

Contamination in newly hatched larvae of the mangrove crab *Ucides cordatus* and a new perspective about trace elements transport

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RESUMO

Manguezais são ecossistemas relevantes devido ao seu papel ecológico, uso econômico e importância social. Uma espécie abundante e importante nos manguezais brasileiros é o explorado caranguejo *Ucides cordatus*. Essa espécie é foco do presente estudo, cujo objetivo foi avaliar a concentração de elementos traço no hepatopâncreas de fêmeas ovígeras, em ovos (estágios de pré-eclosão e de eclosão) e, pela primeira vez, em larvas recém-eclodidas. Fêmeas ovígeras foram capturadas em dois manguezais do sudeste do Brasil (Baía de Guanabara e estuário secundário do Rio Paraíba do Sul) e desovas foram realizadas em tanques, em laboratório. Algumas fêmeas não foram levadas para os tanques, mas congeladas logo após a coleta. Não houve diferença significativa na concentração de elementos traço entre essas fêmeas e aquelas mantidas nos tanques. Elementos traço essenciais e não essenciais foram detectados nas larvas recém-eclodidas. Em comparação com a fêmea adulta, os elementos Zn, Cu e Mn apresentaram as maiores concentrações em larvas e ovos. As concentrações de Vanádio, Cr e Mn foram significativamente maiores nos ovos em estágio de eclosão do que nas larvas recém-eclodidas, indicando a retenção de elementos na casca do ovo. Considerando as concentrações de V (0,17 - 1,17 $\mu\text{g}\cdot\text{g}^{-1}$ peso seco), Cu (14,1 - 41,1 $\mu\text{g}\cdot\text{g}^{-1}$ peso seco) e Zn (235,4 - 263,9 $\mu\text{g}\cdot\text{g}^{-1}$ peso seco) em larvas recém-eclodidas, da mesma ordem de grandeza das observadas em material particulado em suspensão (MPS) e sedimentos em suspensão, a dispersão das larvas planctônicas pode resultar no transporte de elementos para além do manguezal e sua introdução em águas costeiras.

Palavras-chave: metais traço; larva de caranguejo; ovos de caranguejo; hepatopâncreas; Sudeste do Brasil

ABSTRACT

Mangroves are relevant ecosystems due to their ecological role, economic use, and social importance. One of the most abundant and important species in Brazilian mangroves is the exploited crab *Ucides cordatus*. This study focuses on this species and the aim was to evaluate the concentration of trace elements in the hepatopancreas of ovigerous females, eggs (pre-hatching and hatching stages), and for the first time, in newly hatched larvae. Ovigerous females were captured in two Southeastern Brazil mangroves (Guanabara Bay and Paraíba do Sul River's secondary estuary) and spawning was held in spawning tanks, in the laboratory. Some females were not taken to the tanks, but they were frozen right after collection. There was no significant difference in trace element concentration between these females and those kept in the spawning tanks. Both essential and non-essential trace elements were detected in newly hatched larvae. Comparing with the adult female, the elements Zn, Cu, and Mn showed the highest concentrations in larvae and eggs. Vanadium, Cr, and Mn

concentrations were significantly higher in hatching eggs than observed in newly hatched larvae, indicating the retention of elements in the eggshell. Considering the V (0.17 - 1.17 $\mu\text{g}\cdot\text{g}^{-1}$ dry weight), Cu (14.1 - 41.1 $\mu\text{g}\cdot\text{g}^{-1}$ dry weight), and Zn (235.4 - 263.9 $\mu\text{g}\cdot\text{g}^{-1}$ dry weight) concentrations in newly hatched larvae, with the same order of magnitude observed in suspended particulate matter (SPM) and sediments in suspension, the planktonic larval dispersion could result in the transport of elements beyond the mangrove, and their introduction in coastal waters.

Keywords: trace metals; crab larvae; crab eggs; hepatopancreas; Southeastern Brazil

1 INTRODUCTION

Human activities have resulted in a large introduction of trace elements into the environment, and high concentrations of these elements in coastal sediments have been a great concern in recent decades (RAINBOW; FURNESS, 2018). Accumulation of these elements is more notable in low hydrodynamic environments with high deposition rates, such as lagoons, bays, and deltas. Among these environments there are the mangroves, coastal ecosystems with extensive deposition of fine sediments – which are considered geochemical barriers due to the physical retention of particles – and formation of iron plaques in trees' roots, with consequent retention of trace metals (MACHADO *et al.*, 2005). In addition, mangroves are critical ecosystems due to their relevant ecological role, economic use, and social importance (FIELD, 1995).

Crabs are keystone species in mangroves and salt marshes, occupying different trophic levels. In these habitats, it is possible to observe diversified crab feeding habits, such as primary consumers (herbivorous, algivorous), omnivorous, predators, and detritivorous. Due to the crabs' relevance, many studies on trace element contamination have been carried out with these animals. Burrowing crabs are bioturbators that show particularly important participation in trace elements dynamics, as shown by Costa *et al.* (2017). These authors pointed out the significant influence of the mud crab *Neohelice granulata* in the environment's arsenic dynamics. Unfortunately, the trace elements studies were held mainly with adult males. Few studies have been conducted with females, and in Brazil, we can mention Silva *et al.* (2018) in the Amazon region. Contamination analysis in crab eggs is still rare, and we can mention studies with *N. granulata* (BELTRAME *et al.*, 2010, SIMONETTI *et al.*,

2012), *Callinectes* sp. (LAVRADAS *et al.*, 2014), and *Ucides cordatus* (ALMEIDA *et al.*, 2016). Regarding crab larvae, there is a predominance of ecotoxicological studies (LÓPEZ-GRECO *et al.*, 2002; FERRER *et al.*, 2006; AMIN; COMOGLIO 2010; LYLA; KHAN, 2010). In marine species, previous studies were limited to the analysis of maternal tissues and egg contamination, whereas newly hatched larvae were rarely considered. Up to this date, no study has focused on the contamination of newly hatched larvae. When females and early stages are disregarded in contamination studies, valuable data on the relationship between contaminants and crab reproduction are lost.

Ucides cordatus, the focus species of the present study, is known as “uçá crab” or mangrove crab and is one of the main crabs in mangroves of the western South Atlantic Ocean (SAO). Due to its burrowing and detritivorous habits, this crab is considered a keystone species, playing an important role in nutrient cycling (NORDHAUS; WOLFF, 2007). Unfortunately, this crab appears on the list of endangered species, mainly due to overexploitation and frequent environmental issues in Brazilian mangroves such as deforestation, landfill, shrimp farming, and effluent disposal (PINHEIRO; RODRIGUES, 2011). The mangrove crab reproduces in the summer and females carry the egg mass in their abdominal appendages for approximately 20 days (PINHEIRO; HATTORI, 2003). Their spawning is synchronized with nocturnal ebb tides, and the species exhibits a larval export strategy (ALMEIDA *et al.*, 2013). The *U. cordatus* planktonic larvae need salinities above 15 psu (practical salinity unit) to develop successfully, so they usually need to be transported far from the adults' habitat (DIELE; SIMITH, 2006). As fertility rates are high, from 64,000.00 up to

220,800.00 eggs.female⁻¹ (HATTORI; PINHEIRO, 2003), spawning periods result in a massive introduction of larvae into the pelagic environment.

