Mixotrophic ciliate dynamics in two zones of a temperate and highly turbid estuary in South America, Argentina

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Abstract. Mixotrophy is a feeding strategy by which some organisms combine autotrophic and heterotrophic activities. The seasonal dynamics of the five most common mixotrophic ciliates were studied monthly in surface layers of the inner and middle zone of the Bahía Blanca estuary, a nutrient-rich, shallow and highly turbid environment in Argentina, from January to December 2009. Temperature, salinity, turbidity and chlorophyll *a* were recorded, as well as the abundance and biomass of *Strombidium capitatum*, *Strombidium acutum*, *Cyrtostrombidium* sp., *Lohmanniella oviformis* and *Tontonia appendiculariformis*. The highest mixotrophic ciliate abundance was recorded during the austral winter (June-July) in the inner zone, meanwhile in the middle zone of the estuary the presence of these ciliates was almost null. The highest chlorophyll contribution derived from mixotrophic ciliates to total chlorophyll *a* ranged from 6% in the inner zone to 23% in the middle zone, both registered in autumn. The low abundance of mixotrophs in the middle zone of the estuary during the winter, as is usually observed in other coastal ecosystems, could be explained through a higher grazing pressure in this zone (top-down control) by mesozooplankton (e.g., copepods) in comparison to the inner zone. The secondary bloom of phytoplankton consistently observed during the last summers dominated by small sized diatoms and nanoplankton phytoflagelates, could have sustained the high abundance of mixotrophic ciliates registered in the middle zone in autumn and summer.

[Keywords: mixotrophy, chlorophyll concentration, turbidity, Southern Atlantic]

Resumen. Dinámica de los ciliados mixótrofos en dos zonas de un estuario templado y turbio en Sud América, Argentina. La mixotrofía es una estrategia de alimentación por medio de la cual un organismo combina la autotrofia y la heterotrofia. La dinámica estacional de cinco ciliados mixótrofos comúnmente presentes en las aguas superficiales de la zona interna y media del estuario de Bahía Blanca, Argentina, rico en nutrientes, somero y con alta turbidez, se estudió mensualmente desde enero a diciembre de 2009. Se registraron los valores de temperatura, salinidad, turbidez y clorofila *a*, así como también la abundancia y biomasa de *Strombidium capitatum*, *Strombidium acutum*, *Cyrtostrombidium* sp., *Lohmanniella oviformis* y *Tontonia appendiculariformis*. La abundancia más alta de los mixótrofos se registró en el invierno en la zona interna, mientras que en la zona media del estuario la presencia de estos ciliados fue casi nula. La contribución más alta de clorofila proveniente de los mixótrofos a la clorofila *a* total fue 6% en la zona interna y 23% en la zona media (ambas estimaciones registradas en otoño). La baja abundancia de mixótrofos en la zona media del estuario durante el invierno, como usualmente es observado en otros sistemas costeros, podría ser explicada por una presión de pastoreo elevada en esta zona (control de tipo "top-down") por parte de mesozooplancton (e.g., copépodos), en comparación con la zona interna. La floración secundaria del fitoplancton observada durante los últimos veranos, dominada por diatomeas de pequeño tamaño y fitoflagelados del nanoplancton, podría haber sustentado la alta abundancia de ciliados mixótrofos que se registró en la zona media en otoño y verano.

[Palabras clave: mixotrofía, concentración de clorofila, turbidez, Atlántico Sur]

Introduction

In aquatic food webs, mixotrophy represents a spectrum of nutritional strategies from absolute heterotrophy to total autotrophy (Flynn et al. 2012). Whereas acquired phototrophy with endosymbiotic associations generally prevails in nutrient-poor environments, organelle retention is typical of more productive environments (Stoecker et al. 2009; Johnson 2011a-b; Flynn et al. 2012; Caron & Hutchins 2013). Therefore, mixotrophic planktonic protists diversify the flow of energy and nutrients by placing them at different trophic levels. Planktonic ciliates, many of which are mixotrophs (Pérez et al. 1997), are one of the most important ecological groups within microzooplankton, both in abundance and in

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composition in several marine environments. Mixotrophic ciliates are frequent in the water column mixing zone, particularly during stratification periods (Stoecker et al. 2009). Under these conditions, they generally reach their highest abundances, the most common being the kleptoplastidic oligotrichs (that retain algal plastids derived from their prey). In marine surface layers, mixotrophs are generally dominant among oligotrich ciliates (Stoecker et al. 2009), being also common in coastal and estuarine mixed environments. However, knowledge on the ecology of mixotrophic ciliates is still fragmentary (Sanders 1995; Pérez et al. 1997, 2000; Dolan and Marrasé 1995; Modigh 2001; Urrutxurtu et al. 2003; Mironova et al. 2011, 2013, 2014) and data are rather scarce worldwide.

Estuaries are very complex coastal environments. Local physicochemical and biological factors, such as the water column depth, light availability, nutrient turnover, grazing pressure and species-specific interactions, in addition to the particular longitudinal hydrological gradient and seasonal dynamic (temperature, salinity and turbidity), induce high variability in the distribution and structure of planktonic populations (McLusky and Elliott 2004). Primary production usually decreases towards the estuary head where turbidity, nutrient concentration and particulate organic matter are high (Kocum et al. 2002). Phytoplankton development is mainly regulated by light and nutrient availability, nevertheless, in turbid and shallow estuaries of temperate regions light is the chief factor acting on phytoplankton dynamics (Cloern 1987; Kocum et al. 2002). Light availability depends on the water turbidity whose concentration usually varies over the year due to the influence of tides, winds velocity and intensity, and freshwater inputs (Smayda 1983; Cole et al. 1992; Kocum et al. 2002).

