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Phytoplankton assemblages in the Gerlache and Bransfield Straits (Antarctic Peninsula) determined by light microscopy and CHEMTAX analysis of HPLC pigment data

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Abstract

The distribution and composition of phytoplankton assemblages were studied in the Gerlache and Bransfield Straits (Antarctic Peninsula) during the FRUELA 95 (December 1995) and FRUELA 96 (January 1996) cruises, using light microscopy and HPLC pigment analysis. Based on phytoplankton size and composition, two regions could be distinguished. The first region embraced the southwestern part of the Gerlache Strait, including a frontal system in the northeastern area. Chlorophyll (Chl) *a* values were generally high in surface waters (from 3.5 to 26.2 $\mu g l^{-1}$). Phytoplankton assemblages in the stratified waters of the southwestern Gerlache Strait were dominated by large diatoms and the flagellate *Pyramimonas* sp. (mixed with *Phaeocystis* in FRUELA 95). Pigment patterns included Chl *a*, Chl *b*, different Chls *c*, and fucoxanthin as the major carotenoid. The frontal zone was characterized by a bloom of *Pyramimonas*. Following a transect from southwestern Gerlache Strait towards the Bransfield Strait an increased contribution of Chl *b*, violaxanthin, and two unknown carotenoids (tentatively identified as loroxanthin and loroxanthin-ester) was observed which paralleled the *Pyramimonas* distribution. The marker pigment lutein, usually associated with chlorophytes and prasinoxanthin-lacking prasinophyceans, was only detected at very low concentrations. The second region, embracing the Bransfield Strait and one station in the Drake Passage, was characterized by stratified waters and low Chl *a* concentration (from 0.18 to 3.88 $\mu g l^{-1}$). Phytoplankton assemblages were dominated by the nanoplankter *Cryptomonas* sp. (FRUELA 95), the colonial haptophyte *Phaeocystis* cf. *antarctica*, and small flagellates (FRUELA 96). Pigment composition was mainly constituted by Chl *a*, Chl c_2, Chl c_3, alloxanthin, fucoxanthin, 19′-butanoyloxyfucoxanthin, and 19′-hexanoyloxyfucoxanthin. HPLC pigment data were processed using a factorization matrix program (CHEMTAX) to estimate the contribution of different algal classes to total Chl *a*. Four 'algal groups' were included in the chemotaxonomic approach: '*diatoms*', '*Phaeocystis*', '*cryptophytes*', and '*Pyramimonas*'. A fifth '*chemotaxonomic group*' was defined to reconstruct the distribution of an assemblage consisting of autotrophic peridinin-lacking dinoflagellates, some haptophytes, and chrysophytes, which were probably included by cell counting into the single group of 'small flagellates'. The distribution patterns of the CHEMTAX groups were in agreement with cell counts of diatoms, cryptophytes, and *Pyramimonas*. Discrepancies were observed for *P.* cf.

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antarctica as well as for small flagellates and dinoflagellates. Significant positive correlations were found between phytoplankton cell counts and different Chls *c*, suggesting the chemotaxonomic usefulness of Chls *c* as marker pigments for phytoplankton groups in addition to carotenoids. © 2001 Published by Elsevier Science Ltd.

1. Introduction

The phytoplankton composition in coastal and frontal regions of Antarctic waters has been described as a nano- and picoplankton-sized community (Azam et al., 1991; Hewes et al., 1990; Smetacek et al., 1990) on which blooms of diatoms (Bodungen et al., 1986; Detmer and Bathmann, 1997), haptophytes (*Phaeocystis antarctica*; Baumann et al., 1994), prasinophyceans (*Pyramimonas* sp.; Bird and Karl, 1991), and cryptophytes (*Cryptomonas* sp.; Vernet, 1992) are occasionally superimposed.

Bloom formation is generally associated with the stabilization of the upper mixed layer (UML) by melting from receding ice edges (Bodungen et al., 1986; Holm-Hansen et al., 1989) in shelf waters and marginal ice zones (MIZ), as occurs in the Gerlache and Bransfield Straits. Algal blooms also have been reported either associated with ice formation in the southern Weddell Sea (Sakshaug, 1989; Smetacek et al., 1992) or with frontal structures like those found in Bransfield Strait (Mura and Agustí, 1998).

The small-sized phytoplankton in the Southern Ocean hampers the light microscopy identification and counting because these organisms usually lack taxonomically useful morphological features. In addition, many species are very fragile and do not survive sample fixation (Gieskes and Kraay, 1983; Simon et al., 1994). To overcome some of these problems, a chemotaxonomic approach based on chromatographic pigment analysis of taxon-specific marker pigments has been employed to distinguish the main algal classes. Pioneering applications (Jeffrey, 1976; Jeffrey and Hallegraeff, 1980) were based on thin-layer chromatography (TLC); since mid-1980s, the preferred method has been HPLC (Barlow et al., 1993; Bidigare et al., 1990; Gieskes and Kraay, 1986; Goericke and Repeta, 1993; Letelier et al., 1993; Wright et al., 1996). Previous studies of phytoplankton pigment distributions in the Southern Ocean have revealed that taxonomical groups such as haptophytes (Barlow et al., 1998; Buma et al., 1990), green algae (Peeken, 1997; Prezelin et al., 1992), and cryptophytes (Buma et al., 1992; Vernet, 1992) were important components in austral spring and summer blooms.

The use of HPLC for estimating the quantitative contribution of different phytoplankton groups to total chlorophyll (Chl) *a*, using marker pigments, has attracted much attention in recent years (Andersen et al., 1996; Gieskes et al., 1988; Letelier et al., 1993; Wright et al., 1996). However, ideally, the distribution of microalgal groups inferred from marker pigments should be carefully contrasted with microscopy (or flow cytometry) observations because some carotenoids and chlorophylls are shared among different algal classes (Jeffrey et al., 1999). Divinyl (DV) Chl *a* and alloxanthin are specific marker pigments for *Prochlorococcus marinus* and cryptophytes; however, the abundance of diatoms and haptophytes, estimated from fucoxanthin and 19′-hexanoyloxyfucoxanthin, respectively, may be prone to error because other algal classes (chrysophytes, dinoflagellates, etc.) contribute to these carotenoid pools. Moreover, pigment composition and pigment ratios are influenced by environmental factors (Geider et al., 1993; Goericke and Montoya, 1998; van Leeuwe and Stefels, 1998). Pigment distribution can be highly variable between members of a single class (Simon et al., 1994; Zapata and Garrido, 1997), and even between strains from a single species (e.g. *Phaeocystis*, Bidigare et al. (1996) and Vaulot et al. (1994), or *Emiliania huxleyi*, Garrido and Zapata (1998)). All these statements must be borne in mind when interpreting the relative abundance of phytoplankton classes from pigment concentrations.

To date, the most suitable approach for HPLC pigment data interpretation is that achieved by the matrix factorization program CHEMTAX (Mackey et al., 1996). This mathematical technique calculates the relative abundance of algal classes

based on initial guesses of pigment ratios for each class. Its application to field samples in different oceanic regions (Higgins and Mackey, 2000; Mackey et al., 1998; Wright et al., 1996; Wright and van den Enden, 2000), coastal waters (Pinckney et al., 1998), and several lakes (Descy et al., 2000) has shown a sound capability to reconstruct distributions of several algal classes, and even different pigment types from a single class (Wright et al., 1996; Wright and van den Enden, 2000).

HPLC methods usually employed in marine research cannot resolve the diverse array of Chl *c* pigments potentially present in natural samples (Jeffrey et al., 1999). The incorporation of Chls *c* to the chemotaxonomic analysis of phytoplankton could be very useful to improve the description of phytoplankton assemblages obtained from the CHEMTAX program, which is mainly based on carotenoids.

The scope of this study was to describe the spatial distribution of phytoplankton assemblages and compare the results obtained using two techniques: first, the classical method of cell counting by light microscopy, and second, the chemotaxonomic approach based on HPLC pigment analysis and CHEMTAX processing of pigment data. The pigment data presented here were obtained using a new HPLC method, able to separate most taxon-specific carotenoids and chlorophylls (specially Chl *c* pigments) from marine phytoplankton (Zapata et al., 2000).

Cell counts and pigment analysis provided a good agreement with the distribution patterns for microplankton (diatoms), and some nanoplankton-sized algae (*Pyramimonas* sp., *Cryptomonas* sp.). In samples having low Chl *a*, the chemotaxonomic approach allowed the detection of marker pigments associated with small-sized cryptophytes, haptophytes, and chrysophytes grouped as 'small flagellates' by light microscopy.

2. Materials and methods

2.1. Sample collection

Phytoplankton samples were collected at 11 stations in the Gerlache and Bransfield Straits (Eastern area of the Bellingshausen Sea) during the FRUELA 95 (December 1995) and FRUELA 96 (January 1996) cruises on board R.V. *Hespérides* (Fig. 1). Samples were taken from CTD-casts using 12-l PVC Niskin bottles, at depths of 5, 10, 20, 40, and 60 m. For HPLC pigment analysis seawater samples (490–2000 ml) were filtered onto Whatman GFF filters (47 mm diameter) and kept frozen until pigment analysis.

2.2. Phytoplankton counting

Aliquots of 125 ml were preserved with Lugol's solution in plastic bottles (Margalef, 1974). Samples were kept in dark and cool (4°C) conditions until cell counting. Phytoplankton cells were enumerated using the inverted microscope procedures described by Uthermöhl (1958). Sample volumes of 10–50 ml were allowed to settle for 24–48 h, depending on the expected abundance of cells as estimated from Chl *a* concentrations. A Nikon Diaphot TMD inverted microscope with Nomarski system was used. The whole bottom chamber was examined at $40\times$ to enumerate larger and less frequent microplankters, then, $100\times$, $200\times$, $400\times$, and $1000\times$ for identifying and counting smaller organisms.

When possible, the cells were identified to species level, but many of the observed forms had to be placed into taxonomic categories such as small flagellates. In this group were included organisms from different algal classes: Prasinophyceae, Prymnesiophyceae, Cryptophyceae (other than *Cryptomonas* sp.), and Chlorophyceae.

