




Seasonal influences on swimming crab mercury levels in an eutrophic estuary located in southeastern Brazil

Paloma de Almeida Rodrigues¹ · Rafaela Gomes Ferrari²  · Rachel Ann Hauser-Davis³ · Luciano Neves dos Santos⁴ · Carlos Adam Conte-Junior^{1,2,5}

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Abstract

Although significantly impacted, Guanabara Bay (GB), located in southeastern Brazil, is still an important fishery source for the state of Rio de Janeiro. Hg contamination, in particular, is of concern in the area and should be regularly monitored, as Hg bioaccumulation and biomagnification processes may lead public health risks to the local human population due to the consumption of contaminated food items, such as crabs. In this context, the aim of the present study was to determine total Hg (THg) concentrations in swimming crabs from three GB areas and investigate the influence of biotic and abiotic factors on Hg concentrations at the beginning and the end of the rainy season. Crabs and water samples were obtained from three areas, inside the bay, at the mouth of the bay and outside the bay. A clear rainfall effect on the investigated abiotic variables was observed, with increased rainfall and temperatures noted at the end of the study period. Significant statistical correlations were observed between THg concentrations and the assessed abiotic variables at the three study points at the beginning and end of the rainy season. The rainy season was noted as directly affecting THg concentrations at Guanabara Bay and, consequently, swimming crab THg contents. THg concentrations in swimming crabs at Urca and at the Cagarras Islands were higher at the beginning of the rainy season compared to the end, while the opposite was observed for the sampling point outside the bay. Higher Hg concentrations were detected at the outermost point of the bay in relation to the Cagarras Islands, probably due to the local upwelling event. THg values in *Callinectes* sp. were higher than concentrations reported for other areas in Brazil but lower than other reports worldwide. Calculated THg intakes surpassed the maximum National Research Council permissible limits of 0.049 mg/week at all sampling stations during both seasons, raising public health concerns. Further research for longer monitoring periods during different seasons are essential to ascertain which climatic period is most critical regarding Hg availability at this anthropogenically-impacted estuary.

Keywords Hg · Bioavailability · Abiotic parameters · Seafood · Public health risks

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✉ Rafaela Gomes Ferrari
rafaelaferri@yahoo.com.br

- ¹ Molecular and Analytical Laboratory Center, Department of Food Technology, Faculty of Veterinary, Universidade Federal, Fluminense, Niterói, Brazil
- ² Chemistry Institute, Food Science Program, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
- ³ Laboratório de Avaliação e Promoção da Saúde Ambiental, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Av. Brasil, 4.365, Manguinhos, Rio de Janeiro 21040-360, Brazil
- ⁴ Laboratory of Theoretical and Applied Ichthyology, Department of Ecology and Marine Resources, Universidade Federal do Estado do Rio de Janeiro, Rio de Janeiro, Brazil
- ⁵ National Institute of Health Quality Control, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

Introduction

Estuarine environments are extremely complex environments presenting significant economic, social, and ecological importance. These areas display very high habitat diversity and are used as shelter, nurseries, and food sources for several resident and transient marine species, making them relevant socio-economic areas for regional fisheries (Dolbeth et al. 2008).

One of the most important estuaries in Brazil, Guanabara Bay (GB), located in the state of Rio de Janeiro, in southeastern Brazil, is densely populated and surrounded by industrial areas (Soares-Gomes et al. 2016). This area is strongly impacted by organic matter, high sedimentation rates, hydrocarbons, oil and high metal concentrations, including mercury (Hg) (Kehrig et al.

2011). This toxic metal can accumulate in animal tissues, bioaccumulating and biomagnifying throughout the food chain, mainly in its organic form, methylmercury (MeHg) (Arcagni et al. 2018; Ruus et al. 2017; Taylor and Calabrese 2018). The main Hg contamination sources at GB are chlor-alkali plants and the local burning of fossil fuels, as well as domestic sewage (Baptista Neto et al. 2016; Kehrig et al. 2011). However, despite the considerable pollution noted at GB, due to the constant spillage of domestic and industrial sewage (Baptista Neto et al. 2016; Fistarol et al. 2015), the area still exhibits typical tropical estuary characteristics, including a relatively well-preserved mangrove forest, high productivity, and favorable conditions for the growth and reproduction of estuarine species, including fish, crustaceans, mollusks, cetaceans, polychaetes, and algae (Soares-Gomes et al. 2016), and artisanal fishing activities are significant in this area. One of the most abundant benthonic organisms at GB are swimming crabs, which are significantly consumed by the local human population (Soares-Gomes et al. 2016). This may lead to public health concerns regarding Hg ingestion.

During the rainy season, GB is influenced by an annual low-intensity upwelling event occurring from November to March (late spring and summer), where an increase in north-east winds brings the South Atlantic Central Water (SACW) to the surface 100 km north of the bay entrance, establishing subtropical and temperate characteristics for the Rio de Janeiro coast, with temperatures ranging between 10° and 20 °C (Silva et al., 2016). Distinct oceanographic features are observed throughout the rest of the year, due to Brazilian marine currents (Silva et al., 2016). Thus, it is probable that local rainfall regimes, in addition to drainage basin features (rivers and streams) and the input of accumulated and potentially contaminated sediment during the early summer, lead to initially high-contaminant loads in the area, which tend to decrease at the end of the rainy season.