The Bahía Blanca estuary, located on the southwest temperate Atlantic coast in Argentina, is a shallow, highly turbid and eutrophic ecosystem (Freije and Marcovecchio 2004; Guinder et al. 2009). A study carried out by Popovich and Marcovecchio (2008) on the spatial and temporal phytoplankton variability and the associated environmental factors in the main navigation channel of this estuary, showed that both phytoplankton abundance and biomass decrease from the inner to the outer estuary. So, these authors determined three physiographic zones along this spatial gradient (inner, middle and outer zones), concluding that the inner zone displays the maximum production of phytoplankton biomass during winter-early spring. The environmental conditions which promote the phytoplankton bloom seems be the high nutrient concentration in autumn, which coincides with the highest rainfall period for the region (Piccolo and Perillo 1990), the low grazing pressure by mesozooplankton due to low water temperature (Popovich and Marcovecchio 2008; Popovich et al. 2008; Pettigrosso and Popovich 2009) and the marked reduction in water turbidity (Guinder et al. 2009). The middle zone is euhaline, being the phytoplankton biomasses and nutrient contents lower than those registered in the inner zone and showing a less pronounced temporal (seasonal) variability (Popovich and Marcovecchio 2008). The outer estuarine zone is characterized by a higher influence from the shelf region yet, with lower phytoplanktonic biomass and nutrient content. The most representative area of the inner zone of the Bahía Blanca estuary is Puerto Cuatreros (Freije and Marcovecchio 2004), characterized as a semi-closed area with a restricted circulation and low advection, a relatively high residence time and a well-mixed water column all year round due to the effect of tides and winds (Perillo et al. 2001).

Although ciliates are one of the most important links in planktonic food webs, and in the Bahía Blanca estuary several studies on species composition, abundance and biomass of aloricate ciliates have been carried out (Pettigrosso 2003; Barría et al. 2003; Pettigrosso and Popovich 2009), there is no quantitative information to date on the spatial and seasonal variability of mixotrophic ciliates, particularly during an annual cycle. Thus, the objective of this study was to analyse the seasonal dynamics of five mixotrophic ciliates commonly found in the superficial waters of the inner and middle zones of the Bahía Blanca estuary. We hypothesized that a higher availability of food (phytoplankton bloom) in winter at the inner zone of the estuary favours the development and high abundances of mixotrophic ciliates. This fact could have effects on the magnitude of the primary production and efficiency of the plankton food web.

## Materials and Methods

Study area

The Bahía Blanca estuary is located at 38°45'-39°40' S and 61°45'-62°30' W, in a temperate,

semiarid zone on the Southern Atlantic coast in Argentina (Figure 1). The estuary is a mesotidal coastal plain environment formed by a series of NW-SE channels separated by interconnected tidal channels, islands, extensive tidal flats, and low marshes. The main navigation channel is funnel-shaped, with a total length of ~68 km and a width that varies from 200 m at the head (3 m depth) to 3-4 km at the mouth (22 m depth) (Perillo et al. 2001). Based on its physiographic characteristics, the main navigation channel is divided into three sections, namely: inner, middle and outer zone (Popovich and Marcovecchio 2008). The annual average transparency (depth of light penetration measured by Secchi disk) is 0.55 m in the inner zone, 2.04 m in the outer zone and 0.65 m at the middle zone (Popovich and Marcovecchio 2008). The water column is vertically homogeneous throughout the year although it could be partially mixed in the inner zone depending on the intensity of freshwater runoff. Surface water temperature is slightly higher at the head of the estuary. Water temperature seasonally varies between 4 and 26 °C, with an average of 21 and 8.5 °C in summer and in winter, respectively (Piccolo and Perillo 1990).

Mean surface salinity normally increases from the head to the mouth. Historical values of salinity vary throughout the year from 17.3 to 41.9 ups, with an average annual value of 31.61 ups in the inner zone, 32.91 ups in the middle, and 32.80 ups in the outer zone (Freije and Marcovecchio 2004; Popovich and Marcovecchio 2008). Oxidation and remineralization of organic matter is stimulated by the usually high levels of dissolved oxygen which generally are close to saturation level values due to the dynamics of the environment (Popovich et al. 2008). Freshwater input is, in general low, the main tributaries being the Sauce Chico and the Naposta Grande Rivers with a mean annual run-off of 1.9 and 0.8 m^3/s, respectively (Perillo and Piccolo 1991).

Sampling was carried out monthly during daylight hours on the main navigation channel of the estuary from January 2009 to December 2009. Only one sample of both biotic and abiotic variables was taken at each two fixed stations. These were Puerto Cuatreros (PC) in the inner zone of estuary and Boya 26 (B26) located in its middle zone (Figure 1). Surface water samples (1 L) were collected using a 2.5 L Van Dorn bottle to determine chlorophyll a concentration (Chl-a), and to analyse mixotrophic ciliates. Temperature (°C), salinity and turbidity (NTU) were measured with a multisensor HORIBA U-10. Chlorophyll a was determined spectrophotometrically following Lorenzen and Jeffrey (1980). Mixotrophic ciliates were identified following Maeda and Carey (1985), Maeda (1986), Agatha and Riedel-
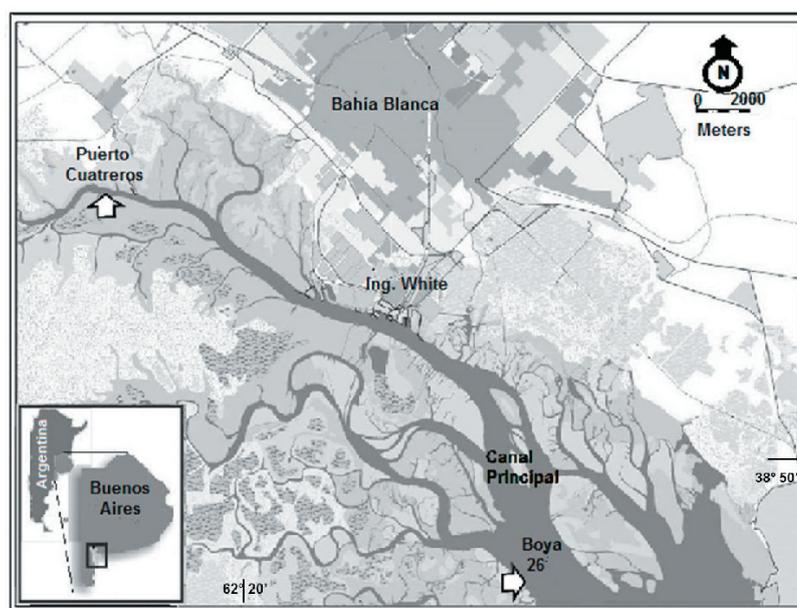
Figure 1. Map of the Bahía Blanca estuary showing the location of the two sampling sites: the inner zone (Puerto Cuatreros) and the middle zone (Boya 26).

