Journal of the Marine Biological Association of the United Kingdom, page 1 of 7.
doi:10.1017/S0025315411000105

Biomasss and microzooplankton seasonal assemblages in the Bahía Blanca Estuary, Argentinean Coast

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We investigated the occurrence and seasonal variation of the biomass of rotifers, tintinnids, the heterotrophic dinoflagellate Gyrodinium fusus *and copepod nauplii in the Bahía Blanca Estuary (38°42′S61°50 ′W), Argentina, during an annual cycle. The rotifers fauna comprised three species, while the tintinnids were represented by sixteen species. The biomass of the rotifers fluctuated between 0.62 and 8.90* μ*gC* l^{-1}*. The biomass of the tintinnids fluctuated between 0.13 and 9.37* μ*gC* l^{-1}*, the biomass of the nauplii stages between 1.78 and 7.65* μ*gC* l^{-1}*; while the biomass of* G. fusus *varied from 0.26 and 7.94* μ*gC* l^{-1}*, these results are compared to estimates of microzooplankton in other regions. We analysed the presence of the different groups in relation to the environmental variables, based on point-biserial correlation. Salinity fluctuated between 25.14 and 36.64; temperature between 7.5 and 23.2°C, solar radiation between 0.9 and 30.8 MJ* $m^{-2}d^{-1}$ *and Secchi distance between 0.25 and 1.43 m. Rotifers were correlated positively with temperature, chlorophyll-*a *and Secchi depth and negatively with salinity. The tintinnids were positively correlated with salinity.* Gyrodinium fusus *was positively correlated with Secchi depth, and chlorophyll-*a*, and negatively with temperature and solar radiation. Nauplii stages were negatively correlated with chlorophyll-*a*. Based on the occurrence of the microzooplankters in relation to the physico-chemical variables, it was possible to establish two seasonal assemblages: (a) the co-occurrence of the rotifers and the heterotrophic dinoflagellate* G. fusus *during the winter–spring; and (b) the tintinnids and nauplii larvae during the summer. We conclude that, in this estuary, physico-chemical variables are the forcing factors that directly, or indirectly, influence the seasonal assemblages of the microzooplankton.*

Keywords: microzooplankton, biomass, seasonal assemblages, estuaries, Argentina

Submitted 23 June 2010; accepted 7 December 2010

INTRODUCTION

The microzooplankton has a key position as a link in the carbon transfer between the pico and nanoplankton and the mesozooplankton. Some microzooplankters such as tintinnids and rotifers which feed on the smaller primary producers, are a food source especially for calanid copepods (Robertson, 1983; Ayukai, 1987; Stoecker & Egloff, 1987).

Rotifers constitute an important group of the zooplankton community in freshwater, marine and estuarine waters. In the sea they are apparently restricted to coastal areas (Heinbokel *et al*., 1988). In some estuaries they can be the dominant herbivores in the plankton food web (Dolan & Gallegos, 1992), competing even with copepods; and they are, in turn, prey to larger zooplankton. Rotifers can be an important component of the microzooplankton during specific periods in estuarine habitats, with a brief but intensive contribution to the total annual microzooplankton biomass, and may play a relevant trophic role by seasonally replacing other microzooplankters (Park & Marshall, 2000). Fradkin (2001) has observed that rotifers presented the densest aggregations associated with estuary outlets, suggesting that estuaries may be important in exporting rotifers to nearby shore coastal waters.

The occurrence of aloricate ciliates, tintinnids and some heterotrophic dinoflagellates has been previously investigated in the Bahía Blanca Estuary (Barría de Cao, 1992; Barría de Cao *et al*., 1997; 2005; Pettigrosso *et al*., 1997; Pettigrosso, 2003; Barría de Cao & Piccolo, 2008; Pettigrosso and Popovich, 2009), but the rotifers have never been studied in this site.

