Abundance, size structure and community composition of phytoplankton in the Southern Ocean in the austral summer 1999/2000

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Abstract: The abundance, size structure and community composition of phytoplankton in the Southern Ocean were studied, using flow cytometry, microscopy and pigment profiles on two transects one latitudinal (N) and one longitudinal (W) during December 1999 and January 2000. In both transects, the concentration of autotrophic eukaryotes of 2–10 μm equivalent spherical diameter (ESD) commonly exceeded those < 2 μm ESD. No cells >10 μm ESD were detected by flow cytometry (however microscopy showed cells>10 μm in length). Throughout transect N, chlorophyll *a* concentrations were generally <0.5 μg l^{-1}. South of the Antarctic Polar Front (APF), chlorophyll *a* concentrations increased southward. CHEMTAX allocation of pigment data (italicized) showed that *Diatoms* contributed most chlorophyll with *Haptophytes* sub-dominant. North of the APF, chlorophyll *a* concentrations tended to increase northward. Here, *Haptophytes* contributed most chlorophyll, followed by *Diatoms, Chlorophytes* and *Cyanobacteria,* except at the northernmost stations where *Cyanobacteria* dominated. In transect W, chlorophyll *a* concentrations were also <0.5 μg l^{-1} in most cases, but variable. Higher concentrations occasionally occurred in the west. In this transect, *Diatoms* contributed most (mean=61 $\pm$ 15%) of the chlorophyll *a*, followed by *Haptophytes.* Nanodiatoms (particularly *Fragilariopsis* spp.) numerically dominated the diatom community. Fecal pellets composed of these nanodiatoms were observed in the Antarctic water, probably originating from heterotrophic dinoflagellates, implying a significant contribution of nanodiatoms to the microbial food web. However they contributed little to total chlorophyll *a* and diatom carbon biomass, particularly when chlorophyll and carbon concentrations were high.

key words: phytoplankton, size structure, community composition, nanoplanktonic diatoms, Southern Ocean

Introduction

Our knowledge of the abundance, and the spatial and temporal distribution of Antarctic phytoplankton taxa is limited. Nanoplankton are difficult to identify, even to class level, by light microscopy due to their small size and poor preservation in conventional fixatives. However, numerous studies have shown that Antarctic phytoplankton communities are often dominated by organisms < 20 μm in size (Hewes *et al.,* 1985, 1990; Kosaki *et al.,* 1985; Weber and El-Sayed, 1987; Marchant and Murphy,

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1994). Haptophytes, prasinophytes, chrysophytes, cryptophytes, dinoflagellates and diatoms comprise the nanophytoplankton in the Antarctic waters (El-Sayed and Fryxell, 1993; Marchant, 1993; Marchant and Thomsen, 1994; Garrison and Mathot, 1996). Of these, haptophytes and diatoms dominate (Marchant, 1993; Marchant and Thomsen, 1994).

Analysis of accessory pigments using high performance liquid chromatography (HPLC) and the chemical taxonomy software "CHEMTAX", has proved useful to assess the taxonomic composition of phytoplankton community in natural samples (Mackey *et al.,* 1996; Wright *et al.,* 1996; Jeffrey *et al.,* 1999). A major advantage of this technique is that it estimates the relative abundance of organisms too small to effectively resolve by light microscopy. Previous studies using CHEMTAX have successfully revealed the phytoplankton community structure in Antarctic waters (Wright *et al.,* 1996; Wright and van den Enden, 2000).

Flow cytometry has also been applied to examine phytoplankton communities (especially smaller phytoplankton) in temperate and tropical waters (*e.g.,* Olson *et al.,* 1985; Shimada *et al.,* 1993; Blanchot and Rodier, 1996; Zubkov *et al.,* 1998). In Antarctic waters, Detmer and Bathmann (1997) showed a good correlation between both cell numbers obtained by flow cytometry and epifluorescence microscopy for autotrophs <20 μm, while counts by the former were always higher than those of the latter.

Using the CHEMTAX in conjunction with flow cytometry and microscopy offers complementary tools to investigate the phytoplankton community structure. In this study, we apply these methods to report abundance, size structure and community composition of phytoplankton across the Southern Ocean and in Antarctic waters during the summer of 1999/2000.

Materials and methods

Field observation and sample collection

Two transects were surveyed from "*RSV Aurora Australis*" during December 1999 to January 2000. A westward (W) transect was conducted between 64°S, 150°E and 66°S, 65°E, and a northward (N) transect from 66°S, 70°E to 44°S, 146°E (Fig. 1). Several sampling sites south of 62°S in Transect N, and all of transect W, were located within the sea-ice zone. Temperature and salinity of surface waters were recorded continuously along both transects using ship-board sensors that were calibrated immediately before the voyage.

Samples for flow cytometry, HPLC analysis and microscopy were obtained from the ship's clean seawater intake located at 7 m depth. For HPLC, 300–2200 m*l* of water were filtered through a Whatman GF/F glass fiber filter (13 mm diameter), which was folded, blotted thrice to remove excess sea water, and immediately transferred to cryovials and stored in liquid nitrogen for subsequent analysis. Approximately 1 *l* of seawater was transferred to glass bottles and fixed with acid Lugol's solution and stored at 4±2°C for subsequent microscopy.

Ship-board flow cytometry

Autotrophic populations were analyzed in the fresh samples using a FACScan flow
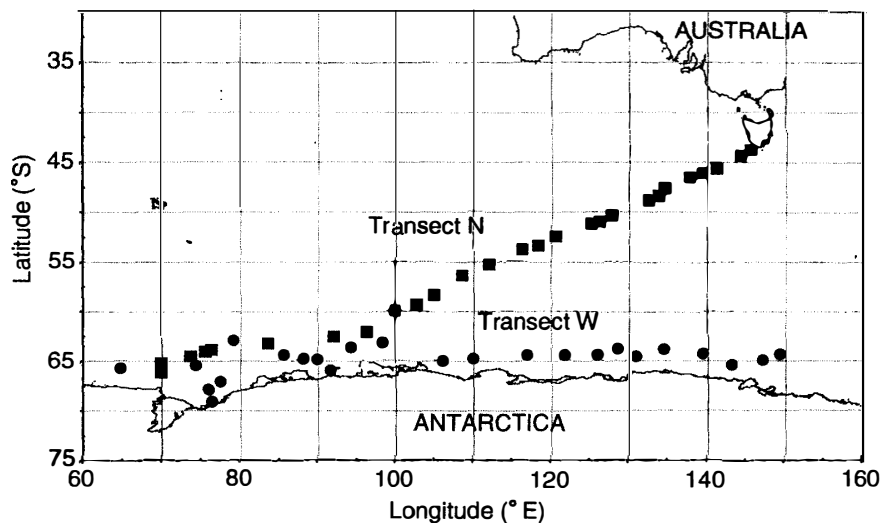
Fig. 1. Location of sampling stations during the westward (Transect W, circles) and northward (Transect N, squares) cruises in the Southern Ocean.

