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Research Article

Integrated analysis of two biomarkers in *Sciades herzbergii* (Ariidae, Siluriformes), to assess the environmental impact at São Marcos' Bay, Maranhão, Brazil

Débora Batista Pinheiro-Sousa[1], Zafira da Silva de Almeida[1] & Raimunda Nonata Fortes Carvalho-Neta[1]

[1]State University of Maranhão, Department of Chemistry and Biology, Laboratory of Fishing Biodiversity and Population Dynamics of Fish, Campus Paulo VI, University City
P.O. Box 09, Tiririca, 65075-000 São Luís, Maranhão, Brazil

ABSTRACT. Guribu catfish (*Sciades herzbergii*) is a resident species in estuaries of Maranhão, Brazil. The aim of this work was to determine the feasibility of integrated analysis of the branchial lesions and gonadosomatic index of *Sciades herzbergii* in order to evaluate the effects of pollutants in São Marcos' Bay. The first site (S1) is located near the Ilha dos Caranguejos and was used as a reference area for being an environmental protection area. The second site (S2) is located near the ALUMAR/ALCOA port, and was used as a potentially polluted area. Fish were collected at each site, forty-eight in S1 and forty in S2. Gills were fixed in 10% formalin, and usual histological techniques were used in the first right gill arch, with inclusion in paraffin and sections of 5 µm thickness. There were no histopathological changes in animals captured at the reference site. However, in those catfish collected in the potentially contaminated area it was observed several branchial lesions, such as lifting of the lamellar epithelium, fusion of some secondary lamellae, hypertrophy of epithelial cells and lamellar aneurysm. The analysis using the gonadosomatic index (GSI) showed significant differences, being higher in fish analyzed from the reference area ($P < 0.05$). The branchial lesions and GSI were sensitive for monitoring environmental impacts of different locations at São Marcos' Bay, Maranhão, Brazil.

Keywords: biomonitoring, branchial lesions, GSI, catfish, Ilha dos Caranguejos, Brazil.

Análisis integrado de dos biomarcadores en *Sciades herzbergii* (Ariidae, Siluriformes) para evaluar el impacto ambiental en la Bahía de San Marcos, Maranhão, Brasil

RESUMEN. El bagre guribú (*Sciades herzbergii*) es una especie residente en los estuarios del Maranhão, Brasil. El objetivo de este trabajo fue determinar la viabilidad de un análisis integrado de las lesiones branquiales y el índice gonadosomático de *Sciades herzbergii*, para evaluar los efectos de los contaminantes en la bahía de San Marcos. El primer sitio (S1), situado cerca de la Ilha dos Caranguejos, fue utilizado como zona de referencia, por ser un área de protección ambiental. El segundo sitio (S2) situado cerca del puerto ALUMAR/ALCOA, fue utilizado como una zona potencialmente contaminada. En cada sitio se recogieron peces, cuarenta y ocho en S1 y cuarenta en S2. Las branquias fueron fijadas en formol al 10% y las técnicas histológicas habituales fueron empleadas en el primer arco branquial derecho, con inclusión en parafina y secciones de 5 µm de espesor. En los peces capturados en el sitio de referencia no hubo ningún cambio histopatológico. Sin embargo, en los bagres de la región contaminada se encontró un amplio rango de alteraciones branquiales, como por ejemplo, el desprendimiento del epitelio, fusión y plegamiento lamelar, hipertrofia del epitelio y aneurisma lamelar. El análisis del índice gonadosomático (GSI), mostró diferencias significativas, siendo mayor en los peces analizados en el área de referencia ($P < 0,05$). Las lesiones branquiales y el GSI fueron sensibles para el monitoreo del impacto ambiental de diferentes lugares en la bahía de San Marcos, Maranhão, Brasil.

Palabras clave: biomonitoreo, lesiones branquiales, GSI, bagre, Ilha dos Caranguejos, Brasil.

