have been scarcely documented for the Southern Ocean (Whitaker 1982; Mitchell and Holm-Hansen 1991; Rivkin 1991), and time-series field investigations of photoadaptive processes are rare. Knowledge of the timescales and rates of these processes is critical when attempting to assess the mechanisms controlling primary productivity and phytoplankton growth. Additionally, identification of photoadaptive indices will provide insight on the light histories of phytoplankton and may yield information of the mixing processes operating on similar timescales (Lewis et al. 1984; Cullen and Lewis, 1988).

Phytoplankton respond to changes in the light intensity and quality by differentially altering the relative amounts of various pigment concentrations and ratios. Pigments serve two major functions in the cell. Chlorophylls and most carotenoids absorb light for photosynthesis, while some carotenoids function largely as photoprotective pigments (Hagar and Stransky 1970; Stransky and Hagar 1970; Bidigare et al. 1987, 1993; Demers et al. 1991; Kerhervé 1991; Frank et al. 1994; Johnsen et al. 1997). In diatoms, the carotenoids diadinoxanthin (DD) and diatoxanthin (DT) function as a photoprotective system, undergoing a light regulated de-epoxidation/epoxidation known as the rapid xanthophyll cycle. These pigments dissipate excess energy by non-photothermal fluorescence quenching, as recently described for marine diatoms (Arsalane et al. 1994; Olaizola et al. 1994) and higher plants (Brugnoli et al. 1994). On timescales of seconds to hours, the DD + DT pool has been found to remain constant (Welschmeyer and Høeppfner 1986; Demers et al. 1991; Caron et al. unpubl.); however, over timescales of hours to days, the pool increases in response to high irradiances (Hagar and Stransky 1970; Stransky and Hagar 1970; Bidigare et al. 1987). Given the changes in in situ light fields in turbulent environments, it has been hypothesized that these pigments could be used as indices of phytoplankton light histories and for potentially determining vertical mixing rates on identical timescales (Lewis et al. 1984; Welschmeyer and Høeppfner 1986; Bidigare et al. 1987). Conceptually, the DT:DD ratio would reflect rapid timescale responses (seconds to hours), while a measure of the total xanthophyll pool (DD + DT) could assess a photoresponse over timescales of hours to days.

Although the DT:DD ratio was found to respond to changes in in situ light fields in the Subarctic, it was unreliable for inferring mixing rates (Olaizola et al. 1992). This could have resulted from the long handling time of the samples relative to the rate of change between DD and DT and the procedural difficulties in quantifying DT in the field. Recognizing this, Claustre et al. (1994) assessed in situ phytoplankton photoadaptation and kinetic rates of photoadaptation in a frontal region in the Mediterranean Sea using DD:Chl a. Here, in order to avoid possible influence of short-term fluctuations in the ratio, the biomass-specific xanthophyll pool, (DD + DT):Chl a (wt/wt), is quantified as a photophysiological index to assess the photoadaptive response of Antarctic diatoms to changing light environments on timescales of hours to weeks. Accessing these phytoplankton responses may further our understanding of the mechanisms controlling biomass and primary production in the Southern Ocean.

From 5 December 1991 until 7 January 1992, a total of 65 discrete water samples were collected at sta. B (Fig. 1; Waters and Smith 1992) as part of the Palmer Long-term Ecological Research (LTER) program (Ross and Quentin 1992). Sampling was conducted from a Mark V Zodiac with an effort to sample near solar noon. Prior to collection, vertical profiles of photosynthetically available radiation (400–700 nm, $Q_{par}$) were measured according to procedures detailed by Moline and Prézelin (1997). Incident $Q_{par}$ was also recorded continuously every 5 min over the 3-month period...
at Palmer Station. A comparison between the two measurements showed that surface $Q_{par}$ readings differed <5%. Temperature and conductivity data were also collected in conjunction with light measurements from a second Zodiac detailed in Smith et al. (1992), from which density profiles ($\sigma T$) were derived (see Moline et al. 1997). The upper mixed layer depths over the season were calculated from the $\sigma T$ profiles, according to Mitchell and Holm-Hansen (1991), as the depth of maximal $\sigma T$ gradient (>0.05 kg m$^{-3}$). Whole-water samples were collected in cleaned 5-liter GoFlo bottles, transferred to dark acid-washed polypropylene bottles, and returned to Palmer Station within 30 min, where samples remained in a cold room (−2°C) less than 20 min until analyses. In situ temperatures during the study ranged from −1.8°C to 1.2°C (Moline and Prézelin 1996).