cytometer (Becton Dickinson, Oxford UK), equipped with a 488 nm argon laser, immediately after they were collected. The flow cytometer data were analyzed using CellQuest software (Immunocytometry Systems, Becton Dickinson, Oxford UK). A known concentration of 2.021 μm diameter yellow/green fluorescent microspheres (Polyscience Inc.) was added as an internal standard to each sample to determine the volume of sample analyzed by the flow cytometer. Particles were divided into regions from bivariate plots of red chlorophyll a (655 ± 5 nm) and orange (585 ± 42 nm) autofluorescence to discriminate the high orange:red fluorescent ratio of cyanobacteria that contained phycoerythrin. The forward scatter (FSC) response was calibrated with beads of 0.5, 2.021, 5.06, 9.6 and 16.7 μm in diameter (Polyscience, Inc.). A highly significant positive correlation was found between the bead diameter and log FSC ($r^2 = 0.91$, $p < 0.02$). Bivariate plots of FSC (approximating equivalent spherical diameter, ESD) against red autofluorescence were then used to quantify the concentrations of autotrophs in three size categories. Thus, four phytoplankton concentrations were determined for each sample in this study namely, picoplankton <2 μm ESD, nanoplankton from 2 to 10 μm ESD, nano- and microplankton >10 μm ESD and cyanobacteria. Autotroph concentrations were computed from the particle counts in each analysis region, using the volume (from 13 to 190 μl) of sample analyzed by the flow cytometer.

Pigment analysis

Filters were sonicated for 1 min in 1.5 ml methanol (100%) for pigment extraction. Apo-8-carotenal (140 ng, Fluka) was added to each sample as an internal standard. Pigments were then analyzed using the HPLC technique of Zapata et al. (2000). Pigment detection, data analysis and pigment identification, thereafter, were the same as described in Wright and van den Enden (2000). Namely, pigments were detected with a Waters 996 photodiode array and Hitachi FT 1000 fluorescence detectors, the data were computed by software (Waters Millennium and custom software) and identified by UV-

visible spectra of pigments and by comparison of retention times with a standard mixture.

Contributions of various phytoplankton classes to the total concentration of chlorophyll *a* were estimated (Mackey *et al.,* 1996; Wright *et al.,* 1996) using CHEMTAX interpretation of HPLC pigment data. Eight major phytoplankton groups were classified: *Cryptophytes, Dinoflagellates, Prasinophytes, Chlorophytes, Cyanobacteria, Diatoms,* and 2 groups of *Haptophytes.* It is important to stress that these groups represent pigment suites typical of the various algal taxa, rather than the taxa themselves. For this reason the group names are italicized throughout to emphasize that they are not conventional taxa. A full discussion of the meaning of these groups is given in Wright and van den Enden (2000). The *Haptophyte* groups are called *Hapto3s* and *Hapto4s* in Wright and van den Enden (2000). *Hapto3s* represent type 3 haptophytes (Jeffrey and Wright, 1994), typically coccolithophorids, while *Hapto4s* represent type 4 haptophytes (Jeffrey and Wright, 1994), including *Phaeocystis antarctica* Karsten, but also chrysophytes including Pelagophyceae and Parmales. In this study, non-polar chlorophyll c_2 was added to the pigment matrix for *Hapto3s* and *Hapto4s* (Zapata, pers. comm.). As pointed out by Wright and van den Enden (2000), CHEMTAX groups do not correspond exactly to taxonomic group as pigments arrays overlap between some taxa. However, here we consider them as representative groups possessing distinctive pigment arrays.

Microscopy

Samples fixed with acid Lugol's solution were sedimented over ≥ 3 days and the supernatant aspirated off to give a final concentrated volume of *ca.* 20 m*l*. A 2–7 m*l* aliquot of the concentrate was further settled in a sedimentation chamber for 1 day, and diatoms were counted and identified at 200–400 × magnification using a Zeiss Axiovert 135 equipped with Nomarski interference contrast optics.

In transect W, the dimensions of 10–50 cells for each diatom taxon were measured at 400 × magnification and cell volumes (μm^3) were calculated. Cell concentrations were then converted to carbon biomass for each diatom species using the conversion statistics: pgC cell^{-1} = 0.288 × volume$^{0.811}$ (Menden-Deuer and Lessard, 2000).

Results and discussion

Physical oceanography

Two major oceanographic fronts were encountered in the northward transect (N) namely, the Antarctic Polar Front (APF) at around 52°S and Subantarctic Front (SAF) between around 47 and 49°S (Fig. 2a). Temperature increased from 4.6 to 7.7°C and salinity rose from 33.83 to 34.05 PSU at the APF. At the SAF, temperature increased from 7.7 to 13.2°C and salinity rose from 33.96 to 34.64 PSU. Although positions of these Fronts can not be precisely determined without vertical profiles of temperature, their positions in this study are not substantially different to those reported from adjacent areas (Nagata *et al.,* 1988; Wright *et al.,* 1996). There was a rapid drop in salinity south of 63°S in transect N, followed by an increase south of 66°S, and a 2°C increase in temperature south of 67°S (Fig. 2a).