Figura 1. Mapa del estuario de Bahía Blanca mostrando la ubicación de los dos sitios de muestreo: zona interna (Puerto Cuatreros) y zona media (Boya 26).

Lorje (1997), Montagnes and Lynn (1991), Montagnes and Taylor (1994), Pettigrosso (2003). The genera and species considered in this study have been documented as mixotrophs from the literature (Pierce and Turner 1992; Bernard and Razouzadegan 1995; Modigh 2001; Stelfox-Widdicombe et al. 2004; Stoecker et al. 2009) and they have already been recognized as mixotrophs in this estuary (López Abbate et al. 2015). Samples for the estimation of abundance and biomass of mixotrophic ciliates were preserved in 2% acidified Lugol's solution (Stoecker et al. 1994). After gently mixing, a subsample of 50 ml was removed and sedimented in a cylinder combined chamber for 24-48 h following the Utermöhl's method after Hasle (1978). The mixotrophs in the chamber were counted and the linear dimensions (length and diameter) of each cell were measured at 400X magnification using a Nikon® Eclipse TE 300 inverted microscope and a Nikon® Sight DS-U2 digital camera. Biovolume of mixotrophs was estimated by associating the shape of each ciliate with standard geometric configurations (Montagnes and Lynn 1991). Carbon biomass (Biom) was estimated by converting cell volume into carbon weight using a factor of 0.19 pg C/μm^3 (Putt and Stoecker 1989), and expressed as μg C/L.

Statistical analysis

To determine spatio-temporal variations in the studied biotic (abundance and biomass of mixotrophic ciliates) and environmental variables (temperature [T°], turbidity [Tu], salinity [S], chlorophyll *a* [Chl *a*] and depth [D]) between sites and seasons, two-way ANOVAs were performed by grouping the data corresponding to the three months of each season (n=24; df=3; df$_{error}$=20) and to the sites (n=24; df=1; df$_{error}$=22), respectively. No interaction between the factors (site and period) was found so variables were compared separately between each factor level (PC vs. B26, and among seasons). In all cases, mean comparisons were performed using ANOVA followed by Fisher's least significant difference (LSD) after checking the assumptions of normality and homoscedasticity. If assumptions were not met for ANOVA, data were log-transformed (Sokal and Rohlf 1999). Pearson correlations considering abundance, biomass, chlorophyll *a*, temperature, salinity and turbidity were performed for each sampling site using INFOSTAT software.

In addition, log transformed compositional mixotrophic ciliate data was converted into a similarity matrix using the Bray-Curtis index. Similarity percentage routines (SIMPER) were performed to detect the species, which contributed most to each sampling site or season. Analysis of similarity (ANOSIM) was applied to detect statistical differences in the species compositions between sampling sites and seasons (Clarke and Warwick 1994). These analyses were made using PRIMER-E6.

For comparison with other studies, the chlorophyll *a* content of mixotrophic ciliates was estimated using the relationship between cell volume and volume-specific chlorophyll *a* content in mixotrophic oligotrichs based on literature reports of direct measurements (Pérez et al. 2000). This relationship was applied to estimate chlorophyll content per cell, based on the cell size of the mixotrophic ciliates found in this study.

Results

Environmental variables

Water surface temperature ranged from 7.4 °C in July to 23.4 °C in December in the inner zone, while at the middle zone temperature varied from 7.4 °C in July to 22.9 °C in March (Figure 2a). There were no significant differences in temperature between the inner and middle zone (ANOVA: F=2.6x10^{-3}; P=0.96).

Water surface salinity fluctuated from 27.24 ups in July and August to 42.92 ups in January at the inner zone, and from 26.7 ups in July to 40.9 ups in January, at the middle zone (Figure 2b). No significant differences were shown in salinity between the sites all along the year (ANOVA: F=0.19; P=0.66), but it was different among the seasons (ANOVA: F=7.33; P<0.05) being significantly lower during winter in comparison with the other seasons, at the two sites studied (LSD Test: P<0.05) (Table 1).

Surface turbidity varied from 23.4 and 35 NTU in July to 136 and 186 NTU in January, at the inner and middle zone respectively (Figure 2c). No differences in turbidity were detected between the sites (ANOVA: F=3.58; P=0.07), but a significant decrease was shown in winter differing with spring and summer (ANOVA: F=7.26; P<0.05; LSD Test: P<0.05) (Table 1).

