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Influences of Sex, Habitat, and Seasonality on Heavy-Metal Concentrations in the Burrowing Crab (*Neohelice Granulata*) From a Coastal Lagoon in Argentina

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Abstract Cadmium, chromium, copper, and manganese concentrations were determined in hepatopancreas of both sexes as well as in eggs at different embryonic development stages of the burrowing crab *Neohelice granulata* (Brachyura, Varunidae) from Mar Chiquita Coastal Lagoon, a biosphere reserve from Argentina, during a period of 1 year, to assess the bioaccumulation of metals associated with sex and seasonality. Furthermore, metal levels in associated superficial sediment samples were also determined. Two different "cangrejales," one in a mudflat and another one in a salt marsh, were studied. The results showed high concentrations of copper within the hepatopancreas, which was considered a strong reflection of high exposure of *N. granulata* to this metal. Metal accumulation in hepatopancreas from both study sites and sexes did not present significant differences, as did seasonality. In this sense, both spring and summer metal levels in hepatopancreas were the greatest. Eggs did not present differences in metal accumulation, with the exception of manganese, between sites or between crabs at different embryonic stages. For this metal, eggs from female crabs inhabiting mudflats showed higher levels than those from inhabiting salt marshes. Moreover, eggs in the late embryonic stage also showed the highest manganese concentrations. Metal levels in sediments, however, were similar in both sites. These are the first results of metal level in biota and sediments in this particular environment. Such results could be used as a baseline for the monitoring of metal levels in future studies in Mar Chiquita Coastal Lagoon.

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Aquatic environments are increasingly being contaminated with different kinds of organic and inorganic pollutants. Many of these dangerous compounds, such as heavy metals, can readily accumulate within crustacean tissues at much higher concentrations than those in the water column and in sediment (Rainbow 2007). This fact could be significant considering that both essential and nonessential metals can become toxic. In addition, natural and anthropogenic metal inputs influence the bioavailable metal supply within aquatic systems. This bioavailable fraction is usually determined by measuring the metal accumulated by organisms, which is the main goal in biomonitoring (e.g., Rainbow 1993; Zhou et al. 2008).

Aquatic invertebrates show a large variability in heavy-metal accumulation among both metals and taxa (Rainbow 1993, 2007). It is well known that heavy metals can be accumulated in tissues of aquatic animals; hence, heavy metals measured in tissues of these organisms can reflect past exposures (Canli and Atli 2003; Yilmaz 2003; Yilmaz and Yilmaz 2007). Metal bioavailability to marine invertebrates is influenced by a number of both physicochemical or extrinsic factors (e.g., metal speciation, salinity, and temperature) and biological or intrinsic factors (e.g., size, age, diet, and sex) (Powell and White 1990; Páez-Osuna and Ruiz-Fernández 1995; Borgmann 2000; Pourang and Amini 2001; Yilmaz and Yilmaz 2007; Barrento et al. 2009). The former affect all biota in almost the same way, depending on the environmental characteristics (Borgmann 2000), whereas biologic factors may act in different ways. Unlike this, other investigators have claimed that these factors are not important, and, if they were, their effect on metals accumulation could be variable (Kannan et al. 1995; Guns et al. 1999).

The semiterrestrial burrowing crab *Neohelice granulata* (Brachyura, Varunidae) is a eurihaline species widely distributed along the Atlantic coast of South America from Rio de Janeiro (Brazil) to Patagonia (Argentina). It is one of the main inhabitants of both the supratidal and intertidal zones of brackish salt marshes, estuaries, and coastal lagoons within the south eastern of South America and builds great cangrejales, which are distributed from the soft bare sediment flats to areas vegetated by the cordgrass *Spartina densiflora*. These crabs excavate and maintain semipermanent open burrows (Spivak et al. 1994). Specimens from the *Spartina*-dominated marsh and mudflats differ in their trophic mode, sediment-processing rate, burrow architecture, and burrow dynamics (Iribarne et al. 1997). This species reproduces during most of spring, all summer, and at the beginning of autumn (Spivak et al. 1994; Ituarte et al. 2004). The abundant populations of this species denote an important linking factor within the corresponding trophic web, and all stages of the crab's life cycle become a relevant food component for many fish, shellfish, and bird species. As a key species within this coastal lagoon, this crab plays a major role in the transference of pollutants to higher trophic levels.

A large population of *N. granulata* inhabits Mar Chiquita Coastal Lagoon and its corresponding wetlands. This is a shallow, unique coastal lagoon in Buenos Aires province (Argentina), located between 37°3′ to 37°43′ S and 57°15′ to 57°30′ W, which has been declared a biosphere reserve by the Man and Biosphere Reserve Program (MAB) from the United Nations Educational Scientific and Cultural Organization (UNESCO). This coastal lagoon provides refuge and food for numerous native and migratory species (e.g., crustaceans, fishes, and birds) and constitutes an estuarine environment with a very particular behavior (Marcovecchio et al. 2006; Beltrame et al. 2009). High concentrations of heavy metals, especially zinc, copper, and lead, in both dissolved and particulate forms, have been reported within this coastal lagoon. Also, manganese and chromium were presented at high concentrations in particulate phase (Beltrame et al. 2008, 2009). Other metals, such as cadmium, were also present but in lower levels.

