SIZE-FRACTION, TROPHIC CATEGORIES AND MORPHOTYPES STRUCTURE OF PLANKTON SMALLER THAN 20 μm DURING THE AUSTRAL SUMMER (ADMIRALTY BAY, KING GEORGE ISLAND, WAP)

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https://doi.org/10.4322/apa.2016.016

Abstract: *The density and distribution of plankton community smaller than 20 μm in Admiralty Bay (King George Island, Antarctica) were studied at three sampling sites during the austral summer of 2013/2014 (two surveys at the beginning – early summer, two at the end – late summer). The aim was to identify the environmental factors that influence their variability. Salinity (34.2 ± 0.1) and temperature (0.47 ± 0.24°C) showed little variation in late summer, but in general, the concentration of dissolved nutrients increased towards this period. Organisms smaller than 10μm showed the higher contribution (74%) for Chlorophyll a concentration. Picoplankton (<2 μm), dominated by basically heterotrophs (98.5%), had density of 3.9 ± 1.8 x10^8 cell L^{-1}. The fraction between 2 and 20 μm, dominated by autotrophs (60%), presented densities up to 3.6 ± 1.2 x10^6 cell L^{-1}. This community was dominated by cocci and spherical morphotypes. Our results suggest that: (i) cell density increase along the study period followed nutrient and organic matter inputs; (ii) lower densities relative to 2009-2011 summers were related to lower temperatures and melting rates, besides predation forces, demonstrating the complex spatial-temporal relationships that take place between plankton community and environmental parameters at Admiralty Bay coastal zone.*

Keywords: Epifluorescence microscopy, Plankton density, West Antarctic Peninsula.

Introduction

The Southern Ocean usually have "High Nutrient, Low Chlorophyll" (HNLC) regime and is classified as a "hypoproductive" ecosystem. Pico- and nanoplankton dominate the community in these waters (Ducklow *et al.,* 2011). Microorganisms smaller than 20 μm are very important in the oceans. While autotrophs are responsible for up to 83% of the total primary production (PP), heterotrophs play an essential role in the microbial loop, with equal or superior rates than the local PP rate (Ducklow *et al.,* 2011). Furthermore, according to Montes-Hugo *et al.,* (2009; and references therein), a study started in the 1970s shows that environmental conditions imposed to the Antarctic Ocean in recent decades have induced variations in planktonic composition, such as the replacement of microplankton by organisms smaller than 20μm.

As Admiralty Bay (King George Island, Antarctica) has been designated an Antarctic Specially Managed Area (ASMA), actions to protect this region should be implemented. Shallow coastal water monitoring (<30 m) was implemented in 2002 under the Brazilian Antarctic Program (PROANTAR) with the purpose to study the long-term effects of natural and anthropogenic impacts on the marine ecosystem. In 2009, the analysis of density and trophic structure of the smallest fractions of the

plankton community (<20µm) was included in the program (Tenenbaum *et al.,* 2011), while the study of picoplankton and nanoplankton biomass was established since the summer of 2010/2011 (Vanzan *et al.,* 2015) in order to evaluate the changes in the planktonic community size-structure. The objective of the present study is to identify the size-fraction, trophic categories and morphotypes structure of plankton smaller than 20 µm and environmental factors that influence their variability during the Austral summer of 2013/2014 in Admiralty Bay.

Materials and Methods

Study Area: Admiralty Bay (AB) is located off the West Antarctic Peninsula - WAP (62°03'–12'S, 58°18'–38'W) and covers an area of 122 km^2. As a typical polar region, it is characterized by wide seasonal oscillations in plankton abundance, driven by environmental abiotic factors, biological interactions and human impacts (Nedzarek and Rakusa-Suszczewski, 2004).

Sampling and Analysis: Four surveys were conducted at AB in the shallow coastal zone (<30 m) during December 2013 (early summer: ES1 and ES2) and during February 2014 (late summer: LS1 and LS2). Samples were collected in Niskin bottles (5 L) at three depths (0 m, 15 m, and 30 m), at three sampling sites: the Comandante Ferraz Brazilian Station (EACF), Machu Picchu (MP), and Arctowski (AR). The Admiralty Bay location and the position of the three sampling stations are the same described in Vanzan *et al.,* (2015). Water Temperature and Salinity were measured *in situ* using a mini-CTD Valeport®. Dissolved inorganic nutrients (nitrate, nitrate, phosphate and silicate) were analyzed by the LabQOM-IOUSP. Processes used to determinate total and fractionated (<2 µm, 2-10 µm, >10 µm, and <20µm) Chlorophyll *a* (Chl*a*) concentration are described in Tenório *et al.,* (2011).