In this context, mercury contamination at GB should be regularly monitored, as Hg bioaccumulation and biomagnification processes may lead to the consumption of contaminated food items, such as fish and crabs, resulting in public health risks to the local human population. In this regard, benthonic animals are considered adequate sentinel organisms regarding environmental contamination, as they exist in direct contact with potentially contaminated substrates (Arcagni et al. 2018; Chen et al. 2014). Therefore, the aim of this study was to assess Hg concentrations in swimming crabs from three different GB areas, sampled in the beginning and end of the rainy season. In addition, abiotic factors can also affect bioaccumulation and biomagnification processes, so it is also important to assess these variables during environmental monitoring assessments (Ando et al. 2010; Anual et al. 2018; Arcagni et al. 2018; Boyd et al. 2017).

Material and methods

Study area

Guanabara Bay (22°24′–22°57′S, 42°33′–43°19′W) comprises 4081 km², drained by 50 rivers and streams, with a water volume of 1.87 billion m³ and high sedimentation rates (0.6 to 4.5 cm year⁻¹), mostly as a result of drainage basin deforestation and river channeling. The bay also presents a bidirectional surface flux towards the ocean and a bottom layer flux towards the continent, undergoing a 50% water renewal rate every 11.4 days, due to tidal (mainly high tides) and seasonal (especially the rainy season, from December to March) influences (Chaves et al. 2018; Fistarol et al. 2015).

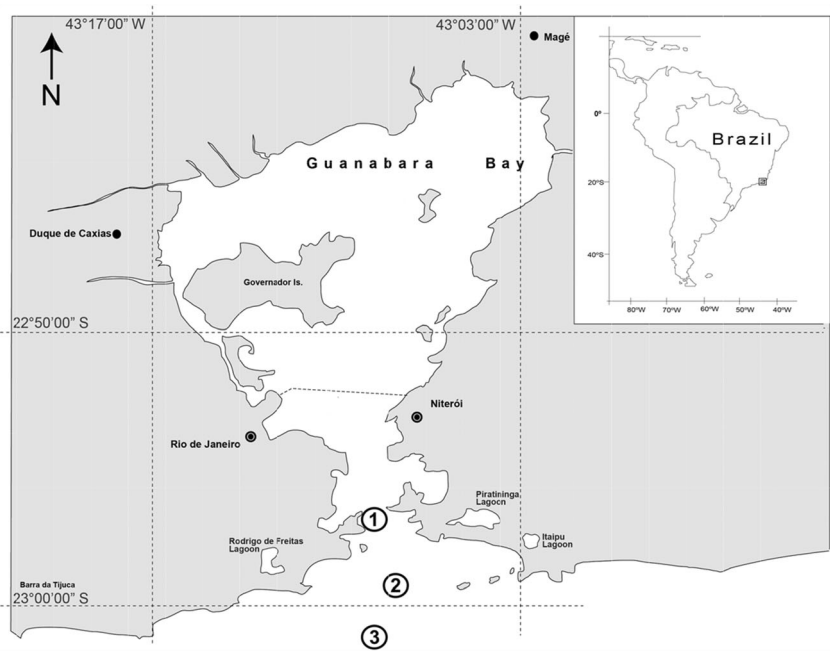
Three sampling points within the bay were investigated herein, P1, Urca Beach; P2, Cagarras Islands; and P3, an area beyond the Cagarras islands (Fig. 1), further out towards the Atlantic Ocean. Urca Beach is located inside but the bay, but near its entrance, in the lower estuary, suffering the influence of both oceanic marine waters and the bay's organic matter content, especially during the rainy season (Barreto et al. 2017; Chaves et al. 2018; Seixas et al. 2016). The other two points are external and undergo significant oceanic water influence and lesser bay influence, although a certain amount of internal bay fluxes is still noted (Fistarol et al. 2015).

Water and swimming crab samplings

Swimming crab samplings were carried out at the beginning and at the end of the rainy season (December 2015 and March 2016, respectively). Two trawling campaigns with a standardized total duration of 20 min each were carried out at the three sampling sites, totaling six trawls, using a single port net (25 m mouth opening and 6 m high, with a 25-mm mesh between adjacent nodes). The first trawl was carried out at the bottom-shore direction of the bay, followed by a laterally conducted trawl for 200 m, while the second trawl was carried out in the bottom-coast direction. Ten animals from each of the three sampling points were sampled, five from the first trawling and five from the second trawling, totaling 30 animals in 2015 and 20 in 2016. Sampled animals were transported on ice to the laboratory.