Chlorophyll *a* concentration showed the highest value at the inner zone (17.33 μg/
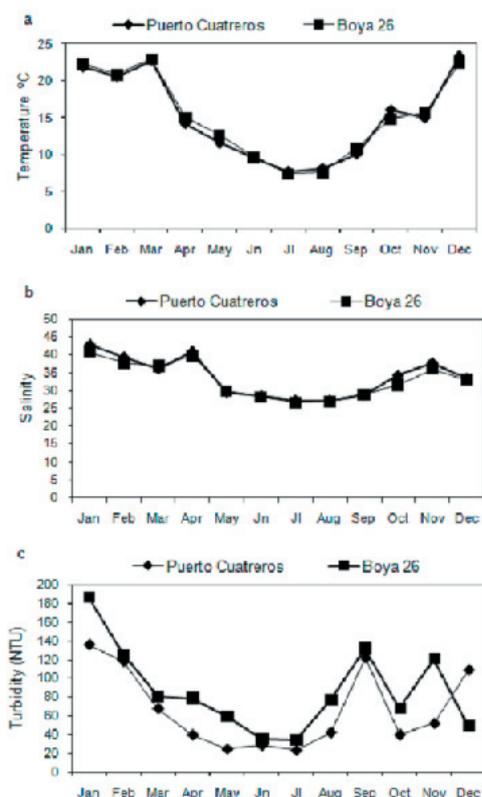
Figure 2. Temporal variability of a) surface water temperature (°C), b) salinity and c) turbidity (NTU) at Puerto Cuatreros and Boya 26 from January 2009 to December 2009.

Figura 2. Variación temporal de a) temperatura superficial del agua (°C), b) salinidad y c) turbidez (NTU) en Puerto Cuatreros y Boya 26, desde enero a diciembre de 2009.

L) in July and the lowest value (1.52 µg/L) in May. At the middle zone, it varied from 2.16 µg/L in April to 8.42 µg/L in December (Figure 3). Chlorophyll *a* was no significantly different between the sites (ANOVA: $F=4.11$; $P=0.06$). It showed significant differences among the seasons (ANOVA: $F=5.55$; $P<0.05$), showing lower values in autumn and higher ones in summer and winter (LSD Test: $P<0.05$) (Table 1).

Temporal and spatial variability and species composition of mixotrophic ciliates

The abundance and biomass of mixotrophic ciliates showed no clear pattern of distribution at any sampling site during the annual cycle (Figure 4a-b). Positive correlations between abundance and biomass were observed both at the inner and middle zone (r=0.89 and 0.72; $P<0.05$, respectively) (Figure 5a-c). The highest abundance value of mixotrophic ciliates was 1.65×10^3 cells/L, recorded at the inner zone during winter (July), along with the lowest values of temperature and turbidity. Significant negative correlation was registered at inner zone between abundance of mixotrophs and turbidity (r=-0.72; $P<0.05$) (Figure 5b). In winter, the maximum total aloricate ciliate abundance was also recorded, of which, mixotrophic ciliates accounted for 39.8%. At the middle zone, mixotrophic ciliates showed the highest abundances in autumn (March) and in summer (December and

Table 1. Seasonal mean and standard deviation (in brackets) of temperature, salinity, turbidity, chlorophyll *a*, abundance and biomass of mixotrophic ciliates in both sampling sites of the Bahía Blanca estuary: Puerto Cuatreros (inner zone) and Boya 26 (middle zone). Letters on the side of each variable indicate ANOVA and LSD test.

Tabla 1. Media estacional y desviación estándar (entre paréntesis) de temperatura, salinidad, turbidez, clorofila *a*, abundancia y biomasa de los ciliados mixotrófos en ambos sitios de muestreo en el Estuario de Bahía Blanca. Puerto Cuatreros (zona interna) y Boya 26 (zona media). Las letras al lado de cada variable indican el resultado de ANOVA y LSD test.

	Temperature (°C)		Salinity (ups)		Turbidity (NTU)		Chlorophyll-*a* (µg/L)		Abundance (ind./L)		Biomass (µg C/L)	
Puerto Cuatreros												
Summer	21.97 (1.40)	c	38.59 (4.8)	c	121 (13.75)	c	9.51 (1.95)	b	333.33 (577.35)	a	1.31 (1.73)	a
Autumn	16.21 (5.87)	b	35.56 (5.73)	bc	44.33 (21.83)	ab	3.25 (2.8)	a	300 (200)	a	1.59 (0.44)	a
Winter	8.47 (0.97)	a	27.67 (0.74)	a	31.47 (9.89)	a	12.22 (5.43)	b	1316.67 (493.29)	a	2.62 (0.45)	a
Spring	13.72 (3.22)	b	33.64 (4.57)	b	71.37 (44.25)	bc	5.96 (2.76)	ab	583.33 (803.64)	a	1.52 (1.53)	a
Boya 26												
Summer	21.87 (0.93)	c	37.32 (3.96)	c	120.33 (68.12)	c	6.18 (1.99)	b	733.33 (461.88)	a	3.13 (1.16)	a
Autumn	16.84 (5.41)	b	35.6 (5.06)	bc	73.33 (11.59)	ab	2.77 (0.85)	a	733.33 (776.75)	a	4.13 (1.07)	a
Winter	8.17 (1.24)	a	27.35 (0.86)	a	49.5 (24.25)	a	6.60 (2.13)	b	300 (519.62)	a	0.64 (1.73)	a
Spring	13.76 (2.57)	b	32.26 (3.57)	b	107.2 (33.88)	bc	5.91 (2.79)	ab	316.67 (170.17)	a	3.12 (0.95)	a
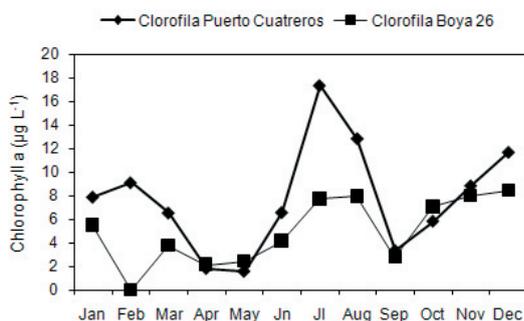
Figure 3. Temporal variability of chlorophyll *a* (µg/L) at Puerto Cuatreros and Boya 26 from January 2009 to December 2009.

