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Trace elements and multibiomarkers in *Sciades herzbergii* (Pisces, Ariidae) for monitoring port areas on the north coast of the Amazon, Brazil

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ABSTRACT

In this study, the objective was to evaluate the effects of trace elements and enzymatic and histological multibiomarkers, on *Sciades herzbergii*, from areas with port influence in the Amazon region, Maranhão. Samples of livers and gills were subjected to histological and enzymatic analyses, physical-chemical parameters of the water and biometric indices were analyzed in two regions, Porto Grande-PG (potentially impacted) and Ilha dos Caranguejos-IC (Conservation Unit). It was observed that fish collected in IC during the rainy season had greater weight and size compared to those from the most impacted area. Histological biomarkers in gills and livers showed higher values in PG, especially in the rainy season. Enzymatic analyzes indicated a significant difference ($p<0.05$) for GST at both times of the year. PCA showed a strong relationship between multibiomarkers and areas and periods. The data found on histological and enzymatic multibiomarkers in fish from port and conservation areas can assist in environmental management and monitoring programs in these areas.

1. Introduction

The increase in port activities is closely associated with human actions that have drastically altered aquatic ecosystems, such as discharges of xenobiotic substances that significantly change resident species (Carvalho-Neta et al., 2012; Moraes Calado et al.2021). In São Marcos Bay, Maranhão, Brazil, the State port complex stands out as the largest interconnection point for the transport of cargo throughout the northern Amazon region and as the second largest port in Latin America. This port region in question, as well as the nearby industrial region, has been associated with several sources of anthropogenic impacts on coastal estuaries (Oliveira et al., 2019).

The main xenobiotic elements recorded in regions with port activities include polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and trace metals (Jesus et al., 2021; Soares et al., 2020). The port is also associated with a considerable increase in other effluents, such as domestic, commercial and agricultural effluents in the surrounding areas (Carvalho-Neta et al., 2014; Yeh et al., 2020). This entire situation has also been worsening naturally, due to the drainage of the Mearim River into the estuary, which increases the transport of xenobiotics (mainly pesticides used in the region), thus contributing to the dispersion of these contaminants, making it difficult to monitor these coastal areas. (Pinheiro-Sousa et al., 2021).

Over the years, the use of biomarkers has increasingly established itself as a viable and useful tool for monitoring the likely impacts of human activities in coastal environments (Gabriel et al., 2020; Noleto et al., 2021; Silva et al., 2020). According to the authors Carvalho-Neta et al. (2014) and Nunes et al. (2020) the most used biomarkers in studies carried out in the port region of São Marcos Bay are catalase (CAT), which is an antioxidant enzyme, and glutathione S-transferase (GST), which plays a significant role in the cellular detoxification process. Furthermore, histological changes, especially gill and liver lesions in fish, have also been investigated and validated as biomarkers (Carvalho-Neta et al., 2014; Nunes et al., 2020; Torres et al., 2023).

The biomarker technique can be applied to different aquatic organisms, such as fish, crustaceans and molluscs. Through this biological

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approach, information is provided that complements the assessment of the specific conditions of the quality of the environment and the health of the individuals who inhabit it (Silva et al., 2020). Monitoring the health of aquatic organisms, especially fish, is of fundamental importance, as they are included in healthy and balanced diets and are considerable sources of proteins, which are essential for maintaining human health (Laso et al., 2022). The species *Sciades herzbergii* (Siluriformes: Ariidae), is well distributed in the northern Amazon region and is an abundant benthic fish on the coast of Maranhão, where it is popularly known as guribu catfish and is part of the diet of the local riverside population (Castro et al.2018). Furthermore, this taxon has been used as a biomonitor species for port environments (Carvalho-Neta et al., 2014; Nunes et al., 2020; Torres et al., 2023). In this context, the present research aimed to evaluate trace elements and enzymatic and histological multibiomarkers in *S. herzbergii* from areas with port influence in the Amazon region (Maranhão coast).

2. Materials and methods

The organisms were collected in two areas in São Marcos Bay - MA. In the first location is the area called Porto Grande (PG) under coordinates 02º39.460′S, 44º21.401′ W, in the São Luís/Maranhão Port Complex. This area is considered most impacted by human activities (Carvalho-Neta et al., 2014; Torres et al., 2023). The second area is on Ilha dos Caranguejos (IC), under coordinates 02∘50.61′ S, 044∘30.614′ W, which is part of a Protected Area. In this work, it is considered the least impacted area (Fig. 1).

In the field, sediments and biological material were collected from 2019 to 2021 in the months of March (rainy) and August (dry), with 19 males and 21 females in IC and 22 males and 18 females in PG. Using a gill net, 80 specimens of *S. herzbergii* were captured, anesthetized with eugenol and euthanized. The following biometric data were measured: total length TL (cm), standard length SL (cm), furcal length FL (cm) and total weight TW (g). They were then dissected to remove the gill and liver organs, for enzymatic analysis (glutathione S-transferase - GST and catalase - CAT) and for histological analysis. All experimental and handling procedures were carried out in accordance with regulatory bioethical requirements and approved by the Research Ethics Committee of the State University of Maranhão (protocol nº 01/2018 CRMV-MA). The study protocol follows the guidelines of the Brazilian Agency Faculty of Animal Experimentation (COBEA, 2015). The environmental authorization (nº 09/2019) was obtained from the State Secretariat for the Environment and Natural Resources.