Corresponding author: Débora Batista (deborabpsousa@hotmail.com)

INTRODUCTION

The monitoring of aquatic ecosystems with biomarkers is responsible for identifying impacts and biologic changes in organisms (Mozeto & Zagato, 2006). Biomarkers are defined as cellular changes, biochemical, molecular or physiological, which are measured in cells, body fluids, tissues or organs within an organism, and are indicative of exposure and doses of xenobiotics that lead to biological effects (Lan & Gray, 2001).

The contaminants effects in fish can be express in various levels of biological organization, including physiological dysfunction, structural changes in organs and tissues and behavioral alteration that lead to impaired growth and reproduction (Adams, 1990). The gill lesions are used as sensitive biomarkers of environmental impacts on fish (Stentiford *et al.*, 2003), and it has been recognized, by many researchers, that histopathological examination is a valuable tool for assessment of environmental impacts on fish populations (Teh *et al.*, 1997). Those morphologic alterations could occur because the gill of the fish is in permanent contact with the environment (Heath, 1995). The detection of early warning signals through branchial lesions is ecologically relevant, economic and faster, and it has the potential to be used as a type of biomarker.

In the south and southeast of Brazil, there are already some studies using different types of biomarkers (Amado *et al.*, 2006; Camargo & Martinez 2006; Umbuzeiro *et al.*, 2006; Zanette *et al.*, 2006; Valdez-Domingos *et al.*, 2007). These researches indicate the need for biomarkers to diagnose the key impacts in aquatic ecosystems. In this context, some biomarkers have been frequently used in programs for evaluating the impact on aquatic ecosystems, because they have well-founded methodology, generating answers in a short time, with low cost of analysis and highly sensitive (Freire *et al.*, 2008).

In São Luís (Maranhão), a region that has the largest port with cargo movement in Brazil, studies using biomarkers in *Sciades herzbergii* from São Marcos' Bay has indicated the necessity of continuing this type of analysis (Carvalho-Neta & Abreu-Silva, 2010). The economic importance of this species and the pollution of the port are factors that suggest the need for biomonitoring this bay. Thus, the aim of this work was to determine the feasibility of integrated analysis of the branchial lesions and gonadosomatic index of *Sciades herzbergii* in order to evaluate the effects of pollutants in São Marcos' Bay.

MATERIALS AND METHODS

Site description and sample collection

Two samples were collected in the seasonal period from August 2010 to April 2011 in two distinct sites in São Marcos' Bay. The first site (S1) is located near the Crabs Island (02°49'06"S, 44°29'05"W) and was used as a reference area for being an environmental protection area. The second site (S2), located near the ALUMAR/ALCOA port (02º43'14"S, 44º23'35"W), was used as a potentially impacted area (Fig. 1).

The catfish were captured in their natural habitat in three points of each area (S1 and S2) using gill nets, approximately twenty-four hours in each sampled area. We collected forty fish in the potentially impacted area and forty-eight fish in the reference area. The collected animals at each sampling location were placed in plastic bags properly labeled, identified and sealed, placed in coolers containing ice and transported to the laboratory, about 40 km. Fish were dissected and their gills were fixed immediately in 10% formalin.

Water chemistry parameters (salinity, pH, temperature, dissolved oxygen, dissolved oxygen saturation and turbidity) were measured directly in the field.

Analysis of biometric data

The total length (LT), fork length (LF), total weight (WT) and gonad weight (WG) were recorded. After measured and weighed, the specimens of fish were opened for macroscopic observation and classification of the gonads, considering the scale of gonadal stages of development given by Vazoller (1996) and modified by Carvalho-Neta & Castro (2008): EG1 (immature), EG2 (in maturation our repose), EG3 (mature) and EG4 (exhausted). Gonadosomatic index (GSI) was calculated as follows: (gonad weight × 100) / total weight (Vazzoler, 1996).

