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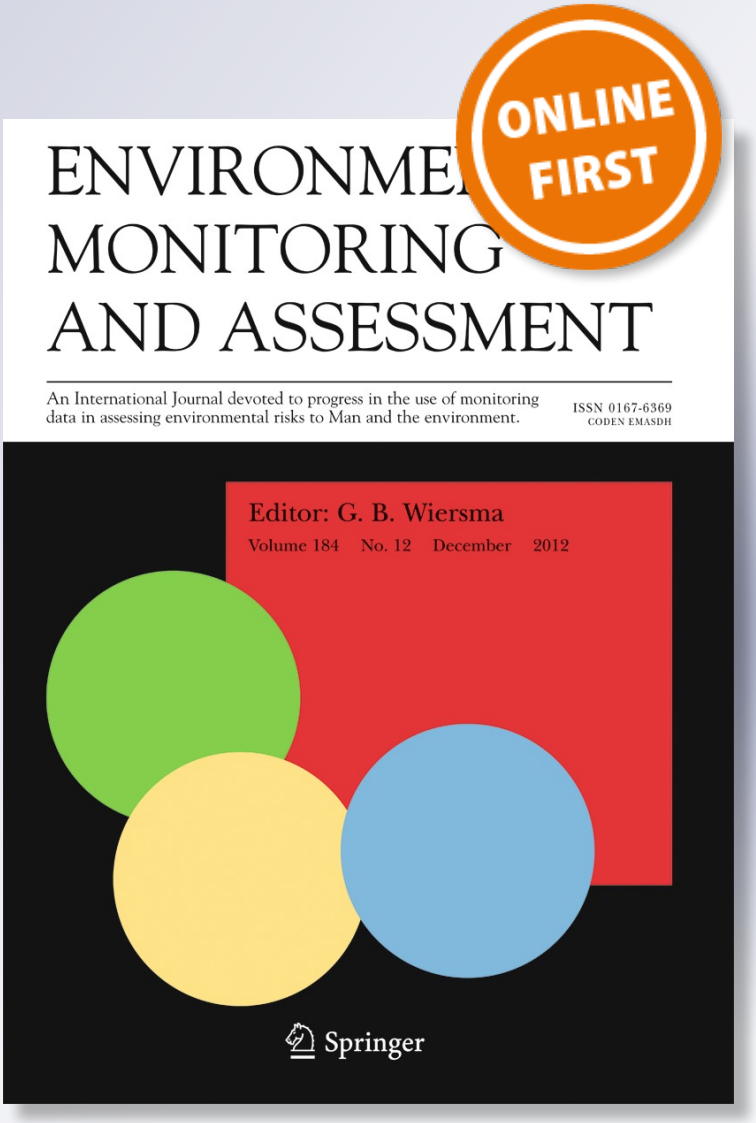

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Abstract The present work includes part of the first studies of metals concentrations in the zooplankton from a heavily industrialized estuary of Argentina, the Bahía Blanca estuary. Cd, Cu, and Pb concentrations in the zooplankton (macro- and mesozooplankton) and the suspended particulate matter were measured at stations with different degree of pollution. Physicochemical variables and zooplankton composition and abundance were also analyzed. Thus, the aim of the present work was to analyze the spatial and temporal distribution of heavy metals in these two different fractions, and the possible relation among them due to their importance in the biogeochemical cycles of marine environments. Samplings were carried out during a year, from March 2005 to April 2006, every 2 months, at stations located near chemical and petrochemical industries, stations far from these points and one station in an intermediate location. In the mesozooplankton, the mean concentrations of Cd, Cu, and Pb were 3.63±1.46, 34.46±5.40, and 11.54±3.04 $\mu g g^{-1}$ dry weight (d.w.) respectively, while in the macrozooplankton, 3.20±2.28, 21.86±4.79, and 8.36±1.85 $\mu g g^{-1}$ d.w. On the other hand, particulate Cd, Cu, and Pb presented a mean concentration of 3.33±1.22, 12.75±2.67, and 12.53±3.20 $\mu g g^{-1}$ d.w., respectively. Metals' levels in both the SPM and zooplankton fluctuated throughout the study time and were relatively high in the particulate phase especially for Cu and Pb. Moreover, zooplankton accumulated important concentrations of the three metals. The sources of them are probably the discharges of the industries and domestic sewages located near the estuary.

Keywords Heavy metals · Estuary · Zooplankton · Suspended particulate matter · Pollution

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Introduction

Heavy metals are persistent contaminants widely distributed in nature, in compartments, such as water,

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soil, air, and different organisms, which can produce damage to the environment and finally jeopardize human health. Some of them, such as Cu, Zn, Mn, Fe, and Cr are essential for the metabolism of the organisms while others like Cd, Pb, Hg, and As are nonessential. However, all such metals, essential or not, are toxic above a threshold biovailability (Rainbow 1993).

Estuarine environments are contaminated by human waste containing elevated concentrations of these trace metals as well as nutrients, organic pollutants, and radionuclides (Kennish 1997). These areas are therefore important for the fate of contaminants and hence are worth studying. Additionally, estuaries are unique ecosystems with dynamic and complex processes of ecological and economic importance, and they are among the most productive environments on Earth due to the presence of high phytoplankton and zooplankton biomass, key components in aquatic food webs (Omori and Ikeda 1984). Particularly, zooplankton is critical to the functioning of ocean food webs because of their sheer abundance and vital ecosystem roles. The most prominent zooplankton, the copepods, are the most abundant multicellular animals on Earth (Schminke 2007) and one of the most important role is as the major grazers in ocean food webs, providing the principal pathway for energy from primary producers to consumers at higher trophic levels (Richardson 2008). Additionally, they play an important role in the biogeochemical cycling of trace metals in marine ecosystems. Thus, zooplankton grazing can strongly influence the fate of carbon and trace metals associated with phytoplankton biomass (Fowler and Knauer 1986). During grazing, metals which are assimilated into grazer biomass enter the organic cycling in the sea and may have longer residence times in the surface waters (Whitfield and Turner 1987; Reinfelder and Fisher 1991). Metals that are egested with sinking fecal pellets will be exported out of the surface waters (Fowler and Knauer 1986), enriching the deep-water dissolved-metal pool through remineralization and release (Fisher et al. 1991). Cellular metals regenerated in the dissolved state during grazing may be recycled many times and re-utilized by the phytoplankton community (Hutchins et al. 1993; Wang and Fisher 1998a, b). The relative importance of these different biogeochemical pathways is metal specific and can vary greatly with grazing conditions. In addition, zooplankton can be used as biomonitors due to their potential role as indicators of the minimal lethal concentration of metals and also their ability to accumulate metals (Fang et al. 2006). Accordingly, metal accumulated in these organisms has also the potential to provide information on the applied relevance in terms of geographic and temporal variation in the bioavailabilities of toxic metals in aquatic systems (Rainbow 2002).

Suspended particulate matter (SPM), comprise sediments and seston which are suspended in the water column, either permanently, or temporarily and in a state of exchange with bed sediment reservoir. Estuarine SPM is derived from continental and coastal erosion, in situ chemical and biological processes, the atmosphere, and industrial activities. These particles are instrumental in controlling the reactivity, transport and biological impacts of substances in aquatic environments, and provide a crucial link for chemical constituents between the water column, bed sediments, and food chain (Turner and Millward 2002). When associated with heavy metals, particulate metals eventually settle to the bottom and may be available to benthic organisms or may be released to the water column through resuspension, adsorption/desorption, reduction/oxidation reactions, or degradation of organisms. Therefore, the SPM has a dominant role in controlling the trace metal composition of aquatic environments (Balls 1990). Although, the behavior of heavy metals in estuaries can be very dynamic, and their concentration may vary over large ranges, temporal distribution in the SPM and zooplankton provides information for defining temporal variations of a specific element, regional concentration differences, and different elemental behaviors. Thus, the main objective of the present study was to analyze metals accumulation of Cd, Cu, and Pb in zooplankton organisms and SPM in different times of the sampling period and the relationship between these fractions. In addition, the study covered sampling stations located near industrial settlements and stations far from these points in order to detect the possible influence of the industrial and urban discharges on the metal concentrations. There are many studies on trace metals in particulate matter and zooplankton; however, few articles analyze these two fractions together (e.g., Nguyen et al. 2005; Fang et al. 2006). Many authors have published valuable information on metal concentrations in different matrixes, but they did so separately without establishing a relation between biotic (organisms) and abiotic (geologic, aquatic, etc.) compartments. In zooplankton of coastal environments of Argentina, the information on metal concentrations is

very scarce (Scarlato et al. 1993, 1997). It is worth noting that the present work is part of the first records of Cd, Cu, and Pb concentrations in meso- and macrozooplankton in association with the SPM. Moreover, no previous reports have analyzed metals concentrations in *Eurytemora americana*, an important copepod of the mesozooplankton. Similarly, there are no results of metals levels in the mysids *Arthromysis magellanica* and *Neomysis americana*, commonly found in the macrozooplankton. Therefore, this work is a contribution to knowledge about the levels of Cd, Cu, and Pb in these key species of estuarine zooplankton.