To obtain physical-chemical data from the water and sediments, the collections were carried out in parallel with the fish captures. In the physical-chemical analysis, the following parameters were evaluated: temperature (ºC), pH, dissolved oxygen (mg/L), and salinity (mg/L) with multiparameter equipment (HI 9829 M, Hanna Instruments, Woonsocket, RI, USA; sensor resolution: temperature (0.1 ºC), pH (0.01), dissolved oxygen (0.1 mg/L) and salinity (0.01–0.1 ppt).

For the analysis of bottom sediments, the samples were dried in an oven at 40 °C, crushed and subsequently stored in polyethylene bags. In duplicate, 1.0 g of the dry weight sample was used for the determination of trace elements after acid digestion with HNO_3 (65 %) and HCl (37 %) in a digester block at 80 °C, followed by neutralization with 15 mL of 0.1 NHCl. Trace element levels: aluminum (Al), arsenic (As), barium (Ba),

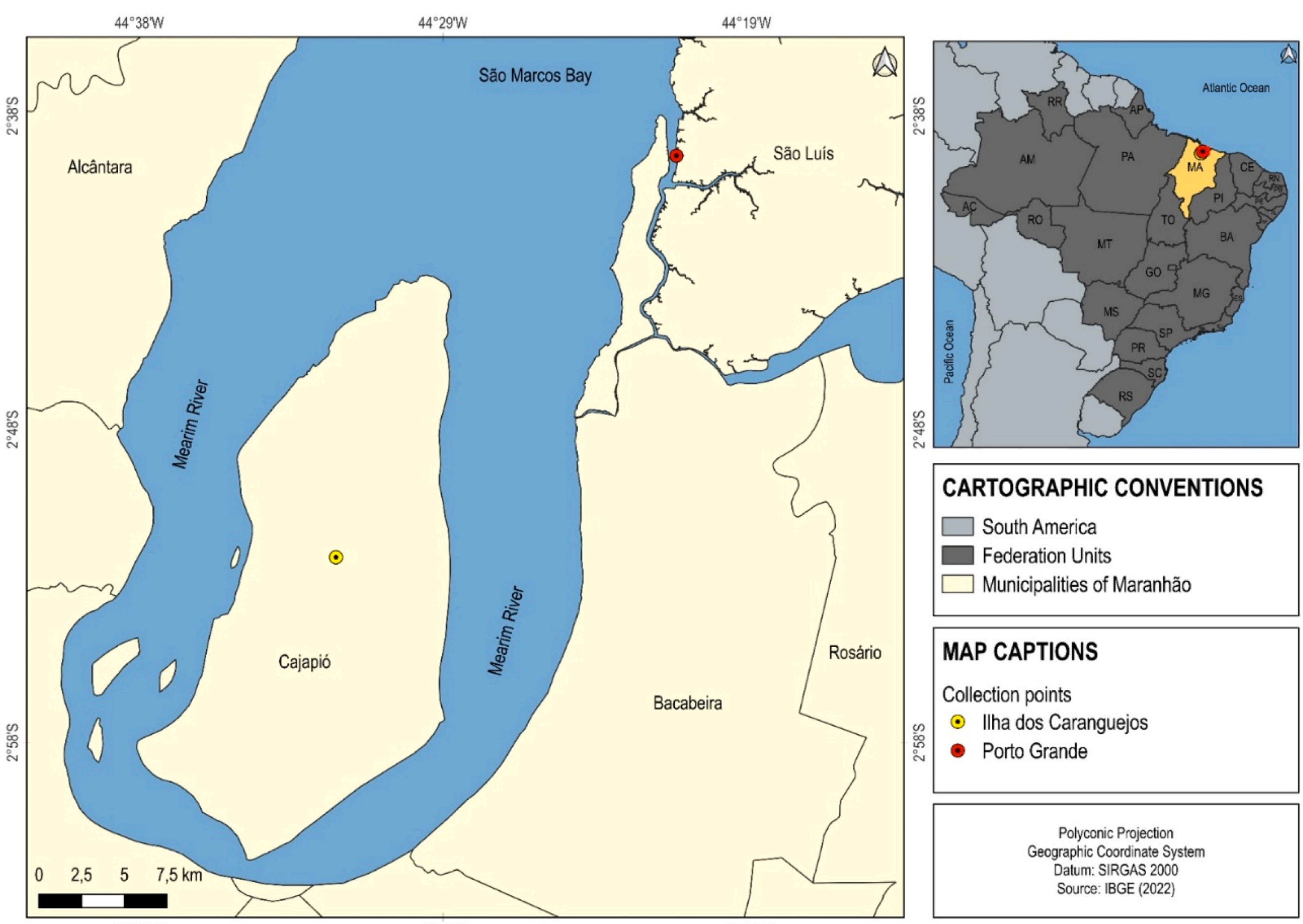

Fig. 1. Location map of *S. herzbergii* sampling points in São Marcos Bay, Maranhão State, Brazil: port site (Porto Grande) and reference site (Ilha dos Caranguejos).

cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), strontium (Sr), vanadium (V), and zinc (Zn) were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) using an Optima 8300 system (PerkinElmer, USA; wavelength range: 165–800 nm, resolution: typically < 0.009 nm to 200 nm, detection limit: in the range of 1–10 parts per billion (ppb) and spectral bandwidth: typically 0.007 nm). According to the method proposed by Malm et al. (1989) and Bastos et al. (1998) the determination of total mercury (Hg) was carried out, where 500 mg of sediment (dry weight) were added to a solution containing HCl/HNO_3 (3:1) (Merck) and 5 % (m/v) of $KMnO_4$. (Merck). After the process in a digester block for 60 min at 60 °C, the samples were titrated with 12 % hydroxylamine hydrochloride solution and 12 mL of ultrapure water were added.

Total mercury quantification was conducted using cold vapor atomic absorption spectroscopy, using PerkinElmer's Flow Mercury 400 injection system. High analytical purity reagents (PA, Merck) and certified sediment standards (SS2 SSP-SCIENCE) were employed to ensure quality control. Ultrapure water (Milli-Q Plus, Millipore) was used to prepare the solutions. The glassware used was immersed in 5 % (v/v) HNO3 (Merck) for 24 hours and subsequently rinsed with ultrapure water. Average trace element recovery rates ranged from 80 % to 120 %. For the analysis of Polycyclic Aromatic Hydrocarbons-PAHs, sediment samples were preserved at −80 °C and processed according to US EPA 3550 C/8270D protocols.

For histological processing, samples of fish gills and livers were preserved in 10 % formalin for 24 hours. Then the gill arches were decalcified for 24 hours in a 10 % nitric acid solution. Liver and gill samples were decalcified and dehydrated through an increasing alcohol gradient, diaphanized in xylene and embedded in paraffin. Transverse sections approximately 5 μm thick were obtained and stained with hematoxylin and eosin. The histological slides were analyzed under an optical microscope (Carl Zeiss, Oberkochen, Germany) and photomicrographed under a microscope with a camera (Olympus BX51). The histological slides were analyzed under an optical microscope (Carl Zeiss, Oberkochen, Germany) and photomicrographed under a microscope with a camera (Olympus BX51). The presence of histological changes for each organ was evaluated semi-quantitatively, according to the classification established by Bernet et al., (1999), in which each change in the investigated organ is categorized into one of five reaction patterns: circulatory disorders, regressive changes, progressive changes, inflammation and tumor. Each of these reaction patterns encompasses several histological changes related to the organ under analysis. An importance factor (w) was assigned to each change, ranging from 1 to 3, where 1 = minimal pathological importance (easily reversible lesions), 2 = moderate pathological importance (reversible lesions in most cases), and 3 = pathological importance severe (generally irreversible injuries). Healthy individuals will be considered those whose sample analyzed shows integrity and tissue organization, and the absence of signs of alterations.

For the analysis of GST and CAT activity, 1.0 g samples of *S. herzbergii* gills and livers were weighed on a precision balance and added to buffer (50 mM Tris-HCl, 0.15 M KCl, pH 7,4) in a sample/solution ratio of 1:4. The material was centrifuged at 9000 x g and 4°C for 30 min. The supernatant obtained was centrifuged at 37,000 × g and 4 °C for 60 min to separate the cytosolic fraction, which was used to analyze the activity of the two enzymes. GST activity was measured as the increase in absorbance at 340 nm and 25 °C, according to the method of Keen et al. (1976), using reduced glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB) as substrates. The method is based on the reaction between CDNB and the –SH group of GSH catalyzed by GST contained in the samples. GST activity is expressed as micromoles per minute per milligram of protein (μmol/min/mg protein). CAT activity was assessed at 240 nm by measuring the decomposition rate of hydrogen peroxide (H_2O_2), as described by Ventura et al. (2002) and Tagliari et al. (2004). CAT activity was expressed as enzymatic units (U)/mg of protein. Protein concentrations in the supernatant were also determined according to the modified method of Jerome et al. (2017), using a commercial biuret kit and bovine serum albumin as standard.

3. Statistical analysis

Physicochemical and biometric data were tested for normality using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Subsequently, physicochemical data were analyzed using ANOVA, while biometric data were analyzed using the Kruskal-Wallis test. Both tests were carried out using the GraphPad Prism 5.0 program to check whether there were significant differences between locations and collection periods. For histological biomarker data, a frequency of changes identified for the two organs (gills and liver) was calculated and the mean and standard deviation were applied. The enzymatic data (CAT and GST) from gills and livers from both areas (IC and PG) and periods (dry and rainy) passed the Shapiro-Wilk normality test and Levene's homogeneity test. The transformation (log 10) of data that did not present a normal distribution was performed. Next, ANOVA was applied and when there was a significant difference ($p<0.05$) between the means, the Tukey test was applied to determine which groups had statistically different means. The set of data used in the Principal Component Analysis (PCA) considered 20 samples and 11 variables that were used to evaluate the proximity of the enzymatic biomarkers variables (GST and CAT), histological (gill changes) in relation to seasonality (rainy and dry) and areas (IC and PG). The data were analyzed in MatLab (Mathworks Laboratory) version 2018, and the PCA toolbox version 1.5 was used for principal component analysis simulation. The ideal number of PCA used in the analysis was estimated (Kaiser, 1958).