The present study reports some ecological aspects of the seasonal assemblages of the microzooplankton in the Bahía Blanca Estuary. Our hypothesis is that the environmental factors influence the occurrence of the various groups of the microzooplankton structuring seasonal assemblages in the estuary. In order to test the hypothesis, we carried out an analysis of the seasonal occurrence and abundance of the different groups of the microzooplankton in relation to physico-chemical variables during an annual cycle in the inner part of the Bahía Blanca Estuary.

MATERIALS AND METHODS

The study was carried out at a fixed station located in the inner part of the estuary, Cuatreros Port, (Figure 1), which is described

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elsewhere (Barría de Cao *et al.*, 2005). Sampling was done at surface level, during daylight hours, with a frequency of approximately two weeks, from March 2003 to March 2004. Samples for taxonomic determinations were obtained with 30 μm plankton net in horizontal tows; while samples for enumeration purposes were collected with a 3 l Van-Dorn bottle. All the samples were fixed in Lugol's iodine solution. Together with sampling, measurements of surface temperature, salinity, water transparency, solar radiation, chlorophyll-*a* concentration and phaeopigments were measured all year round. Incident solar radiation was measured with a SKS 1110 (Skye Instruments) piranometer every 10 minutes. Instantaneous values were integrated in order to calculate the daily radiation (MJ m^{-2}). The average of the values of a week previous to the sampling date was used in the analysis. Transparency was measured with a Secchi disc. Chlorophyll-*a* and phaeopigments were estimated following the technique of Lorenzen (1967).

Net samples were observed *in vivo*, some drops of carbonated water were added to inhibit contraction of the specimens prior to fixation. Rotifers were identified following descriptions by Berzins (1960a, b) and Rougier *et al.* (2000) taking into account features such as general morphology, sizes and shapes of relaxed specimens, toe and trophi. The trophi were separated with sodium hypochlorite. Also, some observations on eggs were made. Enumeration was done in concentrated material from 250 ml bottle subsamples by means of an inverted microscope.

The biomass of the microzooplankters was calculated as the total biovolume of the individuals counted for each sampling date. Volumes were calculated by assigning standard geometric shapes to the organisms. For the tintinnids, only the cell volume was taken into account. The conversion of the biovolume to organic carbon units was done applying appropriate conversion factors for each taxonomic group (Beers & Stewart, 1970; Heinbokel *et al.*, 1988; Putt & Stoecker, 1989; Menden-Deuer & Lessard, 2000). Correlations between the microzooplankters and the environmental variables were calculated in order to analyse the influence of the physico-chemical factors on their presence and the seasonal variation. For those groups present all the year round, such as the tintinnids, the correlations were calculated using Pearson's correlation coefficient r (Sokal & Rohlf, 1981). For those that were absent in some period of the year the Pearson's φ was applied: correlation biserial-punctual, for binary data (Kendall & Stuart, 1973).

RESULTS

Species composition of the microzooplankton community

During the study period, three species of rotifers: *Synchaeta cecilia* Rousselet 1902, *Synchaeta* sp. and *Trichocerca marina* Daday 1890 and sixteen species of tintinnids: *Tintinnidium balechi* Barría de Cao, 1981; *Tintinnidium* sp. aff. *T. semiciliatum* (Sterki, 1879); *Tintinnopsis amphora* Kofoid and Campbell, 1929; *Tintinnopsis baltica* Brandt 1896; *Tintinnopsis beroidea* Stein, 1867; *Tintinnopsis brasiliensis* Kofoid and Campbell 1929; *Tintinnopsis buetschlii mortensenii* (Schmidt 1901); *Tintinnopsis glans* Meunier, 1919; *Tintinnopsis gracilis* Kofoid and Campbell 1929; *Tintinnopsis levigata* Kofoid and Campbell, 1929;

