Deep-Sea Research I 65 (2012) 1–14

Contents lists available at SciVerse ScienceDirect

Deep-Sea Research I

journal homepage: www.elsevier.com/locate/dsri

Dynamics of phytoplankton communities during late summer around the tip of the Antarctic Peninsula

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ARTICLE INFO

Article history:
Received 11 May 2011
Received in revised form
22 February 2012
Accepted 6 March 2012
Available online 20 March 2012

Keywords:
Antarctic Peninsula
Phytoplankton
Pigments
HPLC
CHEMTAX

ABSTRACT

The composition and distribution of phytoplankton assemblages around the tip of the Antarctic Peninsula were studied during two summer cruises (February/March 2008 and 2009). Water samples were collected for HPLC/CHEMTAX pigment and microscopic analysis. A great spatial variability in chlorophyll *a* (Chl *a*) was observed in the study area: highest levels in the vicinity of the James Ross Island (exceeding 7 mg m^{-3} in 2009), intermediate values (0.5 to 2 mg m^{-3}) in the Bransfield Strait, and low concentrations in the Weddell Sea and Drake Passage (below 0.5 mg m^{-3}). Phytoplankton assemblages were generally dominated by diatoms, especially at coastal stations with high Chl *a* concentration, where diatom contribution was above 90% of total Chl *a*. Nanoflagellates, such as cryptophytes and/or *Phaeocystis antarctica,* replaced diatoms in open-ocean areas (e.g., Weddell Sea). Many species of peridinin-lacking autotrophic dinoflagellates (e.g., *Gymnodinium* spp.) were also important to total Chl *a* biomass at well-stratified stations of Bransfield Strait. Generally, water column structure was the most important environmental factor determining phytoplankton communities' biomass and distribution. The HPLC pigment data also allowed the assessment of different physiological responses of phytoplankton to ambient light variation. The present study provides new insights about the dynamics of phytoplankton in an undersampled region of the Southern Ocean highly susceptible to global climate change.

1. Introduction

The Antarctic Peninsula (AP) is experiencing one of the fastest rates of regional climate change on Earth, as ocean surface temperatures at the continental margin of the western AP have undergone a pronounced warming (3–4 °C) over the past century (Turner et al., 2005; Steig et al., 2009). Such changes promote the collapse of ice shelves, retreat of glaciers and exposure of new terrestrial habitats (Clarke et al., 2007). Environmental features, as regional circulation system and seasonal changes in the light regime and sea ice cover, have been shown to determine a latitudinal variation in phytoplankton productivity along the western AP (Garibotti et al., 2003). Moreover, recent studies have shown that changes in phytoplankton biomass and composition along the western shelf of the AP are associated with regional long-term climate alterations (Montes-Hugo et al., 2009).

The Southern Ocean is generally a high-nutrient and low-chlorophyll (HNLC) area, mainly due to the limitation of micronutrients, such as iron. However, high phytoplankton biomass has been observed in particular regions, especially at oceanic fronts, marginal ice zones and nearshore straits, bays, and lees of islands (Prézelin et al., 2000 and references therein). These high biomass regions are considered critical feeding sites for higher trophic levels and play a crucial role on biogeochemical cycling of nutrients. Phytoplankton blooms in those regions (usually dominated by diatoms or haptophytes, such as *Phaeocystis antarctica*) are generally associated with the development of a shallow mixed layer (with increased light levels that enhance phytoplankton growth) and/or iron availability (Prézelin et al., 2000). On the other hand, recent studies have shown an increasing dominance of cryptophytes in the AP region, particularly in areas with glacial melt water (Moline and Prézelin, 1996; Moline et al., 2004). The dominance of cryptophytes instead of diatoms may influence the trophic web, as cryptophytes are more efficiently grazed by salps than by antarctic krill (Moline et al., 2004). Therefore, studies on phytoplankton and the influence of environmental constraints in species/groups composition are relevant to evaluate ecosystem changes, both at short and long-term scales.

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http://dx.doi.org/10.1016/j.dsr.2012.03.002

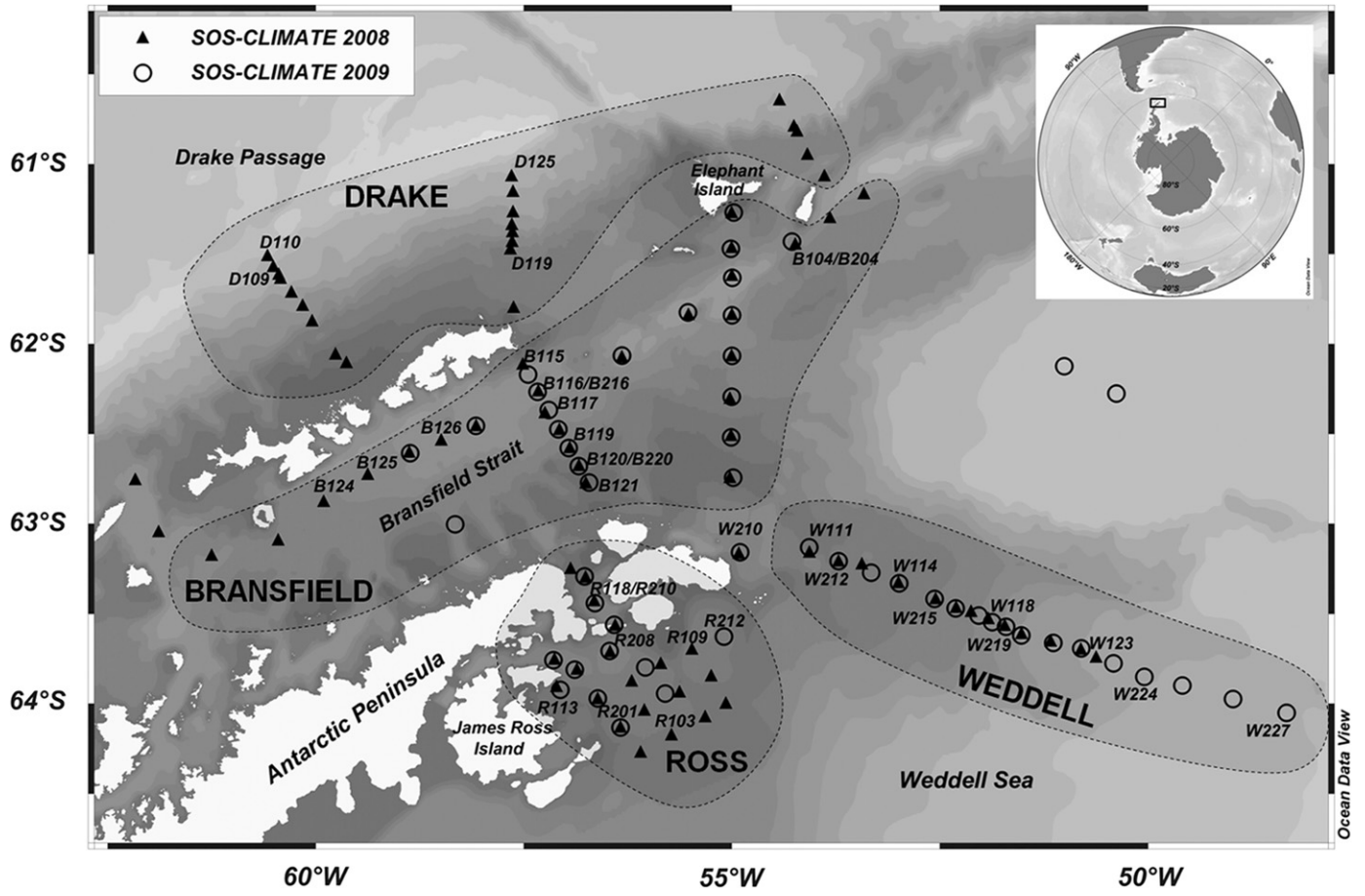

Fig. 1. Study area and stations' locations during SOS-CLIMATE 2008 and 2009 summer cruises. Bounded stations (dashed line) represent the geographical zonation used in this study (non-bounded stations were not used in the discussion of the results). The first letter of the stations' label is related to the surveyed region (D=DRAKE, B=BRANSFIELD, W=WEDDELL, R=ROSS). The number following that letter refers to the sampling period (1=2008 cruise, 2=2009 cruise). Inset map includes the South Polar orthographic projection and the box indicates the magnified region.

The study of phytoplankton community composition has been classically performed with light microscope examination. An alternative way to study the phytoplankton community structure is through chemotaxonomic methods based on High Performance Liquid Chromatography (HPLC) analysis, which rely on the presence and relative concentration of pigments that are characteristic of distinct algal taxonomic groups (Wright and Jeffrey, 2006). Nevertheless, the use of phytoplankton pigments in chemotaxonomic methods has drawbacks, such as non-unique pigment markers and/or potential fluctuations in pigment ratios with physiological stressors (e.g., irradiance and nutrients), and both at species and at cellular level (Wright and Jeffrey, 2006). On the other hand, variations in the relative concentration of those pigments may be used as indicators of the physiological state of phytoplankton communities (Moline, 1998; DiTullio et al., 2007; Van Leeuwe and Stefels, 2007). One of the chemotaxonomic tools that has been developed and continuously improved to minimize errors inherent to fluctuations of pigment ratios is CHEMTAX (Mackey et al., 1996). This approach involves an iterative process of matrix factorization to optimize pigment ratios in order to estimate the contribution of phytoplankton groups to total chlorophyll *a* (Chl *a*). CHEMTAX has been extensively used in phytoplankton communities in the Australian sector of the Southern Ocean (Wright et al., 1996; Wright and van den Enden, 2000; Wright et al., 2009, 2010). In contrast, few studies applied this approach to the AP region (Rodriguez et al., 2002; Kozlowski et al., 2011).