During embryonic development, the *U. cordatus* egg mass remains in contact with water and sediments, being susceptible to contamination (ALMEIDA *et al.*, 2016). The maternal transference during vitellogenesis and the absorption of mangrove pore water and minerals during embryonic development would be the main sources of trace element contamination. However, the analysis of egg mass does not clarify what percentage of the

2 MATERIALS AND METHODS

2.1 STUDY SITES

The *Ucides cordatus* females sampling was made in the same locations and with the same methods seen in Almeida *et al.* (2016). The two study sites were the Caceribu River mangrove (Guanabara Bay - GB) and the Gargaú mangrove (Paraíba do Sul River, secondary estuary - PSR), which are in SE Brazil (Fig. 1). In both areas, a humid tropical climate is predominant, with two defined seasons (wet summer and dry winter), and annual rainfall rates about 900 to 1.300 mm.yr⁻¹. The tide average amplitude in spring periods is ±1.0 m (micro-tidal), with semidiurnal cycles. The choice of these two-collection sites was for two reasons: sampling two different populations of *U. cordatus*, and sampling environments with different degrees/types of human interference.

The Caceribu mangrove is located in the Environmental Protection Area of Guapi-Mirim, in the inner Guanabara Bay. The crabs' sampling took place at coordinates 22°43'32.55"S; 043°00'48.03"W, about two kilometers upstream from the river mouth. The Guanabara Bay is an estuarine complex with about 30 small rivers and channels and a watershed with an area of 4,080 km² (KJERFVE *et al.*, 1997). Fourteen cities surround the bay, with a total population of over 11 million inhabitants, being one of the highest population densities in Southeastern Brazil. The bay is also highly eutrophic and holds the second-largest industrial park in the country, receiving massive amounts of domestic and industrial waste daily (COTOVICZ *et al.*, 2019). Despite the degradation, the mangroves at the inner bay are

contaminants remains in the eggshell (chorion) and what reaches the embryos. Thus, this study aimed to determine for the first time the trace elements concentration in *U. cordatus* newly hatched larvae from two Southeastern Brazilian mangroves (Guanabara Bay and Paraíba do Sul secondary estuary). The study also aimed to evaluate the contamination in the females' hepatopancreas, and in eggs in pre-hatching and hatching stages, in comparison with the larvae. Finally, the study discusses the relevance of planktonic larvae in the trace elements transport beyond the mangrove geochemical barrier.

relatively conserved, especially the ones located in conservation units, which add up to 80 km².

Contamination by trace elements in the sediments of Guanabara Bay has increased significantly in the last century (MONTEIRO *et al.*, 2012). Soares-Gomes *et al.* (2016), in their overview of the bay, stated that the introduction of anthropogenic origin-heavy metals and their accumulation in sediments have been evidenced by several studies. The western portion of the bay and the harbor areas are the most eutrophic and most contaminated by trace elements, mainly due to the contributions of urban effluents to rivers. The distribution of trace elements in the bay's sediments shows a strong relationship with the proximity of anthropic sources, particle size, and organic carbon content (MACHADO *et al.* 2002; BATISTA NETO *et al.* 2006). The remaining mangroves in the bay play an important role in retaining much of the metals and reducing their transport to the waters of the bay (MACHADO *et al.*, 2002). It is known that a large amount of organic matter suspended in the bay affects the bioavailability of some metals. Such fact, known since the 1990s, makes the concentrations of trace elements lower in some organisms, even in areas where the contamination of the environment is greater (CARVALHO; LACERDA, 1992). Cordeiro *et al.* (2015) stated that Zn and Cd are the most labile metals in the bay, while Cr, Cu, Ni, and Hg are the less, due to 62 to 84% of their concentration being associated with residual organic fraction in sediments.

