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Original Article

# Impacted estuaries on the Brazilian Amazon coast near port regions influence histological and enzymatic changes in *Sciades herzbergii* (Ariidae, Bloch, 1794)

## Estuários impactados na costa amazônica brasileira próximos a regiões portuárias influenciam mudanças histológicas e enzimáticas em *Sciades herzbergii* (Ariidae, Bloch, 1794)

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**Abstract**

Enzymatic (glutathione S-transferase, GST and catalase, CAT) and histological biomarkers in *S. herzbergii* are important for the analysis of impacted estuaries in port regions of the Brazilian Amazon coast. Fish specimens were collected in two areas in the rainy and dry seasons: Porto Grande (potentially impacted region) and Ilha dos Caranguejos (less impacted region). Sediment samples were collected for chemical analysis. Morphometric, histological, and enzymatic biomarker analyzes were performed. The analysis of the sediments collected in the potentially impacted region showed levels of iron, aluminum and polycyclic aromatic hydrocarbons above the limits allowed by CONAMA legislation. Histological changes in the gills and liver, as well as GST and CAT activities, were high in fish collected at the port. Analyzes suggest that fish in the potentially impacted region are subject to pollutants that compromise their health.

**Keywords:** ecotoxicology, catfish, histology, organ, pollution.

**Resumo**

Biomarcadores enzimáticos (glutationa S-transferase, GST e catalase, CAT) e histológicos em *S. herzbergii* são importantes para a análise de estuários impactados em regiões portuárias da costa amazônica brasileira. Espécimes de peixes foram coletados em duas áreas nas estações chuvosa e seca: Porto Grande (região potencialmente impactada) e Ilha dos Caranguejos (região menos impactada). Amostras de sedimento foram coletadas para análise química. Análises morfométricas, histológicas e de biomarcadores enzimáticos foram realizadas. A análise dos sedimentos coletados na região potencialmente impactada mostrou teores de ferro, alumínio e hidrocarbonetos aromáticos policíclicos acima dos limites permitidos pela legislação do CONAMA. As alterações histológicas nas brânquias e no fígado, bem como as atividades de GST e CAT, foram elevadas nos peixes coletados no porto. As análises sugerem que os peixes da região potencialmente impactada estão sujeitos a poluentes que comprometem sua saúde.

**Palavras-chave:** ecotoxicologia, bagre, histologia, órgão, poluição.

## 1. Introduction

In Maranhão, the São Marcos Bay estuarine region is subject to anthropogenic impacts resulting from port, domestic, and agricultural activities that generate chemical pollutants, contaminating water bodies, sediments, and aquatic biota. Such effects are mainly seen in typical species of the mangrove ecosystem (Oliveira et al., 2019), necessitating rigorous investigation of environmental impacts in the region (Carvalho Neta et al., 2012). The port complex is a very important undertaking for Maranhão State, ranking among the largest ports for large cargo ships (Pinheiro-Sousa et al., 2022).

Biomarkers are any biological change in organisms that predict in advance the effects of pollutants on molecules, cells, tissues, physiology and behavior of animals in the face of environmental stress (Walker et al., 2010). Biomonitoring in regions that concentrate industrial and port enterprises is essential to reduce anthropogenic impacts, especially environmental impacts caused by

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heavy metals and polycyclic aromatic hydrocarbons (PAHs), which are highly deleterious to aquatic biota in estuarine regions (Pinheiro-Sousa et al., 2022).

In coastal systems, estuaries are ecosystems of high biological and socioeconomic importance inhabited by different populations of aquatic organisms, such as crustaceans, fish, and mollusks (Rodrigues et al., 2016). Some fish species are particularly important to local economy and biodiversity, such as the catfish species *Sciades herzbergii* - Bloch, 1794 and *Bagre bagre* - Linnaeus, 1766 (Castro et al., 2018). The fish known as "guribu" catfish (*S. herzbergii*) is a species found in several water bodies in Maranhão and is commonly captured in artisanal fisheries and consumed by the Maranhão population, having a great commercial value. *S. herzbergii* belongs to the Ariidae family and occurs in tropical and subtropical coastal zones, in marine, estuarine and freshwater environments, being generally abundant in coastal waters with muddy and shallow bottoms (Araújo, 1988; Soares et al., 2020; Pinheiro-Sousa et al., 2022). Studies indicate that fish can bioaccumulate high levels of pollutants present in the environment. Fish easily accumulate pollutants present in contaminated environments, such as via the food chain, feeding on other organisms, as well as being in direct contact with contaminated water (Pinheiro-Sousa et al., 2022).

*S. herzbergii* is considered an excellent model organism for the assessment of protected and impacted areas, serving as a bioindicator of environmental pollution, given its ability to respond to xenobiotics (Carvalho Neta et al., 2012). It is also worth mentioning that this estuarine species is an abundant resource of great relevance for artisanal fisheries in Maranhão, found in legally protected areas as well as in areas historically impacted by heavy metals and PAHs, such as the port complex (Carvalho Neta et al., 2009).

