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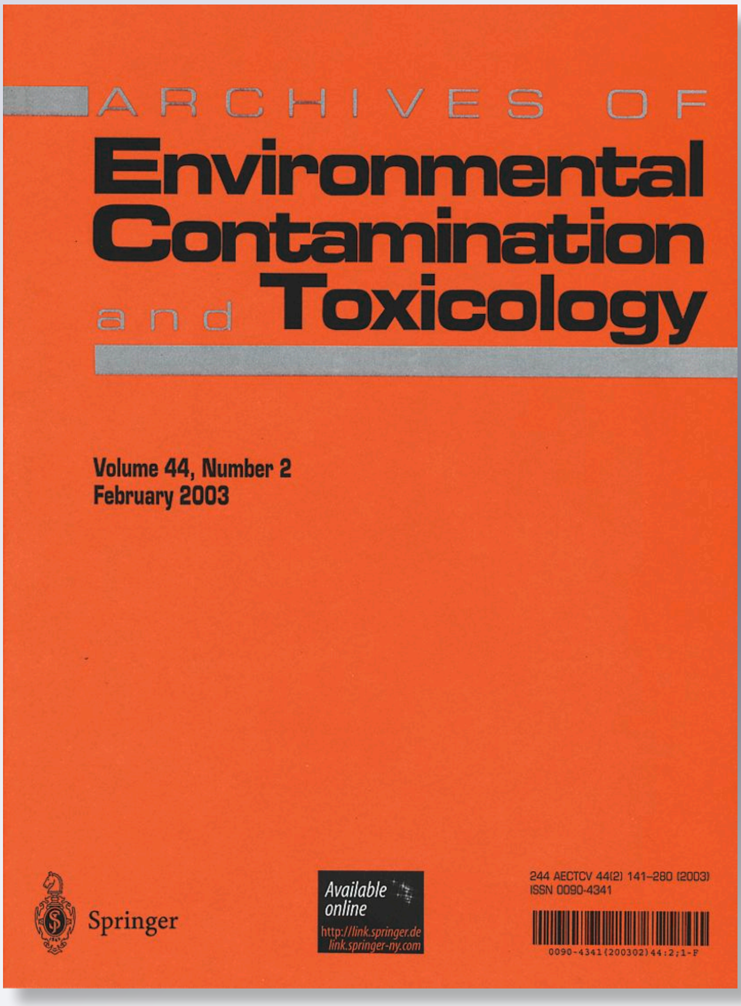

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Heavy-Metal Concentrations in Soft Tissues of the Burrowing Crab *Neohelice granulata* in Bahía Blanca Estuary, Argentina

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Abstract Copper (Cu), cadmium (Cd), and chromium (Cr) in soft tissues of male and female burrowing crab *Neohelice granulate*, as well as their eggs, were measured in two different locations in the Bahía Blanca estuary, a moderately polluted ecosystem, from October 2008 to August 2009. Differences between sexes, sites, and seasonality were assessed. Furthermore, metal levels in eggs were compared with metal levels in female crabs. The results showed no significant differences for Cu and Cd between sexes (Student *t* test $p > 0.25$ for both sites) and sites (two-way analysis of variance: Cu $p = 0.82$ and Cd $p = 0.29$). Nevertheless, seasonality was found, with winter having significantly lower concentrations for both metals. The range of Cu concentrations was between 96.92 and 152.18 $\mu g\ g^{-1}$ dry weight (dw), and the range of Cd concentrations was between 6.09 and 10.41 $\mu g\ g^{-1}$ dw. Cr concentrations could not be assessed because most of the values were lower than the detection limit. Although heavy metals in sediments were not measured in this study, a bioaccumulation process may be occurring for Cd because levels found during the entire sampling period were greater than levels in sediment from previous years. For Cu, a regulation process may be occurring considering that this is an essential metal and levels of Cu in *N. granulata* found in this study were between 1 and 2 orders of magnitude greater than levels in sediments. Finally, Cu and Cd levels in eggs were detectable, but they were lower than levels in female crabs. The importance of these findings is linked to the fact that metal accumulation seems to be occurring before hatch. The presence of heavy metals in soft tissues as well as in eggs of *N. granulata* is of great importance considering that this is a key species within the Bahía Blanca estuary; therefore, it plays a major role in the transference of pollutants to greater trophic levels.

Pollution of marine environments has been progressively increasing over the years due to anthropogenic activities. The economic and demographic development of coastal regions (i.e., increase of urban, industrial and port settlements) generates great loads of wastes, which are discharged into the water. Estuaries can be characterized by a slow rate of water exchange relative to their volume; therefore, they are most susceptible to pollutants inputs (Kennish 1997). These coastal ecosystems are complex and dynamic and have economical as well ecological importance associated with their large marine biodiversity (Constanza et al. 1993; Perillo 1995; Kennish 1997).

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Aquatic invertebrates tend to be highly vulnerable because they are exposed to several toxic organic and inorganic pollutants present in these environments (Kennish 1997, 2002). Because heavy metals can be found in seawater and sediments, benthic invertebrates are permanently exposed to these contaminants. These organisms are known to take up and accumulate heavy metals, both essential and nonessential, from water and sediment as well as from their food supply (Rainbow 1997, 2002; Wang and Fisher 1999). Thus, it is expected that species inhabiting estuarine environments with a moderate anthropogenic impact should bioaccumulate heavy metals within their tissues.

