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Phytoplankton community structures in shelf and oceanic waters off southeast Brazil (20° – 25° S), as determined by pigment signatures



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ABSTRACT

The influences of the hydrological features and environmental conditions in the phytoplankton community found in the Campos Basin area in the Atlantic Ocean (20° to 25°S; 42° to 38°W) were studied using HPLC/CHEMTAX pigment analysis. Samples were collected at 72 stations distributed along the 25-3000 m isobaths at two depths during two seasonal periods (rainy and dry). Seven taxonomic groups of phytoplankton were detected (diatoms, dinoflagellates, prasinophytes, cryptophytes, haptophytes, pelagophytes and cyanobacteria). Redundancy analysis showed that the spatial and temporal patterns observed in the distribution of the phytoplanktonic groups were primarily related to variations in the availability of light and nutrients. Nutrient variations were caused by South Atlantic Central Water seasonal intrusions over the continental shelf region. Cyanobacteria predominated in the rainy season, while diatoms, Haptophyceae and Prasinophyceae, were associated with higher nutrient availability in the dry season. In the inner shelf region, diatoms dominated and were associated with increased conditions of turbulence and nutrient availability. Haptophytes and prasinophytes were predominant on the outer shelf and shelf-break regions associated with high nutrient concentrations and availability of light. Prochlorococcus was related to oceanic waters (in both dry and rainy periods) or to low nutrient/ strongly stratified shelf waters (rainy period). In contrast, Synechococcus was widely distributed in both the shelf and oceanic regions. Variation in the quality of light between coastal and oceanic waters was probably responsible for the distributions observed. Through HPLC/CHEMTAX pigment analysis we have developed a detailed picture of the influence of hydrological regime on the dynamics of the phytoplankton community in an under-studied shelf/ocean system in the tropical southern Atlantic Ocean.

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1. Introduction

Estimating the composition and biomass of phytoplankton is of great importance for understanding the structure and dynamics of pelagic ecosystems (Ediger et al., 2006). As the major primary producers in many aquatic systems and the basis of nearly all food webs in aquatic ecosystems (Arrigo, 2005), phytoplankton are essential for marine ecological and biogeochemical processes.

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http://dx.doi.org/10.1016/j.dsr.2014.03.006 0967-0637/© 2014 Elsevier Ltd. All rights reserved. In tropical marine waters, the phytoplankton communities consist of autotrophic, heterotrophic and mixotrophic organisms of variable size. While diatoms dominate in terms of species diversity in coastal and shelf regions, their relative importance is gradually reduced toward the open ocean, where the contribution of dinoflagellates increases significantly (Fernandes and Brandini, 2004). On continental shelves, the contribution of nanoplanktonic haptophytes is also important (Simon et al., 2009). In open seas, picoplankton (primarily consisting of cyanobacteria and small eukaryotes) dominate both the photosynthetic biomass and production (Vaulot et al., 2008; Simon et al., 2009).

The tropical and subtropical waters of the Atlantic Ocean along the Brazilian, Uruguayan and Argentinean coasts are influenced by a variety of hydrographic processes, which promote great diversity

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in the biological systems and result in several biogeographical provinces, from the oligotrophic waters of the South Atlantic gyre to the highly productive coastal waters at the La Plata river (35°S) and the Patos Lagoon (32°S) fronts (Gonzalez-Silvera et al., 2004).

Microphytoplankton in the Central Region of the Brazilian shelf waters (22–19°S) are typical of oligotrophic tropical oceans, with low density ($< 10^3$ cell L⁻¹) and high diversity and dominance of thermophile species and heterotrophic dinoflagellates (Tenenbaum et al., 2006). Diatoms and flagellated cells of nanoand microplankton belonging to the groups Dynophyceae, Haptophyceae. Cryptophyceae. Prasinophyceae and Chlorophyceae are dominant among the taxonomic groups present on the Brazilian Shelf (Fernandes and Brandini, 2004). Blooms of the filamentous cyanobacteria Trichodesmium are also common (Sato et al., 1963; Brandini, 1988; Gianesella-Galvão et al., 1995). Although the phytoplankton composition has been determined in the Cabo Frio region (23°S) in terms of the size-fractioned chlorophyll *a* biomass (Guenther et al., 2008), and the total pico-, nano- and microplankton biomass in the region between Cabo de São Tomé (22°S) and Salvador (13°S) was analyzed using epifluorescence microscopy (Tenenbaum et al., 2006), a comprehensive assessment encompassing the full phytoplankton size range is still lacking.

Assessments performed via inverted microscopy can underestimate the biomass of small phytoplankton and may not always distinguish photoautotrophic from heterotrophic cells (Seoane et al., 2011). Furthermore, nanoplanktonic flagellates can be difficult to identify using this technique, as is the case for haptophytes of the genus Phaeocystis sp., which display a worldwide distribution and occur in highly diverse marine systems (Schoemann et al., 2005). The development of electron microscopy has allowed unambiguous determination of picoplanktonic species, such as Bathycoccus prasinos or Imantonia rotunda, and the importance of some groups has been established as a result (Vaulot et al., 2008). However, electron microscopy is a difficult and time-consuming technique. Cellular flow cytometry analysis allows determination of the biomass of the three main picophytoplanktonic ($<2-3 \mu m$) groups present in open oceans: cyanobacteria of the Prochlorococcus and Synechococcus genera and eukaryotes belonging to diverse taxa (Grob et al., 2007). However, in many cases, scattering and fluorescence properties are not sufficient to discriminate picoeukaryote taxa, with the exception of cryptophytes (Vaulot et al., 2008). In recent years, the composition of marine picoeukaryote communities has been intensively investigated using molecular approaches, but adequate assessment of the photosynthetic fraction involves very sophisticated methods based on flow cytometry sorting, followed by the construction of 18S rRNA gene clone libraries (Shi et al., 2009). These methods cannot yet be employed on a routine basis for measuring the full size range of phytoplankton (Schlüter et al., 2011).

The presence of photosynthetic pigments does not depend on phytoplankton size and can be analyzed via High performance liquid chromatography/UV-vis absorption Diode array detection (HPLC/DAD), which is a widely applied technique. Some pigments unambiguously identify a certain class or a genus (e.g., prasinoxanthin in some prasinophytes), while others are common to multiple classes (e.g., fucoxanthin). Therefore, although mathematical methods have been developed to express the contribution of taxonomic groups to Chl *a* (Gieskes et al., 1988; Letelier et al., 1993; Obayashi et al., 2001), reconstruction of the phytoplankton composition based on pigment concentrations is not straightforward. Mackey et al. (1997) proposed a data-fitting technique for estimating the Chl *a* content of taxonomic groups using pigment data, the CHEMTAX program. It uses factor analysis and a steepest descent algorithm to find the best fit to the data based on suggested marker pigment/Chlorophyll a ratios for the phytoplankton groups to be determined. Detailed analysis of pigments from natural samples allows the identification of phytoplanktonic taxonomic groups and this approach has been used in many aquatic ecosystems including marine and freshwater habitats (Marinho and Rodrigues, 2003; Carreto et al., 2008; Wright et al., 2010; Gonçalves-Araujo et al., 2012). However, there have been few studies addressing phytoplankton pigments from the tropical South Atlantic, and, to our knowledge, no previous data are available in the region of the present study.

This study was conducted in the Campos Basin. located within the Brazil Shelf Large Marine Ecosystem. The water masses that comprise this region are the Coastal Water (CW). Tropical Water (TW) and the South Atlantic Central Water (SACW), the latter two of oceanic origin, transported by the Brazil Current, Coastal upwelling and intrusions of waters from the oceanic pycnocline (SACW) onto the continental shelf of southeast Brazil are phenomena controlled by various physical processes, which operate at multiple space and time scales (Palóczy et al., 2013). Our hypothesis is that the intrusions of SACW drive biological processes from the coastal to the oceanic region and the responses of biotic communities including the phytoplankton. HPLC/CHEMTAX analysis of pigments was used as a tool to bring insight into the structure of the phytoplankton assemblages, including phytoplankton taxa generally underestimated or overlooked by microscopy. In particular we addressed two questions: (1) How is this structure influenced by the hydrologic features of the region? (2) How do the assemblages correlate with the environmental conditions?

