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Trace elements in *Penaeus* shrimp from two anthropized estuarine systems in Brazil

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Abstract: This study measured concentrations of trace elements (Al, As, Cd, Cu, Fe, Mn, Ni, and Pb) in the muscle of pink shrimps (genus *Penaeus*) from two anthropized estuarine systems in Brazil: Guanabara Bay (GB) and Sepetiba Bay (SB). Concentrations were highest in the less anthropized SB site, where shrimps showed higher assimilation rates that can be explained by their higher trophic position compared to shrimps from GB. These results reinforce the role of food sources as the main route of trace elements for the aquatic animals.

Keywords: Coastal systems, metals, metalloid, pink shrimps, stable isotope.

Portuguese abstract: Este estudo verificou que a concentração de elementos traço (Al, As, Cd, Cu, Fe, Mn, Ni e Pb) no músculo de camarões-rosa (gênero *Penaeus*) é variável entre dois sistemas estuarinos antropizados no Brasil (Baía de Guanabara - BG e Baía de Sepetiba - BS), sendo maior no local menos antropizado (BS). As regressões entre as concentrações de elemento traço e os valores de $\delta^{15}\text{N}$ mostraram maiores taxas de assimilação nos camarões da BS, o que pode ser explicado pela sua posição trófica mais elevada em relação aos camarões da BG. Os resultados reforçam o papel das fontes alimentares como a principal rota de elementos traço para os animais aquáticos.

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INTRODUCTION

Trace elements such as metals and metalloids can accumulate in all compartments of aquatic environments (Brown & Depledge 1998), where their concentrations reflect both natural levels and anthropogenic contamination. When they enter human food chains, elements such as Cd, Hg, and Pb can cause neurological and kidney damage (WHO 2019), while As (metalloid) is linked to several types of cancers (Palma-Lara et al. 2020). Shrimps and other crustaceans accumulate trace elements from water, sediment, and food sources at levels beyond those necessary for nutrition and metabolism (Rainbow 2002; Boudet et al. 2019). Food is the main route of trace elements for both invertebrates and vertebrates (Rainbow 2002; Di Benedetto et al. 2021; Kehrig et al. 2022)

The pink shrimps *Penaeus brasiliensis* Latreille, 1817 and *P. paulensis* Perez Farfante, 1967 are sympatric in the southwestern Atlantic, where juveniles inhabit estuaries while adults live in marine waters (Neto 2011). Carvalho et al. (2021) investigated the stomach contents and the niche breadth of juvenile pink shrimps in two anthropized estuarine systems located in Sepetiba Bay and Guanabara Bay, southeastern Brazil (~23°S) (Figure 1). These areas are proximal coastal nurseries and fishing sites for both species. Niche analysis revealed pink shrimps from Guanabara Bay occupied a lower trophic position and showed greater trophic diversity in comparison to pink shrimps from Sepetiba Bay (Carvalho et al. 2021). The authors verified that interspecific differences in feeding preferences are negligible within the same estuarine system.

Based on findings of Carvalho et al. (2021) and the premise that food sources are the main route of trace elements for the animals, we made two predictions: i) the concentration of trace elements in shrimps *Penaeus* is variable between the two estuarine systems, following the spatial difference of the trophic niche, and ii) pink shrimps from Sepetiba Bay have higher trace element concentrations, since their trophic position is higher than in Guanabara Bay.

METHODS

Since *P. brasiliensis* and *P. paulensis* have similar feeding habits and niches in estuarine systems, we grouped them as *Penaeus* shrimps. Juvenile pink shrimps were sampled through fisheries inside Guanabara Bay (n = 80 individuals) and Sepetiba Bay (n = 67 individuals);

herein referred as GB and SB, respectively (Figure 1). The samplings were done in 2021–2022. In GB, shrimps were caught in a fishing site in the central portion of the bay (8 km², 12–15 m deep), 10 km from the bay entrance (Figure 1). In SB, the fishing site included an area of approximately 6 km² and 10 m deep, 20 km from the bay entrance and 3 km from its northeastern shore, in front of the Guandu River mouth (Figure 1).

GB is more anthropized than SB. It comprises 384 km² with a drainage basin of 4,080 km² (55 small-river inputs). In surrounding areas, there are almost 12 million inhabitants, 6,000 industries and intensive inputs of domestic sewage and industrial effluents (Cordeiro et al. 2021). The SB comprises 450 km² with a drainage basin of 2,065 km². The SB surrounding areas have 400 industries, mainly chemical and metallurgic plants, and a population of approximately 2.0 million (Costa et al. 2011).