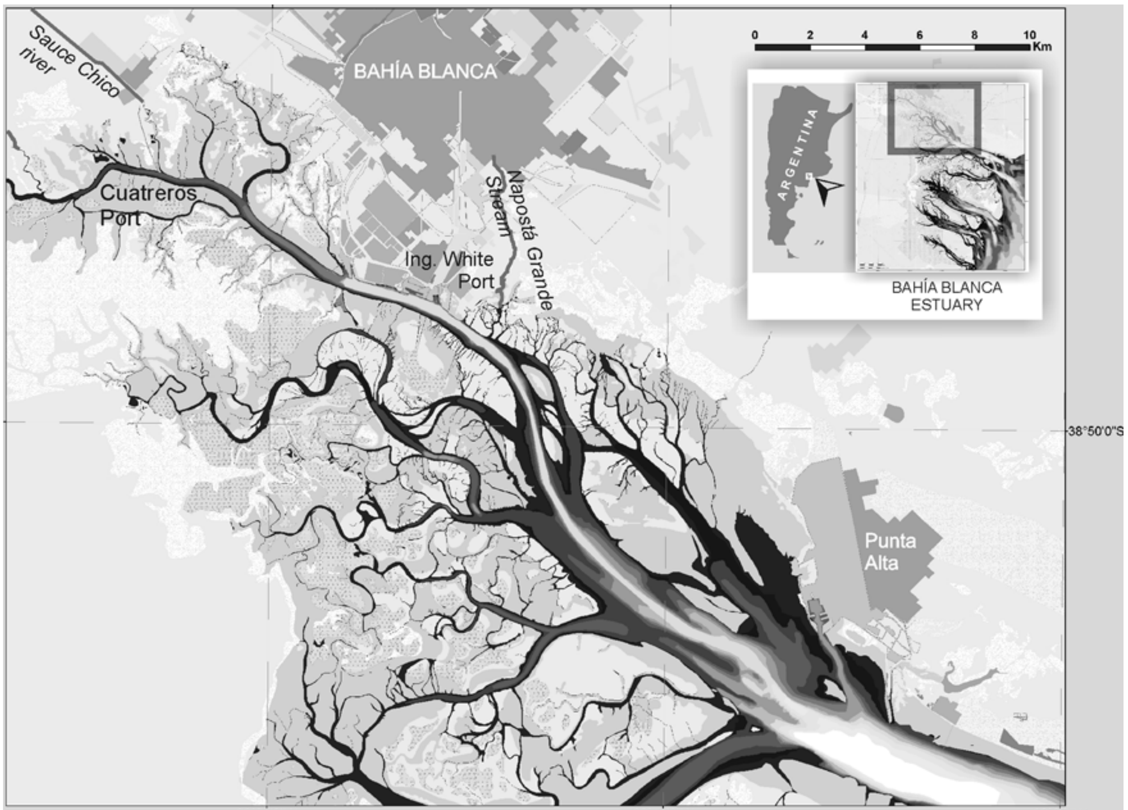

Fig. 1. Location of the sampling station at the Bahía Blanca Estuary.

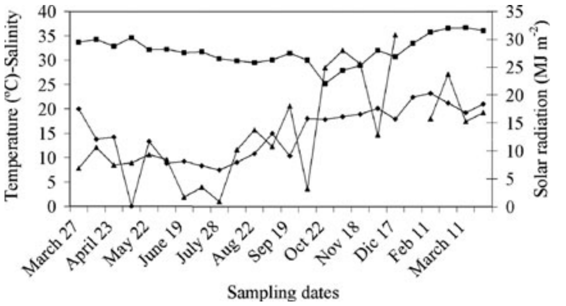

Fig. 2. Temperature, salinity and solar radiation variation at Puerto Cuatreros Station.

Tintinnopsis parva Merkle 1909; *Tintinnopsis parvula* Jörgensen, 1912; *Tintinnopsis* sp.$_1$, *Tintinnopsis sp.*$_2$, *Leprotintinnus pellucidus* and *Codonellopsis lusitanica* Jörgensen 1924, were found. All these species are typical of estuarine and coastal environments.

