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Phytoplankton pigments at the Weddell–Scotia confluence during the 1993 austral spring

A.C.Sigleo, P.J.Neale[1] and A.M.Spector[1]

US Environmental Protection Agency, Western Ecology Division, 2111 SE Marine Science Drive, Newport, OR 97365-5260 and College of Oceanic and Atmospheric Sciences, Oregon State University, Newport, OR 97365 and [1]Smithsonian Environmental Research Center, PO Box 28, Edgewater, MD 20715, USA

Abstract. During a 1993 austral spring cruise, a complex biomass was encountered near South Orkney Island that ranged from a low-biomass, *Chaetoceros tortissimus* assemblage south of the front towards the ice edge, to a high-biomass, *Thalassiosira gravida*-dominated assemblage at the northern edge. The maximum levels of chlorophyll (Chl) *a* (up to 6 mg m^{-3}) were higher than those observed in previous high-performance liquid chromatography-based studies of pigments in the pelagic Southern Ocean. The non-photosynthetic pigment chlorophyllide *a* comprised up to 75% of the chlorophyllous pigments in the southern assemblage, but < 5% in the northern assemblage. Concentrations of the xanthophylls diadinoxanthin (DD) and diatoxanthin (DT), used as indicators of mean irradiance, indicated low-light-adapted populations. Low-light DD + DT/Chl *a* ratios in surface waters indicated that vertical mixing limited phytoplankton residence time in the near-surface layer, and thus limited exposure to maximum irradiance. Deck incubations of natural assemblages indicated that the dark epoxidation reaction (i.e. the return of DT to DD) was a two-step reaction with the initial rate being more rapid ($t^{1/2}$ = 9.5 min) than the second ($t^{1/2}$ = 55 min). Fucoxanthin, a major diatom pigment, was more stable chemically in the water column than Chl *a*, and the vertical profiles of fucoxanthin followed those of chlorophyllide *a* in some cases. The formation and apparent stability of chlorophyllide *a* and fucoxanthin are important considerations when estimating photosynthetically active biomass over large regions of the ocean.

Introduction

Phytoplankton pigments are used to indicate phytoplankton biomass, composition by taxonomic group, and phytoplankton physiological condition or growth stage (Jensen and Sakshaug, 1973; Jeffrey, 1974; Hallegraeff, 1981; Bidigare *et al*., 1986, 1996; Everitt *et al*., 1990; Vernet *et al*., 1994). The following study focused on the pigment compositions in an area of high phytoplankton biomass during the austral spring and summer that characteristically occurs in the region between Elephant Island and South Orkney Island, between the Scotia Sea to the north and the Weddell Sea to the south (El-Sayed and Taguchi, 1981; Nelson *et al*., 1989). In this region, an upper surface layer of cooler, fresher water from the Weddell Sea flows north through the Scotia arc over the slightly warmer, denser water of the Scotia Sea (Nelson *et al*., 1987, 1989; Bianchi *et al*., 1992). High-altitude imagery for the region also indicates large amounts of phytoplankton productivity in this hydrographic region (Sullivan *et al*., 1993).

Austral spring blooms related to increased solar irradiance and the ice retreat reported for the Weddell Sea and Weddell–Scotia Confluence in the vicinity of 60°S are often dominated by diatoms, although the prymnesiophyte *Phaeocystis* sp. can also provide a major portion of the biomass (El-Sayed and Taguchi, 1981).

Rich blooms of diatoms, indicated by chlorophyll (Chl) *a* concentrations up to 7 mg m^{-3}, with exceptional bloom centers up to 25 mg m^{-3}, were observed in Gerlache and Bransfield Straits (Mandelli and Burkholder, 1966). Mandelli and Burkholder also observed a considerable range in biological vitality, with 'old' cells being characterized by low photosynthetic efficiency (Mandelli and Burkholder, 1966).

Pigment studies using high-performance liquid chromatography (HPLC) found that phytoplankton populations, represented by class-specific indicator pigments, were variable throughout Antarctic waters (Bidigare *et al*., 1986, 1996; Bidigare, 1989; Buma *et al*., 1990, 1992; Vernet *et al*., 1994). In the waters west of the Antarctic Peninsula, the presence of the pigment 19′-hexanoyloxyfucoxanthin during November 1991 indicated a prymnesiophyte-dominated population, along with cryptomonads as indicated by alloxanthin (Vernet *et al*., 1994). Bidigare *et al*. found during October–November 1990 that the *Phaeocystis*-dominated community within 50 km of the ice edge was replaced by a diatom-dominated community in the open water 150 km from the ice edge (primarily *Chaetoceros* spp. and *Thalassiosira* spp.) (Bidigare *et al*., 1996). In the Weddell–Scotia Confluence, Buma *et al*. used cluster analysis of accessory pigment concentrations to define four plankton communities that included a diatom-dominated community at the Weddell–Scotia Confluence and three phytoflagellate communities slightly further north and south of the Weddell–Scotia Confluence (Buma *et al*., 1990).

Chromophyte algae live in a dynamic, turbulent environment, frequently under rapidly changing light conditions. To accommodate this variation in light intensity, rapid (of the order of minutes) and reversible changes in the *in vivo* fluorescence per cell occur when diatom cells are transferred from low to high light intensities (Sakshaug *et al*., 1987). The rapid change in the *in vivo* fluorescence per cell is associated with rapid and reversible changes in the concentrations of carotenoid pigments in the xanthophyll cycle (Sakshaug *et al*., 1987; Demers *et al*., 1991; Brunet *et al*., 1993; Olaizola, 1993).

The xanthophyll cycle consists of de-epoxidation/epoxidation reactions that transform xanthophylls from the epoxide or oxygen-bound form to the de-epoxide forms. In diatoms, the de-epoxidation reaction converts diadinoxanthin (DD) to diatoxanthin (DT) under increasing irradiance or strong light, concurrent with changes in cell fluorescence (Demers *et al*., 1991). For this reason, some investigations have considered using DT/DD ratios in the oceans as a potential index of vertical mixing, and as an index of irradiance exposure (Olaizola *et al*., 1992; Brunet *et al*., 1993; Olaizola, 1993). Brunet *et al*. found that measurements of DD and DT provided an indication of the light environment of phytoplankton (Brunet *et al*., 1993). Their data demonstrated a direct relationship between light intensity and DD + DT/Chl *a* ratios, indicating that phytoplankton cells were adapting to local irradiances. We further examined DT/DD ratios in the ocean during a day cycle, along with the rate of the reverse epoxidation reaction.

