Atlantic seabob shrimp as biomonitor of Cu and Zn near port activities: is it really a suitable choice?

Camarão sete-barbas como biomonitor de cobre e zinco no entorno de atividades portuárias: a escolha é realmente adequada?

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ABSTRACT

The trace elements concentration in the muscle of the Atlantic seabob shrimp (*Xiphopenaeus kroyeri*) caught in coastal fishing highlighted copper (Cu) and zinc (Zn), both related to antifouling systems, as the main elements related to the intensity of port activities of southeast Brazil (~20—to 22ºS). The aim of this study is to analyze if the behavior of Cu and Zn in the muscle of this shrimp species is constant among different sampling sites, verifying if the species is suitable as biomonitor for these elements. The shrimps came from fisheries done in 2017 in Vitória, Anchieta, and Farol de São Tomé, southeast Brazil. After sampling, each individual was categorized for gender and maturity stage, measured, and weighted. Bulk muscle samples were freeze-dried for determination of Cu, Zn, and ratios of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). The data analysis verified if the concentration of Cu and Zn in male and female shrimps vary among maturity stages and sampling sites, and how the concentration of Cu and Zn is related to shrimps foraging area and/or trophic position. Both bioaccumulation and growth dilution occurred, but not in the same way for genders and sampling sites, with Cu showing more variability. Relationships between elements and shrimps foraging area and trophic position did not show a clear trend among the sampling sites. Regression models indicated moderate relationships, explaining 51% (Cu) and 60% (Zn) of the association with the foraging area in Anchieta, but up to 8% in Vitória and Farol de São Thomé. For the trophic position, the models explained 33% (Cu) and 34% (Zn) in Anchieta and up to 14% in Vitória and Farol de São Thomé. The results showed that the

RESUMO

A concentração de elementos traço no músculo do camarão sete-barbas (*Xiphopenaeus kroyeri*) capturado na pesca costeira destacou cobre (Cu) e zinco (Zn), ambos relacionados com sistemas anti-incrustantes, como os principais elementos associados à intensidade das atividades portuárias do Sudeste do Brasil (~20–22ºS). O objetivo deste estudo foi analisar se o comportamento de Cu e Zn no músculo dessa espécie de camarão era constante entre os diferentes locais de amostragem, verificando se a espécie era adequada como biomonitor para esses elementos. Os camarões vieram de pescarias realizadas em 2017 em Vitória, Anchieta e Farol de São Thomé, Sudeste do Brasil. Após a amostragem, cada indivíduo foi categorizado quanto a sexo e estágio de maturidade, medido e pesado. Amostras compostas de músculo foram liofilizadas para a determinação de Cu, Zn e razões de isótopos estáveis de carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$). A análise dos dados verificou se a concentração de Cu e Zn em camarões machos e fêmeas variou entre os estágios de maturidade e locais de amostragem, e como a concentração de Cu e Zn estava relacionada à área de forrageamento e/ou posição trófica dos camarões. Tanto a bioacumulação quanto a diluição do crescimento ocorreram, mas não da mesma forma para os gêneros e locais de amostragem, com Cu apresentando maior variabilidade. As relações entre os elementos e a área de forrageamento e a posição trófica dos camarões não mostraram tendência clara entre os locais de amostragem. Modelos de regressão indicaram relações moderadas, explicando 51% (Cu) e 60% (Zn) da associação com a área de forrageamento em Anchieta, mas somente até 8% em Vitória e Farol de São Thomé. Para a posição trófica, os modelos explicaram 33% (Cu) e 34% (Zn) em Anchieta e até 14% em Vitória e Farol de São Thomé. Os resultados

utilization of this shrimp species as biomonitor of marine coastal environments near port activities to monitoring the levels of Cu and Zn is not a suitable choice, at least in the spatial scale considered by this study.

Keywords: trace elements; stable isotopes; coastal shrimp; environmental monitoring; Western Atlantic.

mostraram que a utilização dessa espécie de camarão como biomonitor de ambientes costeiros marinhos próximos às atividades portuárias para monitoramento dos níveis de Cu e Zn não é uma escolha adequada, pelo menos na escala espacial considerada neste estudo.

Palavras-chave: elementos traço; isótopos estáveis; camarão costeiro; monitoramento ambiental; Atlântico Ocidental.