Water from each sampling point was collected during the trawls, with the aid of a Van Dorn bottle at 1 m from the bottom, and pH, salinity (g.L⁻¹), temperature (°C), and dissolved oxygen (DO) (mg L⁻¹) were determined using a multiparameter water quality meter (Multiparameter Probe). Water transparency was also assessed with the use of a Secchi disk, while depth was determined using a GPS. Accumulated rainfall and average temperatures from July 2015 to May 2016 at the Rio de Janeiro station were

Fig. 1 Guanabara Bay sampling points: P1—Urca Beach, P2—Cagarras Islands and P3—beyond the Cagarras Islands



obtained through the National Institute of Meteorology (INMET).

Biometric analysis and dissection

The sampled swimming crabs were identified as *Callinectes* spp. and *Achelous* spp. based on phenotypic characteristics described in the literature (Crab Database 2016; FAO 2018). After identification, the animals were sexed and measured with a caliper.

Twenty-seven males and 33 females were obtained in total, 12 males and 18 females sampled in 2015, and 15 males and 15 females sampled in 2016. Medium-sized males and females (approximately 70–80 mm) were selected, the same size as animals destined for human consumption (Igarashi 2009). After the biometric assessments, the animals were dissected and muscle tissue was removed for the Hg analyses.

Mercury analyses

Total Hg (THg) was determined using a Direct Mercury Analyzer (DMA-80, Milestone, Bergamo, Italy) following the manufacturer’s recommendations. Briefly, about 0.27 g of each sample was inserted in a quartz tube and dried under an oxygen flow at 4 atm, used as both the combustion and carrier gas. Sample evaporation is carried out at 160 °C for 1 min, 650 °C for 2 min, and 650 °C for another 1 min. At the end of this process, the Hg vapor is desorbed for quantification using a gold amalgamation trap. A 1000-mg-L⁻¹ Hg solution (Sigma-Aldrich, São Paulo, Brazil) was used to prepare the calibration curves ranging from 0.5 to 1000 ng g⁻¹. Readings were performed at a 253.7 nm, and Hg quantification was

carried out using a calibration curve ($R^2 = 0.9999$) taking into account sample weight and peak height. The results were expressed as mg kg⁻¹ (Guimarães et al. 2016). All determinations were performed in duplicate. The method limit of quantification is 0.005 ng (DMA-80, Milestone, Bergamo, Italy).

Statistical analyses

The means and standard deviations of the abiotic and biometric data and Hg concentrations were determined. A Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson 2005) was carried out for the abiotic data, where all factors were first evaluated together and then separately.

A principal component analysis (PCA) using the CANOCO 4.5 software package (Leps and Smilauer 2003) was then applied to the abiotic data matrix (log₁₀-transformed) to assess which physical and chemical water variables were the most important to explain the spatial and seasonal patterns. The significance of each environmental variable was tested through a Monte Carlo permutation test (999 permutations).

A covariance analysis (ANCOVA) using the STATISTICA 11.0 software package was also carried out, applying the length of the animals as co-variable. The first aim was to verify statistical differences between Hg concentrations in both swimming crabs (*Callinectes* sp. and *Achelous* sp.) at P1 (Urca). The ANCOVA test was then applied to verify differences between Hg concentrations for each climatic period and study area.

Generalized additive models (GAMs), available in the CANOCO 4.5 software package, were applied correlating

Hg concentrations and abiotic factors. The axis displaying the greatest significance in the PCA, i.e., the one responsible for most of the spatial variation of the environmental variables, was then applied as an independent variable in the GAM analysis. The complexity of the GAM model was chosen by a step-by-step selection procedure using the Akaike Information Criterion (AIC), as available in the CANOCO 4.5 software package. The AIC considers not only the goodness of fit, but also parsimony, penalizing more complex models (Burnham and Anderson 1998). Statistical significance was accepted when $p < 0.05$.