Ecological observations

ENVIRONMENTAL CONDITIONS, PHYSICO-CHEMICAL VARIABLES AND CHLOROPHYLL-*A*

During the annual period analysed, the temperature varied from 7.5 to 23.2°C and followed the trend of the solar radiation, which fluctuated between 0.9 MJm^{-2}d^{-1} in winter and 30.8 MJm^{-2}d^{-1} at the end of the spring; the lowest value of salinity (25.14) was registered during the spring and the highest (36.64) during the summer (Figure 2). Minimum value of Secchi distance was 0.25 m in summer and the highest (1.43 m) during the winter; also the highest value of chlorophyll-*a* (23.25 mg. m^{-3}) was recorded in winter (Figure 3).

OCCURRENCE, BIOMASS AND SEASONAL ASSEMBLAGES OF THE MICROZOOPLANKTON

Among the rotifers, *Synchaeta* sp. was present in nearly all the samples from winter and spring (90.9%), while *Synchaeta cecilia* and *Trichocerca marina* were observed only in 45.5% of the samples. The highest density (320 ind. l^{-1}) corresponded to *Synchaeta* sp. (Figure 4). In the Bahía Blanca Estuary, rotifers were present at temperatures between 7.5 and 18°C, which is below the mean temperature of the sampling period (Figure 5A–F).

Concerning the other environmental variables analysed, the rotifers were present with salinity values between 25.14 and 31.71, which is below the mean salinity for the period; with 0.9 and 30.8 MJm^{-2}d^{-1}, which is below the mean solar radiation for the period; with 40 and 143 cm, over the mean Secchi depth; with chlorophyll values between 1.59 and 23.25 mg. m^{-3}, over the mean chlorophyll for the period, and with values of phaeopigments between 0 and 6.86 mg. m^{-3}, over the mean value for the period (Figure 5A–F).

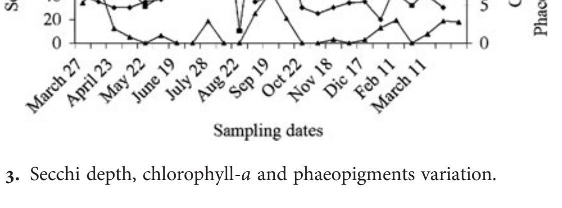

Fig. 3. Secchi depth, chlorophyll-*a* and phaeopigments variation.

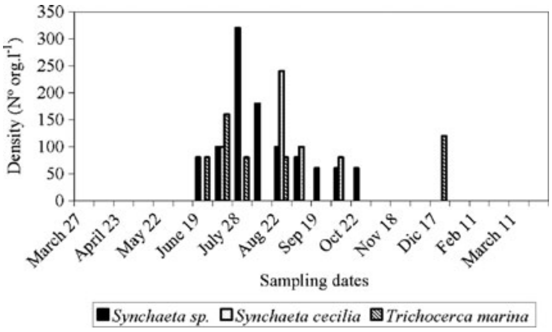

Fig. 4. Density of the different species of rotifers during the annual cycle.

The correlation analysis between the occurrence of rotifers and the environmental variables demonstrated that this group was highly negatively correlated with salinity ($P < 0.001$) and positively correlated with temperature ($P < 0.01$), Secchi depth and chlorophyll ($P < 0.05$) (Table 1).

The occurrence of rotifers was restricted to winter and spring, when peaks in phytoplankton abundance are registered in the estuary (Barría de Cao *et al.*, 1997; Gayoso, 1999). The annual densities of rotifers of the three species together, fluctuated from 60 to 420 ind. l^{-1} (Figure 4). The sum of the biomass of the three species of rotifers varied from $14232 \times 10^3 \mu m^3$ l^{-1}, equivalent to 0.62 μgC l^{-1} at the end of the spring to $171 \times 10^3 \mu m^3$ l^{-1}, 8.90 μgC l^{-1} in mid-winter (Figure 6). The major contribution to the total biomass was due to *Synchaeta* sp., both because of its bigger size and frequency of occurrence. The contribution of the rotifers to the total annual biomass of the microzooplankters analysed was 29%, but this percentage reached 56% during the winter–spring period.