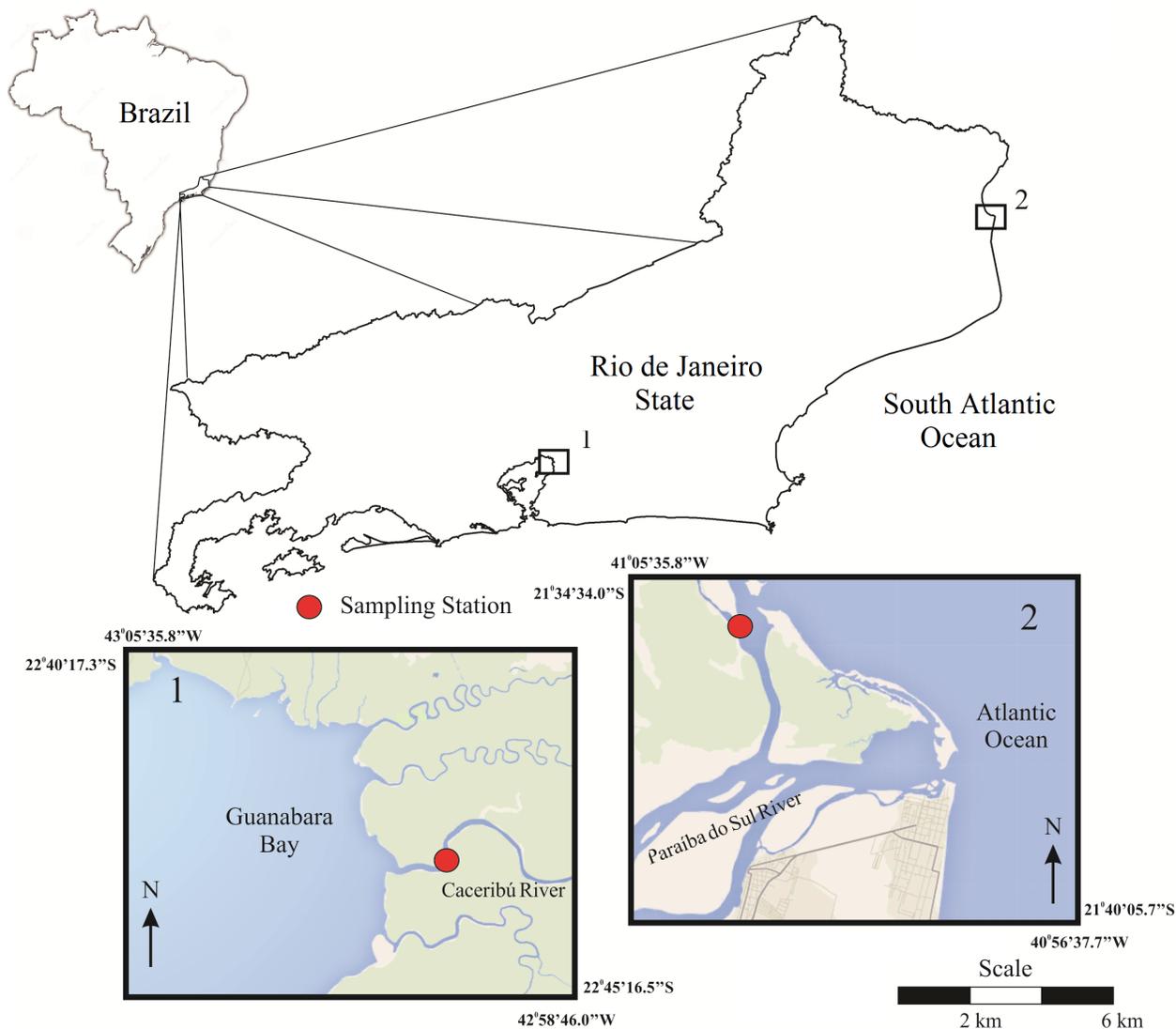


Figure 1
Location of *U. cordatus* ovigerous female sampling sites: 1. Caceribu River mangrove - Guanabara Bay (GB) and 2. Gargaú mangrove - Paraiba do Sul River (PSR), both on Southeastern Brazilian Coast, State of Rio de Janeiro. Modified from Almeida *et al.* (2016).

Gargaú mangrove – the other study site – is in the secondary estuary of the Paraíba do Sul River and it is classified as moderately mesotrophic to eutrophic. The river basin comprehends three states of SE Brazil (São Paulo, Minas Gerais, and Rio de Janeiro), highly urbanized and industrialized. However, in the lower basin area, extensive agriculture prevails, especially the production of sugar cane and pineapple. Comparing this study area with the Guanabara Bay, the Gargaú mangrove region is less urbanized ($\pm 530,000$ inhabitants) and the mangroves occupy an area ten times smaller ($\pm 8.0 \text{ km}^2$). As a result of the land use, deforestation,

and several dams built along this river, the volume of water has decreased in recent decades, as well as the sediments export (ANDRADE; RIBEIRO, 2020). For most of the year, the secondary Paraíba do Sul estuary is homogeneous and shows a predominance of freshwater (MOLISANI *et al.*, 1999).

Studies showed contamination by Zn, Pb, and Cu in the middle course of the Paraíba do Sul River and, in general, the concentrations of these elements in the suspended particulate material and sediments have decreased towards the river mouth (CARVALHO *et al.*; 1999; MOLISANI *et al.*, 1999, 2005). The exception was Cr, which

increased towards the estuary, probably due to its association with suspended organic matter. According to these authors, the concentrations of metals weakly bound to the sediment (fraction <63 μm) were relatively constant throughout the river-estuary complex, except for Mn and Fe, which in the estuarine region showed a decrease, probably explained by anoxic conditions. In general, the decrease in the concentration of metals downstream can be explained by the dilution in less contaminated areas or due to the barriers along the river, which generate sedimentation. However, Meneguelli-Souza *et al.*

2.2 SAMPLING OF OVIGEROUS FEMALES

To obtain newly hatched larvae, we opted to perform spawning in the laboratory. The other alternative, obtaining larvae from plankton sampling, would not allow comparing the larvae with their parents. The sample of ovigerous females occurred in three campaigns, even with the support from professional fishermen and under the license 27753-4 (Brazilian Ministry of the Environment). The first campaign was conducted in the Caceribu mangrove (GB) in January 2013. In February 2014, another campaign was assembled at the same site, and the last one took place in the Gargaú mangrove - Paraíba do Sul River (PSR), by January.

In the 2013 campaign, five ovigerous females were used to obtain eggs in the prehatching stage,

2.3 SPAWNING TANKS

Aquarium with 38.0 liters capacity were used to obtain spawns. They were filled with water from the mangrove main channel close to the female's sample site, with salinities above 20.0 psu. Water with this salinity range was necessary to stimulate the spawn, once the species need saltwater for successful larval development (DIELE; SIMITH, 2006). Salinities both in the field and in the laboratory were evaluated using a refractometer. Before being poured into the aquariums, the water was filtered through a 64 μm mesh. Trace element concentration in the aquarium water was determined with the aid of a Graphite Furnace Atomic Absorption Spectrometer (GFAAS) (Varian 240z).

Each aquarium received one female, equipped with an aerator, and kept out of the light until the spawn. The females were not fed. Check-ups were

(2021) pointed out higher concentrations of As in the sediment of the secondary estuary (our study area) and in flooded areas (5.16 ± 4.78 and $1.23 \pm 0.44 \mu\text{g}\cdot\text{g}^{-1}$, respectively), where the finer particle size fractions (silt and clay) prevailed. Miguens *et al.* (2016) studied metals by X-ray microanalysis in sediment grains from the lower basin of the PSR (including in the secondary estuary). They stated that in sediment grains, the metals occurred in at least one type of soluble compound, corroborating the pollution of the Paraíba do Sul River and the risk of contamination of the food chain.

3 to 4 days until hatching - stage VII according to Pinheiro and Hattori (2003). The eggs were transported to the laboratory and immediately frozen at -18°C . Another 10 females with hatching eggs (1 to 2 days until hatching, stage VIII) were taken to the laboratory and placed in the spawning tanks. In the 2014 campaigns, we obtained 10 females in each study area, which were transported to the spawning aquariums, and another 5 ovigerous females in each site. This last group was used to obtain hepatopancreas and eggs in the pre-hatching stage. Those females were also transported to the laboratory and frozen at -18°C . In all cases, immediately after sampling, the animals were washed to remove the mud surplus before transportation to the laboratory.

carried out every 30 minutes and impurities were drawn from the bottom. To minimize the effects of female permanence in the aquarium, only larvae hatched in the first 24h were used in the experiments. Each spawning was interrupted when 60-70% of the eggs had hatched, and the female was frozen along with the remaining eggs. This way, we obtained eggs in the hatching stage. Immediately after each spawning, the aquarium aeration was switched off. After the decantation of suspended particles, the larvae were attracted to the surface through a light stimulus. In sequence, they were carefully aspirated with the aid of sterilized pipettes. The larvae were concentrated using a sterilized 100 μm opening mesh. While still attached to the mesh, they were swiftly rinsed with distilled water to remove salts. Then, an absorbent paper was placed outside the

mesh to remove excess water. Finally, the larvae pool from each female was individually stored. Egg mass and larval pool from each female were

individually packed and frozen at -18° C until the analysis.