Results and discussion

Abiotic factors

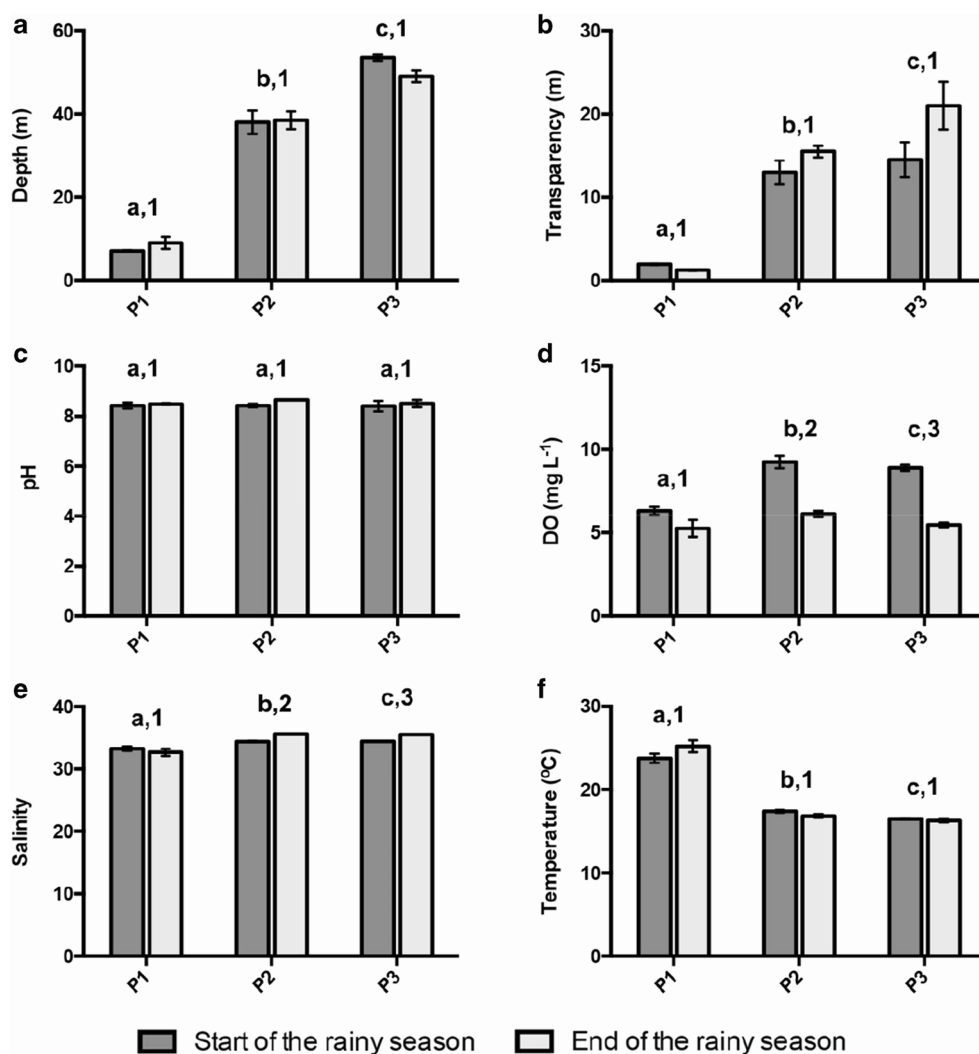
The means and standard deviations of each of the studied abiotic variables (depth, transparency, pH, oxygen, salinity and temperature) are displayed in Fig. 2. The

PERMANOVA analysis performed with all of the assessed parameters indicated significant differences between season and sampling areas, as well as interactions between several abiotic variables, discussed below.

Depth. No statistical difference between seasons ($P = 0.2830$) and a significant difference between sampling areas ($P = 0.0001$) were observed, where the depth of the outermost point of the bay (P3) was higher than at P2 (3 and 2, $P = 0.0005$), which was, in turn, higher than at P1 (3 and 1, $P = 0.0001$; 2 and 1, $P = 0.0001$).

Transparency. A statistical difference between areas ($P = 0.0001$) was observed for transparency, where P3 and P2 displayed similar values at the beginning of the study period and were more transparent than P1, while P3 presented a significantly higher means compared to P2 at the end of the season, both presenting higher means than P1. Although P2 and P3 did not differ statistically from each other, both differed from P1 at the beginning ($P = 0.0014$ and $P = 0.0027$, respectively) and at the end ($P = 0.0002$ and $P = 0.0014$, respectively) of the study period.

Fig. 2 Mean abiotic water parameters at the beginning and end of the study season. **a** Depth (m). **b** transparency (m). **c** pH. **d** DO (mg L^{-1}). **e** salinity (g L^{-1}). **f** Temperature ($^{\circ}\text{C}$). P1: Urca Beach; P2: Cagarras islands; and P3, beyond the Cagarras islands. Different letters (a–c) indicate statistical differences between the sampling points, while different numbers (1–3) indicate statistical differences between climatic period



pH. No statistical difference between areas or season was noted for pH.

DO. Statistical differences between climatic period ($P = 0.0001$) and sampling areas ($P = 0.0005$) were observed for DO. At the beginning of the rainy season, DO concentrations at P2 and P3 did not differ statistically from each other but were different from P1 ($P = 0.0112$ and $P = 0.0075$). At the end of the season, P2 and P3 presented only a very subtle difference ($P = 0.0519$), indicating similar DO concentrations at the three sampling points.

Salinity. Statistically significant differences were observed between season ($P = 0.0179$) and areas ($P = 0.0001$). However, despite the two outer points (P2 and P3) presenting higher means than the inner point (P1), differences were only noted when compared to P1. Regarding climatic period, a subtle increase in salinity from the beginning to the end of the rainy season was observed for P2 and P3. In the beginning, P2 and P3 and P1 and P2 did not differ statistically from each other. Nevertheless, the outermost sampling point (P3) was statistically different from P1, and more saline than P1. At the end of the season, only the P1 displayed statistically significant differences compared to the outer points (P3 compared to P1 and P2 compared to P1), with P1 presenting the lowest salinity compared to P2 and P3.