2.3. HPLC pigment analysis

Frozen filters were extracted in 5 ml of 95% methanol using a spatula for filter grinding and further sonication during 5 min at low temperature (~ 5°C). Extracts were then filtered through Whatman GFF filters to remove cell and filter debris. An aliquot (1 ml) of methanol extract was mixed with 0.4 ml of water to avoid peak distortion (Zapata and Garrido, 1991). Each sample was injected just after the water addition as a decrease in non-polar pigment concentrations was observed when diluted extracts were kept waiting for

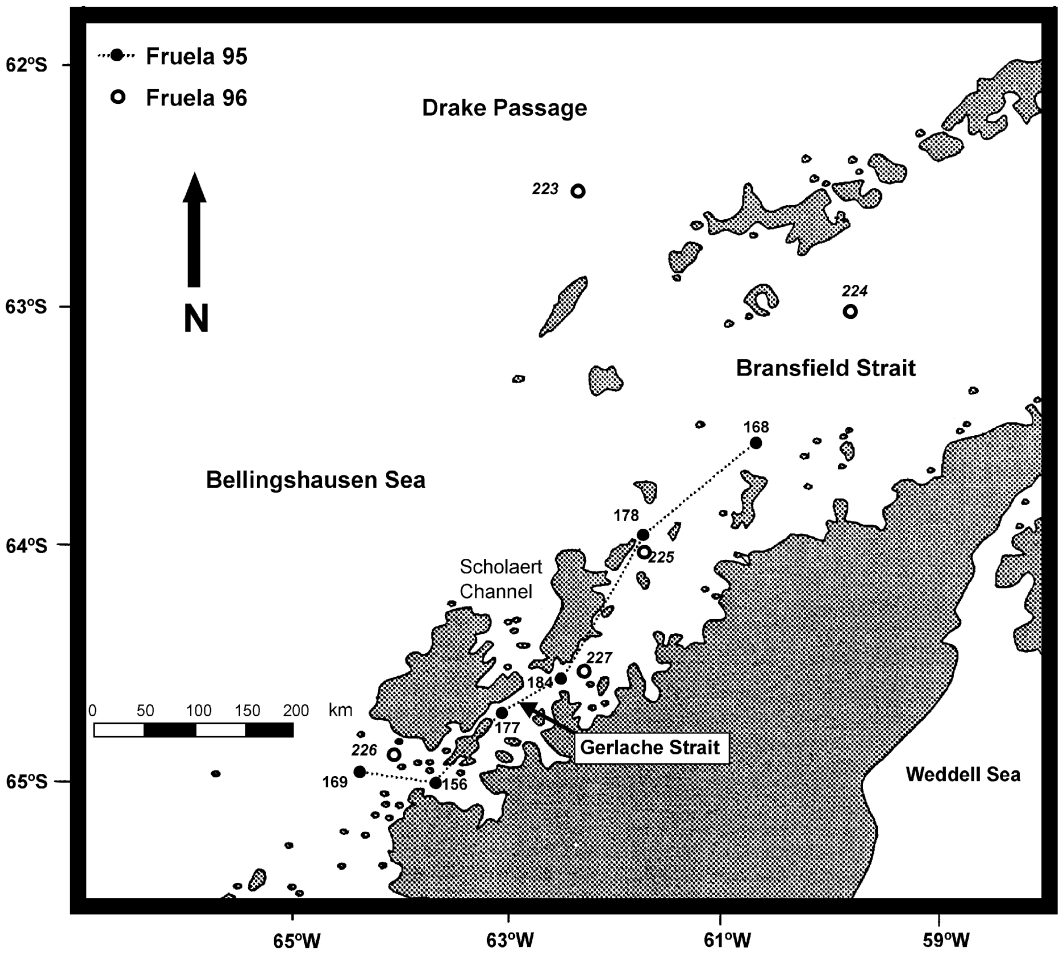

Fig. 1. Area of study and station locations for FRUELA 95 and 96 cruises.

injection inside the refrigerated autosampler (Zapata et al., 2000). A volume of 200 μl was injected into a Waters Alliance HPLC System consisting of a 2690 separations module, a Waters 996 photodiode array detector interfaced with a Waters 474 scanning fluorescence detector by a Sat/in analog interface.

Pigment separation was performed by HPLC according to Zapata et al. (2000). The stationary phase was a C_8 column (Symmetry 150×4.6 mm, 3.5 μm particle size, 100 Å pore size) thermostated at 25°C by means of a refrigerated circulatory water bath. Mobile phases were: A: methanol : acetonitrile : aqueous pyridine solution (0.25 M pyridine, pH adjusted to 5.0 with acetic acid) (50 : 25 : 25 v/v/v), and B: acetonitrile : acetone (80 : 20 v/v). A linear gradient from 0% to 40% B was pumped for 22 min, followed by an increase to 100% at minute 26 and isocratic hold at 100% B for a further 12 min. Initial conditions were reestablished by reversed linear gradient. Flow rate was 1 ml min^{-1}.

Chlorophylls and carotenoids were detected by diode-array spectroscopy (350–750 nm). Chlorophylls were also detected by fluorescence (Ex [excitation]: 440 nm, Em [emission]: 650 nm). Pigments were identified by co-chromatography with authentic standards and by diode-array spectroscopy (wavelength range: 350–750 nm, 1.2 nm spectral resolution). Each peak was checked for

spectral homogeneity using the Millennium software (Waters) algorithms, and the absorption spectrum was compared with a spectral library previously created. Pigments were quantified using external standards and extinction coefficients compiled by Jeffrey (1997).

2.4. CHEMTAX analysis of pigment data

The contributions of phytoplankton classes (or phytoplankton pigment groups) to the total Chl *a* concentration were obtained using CHEMTAX software running under MATLAB™. The basis of calculations and procedures used are fully described in Mackey et al. (1996).

The initial pigment-ratio matrix (Table 1) was a modification of that reported by Mackey et al. (1996). Additional pigments, members of the Chl *c* family, were added to the initial ratio matrix: Chl c_1, Chl c_2, Chl c_3 as well as the non-polar Chl *c* whose molecular structure has been recently described in *E. huxleyi* (Garrido et al., 2000) as a Chl c_2 moiety esterified to a monogalactosyldiacylglyceride (Chl c_2-MGDG). Pigment ratios from *Phaeocystis* sp. strains isolated from Antarctic waters (Culture Collection of Australian Antarctic Division, Kingston, Tasmania, Australia), were used for estimating the '*Phaeocystis*' distribution. The absence of peridinin (the marker pigment for autotrophic dinoflagellates), even in samples where dinoflagellates were detected by microscopy, precluded the evaluation of the contribution of peridinin-containing dinoflagellates to total Chl *a*. A '*chemotaxonomic group*' with a pigment signature including Chl c_3, Chl c_2, fucoxanthin (Fuco), 19′-butanoyloxyfucoxanthin (But-fuco), and 19′-hexanoyloxyfucoxanthin (Hex-fuco) was created to describe a pigment group that could

Table 1
Peak identification, retention times, and spectral absorbance maxima of phytoplankton pigments detected in seawater samples from cruises FRUELA 95 and 96

No.	Pigment	Retention time (min)	Maxima in eluant (nm)		
1	Chlorophyll c_3	7.65	457	588	628
2	Unknown chlorophyll *c*	8.91	450	583	631
3	Chlorophyllide *a*	10.14	430	581	663
4	MgDVP	10.67	438	575	627
5	Chlorophyll c_2	11.01	452	583	633
6	Chlorophyll c_1	11.72	448	580	631
7	19′-Butanoyloxyfucoxanthin	17.27		446	469
8	Fucoxanthin	18.17		449	
9	Neoxanthin	18.70	(416)	438	466
10	Unknown carotenoid 1 (loroxanthin)	18.85	(420)	444	472
11	Violaxanthin	20.68	416	440	470
12	19′-Hexanoyloxyfucoxanthin	21.19		446	469
13	Diadinoxanthin	23.23	(422)	446	476
14	Alloxanthin	25.70	(426)	452	482
15	Monadoxanthin	26.88	(423)	447	476
16	Zeaxanthin	26.95	(426)	453	478
17	Lutein	27.10	(422)	446	475
18	Unknown carotenoid 2 (loroxanthin ester)	29.27	(421)	448	475
19	Crocoxanthin	29.89	(422)	447	476
20	Chlorophyll *b*	30.32	462	599	648
21	Chlorophyll c_2-MGDG	30.87	455	584	633
22	Chlorophyll *a* allomer	31.28	430	615	662
23	Chlorophyll *a*	31.68	431	617	662
24	Chlorophyll *a* epimer	32.12	430	615	664
25	β-ε carotene	34.97	(422)	447	475
26	β-β carotene	35.39	(426)	452	477

account for the contribution of peridinin-lacking autotrophic dinoflagellates such as *Gymnodinium galatheanum* (Johnsen and Sakshaug, 1993) or *G. breve* (Zapata et al., 1998), and other algal groups whose pigment composition has not yet been exhaustively analyzed (e.g. Parmales, Chrysophyta). In the optical microscopy observations many of these organisms would be included into the 'small flagellates' group.

A culture of the prasinophycean algae *Pyramimonas gelidicola* (CS-129), isolated from Antarctic waters (CSIRO Algal Culture Collection, Tasmania, Australia) was studied to compare its pigment pattern with natural samples where *Pyramimonas* sp. was dominant.

3. Results

3.1. A brief description of oceanographic features from the study area

Physico-chemical gradients during the FRUELA cruises have been presented and discussed by Castro et al. (2002), García et al. (2002), and Rodríguez et al. (2002). Relevant hydrographic features were (i) the stratified water column found at both ends of the Gerlache Strait and (ii) the vertical mixed central region (Rodríguez et al., 2002) located in Scholaert Channel (St. 184) during FRUELA 95. The southwestern part of the Gerlache Strait was characterized by an upper layer of cold and low salinity water from melting ice, whereas the northeastern part showed a warmer and saltier surface layer (Fig. 2 from Rodríguez et al., 2002). A frontal region located between the mixed waters and the northeastern stratified side of the Gerlache Strait (Rodríguez et al., 2002) bounded different phytoplankton assemblages.

3.2. Spatial distribution of phytoplankton assemblages

3.2.1. Phytoplankton composition during the FRUELA 95 cruise

According to the phytoplankton distribution obtained by light microscopy, two regions could be distinguished in the study area: (i) from the southwestern end to the middle of the Gerlache

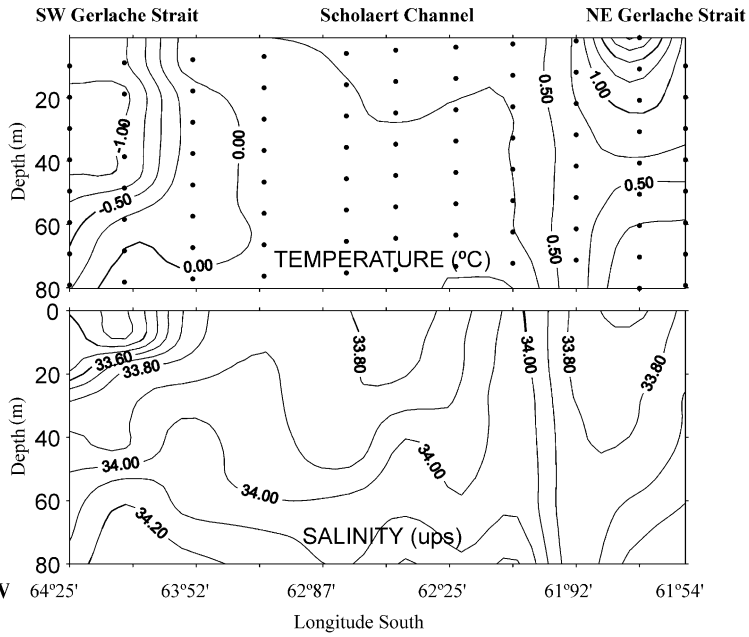

Fig. 2. Temperature (°C) and salinity (psu) distribution along FRUELA 95 cruise.

Strait (Sts. 169–184) and (ii) Bransfield Strait (Sts. 178 and 168). Fig. 3 shows cell counts of distinct algal groups in both regions, separated by the frontal region (St. 184).

The phytoplankton assemblages in the first region were characterized by *Pyramimonas* sp., chain-forming diatoms *Eucampia antarctica*, *Chaetoceros socialis* and *Odontella weissflogii*, and *P.* cf. *antarctica* (Fig. 3). The frontal region showed a surface bloom of *Pyramimonas* sp. (1.90×10^3 cells ml^{-1} at 2 m depth), and high numbers of small flagellates (9.53×10^3 cells ml^{-1}).

