Temporal variability of diversity and biomass of tintinnids (Ciliophora) in a southwestern Atlantic temperate estuary

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Seasonal variations in diversity and biomass of tintinnids (Ciliophora: Tintinnida) were investigated at two fixed stations in the inner part of the Bahía Blanca Estuary (38° 42′ S, 61° 50′ W) during an annual cycle. The variations were analysed in relation to surface temperature, salinity, transparency, solar radiation and chlorophyll a (Chl a)concentration. Biomass was calculated in terms of biovolume and carbon units. Diversity was estimated as the number of species and the Shannon Index (H', In based). Density of tintinnids ranged from 100 to 7800 individuals L^{-1} H' ranged from 0 to 1.81. The biomass varied from 0.3 to 127.78 × 10⁶ µm³ L^{-1} (0.02–39.4 µg C L^{-1}). Density was significantly related to temperature, solar radiation and Secchi distance (P < 0.01); diversity was significantly related to temperature (P < 0.01) and solar radiation (P < 0.05). Biomass was significantly related only to temperature (P < 0.01) in one of the stations. According to principal components analysis (PCA) tintinnids exhibited a segregation of three groups: winter, spring–summer and autumn for the most internal station and winter, spring and summer–autumn for the most external station. H' values were lower than those observed in other coastal systems found at about the same latitude in the northern hemisphere.

INTRODUCTION

Temperate estuaries are characterized as highly variable ecosystems where insolation as well as temperature exhibit a large annual fluctuation. Also, the typical estuarine mixture of sea water with fresh water derived from land drainage determine large salinity gradients. Most estuaries present a maximum turbidity near the head, where there is a zone of high suspended sediment concentration.

Estuaries are habitats with low diversity. High variations of physical and chemical factors convert the estuaries in adverse environments for some organisms while a few others able to prosper in those conditions, prosper exhibiting a high dominance. The biodiversity of estuaries depends on a variety of factors such as the complexity of its hydrodynamics, the type of sediments, the tidal range, nutrient supply and the amount of freshwater inflows among others. This research was conducted in the Bahía Blanca Estuary located at $38^{\circ}42'-39^{\circ}25'$ S, $61^{\circ}50'-62^{\circ}22'$ W on the Argentine Coast. Some quantitative studies of the microzooplankton of Bahía Blanca Estuary have been carried out (Barría de Cao, 1992; Barría de Cao *et al.*, 1997; Pettigrosso *et al.*, 1997), but none has focused on diversity.

The aim of this study was to report on the seasonal distribution of tintinnids in the Bahía Blanca Estuary with emphasis on the temporal changes in biomass and diversity and to determine the factors that exert major influence on them. We explored the relationship between variations of tintinnids diversity and biomass and the variations of surface temperature, salinity, transparency, solar radiation, and chlorophyll a (Chl a) concentration. These results are compared with those obtained in other coastal ecosystems of the world.

The Bahía Blanca Estuary comprises an area of 2300 km². It is a mesotidal estuary formed by a complex system of tidal channels of variable size, islands and extended tidal flats (Angeles, 2001). These are large subhorizontal, clayey–silty surfaces with a gentle slope that are flooded twice a day by the tides. The sampling sites are located at two different tidal flats. Cuatreros Port (CuP) (38°45′ 05″ S, 62°22′ 50″ W) is situated near the estuary head, and Rosales Port (RoP) (38°55′ 16″ S, 62°04′ 36″ W) is 32.6 km away toward the mouth (Fig. 1).

Freshwater input is provided by the Sauce Chico River (with a basin of 1600 km²), the Napostá Grande Stream (920 km²) and other minor creeks which discharge in the estuary only during rainfall periods (Pettigrosso *et al.*, 1997). Annual mean runoffs are low (1.9 and 0.8 m³ s⁻¹, Sauce Chico and Napostá Grande, respectively), although the Sauce Chico River may reach peaks of 10–50 m³ s⁻¹.

The Bahía Blanca Estuary has usually been partitioned into two different areas separated by an imaginary line across Ingeniero White Port (Mianzán, 1986; Piccolo *et al.*, 1987), where estuarine and marine influences meet. Piccolo *et al.* (Piccolo *et al.*, 1987) performed an estuary division according to the vertical distribution of salinity. The inner area (where CuP is located) was described as partially mixed during normal runoff conditions, with a strong tendency to become sectionally homogeneous during low runoff. The middleouter area (where RoP is situated) is sectionally homogeneous. Freshwater presence cannot be identified and mean salinities are similar to those of the adjacent continental shelf. The physical and chemical variables suffer large fluctuations at the inner zone. The succeeding species in this area are those adapted to an estuarine environment. The area is characterized by shallow waters which are constantly mixed by wind and tidal currents. In the area of CuP the channel is ~5 m deep. It is a highly turbid environment with scarce light penetration due to a great amount of organic and inorganic suspended material.