In the present paper, primary and accessory phytoplankton pigments from a series of stations near 60°S, 51°W (Table I) were used to estimate phytoplankton composition, biomass and phytoplankton physiological condition. The pigments

Table I. Station locations and 1993 sample dates

Station	Date	Latitude (degrees:minutes)	Longitude
A	October 18	58:47.00	49:49.00
B	October 18	59:40.00	50:00.00
C	October 18	60:10.00	50:00.00
M, M′	October 20, 21	59:34.60	50:00.00
N	October 24	60:03.55	52:09.41
P	October 26	50:55.00	52:09.34
R, R′	October 29, November 5	59:36.99	52:09.92

were measured across a zone of high biomass at the confluence of the Scotia and Weddell Seas ~100 km north of the marginal ice zone as part of a larger investigation on the effects of stratospheric ozone depletion and the concomitant increase in UVB (280–320 nm) radiation on phytoplankton photosynthetic processes (Neale and Spector, 1994; Sigleo and Neale, 1994; Ferreyra, 1995; Neale *et al*., 1998). The standard method for determining Chl *a* and phaeopigment concentrations using a fluorometer (Strickland and Parsons, 1972) also was used as a rapid shipboard method for estimating plankton biomass. The physiological status of phytoplankton assemblages was estimated from the relative proportions of Chl *a* and chlorophyllide *a* (Jensen and Sakshaug, 1973; Gowen *et al*., 1983; Ridout and Morris, 1985; Klein and Sournia, 1987). Chlorophyllide *a* is formed by chlorophyllase activity that hydrolyzes the phytol from the Chl *a* molecule in mature or senescent diatoms (Jeffrey, 1974; Simpson *et al*., 1976). Chlorophyll *a* molecules that have degraded to chlorophyllide *a* are not photosynthetically active (Simpson *et al*., 1976).

Method

Water samples were collected from the upper 200 m at the confluence of the Weddell–Scotia water masses near 60°S, 51°W (Figure 1) between 18 October and 5 November 1993 from the R/V 'Nathaniel B. Palmer'. The vessel was equipped with a General Oceanics 12 Niskin bottle rosette sampler surrounding a Sea Bird Electronics Model SBE-9 conductivity, temperature and depth (CTD) profiler designed for real-time operation. CTD data were logged on a Hewlett Packard Vectra PC using standard Sea Bird software.

The formation of DT from the parent DD with variations in irradiance was examined over a day cycle in samples collected by bucket over the port rail, and immediately syringe filtered (100 ml) through 25 mm GF/F filters. The filters were submerged in 100% acetone within 2 min from the time the bucket surfaced in the water, as monitored by stopwatch, and analyzed as described below. The time course of the reverse dark epoxidation reaction (DT → DD) was estimated by filling a brown glass bottle after a 15 min deck exposure to sunlight. The bottle was capped, wrapped in black plastic and stored in the dark. Samples were withdrawn from the bottle with a 100 ml syringe at ~15 min intervals.

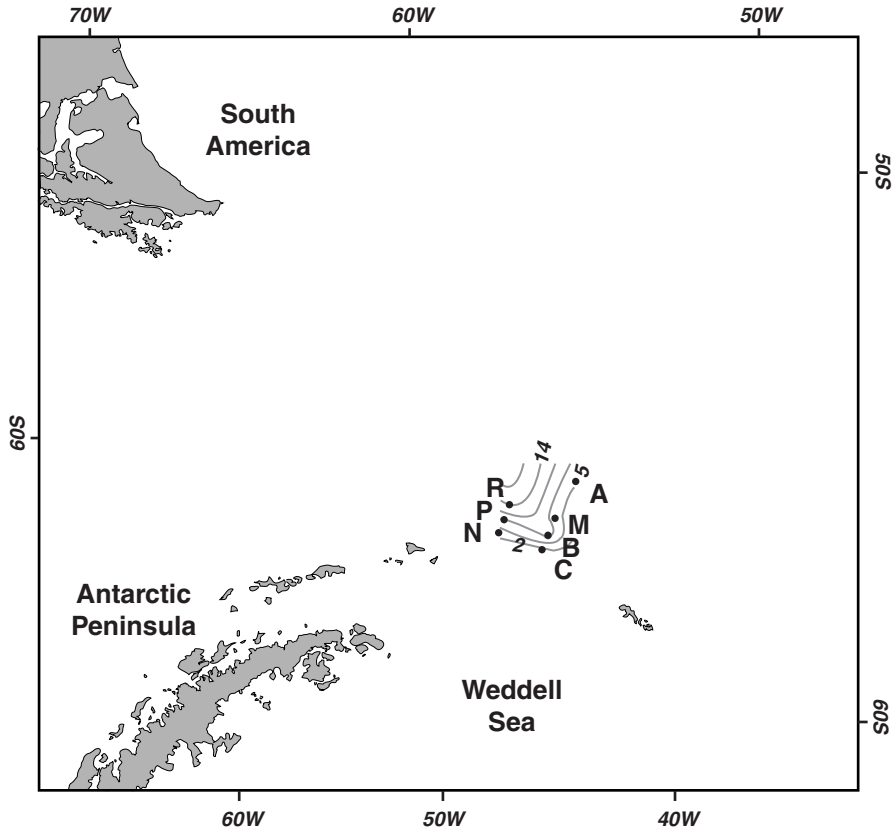

Fig. 1. Location map for stations listed in Table I. Contour lines indicate total pigment concentrations in mg m^{-3}.