2.4 TRACE ELEMENTS ANALYSIS

All materials used in dissections, sample manipulation, and elements analysis were thoroughly acid cleaned and washed with deionized water before use. For female trace elements analysis, crabs were unfrozen, washed carefully, dried, and dissected to obtain the hepatopancreas. Pre-hatching eggs in the 2013 experiment came from the females that did not spawn, and the new larvae came from the females placed in spawning tanks. In 2014, hatching eggs and new larvae were obtained from each female of the spawning tanks. In 2014, we also obtained pre-hatching eggs from field females. In summary, five types of samples were obtained: 1. spawning females' hepatopancreas, 2. non-spawning females' hepatopancreas, 3. eggs in pre-hatching stage, 3. eggs in hatching stage, and 4. newly hatched larvae.

Each egg mass or larval pool was used as a composite sample. Eggs or larvae from different females were kept separated. Before the trace elements analysis, all samples were freeze-dried and homogenized. From each sample we derived three 0.250 g subsamples, that were digested in supra pure nitric acid (HNO₃), using a microwave (SpeedWave - Berghof). The trace element analysis was performed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Thermo Scientific® XSERIES 2). The accuracy of the analysis was evaluated by the addition of an internal standard (Rh) to all samples, and the relative error was less than 2%. The accuracy of the method was checked using the SRM-1566B (oyster tissue), a certified reference material considered adequate. The detection limits of trace elements analysis methods and the recoveries are presented in Table 1.

Table 1 - Detection limits on trace elements analysis ($\mu\text{g}\cdot\text{L}^{-1}$) and recovery percentages (%) in the 2013 and 2014 campaigns – Oyster Tissue Standard Reference Material (SRM 1566B). LoD: Limit of Detection ($\mu\text{g}\cdot\text{L}^{-1}$), LoQ: Limit of Quantitation ($\mu\text{g}\cdot\text{L}^{-1}$).

Trace elements	GFAAS		ICP-MS		Recovery (%)	
	LoD	LoQ	LoD	LoQ	2013	2014
V	1.9	6.3	5×10^{-4}	5.0	68.8	72.3
Cr	-	-	6×10^{-3}	5.0	-	-
Mn	0.091	0.30	1×10^{-3}	5.0	96.1	94.2
Ni	0.72	2.4	5×10^{-3}	5.0	67.5	73.9
Cu	0.90	3.0	3×10^{-3}	5.0	97.2	84.7
Zn	6.5	22	1×10^{-2}	5.0	109.2	107.0
As	-	-	8×10^{-3}	5.0	106.4	104.4
Cd	0.033	0.10	4×10^{-4}	5.0	114.3	130.1
Pb	0.81	2.7	2×10^{-3}	5.0	124.3	127.4

2.5 BIOMETRY AND FECUNDITY

The females' carapace width (CW) was evaluated by calipers (± 1.0 mm). Fecundity rates were estimated in 10 females from each study area (only 2014 campaign). The eggs were removed from the pleopods and dried with absorbent paper to remove the water surplus.

Then, the egg mass was weighed on an analytical balance (0.0001 g). The number of eggs.female⁻¹ was estimated by sub-sampling, counting the eggs in three 0.5 g fractions from each egg mass, with the aid of a stereoscope (80x).

2.6 STATISTICAL ANALYSIS

To assess differences in trace element concentrations, firstly we performed assumption checks on the homogeneity of variances (Levene's test) and normality (Shapiro-Wilk test). When the data did not show homogeneity and normality, non-parametric tests were performed (Mann-Whitney U test). When the opposite occurred, Student's t-tests were applied. These tests were performed to assess differences in trace element concentration between: 1. the two

3 RESULTS AND DISCUSSION

In the Caceribu mangrove (GB), the *Ucides cordatus* female's average carapace width (CW) was 69.0 ± 3.0 mm in 2013, and 70.0 ± 5.0 mm in 2014. In the Gargaú mangrove (PSR) the average CW was 63.8 ± 4.9 mm. These average CWs are equivalent to adult specimens within the commercial size, according to Brazilian law. *Ucides cordatus* presents slow growth, with a frequency of one single ecdysis per year after reaching CW of 50.0 mm. It reaches commercial size (CW ≥ 60.0 mm) at approximately three years, while the maximum asymptotic size (CW 80.0 mm) is reached at about 10 years (Pinheiro *et al.* 2005).

In the Caceribu mangrove (GB), fecundity rates ranged from 53,624.0 to 173,931.0 ($118,411.0 \pm 36,624.0$ eggs.female⁻¹). In the Gargaú mangrove, they ranged from 66,005.0 to 149,134.0 ($110,355.0 \pm 28,070.0$ eggs.female⁻¹). These values are higher than those observed in *U. cordatus* by Almeida *et al.* (2016) at the same locations: $39,297.0 \pm 18,783.0$ eggs.female⁻¹ in the Caceribu mangrove and $84,804.0 \pm 36,097.0$ eggs female⁻¹ in the Gargaú mangrove. In other Southeastern Brazilian mangroves, Pinheiro *et al.* (2003) observed *U. cordatus* fecundity from 36,081.0 to 250,565.0 and, João and Pinheiro (2018) from 52,334.0 to 212,801.0 eggs.female⁻¹, values like the observed in the present study. Also in SE Brazil, Hattori & Pinheiro (2003) observed *U. cordatus* fertility rates ranging from 71,200 to 220,800.0 larvae.female⁻¹. In crabs and other decapods, variations in fecundity/fertility are expected even in nearby populations.. Bas *et al.* (2007) suggested changes in salinity levels, quality or quantity of benthic food sources, and productivity as co-responsible factors for the

populations (GB and PSR), 2. hepatopancreas from spawning females and non-spawning females (from the same population), 3. pre-hatching and hatching eggs (from the same population), 4. pre-hatching eggs and newly hatched larvae (2013, only GB), and 4. hatching eggs and larvae (2014, GB and PSR). In all tests, results were considered significant at the 0.05 level. All analyses were performed using the software Jamovi (2020).

fecundity variations in subsequent years and in nearby populations of *Neohelice granulata* crab.

The spawning success rate from females of the Caceribu mangrove (GB) was 50% in 2013 (n = 5 females). In 2014, the success rate was 40% in both study sites. These success rates were like those observed by Pinheiro and Hattori (2003) (44%), as was the occurrence of nocturnal spawning. In nature, *U. cordatus* spawns preferentially in nocturnal spring tides (CASTILHO-WESTPHAL *et al.*, 2013). This strategy is commonly observed among mangrove crabs and related to an improvement in dispersion and larval survival (MORGAN, 1995).