In transect W (Fig. 2b), temperature varied from −1.8 to 1.0°C and salinity from around 33 to 34.5 PSU, respectively, probably due to the effects of sea ice.
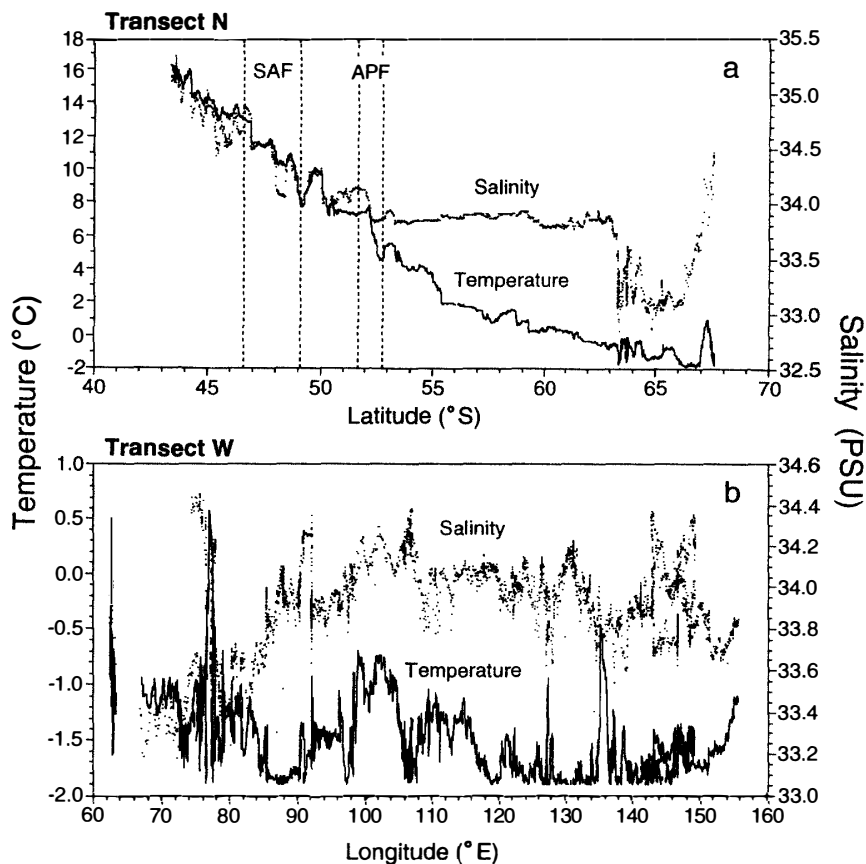
Fig. 2.   Surface distributions of water temperature and salinity in (a) transect N and
(b) transect W. APF = Antarctic Polar Front; SAF =Subantarctic Front.

Flow cytometry

Phytoplankton populations detected by flow cytometry fell into 3 classes; autotrophic picoeukaryotes (APE, <2 μm ESD), autotrophic nanoeukaryotes (ANE, 2–10 μm ESD) and cyanobacteria (only north of APF) (Figs. 3a, b). Flow cytometry detected no cells in either transect with an ESD > 10 μm.

Concentrations of APE ranged between the orders of 10^5 and 10^6 cells l^{-1} while ANE were in the order of 10^6 cells l^{-1}, except for one site (1.14×10^7 cells l^{-1}) at 77°31'E in transect W.   Changes in their concentrations commonly correlated in both transects. Concentrations of ANE and APE in the Antarctic waters (in transect W and south of the APF in transect N) were similar to values from other studies in the area (Odate and Fukuchi, 1995; Detmer and Bathmann, 1997 and references therein).   Autotroph (ANE + APE) concentrations in the Antarctic waters (1.6×10^6 up to 1.7×10^7 cells l^{-1}) were similar to those of the flow cytometric study by Detmer and Bathmann (1997) in the Atlantic Sector south of the APF.   The mean concentrations of autotrophs south of the APF ($4.8 \pm 2.7 \times 10^6$ cells l^{-1}) were commonly lower than those reported by Detmer and Bathmann (1997) but were higher than their hypothesized "background" concentration of $2–4 \times 10^6$ cells l^{-1} during spring and early summer.   This suggests there had been some development of the phytoplankton community towards its summer maximum in this study.
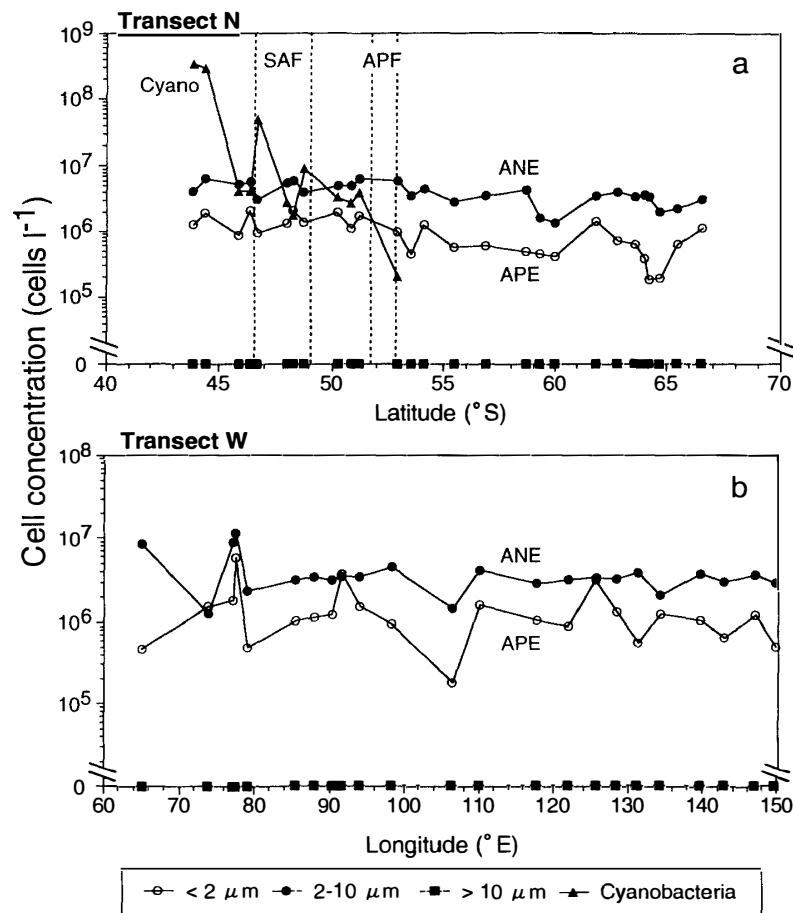
Fig. 3. Surface distributions of phytoplankton (cyanobacteria, and eukaryotes in the size ranges of<2 μm ESD, 2–10 μm ESD and>10 μm ESD) in (a) transect N and (b) transect W. APF=Antarctic Polar Front; SAF= Subantarctic Front; Cyano=Cyanobacteria; APE=autotrophic picoeukaryotes (<2 μm ESD); ANE=autotrophic nanoeukaryotes of 2–10 μm ESD.