The second region was characterized by an increase in the relative abundance of nanoplankton-sized organisms (2–20 μm). At St. 178, the surface populations were dominated by the cryptophyte *Cryptomonas* sp. (6.36×10^3 cells ml^{-1}) and small flagellates (9.1×10^3 cells ml^{-1}), with

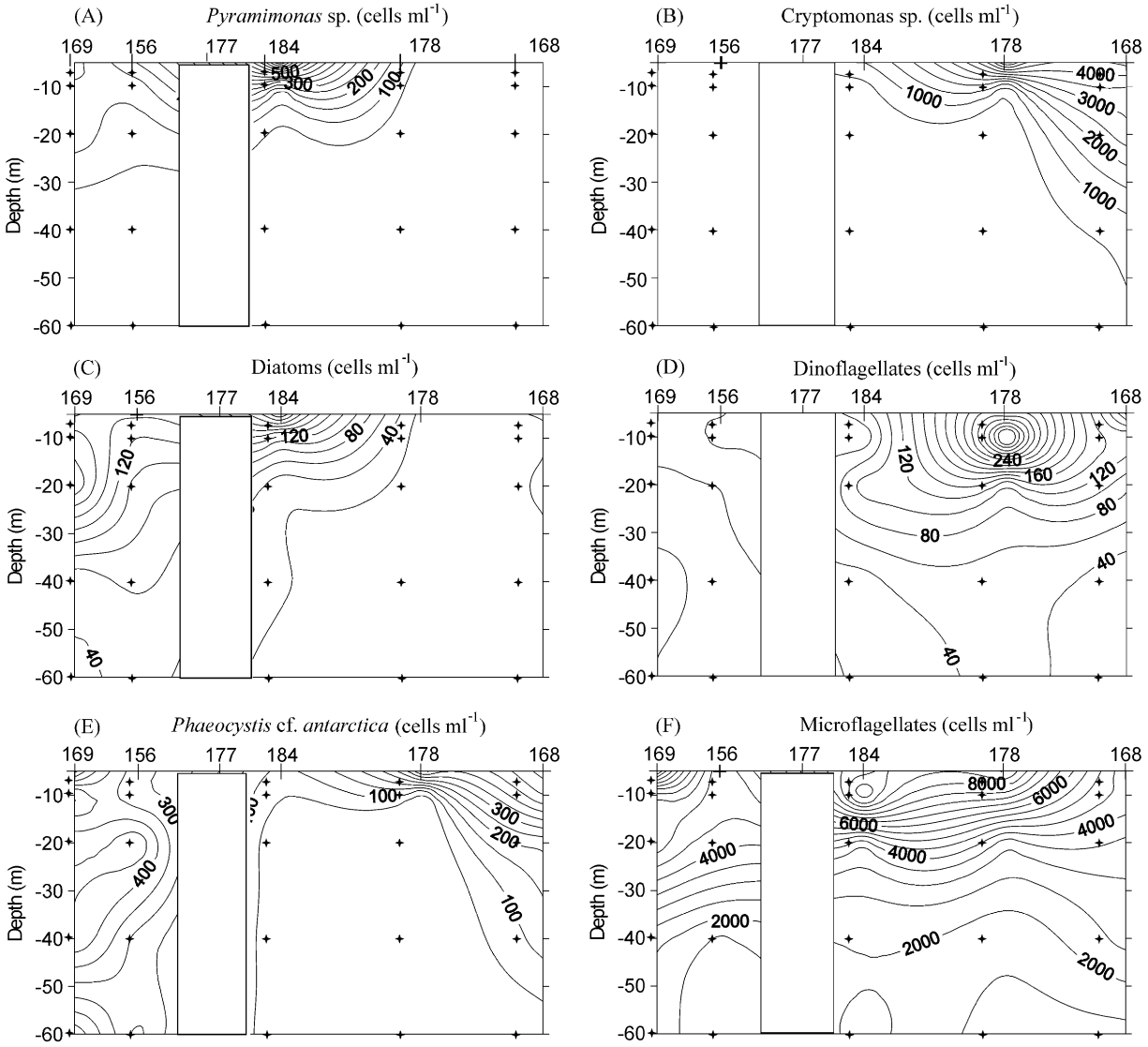

Fig. 3. Abundance of phytoplankton groups (cells ml^{-1}) during FRUELA 95 cruise: (a) *Pyramimonas gelidicola* (Prasinophyceae), (b) *Cryptomonas* sp. (cryptophyte), (c) diatoms, (d) dinoflagellates, (e) *Phaeocystis antarctica* (haptophyte), and (f) microflagellates.

highest abundance of dinoflagellates at surface waters (400 cells ml^{-1}). At St. 168 located in Bransfield Strait high numbers of *Cryptomonas* sp. were observed in the upper 40 m depth, together with a surface maximum of *P.* cf. *antarctica* (707 cells ml^{-1}).

3.2.2. Phytoplankton composition during the FRUELA 96 cruise

Stations sampled during FRUELA 96 were not distributed along a linear section, and the results have been plotted as individual stations in each area (Gerlache Strait, Bransfield Strait, and Drake Passage, Fig. 4). Diatoms and *Pyramimonas* sp. were dominant in the Gerlache Strait (Fig. 4, Sts. 226, 227, 225), whereas nanoplankton-sized phytoplankters (e.g. flagellated forms of *P.* cf. *antarctica*) were dominant in the Bransfield Strait and Drake Passage (Fig. 4, Sts. 224 and 223). In the Gerlache Strait (Sts. 226 and 227), diatoms like *Eucampia antarctica*, *Odontella weissflogii*, *and C. socialis* constituted the main component of the microplankton and showed a surface distribution restricted to the upper 20 m depth. The main feature was again a surface bloom of *Pyramimonas* located at NE Gerlache Strait (St. 225), with densities up to 1.73×10^3 cells ml^{-1}, similar to those observed in the previous cruise. In this area and in the Drake Passage (St. 223), the phytoplankton biomass was lower than in the FRUELA 95 cruise. In the Bransfield Strait (St. 224), C*ryptomonas* sp. (dominant during FRUELA 95) was substituted by *Phaeocystis* populations, mainly free cells, and large diatoms.

3.3. Spatial distribution of phytoplankton pigments

3.3.1. HPLC pigment patterns during the FRUELA 95 cruise

Based on the obtained chromatograms (Fig. 5) we distinguished four pigment patterns linked to the pigment (Figs. 6 and 7) and phytoplankton distributions observed in the study area.

The first two pigment patterns (Fig. 5a and b) occurred in the southwestern Gerlache Strait and the frontal region (Sts. 169–184); both were characterized by high Chl concentrations, particularly the second one, corresponding to the *Pyramimonas* bloom (20 μg Chl $a\,l^{-1}$ and 12.6 μg Chl $b\,l^{-1}$, Fig. 4a and b). The first pigment pattern was mainly contributed by diatoms and some *Pyramimonas* in the southwestern Gerlache Strait (Sts. 169–177). Chl c_3, Chl c_2, and Chl c_1 were the major Chl *c* pigments (Fig. 6), and Fuco the dominant carotenoid (Fig. 7). In particular, Chl c_2 and Chl c_1 attained their highest concentrations (1.23 μg Chl $c_2\,l^{-1}$ and 0.135 μg Chl $c_1\,l^{-1}$) at the southern boundary of the frontal region (St. 177, Fig. 6). The lack of cell counts at St. 177 precludes the comparison with phytoplankton pigments, but the subsequent CHEMTAX analysis of pigment data noticed a diatom maximum at this station. Minor contributions by haptophytes were also detected at Sts. 169 and 177, where a maximum of the Chl c_2-MGDG (Fig. 6) was associated with Chl c_3 and Hex-fuco, corresponding with high abundance of *P.* cf. *antarctica*. Chl c_3 registered a second maximum at St. 184 (0.160 μg Chl $c_3\,l^{-1}$) associated with Fuco as the major carotenoid and a maximum of small flagellates.

The second pigment pattern was observed in association with the *Pyramimonas* sp. bloom in the frontal region of Gerlache Strait (Fig. 5b). Chl *b* was the dominant accessory chlorophyll together with the highest concentrations detected of violaxanthin (Viola) and two unknown carotenoids (Table 2, peaks 10 and 18). The first unknown carotenoid practically coeluted with 9′-*cis*-neoxanthin (Neo) in our HPLC system, and both pigments were highly correlated to Chl *b* (peak 10, $r = 0.93$, $P < 0.001$, $n = 30$, and peak 18, $r = 0.94$, $P < 0.001$, $n = 30$).

The second pigment pattern was compared with that obtained for the prasinophycean *P. gelidicola* (CS-139) using the method of Wright et al. (1991). The resulting chromatogram showed a carotenoid pool constituted by Neo, Viola, and two unknown carotenoids as major peaks, with minor contribution of lutein (Lut). The first unknown was spectrally similar to the unknown peak 10 (Table 2), and considering its retention time and spectral characteristics was tentatively identified as loroxanthin (Loro). This pigment has been previously reported in several members of green algae like Chlorophyceae, Micromonadophyceae (Prasinophyceae), Ulvophyceae (see Fawley, 1991), as well