The anthropic influence is mainly due to the socioeconomic activities generated by industries, harbors and nearby cities that develop at the northern coast of the Bahía Blanca Estuary.

METHOD

Sampling was carried out at two fixed stations located in the inner part of the estuary, CuP and RoP (Fig. 1), approximately every 15 days from 8 March 2002 to 12 March 2003 during daylight hours and between low and high tide. Microzooplankton was collected at subsurface level using a 3 L Van-Dorn bottle and 250 mL samples of whole water were preserved with Lugol's solution. As



Fig. 1. Location of sampling stations at the Bahía Blanca Estuary.

no differences in the species composition or abundance were observed at different depths at the studied sites (Barría, 2002), due to the shallowness of the ecosystem, samples were taken only at the subsurface level.

Surface temperature, salinity and water transparency were measured at both stations on each sampling date. Solar radiation, Chl *a* concentration and phaeopigments were able to be measured only at CuP. Incident solar radiation was measured with a SKS 1110 (Skye Instruments) piranometer located at CuP, every 10 min. Instantaneous values were integrated in order to calculate the daily radiation (MJ m⁻²). The average of the values of a week previous to and on the sampling date was used in the analysis. Transparency was measured with a Secchi disk. Chl *a* and phaeopigments were measured following the technique of Lorenzen (Lorenzen, 1967).

For tintinnids enumeration, 50 mL subsamples representing material from 250 mL of well-shaken water samples were concentrated after settling in a combined plate chamber. Subsequently, the entire bottom chamber was scanned for each subsample using an inverted microscope following the Utermöhl's method after Hasle (Hasle, 1978).

Biomass was calculated as the total biovolume of the tintinnid community for each sampling date. Both loricae and cells were measured, and volumes were calculated by assigning standard geometric configurations. As most of the tintinnids possessed heavily agglutinated loricae, xylene was utilized to clear the loricae in order to visualize the cells suitably and to be able to measure them. The carbon biomass for each species was also estimated considering both cell and loricae volumes. Biomass in terms of cell carbon was calculated using a factor of 0.19 pg μ m⁻³ (for cells fixed in Lugol's iodine) following Putt and Stoecker (Putt and Stoecker, 1989). Lorica carbon was calculated using a linear regression equation: C (pg) = 444.5 + 0.053 lorica volume (μ m³), after Verity and Langdon (Verity and Langdon, 1984).

The Shannon Index $(H', \ln based)$ and the total biomass of tintinnids were calculated for each sample.

Correlations were calculated using Pearson's correlation coefficient (Sokal and Rohlf, 1981) to analyse the relationships among all the variables for each station, and the correlation matrix was used to generate the vector ordinates to perform the principal components analysis (PCA), assigning equal weight to all variables (Gould *et al.*, 1986). Numerical abundance was considered as the number of individuals L^{-1} (logarithmic transformation + 1), and biomass was considered in terms of $\mu m^3 L^{-1}$ (logarithmic transformation) and in terms of $\mu g C L^{-1}$. Hierarchical cluster analysis (Anderberg, 1973) was applied to group sampling dates.

RESULTS

Environmental variables and species composition

Temperature values varied from 6 to 21.7°C at CuP and from 6 to 22.8°C at RoP. Salinity values varied from 13.65 to 34.39 at CuP and from 27.31 to 33.62 at RoP. Secchi distance fluctuated from 0.25 to 1.26 m at CuP and from 0.20 to 1.18 m at RoP. Solar radiation measured at CuP fluctuated between 4.1 MJ m⁻² day⁻¹ in late autumn and 27.8 MJ m⁻² day⁻¹ at the end of the summer (Figs 2, 3 and 4). Maximum value of Chl *a*, 37.47 μ g L⁻¹, was registered at the beginning of the winter and maximum value of phaeopigments, 8.57 μ g L⁻¹, in spring (Fig. 3).

The community of tintinnids comprised 15 species belonging to four genera (Table I), among which *Tintinnopsis*



Fig. 2. Seasonal variations of temperature, salinity and solar radiation at Cuatreros station.



Fig. 3. Seasonal variations of chlorophyll, phaeopigments and Secchi distance at Cuatreros station.