The present work studied the spatial pattern of phytoplankton communities around the tip of the AP, encompassing the Bransfield Strait, part of the Drake Passage and a northwestern section in the Weddell Sea. On the eastern side of the AP, particularly in the ice edge zone of the Weddell Sea, extensive phytoplankton blooms have been detected during spring and summer (Sullivan et al., 1993; Park et al., 1999; Kang et al., 2001), which form an important feeding ground for grazers. In addition, the shallows and bays of southwestern Bransfield Strait are breeding grounds for a host of biota, especially krill (Zhou et al., 1994), as a result of high surface phytoplankton biomass associated with seasonal blooms (Karl et al., 1991; Castro et al., 2002). The main objective of this study was to understand phytoplankton biomass variation and assemblage distribution during two late summers around the tip of AP by chemotaxonomic analysis and complemented with microscopic observations. The specific question addressed in this study was: what is the relationship between the thermohaline structure, water-column physicochemical properties and the phytoplankton communities in different areas of the study region?

2. Material and methods

2.1. Study area and sampling collection

Oceanographic cruises were conducted in the adjacent waters of the tip of AP, from 60.6°S to 64.3°S and from 48.3°W to 62.2°W (Fig. 1), as part of the SOS-CLIMATE (Southern Ocean Studies for Understanding Global-CLIMATE Issues) project. Sampling was

Table 1
Concentrations (mg m^{-3}) of pigments (average and minimum-maximum concentrations for each geographic region). Chl *a*=chlorophyll *a*; Chlide *a*=chlorophyllide *a*; Phytin *a*=pheophythin *a*; Phide *a*=pheophorbide *a*; Chl *b*=chlorophyll *b*; Chl c_2=chlorophyll c_2; Chl c_3=chlorophyll c_3; Allo=alloxanthin; Fuco=fucoxanthin; Hex-fuco=19′-hexanoyloxyfucoxanthin; But-fuco=19′-butanoyloxyfucoxanthin; Diadino=diadinoxanthin; Diato=diatoxanthin; Perid=peridinin.

Pigment	2008				2009		
	Drake	Bransfield	Ross	Weddell	Bransfield	Ross	Weddell
Chl *a*	0.39 (0.04–0.89)	0.55 (0.12–1.08)	1.76 (0.25–4.50)	0.15 (0.04–0.15)	0.92 (0.35–1.98)	3.73 (0.36–7.61)	0.27 (0.02–0.27)
Chlide *a*	0.01 (0.00–0.03)	0.02 (0.00–0.05)	0.24 (0.00–0.87)	0.01 (0.00–0.01)	0.03 (0.00–0.14)	0.33 (0.00–0.90)	0.01 (0.00–0.03)
Phytin *a*	0.02 (0.00–0.05)	0.02 (0.01–0.05)	0.07 (0.01–0.26)	0.01 (0.00–0.01)	0.04 (0.01–0.07)	0.08 (0.01–0.18)	0.01 (0.00–0.03)
Phide *a*	0.04 (0.00–0.10)	0.08 (0.01–0.22)	0.19 (0.02–0.61)	0.01 (0.00–0.03)	0.09 (0.01–0.29)	0.26 (0.03–0.55)	0.01 (0.00–0.04)
Chl *b*	0.01 (0.00–0.02)	0.01 (0.00–0.04)	0.03 (0.02–0.09)	0.01 (0.00–0.03)	0.01 (0.00–0.03)	0.02 (0.00–0.03)	0.01 (0.00–0.03)
Chl c_2	0.07 (0.01–0.20)	0.11 (0.03–0.24)	0.50 (0.03–1.27)	0.02 (0.01–0.05)	0.13 (0.03–0.28)	0.73 (0.03–1.71)	0.04 (0.00–0.13)
Chl c_3	0.10 (0.00–0.28)	0.15 (0.00–0.37)	0.06 (0.00–0.21)	0.01 (0.00–0.03)	0.09 (0.01–0.28)	0.11 (0.00–0.30)	0.02 (0.00–0.05)
Allo	0.01 (0.00–0.07)	0.02 (0.00–0.23)	0.00 (0.00–0.00)	0.02 (0.00–0.06)	0.01 (0.00–0.03)	0.00 (0.00–0.01)	0.03 (0.00–0.13)
Fuco	0.31 (0.03–0.72)	0.44 (0.10–0.96)	1.60 (0.17–3.47)	0.05 (0.02–0.14)	0.59 (0.10–1.42)	3.00 (0.2–6.95)	0.11 (0.02–0.60)
Hex-fuco	0.07 (0.02–0.12)	0.08 (0.02–0.14)	0.02 (0.00–0.07)	0.05 (0.02–0.08)	0.02 (0.01–0.06)	0.01 (0.00–0.03)	0.06 (0.01–0.14)
But-fuco	0.06 (0.01–0.19)	0.07 (0.01–0.18)	0.01 (0.00–0.02)	0.01 (0.00–0.02)	0.02 (0.01–0.06)	0.00 (0.00–0.01)	0.01 (0.00–0.02)
Diadino	0.10 (0.01–0.23)	0.13 (0.02–0.29)	0.13 (0.02–0.28)	0.02 (0.01–0.03)	0.09 (0.01–0.22)	0.29 (0.02–0.60)	0.03 (0.00–0.09)
Diato	0.01 (0.00–0.04)	0.02 (0.00–0.06)	0.02 (0.00–0.05)	0.00 (0.00–0.00)	0.02 (0.00–0.06)	0.06 (0.00–0.16)	0.00 (0.00–0.01)
Perid	0.02 (0.00–0.05)	0.05 (0.00–0.13)	0.06 (0.00–0.17)	0.00 (0.00–0.00)	0.03 (0.00–0.09)	0.03 (0.00–0.06)	0.01 (0.00–0.02)

performed during late summer of 2008 (February/March) and 2009 (February/March). Surface water samples were taken in all CTD (conductivity–temperature–depth) stations for phytoplankton pigments and microscopic analyses (phytoplanktonic cell abundance and carbon biomass). No microscopic analyses were made for the Weddell Sea samples due to very low phytoplankton concentrations. Both physical data (conductivity, temperature and salinity) and water samples were collected using a combined Sea-Bird CTD/Carrousel 911+system® equipped with 24 five-liter Niskin bottles. Density (kg m^{-3}) was calculated for evaluation of the water column physical structure based on temperature, salinity and pressure data. The upper mixed layer (UML) depth was determined as the depth where a change of 0.05 kg m^{-3} occurred over a 5 m depth interval (Mitchell and Holm-Hansen, 1991). At some stations, chosen according with the fluorescence profiles (WetLabs® profiling fluorometer), water samples were taken from several depths for phytoplankton pigment analysis. Phytoplankton cell abundance and carbon biomass data were calculated for surface samples, as they are representative of the UML (Garibotti et al., 2003).

2.2. HPLC pigment analysis

Immediately after sampling, seawater samples (1–2 L) were filtered onto Whatman GF/F filters (nominal pore size of 0.7 μm and 25 mm in diameter), under vacuum pressure (<5 in Hg) and filters were immediately stored in liquid nitrogen. Phytoplankton pigments were extracted in the dark with 2 mL of 95% cold-buffered methanol (2% ammonium acetate) for 30 min at -20 °C. Samples were sonicated (Bransonic, model 1210, w: 80, Hz: 47) for 1 min at the beginning of the extraction period. Samples were then centrifuged at 1100 g for 15 min at 4 °C. Extracts were filtered (Fluoropore PTFE membrane filters, 0.2 μm pore size) and immediately injected in the HPLC instrument. Pigment extracts were analyzed using a Shimadzu HPLC comprised of a solvent delivery module (LC-10ADVP) with system controller (SCL-10AVP), a photodiode array (SPD-M10ADVP), and a fluorescence detector (RF-10AXL). The chromatographic separation of pigments was achieved using a monomeric OS C8 column (Symmetry C8, 15 cm long, 4.6 mm in diameter, and 3.5 μm particle size). Mobile phases were: (A) methanol:acetonitrile:aqueous pyridine solution (0.25 M, pH adjusted to 5.0 with acetic acid) (50:25:25, v/v/v), and (B) methanol:acetonitrile:acetone (20:60:20, v/v/v). The solvent gradient followed Zapata et al. (2000) with a flow rate of 1 mL min^{-1}, with an injection volume of 100 μL, and 40 min runs. The limit of detection and limit of quantification of this method were calculated and discussed in Mendes et al. (2007). Pigments were identified from both absorbance spectra and retention times and concentrations calculated from the signals in the photodiode array detector or fluorescence detector (Ex. 430 nm; Em. 670 nm). The HPLC system was previously calibrated with pigment standards from Sigma (chlorophyll *a*, *b* and β-carotene) and DHI (for other pigments). Table 1 lists all pigments detected above the limit of quantification and that were considered in this study.

2.3. CHEMTAX analysis of pigment data

The relative abundance of microalgal groups contributing to total Chl *a* biomass was calculated by pigment concentration data using CHEMTAX v1.95 chemical taxonomy software (Mackey et al., 1996; Wright et al., 1996; Wright et al., 2009). CHEMTAX uses a factor analysis and steepest-descent algorithm to best fit the data on to an initial pigment ratio matrix. The basis for calculations and procedures are fully described in Mackey et al. (1996). The initial pigment ratios of major algal classes were based on pigment matrices used in studies from the western AP region (Rodriguez et al., 2002; Kozlowski et al., 2011) (Table 2(a)). Based on the identified diagnostic pigments and confirmation of the higher taxonomic groups by microscopic analysis, six algal groups were loaded on CHEMTAX: diatoms, dinoflagellates-1 (peridinin-containing dinoflagellates), "*Phaeocystis antarctica*", cryptophytes, green flagellates (with Chl *b*) and "chemotaxonomic group". The loaded pigments were chlorophyll c_3 (Chl c_3), chlorophyll c_2 (Chl c_2), peridinin (Perid), 19′-butanoyloxyfucoxanthin (But-fuco), fucoxanthin (Fuco), 19′-hexanoyloxyfucoxanthin (Hex-fuco), alloxanthin (Allo), chlorophyll *b* (Chl *b*) and chlorophyll *a* (Chl *a*) (see Table 2(a)). The "chemotaxonomic group" was characterized by a pigment signature that includes Chl c_3, Chl c_2, But-fuco, Fuco and Hex-fuco, relative to a group including peridinin-lacking autotrophic dinoflagellates and diatoms with Chl c_3 (Wright and Jeffrey, 2006), and other algal groups whose pigment composition has not yet been exhaustively analyzed (e.g., Parmales and Chrysophytes).