Introduction

The Atlantic seabob shrimp, *Xiphopenaeus kroyeri* (Heller 1862), is a penaeid from Western Atlantic Ocean (36°N to 30°S) widely caught in coastal commercial fisheries along its distribution range (FAO, 2018). The species is an omnivorous consumer that spends its entire life cycle in marine coastal waters feeding on a broad spectrum of food items, such as primary sources (phytoplankton and macroalgae) and small animals from both seabed and water column. In this species, males and females have similar diets, whereas juveniles feed on smaller prey when compared to adults, which are in a higher trophic position (Branco and Moritz-Júnior, 2001; Willems et al., 2016). Like other penaeid shrimps, the Atlantic seabob shrimp presents sexual dimorphism in body size, with females larger and heavier than males, and adult males comparable to juvenile females in size (Hartnoll, 1982).

In general, shrimps are recognized as biomonitors of trace elements contamination in coastal environments due to their abundance, easy sampling, and strong association with the benthic environment (Stentiford and Feist, 2005; Fry et al., 2016). Biomonitors are sentinel organisms that provide quantitative information on the environmental quality because they accumulate contaminants in their tissues, yielding a relative measure of the total amount of these contaminants in the environment (Hatje, 2016). Crustaceans accumulate trace elements from the environment, mainly from water and diet, whether they are essential or not to their body functions. The accumulation pathway in crustaceans may vary among species, gender, maturity stage, and organs (Rainbow, 2002; Pourang et al., 2004; Yilmaz and Yilmaz, 2007). In animal ecotoxicological studies, the relationships between the concentration of trace elements and the ratios of stable isotopes of carbon and nitrogen allow evaluating the assimilation of trace elements from a foraging area, measured by $\delta^{13}C$ values, and from a trophic position, estimated by $\delta^{15}N$ values (Asante et al., 2008; Liu et al., 2018; Liu et al., 2019). As a common sense, $\delta^{13}C$ values indicate the food source origin, being more depleted (more negatives values) in pelagic than benthic areas; and $\delta^{15}N$ values are usually more enriched at higher trophic levels (Fry, 2008).

The concentration of 12 trace elements in the muscle (edible portion) of the Atlantic seabob shrimp caught in coastal fishing highlighted copper (Cu) and zinc (Zn) as the main elements related to the intensity of port activities of southeast Brazil (~20–22°S) (Di Beneditto

et al., 2020). The tributyltin (TBT) antifouling systems used in ships and boats were worldwide banned in 2008, and they were replaced by antifouling products with Cu metal oxides in combination with other co-biocides, such as Zn pyrithione or the polymer zineb (Dafforn et al., 2011; Amara et al., 2018). These antifouling products increased levels of Cu and Zn in fishes, as demonstrated by Nikolaou et al. (2014) in aquaculture farms. Di Beneditto et al. (2020) considered the same explanation to Cu and Zn concentrations in shrimps caught near large port activities of southeast Brazil. It is noteworthy that both elements are essential in crustaceans' metabolism (and in most animals), as key components of many enzymes (Zn) and constituent of hemocyanin (Cu) (Rainbow, 2002; 2007). However, Cu and Zn can cause hazardous effects in animal species, including humans, at high concentrations (Ali and Khan, 2019).

Based on the previous results of Di Beneditto et al. (2020) and because shrimps are recognized as good biomonitors of trace element contamination in coastal environments (Stentiford and Feist, 2005; Fry et al., 2016), the aim of this study is to analyze if the behavior of Cu and Zn in the muscle of the Atlantic seabob shrimp (herein referred to as shrimp) is constant among different sampling sites, verifying if the species is suitable as biomonitor for these elements. The study raised two questions to understand the presence of Cu and Zn in the shrimps, considering its population structure and habitat: Does the concentration of Cu and Zn in male and female shrimps vary among maturity stages and sampling sites? and How is the concentration of Cu and Zn related to shrimps foraging area and/or trophic position? This study may contribute for future decisions on environmental quality monitoring near port activities, since the Atlantic seabob shrimp is widely distributed in marine coastal waters along the Western Atlantic Ocean, and both Cu and Zn are elements present in most antifouling systems applied in vessels and boats.