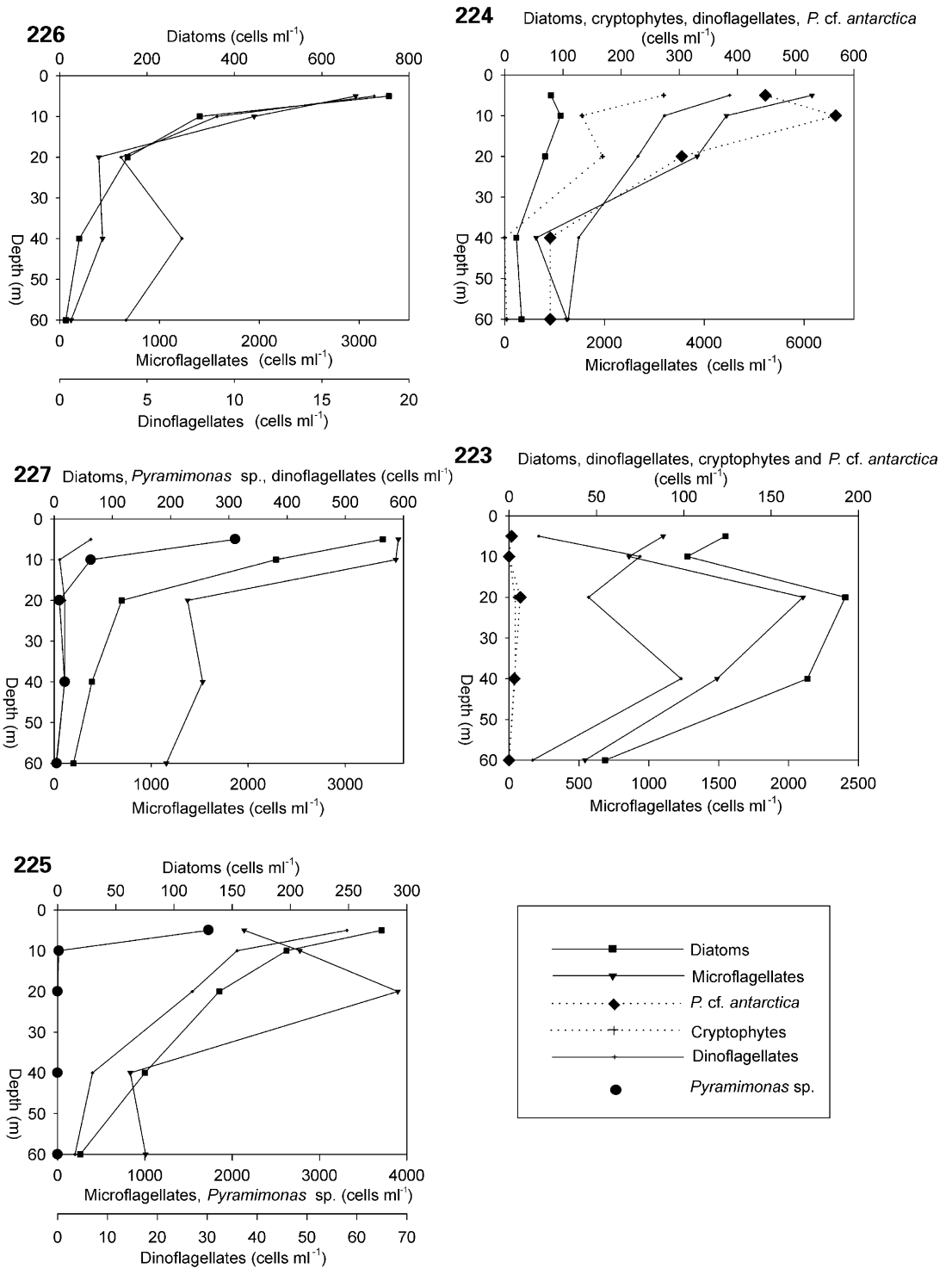

Fig. 4. Abundance of phytoplankton groups (cells ml^{-1}) during FRUELA 96 cruise: Sts. 226, 227, 225, 224, 223.

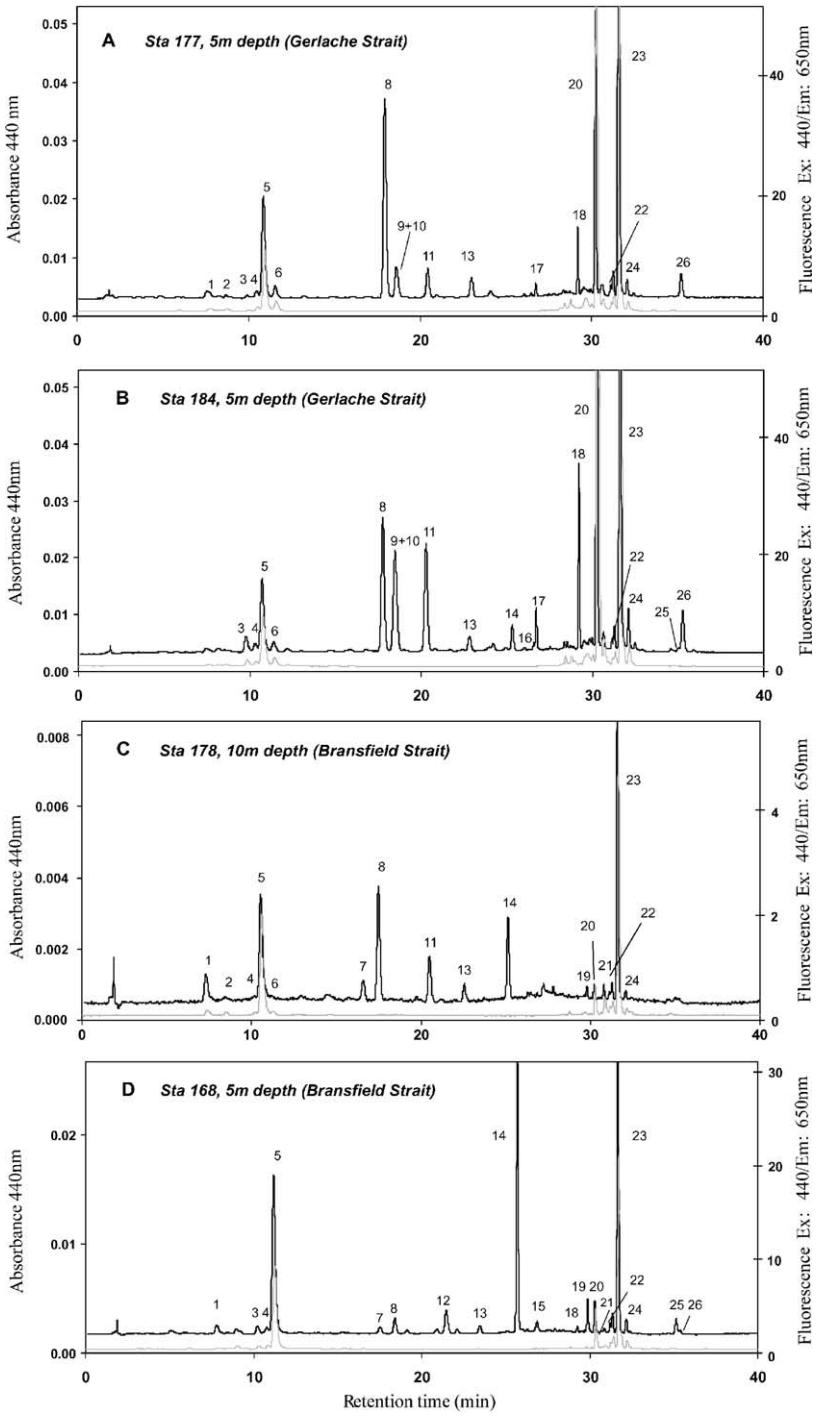

Fig. 5. Selected HPLC chromatograms showing pigment patterns associated to the main phytoplankton assemblages during FRUELA 95 cruise: (a) diatoms–*Pyramimonas gelidicola* at SW Gerlache Strait (FRUELA 95); (b) *Pyramimonas gelidicola* bloom at the frontal zone between Gerlache and Bransfield Straits (FRUELA 95); (c) *Phaeocystis antarctica–Cryptomonas* sp. at NE Gerlache Strait (FRUELA 95); and (d) *Cryptomonas* sp. at Bransfield Strait (FRUELA 95). Peak identifications are as in Table 1.

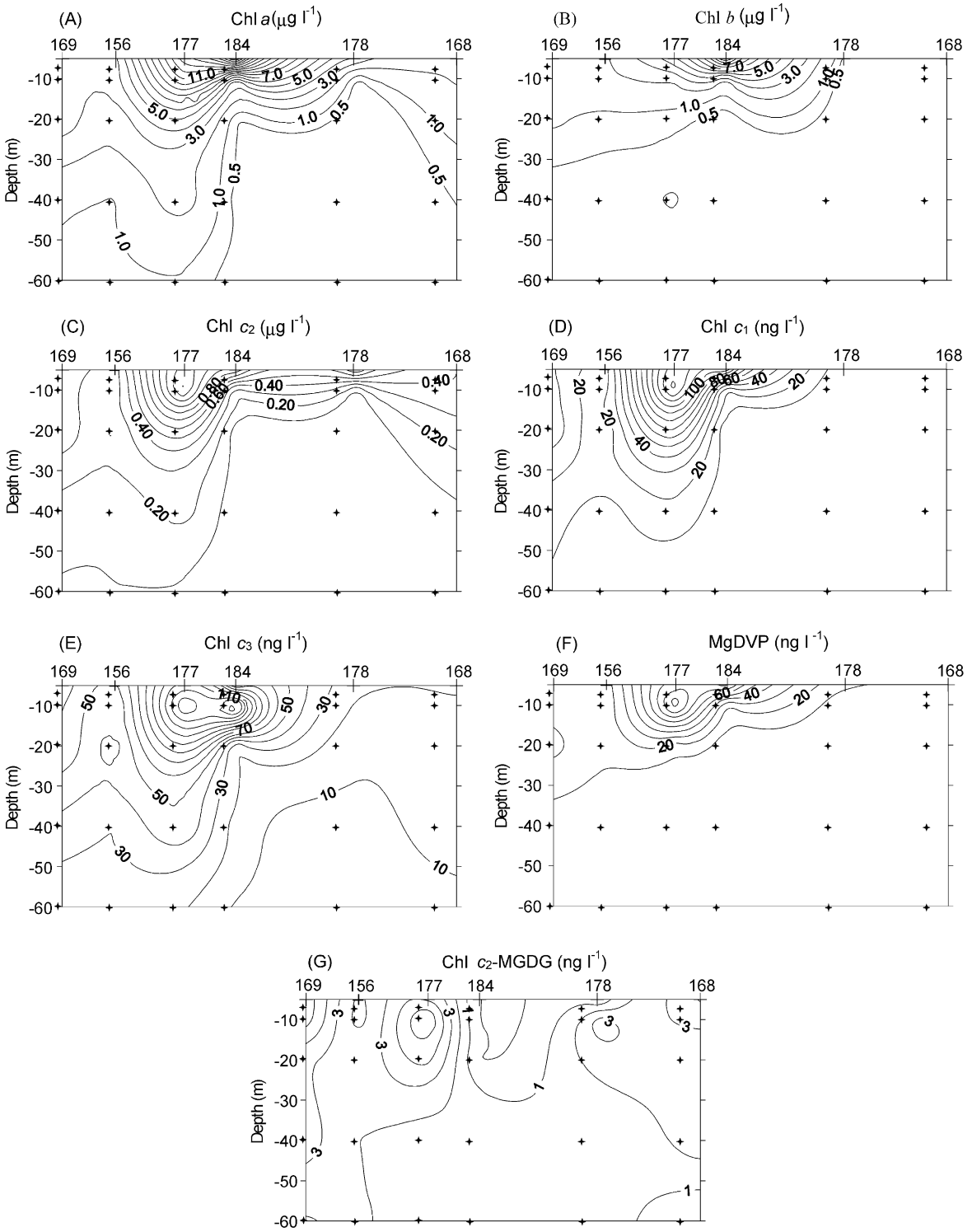

Fig. 6. Concentrations of chlorophylls from FRUELA 95 cruise: (a) Chl *a*, (b) Chl *b*, (c) Chl c_2 ($\mu g\,l^{-1}$), and (d) Chl c_1, (e) Chl c_3, (f) MgDVP, and (g) Chl c_2-MGDG ($ng\,l^{-1}$).