The same initial ratio was used in data from both study years, but data from each cruise were run separately in order to detect potential variations in optimization of CHEMTAX procedures. In order to account for pigment ratios' variation with irradiance and/or nutrient availability, data from each cruise were also split into three bins according to sample depth (0–50 m, 50–100 m and >100 m).

A series of 60 pigment ratio matrices were generated by multiplying each ratio from the initial matrix by a random function, as

Table 2
Pigment to chlorophyll *a* ratios used for CHEMTAX analysis. Initial ratios before analysis (a), 2008 optimized ratios (for 0–50 m bin) after analysis (b), and 2009 optimized ratios (for 0–50 m bin) after analysis (c).

	Chl c_3	Chl c_2	Perid	But-fuco	Fuco	Hex-fuco	Allo	Chl *b*	Chl *a*
(a) Input matrix									
Diatoms	0	0.110	0	0	0.754	0	0	0	1
Dinoflagellates-1	0	0.320	0.720	0	0	0	0	0	1
Chemotaxonomic group	0.067	0.126	0	0.122	0.290	0.248	0	0	1
Phaeocystis antarctica	0.141	0.144	0	0.080	0.011	0.916	0	0	1
Cryptophytes	0	0.174	0	0	0	0	0.228	0	1
Green flagellates	0	0	0	0	0	0	0	0.945	1
(b) Output matrix: 0–50 m (2008 data)									
Diatoms	0	0.225	0	0	0.940	0	0	0	1
Dinoflagellates-1	0	0.274	0.926	0	0	0	0	0	1
Chemotaxonomic group	0.501	0.184	0	0.337	0.821	0.353	0	0	1
Phaeocystis antarctica	0.209	0.128	0	0.135	0.023	0.982	0	0	1
Cryptophytes	0	0.191	0	0	0	0	0.428	0	1
Green flagellates	0	0	0	0	0	0	0	0.932	1
(c) Output matrix: 0–50 m (2009 data)									
Diatoms	0	0.149	0	0	0.821	0	0	0	1
Dinoflagellates-1	0	0.381	0.898	0	0	0	0	0	1
Chemotaxonomic group	0.249	0.118	0	0.093	0.401	0.037	0	0	1
Phaeocystis antarctica	0.208	0.128	0	0.080	0.011	1.237	0	0	1
Cryptophytes	0	0.192	0	0	0	0	0.362	0	1
Green flagellates	0	0	0	0	0	0	0	0.879	1

described by Kozlowski et al. (2011) to optimize the input matrix. The average of the best six output matrices (with the lowest residual or root mean square error) were taken as the optimized results. The optimized pigment ratio matrix derived by CHEMTAX for the 0–50 m is presented in Table 2(b) and (c) (data from 2008 to 2009, respectively). The output data are presented as absolute amounts (mg m^{-3}) of Chl *a* attributed to each phytoplankton group, and as a relative amount (percentage) of the total Chl *a* in a sample.

2.4. Microscopic analysis

In order to determine the species composition, water samples were preserved in amber glass flasks (~250 mL) with 2% alkaline Lugol's iodine solution for phytoplankton identification and counting. Settling chambers (from 50 to 100 mL settling volume) were inspected on an Axiovert 135 ZEISS inverted microscope (Utermöhl, 1958; Sournia, 1978) at 200×, 400× and 1000× magnification, according to specific literature (mainly, Hasle and Syvertsen,1996; Scott and Marchant, 2005). Staining cells with Lugol's solution allows recognition of chloroplasts and pyrenoids and provides a clear picture of the cell outline, which favors recognition of shape and size under the microscope (Sournia, 1978). Distinction between autotrophic and heterotrophic dinoflagellates was made on either the known taxonomic trophic mode or the presence/absence of chloroplasts. Species-specific cell biovolumes were estimated by measuring cell dimensions (from microscope images – Spot Insight QE camera) and by applying volume calculations based on the most similar geometric shapes as in Hillebrand et al. (1999). At least 30 specimens of each species or major taxa were randomly chosen for measurements. Cell carbon content (carbon biomass) was then calculated using specific carbon-to-volume ratios for diatoms and dinoflagellates (Montagnes et al., 1994); and for all other algae groups (Menden-Deuer and Lessard, 2000).

3. Results

3.1. Spatial distribution of phytoplankton pigments

Three spatial features were observed in Chl *a* distribution (Fig. 2) around the tip of the AP: (i) high Chl *a* region (exceeding 7 mg m^{-3} in 2009) in the vicinities of James Ross Island; (ii) intermediate Chl *a* levels (0.5 to 2 mg m^{-3}) in the Bransfield Strait, and (iii) very low Chl *a* concentration (below 0.5 mg m^{-3}) in the Weddell Sea section and stations located offshore the Drake Passage (only sampled in 2008).

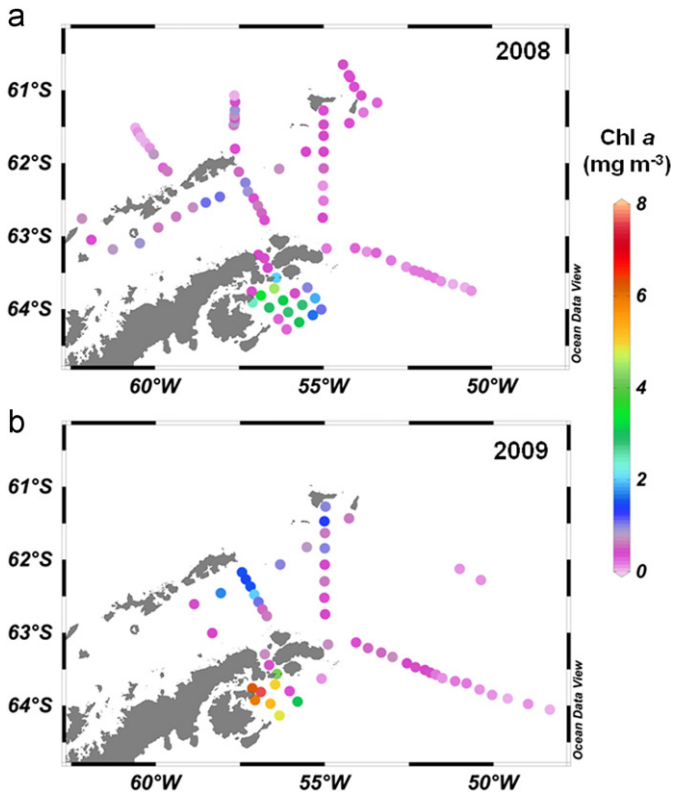

Fig. 2. Surface distribution of total chlorophyll *a* (mg m^{-3}) for SOS-CLIMATE 2008 (a) and 2009 (b).

Besides Chl *a*, the most abundant pigments (with maximum concentrations >0.5 mg m^{-3}) were Fuco, Chl c_2, diadinoxanthin (Diadino) and some degradation products of Chl *a* (see Table 1). The highest concentrations of these pigments were observed near James

Ross Island. Bransfield Strait (particularly in 2008) and Drake Passage also presented relatively high values (>0.05 mg m^{-3}) of Chl c_3, Hex-fuco and But-fuco. In the Weddell Sea region, where the lowest pigment concentrations were observed, Fuco was the main accessory pigment at coastal stations, while Allo and Hex-fuco appeared as the major carotenoids at some offshore stations.

Relationships between particular accessory pigments can be used to reveal the dominance of specific taxonomic groups. As observed in Fig. 3, the highest values of Chl c_3:Fuco (slope=0.38) were registered in 2008 for the Bransfield Strait and Drake Passage. Intermediate values (slope=0.16) were recorded in 2009 for the Bransfield Strait and the lowest values (slope=0.037), for both years, were observed near James Ross Island. The different slopes of this ratio were associated with relative diatom contribution to phytoplankton community, as observed in Ross stations where higher diatom contributions were associated with a lower slope (further information on next section).

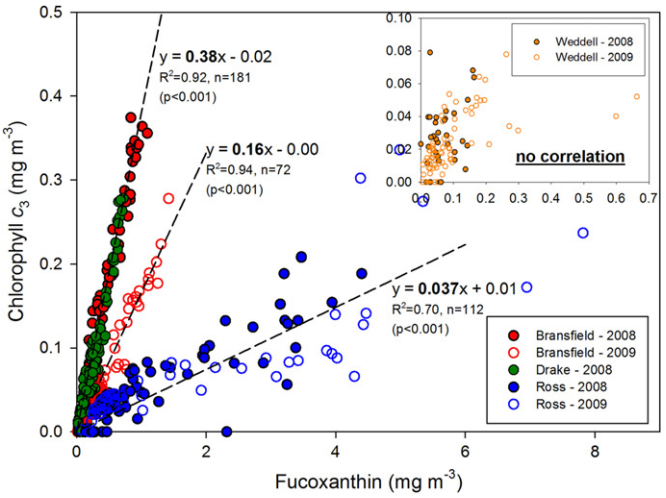

Fig. 3. Relationship between chlorophyll c_3 and fucoxanthin for the different regions and sampling periods.

3.2. Distribution of taxonomic groups in relation to oceanography

3.2.1. Spatial distribution

The relative contribution of the main phytoplankton groups to surface Chl *a*, calculated by CHEMTAX, is shown in Fig. 4. Phytoplankton assemblages were generally dominated by diatoms in both years (Fig. 4(a) and (b)), especially at stations with high Chl *a* concentration (mainly near James Ross Island) where diatom contribution was above 90% of total Chl *a*. Other groups were also abundant at distinct areas around the tip of the AP. Cryptophytes dominated the Weddell Sea region, particularly stations with low diatom contribution, and at one station in the Bransfield Strait, in 2008 (Fig. 4(c)). The haptophyte *P. antarctica* showed the greatest contributions to total biomass in the Drake Passage region (only sampled in 2008) and at some Weddell Sea stations (Fig. 4(e) and (f)). The "chemotaxonomic group" was more dominant in the Bransfield Strait comparing to other regions (Fig. 4(g) and (h)). Dinoflagellates-1, more abundant in the Bransfield Strait, were always below 10% of total Chl *a*, and green flagellates never represented more than 8% of biomass (data not shown).