Analysis of histological and enzymatic biomarkers in *S. herzbergii* is an important tool for identification of anthropogenic impacts on the fish assemblage of São Marcos Bay (Sousa et al., 2013; Pinheiro-Sousa et al., 2022).

Previous studies assessed histological and enzymatic biomarkers in fish gills and liver to understand environmental impacts on fish populations (Pinheiro-Sousa et al., 2022). The gills are in permanent contact with the environment. Therefore, alterations in this organ serve as an early warning of the presence of environmental pollutants. It is also important to consider that gill alterations may cause metabolic imbalance and compromise the survival and performance of fish (Saleh and Marie, 2016). In fish, the liver has digestive and secretory functions and participates in the biotransformation and excretion of xenobiotics, being responsible for detoxification of the organism (Matos et al., 2007).

Studies on organ alterations provide punctual information that may reveal potential harm to animal health (Pinheiro-Sousa et al., 2022). A great advantage of using fish histological and enzymatic biomarkers in environmental monitoring is that these parameters allow examining target organs, including the gills and liver, which are responsible for vital functions such as respiration and excretion as well as accumulation and biotransformation of xenobiotics (Soares et al., 2020). Thus, biomarkers are important methodologies to measure the biological responses that fish are submitted.

This study aimed to analyze histological (gill and liver) and enzymatic (glutathione *S*-transferase, GST, and catalase, CAT) biomarkers in *S. herzbergii* from estuaries near the port complex of São Luís, Maranhão, Brazilian Amazon coast.

## 2. Materials and Methods

### *2.1. Study area*

Fish specimens (*S. herzbergii*) were captured at two sites in São Marcos Bay (Figure 1). The first site is located near the Alumar/Alcoa port of Porto Grande (PG - 02º39.460′ S, 44º21.401′ W), an area considered potentially impacted by anthropogenic activities. The other site is located on Ilha dos Caranguejos (IC 02 ◦ 50.61′ S, 044 ◦ 30.614′ W) in a State Environmental Protection Area. IC is less subject to anthropogenic activities and was therefore used as a reference site in the current study (Figure 1).

### *2.2. Sampling of S. herzbergii*

Fish specimens were captured by using a gillnet with the help of local fishers in March ($n = 20$) and July ($n = 20$) 2019, corresponding to the rainy and dry seasons, respectively. In loco, specimens were processed to collect biological material such as gills and liver for enzymatic analysis (GST and CAT) and for histology analysis. Biometric data (measurements of the size and weight of each specimen) was also recorded. All experimental and handling procedures were performed in accordance with regulatory bioethical requirements and were approved by the Research Ethics Committee at Maranhão State University (protocol No. 01/2018 CRMV-MA). The study protocol follows the guidelines of the Brazilian College of Animal Experimentation (COBEA, 2015). Authorization for fish collection was obtained from the Maranhão State Secretariat for the Environment and Natural Resources (SEMA, 09/2019).

### *2.3. Water physicochemical parameters and trace elements and PAH levels in sediment samples*

At the time of fish sampling, water physicochemical parameters (temperature, pH, and dissolved oxygen) were measured using a multiparameter analyzer (HI 9829M, Hanna Instruments, Woonsocket, RI, USA).

Bottom sediment samples were standardized granulometrically to 200 m depth (74 µm), oven-dried at 40 °C, ground, and stored in propylene flasks. Approximately 1.0 g (dry weight) of sample was used, in duplicate, to determine trace elements after acid digestion with $HNO_3$ (65%) and HCl (37%) in a block digester at 80 °C and neutralization with 15 mL of 0.1 N HCl. Metals levels (Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sr, V, and Zn) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) using an Optima 8300 system (PerkinElmer, USA). Total Hg (THg) was determined by the methods described by Malm et al. (1989) and Bastos et al. (1998). Briefly, 500 mg of bottom sediment (dry weight) were mixed with a solution containing $HCl/HNO_3$ (3:1) (Merck) and 5% (w/v) $KMnO_4$ (Merck).