With very few exceptions, the concentration of ANE exceeded that of APE in both transects. These data agree with studies using size-fractionated chlorophyll *a* showing that nanoplankton dominate picoplankton in the Antarctic waters (Kosaki *et al.,* 1985; Weber and El-Sayed, 1987). Our data suggest that ANE dominate autotrophic eukaryote populations throughout the Southern Ocean.

There are four possible explanations for the absence of >10 μm sized cells. The small volume of sample analyzed by the flow cytometer (between 13 and 190 μl) may preclude measurement of infrequent large phytoplankton cells. Even in the sample that contained 8.84 μg l^{-1} of chlorophyll *a* (see below), only 5 cells of large centric diatoms (*Thalassiosira* spp. in this case; also see below) would have been expected in the 17 μl subsample analyzed in this time. For less eutrophic samples, no such large cells would have been expected. Discoid shaped cells of exceeding 100 μm in diameter, such as *Coscinodiscus,* would be excluded from the sample tube. The size of a particle detected by the flow cytometry best equates to the ESD. Narrow cells, such as Nitzschioid

pennate diatoms, can substantially exceed 10 μm in length but have an ESD < 10 μm. Furthermore, such phytoplankton as pennate diatoms are likely to pass through the sample capillary as their passage is determined by particle width rather than length. However, the sample area illuminated by the laser is only 20 μm high and 64 μm wide with peak excitation substantially less than these dimensions. Thus, the ESD of such cells may be poorly estimated (Olson *et al.*, 1989).

Cyanobacteria were restricted to waters warmer than $5°C$, similar to microscopic observations by Waterbury *et al.* (1986). Cyanobacteria were detected from immediately south of APF and their abundance dramatically increased northward (from 2.1×10^5 to 3.4×10^8 cells l^{-1}) (Fig. 3a). Our findings correspond to the results of other studies conducted in adjacent areas of the Southern Ocean (Marchant *et al.*, 1987; Odate and Fukuchi, 1995; Wright *et al.*, 1996) except that concentrations in the northern-most samples were higher than literature values but were similar to those found in austral autumn off South America (Waterbury *et al.*, 1986).

Particles with high orange : low red fluorescence generally associated with phycoerythrin-containing cyanobacteria were also detected by flow cytometry in the samples from Antarctic waters. However, counts were unreliable since at low concentration instrument noise became significant. Hence these particles were excluded from the cyanobacterial counts. Other studies using epifluorescence microscopy have reported the presence of cyanobacteria in Antarctic waters below $5°C$ but at concentrations commonly < 10^5 cells l^{-1} (Marchant *et al.*, 1987; Walker and Marchant, 1989; Odate and Fukuchi, 1995).

Phytoplankton pigments

Chlorophyll *a*: Chlorophyll *a* concentrations were generally low throughout, being less than 0.5 μg l^{-1} in all but one site of transect N and for most of transect W (Figs. 4a,b). In transect N, chlorophyll *a* concentrations decreased northward from a maximum of 0.76 μg l^{-1} at the southmost site to 0.07 μg l^{-1} at the APF. Concentrations approximately doubled to around 0.2 μg l^{-1} between the APF and SAF, declined again to approximately 0.1 μg l^{-1} at the SAF, then increased northward, reaching 0.44 μg l^{-1} at the northern end of the transect. In transect W, concentrations of chlorophyll *a* in the region west of $120°E$ were occasionally high (0.66–2.1 μg l^{-1} but reaching 8.84 μg l^{-1} at $91°37'E$) while concentrations in the east of $120°E$ were low (commonly < 0.4 μg l^{-1}). This is consistent with previous observations showing increase of chlorophyll *a* concentration (Suzuki and Fukuchi, 1997; Wright and van den Enden, 2000) and of productivity (Strutton *et al.*, 2000) in the Indian sector of Antarctic Ocean.

The low chlorophyll *a* concentrations we observed south of APF are commonly reported from oceanic waters around Antarctica, despite of high nutrient concentrations (*e.g.*, Odate and Fukuchi, 1995; Chiba *et al.*, 2000). Biological, chemical and physical factors, including grazing, micronutrients availability and mixed depth are considered responsible for the low phytoplankton biomass (Hewes *et al.*, 1985; Smith and Nelson, 1985; Martine and Fitzwater, 1988; Martine *et al.*, 1990; Holm-Hansen and Mitchell, 1991; Lancelot *et al.*, 1993; Chiba *et al.*, 2000). However high concentrations of chlorophyll *a*, >3 μg l^{-1} have also been reported from coastal and open ocean Antarctic waters (*e.g.*, Holm-Hansen *et al.*, 1989; Hewes *et al.*, 1990; Sullivan *et al.*, 1990;
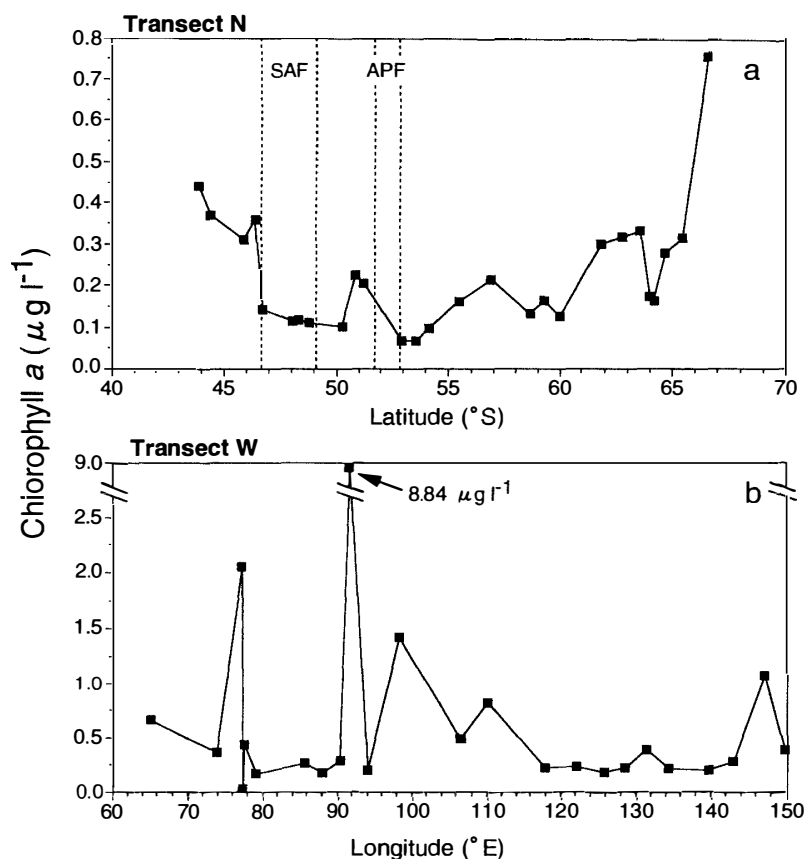
Fig. 4. Surface distributions of chlorophyll a concentrations in (a) transect N and
(b) transect W. APF=Antarctic Polar Front; SAF=Subantarctic Front.