Fig. 4. Seasonal variations of temperature, salinity and Secchi distance at Rosales station.

 Table I: Tintinnid species encountered at each
 Bahía Blanca Estuary station

Species	Station	
	Cuatreros	Rosales
Tintinnidium balechi	Х	Х
Tintinnidium sp. aff. semiciliatum	Х	Х
Leprotintinnus pellucidus	Х	
Tintinnopsis amphora	Х	Х
Tintinnopsis baltica	Х	Х
Tintinnopsis beroidea	Х	Х
Tintinnopsis brasiliensis	Х	Х
Tintinnopsis glans	Х	Х
Tintinnopsis gracilis	Х	Х
Tintinnopsis lavalae	Х	Х
Tintinnopsis levigata	Х	
Tintinnopsis parva	Х	Х
Tintinnopsis parvula	Х	
<i>Tintinnopsis</i> sp.		Х
Codonellopsis lusitanica	Х	Х

Presence is shown by 'X'.

was the most numerous genus in terms of number of species comprising 73% of the total recorded species. Description of species has been done in previous works (Barría de Cao, 1981; Barría, 2002).

Influence of environmental factors on abundance, diversity and biomass of tintinnids

The numerical abundances of tintinnids recorded during the year ranged from 100 individuals L^{-1} in winter to 7800 individuals L^{-1} at the beginning of the spring for CuP and from 20 individuals L^{-1} in winter to 12 400 individuals L^{-1} at the beginning of the spring for RoP. Mean annual abundances were 1773 and 2419 individuals L^{-1} at CuP and at RoP, respectively. Values of diversity measures (H') ranged from 0 (only one species present) to 1.81 at CuP and from 0 to 1.59 at RoP (Figs 5 and 6). For both stations minimum values were recorded in winter and maximum values were recorded in summer. The values of biomass varied from 0.64 to 127.78×10^6 $\mu m^3 L^{-1}$, equivalent to 0.3 and 39.4 $\mu g C L^{-1}$, respectively, at CuP and from 0.03 to $101.52 \times 10^6 \ \mu m^3 \ L^{-1}$ equivalent to 0.02 and 34.9 μ g C L⁻¹, respectively, at RoP (Figs 5 and 6). Annual average values of biomass were $23.54 \times 10^6 \ \mu m^3 \ L^{-1}$ for CuP and 17.68×10^6 $\mu m^3 L^{-1}$ for RoP. Minimum values were recorded in autumn and maximum values were recorded in winter in CuP; on the contrary, maximum values were recorded in summer-autumn and minimum in winter in RoP.

Correlation between numerical abundance at CuP and numerical abundance at RoP was highly significant



Fig. 5. Seasonal variations in numerical abundance, biomass and diversity of tintinnids at Cuatreros station.



Fig. 6. Seasonal variations in numerical abundance, biomass and diversity of tintinnids at Rosales station.

(P < 0.01), also between diversity at CuP and diversity at RoP; while correlation between biomass at CuP and biomass at RoP was not significant (P > 0.05). At CuP, numerical abundance was significantly related to temperature, solar radiation and Secchi distance (P < 0.01); diversity was significantly related to temperature (P < 0.05), biomass did not correlate with any of the parameters measured (Table II). At RoP, biomass was significantly related to numerical abundance and temperature (P < 0.01); diversity was significantly related to salinity (P < 0.01); diversity was significantly related to salinity (P < 0.01).

PCA (Fig. 7a), determined two main variables that explained 62.5% of the total variance at CuP. The first principal component (PC) was associated with radiation, temperature, abundance, H', Secchi distance and chlorophyll. Radiation, temperature, abundance and H' were distributed on the positive part of the PC1 axis while Secchi distance and chlorophyll were distributed on the negative part of the axis. The second PC was associated with biomass and salinity. Along this axis the biomass was opposed to salinity. For RoP, PCA determined two main variables that explained 65.4% of the total variance (Fig. 8a). The first PC was associated with temperature, biomass, abundance and H', distributed on the positive part of the axis. The second PC was associated with salinity and Secchi distance.

Seasonal variations

Based on the results of a cluster analysis of the sampling dates and interpreted in the PC1 and PC2 space using the correlations between the variables and the components, it was possible to obtain a segregation of the seasons. The distribution of sampling data in the plane defined by the two first PCs exhibited a clear segregation of three groups: winter, spring–summer and autumn for CuP (Fig. 7b) and winter, spring and summer–autumn for RoP (Fig. 8b).