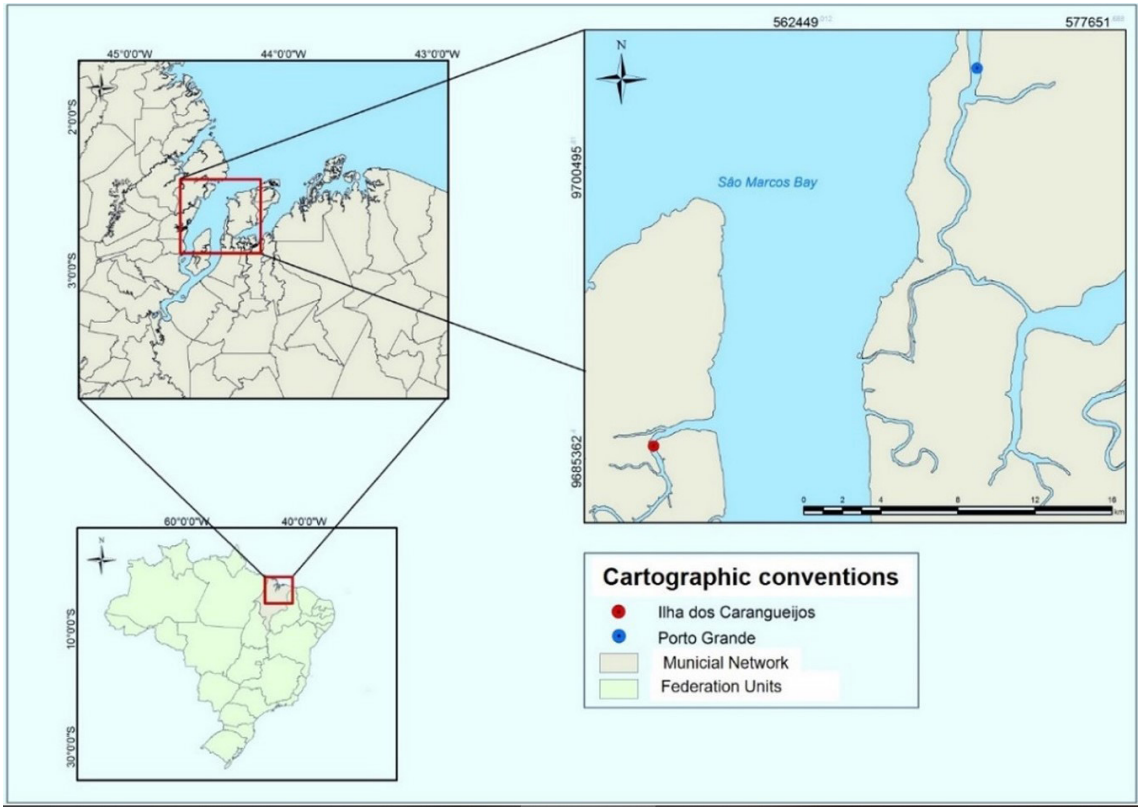


**Figure 1.** Location map of *S. herzbergii* sampling points in São Marcos Bay, Maranhão State, Brazil: reference site (Ilha dos Caranguejos) and port site (Porto Grande).

After digestion in a block digester for 60 min at 60 °C, samples were titrated with 12% (w/v) hydroxylamine hydrochloride (Merck) and mixed with 12 mL of ultrapure water. THg measurements were performed by cold vapor atomic absorption spectroscopy using a Flow Mercury Injection System 400 (PerkinElmer). For quality control purposes, reagents with a high degree of analytical purity (PA, Merck) and certified sediment standards (SS2 SSP-SCIENCE) were used. Solutions were prepared in ultrapure water (Milli-Q Plus, Millipore). All glassware was immersed in 5% (v/v) $HNO_3$ (Merck) for 24 h and rinsed with ultrapure water before use. Mean recovery percentages of trace elements ranged from 80% to 120%. For PAH analysis, sediment samples were stored at 80 °C and processed according to US EPA3550C/8270D protocols. Analysis was performed by ICP-OES (Optima 8300, PerkinElmer).

### *2.4. Biometric measurements of S. herzbergii*

In the field, fish were anesthetized with clove oil (eugenol) and euthanized. Specimens were evaluated for total length (cm), standard length (cm), fork length (cm), and total weight (g). Liver and gill samples were preserved in 10% formalin for 24 h for histological analysis.

### *2.5. Histological processing of S. herzbergii gills and liver*

The gill arches of fish were decalcified for 24 h in a 10% nitric acid solution. Liver and decalcified gill samples were dehydrated through an increasing alcohol gradient, diaphanized in xylene, and embedded in paraffin. Cross sections (approximately 5 µm thick) were stained with hematoxylin and eosin. The histological slides were analyzed in microscopy (Carl Zeiss, Oberkochen, Germany) and photomicrographed in an Olympus BX51 microscope. Histological alterations in gills and in liver tissues were evaluated semi-quantitatively according to Bernet et al. (1999).

### *2.6. Bernet index for S. herzbergii gills and liver*

When a histological alteration was identified, the importance factor ($w$) of such an alteration was categorized into one of the following three levels: 1, minimal or reversible damage; 2, moderate or mostly reversible damage; and 3, marked or irreversible damage (Viana et al., 2021). We also recorded the extent of histological alterations ($a$), which were classified as follows: 0, no alteration; 2, mild occurrence; 4, moderate occurrence; and 6, severe occurrence. Gill and liver alterations were quantified separately. Organ indices ($I_{org}$) were calculated as proposed by Bernet et al. (1999), in the Equation 1 below.

$$I_{org} = \sum_{rp}\sum_{alt}\left(a_{orgrpalt} \times W_{orgrpalt}\right) \quad (1)$$

where org is the organ (constant), rp is the reaction pattern, alt is the alteration, a is the score value, and w is the importance factor.