Material and Methods

Sampling

The shrimps analyzed in this study came from the same dataset considered in Di Beneditto et al. (2020). The samplings were done in June–July 2017, during landings from commercial fisheries in three fishing sites from southeast Brazil: Vitória (20°31′S; 40°30′W), near Vitória and Tubarão ports; Anchieta (20°48′S; 40°38′W), near Ponta de

Ubu maritime terminal; and Farol de São Thomé (22°02′S, 41°02′W), near Açu superport. The first two sites are located in the state of Espírito Santo (ES) and the last one in the state of Rio de Janeiro (RJ) (Figure 1). Details regarding cargo activities in Vitória and Tubarão ports (largest ports), Ponta de Ubu maritime terminal, and Açu superport are in Di Beneditto et al. (2020).

Each shrimp was categorized macroscopically for gender (male or female) and maturity stage (juvenile or adult), as described in Campos et al. (2009). Each individual was measured to carapace size with a caliper (± 0.1 mm) and weighted in a digital scale (± 0.1 g). Carapace, gills, hepatopancreas, gonads, and intestine were removed before analysis and only abdominal muscle was considered for the quantification of Cu, Zn, and stable isotopes. Muscle is a low metabolic tissue with low turnover rate (Madigan et al., 2012), being a good tissue to represent trace elements incorporation in the shrimps' body (Di Beneditto et al., 2020). Muscle samples were kept frozen (−20°C) in transparent and clean plastic bags prior to analysis.

The sample size and number of bulk samples from each sampling site were 115 individuals from Vitória with 49 bulk samples (12 juvenile males, 13 adult males, 10 juvenile females, and 14 adult females); 116 individuals from Anchieta with 49 bulk samples (11 juvenile males, 14 adult males, 9 juvenile females, and 15 adult females); and 119 individuals from Farol de São Tomé with 57 bulk samples (13 juvenile males, 14 adult males, 15 juvenile females, and 15 adult females). According to the end mass after freeze-dried, each bulk sample was composed by one to four individuals of similar size (carapace length and total weight), and same gender (male or female) and maturity stage (juvenile or adult).

Figure 1 – Fishing areas of the Atlantic seabob shrimp (gray ellipses) and the fishing ports of Vitória and Anchieta, state of Espírito Santo (ES), and Farol de São Thomé, state of Rio de Janeiro (RJ).

Cu and Zn determination and stable isotope analysis

Muscle samples were freeze-dried and homogenized into a fine powder using a mortar and pestle. Each bulk sample (0.3 g dry weight) was analyzed for the concentration of Cu and Zn. For the determination of each element, dry samples were solubilized in 10 mL of 65% HNO_3, and then heated in a digestion block at 150°C until drying the extract. The samples were resuspended with 5 mL of 0.5 N HNO_3 at 60°C in a digestion block. Subsequently, the samples were filtered on filter paper (Whatman 40) and completed to 20 mL with 0.5 N HNO_3. Analytical blanks controls were prepared for each 20 samples to check for solution contamination. Certified reference material (Standard Reference Material DORM 4) was analyzed, with recovery of 110% and 70% for Cu and Zn, respectively, and coefficients of variation of triplicates < 10%. The certificate reference material recovery showed good results, and we assumed that the possible trace elements loss during the filtering processes did not significantly affect the results. No reference is available for shrimps; but there are references for fish tissues that show it (Maurya et al., 2019; Lacerda et al., 2020).

The trace elements were determined using an inductively coupled plasma-optical emission spectrometer (ICP-OES 720 ES, Varian). The operating conditions for ICP-OES were detailed in Di Beneditto et al. (2020). To avoid measurement errors, we performed the ICP-OES calibration every 30 samples and the quantification control with a calibration standard of 0.05 mg L⁻¹ for 15 samples. Cupper and Zn concentrations were expressed in µg g⁻¹ of dry weight. The limit of detection (LOD) and limit of quantification (LOQ) of ICP-OES followed the calculation presented in Skoog and Leary (1992): LOD = 3× standard deviation of blanks divided by the slope of the calibration curve. The LOQ were obtained by 3.3× LOD, coinciding with the first point of the calibration curve after the control blanks (Thomsen et al., 2003). The LOD (µg g⁻¹) and LOQ (µg g⁻¹) for each trace element are, respectively: Cu (< 0.27 and 0.88) and Zn (< 0.20 and 0.66). Determinations for all samples had results above the LOD and LOQ.