Materials and methods

Study site

The Bahía Blanca estuary is a mesotidal coastal plain estuary located in the southwest of the Buenos Aires Province in Argentina (38°45′–39°40′ S and 61°45′–62°30′ W). It extends over about 2,300 km^2 and comprises several tidal channels, extensive tidal flats (1,150 km^2) with patches of low salt marshes, and islands (410 km^2) (Fig. 1). It is a highly turbid estuary with sediments mainly represented by silts and clays (Perillo and Piccolo 1999). Based on the salinity and temperature distribution, the estuary is divided in two zones. The inner one (from the mouth of the Sauce Chico River to Ingeniero White Harbor) is classified as a partially mixed estuary during normal runoff conditions, but with a strong tendency to become vertically and even sectionally homogeneous during low runoff. The outer zone (from Ingeniero White Harbor to the mouth of the estuary) is sectionally homogeneous (Piccolo et al. 2008). The estuary is among the most productive marine ecosystems in Argentina, presenting a high phytoplankton density, as well as high nutrient concentrations. On the other hand, the northern boundary of the Bahía Blanca estuary is affected by different anthropogenic activities due to the presence of the urban, industrial, and harbor areas. The main urban discharges correspond to the Bahía Blanca city and the towns of General Cerri, Ingeniero White, and Punta Alta (350,000 inhabitants). Also, oil refineries and terminal, petrochemical industries, fish and leather factories, textile plants and wool washing plants, silos, and cereal mills discharge their processing residues into the streams or directly into the estuary. It is extensively used by fishing boats, oil tankers, and cargo vessels and thus requires regular dredging. Therefore, this coastal marine ecosystem receives inputs from urban wastewaters, direct industrial discharges, harbor-related operations, and runoff water that carry material from land development areas and aerial fallout from atmospheric pollutants.

Field sampling

Samples were collected from March 2005 to April 2006, at five sampling stations, approximately every 2 months and always during ebb tide. Stations 1 and 2 are located near the industrial area represented by oil petrochemical and chemical industries. Periodical dredging, artisanal and commercial fisheries, and oil and cereal cargo vessel traffic usually affect this area, as well as the sewage discharges of Bahía Blanca, Ingeniero White, Punta Alta, and General Cerri cities. Station 3 is located 7 km far from this industrial zone and is near a landfill of no organic garbage. The other sampling sites, stations 4 and 5 are located 11 and 13 km far from the industrial area, and they are expected to present less or at least different anthropogenic pressure due to their location (Fig. 1). All these stations were selected with the aim to detect differences between areas with human influence from those with less pressure. Water and zooplankton samples (1 m deep from the water surface) were taken aboard the R/V Buen día Señor at each sampling station. The physicochemical variables like *T*°, pH, conductivity, and dissolved oxygen were recorded at 1 m depth with a water quality checker U-10 (Horiba Ltd., Japan). Salinity was calculated from the conductivity data and in this study was reported using the practical salinity unit.

Cleaning procedures

Cleaning procedures followed internationally recommended protocols (APHA 1998). Before use, all the equipment for the collection, storage, and filtration of the samples was soaked in ultrapure 0.7 % HNO_3 for 48 h and rinsed four times with deionized water. Millipore HA filters (0.45 μm pore size) for metal analyses in the SPM were also soaked in 0.7 % HNO_3 for 48 h, rinsed with deionized water, dried to constant weight in individual Petri dishes in a laboratory stove at 50±5 °C during 56 h, and then weighed in analytical balance (OHAUS, Adventurer™).

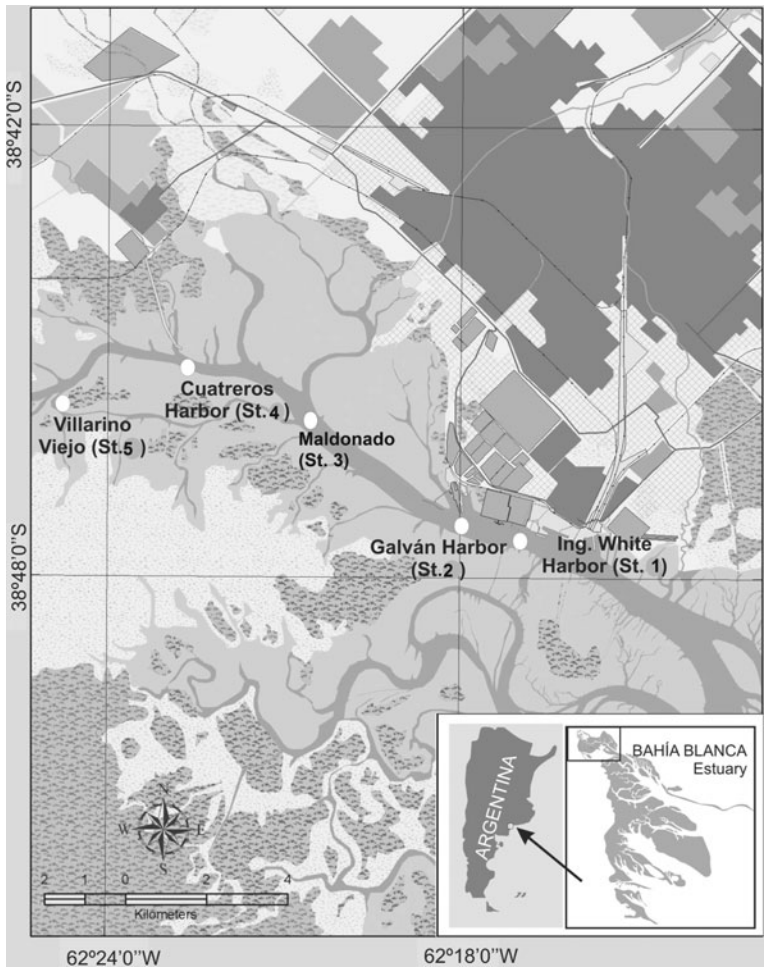

Fig. 1 Map of the study area, the Bahía Blanca estuary, with the location of the sampling stations

SPM analysis

Water samples for the determination of metals in the SPM were collected by hand at 1 m depth with 1.5 l polyethylene-tereftalate bottles previously cleaned with 0.7 % HNO_3. At each sampling site, two subsamples of 750 ml were considered, obtained from the homogenization of the bottle and dividing the volume (1.5 l) into two equal parts. All the samples were stored in cooler cases and immediately transported to the laboratory. Then, they were filtered through a 200-μm mesh to avoid organisms like zooplankton in the sample. Later, the samples were filtered with vacuum through dry pre-weighted Millipore HA filters (0.45 μm pore size) and pre-cleaned with ultrapure 0.7 % HNO_3 to separate the particulate fraction. The filters with the retained SPM were dried in a laboratory stove at 50±5 °C during 56 h to constant weight, then weighed in analytical balance (OHAUS, Adventurer™) and stored in a desiccator until their analytical treatment. The content of SPM (in grams) in each sample was calculated as: (weight of filter with SPM)−(weight of the same filter but without SPM, previous the filtration proceeding). Then the filters were subjected to acid mineralization, following the methodology of Marcovecchio and Ferrer (2005). Each sample was mineralized with a mixture of 3 ml of HNO_3 and 1 ml of concentrated $HClO_4$ in a bath of glycerin at 110±10 °C to obtain an extract of about 1 ml. Then, each of them was transferred to a graduated tube and filled with 0.7 % HNO_3 to a volume of 10 ml. The same digestion procedure was performed for filters without particles to act as blanks. All the fractions were analyzed in duplicate to ensure the reproducibility of the method. Metals concentrations were measured with a Perkin–Elmer AA-2380 atomic absorption spectrophotometer with an air/acetylene flame.

Analytical grade reagents were used to make the relevant blanks and calibration curves, and the analytical quality was tested against reference materials (mussel tissue flour R.M. No. 6) provided by the National Institute for Environmental Studies, from Tsukuba (Japan) as well as from the United Nations Environment Programme and estuarine water BCR-505 by Institute for Reference Materials and Measurements, Geel, Belgium. The obtained values from the analysis of the reference materials were within the range of the certified ones. Within the scope of an analytical assessment quality, percentages of recovery of same materials were higher than 90 % (Table 1). Analytical precision expressed as coefficients of variance was <10 % based on replicate analysis. The method detection limits (MDL) for Cd, Cu, and Pb in SPM and zooplankton are: 0.01, 0.04, and 0.50 $\mu g g^{-1}$ d.w., respectively.