*2.7. Analysis of GST and CAT activity in S. herzbergii gills and liver*

Gill and liver samples were weighed (1 g) on a precision balance and mixed with buffer (50 mM Tris-HCl, 0.15 M KCl, pH 7.4) at a sample/solution ratio of 1:4. The material was centrifuged at 9,000 × *g* and 4 °C for 30 min. The supernatant was centrifuged at 37,000 × *g* and 4 °C for 60 min to separate the cytosolic fraction, which was used in the analysis of GST and CAT activities. 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 the GST contained in samples. GST activity is expressed as micromoles per minute per milligram of protein (µmol/min/mg protein). CAT activity was evaluated 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 is expressed in enzyme units (U)/mg protein. Protein concentrations in the supernatant were also determined according to the modified method of Jerome et al. (2017), using a commercial kit of biuret and bovine serum albumin as standard.

*2.8. Statistical analysis*

Abiotic, biometric, histological, and enzymatic data were tested for normality by the Kolmogorov-Smirnov test ($p < 0.05$). Subsequently, Student's *t*-test was applied using Statistica 7.0 software (StatSoft Inc., 2005, Tulsa, OK, USA) to determine significant differences between less impacted (IC) and potentially impacted (PG) sites. Values of $p < 0.05$ were considered significant.

## 3. Results

*3.1. Water physicochemical parameters and trace elements and PAH levels in sediment samples*

The physical-chemical parameters are similar between the two areas. Apparently IC showed, in general, similar and/or higher values for trace elements than PG, so much so that no significant differences are seen between sites. On the other hand, for PAH the values were really higher in PG (Table 1). Trace element concentrations in sediments were detected in the following order of magnitude: Fe > Al > Mn > Sr > Cr > Ba > V > Zn > Ni > Cu > Pb > As > Cd > THg (Table 1). Values for Fe and Al in PG and IC were above the maximum levels defined by Brazilian environmental legislation (CONAMA, 2004). Different PAHs (2-4-Ring, 2-Meth, AceP, AceN, Chri , DibenA , Phen , Fluoran, Fluore, Naph, Py, BaA, 5-6-Ring, BaP, BbF, BghiP) were detected at different concentrations in sediments, varying according to site and season (Table 1). 5-6-Ring PAHs were detected above regulatory limits (CONAMA, 2004) in all sampling season.

**Table 1.** Physicochemical parameters of water samples, trace element and polycyclic aromatic hydrocarbon (PAH) levels in sediment samples collected from estuaries in the Ilha dos Caranguejos and Porto Grande sites, São Marcos Bay, Brazil.

| Physicochemical parameter | Ilha dos Caranguejos | | Porto Grande | | Values of reference's |
|---|---|---|---|---|---|
| | Rainy season | Dry season | Rainy season | Dry season | |
| Temperature (°C) | 28.7 | 28.2 | 28.2 | 30.3 | 28-32 º C |
| Salinity | 13.3 | 14.2 | 15.3 | 15.5 | 0,5- 30‰ |
| pH | 7.41 | 7.77 | 7.53 | 6.79 | 5-9 |
| Dissolved $O_2$ (mg/L) | **6.1**$^{*a}$ | **6.3**$^{*a}$ | **5.7**$^{*b}$ | **5.8**$^{*b}$ | >3mg/L |
| **Trace element** | **Ilha dos Caranguejos** | | **Porto Grande** | | |
| | **Rainy season** | **Dry season** | **Rainy season** | **Dry season** | |
| Hg | 0,0260 ± 0,0011 | 0,0314 ± 0,0028 | 0,0292 ± 0,0032 | 0,0305 ± 0,0035 | |
| Al | **10,076.31** ± 41.75$^{*a}$ | **11,949.47** ± 818.33$^{*}$ | **10,252.33** ± 739.50$^{*b}$ | **12,365.58** ± 2,593.15$^{*}$ | |
| As | 6.05 ± 0.06 | 7. 01 ± 0.52 | 5.60 ± 0.08 | 7. 30 ± 0.10 | |
| 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 | |
| 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 | |
| Cu | 7.95 ± 0,09 | 7. 50 ± 0.03 | 7. 57 ± 0.16 | 8. 14 ± 0.07 | |
| Fe | **20,611.10** ± 80.30$^{*}$ | **21,481.52** ± 677,14$^{*}$ | **19,381.87** ± 877,96$^{*}$ | **21,764.62** ± 2,016,63$^{*}$ | |
| 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 | |
| Pb | 6. 60 ± 0.13 | 5. 06 ± 0.15 | 5. 15 ± 0.13 | 5. 74 ± 0.22 | |
| 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 | |