One-liter samples were filtered and analyzed at Palmer Station for algal pigments using modified reverse-phase HPLC procedures described by Bidigare et al. (1989; see Moline and Prézelin 1997). Peak identities of algal extracts were determined by comparing their retention times with pure pigments provided by R. Bidigare. Quantification of specific pigments from the algal extracts were made by comparing peak areas with those from known amounts of pigment standards.

Relatively low wind speeds after the break up of the coastal fast ice resulted in a stable water column during the beginning of December 1991. This stability was enhanced with freshwater input into the local area from glacial melting and the depth of the upper mixed layer (UML) shallowed to ~20 m (Fig. 2A; Moline et al. 1997). Brunt-Väisälä frequencies (Pond and Pickard 1983) showed increased stability with time and depth over the study period with mean frequencies within the upper 20 m increasing from 1.5 cph on day 339 to 11.8 cph on day 7 (of 1992), high but within the range for marine systems (D. Siegel pers. comm.). With the combination of stability, increased light penetration, and the release of concentrated phytoplankton from the sea ice into the water column, a large bloom developed at Sta. B (Fig. 2B). This near unialgal bloom of Coscinodiscus spp., as confirmed by microscopic examination, persisted for 4 weeks and accounted for >95% of the carotenoid pigmentation (as fucoxanthin) at Sta. B (Moline and Prézelin 1997). Chl $a$ concentrations ranged from 0.3 to ~30 mg Chl $a$ m$^{-2}$. Integrated water column Chl $a$ biomass peaked at 612 mg Chl $a$ m$^{-2}$ the last day of 1991 (Moline et al. 1997).

In January 1992, there was a rapid community transition in the bloom from diatoms to a cryptophyte-dominated community (Moline and Prézelin 1996). Unlike diatoms, cryptophytes do not have a xanthophyll cycle, and pigments that function as photoprotectants in the visible spectrum in this group are not specifically known. Experiments have shown that the primary cryptophyte-specific carotenoid, alloxanthin, functions largely as a photosynthetic pigment, with low transfer efficiency, but also as a photoprotective pigment (G. Johnsen and M. Vernet pers. comm.). The vertical distribution of alloxanthin: Chl $a$ at Sta. B during January 1992 showed a subsurface maximum occurring at the 5–10% $Q_{par}$ (0+) light levels, suggesting a photosynthetic function (data not shown). Because of the shift in community composition from diatoms to a taxonomic group where the photoprotective pigmentation is uncertain, this study focused on the period from day 339 of 1991 to day 7 of 1992, when diatoms were dominant and the major photoprotective pigments were the xanthophyll pigments DD and DT, with maximum concentrations of 2.7 mg m$^{-3}$ and 0.8 mg m$^{-3}$, respectively, during the bloom. While diatoms are a primary bloom forming phytoplankton group in the Antarctic, note that other groups containing xanthophyll pigments, such as prymnesiophytes (e.g. Phaeocystis spp.), also contribute significantly to phytoplankton biomass in the Southern Ocean (El-Sayed and Fryxell 1993).

