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CHLOROPHYLL *a* BIOMASS AND ACCESSORY CHLOROPHYLL PIGMENTS IN THE SHALLOW COASTAL ZONE AT ADMIRALTY BAY, ANTARCTICA: COMPARISON BETWEEN TWO CONSECUTIVE AUSTRAL SUMMERS

Márcio Murilo Barboza Tenório[1*], Fernanda de Alcântara Vaz Pinto[1],
José Juan Barrera-Alba[1,2**], Jacques Neveux[3***] & Denise Rivera Tenenbaum[1#]

[1]Laboratório de Fitoplâncton Marinho, Instituto de Biologia, Universidade Federal do Rio de Janeiro – UFRJ, Av. Carlos Chagas Filho, 373, CCS, sala A-61, Ilha do Fundão, Cidade Universitária, CEP 21949-902, Rio de Janeiro – RJ, Brasil
[2]Departamento de Ciências do Mar, Campus Baixada Santista, Universidade Federal de São Paulo – UFRJ, Av. Saldanha da Gama, 89, Ponta da Praia, CEP 11030-400, Santos – SP, Brasil
[3]UMR 7621, LOMIC – CNRS, Université Pierre et Marie Curie, Laboratoire Arago, 66650 Banyuls-sur-mer, France
*e-mails: *marcio.tenorio@biologia.ufrj.br, **juanalba@biologia.ufrj.br, ***neveux.jacques@orange.fr, #deniser@biologia.ufrj.br*

Abstract: *Chlorophyll a and accessory chlorophyll concentrations of the phytoplankton community were studied in Admiralty Bay in early and late summer of 2009/2010 and 2010/2011, using spectrofluorometry. Chlorophyll a biomass increased from early to late summer and was higher in 2010/2011 than in 2009/2010. The ratios of accessory pigments to Chlorophyll a showed a greater presence of chlorophytes relative to chromophytes in 2009/2010 than in 2010/2011 summer. The variations observed in chlorophyll biomass and accessory pigments to Chlorophyll a ratios was associated to increase in water temperature during austral summer.*

Keywords: Chlorophylls, Spectrofluorometry, Western Antarctic Peninsula, Admiralty Bay

Introduction

The chlorophyll *a* (Chl *a*) concentration is one of the most measured oceanographic variables to determine phytoplankton biomass and primary production in waters. Although C/Chl *a* ratio shows important variations according to environmental changes, Chl *a* remains the best proxy of phytoplankton biomass for studying spatial and temporal variability of primary productivity (Huot *et al.*, 2007). Associated with Chl *a* in more specific phytoplankton taxa, accessory pigments (like chlorophyll *b* and chlorophyll *c*, amongst others) provides useful information concerning taxonomic composition, photoadaptability and the physiological status of the algal community (Neveux & De Billy, 1986). However, there is still no information about these variables in Admiralty Bay, King George Island.

The Chl *a* biomass is generally low in the Southern Ocean despite the presence of high macronutrient concentration (Martin *et al.*, 1991; Rose *et al.*, 2009). Research studies in Admiralty Bay since the 80's reported Chl *a* concentrations during summer periods usually below 1 μg L^{-1} (Kopczynska, 1980; Lipski, 1987; Lange *et al.*, 2007). Phytoplankton biomass in Antarctic Waters shows high variability at different time scales (weekly to interannual) that is driven by physical, chemical and biological forcing like water column stabilization which is affected by local wind stress, temperature, nutrients, light availability, grazing pressure, dynamics related to sea ice melt and glacial melt water, among others (Moline & Prézelin, 1996; Smith *et al.*, 2008).

In this study we present the temporal and spatial variability of chlorophylls concentration in Admiralty Bay, King George Island, during 2009/2010 and 2010/2011 summers.

Materials and methods

Study area and sampling

Admiralty Bay (62°03' – 12'S, 58°18' – 38'W), located at King George Island, has been the focus of a Brazilian National Monitoring Program from the National Institute of Science and Technology - Antarctic Environmental Research (INCT-APA) since 2008. The sampling methodology follows the standard procedures for hydrological (Cascaes *et al.*, 2012) and plankton studies (Tenório *et al.*, 2011; Tenenbaum *et al.*, 2011). Measurements of Chl *a* were obtained from surface, 15 m and near the bottom (≈ 29 m) at five stations in early (ES) and late (LS) summers (2009/2010 and 2010/2011) during 12 surveys from Antarctic Operations (AO) XXIX and XXIX (n = 180 samples). Temperature was measured *in situ* with a Seamon Mini sensor. Salinity and nutrients chemical analyses was presented on Cascaes *et al.* (2012). Spearman´s rank correlation coefficient (r) was used to measure the degree of association between pigments and physical and chemical variables.