$^{a, b}$ Indicates significant differences between sites ($p < 0.05$). $^{*}$ Indicates significant differences between seasons ($p < 0.05$). LD: limit of detection; LQ: limit of quantification. Data for trace elements are expressed as mean ± standard deviation. Physicochemical parameters above the maximum thresholds defined by the Brazilian Environmental Agency (CONAMA, 2011) are shown in bold. Trace elements above the maximum thresholds defined by the Brazilian Environmental Agency (CONAMA, 2004) are shown in bold. PAHs above the maximum thresholds defined by the Brazilian Environmental Agency (CONAMA, 2004) are shown in bold.

**Table 1.** Continued...

| PAHs | Ilha dos Caranguejos | | Porto Grande | |
|---|---|---|---|---|
| | Rainy season | Dry season | Rainy season | Dry season |
| 2-4-Ring | | | | |
| 2-Meth | 5.65 | <LQ | **99.6** | 19.05 |
| AceP | <LQ | <LQ | <LQ | <LQ |
| AceN | 6.78 | <LQ | <LQ | <LQ |
| Chri | <LQ | <LQ | <LQ | <LQ |
| DibenA | <LQ | <LQ | 50.87 | 32.09 |
| Phen | <LQ | <LQ | <LQ | <LQ |
| Fluoran | <LQ | <LQ | <LQ | <LQ |
| Fluore | <LQ | <LQ | <LQ | <LQ |
| Naph | <LQ | <LQ | <LQ | <LQ |
| Py | 2.05 | <LQ | <LQ | <LQ |
| BaA | <LQ | <LQ | <LQ | <LQ |
| 5-6-Ring | | | | |
| BaP | <LD | <LD | **415.2** | **331.1** |
| BbF | <LD | <LD | **348.1** | **270.7** |
| BghiP | <LD | <LD | **329.0** | **303.4** |

[a, b] Indicates significant differences between sites ($p < 0.05$). * Indicates significant differences between seasons ($p < 0.05$). LD: limit of detection; LQ: limit of quantification. Data for trace elements are expressed as mean ± standard deviation. Physicochemical parameters above the maximum thresholds defined by the Brazilian Environmental Agency (CONAMA, 2011) are shown in bold. Trace elements above the maximum thresholds defined by the Brazilian Environmental Agency (CONAMA, 2004) are shown in bold. PAHs above the maximum thresholds defined by the Brazilian Environmental Agency (CONAMA, 2004) are shown in bold.

**Table 2.** Mean ± standard deviation of biometric measurements of *S. herzbergii* captured at two sites in the São Marcos Bay, Brazil.

| Parameter | IC | | PG | |
|---|---|---|---|---|
| | Rainy season | Dry season | Rainy season | Dry season |
| Total weight (g) | 204.2*± 18.41 | 204.2 ± 105.44 | 131.2* ± 25.77 | 88.22 ± 23.86 |
| Total length (cm) | 28.8* ± 0.71 | 11.63 ± 5.25 | 25.45* ± 1.77 | 22.27 ± 2.13 |
| Standard length (cm) | 24.39* ± 0.71 | 9.69 ± 1.18 | 20.65* ± 1.79 | 18.77 ± 1.67 |
| Fork length (cm) | 25.26* ± 0.68 | 10.09 ± 1.18 | 21.47* ± 1.74 | 19.27 ± 1.67 |

* significant differences at p < 0.05. IC: Ilha dos Caranguejos; PG: Porto Grande.

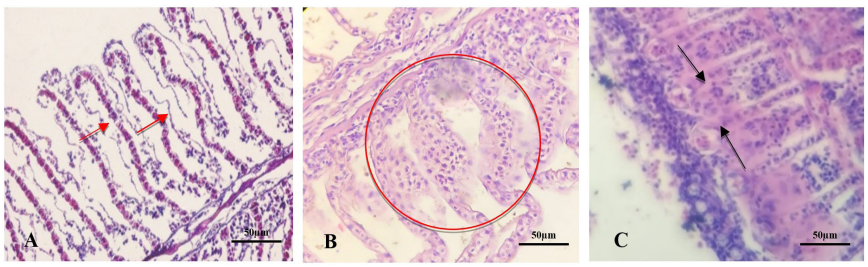


**Figure 2.** Representative images of gill alterations in *S. herzbergii*. (A) Displacement of secondary lamellae (thin red arrows), (B) hyperplasia (red circle) and (C) necrosis (thick black arrows).

### *3.2. Biometric measurements*

Table 2 presents the mean and standard deviation of biometric measurements of fish caught at both study sites (IC and PG) at different times of the year (rainy and dry seasons).