The ratios of stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) were determined in 0.4 mg of each bulk sample 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). Reference values for C and N were Pee Dee Belemnite (PDB) and atmospheric nitrogen, respectively. 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 Microanalysis Protein Standard OAS) and based on triplicates for every 10 samples (± 0.2‰ for $\delta^{13}C$; ± 0.3‰ for $\delta^{15}N$), respectively. There was no prior extraction of lipids from the muscle samples; however, the C/N ratios were lower than 3.5, indicating low lipid levels that do not compromise the interpretation of $\delta^{13}C$ values (Post et al.,
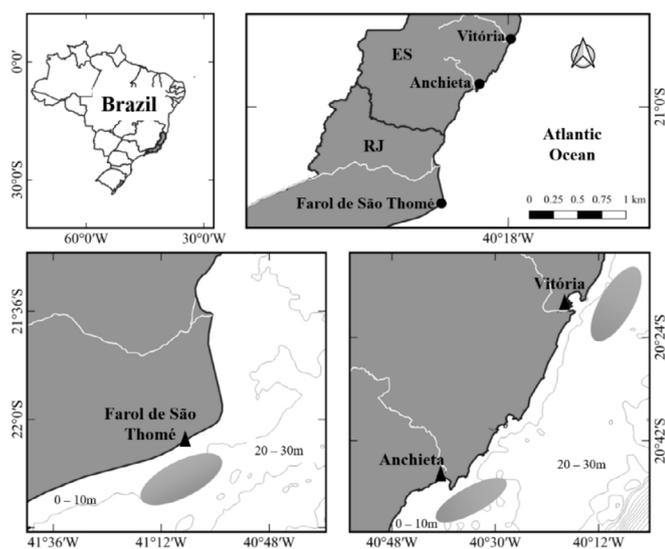
2007). The isotopic results were presented as parts per thousand (‰). All analysis (trace elements and stable isotopes) were done at Laboratório de Ciências Ambientais from Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF).

Data analysis

All statistical analyses were done in the R program (R Core Team, 2020). The concentration of Cu and Zn was normalized by the shrimp carapace length to remove intraspecific bias in bioaccumulation or growth dilution. Karimi et al. (2007; 2010) defined somatic growth dilution (SGD) (or just growth dilution) as a reduction in the somatic concentration of an element during rapid growth that normally happens in aquatic invertebrates. In general, SGD of an ingested element occurs when total biomass gain outpaces element gain from food, thereby diluting mass-specific element concentration in the body.

The relationship between the square root of shrimp's total weight ($\sqrt{W}$) and carapace length (CL) was adjusted for all bulk samples (mean values): $\sqrt{W} = 0.142 \cdot CL - 0.466$ ($R^2 = 0.95$, $p < 0.000001$). Since these measures have a strong relationship with each other, as demonstrated by the R^2 value, both could be applied as a measure of shrimp size in data normalization. Then, we evaluated how gender, maturity stage, foraging area ($\delta^{13}C$) and/or trophic position ($\delta^{15}N$) might influence the elements concentration in the shrimp muscle.

To answer the first question (Does the concentration of Cu and Zn in male and female shrimps vary among maturity stages and sampling sites?), the concentration of each element was used to calculate the ratio between the normalized concentrations in adults per normalized concentration in juveniles, as indicated below. Thus, when Ratio$_{Adults,Juveniles}$ is > 1, there is trace element bioaccumulation between the maturity stages, whereas values < 1 indicate a growth dilution effect (Equation 1).

$$\text{Ratio}_{Adults,Juveniles} = \frac{\text{Normalized elements concentration by carapace size in adults}}{\text{Normalized elements concentration by carapace size in juveniles}} \quad (1)$$

All combinations of Cu and Zn concentration in adults and juveniles were calculated through empirical combinatorial analyses (Monte Carlo method; expand.grid function, BASE package, Khitalishvili, 2016; R Core Team, 2020). This method generates all possible scenarios among individuals sampled in each sampling site, and correctly propagates the error associated with the ratios of trace elements between maturity stages into the results. The number of combinations performed did not exceed X·Y, with X and Y being the count of values for adults and juveniles, respectively. Only unique combinations were used to prevent biased results.