The estuarine burrowing crab *Neohelice granulata* (Brachyura, Varunidae) is ubiquitous along the Atlantic coast of South America from southern Brazil (23°S) to the northern Argentine Patagonia (41°S) (Spivak et al. 1994). These benthic organisms inhabit the intertidal zone in tidal flats as well as salt marsh areas vegetated by *Spartina densiflora*, *S. alterniflora*, and *Sarcocornia perennis* (Spivak et al. 1994; Escapa et al. 2008). Individuals of *N. granulata* from the *Spartina*- and *Sarcocornia*-dominated marshes and mud flats have a specific trophic mode, sediment-processing rate, burrow architecture, and burrow dynamics. Crabs are herbivorous–detritivorous when associated with salt marshes and deposit feeders when living in mud flats (Iribarne et al. 1997). This species breeds from middle of spring to the beginning of autumn (Ituarte et al. 2004). This crab is one of the most abundant macroinvertebrates inhabiting these estuarine environments (Boschi 1964; Spivak 1997) and represents an important link in the trophic web because all stages in the crab's life cycle are a relevant food component for other species, such as fishes, shellfishes, and birds. As a key species within this ecosystem, *N. granulata* could play a major role in the transference of pollutants to greater trophic levels.

An extensive population of *N. granulata* inhabits the intertidal areas of the Bahia Blanca Estuary, Argentina. It is large area and the fact that it is partially exposed to the open sea makes it an excellent feeding and breeding site for a variety of species, including shorebirds (Delhey et al. 2001; Petracci 2002). The estuary is under constant and increasing anthropogenic pressure. There are several urban settlements (350,000 habitants) with industrial developments (petrochemical complex, industrial park, oil refinery) and harbors, which produce an impact on the environment through the discharge of their sewage, with insufficient treatment and purification, into the estuary (Andrade et al. 2000; Ferrer et al. 2000a, b; Tombesi et al. 2000; Marcovecchio et al. 2008). Raw sewage and runoff from the intensively used agricultural areas also generates an impact on the environment (Perillo et al. 2001). Aerial fallout from atmospheric pollutants also must be considered (Botte et al. 2007). Several studies made within the estuary show low to medium heavy-metal concentrations in sediments, water, and particulate matter (i.e., Andrade et al. 2000; Marcovecchio and Ferrer 2005; Botte et al. 2007, 2010; Fernández Severini et al. 2009).

There is also information about the presence of these pollutants in certain organisms in the estuary, for example fishes (sharks, soles), crustaceans (shrimp, king prawn, crab), diatoms, and some halophyte species (Marcovecchio 1994; Marcovecchio et al. 1988a, b, 1996; Ferrer et al. 2000a, b; Botté 2005; Fernández Severini et al. 2009). This indicates that the transference of several heavy metals occurs between abiotic and biotic components.

The objective of this study was focused on determining levels of cadmium (Cd), copper (Cu) and chromium (Cr) in soft tissues of the burrowing crab *N. granulata* in the Bahia Blanca estuary. Differences between sex, location, and season were analyzed for these heavy metals. Finally, the presence of these metals was measured in crab's eggs and compared with levels detected in female crabs.

Materials and Methods

Study Area

The Bahía Blanca estuary is located southwest of the coast of Buenos Aires Province, Argentina (38°45′ to 39°40′ S and 61°45′ to 62°30′ W). It is a mesotidal coastal plain estuary that extends over approximately 2300 km^2 and is formed by several tidal channels, extensive tidal flats with patches of low salt marshes, and islands (Piccolo et al. 2008).

The Blanca Bay, Falsa Bay, and Verde Bay Nature Reserve is located within the estuary (180,000 ha) and includes several islands, tidal channels, and tidal flats (Fig. 1). This reserve was created in 1998 with the primary purpose of safeguarding the marine ecosystem as a whole as well as to protect and preserve an important refuge and reproduction site for several terrestrial and marine species. In 2006, a new coastal reserve (319 ha) was created in the inner part of the Bahia Blanca estuary, showing the growing concern about the quality of this environment, which is under anthropogenic pressure. This new reserve is located in the northern coast of the estuary, where urban zones, ports, and industries are located (Fig. 1).

At the head to the mouth of the estuary, several physicochemical characteristics (e.g., water salinity, water temperature, sediment composition, organic matter content, and pH) vary (Freije and Marcovecchio 2004; Freije et al. 2008; Piccolo et al. 2008). This is significant for our research because heavy-metal content in sediment and water in estuaries is related to these parameters (Förstner and Wittmann 1983; Salomons and Förstner 1984).

