The planktonic ciliate community and its relationship with the environmental conditions and water quality in two bays of the Beagle Channel, Argentina

M.S. BARRÍA DE CAO^{1,2}, M.C. LÓPEZ ABBATE¹, R.E. PETTIGROSSO² AND M.S. HOFFMEYER^{1,3} ¹IADO (CONICET - UNS) Florida 4750, B8000FWB-Bahía Blanca, Argentina, ²DBByF - UNS, San Juan 670-Bahía Blanca, Argentina, ³Universidad Tecnológica Nacional, Facultad Regional Bahía Blanca, 11 de abril 461, B8001LMI-Bahía Blanca, Argentina

The relationship between the ciliate community and the environmental variables in Ushuaia and Golondrina bays (54°79'S 68°22′W and 54°85′S 68°36′W, respectively) in the Beagle Channel, Argentina was investigated. The study was performed in three zones within the bays, previously delimited on the basis of their water quality. The most perturbed sites were located inshore. In order to analyse the contribution of each species to the similarity or dissimilarity between zones, similarity percentages analysis was undertaken using the Bray - Curtis similarity index. The variations in species composition and dominance in the selected zones were examined by the abundance-biomass comparison plot. We also studied the relationship between environmental and ciliates variability. The ciliate community comprised a total of 43 species belonging to 15 genera. Ciliate abundance and biomass varied temporally and spatially. A more diverse community dominated by small and opportunistic species tolerant to environmental changes was found in the most perturbed zone, while in the less stressed zone the community comprised bigger species, probably adapted to more stable environmental conditions. A community comprising species from both zones was found in a transitional area. We conclude that the structure of the community varied closely with environmental conditions.

Keywords: planktonic ciliates, biomass, diversity, water quality, Beagle Channel, Argentina

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INTRODUCTION

Ciliates are among the most important marine micro-organisms, as they play a major role in the microbial food web (Azam et al., 1983). Furthermore, ciliates exhibit a variety of nutritional habits and metabolic rates which significantly contribute to nutrient cycling (Fenchel, 1980; Pierce & Turner, 1992). They have been reported to be ubiquitous and abundant in coastal as well as oceanic environments (Garrison et al., 2000), and show widespread distribution patterns (Agatha, 2011).

biomass in high latitudes (e.g. Garrison, 1991; Froneman & Perissinotto, 1996; Nielsen & Andersen, 2002) and are thought to be key remineralizers of primary production (Burkill et al., 1995; Archer et al., 2000; Putland, 2000; Gaul & Antia, 2001). It is accepted that copepods dominate the mesozooplankton community in the southern Patagonian shelf (Sabatini et al., 2001, 2004) and in the Beagle Channel (Biancalana et al., 2007). Studies on ciliate diversity and temporal dynamics in the southern Atlantic Ocean are relatively scarce (Agatha, 2011). Moreover, the only reference on ciliates

Ciliates represent a substantial fraction of zooplankton

in the Beagle Channel is about the species composition during wintertime (Biancalana et al., 2007), but there are no available data either on their biomass or about their relationship with environmental variables.

On the other hand, it was pointed out that the growing population (~ 57000 inhabitants in 2010) of Ushuaia city, located on the north-western shore of Ushuaia Bay and the associated industrial and port activities have direct effects in the adjacent coastal area. A lower seawater quality resulting from high nutrient and organic loading was found near the urban and industrial centre (Amín et al., 1996; Commendatore & Esteves, 2001; Esteves et al., 2003). Also, a significantly higher heavy metal concentration was detected in this area (Amín & Comoglio, 2007). Gil et al. (2010), performed a detailed analysis of water and sediment quality in Ushuaia and Golondrina Bays, and concluded that the northwestern area of Ushuaia Bay has hypertrophic conditions, in contrast with the oligotrophic nature of the Beagle Channel. The main sources of these pollutants were sewage drainage and industrial effluent. A study on the structure and spatial and temporal dynamics of the mesozooplankton corroborated the occurrence of eutrophic conditions in the inner Ushuaia Bay (Biancalana, 2008). Consistent differences in mesozooplankton assemblages were found, probably related to poor water quality (Biancalana et al., 2007). Studies relative to phytoplankton biomass and composition during an annual cycle in relation to environmental variables, demonstrated low

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Corresponding author: M.S. Barría de Cao Email: sbarria@criba.edu.ar density and biomass during autumn-winter and a significant increase in spring-summer (Almandoz *et al.*, 2011). The mesozooplankton in the Beagle Channel exhibits a temporal pattern that is more dependent on primary production than on physical factors (Aguirre *et al.*, 2012).