Figure 3 shows the photophysiological index, (DD + DT): Chl $a$ (wt/wt), with depth over the study period. First, what is clearly evident is that the ratio decreased with depth for each sampling date, suggesting that the response rate of the photoprotective pigments was always greater than the rates of vertical mixing. Given this, the time evolution of the (DD + DT): Chl $a$ ratio also illustrated the response to changes in the incident light fields and the apparent optical properties of the water column. The 100-fold variation in phytoplankton biomass over the course of the bloom had a measurable effect on the attenuation of $Q_{par}$ in the water column. The 1% $Q_{par}$ light level shallowed from 60 m in early December to ~10 m at the peak of the bloom (Fig. 3). The contours of the (DD + DT): Chl $a$ ratio paralleled the increasing attenuation over the season with 1% $Q_{par}$ depth significantly correlating to an absolute (DD + DT): Chl $a$ ratio between 0.05 and 0.06 (R$^2$ = 0.98), indicating the diatom bloom was potentially light limited from self-shading. Photosynthesis-irradiance data revealed that, in fact, the depth of light limitation during the peak of the diatom bloom shallowed to ~5 m (data not shown; Moline et al. 1997).

Reports have documented the effect of nutrient stress on the accumulation of xanthophyll pigments in cultures (Geider et al. 1993; Olaizola 1993) and suggested a similar relationship in field measurements (Olaizola 1993; Babin et al. 1996). The production of xanthophyll pigments under nutrient-starved conditions is thought to be analogous to responses of phytoplankters to high light stress, increasing photoprotection by nonphotochemical quenching of excitation energy. While NO$_3^-$ and PO$_4^{3-}$ were significantly depleted (>25 < 0.05 µmol m$^{-3}$ NO$_3^-$ and >2.5 < 0.03 µmol m$^{-3}$ PO$_4^{3-}$) during the development of the bloom (Moline et al. 1997), associated increases in the (DD + DT): Chl $a$ ratio with either time or with depth were not detected (Fig. 3). Changes in nutrient concentrations and nutrient ratios were, however, timed to the shift in taxonomic composition of the phytoplankton at Sta. B (Moline et al. 1997).

In addition to tracking the increases in attenuation resulting from the bloom, the (DD + DT): Chl $a$ ratio was also significantly correlated (P < 0.001) to the light intensity at each depth and reflected the general decrease in the incident surface $Q_{par}$ measured over the 33-d period (Moline and Prézelin 1996). This agrees with in situ (Claustre et al. 1994) and laboratory studies (Demers et al. 1991; Claustre et al. 1994) showing the xanthophyll pool to be light dependent on daily timescales. Interestingly, the most significant correlation between the ratio and $Q_{par}$ was using the $Q_{par}$ integrated 6–8 h prior to collection and close to dawn [$R^2 = 0.86$; (DD + DT): Chl $a$ = 0.003($Q_{par}$) + 0.046; n = 65].
Fig. 2. A. Seasonal change in the depth distribution of $\sigma t$ (kg m$^{-3}$) at LTER Sta. B from 5 December 1991 to 7 January 1992. Profile dates are marked with filled arrows. The depths of the upper mixed layer (■) were calculated according to Mitchell and Holm-Hansen (1991). B. The seasonal change in the depth distribution of Chl $a$ (mg Chl $a$ m$^{-3}$) at LTER Sta. B from 5 December 1991 to 7 January 1992. Contours of Chl $a$ values in excess of 8 mg m$^{-3}$ are not shown. The distribution of discrete samples collected for HPLC determinations is shown by open circles. Contour plots were generated using the Delaunay triangulation method.

When integrated light from the previous day (18–24 h prior to collection) was included, the regression significance rapidly decreased, indicating the photoprotective response was not responding the light of the previous photoperiod. These results show a 6–8-h delay in the response of the (DD + DT): Chl $a$ ratio to the in situ light field and indicate there was little convective or wind-driven mixing over the course of the study.

The ratio of (DD + DT): Chl $a$ for each depth normalized to the surface values showed a significant linear relationship for light levels between 0.1 and 100% of the $Q_{par}$ (0°) value (Fig. 4). For light levels below 0.1%, the normalized (DD
Fig. 3. Seasonal change in the depth distribution of the (diadinoxanthin + diadinoxanthin): Chl a ratio (wt/wt) [(DD + DT): Chl a] from 5 December 1991 to 7 January 1992. The distribution of discrete samples collected for HPLC determinations is shown by open circles. The depths of the 1% surface Q\text{PAR} are indicated by closed circles.