Temperature. No difference was identified between the beginning and end of the season, while a significant difference was noted between areas ($P = 0.0001$). At the beginning of the season, P2 and P3 presented similar temperatures, both lower than P1, with differences noted between P3 and P1 ($P = 0.0026$) and P2 and P1 ($P = 0.0032$). Temperatures did not differ statistically between the sampling points at the end of the season. However, according to the means, P1 displayed a subtle increase in temperature in relation to the beginning of the season.

The PCA results are displayed in Fig. 3. pH, oxygen, and temperature were negatively correlated to the other assessed abiotic parameters. Data variation was mostly explained by PCA1 (65.92%). Urca presented higher temperatures, both in the beginning and the end of the season, with negative correlations to the other assessed parameters. At the beginning of the season, the two external sampling points presented low DO concentrations and higher pH, while the opposite was observed at the end of the season.

Concerning rainfall, the Rio de Janeiro meteorological station (INMET) clearly indicates the beginning and end of the rainy season (December to March), based on increased precipitation rates (mm). According to these data, November 2015 and January and March 2016 surpassed rainfall expectations, while the other months displayed lower than expected rainfall values, based on the climatological normal values from 1961 to 1990.

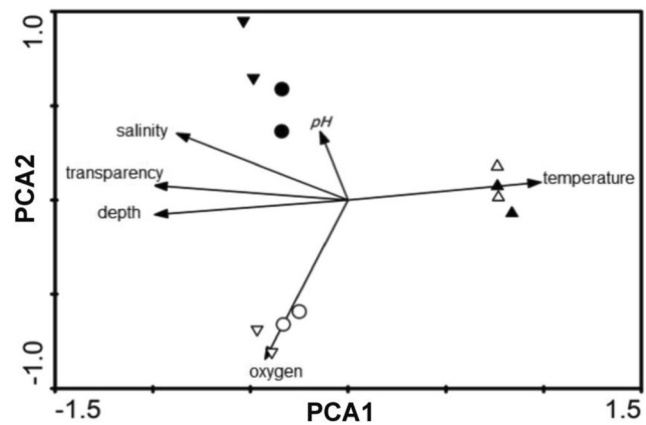


Fig. 3 Principal component analysis (PCA) concerning the abiotic variables assessed at the three study points. Axis 1 = 65.92%; Axis 2 = 22.95%. White shapes indicate sampling carried out at the beginning of the study period: white triangle Urca beach; white circle Cagarras island; white inverted triangle Beyond the Cagarras islands, while black shapes indicate sampling carried out at the end of the study period: black triangle Urca beach; black circle Cagarras island; black inverted triangle Beyond the Cagarras islands—end of the study season

The abiotic results indicate differences between the external (P2 and P3) and inner (P1) sampling points, probably due to the influence of continental drainage and coastal waters. The former is a significant influence in the inner zone of the bay, due to the extensive local drainage basin, while coastal waters act on the outermost region through tidal currents (Silva et al., 2016).

An expected depth gradient was noted, with deeper external bay zones compared to the internal region, due to higher sedimentation rates and sediment accumulation in the internal zone compared to the oceanic region (Fistarol et al. 2015; Vieira et al. 2015).

A transparency gradient was also observed, especially at the end of the season, following the order $P3 > P2 > P1$, due to the bay’s pollution gradient, where low transparency due to excess dissolved organic matter is observed in the bay’s internal zones, whereas zones undergoing greater oceanic influence, such as P2 and P3, tend to be more transparent, due to the local tidal regime and dilution caused by marine waters (Fistarol et al. 2015). At the beginning of the season, the effect of the drainage basin combined with rainfall, which leads to the transport of contaminated sediments, reached only Urca, as similar and higher transparency values were observed at external points. At the end of the season, the sediment plume spread to the outer points, leading to a more evident difference in the transparency gradient, although P2 and P3 remained statistically similar.

Regarding pH, values remained alkaline at all sampling points, corroborating previous assessments (Chaves et al. 2018; Souza et al. 2018).

Concerning DO, P2 and P3 presented similar values at the beginning of the season, both higher and statistically different

from Urca, as corroborated by the PCA. At the end of the season, DO values were similar at all three points, with an evident decrease from the beginning to the end of the season. This indicates that the eutrophication process, initially restricted to Urca, spread to the outer areas due to rainfall and drainage basin action. Fistarol et al. (2015) and Souza et al. (2018) also report the presence of a DO concentration gradient in the bay due to local eutrophication processes.

Lower salinity values were observed in the inner bay area due to drainage basin influence, anthropogenic action and increased rainstorm frequencies, common in Rio de Janeiro during the summer, and higher values were observed in the oceanic region when compared to Urca, also corroborating previous assessments, due to the influence of oceanic waters (Chaves et al. 2018; Souza et al. 2018; Fistarol et al. 2015).

Similar temperature values were observed for the external sampling points, significantly different from Urca, and lower at the beginning and end of the season. However, P2 presented a slight increase in temperature at the end of the season in relation to the beginning, which indicates the arrival of the plume from the inner bay, spreading abiotic bay conditions, as well as contaminated sediment, to the external zone. These results are in agreement with those reported by Fistarol et al. (2015), who also indicated a temperature gradient along the bay, and in agreement with what is expected for the region, due to the influence of the South Atlantic Central Water (SACW), which establishes subtropical and temperate characteristics for the Rio de Janeiro coast (Silva et al., 2016).