The biomass of tintinnids varied from 0.13 during the spring, to 9.37 μgC l^{-1} in summer.

The tintinnids were present during all the sampling period. This group was correlated positively ($P < 0.05$) with salinity (Table 1).

The biomass of *Gyrodinium fusus* fluctuated from 0.26 to 7.94 μgC l^{-1}. *Gyrodinium fusus* was present at temperatures between 7.5 and 18°C, under the mean temperature for the period; salinity between 29.86 and 31.71, under the mean value of salinity; solar radiation between 0.2 and 10.2 MJm^{-2}d^{-1}, under the mean radiation value for the period; Secchi depth between 40 and 143 cm, over the mean Secchi depth value for the period; chlorophyll between 8.57 and 23.25 mg. m^{-3}, over the mean chlorophyll concentration for the period; phaeopigments concentration between 0 and 3.26 mg. m^{-3}, under the mean phaeopigments concentration for the period (Figure 5A–F). Its occurrence was highly correlated with Secchi depth and chlorophyll ($P < 0.001$), and negatively correlated with temperature ($P < 0.001$) and solar radiation ($P < 0.05$) (Table 1).

The biomass of nauplii stages varied from 1.17 to 7.64 μgCl^{-1}. Nauplii stages were observed with temperature values between 9.2 and 23.2°C, over the mean temperature for the period; salinity