4. Results

Most seawater abiotic parameters, presented in Table 1, demonstrated variation between different areas and collection periods. Specifically, salinity was the factor that varied the most. However, it was not possible to identify statistically significant differences ($p > 0.05$).

The individuals (males and females) collected on IC during the rainy season showed higher total length and total weight values compared to organisms from the most impacted area in PG (Table 2); however, there was no statistically significant difference ($p > 0.05$).

Sediment analysis (Table 3) showed that the majority of trace elements researched are in compliance with limits established by Brazilian environmental legislation (CONAMA nº 454/2012). However, it is important to highlight that some elements analyzed do not contain limits defined in this legislation. Among these, Al, Fe, Mn and Sr presented the highest concentrations in the sediment and indicated a statistically significant difference ($p < 0.05$) between collection sites and between seasonal periods.

In this study, the following changes were identified in *S. herzbergii*: branchial - Aneurysm (gANR), necrosis (gNC), displacement of the epithelium (gDE), lamellar fusion (gLF), congestion (gCG), hyperplasia

Table 1
Physicochemical parameters of the water, obtained in the two areas sampled in São Marcos Bay, Brazil.

	IC		PG		
Parameters	Rainy	Dry	Rainy	Dry	CONAMA 357/2005[a]
pH	7.46	7.18	7.34	6.64	5–9
Dissolved oxygen (mg/L)	4.90	7.63	6.20	5.70	not less than 3 mg L^{-1}
Temperature (°C)	29.10	29.00	29.10	29.20	28–32 (ºC)
Salinity (mg/L)	13.10	29.00	25.20	32.50	0.5–30 ‰

IC =Ilha dos Caranguejos; PG = Porto Grande. [a]Limits established by the National Environmental Council
(CONAMA) in Resolution No. 357 of 2005 for class 3 brackish Waters.

Table 2
Morphometric data of *S. herzbergii* in São Marcos Bay, Brazil.

Biometric Parameters		IC		PG	
		Rainy	Dry	Rainy	Dry
TW	M	283.14 ± 83.12	196.33 ± 48.87	176.25 ± 22.69	282.67 ± 10.50
	F	350.67 ± 107.64	202.25 ± 112.05	259.67 ± 210.31	274.29 ± 89.84
TL	M	12.96 ± 1.17	11.58 ± 1.00	11.78 ± 0.45	12.65 ± 0.15
	F	13.47 ± 1.04	11.55 ± 2.05	12.22 ± 2.39	12.26 ± 1.33
SL	M	10.47 ± 0.81	9.53 ± 1.00	8.98 ± 0.53	10.65 ± 0.35
	F	10.90 ± 1.35	9.45 ± 1.84	9.48 ± 1.97	10.44 ± 0.94
FL	M	11.37 ± 0.91	10.17 ± 0.92	10.1 ± 0.56	11.45 ± 0.05
	F	11.90 ± 1.04	10.00 ± 1.79	10.63 ± 2.22	10.74 ± 1.27

IC =Ilha dos Caranguejos; PG = Porto Grande

Table 3
Levels of trace elements in sediments collected at two points located in São Marcos Bay, Brazil.

Trace elements	IC		PG		CONAMA 454/2012 (mg kg^{-1})[a]	
	Rainy	Dry	Rainy	Dry	L1	L2
Al	10,076.31 ± 41.75*a	11,949.47 ± 818.33*	10,252.33 ± 739.50*b	12,365.58 ± 2593.15*	-	
As	6.05 ± 0.06	7. 01 ± 0.52	5.60 ± 0.08	7. 30 ± 0.10	19–70	
Ba	21.95 ± 0.19	16.54 ± 0.57	15. 83 ± 0.41	18.92 ± 1.66	-	
Cd	0. 19 ± 0.00	0.23 ± 0.00	0.18 ± 0.01	0.23 ± 0.01	1.2–7.2	
Co	4. 84 ± 0.04	3. 49 ± 0.03	3. 76 ± 0.12	3. 90 ± 0.35	-	
Cr	27.65 ± 0.51	24. 46 ± 0.39	22.79 ± 0.64	26.74 ± 2.21	81–370	
Cu	7.95 ± 0.09	7. 50 ± 0.03	7. 57 ± 0.16	8. 14 ± 0.07	34–270	
Fe	20,611.10 ± 80.30*	21,481.52 ± 677,14*	19,381.87 ± 877,96*	21,764.62 ± 2016,63*	-	
Hg	0.0260 ± 0.0011	0.0314 ± 0.0028	0.0292 ± 0.0032	0.0305 ± 0.0035	0.3–1.0	
Mn	338.25 ± 0.86*	308. 63 ± 4.73*	125. 06 ± 6.25*	301.10 ± 44.93*	-	
Ni	9. 54 ± 0.11	7. 40 ± 0.19	7.26 ± 0.20	8. 12 ± 0.56	20.9 – 51.6	
Pb	6. 60 ± 0.13	5. 06 ± 0.15	5. 15 ± 0.13	5. 74 ± 0.22	46.7–218	
Sr	113. 43 ± 0.79*	363. 81 ± 4,77*	222. 12 ± 6. 59*	281. 32 ± 12. 58*	-	
V	21. 25 ± 0.04	21. 46 ± 0.70	20. 26 ± 0.74	23. 57 ± 1. 27	-	
Zn	18. 62 ± 0.11	13.88 ± 0.62	14.76 ± 0,22	16. 27 ± 0.56	150–410	