The PCA also clearly demonstrates the discussed abiotic characterization for Urca, where higher temperature and lower salinity, transparency, pH, oxygen, and depth values have been reported by other authors (Fistarol et al. 2015; Seixas et al. 2016; Souza et al. 2018; Vieira et al. 2015). A clear rainfall effect on abiotic variable alterations is also noted, corroborating meteorological data, which indicates increased rainfall and temperature at the end of the study period.

Regarding Hg, differences between sampling points and season were observed. THg concentrations were similar between Urca (P1) and beyond the Cagarras islands (P3), and both were different and higher than at the Cagarras islands themselves (P2). At the beginning of the season, local rainfall and drainage basin effects result in sediment mixing, leading to Hg bioavailability through methylation, corroborated by the high Hg concentrations found in swimming crabs at Urca. However, the contamination plume did not reach the island region. Higher Hg concentrations were also observed at the outermost point the bay (P3) in relation to P2, probably due to the aforementioned upwelling event. This upwelling event is induced by local winds and leads to coldwater inversion from the bottom to the surface, also mixing contaminated sediment, also increasing Hg bioavailability (Vieira et al. 2015).

At the end of the season, Hg concentrations at P1 and P2 were similar and higher than at P3, in agreement with Franco et al. (2018), who consider that more intense upwelling effects occur during the late spring and beginning of summer near the GB entrance compared to the end of summer. Although this phenomenon is no longer as evident at the end of the season, it is assumed that the lesser influence of currents and winds, at the end of the season, leads to lower Hg bioavailability, resulting in decreased Hg concentrations at P3, as observed in the GAM graph displayed in Fig. 4, displaying the correlation of Hg concentrations with the abiotic variables assessed at the three study points in the beginning and end of the season. On the other hand, the plume spread from the inland region to the islands (P2) at the end of the season, leading to higher Hg contamination in this region. The plume that reaches the island region is also responsible for similar Hg concentrations at Urca. These results indicate that, regardless of season, Urca presents high and continuous Hg contamination promoted by the assessed abiotic variables (Chaves et al. 2018).

Biotic factors

Regarding swimming crab length, no statistical difference between the beginning and end of the rainy season was noted, nor between the three sampling points. The ANCOVA test

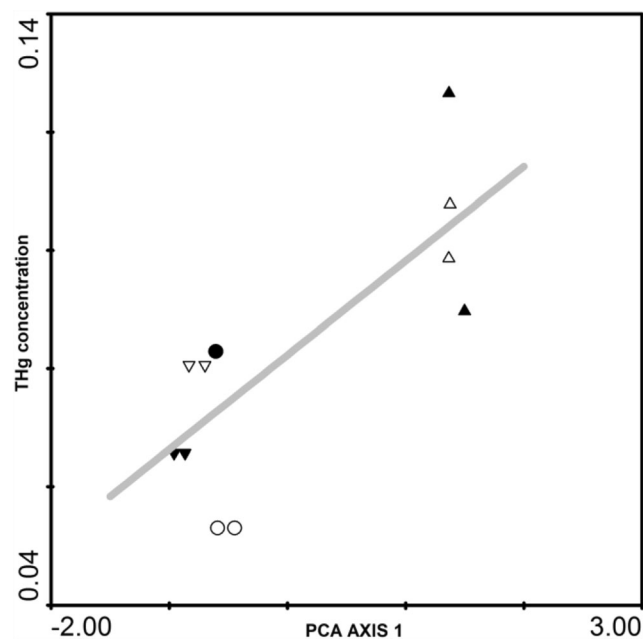


Fig. 4 Generalized Additive Models (GAM) correlating Hg concentrations with abiotic variables at the three study points at the beginning and end of the season. White shapes indicate sampling carried out at the beginning of the study period: white triangle Urca beach; white circle Cagarras island; white inverted triangle Beyond the Cagarras islands, while black shapes indicate sampling carried out at the end of the study period: black triangle Urca beach; black circle Cagarras island; black inverted triangle Beyond the Cagarras islands—end of the study season