Although copper, chromium, and manganese are essential elements required for the normal growth and metabolism of aquatic organisms, increased levels of these metals in aquatic ecosystems as a result of anthropogenic activities can lead to deleterious effects, which may be persistent and bioaccumulated in the environment (Paez-Osuna et al. 1998). Nonessential metals, such as cadmium, also have the potential to cause ecotoxicological damage in small amounts. Numerous effects of heavy metals on aquatic invertebrates have been observed. For example, cadmium has been shown to inhibit molting of the crab *N. granulata* (Rodríguez Moreno et al. 2003) and to produce histopathological injury in the white shrimp *Litopenaeus vannamei* (Wu et al. 2008). Manganese has been shown to significantly induce apoptosis in haematopoietic cells (Oweson et al. 2006) and to affect parts of food-search behaviour of the lobster *Nephrops norvegicus* (Krång and Rosenqvist 2006). Chromium has been shown to produce endocrine disruption in the crab *Ucides cordatus* as well as glycemia (Dias Corrêa et al. 2006) and decreased oxygen consumption in the mussel *Perna viridis* (Vijayavel et al. 2007). Copper exposure has been shown to produce increased oxidative stress response (Sabatini et al. 2009) and several morphological abnormalities in hatched larvae (Lavolpe et al. 2004) in the crab *N. granulata*. It is therefore essential to monitor the levels of these metals in aquatic systems. However, the bioaccumulation of heavy metals has still not been studied within this environment.

Considering the potential bioavailability of metals in this unique ecosystem, this study focused on determining seasonal, sexual, and habitat variations of cadmium, manganese, chromium, and copper levels within hepatopancreas and eggs from the burrowing crab *N. granulate* and in superficial sediments from Mar Chiquita Coastal Lagoon. Furthermore, heavy-metal concentrations within eggs in different embryonic development stages were compared. Moreover, a comparative study of the results against international reports was performed.

Materials and Methods

Study Site

Mar Chiquita Coastal Lagoon is located between 37°33′ and 37°43′ S and 57°15′ and 57°30′ W on the Atlantic coast of Buenos Aires Province, Argentina (Fig. 1). It has recently been declared a biosphere reserve under the UNESCO MAB. This coastal lagoon constitutes an estuarine environment with a very particular behavior. It has an area of approximately 60 km^2 with a tributary basin of 10,000 km^2. Its shape is irregular; the bottom topography is very smooth; and it reaches a maximum depth of 1.50 m (Lanfredi et al. 1987).

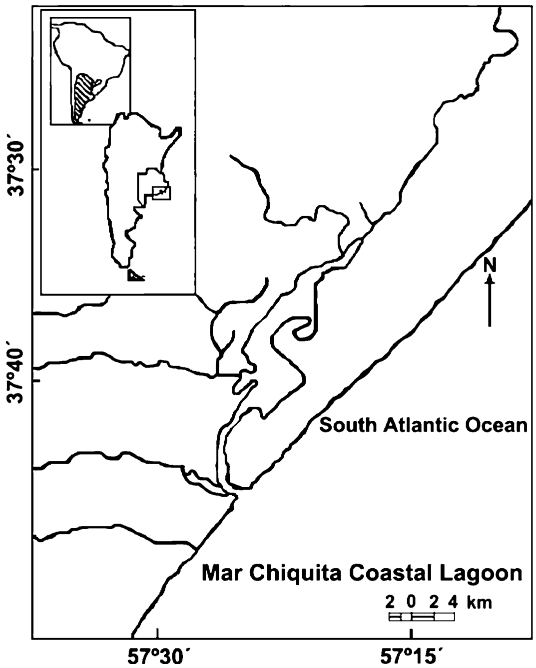

Fig. 1 Location of Mar Chiquita Coastal Lagoon

The lagoon is connected to the sea through an elongated inlet channel approximately 6 km long and >200 m wide. Freshwater influence is quantitatively more important than that of seawater (Marcovecchio et al. 2006). Its main input is continental drainage, which collects rainwater from a large basin, including the Tandilia orographic system, where an important agricultural land use is developed, and different residues are incidentally discharged within its body (Menone et al. 2000). Average rainfall for this area is approximately 900 mm year^{-1}, with a homogeneous distribution throughout the year. Although the greatest rainfall historically has been recorded during summer (December–March), significant rains occurred not only during summer (particularly in February in both years of study) but also during August (winter) during the study period. Moreover, the role of the phreatic reservoir, which regulates not only the lagoon water level but also the standard meteorological conditions within the area, has been fully recognized (Fasano et al. 1982). This coastal lagoon is a microtidal estuary, characterized by semidiurnal tides and shows strong salinity fluctuations (Marcovecchio et al. 2006; Beltrame et al. 2009). In addition it provides refuge and food for numerous native and migratory species (e.g., crustaceans, fishes, and birds).