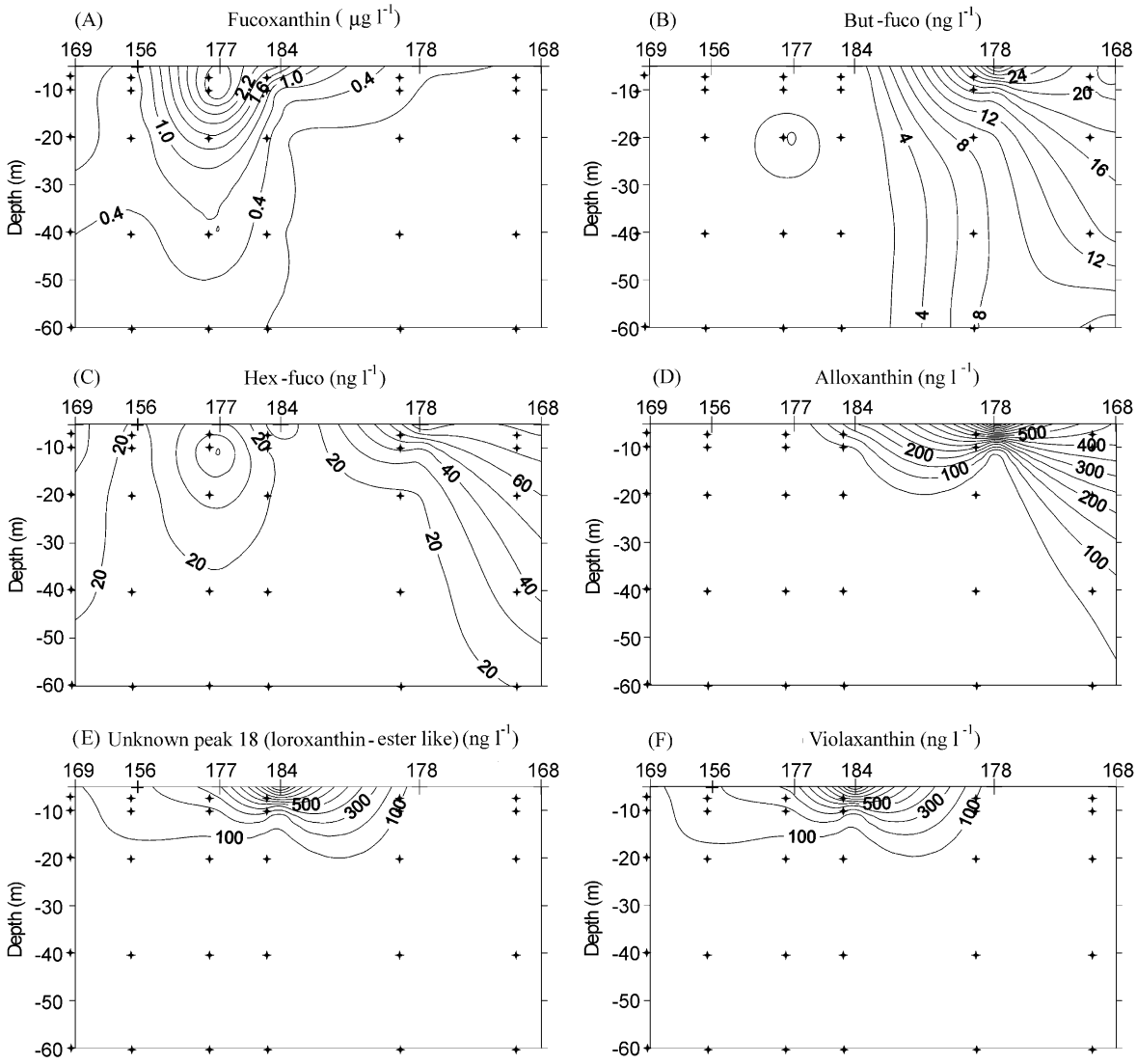

Fig. 7. Concentrations of carotenoids from FRUELA 95 cruise: (a) fucoxanthin ($\mu g\,l^{-1}$), and (b) 19′-butanoyloxyfucoxanthin, (c) 19′-hexanoyloxyfucoxanthin, (d) alloxanthin, (e) unknown peak 18 (loroxanthin ester-like), and (f) violaxanthin ($ng\,l^{-1}$).

as in *Pyramimonas parkeae* (Kohata and Watanabe, 1989), although Loro was not detected in other five species of *Pyramimonas* (Brown and Jeffrey, 1992; Egeland et al., 1997). The second unknown carotenoid from *P. gelidicola* (CS-139) showed similar retention and spectral characteristics to the unknown peak 18 (Table 1). Both the spectral similarity with respect to Loro and its higher retention time are consistent with a loroxanthin-ester previously described in *Pyramimonas parkeae* (Kohata and Watanabe, 1989).

A third pigment pattern (Fig. 5c), contributed by *Phaeocystis* cf. *antarctica* and *Cryptomonas* sp. (minor groups as e.g. dinoflagellates and chrysophytes), was observed at Bransfield Strait (St. 178). This pigment pattern showed lower pigment

Table 2
Initial pigment ratios and calculated pigment ratios for FRUELA 95 and 96 cruises analyzed by CHEMTAX

	Chl c_3	Chl c_2	Chl c_1	Fuco	But-fuco	Hex-fuco	Allo	Viola	Chl b	Chl c_2-MGDG
Initial ratio matrix										
Pyramimonas	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.055	0.945	0.000
Cryptophytes	0.000	0.174	0.000	0.000	0.000	0.000	0.228	0.000	0.000	0.000
Chemotaxonomic group	0.067	0.126	0.000	0.290	0.122	0.248	0.000	0.000	0.000	0.000
Diatoms	0.000	0.110	0.073	0.754	0.000	0.000	0.000	0.000	0.000	0.000
Phaeocystis	0.141	0.144	0.000	0.011	0.080	0.916	0.000	0.000	0.000	0.054
Output ratio matrix										
Pyramimonas	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.069	0.977	0.000
Cryptophytes	0.000	0.184	0.000	0.000	0.000	0.000	0.228	0.000	0.000	0.000
Chemotaxonomic group	0.302	0.248	0.000	0.571	0.219	0.054	0.000	0.000	0.000	0.000
Diatoms	0.000	0.149	0.012	0.425	0.000	0.000	0.000	0.000	0.000	0.000
Phaeocystis	0.141	0.144	0.000	0.011	0.099	0.916	0.000	0.000	0.000	0.054

concentrations and was constituted by Chl c_2, Chl c_3, Chl c_2-MGDG, and the carotenoids But-fuco, Hex-fuco, and Allo as major compounds.

A fourth pigment pattern (Fig. 5d) dominated by cryptophytes was distinguished at St. 178 and specially at St. 168 located in the Bransfield Strait. Changes in vertical pigment distribution were observed at St. 178, with a cryptophyte pigment pattern in the surface layer, and Chl c_3, But-fuco, and Hex-fuco at deeper samples. However, single cells (or colonies) of *P.* cf. *antarctica* were only detected by microscopy for samples at 5 m depth. At St. 168, the *Cryptomonas* species pigment profile was dominant throughout the sampled water column (up to 60 m).

3.3.2. HPLC pigment patterns during the FRUELA 96 cruise

Four pigment patterns (Fig. 8) were distinguished in association with the dominant phytoplankton assemblages present in the study area. The first pigment pattern (Fig. 8a) was mainly contributed by diatoms in the southwestern Gerlache Strait (Sts. 226 and 227), characterized by Chls c_2 and c_1 (Fig. 9), and Fuco (Fig. 10) as the major carotenoid. The relationship between diatom numbers and these marker pigments was confirmed by significant correlation (Chl c_1, $r = 0.77$, $P < 0.001$, $n = 50$; Chl c_2, $r = 0.80$, $P < 0.001$, $n = 50$; Fuco, $r = 0.89$, $P < 0.001$, $n = 50$).

The second pigment pattern (Fig. 8b) was observed in the frontal region in the Gerlache Strait and the Bransfield Strait confluence, associated with the *Pyramimonas* bloom. The highest concentrations of Chl *a* ($26.1\,\mu g\,l^{-1}$) and Chl *b* ($21.2\,\mu g\,l^{-1}$) found during the study (Fig. 9) were reported during this event.

The third pigment pattern (Fig. 8c) was observed in the Bransfield Strait (St. 224) and Drake Passage (St. 223); it was characterized by low Chl *a* concentrations ($< 1.0\,\mu g\,l^{-1}$) associated with *P. antarctica* and small flagellates. The major chlorophylls were Chl *a*, Chl c_2, Chl c_3, and Chl c_2-MGDG, and the dominant carotenoids were Fuco, But-fuco, and Hex-fuco (Fig. 10). Chl *b* and Allo were only detected at St. 224. *Phaeocystis* counts showed a significant correlation with the Chl c_2-MGDG ($r = 0.47$, $P < 0.001$, $n = 50$) and Hex-fuco ($r = 0.47$, $P < 0.001$, $n = 50$). Unidentified small flagellates were significantly correlated with Chl c_3 ($r = 0.47$, $P < 0.001$, $n = 50$) and Allo ($r = 0.42$, $P < 0.001$, $n = 50$), the latter showing that some cryptophytes were probably included into this group by light microscopy.

A fourth pigment pattern observed at St. 223, located further north in the Drake Passage, was characterized by Chl c_3, Chl c_2, Fuco, But-fuco, and Hex-fuco. Small flagellates were the dominant group as identified by cell counts, and the lack of Chl c_2-MGDG distinguished this pigment pattern

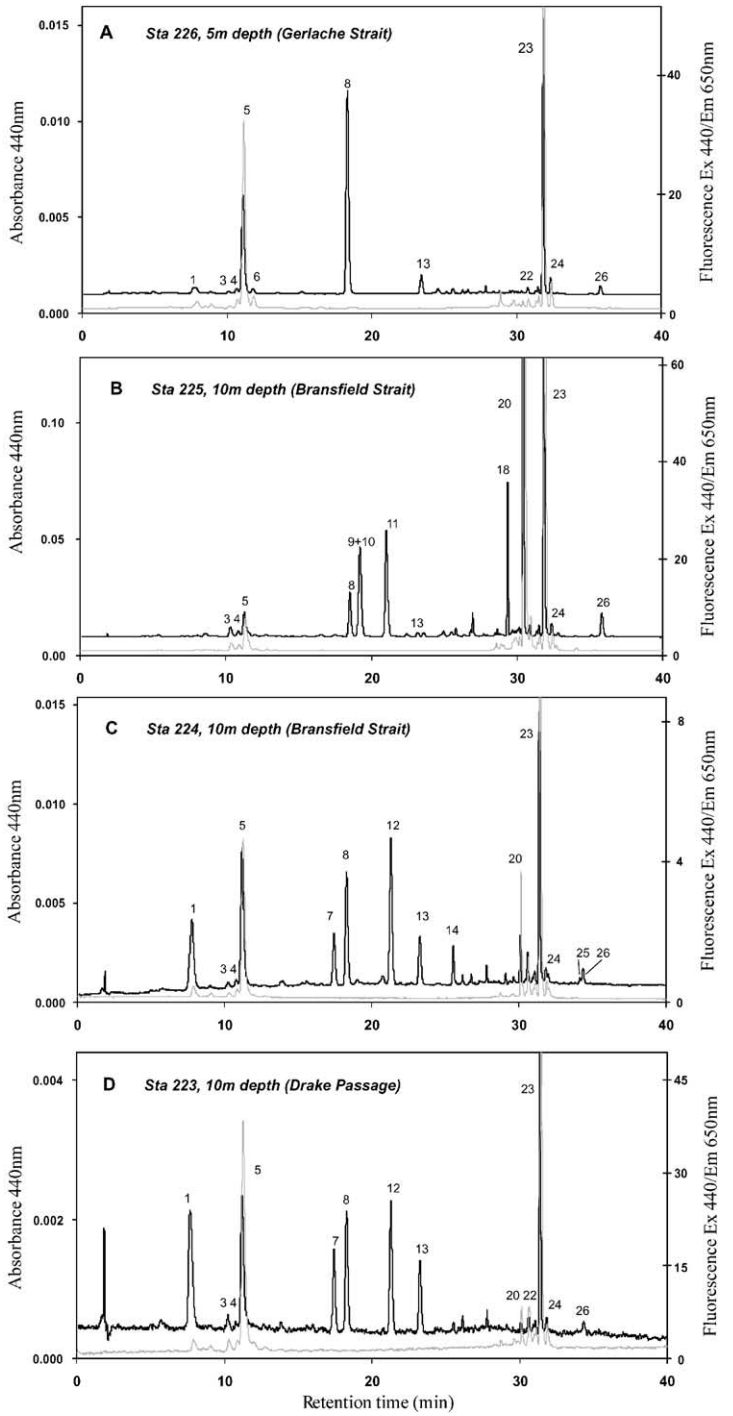

Fig. 8. Selected HPLC chromatograms showing pigment patterns associated to the main phytoplankton assemblages during FRUELA 96 cruise: (a) diatoms at SW Gerlache Strait (FRUELA 95); (b) *Pyramimonas gelidicola* bloom at the frontal zone in Bransfield Strait; (c) *P. antarctica* in Bransfield Strait, and (d) microflagellates at Drake Passage (FRUELA 96). Peak identifications are as in Table 1.