2. Methodology

2.1. Study area

This study was conducted in the Campos Basin region, located in the Atlantic Ocean (20° to 25°S; 42° to 38°W), adjacent to the southeastern coast of Brazil (Fig. 1). This region is located within two biogeographical provinces, one characterized by oligotrophic waters with coastal influence and the other by upwelling phenomena, located in Cabo Frio (Gonzalez-Silvera et al., 2004). The Campos Basin is divided between the eastern and southern portions of the Brazil Shelf Large Marine Ecosystem. Samples were collected at stations distributed along the 25, 50, 75, 150, 400, 1000, 1900 and 3000 m isobaths, along nine transects ranging from south to north (designated A–I), during two campaigns, one



Fig. 1. Study Area: Transects (A–I), isobaths (25–3000 m). Black circles represent sampling stations.

of which was performed in the rainy season (between March 5th and April 13th, 2009) and the other in the dry season (between August 7th and September 16th, 2009). At each station, samples were collected from the sub-surface (1 m) and at a second depth defined for the 25, 50 and 75 m isobaths as the middle of the water column, or as the 20 °C isotherm (typical of the South Atlantic Central Water), when it was detected. For the stations at the isobaths of 150, 400, 1000, 1900 and 3000 m, it was the depth at which the water temperature was 0.5 °C below that at the surface, which typically corresponded to the depth of the upper mixed layer (Fig. 1). On the continental shelf, the second depth ranged from 10 to 56 m during the rainy season and from 11 to 53 m during the dry season. At the oceanic region, it varied from 18 to 60 m and from 17 to 130 m in the rainy and dry seasons, respectively.

2.2. Environmental data

The environmental data used in our analysis included physical (water temperature and salinity obtained by a CTD) and chemical (inorganic nutrients analyzed by standard oceanographic methods (Grasshoff et al., 1999)) variables. The euphotic zone (Z_{euf}) was estimated as 3 times the Secchi disk extinction depth (Cole, 1994). Detailed discussion about the hydrochemistry of the study area is presented elsewhere (Suzuki et al., in press).

2.3. HPLC analysis of pigments

Samples for the analysis of phytoplankton pigments were obtained by filtering 6 L of seawater through GF/F 47 mm membranes (Whatman, UK) under reduced light at a minimum pressure of 250 mmHg. The filters were immediately placed in cryovials and stored in liquid nitrogen until analysis. The pigments were extracted using the method described by Wright and Jeffrey (1997), with some modification: trans-beta-apo-8'-carotenal (Sigma Aldrich, USA), which is often used as an internal standard (Wright et al., 2010), was added immediately before the ultrasound-assisted extraction step (using a Bandelin Sonoplus probe, Berlin, Germany). The samples were then analyzed according to Van Heukelem and Thomas (2001), (Method 1), and Brotas and Plante-Cuny (2003), (Method 2), using a Thermo Accella model 600 chromatograph with a diode array detector (Thermo Scientific, USA) and a Bischoff Analysentechnik chromatograph (Leonberg, Germany) coupled to a SPD-M10A VP diode array detector (Shimadzu, Kyoto, Japan). In Method 1, the Eclipse C8 150 mm, 3 µm (Agilent, Technologies, Waldbronn, Germany) column used by Van Heukelem and Thomas (2001) was replaced with an ACE C8 150 mm, 3 µm column (Advanced Chromatography Technologies Ltd, UK), and the flow rate was changed to 1.2 mL min⁻¹. The use of an ACE C8 column was advantageous for improving the separation of the pairs chlorophyll b/ divinyl-chlorophyll b and chlorophyll a/divinyl-chlorophyll a. However, the carotenoids 19'hexanoyloxifucoxanthin, prasinoxanthin and violaxanthin were coeluted, and B,E- and B,B-carotene were not entirely resolved (Fig. 2a). Method 2 did not resolve the monovinyl/divinyl pairs of chlorophylls a and b (Fig. 2b). All samples were analyzed in parallel with Methods 1 and 2. The quality of the extraction was controlled by the recovery of trans-beta-apo-8'carotenal (ApoCar) added in the extraction medium. ApoCar was not used as an internal standard, to avoid confusing the efficiency of the extraction of individual pigments from the cells with the recovery of the free added carotenoid. Furthermore, the fact that the extracts were analyzed by two parallel HPLC methods helped to verify whether there had been an extraction or an injection problem (i.e., if the area of ApoCar was low by only one method, an injection problem was detected). The pigment contents were quantified using standards for chlorophyll c3 (Chl c3), chlorophyll c2 (Chl c2),



Fig. 2. Separation of pigments from sample B3, second depth, rainy period. Method: (a) modified from Van Heukelem and Thomas (2001); (b) **Brotas and Plante-Cuny (2003)**. Pigments: 1–Chl *c*3, 2–MV Chl *c*3, 3–Chl *c*2, 4–Chl *c*1, 5–Per, 6–19'But, 7–Fuco, 8–Neo, 9–19'Hex, 10–Pras, 11–Viola, 12–Diadino, 13–Allo, 14–Zea, 15–LS. (Trans-beta-Apo 8'-Carotenal), 16–DV Chl *b*, 17–Chl *b*, 18–DV Chl *a*, 19–Chl *a*, 20– β ,e⁻Car, 21– β -Car.

chlorophyll *b* (Chl *b*), divinyl-chlorophll *a* (DV Chl *a*), chlorophyll *a* (Chl *a*), peridinin (Per), 19'butanoyloxifucoxanthin (19'But), fucoxanthin (Fuco), 19'hexanoyloxifucoxanthin (19'Hex), zeaxanthin (Zea), lutein (Lut), prasinoxanthin (Pras), neoxanthin (Neox), violaxanthin (Viola), diadinoxanthin (Diad), diatoxanthin (Diat), alloxanthin (Allo), mixoxantophyll (Mixo), $\beta_{,\varepsilon}$ -carotene ($\beta_{,\varepsilon}$ -Car) and $\beta_{,\beta}$ -carotene ($\beta_{,\beta}$ -Car), obtained from DHI-Water and Environment (Hørsholm, Denmark). All of the pigments were quantified at 440 nm, except mixoxantophyll, which was analyzed at 503 nm. The application of the two methods enabled the quantification of 21 pigments, at detection limits between 0.1 and 2 µg m⁻³ (calculated according to Miller and Miller, 1988). Total chlorophyll *a* (TChl *a*) concentration, which was used as an indicator of phytoplankton biomass, was estimated as the sum of Chl *a* and DV Chl *a* concentrations in each sample.

Chl *a*, DV Chl *a*, Chl *b*, and DV Chl *b* concentrations were taken from HPLC Method 1 because Method 2 did not resolve the monovinyl/divinyl pairs. Mixo was quantified by Method 1, because of the higher sensitivity observed. 19'Hex, Pras and Viola were quantified by Method 2 because they were not resolved by Method 1. Per was also quantified by Method 2 because peak purity was higher than by Method 1. Since the average difference between the concentrations obtained from the two methods was 10–20%, concentrations of well resolved pigments could be taken from either of the methods. In the rainy period, Chl *c3*, Chl *c2*, 19'But, Fuco, Neo, Allo, and Mixo were also taken from Method 1; in the dry period only the mono/divinyl pairs were taken from Method 1.

2.4. Processing of pigment data-CHEMTAX analysis

The relative contributions of different phytoplankton groups to the observed chlorophyll *a* content was calculated for each

sampling station using CHEMTAX software, version 1.95 (Mackey et al., 1997). The following pigments were included in the matrices: Chl c3, Chl c2, Chl b, DV Chl a, Chl a, Per, 19'But, Fuco, 19'Hex, Zea, Lut, Pras, Neox, Viola, Allo and Mixo. The initial pigment ratios in the algal taxa used in the chemotaxonomic analysis were obtained from the literature (Carreto et al., 2008; Schlüter et al., 2011) because pigment ratios from local phytoplankton were not determined in the present study. Pigment ratios for diatoms were taken from Carreto et al. (2003). For Synechococcus. prasinoxanthin-containing prasinophytes (Pras 1), violaxanthin-containing prasinophytes (Pras 2), cryptophytes (Crypto), pelagophytes (Pelago) and dinoflagellates (Dino) the mean pigment ratios of unialgal cultures of Synechococcus. Pycnococcus provasolii (Pras 1), Pyramimonas disomata (Pras 2), Rhodomonas salina (Crypto), Pelagococcus subviridis (Pelago) and Prorocentrum micans (Dino), grown under different light intensities (Schlüter et al., 2000), were used. The only reference for pigment ratios of filamentous cyanobacteria were the Trichodesmium ratios found in Mackey et al. (1997). This study was also used for the pigment ratio of Prochlorococcus (Zea/DV Chl a). The inclusion of three types of haptophytes in the input matrix was based on the detected pigments, on previous findings in the south of Brazil (Bergesch et al., 2008) and on the high complexity of haptophytes, which have eight subgroups with different pigment profiles (Zapata et al., 2004); the respective pigment profiles were taken from the values obtained by Carreto et al. (2008) in the Southern Atlantic.