3.2.2. Microscopy vs. CHEMTAX

Direct comparisons of the estimated biomass using microscopy data and CHEMTAX showed significant relationships for total phytoplankton (Fig. 5(a)) and diatom biomass (Fig. 5(b)). The significant correlation between microscope-derived carbon biomass and diatom-allocated Chl *a* calculated through CHEMTAX (Fig. 5(b)) mirrored the correlation for the total autotrophic community (Fig. 5(a)), which denotes a clear dominance of diatoms. A conspicuous dominance of diatom carbon biomass was observed in both years, with higher values found near James Ross Island (>100 μg C l^{-1}, in average), which agrees with CHEMTAX results (see Fig. 4(a) and (b)). Differences for the haptophyte *P. antarctica* varied with the study period. In 2009, this organism was rarely recorded in microscope observations, partly due to the lack of microscope data from Weddell Sea, where the contribution of *P. antarctica* to Chl *a* was 20–40% (determined by CHEMTAX). In 2008, with the additional data of Drake Passage, a significant correlation was observed between the two methods (Fig. 5(c)). Other groups (not shown in Fig. 5), such as cryptophytes, were barely separated from other small flagellates by microscopic analysis, except at one station in the Bransfield Strait (in 2008), where CHEMTAX data also showed a higher contribution of cryptophytes to biomass. The "chemotaxonomic group" was correlated with small flagellate biomass in the Drake Passage ($R^2=0.52$; $p<0.05$). Significant correlations were observed in the Bransfield Strait between the "chemotaxonomic group" and dinoflagellates for both years ($R^2=0.62$; $p<0.05$). This correlation observed in the Bransfield Strait may indicate the presence of other types of dinoflagellates that contain combinations of pigments without peridinin.

3.2.3. Drake passage (DRAKE)

Fig. 6 shows the vertical profiles of Chl *a* biomass of the taxonomic groups determined by CHEMTAX at a typical coastal and offshore station from DRAKE. Increased water column stratification was generally observed from coastal to offshore stations, as observed in Fig. 6. A coastal-offshore gradient was also observed for biomass and relative distribution of taxonomic groups, with higher Chl *a* concentration at the coastal stations and decreasing towards offshore. At the coastal station (Fig. 6(a)), a dominance of diatoms was observed but no deep chlorophyll maximum (DCM), which was present at the offshore station (Fig. 6(b)). Relatively low diatom contributions were found at the surface layers of the offshore stations (below 60 m diatoms became dominant) as they were replaced by nanoplankton (<20 μm in greatest axial linear dimension), such as *P. antarctica*, cryptophytes and green flagellates (Fig. 6(b)). Although many flagellates could not be identified by microscope observations, the most representative phytoplankton species in DRAKE were the large centric diatom *Corethron pennatum*, the haptophyte *P. antarctica* and nanoflagellates, comprising dinoflagellates (e.g., *Gymnodinium* spp.).

3.2.4. Bransfield strait (BRANSFIELD)

Great spatial and temporal variability were observed for both biomass and distribution of taxonomic groups and between the two surveyed years (Fig. 7). Higher biomass was observed in 2009 (see also Fig. 2) compared with 2008, coupled with an increase in the relative contribution of diatoms (mainly the centric *Thalassiosira* spp., *Corethron pennatum*, the nano-sized *Chaetoceros neglectus* and the pennate *Pseudonitzschia* spp.). The highest biomass levels within the UML were generally registered at the deep stations in the central basin and characterized by a major contribution of the "chemotaxonomic group" (associated with high densities of *Gymnodinium* spp.) and diatoms (Fig. 7(b) and (e)). Biomass levels decreased from offshore to coastal stations, where diatoms and/or cryptophytes dominated the phytoplankton community (Fig. 7(a) and (d)). In 2008, a negative correlation was found between surface Chl *a* and the UML depth ($R^2=0.50$,

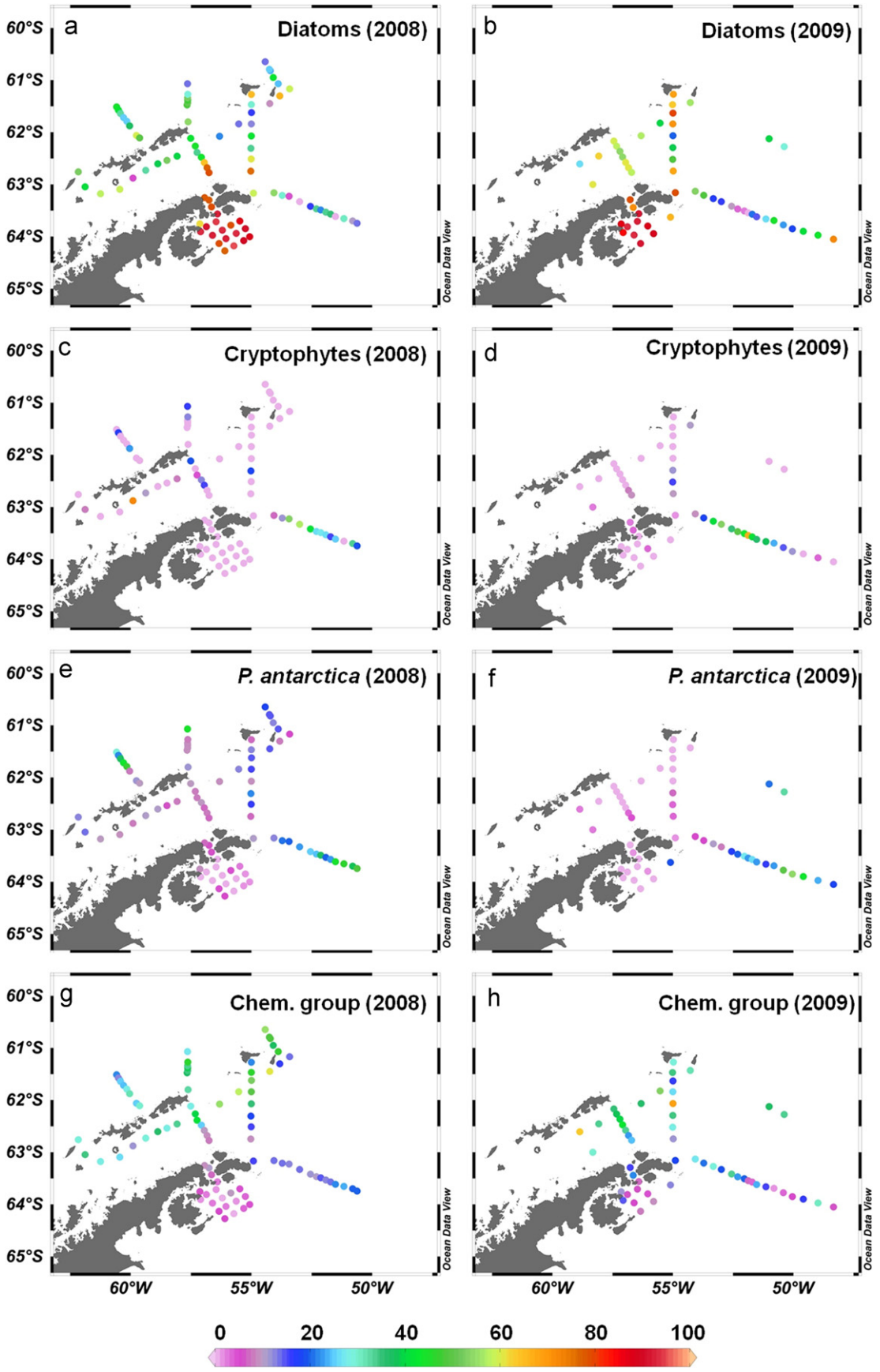

Fig. 4. Surface distribution of the relative contribution (%) of main phytoplankton groups to total Chlorophyll *a* estimated by CHEMTAX using HPLC pigment data: diatoms in 2008 (a) and 2009 (b); cryptophytes in 2008 (c) and 2009 (d); *Phaeocystis antarctica* in 2008 (e) and 2009 (f); "Chemotaxonomic group" in 2008 (g) and 2009 (h).

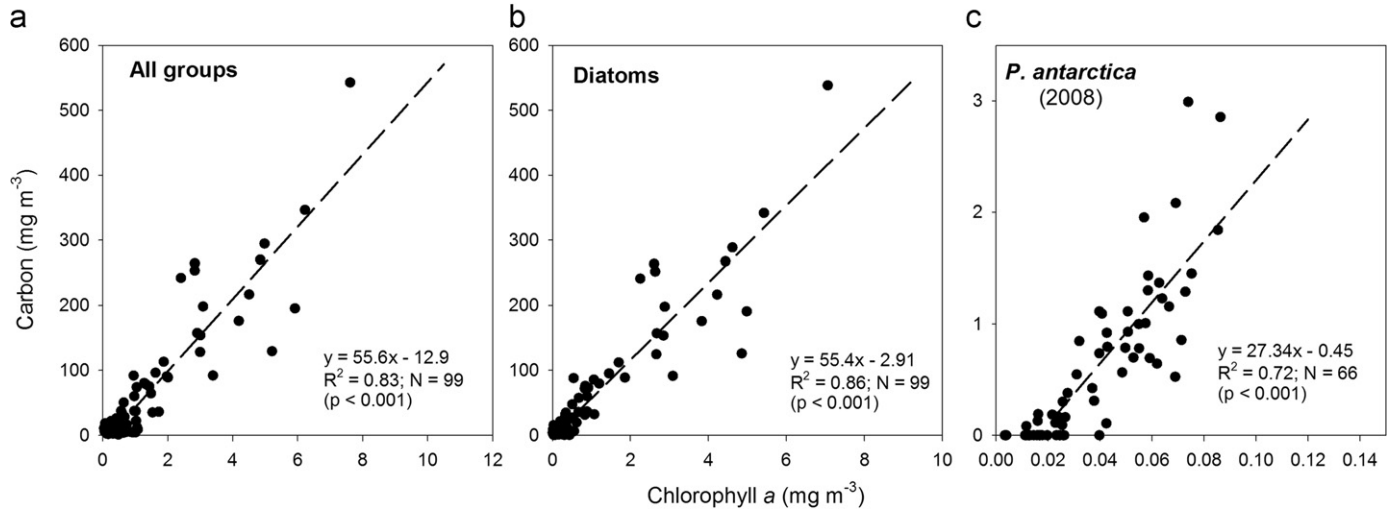

Fig. 5. Relationship between Chlorophyll *a* biomass estimated from CHEMTAX/HPLC pigment data and carbon biomass obtained from microscopy data. (a) All groups (2008 and 2009), (b) diatoms (2008 and 2009) and (c) *Phaeocystis antarctica* (only 2008).