The studies of Biancalana *et al.* (2012) showed that the enrichment of the water by an increase of nutrients indirectly contributed to modulate the spatial-seasonal distribution patterns of mesozooplankton.

The aim of this work is to report, for the first time, on the species composition, diversity and abundance of aloricate ciliates and tintinnids in the Beagle Channel in all the seasons, in relation to environmental variability and water quality.

MATERIALS AND METHODS

Study area

Ushuaia Bay (UB) and Golondrina Bay (GB) are located on the northern side of the Beagle Channel, Argentina (54°79'S 68°22′W and 54°85′S 68°36′W, respectively; Figure 1). These bays display different physical and hydrological features. Both their extent and bathymetry are markedly different. UB is 9 km long while GB is 2.2 km long. UB is deeper eastwards and towards the Beagle Channel, reaching 130 m depth, whereas GB is shallower (20 m approximately). The bays are also different in the composition of the bottom. GB displays a soft bottom surface, whereas UB presents a more consolidated soft bottom with stones and shells (Fernández Severini & Hoffmeyer, 2005, and references therein). Balestrini et al. (1998) reported for GB and Bridges Island areas, permanent currents flowing from the south-west with maximal velocities of 2.6 and 15.6 cm s⁻¹, respectively. In UB a permanent strong current moves west along the northern coast of the bay at 2 cm s⁻¹, and then progresses to the south-east along the southern coast at 16.3 cm s⁻¹. The strong wind gusts coming mostly from the south-east can drive water mass transport in shallow areas and destabilize the structure of the water column (Isla et al., 1999). Both

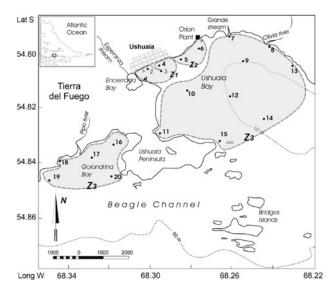


Fig. 1. Location of sampling stations in Ushuaia and Golondrina Bays in the Beagle Channel.

bays receive effluent from Ushuaia city and the industries situated in the surroundings, mainly in the north-north-west area of UB. As a result, coastal waters are slightly polluted (Amín *et al.*, 1996; Commendatore & Esteves, 2001).

Sampling strategy and laboratory procedures

The sampling area comprised 15 stations in UB and five stations in GB (Figure 1). The environmental analysis made by Gil et al. (2010) yielded the delimitation of three zones within these two bays: zone 1 (Z1), 2 (Z2) and 3 (Z3) which were taken into account for analysis in the present study. Z1 (NW area of UB) was the most perturbed by organic matter and nutrient enrichment due to the urban pressure and industrial effluents. Z2 comprised the sites on the northern coast of UB and Z₃, and included those stations less influenced by anthropogenic activities. Z₃ was formed by three sub-zones, one including the rest of stations located in UB, the second was located in GB, and a third sub-zone comprised the stations located in front of Olivia and Pipo rivers, within UB and GB, respectively. In accordance with the latter criterion, sampling Stations 1-4 belonged to Z1, Stations 5 and 6 to Z2 and Stations 7-20 to Z3, being Stations 8, 17 and 18 in a sub zone with freshwater influence (Figure 1).

Sampling was carried out in the four seasons from 2004 to 2005. Microplankton including ciliates was collected from surface water with a Van Dorn bottle and preserved in Lugol's solution.

Temperature, salinity, pH, dissolved oxygen and turbidity were measured by means of a multi-parametric sensor Horiba U-10. Chlorophyll-*a* and phaeopigments were estimated following Strickland & Parsons (1968), whereas nutrient concentration was analysed following the methods described by Grasshoff *et al.* (1983), using a Skalar auto-analyser.