Particulate organic matter, nutrients, and pigments analyses

Additional water samples (500 ml) were collected to determine inorganic nutrients, photosynthetic pigments, and particulate organic matter (POM). For POM, water was filtered through previously dried 1.2 μm Whatman GF/C filters, then 4 ml of sodium sulfate was added and finally the filters were stored at 4 °C until the analysis. Later, the POM concentrations were determined following the methodology of Strickland and Parsons (1968) and using an UV–visible spectrophotometer. Water samples were also filtered through Whatman GF/C for the study of dissolved inorganic nutrients, and frozen until they were analyzed. Nitrate, nitrite, ammonium, phosphate, and silicate were determined by standardized methods (Eberlein and Kattner 1987; Technicon Autoanalyzer II 1973; Treguer and Le Corre 1975) using a Technicon AAII autoanalyser. Chlorophyll *a* and phaeopigments were spectrophotometrically determined according to Lorenzen and Jeffrey (1980) from 250-ml water sample aliquots filtered through Whatman GF/C filters, which were stored at 20±1 °C until they were used for analytical treatment.

Table 1 Percentages of recovery in the analysis of reference materials (NIES) and (UNEP) to assess analytical quality (AQ)

Metal analyzed	Estuarine water (%)	Seawater (%)	Organisms (%)
Cadmium	91.1–95.3	93.1–95.7	92.4–98.7
Copper	92.9–96.5	92.2–97.5	93.1–97.9
Lead	92.9–96.5	92.2–97.5	95.3–99.8

Zooplankton analysis

Each meso- (200 μm–2 mm) and macrozooplankton (2–20 mm) samples consisted of two replicates of approximately 750 ml at each sampling station. Two Nansen nets (200 μm pore size and 30 cm mouth diameter) and a Bongo net (500 μm pore size and 40 cm mouth diameter) for meso- and macrozooplankton were respectively collected. The nets were equipped with a General Oceanics Model 2030 flowmeter. The tows were oblique (0.5–1 m depth) and performed at a constant speed (2 knots) during 5 min. The samples were then stored in cooler boxes and transported to the laboratory. For metal determination, mesozooplankton samples were prefiltered using a 2-mm mesh size net to retain unwanted particles and then this samples were filtered using a 20-μm mesh size net to obtain mesozooplankton fraction. For macrozooplankton, nets of 2 mm mesh size were used. Then, all the samples were filtered with low vacuum (<20 cmHg) and then were subjected to acid mineralization, following the same procedure as the SPM.

Qualitative and quantitative zooplankton analyses

To determine the composition and abundance of the zooplankton, sub-samples of 100 ml were obtained from the original samples of 750 ml and then preserved in 10 % formaldehyde until their analysis. Once in the laboratory, the meso- and macrozooplankton were counted and identified to the lowest taxonomic level as possible, using a stereoscopic microscope (Nikon SMZ 645) and microscope (Nikon Eclipse 80 i).

Statistical analyses

Blocked nested analysis of variance (ANOVA) and Bonferroni test were used to detect differences between the sampling stations in relation to both the physicochemical variables and metals concentrations in the different fractions (SPM, mesozooplankton, and macrozooplankton) according to Zar (1999). Previously,

some of the variables were transformed to the square root to meet the assumptions of normality and homocedasticity required for the ANOVA tests. When more than 40 % of the censored data of metals concentrations were below the MDL, the results were substituted by one half the detection limit (Clarke 1998). Statistical analyses were performed using a software package provided by the Statistical Area of the Department of Mathematics, at Universidad Nacional del Sur (Argentina).

Results and discussion

Physicochemical variables

The temporal and spatial distribution of the physicochemical variables are shown in the Fig. 2 and the blocked ANOVA and Bonferroni tests to detect differences of these variables among the sampling stations are detailed in Table 2. In this table, sampling stations with different letters present significant statistical differences between them.

Temperature in the subsurface layer of the water column (0–1 m) varied from 7.6 to 24.1 °C showing the regular seasonal cycle, with low values in July (winter) and high in February (summer). In addition, no significant statistical differences were detected between the sampling stations since temperature values were very similar among the stations, despite their location (Fig. 2a). Also, the pH values were very homogeneous and varied between 7.9 and 9.3 with no significant differences (Fig. 2b). Salinity presented a range of 25.7 and 37.1 with temporal variations (Fig. 2c). The low values were registered in May, July (winter), and September (spring) and the high ones in November (late spring), December and February (summer). This temporal behavior is typical in the Bahía Blanca estuary (Piccolo et al. 2008). This study area becomes hypersaline during warmer months as a consequence of the high temperature, which favors water evaporation and hence an increase of salinity and also due to the low rainfalls during summer. Taking into account the sampling stations, some statistical differences were detected. The stations located far from the industrial area (stations 5, 4, and 3) presented lower salinity values than those near the industries. These results coincide with Piccolo et al. (2008) which found that the mean surface salinity increases from the head of the estuary, where stations 5 and 4 are located, to the middle reach of the estuary.

Dissolved oxygen presented a range of 5.6 and 14.62 mgl^{-1} and significant differences among the stations (Fig. 2d). Particularly, the farthest station from the industrial area (station 5) presented the highest concentrations. On the other hand, high values recorded in April, May, and July (autumn–winter) are related to the low temperatures which favor oxygen solubility and also to the phytoplankton bloom thorough the photosynthesis process (Freije and Gayoso 1988). During winter an important phytoplankton bloom develops in the estuary which produces variations in some of the physicochemical variables, such as dissolved oxygen, nutrients, and pigments. On the other hand, the low values of dissolved oxygen in November, December, and February (late spring–summer) are due to the temperature increases as well as to the phytoplankton decrease caused by low phytoplankton abundance. In addition, the recorded concentrations and the temporal distribution are consistent with previous studies in the estuary (e.g., Ferrer et al. 2003; Marcovecchio et al. 2008) and are indicative of well-oxygenated estuarine waters.

With regard to photosynthetic pigments, chlorophyll *a* presented a minimum of 0.29 and a maximum of 28.86 μgl^{-1} with a definite trend during the study period and statistical differences among the sampling stations (Fig. 2e). In July and September (winter–early spring), higher concentrations were recorded (18.91–28.86 μgl^{-1}) as a consequence of the phytoplankton bloom which increase chlorophyll *a* levels. In February, a small pulse of phytoplankton was also detected, since chlorophyll *a* levels increase in this month with a maximum of 15.71 μgl^{-1}. During the rest of the period, the concentrations were remarkably lower (0.29–6.20 μgl^{-1}). All this temporal pattern coincide with historical data for the Bahía Blanca estuary (Marcovecchio and Freije 2004; Marcovecchio et al. 2008; Popovich et al. 2008), and it is worth noting that this estuary is very productive since chlorophyll *a* levels have never been null. On the other hand, phaeopigments presented a range of 0 and 17.79 μgl^{-1} and an opposite behavior to chlorophyll *a* (Fig. 2f). During the phytoplankton bloom, phaeopigments showed low to null concentrations in most of the sampling stations, and after this bloom phaeopigments began to increase as a consequence of the degradation of phytoplankton. This behavior is typical in the estuary and Botté (2005) and