Samples collected for microplankton microscopic analysis were consistent with the constructed CHEMTAX input matrix: diatoms were mainly represented by *Chaetoceros, Rhizosolenia, Dactyliosolen, Leptocylindrus* and *Pseudo-nitzschia* in the rainy period and *Dactyliosolen* and *Guinardia* in the dry period; dinoflagellates consisted mainly of the genus *Prorocentrum* in the south of the Campos Basin in the rainy period and of unidentified tecate species in the shelf in both periods; filamentous cyanobacteria were important in the rainy period (Tenenbaum et al., in press).

For chemotaxonomic analysis, samples were divided in groups according to both the depth and spatial distribution observed for the pigments. In each campaign, four groups were defined: Group 1: samples collected at 1 m in the 25, 50 and 75 m isobaths; Group 2: samples collected at the second depth in the 25, 50 and 75 m isobaths (second depth); Group 3: samples collected at 1 m in the 150, 400, 1000, 1900, 3000 m isobaths; and Group 4: samples collected at the second depth in the 150, 400, 1000, 1900, 3000 m isobaths (second depth). The CHEMTAX analysis followed the procedure adopted by Wright et al. (2009): 60 ratio matrixes were generated by multiplying each cell of the initial ratio matrix by a randomly determined factor, *F*, where $F = 1 + S \times (R + 0.5)$; *S* is a scaling factor (normally 0.7); and *R* is a random number between 0 and 1. Each of the 60 ratio tables was employed as the starting point for a CHEMTAX optimization, resulting in 60 solutions. To generate the final results, the six best solutions (those with the smallest residuals) were averaged. The biomasses of taxonomic groups were expressed in terms of their Chl a content, except Prochlorococcus, which was expressed in terms of the concentration of DV Chl a.

2.5. Statistical analysis

To verify the similarities along transects (latitudinal pattern), a permutational multivariate analysis of variance (PERMANOVA) for models with multiple factors (Anderson, 2001) was performed. We analyzed two factors (transects and isobaths), and the pigment values obtained at the two depths were considered as replicates. The Bray–Curtis distance was chosen as a measure of dissimilarity for all analyses, and 9999 permutations were run for each analysis

for tests with a significance level of 0.05. In the case of a significant result, affinity patterns were identified through a posteriori tests using 999 permutations. Similarities between the isobaths (bathymetric profile) and between the depths (vertical profile) sampled were verified through analysis of similarity (ANOSIM) and via a nonparametric test of significance (applying a Bonferroni correction to the resulting p values) in similarity matrices, using the Bray-Curtis index (Clarke, 1993). When differences were identified, non-parametric analysis of similarity percentages (SIMPER) was employed to determine which taxonomic groups contributed most to the observed similarities and dissimilarities (Clarke and Warwick, 2001), ANOSIM and SIMPER were performed using PAST software, version 2.0 (Hammer et al., 2001), PERMANOVA was realized using PERMANOVA software, version 1.6 (Anderson, 2005). Kruskal-Wallis one-way analysis of variance on ranks was used to compare the values of photosynthetic pigments/TChl a ratio among depths and campaigns. Redundancy analysis (RDA) was conducted to examine the influence of environmental variables on phytoplankton (Ter Braak and Smilauer, 2002). Biomass data were transformed by applying the function $\log 10 (x+1)$. Forward selection was performed to determine the minimum number of factors that could explain a statistically significant proportion (p < 0.05) of the variation in phytoplankton biomass. The selected variables were water temperature, salinity, euphotic zone (Z_{euf}) and nitrate, silicate and phosphate concentrations. The significance of these variables was assessed using Monte Carlo permutation tests with 999 unrestricted permutations.

3. Results

3.1. Oceanographic conditions

During both campaigns, South Atlantic Central Water (SACW) intruded into the continental shelf region. In the rainy season, this intrusion caused Tropical Water (TW) and SACW to mix, especially at the second depth; during the dry season, a mixture composed mainly of CW and SACW was observed at the surface, and SACW intruded into a significant portion of the continental shelf area (Foloni-Neto, 2010).

Light penetration was evaluated through the depth of euphotic zone (Z_{euf}) and was more pronounced in the ocean, especially in the rainy season. On the continental shelf, Z_{euf} varied from 6 to 60 m (mean=29 m) in the rainy period and from 18 to 48 m (mean=37 m) during the dry period. In the oceanic region, Z_{euf} varied from 30 to 75 m (mean=53 m) in the rainy season and from 18 to 60 m (mean=41 m) during the dry season.

In this study, the concentrations of all inorganic nutrients were lower in surface waters (1 m). The average orthophosphate values for the dry and rainy periods were 0.09 and 0.18 μ mol L⁻¹ at the second depth and 0.04 and 0.15 μ mol L⁻¹ at the surface, respectively. Nitrate was also more depleted at the surface and in the dry season, with average values of 1.18 and 1.37 μ mol L⁻¹ at the surface, and 1.17 and 1.49 μ mol L⁻¹ at the second depth in the dry and rainy seasons, respectively. The same patterns were observed for silicate, with an average of 1.16 and 1.57 μ mol L⁻¹ for surface waters and 1.47 and 1.62 μ mol L⁻¹ at the second depth in the dry and rainy seasons.

3.2. Spatial and temporal distribution of pigments

The maximum, minimum and median pigment concentrations recorded in the rainy and dry periods are presented in Tables 1 and 2, respectively. TChl *a* decreased from the coast to the oceanic region (Tables 1 and 2 and Fig. 3). On the continental shelf the obtained TChl *a* values were typical of coastal areas (maximum of

Table 1

Maximum (Max.), median (Med.) and minimum (Min.) concentrations ($\mu g m^{-3}$) of the main pigments found in the rainy period on the continental shelf (isobaths 25, 50 and 75 m) and in the oceanic region (isobaths 150, 400, 1000, 1900 and 3000 m), at both depths.

	(n=26)	depth ($n=26$)	1 m (n=45)	depth $(n=45)$
Max.	1134	1856	343	496
Med.	268	521	151	166
Min	104	204	33	54
Max.	1049	1856	276	383
Med.	234	475	104	112
Min.	104	124	22	38
Мах.	312	588	28	27
Med.	23	54	4	6
Min.	6	14	1	3
Max.	90	336	21	89
Med.	16	80	4	8
Min.	5	27	0	1
Мах.	61	217	38	89
Med.	29	95	18	31
Min.	17	31	6	12
Max.	147	195	131	136
Med.	104	61	86	85
Min.	39	23	29	39
Мах.	104	191	22	40
Med.	18	58	7	13
Min.	2	16	2	3
Max.	43	165	19	47
Med.	9	61	4	9
Min.	0	13	0	3
Мах.	15	161	17	50
Med.	8	35	5	8
Min.	5	9	1	3
Max.	85	153	91	118
Med.	34	32	48	61
Min.	0	153	11	15
Max.	69	147	14	10
Med.	7	12	3	5
Min.	0	0	0	0
Max.	25	87	10	6
Med.	3	13	0	0
Min.	0	0	0	0
Max.	16	49	2	11
Med.	2	11	0	0
Min.	0	49	0	0
Мах.	14	49	4	6
Med.	4	8	1	2
Min.	0	3	0	0
Max.	18	10	16	11
Med.	6	0	4	0
Min.	0	0	0	0
Мах.	13	19	0	1
Med.	1	1	0	0
Min.	0	0	0	0
	Max. Med. Min Max. Med. Max. Med. Min. Med. Min. Min. Min. Min. Min. Min. Min. Min	Max. 1134 Med. 268 Min 104 Max. 1049 Med. 234 Min. 104 Max. 312 Med. 23 Min. 6 Max. 312 Med. 23 Min. 6 Max. 90 Med. 16 Min. 5 Max. 61 Med. 29 Min. 17 Max. 147 Med. 104 Min. 39 Max. 104 Min. 2 Max. 104 Min. 2 Max. 104 Min. 2 Max. 104 Min. 2 Max. 104 Min. 2 Max. 104 Min. 5 Max. 43 Med. 8 Min. 0 Max. 15 Med. 8 Min. 0 Max. 5 Med. 34 Min. 0 Max. 16 Max. 16 Med. 2 Min. 0 Max. 16 Med. 2 Min. 0 Max. 16 Med. 2 Min. 0 Max. 16 Med. 3 Min. 0 Max. 16 Med. 4 Min. 0 Max. 18 Med. 4 Min. 0 Max. 18 Med. 6 Min. 0 Max. 13 Med. 1 Min. 0	Max. 1134 1336 Med. 268 521 Min 104 204 Max. 1049 1856 Med. 234 475 Min. 104 124 Max. 312 588 Med. 23 54 Min. 6 14 Max. 90 336 Med. 16 80 Min. 5 27 Max. 61 217 Med. 29 95 Min. 17 31 Max. 147 195 Med. 104 61 Min. 39 23 Max. 104 191 Med. 18 58 Min. 0 13 Max. 104 191 Med. 9 61 Min. 0 13 Max. 15 161 Med. 3 35 Min. 0 153 <td>Max. 1134 1836 343 Med. 268 521 151 Min. 104 204 33 Max. 1049 1856 276 Med. 234 475 104 Min. 104 124 22 Max. 312 588 28 Med. 23 54 4 Min. 6 14 1 Max. 90 336 21 Med. 16 80 4 Min. 5 27 0 Max. 61 217 38 Med. 29 95 18 Min. 17 31 6 Max. 147 195 131 Med. 104 61 86 Min. 39 23 29 Max. 104 191 22 Max. 104 191 22 Max. 104 161 17 Med. 9 <</td>	Max. 1134 1836 343 Med. 268 521 151 Min. 104 204 33 Max. 1049 1856 276 Med. 234 475 104 Min. 104 124 22 Max. 312 588 28 Med. 23 54 4 Min. 6 14 1 Max. 90 336 21 Med. 16 80 4 Min. 5 27 0 Max. 61 217 38 Med. 29 95 18 Min. 17 31 6 Max. 147 195 131 Med. 104 61 86 Min. 39 23 29 Max. 104 191 22 Max. 104 191 22 Max. 104 161 17 Med. 9 <