Tréguer and Jacques, 1992; Laubscher *et al.*, 1993; Wright and van den Enden, 2000)
and microplanktonic diatoms reportedly contribute most of the chlorophyll at these sites
(Holm-Hansen *et al.*, 1989; Hewes *et al.*, 1990; Detmer and Bathmann, 1997).   In this
study, the highest chlorophyll a concentration (8.84 μg l^{-1}) was also contributed by large
diatoms (see below).   In addition, 2 of 4 other elevated chlorophyll a peaks exceeding
0.8 μg l^{-1} in transect W (110°04′E and 147°04′E, Fig. 4b) correlated with elevated
concentrations of large diatoms.

HPLC CHEMTAX: Contributions of various phytoplankton classes to the total
concentration of chlorophyll a were calculated from pigment HPLC data using
CHEMTAX software. In this study, CHEMTAX explained 99% of the total chlorophyll a
(data not shown).   Furthermore, we found a strong correlation ($r^2 = 0.85$, $n = 12$, $p < 0.001$)
between the CHEMTAX estimate of cyanobacterial chlorophyll a and the concentration of
cyanobacteria obtained by flow cytometry.   Other taxa cannot be correlated directly as
they do not display a unique flow cytometric signature, and microscopic statistics were
inadequate.

The chlorophyll a concentration and percent total chlorophyll a attributable to each
of the 8 main phytoplankton classes are shown in Figs. 5a, b and Figs. 5c, d, respectively.
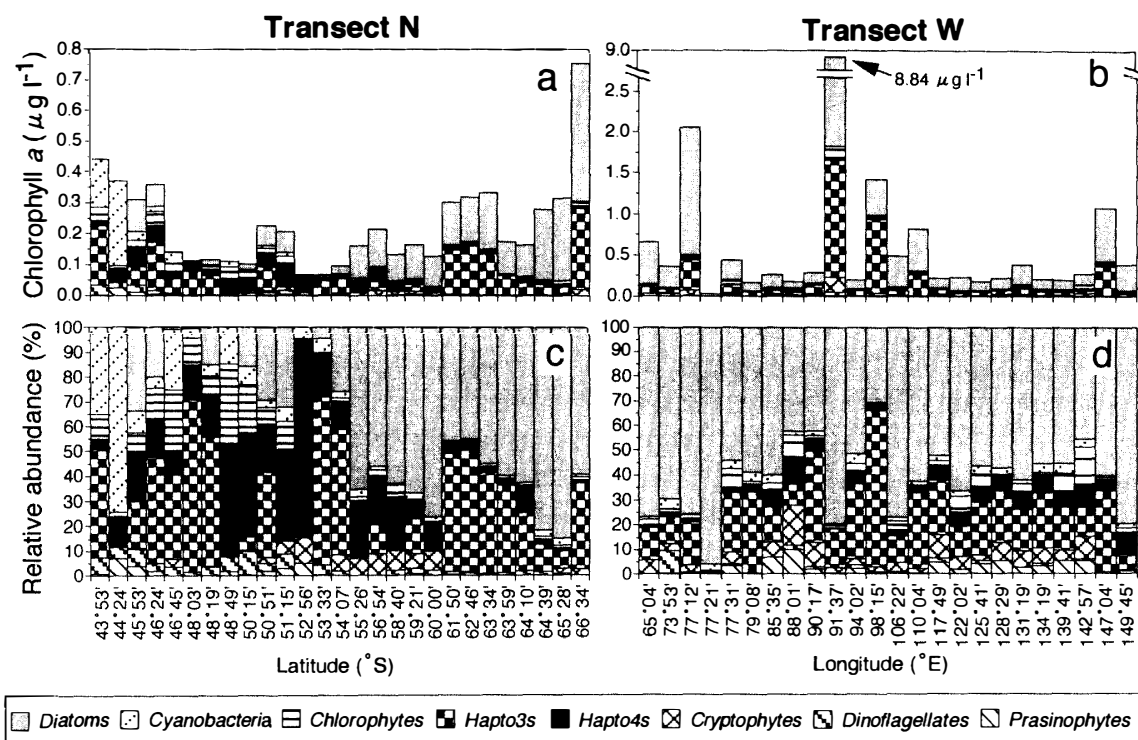The ratios of marker pigments:chlorophyll a for each algal group after optimization by

Fig. 5. Surface distributions of (a, b) contributions of phytoplankton classes to total chlorophyll *a* concentrations, as determined by CHEMTAX analysis, and (c, d) their percent contributions in transect N (left panels) and transect W (right panels).

CHEMTAX are shown in Table 1. In transect N, composition of the phytoplankton community changed greatly from south to north (Fig. 5c). North of 54°S, the phytoplankton community was dominated by *Haptophytes*. *Hapto3s* commonly contributed a higher percentage of the chlorophyll *a* than *Hapto4s* throughout transect N. *Diatoms* were sub-dominant and *Chlorophytes* and *Cyanobacteria* were occasionally abundant except at 44°S where *Cyanobacteria* dominated (74%). South of 54°S in transect N, *Diatoms* dominated, contributing between 45 and 85% of the chlorophyll *a* and followed by *Haptophytes* (*Hapto3s* and *Hapto4s*). *Cyanobacteria* and *Chlorophytes* were only minor components south of the APF, as were *Cryptophytes*, *Dinoflagellates* and *Prasinophytes* throughout the transect.