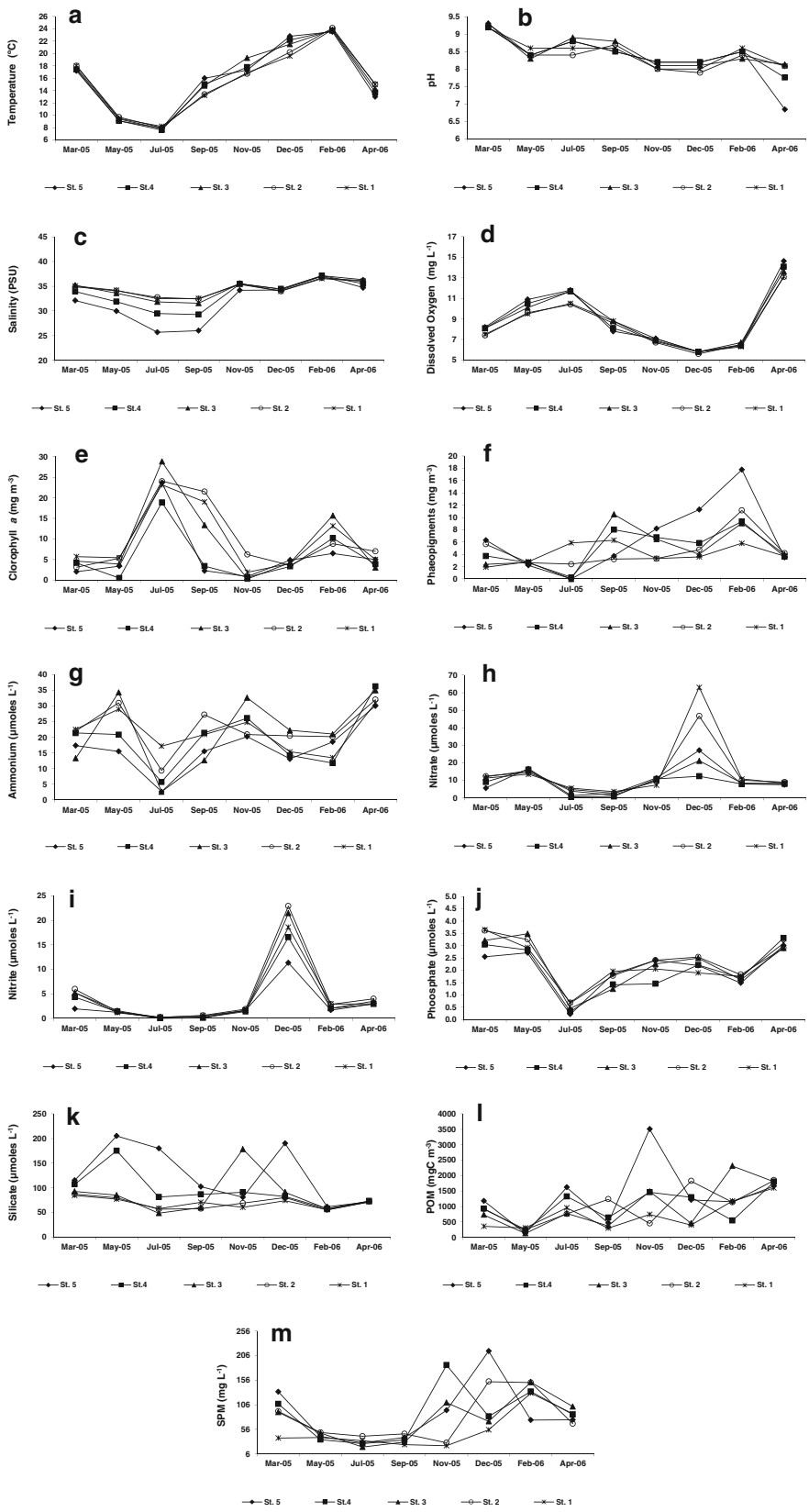

Fig. 2 Temporal and spatial distribution of the physico-chemical variables in the Bahía Blanca estuary. *POM* particulate organic matter, *SPM* suspended particulate matter

Table 2 Blocked nested ANOVA and Bonferroni test of the physicochemical variables

ANOVA test		Bonferroni test				
		Groups				
Variables	*p*	Station 1	Station 2	Station 3	Station 4	Station 5
T°	0.76 ns	–	–	–	–	–
Salinity	0.002[a]	b	b	b	ab	a
pH	0.76 ns	–	–	–	–	–
Dissolved Oxygen[c]	0.009[a]	a, b	a	a, b	a, b	b
Chlorophyll *a*[c]	0.02[b]	c	c	b, c	a	a, b
Phaeopigment[c]	0.69 ns	–	–	–	–	–
Ammonium	0.11 ns	–	–	–	–	–
Nitrate[d]	0.03[b]	b	a, b	a, b	A	a, b
Nitrite[d]	0.004[a]	b	b	a, b	a, b	a
Phosfate[c]	0.03[b]	a, b	b	a, b	a	a
Silicates[d]	0.003[a]	a	a	a, b	a, b	b
POM[c]	0.29 ns	–	–	–	–	–
SPM[c]	0.3 ns	–	–	–	–	–

The sampling stations with different letters present significant statistical differences

POM particulate organic matter, *SPM* suspended particulate matter, *ns* not significant differences

[a] Highly significant differences

[b] Significant differences

[c] Square root transformation

[d] Natural logarithm transformation

Spetter (2006) among other authors have already described it. In addition, no significant differences were detected among the stations, indicating a similar trend.

Nutrients like ammonium, nitrate, nitrite, phosphate, and silicate were also analyzed. The first of these nutrients, the ammonium, presented a range of 2.60 and 36.25 $\mu mol l^{-1}$, and the sampling stations showed similar levels since no statistical differences were detected among them (Fig. 2g). In coincidence with the phaeopigments, this nutrient strongly decreased in July (winter), when the phytoplankton bloom occurred, and this behavior is very common since the phytoplankton needs this nutrient to survive (Spetter 2006). On the other hand, the maximum concentrations coincide with high zooplankton abundances, thus these organism could be sources of ammonium through both the grazing of phytoplankton and excretion mechanisms. In the case of nitrate, the concentrations were between 0.47 and 63.05 $\mu mol l^{-1}$ and some stations differed statistically (Fig. 2h). Again, low nitrate were recorded in July indicating the influence of the phytoplankton on this nutrient. After this bloom, the levels began to increase reaching the maximum in December (summer). Later, in February (summer) due to another bloom but smaller than the winter, nitrate decreased due to the consumption of this nutrient by the phytoplankton. This temporal behavior coincided with previous studies which described the temporal cycle of nutrients in the estuary (Spetter 2006; Popovich et al. 2008). Nitrite presented the same trend as nitrate, being the phytoplankton responsible of the temporal variations of this nutrient. The recorded range was 0.03 and 22.90 $\mu mol l^{-1}$ and statistical differences were detected between the stations (Fig. 2i). Phosphate also presented the lower values in July and the same behavior as the previous mentioned nutrients. The range was 0.21–3.64 $\mu mol l^{-1}$ and statistical differences were detected (Fig. 2j). Besides, this result coincides with the studies of Spetter (2006). On the other hand, silicate presented a different behavior. The concentrations decreased in July but also in September and November (Fig. 2k). Although phytoplankton consumes Si, mainly for their frustules, the

regeneration of this important nutrient is slower than the other ones.

In this study, we considered the concentrations of two types of particulate matter: the POM and the SPM. The range of the POM was: 130–3,505 mg Cl^{-1}, and no statistical differences were detected among the stations (Fig. 2l). The results showed that the maximum concentrations were recorded in July, November, December, and February. But particularly the levels in July and February coincide with high phytoplankton abundances, indicating a relation between them. Most of the POM in these months was originated from the phytoplankton. In addition, other sources like detritus of halophyte vegetation and discharges of wastewaters may contribute to organic matter levels (Botté 2005). The concentrations of SPM were within the normal values recorded in the Bahía Blanca estuary, with a minimum of 22.54 and a maximum of 214.92 mgl^{-1} (Fig. 2m). Considering the stations, no statistical differences were detected. This environment is very turbid as a consequence of the resuspension of silt and clay, fine sediments which are strongly influence by tidal currents and winds from the north and northwest. Besides, the main navigation channel of the estuary is regularly dredged which increases the resuspension of particulate matter (Marcovecchio and Freije 2004).

The Bahía Blanca estuary is a natural nutrient-enriched environment, maintaining significant levels of inorganic compounds during most of the year and low concentrations during the presence of the phytoplankton bloom (Marcovecchio and Freije 2004). Our results of the dissolved nutrients showed the typical seasonal behavior, with maximum values during autumn and spring–summer and minimum during winter. Ammonium, nitrite, nitrate, and phosphate concentrations decrease abruptly in July, during the phytoplankton bloom, due to the consumption of these primary producers. The high levels recorded in the autumn months (March–May) are related to the rains which take place in the estuary during this period. In spring, after the bloom, the concentration increases and a "recovery" phase take place (Marcovecchio and Freije 2004; Spetter 2006; Marcovecchio et al. 2008). This phase is associated to the re-mineralization of the organic matter generated in the bloom and also to the freshwater and rainfalls which supply nutrients. In addition, nutrient regeneration processes resulting from water–sediment interactions, the increase of zooplankton activity during post-bloom period (Hoffmeyer 1994), and presumably other factors (such as adjacent salt marshes) may also contribute to the nutrient increase that occurs during spring and summer (Popovich et al. 2008). Recent studies have demonstrate that the winter/early spring phytoplankton bloom starts in June with the consumption of NO_3^- as a first source of nitrogen and depleting all dissolved inorganic nutrients until August (Popovich et al. 2008). The ammonium seemed to be the main source of nitrogen (Spetter 2006). Finally, the annual averages of all these parameters were within the expected range for the Bahía Blanca estuary and did not show any signs of abnormality (Marcovecchio and Freije 2004; Marcovecchio et al. 2008).