The value of (DD + DT): Chl a was maximal. This corresponds well with the lagged response in the ratio found for the integrated in situ irradiance measurements and confirms the response time of the photoprotective pigment pool for this diatom bloom. This response is significantly slower than that found in kinetic experiments conducted in the Mediterranean Sea, where phytoplankton responded to changes in light intensity in <1 h (Claustre et al. 1994). In addition to differences in community composition between the two locations, the longer response times for Antarctic phytoplankton in this study may be a function of lower temperatures, slowing enzyme rate kinetics and the de novo synthesis of the xanthophyll pool. Temperature has been found to affect photosynthesis (Tilzer et al. 1986), respiration (Tilzer and Dubinsky 1987), growth rates (Sakshaug and Holm-Hansen 1986), and enzymatic activity (Li et al. 1984) in high latitude environments and therefore may similarly affect the turnover rates of the xanthophyll pool in natural populations.
The delayed response of the DD + DT pool to the in situ light field may have significant implications for primary productivity in this coastal environment. The peak accumulation of the photoprotective pigments occurred significantly later than the peak solar insolation and therefore may have potentially increase the susceptibility of the cell to photoinhibition during the midday hours. However, photoinhibition (measured by $P$ vs. $I$ relationships) was not detected at high irradiances, with the saturation irradiances for photosynthesis stable over the day and high light-saturated photosynthetic potentials ($3.5-3.6$ mg C mg Chl a m$^{-3}$ h$^{-1}$) with maximums centered within an hour of solar noon (Moline and Prézelin 1997). This, however, is circumstantial evidence, given the complicated nature of photoinhibition (see Baker and Bowyer 1994). Daily minimum values of (DD + DT):Chl a for the diel experiments were $0.15 \pm 0.03$ for surface samples and $0.07 \pm 0.01$ for Chl a max. samples. These levels of photoprotective pigmentation may have been sufficient to dissipate excess energy in the hours up to and following solar noon, with the synthesis of additional pigmentation ($\sim 30\%$) responding to the increasing sensitivity to high light and susceptibility to photoinhibition later in the day (Marra 1978). This mechanism is probable for the diatoms in this study, which were isolated in the stable surface waters at Sta. B for more than a month by stratification and exposed to high irradiances. More than 70% of the water column production was light saturated (Claustre et al. 1997), and 85% of production was light saturated in the upper 5 m (unpubl. data), further suggesting that these surface populations were susceptible to high light stress.

With (DD + DT):Chl a responding in a predictable manner to the light fields in stable in situ and in vitro conditions, the ratio may provide additional information on the vertical stability of the water column and rates of mixing, given that most phytoplankton contain xanthophyll pigmentation. Traditional interpretation of density profiles provides the depth of mixing, but provides no information on the timescales of the mixing processes. The use of the photoadaptive index (DD + DT):Chl a is illustrated in two profiles collected in November 1991 off the Antarctic Peninsula (Fig. 5). Fig. 5A shows the profile collected at 1030 h on day 317 from a protected nearshore coastal station in Dallmann Bay. The mixed layer was $\sim 60$ m, typical for this area (Mitchell and Holm-Hansen 1991), and the incident solar irradiance was $\sim 1,975 \mu$mol m$^{-2}$ s$^{-1}$. Along with density, the (DD + DT):Chl a ratio showed uniform distribution with depth. The ratio within the layer was higher compared to samples taken at or below the pycnocline. The added physiological response information clearly shows that this mixed layer, as defined by physical data, was mixing or had been recently mixing faster than the photophysiological response. The high ratio within the layer also indicates exposure to high surface light intensities. A second profile, collected from an offshore station ($\sim 100$ km from Anvers Island) on day 322 with a surface irradiance of $\sim 775 \mu$mol m$^{-2}$ s$^{-1}$, showed a uniform density distribution to $\sim 100$ m (Fig. 5B). Unlike the nearshore station, however, the (DD + DT):Chl a ratio decreased exponentially with depth, implying that the rate of vertical mixing was in fact slower than the photoprotective response.