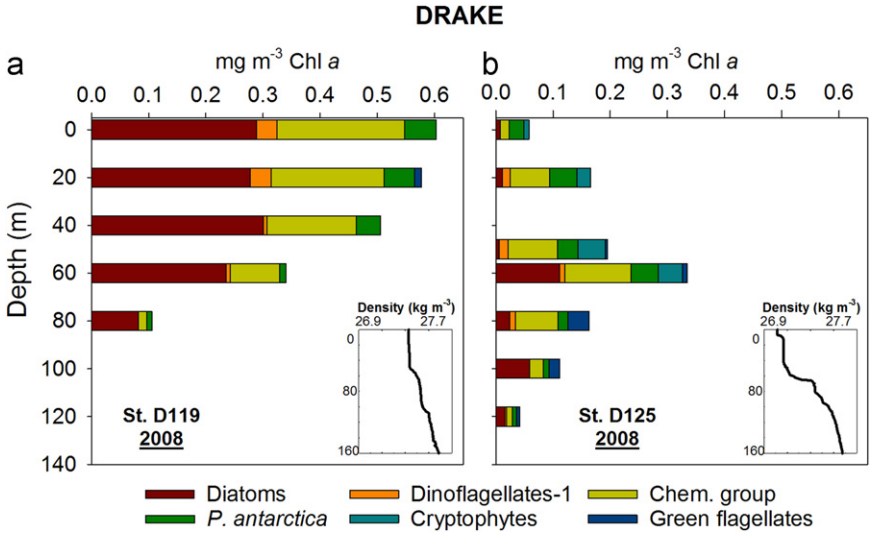

Fig. 6. Depth distribution of phytoplankton groups' biomass (as Chlorophyll *a* concentration) calculated by CHEMTAX at: (a) a coastal station (D119) and (b) offshore station (D125) in the Drake Passage region. Insets: density profiles of the respective stations (see Fig. 1 for stations' locations).

$p < 0.01$), and the deepest UML reached 155 m near the Elephant Island (station B104). Lower biomass and an increase contribution of small flagellates over diatoms were observed at stations with deeper UML (see Fig. 7(c)). This physical feature was not observed in 2009 (UML was always less than 100 m) and biomass levels were similar to those observed at other BRANSFIELD stations, which were characterized by diatoms dominance (see Fig. 7(f)).

3.2.5. *Weddell sea (WEDDELL)*

The phytoplankton community was mainly composed by diatoms, cryptophytes and *P. antarctica* (see Fig. 4), and was associated with low biomass values during both years (Chl *a* always below 0.5 mg m^{-3}; see Fig. 2). A particular strong coastal-offshore gradient in water column stratification was observed at WEDDELL (Fig. 8). The phytoplankton community composition displayed a neat succession along this gradient (insets in Fig. 8). Diatoms were dominant in the well-mixed water column at coastal stations, were associated with highest biomass (> 0.2 mg m^{-3}) observations, and were gradually replaced by cryptophytes at stations with intermediate stratification. During both studied years, offshore stations were strongly stratified, which were associated with very low biomass (< 0.1 mg m^{-3}).

Fig. 9 shows the vertical profiles of the phytoplankton community composition at six stations (Fig. 9(a)–(f)), including vertical density profiles (insets in Fig. 9). The dominance of diatoms at the coastal stations was associated with homogeneous water columns and, therefore the distribution of their biomass was also fairly uniform with depth (e.g., station W111, Fig. 9(a)). At stations with intermediate stratification (stations W114 and W215, Fig. 9(b) and (e)), a major contribution of cryptophytes was observed, mainly within the upper layers (20–50 m). Although both stations were located in a similar position and showed a similar density profile, the intermediate stratified station W114 (Fig. 9(b)) showed a different biomass profile compared to station W215 (Fig. 9(e)). The contribution of *P. antarctica* to total biomass was more important at the farthest offshore stations (Fig. 9(c) and (f)), where a strong stratification was recorded associated with low biomass and an evident DCM close to 50 m depth. Despite the similar biological pattern between sampling years, there was an evident interannual difference in the Weddell Sea region, with particularly higher biomass in 2009 (Fig. 9(d)–(f)).

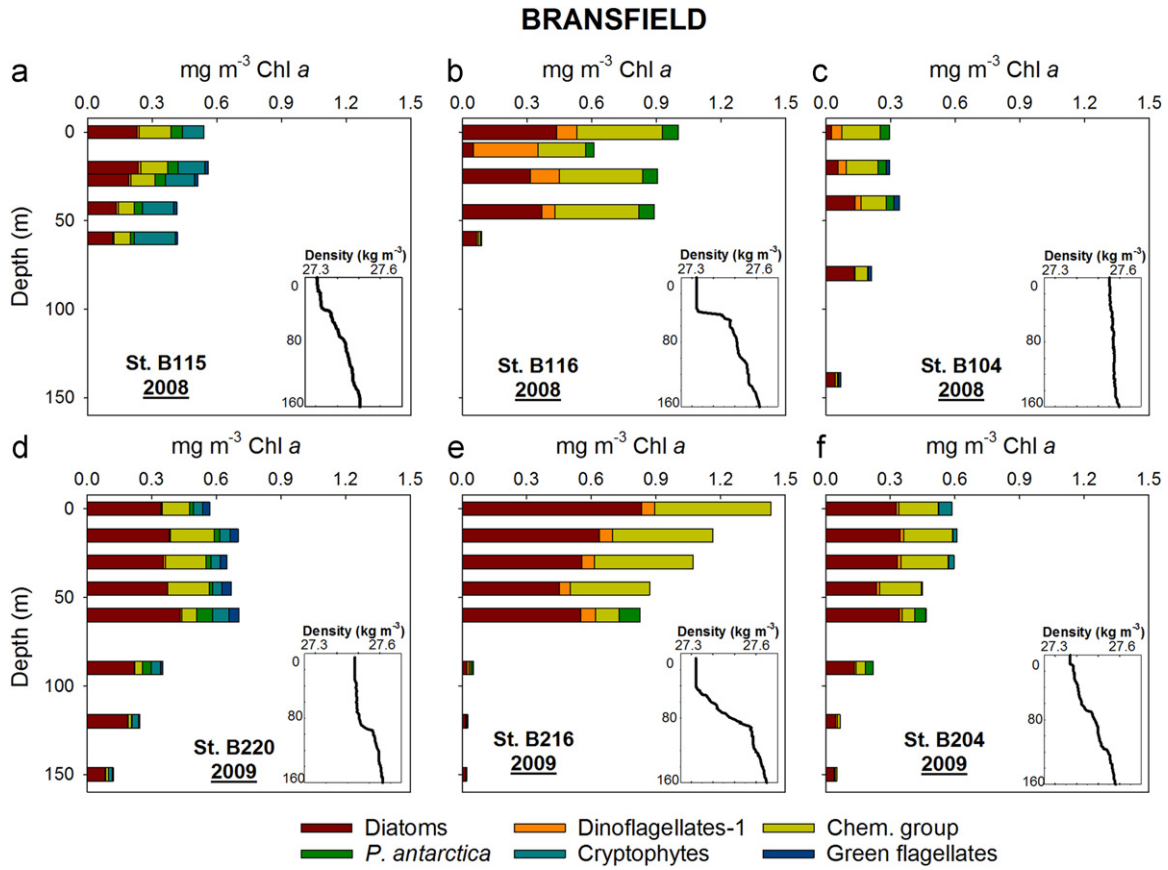

Fig. 7. Depth distribution of phytoplankton groups' biomass (as Chlorophyll *a* concentration) calculated by CHEMTAX at stations (a) B115, (b) B116 and (c) B104 (occupied in 2008); and at stations (d) B220, (e) B216 and (f) B204 (occupied in 2009) in the Bransfield Strait. Insets: density profiles of the respective stations (see Fig. 1 for stations' locations).

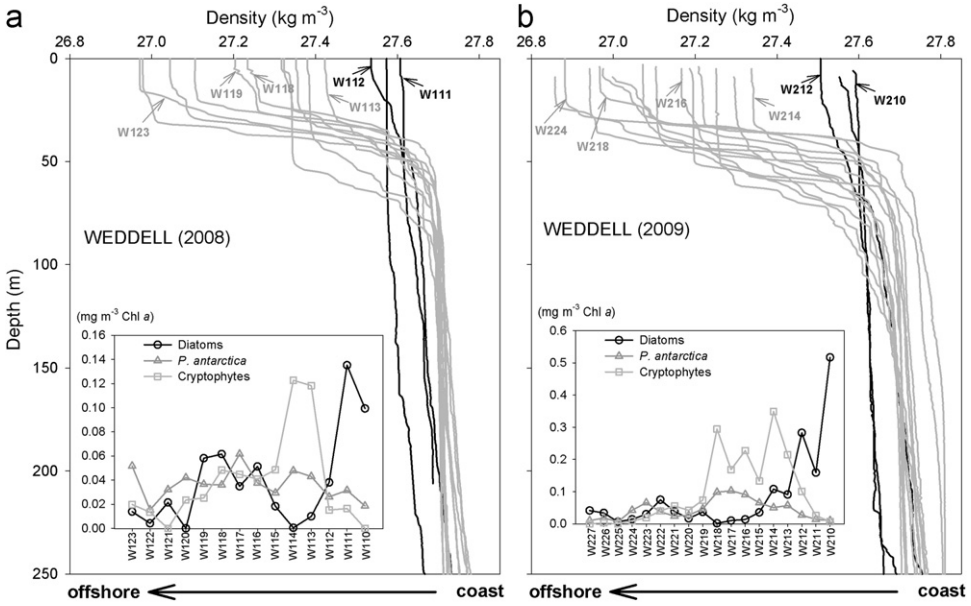

Fig. 8. Vertical profiles of water column density for the Weddell Sea transect during (a) 2008 and (b) 2009. Insets: absolute contribution (mg m^{-3} of chlorophyll *a*) of major taxonomic groups along the longitude *W* (coastal-offshore gradient displayed by arrows) for the same stations on the main graphs (see Fig. 1 for stations' location). Labels of some stations are displayed in order to assist the geographical localization. Density profiles of more coastal stations are highlighted in black lines.