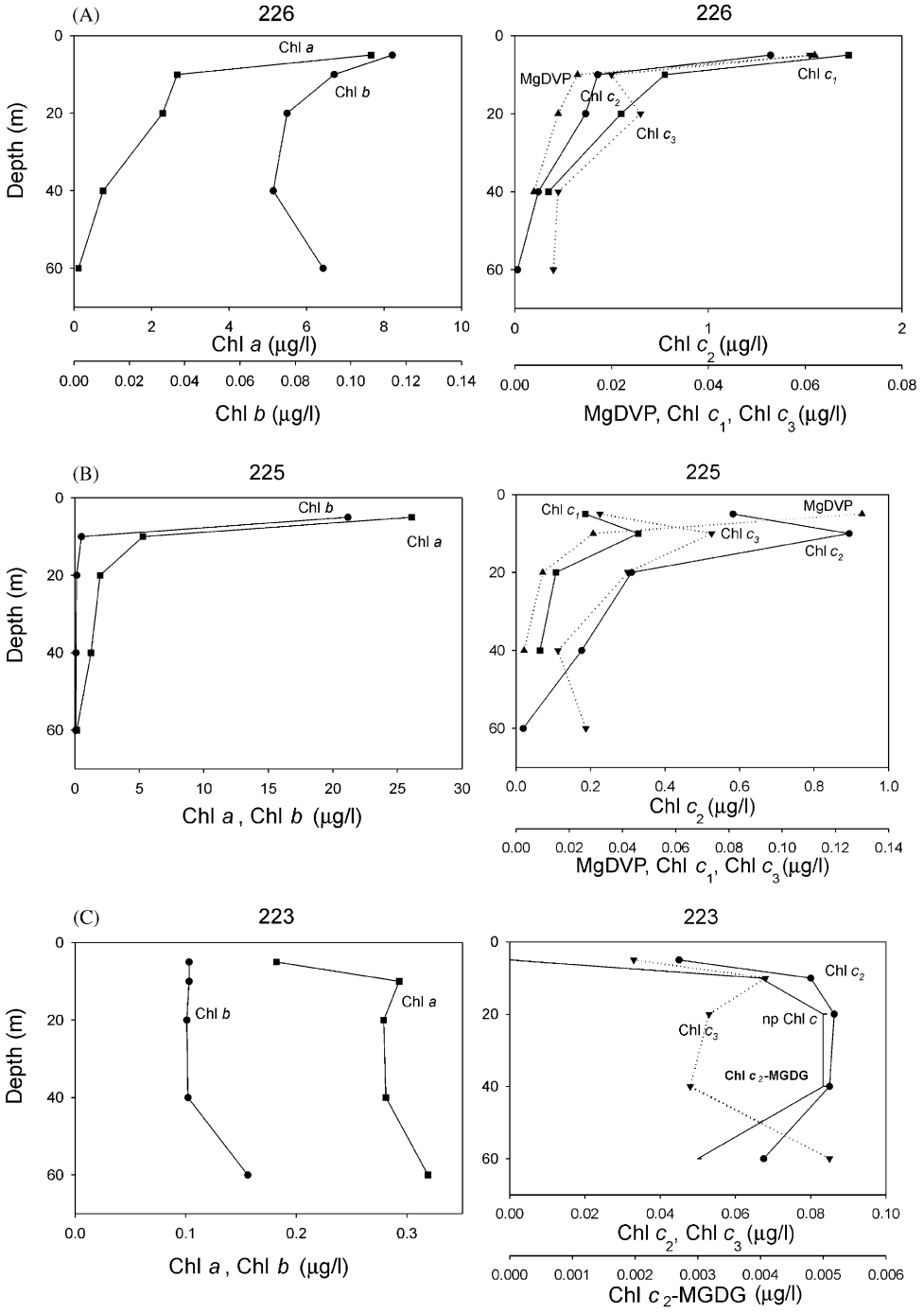

Fig. 9. Concentrations of chlorophylls from FRUELA 96 cruise: (a) St. 226, (b) St. 225, and (c) St. 223.

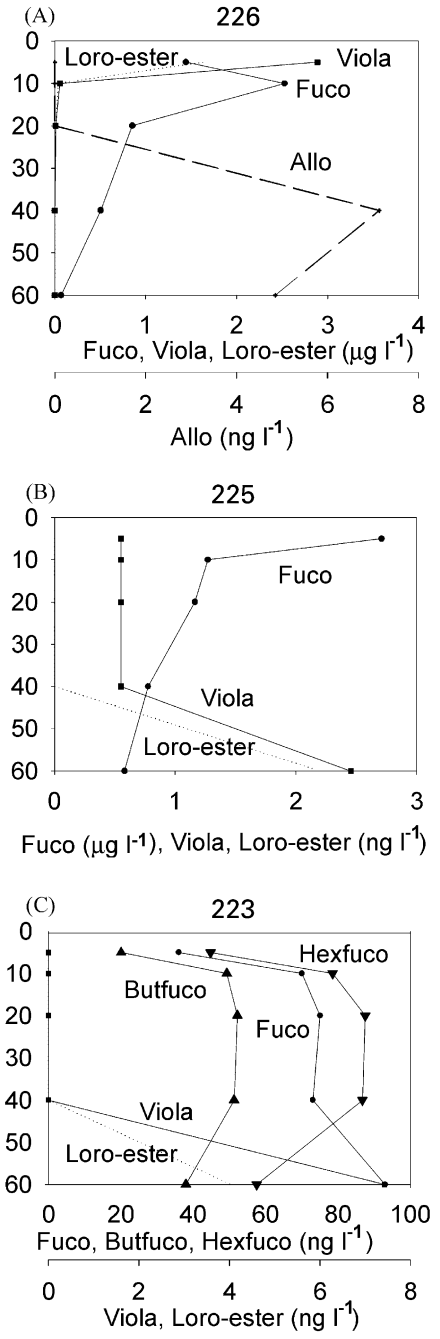

Fig. 10. Concentrations of carotenoids from FRUELA 96 cruise: (a) St. 226, (b) St. 225, and (c) St. 223.

from that observed in samples with *P.* cf. *antarctica*.

3.3.3. Interpretation of HPLC pigment data by CHEMTAX program

The initial pigment-ratio matrix and the final pigment ratios resulting from the fitting procedure are shown in Table 1. Initial and calculated pigment ratios were almost identical for '*Pyramimonas*', '*cryptophytes*', and '*Phaeocystis*', while larger differences were observed in the case of '*diatoms*' and the '*chemotaxonomic group*'.

3.3.4. FRUELA 95 cruise

The CHEMTAX-derived distribution of phytoplankton groups during FRUELA 95 (Fig. 11) showed mixed populations of '*diatoms*' and '*Pyramimonas*' at Gerlache Strait, with a minor contribution of '*chemotaxonomic group*' and '*Phaeocystis*'. The highest values of Chl *a* attributed to '*diatoms*' and '*Phaeocystis*' were obtained at St. 177, but could not be contrasted with cell counts due to the lack of data for this station.

CHEMTAX results in the frontal region (St. 184) reported high values of '*Pyramimonas*' and also a maximum for the '*chemotaxonomic group*'. The distribution of the '*chemotaxonomic group*' was similar to that of Chl c_3, showing its highest values at surface waters in the Gerlache Strait during the FRUELA 95 cruise, coinciding with a small flagellate maximum at St. 184.

In the Bransfield Strait zone, phytoplankton was dominated by mixed populations of '*cryptophytes*' and '*Phaeocystis*', resembling the distribution pattern based on cell counts (Fig. 3).

3.3.5. FRUELA 96 cruise

A similar taxonomical segregation associated with the hydrographical conditions was observed (Fig. 12): '*diatoms*' were mainly restricted to the Gerlache Strait (Sts. 226 and 225), while '*chemotaxonomic group*', '*cryptophytes*', and '*Phaeocystis*' were dominant in the Bransfield Strait and Drake Passage (Sts. 224 and 223). A maximum of '*diatoms*' was shown by CHEMTAX at surface in St. 225, but this feature could not be explained by diatom abundance patterns or by changes in diatom species. As the pigment pattern was by far