Strombidid ciliates were identified according to Maeda & Carey (1985) and tintinnids according to Kofoid & Campbell (1929). For enumeration, a 50 ml subsample was concentrated after settling in a combined plate chamber; the entire bottom chamber was scanned for each subsample using a Wild M40 inverted microscope following the Utermöhl method after Hasle (1978). Biomass in terms of carbon was calculated using a factor of 0.19 pg μ m⁻³ for the naked ciliates following Putt & Stoecker (1989), and a linear regression equation: C (pg) = 444.5 + 0.053 lorica volume (μ m³), for tintinnids (Verity & Langdon, 1984).

Data analysis

The Shannon diversity index (H', ln based) and total biomass of ciliates (μg C l⁻¹) were calculated for each sample. For statistical analysis, the data set were transformed using the \log_{10} (x + 1) (Clarke & Warwick, 1994). The ANOVA and multiple comparisons LSD test were used to detect differences in chlorophyll-a and ciliate abundance between sampling dates and zones (Sokal & Rohlf, 1981). In order to analyse the contribution of each ciliate taxa to the similarity within zones and to the dissimilarity between zones delimited by Gil *et al.* (2010), similarity percentage analysis (SIMPER) was carried out using the Bray–Curtis similarity index. The ANOSIM was later applied with the purpose of detecting significant differences in ciliate composition along the previously selected zones.

The variation in species composition and dominance in the three zones was examined through the abundance-biomass

comparison (ABC) plot (Warwick, 1986). For the ABC curves, a total of 43 species of ciliates were considered. This representation has the advantage of easily comparing the distribution of species abundances among individuals and the distribution of species biomasses among individuals on the same terms, taking into consideration the species size. Species are ranked in order of importance of numerical abundance and biomass on the x-axis, and the percentage of dominance on the y-axis. The W statistic proposed by Warwick (1986), allows testing for the statistical significance in different ABC plots, and ranges between -1 and 1. When the curve for biomass lies above the curve for abundance (W > 1), the biomass is dominated by one or few large species and numerical dominants are usually small opportunistic species which do not represent a large proportion of total biomass.

Conversely, when the curve for abundance lies above the curve for biomass, large competitive dominants are gradually eliminated and the community biomass and numerical abundance become dominated by one or few very small species (W < 1).

The general trend of ciliate abundance, biomass, species richness and chlorophyll-concentration, as a proxy of phytoplankton, and the environmental variability (temperature, salinity and nutrients content) was studied using principal component analysis (PCA). The strength of the link between ciliates and environmental variability was investigated in the selected zones by comparing the first PC of both data sets through sliding correlation using bootstrap which involves random pairwise sampling with replacement. Each data set was resampled 1000 times. The probability density

Table 1. Mean species seasonal abundance (No. ind. l^{-1}) for each zone in Ushuaia and Golondrina Bays.