Locations and Samples

Two different cangrejales located in different tidal flat sites from Mar Chiquita Coastal Lagoon were selected: one in a salt marsh and another in a mudflat. These sites were selected because they are fully representative of this system since they have been indicated as such in previous studies describing them (De Marco et al. 2005; Marcovecchio et al. 2006; Beltrame et al. 2009, among others) as well as in specific studies on these cangrejales (Iribarne et al. 1997; Ituarte et al. 2004; among others). This allows us to extrapolate the present results to the entire ecosystem.

A total of 70 specimens of *N. granulata* (35 female and 35 male crabs) were handpicked four times, representing different seasons, during low tide at the cangrejales in each site. Samples were collected during the period of November 2006–August 2007. Sluggish crabs, or those lacking one or more appendixes, were discarded. Crabs were transported to the laboratory in thermally isolated boxes with in situ—collected water. Mature crabs and those in intermolt stage with carapace width (maximum distance between the two prominent lateral spines) between 20 and 30 mm for female crabs and between 25 and 35 mm for male crabs were selected.

Once in the laboratory, crabs were anesthetized by freezing, washed with double-distilled deionised water, and their carapace width measured. Precautionary measures to prevent contamination during collection, dissection, and analysis were taken by cleaning all glassware and equipment in diluted nitric acid (0.7% v/v) (Clesceri et al. 1999). Samples were dissected out carefully to collect the hepatopancreas. Each hepatopancreas was placed on a filter paper to drain off any blood; they were then dried in an oven at 60°C until constant weight. The hepatopancreas from five crabs were then pooled according to sex, sampling site, and season and then homogenized by crushing them in a porcelain mortar.

In addition, ovigerous female crabs were collected from each site to determine heavy-metal levels in egg samples. After collection, female crabs were transported to the laboratory. An egg sample was taken from each ovigerous female crab, inspected under an optical microscope, and staged according to Bas and Spivak (2000). According to this system, eggs were classified in early embryonic stages (stages 1 through 3), intermediate embryonic stages (stages 4 through 7), or late embryonic stages (stages 8 and 9). Egg masses were removed from the abdomen and cleaned with double-distilled deionised water. Then egg masses were dried until constant weight. The egg masses from two female crabs were pooled according to embryonic development stage and sampling site. Pooled samples of each development stage from each site were homogenized. Finally, egg masses were grinded in a porcelain mortar and prepared for extraction.

A total of seven to eight pooled samples of hepatopancreas were prepared for each study site and for each season for both sexes. The total samples analyzed were 56 to 58 for each sex. The total of pooled egg samples were 7 for each embryonic stage group and each study site.

Furthermore, samples of sediments (approximately 10 to 15 cm thickness of the surface sediment) were collected bimonthly during 2 years using a corer sampler. A single core was collected randomly in each site. All bioclasts were extracted carefully and dried at 60°C until constant weight.

Analytical Procedures

All materials associated with trace-metal extraction were thoroughly acid-cleaned and rinsed with deionised water before use according to internationally recommended protocols (Clesceri et al. 1999). Samples were digested in a mixture of concentrated acids according to the method described by Marcovecchio and Ferrer (2005). Subsamples were removed and mineralized with a 1:3 perchloric-to-nitric acid mixture in a thermostatic bath (at 120 ± 10°C) up to minimum volume. Samples of eggs and hepatopancreas were pooled to reach a weight of 250 mg for each replicate. Solutions were made up to 10 ml with 0.7% nitric acid. Samples digestion was carried out in duplicate to ensure the reproducibility of the method.

Heavy-metal concentrations (cadmium, copper, manganese, and chromium) were determined using a Perkin-Elmer AA-2380® (PerkinElmer Inc., Boston, MA) atomic absorption spectrophotometer with air/acetylene flame. All concentrations are expressed in parts per million ($\mu g\ g^{-1}$) on a dry weight (dw) basis. Analytical grade reagents were used to build up the relevant blanks and calibration curves, and analytical quality was tested against reference materials (mussel tissue flour, Reference Material N° 6) was provided by The National Institute for Environmental Studies (Tsukuba Japan). The obtained values from the analysis of the reference materials were within the range of certified ones. The analytical, which as precision expressed as coefficients of variance, are <10% for all metals based on replicate analysis. Detection limits of the applied analytical method ($\mu g\ g^{-1}$) were as follows: cadmium 0.20, copper 0.77 chromium 0.20, and manganese 0.58. Percentage ranges of recovery within the analysis of reference materials to assess analytical quality were between 91.3 and 100.3% for all metals considered.