The phytoplankton community composition on transect W (Fig. 5d) was similar to that south of 54°S in transect N. *Diatoms* were always dominant (mean=61 ± 15% of chlorophyll *a*), followed by *Haptophytes* (mean=25 ± 13% of chlorophyll *a*) with *Hapto3s* often >*Hapto4s*. *Cyanobacteria* and *Chlorophytes*, *Cryptophytes*, *Dinoflagellates* and *Prasinophytes* were minor components of the phytoplankton community. Our results are similar to a previous study using CHEMTAX conducted in the Indian sector of Antarctic waters (80 to 150°E) (Wright and van den Enden, 2000).

Previous studies found that diatoms and haptophytes (mainly *Phaeocystis*) numerically dominate the nanoplanktonic phytoplankton in Antarctic waters (*e.g.*, Davidson and Marchant, 1992; Marchant, 1993; Marchant and Thomsen 1994; Detmer and Bathmann, 1997; Waters *et al.*, 2000), although cryptophytes and prasinophytes are

Table 1. Final pigment ratios (w/w Chl *a*) after optimization by CHEMTAX for each algal group.

Algal group	Pigment							
	Chl c_3	Chl c_2	Perid	19′-but	Fucox	Neox	Prasinox	Violax
Prasinophytes	–	–	–	–	–	0.120	0.315	0.160
Dinoflagellates	–	0.300	1.063	–	–	–	–	–
Cryptophytes	–	0.200	–	–	–	–	–	–
Hapto3s	0.136	0.150	–	–	–	–	–	–
Hapto4s	0.258	0.413	–	0.310	0.441	–	–	–
Chlorophytes	–	–	–	–	–	0.020	–	0.040
Cyanobacteria	–	–	–	–	–	–	–	–
Diatoms	–	0.200	–	–	0.755	–	–	–

Algal group	Pigment							
	19′-hex	Diadinox	Allox	Zeax	Lutein	Chl *b*	Np chl c_2	Chl *a*
Prasinophytes	–	–	–	–	0.009	0.945	–	1.000
Dinoflagellates	–	0.240	–	–	–	–	–	1.000
Cryptophytes	–	–	0.229	–	–	–	–	1.000
Hapto3s	0.500	0.140	–	–	–	–	0.119	1.000
Hapto4s	0.676	0.250	–	–	–	–	0.106	1.000
Chlorophytes	–	–	–	0.009	0.203	0.263	–	1.000
Cyanobacteria	–	–	–	0.348	–	–	–	1.000
Diatoms	–	0.136	–	–	–	–	–	1.000

Pigment abbreviations: Chl, chlorophyll; Perid, peridinin; 19′-but, anoyloxyfucoxanthin; Fucox, fucoxanthin; Neox, neoxanthin; Prasinox, prasinoxanthin; Violax, violaxanthin; 19′-hex, 19′-hexanoyloxyfucoxanthin; Diadinox, diadinoxanthin; Allox, alloxanthin; Zeax, zeaxanthin; Np, non-polar.

occasionally abundant (Buma *et al.,* 1992; Nöthig *et al.,* 1991). Coccolithophorids are essentially absent south of 60°S (Nishida, 1986). In this study, the abundance of *Hapto3s* was commonly higher than *Hapto4s* (representing Pelagophyceae, Parmales and mainly *P. antarctica*), suggesting that coccolithophorids or similarly pigmented cells dominated among haptophytes in Antarctic waters. However, multiple pigment patterns exist within the haptophyceae (Zapata, pers. comm.) and the species composition of *Hapto3s* and *4s* is still equivocal (Wright and van den Enden, 2000). Marchant and Thomsen (1994) have shown an unknown species of haptophyte, which is similar to *Phaeocystis* and only distinguishable by the swimming pattern, indicating the difficulty of study in the field of haptophytes. Further studies of pigments and taxonomy on haptophytes inhabiting Antarctic waters are needed.

Microscopy of diatoms

Diatoms were more abundant (commonly $>2 \times 10^5$ cells l^{-1}) south of 54°S than to the north ($<1.3 \times 10^5$ cells l^{-1}) (Fig. 6a). The maximum concentration was 8.2×10^5 cells l^{-1} at 61°50′S in transect N. In transect W, the concentration was almost consistently 1 to 2×10^5 cells l^{-1} east of 85°E but fluctuated from 4.3×10^3 cells l^{-1} to the maximum of 4.5×10^6 cells l^{-1} west of 85°E (Fig. 6b). With one exception, the concentration of pennate species was always higher than that of centric species throughout both transects (Figs. 6c, d). Pennate diatoms contributed 65–100% of the total diatom cell concentration, except at 91°37′E in transect W, where *Thalassiosira* spp. comprised 48%
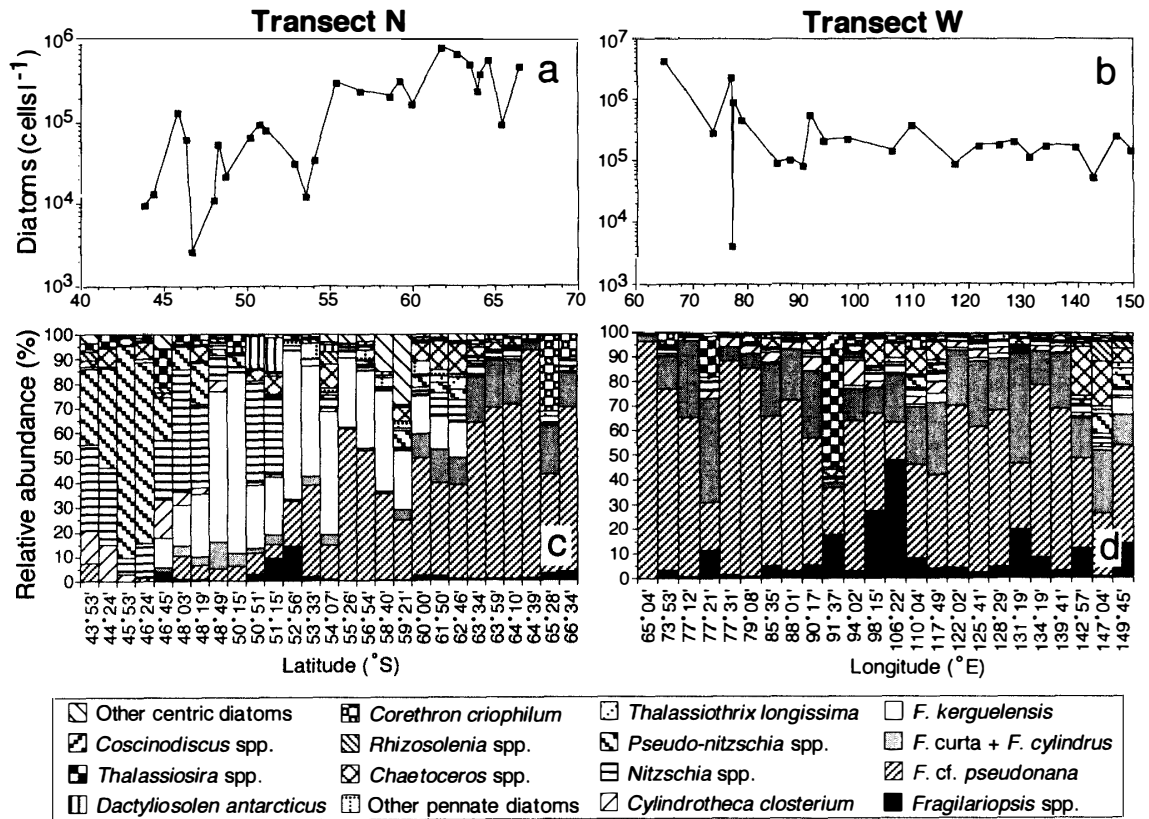
Fig. 6. Surface distributions of (a, b) diatom concentrations and (c, d) percent contributions of its taxa in transect N (left panels) and transect W (right panels). *Rhizosolenia* spp. includes *Proboscia alata* here.