Figura 3. Variación temporal de la concentración de clorofila *a* (µg/L) en Puerto Cuatreros y Boya 26, desde enero a diciembre de 2009.
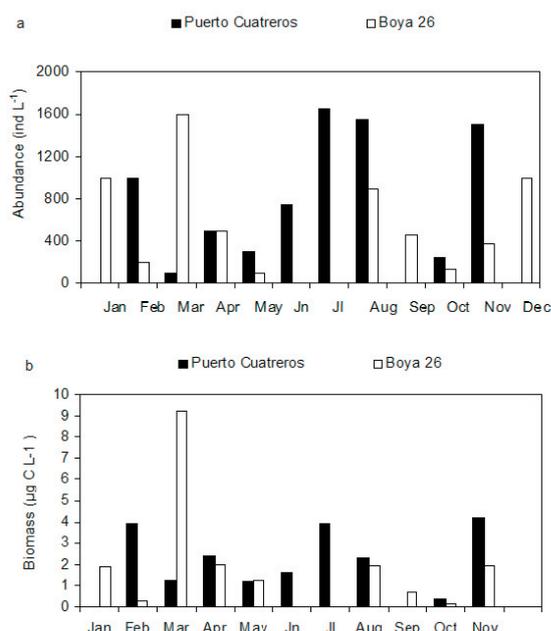
Figure 4. a) Temporal variability of mixotrophic ciliates abundance (ind./L) and b) biomass (µg C/L) at Puerto Cuatreros and Boya 26 from January 2009 to December 2009.

Figura 4. a) Variación temporal de la abundancia (ind./L) y b) de la biomasa (µg C/L) de los ciliados mixótrofos en Puerto Cuatreros y Boya 26 desde enero a diciembre de 2009.

January), reaching 1.6×10^3 cells/L and 1×10^3 cells/L, respectively. A significantly positive correlation between abundance with turbidity and temperature was registered (r=0.69 and 0.61; *P*<0.05 respectively) (Figure 5d-e).

Regarding mixotrophic biomass, at the inner zone, the highest values were recorded, in spring (November) representing 28% in relation to total aloricate biomass. At the middle zone, the maximum pick of biomass was registered in autumn (March), represent-

ing 90% of the total aloricate biomass. Positive correlation between mixotrophic ciliates biomass and temperature (r=0.58; *P*<0.05) was registered (Figure 5f). There were not statistical differences between sites and among seasons neither in abundance nor in biomass (ANOVA: *P*>0.05).

The mixotrophic genera and species studied were *Cyrtostrombidium* sp. Lynn and Gilron 1993; *Strombidium acutum* Leegaard 1915; *Tontonia appendiculariformis* Fauré-Fremiet 1914; *Strombidium capitatum* (Leegaard 1915) Kahl 1932, and *Lohmanniella oviformis* Leegaard 1915. In terms of species composition, ANOSIM showed no difference between the sampling sites (Global R=-0.031; *P*=0.054), whereas differences were found among the seasons (ANOSIM, Global R=0.179; *P*<0.05). According to SIMPER, *Cyrtostrombidium* sp. was the only species which contributed to summer (100%), while *S. capitatum* was the main contributor to autumn (71.97%), and *T. appendiculariformis* contributed (100%) to winter and spring, of total aloricate ciliates.

### Estimation of chlorophyll content in mixotrophic ciliates

The chlorophyll content of *L. oviformis, S. capitatum, S. acutum, Cyrtostrombidium* sp. and *T. appendiculariformis* in this ecosystem were 3.62×10^{-3}, 1.4×10^{-3}, 3.5×10^{-3}, 3.8×10^{-3} and 1.5×10^{-3} pg chlorophyll *a*/µm³, respectively. The values were then transformed to µg Chl/L to estimate the contribution of chlorophyll content in mixotrophic ciliates to total chlorophyll.

In the inner zone, the highest value of chlorophyll derived from mixotrophic ciliates was observed in winter and in the middle zone, in autumn. In relation to the contribution of the chlorophyll derived from mixotrophic ciliates to the total phytoplankton chlorophyll, the highest values were observed in autumn at both sites. In the inner zone, the highest value (6.5%) of chlorophyll was reached mainly by the presence of *S. capitatum* and *T. appendiculariformis* sp., whereas in the middle zone (23%), by the contribution of *S. capitatum* and *Cyrtostrombidium* sp.