Data Analysis

A statistical package (Sigmastat Software 3.0®, Systat Software Inc., Point Richmond, CA) was used to analyze the results. Data of hepatopancreas samples were analyzed by one-way analysis of variance (ANOVA). When data did not satisfy parametric criteria Kruskal–Wallis test was used. Also, two-way ANOVA was performed to analyze interactions between seasons and sites. Multiple comparisons were tested with Dunn's or Holm-Sidak methods. Single differences were tested using Student *t* test. Regarding egg samples, data were analyzed by two-way ANOVA (embryonic developments and sites interaction analysis) followed my multiple comparison test (Holm-Sidak method).

In the case of samples that were lower than the detection limits of the applied analytical method, a value of one-half the detection limit was assigned and was included within the data set for statistical treatment.

Results

Sediments

Table 1 lists the distribution of copper, chromium, cadmium, and manganese in sediment samples from the two study sites (mudflat and salt marsh) from Mar Chiquita Coastal Lagoon. No statistical differences between both study sites were recorded for all metals analyzed ($p < 0.05$). Copper concentrations ranged from 3.62 to 8.01 $\mu g\ g^{-1}$; cadmium ranged from 0.95 to 2.66 $\mu g\ g^{-1}$; chromium

Table 1 Concentrations of heavy metals ($\mu g\ g^{-1}$ dw) in sediments collected from Mar Chiquita Coastal Lagoon

Metal	Site	Sediment ($\mu g\ g^{-1}$ dw)												Mean ± SD
		Aug 2004	Oct 2004	Dec 2004	Feb 2005	April 2005	Jun 2005	Aug 2005	Oct 2005	Dec 2005	Feb 2006	April 2006	Jun 2006	
Cd	SM	0.98	1.41	1.32	1.50	1.34	1.95	2.20	2.27	2.13	1.42	2.51	2.66	1.73 ± 0.54
	MF	1.08	0.95	1.05	1.43	1.06	1.98	0.93	2.35	2.36	1.32	2.21	2.64	1.5 ± 0.64
Cu	SM	7.1	5.68	5.59	5.25	6.1	6.27	5.97	5.43	5.72	6.25	7.74	3.62	5.81 ± 1.0
	MF	4.5	6.35	4.84	5.22	5.76	7.23	6.63	8.01	5.56	6.85	7.68	7.17	6.23 ± 1.14
Cr	SM	3.6	3.28	3.43	3.76	3.56	5.45	2.41	4.20	6.03	0.53	5.51	0.58	2.86 ± 1.74
	MF	2.36	6.59	3.85	3.74	3.72	5.40	3.6	3.60	7.25	0.34	7.41	2.75	3.46 ± 2.09
Mn	SM	63.15	67.03	72.53	58.76	80.61	96.06	84.8	87.58	84.54	634.86	148.83	125.75	100.34 ± 159.94
	MF	69.77	214.09	81.09	73.56	76.19	107.5	75.86	152.28	84.79	654.96	168.3	283.19	129.53 ± 166.88

MF mudflat, *SM* salt marsh

ranged from 0.34 to 7.25 μg g^{-1}; and manganese ranged from 58.76 to 654.96 μg g^{-1}. In addition, the local distribution of metals in sediments showed a similar pattern in both study sites.

Metal Concentrations in Hepatopancreas and Eggs

Mean concentrations and associated SDs of manganese, cadmium, chromium, and copper in hepatopancreas of male and female specimens of *N. granulata* collected during four seasons from salt marsh and mudflat in Mar Chiquita Coastal Lagoon are listed in Tables 2 and 3. Metal concentrations were calculated in μg g^{-1} on a dw basis.

There were no significant differences in cadmium levels in hepatopancreas between different sexes and sites. Moreover, cadmium concentrations varied significantly among seasons within the studied crab tissues (Table 2). Higher concentrations of cadmium were observed in spring and summer than in autumn and winter. Also, there was no statistically significant interaction between seasons and sites in both sexes. Cadmium concentrations were similar in hepatopancreas and eggs. Cadmium concentrations in hepatopancreas ranged from not detectable (ND) to 2.13 μg g^{-1} dw, whereas in eggs cadmium concentrations ranged from ND top 1.1 μg g^{-1} dw. In the egg analysis, there were no significant differences among embryonic stages ($p = 0.242$) or between sites ($p = 0.925$), and there was no interaction between both levels ($p = 0.339$).

Mean chromium concentrations were similar in hepatopancreas of female and male crabs, and they were also similar between both sites (Table 3). There were significant differences among the analyzed seasons in both study sites and sexes ($p < 0.05$) (Table 2). The highest chromium concentrations were detected in spring for both salt marsh and mudflat study sites. Interaction between seasons and sites was not observed. The concentration range in hepatopancreas ranged from ND to 4.71 μg g^{-1} dw. Regarding the eggs, no significant differences were found in mean chromium concentrations between both sites ($p = 0.638$) and among the studied embryonic stages ($p = 0.587$); also, there was no statistically significant interaction between embryonic stages and sites ($p = 0.2$). The concentration range in eggs ranged from ND to 3.08 μg g^{-1} dw (Table 4). Chromium levels in hepatopancreas and eggs were similar.