3.2.6. *Vicinity of James Ross Island (ROSS)*

High Chl *a* concentration was generally recorded around ROSS. On the other hand, at the Antarctic Sound stations (e.g., stations R118 and R210; see Fig. 1 for their location) surface Chl *a* was always below 0.5 mg m^{-3} (see Fig. 2). Fig. 10 presents the vertical profiles of the phytoplankton community at six stations (Fig. 10(a)–(f)) that represent high (Fig. 10(a) and (d)), intermediate (Fig. 10(b) and (e)) and low (Fig. 10(c) and (f)) Chl *a* values during both years. Despite this relatively large biomass range, there was an absolute dominance of diatoms at all stations (> 90% contribution to total biomass).

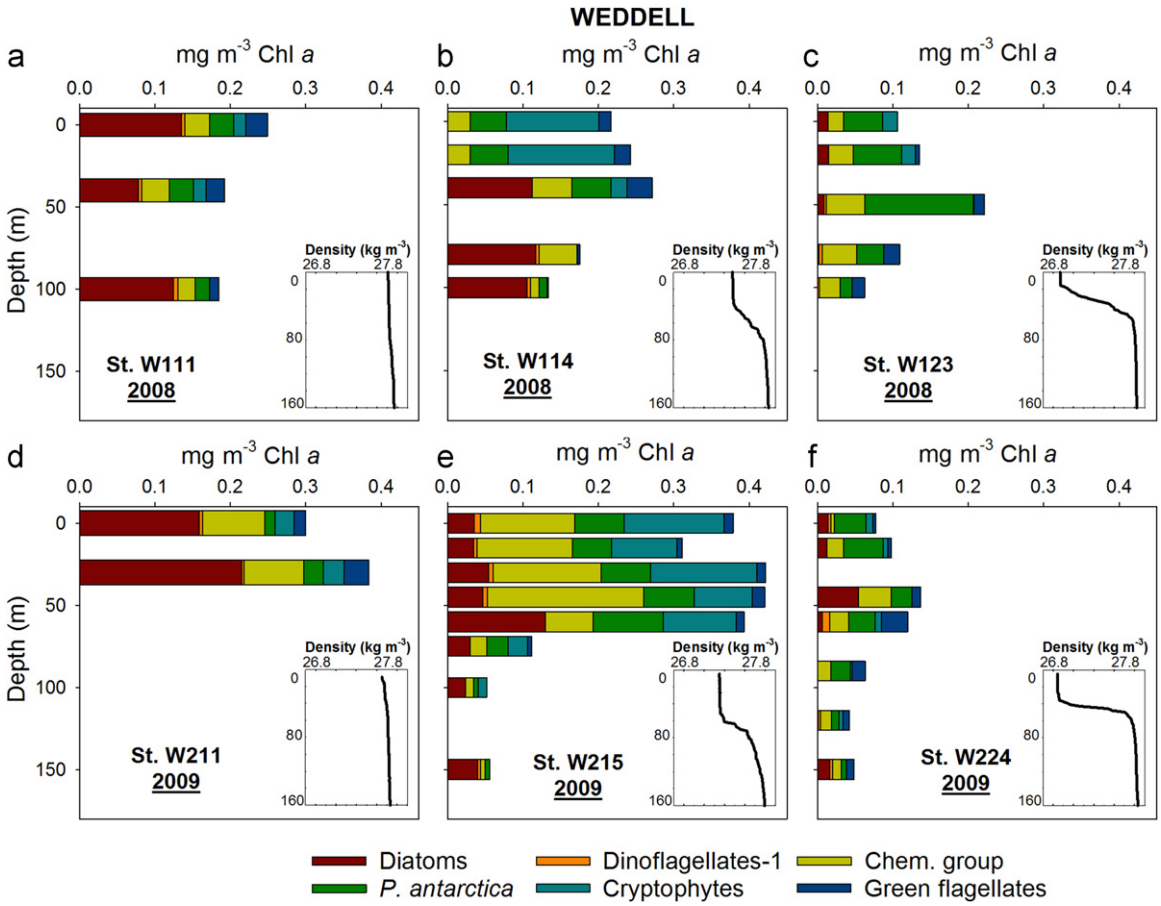

Fig. 9. Depth distribution of phytoplankton groups' biomass (as chlorophyll *a* concentration) calculated by CHEMTAX at the selected stations from the Weddell Sea. The order of appearance of stations corresponds to the onshore-offshore gradient in 2008 (a)–(c) and 2009 (d)–(f). Insets: density profiles of the respective stations (see Fig. 1. for stations' locations).

On a decreasing level of importance, the main diatom species were *Odontella weissflogii* (>70 μm in length), an assembly of moderately large centric diatoms (from 20 to 100 μm in diameter) and *Eucampia antarctica*. Areas with relatively high biomass were associated with a shallow UML, and generally comprised the stations closer to land (e.g., Fig. 10(a) and (d); stations R113 and R208, respectively). On the other hand, relatively low biomass (Fig. 10(c) and (f); stations R118 and R210, respectively) was observed at deep UML (insets in Fig. 10(c) and (f)) Antarctic Sound stations. Maximum and average Chl *a* levels in 2009 were twofold greater than those observed in 2008 (see Table 1).

3.3. Other pigment information

3.3.1. Pigment degradation products

The HPLC analysis allowed the separation, identification and quantification of three types of Chl *a* degradation products: chlorophyllide *a* (Chlide *a*), pheophytin *a* (Phytin *a*) and pheophorbide *a* (Phide *a*). Apart from the area around ROSS, where a typical diatom-bloom situation was observed, the concentration of the degradation products were always below 0.1 mg m^{-3}. The main degradation product of Chl *a* for the whole surveyed region was pheophorbide *a* (see Table 1). The concentrations of degradation products, particularly chlorophyllide *a*, were higher at ROSS than in other areas. Fig. 11 shows the linear relationships observed between degradation products and total Chl *a* in this region, and indicates a significant difference between both study years. In 2008, all degradation products were present at significantly higher concentrations than in 2009, with an average proportion (degradation product/total degradation products plus Chl *a*) of 11% for chlorophyllide *a*, 9% for pheophorbide *a* and 3% for pheophytin *a*. In 2009 the average proportions were 7, 6 and 2%, respectively.

3.3.2. Photosynthetic and photoprotective pigments

The array of phytoplankton pigments found in this study include photosynthetic and photoprotective carotenoids. The ratio of the sum of photoprotective carotenoids (PPC; alloxanthin, diadinoxanthin and diatoxanthin in our study) to the sum of total pigments (TP) indicated the physiological adaptation of the phytoplankton community to the prevailing ambient light. An evident difference in those indices was found between samples taken during day and at nighttime in the diatom-dominated ROSS region. For instance, Fig. 12 shows vertical profiles of PPC:TP for ROSS and DRAKE regions. The PPC:TP ratios at the nighttime stations in ROSS were about twofold smaller than those at daytime, especially on the surface layer (Fig. 12(a) and (b)), which indicates that relative PPC concentrations may change over a day. In the stratified water column conditions observed at DRAKE, the PPC:TP ratios within the upper mixed layer were five-times higher than those at or below the pycnocline during daytime (Fig. 12(c)). However, no noteworthy differences were found at stratified stations between day and nighttime stations or with depth variation for the ratio of pooled photosynthetic carotenoids (PSC; 19′-butanoyloxyfucoxanthin, 19′-hexanoyloxyfucoxanthin, fucoxanthin and peridinin) to TP (insets in Fig. 12).

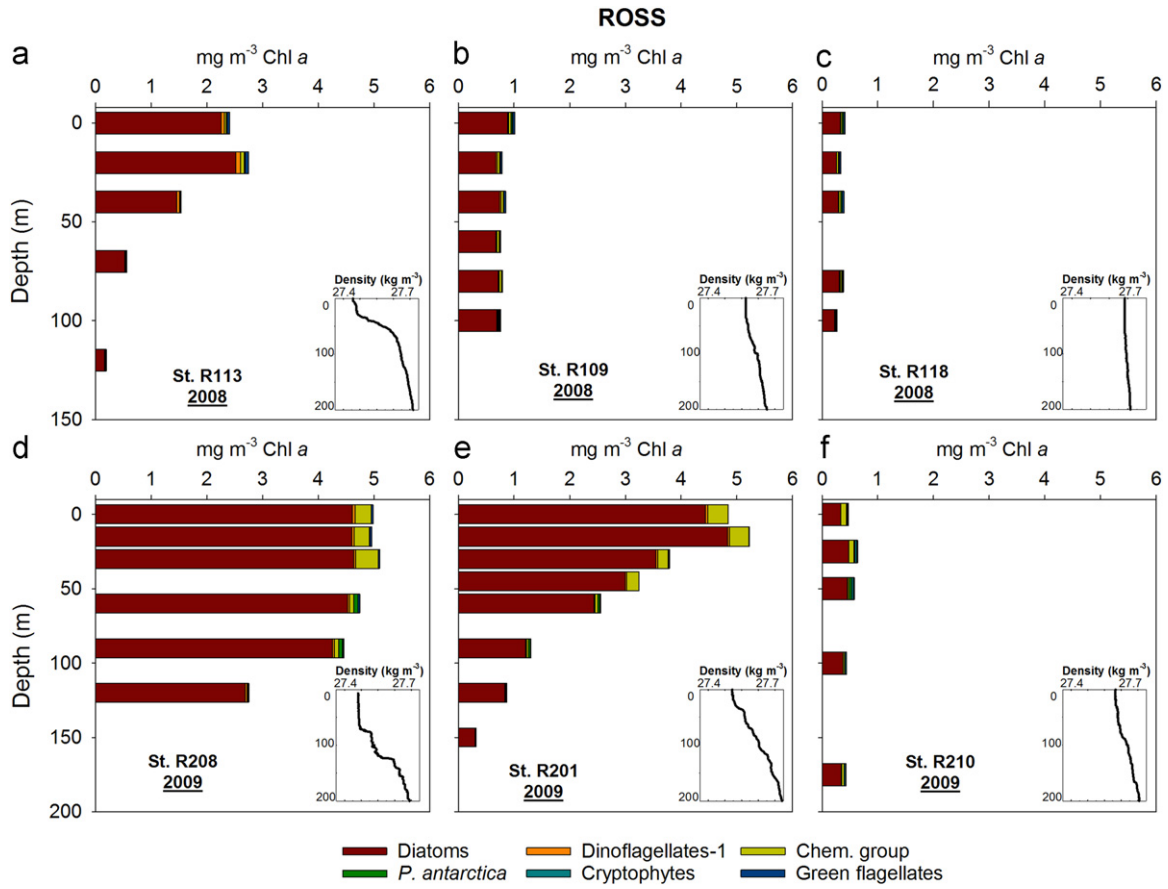

Fig. 10. Depth distribution of phytoplankton groups' biomass (as chlorophyll *a* concentration) calculated by CHEMTAX at the selected stations in the vicinities of James Ross Island (a)–(f). (a) Coastal station, 2008; (b) Non-coastal station, 2008; (c) Antarctic Sound station, 2008; (d) Coastal station, 2009; (e) Non-coastal station, 2009 and (f) Antarctic Sound station, 2009. Insets: density profiles of the respective stations (see Fig. 1 for stations' locations).