Plankton: Size-fraction structure, trophic categories and morphotypes: Aliquots of 5 mL for picoplankton and 30 mL for nanoplankton were fixed (glutaraldehyde 2% f.c.), stained with DAPI (0.01 µgL^{-1} f.c.), filtered through 0.22 µm and 1.0 µm polycarbonate black membrane filters (Poretics®), respectively, mounted on slides, and stored at -20°C. Analyses were performed via epifluorescence microscopy (1000x Olympus BX51®), by counting random fields and expressed by cell.L^{-1}. Heterotrophs were calculated based on the count of total density (UV filter combination λ= 420-460nm, via DAPI) minus the autotrophs (blue filter combination λ= 475nm, via autofluorescence). Organisms were classified according to (i) trophic category and size: auto and heterotrophic picoplankton (APICO and HPICO, 0.2-2µm), auto and heterotrophic nanoplankton (ANAN and HNAN; sizes >2-10 µm and >10-20 µm); (ii) morphotype: cocci, rods or curved (picoplankton); sphere, conic sphere, ellipsoid or cylinder (nanoplankton).

Data treatment: Temporal variations were presented by using integrated values in the water column (whole sample), while spatial variability was shown throughout depth (mean values of all sampling sites and surveys). Morphotype contributions were shown by mean values of all surveys.

Results

Values of salinity and temperature were not registered in December (ES). In February (LS), salinity exhibited a spatial-temporally homogeneous distribution (34.2 ± 0.1). The temperature increased from LS1 (0.26 ± 0.1°C) to LS2 (0.69 ± 0.1°C), but did not exhibit variability between depths and sampling sites. Total Chlorophyll *a* concentration varied between 0.39 and 2.33 µg L^{-1}, and Chl*a* <20µm varied between 0.33 and 1.74 µg L^{-1}. On average, Chl*a* was dominated by 2-10µm fraction (45%), followed by < 2µm (29%) and > 10 µm (26%). Differences between concentrations of total Chl*a* and <20µm fraction at ES2 were influenced by a gradient among sampling sites and variances throughout the water column (Figure 1a). Except for phosphate at ES1 (1.41 ± 0.31 µM), dissolved inorganic nutrients exhibited an increase from ES to LS (Figure 1b).

Cellular densities varied between trophic levels, surveys and depth. Minimal differences were observed between sampling sites and, therefore,mean spatial values were presented. APICO's densities varied from 2.2 to 12.4 x 10^6 cell L^{-1} and HPICO's varied from 1.9 to 11 x 10^8 cell L^{-1} (Figure 2a,b). Higher densities were observed at LS2 (Figure 2b) and these usually decreased from the surface (maximum 11.1 x 10^8 cell L^{-1}) to the bottom depth (Figure 2c). Picoplankton was dominated by cocci forms (83%) and by heterotrophs (98.5%) (Figure 2d). Nanoplankton densities presented an increasing trend from ES to LS (Figure 2a,b). ANAN's densities varied between 0.7 and 4.9 x 10^6 cell L^{-1} and HNAN's, from 0.2 to

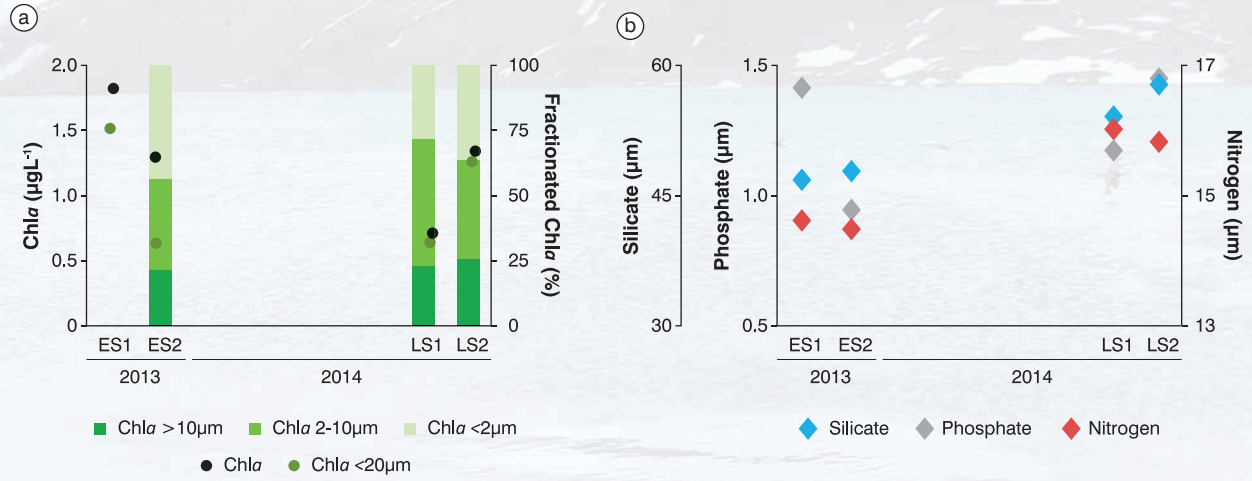

Figure 1. Temporal and spatial variations of: **(a)** Chlorophyll *a* (µgL^{-1}) in the whole sample (black dots) and in the < 20 µm fractions (green dots), and relative contribution (%) of different size classes (bars) to total Chlorophyll; **(b)** Silicate, Phosphate and Nitrogen (Nitrate + Nitrite) (µM) in Admiralty Bay in Early and Late Summer 2013/2014 (mean values of each survey).