There was no significant difference in the hepatopancreas' elements concentration among spawning and non-spawning females. The most concentrated elements in this organ were Zn, Cu, and Mn (Tab. 2), which was within expectations, since Zn and Cu are essential metals for crustaceans (RAINBOW, 2007), and Mn is highly abundant in the mangrove crab's main food source, the mangrove leaves. These leaves present relatively high Mn concentrations, which is an essential element for higher plants, including mangrove trees (MACHADO *et al.*, 2002; KABATA-PENDIAS, 2010; PINHEIRO *et al.*, 2012). The hepatopancreas can retain trace elements in amorphous granules and is highly related to alimentation, due to its storage function (CORRÊA-JUNIOR *et al.*, 2000). In crustaceans, there are records of Zn, Cu, Fe, Cd, Hg, and Ag retention in granules, but other ions may be also immobilized (AHEARN *et al.*, 2004).

In eggs and newly hatched larvae, Zn, Cu and Mn also showed the highest concentrations, in all campaigns and study areas (Tabs. 3 and 4). Com-

paring pre-hatching and hatching eggs from the 2014 campaigns, V, Cr, and Mn concentrations were significantly higher in hatching eggs, in both study sites (Fig. 2). Almeida *et al.* (2016) also reported an increase in the trace elements concentration in *U. cordatus* eggs throughout development. These authors suggested the adsorption of trace elements in the eggshell, especially due to the assimilation of water and salts during embryonic development. The eggs of several crab species, including *U. cordatus*, absorb water and salts from the environment,

especially during the days and hours before hatching (PANDIAN, 1967; DEVRIES; FORWARD, 1991; PETERSEN; ANGER, 1997; GIMÉNEZ; ANGER 2001; PINHEIRO; HATTORI, 2003; GARCÍA-GUERRERO; HENDRICKX, 2006). The high-water absorption rates before hatching are explained by the contribution of the water in the eggshell disruption (PANDIAN, 1970). This swift absorption of water probably increases the trace elements concentration in the eggs.

Table 2 - Average \pm standard deviation of trace elements concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ dry weight) in the hepatopancreas of *U. cordatus* females from the Caceribu mangrove - Guanabara Bay (2013 and 2014) and from the Gargaú mangrove - Paraíba do Sul secondary estuary (2014), SE Brazil. The *n* symbol represents the total number of females of each group, campaign, and study site, where: non-spawning group – ovigerous females frozen immediately after sampling; spawning group – ovigerous females transported to the spawning tanks in the laboratory.

Trace Element	Caceribu mangrove (GB)				Gargaú mangrove (PSR)	
	non-spawning group		spawning group		non-spawning group	spawning group
	2013 (n=5)	2014 (n=5)	2013 (n=5)	2014 (n=4)	2014 (n = 5)	2014 (n = 4)
V	0.63 \pm 0.05	0.83 \pm 0.21	0.04 \pm 0.01	0.05 \pm 0.03	0.96 \pm 0.60	0.98 \pm 0.10
Cr	0.46 \pm 0.07	0.46 \pm 0.05	0.10 \pm 0.03	0.08 \pm 0.03	0.66 \pm 0.18	0.47 \pm 0.04
Mn	4.53 \pm 0.81	4.93 \pm 1.75	1.28 \pm 0.42	1.19 \pm 0.50	16.55 \pm 8.30	14.98 \pm 6.89
Ni	1.85 \pm 0.88	1.89 \pm 0.84	0.19 \pm 0.08	0.16 \pm 0.04	3.33 \pm 1.98	2.67 \pm 1.21
Cu	55.50 \pm 10.40	65.20 \pm 6.59	6.47 \pm 1.19	4.73 \pm 1.81	226.42 \pm 54.17	207.95 \pm 112.31
Zn	89.90 \pm 19.20	89.40 \pm 22.60	113.60 \pm 32.90	101.30 \pm 78.70	159.48 \pm 55.44	112.35 \pm 36.96
As	0.98 \pm 0.19	1.00 \pm 0.24	0.17 \pm 0.09	0.10 \pm 0.13	1.39 \pm 0.71	1.11 \pm 0.13
Cd	0.09 \pm 0.03	0.12 \pm 0.07	0.03 \pm 0.01	0.02 \pm 0.01	0.29 \pm 0.14	0.25 \pm 0.15
Pb	0.19 \pm 0.05	0.15 \pm 0.05	0.11 \pm 0.10	0.03 \pm 0.03	0.20 \pm 0.03	0.13 \pm 0.03

Table 3 - Average \pm standard deviation of trace elements concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ dry weight) in pre-hatching eggs (pre-h. eggs) and in hatching eggs of *U. cordatus* from the Caceribu mangrove - Guanabara Bay (GB - 2013 and 2014) and from the Gargaú mangrove - Paraíba do Sul secondary estuary (PSR - 2014), SE Brazil. The “n” represents the total number of composite samples with eggs from each ovigerous female.

Trace Element	Caceribu mangrove (GB)			Gargaú mangrove (PSR)	
	pre-h. eggs	pre-h. eggs	hatching eggs	pre-h. eggs	hatching eggs
	2013 (n=5)	2014 (n=5)	2014 (n=5)	2014 (n=5)	2014 (n=5)
V	3.05 \pm 3.37	1.57 \pm 1.03	1.57 \pm 0.92	1.21 \pm 1.40	7.82 \pm 1.99
Cr	1.26 \pm 1.11	0.65 \pm 0.41	0.65 \pm 0.37	0.59 \pm 0.58	1.90 \pm 0.47
Mn	19.40 \pm 14.40	5.26 \pm 1.03	5.26 \pm 0.92	4.70 \pm 4.24	18.59 \pm 12.57
Ni	0.71 \pm 0.42	0.36 \pm 0.14	0.36 \pm 0.13	0.38 \pm 0.19	0.83 \pm 0.21
Cu	23.40 \pm 7.97	36.11 \pm 24.93	36.10 \pm 22.30	82.66 \pm 28.21	30.29 \pm 4.85
Zn	221.70 \pm 16.50	254.01 \pm 25.89	254.00 \pm 23.20	301.41 \pm 37.89	239.50 \pm 20.02
As	11.80 \pm 3.21	1.63 \pm 0.25	1.63 \pm 0.22	1.98 \pm 0.73	2.15 \pm 0.39
Cd	0.01 \pm 0.01	0.01 \pm 0.02	0.01 \pm 0.02	0.03 \pm 0.03	0.06 \pm 0.01
Pb	3.79 \pm 3.19	0.78 \pm 0.57	0.78 \pm 0.51	0.34 \pm 0.30	1.55 \pm 0.16

Table 4 - Average \pm standard deviation of trace elements concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ dry weight) in newly hatched larvae of *U. cordatus* from the Caceribu mangrove - Guanabara Bay (GB - 2013 and 2014) and from the Gargaú mangrove - Paraíba do Sul secondary estuary (PSR - 2014), SE Brazil. The “n” represents the total number of composite samples made with larvae from each spawning female.