Species	Zı		Z 2		Z ₃	
	Mean	± SE	Mean	± SE	Mean	± SE
Strombidium conicum (Lohman, 1908) Wulff, 1919	106.25	49.991	27.50	9.210	42.14	16.009
S. pulchrum (Leegaard, 1915) Kahl, 1932	0.00	0.000	0.00	0.000	2.86	1.480
S. typicum (Lankester, 1874) Bütschli, 1889	0.00	0.000	0.00	0.000	0.71	0.714
S. sp. aff. sulcatum Claparède & Lachmann, 1859	3.75	2.720	15.00	8.238	4.29	1.668
S. sp. aff. acutum Leegaard, 1915	1.25	1.250	0.00	0.000	0.71	0.714
S. sp. aff. crassulum (Leegaard, 1915) Kahl, 1932	1.25	1.250	2.50	2.500	0.36	0.357
S. sp. aff. cornucopiae (Wailes, 1929) Kahl, 1932	17.50	12.633	5.00	5.000	0.71	0.714
S. sp. aff. minor (Kahl, 1935) Maeda & Carey, 1985	0.00	0.000	0.00	0.000	1.07	1.071
S. sp. aff. capitatum (Leegaard, 1915) Kahl, 1932	1.25	1.250	0.00	0.000	0.00	0.000
S. sp. aff. reticulatum (Leegaard, 1915) Busch, 1921	12.50	4.787	152.50	141.16	25.36	8.879
S. sp. aff. vestitum (Leegaard, 1915) Kahl, 1932	0.00	0.000	0.00	0.000	0.36	0.357
Strombidium sp. 1	257.50	234.34	7.50	3.660	3.93	1.071
Strombidium sp. 2	98.75	79.947	7.50	7.500	1.07	1.071
Strombidium sp. 3	0.00	0.000	0.00	0.000	0.36	0.357
Strombidium sp. 4	1.25	1.250	0.00	0.000	1.79	0.923
Strombidinopsis sp. 5	3.75	2.720	0.00	0.000	0.36	0.357
Strombidinopsis sp. 2	3.75	2.016	0.00	0.000	0.36	0.357
Strobilidium sp.	6.25	5.072	2.50	2.500	0.00	0.000
Laboea strobila Lohman, 1908	8.75	4.460	20.00	20.00	6.79	4.679
Leegardiella sp.	0.00	0.000	0.00	0.000	0.71	0.500
Cyrtostrombidium sp.	1.25	1.250	0.00	0.000	0.00	0.000
Lohmaniella sp.	0.00	0.000	2.50	2.500	0.00	0.000
Holophrya sp.	0.00	0.000	0.00	0.000	0.36	0.357
Tintinnopsis baltica Brandt, 1896	0.00	0.000	0.00	0.000	1.96	0.861
Tintinnopsis beroidea Stein, 1867	1.25	1.250	0.00	0.000	2.14	1.501
Tintinnopsis brasiliensis Kofoid & Campbell, 1929	6.25	6.250	0.00	0.000	0.00	0.000
Tintinnopsis glans Meunier, 1919	2.50	2.500	0.00	0.000	0.54	0.396
Tintinnopsis gracilis Kofoid & Campbell, 1929	6.25	3.521	47.50	28.769	7.68	2.523
Tintinnopsis levigata Kofoid & Campbell, 1929	21.25	13.598	2.50	2.500	2.32	0.987
Tintinnopsis parvula Jörgensen, 1912	1.25	1.250	0.00	0.000	0.00	0.000
Tintinnopsis sp. 1	3.75	2.720	0.00	0.000	2.14	1.214
Tintinnopsis sp. 2	4.38	2.410	0.00	0.000	0.00	0.000
Codonellopsis lusitanica Jörgensen, 1924	1.25	1.250	2.50	2.500	0.09	0.089
C. morchella forma schabi Brandt, 1906 (Cleve) Jörgensen, 1924	4.38	3.287	2.50	2.500	0.36	0.357
Codonellopsis pusilla (Cleve, 1900) Jörgensen, 1924	19.38	7.330	45.00	30.414	4.64	2.883
Amphorides aff. amphora (Claparède & Lachmann, 1858–1859) Strand	0.00	0.000	0.00	0.000	0.18	0.179
Favella taraikaensis Hada, 1932	0.00	0.000	2.50	2.500	1.07	1.071
Helicostomella subulata (Ehrenberg, 1833) Jörgensen 1924	1.25	1.250	0.00	0.000	3.57	2.932
Eutintinnus rugosus Kofoid & Campbell, 1939	13.75	7.238	2.50	2.500	19.46	19.104
Eutintinnus sp.	10.00	0.000	0.00	0.000	0.00	0.000
Undella sp. aff. Claparedei (Entz Sr., 1885)	2.50	1.708	0.00	0.000	0.00	0.000
Aloricate cil. aff. Strombidium (20 µm)	2.50	1.708	0.00	0.000	2.68	1.539
Aloricate cil. aff. Strombidium (40 µm)	0.00	0.000	0.00	0.000	0.36	0.357

distribution of the corresponding correlation coefficients was then computed using non-parametric Kernel smoothing (Casini *et al.*, 2009). PRIMER 5 and Statistica 7 (Statsoft, Tulsa, OK, USA) packages were used to carry out the above mentioned analysis.