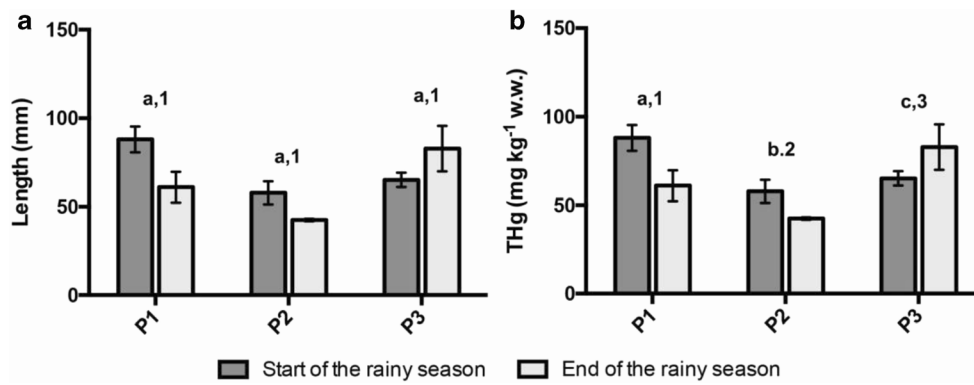


Fig. 5 Mean length and THg concentrations in swimming crabs at the beginning and end of the study season at each sampling point. P1 (Urca beach), P2 (Cagarras islands), and P3 (beyond the Cagarras islands). **a**

Lengths (mm). **b** Mean THg concentrations (mg kg⁻¹). Different letters (a–c) indicate statistical differences between points, while different numbers (1–3) indicate statistical differences between climatic period

indicated a subtle interaction between season and zone (Fig. 5). Higher THg values were observed at P1 and P2 at the beginning of the rainy season, while the opposite was observed at P3.

Figure 6 displays differences between THg concentrations between *Callinectes* sp. and *Achelous* sp. for P1 only, as this was the only area where animals from both genera were collected. The means and ANCOVA test were performed with the data from this point only.

The ANCOVA analysis indicated no statistical difference between length, genera, season, and sex. Likewise, applying length as the co-variable, no statistical difference was noted between the two genera, sex and climatic period. Finally, no correlations between genus versus season, genus versus sex, and season versus sex were observed.

No differences were observed between Hg levels in the two swimming crab genera from Urca between either study period. This is expected, as both swimming crab genera differ only in genus and occupy the same trophic level, while differences in Hg concentrations are most evident between different species and trophic levels (Azevedo-Silva et al. 2016; Chen et al. 2014; Ruus et al. 2017; Sadhu et al. 2015; Taylor and Calabrese 2018).

Although some studies postulate that females, in order to meet reproduction demands, present higher

concentrations due to increased food consumption during the reproductive period (Murphy et al., 2011), no statistical differences between Hg concentrations between male and females was observed, corroborating previous studies in swimming crabs and fish (Adams and Engel 2014; Bastos et al. 2015; Ordiano-Flores et al. 2011).

Swimming crabs are benthic, living in close contact with contaminated sediment, which could favor Hg bioaccumulation in contaminated areas. However, they occupy low trophic levels and feed on other organisms belonging to the same trophic level, leading to low Hg biomagnification (Arcagni et al. 2018). These factors lead to variable Hg concentrations in these animals, depending on the influence of external factors (abiotic factors).

Hg concentrations in the present study were similar to other studies that indicate low Hg values in swimming crab and other crustaceans (Annual et al., 2018; Arcagni et al. 2018; Bisi et al. 2012; Ferreira et al. 2012). Values in swimming crabs in the Urca beach were the highest among the three points (0.1032 ± 0.04 to 0.1007 ± 0.05 mg kg⁻¹ THg), yet all individuals presented concentrations below the safety limit established by the Brazilian (0.5 mg kg⁻¹) and international (1.0 mg kg⁻¹) guidelines (Brasil 2013; European Union (EU) 2006).

Fig. 6 Length and THg concentrations for *Callinectes* sp. and *Achelous* sp. sampled from Urca (P1). **a** Mean length (mm). **b** Mean THg (mg.kg⁻¹). The same letters (a–c) indicate no statistical difference between crab genera, while the same numbers (1–3) indicate no statistical difference between the season

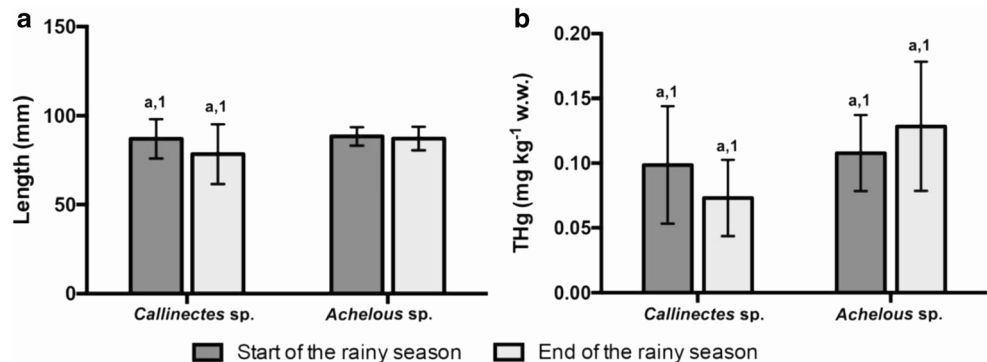


Table 1 Comparisons between total mercury (THg) concentrations in *Callinectes* sp. muscle tissue in other estuaries worldwide. Data are expressed as mg kg⁻¹