Trace Element.	Caceribu mangrove (GB)		Gargau mangrove (PSR)
	2013 (n=5)	2014 (n=4)	2014 (n=4)
V	0.82 \pm 0.18	1.17 \pm 0.64	0.17 \pm 0.05
Cr	0.40 \pm 0.11	0.35 \pm 0.10	0.29 \pm 0.03
Mn	12.00 \pm 9.99	19.20 \pm 2.39	3.88 \pm 0.93
Ni	0.24 \pm 0.06	0.18 \pm 0.09	0.06 \pm 0.03
Cu	21.70 \pm 2.00	14.10 \pm 2.22	41.08 \pm 6.58
Zn	235.40 \pm 21.10	263.90 \pm 23.00	263.36 \pm 10.31
As	4.37 \pm 0.17	1.84 \pm 0.40	1.64 \pm 0.38
Cd	0.01 \pm 0.01	0.03 \pm 0.03	0.04 \pm 0.01
Pb	3.68 \pm 2.57	1.07 \pm 0.98	0.005 \pm 0.01

As previously mentioned, there are few studies on the trace elements concentration in crab eggs. Table 5 compares trace element concentrations in crab eggs from the present study and others, including two common species in SAO. In general, the trace element concentrations were the same order of magnitude, even among different species. The only exception was Cd in *N. granulata* from Bahía Blanca (Argentina), which demonstrated higher values. Cadmium concentrations in the Bahía Blanca sediments (BOTTÉ *et al.*, 2010) are higher than those observed in the Guanabara Bay mangroves (FARIAS *et al.*, 2007). Also, considering the hydrological differences among these locations, this pollutant may be more bioavailable in Bahía Blanca.

Comparing pre-hatching eggs and larvae from different females (GB 2013 only), the Ni and As concentrations were significantly higher in eggs. Comparing hatching eggs and larvae from the same female (2014 campaigns), V, Cr, Mn, and Zn concentrations were significantly higher in eggs (Fig. 1). The same happened for Ni and Cu concentrations in the Gargau mangrove. This means that the eggshell can retain part of the trace elements, partially protecting the embryo. Besides, previous studies on contaminants in crab egg masses could have overestimated the embryo's contamination. It remains unknown if other contaminants, such as organochlorines, polycyclic aromatic hydrocarbons, and others, go through the same process.

Considering the porosity of the eggshell, it is unlikely that it will completely block the passage

of trace elements. Therefore, it is likely that part of the trace elements present in the newly hatched larvae comes from the environment, absorbed during the egg's development. This fact is of great concern because trace elements can be toxic to adult crabs and larvae. Duarte *et al.* (2017), while studying adult *U. cordatus*, observed positive and significant correlations between the number of micronucleated cells (MN%) and concentration of Hg (sediment), Pb (water and green leaves), and Cu (hepatopancreas). In other words, the mean concentration of these elements was significantly associated with *U. cordatus* crab genotoxicity. In *N. granulata* adult crabs, cadmium inhibits key metabolic enzymes, interfering with ionic regulation as well as other vital functions, and reducing the resistance against osmotic and other stresses (VITALE *et al.* 1999). The same physiological trends should occur with larvae. In larvae of the same species, Ferrer *et al.* (2006) pointed out that the toxicity of metals followed the order cadmium > lead > copper > zinc. Rodríguez and Medesani (1994) demonstrated the occurrence of morphological abnormalities in larvae of *N. granulata* when ovigerous females are exposed to cadmium. In the crab *Parasesarma pictum* the larval metamorphosis delay was observed after exposition to Zn, even in low concentrations (PASUPATHI; KANNUPANDI, 1989). In larvae of the *Portunus pelagicus* swimming crab, Cu, Ni, and Cr inhibited molting, increased development duration, and decreased average juvenile body size (MORTIMER; MILLER, 1994). Mendoza-

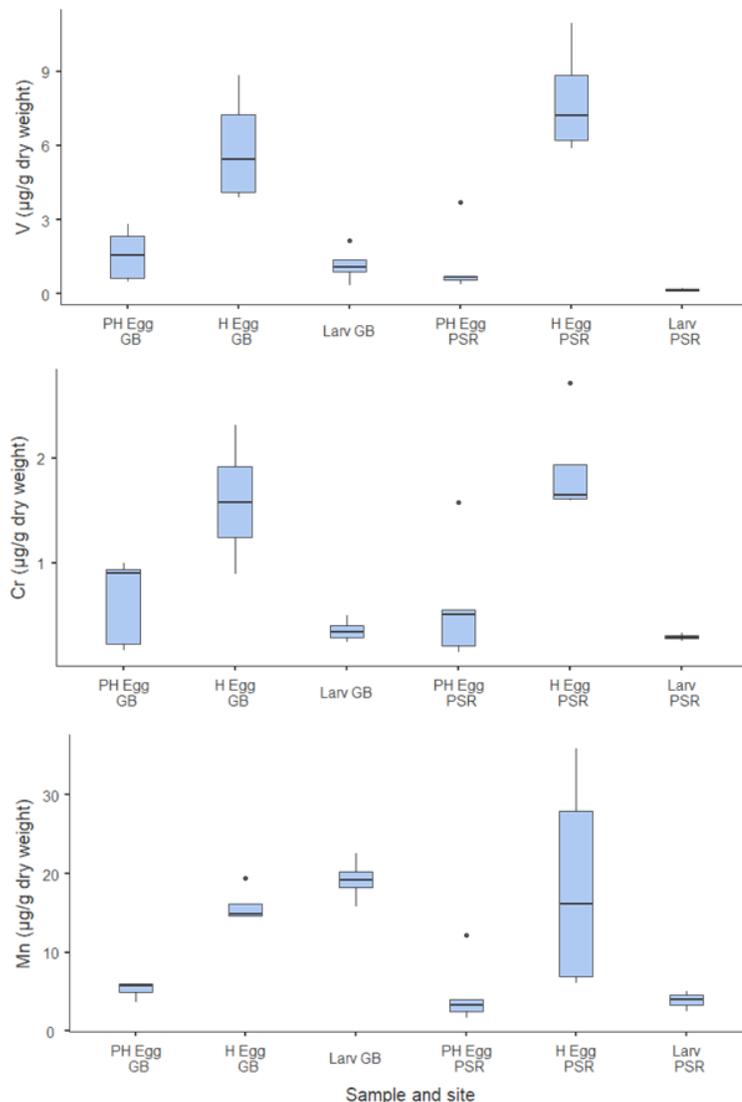


Figure 2 Box plots (showing median, quartiles, minimum-maximum and outliers) of V, Cr, and Mn concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ dry weight) in pre-hatching eggs (PH Egg), hatching eggs (H Egg), and newly hatched larvae (Larv) of *U. cordatus* crabs (2014 campaigns) from the Caceribu River - Guanabara Bay (GB) and from the Gargau mangrove - Paraíba do Sul River (PSR), Southeastern Brazil.