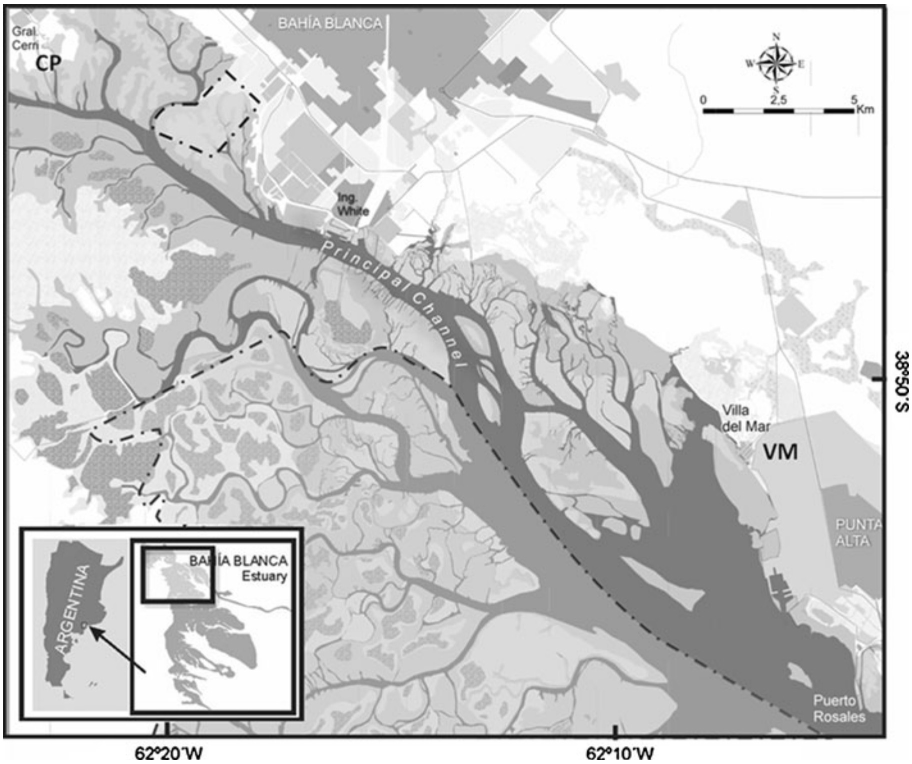

Fig. 1 Map of the study area including both sampling sites: CP and VM. *Dashed* areas represent the Blanca Bay, Falsa Bay, and Verde Bay Nature Reserve and the coastal reserve (the smaller area in the *upper left* corner)

Moreover, these extrinsic factors may influence metal concentrations in crustaceans (Swaileh and Adelung 1995; Turoczy et al. 2001).

Two locations were selected when considering the contrasting position within the Bahía Blanca estuary (Fig. 1). One of them, Cuatreros Port (CP), is located in the inner part of the estuary and influenced by the town of General Cerri. This area is influenced by the Sauce Chico River and the Saladillo de Garcia stream, which drain large extensions of agricultural fields (Perillo et al. 2001; Limbozzi and Leitao 2008). This site is characterized by a water salinity range of 20.8 to 31.9 psu, a water temperature range of 8.5 and 23.3°C, and a sediment composition of mainly silt–clay (96.3%) (Botté 2005).

The other selected site is Villa del Mar (VM), a small village placed near the city of Punta Alta. It is located in the middle-outer part of the estuary and is further away from the industrial area. This site has a water salinity range of 30.88 and 36.57 psu, a water temperature water range of 6.2 and 22.6°C (Negrin 2010), and a sediment composition of a mixture of mud and sand (87 and 13%, respectively) (Pratolongo et al. 2010).

Sampling

Samples from the CP and VM sites were collected bimonthly from October 2008 to August 2009. For CP, one sampling was taken in spring; two bimonthly samplings were taken in summer; one sampling was taken in autumn; and two bimonthly samplings were taken in winter. For VM, one sampling was taken in spring; two bimonthly samplings were taken in summer; one sampling was taken in autumn; and one sampling was taken in winter. Crabs of both sexes were handpicked during low tide at the crab beds in the nude mud flats (no samples were collected in the vegetated areas). At the laboratory they were washed with distilled water and preserved at −20°C until analysis. Mature crabs with maximum width carapace (maximum distance between the two prominent spines), namely >15 mm for male and >17 mm for female crabs, were selected according to Luppi et al. (2004). When available, both ovigerous and nonovigerous female crabs were treated separately. At VM, ovigerous female crabs were only found in October and December 2008; at CP, ovigerous female crabs were available in October and December 2008 and February 2009.

All of the equipment used for dissection and drying of samples had been previously cleaned with diluted nitric acid (0.7% v/v) to prevent contamination. Crab samples were dissected to separate soft tissue from the carapace; then pools of soft tissues from five or six specimens, according to sex and location, were placed in Petri dishes in an oven at 50°C until constant weight was reached. Finally, dried samples were homogenized by crushing them in a porcelain mortar.

Female crabs' eggs were removed from the pleon and cleaned with distilled water. Then egg masses were dried until constant weight and were pooled according to sampling site. Pooled samples of each site were homogenized ($n = 3$ to 4 individuals). Finally, egg masses were grinded in a porcelain mortar and prepared for extraction.

Acid digestion of crab tissues and egg samples was performed according to the methodology described by Marcovecchio and Ferrer (2005). Subsamples of 250 and 500 mg were separated into two tubes and mineralized with 3 ml concentrated nitric acid (65%) and 1 ml concentrated perchloric acid (70–72%) and placed in a heated glycerin bath at 120 ± 10°C. When the acid digestion finished, the residue was transferred to centrifuge tubes and completed with diluted nitric acid (0.7%) up to 10 ml. Sample digestion was performed in duplicate to ensure reproducibility of the results. Heavy-metal concentrations (Cu, Cd, and Cr) were determined by atomic absorption spectroscopy with air-acetylene flame using a Perkin-Elmer AA-2380® (PerkinElmer Inc., Boston, MA). All concentrations are expressed in parts per million ($\mu g\ g^{-1}$) on a dry weight (dw) basis.