Metals in the particulate fraction (SPM)

The mean concentrations of Cd in the SPM were variable during the sampling period as well as at the different sampling stations (Fig. 3a). The general range comprised concentrations below the MDL: <0.01 and 32.83 $\mu g g^{-1}$ d.w. and considering the stations, those located in the industrial area presented higher levels than the other stations far from these points. At stations 1 and 2, the mean concentrations were 5.97 and 5 $\mu g g^{-1}$ d.w., respectively, while at stations 4, 3.37 and at station 5, 1.2 $\mu g g^{-1}$ d.w. Besides, station 3 located in an intermediate distance between these stations, presented the lowest mean concentration, 1 $\mu g g^{-1}$ d.w. However, from a statistical point of view, no significant differences were detected (blocked nested ANOVA, $p>0.25$). When considering the temporal distribution, some variations could be observed. The higher concentrations were always recorded in May (autumn) and November (spring), and in the particular case of this latter month the high concentrations could be related to the rise in SPM and POM, also recorded in this month. It is well known that the increase of the particulate fraction could favor the adsorption of Cd upon particles (Bibby and Webster-Brown 2006). Taking into account previous results recorded in the estuary, Cd concentrations are similar. In general, the levels reported by Andrade (2001) and Botté (2005) coincide with this study. For example, the first author recorded a range of 0.2–8 $\mu g g^{-1}$ d.w. during 1997–1998 and the other author a range of 0.9–4.9 during 2000–2001. However, the concentrations recorded in autumn (22.26 $\mu g g^{-1}$ d.w.) and spring (32.83 $\mu g g^{-1}$ d.w.) in our study, are the

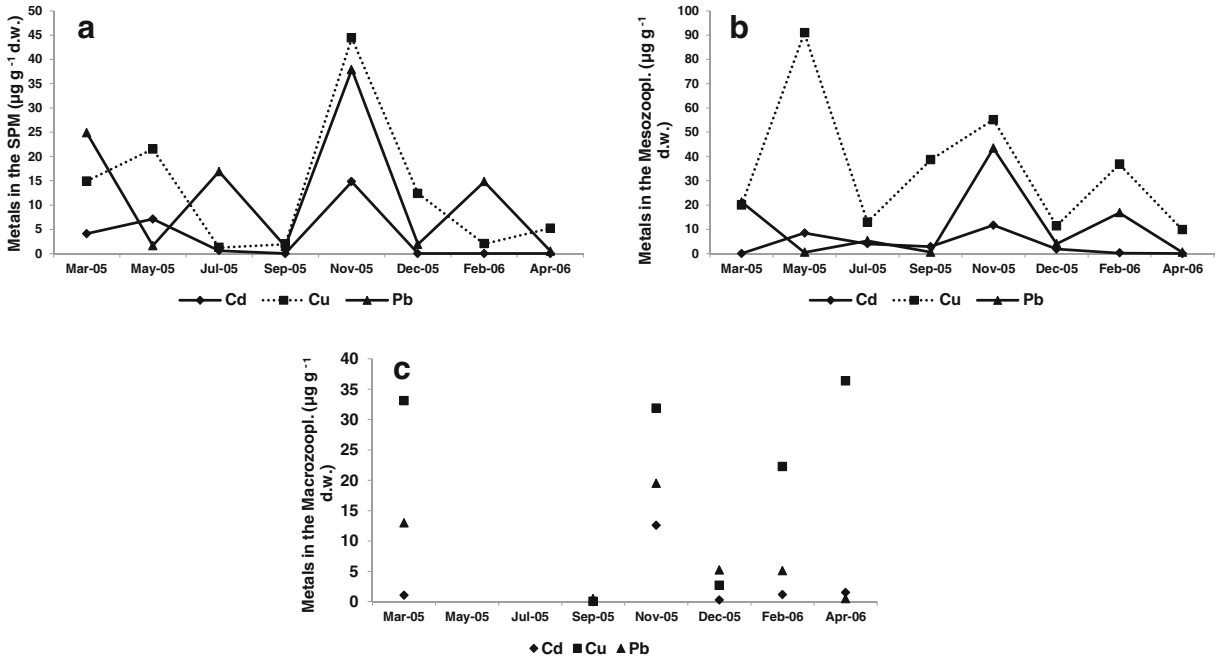

Fig. 3 Temporal distribution of Cd, Cu, and Pb concentrations (in mmicrograms per gram d.w.) in the SPM (**a**), mesozooplankton (**b**), and macrozooplankton (**c**). No lines in figure (**c**) because no samples were collected in May and July

maximum levels ever recorded in the Bahía Blanca estuary. In other estuarine or coastal ecosystems of the world, the concentrations are lower. Gavriil and Angelidis (2005) in the Kalloni Bay (Greece) reported a range of 0.2 and 1.2 $\mu g g^{-1}$ d.w. and Yurkovskis (2004) in the Daugava estuary (Latvia) a range of 0.5 and 4.7 $\mu g g^{-1}$ d.w. In the Gironde estuary (France), Masson et al. (2006) recorded a maximum of 27.9 $\mu g g^{-1}$ d.w. and mean concentrations of 4.7 $\mu g g^{-1}$ d.w., and in the Penzé estuary (France), Cd ranged between 0.4 and 2.4 $\mu g g^{-1}$ d.w. (Waeles et al. 2005). Recently, Vicente-Martorell et al. (2009) reported a range of 0.35–1.91 $\mu g g^{-1}$ d.w. in the Ría de Huelva estuary (Spain) a heavily polluted environment.

The mean concentrations of Cu in the SPM during the sampling period are plotted in Fig. 3a and some temporal variations could be observed. The general range presented values below the MDL (<0.04 $\mu g g^{-1}$ d.w.) and 62.78 $\mu g g^{-1}$ d.w. and again, the maximum levels were recorded in May (autumn) and November (spring) as in the case of Cd. Moreover, a significant correlation was found between particulate Cd and particulate Cu (r=0.7, p<0.01; N=40). Thus, Cd and Cu seemed to have the same behavior and therefore they could have the same source of origin. On the other hand, the decrease of particulate Cu in July could be related to the previously mentioned phytoplankton bloom. This relation in which Cu in the SPM decrease with phytoplankton increase has been reported in other estuaries (Balls 1990; Zwolsman and van Eck 1999; Hatje et al. 2001) and is due to strongly complexing hydrophobic phytoplankton-released ligands which favor Cu desorption from SPM. The sampling stations presented similar mean concentrations and therefore no statistical differences were detected among them (blocked nested ANOVA, p>0.25).

Previous results of Cu in the SPM in the Bahía Blanca estuary coincide with this study. Andrade (2001) recorded a range of 12 and 60 $\mu g g^{-1}$ d.w. and a mean concentration of 30 $\mu g g^{-1}$ d.w. Later, Botté (2005) reported a range of 13.3 and 31 $\mu g g^{-1}$ d.w. and a mean concentration of 20 $\mu g g^{-1}$ d.w. Other estuarine or coastal areas of the world presented similar or higher levels than the Bahía Blanca estuary. In Port Jackson estuary, for example, the concentrations reported by Hatje et al. (2001) were between 59.1 and 95.7 $\mu g g^{-1}$ d.w., and in the northern Taiwan Coast, Fang et al. (2006) recorded a range of 13.4 and 42.8 $\mu g g^{-1}$ d.w., similar to the range of Gavriil and Angelidis (2005) in the Kalloni Bay (10–35 $\mu g g^{-1}$

d.w.). Also, Vicente-Martorell et al. (2009) registered values between 21.2 and 33.1 $\mu g g^{-1}$ d.w.

The concentrations of Pb in the SPM were highly variable from a temporal point of view. The minimum value was <0.5 (MDL) while the maximum, 68.6 $\mu g g^{-1}$ d.w. (Fig. 3a). However, when considering the sampling stations, no statistical differences were detected among them (blocked nested ANOVA, $p>0.25$) which indicates similar levels of Pb. In accordance with the other metals, the high concentrations were recorded in November (spring) but also in March (autumn), July (winter), and February (summer). Besides, the peak concentration recorded in July coincided with the phytoplankton bloom, thus part of the SPM with high Pb levels may be represented by the phytoplankton, which took in Pb in the dissolved form. Botté (2005) also found important concentrations of particulate Pb in July 2000–2001 in the Bahía Blanca estuary. In February, the concentrations of particulate Pb also increased and the phytoplankton could be the responsible since in this month it developed a small phytoplankton bloom which could take in dissolved Pb. Additionally, the high levels of particulate Pb in March and November coincide with high levels of dissolved Pb also recorded in the estuary, indicating a continuous input of dissolved Pb that is rapidly adsorbed to the SPM. This high particle reactivity and its removal through metal adsorption on suspended particles have been observed in many estuaries (e.g., Elbaz-Poulichet et al. 1984; Waeles et al. 2005). Besides, the increase of SPM concentrations recorded in March and November favor Pb adsorption onto this particulate fraction.