Carranza *et al.* (2016), in one of the few published studies about trace element concentration in crab larvae, observed concentrations of $4.75 \mu\text{g Cr}\cdot\text{g}^{-1}$, $5.37 \mu\text{g Ni}\cdot\text{g}^{-1}$, and $112.2 \mu\text{g Zn}\cdot\text{g}^{-1}$ in planktivorous brachyuran larvae obtained from plankton samples in a Mexico lagoon. Although these authors did not specify the larval stage or species, Cr and Ni concentrations were 10 to 30 times higher than those observed in the present study. However, Zn concentrations were lower, about half the concentrations observed for *U. cordatus* newly hatched larvae. One of the few works about trace elements concentration in marine invertebrates newly hatched larvae is

Weng and Wang (2017). Comparing our results with that work, performed with oyster newly hatched larvae (*Crassostrea hongkongensis*) from two polluted estuaries in Southern China, Cr, Ni, Cu, As, and Cd concentrations were higher in oyster larvae (two to ten times). On the other hand, Zn and Pb (except PSR) concentrations were higher in the *U. cordatus* larvae. The difference in concentrations among species and sampling sites may reflect the larval instar or stage, environmental contamination levels (especially in the water), and trace elements bioavailability.

Table 5 - Average trace element concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ dry weight) in crab eggs from South Atlantic Ocean species. (-): not analyzed, nd: not detected.

Species	Study area	Egg stage	Trace elements						References
			Cr	Mn	Cu	Zn	Cd	Pb	
<i>Ucides cordatus</i>	Guanabara Bay, SE Brazil	pre-hatching	1.6	15.8	27.0	171.0	0.06	6.1	ALMEIDA <i>et al.</i> (2016)
		pre-hatching	1.3	19.4	23.4	221.7	0.01	3.8	This study (2013 campaign)
		pre-hatching	0.6	5.3	36.1	254.0	0.01	0.8	This study (2014 campaign)
		hatching eggs	0.7	5.3	36.1	254.0	0.01	0.8	This study (2014 campaign)
	Paraíba do Sul River, SE Brazil	pre-hatching	2.9	29.0	25.0	230.0	0.08	6.6	ALMEIDA <i>et al.</i> (2016)
		pre-hatching	0.6	4.7	82.7	301.4	0.03	0.3	This study (2014 campaign)
<i>Callinectes</i> sp.	Ilha Grande, SE Brazil	hatching eggs	1.9	18.6	30.3	239.5	0.06	1.6	This study (2014 campaign)
		not informed	-	-	44.1	290.2	0.05	1.4	LAVRADAs <i>et al.</i> (2014)
<i>Neohelice granulata</i>	Mar Chiquita, Argentina	pre-hatching/hatching	1.0	53.8	53.2	-	0.50	-	BELTRAME <i>et al.</i> (2010)
		pre-hatching/hatching	1.5	23.4	48.9	-	0.71	-	BELTRAME <i>et al.</i> (2010)
	Bahía Blanca, Argentina	not informed	nd	-	62.5	-	6.38	-	SIMONETTI <i>et al.</i> (2012)
		not informed	nd	-	51.3	-	3.76	-	SIMONETTI <i>et al.</i> (2012)

In the present study, when comparing female hepatopancreas and their newly hatched larvae, some trace elements showed significantly different concentrations. In the 2013 campaign in the Caceribu mangrove (GB), the trace elements concentration was significantly higher in larvae, except for Ni and Cr. In 2014, at the same site, Mn and Zn were also significantly higher in larvae. In the Gargaú mangrove (PSR) the same pattern was observed with Zn. The higher concentration of zinc, an essential metal for crustaceans (RAINBOW, 2007), may be associated with the maternal transfer, female depuration, or natural absorption of this metal. Lavradas *et al.* (2014) found higher Cd and Zn concentrations in eggs of the swimming crab *Callinectes* sp., when compared to the females' hepatopancreas. These authors, analyzing metallothioneins and glutathione concentrations, observed a strong correlation, which indicates the transfer of Pb and Zn from females to embryos. Regarding the higher concentration of Mn and other elements in *U. cordatus* larvae, it could indicate depuration of trace metals or metalloids in females. Tsui and Wang (2007) detected maternal transfer as a route for metal elimination in *Daphnia magna* (Crustacea: Branchiopoda), especially for Se, Zn, and MeHg. For a better understanding of the trace elements transfer among females and embryos, an efficient method was described in Weng and Wang (2017). These authors, while analyzing

trace elements transfer between female oysters and their embryos, obtained eggs directly from the gonadal tissue. In studies with crabs, this method will be considered, once would prevent the contact of the egg with the environment and the absorption or adsorption of trace elements.

Concentrations of Mn, Cu, and Cd were significantly higher in the females of Gargaú ($p < 0.005$). In previous studies, in the same regions of the present study, these elements were more concentrated in the sediments of the PSR secondary estuary. As an example, Cd showed a concentration of $0.2 \mu\text{g}\cdot\text{g}^{-1}$ in BG mangrove sediments, west of the Caceribu River (FARIAS *et al.*, 2007). In the PSR secondary estuary, where the Gargaú Mangrove is located, Molisani *et al.* (2005) recorded higher concentrations, from 0.7 to $1.8 \mu\text{gCd}\cdot\text{g}^{-1}$. In addition to higher concentrations in the PSR secondary estuary, probably these elements are more bioavailable in the region. Bosco-Santos *et al.* (2017), analyzing muscle tissue of male *U. cordatus* from Southeastern Brazil, found a positive correlation between concentrations of Cu, Zn, and Pb in surface sediments and crabs. These authors stressed the importance of crab feeding as a contamination pathway since there is a small but constant percentage of sediment ingestion. As previously mentioned, Cu is an essential metal for crustaceans and the main food of *U. cordatus* crabs is Mn-enriched. In areas with the anthropic

introduction of these elements, it is expected that the most abundant in sediments appear in higher concentrations in crabs. Comparing GB and PSR crab eggs, there was no significant difference. On the other hand, in the newly hatched larvae from GB, significantly higher concentrations of V, Mn, and Ni were observed. This could mean that either the female-embryo transfer is different between the study areas or that the conditions of sediment and pore water of the Rio Caceribu mangrove (GB) support the contamination of the embryos during the incubation of eggs. Further studies are needed to clarify these facts.