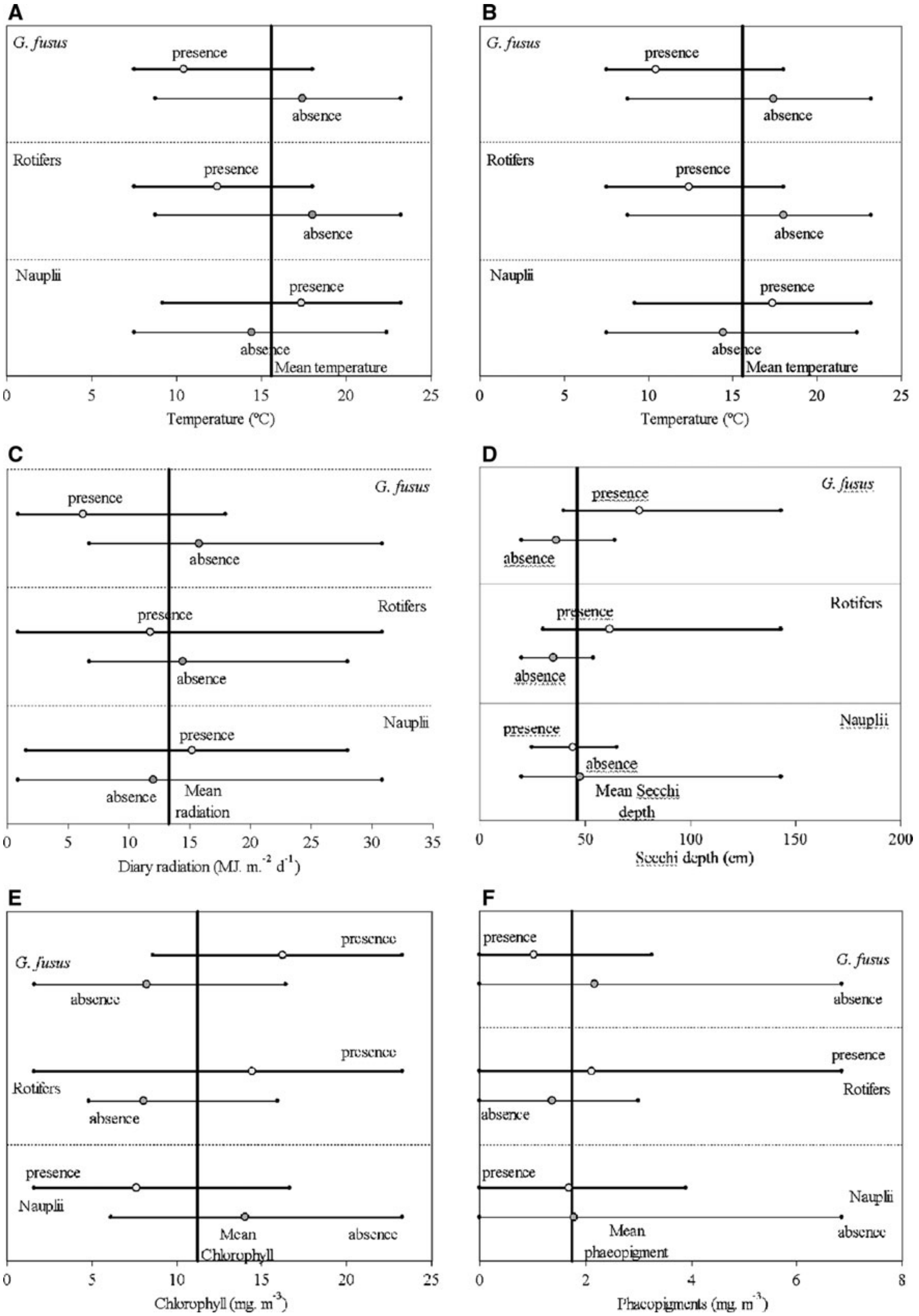

Fig. 5. (A–F) Presence–absence (circles) of the microzooplankters seasonally restricted, in relation to the media and range of the physico-biochemical variables. The horizontal bars represent the range of the parameter and the vertical bars, the mean. A: temperature; B: salinity; C: solar radiation; D: Secchi depth; E: chlorophyll; F: phaeopigments.

between 27.88 and 36.69, over the mean salinity; solar radiation between 1.6 and 23.7 $MJm^{-2}d^{-1}$, over the mean radiation value for the period; Secchi depth between 25 and 65 cm, very close to the mean Secchi depth for the period; chlorophyll between 1.59 and 16.62 mg. m^{-3}, under the mean chlorophyll concentration for the period; phaeopigments concentration between 0 and

Table 1. Correlation between the microzooplankton and the physico-biochemical variables and correlation among the different groups. **significant ($P < 0.01$); *significant ($P < 0.05$); Chl, chlorophyll; ns, not significant ($P > 0.05$); Gf, *Gyrodinium fusus*; R, rotifers; N, nauplii; Ph, phaeopigments; Rad, solar radiation; Sal, salinity; Tin, tintinnids; SD, Secchi depth; T, temperature.

	T	Sal	Rad	SD	Chl	Ph	Gf	R	N	Tin
T	1									
Sal	0.26ns	1								
Rad	0.62**	−0.17ns	1							
SD	−0.45*	−0.05ns	−0.51**	1						
Chl	−0.23ns	−0.33ns	−0.20ns	0.28ns	1					
Ph	0.02ns	0.08ns	0.01ns	0.10ns	0.01ns	1				
Gf	−0.62**	−0.24ns	−0.45*	0.65**	0.63**	−0.28ns	1			
R	−0.57**	−0.56**	−0.16ns	0.51*	0.52*	0.19ns	0.68**	1		
N	0.29ns	0.32ns	0.18ns	−0.06ns	−0.52*	−0.02ns	−0.25ns	−0.30ns	1	
Tin	0.26ns	0.41*	0.36ns	−0.05ns	−0.20ns	−0.18ns	−0.14ns	−0.22ns	0.40*	1

3.9 mg. m^{-3}, very close to the mean phaeopigments concentration for the period (Figure 5A–F). The occurrence of nauplii was negatively correlated with chlorophyll (Table 1).