For pigment analyses, 500 ml–2 l seawater samples were filtered through 47 mm GF/F glass fiber filters. The filters were immediately submerged in 1.5 ml of 100% acetone and extracted at –20°C in the dark for 18–24 h. Extracted samples were centrifuged for 5 min to remove cellular debris and analyzed at sea by reverse-phase HPLC using a St Johns Associates liquid chromatograph with a Microsorb-MV C_{18} column (4.6 × 100 mm, 3 µm particle size; Rainin Instrument Corp.). The pigments were separated using a three-step solvent gradient based on the solvent compositions and ion-pairing solution of Mantoura and Llewellyn (Mantoura and Llewellyn, 1983). After injection (Alcott Model 728 autosampler with a 20 µl loop), mobile phase A (80:15:5, methanol:water:ion-pairing solution) was programmed for 2 min, phase B (90:8:2, methanol:water:ion-pairing solution) for 8 min and mobile phase C (100% methanol) for 32 min, all at a flow rate of 0.5 ml min^{-1}. Pigments were detected sequentially with a Waters Model 420 fluorescence detector (excitation 400–460 nm, emission >600 nm) for chlorophylls and phycoerythrin, and a Hewlett Packard 1050 variable wavelength UV–visible

absorbance detector programmed at 440 nm for carotenoid pigments. Eluting peaks were quantified with Hewlett Packard Model 3392A integrators.

The identity and relative retention time for each pigment were determined after extraction from pure cultures of phytoplankton with well-established pigment contents (Wright *et al.*, 1991). The retention times and response factors were confirmed with standards separated in the above manner by Welschmeyer [Environmental Protection Agency (EPA) contract, EPA, Cincinnati]. The chlorophyllides and phaeopigments for HPLC standards were derived chemically from Chl *a* according to the procedures of Mantoura and Llewellyn (Mantoura and Llewellyn, 1983). All pigment data are arithmetic means of duplicate injections. The analytical precision, expressed as the standard deviation for multiple standard injections, was ±7% (n = 19) for the Antarctic cruise. Standards were analyzed before each sample group, and after each 10 samples. Reagent blank concentrations were three orders of magnitude below sample concentrations for Chl *a* and undetectable for all other pigments.

Chlorophyll *a* and phaeopigment concentrations were also determined on a Turner Designs Model 10 fluorometer calibrated with authentic Chl *a* (Sigma) in 90% acetone (Strickland and Parsons, 1972). The concentration of Chl *a* in the standard was determined spectrophotometrically (Jeffrey and Humphrey, 1975). The samples (34 ml) were prepared for analysis by filtration through Whatman GF/F 25 mm filters and extracted in 10 ml of 90% acetone overnight at –20°C in the dark. Turner fluorometer measurements were taken before and after acidification to determine both Chl *a* and total phaeopigments (Strickland and Parsons, 1972). Samples for phytoplankton counts were preserved with Lugol's solution and identified using the Utermöhl method with an inverted Zeiss microscope (Utermöhl, 1958).

Results

During October–November 1993, surface phytoplankton abundance increased sharply northward from 60°S, 50°W, ~100 km north of the ice edge (Figure 1; Table I). At stations north of 60°S, there was a well-defined surface upper mixed layer as illustrated by the sharp decline in pigment concentrations at depths of 60–100 m (Figure 2) that coincided with a pronounced pycnocline (Neale *et al.*, 1998). Experimental work was carried out primarily at Station P (26–28 October) and Station R (29 October–8 November). During the latter period, westerly winds continued at 10–15 m s^{-1}, maintaining active and deep vertical mixing. Depression of fluorescence near the surface was absent (Neale *et al.*, 1998). Samples were collected for pigment analyses in multiple parts of the biomass, processed identically, and analyzed at sea within 24 h of collection to eliminate problems of sample degradation. Jeffrey and Hallegraeff (Jeffrey and Hallegraeff, 1987) suggested that chlorophyllase may be released during filtration, particularly of mature cells, and recommended pigment extractions in 100% acetone to inactivate the chlorophyllase, a procedure followed in the present study. Also, we found that 2 min (or less) for filtration and submergence in acetone minimized the effects of chlorophyllase activity. The differences in sample pigment concentrations,

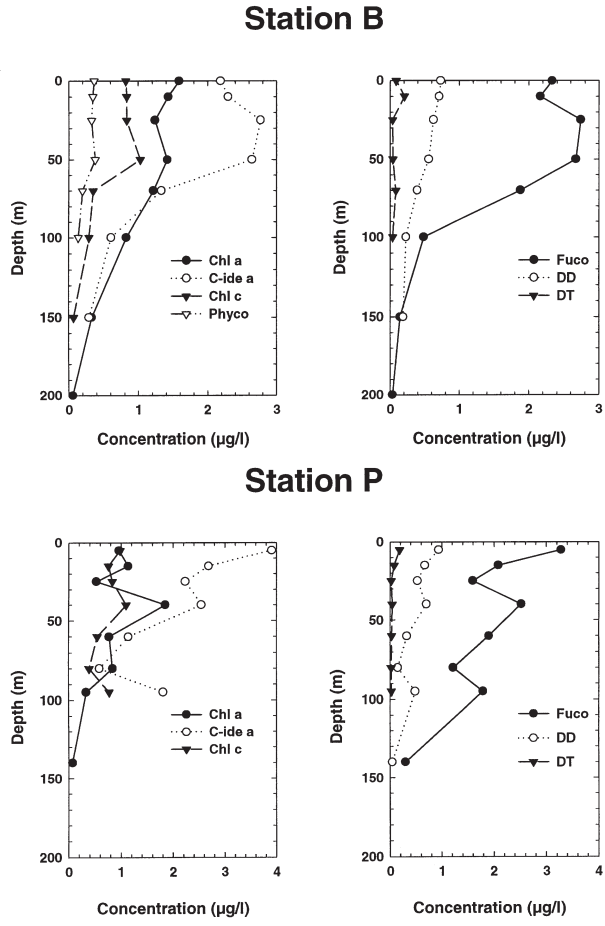

Fig. 2. Vertical distributions of chlorophylls (left panels) and carotenoids (right panels) measured at Stations B (18 October) and P (26 October). High concentrations of chlorophyllide *a* open squares are evident. Fucoxanthin profiles (right panels) follow those of chlorophyllide *a*.

therefore, were indicative of phytoplankton abundance and physiological condition, and were not artifacts from differences in sample treatment, handling or storage. Despite these precautions, there is still a possibility that some chlorophyllase was released during filtration.