THg (mg kg ⁻¹)	Location	Reference
0.41288 to 0.40300 dry weigh (0.10322 to 0.10075 w.w)	Urca, Guanabara Bay	The present study
0.21216 to 0.33180 dry weigh (0.05304 to 0.08295 w.w)	Cagarras Islands, Guanabara Bay	
0.32240 to 0.26288 dry weigh (0.0806 to 0.06572 w.w)	Beyond the Cagarras Islands, Guanabara Bay	
0.226 ± 0.114 dry weight (mid-bay) 0.208 ± 0.074 dry weight (upper-bay)	Narragansett Bay (Rhode Island/Massachusetts, USA)	Taylor and Calabrese (2018)
0.035 ± 0.007 dry weight (fluvial dominated area) 0.103 ± 0.038 dry weight (marine dominated area)	Jaguaribe River Basin (Ceará, Brazil)	Moura and Lacerda (2018)
0.090 ± 0.01 dry weight	Köyceğiz Lagoon System -Estuary	Genç and Yulmaz (2017)
Average: 0.32 dry weight (dry season and rainy season/2011) 0.16 dry weight (rainy season/2013)	Santos Estuarine System (São Paulo, Brazil)	Bordon et al. (2016)
Average: 0.28 dry weight (rainy season/2010)	Santos Estuarine System (São Paulo, Brazil)	Bordon et al. (2012)
0.230 ± 0.03 dry weight	Narragansett Bay (Rhode Island/Massachusetts, USA)	Taylor et al. (2012)
≈ 0.45 ± 1 dry weight ≈ 0.15 ± 1 dry weight	- Hackensack Meadowlands-Hudson-Raritan estuary - Mullica River-Great Bay estuary (New Jersey, USA)	Reichmuth et al. (2010)
0.496 ± 0.044 dry weight	Cartagena Bay (Colombia)	Olivero-Verbel et al. (2008)

Table 1 presents the total Hg values found in *Callinectes* sp. from other estuaries worldwide. *Achelous* crabs are not routinely assessed, and no studies were found in the literature. Compared to the listed studies, Hg values observed in the present study were higher than those found in other areas in Brazil and lower than other reports worldwide.

Public health concerns regarding Hg ingestion

It is important to emphasize that studies have reported that almost 100% of Hg is found in its methylated form in animals, the most toxic form of this metal (Adams and Engel 2014; Souza-Araujo et al., 2016; Taylor and Calabrese 2018).

Although all Hg values in the assessed crabs were below the stipulated limit of 1.0 mg kg⁻¹ THg in seafood for the European Union and 0.5 mg kg⁻¹ in crustaceans by the Brazilian regulatory agency ANVISA (Brasil 2013; European Union (EU) 2006 depending on the frequency and amount of local consumption, contaminated swimming crab intake may reflect in risks to consumer health in the long term.

Thus, the maximum limits stipulated for human Hg intake per week should be taken into account. The provisional tolerable weekly intake (PTWI) for THg is of 4 µg/kg bw/week according to the JECFA/WHO (JECFA; WHO, 2004), while the (US) National Research Council (NRC) has established an intake limit of 0.7 µg/kg bw/week (NRC, 2000), 2.3-fold lower than the one allowed by JECFA/WHO. Weekly Hg levels were calculated based on the annual average consumption of seafood reported for the metropolitan region of Rio de Janeiro of 18.5 kg (Barroso and Wiefels, 2010), or a weekly

consumption of 0.356 kg. The results of 0.147 and 0.143 mg kg⁻¹ for the beginning and end of the dry season at Urca, respectively; 0.075 and 0.0118 mg kg⁻¹ Hg for the beginning and end of the dry season at the Cagarras Islands, respectively; and 0.0114 and 0.0093 mg kg⁻¹ Hg for the beginning and end of the dry season at beyond the Cagarras Islands, respectively indicate that Hg intakes were within JECFA guidelines JECFA (0.28 mg/week) for all sampling locations and seasons but surpassed NRC recommended guidelines (0.049 mg/week) at all sampling stations during both seasons, raising public health concerns.

Conclusions

The findings reported herein indicate that the rainy season directly affects Hg concentrations at Guanabara Bay and, consequently, swimming crab THg contents. A clear rainfall effect on abiotic variable alterations was observed, with increased rainfall and temperatures at the end of the study period. Significant correlations between THg concentrations and the abiotic variables were observed at the three study points at the beginning and end of the season.

THg concentrations in swimming crabs at Urca and at the Cagarras Islands were higher at the beginning of the rainy season compared to the end, while the opposite was observed for beyond the Cagarras Islands. Higher Hg concentrations were observed at the outermost point the bay in relation to the Cagarras Islands, probably due to the local upwelling event. The Hg values detected in *Callinectes* sp. were higher

than those reported for other areas in Brazil, but lower than other reports worldwide. Calculated Hg intakes surpassed NRC recommended guidelines at all sampling stations during both seasons, raising public health concerns.

Further research taking into account different seasons and longer monitoring periods are essential in order to ascertain which climatic period is most critical regarding Hg availability at this anthropogenically impacted estuary.

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