4. Discussion

4.1. Application of CHEMTAX in the study of phytoplankton communities

Several studies in the western AP region already suggested that both microscopy and HPLC techniques should be simultaneously used (e.g., Rodriguez et al., 2002; Kozlowski et al., 2011). Microscope observations provide important taxonomical information (to species or genus), which provides a better taxonomic resolution than HPLC, particularly for large and recognizable organisms. Small-sized organisms, such as nanoplanktonic cells that were common in low biomass areas of the studied region, are often difficult to preserve and recognize by light microscopy. HPLC-CHEMTAX provides valuable information about the whole phytoplankton community, including small-size groups. The good relationship observed between HPLC-CHEMTAX and microscope derived biomass of representative taxonomic groups (diatoms and *P. antarctica*) (Fig. 5) supports the reliability of the HPLC. Additionally, a high number of pigment samples were analyzed with HPLC-CHEMTAX during these oceanographic surveys, which would be impractical to study only by microscopic analysis.

The CHEMTAX software (Mackey et al., 1996) has been successfully used in many worldwide investigations (e.g., Mackey et al., 1998; Schlüter et al., 2000; Carreto et al., 2008; Wright et al., 2009, 2010). However, regarding to matrix optimization procedures, it would be advisable to apply different approaches when using the CHEMTAX tool, as either described by Latasa (2007) and Wright et al. (2009), and/or by using a combination of different approaches in order to improve the results (Mendes et al., 2011; Schlüter et al., 2011; de Souza et al., 2012). In the present study, we used the Wright's method (Wright et al., 2009) to obtain the output data from CHEMTAX. This method is appropriate for regions with low pigment concentrations (Wright et al., 2009), which was observed in the Weddell Sea and Drake Passage.

The output pigment ratios (see Table 2(b) and (c)) were generally equivalent to values available in literature (e.g., Rodriguez et al., 2002; Kozlowski et al., 2011) for the AP region. The average Fuco:Chl *a* (diatom) output ratio was lower in Rodriguez et al. (2002) (0.425 in 1995/1996) as compared to our study (0.940 for 2008 and 0.822 for 2009), but it was similar to the maximum value (0.714 ± 0.160 from 1995 to 2007) observed by Kozlowski et al. (2011). The cryptophyte Allo:Chl *a* ratio (0.428 for 2008 and 0.362 for 2009) was also higher than the observations made by Rodriguez et al. (2002) (0.228 in 1995/1996), but still within the range observed by Kozlowski et al. (2011) (0.443 ± 0.125 from 1995 to 2007). On the other hand, we have observed negligible variations between our study and results presented by both Rodriguez et al. (2002) and Kozlowski et al. (2011) for the ratios Hex-fuco:Chl *a* and But-fuco:Chl *a* of *P. antarctica*. These slight differences found in pigment ratios between this study and literature data may be associated with a light regime variation, nutrients availability and changes in algal populations (Schlüter et al., 2000). The different CHEMTAX approaches used by the different authors may also affect these final ratios. The output ratios we observed for the "chemotaxonomic group" varied between the two sampling years and also according to the literature previously mentioned. Such differences are associated with the taxonomic composition of the

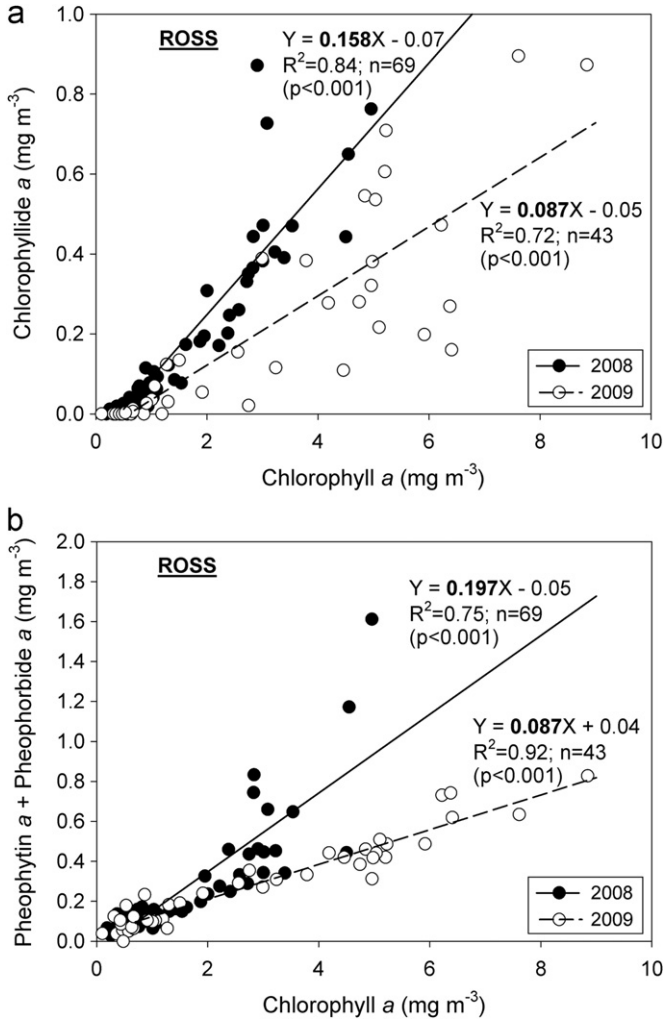

Fig. 11. Relationship between chlorophyll *a* concentration and (a) chlorophyllide *a* concentration and (b) pheophytin *a* plus pheophorbide *a* concentration, for stations near James Ross Island.

"chemotaxonomic group", which encompasses different taxa. For instance, the high Chl c_3:Chl *a* ratio (0.501) observed in 2008 may be related to the presence of *Gymnodinium* spp. (detected by microscopy), as higher concentrations of Chl c_3 were registered only at stations with high dinoflagellates abundance. Moreover, the high abundance of Chl c_3-containing *Pseudonitzschia* spp. may have contributed to this ratio, particularly in the Bransfield Strait.

4.2. Phytoplankton communities in relation to oceanographic parameters

A great spatial variability (horizontal and vertical) in the phytoplankton community was observed in the AP for both biomass and composition. This variation was mainly associated with water column structure, which can determine light availability and/or iron limitation within the UML. Stratification in the study area may be associated with several physical processes, such as coastal ice melting (characteristic of ROSS region) and seasonal warming of surface layers (evident in WEDDELL and DRAKE regions). For instance, a remnant cold and salty Winter water is usually found below warmer and fresher Antarctic Surface Water commonly formed during summer, particularly in offshore areas (Gordon and Huber, 1984).

We sampled during the late summer, when the existing phytoplankton community result from the succession associated with timing and extent of ice melting during the summer (Garibotti et al., 2005 and references therein). Although in the present study a seasonal variation was not evaluated, the great spatial heterogeneity allowed us to understand some processes related to the dynamic of phytoplankton communities around the tip of the AP. For instance, the low phytoplankton biomass observed in WEDDELL may be associated with a post bloom stage, as frequent blooms are often observed in this region during summer (Sullivan et al., 1993; Park et al., 1999; Kang et al., 2001). On the other hand, a clear diatom bloom situation was observed in ROSS. In this region we also observed a reasonably well-stratified water column due to ice melt and runoff from glaciers at James Ross Island. Both ice melt and runoff are likely noteworthy sources of iron that may have triggered the diatom-

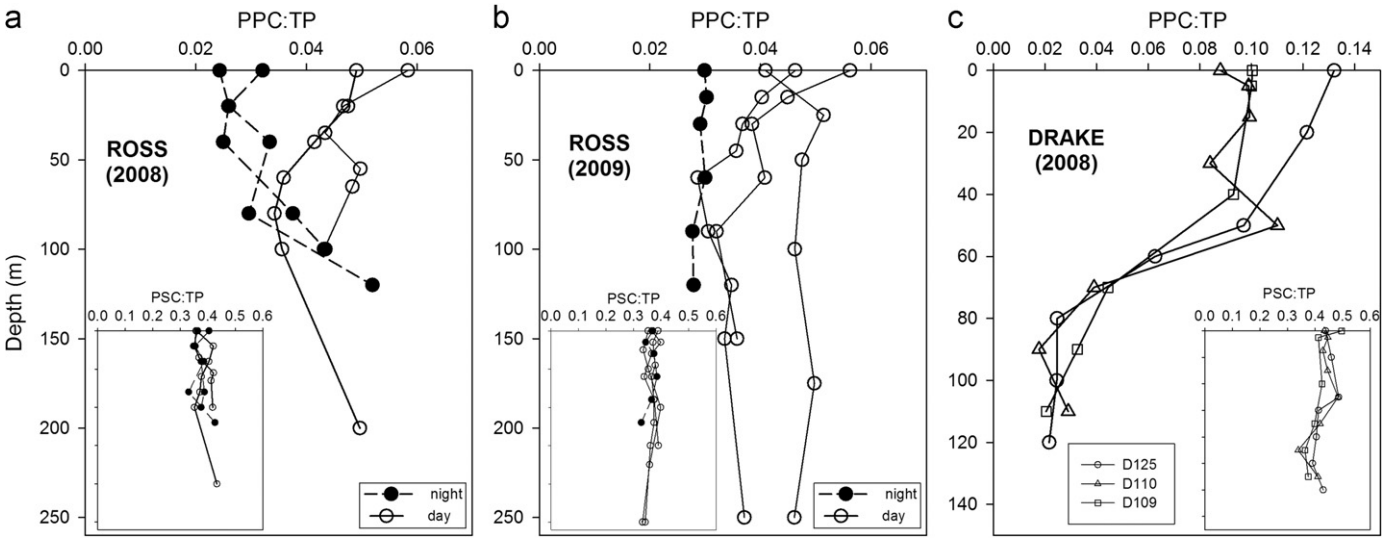

Fig. 12. Vertical profiles of photoprotective carotenoids (PPC) to total pigments (TP) ratios for available night (R103 and R109) and day (R113 and R118) stations at Ross region in 2008 (a); night (R208) and day (R201, R210 and R212) stations at Ross region in 2009 (b) and stratified daytime stations in Drake Passage (c). Insets: Profiles of the PSC:TP ratios for the same stations. Note the different scales between main graphs and insets.