Similar concentrations of copper were observed in the hepatopancreas of all female and male crabs (Table 3) in both salt marsh and mudflat. Also, no significant differences were observed between both sites. Copper concentrations varied significantly ($p < 0.05$) in hepatopancreas among seasons. Concentrations during spring were higher than those during other seasons. There was no statistically significant interaction between sites and seasons ($p < 0.005$).

Table 2 Seasonal heavy-metal concentrations (mean value ± SD) (μg g^{-1} dw) in hepatopancreas of *N. granulata* from Mar Chiquita Coastal Lagoon

Metal	Sex	Site	Seasons			
			Spring	Summer	Autumn	Winter
Cd	Male	MF	0.71 ± 0.51^{C}	1.22 ± 0.59^{A}	$0.42 \pm 0.24^{B,C}$	0.11 ± 0.14^{B}
		SM	0.34 ± 0.29^{A}	0.81 ± 0.42^{B}	0.38 ± 0.17^{A}	0.12 ± 0.067^{A}
	Female	MF	1.10 ± 0.66^{A}	0.83 ± 0.60^{A}	0.47 ± 0.086^{A}	0.12 ± 0.25^{B}
		SM	0.82 ± 0.75	0.92 ± 0.48	0.33 ± 0.29	0.34 ± 0.17
Cu	Male	MF	1442.10 ± 1183.66^{A}	241.44 ± 101.82^{B}	44.98 ± 32.74^{B}	55.70 ± 18.45^{B}
		SM	56.79 ± 27.98	69.96 ± 25.62	82.99 ± 46.80	49.53 ± 17.18
	Female	MF	555.35 ± 234.46^{A}	88.21 ± 22.98^{B}	37.73 ± 20.27^{B}	91.08 ± 33.31^{B}
		SM	1672.34 ± 1868.24^{A}	71.76 ± 33.90^{B}	61.85 ± 25.41^{B}	86.17 ± 17.47^{B}
Mn	Male	MF	10.21 ± 4.64^{A}	$9.25 \pm 2.29^{A,C}$	ND^{B}	5.80 ± 2.26^{C}
		SM	7.51 ± 1.72^{A}	8.64 ± 3.25^{A}	ND^{B}	3.79 ± 1.73^{C}
	Female	MF	17.58 ± 6.47^{A}	14.80 ± 6.84^{A}	ND^{B}	4.01 ± 1.72^{B}
		SM	15.87 ± 3.04^{A}	12.47 ± 2.47^{B}	ND^{C}	4.27 ± 1.48^{D}
Cr	Male	MF	1.87 ± 1.18^{A}	0.49 ± 0.47^{B}	ND^{B}	0.07 ± 0.08^{B}
		SM	1.98 ± 1.22^{A}	0.30 ± 0.24^{B}	ND^{B}	0.73 ± 0.51^{B}
	Female	MF	3.12 ± 1.54^{A}	1.27 ± 1.97^{B}	ND^{B}	0.31 ± 0.48^{B}
		SM	2.56 ± 1.45^{A}	0.44 ± 0.36^{B}	ND^{B}	0.61 ± 0.42^{B}

MF mudflat, *SM* salt marsh, *ND* not detected

Means in the same line with different superscripts were significantly different among seasons ($p < 0.05$)

N = Seven to eight (pooled samples)

Table 3 Annual heavy-metal concentrations (mean value ± SD) (μg g^{-1} dw) in hepatopancreas of male and female crabs *N. granulata* from Mar Chiquita Coastal Lagoon

Metal	Saltmarsh			Mudflat		
	Male ($N = 29$)	Female ($N = 27$)	Mean	Male ($N = 30$)	Female ($N = 27$)	Mean
Cd	0.39 ± 0.35	0.58 ± 0.53	0.49 ± 0.45	0.62 ± 0.57	0.62 ± 0.57	0.59 ± 0.57
Cu	64.59 ± 32.24	560.06 ± 1232.16	312.33 ± 897.49	485.89 ± 853.93	171.96 ± 220.47	335.47 ± 93.57
Mn	4.82 ± 3.90	8.11 ± 6.96	6.47 ± 5.82	6.48 ± 4.92	8.85 ± 8.68	7.62 ± 7.00
Cr	0.77 ± 1.01	1.01 ± 1.33	0.89 ± 1.18	0.66 ± 1.01	1.17 ± 1.71	0.90 ± 1.40

Statistical differences between sexes were not observed

Table 4 Mean heavy-metal concentrations (μg g^{-1} dw) and SDs in eggs of *N. granulata* from Mar Chiquita Coastal Lagoon