A two-way analysis of variance (ANOVA) compared the Ratio$_{Adults,Juveniles}$, isolating the effects of each factor (gender and sampling site) separately, and measuring the interactions between them. Because the number of combinations generated by the Monte Carlo method were

reasonably large (Anchieta = 289 combinations, Farol de São Thomé = 407 combinations, and Vitória = 296 combinations), the p values associated with ANOVA were all significant, even when corrected by the Bonferroni method (Signorell, 2020). Therefore, we report the effect size without p values to avoid redundancy.

To answer the second question (How is the concentration of Cu and Zn related to shrimps foraging area ($\delta^{13}C$) and/or trophic position ($\delta^{15}N$)?), a linear regression between each trace element (normalized concentrations) and stable isotopes values was done considering all shrimps sampled in a given sampling site (R^2 and p values are in Table 1). The slopes of regression equations from different sampling sites were compared by ANCOVA followed by Tukey multiple comparison test (Lenth, 2019).

When it was necessary, a maximum likelihood function (boxcox, MASS package; Venables and Ripley, 2002) was used for variable transformation to meet the parametric tests assumptions (linearity, normality, and homoscedasticity). Data transformations were done when necessary, as indicated in the figure and table legends. In addition, the type I *a priori* error was $\alpha = 0.05$ for all hypothesis tests.

The main steps described in Material and Methods section to reach the aim of this study are summarized in Figure 2.

Results

For Cu, both bioaccumulation and growth dilution were recorded in males and females, while for Zn only growth dilution was noted (Figure 3). The Cu behavior in the shrimp's muscle was variable among the sampling sites. In Vitória, males and females showed similar Ratio$_{Adults,Juveniles}$. In Anchieta, males had higher Ratio$_{Adults,Juveniles}$ than females, and in Farol de São Thomé females had higher Ratio$_{Adults,Juveniles}$ than males. For males, an increasing latitudinal gradient was noted for Cu Ratio$_{Adults,Juveniles}$: Vitória (0.59) < Anchieta (0.78) < Farol de São Thomé (0.95). For females, there were two groups: Vitória and Anchieta with Cu concentrations at least 40% higher in juveniles than adults, and Farol de São Thomé with an opposite trend (Figure 3).

Regarding Zn, males showed higher Ratio$_{Adults,Juveniles}$ than females in Vitória and Anchieta, but not in Farol de São Thomé. The Ratio$_{Adults,Juveniles}$ was similar for males among the sampling sites, and for females, there was an increasing latitudinal gradient: Vitória (0.46) < Anchieta (0.61) < Farol de São Thomé (0.95) (Figure 3).

The relationships of Cu and Zn with the shrimps foraging area (measured by $\delta^{13}C$ values) and trophic position (measured by $\delta^{15}N$ values) were stronger in Anchieta compared to the other sampling sites (Table 1). In Anchieta, the concentrations of Cu and Zn decreased with the enrichment of $\delta^{13}C$ and $\delta^{15}N$ (negative slope). The regression models indicated moderate relationships, explaining 51% (Cu) and 60% (Zn) of the relationship with the foraging area in Anchieta (Table 1). In Vitória and Farol de São Thomé, the models explained up to 8% (Table 1). A similar trend was noted for shrimp's trophic position. The mod-

Table 1 – Associations of Cu and Zn concentration normalized by shrimp carapace size (Cu$_{Norm}$ and Zn$_{Norm}$) with foraging area (δ^{13}C) and trophic position (δ^{15}N) in the sampling sites*.

| Elements | Sampling Sites | δ^{13}C | | | | |
| | | Regression Models | | | Slope Comparisons | |
		Equations	R²	p-value	Slope (Confidence Interval)	Multiple comparisons (p-value)
Cu	Vitória	Cu$_{Norm}$ = −0.23·δ^{13}C − 2.59	0.07	0.05	-0.23 (-0.47, 0.008)	Anchieta–Farol de São Thomé = 0.01
	Anchieta	Cu$_{Norm}$ = −0.60·δ^{13}C − 8.46	0.51	< 0.0001	-0.60 (-0.77, -0.43)	Anchieta–Vitória = <0.0001
	Farol de São Thomé	Cu$_{Norm}$ = −0.03·δ^{13}C − 0.10	<0.0001	0.59	-0.03 (-0.15, 0.08)	Farol de São Thomé–Vitória = 0.57
Zn	Vitória	Zn$_{Norm}$ = −0.56·δ^{13}C − 6.80	0.07	0.05	-0.56 (-1.14, 0.002)	Anchieta–Farol de São Thomé = 0.62
	Anchieta	Zn$_{Norm}$ = −0.76·δ^{13}C − 10.09	0.60	< 0.0001	-0.76 (-0.94, -0.58)	Anchieta–Vitória = 0.69
	Farol de São Thomé	Zn$_{Norm}$ = −0.39·δ^{13}C − 5.20	0.08	0.02	0.39 (-0.73, -0.04)	Farol de São Thomé–Vitória = 0.90