As previously stated, *U. cordatus* is a large mangrove crab with high fecundity rates and synchronized spawning, which exhibits a planktonic larval export strategy. This strategy enables the larvae transportation to areas with saltwater (salinity > 20 psu), essential for their successful development (DIELE; SIMITH, 2006). Commonly, the exportation mechanism results in larval transport to distant waters and high connectivity between populations (BRITTO *et al.*, 2011). In the present study, we registered

trace element concentrations in newly hatched larvae, including some non-essential elements. Considering the *U. cordatus* reproductive dynamics, are the planktonic larvae means of transportation of trace elements beyond mangroves? Comparing trace elements in *U. cordatus* newly hatched larvae with concentrations in suspended particulate matter (SPM) and suspended sediments, it was observed that the concentrations of V, Cu, Zn, and As (only PSR) in larvae represent a significant percentage of these elements in the water column as a whole (Tab. 6). If, on the one hand, the introduction of larvae does not occur throughout the whole year, it is noteworthy that the elements present in the larvae enter much more easily in the food chain, when compared to those in the suspended sediments. Crab larvae from several species are predated by a large variety of pelagic and benthonic animals (MORGAN, 1992). Moreover, unlike the larvae, the suspended mineral materials tend to flocculate and precipitate in salinity gradients.

Table 6 - Trace elements concentration ($\mu\text{g}\cdot\text{g}^{-1}$ dry weight) in suspended particulate matter (SPM) from the Paraíba do Sul River (PSR), in suspended sediments from anthropized Guanabara (GB) and Sepetiba bays (SE Brazil), and in *U. cordatus* newly hatched larvae (present study), Southeastern Brazil.

Trace Element	MPS		Suspended sediment		Newly hatched larvae		
	Paraíba do Sul River (Carvalho <i>et al.</i> 2002)	(Meneguelli-Souza <i>et al.</i> 2021)	Sepetiba bay (Lacerda <i>et al.</i> 1988)	Guanabara bay (Melo <i>et al.</i> 2014)	GB 2013	2014	PSR 2014
As	-	1.98 - 2.60	-	-	4.37	1.84	1.64
V	-	-	-	≈ 0.6	0.82	1.17	0.17
Cr	47.0 - 79.0	-	≈ 25.0 - 100.0	≈ 200.0	0.40	0.35	0.29
Mn	1,348.0 - 1,913.0	-	≈ 250.0 - 1,000.0	≈ 3,000.0	12.0	19.2	3.88
Ni	-	-	≈ 20.0 - 60.0	-	0.24	0.18	0.06
Cu	125.0 - 134.0	-	≈ 30.0 - 250.0	-	21.7	14.1	41.1
Zn	129.0 - 353.0	-	≈ 250.0 - 800.0	≈ 750.0 - 800.0	235.4	263.9	263.4
Cd	-	-	≈ 1.5 - 7.0	≈ 10.0	0.01	0.03	0.04
Pb	-	-	≈ 30.0 - 130.0	≈ 120.0	3.68	1.07	0.005

When comparing the trace element concentrations in *U. cordatus* newly hatched larvae with the *Laguncularia racemosa* tree leaves from the Guapimirim EPA (Guanabara Bay) (MACHADO *et al.* 2002), the elements Zn and Cu showed concentrations 18 and 2.2 times higher in the larvae. According to Ramos e Silva *et al.* (2006), 7% of fallen leaves (mangrove trees)

are exported to the marginal marine system in the Potengi River, NE Brazil. The average exportation rates of heavy metals through leaf litter were: 1.31 (Zn), 0.21 (Cr), 0.64 (Cu), 17.61 (Ni) and 0.112 (Cd) $\mu\text{g}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$. However, to better understand the participation of crab larvae and other organisms in the annual exportation of elements, it is necessary to go further in future

investigations and estimate how much of the larval biomass is exported in each spawning season, for each mangrove. In addition, it will be imperative to characterize the flow and transport of the newly hatched larvae and to determine the main predators in each region, as well as the intensity of predation. To confirm the transfer in the food web will be necessary to coupling trace elements analysis with the stable isotopes study at different trophic levels, as suggested by Wang (2002).

The massive larval introduction in coastal waters during synchronized spawning generates high plankton densities. In the SE Brazilian summer, Almeida *et al.* (2013) registered maximum larval densities of 245.0 larvae.m⁻³ in the Macaé River (SE Brazil) and Almeida *et al.* (2017) registered 107.0 larvae.m⁻³ in the Guanabara Bay, although Schwamborn *et al.*

4 CONCLUSIONS

Both essential and non-essential trace elements were found in newly hatched larvae, eggs, and hepatopancreas of *U. cordatus* females. The elements with the highest concentrations were Zn, Cu, and Mn. There was a higher concentration of some elements in the eggs when compared to the newly hatched larvae. This indicates the retention of part of the elements in the eggshell. Considering that the species has a larval export mechanism, the dispersion of the planktonic larvae may be responsible for the trace elements transport beyond the mangroves.

Like the *U. cordatus*, there are many other mangrove and salt marsh crab species that live in the mud, present high fecundity rates, and synchronized spawning. Examples include the crab *Ucides occidentalis*, various fiddler crabs

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(2001) observed higher densities (1,778.0 larvae.m⁻³) in the Itamaracá mangrove (NE Brazil). The values during synchronized spawning could be even higher. In the Caceribu River and Paraíba do Sul River, Almeida observed maximums of 332.0 and 1,892.0 larvae.m⁻³, respectively (unpubl. data). It is worth mentioning that in some regions, female migration could precede spawning. We recently heard reports from Guanabara Bay fishermen regarding female migrations before spawning. The fishermen have related migrations 1.0-2.0 km away from the mangroves, leading to the concentration of females in muddy areas at the bottom of the bay, where the salinity is higher. If that behavior is scientifically proven, this phenomenon would indicate an increase in larval transport in the bay.

(genera *Leptuca*, *Minuca*, *Uca*, etc.), mud crabs such as *Neohelice granulata*, *Goniopsis cruentata*, *Scylla serrata*, among others. The *Dotilla fenestrata* and *Dotilla myctiroides* soldier crabs also deserve attention, due to their abundance in coastal habitats in Africa and Asia, and to their continuous reproduction throughout the year (HAILS; YAZIZ 1982; LITULO *et al.* 2005). All these species deserve greater attention regarding the contamination of their embryos and newly hatched larvae and on their influence on the trace elements dynamics. In future studies on trace elements and other pollutants in marine invertebrates, it is particularly important to consider female transference and the influence of water and salts absorption during the embryo's development.

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