2.9 x 10^6 cell L^{-1}, and were dominated by spherical (44%) and ellipsoid (37%) forms, 2-10µm size cells (93%) and autotrophs (60%) (Figure 2d). No spatial differences were observed between sampling sites, but vertical stratification in cell density of this size-fraction was indicated by a decrease of autotrophs with depth (Figure 2c).

Discussion

HPICO's (10^8 cell L^{-1}) and APICO's (10^6 cell L^{-1}) densities were 10-fold lower than those recorded in the same region in the summers of 2009/2010 and 2010/2011 (~10^9 cell L^{-1} and 10^7 cell L^{-1}, respectively), but were similar to those measured in AB and WAP during the 1990s (see Table 3 in Vanzan *et al.,* 2015). Nevertheless, as suggested in these previous studies, the predominance of heterotrophic and cocci forms in this size fraction could be related to lack of nutrient limitation, because this has the lowest surface/volume ratio (Vanzan *et al.,* 2015). The decrease that took place with depth could be related to the positive correlation between HPICO, Chl*a* and PP (White *et al.*, 1991).

The predominance of spherical forms (of the 2-10µm size-fraction) and autotrophs, as well as the densities (~10^6 cell L^{-1}) registered overall for the nanoplankton, are in line with the results obtained in previous studies in AB and other Antarctic regions (Vanzan *et al.*, 2015). Leakey *et al.,* (1996) showed that 3 to 12% of the picoplankton biomass are daily grazed by these spherical and small HNAN. The morphotype and size-fraction structure of the nanoplankton is an important aspect to be considered in order to evaluate the carbon biomass and the planktonic community structure, especially in studies addressing trophic dynamics, and studies modeling and monitoring ecosystems (Hillebrand *et al.,* 1999) such as the AB coastal zone.

The lower densities observed during the summer of 2013/2014 compared to 2009/2010 and 2010/2011 can be explained by the anomalous low water temperature observed in the late summer (<1.13°C), which could have affected plankton grow rates (Vanzan *et al.,* 2015; Kejna *et al.,* 2013). These low late summer temperature could also lead to lower ice melting rates, affecting the entire planktonic community by decreasing the input of nutrients and epontic algae inputs (Vanzan *et al.,* 2015; Kejna *et al.,* 2013; Montes-Hugo *et al.,* 2009).

On the other hand, throughout the summer of 2013/2014, the observed increase in autotrophs could be a result of the rising nutrient concentrations from ES towards LS. This could promote high PP when absorbed by organisms (Leakey *et al.,* 1996). The similar pattern observed for the total and <20 µm fraction of Chl*a* suggests that plankton smaller than 20µm (especially <2-10 µm) are the main contributors to the phytoplanktonic biomass in this region (Vanzan *et al.,* 2015; Barrera-Alba *et al.,* 2012).