Total pigment concentrations in the euphotic zone ranged from 0.78 µg l^{-1} (or mg m^{-3}) at Station C on 18 October to >14 µg l^{-1} on 5 November at Station R (Table II). Chlorophyll *a* concentrations ranged from 0.4 to 6.4 µg l^{-1}, and comprised between 16 and 46% by weight (10–36 mol%) of the total pigments (Table II). High concentrations of the accessory pigments fucoxanthin (up to 3.8 µg l^{-1}), Chl *c* (up to 1.5 µg l^{-1}) and the photoprotective carotenoids, DD and DT were also present (Figure 3). Chlorophyll *b* concentrations were low (20 ng l^{-1}) or below the detection limit. Phycoerythrin, indicative of cyanobacteria, was

Table II. Major and accessory pigment concentrations in micrograms per liter averaged over the upper mixed layer (upper 40–80 m to the surface)

Station and date	Pigments													
	Chl *a*	C-ide *a*	Me-ide	Chl *c*	Phyco	Fuco	DD	DT	Lutein	Zea	19′ Hexa	Peri	Viola	Total
A Oct. 18	0.85 ± 0.26	0.80 ± 0.15	0.27 ± 0.05	0.46 ± 0.08	0.18 ± 0.03	1.85 ± 0.45	0.88 ± 0.16	–	–	–	–	–	–	5.3
B Oct. 18	1.38 ± 0.15	2.24 ± 0.56	0.52 ± 0.17	0.77 ± 0.25	0.32 ± 0.07	2.36 ± 0.36	0.60 ± 0.14	0.09 ± 0.07	0.05 ± 0.02	0.12 ± 0.04	0.15 ± 0.03	_	0.06 ± 0.02	8.6
C Oct. 18	0.49 ± 0.10	–	–	–	0.15 ± 0.03	0.07 ± 0.01	0.05 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	–	0.03 ± 0.01	_	_	0.8
M Oct. 20	1.12 ± 0.32	1.06 ± 0.47	0.45 ± 0.13	0.67 ± 0.12	0.22 ± 0.06	1.55 ± 0.45	0.33 ± 0.08	0.04 ± 0.01	0.03 ± 0.01	0.08 ± 0.03	0.08 ± 0.02	0.09 ± 0.04	–	5.7
M Oct. 21	1.50 ± 0.58	0.93 ± 0.19	0.40 ± 0.05	0.46 ± 0.07	0.28 ± 0.05	1.83 ± 0.44	0.58 ± 0.33	0.06 ± 0.03	0.06 ± 0.02	0.10 ± 0.02	0.13 ± 0.02	0.05 ± 0.01	0.07 ± 0.02	6.5
N Oct. 24	0.46 ± 0.09	0.50 ± 0.19	0.21 ± 0.01	0.26 ± 0.05	0.14 ± 0.05	0.58 ± 0.09	0.22 ± 0.14	0.02 ± 0.01	–	–	0.05 ± 0.01	–	–	2.4
P Oct. 26	1.01 ± 0.50	2.50 ± 0.99	0.50 ± 0.16	0.77 ± 0.23	0.30 ± 0.09	2.10 ± 0.73	0.55 ± 0.28	0.08 ± 0.07	0.06 ± 0.04	–	–	–	–	7.9
R Oct. 29	2.60 ± 0.62	0.56 ± 0.22	0.47 ± 0.07	0.62 ± 0.11	0.25 ± 0.04	2.01 ± 0.47	0.53 ± 0.09	0.04 ± 0.01	0.19 ± 0.19	–	–	–	–	7.3
R Nov. 5	6.40 ± 0.21	0.67 ± 0.01	–	1.50 ± 0.51	–	3.75 ± 0.20	1.30 ± 0.24	0.27 ± 0.13	0.12 ± 0.01	–	–	–	–	14.0

Chl *a*, chlorophyll *a*; C-ide *a*, chlorophyllide *a*; Me-ide, methylchlorophyllide *a*; Chl *c*, chlorophyll $c_{1\&2}$; Phyco, phycoerythrin; Fuco, fucoxanthin; DD, diadinoxanthin; DT, diatoxanthin; Zea, zeaxanthin; 19′ Hexa, 19′-hexanoyloxyfucoxanthin; Peri, peridinin; Viola, violaxanthin; –, not detected.

Table III. Ratios (w:w) of major and accessory pigments in the upper mixed layer (upper 40–80 m to the surface)

Station	Date	Pigment ratios				
		C-ide *a*	Fuco	Fuco	Chl *c*	DD + DT
		Chl *a*	Chl *a*	Chl *a* + C-ide *a* + Me-ide	Chl *a*	Chl *a*
A	October 18	1.02 ± 0.34	2.34 ± 0.20	0.97 ± 0.20	0.57 ± 0.14	–
B	October 18	1.63 ± 0.39	1.93 ± 0.29	0.58 ± 0.05	0.56 ± 0.15	0.50 ± 0.09
C	October 18	0.58 ± 0.29	0.15 ± 0.05	–	0.33 + 0.11	–
M	October 20	1.05 ± 0.59	1.49 + 0.55	0.59 ± 0.05	0.64 ± 0.21	0.33 + 0.09
M′	October 21	0.62 ± 0.29	1.41 ± 0.77	0.67 ± 0.19	0.34 + 0.11	0.31 ± 0.04
N	October 24	1.13 ± 0.43	1.29 ± 0.23	0.50 ± 0.01	0.56 ± 0.03	0.55 ± 0.32
P	October 26	2.71 ± 1.2	2.43 ± 0.76	0.62 ± 0.09	0.91 ± 0.37	0.74 ± 0.31
R	October 29	0.22 ± 0.08	0.77 ± 0.03	0.55 ± 0.02	0.24 ± 0.03	0.22 ± 0.02
R′	November 5	0.11 ± 0.01	0.59 ± 0.04	0.53 ± 0.03	0.23 ± 0.05	0.25 ± 0.01

Chl *a*, chlorophyll *a*; C-ide *a*, chlorophyllide *a*; Me-ide, methylchlorophyllide *a*; Chl *c*, chlorophyll $c_{1\&2}$; Fuco,

minor (~200 ng l^{-1}) but persistent (<4%) at most stations. 19′-Hexanoyloxyfucoxanthin, characteristic of prymnesiophytes such as *Phaeocystis* sp., was present at Stations B, M and N in the range 20–150 ng l^{-1}, or <5 mol% of the total. Peridinin and violaxanthin, indicative of dinoflagellates and chlorophytes, respectively, were detected (~20 ng l^{-1}) only in a few samples (Table II).