Histopathological analysis

In the laboratory, the gills were fixed in 10% formalin and kept in 70% alcohol until histological processing. For this, the first right gill arch was dehydrated in ascending series of alcohols, cleared in xylene,

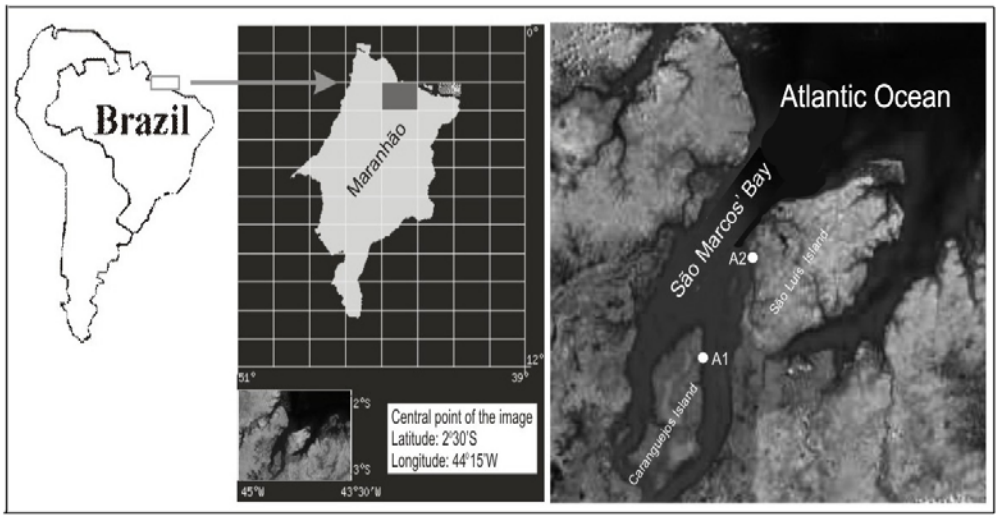

Figure 1. Sampling locations for *Sciades herzbergii* in São Marcos' Bay, indicating the reference area (S1), and the potentially contaminated site (S2).

impregnated and embedded in paraffin. The tissue sections were stained with hematoxylin-eosin. Four tissue sections from each fish were examined by Zeiss light photomicroscope. Histopathological lesions were classified according to the diagnostic criteria of Bernet *et al.* (1999).

Statistical analysis

The analysis of environmental parameters was made by comparing seasonal data (dry season and rainy season) in the potentially contaminated area with the reference site. The results of the fish biometric data were expressed as mean ± standard deviation for males and females, and compared using the Student t-test. The level of significance was 0.05.

RESULTS

Average values of abiotic variables registered from São Marcos' Bay during the two collections were grouped into "dry season" and "rainy season", as is shown in Table 1. The salinity was found to be uniform in both sampling sites, decreasing during the rainy season. The dissolved oxygen and oxygen saturation were always lower at the potentially contaminated area (S2). The values for pH and turbidity were constants for both areas, demonstrating the homogeneity of these abiotic factors in both areas.

Results of the statistical analysis of the biometric data for males and females of *Sciades herzbergii*, during the dry (August 2010) and rainy (April 2011) seasons, in the two sites (S1 and S2) in São Marcos' Bay, can be seen in Tables 2 and 3 respectively. The data indicate that total and fork length of fish caught in the potentially contaminated site (S2) were significantly lower ($P < 0.05$) than those of the reference site (S1). However, the gonadosomatic index (GSI) showed significant differences between the two fish groups. The GSI in fish from the contaminated site was significantly lower ($P < 0.05$) than in control fish during all phases of the gonadal cycle.

The results of gonadal stages of fish captured during the rainy and dry season are shown in Table 4. The data showed fish from the reference area in all gonadal stages, but in potentially contaminated site juveniles (EG1) were not found.

The histopathological analysis in *Sciades herzbergii* sampled during the dry (August 2010) and rainy (April 2011) seasons from Ilha dos Caranguejos (reference area) showed no morphological changes in gills of the catfish (Fig. 2). However, individuals caught in the potentially contaminated area showed several histopathological changes (Fig. 3). The most important change found in the gills of *S. herzbergii* was lamellar narrowing and epithelial lifting of the primary lamella (Table 5). Histopathological seasonal variation was not detected in the potentially contaminated site ($P < 0.05$).