of the diatom community (Fig. 6d) and the highest concentration of chlorophyll *a* was observed (Fig. 4b). Among the pennate diatoms, small species of *Fragilariopsis* including *F.* cf. *pseudonana* (Hasle) Hasle, *F. curta* (Van Heurck) Hustedt and *F. cylindrus* (Grunow) Krieger were dominant components in transect W and in most sites south of 54°S in transect N. They are reportedly common and often dominate in fast ice, pack ice and open water regions of Antarctic (*e.g.*, Hewes *et al.*, 1985, 1990; Garrison *et al.*, 1986, 1987; Wilson *et al.*, 1986; Weber and El-Sayed, 1987; Fryxell, 1989; Ishikawa *et al.*, 2001). In this study, since *F. curta* and *F. cylindrus* could not be reliably distinguished by light microscopy, they were counted as a single taxon (*F. curta* plus *F. cylindrus*). Furthermore, *F.* cf. *pseudonana* might include the cells of *F. curta* plus *F. cylindrus* as they were often observed in girdle view during the counts. North of 55°S in transect N, *F. kerguelensis* (O'Meara) Hustedt, *Nitzschia* spp. and *Pseudo-nitzschia* spp. became dominant.

Despite of the high concentrations of small *Fragilariopsis* species (excluding *F. kerguelensis*) in Antarctic waters, they only contributed $26 \pm 17\%$ of the total diatom carbon throughout transect W (Fig. 7b). In contrast, concentrations of large centric diatoms such as *Corethron criophilum* Castracane, *Coscinodiscus* spp., *Rhizosolenia* spp. [including *Proboscia alata* (Brightwell) Sundström] and *Thalassiosira* spp. were generally low in this study but contributed $51 \pm 20\%$ of the total biomass in transect W.
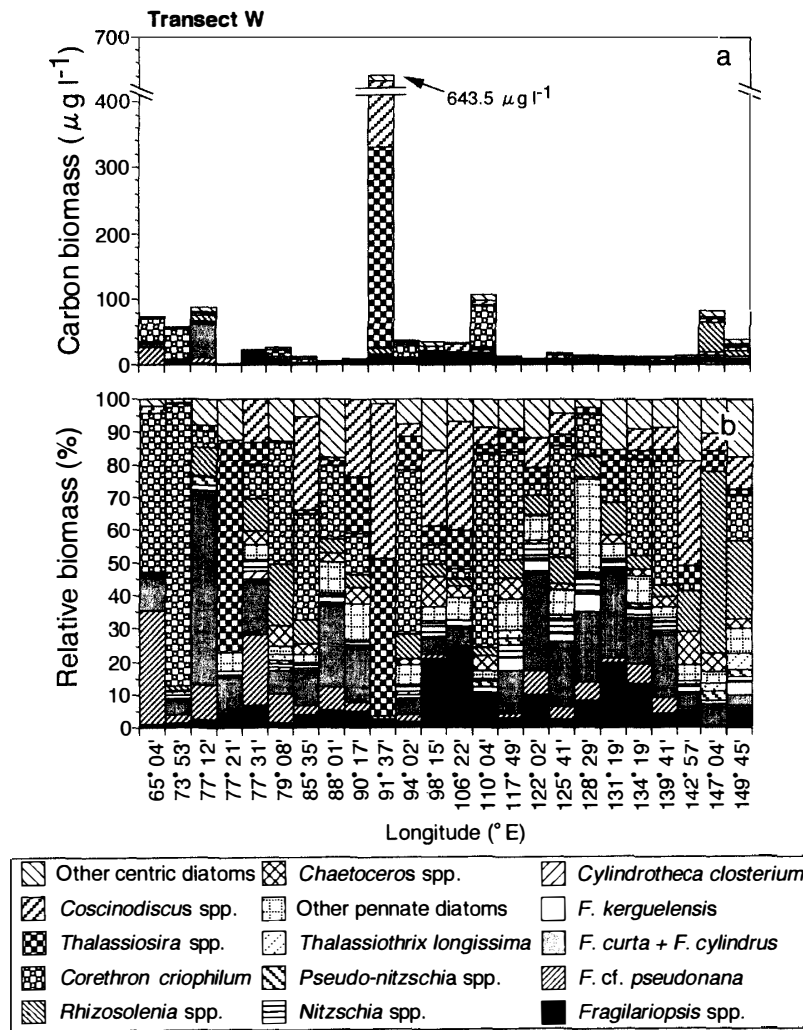
Fig. 7. Surface distributions of (a) contributions of diatom taxa to total carbon biomass and (b) their percent contributions in transect W. *Rhizosolenia* spp. includes *Proboscia alata* here.