RESULTS

Species composition and diversity

The ciliate community in UB and GB comprised a total of 43 species of which 25 were aloricate and 18 tintinnids belonging to eight and seven genera, respectively (Table 1). The mean diversity value for all the seasons for Z1 was 0.997, 1.065 for Z2 and 0.641 for Z3. Z1 and Z3 had the highest species richness but diversity values were significantly different (P = 0.05). Z2 presented a diversity value similar to Z1 and shared with the other two zones 11 of the 15 total species. In terms of seasonal mean diversity, the highest value was observed in Z1 in March, while in Z3, the highest average value of diversity was observed in August and the lowest in December and March. Strombidium and Tintinnopsis were the best represented genera of aloricate and tintinnid ciliates, respectively.

Seasonal variation and spatial distribution of ciliates, chlorophyll-a and environmental variables

The highest mean value of the numerical abundance of ciliates for the three zones was registered during summer, following the chlorophyll-a trend (Figures 2 & 3), although the maximum value for the abundance of a species, *Strombidium* sp. 1 was 3760 ind. l^{-1} in winter in Z1. The minimum values were registered in autumn. The highest seasonal mean value of the numerical abundance of ciliates and chlorophyll-a were registered in Z1 (Figures 2 & 3).

Spatial patterns of total ciliates biomass are summarized in Figure 4. The highest mean annual value was found in Station 7 (Z₃). The maximum value of biomass registered was 41.2 μ gC. l^{-1} in spring in Z₃ and was due to tintinnids which possessed big loricae such as *Eutintinnus rugosus*.

Regarding to species contribution to the average similarity within zones (SIMPER analysis), the main contributing species to the similarity within each zone were *Codonellopsis pusilla*, *Strombidium conicum* and *Srombidium* sp. *aff. reticulatum* in Z1; *Strombidium conicum*, *Srombidium* sp. *aff. reticulatum* and *Strombidium sulcatum* in Z2; and *Strombidium conicum*, *Srombidium* sp. *aff. reticulatum* and *Strombidium* sp. 1 in Z3. The average similarity within each zone was 14.04, 27.57 and 16.64% for Z1, Z2 and Z3, respectively. The average dissimilarity among zones was mostly given by *Strombidium conicum*. The dissimilarity found between Z1 and Z3 was the highest (86.24%). However, the contrast was not statistically significant.

The ABC plot curves for the ciliates abundance–biomass comparison and W values are shown in Figure 5. The abundance curve lies above the biomass curve in the polluted area (Z_1), while the opposite trend occurred in the unpolluted area (Z_3). The curves were closely coincident in Z_2 which showed a moderate pollution.

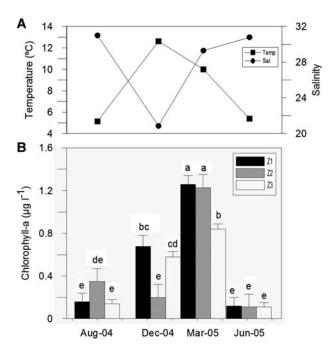


Fig. 2. Hydrological and chlorophyll-a dynamics in Ushuaia and Golongrina Bays during the four surveys: (A) variation of temperature and salinity; (B) variation of chlorophyll-a. Bars show mean + SE. Mean values are presented alphabetically in decreasing order, those that share a letter do not differ at P=0.05.

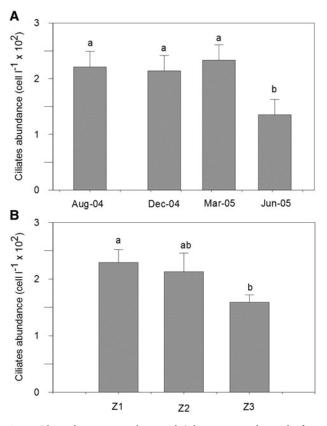


Fig. 3. Ciliates dynamics in Ushuaia and Golongrina Bays during the four surveys: (A) mean ciliates abundance in the different seasons; (B) mean ciliates abundance in the different zones. Bars show mean + SE. Mean values are presented alphabetically in decreasing order, those that share a letter do not differ at P=0.05.