With respect to the temporal variation of the biomass, a *G. fusus* biomass peak (7.94 μgC l^{-1}) was also found in winter. Conversely, biomass peaks of the tintinnids and nauplii stages (9.37 μgC l^{-1} and 7.65 μgC l^{-1}, respectively), were observed during the summer (Figure 6).

Positive correlation was found between the variation of the biomass of rotifers and the biomass of *G. fusus* ($P < 0.05$) and between the biomass of tintinnids and nauplii ($P < 0.01$) (Table 1). These results showed a co-occurrence of these groups and a segregation of the microzooplankton into two seasonal assemblages: the rotifers and *G. fusus* during winter–spring and the tintinnids and nauplii larvae during the summer.

DISCUSSION

The presence of rotifers was restricted to winter and spring. A winter–early spring diatom bloom has been reported for more than two decades in the estuary and has been one of the characteristics of the seasonal pattern of the phytoplankton (Gayoso, 1988, 1999). Gayoso (1988) suggested to carry out autoecological studies of the blooming species in order to better understand this phenomenon, unusual for its early beginning. Even if the succession pattern of the phytoplankton has suffered changes since 2003 (Popovich *et al.*, 2008), concerning the timing, abundance of the blooming species and magnitude of the bloom, during the period of our study, the maximum values of chlorophyll-*a* were registered in winter, showing the existence of a peak of phytoplankton—possibly of a minor magnitude-, during that time. The positive correlation between the abundance of rotifers and the concentration of chlorophyll-*a*, could indicate a trophic dependence upon some of the phytoplankton species present during that peak such as diatoms of the genus *Thalassiosira*.

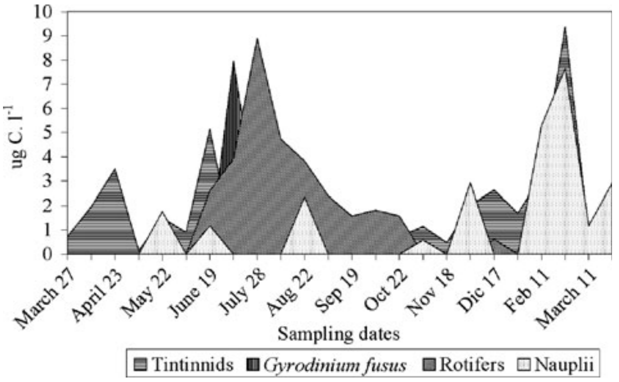

Fig. 6. Seasonal variation of the biomass of the different microzooplankton groups in terms of carbon.

The genera *Synchaeta* and *Trichocerca* seem to be restricted to coastal waters. Fradkin (2001) has found these genera within 16 km of the shore and with increasing abundance closer to shore in the north-east Pacific Ocean.

The dynamics of rotifer populations in the estuary must also be related to the strategy of diapausal egg production, which has not been studied here. Diodato *et al.* (2006), observed eggs in the sediments of Cuatreros Port whose characteristics agree with diapausal eggs of the species *Synchaeta* sp. These eggs, found mainly in samples collected during the warm months could explain, in part, the dynamics of the rotifers populations. In our study, all the individuals of *Synchaeta cecilia* and *S.* sp. observed were females, carrying eggs attached to the foot, or with eggs inside the body. In the case of *Trichocerca marina*, few eggs were encountered, always free in the samples, not attached to the bodies. These eggs were probably close to hatching point, as the embryos were well developed and visible inside them.

During the phytoplankton peak, the other group of the microzooplankton present, the tintinnids, are rather scarce (Barría de Cao, 1992), and this may be due to the lack of adequate food (Barría de Cao et al., 1997, 2005). The aloricate ciliates do not have a grazing impact on the phytoplankton during the diatom bloom either (Pettigrosso & Popovich, 2009); so, perhaps, the trophic niche may be occupied by the rotifers. Concerning *G. fusus*, this heterotrophic dinoflagellate could be competing with the rotifers for the same food source since its presence was restricted to the same season and we have observed some *Thalassiosira* cells inside the food vacuoles. Comparing the seasonal variation of the biomass of the microzooplankters analysed with other coastal waters of the world, the contribution of the rotifers to the total biomass of microzooplankton was very high in winter–spring; which coincides with the observations of Fradkin (2001) about a brief but intensive contribution of the rotifers to the annual biomass in the coastal waters of the north-east Pacific Ocean.