*S. herzbergii* specimens captured in IC (less impacted site) weighed considerably more than those captured in PG (potentially impacted site) in both seasons. Fish from IC had higher total length than fish from PG in the rainy season. In the dry season, by contrast, fish from PG had higher total length than fish from IC.

### *3.3. Histological biomarkers in gills and livers of S. herzbergii*

The gill alterations identified in *S. herzbergii* captured in IC were lamellar fusion (34%), epithelial displacement (32%), tissue degeneration (17%), and hyperplasia (7%). In individuals sampled in PG, the main gill alterations were epithelial displacement (47%), lamellar fusion (15%), hyperplasia (12%), necrosis (5%), and lamellar narrowing (6%). Figure 2 depicts the main gill alterations observed in *S. herzbergii* collected at the study sites (IC and PG).

The hepatic alterations identified in *S. herzbergii* collected in IC were fibrosis, necrosis, and melanomacrophage

centers and those of fish collected in PG were fibrosis, necrosis, melanomacrophage centers, and hepatocyte vacuolization (Figure 3).

### 3.4. Bernet indices for S. herzbergii gills and liver

The organ indices for the gills and liver of fish collected from IC and PG (Table 3) are described according to Bernet et al. (1999). The lesion indices of gills were higher in IC in both seasons. Liver lesion indices, on the other hand, were higher in PG in both seasons, demonstrating that, although the region is more impacted, it contains aquatic contaminants that influence the response of *S. herzbergii*. Despite the higher frequency of overall histological alterations in fish from PG, gill lesions were more intense in the region with lower impact (IC).

### 3.5. Enzyme biomarkers (GST and CAT) in gills and liver of S. herzbergii

The results of GST and CAT activities in the gills and liver of *S. herzbergii* captured in IC and PG at different times of the year (rainy and dry seasons) are shown in Figure 4. Student's *t*-test revealed no significant differences in GST and CAT activities between study sites and seasons ($p > 0.05$).

Gill GST activity of fish collected in IC was high in the rainy season (Figure 4A). The same behavior is seen for GST activity in fish liver collected in IC (Figure 4B).

Gill CAT activity was higher in the rainy season than in the dry season for both sites. Furthermore, CAT activity was higher in IC than in PG (Figure 4C). Liver CAT activity was also higher in the rainy season in IC than in PG. In the dry season, liver CAT activity was low in both IC and PG (Fig.4D).

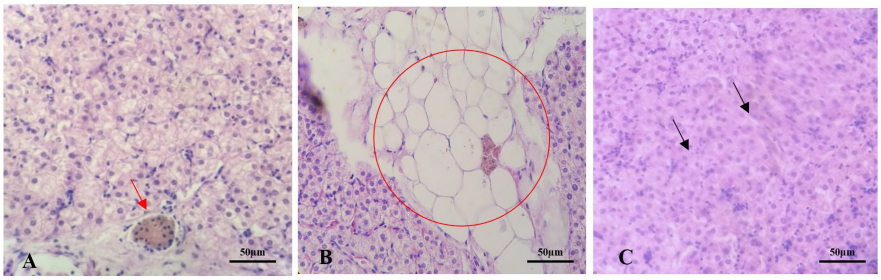


**Figure 3.** Representative images of liver alterations in *S. herzbergii*. (A) Melanomacrophage center (thin red arrow), (B) vacuolization (red circle) (C), necrosis (thin black arrow).