Data Analysis

Two statistical packages were selected: SPSS 15.0® for Windows (SPSS Inc., Chicago, Ill) and Infostat 5.1® for Windows (Grupo Infostat Professional, FCA, Universidad Nacional de Cordoba, Argentina). Student *t* test was used to check statistical differences between sexes in both locations. This test was also used to compare Cu and Cd levels between ovigerous and nonovigerous female crabs. For Cu and Cd, two-way analysis of variance (ANOVA) was performed to analyze interactions between locations and seasons, and Fisher's least significant difference test was used for multiple comparisons.

The method detection limits (MDLs) were experimentally calculated as the SD of 12 blank replicates. The MDLs, expressed in $\mu g\ g^{-1}$, were as follows: Cu 0.77, Cd 0.27, and Cr 0.29. All %RSD (percentage of relative SD) of the replicate samples was between 20 and 30%. Although these percentages are high, this is due to the lack of large available quantities of biological material that could be used in case a sample must be repeated. Because obtaining the biological material is a laborious job, we decided to collect only enough for one sample with its duplicate. Certified reference materials (CRMs: mussel tissue flour, reference material No. 6 [National Institute for Environmental Studies, Tsukuba, Japan]) and analytical-grade reagents Merck® (Merck & CO., Inc., Whitehouse Station, NJ) or Baker® (Mallinckrodt Baker Inc., JTBaker Chemical CO., Phillipsburg, NJ) were used for both analytical quality control and build up of reagent blanks and calibration curves. Recovery percentages for the three metals in CRM were >90%. For samples that were lower than the detection limit of the applied analytical method, a value of one half the detection limit was assigned, and the sample was included within the data set for statistical treatment (Jones and Clarke 2005).

Results

Metal Concentrations in Soft Tissues

The distributions of Cu, Cd, and Cr in soft tissues of *N. granulata* from the two sampling sites (VM and CP)

Table 1 Heavy-metal concentrations ($\mu g\ g^{-1}$ dw) (mean value ± SDs) in soft tissues from *N. granulata* crabs according to sex, location, and season

Metal	Location	Sex	Season			
			Spring	Summer	Autumn	Winter
Cu	VM	F	121.91 ± 19.70	138.30 ± 1.71	155.99 ± 13.27	106.08 ± 20.56
		M	155.64 ± 20.02	150.04 ± 6.42	149.84 ± 3.64	99.59 ± 7.27
	CP	F	136.07 ± 19.29	151.14 ± 4.09	155.36 ± 4.62	88.80 ± 1.17
		M	172.52 ± 67.77	169.26 ± 4.63	137.79 ± 10.26	81.40 ± 5.33
Cd	VM	F	9.04 ± 0.74	11.94 ± 0.97	9.98 ± 0.70	5.56 ± 0.39
		M	8.69 ± 0.36	10.58 ± 0.48	9.70 ± 0.25	7.45 ± 1.09
	CP	F	7.79 ± 0.59	8.61 ± 0.46	10.40 ± 0.03	4.89 ± 0.20
		M	10.92 ± 0.48	10.52 ± 0.14	6.50 ± 0.14	5.63 ± 0.34
Cr	VM	F	0.66 ± 0.40	ND	ND	ND
		M	ND	ND	ND	ND
	CP	F	1.93 ± 0.03	ND	ND	ND
		M	0.48 ± 0.001	ND	ND	ND

ND not detected, $N = 5$–6 individuals (pooled samples) through the seasons (spring, summer, autumn, and winter) and from both sexes are listed in Table 1. Throughout this research we have worked with dry weight, which represents between 21 and 25% of total weight. In the case of Cr, because most of the values were lower than the detection limit, no statistical analysis was performed. Cr concentrations were detected exclusively during the spring (except for male crabs from VM). During the rest of the seasons, the values were none detectable for both sexes.

In the case of the other heavy metals, first, levels of Cu and Cd in ovigerous and nonovigerous female crabs were tested, and no significant differences were found for either of the two metals (Student *t* test $p = 0.18$ and $p = 0.37$, respectively). Therefore, date ovigerous and nonovigerous female crabs were combined and renamed "females."

Comparing between seasons within the same sex, we observed that female crabs from both locations showed the highest Cu and Cd values in autumn and summer. Meanwhile, for almost all male crabs, the highest values of these heavy metals occurred in spring and summer. For Cd, this same phenomenon was only observed in crabs from CP. Meanwhile, in VM crabs, the highest values were in summer and autumn.

In addition, comparing between sexes within the same season, the results showed that within the temperate months, male crabs presented greater Cu values than female crabs. This tendency was reversed during the coldest seasons, when the values in the female crabs were greater. For Cd, the relation between sexes depends on the site. In VM crabs, concentrations were mostly greater in females (except for winter) but in CP crabs were mostly greater in males (except for autumn).

Nevertheless, despite these observed differences, Cu and Cd levels within the studied crab tissues did not significantly vary between sexes in any of the sampled locations (Student *t* test $p > 0.25$ for both metals). Therefore, sex differentiation was omitted in subsequent analysis.