After sampling, shrimps were stored in transparent clean plastic bags and kept in cold storage during transportation. In the laboratory, the abdominal muscle (edible portion) of each shrimp was removed, stored in a dry sterile bottle, frozen (–20°C), freeze-dried and homogenized to a fine powder using a mortar and pestle.

The trace elements considered in this study are Al, As, Cd, Cu, Fe, Mn, Ni, and Pb, which were determined using ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) 720 ES (Varian Liberty Series II). Briefly, freeze-dried muscle (0.5 g) was solubilized in 10 mL of 65% HNO₃ and heated in a digester block. The samples were resuspended in 5 mL of 0.5% HNO₃ at 60 °C, filtered and brought to a final volume of 20 mL with 0.5% HNO₃. An analytical control solution was prepared to check for contamination. A reference material (DORM-4 fish protein, National Research Council of Canada) was analyzed to test the precision and accuracy, and the recovery values were above 95%. The coefficients of variation among analytical replicates were < 10%. The concentrations were determined in mg kg^{–1} of dry weight.

We used the ratio of nitrogen stable isotope (δ¹⁵N) to evaluate the trophic position of the pink shrimps. Dry muscle sample (0.4 mg) of each shrimp was analysed using an organic elemental analyzer (Flash 2000, Thermo Scientific) coupled to a mass spectrometer (Delta V Advantage Isotope Ratio Mass Spectrometer, Thermo Scientific) through the ConFlo-VI interface (Model BR30140, Thermo Scientific). The reference value for nitrogen was the atmospheric nitrogen. Samples were analyzed using analytical blanks and urea analytical standards (IVA Analysentechnik-330802174). Analytical control and reproducibility were done for every 10 samples using a certified isotopic standard (Elemental