| Elements | Fishing Areas | δ^{15}N | | | | |
| | | Regression Models | | | Slope Comparisons | |
		Equations	R²	p-value	Slope (Confidence Interval)	Multiple Comparisons (p-value)
Cu	Vitória	Cu$_{Norm}$ = −0.23·δ^{15}N + 3.77	0.08	0.04	-0.23 (-0.45, -0.01)	Anchieta–Farol de São Thomé = < 0.0001
	Anchieta	Cu$_{Norm}$ = −1.16·δ^{15}N + 14.08	0.33	< 0.0001	-1.16 (-1.65, -0.68)	Anchieta–Vitória = < 0.0001
	Farol de São Thomé	Cu$_{Norm}$ = 0.16·δ^{15}N − 1.36	0.14	0.004	0.16 (0.05, 0.26)	Farol de São Thomé–Vitória = 0.12
Zn	Vitória	Zn$_{Norm}$ = −0.49·δ^{15}N + 8.02	0.06	0.06	-0.49 (-1.03, 0.03)	Anchieta–Farol de São Thomé = 0.005
	Anchieta	Zn$_{Norm}$ = −1.39·δ^{15}N + 17.63	0.34	< 0.0001	-1.39 (-1.95, -0.83)	Anchieta–Vitória = 0.11
	Farol de São Thomé	Zn$_{Norm}$ = 0.33·δ^{15}N − 2.47	0.07	0.04	0.33 (0.007, 0.66)	Farol de São Thomé–Vitória = 0.09

*Regression models were fitted for each element and area. R², p values, and slope comparisons (Lenth, 2019) are shown.
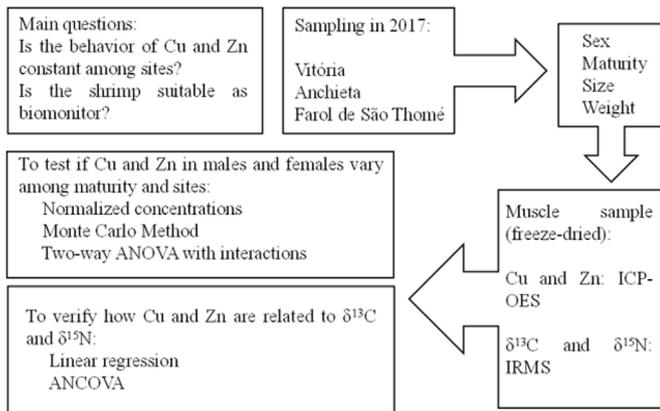
Figure 2 – Flowchart of the methodological steps of this study.

els explained 33% (Cu) and 34% (Zn) in Anchieta, and up to 14% in Vitória and Farol de São Thomé (Table 1).

Discussion

The concentrations of Cu and Zn in the Atlantic seabob shrimp varied between genders and maturity stages among the sampling sites. Indeed, the trace elements pathway in crustaceans may vary between males and females and juveniles and adults, as demonstrated in the pre-
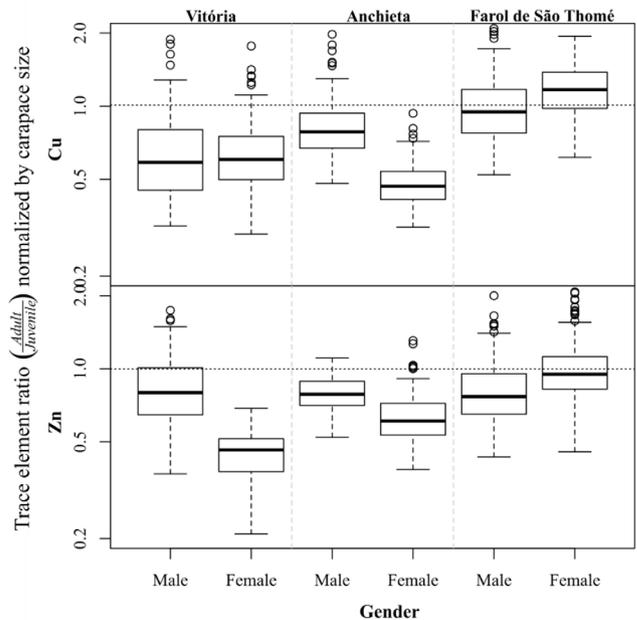
Figure 3 – Ratios of Cu and Zn concentration (adult/juvenile) normalized by shrimps' size among sampling sites and genders. Horizontal dashed lines indicate that trace elements concentration between maturity stages is the same (Ratio$_{Adults,Juveniles}$ = 1). Values > 1.0: higher concentrations in adults (bioaccumulation) and values < 1.0: higher concentrations in juveniles (growth dilution). The distances between the y-axis values were log-transformed.