Variations (media and rank) of the variables for each group for both stations are shown on Figs 9 and 10.

Table II: Correlation matrix showing relationships between species abundances, diversity and biomass and environmental parameters for Cuatreros Port (CuP)

	H′	Biomass	Abundance	Surface temperature	Salinity	Secchi distance	Solar radiation	Chlorophyll a
H′	1	NS	NS	**	NS	NS	*	NS
Biomass	0.142	1	NS	NS	NS	NS	NS	NS
Abundance	0.367	-0.131	1	* *	NS	* *	* *	NS
Surface temperature	0.533	-0.251	0.562	1	NS	* *	* *	×
Salinity	0.067	0.172	-0.172	0.134	1	NS	NS	NS
Secchi distance	-0.252	0.305	0.566	-0.771	-0.107	1	* *	NS
Solar radiation	0.501	0.103	0.566	0.809	0.03	-0.675	1	NS
Chlorophyll a	-0.222	0.395	-0.576	-0.438	0.208	0.282	-0.195	1

For all comparisons, n = 24. NS, not significant. *0.05, **0.01.

Table III: Correlation matrix showing relationships between species abundances, diversity and biomass and environmental parameters for Rosales Port (RoP)

	H′	Biomass	Abundance	Surface temperature	Salinity	Secchi distance
H′	1	NS	NS	NS	* *	NS
Biomass	0.29	1	* *	* *	NS	NS
Abundance	-0.048	0.732	1	NS	NS	NS
Surface temperature	0.253	0.552	-0.237	1	*	NS
Salinity	0.567	-0.395	0.145	0.482	1	NS
Secchi distance	0.024	-0.049	0.213	-0.249	0.0.29	1

For all comparisons, n = 23. NS, not significant. *0.05, **0.01.



Fig. 7. (a) Principal components analysis of environmental variables and numerical abundance and biomass and diversity of tintinnids at Cuatreros station. (b) Distribution of sampling data in principal component 1 (PC1) versus PC2 space at Cuatreros Port (CuP).

DISCUSSION

With the exception of some differences in salinity variations (Figs 2 and 4), physicochemical variables showed similar trends at both stations. The studied stations were very similar from a biological point of view, at least in that which concerns the seasonal variations of abundance and diversity of the tintinnids, which are an important component of microzooplankton.

The species composition showed little variation with respect to that described previously for this part of the estuary (Barría de Cao, 1981; Barría, 2002). The numerical abundance of tintinnids was similar at both stations, with peaks in abundance at the beginning of the spring and the lowest values registered during the winter (Figs 5 and 6).

Seasonal variation in numerical abundance of tintinnids has been analysed for CuP during an annual cycle and during the period of the phytoplankton bloom (Barría de Cao, 1992; Barría de Cao *et al.*, 1997), hence it is possible to compare the present results with the historic ones. Present values in numerical abundance



Fig. 8. (a) Principal components analysis of environmental variables and numerical abundance and biomass and diversity of tintinnids at Rosales station. (b) Distribution of sampling data in principal component 1 (PC1) versus PC2 space at Rosales Port (RoP).

for CuP are slightly lower than those registered previously (Barría de Cao, 1992) when a minimum of 0.7×10^3 individuals L^{-1} was registered in winter; a maximum of 11.3×10^3 individuals L^{-1} and the annual media was 2.5×10^3 individuals L^{-1} ; on that occasion the peak in abundance was observed in summer. Also, species richness is slightly lower; 19 species of tintinnids were recorded for the inner part of the Bahía Blanca Estuary in 1986–1987 (Barría de Cao, 1992) while only 15 species were recorded in the present study. The reduction of species may be due to two causes, one is the synonymy made for some species based on cytological and morphological studies (Barría, 2002) that was used in this study, and the other, the growing anthropogenic action on the environment that could be affecting the biodiversity.

Values of the Shannon Index, H' varied from 0 (one species present) to 1.81 at CuP and from 0 to 1.59 at RoP. Maximum values were registered at the same sampling date during the summer at both stations (Figs 5 and 6). Both stations showed high correspondence between numerical abundance and diversity, but concerning the biomass, trends were different (Figs 5 and 6). The differences in the biomass could be explained by the presence





Fig. 9. Media and rank of seasonal variations of all variables at Cuatreros station

of a big species: Tintinnopsis lavalae that attained high numbers during the winter in CuP.