Figure 2 shows the seasonal distribution of Cu and Cd in VM and CP crabs. Mean Cu concentrations ranged from 85.10 ± 5.23 (in CP) to 160.20 ± 23.45 (in VM) µg g^{-1} dw. There was no significant interaction between locations and seasons (two-way ANOVA, $p = 0.35$). Levels of Cu between locations did not significantly vary ($p = 0.82$). VM and CP showed similar mean concentrations in each season, with the lowest values occurring in winter. Values in winter were significantly lower than in the rest of the seasons at both sites ($p \leq 0.0002$). In the case of Cd, similar results were found. There was no significant interaction between locations and seasons (two-way ANOVA, $p = 0.83$). Mean Cd concentrations ranged from 5.26 ± 0.53 (in CP) to 11.26 ± 1.28 (in VM) µg g^{-1} dw. As for Cu, Cd levels between locations did not differ significantly ($p = 0.29$). In addition, for Cd there were significant differences between seasons ($p = 0.0081$), with significantly lower values during winter than during the remaining seasons at both sites. Because there were no significant differences in Cu and Cd levels in crabs between locations, data from both sites were joined and renamed "crabs from estuary." Values of each season in both locations were averaged, thus obtaining a mean value for each season with a corresponding SD. Figure 3 shows the seasonal distribution for Cu and Cd in Crabs from Estuary. Winter showed significantly lower mean Cu and Cd concentrations than spring, summer, and autumn, when the levels for these two metals were similar.

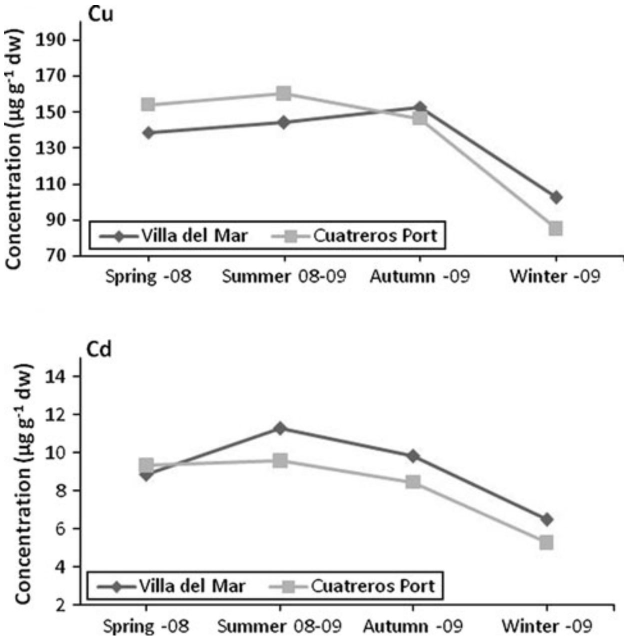

Fig. 2 Mean Cu and Cd concentrations (µg g^{-1} dw) for each season at VM and CP

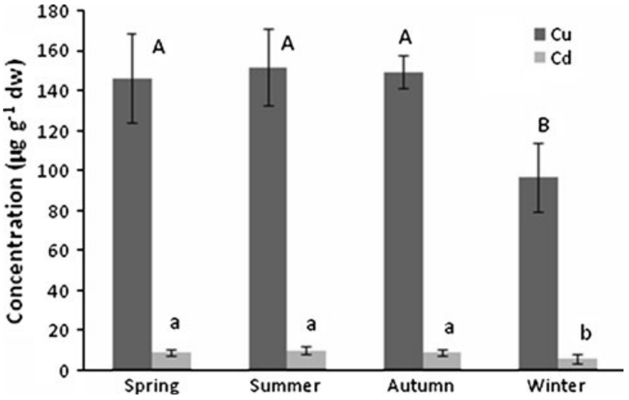

Fig. 3 Seasonal mean concentrations of Cu and Cd (µg g^{-1} dw) in soft tissues of *N. granulata* from the estuary. *Different letters* for each metal indicates significant difference ($p < 0.05$)

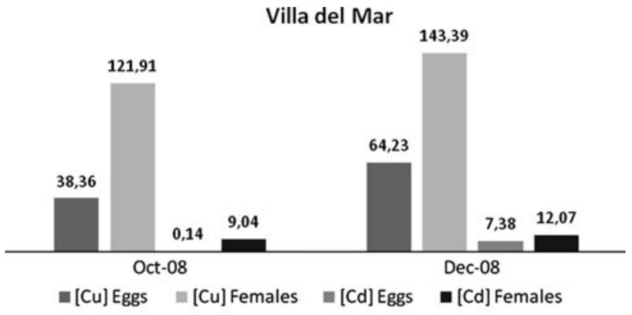

Fig. 4 Cu and Cd concentrations (μg g^{-1} dw) (mean value in *bars*) in eggs and female crabs from VM and CP

Metal Concentration in Eggs

Cu and Cd levels in eggs and female crabs from VM and CP are displayed in Fig. 4. Because no ovigerous female crabs were found in February 2009 at VM, comparison between eggs and female crabs during February was performed only for CP. In all cases, levels in female crabs were greater than levels in eggs; moreover, for Cu this increase was 1 order of magnitude greater. Mean concentrations of Cr were not included in the figure because most of values were lower than the detection limit, except for female crabs from VM and CP in spring.