vious studies (Rainbow, 2002; Pourang et al., 2004; Yilmaz and Yilmaz, 2007); however, a constant behavior of both elements in the shrimp's muscle regardless of the sampling site would be expected. Both variables (gender and maturity stage) were poor predictors for elements concentrations among sampling sites, especially for Cu. For Cu, the comparisons within the same gender indicated both bioaccumulation (Ratio$_{Adults,Juveniles}$ > 1) and growth dilution (Ratio$_{Adults,Juveniles}$ < 1).

Asante et al. (2008) analyzed nine crustacean species and verified that Cu concentrations were lower in higher trophic levels. For the Atlantic seabob shrimp, adult individuals of both genders are in higher trophic position than juveniles, such as demonstrated by Branco and Moritz-Júnior (2001) in the state of Santa Catarina, southern Brazil, and Willems et al. (2016) in Suriname coastal waters. Then, it would be expected lower concentrations of Cu in adult shrimps of our sampling. This was true for Anchieta and Vitória shrimps, but not for Farol de São Thomé. Shrimps caught in Farol de São Thomé showed bioaccumulation of Cu; that is, concentration was higher in adult shrimps. The feeding habits of the Atlantic seabob shrimp stock from Farol de São Thomé could differ from the above pattern, in which adults are in higher trophic position than juveniles; however, there are no data on the species feeding habits in this sampling site for further discussion.

Crustaceans accumulate trace elements in proportion to their bioavailability in the environment, mainly from water (gills breathing) and diet (Rainbow, 2002). The stable isotopes applied in this study are chemical proxies that track trace elements uptake from diet (Fry, 2008; Fry et al., 2016). The relationships between Cu and Zn and shrimps foraging area (δ^{13}C) and trophic position (δ^{15}N) did not show the same trend among sampling sites. In Anchieta, the concentration of trace elements in the shrimp muscle was lower the stronger its association with foraging areas on the seabed (δ^{13}C more enriched) and the higher its trophic position (δ^{15}N more enriched). Since this shrimp species is an omnivorous consumer with high food plasticity (Willems et al., 2016), it is possible that the main food sources responsible for the transfer of Cu and Zn in Anchieta are pelagic, not benthic. In Vitória and Farol de São Thomé, in turn, both foraging area and trophic position had a negligible influence on the concentrations of these trace elements. The negligible influences indicate that both benthic and pelagic food sources contribute to the transfer of these trace elements to the consumer, and that the shrimp trophic position did not drive this transfer. Probably, the shrimps share the foraging area and trophic level in these sampling sites, regardless of their maturity stage.

The relationship between trace elements and stable isotopes to understand the elements trophic pathway is quite variable among marine organisms, often making data interpretation difficult. Asante et al. (2008), for instance, analyzed 22 elements in marine organisms (invertebrates to fish from shallow to deep waters) sampled in China Sea, including Cu and Zn. The relationships between them and δ^{15}N values were negative (Cu) and negligible (Zn), while between them and

δ^{13}C values were positive (Cu) and negligible (Zn). Liu et al. (2019) investigated the relationship between eight trace elements, including Cu and Zn, and carbon and nitrogen stable isotopes in crustaceans, shellfish and fish from Chinese coastal waters, and did not record any significant relationship among them.