Metal	Site	*N*	Embryonic development stage			
			Early	Intermediate	Late	Mean concentration
Cd	MF	7	0.40 ± 0.18	0.61 ± 0.16	0.50 ± 0.49	0.51 ± 0.30
	SM	7	0.41 ± 0.12	0.44 ± 0.28	0.71 ± 0.25	0.52 ± 0.25
Cu	MF	7	47.20 ± 8.40	40.65 ± 7.40	53.22 ± 13.78	47.025 ± 10.88
	SM	7	45.50 ± 7.45	46.00 ± 8.77	48.98 ± 14.84	46.83 ± 10.16
Mn	MF	7	28.07 ± 3.95^{A}	56.01 ± 14.30^{B}	53.79 ± 14.22^{B}	**45.96 ± 17.12**
	SM	7	9.72 ± 1.58	14.55 ± 1.61	23.43 ± 13.82	**15.90 ± 9.52**
Cr	MF	7	1.21 ± 0.72	1.62 ± 1.00	0.96 ± 0.61	1.26 ± 0.79
	SM	7	0.80 ± 0.22	1.07 ± 0.68	1.53 ± 0.92	1.13 ± 0.70

MF mudflat, *SM* salt marsh, *N* pooled samples

Means in the same line with different superscripts were significantly different ($p < 0.05$) between eggs embryonic development stage

Mean concentrations in bold text presented significant differences between both study sites

No significant differences in egg copper concentrations were found between embryonic stages ($p = 0.271$) or study sites ($p = 0.959$); moreover, interaction between both levels were not observed ($p = 581$) (Table 4). Copper concentrations in hepatopancreas largely varied (between 13.87 and 4443.98 μg g^{-1} dw) in both sexes and study sites. Copper levels in eggs were more constant and ranged between 35.53 and 61.69 μg g^{-1} dw. In all cases, copper levels in hepatopancreas were greater than in eggs.

No significant differences were observed between manganese concentrations in hepatopancreas crabs from salt marsh versus mudflat or between both sexes (Table 3). Significant differences ($p < 0.05$) in manganese concentrations in hepatopancreas were found among seasons. During spring and summer, detected levels were greater than those in autumn and winter in both study sites. Interaction between seasons and sites was not observed ($p < 0.005$). Manganese concentrations in hepatopancreas of male and female crabs varied between ND and 25.83 μg g^{-1} dw. Concentrations of manganese in eggs from mudflat were higher than those from salt marsh ($p < 0.001$). Mean concentrations were 45.96 ± 17.12 and 15.90 ± 9.52 μg g^{-1} dw, respectively, for mudflat and salt marsh. Furthermore, there was a statistically significant difference between embryonic stages ($p = 0.02$). Concentrations of this metal measured in crabs in early embryonic stages were lower than in crabs in intermediate and later stages (Table 4). There was no statistically significant interaction between embryonic stages and sites ($p = 0.563$).

Discussion

Results of the present study clearly indicated that a copper bioconcentration occurred in *N. granulate*: The concentrations in eggs and hepatopancreas were two to three orders of magnitude greater than those within the sediment. In contrast, cadmium and manganese levels were close to one order of magnitude greater in sediment than those measured in *N. granulata*. The comparative assessment between salt marsh and mudflat showed that the concentration of metals in sediments was relatively uniform in both sites.

Moreover, a comparison of mean concentrations of trace metals reported in decapod crustaceans for other areas within the world are listed in Table 5. Concentrations of the four metals in hepatopancreas of *N. granulata* fall within the range of values included in the mentioned investigations. Copper presented the highest concentration among the four metals included in the present study, although the measured concentrations were lower than the values reported for crabs in Óbidos Lagoon, Portugal (Pereira et al. 2009). Cadmium concentrations in *N. granulata* hepatopancreas were low, and these values were comparable to those reported for *Carcinus maenas* (Pereira et al. 2009). In contrast, chromium concentrations of *N. granulata* were similar to values recorded within hepatopancreas from *Paralithodes camtschaticus* and *C. maenas* (Chou et al. 2002; Pereira et al. 2009). However, manganese concentrations in *N. granulata* were lower than those reported for *C. maenas* (Pereira et al. 2009) as well as similar to those from other species (Table 5).

Previous studies have shown that copper accumulation in aquatic invertebrates is organ- and species-specific as well as highly dependent on the water quality in which copper exposure occurs (Perkins et al. 1997). Decapod crustaceans have been reported to have relatively higher concentrations of copper in their bodies because this metal is a component of the respiratory pigment haemocyanin. White and Rainbow (1982) reported that decapod crustaceans can regulate their body copper concentrations, which is required for haemocyanin synthesis. However, above a certain concentration of copper in the external medium, the regulation breaks down, and decapod crustaceans accumulate copper (Scott-Fordsmand and Depledge 1997). Therefore, high copper concentrations in hepatopancreas it is not surprising because this tissue is thought to serve as a natural copper store (Brouwer and Brouwer 1998; Djangmah and Grove 1970; Engel 1987).

Metal bioavailability to marine invertebrates can be influenced by a number of intrinsic and extrinsic factors. However, some studies have shown that these factors are not important, and, if they were, their effect on metal accumulations can be variable (Kannan et al. 1995; Guns et al. 1999). An exhaustive analysis directed to estimate the key parameters controlling metal accumulation within *N. granulata* in Mar Chiquita Coastal Lagoon has been shown to be necessary. Thus, several endogenous (sex, organ) and exogenous factors (season, habitat) were analyzed in the present study.