DISCUSSION

Mixotrophic ciliates and environmental variables

The variables analyzed in the present study (temperature, salinity, turbidity and chlorophyll *a*) were not significantly different at the two sampling sites, but along the year, chlo-
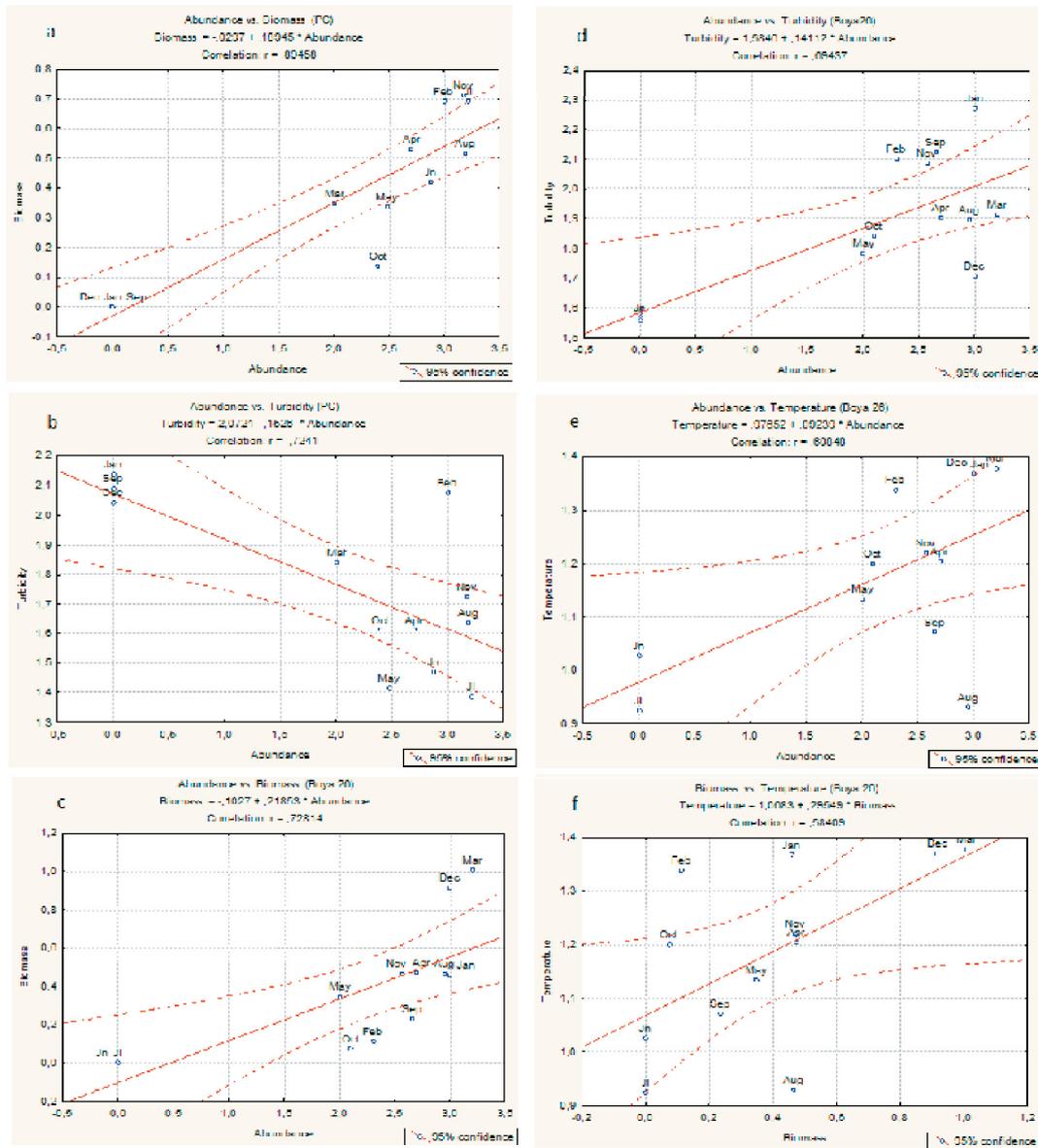
Figure 5. Main statistically significant (*p 0.05*) Pearson correlations between variables, (N = 24, f.deg = 22). PC: a) abundance vs. biomass. b) abundance vs. turbidity. Boya 26: c) abundance vs. biomass. d) abundance vs. turbidity. e) abundance vs. temperature. f) biomass vs. temperature.

Figura 5. Principales correlaciones de Pearson estadísticamente significativas (*p 0,05*) entre las variables, (N =24, g. l. = 22). PC: a) abundancia vs. biomasa. b) abundancia vs. turbidez. Boya 26: c) abundancia vs. biomasa. d) abundancia vs. turbidez. e) abundancia vs. temperatura. f) biomasa vs. temperatura.

rophyll *a* concentration showed its highest value in winter along with the lowest turbidity, especially at the inner zone. The latter is consistent with findings from previous reports in the estuary (Popovich et al. 2008; Popovich and Marcovecchio 2008; Guinder et al. 2012). Following the trend of the cholorophyll *a*, mixotrophic ciliates showed their highest abundance in the inner zone in winter, even though correlations between these variables were not significant. Even though in estuaries there are

no reports, to our knowledge, in some of early studies carried out in non-estuarine environments (Catalan Sea), the lack of correlation between mixotrophic ciliates and chlorophyll *a* was considered a reasonable result because mixotrophs appear to be mainly restricted to the surface and show distributional patterns affected by vertical migrations which may vary at different hours of the day and from ecosystem to ecosystem (Dolan and Marrasé 1995). On the other hand, in the Liguria Sea

(NW Mediterranean), Pérez et al. (2000) studied the vertical distribution of planktonic ciliates in relation to vertical distribution of chlorophyll and found positive correlation between mixotrophs and chlorophyll only in discrete samples, but reported a lack of correlation when samples were integrated through the water column, although a positive relationship was found for some species when considered independently.