Concerning the tintinnids, we registered the highest biomass in summer, while Tillmann & Hesse (1998) observed the dominance of tintinnids in winter in the Wadden Sea, a shallow basin with similar characteristics of turbidity and eutrophication.

The maximum biomass of aloricate ciliates occurred during winter in this estuary, fluctuating from an average of 8.18 in summer, to 16.6 μgC l^{-1} in winter (Pettigrosso & Popovich, 2009). For the northern hemisphere, Pilling *et al.* (1992) estimated a similar value of biomass, 12 μgC l^{-1} in summer, for aloricate ciliates from surface waters of Plymouth coastal waters. Also, Tillmann & Hesse (1998), reported the largest contribution of aloricate ciliates and dinoflagellates in the Wadden Sea in spring and summer.

About the biomass of heterotrophic dinoflagellates from other areas, Verity *et al.* (1993) have reported an average value of 0.5 to 10.9 μgC l^{-1} for two years of study in oceanic waters in the North Atlantic, which included aplastidic dinoflagellates comprising two size-classes: cells $<$20 μm and cells $>$20 μm. In comparison, *G. fusus* alone, contribute highly to the total biomass in the Bahía Blanca Estuary during the season of its occurrence.

Some reported data on biomass of copepod nauplii are in the range of 0–7μgCl^{-1} (Laizhou Bay, Bohai Sea, China; Zhang & Wang, 2000) which are similar to those found in the Bahía Blanca Estuary.

Based on the occurrence of the microzooplankters in relation to the physico-chemical variables, the annual variation of the biomass of each group, and the correlation of the presence among the different groups, it was possible to establish two seasonal assemblages in the microzooplankton: (a) the co-occurrence of the rotifers and the heterotrophic dinoflagellate *G. fusus* during the winter–spring; and (b) the tintinnids and nauplii larvae during the summer; although if the tintinnids are present all the year round, their abundance was more strongly related to the environmental conditions of the summer season.

In this study, we found that salinity was the main variable associated with the tintinnids seasonal assemblages; however, in prior studies (Barría de Cao *et al.*, 2005) it was observed that, also the temperature and the solar radiation explained the seasonal variations. Turbidity represented an important factor for the presence of *G. fusus*, as was previously reported (Barría de Cao & Piccolo, 2008).

Another factor that should be investigated in the structuring of seasonal assemblages is the predation upon the microzooplankton. Grazing experiments carried out in the estuary have shown that mesozooplankton consumers presented high filtration rates on microzooplankton. Diodato & Hoffmeyer (2008) demonstrated that 88% of the available microzooplankton was consumed by the mesozooplankton, against only 51% of the phytoplankton.

It may be concluded that the physico-chemical variables are the forcing factors that influence directly, based on specific preferences, or indirectly, affecting the quality and abundance of the food resources, on the seasonal assemblages structuring the microzooplankton community in the Bahía Blanca Estuary.

ACKNOWLEDGEMENTS

We are grateful to Oceanólogo Eder dos Santos for his help with sample collection, to the Marine Chemistry Laboratory of the Instituto Argentino de Oceanografía for chlorophyll determinations, and to Dr Walter Melo for the map and the editing of the figures. The authors thank the referees for their constructive criticism of the manuscript. This work was supported by the Agencia Nacional de Promoción Científica, Tecnológica y de Innovación, ANPCYT, Government of Argentina (PICT 21412) and the Universidad Nacional del Sur (PGI 24/H/082).

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