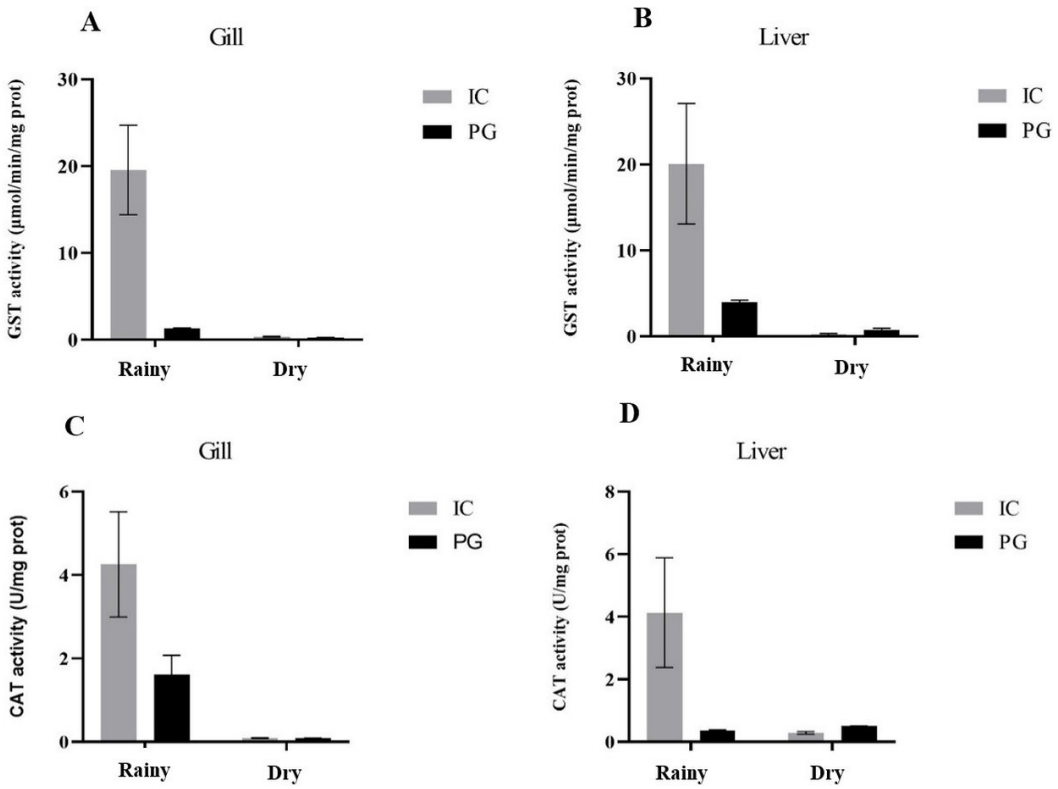


**Figure 4.** Glutathione *S*-transferase (GST) and catalase (CAT) activity in gills and liver of *S. herzbergii* captured at different sites (IC and PG) and times of the year (rainy and dry seasons) in the São Marcos Bay, Brazil.

**Table 3.** Lesion indices for gills and liver of *S. herzbergii* sampled at different sites in rainy and dry seasons in the Ilha dos Caranguejos (IC) and Porto Grande (PG).

| Organ | Type of alteration | Alteration | Importance factor (*w*) | Rainy season (log) | | Dry season (log) | |
|---|---|---|---|---|---|---|---|
| | | | | IC | PG | IC | PG |
| Gill | Circulatory disorders | Hemorrhage | 1 | | | | |
| | | Aneurysm | 1 | | | | |
| | | Congestion | 1 | | | | |
| | | Epithelial displacement | 1 | | | | |
| | Regressive alterations | Necrosis | 3 | 203 | 184 | 220 | 168 |
| | | Lamellar narrowing | 1 | | | | |
| | | Tissue degeneration | 2 | | | | |
| | | Fusion | 2 | | | | |
| | Progressive alterations | Hyperplasia | 2 | | | | |
| Liver | Circulatory alterations | Hemorrhage | 1 | | | | |
| | | Necrosis | 3 | | | | |
| | | Fibrosis | 2 | | | | |
| | | Steatosis | 2 | | | | |
| | Regressive alterations | Tissue degeneration | 2 | | | | |
| | | Hepatocytes vacuolization | 2 | 40 | 86 | 70 | 80 |
| | Inflammatory responses | Melanomacrophage centers | 2 | | | | |
| | | Leukocyte infiltration | 2 | | | | |
| | | Differentiating cells | 1 | | | | |
| | | Encapsulated amorphous mass | 2 | | | | |

Gill and liver histological indices of *Sciades herzbergii* collected in estuarine regions (IC and PG) of São Marcos Bay, Maranhão. IC: Ilha dos Caranguejos; PG: Porto Grande; w: Importance factor; log: Organ index.

## 4. Discussion

Abiotic data (water pH, dissolved oxygen, salinity, and temperature) for the study sites were in accordance with CONAMA (2011) resolution for seawater. Mean pH, salinity, and temperature values did not differ between seasons at both collection sites. However, dissolved oxygen was lower in PG in the dry and rainy seasons. Similar values of temperature, salinity, and dissolved oxygen were reported by Pinheiro-Sousa et al. (2022) for São Marcos Bay. Castro et al. (2018), however, reported different results for the port region, observing high values of dissolved oxygen for the same region. Dissolved oxygen is essential for vital physiological processes in fish, also serving as an important indicator of environmental quality (Yu et al., 2023). High input of contaminants from industrial and domestic wastewaters may lead to imbalances in hydrological parameters, especially those related to a decrease in dissolved oxygen (Santos et al., 2009). Dissolved oxygen is considered one of the main parameters for characterizing the effects of water pollution from effluents and contaminants (Oliveira et al., 2010).

Pinheiro-Sousa et al. (2022), when evaluating *S. herzbergii*, and Jesus et al. (2020), evaluating the invertebrate *Ucides cordatus* (Linnaeus, 1763), determined the concentrations of Al, As, Cd, Fe, Mn, Pb, Ni and Hg in sediments in São Marcos Bay. Element levels were found above the limits established by law (CONAMA, 2004), a situation also observed in the present study and reported by Carvalho Neta et al. (2012), Carvalho Neta et al. (2009) and Castro et al. (2018). Sediments are long-term storage sites for metals, and their profile is an important indicator of environmental quality (Qu et al., 2017).