Previous results in the estuary showed that the levels of particulate Pb are lower than the present study. Ferrer et al. (2000) found 3.3 $\mu g g^{-1}$ d.w. at station 4 during autumn 1997–winter 1998. Later, Andrade (2001) reported a range of non detected values and 5.5 $\mu g g^{-1}$ d.w. between autumn 1997 and spring 1999 at station 4. In addition, Botté (2005) found concentrations below the detection limit and 50 $\mu g g^{-1}$ d.w. at stations 2 and 4, during winter 2000–summer 2002. Thus, particulate Pb concentrations are among the highest values ever recorded in the estuary and should be considered in future studies in order to detect possible increases with time. However, taking into account other estuaries or coasts with human impact, the concentrations in this study are in general lower. For example, Fang et al. (2006) in the coast of Taiwan recorded a range between 10.3 and 24.6 $\mu g g^{-1}$ d.w. but in Port Jackson Estuary (Australia), Hatje et al. (2001) found a mean concentration of 220 $\mu g g^{-1}$ d.w. Also, in the coast of China, Zhang and Liu (2002) reported a range of 54.8 and 82 $\mu g g^{-1}$ d.w. while Zhou et al. (2003) in the Conwy estuary, North Wales recorded maximum values of 300 $\mu g g^{-1}$ d.w. In addition, Masson et al. (2006) in the Gironde Estuary (France) recorded a range of 32 and 338 $\mu g g^{-1}$ d.w. and Gavriil and Angelidis (2005) in the Kalloni Bay (Greece) found concentrations between 20 and 551 $\mu g g^{-1}$ d.w.

Metals in the mesozooplankton

The temporal distribution of Cd concentrations in the mesozooplankton is shown in Fig. 3b and in this case, the levels were between <0.01 (MDL) and 41.87 $\mu g g^{-1}$ d.w. The stations presented variable concentrations. At stations 1 and 2, the mean concentrations were 0.35 and 5.51 $\mu g g^{-1}$ d.w. respectively, while at station 4, 5.48 $\mu g g^{-1}$ d.w. and at station 5, 4.53 $\mu g g^{-1}$ d.w. Besides, station 3 located in an intermediate distance between these stations, presented a mean concentration of 2.27 $\mu g g^{-1}$ d.w. However, no clear pattern was observed and no statistical differences were detected among them (blocked nested ANOVA, $p>0.25$). On the other hand, the higher concentrations were recorded in May (autumn) and November (spring) in coincidence with the Cd levels in the particulate fraction. Therefore, the levels of Cd in the mesozooplankton could be related to the Cd concentrations in the SPM because part of the diet of the mesozooplankton is SPM. Moreover, the high correlation ($r=0.9$, $p<0.01$; $N=40$) coefficient detected between Cd concentrations in both mesozooplankton and SPM supports this explanation. In accordance to these results, the mesozooplankton ingested Cd through SPM rich in Cd. In this sense, plankton accumulates metals from the dissolved phase as well as from the ingestion of food and both pathways are very important in the metals' uptake (Reinfelder and Fisher 1991; Wang and Fisher 1998a). And also, it is well known that marine zooplankton have the ability to accumulate metals from the environment where they live at much higher concentrations, which could be the implication of this high concentration found in the biological system in the present study. As the zooplankton may be slow to metabolize or excrete the metals, the pollutant may bioaccumulate within the organism. In this sense, most aquatic invertebrates show accumulation patterns for

most trace metals that include some storage of accumulated metal in detoxified form somewhere in the body (Rainbow 2002). A final point to consider on the accumulation of metals by zooplankton, is the knowledge of the accumulation pattern used by each zooplankton species (Rainbow 2002).

The different species of the mesozooplankton which were present during the sampling period is another essential point to consider. The copepod *Acartia tonsa* was present throughout the study period but with high densities in March (autumn), November (spring), December (summer), and February (summer). This copepod is omnivorous which means that its diet is varied and comprised SPM, POM, detritus, phytoplankton, etc. *E. americana* was the other copepod which presented high densities only in July and September (winter–early spring). This species is herbivorous so its diet is phytoplankton. The larval forms of the cirripeds *Balanus* spp. and Decapoda were also important mainly in July–September and November–December, respectively, and like *A. tonsa*, they are omnivorous. Therefore, the months when the high levels of Cd were recorded (May and November), *A. tonsa* and larvae of Decapoda were the most important species which mainly incorporated Cd through the SPM.

In the Bahía Blanca estuary, there is no historical information about the levels of metals in the zooplankton. The first studies started in 2004 and the results of the present study are part of them. In other marine environments of the world, there is much information on metals content in zooplankton, especially on different species of copepods as well as mixed zooplankton samples. But in the particular case of the copepod *A. tonsa*, the information is scarce. Table 3 describes metals concentrations of mesozooplankton organisms in different marine environments. In general, our results are similar to these concentrations; however, in some cases the levels in the Bahía Blanca estuary are higher, for example, when comparing with pristine environments, such as Antarctica. In addition, it is worth noting the importance of the different factors that determine metals' concentrations in the organisms such as source of pollution, accumulation strategies, assimilation efficiency, life cycle, efflux rate, detoxification strategies, etc. (Wang and Fisher 1998a; Marsden and Rainbow 2004).

The temporal distribution of the mean Cu concentrations in the mesozooplankton is plotted in Fig. 3b and these levels ranged between <0.04 (MDL) and 148.9 $\mu g g^{-1}$ d.w. Besides, the sampling stations located near the industrial area (stations 1 and 2) presented similar mean concentrations with 38.17 and 37.64 $\mu g g^{-1}$ d.w., respectively. The intermediate station (station 3) located 7 km far from the industrial area presented a mean concentration of 28.74 $\mu g g^{-1}$ d.w. while the other ones (stations 5 and 4): 27.63 and 40.80 $\mu g g^{-1}$ d.w., respectively. These results seemed to indicate a Cu decrease from the industrial zone to the stations located a few kilometers far from this zone. However, the more distant station (station 5) presented one of the higher levels. Therefore, no clear spatial pattern could be distinguished. Besides, these stations did not differ statistically (blocked nested ANOVA, $p > 0.25$). In coincidence with Cd in the mesozooplankton, the maximum values were recorded in May (autumn) and November (spring), and moreover, the maximum levels of Cu in the SPM were also recorded in May and November. Therefore, the mesozooplankton could have ingested Cu through the SPM also enriched with this metal. In addition, the mesozooplankton was represented by *A. tonsa* in May and in December by larvae of decapods and *A. tonsa*, both omnivorous organisms which feed on SPM, detritus, etc. In February (summer), important levels of Cu were also recorded; however, the concentrations in the particulate fraction were low. Thus, the source of Cu for the mesozooplankton could be the phytoplankton, which presented a small pulse in this month and could have taken Cu from the dissolved phase. In this case, the copepod which dominated the mesozooplankton in February was *A. tonsa*. Copper is an essential metal, generally required by crustaceans as a component of enzymes and hemocyanins. However, it is believed that copepods do not have this respiratory pigment (hemocyanin), therefore the requirements for copper would depend on enzyme activities related to the processes of growth and egg production (Kahle and Zauke 2003). In addition, the dissolved copper could also be an important source. Studies suggest that copper accumulation in marine zooplankton is incorporated through the diet but the incorporation through the dissolved route may be important in polluted waters (Chang and Reinfelder 2002). Furthermore, these authors argue that this is because copper bioaccumulation in copepods is characterized by low rates of efflux and rates of assimilation efficiency relatively high. Regarding physiological requirements in copepods, Zauke and Schmalenbach (2006) argue that could be compared

Table 3 Metals concentrations (in micrograms per gram d.w.) in mesozooplankton and macrozooplankton of the Bahía Blanca estuary and different marine environments of the world