In our study, turbidity showed a great variability throughout the year, however, at both sampling sites, inner and middle zone turbidity showed similar values during winter but slightly higher in the middle estuary zone. In shallow estuaries, turbidity is considered one of the main factors influencing the seasonal variation of the phytoplankton community. Studies carried out by Popovich and Marcovecchio (2008) and Guinder et al. (2012), showed that under similar conditions of turbidity in the inner and middle zones of the Bahía Blanca estuary, phytoplankton bloom develops only in the inner zone. In the inner zone, phytoplankton blooms occur even in a thin euphotic zones in a completely mixed water column due to the shallowness of the area and the decreasing turbidity, the phytoplankton cells remain below the euphotic zone during short periods of time as they are constantly resuspended into the surface layers by vertical mixing processes (Popovich and Marcoveccio 2008; Guinder et al. 2012). Given that mixotrophic ciliates are in general restricted to the euphotic zone of the water column (Stoecker et al. 2009; Dolan and Marrasé 1995), the food availability in the water surface layer, represented by the chlorophyll concentration, could be one of the main conditions that sustained the high abundance of mixotrophic ciliates during winter in the inner zone. On the other hand, although in this study mesozooplankton abundance was neither addressed nor estimated, a decrease in the number of planktonic metazoans (e.g., copepods) is usually observed in this estuary in winter (Hoffmeyer 2004; Pettigrosso and Popovich 2009). The consequent low predation pressure (e.g., from copepods and invertebrate larvae) may have supported the increase in the ciliates abundance.

In contrast, in the middle zone during winter, a low abundance of mixotrophic ciliates was observed along with a low chlorophyll *a* concentration. In this zone, a strong decrease of phytoplankton biomass was recorded as a consequence of both, deep-mixed depths and a low euphotic/mixing depth ratio (Popovich

and Marcovechio 2008; Guinder et al. 2012). Considering that mixotrophic ciliates are selective consumers, retain functional plastids from ingested algal, that there is no evidence of plastid reproduction inside ciliate cytoplasm and are dependent on algal food to replace their plastids, the scarce number of mixotrophs in winter may be related to the lower availability of suitable food. On the other hand, in the last years, a secondary bloom of phytoplankton was consistently observed during summer dominated by small sized diatoms and nanoplankton phytoflagellates (Guinder et al. 2012; Popovich and Marcovecchio 2008) which could have sustained the high abundance of mixotrophic ciliates observed in the middle zone in autumn and summer.

Although phytoplankton and mixotrophic ciliates have different nutrition modes, and being that food source is one of the main factors that modulates the community structure of ciliates, we consider that peculiar features between the inner and middle zone of the Bahía Blanca estuary, which affect the pelagic distribution of phytoplankton communities could also have influenced indirectly the distribution of mixotrophic ciliates. Thus, the greatest biomass of ciliates in temperate estuaries and coastal environments follows the pattern of the phytoplankton bloom (Lynn et al. 1991; Johansson et al. 2004). In the inner estuary, previous studies have shown that the maximum biomass of total aloricate ciliates occurred during winter, immediately after the phytoplankton peak (Pettigrosso & Popovich 2009) which agrees with our findings. However, it is noteworthy the almost null abundance of mixotrophs found in the middle zone of the estuary during the winter, as is usually observed in other estuaries and coastal ecosystems where mixotrophs commonly decrease in abundance and biomass during the coldest season (see Table 2). Even though we did not find significant differences in abundance and biomass between both sampling sites, the almost null abundance of mixotrophic ciliates in the middle zone during winter could be explained through a higher grazing pressure (top-down control) by mesozooplankton (e.g., copepods) in this zone, in comparison to the inner zone.

Mixotrophic ciliates contribution to the abundance and biomass of aloricate ciliates and species composition

Mixotrophic ciliates contribution to total aloricate ciliates during the winter was higher than the relative values reported in

Table 2. Mixotrophic ciliates in different marine ecosystems. Abundance in ind./L and biomass in µg C/L.
Tabla 2. Ciliados mixótrofos en diferentes ecosistemas marinos. Abundancia en ind./L y biomasa en µg C/L.

Marine ecosystem	Depth (m)	Season	Mixotroph abundance	Mixotroph biomass	Ciliate abundance (%)	Ciliate Biomass (%)	Mixotrophic taxa	Reference
Great Harbor (NW Mediterranean)	0	1 year	1167	2.0	-	-	All plastidic oligotrichs	Stoecker et al. 1987
MA Coastal (NW Atlantic coast)	0-9	Summer	2380		52,4	-	All plastidic oligotrichs	Stoecker et al. 1987
Georges Bank (NW Atlantic)	0-35	Summer	978	1.9	33.5	-	All plastidic oligotrichs	Stoecker et al. 1989
Adriatic Sea	0-30	Summer			29	-	*Strombidium conicum, S. delicatisimun*	Revelante & Gilmartin 1990
Nordic Sea	0	Summer	373		54	-	All plastidic oligotrichs	Putt 1990
Lame Cay (Caribean Coast)	5	1 year	-	-	12	25	*Loboea* sp., *Tontonia* sp.	Lynn et al. 1991
N.E Pacific Seamount	0-24	Summer	-		-	76.9	All plastidic oligotrichs	Sime-Ngando 1992
Villefranche Bay	0	1 year	-		-	51	All plastidic oligotrichs	Bernard & Rassoulzadegan 1993
E. Pacific fjord	2	Summer	57		1.6	7.1	*Loboea* sp., *Tontonia* sp., *Strombidium capitatum*	Martin & Montagnes 1993
N Atlantic NABE (NW Atlantic coast)	0-20	Spring-Summer	1774	1.8	60	58.5	All plastidic oligotrichs	Stoecker et al. 1994
Ligurian Sea	0	1 year	-	-	-		All plastidic oligotrichs	Bernard & Rassoulzadegan 1994
Catalan Sea	0-20	Summer	70	-	18	48	*Tontonia* sp., *Loboea* sp.	Dolan & Marrasé 1995
Ligurian Sea	5-50	Spring	1120	0.59	46	39	All plastidic oligotrichs	Perez et al. 2000
Nervión River, Bay of Biscay	0	Summer and early autumn	-	-	-	-	*Loboea* sp., *Tontonia* spp., *Strombidium*	Urrutxurtu et al. 2003
East China Sea	5-10	Winter-Summer-Autumn	390	-	-	-	*Strombidium* spp., *Tontonia* spp.	Ota & Taniguchi 2003
Neva Estuary	0.8	Summer	-	-	34	-	All plastidic oligotrichs	Mironova, et al. 2011
Neva Estuary (Baltic Sea)	0-8	Summer	1900	2.4	67	-	*Strombidium* spp., *Myrionecta rubra*	Mironova et al. 2013
Bahía Blanca Estuary (South Atlantic)	0	Winter	1650	3.9	39	29	*Cyrtostrombidium* sp., *T. appendiculariformis, S. acutum, S. cuminatum, L. oviformis*	This study