It is well known that dietary exposure is the major route for metal bioaccumulation in many marine animals (Borgmann 2000; Wang 2002). This fact pointed out the assimilation efficiency of contaminants, which is critical for understanding both their bioaccumulation and trophic transfer ability in aquatic invertebrates (Wang and Fisher 1999). *N. granulata* specimens from the *Spartina*-dominated marsh and mudflats differ in their trophic mode (Iribarne et al. 1997). Crabs were herbivorous when associated with the cordgrass *S. densiflora* at the salt marsh as were deposit feeders when living in tidal creeks and channels. However, differences in hepatopancreas metal levels between both study sites were not observed in the present study. This suggests that metal bioavailability in both sites was the same or even that the diet in this species is an unimportant factor within the mentioned process. Certain studies have shown that in some aquatic invertebrates, heavy-metal uptake through the dissolved phase is greater than that obtained by food in some aquatic invertebrates (Wang et al. 1996a, b; Chong and Wang 2000).

Season may influence body burdens of heavy metals. This seasonal variability may result from either internal biological cycles of the organism or from changes in the

Table 5 Maximum concentrations values (μg g^{-1} dw) and SDs of trace metals reported in hepatopancreas of decapod crustaceans from other areas of the world

Species	Location	Cd	Cu	Cr	Mn	References
Portunus pelagicus	Kuwait Gulf	–	52.45 ± 2.92	0.52 ± 0.02	1.62 ± 0.07	Al-Mohanna and Subrahmanyam (2001)
Pseudocarcinus gigas	Southeast Australian	22.4 ± 17.5	52 ± 6	–	–	Turoczy et al. (2001)
Carcinus maenas	Óbidos Lagoon, Portugal	0.17 ± 0.02	478 ± 109	1.1 ± 0.21	26 ± 3.8	Pereira et al. (2009)
Paralithodes camtschaticus	Northeastern Bering Sea, Arctic Alaska	13.52 ± 3.48	127.48 ± 48.60	0.91 ± 0.11	–	Jewett and Naidu (2000)
Cancer irroratus	Bay of Fundy, Atlantic Canada	48.8 ± 41.4	165 ± 95.3	–	2.78 ± 0.52	Chou et al. (2002)
N. granulata	Mar Chiquita, Argentina	0.59 ± 0.57	335.47 ± 93.57	0.90 ± 1.40	7.62 ± 7.00	This study

availability of metals in the organism's environment. In this sense, it is well known that the seasonal spawning of gametes from many benthic invertebrates usually changes the relative proportion of body tissue weight within different organs (Pourang et al. 2004). Moreover, Steenkamp et al. (1994) reported that significant differences in metal levels were detected during several months for most tissues (except the gills) in the crab *Potamonautes warreni* sampled from the Natalspruit River (South Africa). Joseph and Srivastava (1992) showed that heavy-metals level exhibited seasonality in the prawn *Penaeus indicus*, and relatively high concentrations were observed in prawns collected during November (autumn in the North Hemisphere). The present study has shown a significant seasonal variation ($p < 0.05$) in the concentration of the studied metals in the selected organs of this burrowing crab. The highest concentrations were detected in individuals collected during spring and summer (which are the seasons fullest of biological activities), in agreement with the temperature peak of seawater within the studied region (Beltrame et al. 2009). This could produce an increased metabolic rate, which induces increased oxygen consumption and uptake of dissolved metals by gills. Seasonality could be associated also with reproduction stage concordant with the months (i.e., September through April) of the reproductive period (Ituarte et al. 2004). The reproductive period could generate greater energy consumption, thus producing decreased detoxification ability and therefore increasing metal bioaccumulation. Seasonality may also produce a different metals bioavailability due to different seasonal conditions.

Specimen size is a factor that has been demonstrated to influence the concentration of trace metals in crustaceans (Chen et al. 2005; MacFarlane et al. 2000). The total length of *N. granulata* ranged from 20 to 30 mm and 25 to 35 mm for female and male crabs, respectively, at both sites in this study. This range was chosen because at this size individuals are functionally mature (López Greco and Rodríguez 1998) and are the greater size ranges encountered in the environment in both sexes. Significant differences in size among seasons were not recorded for both sexes. Therefore, this factor could not be the responsible for the observed seasonal bioaccumulation patterns of the studied metals in male and female crabs within *N. granulata* from Mar Chiquita Coastal Lagoon.

Salinity is another factor that can influence metal bioaccumulation patterns in aquatic invertebrates. Decreased salinity is known to increase both the bioavailability and toxicity of metals (McLusky et al. 1986; Wright 1995; Beltrame et al. 2008); thereby this fact could explain increased body concentrations or toxicity of metals at lower salinities. However, greater metal bioaccumulation during spring or summer was observed in the present study, when salinity was higher mainly because of higher temperatures and increasing evaporation (Beltrame et al. 2009). Thus, the present results confirmed that salinity has not influenced metals bioaccumulation within the studied crab.