Discussion

Sex is one of the biological intrinsic factors that may influence the bioconcentration of heavy metals in decapods crustaceans (Devescovi and Lucu 1995; Swaileh and Adelung 1995; MacFarlane et al. 2000; Turoczy et al. 2001; Barrento et al. 2009). Nevertheless, according to the literature, there is no general consensus about sex as a factor that may influence metal accumulation in this group. Although for some species differences between sexes are not significant, others do show sexual differences in trace metal accumulation. For the marine crab *Tachypleus tridentatus*, Kannan et al. (1995) did not find sex differences for Cu, Cd, cobalt, nickel, manganese (Mn), Hg, iron, and lead accumulation. Moreover, Turoczy et al. (2001), who worked with king crab *Pseudocarcinus gigas*, found that metal concentrations (Cd, Cu, Hg, and zinc (Zn)) did not depend significantly on the sex of the crab. Meanwhile, in the case of the shrimp, *Pleoticus muelleri*, Jeckel et al. (1996) found differences between male and female shrimp regarding Cu and Zn concentrations in digestive gland (for Cu 82.9 and 30.8 μg g^{-1} wet wt, respectively) and Zn, Mn, and Cd in reproductive system (for Cd 0.29 and 0.58 μg g^{-1} wet weight, respectively).

The obtained results showed that male and female *N. granulata* from VM and CP did not significantly differ with respect to Cu and Cd levels in any season. Cr concentrations could not be determined because the obtained values were lower than the detection limit in most of the samples in both sexes.

Previous studies in soft tissues of *N. granulata* from CP did present sex differences for Cu but not for Cd, with levels in male crabs being significantly greater than in female crabs (Ferrer 2001). Meanwhile, Beltrame et al. (2011) did not find sex differences for any of the three metals in hepatopancreas of *N. granulata* from Mar Chiquita Coastal Lagoon in the northern coast of Buenos Aires Province (Argentina). Cu is an essential metal for decapods crustaceans because they are an integral part of the respiratory pigment haemocyanin (Rainbow 1997). Several studies have shown that Cu does not accumulate but is regulated until it reaches a threshold exposure level beyond which Cu is accumulated proportionally to its external levels. Meanwhile, for Cd, a nonessential metal, this phenomenon has not been observed (White and Rainbow 1982; Rainbow and White 1989; MacFarlane et al. 2000; Rainbow 2002). White and Rainbow (1982) found that Cu concentrations in the intertidal shrimp *Palaemon elegans* were regulated at approximately 110 μg g^{-1} dw until certain exposure levels. In the case of Cd, levels in this species were directly proportional to external exposure. Moreover, MacFarlane et al. (2000) working with the semaphore crab, *Heloecius cordiformis,* found that Cu was regulated at concentrations between 200 and 600 μg g^{-1} wet weight. For *N. granulate*, the threshold level for Cu is still unknown. Because Cu is an essential element for blood pigments in crustaceans, the lack of differences between male and female crabs with respect to Cu concentrations may be because its demands do not differ between sexes.

Notwithstanding, as was mentioned in the Results section, there is a difference between male and female crabs regarding which seasons show greater Cu and Cd levels. For female crabs, the highest values were in autumn and summer; for male crabs, the highest values were in spring and summer. Although the statistics did not show this difference, it is something that must be considered in further studies because this could be related to the months with fullest activity, including reproduction, for this species (see later text).

Because bioconcentration of heavy metals in crustaceans may be also influenced by physicochemical extrinsic factors (e.g., water temperature and salinity) (Powell and White 1990; Pourang and Amini 2001), significant differences were expected to be observed in crabs from both locations. As already mentioned, VM and CP show differences in several physicochemical characteristics. Nevertheless, for the three analyzed metals these differences were not found. Moreover, for Cu and Cd, the seasonal distribution pattern in both locations was similar, with levels in winter being significantly lower than values during the remaining seasons.

Seasonal variations in metal concentrations in crustaceans has been described by several investigators (Swaileh and Adelung 1995; Devescovi and Lucu 1995; Cain and Luoma 1990; Bjerregaard et al. 2005; Beltrame et al. 2009). In general, it is expected to find a decrease in levels of heavy metals in winter. This may be related to varying seasonal growth rate, reproductive cycle, water salinity, and temperature. Devescovi and Lucu (1995) also found a decrease in Cu levels during winter in *Carcinus mediterraneus*, and they attributed this decrease to metabolic reactions induced by nutritional status. Beltrame et al. (2009) also found a seasonal variation for Cu, Cd, Cr, and Mn in *N. granulata* from Mar Chiquita Lagoon, Argentina, with the highest concentrations in spring and summer. These two are the seasons with the greatest biological activities, as opposed to winter months when crabs spend most of their time borrowed deep in crab beds (Ituarte et al. 2004). It is worth noting that Botte et al. (2010), working with four metals (Hg, Cd, Pb, and Cr) in tidal flats sediments from the Bahía Blanca estuary, found that levels of these metals were not associated with season. This supports the idea that seasonal variations in heavy-metal levels in *N. granulata* may be more related to internal than external conditions.