Table 2

Maximum (Max.), median (Med.) and minimum (Min.) concentrations ($\mu g m^{-3}$) of the main pigments found in the dry period on the continental shelf (isobaths 25, 50 and 75 m) and in the oceanic region (isobaths 150, 400, 1000, 1900 and 3000 m), at both depths.

Pigment		Shelf 1 m $(n=26)$	Shelf 2nd depth $(n=26)$	Ocean. Reg. 1 m (n=42)	Ocean. Reg. 2nd depth $(n=41)$
TChl a	Max.	2156	5507	2025	1668
	Med.	520	767	178	285
	Min.	117	184	57	88
Chl a	Max.	2156	5507	2025	1656
	Med.	492	760	114	244
	Min.	102	179	36	58
Fuco	Max.	2301	3277	195	1249
	Med.	180	338	17	33
	Min.	16	9	5	2
Chl b	Max.	401	468	135	156
	Med.	68	98	13	50
	Min.	401	15	0	5
19'Hex	Max.	139	251	212	211
	Med.	53	84	31	77
-	Mın.	20	21	11	22
Zea	Max.	147	145	95	77
	Med.	29	16	49	41
Chl al	Man.	11	071	14	5
$Chi c_2$	Wad	404	971	150	317
	Min	02	108	15	57
Chl a2	IVIIII.	212	14	5 110	200
	Mod	512	338 102	15	209
	Min	55 10	105	15	55 10
19' Rut	May	56	92	63	109
15 Dui	Med	12	22	12	40
	Min	56	10	4	9
DV Chl a	Max.	30	38	100	109
21 011 0	Med.	0	0	34	40
	Min.	0	0	100	109
Per	Max.	28	44	18	16
	Med.	8	11	4	6
	Min.	3	0	0	0
Allo	Max.	102	60	543	41
	Med.	17	17	1	5
	Min.	0	3	0	0
Pras	Max.	102	103	31	33
	Med.	12	17	2	7
	Min.	0	0	0	0
Viola	Max.	57	60	21	22
	Med.	10	12	3	4
	Min.	57	2	21	0
Neo	Max.	59	39	19	61
	Med.	17	7	4	2
_	Min.	59	1	0	0
Lut	Max.	27	70	6	4
	Med.	4	4	1	0
	Min.	0	U	0	0

1.86 mg m⁻³ in the rainy period and 5.51 mg m⁻³ in the dry period). In the oceanic region the TChl *a* values were typical of oligotrophic conditions (0.03 to 0.3 mg m⁻³ in the rainy period and 0.06 to 2.0 mg m⁻³ in the dry period), with little variation being observed between periods. However, in the region of the shelf break (isobaths of 150 and 400 m), the biomasses observed in the northern Campos Basin (Transects H and I) were higher in the dry period (Fig. 3). Regardless of the period and bathymetry, the highest TChl *a* values were registered at the second depth.

Fuco, a pigment associated with diatoms and haptophytes, was found to be the most abundant pigment after Chl a (Tables 1 and 2). Higher concentrations of Fuco were registered in the dry period, especially closer to the coast. In contrast, 19'Hex and 19'But (marker pigments for haptophytes) were distributed along the bathymetric profile, and no marked temporal variation in their concentrations was apparent. The 19'But/19'Hex and

19'But/(19'But+Fuco+19'Hex) ratios varied from 0.07 to 0.25 and 0.24 to 0.52, respectively; although no temporal or spatial variations were observed, the values were usually higher for the second depth (Table 3). Chl c3, which is also present in haptophytes, was found at higher concentrations at the first three isobaths in the rainy period and extended to the oceanic region in the dry period. After Fuco, Chl b was the most abundant pigment $(0-0.47 \text{ mg m}^{-3})$, indicating the importance of green algae in this environment (Tables 1 and 2). We also detected Lut, Neo, Viola and Pras, which are characteristic carotenoids of green algae. Zea, a marker of cyanobacteria, was evenly distributed across the Campos Basin, with slightly higher concentrations being recorded in the rainy period $(0.02-0.2 \text{ mg m}^{-3})$. The high Zea/Chl a ratios observed (maximum ratios of 1.4 and 1.1 in the rainy and dry periods, respectively) suggested the presence of picoplanktonic cyanobacteria of the genus Synechococcus (Carreto et al., 2003).



Fig. 3. Spatial distribution of TChl a (mg m⁻³) in Campos Basin during the two sampling periods, at the two sampled depths. (a) Rainy period, 1 m; (b) dry period, 1 m; (c) rainy period, second depth; (d) dry period, second depth. Notice the different scales.

DV Chl *a*, an unequivocal *Prochlorococcus* pigment, was detected at significant concentrations in both periods (0–0.15 mg m⁻³). Mixo, which is present in the filamentous cyanobacteria of the genus *Trichodesmium* (Mackey et al., 1997), was detected only in the rainy period. Per, an unequivocal dinoflagellate-associated pigment, was found at low concentrations in both studied periods, as was Allo, an unequivocal indicator of the presence of cryptophytes. However, higher concentrations of the later pigment were detected in the dry period, especially at the 150 m isobath (0.543 mg m⁻³).

3.3. Photosynthetic pigments/TChl a ratio

The ratio between the examined Photosynthetic pigments (Per, 19'But, Fuco, 19'Hex, Pras, Allo, Chl c2, Chl c3, DV Chl b and Ch b) and TChl a was used as a proxy to evaluate photoacclimation in the phytoplankton populations (Carreto et al., 2008). This ratio was calculated for the two sampled depths on the shelf (first three isobaths) and in the oceanic region (remaining isobaths) of the Basin; the regions were defined according to the observed spatial

distributions of the pigment concentrations. Photosynthetic pigments/TChl a ratios were significantly higher at the second depth on the shelf in the rainy period and in the oceanic region in the dry period (Fig. 4). The ratios were also significantly higher in the dry period than the rainy period.

3.4. CHEMTAX analysis

The presence of seven classes of phytoplankton (Bacillariophyceae, Haptophyceae, Prasinophyceae, Dinophyceae, Pelagophyceae, Cryptophyceae and Cyanobacteria) was detected in both campaigns. Two groups of prasinophytes, three groups of haptophytes and three genera of cyanobacteria (*Trichodesmium, Synechococcus* and *Prochlorococcus*) were distinguished. With the exception of *Trichodesmium*, which was recorded only during the rainy period campaign, all of these groups/ classes were present in both campaigns.