Fucoxanthin was the most abundant carotenoid and generally co-varied with Chl *a*, chlorophyllide *a*, or the sum of the two (Table III; Figure 2). The ratio of chlorophyllide *a*:Chl *a* varied from 0.1 at Station R to 2.5 in the community at Station P. The fucoxanthin:Chl *a* ratio also varied widely from ~0.6 to 2.4 (Table III). The ratio of fucoxanthin to the arithmetic sum of Chl *a* + chlorophyllide *a* + methylchlorophyllide *a*, however, varied only between 0.5 and 0.6 at Stations R and P, respectively (Table III). The concentration of chlorophyllide *a* varied from 0.5 μg l^{-1} at Station R to >3 μg l^{-1} at Stations B and P (Figure 2). At Stations B and P, the concentrations of chlorophyllide *a* increased when those of Chl *a* decreased, as expected of a secondary product formed from a primary one.

Carotenoids, or accessory pigment to Chl *a* ratios, are used frequently as indicators of phytoplankton taxa in natural waters (Everitt *et al*., 1990; Letelier *et al*., 1993; Vernet *et al*., 1994; Bidigare *et al*., 1996). High concentrations of the indicator pigment fucoxanthin (22–28% of the total pigments) suggest that diatoms were the primary class of phytoplankton observed during this sampling. Using the calculation factor of Everitt *et al*. (Everitt *et al*., 1990), the diatom contribution to total Chl *a* was found by multiplying the diatom-derived fucoxanthin concentration by 1.4 to give a value of up to 82% of the Chl *a* as being diatom derived.

Algal communities examined by inverted microscopy from the confluence of the Scotia and Weddell Seas during the 1993 austral spring were dominated by *Chaetoceros tortissimus* (45% of the total cell number) at Station P, whereas *Thalassiosira gravida* was the most important species at Station R (96% of the total cell number) (Irene Schloss, personal communication) (Table IV). *Thalassiosira gravida* occurred in large, particularly conspicuous colonies up to several millimeters in length, consisting of single cells embedded in a gelatinous matrix.

Table IV. Phytoplankton species abundance at the Weddell–Scotia Confluence, 1993

Station and date	Phytoplankton	Cells ml^{-1}
P October 26	*Chaetoceros tortissimus*	16,800
	Thalassiosira gravida	6,740
	Miscellaneous diatoms	3,400
	Miscellaneous flagellates	10,900
R November 1	*Thalassiosira gravida*	735,000
	Chaetoceros tortissimus	10,200
	Miscellaneous diatoms	94,400
	Phytoflagellates	35,700

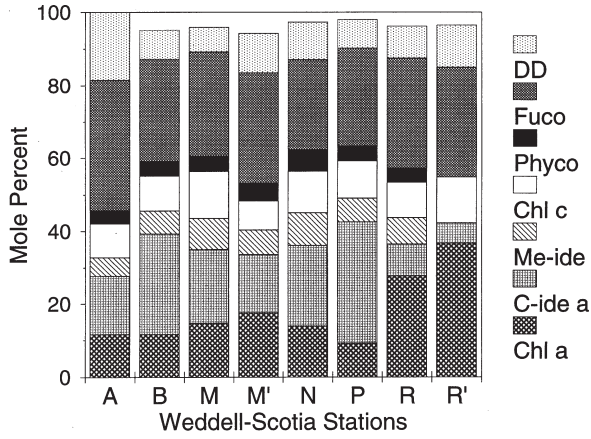

Fig. 3. Relative abundances in mole percent of major and accessory pigments, and chlorophyll *a* degradation products at stations listed in Table I. Chl *a*, chlorophyll *a*; C-ide *a*, chlorophyllide *a*; Me-ide, methylchlorophyllide *a*; Chl *c*, chlorophyll *c*; Phyco, phycoerythrin; Fuco, fucoxanthin; DD, diadinoxanthin.

Fluorescence comparison

Samples analyzed by both HPLC with the fluorescence detector and the Turner fluorometer showed higher Chl *a* by the conventional fluorometric method, with the exception of one station (Figure 4). At that station, Station R, diatoms were present as large aggregates that may have been more plentiful in the samples used for HPLC, causing a bias in the data set. For the other stations in Figure 4 (B, M, N, P and R, 29 October), the linear regression of fluorometer Chl *a* versus HPLC Chl *a* has a slope of 2.6 and an intercept of 0.2, a value not significantly different from zero ($R^2 = 0.75$, $n = 26$). The 25 m samples from Station P and Station R were outliers from this relationship and were omitted from the regression. The regression of fluorometer Chl *a* versus total Chl *a*-derived pigments measured with HPLC (Chl *a* + chlorophyllide *a* + methylchlorophyllide *a*) using the same points had a slope of 1.06 when forced through the origin ($R^2 = 0.45$).