In the present study, IC fish had higher weights in both seasons than PG specimens. Regarding length, individuals sampled from PG were larger only in the dry season. Similar results were described by Soares et al. (2020) for the same locations: IC fish were heavier than PG fish. The authors attributed these results to the fact that fish from the CI probably had more adequate feeding conditions. The findings are in line with those of Carvalho-Neta et al. (2012) and Viana et al. (2021).

IC is part of an Environmental Protection Area, suffering less impact than the port site. Environmental conditions may influence fish weight and size (Pinheiro-Sousa et al., 2022). According to Soares et al. (2020), exposure of organisms to contaminants and high pollutant concentrations may compromise vital biological, biochemical, physiological, and behavioral systems, leading to redirection of energy to detoxification processes (Sousa et al., 2013). The high traffic of vessels in the port complex is likely causing an increase in toxic substances that might be associated with induction of gill and liver lesions in fish and damage to aquatic health (Castro et al., 2018; Soares et al., 2020).

The frequency of histological alterations in the gills and liver of *S. herzbergii* specimens collected at the port site indicates that these organisms are under stress caused by metal and PAH contamination. Environmental contamination by human activities causes functional damage to fish. The alterations observed in the present study were also described by Castro et al. (2018) in a study on the same species occurring in São Marcos Bay. The authors found that epithelial displacement was the most frequent alteration. Fusion of secondary lamellae and epithelial displacement in gills were also described by Sousa et al. (2013).

According to Soares et al. (2020) identified lamellar fusion and epithelial displacement in the tissues of *S. herzbergii* sampled in São Marcos Bay. Lamellar fusion is a type of injury that occurs as a defense mechanism against direct contact between contaminants and the lamellar epithelium; however, these changes also reduce the respiratory surface, which may result in death (Pereira et al., 2020).

According to Pereira et al. (2020), epithelial displacement is one of the most common biomarkers observed in the gills of fish exposed to acute toxicity. The authors argued that fusion of secondary lamellae is also frequently found in organisms affected by contaminants. Fish are closely associated with their aqueous environment, and changes in the ecosystem are quickly reflected in quantifiable physiological alterations. Analysis of gill lesions may demonstrate the degree of stress to which organisms are subjected (Sousa et al., 2013).

Epithelial displacement, fusion of secondary lamellae, aneurysm, and hyperplasia, the most common alterations observed in gills in the current study, were also reported by Flores- Sousa et al. (2013); Pinheiro-Sousa et al. (2021). These studies demonstrate the importance of identifying gill lesions as a strategy to assess water quality and aquatic species health. Gills are important indicators of water quality because of their sensitivity to water conditions and direct contact with the impacted environment.

Histological evaluation of *S. herzbergii* liver specimens revealed a high frequency of melanomacrophage centers, necrosis, hepatocyte vacuolization, and fibrosis in the study sample. This result is in agreement with the findings of Carvalho-Neta et al. (2014), who stated that melanomacrophage centers are examples of defense mechanisms. According to Bombonato et al. (2007), melanomacrophage centers act as a form of natural defense in fish. Santos Filho et al. (2014), in studying histological biomarkers in bony fish, observed the presence of these defense structures.

The liver is an important organ, as it performs vital functions related to basal metabolism and participates in detoxification, bioactivation, accumulation, and excretion of xenobiotics (Figueiredo-Fernandes et al., 2006). Therefore, the organ is of high interest to investigate the exposure of fish to contaminants (Bernet et al., 1999). Alterations such as necrosis appear after irrevocable loss of cell function (Bernet et al., 1999). These pathologies constitute a common response of fish in degraded aquatic environments (Soares et al., 2020).

The gills and liver of harbor fish showed reversible and irreversible damage. Alterations described as moderate to mild may allow regeneration and recovery of the function of the affected structure, if there is a decrease in exposure to xenobiotics present in the environment (Bernet et al., 1999). Histopathological changes in fish tissues are biomarkers of exposure to environmental stressors, such as one or more toxic agents (Bernet et al., 1999).

Antioxidant enzymes are considered the first line of cellular defense against oxidative damage (Van der Oost et al., 2003). Biochemically, cells try to eliminate free radicals formed during metabolization of chemical compounds by using specific enzymes, such as CAT and GST (Di Giulio et al., 1989). The high CAT and GST activities observed at different collection season might have been a physiological response to prevent significant lipid damage. On the other hand, according to Freire et al. (2015), these differences may simply be seasonal. However, the increased induction of GST in contaminated sites suggests that this type of biotransforming enzyme responds strongly to environmental contamination (Santana et al., 2018).

Histological changes are increasing in fish sampled in the most impacted region, showing that the health of *S. herzbergii* is compromised. We emphasize the importance of carrying out molecular studies on *S. herzbergii* and the use of multibiomarkers to subsidize effective measures for the biomonitoring of the area and the health of the local biota.

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