DISCUSSION

The environmental parameters of the two sample sites indicated that waters of the São Marcos' Bay are uniform in terms of salinity and pH. This characteristic pattern of the salinity and pH was found by Lopes (2005) in the same region.

The lowest concentrations of dissolved oxygen were found in the area of influence of the port. The low levels of dissolved oxygen are considered unsuitable for estuarine waters according to the Brazilian Agency for Water Quality Legislation (CONAMA,

Table 1. Environmental parameters analyzed at collection site in each region of São Marcos' Bay, Maranhão, during dry (August 2010) and rainy (April 2011) seasons.

Parameter	Reference		Potentially contaminated	
	Dry season	Rainy season	Dry season	Rainy season
Temperature (°C)	29.0	29.1	29.0	29.0
Salinity (UPS)	15.0	15.0	14.0	10.0
pH	8.1	8.2	8.2	8.1
Dissolved oxygen (mL L^{-1})	6.0	4.9	5.1	6.1
% Saturation of dissolved oxygen	88.6	86.2	86.4	88.8
Turbidity (NTU)	13.0	12.3	13.3	13.0

Table 2. Biometric data of males and females of *Sciades herzbergii* collected in reference area and potentially contaminated area of São Marcos' Bay during dry (August 2010) season.

Parameters	Mean ± Standard deviation			
	Reference		Potentially contaminated	
	(Dry season)		(Dry season)	
	Females	Males	Females	Males
LT (cm)	24.49 ± 6.64*	20.48 ± 4.14	20.27 ± 2.56	20.28 ± 2.54
LF (cm)	21.35 ± 5.70*	16.87 ± 3.62	17.06 ± 2.46	18.15 ± 2.36
WT (g)	160.20 ± 45.10*	291.70 ± 70.19*	72.49 ± 31.59	60.34 ± 25.98
Wg (g)	6.25 ± 0.19	1.58 ± 0.16	6.72 ± 13.37	0.95 ± 0.95
GSI	2.46 ± 1.28*	1.80 ± 0.09*	0.37 ± 0.25	0.09 ± 0.07

*indicates significant difference relative to the contaminated site ($P < 0.05$). Total number of animals: 88. Number of females in: S1: 18; S2: 25. Number of males in: S1: 22; S2: 23. Biometric data: LT: total length; LF: fork length; WT: total weight; Wg: gonad weight, and GSI: gonadosomatic index.

Table 3. Biometric data of males and females of *Sciades herzbergii* collected in reference area and potentially contaminated area of São Marcos' Bay during rainy (April 2011) season.

Parameter	Mean ± Standard deviation			
	Reference		Potentially contaminated	
	(Rainy season)		(Rainy season)	
	Females	Males	Females	Males
LT (cm)	24.43 ± 2.4*	20.46 ± 6.87	20.43 ± 4.41	20.46 ± 6.87
LF (cm)	29.86 ± 2.04*	20.28 ± 3.99	16.43 ± 4.40	13.45 ± 6.06
WT (g)	113.64 ± 36.10*	122.35 ± 14.17*	27.67 ± 2.17	14.05 ± 3.35
Wg (g)	19.72 ± 1.95*	9.14 ± 4.04*	5.41 ± 1.44	3.57 ± 1.59
GSI	1.53 ± 0.86*	1.16 ± 0.11*	0.56 ± 0.41	0.13 ± 0.42

* Indicates significant difference in relation to the contaminated site ($P < 0.05$). Total number of animals: 88; Number of females in: S1: 18; S2: 25; Number of males in: S1: 22; S2: 23. Biometric data: LT: total length; LF: fork length; WT: total weight; Wg: gonad weight, and GSI: gonadosomatic index.

Table 4. Gonad stage (males and females) of *Sciades herzbergii* captured São Marcos' Bay during dry (August 2010) and rainy (April 2011) season.