In temperate coastal waters, the microzooplankton community shows marked seasonal variations in abundance and biomass (Shin-Ichi et al., 1996). Concerning the seasonal distribution patterns of tintinnids, our results showed also distinct associations: winter, springsummer and autumn groups for CuP, and winter, spring and summer-autumn associations for RoP (Figs 7b and 8b). Different assemblages could be linked to changes in resources. There is a clear segregation of winter season at both stations. Although most of the results about the abundance of ciliates suggest that they may 'track' the

et al., 1988), the situation in the Bahía Blanca Estuary is different. During the bloom of phytoplankton the lowest density of tintinnids was registered and the values of diversity were also low.

The PCA results revealed that the variables that explained most of the variations were the temperature and the solar radiation. Chl a was negatively related to the abundance of tintinnids. The highest values of Chl a were obtained in winter (Fig. 3) during the bloom of phytoplankton formed by diatoms that occurs every year (Freije et al., 1981; Gayoso, 1988, 1998, 1999).

The low densities of tintinnids during this period may be due to the lack of adequate food. Peaks in ciliate abundance are related to cell size phytoplankton prey (Lynn and Montagnes, 1991). Few species of tintinnids (only those that have large peristome and oral lorica diameters) are able to graze on the phytoplankton during the bloom in the Bahía Blanca Estuary (Barría de Cao et al., 1997). The bloom is dominated by chain forming and projections bearing centric diatoms of the genus Thalassiosira and Chaetoceros. Thread extrusion enlarges phytoplankton cell size making it difficult to be ingested by some tintinnids (Verity and Villareal, 1986). The peaks in abundance and the highest values of diversity of tintinnids were registered at the end of the bloom. A trophic coupling may exist between the other seasonal associations and a more diverse phytoplankton which includes short peaks of phytoflagellates principally during late spring and summer (Gayoso, 1998).

The densities of tintinnids recorded in the inner part of the Bahía Blanca are similar to those observed in other estuaries, such as the Nervión River estuary (Urrutxurtu, 2004); Damariscotta River estuary (Sanders, 1987); Narragansett Bay (Hargraves, 1981; Verity, 1987); Seto Inland Sea of Japan (Kamiyama and Tsujino, 1996) and higher than those obtained in some tropical estuaries (Godhantaraman, 2002). The values of biomass obtained in the Bahía Blanca Estuary are lower than those registered in other temperate coastal areas. Verity (Verity, 1987) observed in Narragansett Bay a maximum of 87 μ g C L⁻¹ in spring and summer and a media of 8 μ g C L⁻¹. In Bahía Blanca Estuary, the highest biomass was observed in autumn; however, the lowest values were also obtained during the winter. In comparison with the values of biomass observed in tropical estuaries, biomass in this estuary is quite higher; Godhantaraman (Godhantaraman, 2002) reported a maximum value of only $3.01 \ \mu g \ C \ L^{-1}$ during the summer for estuarine waters in the southeast coast of India.

Dolan and Gallegos (Dolan and Gallegos, 2001) suggest that some factor associated with latitude may determine species richness and species abundances of tintinnids and that a latitudinal gradient of diversity could exist; however, regarding the results obtained, this does not appear so clearly for the Bahía Blanca Estuary. The species richness (15 species) found in the Bahía Blanca Estuary was similar to that found in the Bay of Büyükçekmece, Sea of Marmara, 41° N (14 species) (Balkis, 2004), the Damariscotta River estuary, 44° N (20 species) (Sanders, 1987) and the Chesapeake Bay, 37-39° N (20-25 species) (Dolan and Gallegos, 2001) but was lower than those found in the Bay of Villefranche (32 and 39 species), 43° N (Cariou et al., 1999), and higher than those obtained in some other temperate estuaries of the southern hemisphere such as the Wilson Inlet, Australia, 35° S (7 species) (Gaughan and Potter, 1995). The maximum values of the Shannon Index, H', were also lower than those observed in other coastal systems found at about the same latitude in the northern hemisphere, such as the Chesapeake Bay (Dolan and Gallegos, 2001), where H'reached a value of 2.5 at the end of the summer and the Bay of Villefranche (Cariou et al., 1999), where H' reached a value of 3 during the spring.

In comparison with the temperate eutrophic estuary of Chesapeake Bay, one difference that could be pointed out concerns the salinity, whose values were markedly lower than those registered in the Bahía Blanca Estuary. Anyway, more information about the biomass and species richness of tintinnids from other locations is necessary, especially from the southern hemisphere, and, if possible, following the same sampling, fixation and enumeration protocols to better understand the factors influencing its diversity and species abundance.

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