Furthermore, at the site (91°37′E) where the highest concentration of chlorophyll *a* was observed, cell carbon biomass reached 643.5 μg l^{-1} (Fig. 7a), 95% of which was contributed by only two large centric diatom species (*Coscinodiscus* spp. and *Thalassiosira* spp.). Our results indicate the importance of microplankton for carbon cycling in Antarctic waters.

Microheterotroph grazing

In Antarctic waters, heterotrophic flagellates, such as dinoflagellates and other nanoflagellates, are common and sometimes abundant (see Garrison and Mathot, 1996). It has been found that they can graze up to 25% of daily primary production in Antarctic waters (Archer *et al.,* 1996). Some of heterotrophic dinoflagellates in Antarctic waters and sea ice are likely to produce fecal pellets composed of diatoms, including *Fragilariopsis* spp. (Nöthig and Bodungen, 1989; Buck *et al.,* 1990; Gonzáles, 1992). We frequently observed fecal pellets that contained *Fragilariopsis* spp. cells in
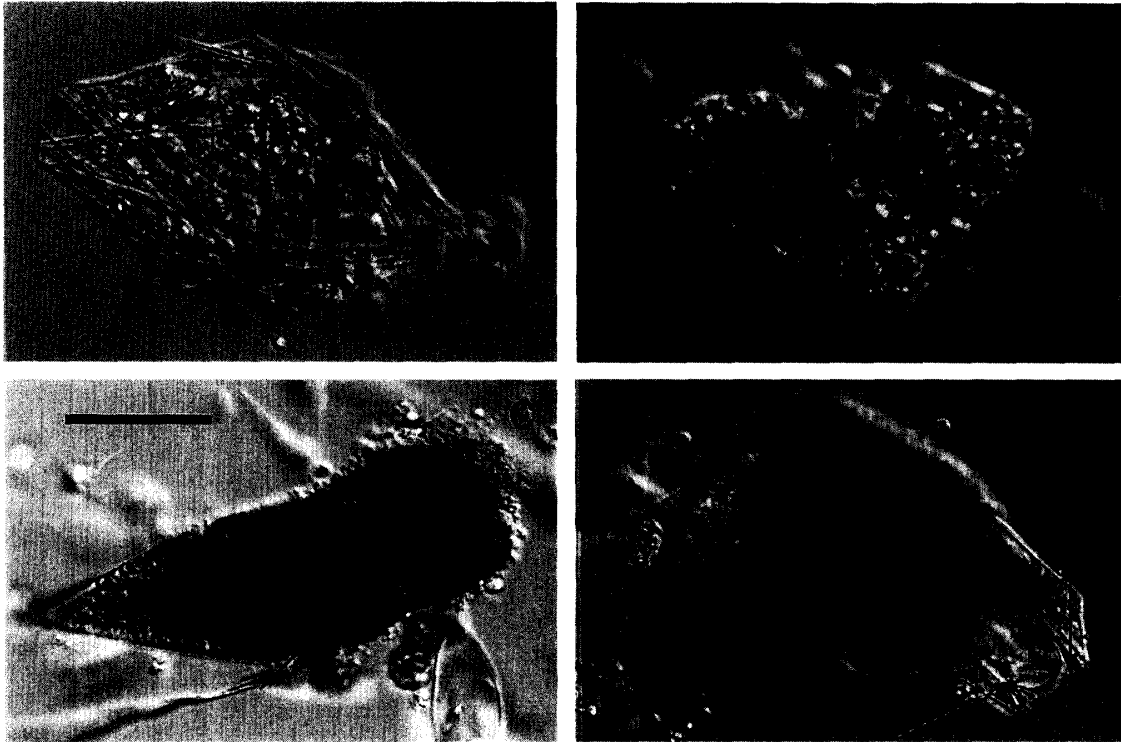
Fig. 8. Light microphotographs obtained during transect W showing (a, b) membrane bound fecal pellets, probably produced by heterotrophic dinoflagellates, containing many (a) *Fragilariopsis* spp., *Nitzschia* spp. and *Pseudo-nitzschia* spp. cells and (b) *Fragilariopsis* spp. cells, (c) *Gyrodinium* sp. with ingested *Fragilariopsis* spp. cells (arrow) and (d) *Gyrodinium* sp. expelling a membrane bound (arrow with solid line) fecal pellet containing *Corethron criophilum* (arrow with dotted line). Scale bars 50 μm.

transect W, probably produced by heterotrophic dinoflagellates (Figs. 8a, b). Furthermore, *Gyrodinium* sp. was observed that had ingested *Fragilariopsis* spp. cells (Fig. 8c). This suggests that nanoplanktonic diatoms are an important food source for dinoflagellates and possibly play a significant role in the microbial food web in the Antarctic waters. However, Buck and Newton (1995) found dinoflagellate fecal pellets from Puget Sound, North America, containing large *Thalassiosira* spp. In addition, Gonzáles (1992) found small fecal pellets containing large centric diatoms, such as *C. criophilum,* in Antarctic waters. We also found that *Nitzschia* spp. and *Pseudo-nitzschia* spp. were contained in the fecal pellets in this study (Fig. 8a) and *Gyrodinium* sp. was observed expelling fecal pellets containing diatoms as large as *C. criophilum* (Fig. 8d). Thus dinoflagellates are not limited to grazing the numerically dominant nanoplankton but can ingest the large diatoms that commonly comprise most of carbon and chlorophyll in Antarctic waters (see above).

Conclusions

This study confirmed that cyanobacteria were rare south of APF and showed that the abundance of ANE was generally greater than APE across the Southern Ocean and in

Antarctic waters. While the relative contributions of pico- and nanophytoplankton to the total phytoplankton standing stock vary with both time and space (Weber and El-Sayed, 1987), the scarcity of autotrophic picoplankton is considered to be a key feature of the Antarctic waters (Garrison and Mathot, 1996). There were major differences in phytoplankton community composition across the Southern Ocean. Nanoplanktonic *Diatoms* dominated south of the APF, *Haptophytes* north of it and cyanobacteria at northern end, suggesting that carbon flow in the ecosystem may vary latitudinally. In Antarctic waters, grazing by microheterotrophs, particularly dinoflagellates, appears to be important in packaging small cells as larger, presumably faster sinking, aggregates. Consequently, as suggested by Marchant (1993), there appears to be a substantial difference in the microbial community and therefore community interactions between Antarctic and warmer waters.

Acknowledgments

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