The input matrix (Table 4) appeared to describe the environment well because in both periods and at both depths, the output ratios (Tables 5 and 6) generally did not differ dramatically from the initial input values. In the following cases, differences between the output and input ratios should be noted: in the rainy period, the Fuco/Chl a ratio for diatoms was lower in the shelf region, and the Per/Chl a ratio for dinoflagellates on the shelf was higher for the second depth. For pelagophytes, during the rainy period, 19'But/Chl a increased, while Fuco/Chl a decreased with depth. The ratios obtained for type 8 haptophytes were stable, except for on the shelf in the dry period, where the 19'Hex/Chl a values were found to be much lower for the second depth. The Chl c3/Chl a ratios were stable, except for those of type 8 haptophytes on the shelf in the dry period. The Zea/Chl a ratio obtained for Synechococcus was quite stable, showing a tendency towards somewhat lower values in the dry period, while Zea/Chl a for Prochlorococcus displayed little variation across the sampling periods and depths (0.32-0.44). Beyond the shelf break, Pras/Chl a decreased while Chl b/Chl a decreased for prasinophytes (type 1).

3.5. Spatial and temporal distribution of phytoplankton groups

No spatial patterns in the distribution of phytoplankton with respect to the latitudinal profile were identified. Statistical analyses (PERMANOVA) revealed significant differences only in the shelf region, for transect H, which differed from transects A and C in the southern Campos Basin in the rainy period (p < 0.05) and from all transects except A in the dry period (p < 0.05). SIMPER analysis showed that the difference observed in the dry period was due to a greater prasinophyte and haptophyte contribution and a low cryptophyte and diatom biomass (data not shown).

Distinct spatial patterns were observed along the bathymetric profile for the phytoplankton groups. The diatom biomass was high at the 25 and 50 m isobaths and then declined dramatically with the distance from the shore (Fig. 5). The biomass of nanoplanktonic flagellate organisms (prasinophytes, haptophytes and cryptophytes) generally decreased with the distance from the shore (Figs. 5-7). The spatial distribution pattern observed for pelagophytes (eukaryotic picoplankton) was distinct: pelagophyte biomass increased toward the 400 m isobath and declined in the oceanic region (Fig. 7). Among the identified cyanobacteria (Fig. 8), Trichodesmium was recorded only in the rainy period and was widely distributed in both the shelf and oceanic regions, displaying higher biomasses at the surface. Synechococcus was also present in both periods, but no spatial distribution pattern was found for this genus at either sampling depth. During the rainy period campaign, the biomass of the picoplanktonic Prochlorococcus increased from coastal areas toward the 400 m isobath and was equally distributed in the oceanic region. However, during the dry period, a distinct gradient was apparent, with *Prochlorococcus* biomass increasing from the 150 m isobath to the oceanic region.

With respect to the vertical distribution, ANOSIM revealed a significant difference (p < 0.05) between the phytoplankton assemblages at the two depths in both the shelf and oceanic regions. SIMPER analysis showed that the vertical patterns observed were related to both the sampling period (rainy or dry) and the region (shelf or oceanic). Diatoms were the group that was most responsible for the dissimilarity observed in the shelf region, where the highest diatom biomass was measured, at the second depth (Fig. 5). In the oceanic region, cyanobacteria and haptophytes showed greater variability

Table 3

Mean and standard deviations of the 19'But/(19'But+Fuco+19'Hex) and 19'But/19'Hex ratios in the continental shelf and oceanic regions in the rainy and dry periods at the two sampled depths.

Ratio	Rainy period		Dry period				
	Continental shelf $(n=27)$	Oceanic region $(n=45)$	Continental shelf $(n=27)$	Oceanic region $(n=42)$			
19'But/(19'But+Fuco+19	/Hex)						
1 m	0.13 ± 0.06	0.16 ± 0.02	0.07 ± 0.06	0.18 ± 0.06			
2nd Depth	0.20 ± 0.12	0.19 ± 0.03	0.10 ± 0.09	0.25 ± 0.09			
19'But/19'Hex							
1 m	0.28 ± 0.07	0.25 ± 0.05	0.24 ± 0.06	0.37 ± 0.10			
2nd Depth	0.50 ± 0.20	0.30 ± 0.07	0.35 ± 0.18	0.52 ± 0.16			



Fig. 4. Photosynthetic pigments/TChl *a* ratios at the two sampled depths, in the (a) shelf region (25 to 75 m isobaths); (b) oceanic region (150 to 3000 m isobaths) during the rainy and dry periods. Different symbols ((a)–(c)) denote statistically significant differences (p < 0.05).

Table 4

CHEMTAX Input ratio-matrix (pigment/chlorophyll *a*).

Class/Pigment	per	19but	fuco	19hex	neox	pras	violax	allo	lut	zea	Mixo	chl_b	chlc2	chlc3
Pras1 Pras2 Dinofl Crypt Hapt6 Hapt7 Hapt8 Syn Tric Proc Pel Diat	0.411	0.021 0.062 0.220 0.510	0.100 0.360 0.480 0.732 0.863	1.060 0.720 0.450	0.113 0.079	0.458 0.000	0.000 0.138	0.261	0.018 0.049	0.079 0.043 1.245 0.048 0.327	0.034	0.679 0.532	0.099 0.057 0.230 0.110 0.100 0.397 0.208	0.240 0.090 0.310 0.174

 Table 5

 CHEMTAX output matrices for the rainy period. (Means of the 6 matrices that generated the lowest residuals. The mean coefficient of variation of the pigment ratios in the

matrices ranged from 13 to 20%).

	Per	19but	fuco	19hex	neox	pras	violax	allo	lut	zea	Mixo	chl_b	chlc2	chlc3
Shelf-1	m													
Pras1					0.113	0.454			0.020	0.080		0.662		
Pras2					0.074	0	0.147		0.041	0.040		0.544		
Dinofl	0.391							0.070					0.097	
Crypt		0.021	0.005	0 772				0.272					0.056	0.252
Hapto Hapt7		0.021	0.095	0.772									0.217	0.253
Hant8		0.035	0.303	0.375									0.090	0.082
Svn		0.210	0.407	0.140						1 689			0.050	0.500
Tric										0.095	0.091			
Proc										0.381				
Pel		0.588	0.555										0.391	0.167
Diat			0.578										0.169	
Shelf-2n	nd depth													
Pras1					0.112	0.428	0.112		0.021	0.070		0.725		
PIdS2 Dipofl	0.649				0.082	0	0.112		0.026	0.047		0.614	0.114	
Crypt	0.040							0.252					0.058	
Hapt6		0.021	0 101	0 969				0.252					0.050	0254
Hapto Hapt7		0.058	0.361	0.764									0.110	0.090
Hapt8		0.182	0.301	0.456									0.112	0.409
Syn										1.533				
Tric										0.042	0.033			
Proc										0.369				
Pel		0.881	0.397										0.353	0.190
Diat			0.684										0.200	
Oceanic	region—1 n	1			0.12.4	0.426			0.017	0.070		0.700		
Pras1					0.124	0.436	0.120		0.017	0.078		0.706		
PrdS2 Dipofl	0 272				0.078		0.139		0.047	0.043		0.600	0.006	
Crypt	0.372							0 2 3 7					0.090	
Hapt6		0.021	0.068	1.182				0.237					0.286	0.205
Hapt7		0.067	0.336	0.687									0.113	0.085
Hapt8		0.181	0.413	0.470									0.107	0.360
Syn										1.656				
Tric										0.087	0.102			
Proc										0.325				
Pel		0.643	0.354										0.349	0.156
Diat			0.781										0.222	
Oceanic I	region—2nd	l depth			0.125	0.249			0.017	0.076		0.000		
Pras 2					0.125	0.240	0 137		0.017	0.070		0.909		
Dinofl	0 360				0.005	0	0.157		0.015	0.044		0.751	0 101	
Crypt	0.500							0.252					0.061	
Hapt6		0.019	0.054	1.056									0.267	0.264
Hapt7		0.065	0.339	0.874									0.101	0.088
Hapt8		0.209	0.469	0.411									0.103	0.303
Syn										1.562				
Tric										0.113	0.076			
Proc										0.443				
Pel		0.874	0.325										0.378	0.170
Diat			0.827										0.207	

Table 6

CHEMTAX output matrices for the dry period. (Means of the 6 matrices which generated the lowest residuals. The mean coefficient of variation of the pigment ratios in the matrices ranged from 13 to 20%).