Metabolic processes in crustaceans (and other animals) that vary among species, gender, maturity stage, and organs, together with spatial-temporal variations in trace elements availability, influence the elements accumulation (Rainbow, 2002; Pourang et al., 2004). The longevity of the Atlantic seabob shrimp varies from 2 to 3 years (Jardim et al., 2011) and the muscle represents food assimilation (and trace elements accumulation) over the last months, being more consistent than internal organs, such as gills, gonads, and hepatopancreas, to represent the feeding site and elements pathway in a medium-long term (Di Beneditto et al., 2020; Ferreira et al., 2021). The trace elements behavior in the trophic pathway, measured through δ^{13}C and δ^{15}N, can vary among elements and sampling sites, as recorded here. For the Atlantic seabob shrimp, this behavior was intra-specifically variable within same gender and maturity stage, which was an unexpected finding.

The Atlantic seabob shrimp has features of a putatively good biomonitor of trace elements pollution in coastal waters, as reported for other shrimp species in Stentiford and Feist (2005) and Fry et al. (2016). The species is widely and continuously distributed along Western Atlantic Ocean (36°N to 30°S) (FAO, 2018), allowing spatial comparisons in large scale. The species has site-fidelity (Bissaro et al., 2013; Boos et al., 2016), allowing long-term monitoring in the same environment. This shrimp is easy to sample because it lives in coastal waters, and it is found in high abundance because it is a commercial species targeted by fisheries (FAO, 2018). Like all penaeid shrimps, the species has a strong association with the seabed (Boos et al., 2016), which is the marine compartment with the highest accumulation capacity for trace elements (Di Leonardo et al., 2017).

Fry et al. (2016) showed how marine shrimps over a broad range of sampling sites along coastlines of Asia-Pacific countries could serve as biomonitors of emerging anthropogenic pollution trends. Rainbow (2002) highlighted that any meaningful comparison on the concentration of trace elements in aquatic invertebrates should only be done intra-specifically to reach reliable results in monitoring programs. Our comparisons were done within the same species and caught in the same time interval (June–July 2017) in the three sampling sites. Meanwhile, our results showed that during the shrimp development (juvenile vs. adult) both bioaccumulation and growth dilution were recorded, but not in the same way between genders and sampling sites. Variations in trace elements concentration between genders and maturity stages are expected; however, for environmental quality monitoring, the biomonitor or sentinel species should follow the same trend among the sampling sites.

Conclusions

The results showed that the behavior of Cu and Zn in the muscle of the Atlantic seabob shrimp was not constant among Vitória, Anchieta, and Farol de São Thomé, which are fishing sites near port activities in southeast Brazil. Therefore, the utilization of this shrimp species as biomonitor of marine coastal environment near port activities to monitoring the levels of Cu and Zn, elements related to antifouling systems applied in vessels and boats, is not a suitable choice, at least in the spatial scale considered by this study.

The study raised two questions to understand the presence of Cu and Zn in the shrimps (Does the concentration of Cu and Zn in male and female shrimps vary among maturity stages and sampling sites? How is the concentration of Cu and Zn related to shrimps foraging area and/or trophic position?). The answers to both questions did not reveal clear trends about the presence of Cu and Zn in the shrimp's muscle. In the first question, the results showed variations among genders, maturity stages, and sampling sites. Even removing intraspecific bias in bioaccumulation or growth dilution and selecting only individuals from the same gender and maturity stage (e.g., adult males), the comparisons among the sampling sites did not show a clear trend, especially for Cu. Regarding the second question, the results demonstrated that both benthic and pelagic food sources contribute to the transfer of Cu and Zn to the shrimp, and shrimp's trophic position did not drive the transfer of these elements to the individuals.

This study may contribute for future decisions on environmental quality monitoring near port activities. The Atlantic seabob shrimp is an easy sampling species widely distributed in marine coastal waters along the Western Atlantic Ocean and might be one of the first choices for biomonitoring purposes along this area. Meanwhile, the limitations raised in this study must be considered for decisions, since the results showed that the choice is not suitable at all.

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Contribution of authors:

Di Beneditto, A.P.M.: Project administration, Funding acquisition, Conceptualization, Investigation, Writing – original draft, Writing – review and editing; Ferreira, K.A.: Methodology; Oliveira, B.C.V.: Methodology; Rezende, C.E.: Funding acquisition, Writing – review and editing; Pestana, I.A.: Formal analysis, Writing – review and editing.

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