other studies (Table 2). In Atlantic coastal waters, mixotrophs represent only 10% of the total ciliates abundance in the cold seasons (Stoecker et al. 2009). Except for winter, similar relative values of mixotrophic ciliates recorded in this study were observed in other environments, (Stoecker et al. 1996; Ota and Taniguchi 2003; Stoecker et al. 2009; Mironova et al. 2013).

Although previous research has reported low percentages of mixotrophic ciliates in nutrient-

rich environments and higher abundances of mixotrophic ciliates in oligo-to-meso-oligotrophic waters (Bernard and Rassoul-zadegan 1995), our findings confirm recent studies on the presence of this type of ciliates in nutrient-rich environments (Stoecker et al. 2009; Mironova et al. 2013; López Abbate et al. 2015), such as the Bahía Blanca estuary (Freije and Marcovecchio 2004).

The mixotrophic oligotrichs and choreotrichs studied belong to the genera *Cyrtostrombidium, Tontonia, Strombidium* and *Lohmanniella*. These genera include the most common and abundant mixotrophic ciliates in the mixed layer of marine and estuarine waters (Bernard and Rassoulzadegan 1995; Stoecker et al. 2009). According to Agatha (2011), *Cyrtostrombidium, Tontonia* and *Lohmanniella* genera occur exclusively in marine and brackish sea waters, whereas *Strombidium* includes both marine and freshwater species. On the other hand, *Tontonia, Lohmanniella* and *Strombidium* have been reported showing a cosmopolitan distribution (Agatha 2011), while *Cyrtostrombidium* has been reported to be restricted to the northern hemisphere. Nevertheless, previous research has reported *Cyrtostrombidium* genus in the Bahía Blanca estuary (Pettigrosso 2003; Barría et al. 2003; Pettigrosso and Popovich 2009).

The association of the five species of mixotrophic ciliates reported in this study showed some seasonal differences in composition and it could be a response to the particular environmental characteristics of the Bahía Blanca estuary. This was evident, for example in the inner zone in winter when the biomass peak was dictated by a predominance of *T. appendiculariformis*. In the Catalan Sea, Dolan & Marrase (1995) considered to *T. appendiculariformis* one of the commonest type of the larger mixotrophic species which was generally restricted to 0 to 20 m in depth. Several studies on *T. appendiculariformis* showed a rapid response to the increase of nutrients in mesocosm experiments (Gismervik et al. 2002). This species alone represented 74.1% of the ciliate biomass and 51.9% of the ciliate abundance in a coastal area of the eastern English Channel (Grattepanche et al. 2011). Furthermore, *T. appendiculariformis* proved to be an important contribution of carbon ingested by copepods in Oregon coastal Waters (Fessenden and Cowles 1994).

Contribution of mixotrophic ciliates to chlorophyll a

Given that literature data on the contribution of the chlorophyll derived from mixotrophs are sparse and variable, and the contribution of ciliates to chlorophyll is very sensitive to the factors used to estimate chlorophyll per biovolume unit and to the mix of occurring species, it is difficult to make comparisons (Stoecker et al. 2014). However our findings are in the range of those reported in the literature (Stoecker et al. 2009).

The estimated chlorophyll *a* contribution from the mixotrophic ciliates to the total phytoplankton chlorophyll *a* showed that in both, the inner and middle zones of the Bahía Blanca estuary, the highest values (6% and 23%, respectively) were observed in autumn, when the average of total chlorophyll *a* at both sites was the lowest: 3.2 µg Chl/L. Phytoplankton is composed by phytoflagel-lates, commonly documented in the inner and middle zone of the estuary during these seasons by Popovich and Marcovecchio (2008). Under these conditions photosynthesis from mixotrophs in the > 20 microns size class of the upper layer of the water column can be impor-tant (Stoecker et al. 1989-2009; Putt 1990).

On the other hand, the size fraction of the mixotrophic ciliates observed in this estuary are particularly important as prey for copepods (Dutz and Peters 2008) and young fish larvae (Figueiredo et al. 2007). Studies have demon-strated that photosynthesis in ciliates may promote the microzooplankton efficiency and could represent a significant flow of carbon to higher trophic levels. In addition, an increase in the gross growth efficiency in planktonic ciliates may increase nutrient regeneration (Dolan and Marrasé 1995; Dolan and Pérez 2000; Stoecker et al. 2009). According to Hammer and Pitchford (2005), mixotrophs due to their capacity of being both producers and consumers, would influence the structure and productivity of microbial food webs and the coupling to metazoan production.

If we consider that the estimations of chlorophyll of mixotrophs registered in this study were obtained only from five mixotrophic species of the superficial layer, the contribution of the chlorophyll from the mixotrophic ciliates in the Bahía Blanca estuary would be underestimated, and the

phytoplankton biomass would be probably overestimated.

Despite the preliminary character of this study, our findings showed that the environmental factors in the time scale analyzed did not determine spatial differences in the abundance and biomass of mixotrophic ciliates. Thus, the planted hypothesis could not be verified. Further research is required to better understand the dynamic of mixtrophic ciliates and its environmental response in this turbid and mixed estuary.

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