Vertical displacement rates were estimated for these profiles following the approach introduced by Falkowski (1983). Using the measured (DD + DT):Chl a ratios of the surface compared with those at the 1% $Q_{pr}$ light depths in Fig. 5, the vertical displacement velocity in the euphotic zones were calculated as

$$\frac{\Delta z}{\Delta t} = \frac{\Delta z(-k)}{\ln[(R_i - R_0)/(R_0 - R)]},$$

where $k$ is the first-order rate constant of 0.5 h$^{-1}$ from Claustre et al. (1994), $R_i$ is the (DD + DT):Chl a ratio at time $t$, $R_0$ is the ratio at time zero, $R$ is the ratio after at infinite time after adaptation, and $\Delta z$ is the vertical distance between the surface and the depth of the 1% $Q_{pr}$ (see Falkowski 1983). Vertical displacement estimates and mixed layer turnover times were within previous reported values (Falkowski 1983; Olazábal et al. 1992) and consistent with the profiles in Fig. 5, with the highest rates for the nearshore station and virtually no vertical movement for the offshore station where the photoprotected response was most pronounced (Table 1).

While enhanced stratification does increase the overall stability of the water column, as seen during this study as the diatom bloom progressed (Fig. 2), the absence of a pycnocline can not be used to infer mixing, at least on the timescales of physiological response. The discrepancies between stratification and stability have been emphasized in previous studies (Ryther and Hulburt 1960; Townsend et al. 1992); however, this point is often overlooked. The degree of stability and the residence time of phytoplankton in the eu-
photic zone is critical for photoadaptation, growth, and productivity (Smetacek and Passow 1990; Nelson and Smith 1991), and therefore interpretations of these indices within the water column based solely on physical data may be limited. The two profiles in Fig. 5 illustrate the utility of (DD + DT):Chl α in assessing water column stability. This index may be of particular interest for programs that utilize long-term (12–24 h) static bottle incubation measurements to determine in situ rates of primary productivity (e.g. Southern Ocean JGOFS). Had 12-h static bottle incubations been made in the water column of Fig. 5A, the water surrounding the incubation bottles would be displaced >12.5 m over the course of the incubation and may not have accurately reflected the in situ productivity rates. Under these conditions the (DD + DT):Chl α index may be valuable in providing additional information toward better data interpretation.

The stable conditions during this study allowed validation of the photoadaptive index (DD + DT):Chl α for interpreting phytoplankton light histories. On scales of hours to weeks, there was a strong coherence of the photoprotective pigment response to the changing light field. While the pool size of DD + DT responded within hours to the instantaneous light field, there is a longer term response to the day-to-day changes in solar insulation. The kinetic response of the xanthophyll pool occurred on similar timescales of vertical mixing (Denman and Gargett 1983), demonstrating the usefulness in (DD + DT):Chl α for improving interpretation of water column stability in diatom-dominated waters. Further studies refining the rate kinetics of these photoprotective pigments and the use of (DD + DT):Chl α as an index of vertical stability (much like the recent model by Doney et al. [1995]) for photochemical species will provide a better understanding of the effects of high vertical mixing on limiting primary production in the Southern Ocean.

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Table 1. Estimates of vertical displacement rates and turnover times in the mixed layer from profiles in Fig. 5.

<table>
<thead>
<tr>
<th>Sta.</th>
<th>z_e (m)</th>
<th>z_w (m)</th>
<th>Estimated vertical displacement rate (cm s⁻¹)</th>
<th>Estimated mixed layer turnover rate (d⁻¹)</th>
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<tr>
<td>700.040</td>
<td>75</td>
<td>62</td>
<td>≥2.9×10⁻²</td>
<td>≥0.4</td>
</tr>
<tr>
<td>600.140</td>
<td>68</td>
<td>115</td>
<td>≤2.8×10⁻⁵</td>
<td>—</td>
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</tbody>
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* Depth of the euphotic zone (1% Q_10).
† Depth of the mixed layer.

References


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