dominated (e.g., *Odontella weissflogii*) phytoplankton bloom. Moreover, a biological-physical gradient was observed, as higher diatom biomass was generally associated with stratified nearshore areas (see Fig. 10). This trend has already been described, and highlights the importance of a shallow UML depth (Mitchell and Holm-Hansen, 1991; Garibotti et al., 2005) and associated stratification as a result of ice melting on phytoplankton development. This feature has been observed predominantly in coastal areas, as these regions are apparently protected from strong winds (Ducklow et al., 2007). Even though the lowest biomass levels in ROSS were measured in the Antarctic Sound area (associated with a deep UML), the phytoplankton composition was similar to other stations of the same region. This could be associated with advection processes in the Sound area, which prevented the accumulation of phytoplankton biomass (e.g., Moline and Prézelin, 1996).

The phytoplankton community observed in WEDDELL and DRAKE regions was characterized by low biomass and dominance of flagellates, including *P. antarctica* and cryptophytes at the stratified offshore stations. These stratification situations were probably a major physical feature affecting phytoplankton assemblages by a supposed limitation of Fe input into the upper surface layer, leading to development of a deep chlorophyll maximum (Ducklow et al., 2007). Previous studies have reported a limitation of primary production and biomass associated with low iron concentration in the northernmost sector of the studied region (Holm-Hansen and Hewes, 2004), which encompasses the Drake Passage and the western Weddell Sea (where low biomass values were observed). Additionally, Sañudo-Wilhelmy et al. (2002) described a coastal-offshore gradient in trace metal concentration (including Fe) in the AP region, from coastal waters with high metal concentrations to offshore waters with low metal levels. This onshore-offshore gradient in iron concentration may have been responsible for the changes observed in the phytoplankton community: dominance of diatoms in coastal regions, cryptophytes dominance in middle sites, and very low biomass (dominated by smaller flagellates such as *P. antarctica*) in far offshore stations (see Fig. 8). These phytoplankton composition shifts are possibly associated with competition for nutrient resources, particularly iron, and different nutrient-uptake abilities of the different phytoplankton groups found across the WEDDELL transect. Other factors, such as senescence and/or grazing, may have also contributed to the low biomass observed in those regions. The dominance of *P. antarctica* at shallow UML stations was not observed in other Antarctic regions, such as the Ross Sea (Arrigo et al., 1999, 2000), where this organism is commonly associated with deep UML due to photophysiological abilities (Kropuenske et al., 2010; Mills et al., 2010). In this study, this haptophyte was found in very low biomass at shallow UML layers and therefore was able to thrive under apparently low iron conditions. Those oligotrophic conditions (mainly in WEDDELL) may reflect the timing of our sampling period (late summer).

The BRANSFIELD region is hydrographically complex, comprising water masses that progressively change from Bellingshausen Sea to Weddell Sea influence (Sangrà et al., 2011 and references therein). This complexity may explain the great temporal (interannual) and spatial variability in phytoplankton biomass and composition. Briefly, higher biomass levels were recorded in 2009 mainly associated with diatoms and a shallower UML. At the northernmost part of this region (near Elephant Island), particularly in 2008, a low-biomass community composed by small flagellates was observed, coupled with a UML deeper than in 2009 (presumably leading to light limitation; see Fig. 7(c)). At coastal sites, diatoms and/or cryptophytes were the major groups contributing to phytoplankton biomass. On the other hand, the "chemotaxonomic group" was very important at the central channel (in the southernmost portion of the Strait). This group was dominated by *Gymnodinium* spp., which is known to contain carotenoids other than peridinin (Carreto et al., 2001). As demonstrated by classical ecological theories (Margalef, 1958; Smayda and Reynolds, 2001), we found the high abundance of *Gymnodinium* spp. and other dinoflagellates correlated with well-stratified water masses at the central channel in BRANSFIELD. Another interesting feature was the conspicuous dominance of cryptophytes at station B124, characterized by an intermediate stratification condition. One possible explanation for this particular area is the occurrence of a topographically induced upwelling of Weddell Sea water, inferred from the temperature–salinity profile (data not shown). However, few studies have reported episodic upwelling caused by topographic characteristics in other regions close to AP (Ducklow et al., 2007 and references therein) as well as intrusions of Weddell Sea water from the southwest into the Bransfield Strait (Sangrà et al., 2011).

4.3. Pigments as indicators of community physiological state

Pigment information can be used not only as a taxonomic tool to describe the phytoplankton community but also as a proxy for physiological responses to distinct environmental factors, such as light availability and grazing pressure. Among other environmental factors controlling the phytoplankton community, grazing pressure must also be considered (Ross et al., 1998; Anadón et al., 2002). Despite the lack of zooplankton data in this work, the relative content of Chl *a* degradation products can be used as a proxy for grazing pressure and for senescence of phytoplankton cells (Jeffrey et al., 1997). Apart from ROSS, where a diatom bloom was observed, low concentrations of these degradation products were generally observed (see Table 1). Higher proportions of all those products were observed in ROSS in 2008 than in 2009, which may suggest that, relatively to the scenario found in 2009, the 2008 diatom-bloom was in an advanced senescence stage and under higher grazing pressure (see Fig. 11).

Considerable differences were observed in the proportions of specific (photosynthetic or photoprotective) carotenoids over the total amount of pigments. Contrasting differences between the response of PPC and PSC to irradiance variation were detected across vertical profiles within the diatom-bloom at ROSS and at well-stratified offshore DRAKE stations (see Fig. 12). While a noteworthy difference in PPC:TP ratios were observed between day (higher values) and night (lower values) stations at ROSS, no detectable differences were found between day and night PSC:TP ratios. In addition, the PPC:TP proportion was significantly higher in the upper surface layer than in depth at the well-stratified DRAKE stations, although this pattern was not evident for the PSC:TP ratios. These results may be explained by the carotenoids' key functions in photosynthesis: (i) PSC have a significant role in extending the phytoplankton light-harvesting spectrum, thus ensuring optimal absorption efficiencies, and (ii) PPC acts as a protector of microalgal cells against high irradiances that may damage the photosynthetic apparatus (Kirk, 1994). Furthermore, the ratios of PPC:TP and PSC:TP have been considered remarkably robust for assessing the physiological state of a phytoplankton community (Barlow et al., 2008 and references therein). Information on PPC:TP ratios may thus indicate phytoplankton light histories (e.g., day vs. night, as in our study) and water column stability (Moline, 1998). Nonetheless, the PSC:TP ratios did not show an apparent response to short-term light changes, associated with neither daily-varying light field nor depth profiles or water column stratification. Our results support the assumption that photosynthetic pigments and respective ratios are rather adequate as taxonomic biomarkers.

5. Concluding remarks

This study shows that the spatial distribution of phytoplankton communities around the AP, particularly in the northernmost regions, is very complex and subject to several environmental factors, which may determine their composition and succession stage. Diatoms were the main contributors to Chl *a* biomass in areas presumably affected by ice melting processes, as observed at ROSS. Ice melting processes probably enhanced iron input into seawater, which triggered growth of large diatoms (both isolated cells and colonies). In open-ocean areas such as DRAKE and WEDDELL, where stratification was observed, nanoflagellates replaced diatoms as the dominant phytoplankton group. *P. antarctica* was the dominant organism among flagellates. Cryptophytes were persistently found at intermediate stratification conditions, i.e., between diatom-dominated coastal stations and offshore low biomass stations. At both BRANSFIELD and DRAKE coastal stations, many species of dinoflagellates (dominant taxa of the "chemotaxonomic group" that contain carotenoids rather than peridinin) were also important to total Chl *a*. Based on the spatial distribution of phytoplankton community composition and associated environmental factors, it seems that flagellates may replace diatoms in particular conditions (intermediate to strong stratification probably leading to iron limitation). Finally, this study highlights the usefulness of HPLC pigment data as biotic indicators of physiological responses to environmental conditions, such as ambient light and/or grazing pressure.

Acknowledgments

This study was conducted within the activities of the SOS-CLIMATE (Southern Ocean Studies for Understanding Climate Changes Issues) project. This is a multidisciplinary program as part of the GOAL (Group of High Latitude Oceanography) activities in the Brazilian Antarctic Program, coordinated by SECIRM. Financial support was provided by CNPq (National Council for Research and Development).

The authors thank the crew of the Brazilian Navy RV "Ary Rongel" and several investigators participating in the cruises for their valuable help during the collection of samples. We are grateful to Simon Wright, from the Australian Antarctic Division, for providing CHEMTAX v.1.95 and to Megan Thompson, for manuscript revision and helpful comments. C.R. Mendes and M.C. Leal were funded by a PhD grant from FCT (SFRH/BD/36336/2007 and SFRH/BD/63783/2009, respectively). A PhD fellowship from CAPES (Brazil) was granted to M.S.de Souza. V.M. Garcia and C.A. Garcia are granted with science fellowships from CNPq. We are thankful for the constructive criticism of three anonymous reviewers, which helped to improve the manuscript.

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