Study area	Zooplankton fraction	Taxa	Cd ($\mu g g^{-1}$ d.w.; mean (range))	Cu ($\mu g g^{-1}$ d.w.; mean (range))	Pb ($\mu g g^{-1}$ d.w.; mean (range))	References
Bahía Blanca estuary	Mezoopl.	*Acartia tonsa* (C)	3.7 (<0.01–41.9)	37.34 (<0.04–148.9)	13.4 (<0.5–71.8)	This study
		Eurytemora americana (C)	3.4(<0.01–19.91)	25.8 (<0.04–89.3)	2.9 (<0.5–24.4)	
Banc d´Aguin	Mesozoopl.	Copepods	7.5	55	33	Everaarts et al. (1993)
Mauretania						
North Sea	Mesozoopl.	*Acartia* sp. (C)	1.7	15	2	Zauke et al. (1996)
Seine estuary (France)	Mesozoopl.	*Eurytemora affinis* (C)	(0.13-1.1)	(7–134.7)	(8.5–48.8)	Miramand et al. (2001)
Cheesequake Creek (USA)	Mesozoopl.	*Acartia* sp. (C)	–	20–30	–	Chang and Reinfelder (2002)
Australia and New Zealand Coasts	Mesozoopl.	Copepods	5.1	46	0.94	Barwick and Maher (2003)
Northern Taiwan Coast	Mesozoopl.	*Temora turbinata* (C)	(0.5–2.5)	(14–110)	(9–45)	Fang et al. (2006)
		Oncaea venusta (C)	(0.75–2.10)	(45–95)	(2.6–20)	
		Euchaeta rimana (C)	(0.25–2.2)	(14–160)	(2.6–10)	
Baltic Sea	Mesozoopl.	Copepods	(0.8–2.5)	(3.5–20.5)	(0.7–12.9)	Pempkowiak et al. (2006)
Weddell Sea	Mesozoopl.	*Rincalanus gigas* (C)	3.7 (2.3–6)	11 (10–11)	0.17 (0.09–0.24)	Kahle and Zauke (2003)
Weddell Sea (Antarctica)	Mesozoopl.	*Calanus propinqus* (C)	5.6 (2.7–11)	26 (6–52)	0.51 (0.17–1.07)	
		Crocodylus acutus (C)	4.6 (2.1–7.5)	10 (6–14)	0.31 (0.14–0.59)	
		Metridia curticauda (C)	9.6 (8.4–10.7)	21 (8–48)	0.48 (0.26–0.73)	
		Metridia gerlachei (C)	10.2 (4.6–14.4)	26 (10–51)	0.72 (0.29–1.67)	
		Metridia longa (C)	2.4	7	–	
Barents Sea	Mesozoopl.	*Euchaeta norvegica* (C)	1.2	9	–	Kahle and Zauke (2003)
		Calanus hyperboreus (C)	4.2	6	–	
		Calanus finmarchicus (C)	6.3	7	–	
SE Gulf of California Coast (USA)	Mesozoopl.	Copepods	–	–	(2–7.6)	Soto-Giménez et al. (2008)
Guanabara Bay (Brazil)	Mesozoopl.	Copepods	(0.008–0.06)	(1.50–6.3)	(1.75–5.3)	Kehrig et al. (2009)
Bahía Blanca estuary	Macrozoopl.	*Arthromysis magellanica* (M)	3.4 (<0.01–45.3)	29.8 (<0.04–77.7)	9.8 (<0.5–27.2)	This study
		Neomysis americana (M)				
Loire estuary (France)	Macrozoopl.	*Neomysis integer* (M)	0.07	7.4	4.7	Amiard et al. (1980)
		Mesopodopsis slabberi (M)	0.1	22.4	5.7	
Atlantic Ocean	Macrozoopl.	*Mysidacea* (M)	15.8	18.2	2.6	Schulz-Baldes (1992)
Seine estuary (France)	Macrozoopl.	*Neomysis integer* (M)	0.3	19	3.2	Miramand et al. (2001)
		Mesopodopsis slabberi (M)	0.08	20	2.3	

Mesozoopl. mesozooplankton, *macrozoopl.* macrozooplankton, *C* copepod, *M* mysid

with those of decapod which need between 7 and 15 μg g^{-1} d.w. of copper for enzyme activities and 25 μg g^{-1} d.w. for the respiratory pigment hemocyanin (Depledge and Bjerregaard 1989). In *A. tonsa*, Cu concentrations were higher, taking into account that the copepods do not have hemocyanin, thus, enzymatic requirements would be complete. High concentrations indicate that the requirements were more than satisfied and *A. tonsa* may be accumulating significant amounts of copper as a consequence of a considerable anthropogenic input. These results were higher or within the range reported by other authors in different marine ecosystems (Table 3). However, it has to be taken into account the interespecific differences and the factors that determine metal concentrations in the organisms, such as assimilation strategies, life cycle, detoxification mechanisms, among others factors previously mentioned.

Mean Pb concentrations in the mesozooplankton are shown in Fig. 3b and the range was <0.5 (MDL) and 71.85 μg g^{-1} d.w. The sampling stations presented some differences; however, the spatial distribution was unclear and no statistical differences were detected (blocked nested ANOVA, $p>0.25$). The stations located near the industrial area: stations 1 and 2 presented a mean concentration of 3.11 and 14.64 μg g^{-1} d.w. The station located in an intermediate position station 3, 8.46 μg g^{-1} d.w., while the farther stations, station 4, 11.78 μg g^{-1} d.w. and station 5, 19.72 μg g^{-1} d.w. Regarding temporal distribution, the higher levels were detected in March (autumn), November (spring), and February (summer) at all the sampling stations, and this behavior coincide with temporal distribution of Pb in the SPM. Accordingly, the mesozooplankton ingested SPM with Pb, which led to high concentrations of Pb in these planktonic organisms. It is worth noting that in these months the mesozooplankton is represented by omnivorous organisms in which part of its diet is SPM.

Table 3 show the concentration of Pb in different marine zooplankton of the world with or without human impact such as Antarctica. These results are lower than the recorded in the Bahía Blanca estuary which could indicate that this estuary presents a certain degree of pollution. Industrial as well as the urban discharges could be considered as potential sources of Pb in the estuary; however, long-term studies are necessary to analyze how contaminated is the environment in which they live.

Metals in the macrozooplankton

Cadmium distribution in the macrozooplankton is represented in Fig. 3c, and the concentrations were between <0.01 (MDL) and 45.3 μg g^{-1} d.w. In general, the concentrations were low and the sampling stations showed similar values. Station 2 located near the industries presented the highest mean concentration (11.76 μg g^{-1} d.w.); however, no statistical differences were detected among the sampling stations (blocked nested ANOVA, $p>0.25$). In May and July, no organism was recorded so the levels of metals could not be performed in these months. On the other hand, again in November, there were recorded the higher Cd concentrations in coincidence with high levels of Cd in the SPM and also in the mesozooplankton. The macrozooplankton was mainly composed by mysids like *Artrhomysis magellanica* and *N. americana*, both omnivorous species which feed on SPM, POM, mesozooplankton, detritus, small crustaceans, diatoms, etc. In mysids, the main route of uptake of metals is through the diet (Ridout et al. 1989; Wang 2002). For this reason, Cd content in the macrozooplankton can be a direct result of Cd in both, the SPM and mesozooplankton since they are part of its diet.

There are no previous studies on metal concentrations in mysids like *A. magellanica* and *N. americana* in the Bahía Blanca estuary, neither in other estuarine environments. However, Table 3 shows some metals concentrations in macrozooplankton species of other marine ecosystems around the world, and these results are similar to concentrations recorded in the present study.

The mean concentrations of Cu in the macrozooplankton varied between <0.04 (MDL) and 77.7 μg g^{-1} d.w., and the temporal distribution is plotted in Fig. 3c. High concentrations were recorded in nearly all the months of the sampling period. September (spring) and the months when no macrozooplankton samples were obtained were the exception. Like cadmium, these high levels may be due to food with high concentrations of copper, since both the SPM and the mesozooplankton had significant levels of this metal. In addition, since mysids have hemocyanin as respiratory protein, they need a certain amount of copper to bind to the active sites of this protein and thereby meet the metabolic requirements. In this sense, it is estimated that between 40 and 50 % of total copper in these organisms is associated with the hemolymph (Erk et al. 2008) and

decapods for example, need about 25 $\mu g\,g^{-1}$ d.w. of Cu to join the hemocyanin and thus participate in the transport of oxygen. Moreover, mysids also present metallothioneins, enabling them to accumulate large amounts of copper in the detoxified form, as they participate in the homeostatic control of essential metals such as copper and zinc (Amiard et al. 2006). The sampling stations presented high concentrations but no clear pattern was observed; moreover, no statistical differences were detected among them (blocked nested ANOVA, $p>0.25$).