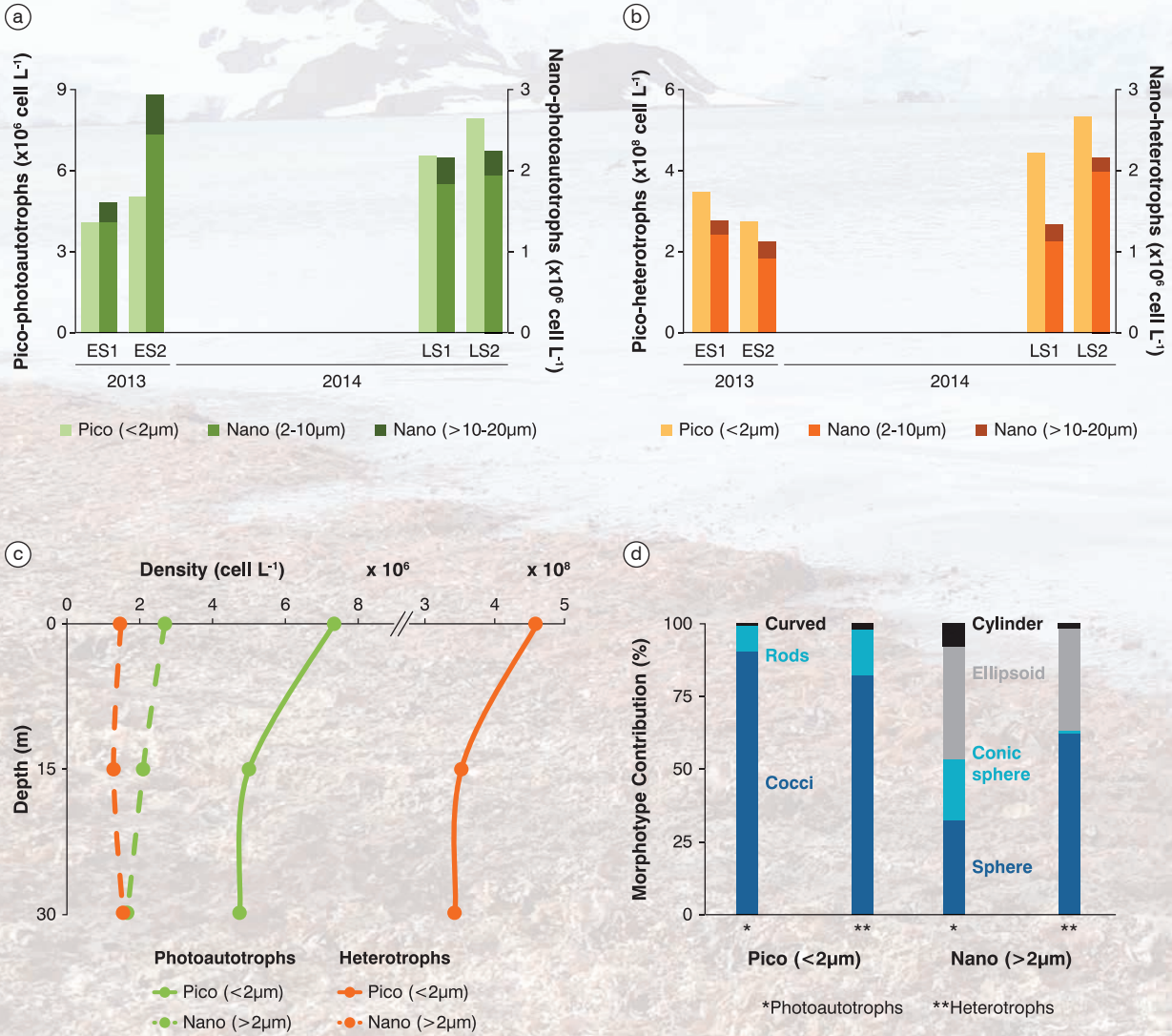

Figure 2. Temporal and spatial variations of plankton size fraction (cell L⁻¹) in Admiralty Bay (Early –EL and Late Summer –LS of 2013/2014): **(a)** autotrophs and **(b)** heterotrophs - mean values of each survey, (**c**) auto- and heterotrophs, pico- and nanoplankton, throughout depth (mean values of all sampling sites and surveys), **(d)** morphotype contribution (%) (mean values of all surveys).

Furthermore, the increase in heterotrophs density throughout the summer may have been a response to the growth in APICO because this: (i) generates high amounts of dissolved and particulate organic matter that can be rapidly absorbed by HPICO; (ii) can serve as prey for HNAN (Vanzan *et al.*, 2015; Leakey *et al.*, 1996).

Conclusion

Compared to previous years (2009-2011), the lower pico and nanoplankton abundance observed in Admiralty Bay in the summer of 2013/2014 can be related to lower temperatures, low melting rates and low nutrient input, as well as to predation.

The increase in the density of plankton smaller than 20 µm from the early to the late summer of 2013/2014, as well as the dominance of cocci and sphere forms, could be explained by the accumulation of nutrients and dissolved and particulate organic matter in the water column.

Knowledge on morphotypes and size-structure contributions to the total plankton biomass is still fragmenred in the Southern Ocean literature. Therefore,

more studies are necessary to understand the impacts on biochemical processes, carbon fluxes throughout the water column, and the relation between each of the different size-fraction categories.

Acknowledgements

This work is part of the National Institute of Science and Technology Antarctic Environmental Research (INCT-APA) that receives scientific and financial support from the National Council for Research and Development (CNPq process: n° 574018/2008-5) and from the Carlos Chagas Research Support Foundation of the State of Rio de Janeiro (FAPERJ n° E-16/170.023/2008). The authors also acknowledge the support of the Brazilian Ministry of Science, Technology and Innovation (MCTI), Ministry of Environment (MMA), Inter-Ministry Commission for Sea Resources (CIRM) and Marine Oceanographic Institute of São Paulo University (LabQOM-IOUSP).

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