Chlorophyll pigments

Water filtration, sample storage and extraction of chlorophyll pigments were described in Tenório *et al.* (2011). Fluorescence properties of the acetonic extracts were measured on a Varian Cary Eclipse® spectrofluorometer. Concentrations of Chl *a*, *b* and *c* were assessed using a modified version of Neveux & Lantoine's (1993) method as described by Tenório *et al.* (2005). The significance of spectrofluorometric results is related both to the relative concentrations of the pigment in the extracts and to its quantum yield in the solvent used. Only an accessory chlorophyll concentration which represented at least 5% (weight to weight) of Chl *a* will be considered significant.

Table 1. Physical, chemical and biological variables at Admiralty Bay during the 2009/2010 and 2010/2011 surveys. Pigment ratios are weight to weight ratios.

Surveys	Periods		Temperature (°C)	Salinity	Chl *a* (µg L^{-1})	Chl *a* (mg m^{-2})	Chl *b*/ Chl *a*	Chl *c*/ Chl *a*
2009/2010	Early Summer	Min	-0.63	33.77	0.13	4.36	0.07	0.11
		Max	1.16	34.43	0.67	17.70	0.19	0.20
		Av	0.06	34.16	0.34	10.07	0.12	0.15
		SD	0.40	0.14	0.11	3.24	0.03	0.02
		n	60	60	60	60	60	60
	Late Summer	Min	0.38	33.64	0.15	6.09	0.04	0.13
		Max	1.35	34.30	0.92	24.50	0.12	0.26
		Av	0.82	34.09	0.48	14.05	0.08	0.18
		SD	0.23	0.17	0.24	6.81	0.02	0.03
		n	45	45	45	45	45	45
2010/2011	Early Summer	Min	0.13	33.38	0.36	12.98	0.02	0.11
		Max	1.49	34.87	3.72	45.27	0.10	0.18
		Av	0.62	34.18	0.84	24.60	0.05	0.15
		SD	0.28	0.23	0.51	8.81	0.02	0.01
		n	45	45	45	45	45	45
	Late Summer	Min	1.39	33.4	0.40	23.05	0.04	0.16
		Max	1.92	34.31	6.11	166.56	0.11	0.19
		Av	1.62	34.12	2.84	82.23	0.05	0.18
		SD	0.14	0.21	1.73	48.65	0.02	0.01
		n	30	30	30	30	30	30

minimum (Min), maximum (Max), average (Av) , standard deviation (SD) , sample numbers (n)

This does not mean necessarily that Chl *b* or Chl *c* are absent, but that the accuracy of its determination is poor below this threshold (Neveux *et al.*, 2009).

Results

Due to the spatial and vertical homogeneity of thermohaline structure in shallow waters of Admiralty Bay, the environment variables and chlorophyll pigments concentrations are averaged (n = 15) for each survey (Figures 1 and 2).

The results showed a gradual increase of temperature in Admiralty Bay waters from the beginning of summer through its end (Figure 1a, Table 1). Moreover, a strong interannual thermal variation of averaged temperature was observed with values 2.5 times lower during the 2009/2010 summer (0.40 ± 0.47 °C) than during the 2010/2011 summer (1.02 ± 0.54 °C). The haline structure exhibited slight temporal variations (Figure 1, Table 1), and similar averaged values for both summers 2009/2010 (34.13 ±0.15) and 2010/2011 (34.16 ± 0.23).

Chlorophyll a, an indicator of overall phytoplankton abundance

During 2009/2010 Chl *a* concentration was low (0.48 ± 0.24 $\mu g\ L^{-1}$) showing values lower than 0.5 $\mu g\ L^{-1}$ in 93% of the samples during ES and 53% in LS. Integrated Chl *a* (0-29m) increased ~ 28% from early (10.07 ± 3.24 $mg\ m^{-2}$) to late summer (14.05 ± 6.81 $mg\ m^{-2}$) (Figure 2a; Table 1). Temperature (r = 0.51; $p = 0.001$) and SiO_4 concentrations (r = -0.38; $p = 0.02$) were the variables that seemed to have an influence on chlorophyll biomass variation. In 2010/2011, Chl *a* values were lower than 1 $\mu g\ L^{-1}$ in 58% of the samples during ES and only 16% during LS. In 2009/2010, integrated Chl *a* also increased but with an higher amplitude, approximately three times from ES to LS (24.60 ± 8.81 $mg\ m^{-2}$ to 82.23 ± 48.65 $mg\ m^{-2}$), and were six times higher than during 2009/2010 (Figure 2a; Table 1). Similarly to that observed in the previous survey Chl *a* variations were influenced by water temperature (r = 0.86; $p < 0.001$) and SiO_4 (r = -0.53; $p = 0.003$). However, in this case, NO_3 concentrations also showed a significant influence (r = -0.47; $p = 0.01$).