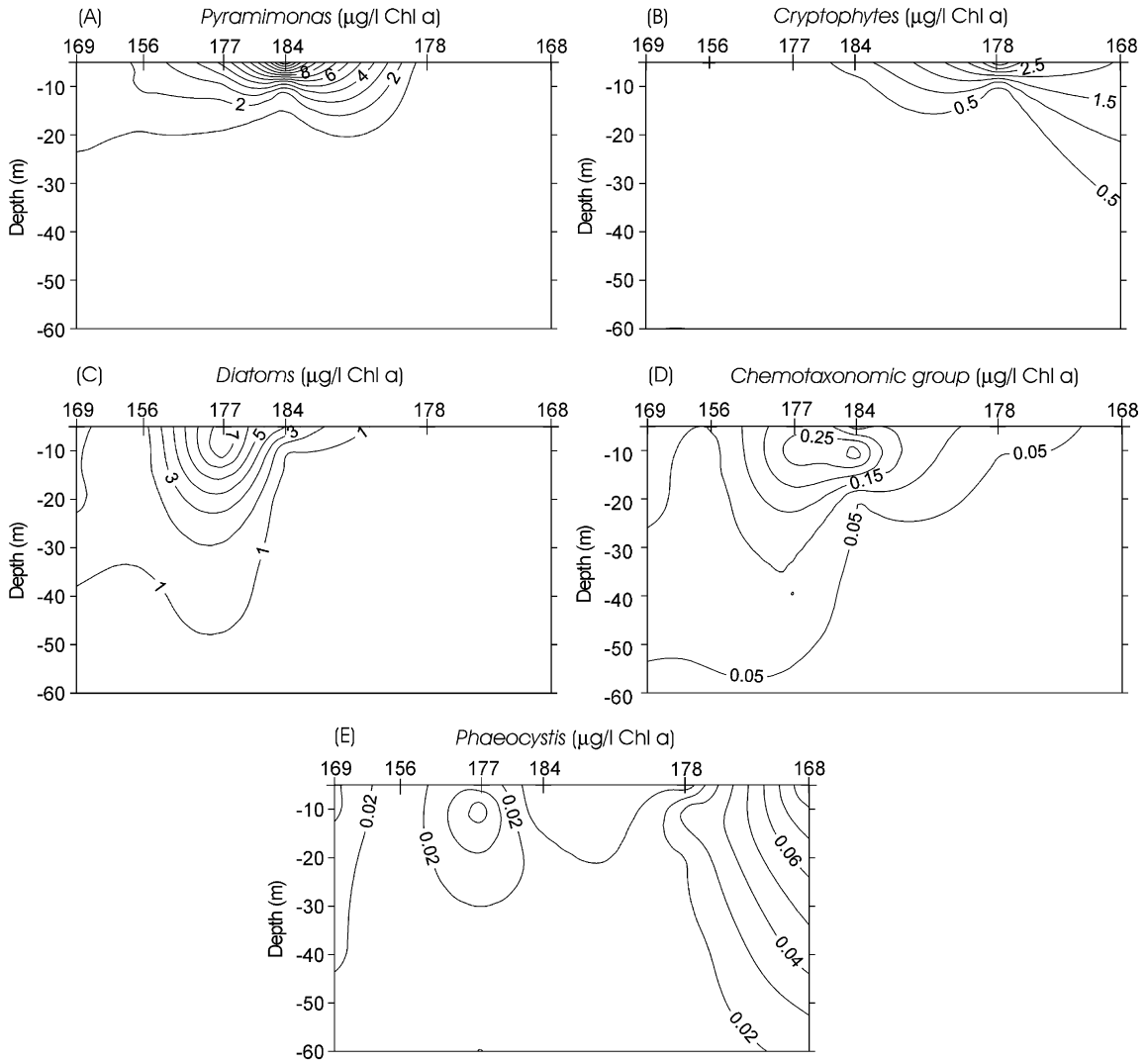

Fig. 11. CHEMTAX estimates of phytoplankton pigment groups to total Chl *a* concentrations during FRUELA 95 cruise: (a) '*Pyramimonas*' ($\mu g\,l^{-1}$), (b) '*diatoms*' ($\mu g\,l^{-1}$), (c) '*Phaeocystis*' ($ng\,l^{-1}$), and (d) '*cryptophytes*' ($\mu g\,l^{-1}$), (e) '*chemotaxonomic group*'.

dominated by diatoms, we can hypothesize that the CHEMTAX maximum was due to discrepancies between the actual and calculated pigment ratios. On the other hand, a maximum ($150\,cells\,ml^{-1}$) of unidentified and very small diatoms ($<10\,\mu m$) at St. 223 was not reconstructed by CHEMTAX. This fact could be explained either by Chl c_1-lacking diatom species or by very low Chl c_1/Chl c_2 ratios yielding undetectable levels of Chl c_1.

CHEMTAX results described a wider spatial distribution of '*cryptophytes*' as compared to that detected by microscopic counts (Fig. 3). This feature seems to be associated with the significant correlation between small flagellates abundance and Allo. Thus, thanks to the detection of Allo, the chemotaxonomic approach can highlight a different distribution, from the microscopic approach, for this microalgal group. '*Pyramimonas*' presented a distribution pattern similar to that of

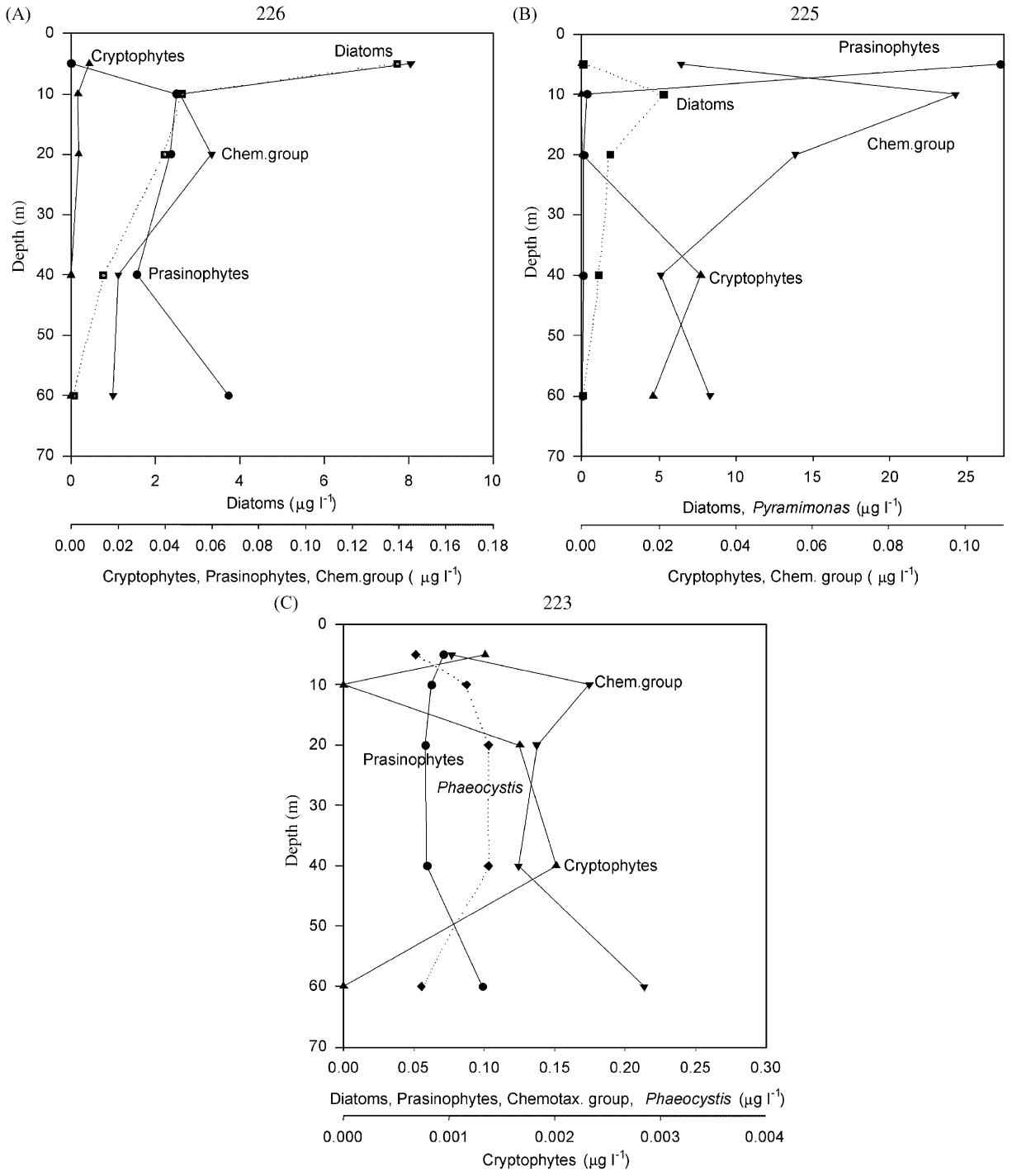

Fig. 12. CHEMTAX estimates of phytoplankton pigment groups to total Chl *a* concentrations during FRUELA 95 cruise: (a) St. 226 (b) St. 225, and (c) St. 223.

FRUELA 95, with the highest abundance at the southwestern Gerlache Strait and the frontal area (St. 225, Fig. 12).

The distribution pattern of '*chemotaxonomic group*' (Fig. 12) was not paralleled by cell counts of a single algal class, although it resembled that described for Chl c_3, as well as the combined patterns of dinoflagellates and small flagellates cell numbers.

3.4. CHEMTAX estimates vs. cell counting

Direct comparisons of CHEMTAX estimates of Chl *a* contributed by different chemotaxonomic groups and phytoplankton cell counts showed a good agreement for diatoms, cryptophytes, and *Pyramimonas* sp. (Figs. 13 and 14). Discrepancies were observed for *P.* cf. *antarctica* and small flagellates and dinoflagellates as compared with '*chemotaxonomic group*'. In FRUELA 95, estimates of Chl *a* contributed by '*Phaeocystis*' yielded a poor correlation with cell counts ($r^2 = 0.12$, $P > 0.05$, $n = 50$), whereas in FRUELA 96 a significant correlation was observed but only a few samples showed presence of *P.* cf. *antarctica* ($n = 7$). Estimates of small flagellate numbers were significantly correlated with '*chemotaxonomic group*' (Fig. 13c) during the FRUELA 95 cruise, but the '*chemotaxonomic group*' in the FRUELA 96 cruise appeared to be significantly related to dinoflagellate abundance (Fig. 14d) and showed no significant relationship with the small flagellate distribution.

4. Discussion

4.1. Phytoplankton assemblages

Two distinct phytoplankton assemblages can be delineated using light microscopy observations: (i) microplankton-sized cells (diatoms and

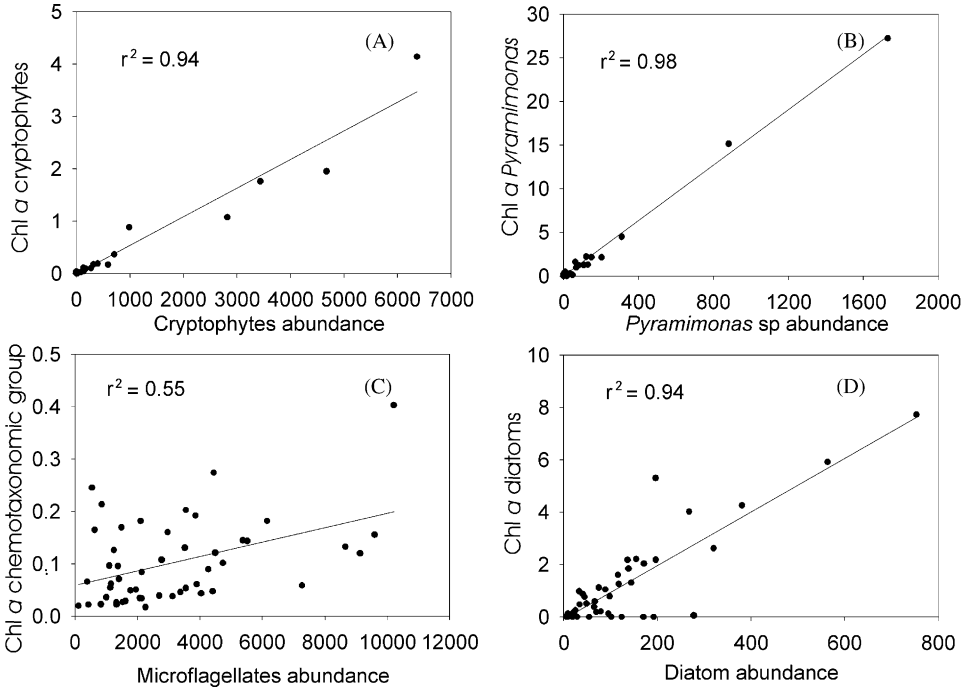

Fig. 13. Contribution to Chl *a* in FRUELA 95 cruise for each group calculated by CHEMTAX against cell numbers (cells ml^{-1}) in the corresponding phytoplankton classes identified by light microscopy: (a) '*cryptophytes*', (b) '*Pyramimonas*', (c) '*chemotaxonomic group*', and (d) '*diatoms*'.