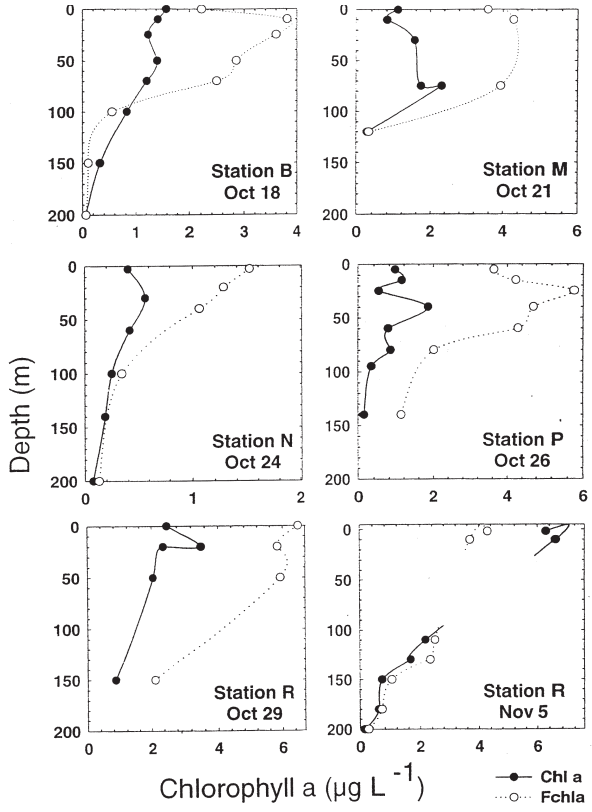

Fig. 4. Comparison of Chl *a* concentrations determined by HPLC (filled circles) and Turner fluorometer (open circles, dashed lines) from stations at the Weddell–Scotia Confluence.

Xanthophyll cycle

Surface water samples collected through the day at Station M showed DT/DD ratios of 0.15–0.3, or ~13% DT (Figure 5). A CTD profile near noon at Station M showed an upper mixed layer depth near 100 m, as illustrated by the pigment profile (Figure 6). Samples from simultaneous deck incubations at Station M had ratios of DT/DD of between 1 and 2 (i.e. >50–75% conversion to DT). The rate of the dark epoxidation reaction (i.e. the return of DT to DD) was estimated from natural seawater samples collected at Station R (6 November). The initial sample had a DT/DD ratio of 0.8, or 44% DT. After 100 min, the remaining DT had decreased to 7.6% (Figure 7).

Discussion

During the 1993 austral spring, the research vessel passed through a complex biomass near South Orkney island that ranged from a *Chaetoceros*-dominated assemblage south of the front towards the ice edge to a *Thalassiosira*-dominated

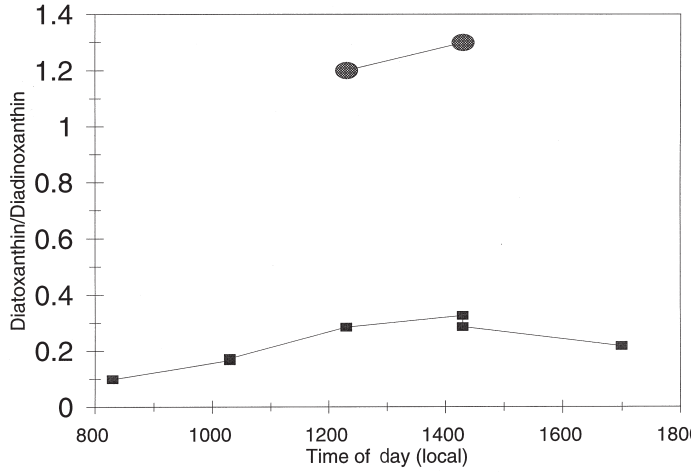

Fig. 5. DT:DD ratios in surface waters at Station M during daylight hours on 22 October. Midday DT:DD ratios (hatched circles) from deck incubations at Station M are included to illustrate the effects of surface irradiance intensities.

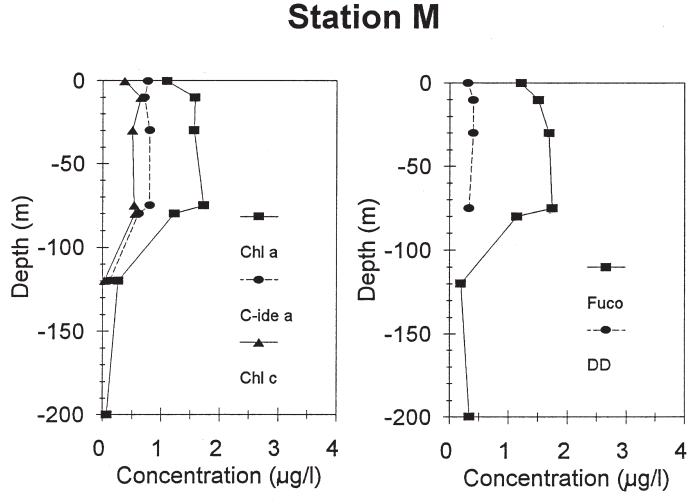

Fig. 6. Vertical distributions of chlorophylls (left panel) and carotenoids (right panel) measured at Station M (21 October 21, 14:00 h GMT) illustrating the upper mixed layer as defined by phytoplankton pigments.

assemblage at the northwestern edge. In this region, the stable layer of less saline water allowed the algal species that were growing most rapidly to accumulate and form local biomass maxima (Smith and Nelson, 1985). Nelson *et al.* also noted that increased biomass levels in the Weddell–Scotia water mass were enhanced

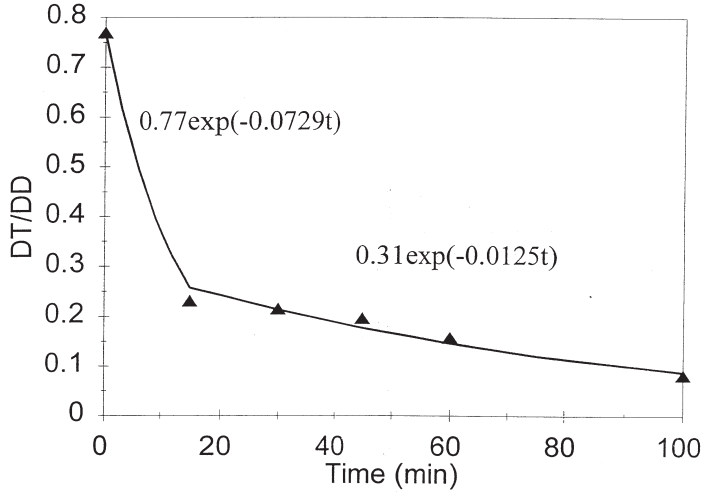

Fig. 7. DT:DD epoxidation reaction as a function of dark incubation time. Sample was taken at Station R (6 November, 15:15 h local time) and exposed to 15 min of surface irradiance on deck before beginning dark incubation.

initially in early spring by a period of delay in zooplankton development, as sometimes occurs during upwelling events in other areas (Nelson *et al.*, 1987). Sediment trap data indicate that a significant amount of surface-derived material sinks as large aggregates after phytoplankton blooms (Alldredge *et al.*, 1995). These aggregates often contain relatively intact diatoms that reach the sea floor without being ingested by zooplankton (Smetacek, 1985). Because zooplankton were not found in tows at these stations, diatom senescence rather than zooplankton ingestion appears to have been the fate of these phytoplankton.