	per	19but	fuco	19hex	neox	pras	violax	allo	lut	zea	chl_b	chlc2	chlc3
Shelf—1 m Pras1 Pras2 Dinofl	0.251				0.090 0.146	0.481 0	0.151		0.016 0.056	0.081 0.049	0.782 0.597	0.086	
Crypt Hapt6 Hapt7 Hapt8 Syn Proc	0.351	0.023 0.065 0.129	0.101 0.404 0.517	1.010 0.706 0.437				0.244		1.313		0.063 0.209 0.107 0.095	0.292 0.092 0.602
Pel Diat		0.619	0.696 1.009							0.570		0.384 0.254	0.179
Shelf-2nd	l depth												
Pras1 Pras2 Dinofl Crypt	0.365				0.115 0.065	0.480 0	0.157	0.280	0.017 0.042	0.089 0.040	0.642 0.832	0.102 0.076	
Hapt6 Hapt7 Hapt8 Syn Proc		0.026 0.056 0.037	0.119 0.331 0.589	0.593 0.142						1.611		0.255 0.114 0.113	0.264 0.092 0.709
Pel Diat		0.703	0.778 0.868							0.205		0.416 0.231	0.208
Oceanic re	egion—1 m												
Pras1 Pras2 Dinofl	0.260				0.136 0.190	0.324	0.143		0.018 0.040	0.083 0.034	0.906 0.542	0.095	
Crypt Hapt6 Hapt7 Hapt8 Syn Proc	0.200	0.024 0.069 0.202	0.089 0.367 0.509	1.075 0.683 0.412				0.275		1.370 0.362		0.081 0.229 0.116 0.090	0.273 0.088 0.359
Pel Diat		0.559	0.610 0.762									0.396 0.203	0.179
Oceanic re Pras1 Pras2	egion—2nd d	epth			0.078 0.435	0.227	0.032		0.016 0.039	0.082 0.076	1.159 0.255		
Dinofl Crypt Hapt6	0.389		0.093	1.240				0.239				0.090 0.057 0.227	0.262
Hapt7		0.021	0 357	0 772								0.105	0.080
Hapt/		0.061	0.337	0.772								0.100	0.005
riapt8 Svn		0.245	0.404	0.465						1.200		0.100	0.313
Proc Pel			0.554							0.304		0.353	0.198
Diat		0.617	0.890									0.213	

between depths (Figs. 6 and 8). In the rainy period, *Trichodesmium* preferred the layers nearer the water's surface, whereas the Prochlorococcus and haptophyte biomasses were higher at the second depth. In contrast, during the dry period, prasinophytes were evenly distributed between the sampling depths, while the cryptophyte biomass was greater at 1 m (Figs. 5 and 7). *Synechococcus* biomass was higher at the surface (1 m).

3.6. Structure of phytoplankton assemblages

Because no statistically significant differences between transects were identified, the median of the values obtained for all transects for a particular isobath was considered a good representation of the phytoplankton assemblage at that isobath (Fig. 9). In the rainy season, different assemblage structures were observed for the two sampling depths. At 1 m (Fig. 9a), the region closest to the coast (25 and 50 m isobaths) was distinct from the remaining isobaths (ANOSIM, p < 0.05). This region was characterized by equal contributions from diatoms, cyanobacteria and haptophytes (approximately 24% from each), with 16% of the biomass being composed of prasinophytes. From the 75 to the 3000 m isobath, there was no significant difference observed between the plankton assemblages: micro- and picoplanktonic cyanobacteria contributed 70% of the total biomass on average (29% *Prochlorococcus*, 22% *Synechococcus* and 19% *Trichodesmium*). At the second depth (Fig. 9b), two regions could be discerned: one on the shelf, from the 25 to the 75 m isobath, and another beginning



Fig. 5. Biomass variation (expressed in mg m⁻³ Chl *a*) for diatoms and prasinophytes (with prasinoxanthin: Pras1, and without prasinoxanthin: Pras2) in samples collected at 1 m and at second depth on different isobaths in the (a) rainy and (b) dry period collection campaigns. The lines within the boxes are medians; the boxes correspond to 25–75% of the data and the bars to 90%.



Fig. 6. Biomass variation (expressed in mg m⁻³ Chl *a*) for haptophytes (types 6–8) in samples collected at 1 m and at second depth on different isobaths in the (a) rainy and (b) dry period collection campaigns. The lines within the boxes are medians; the boxes correspond to 25–75% of the data and the bars to 90%.

on the shelf at the 150 m isobath and extending throughout the oceanic region (ANOSIM, p < 0.05). Diatoms predominated (35% of biomass) at the second depth near the coast (25 m isobath), but in this

case, the high relative importance of haptophytes and prasinophytes (15–40% and 18–34%, respectively) extended into the 75 m isobath. From the 150 to the 3000 m isobath, picoplanktonic cyanobacteria



Fig. 7. Biomass variation (expressed in mg m⁻³ Chl *a*) for dinoflagellates, pelagophytes and cryptophytes in samples collected at 1 m and at second depth on different isobaths in the (a) rainy and (b) dry period collection campaigns. The lines within the boxes are medians; the boxes correspond to 25–75% of the data and the bars to 90%.



Fig. 8. Biomass variation (expressed in mg m⁻³ Chl *a*) for *Synechococcus, Prochlorococcus*, and *Trichodesmium* in samples collected at 1 m and at second depth on different isobaths in the (a) rainy and (b) dry period collection campaigns (*Trichodesmium* was not detected in the dry period). The lines within the boxes are medians; the boxes correspond to 25–75% of the data and the bars to 90%.



Fig. 9. Proportional contributions of algal groups to samples collected during the rainy period (left panel: (a) 1 m, (b) second depth) and dry period (right panel: (c) 1 m, (d) second depth). Values are medians for isobaths.

(36% *Prochlorococcus* and 20% *Synechococcus*) dominated; the contribution of *Trichodesmium* was low relative to the surface layer; and haptophytes accounted for approximately 17% of the plankton biomass.

In the dry period (Fig. 9c and d), the coastal region (25 and 50 m isobaths) was still distinct due to the large contributions of diatoms and nanoflagellates to total biomass (ANOSIM, p < 0.05). Moving away from the coast, from the 150 to the 3000 m isobath, there was a gradual change in the assemblage structure from dominance of nanoplanktonic groups (haptophytes, prasinophytes and cryptophytes) to picoplanktonic cyanobacteria (mainly Prochlorococcus). At 1 m, the planktonic biomass in the more coastal isobaths (25 and 50 m) was, on average, composed of 41% diatoms, 17% haptophytes, 21% prasinophytes and 12% cryptophytes; thereafter, a gradient from 26% haptophytes, 23% prasinophytes and 13% diatoms at the 75 m isobath to 44% Prochlorococcus, 18% Synechococcus and 18% haptophytes at the 3000 m isobath was observed. For the 25 and 50 m isobaths, the second depth showed a similar composition. However, the proportional contribution of Synechococcus decreased, while that of nanoflagellates and picoplanktonic pelagophytes was higher than for the surface waters. At the 3000 m isobath, dominance of Prochlorococcus was observed (51%), with important contributions from prasinophytes (20%) and haptophytes (15%).

3.7. Correlation between phytoplankton groups and environmental variables

A Monte Carlo test of *F*-ratios indicated that the six environmental variables considered contributed significantly to explaining the spatial and temporal distributions of the phytoplankton groups (salinity: F=59.34, p < 0.01; water temperature: F=32.49, p < 0.01; silicate concentration: F=3.70, p < 0.01; nitrate concentration: F=2.58, p < 0.05; phosphate concentration: F=3.39, p < 0.01; depth of the euphotic zone: F=1.97, p < 0.05). The first two ordination axes from the RDA explained 94% (F=18.89, p < 0.01) of the spatial/temporal distribution of phytoplankton groups, with 69% being explained by the first canonical axis and 25% by the second (Fig. 10). A temporal pattern defined by higher salinities and temperatures in the rainy period and

higher nutrient concentrations in the dry period was detected. A spatial gradient associated with the bathymetric profile was also apparent, with higher concentrations of nutrients, particularly silicate, being found in samples from the shelf region and higher salinities and greater euphotic zone depths being observed within the oceanic region.

The identified phytoplankton groups displayed the following patterns. Cyanobacteria predominated in rainy season samples, and *Prochlorococcus* was associated with oceanic waters. Moreover, diatoms and nanoflagellates (Haptophyceae, Prasinophyceae and Cryptophyceae) were associated with higher nutrient availability in the dry season. In addition, there was a distinct association of picoplanktonic eukaryotes (Pelagophyceae) with the oceanic region in the dry period.

4. Discussion

4.1. Pigment data and definition of the taxonomic groups

The use of two parallel HPLC methods served as a form of quality control, as the two quantification methods were compared to detect inconsistencies. This procedure allowed the detection and quantification of 21 pigments.