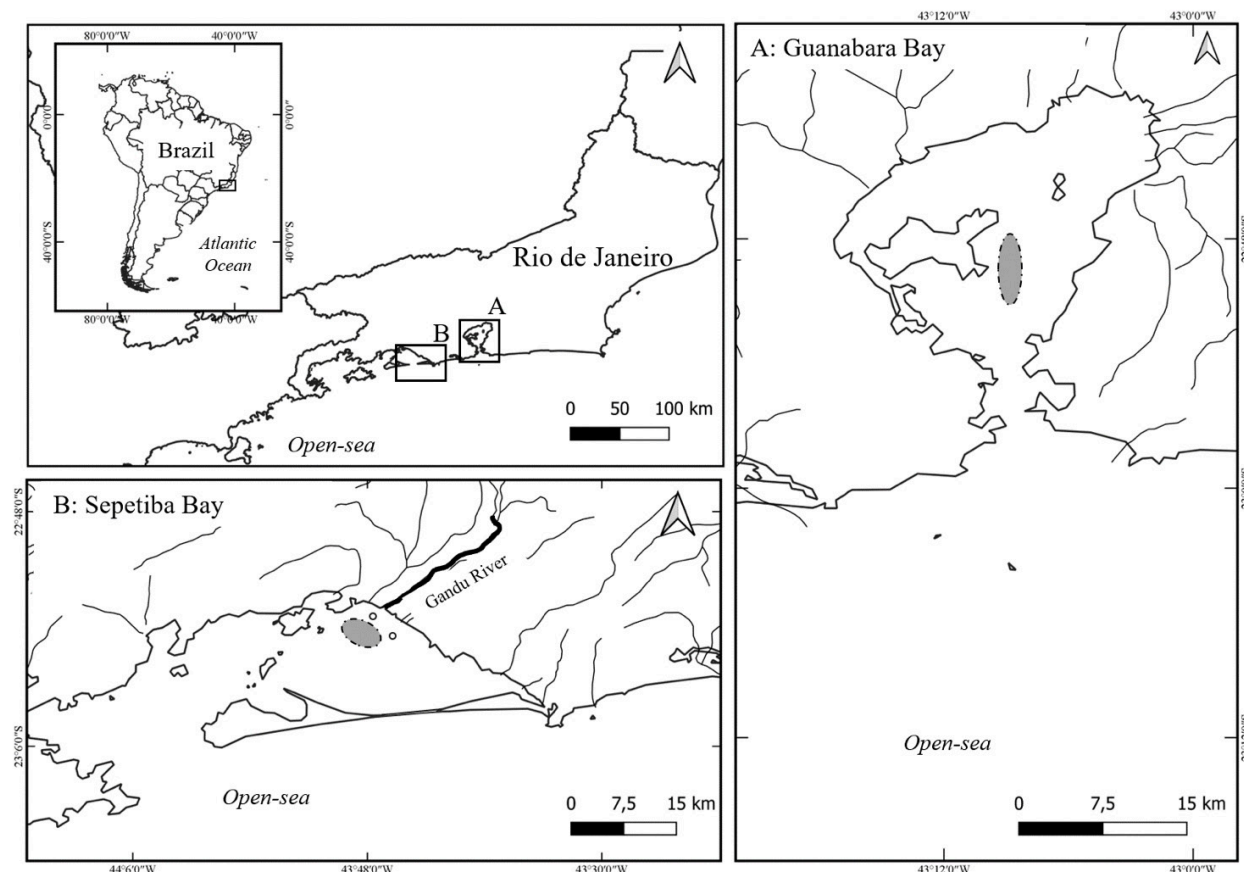


Figure 1. Guanabara Bay (A) and Sepetiba (B) Bay, southeastern Brazil, and the fishing sites of the pink shrimps inside the bays (gray ellipse areas). Extract from Di Benedetto et al. (2021).

Microanalysis Protein Standard OAS) and based on triplicates for every 10 samples ($\pm 0.3\%$ for $\delta^{15}\text{N}$). The isotopic result was presented as parts per thousand (‰).

RESULTS AND DISCUSSION

The concentrations of Al, As, Cd, Cu, Fe, Mn, Ni, and Pb, and the values of $\delta^{15}\text{N}$ in the muscle of the pink shrimps were greater in SB than GB (Table 1). This finding was statistically supported by the ANOVA results (R Core Team 2022) for most elements (Table 1). ANVISA (2021) and FAO/WHO (1991) have established the maximum tolerable limits of some trace elements (e.g., As, Cd, Cr, Cu, Hg and Pb) for food products. In this study, the trace elements determined in the pink shrimps were below these limits.

The difference in $\delta^{15}\text{N}$ values reinforces the findings of Carvalho et al. (2021): pink shrimps from SB are in higher trophic position than GB ($p < 0.001$). The isotopic difference between the pink shrimps from the two estuarine systems was 4.7%, which is high enough

Table 1. Median \pm interquartile range values of Al, As, Cd, Cu, Fe, Mn, Ni, and Pb (mg·kg⁻¹ dry weight), and $\delta^{15}\text{N}$ (‰) in the muscle of pink shrimps from two anthropized estuarine systems in Brazil. Lowercase letters a and b indicate ANOVA differences at $p < 0.05$.

Estuarine system Elements	Guanabara Bay (GB)	Sepetiba Bay (SB)
Al	16.8 \pm 25.5 ^b	42.2 \pm 47.3 ^a
As	3.1 \pm 2.1 ^b	5.1 \pm 3.6 ^a
Cd	0.02 \pm 0.01 ^a	0.02 \pm 0.01 ^a
Cu	8.5 \pm 4.8 ^a	9.6 \pm 6.2 ^a
Fe	10.8 \pm 16.9 ^b	35.8 \pm 31.4 ^a
Mn	0.8 \pm 1.0 ^b	1.5 \pm 1.3 ^a
Ni	0.1 \pm 0.2 ^b	0.2 \pm 0.3 ^a
Pb	0.1 \pm 0.04 ^a	0.1 \pm 0.1 ^a
$\delta^{15}\text{N}$	7.5 \pm 6.6 ^b	12.2 \pm 0.9 ^a

to distinguish different trophic levels between them. According to Post (2002), differences in $\delta^{15}\text{N}$ values ranged from 2 to 5% indicate different trophic levels.