IC = Ilha dos Caranguejos; PG = Porto Grande, [a]Limits established by the National Council for the Environment (CONAMA) in Resolution No. 454 of 2012 for sediments in the waterbed of level 1 (saline water) and level 2 (brackish water). * Indicates statistical seasonal differences between the periods (rainy and dry) ($p < 0.05$).

(gHYP), bleeding (gBLE), tissue degeneration (gTD) and lamellar narrowing (gLN). The greatest occurrence of these changes in PG were observed in both seasonal periods. Liver - Fibrosis (FBl), necrosis (lNC), melanomacrophage center (lMC), encapsulated amorphous material (lEAM), vacuolation of hepatocytes (lVH), bleeding (lBLE), steatosis (lST), hemocytic infiltration (lHI), differentiating cells (lDC) and tissue degeneration (lTD). The high average occurrence of these changes occurred during the rainy season in PG.

The analysis of enzymatic biomarkers in the gills of *S. herzbergii* for CAT showed no significant difference, but the highest average value occurred in PG, in the rainy season (Fig. 2a). The GST activity of the IC gills differed significantly from that of the PG in the same period ($p= 0.0101$), and in the PG the average value was higher than in the IC (Fig. 2b). In the liver, CAT activity differed significantly between periods and areas ($p=0.0338$), since GP results were low in both periods (Fig. 2c). For GST, the enzymatic activity in the liver also showed a significant difference ($p= 0.0091$) in IC in the rainy season and in PG in the dry season (Fig. 2d).

Fig. 3a and b explain the similarity of 48 % of the data, that is, this means the similarity that the data on multibiomarkers, enzymatic activities (CAT and GST), histological changes (gill and liver) are related between the periods of the year (rainy and drought) and study areas. The rPG samples (Fig. 3a) correspond to the enzymatic biomarkers GST and CAT (Fig. 3b), indicating that in most samples there are high values (above normal, according to the scores on the graph) of these activities in PG individuals during the rainy season. In relation to histological biomarkers in the gills of *S. herzbergii*, the change in gANR presents a higher value (above normal, according to the scores on the graph) in individuals of fish collected at dPG. It is also possible to verify the relationship of this change (gANR) with the activities of CAT and GST, as they form clusters. In most dPG fish samples there are very high gHYP values (above normal, according to the scores on the graph). The highest gLF value is found in RIC (above normal, according to the scores on the graph). From the Loads graph (Fig. 3b) we can observe that the gTD index is contrary to the CAT, which means that in the samples, the higher the CAT, the lower the gTD and vice versa. Something similar is evident between gTD and GST, but in fewer samples.

In Fig. 3c and d, the two PCAs explain approximately 46 % of the data. Through the charge graph (d) it is evident that no sample presented encapsulated amorphous changes (lEAM). There are higher CAT values (above normal, according to the scores on the graph) in dIC. Furthermore, lTD and lST are opposites, which means that when one increases the other decreases and vice versa. The same analysis is similar for CAT with lHI and IVH with lNC. lST and lHI are more common in samples from Porto Grande during the rainy season. The IFB presents high values (above normal, according to the scores on the graph) of PG during the drought.

rPG = Porto Grande rainy, dPG = Porto Grande dry, rIC = Ilha dos Caranguejos rainy, dIC= Ilha dos Caranguejos dry.

5. Discussion

Among the physical-chemical parameters of the water recorded in two areas of São Marcos Bay, only the salinity in the Porto Grande area, in the dry period, was in disagreement with the standards of current environmental legislation (for brackish waters class 3, CONAMA 357/2005). High salinity can affect fish health, generating everything from changes in osmotic balance to physiological and metabolic stress (Christensen et al., 2019), resulting in the production of a large number of reactive oxygen species. This disturbance affects the antioxidant capacity, which leads to oxidative stress, negatively influencing the health of animals (Jiang et al., 2022). The high salinity value may be due to the significant decrease in water volume due to the lack of rain, typical of this period. Cavalcanti et al. (2018) also found a similar situation during the dry period in the same region and associated it with the decrease in river flow (from the Mearim and Pindaré Rivers) and the influence of marine waters in São Marcos Bay, due to the decrease in aspect ratio (area where freshwater and saltwater mix) determined by the variation of the free surface and the movement of surface gravitational waves. Macedo et al. (2024) recorded temperature, salinity and pH values in