Accessory chlorophylls ratio: relative indices of eukaryotic components of phytoplankton communities.

During 2009/2010, the Chl *b*/Chl *a* ratio (Figure 2b; Table 1) decreased from ES (0.12 ± 0.03) to LS (0.08 ± 0.02). However, in the last sampling of LS, the ratio increased to an intermediate level between the last two ES samplings (Figure 2b; Table 1). On the other hand, Chl *c*/Chl *a* ratio increased from 0.15 ± 0.02 (ES) to 0.18 ± 0.03 (LS). This ratio was higher than Chl *b*/Chl *a*, except at the beginning of the sampling period. The two ratios showed a significant negative correlation (r = -0.43; $p = 0.007$). The Chl *c*/Chl *a* ratio was positively correlated with Chl *a* (r = 0.51; $p = 0.001$)

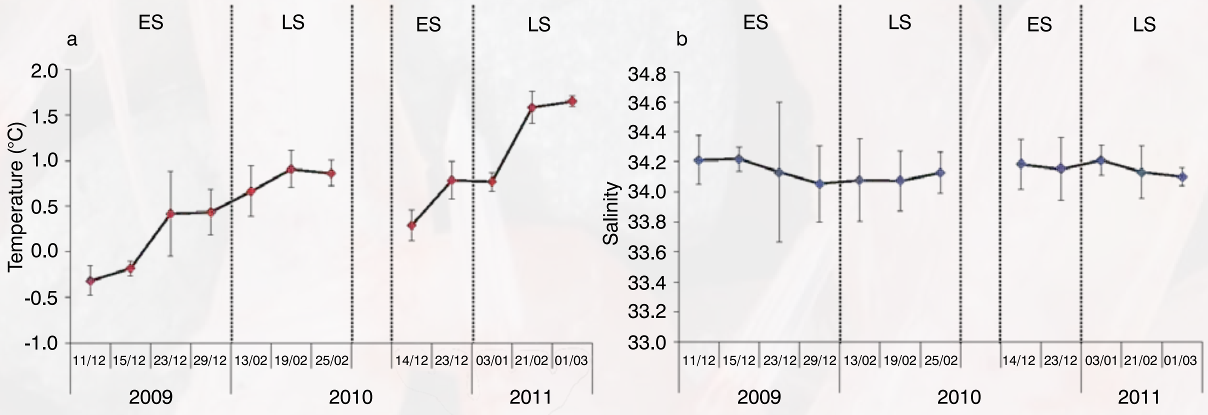

Figure 1. Temporal variation of water temperature (a) and salinity (b) in Admiralty Bay during 2009/2010 and 2010/2011 surveys. ES – Early Summer, LS – Late Summer.

showing that Chl *a* concentrations were rather associated to chromophytes. The variations of the Chl *c*/Chl *a* ratio seems to be influenced by temperature (r = 0.60; $p < 0.001$) and SiO_4 concentrations (r = -0.48; $p = 0.03$) and suggests variations of the relative importance of diatoms in the communities. The Chl *b*/Chl *a* ratio was negatively correlated to water temperature (r = -0.76; $p < 0.001$), suggesting a relative preference of chlorophytes for colder waters.

In the summer 2010/2011 Chl *b* concentrations were low and significant values were not observed in 60% of the samples (Figure 2b). Chl *b* content was found in significant quantities in the last ES sampling (Chl *b*/Chl *a* = 0.06), and at the LS start (Chl *b*/Chl *a* = 0.05) (Figure 2b). The Chl *b*/Chl *a* ratio was clearly lower in 2010/2011 than in 2009/2010 (Figure 2a; Table 1). The Chl *c*/Chl *a* ratio was at similar levels compared to 2009/2010. It was positively correlated with integrated Chl *a* concentrations (r = 0.57; $p = 0.001$) showing the importance of Chromophytes in chlorophyll biomass. Furthermore, water temperature (r = 0.60; $p < 0.001$) and SiO_4 concentrations (r = -0.48; $p = 0.004$) also seemed to influence the variation of this group during this summer.

Discussion

Temporal variation of thermohaline structure was similar to those reported in previous studies (Lipski, 1987; Lange et al., 2007). However, water temperatures recorded on December 2009 were lower to those usually reported, probably due to the influence of El Niño (National Oceanic and Atmospheric Administration - NOAA, EUA). In general, Chl *a* concentrations were low (1- 2 µg L-1) in spite of high dissolved inorganic nutrients concentrations (Cascaes et al., 2012), as commonly reported in Admiralty Bay (Lipski, 1987; Lange et al., 2007; Kopczynska, 2008). One of the explanations for this paradox is usually associated to the low micronutrient iron availability, which is considered as one of the main factors limiting phytoplankton growth under high macronutrients conditions (Martin et al., 1991; Rose et al., 2009). Studies developed on Antarctic phytoplankton cultures showed that both iron addition and increase in water temperature, lead to rise of chlorophyll biomass (Rose et al., 2009). In this way, the increase of biomass in LS was mainly conditioned by rise of temperature, which melts the ice providing the micronutrient iron to phytoplankton growth (Martin et al., 1991). So, the low water temperatures observed during the 2009/2010 atypical summer (three times lower than summer 2010/2011) could explain the Chl *a* concentrations six times lower than during the 2010/2011 summer.