The positive correlation (r=0.540, p < 0.01, n=52 for logtransformed data) of Mixo with *Trichodesmium* biomass (data obtained via microscopy, Tenenbaum, per. comm.) supported its inclusion as a marker for this taxon. Although they were not included in the CHEMTAX input ratios, the pigments monovinyl-Chl c3 and Chl *c2*-monogalactosyldiacylglyceride ester, from *Chrysochromulina* (Seoane et al., 2009), which occur in haptophyte types 6 and 7 (sensu Zapata et al., 2004), were detected in some samples in small amounts, confirming the presence of these two taxonomic groups.

The 19'But/19'Hex and 19'But/(19'But+Fuco+19'Hex) ratios can aid in the interpretation of pigment data and in recognition of the taxonomic groups of haptophytes present. The calculated 19'But/19'Hex and 19'But/(Fuco+9'But+19'Hex) ratios (Table 3) were compared with those found by Seoane et al. (2009) for haptophytes, after transforming the molar ratios reported by these



Fig. 10. RDA ordination diagram with vectors for environmental variables (T–water temperature, S–salinity, Z_{euf}–euphotic zone, NO3–nitrate, PO4–phosphate, SIO2–silicate) and phytoplankton groups (Proc–*Prochlorococcus*, Synec–*Synechococcus*, Tric–*Trichodesmium*, Hapto6/Hapto7/Hapto8–haptophytes types 6–8, Pras1/Pras2–prasinophytes with and without prasinoxanthin, Dino–dinoflagellates, Bacil–diatoms, Crypto–cryptophytes, Pelago–pelagophytes). White dots represent samples from the continental shelf and gray dots represent samples from the oceanic region. The arrow indicates the spatial gradient from the shelf to the oceanic region.

authors into mass ratios. With the exception of the ratios calculated for the continental shelf in the dry period, the average 19'But/(Fuco+9'But+19'Hex) values obtained in this study were higher than the maximum of 0.12 reported in the literature (Laza-Martinez et al., 2007; Seoane et al., 2009) for haptophyte types 1, 2, 3, 6, 7 and 8 (sensu Zapata et al., 2004). Moreover, all of the mean values of the 19'But/19'Hex ratio calculated here were greater than 0.23, which was the maximum value obtained by Seoane et al. (2009) for haptophytes. The pigment 19'But is present in multiple types of haptophytes as well as in picoplanktonic pelagophytes (Dimier et al., 2009). Thus, haptophytes type 8 and pelagophytes, which display a high 19'But content, were included in the present study.

The detection of Pras indicated the presence of prasinophytes of type I (sensu Latasa and Scharek, 2004) in the Campos Basin. Studies conducted using cultures of type I prasinophytes indicate that the Pras/Chl b ratio can vary from 0.17 to 0.77 (Schlüter et al., 2003). Lower ratios indicate the presence of type II prasinophytes and/or Chlorophyceae. Because ratios below 0.17 were observed in the Campos Basin, it can be assumed that type II prasinophytes or Chlorophyceae are additional sources of Chl b in this area. However, the low Lut/Chl b ratios observed (0.01 to 0.13 in the rainy period and 0.00 to 0.20 in the dry period) argue against the presence of Chlorophyceae, which exhibit Lut/Chl b ratios higher than 0.30 (Schlüter et al., 2003).

4.2. CHEMTAX analysis

Due to the large number of stations sampled (72), it was possible to perform CHEMTAX analyses separately for the shelf and oceanic regions and for the two depths in each sampling period. After running CHEMTAX with 60 different input matrices and taking the mean of the six best results, as suggested by Wright et al. (2009), we did not consider it necessary to run CHEMTAX successively for each of the six best output matrices, as some authors have performed (De Souza et al., 2012; Schlüter et al., 2011). We observed that the output ratios of the main pigments of the dominant taxonomic groups generally tended to stabilize with the reduction of residuals, which was the main factor involved in the stabilization of taxonomic group biomass.

The observed decrease in the 19'Hex/Chl a output ratio observed at the second depth for type 8 haptophytes on the shelf during the dry period could be explained by a change in the species composition of this group. For diatoms, a higher Fuco/Chl a content was observed in the dry period, which may reflect different physiological states of the cells (Schlüter et al., 2011) or the presence of other fucoxanthin-containing taxonomic groups, such as type 1–5 haptophytes (which do not contain 19'Hex and 19'But) or dinoflagellate species, such as *Gymnodinium* sp., which contain fucoxanthin and related pigments, but no peridinin (Zapata et al., 1998; Carreto et al., 2001).

4.3. Spatio-temporal distribution of the phytoplanktonic groups

The phytoplankton biomass estimated by TChl a showed high variability in the Campos Basin (Tables 1 and 2, Fig. 3). The observed TChl *a* values in waters over the shelf were typical of Brazilian coastal regions (Gonzalez-Rodriguez et al., 1992; Knoppers et al., 1999), while the concentrations recorded in the oceanic region were characteristic of oligotrophic waters found in systems such as the Brazil Current (Gonzalez-Silvera et al., 2004; Carreto et al., 2008). The observed gradient in TChl *a* concentration was coincident with changes in phytoplankton composition (Fig. 9). Gradients of TChl *a* are known to be associated with shifts in composition of phytoplankton assemblages from micro- and nanoplankton predominance in eutrophic and mesotrophic waters, respectively, to nearly equal pico- and nanoplankton contributions in oligotrophic waters (Uitz et al., 2006).

Over the shelf, the intrusion of SACW (Foloni-Neto, 2010) was responsible for the high nutrient concentrations observed. These intrusions of nutrient rich waters can promote algal growth on photic zone. Based on the significant correlations found with silicate (r=0.44, p < 0.01) and phosphate (r=0.65, p < 0.01) concentrations in the rainy period and phosphate concentrations (r=0.33, p < 0.01) in the dry period, the observed variability in the spatial (bathymetric profile) and temporal (dry and rainy season) biomass patterns may be partly related to the availability of nutrients, particularly phosphorus and silica (Fig. 10).

Although this result is unexpected, since nitrogen is considered the main driver of phytoplankton biomass in marine waters (e.g., Howarth and Marino, 2006; Carlsson et al., 2012), phosphorus limitation is also common in marine systems (Downing et al., 1999; Elser et al., 2007). Furthermore, although no significant correlations between phytoplankton biomass and nitrate were found, low dissolved inorganic nitrogen values were also observed ($<7 \mu$ mol L⁻¹), which could be indicative of a potential nitrogen limitation (Reynolds, 2006). Co-limitation by nitrogen and phosphorus is also reported in many studies (Arrigo, 2005; Zohary et al., 2005).

The vertical distribution of inorganic nutrients showed patterns which are typical of permanently stratified tropical waters, with higher values found in the continental shelf. Nutrient concentrations observed at the second depth were always higher than those obtained at surface waters, and were consistent with contribution/ intrusions of SACW to this depth. Phosphorus limitation was potentially more severe in surface waters with an average N/P ratio of 32 (1 m) vs. 20 (2nd depth). Potential P limitation was more intense during the rainy season, as P levels were the lowest. The vertical distribution pattern of some phytoplankton groups, showing greater biomass at the second depth, was related to a higher nutrient availability (Figs. 5–8).

However, light availability can also be an important driver in the Campos Basin region. Although most of the time the 2nd depth was in the euphotic zone, in many cases the samples were collected at depths greater than Zeu. This was the case during the rainy period at the shelf and for almost all the samples collected in the oceanic region during the dry period (data not shown). Physiological adaptations in terms of pigment composition allow populations to have greater access to nutrients, without suffering light limitation. The increase in the Photosynthetic Pigments/TChl a ratio observed at the second depth indicates this type of photoacclimation response (Carreto et al., 2008) and was observed for the entire Campos Basin region. The disparity was more distinct in the rainy season, when large differences between the surface (Photosynthetic Pigments/TChl a=0.45) and the second depth (Photosynthetic Pigments/TChl a=0.8) were recorded. This reflects the period of greatest vertical stratification of the water column.

Diatoms, nanoflagellates and picoplanktonic cyanobacteria dominated the phytoplankton assemblages with respect to total biomass. Diatoms were more prevalent in the coastal region (25– 50 m isobaths), especially in periods of increased nutrient availability. In the Abrolhos region (northeast coast of Brazil), HPLC/ CHEMTAX analysis also detected important contributions of diatoms to plankton assemblages near the coast, which was not observed in the oceanic region (Knoppers et al., 1999).