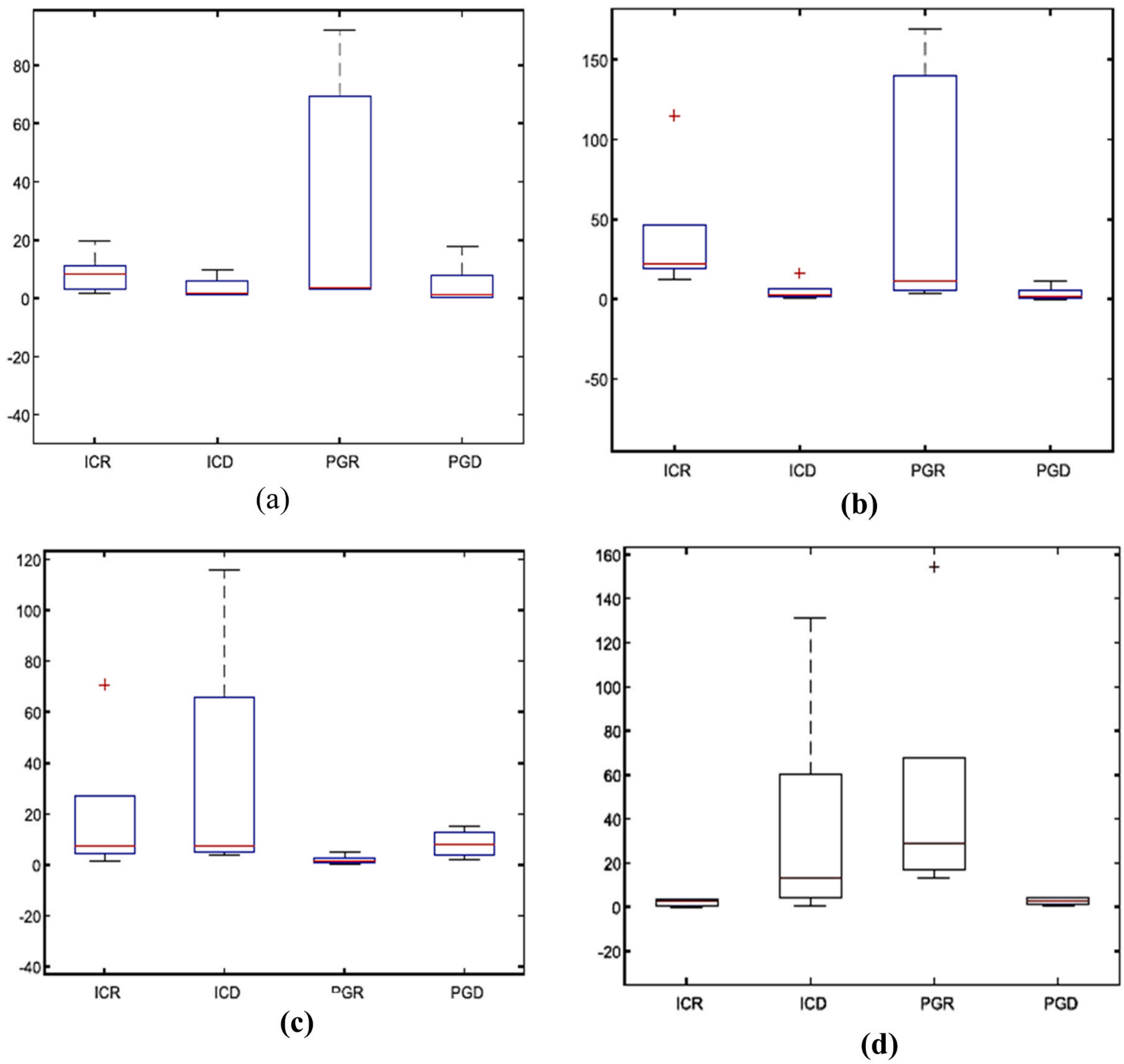

Fig. 2. Statistical analysis of enzymatic multibiomarkers in gills and liver of *S. herzbergii* in the study areas and periods of the year (Rainy and Dry). a) CAT in gills. b) GST in gills; c) CAT in liver; and d) GST in liver.

two locations in the São Marcos Estuarine Complex, within regulatory limits, except for dissolved oxygen levels, which in the Porto Grande location, especially in the dry season, were below the standard of environmental legislation.

Most of the trace elements in the sediments investigated in this research were in accordance with the levels recommended by Brazilian regulatory standards. However, some trace elements (Al, Fe, Mn and Sr) showed differences between the sampled locations. Previous studies in the same region report the presence of trace elements in various environmental matrices. Studies by Montes et al. (2023) evaluated 352 specimens of *S. herzbergii* in the tidal channels of Irinema and Buenos Aires (São Marcos Bay) and in the Caeté estuary (Pará) and found a condition factor of low value, with the highest concentrations of Al, Cd and Hg in muscle of this species at the first study site. While studies by Nunes et al. (2020) found a higher concentration of Fe in the muscles of fish caught in São Marcos Bay and a higher concentration of Al in the sediment (46,645.4 ± 8815.1 μg g^{-1}) than in the present study. Oliveira et al. (2023) recorded concentrations of zinc and manganese in the waters, along the same Bay, above the value established by current Brazilian legislation. The presence of high levels of metals in the estuarine environment can amplify the toxic effects on organisms, as they interfere with osmoregulation processes and can also negatively affect gas exchange (Nunes et al., 2020).