The absolute levels of Chl *a* (up to 6 μg l^{-1} or 6 mg m^{-3}) encountered in the bloom were higher than those observed in many previous studies using HPLC in the Southern Ocean (Bidigare *et al.*, 1986; Buma *et al.*, 1990, 1992), but are typical of values obtained during blooms in productive coastal waters in temperate regions (Klein and Sournia, 1987). In agreement with many previous Antarctic observations (Mandelli and Burkholder, 1966; El-Sayed and Taguchi, 1981; Bidigare *et al.*, 1996), diatom species formed a significant part (up to 96%) of the austral spring biomass at the Weddell–Scotia Confluence during October–November 1993, although small numbers of flagellates also were observed.

Fluorescence comparison

The standard method for determining Chl *a* and phaeopigment concentrations using a fluorometer (Strickland and Parsons, 1972) can under- or overestimate Chl *a* concentrations since this method does not separate the pigments into individual compounds, as does chromatography (Jensen and Sakshaug, 1973; Gowen *et al.*, 1983; Buma *et al.*, 1992; Jeffrey and Mantoura, 1997). Because absorption

and fluorescence bands of non-chlorophyllous accessory pigments and Chl degradation products overlap with those of Chl *a*, Trees *et al.* found an error range of +53 to –69% with an average underestimation of 39% ($n = 300$) in comparing the two methods in ocean waters (Trees *et al.*, 1985). Individual pigment measurements indicated that chlorophyllide *a* alone produces a strong signal for Chl *a* with the fluorometer (Trees *et al.*, 1985). Shipboard measurements of Chl *a* with a spectrofluorometric method also gave higher levels (18%) of Chl *a* as compared to Chl *a* measured by HPLC (Buma *et al.*, 1992). Neither the Turner fluorometer nor the spectrofluorometer differentiates between the chlorophylls and Chl degradation products, which can lead to errors in calculated pigment concentrations. HPLC, on the other hand, separates and then accurately quantifies all major pigments and their degradation products (Bidigare *et al.*, 1985). Our results support the conclusions of Gowen *et al.* (Gowen *et al.*, 1983) and Trees *et al.* (Trees *et al.*, 1985), and indicate that HPLC separation of pigments is recommended over bulk fluorometric methods for phytoplankton pigment determinations.

Physiological status

The relative proportions of the porphyrin pigments vary with the physiological state or growth stage of the algae (Jensen and Sakshaug, 1973). Plankton in early growth stages contain up to 50% of porphyrins as Chl *a* (Jensen and Sakshaug, 1973). At maturity, chlorophyllide *a* (a degradation product) can increase to 40–50% of the total (Hallegraeff, 1981; Gowen *et al.*, 1983; Ridout and Morris, 1985; Klein and Sournia, 1987). Post-bloom biomass can contain 40–60% phaeophorbide *a*, a pigment formed by acidic conditions that can occur by passage through zooplankton or by the last phase of *in situ* cellular senescence (Hallegraeff, 1981; Gowen *et al.*, 1983; Bidigare *et al.*, 1996).

Data from closed-chamber experiments at Stations P and R during the Antarctic cruise (Ferreyra, 1995) further support the differences in physiological status of the phytoplankton at the two stations. In the experiment at Station P, the concentrations of Chl *a* decreased from 1.2 μg l^{-1} to 0.86 ± 0.5 μg l^{-1} ($n = 7$) in 6 days (26 October–1 November), indicating a rather mature assemblage. At Station R (2–8 November), however, the results were quite different and Chl *a* increased from 5.03 μg l^{-1} to 7.4 ± 1.1 μg l^{-1} ($n = 9$; A.C.Sigleo, unpublished data). These data indicate that the diatoms at Station R were in an active growth stage, whereas the diatoms at Station P were in a more mature condition. The difference in physiological status at the two stations, as indicated by the Chl pigment data, is sustained by Chl *a*-specific growth.

Since phaeophorbide *a* is formed by zooplankton grazing (Bidigare *et al.*, 1986), its absence in these samples suggests that zooplankton grazing was minimal at that time, as also indicated by the lack of zooplankton in net tows. During this sampling, the major fate of phytoplankton Chl *a* appears to have been cellular maturity rather than predation. These results (October–November) differ from those of Bidigare *et al.*, who observed that the primary degraded porphyrin in this region was phaeophorbide *a*, and concluded that summertime (January–

February) phytoplankton concentrations were controlled in the Southern Ocean by zooplankton grazing (Bidigare *et al*., 1986).

Because chlorophyllide *a* concentrations exceeded those of Chl *a* by factors of 2–3 (up to 90% of the porphyrins) in cells from Stations B and P, these results from the Southern Ocean emphasize the need for complete chromatographic pigment analyses to avoid errors in estimations of photosynthetically active biomass using chlorophyllous pigments (Gowen *et al*., 1983; Bidigare *et al*., 1985; Trees *et al*., 1985).

Fucoxanthin

Comparison of the vertical profiles for fucoxanthin and chlorophyllide *a* at Stations B and P (Figure 2) indicates a strong covariance of the two pigments. The profiles suggest that the carotenoid, fucoxanthin, is more stable than Chl *a*, and as Chl *a* degrades to chlorophyllide *a*, fucoxanthin remains and can accumulate along with chlorophyllide *a* (Figure 2). Data from higher plant studies support this conclusion, most notably during autumn pigment degradation prior to leaf fall in which carotenoids decline at a visibly slower rate than Chl *a*, thus producing autumn colors (Simpson *et al*., 1976). UVB exposure studies of Antarctic phytoplankton (El Sayed *et al*., 1990) also found that Chl *a* decreased selectively relative to other pigments, whereas fucoxanthin remained constant over long exposures. Poister *et al*. note from sediment trap studies that carotenoid pigments in sedimenting algae were degraded to a lesser extent than Chl *a* (Poister *et al*., 1999).