The relatively high Chl *b*/Chl *a* ratio (0.10 ±0.03) in 2009/2010 survey pointed out a higher contribution of Chl *b*-containing classes of the green algal lineage associated to low water temperatures (0.40 ± 0.48 ºC). At much higher water temperatures (1.10 ± 0.56 ºC), as observed during 2010/2011 summer, the pigment ratios were lower (<0.05). These results were coherent with those of Hashihama et al. (2008), showing higher densities of chlorophytes associated to colder waters in the Antarctic marginal ice zone along

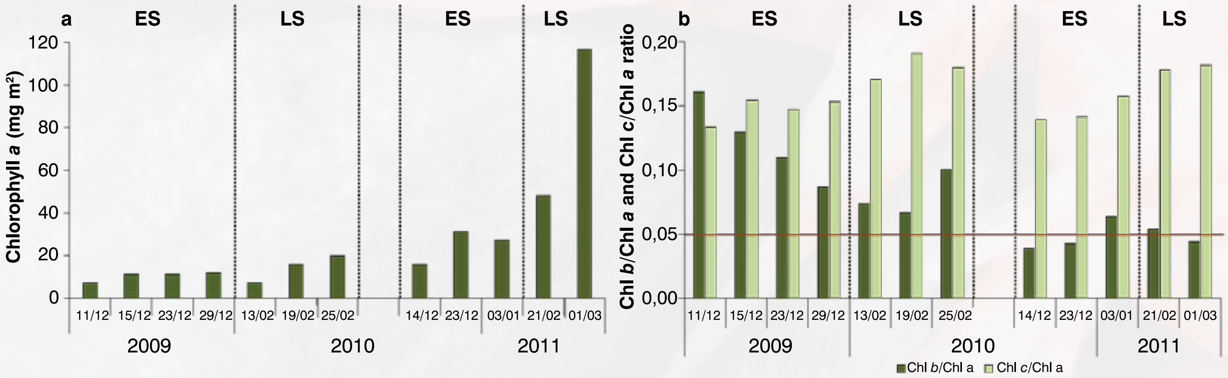

Figure 2. Temporal variation of Chlorophyll pigments: a) Chl *a* concentration, b) Chl *b*/ Chl *a* and Chl *c*/Chl *a* ratio in Admiralty Bay during 2009/2010 and 2010/2011 surveys. ES – Early Summer, LS – Late Summer. Red line represents thresholds for significant values of Chl *b* and Chl *c*.

the 140° E meridian. Relatively high Chl *c*/Chl *a* during 2009/2010 survey could be related to elevated densities of cryptophytes single-celled flagellated algae lower than 10 µm (Moline et al., 2004), identified at the same period in an adjacent region through HPLC analysis (Mendes et al., 2013). In this sense, the dominance of phytoplankton < 10 µm, which represented ~80% of biomass in this survey, had already been highlighted by Tenório et al. (2011). Besides cryptophytes, other chromophytes, like microplanktonic dinoflagellates and diatoms present in Admiralty Bay (Tenenbaum et al., 2011), also contributed to the increase of Chl *c*/Chl *a* ratios during 2009/2010 summer.

Conclusion

Temporal variation in water temperature, chlorophyll biomass and accessory chlorophyll/chlorophyll *a* ratios show important interannual variations. The increase in water temperature during the austral summer lead to enhance of chlorophyll biomass and to variation of accessory pigments to Chl *a* ratios, showing significant changes in the phytoplankton community structure. Nevertheless, similar Chl *c*/Chl *a* ratio between summers and higher Chl *b*/ Chl *a* in 2009/2010 possibly suggests: 1) higher contribution of other taxonomic groups on chlorophyll biomass (*e.g.* cyanobacteria) instead of chlorophytes in the second survey 2) reduction in Chl *c* per cell during 2010/2011 summer due to variations in the composition of chromophytes or/and light availability.

Pigment concentrations studies in the Admiralty Bay proved to be a good tool for monitoring the effects of global changes both on phytoplankton biomass and on the relative proportions of chlorophyll *b* (chlorophytes) and chlorophyll *c* (chromophytes) containing eukaryotes.

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