The fish collected in the rainy season were larger and heavier on Ilha dos Caranguejos. This suggests that less impacted locations may provide more favorable conditions for fish growth, resulting in more robust individuals compared to more impacted areas. The results of the present study were similar to those obtained by Torres et al. (2023) who evaluated the same species in the same region. Pinheiro-Sousa et al. (2021) also found males and females of *S. herzbergii* from this same area, with greater total weight. Ribeiro et al. (2023) found that although the fish in the port area were longer, the specimens collected in the less impacted area were heavier, which was considered consistent with the values obtained for the condition factor and the gonadosomatic index.

The use of biomarkers is a strong methodology capable of identifying a biological response in a specific organ of animals subjected to pressure by toxic agents in the environment (Carvalho-Neta et al., 2014). In this study, different biomarkers were analyzed in which it was possible to identify biological responses at a physiological (enzymatic changes) and morphological level (gill and liver changes) in gills and livers of fish captured in different regions, one of them under strong anthropic influence, and next to industrial and port enterprises (PG) and the other influenced by subsistence activities of fishermen and crab collectors (IC).

To better understand the impacts of these different environments, it was necessary to classify the histological lesions observed and assess

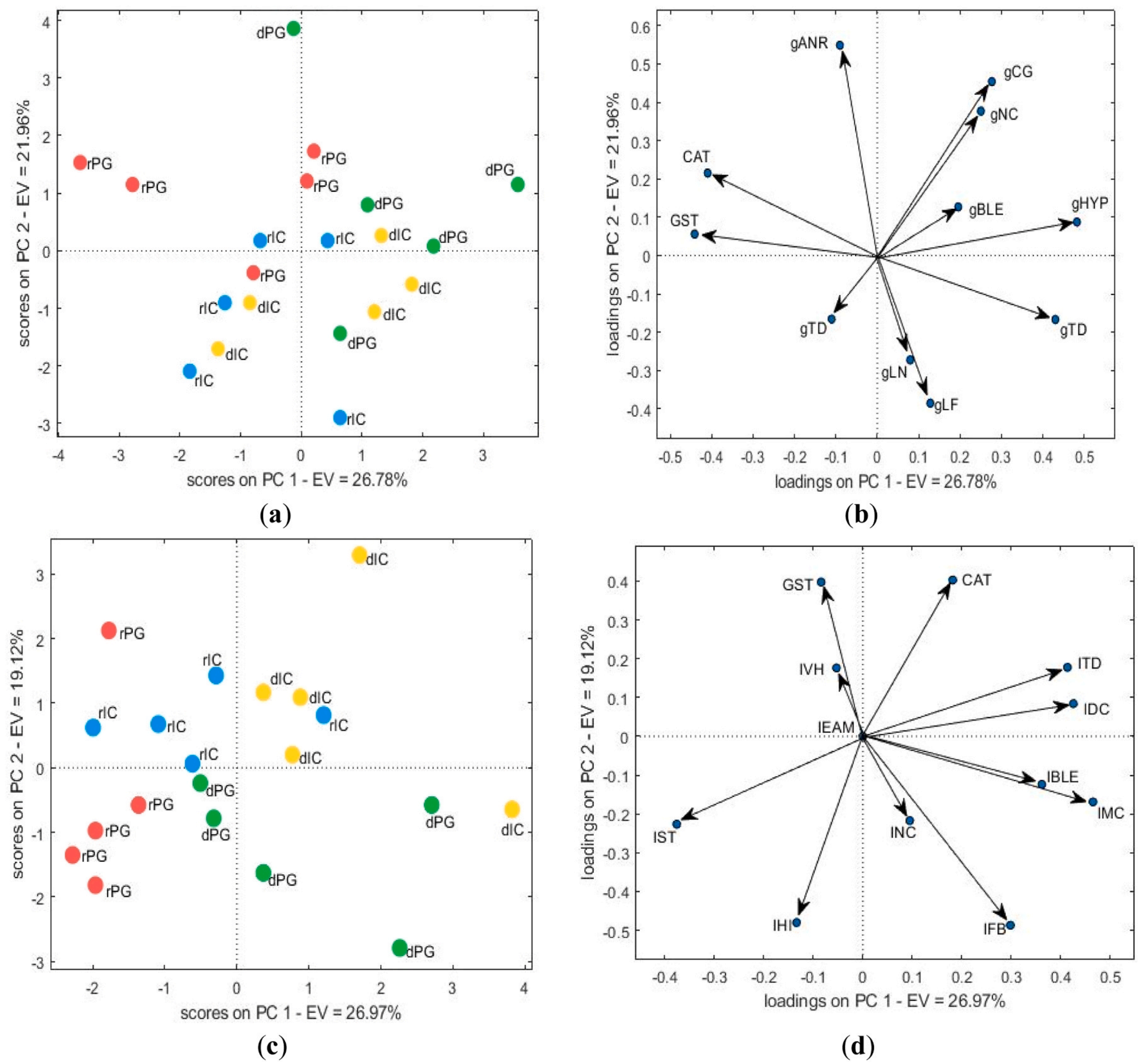

Fig. 3. Multivariate analyzes of biomarkers in gills of *S. herzbergii* from IC and PG during the rainy and dry periods, 2021. a) score graph showing study areas and seasonality. b) loading graph showing enzymatic and histological biomarkers in gills. c) score graph showing study areas and seasonality. d) loading graph showing enzymatic and histological biomarkers in the liver.

their severity according to the criteria established by Bernet et al. (1999). The reaction patterns identified for gill lesions included circulatory disorders (imbalances in the blood and interstitial fluid), regressive changes (capable of reducing the function of the organ) and progressive changes (processes that lead to an increase in the activity of cells or tissues). In addition to the three reaction patterns mentioned, liver injuries also presented inflammation, characterized by inflammatory processes that are often related to other reaction patterns, as described by Bernet et al. (1999).