Table 2 lists mean concentrations of the three analyzed metals from several locations around the world, including Argentina. Concentrations of Cu, Cd, and Cr in soft tissues of *N. granulata* from Bahia Blanca estuary fall within the reported values for other marine crabs. Cu concentrations were comparable with those reported for *P. elegans* in Scotland (White and Rainbow 1985), *P. camtschaticus* in arctic Alaska (Jewett and Naidu 2000), and previous studies of *N. granulata* in the Bahia Blanca estuary (Ferrer 2001). Meanwhile, Cd levels obtained were greater than previous studies for this species and other crab species, except for *P. camtschaticus* in the Bering Sea (Jewett and Naidu 2000).

Heavy metals in superficial sediments were not measured in this study. Nevertheless, compared with previous studies of the estuary, Cu levels in *N. granulata* in this study were between 1 and 2 orders of magnitude greater than levels found in the environment during the period 2004 to 2006. In CP, the Cu sediment range was 6.45–23.57 $\mu g\ g^{-1}$ dw (Botté et al. 2009). In VM, the mean Cu concentration was $10.0 \pm 1.8\ \mu g\ g^{-1}$ dw (Hempel et al. 2008). Although the periods are not coincident, it is interesting to consider that this species is a deposit feeder when living in mudflats. Wang (2002) described that dietary uptake is the major route for metal accumulation for marine animals. Therefore, these results suggests that *N. granulata* in the Bahia Blanca estuary may regulate (and not bioaccumulate, considering what was mentioned previously) Cu concentrations within a certain range according to metabolic requirements. Because the relevance of the threshold value for Cu lies in the fact that essential metals may become toxic in excess, further studies should focus on determining the value for this metal in *N. granulata*.

When doing the same comparison for Cd, we found again that levels in crabs from the estuary were greater than levels described for superficial sediment, although this time

Table 2 Comparison of metal concentrations ($\mu g\ g^{-1}$ dw) (mean value ± SDs) measured in *N. granulata* with mean concentrations in reported in marine crabs in other studies from Argentina and the world

Species	Location	Tissue	Cu	Cd	Cr	Reference
N. granulata	Bahía Blanca, Argentina	ST	136.35 ± 26.38	8.25 ± 1.91	ND—1.11	This study
N. granulata	Bahía Blanca, Argentina	ST	155.62 ± 10.67	1.34 ± 0.37	ND	Ferrer (2001)
N. granulata	San Antonio Bay, Argentina	ST	92.98 ± 6.83	ND	–	Gil et al. (1996)
N. granulata	Mar Chiquita, Argentina	HP	335.47 ± 93.57	0.59 ± 0.57	0.90 ± 1.40	Beltrame et al. (2009)
P. elegans	Millport, Scotland	HP	110	0.9	–	White and Rainbow (1985)
C. maenas	Óbidos Lagoon, Portugal	HP	478 ± 109	0.17 ± 0.02	1.10 ± 0.21	Pereira et al. (2009)
Callinectes sapidus	Swan Lake, USA	ST	61.1	0.09	–	Park and Presley (1997)
Portunus pelagicus	Kuwait Gulf	HP	52.45 ± 2.92	–	0.52 ± 0.02	Al-Mohanna and Subrahmanyam (2001)
Paralithodes camtschaticus	Northeastern Bering Sea, Arctic Alaska	HP	127.48 ± 48.60	13.52 ± 3.48	0.91 ± 0.11	Jewett and Naidu (2000)

HP hepatopancreas, *ST* soft tissue, *ND* not detected only 1 order of magnitude greater. At CP, the Cd range was 0.56–4.44 μg g^{-1} dw (Botté et al. 2009), and for VM, the mean Cd concentration was 0.4 ± 0.1 μg g^{-1} dw (Hempel et al. 2008). In this case, a bioaccumulation process may be occurring because Cd is a nonessential metal (see later text).

This study showed that levels of Cr in *N. granulata* from CP ranged from not detectable to 1.20 μg g^{-1} dw, although levels of Cr in sediment measured by Botté et al. (2009) ranged from to 6.20 to 42.61 μg g^{-1} dw. At VM, values in crabs ranged from not detectable to 0.4 μg g^{-1} dw, whereas in sediment mean Cr concentrations were 3.6 ± 0.5 g^{-1} dw (Hempel et al. 2008). Beltrame et al. (2009) also found that levels of Cr in the environment were greater than levels in hepatopancreas of *N. granulata*. Moreover, Ferrer (2001) found values lower than the detection limit for Cr (Table 2). In addition, the same investigator described, after studying the geochemical partitioning of Cr in subtidal sediment, that $<10\%$ was in the potentially bioavailable fraction (PBF). This could be a good explanation for the levels of Cr in *N. granulata* that were lower than the detection limit. Nevertheless, Botté et al. (2008) found increased Cr in three fractions (including the exchangeable one, which belongs to the PBF) compared with previous results. These investigators suggested a possible recent input as a result of a new source in the area. Therefore, it is not clear why levels of Cr, a nonessential metal, are in most cases lower than the detection limit. Further investigation on this subject must be performed to clearly understand the behavior of this species with regard to Cr.