Microplanktonic diatoms are associated with conditions of high turbulence and nutrient availability (Margalef, 1978; Falkowski, 1980). Many diatom species can be considered R-strategists (disturbance tolerant, or ruderal), presenting a high Surface/ Volume ratio that affords them the ability to harvest light energy under strong mixing conditions, but with high nutrient concentrations (Reynolds, 1997; Alves-de-Souza et al., 2008). The generally high maximum uptake rates observed in diatoms may be advantageous under conditions of high or fluctuating nutrient availability (Turpin, 1988; Grover, 1991; Litchman et al., 2007).

Planktonic nanoflagellates, especially haptophytes and prasinophytes, were found to be important in the Campos Basin region. Phytoplanktonic assemblages were characterized by the dominance of these organisms in regions that extend from areas near the coast to the shelf break (150-400 m isobaths; Fig. 9). The importance of prasinophytes in the Brazil Current was also noted by Carreto et al. (2008), particularly at the base of the euphotic zone. In the present work, pigment analysis was very important in quantifying the contributions of these groups. Pras 2 (violaxanthin-containing prasinophytes), detected primarily on the shelf in the Campos Basin, has been previously reported in coastal areas (Ruivo et al., 2011). The prasinoxanthin-containing prasinophytes (Egeland et al., 1995) (Pras1 in this work) found both on the shelf and in the oceanic region, are picoplanktonic species that appear to be the most abundant picoeukaryotes in coastal waters (Meakin and Wyman, 2011) and can be ubiquitous in oceanic regions around the world (Worden and Not, 2008).

The three types of haptophytes detected in the present study have also been reported in the Brazil Current (Carreto et al., 2008). The type 6 haptophytes include Emiliania huxleyi, which is the most abundant and cosmopolitan coccolithophore species, frequently constituting \sim 50% of the coccolithophore flora (McIntyre and Bé, 1967; Okada and Honjo, 1973). Type 8 includes *Phaeocystis*, which displays a worldwide distribution and is an important component of the haptophycean assemblage (Thomsen et al., 1994; Schoemann et al., 2005). Moreover, Chrysochromulina (type 7) and Phaeocystis and Imantonia (type 8) were found on the Brazilian coast at the maritime front of Lagoa dos Patos (Bergesch et al., 2008). In the Campos Basin, type 6 haptophytes were found primarily in the outer shelf region, while type 8 haptophytes showed a preference for the inner shelf. The biomass of each group was higher at the second depth. Carreto et al. (2008) also observed greater concentrations of haptophytes types 6 and 8 at a depth of 30 m than at the surface in the shelf break area of the Rio de la Plata front. The change in species composition observed for the type 8 group in the shelf at the second depth in the dry period was most likely related to the incidence of SACW at the second depth, while a mixture of TW with SACW was present at 1 m.

Carreto et al. (2008) and Mendes et al. (2011) attributed the presence of nanoflagellate species with rapid growth rates (*r*-selected species) in the shelf break region to circulation patterns that produce internal waves resulting in nutrient inputs. Both haptophytes and prasinophytes can be considered C-strategists (competitors, opportunistic colonizers), which are expected to dominate in waters with high nutrient concentrations and light availability (Reynolds, 1997). According to a classification proposed for phytoplankton by Sommer (1984), prasinophytes appear to exhibit a 'velocity-adapted' strategy, as they exhibit high maximum growth rates (Litchman et al., 2007). In fact, the greatest abundance of haptophytes and prasinophytes was observed during the dry period, when the SACW reached the shelf, bringing nutrients. In addition to being small organisms with rapid growth rates, haptophytes exhibit low half-saturation constants for nutrient uptake (Litchman et al., 2007), which may optimize nutrient acquisition under low nutrient conditions (Turpin, 1988; Grover, 1991). Haptophytes are well adapted not only to low nutrient conditions but also to the high irradiance levels that are often associated with such conditions (Iglesias-Rodriguez et al., 2002). This 'affinity' strategy (Sommer, 1984) was evident during the dry period, when the haptophyte contributions to planktonic assemblages in the oceanic region of the Campos Basin were significant, particularly at the second depth.

The oceanic region of the Campos Basin was characterized by dominance of picoplanktonic cyanobacteria (*Prochlorococcus* and *Synechococcus*). Similar results were obtained through pigment/ CHEMTAX analysis in the warm, highly stratified, nutrient-poor oceanic waters of the Brazil Current at the Maritime Front of the Rio de la Plata (Carreto et al., 2003, 2008). *Synechococcus* is found in nearly all oceanic surface waters (Partensky et al., 1999), whereas the distribution of *Prochlorococcus* is restricted to strongly stratified oligotrophic waters (Bouman et al., 2006) in tropical or subtropical regions. Moreover, *Prochlorococcus* is often present at a low abundance or is absent from coastal waters where *Synechococcus* thrives (Partensky et al., 1999; Gibb et al., 2000).

Our results showed that *Prochlorococcus* was present almost exclusively in oceanic waters (in both the dry and rainy periods), or in the shelf area during periods of low nutrient concentrations and strong stratification (rainy period). In contrast, Synechococcus was widely distributed in both the shelf and oceanic regions (Figs. 8 and 9). These two genera exhibit distinct ecological niches; their requirements for key environmental factors (light and nitrogen) are determined by their light-harvesting antennae and their intracellular nitrogen requirements, which are lower in Prochlorococcus (Ting et al., 2002). Niche differentiation in these cyanobacteria can be linked to the variation in light quality between coastal and oceanic waters. *Synechococcus* is equipped for growth in green light, which predominates in coastal waters, whereas Prochlorococcus is particularly efficient at absorbing the blue wavelengths of light that penetrate to depths of 100-200 m in the ocean (Moore et al., 1998; Partensky et al., 1999). In open ocean regions where Prochlorococcus and Synechococcus co-occur, Prochlorococcus has generally been found to extend deeper in the water column than Synechococcus (Partensky et al., 1999). Photoadapted phytoplankton populations were previously found in the Brazil Current at the sea front of the River de la Plata by Carreto et al. (2008). These authors also highlighted the presence of two distinct populations of *Prochlorococcus*, one growing at the surface, reflecting an ecotype adapted to high light intensities (low DV Chl b/DV Chl a ratios), and one growing in deeper layers, representing an ecotype with photoadapted ratio values > 2 (Ting et al., 2002; Carreto et al., 2008). The data obtained in the Campos Basin also

suggest that the identified *Prochlorococcus* populations exhibit physiological responses that indicate photoadaptation, with higher DV Chl b/DV Chl a ratio values being found at the second depth. However, we were unable to distinguish the presence of different ecotypes because the obtained DV Chl b/DV Chl a ratios were always below 1 (Moore et al., 1995; Ting et al., 2002).

In addition to the picoplanktonic species discussed above, species of the genus *Trichodesmium* were also important in the Campos Basin. This genus was recorded only during the rainy period and was widely distributed in both the shelf and oceanic regions, with higher biomasses being recorded at the surface. The significant contribution of *Trichodesmium* to the cyanobacterial biomass was consistent with previous reports describing the occurrence of this genus throughout oligotrophic tropical and subtropical oceans (Capone et al., 1997; Bergman et al., 2013). The fact that this genus was not registered in the dry period may be related to the lower water temperatures recorded during this period (19–24 °C). *Trichodesmium* abundance is roughly limited to waters warmer than 20 °C, and the temperature tolerance of these species with respect to growth ranges from 20 to 34 °C (Breitbarth et al., 2007; Chappell and Webb, 2010).

5. Conclusion

The pigment analysis using HPLC in conjunction with CHEMTAX software performed here made it possible to characterize the composition and structure of phytoplankton assemblages in the Campos Basin. SACW seasonal intrusions over the continental shelf not only influence the stratification of the water column, but particularly influence the availability of nutrients in the continental shelf region. This hydrological regime was crucial to the dynamics of the phytoplankton community. On the continental shelf, diatoms (R-strategists) were more prevalent in the coastal region in periods of increased availability of nutrients, associated with increased conditions of turbulence. Nanoplanktonic groups (C-strategists-haptophytes and prasinophytes) predominated on the outer shelf and the shelf break, associated with high nutrient concentrations and availability of light. The variation in light quality between coastal and oceanic waters was probably responsible for the distributions of Prochlorococcus and Synechococcus observed. Prochlorococcus was present almost exclusively in oceanic waters (in both dry and rainy periods) or in the shelf area during periods of low nutrient concentrations and strong stratification (rainy period). In contrast, Synechococcus was widely distributed in both the shelf and oceanic regions.

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