Xanthophyll cycle

The xanthophyll de-epoxidation/epoxidation reaction transforms DD to DT under increasing irradiance or strong light (Demers *et al*., 1991). For the diatom *Chaetoceros muelleri*, high-light-induced xanthophyll de-epoxidation occurred with a first order rate constant of 1.6 min^{-1} (Olaizola, 1993). The conversion of DD to DT appeared complete within 5 min (Olaizola, 1993). Under low light, DT is converted back to DD by enzymatic epoxidation.

The DD/DT cycle in diatoms and other phytoplankton is reported to have a photoprotective role by dissipating excess excitation energy in the antennae, thus preventing damage to the photosynthetic system (Sakshaug *et al*., 1987; Demers *et al*., 1991). In the work of Demers *et al*. (Demers *et al*., 1991), the sum of the two pigments remained constant, indicating that no new DD synthesis occurred. Latasa confirmed the photoprotective role by demonstrating that total DD concentrations, i.e. the sum of DD + DT, were higher in diatom cells cultured under high light irradiances, relative to diatoms cultured under lower irradiances (Latasa, 1995). In other words, Latasa's results indicated that additional DD was synthesized in high-light-cultured diatoms, and then became available for conversion to DT. In the present study, the xanthophylls DD and DT increased during deck incubations at Station R, indicating new synthesis of DD. In the incubation studies at Station R (2–6 November), the DD + DT sum increased from

1.09 μg l^{-1} in the water column (sample T_0) to >4.4 μg l^{-1} after 4 days (A.C.Sigleo, unpublished data). The DD + DT/Chl *a* ratios were 0.22 initially, and increased to 0.66 ± 0.08 (n = 9) after 4 days, indicating a 3-fold increase of these protective pigments relative to Chl *a*. Thus, the deck-incubated samples at Station R were transformed from a water column low-light-adapted community to a high-light-adapted community. These results indicate that natural diatom assemblages in the Southern Ocean, although generally low-light adapted for a low-light environment, can synthesize additional protective xanthophylls within days when light conditions change.

Although DD + DT/Chl *a* ratios were slightly higher at Station P (Table III), Olaizola *et al.* (Olaizola *et al*., 1992) and Brunet *et al.* (Brunet *et al*., 1993) have shown that these ratios are valuable only during log growth stages and are unreliable when large quantities of chlorophyllides and phaeophorbides are present. The work of these authors suggests that DD does not degrade as readily as Chl *a*, and, like fucoxanthin, may accumulate (see Figure 3, Station A).

DT/DD or DT + DD/Chl *a* ratios have been used as a potential index of vertical mixing in oceanic environments (Olaizola *et al*., 1992; Brunet *et al*., 1993; Olaizola, 1993). Samples during this cruise collected from surface waters during the day indicated that surface-collected water samples contained low-light-adapted phytoplankton with DT/DD ratios of 0.15–0.3, or ~13% DT (Figure 6). Samples from simultaneous deck incubations at Station M had ratios of DT/DD of between 1 and 2 (i.e. >50–75% conversion to DT), indicating that there was sufficient light intensity above the surface of the water to form maximum ratios. In temperate waters, Brunet *et al.* interpreted the lack of variation in DT/DD ratios in vertical profiles with respect to surface waters as an indicator of recent vertical transport (Brunet *et al*., 1993). Thus, we interpret the low-light adaptation of surface water pigments as an indication of recent vertical transport of the phytoplankton. We conclude that phytoplankton pigments from this experiment indicate that the phytoplankton were acclimated to fluctuating, or low-light conditions.

Epoxidation reaction

The dark epoxidation reaction (i.e. the return of DT to DD), estimated from natural seawater samples collected at Station R (6 November), was found to be a two-step reaction with a rapid initial phase ($t^{1/2}$ = 9.5 min) and a slower second phase ($t^{1/2}$ = 55 min) (Figure 7). Olaizola also observed a probable two-step process, but did not indicate a rate (Olaizola, 1993). Brunet *et al.* measured a time of 3.5 h for the decrease or disappearance of DT after dark (Brunet *et al*., 1993). The intermediate values in bucket-collected surface water samples suggest that the profile samples were adapted to fluctuating low-light conditions, as found in a vertically mixed water column (Demers *et al*., 1991). Vertical mixing may have been a factor limiting phytoplankton residence time in the near-surface layer since under high-light, surface-water conditions DT is formed from DD. Profiles of the pigments indicated that they were homogeneously distributed within the upper mixed layer at all stations (see Figure 6), except Station P (26 October),

which showed a disintegrating mixed layer (Figure 2). These results are consistent with the conclusions of Keller *et al.* that under natural conditions vertical mixing may provide refuge from rapidly attenuated UVB in the water column (Keller *et al.*, 1997).

In summary, Chl *a* concentrations of up to 6.5 μg l^{-1} within the biomass bloom are comparable to those for major algal blooms in temperate waters, and indicate that these are highly productive waters, capable of producing immense quantities of biomass. The high Chl *a* concentrations reinforce concerns about the potentially harmful effects of increased UVB during highly productive periods in Antarctic waters. During this austral spring bloom, however, dynamic hydrographic conditions, as indicated by low-light DD + DT/Chl *a* totals in surface waters, suggest that turbulence and vertical mixing within the water column may minimize phytoplankton residence time in surface waters, thereby decreasing exposure to UVB radiation. To examine this hypothesis further, future work needs to include UVB exposure biomarkers along with vertical mixing data.

Chlorophyllide *a* concentrations greatly exceeded those of Chl *a* towards the termination of a phytoplankton bloom, introducing a potential bias of up to 75% in estimates of photosynthetically active biomass at one station. Fucoxanthin, a major diatom pigment, shows evidence of greater chemical stability in the water column than Chl *a*. The formation and chemical stability of these two pigments are important considerations when high-altitude or remote sensing surveys are used to determine primary productivity over large regions of the ocean. The results emphasize the need for field measurements to confirm actual plankton vitality.

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