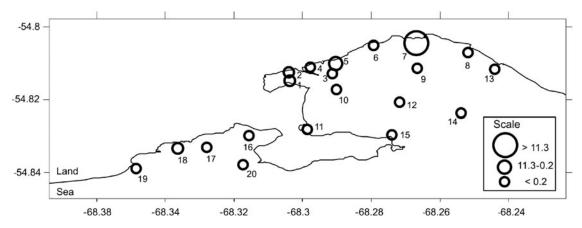


Fig. 4. Mean seasonal biomass (μg C l⁻¹) along the sampling stations in Ushuaia and Golondrina Bays.

The frequency distribution of the correlation coefficients obtained after bootstrap resampling (1000 times) of microplankton variability and environmental variability is shown in Figure 6. The correlation was significant in the impacted zones, and changed to negative in the most polluted zone (Z1) to positive in the moderately polluted zone (Z2). In the less polluted zone (Z₃), no correlation between microplankton and environment was found. The variance extracted from microplankton variability after PCA was 68, 81 and 65% for Z1, Z2 and Z3 respectively, while from environmental variability was 64, 64 and 54% for Z1, Z2 and Z3, respectively. The link between the ciliates and chlorophyll-a and environmental variability was only significant in the polluted zone (Z1), and the correlation between pairs was negative (r = -0.49; P = 0.05). The correlation between the mentioned pairs in the moderately polluted zone (Z2) was positive, although the results were not significant (r = 0.44; P = 0.28). No correlation was found in the unpolluted zone (Z₃) during the studied period (r = -0.04; P = 0.80). The main contributors among zones to ciliates variability were the abundance, biomass and species richness. As for environmental variability, temperature, nitrate + nitrite and phosphate showed the highest scores (Figure 6).

DISCUSSION

Ciliates show high local diversity and are widely distributed, although a recent review made by Agatha (2011) showed that ciliates diversity tend to be lower in the southern hemisphere. The author suggested that the southern diversity is actually underestimated as result of restricted sampling frequency. Our results help to reinforce the above mentioned hypothesis of misleading low southern diversity, given the high species diversity recorded in Ushuaia and Golondrina Bays (43 species).

Studies on the dynamics of planktonic ciliates and their importance in marine food webs have generally been conducted in temperate or tropical environments; however, several studies have highlighted the presence of conspicuous ciliate populations in cold and highly seasonal environments (e.g. Leakey *et al.*, 1994). The number of ciliate species registered in the study area was similar to that found in some temperate coastal waters (Montagnes *et al.*, 1988; Dolan, 1991; Leakey *et al.*, 1992).

In this community, aloricate oligotrich ciliates dominated over tintinnids. The genus *Strombidium* predominated in the three zones; although the tintinnids had some importance in Z2, they were always more scarce. These results are in accordance with those from the Bering Sea and North Pacific in the northern hemisphere (42°N132°W 66°N 156°W) (Sorokin *et al.*, 1996). Regarding the species composition of the three zones, eight species were found only in Z1, nine were present only in Z3 and only one species was unique to Z2. It is worth noting that those specific species of Z1 are also frequently found in other coastal areas of temperate regions (Balech, 1948; Montagnes, *et al.*, 1988; Pettigrosso, 2003; Pettigrosso & Popovich, 2009; Barría de Cao *et al.*, 2005).

Ciliates abundance and biomass varied temporally and spatially. The highest abundance value was registered during the summer and the lowest in autumn. According to Gil et al. (2010) the maximum values of phytoplankton biomass, temperature and solar radiation are registered during the summer, while the autumn is characterized by minimum values of nitrate and phosphate. During the summer, the most important relative contribution to the biomass of the phytoplankton is due mainly to diatoms of the genera Thalassiosira and Pseudo-nitzschia, dinoflagellates of the genus Scrippsiella and tiny unidentified phytoflagellates (Almandoz et al., 2011). We found that the abundance and biomass of ciliates were significantly dependent on chlorophyll-a concentration and also chlorophyll-a was negatively correlated with nitrate + nitrite and phosphate content. Concerning the spatial distribution of ciliates and taking into account the zones environmentally established by Gil et al. (2010), the highest mean abundance was found in Z1. However, the highest mean value of carbon derived from ciliates was registered in a station located in the mouth of the Grande Stream, within the Z₃, where the allocthonous NH₄ was the prevailing nutrient. Comparing the mean ciliate abundances found in the Beagle Channel with those in the northern hemisphere at approximately the same latitude, we found that our results were similar to those from Ito & Taniguchi (2001) but one order of magnitude lower than those reported by Sorokin *et al.* (1996).