Bioaccumulation of metals in aquatic invertebrates may result from metal taken up from both dissolved and particulate phases (Wang and Fisher 1999; Rainbow 2007). In the first case, metals from water may enter by passive diffusion through the exoskeleton, e.g., in crustaceans (Rainbow 2007). Meanwhile, diet seems to be the major source of metals for several marine invertebrates because particulate metals from sediment and suspended particulate matter (SPM) may accumulate after food intake (Wang and Fisher 1999; Wang 2002).

Because *N. granulata* are intertidal organisms that are primarily deposit feeders when inhabiting mud flats (Iribarne et al. 1997), they are exposed and have the potential to bioaccumulate those metals, which are present in both phases (water and sediment). In the present study, we could not calculate the bioaccumulation of Cu, Cd, and Cr because of the lack of heavy-metal data in sediment and water from the same period of time. Nevertheless, as mentioned previously, in the case of Cu a regulation process may be occurring because levels found in crabs were greater than levels found in sediment. Cd levels in crabs were detectable during the entire sampling period and were greater than levels in sediment from previous years. Considering that Cd is a nonessential metal, it may be assumed that there is positive bioaccumulation of Cd in *N. granulata* from the Bahia Blanca estuary. In the case of Cr, is not so clear because the values were lower than the detection limit. Therefore, the behavior of *N. granulata* with regard to Cr must be investigated. Furthermore, in future studies, superficial sediment and water samples should be taken simultaneously with crab samples to determine bioaccumulation and biomagnification values.

Finally, the three metals were also measured in eggs from both locations and were compared with levels in corresponding female crabs. Although levels of Cu and Cd in eggs were always lower than levels in female crabs, these values were in fact detectable.

These findings are of great importance considering that there appears to be a bioaccumulation in this species before hatch. Several investigators have proposed that the increase of Cu with age could be due to the increase of the concentration of haemocyanin (White and Rainbow 1987; Swaileh and Adelung 1995). This could be a good explanation of why levels of Cu found in eggs of *N. granulata* were 1 order of magnitude lower than in female crabs. Therefore, levels of heavy metals found in eggs may be

Table 3 Comparison of mean heavy-metal concentrations (μg g^{-1} dw) (mean value ± SDs) in eggs from *N. granulata* from this study with other values reported in the literature

Species	Location	Cu	Cd	Cr	Moisture content (%)	Reference
N. granulata	VM	51.30 ± 18.29	3.76 ± 5.12	ND	75.5	This study
	CP	62.47 ± 4.59	6.38 ± 1.34	ND	76.67	
N. granulata	Mar Chiquita Lagoon	46.93 ± 0.14	0.52 ± 0.01	1.20 ± 0.09	–	Beltrame et al. (2009)
L. polyphemus	Maine, Florida	–	0.024 ± 0.007	0.25 ± 0.022	–	Burger et al. (2002)[a]
	Delaware Bay (1995)	–	0.07 ± 0.014	0.046 ± 0.004	–	Burger (1997)[a]
T. tridentatus	Hakata Bay, Japan	23.1	<0.001	–	–	Kannan et al. (1995)[a]

ND not detected

[a] Values expressed in μg g^{-1} wet weight

associated with the direct transference of these metals by the mother and by an external route (considering that the incubation of the eggs is external) because they are in direct contact with sediment and water since the time they are laid (Ituarte et al. 2004).

Table 3 lists comparisons between the values obtained in the present study with those found in the literature. There is scarce information about trace metals in crab eggs, and most of them are studies of toxicity on embryos and larvae (Ramachadran et al. 1997; Botton et al. 1998; Botton 2000; López Greco et al. 2001; Ferrer et al. 2006).

The results obtained for the crab *Limulus polyphemus* (Burger 1997; Burger et al. 2002; Table 3) showed detectable levels of Cd and Cr (Cu was not analyzed). Kannan et al. (1995) found detectable levels of Cu and low levels of Cd in eggs of the crab *Tachypleus tridentatus*. Meanwhile, Beltrame et al. (2009) measured several heavy metals (Cu, Cd, Cr, and Mn) in eggs of *N. granulata* in three different embryonic development stages. They found that there were no significant differences between stages. Except for Cr, the mean concentrations of the other two metals were lower than mean concentrations obtained in the present work.

Conclusion

Previous studies have shown that the Bahía Blanca estuary is a moderately polluted ecosystem because of increasing anthropogenic activities. The presence of heavy metals in the environment is of great importance because they can be transferred to the biota. In this study, three heavy metals (Cu, Cd, and Cr), which were previously described in the estuary, were measured in the crab *N. granulata*. According to the results, there were no significant differences between sexes with regard to heavy-metal accumulation. However, we believe that it would be better to keep them separated in further analysis. In contrast, there were no significant differences between locations regarding levels of heavy metals in crabs. Moreover, there were found seasonal variations in Cu and Cd levels, but according to the literature, these variations may be associated with internal conditions of the crabs. These results are surprising because it could allow us to use *N. granulata* as a bioindicator/biomonitor of this ecosystem in the future. The use of a bioindicator/biomonitor would be an excellent tool to assess the quality of this ecosystem. Finally, the presence of heavy metals found in eggs must be considered because crab's eggs are eaten by several species, such as shorebirds, seabirds, and fishes. The knowledge of heavy-metal accumulation not only in soft tissues but also in eggs is of great importance because *N. granulata* is a key species within the Bahia Blanca estuary, playing a major role in the transference of pollutants to greater trophic levels.

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