The pathological relevance of the branchial and hepatic changes found in the specimens in both areas ranged from mild to severe. Mild changes are usually easily reversible, while moderate changes are largely reversible in most cases as long as the source of stress is eliminated. However, accentuated changes are the most serious and are therefore irreversible and can result in substantial damage and even complete or partial loss of organ function (Bernet et al., 1999).

Different changes in the gills were evident in the two study areas in both periods of the year (rainy and dry). However, aneurysm (mild severity) was the most frequent response in *S. herzbergii* in both study areas, in addition, it presented a strong relationship in the PCA analysis, demonstrating that this change is present in this organ, especially during the dry period in PG. These responses indicate that fish are suffering impacts resulting from anthropogenic port and industrial activities, as the presence of trace elements and PAHs have already been identified in these areas (Pinheiro Sousa et al., 2021, Macedo et al., 2024).

On the other hand, necrosis (severe severity) was the most recurrent biological response in the livers of specimens captured in both study areas. According to Manahan (1991), the high occurrence of necrosis in different degrees of severity may be related to high concentrations of xenobiotics during the detoxification process. The occurrence of necrosis can also be a consequence of enzyme inhibition, damage to the integrity of the cell membrane and disturbances in the synthesis of protein and carbohydrate metabolism (Wolf and Wheeler, 2018).

Antioxidant enzymes are the first in the cell's line of defense against oxidative damage (Sturve et al., 2006). CAT functions in the cell to eliminate reactive oxygen species (Santana et al., 2018). Regarding the enzymatic activities in this study, CAT in the gills of *S. herzbergii* did not show a significant difference, although it showed a higher average value of this activity in the rainy season in both areas.

GST also showed high activity in the rainy season in PG. These data corroborate those found by Torres et al. (2023), for this same species in the same region. This result may be a consequence of the greater interaction of fish with contaminating substances from transport promoted by the increase in water flow during this period. In a study carried out on the quality of rainwater in Natal (Rio Grande do Norte) Righetto et al. (2017) found that surface runoff has great potential to pollute water bodies by encountering high pollutant concentration rates at different rainfall intensities.

In the livers of the fish analyzed, it was observed that the highest CAT values were recorded mainly in the IC in the dry period. These data are different from those obtained by Pinheiro Sousa et al. (2021) who observed high values of this enzyme in specimens from the rainy season in PG in 2018 and 2019. GST presented higher values in PG fish in the rainy season, that is, these data indicate that seasonality may be directly influencing the biological responses in these organisms, regardless of the collection area. When comparing the results of statistical analyzes of CAT and GST enzymes in gills through PCA, we noticed that these analyzes have a strong relationship, as in PCA CAT and GST are grouped and related to PG in the dry period. In livers, CAT and GST are close to the dIC, that is, these data are strongly related to each other.

6. Conclusion

Based on multibiomarker assessment, the histological changes in the gills and liver observed in *S. herzbergii* specimens are more susceptible in individuals found in the potentially impacted region. In relation to enzymatic multibiomarkers, the highest levels of oxidative stress and metabolic activity occur mainly in individuals collected in the most impacted area (Porto Grande) during the rainy season. Sediment analysis demonstrated that the majority of trace elements identified in the study areas in both periods of the year are in accordance with available regulations. although they present similar values in both areas. On the other hand, the values of Mn and Sr were different in the two areas of study. Therefore, studies using histological and enzymatic multibiomarkers in fish caught in port and environmental conservation areas are important to understand the dynamics of trace elements in aquatic environments and their impact on aquatic biota. Therefore, environmental monitoring in these areas is relevant for creating management and conservation programs in regions impacted by port projects.

Ethics approval

Ethical approval was obtained by the Research Ethics Committee of the State University of Maranhão (protocol nº 01/2018 CRMV-MA)

Consent to participate

The manuscript has been read and approved by all listed authors.

Consent for publication

All authors are informed and agree to the study.

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CRediT authorship contribution statement

HETTY SALVINO TORRES: Writing – review & editing, Writing – original draft, Software, Methodology, Conceptualization. **Eliane Braga Ribeiro:** Writing – original draft, Methodology. **Wanda Batista de Jesus:** Writing – original draft, Methodology. **Raimundo Nonato Diniz Costa Filho:** Methodology, Formal analysis. **Débora Batista Pinheiro-Sousa:** Writing – review & editing, Methodology. **Raimunda Nonata Fortes Carvalho Neta:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request. The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

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