Regressions between trace element concentrations

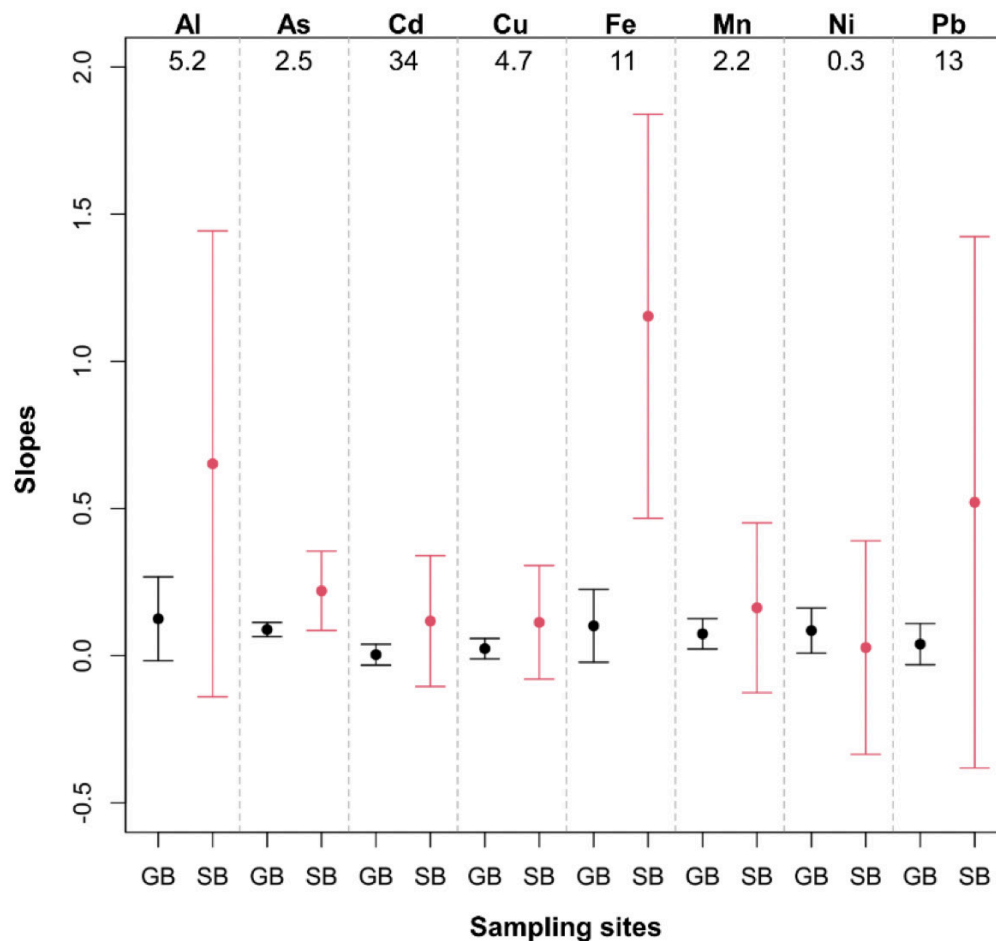


Figure 2. Slopes obtained from regressions between trace elements concentrations and $\delta^{15}\text{N}$ values in pink shrimps from two anthropized estuarine systems in Brazil. The symbols (black circle to Guanabara Bay - GB, and red circle to Sepetiba Bay - SB) and bars represent each slope and its 95% confidence interval, respectively. Numbers below the trace elements are the slope ratio between the estuarine systems (SB/GB).

and $\delta^{15}\text{N}$ values were adjusted (R Core Team 2022) to verify the assimilation rate of each element according to the trophic position (i.e., their slopes). Mathematical transformations were done whenever necessary to meet the regression assumptions (normality, linearity, homoscedasticity) using a maximum likelihood function (Venables & Ripley 2002). The absence of overlapping in the 95% confidence intervals of the slopes can be interpreted as a significant difference between the two estuarine systems ($p < 0.05$). Meanwhile, the high variance in the regressions associated with SB, as showed by the confidence intervals (Figure 2), made it difficult to detect significant differences (except for Fe). Since this is a statistical problem and not a conceptual one, the data interpretation was based on the magnitude of difference in slopes (numbers at the top of Figure 2). The slope ratios between the estuarine systems (SB/GB) were greater than 1 (except for Ni), revealing a greater assimilation rate of the elements in SB. Considering all elements, the

average assimilation rate was 9.2 ± 11.0 times greater in SB compared to GB (Figure 2). The results confirmed the two-hypothesis: the concentration of trace elements is variable between the pink shrimps, following the spatial difference of their trophic niches; and pink shrimps from SB have higher trace element concentrations than in GB due to their higher trophic position.

Trace elements have different availability in the environment and different physiological pathways in the consumers (Boudet et al. 2019; Kolarova & Napiórkowski 2021), which may explain different concentrations in the pink shrimps between and within estuaries. The level of anthropization is higher in GB than SB (Costa et al. 2011; Cordeiro et al. 2021); thus, it could be expected that both the trace element concentrations and assimilation rates are higher in the pink shrimps from GB. However, the trophic position drove the assimilation rates, reinforcing the role of food sources as the main route of trace elements to the aquatic animals (Rainbow 2002; Di

Beneditto et al. 2012; Kehrig et al. 2022).

Carvalho et al. (2021) recommended attention to monitoring the pink shrimps from SB due to their smaller trophic breadth and lower $\delta^{15}\text{N}$ range, which may increase their sensitivity to changing habitat. The data on trace elements in the shrimps' muscle reinforce this recommendation, once their exposure to trace elements, including hazardous elements, can increase in a changing habitat.

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