Sex is another intrinsic factor that was considered in this study. Significant differences in heavy-metal accumulation between both sexes was not been observed. Kannan et al. (1995), who worked with the marine crab *Tachypleus tridentatus*, did not observe differences in accumulation of cadmium, cobalt, copper, iron, mercury, manganese, nickel, or lead between sexes. In a similar way, Sastre et al. (1999) did not observe sex differences in accumulation processes of cadmium, mercury, lead, and copper within the crab *Callinectes* spp.

In addition, several studies (i.e., Rainbow 2002) have indicated that essential metals, such as copper and zinc, can be regulated and do not accumulate in decapods crustaceans until certain environmental threshold levels are reached. In this study, *N. granulata* showed copper and manganese differences during the year, and therefore, no regulation of these metals in this species. This suggests that the bioavailability of these metals were higher than threshold levels or that this species may have not the intrinsic metabolic ability to regulate these elements. In contrast, no crustacean appears to regulate the body concentration of nonessential metals, such as cadmium and chromium (Rainbow 1985; Rainbow and White 1989). Therefore, the yearly variation in total body concentrations of these metals is not surprising.

Cadmium, copper, and chromium levels in eggs were similar in both study sites and among crabs in different embryonic stages. Nevertheless, manganese showed significant differences. Eggshell consists of a trichromatic outer membrane and an inner chitinous membrane. Egg membranes of the spider crab *Hyas araneus* have been shown to dramatically increase in permeability to water and minerals just before the hatching process (Pandian 1970a, b; Petersen and Anger 1997). Increased permeability of the shell could result in increased manganese levels during the intermediate and late embryonic stages. However, other metals did not increase with egg development, which could indicate a special requirement of manganese by embryos during development.

Eriksson (2000) observed increased manganese accumulation in eggs of the lobster *Nephrops norvegicus* at the end of development. Metal concentrations reported in eggs of the crab *Limulus polyphemus* were as follows: cadmium 70 ng g^{-1}; chromium 46 ng g^{-1}, and manganese 2210 ng g^{-1} wet weight (Burger et al. 2002). Although results as obtained in this study are measured in dw, they are nevertheless higher, which indicates greater metal bioavailability. The increased manganese levels during the late stages of embryonic development are an interesting point.

Most of this metal in aquatic crustaceans is incorporated into calcified regions, such as the exoskeleton and the ossicles and teeth of the gastric mill, due to the fact that manganese readily replaces calcium in its carbonate form (Bryan and Ward 1965). For this reason, Eriksson (2000) suggested that because crustacean larvae are thought not to have a calcified cuticle, this could not explain the dramatically increased manganese concentration found in the eggs. However, the hatched lobster embryos develop into carnivorous zoea larvae with a completely functional alimentary canal, and it is therefore more likely that the suddenly increased egg manganese concentrations might be explained by the development of the gastric mill. This is an interesting point to be studied in future works.

Because female crabs at both sites presented similar levels of manganese, the difference in eggs levels could not be related with the direct transference of this metal by the mother. In contrast, it could be related to the direct uptake of manganese from pore water because ovigerous female crabs carry their eggs externally; thus, embryos are in direct contact with the sediment. The bioavailability of manganese (in its reduced form [Mn^{2+}]) could be greater in salt marsh than in mudflat.

Conclusion

The results of the present study give baseline information on the concentrations of four heavy metals—chromium, cadmium, copper, and manganese—in sediment and within hepatopancreas and eggs of the burrowing crab *N. granulata*, which could be useful to programs directed at the conservation of Mar Chiquita Coastal Lagoon biodiversity. This is of great importance considering this is a UNESCO natural reserve due to its particular biological and ecological characteristics.

Interpreting metal levels in this species involves understanding both the effects on the crabs themselves and those on the organisms that consume the crabs or their eggs. From an ecotoxicological point of view, metal concentrations within this species are of great concern within this biosphere reserve, especially because this crab is a key species within the estuarine environment, and consequently they play a major role in the transference of pollutants toward higher trophic levels.

Contaminant concentrations reported in this study in sediments and crabs from Mar Chiquita Coastal Lagoon were, in general terms, similar or even higher than those reported in studies of other estuarine systems.

This results of this study indicate that hepatopancreas of *N. granulata* did not collect different levels of cadmium, copper, chromium, and manganese in this environment on a spatial scale (i.e., between sites), but it did show such on a temporal scale (i.e., among seasons). Furthermore, eggs did not show spatial differences, except for manganese, which presents different levels between salt marsh and mudflat. Moreover, eggs can accumulate greater manganese levels in later embryonic developmental stages.

A general conclusion is that the concentrations of some metals in *N. granulata* exhibited seasonal variability, and this is a significant factor to be considered in monitoring programs. Moreover, there is no remarkable degree of sex influence on the level of bioaccumulated metals, suggesting that samples of both sexes could be used in monitoring programs.

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