The levels of Cu in other macrozooplankton species of the world are describe in Table 3, and in general these results coincide with the recorded in the present study. The only exceptions are some values recorded in the intermediate station, station 3 which presented very high concentrations in November and April.

The range of Pb was <0.5 (MDL) and 27.20 $\mu g\,g^{-1}$ d.w., and the highest values were recorded in March (autumn) and November (spring), when high concentrations of Pb in the SPM and mesozooplankton were also recorded. Thus, the levels of Pb in the macrozooplankton were mainly associated with the Pb concentrations in their food since the SPM and mesozooplankton are part of their diet. On the other hand, Pb seemed to decrease towards the farthest stations from the industrial area; however, no statistical differences were detected that indicate such spatial pattern among the stations (blocked nested ANOVA, $p>0.25$).

When comparing with other species of marine environments, the macrozooplankton of the Bahía Blanca estuary presented higher concentrations (Table 3). Therefore the results in different study areas could indicate different degrees of pollution. However, these comparisons need to take into account the particular physiology and morphology of each species, feeding behavior, metal-accumulation strategies, uptake rate, assimilation efficiencies, detoxification mechanisms, etc.

Qualitative and quantitative analyses of the meso- and macrozooplankton

During the sampling period from March 2005–April 2006, the mesozooplankton was mainly composed of copepods, which comprised the 92 % of the total abundance. The highest abundances were recorded in March (autumn) and February (summer) with 5,576.3 and 5,442.1 ind.m^{-3}, respectively (Fig. 4a). Within the copepods, *A. tonsa* was the most important species since it was present during all the sampling period and also presented the highest abundances. In addition, the most important abundances were recorded in March (autumn), November (spring), December (summer), February (summer), and April (autumn) (Fig. 4a), in coincidence with historical studies in the Bahía Blanca estuary. For example, Hoffmeyer (2004a, b) described *A. tonsa* as a species that is present during all the annual cycle of the mesozooplankton in the estuary, with high abundances in summer and autumn. Besides, this author considers *A. tonsa* a key copepod in the estuarine food chain since presents an important biomass, abundance and dominance. On the other hand, the stations had similar abundances of *A. tonsa*, and no statistical differences were detected among them (one-way ANOVA, $p>0.25$).

Another important copepod was *E. americana* that presented the maximum abundances in July (144.8 ind.m^{-3}) and September (48.9 indm^{-3}), when the phytoplankton bloom developed and the abundances of *A. tonsa* decrease (Fig. 4a). This relation between *E. americana* and the bloom has already been described and is due to the food habits of *E. americana* that feed on phytoplankton. After this bloom, *E. americana* disappeared from the water column but remained as resting eggs in bottom sediments, while the population of *A. tonsa* began to increase. This behavior of *E. americana* is due to both, the food limitation and the temperature's increase, which impact negatively on its population (Hoffmeyer 2004a). In addition, taking into account the abundances of *E. americana* at each sampling stations, no statistical differences were detected among them (one-way ANOVA, $p>0.25$).

The larvae of the cirripeds *Balanus* spp. (Fig. 4a) were also present during the sampling period but with lower abundances than *A. tonsa*. In accordance with *E. americana*, the cirripeds larvae of *Balanus* spp. presented the higher abundances in July (132.8 ind.m^{-3}) and September (197.9 ind.m^{-3}). On the other hand, larval forms of Decapoda were less abundant but with maximum values in November (194.2 ind.m^{-3}), December (97.1 ind.m^{-3}), and February (215.1 ind.m^{-3}) (Fig. 4a). In both taxa (*Balanus* spp. and Decapoda), no statistical differences were detected among the stations (one-way ANOVA, $p>0.25$). In coincidence with the temporal behavior of *A. tonsa* and *E. americana*, the cirriped of *Balanus* spp. as well as larval forms of Decapoda presented the normal pattern

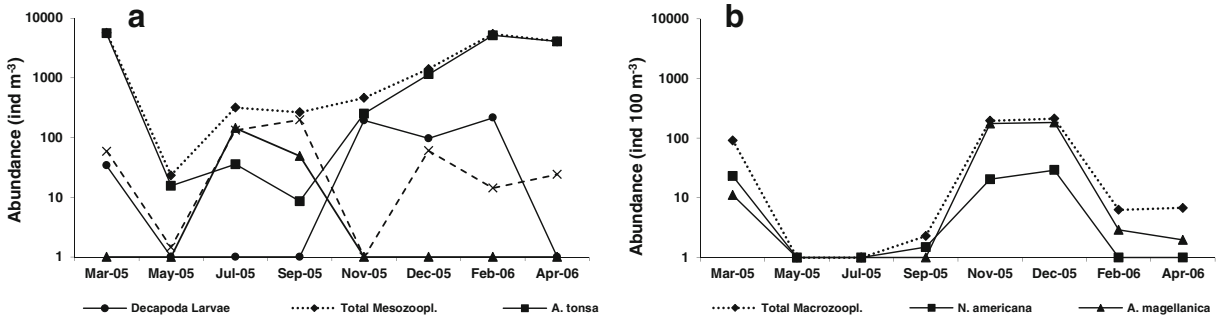

Fig. 4 Temporal distribution of the mesozooplankton (**a**) and macrozooplankton (**b**) abundance

previously described by Hoffmeyer (2004a, b) and Hoffmeyer et al. (2008) in the Bahía Blanca estuary. In the present study, the mesozooplankton presented the typical temporal pattern, with maximum abundances in autumn, spring, and summer. Additionally, the taxa and their spatial distribution coincided with historical information (Hoffmeyer 1994, 2004a, b; Hoffmeyer et al. 2008).

The macrozooplankton was mainly composed of the mysids *N. americana* and *A. magellanica* which represented the 87 % of the total abundance. Both species presented the maximum abundances in March, November, and December (Fig. 4b). Besides, *A. magellanica* was the most abundant species with 174.93 ind. 100 m^{-3} in November, and 183.90 ind. 100 m^{-3} in December. It is worth noting that during winter (May and July) and early spring (September), the macrozooplankton was absent or the abundances were very low. These results agreed with previous studies in the estuary: high abundances in late spring–summer and low in winter, with increasing abundances towards the inner zone of the estuary (Hoffmeyer and Mianzan 2004; Cardelli et al. 2006).

Conclusions

This study includes part of the first results of Cd, Cu, and Pb concentrations in the meso- and macrozooplankton of an important southwestern Atlantic temperate estuary. It considers metal levels in the SPM, which together with the zooplankton are key components in the biogeochemical cycles of metals in estuarine environments. The temporal and spatial behaviors of Cd, Cu, and Pb in these different fractions were analyzed as well as the relation among them. Besides, the main physicochemical variables were also taken into account. From these results, interesting conclusions could be drawn. All the physicochemical variables presented the normal values found in the Bahía Blanca estuary and therefore did not show any sign of abnormality that could influence metals concentrations. In addition, it was not possible to clearly detect zones with anthropogenic pressure from a less impacted one, and this coincides with the metals concentrations distribution in the SPM and zooplankton. Despite the location of the sampling stations, no significant differences were detected in the metals concentrations. Although some stations were located near the industrial discharges and others far from these points, no clear pattern was distinguished. These behaviors can be linked to the tidal dynamics of the estuary. Even though all the sampling campaigns were carried out during ebb tide, water displacement during the samplings cannot be neglected. The SPM presented in some months, important concentrations of Cd, Cu, and Pb that should be taken into account in future studies to analyze their behavior and possible increases of these metals over time. In addition, metals in the mesozooplankton also presented in some cases, high levels and the source of the metals is the SPM with high metal concentrations. In the case of the macrozooplankton, both the SPM and mesozooplankton are believed to be responsible for metal content. The taxonomic composition and abundance of the zooplankton was the commonly found in the estuary, and thus no unusual pattern was detected. The absolute and relative abundance of the zooplankton taxa varied with the season of the year, hence the metal concentrations in these organisms varied in relation to the season as well as to the variations emerging from the metals concentrations in the SPM, consumed by zooplankton as food. In this sense, metals in the particulate fraction as well as

in the meso- and macrozooplankton presented marked temporal variations, therefore the Bahía Blanca estuary can be considered as a very dynamic estuary with important metals fluctuations. The high levels of Cd, Cu, and Pb detected in some months can be related to the industrial and urban discharges and must be consider a warning, however this work is the starting point and further studies are necessary to reinforce this assertion as well as to analyze other possible sources. Additionally, the study of metals in the zooplankton in the present work has been proven to be an additional tool for researches on metal biogeochemistry in estuarine environments. These results demonstrate the value of investigating lower trophic levels to understand the fate of metal contaminants in aquatic food webs.

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