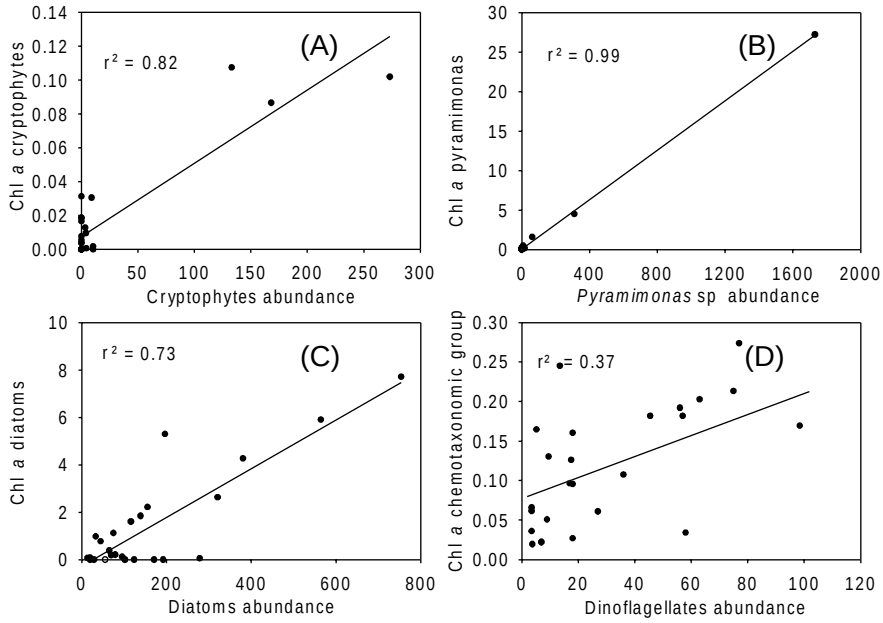

Fig. 14. Contribution to Chl *a* in FRUELA 96 cruise for each group calculated by CHEMTAX against cell numbers (cells ml^{-1}) in the corresponding phytoplankton classes identified by light microscopy: (a) '*cryptophytes*', (b) '*Pyramimonas*', (c) '*chemotaxonomic group*', and (d) '*diatoms*'.

Pyramimonas sp.) at the southwestern Gerlache Strait and its frontal zone and (ii) cryptophytes (FRUELA 95) and *P.* cf. *antarctica* (FRUELA 96) at the Bransfield Strait. Bird and Karl (1991) had already detected a massive bloom of *Pyramimonas* sp. reaching 25 μg Chl $a l^{-1}$, a similar value to those obtained in this study (19.9 μg Chl $a l^{-1}$ in FRUELA 95 and 26.1 μg Chl $a l^{-1}$ in FRUELA 96) at the frontal zone of the northern Gerlache Strait. Vernet (1992) distinguished pigment patterns belonging to diatoms and *Pyramimonas*-like cells at the southern end of the Gerlache Strait and cryptomonads farther north. The occurrence of cryptophytes in Antarctic waters is also well documented (Schloss and Estrada, 1994; Detmer and Bathmann, 1997). In particular, bloom densities have been reported at the Gerlache (Ferrario and Sar, 1992) and Bransfield Straits (Mura and Agustí, 1998).

The variability of the phytoplankton assemblages in the studied coastal region appears to be related to the interaction of different oceanographic processes (like stabilization of the upper mixed layer by ice melting or development of frontal systems) that trigger recurrent blooms in the spring and summer months (Holm-Hansen and Mitchell, 1991; Moline and Prezelin, 1996; Prezelin et al., 1992). In this context, the presence of *P.* cf. *antarctica* (reported as a typical ice alga) and diatoms in the southwestern Gerlache Strait seems to be favored by the melting of ice and the development of stratified conditions in this area (Varela et al., 2002). On the other hand, the frontal area in the northeastern Gerlache Strait represents a boundary region favorable to the development and establishment of large-sized phytoplankton populations (Lütjeharms et al., 1985; Smetacek et al., 1997; Turner and Owens, 1995), such as those of *Pyramimonas* sp., and the diatoms registered in this study. The dominance of cryptophytes and *P.* cf. *antarctica* in the stratified Bransfield Strait waters could be explained by two non-excluding mechanisms: first, these flagellated algae should be favored during enhanced water column stability periods (Kiørboe, 1993; Margalef, 1978), and second, krill grazing pressure also could

be determining the phytoplankton size distribution by removing selectively larger organisms from the water column (Varela et al., 2002).

4.2. Interpretation of pigment data

Pigment analysis during the FRUELA 96 cruise allowed the detection of significant amounts of alloxanthin, which was attributed to cryptophytes. However, the light-microscopy observations did not corroborate the presence of this algal group. A possible explanation for such a disagreement could be (i) the presence of small free-living cryptophytes included into the small flagellates group, (ii) problem of sample preservation, or (iii) the presence of the ciliate *Mesodinium rubrum*, which could contain cryptophytes as endosymbionts, as described by Gieskes and Kraay (1983). The first of hypothesis seems more plausible, given that cryptophyte cell may have been included into the small flagellates group. This also seems confirmed by the significant correlation found between small flagellate abundance and Allo. The other two explanations have been discarded, as samples were adequately preserved and analyzed in a short-time period (4 months), and presence of *M. rubrum* was not detected by cell counts.

We calculated the presence of diatoms using a more specific pigment pattern, which also included Chl c_1, a pigment that showed an exclusive and high correlation with the distribution of diatoms during this study. The CHEMTAX output ratio matrix lowered the Chl c_1/Chl *a* ratio with respect to the initial guess, and this trend also could be confirmed in chromatograms from samples dominated by diatoms (Fig. 8d). The discrepancies found between CHEMTAX-derived distributions and diatom cell counts at St. 225 could be explained by the variability of in situ pigment ratios with respect to those calculated by the CHEMTAX program, or by changes in the average diatom cell size (there was a higher proportion of large species, such as *Odontella weissflogii*, at this station). The lack of pigment contribution by the diatoms at St. 223 could be due to the small size of the diatom cells at this station.

The distribution of Chl c_2-MGDG deserves special significance due to its chemotaxonomical value regarding haptophyte populations. This singular pigment (Garrido et al., 2000) seems to be a useful marker (as shown by its specific correlation with *P.* cf. *antarctica* numbers) to discriminate some haptophytes from members of other taxonomic groups (dinoflagellates, chrysophytes) sharing some chlorophylls (e.g. Chl c_3), and/or carotenoids (e.g. Fuco, But-fuco, Hex-fuco). Considering the Chl *c* pigment pattern observed at Sts. 177 and 184, the presence of different algal groups could be inferred. For instance, at St. 177 the high concentrations of Hex-fuco and Chl c_2-MGDG denoted the presence of 'typical' haptophytes (*P. antarctica*). However, at St. 184 the Hex-fuco concentration decreased around 50% and Chl c_2-MGDG was nearly absent, whereas a higher Chl c_3 concentration was observed. This latter pigment pattern was in agreement with that obtained for the '*chemotaxonomic group*' (higher proportions of Chl c_2, Chl c_3, and But-fuco, with minor contributions of Hex-fuco), which represented approximately the mixed distribution of dinoflagellates and small flagellates during the FRUELA 95 and 96 cruises.

The '*chemotaxonomic group*' in CHEMTAX analysis was created to describe the hypothetical distribution of phytoplankton contributing those pigments that could not be explained by microscopic counts of specific groups. As previously mentioned, a *Phaeocystis*-like pigment pattern was found at several stations (e.g. 184 and 178 (FRUELA 95) and 223 (FRUELA 96)) where no *P.* cf. *antarctica* cells were observed. The discrepancy could be explained by flagellated stages of *P.* cf. *antarctica*, which would be included into the small flagellate group or by other groups contributing to this *Phaeocystis*-like pigment pattern (e.g. dinoflagellates, chrysophytes). We chose a pigment profile including Chl c_3, Chl c_2, Fuco, But-fuco, and Hex-fuco resembling the pattern observed in chromatograms of those stations. The pigment ratios were selected using the dinoflagellate *G. galatheanum* (CS 310), which resembled the pigment relationships for the proposed unknown group. The output ratios obtained by CHEMTAX significantly lowered the initial Hex-fuco : Chl *a*

ratio, increasing those relative to But-fuco and Fuco, which supports the presence of chrysophytes as a component of the '*chemotaxonomic group*'. During the FRUELA 95 cruise, the dominant pigment pattern associated with small flagellate populations matched the pigment composition attributed to the '*chemotaxonomic group*'. However, in the FRUELA 96 cruise, a significant relationship was observed between dinoflagellate abundance and the distribution of the '*chemotaxonomic group*', which could reflect a change in dinoflagellate species composition. An important limitation of our HPLC work was that the dinoflagellate distribution could not be described separately because peridinin was not detected in any samples. Autotrophic dinoflagellates present in these samples were probably included in the '*chemotaxonomical group*' of the CHEMTAX analysis, as we assume, in these peridinin-lacking dinoflagellates, a pigment composition consisting of fucoxanthins and its derivatives But-fuco and Hex-fuco as is the case for some *Gymnodinium* spp. (e.g. *G. galatheanum*, Johnsen and Sakshaug (1993); *G. breve*, Zapata et al. (1998)).

It must be considered that the CHEMTAX output is highly dependent upon the initial estimates of pigment ratios, and that it requires constant pigment : Chl *a* ratios for each algal class and a significant number of samples to obtain meaningful results. We analyzed only a reduced number of samples ($n = 55$), and employed a single pigment pattern, selecting the pigment composition (and pigment ratios) obtained from *Phaeocystis* (RG 2.2 strain), in order to reconstruct the haptophyte distribution. Carotenoid : Chl *a* ratios among *Phaeocystis* species (Vaulot et al., 1994) and *P. antarctica* strains can be highly variable (Zapata et al., in preparation), and differences between colonies and flagellate stages of a same strain also have been reported (Bidigare et al., 1996).

Moreover, the presence of dinoflagellates (and other members of the small flagellate group) sharing a similar pigment pattern (Chl c_3, But-fuco, and Hex-fuco) could indicate the algal distribution patterns inferred by CHEMTAX. This seems the case at St. 178 (FRUELA 95), where *P.* cf. *antarctica* cells observed in surface samples have been assigned to the '*chemotaxonomic group*'.

The chlorophylls detected in this study constituted a complex mixture of polar and non-polar accessory pigments (Chl c_1, Chl c_2, Chl c_3, MgDVP, Chl c_2-MGDG, and Chl *b*) and represent, as far as we know, the first detailed description of the distribution pattern of Chl *c* pigments in the Southern Ocean. We employed this additional information to enhance the number of marker pigments included in the CHEMTAX analysis with Chls *c* contributed by different algal groups (Chl c_1, '*diatoms*'; Chl c_3, '*Phaeocystis*' and '*chemotaxonomic group*'; Chl c_2-MGDG, '*Phaeocystis*'). The results obtained in this work show the importance of achieving high resolution of Chls *c*, which provide chemotaxonomically important markers for some specific groups (e.g. Chl c_2-MGDG in haptophytes and Chl c_1 for diatoms).

New advances of the chemotaxonomic approach will depend on: (i) improvement of present day knowledge about pigment patterns from algal classes, (ii) investigation of pigment pattern variability for members of a single genus or species, (iii) detection of new marker pigments and development of improved HPLC methods for pigment analysis, and (iv) knowledge of mechanisms underlying changes affecting pigment ratios in the photosynthetic apparatus of phytoplankton species.

The results presented here highlight the importance of isolating different typical Antarctic species and characterizing their pigment patterns to improve the usefulness of HPLC pigment analysis in ascertaining phytoplankton composition. In spite of their limitations, HPLC pigment analysis and CHEMTAX data processing represent a powerful approach to study the taxonomic composition of phytoplankton assemblages, specially when the smallest groups contribute significantly to the overall community.

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