From the point of view of the relationship between the ciliate community and the environmental quality and the stressing conditions within Ushuaia and Golondrina Bays, there was spatial variation in the community structure of

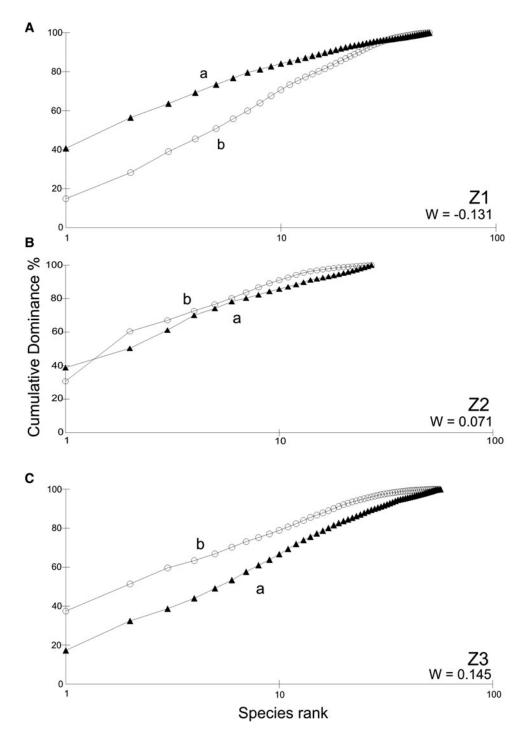


Fig. 5. K-dominance curves for ciliates abundance and biomass in the studied zones within the Ushuaia and Golondrina Bays: (A) Z1; (B) Z2; (C) Z3. a, abundance (solid triangles); b, biomass (circles); W, statistics (see text).

ciliates that matches with the characteristics of seawater in the different zones delimited by Gil *et al.* (2010) within the studied area. The greater dissimilarity was found between Z1 and Z3. The SIMPER analysis highlighted the relevance of *Strombidium conicum* as a key species in the planktonic community of the Beagle Channel, and thus this species should be taken into account for future ecological studies.

The sliding correlation analysis between environmental and planktonic variability was significant only in Z1. The higher environmental variability found at the disturbed Z1 favoured the presence of a diverse community of ciliates

dominated by small and opportunistic species, tolerant to unpredictable changes in the water column. In Z₃, the lack of correlation suggests that the ciliate community in this area was not mainly influenced by environmental changes; likely, it was influenced by specific intrinsic mechanisms and in a lesser extent by environmental conditions, which in addition showed lower variability than in Z₁. This less stressed zone presented a community comprising a smaller number of bigger species, probably related to the more stable environmental conditions. An intermediate situation was found in Z₂, denoting a transitional nature of this zone, as the

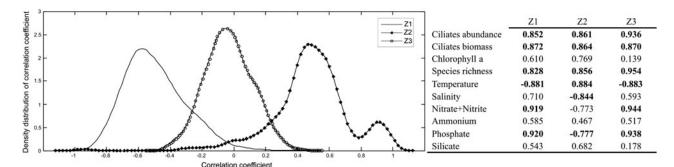


Fig. 6. Correlation between ciliate community and chlorophyll-a and environmental variability in Ushuaia and Golondrina Bays. Left, density distribution of correlation coefficients estimated by Kernel smoothing techniques between the first PC (ciliates and chlorophyll-a) and environmental conditions in the studied zones (Z1, Z2 and Z3). Z1, r = -0.49; P = 0.05; mean P = 0.05; mean

community composition of ciliates found in this area was composed of species present in both Z₁ as in Z₃.

The results showed that the structure and composition of the ciliate community in the two bays of the Beagle Channel were tightly related to the environmental characteristics. Our findings clearly have shown how this pelagic community can be shaped by environmental conditions.

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Correspondence should be addressed to:

M.S. Barría de Cao IADO (CONICET – UNS) Florida 4750 B8000FWB-Bahía Blanca, Argentina email: sbarria@criba.edu.ar