Gonad stage	Reference				Potentially contamined			
	Dry season		Rainy season		Dry season		Rainy season	
	%F	%M	%F	%M	%F	%M	%F	%M
EG1	7	9	7	9	0	0	0	0
EG2	8	13	6	6	50	55	20	15
EG3	20	20	2	11	3	4	2	1
EG4	0	4	50	27	9	10	11	16

Total number of animals: 88. Number of females in: S1: 18; S2: 25. Number of males in: S1: 22; S2: 23. Gonadal stages: EG1: immature, EG2: in maturation our repose, EG3: mature, and EG4: exhausted, F: females; M: males.

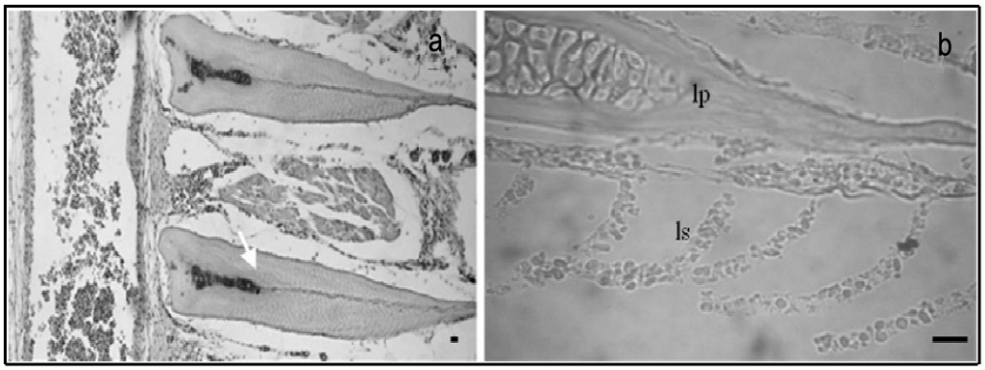

Figure 2. a) Photomicrograph of the gill of *Sciades herzbergii* sampled during the dry season (August 2010) and rainy season (April 2011), in São Marcos' Bay with no morphological changes (arrow), b) detail of gill filaments showing one primary (lp) and secondary lamellae (ls). Scale bar = 20 µm.

2005). In the Ilha dos Caranguejos values were registered inside of the limits considered normal in an estuary (CONAMA, 2005).

The abiotic data, such as water temperature, conductivity, pH, dissolved oxygen and turbidity can change the fish richness and assemblage composition (Fialho *et al.*, 2008). These can also be affected by anthropogenic impacts (Penczac *et al.*, 1994). The similar data for turbidity, salinity and pH recorded for the two analyzed areas indicate a dynamic region, where the winds, tides and river discharges determine a high load of particulate matter. On the other hand, previous studies on sediment and water in the potentially contaminated area showed significantly higher levels of mercury and chrome which confirms that port area in São Marcos' Bay is a site with high exposure risks for some contaminants (Carvalho-Neta *et al.*, 2012).

The results of the biometric data of *S. herzbergii* in São Marcos' Bay showed significant differences between the potentially contaminated site (S2) and the reference area (S1). In the same period, both males and females of S1 were higher in total weight, total length, and fork length, when compared with individuals of S2 ($P < 0.05$). The gonadosomatic index (GSI) was also higher in the S1 than the one from the S2 in both periods analyzed. These results indicate a greater reproductive activity in S1, since higher values of GSI expressed appropriate maturation of the gonads. In a similar study carried out by Carvalho-Neta & Abreu-Silva (2010), the GSI in *S. herzbergii*, from the potentially contaminated site (São Marcos' Bay), was significantly lower than in control fish during all phases of the gonadal cycle. Several studies have showed a decrease in the gonadosomatic index of fish from the contaminated site (Mayon *et al.*, 2006). Xenobiotics cause problems to the endocrine and reproductive system of fish, directly affecting the development of gametes and their viability (Kime, 2000). Intersex and atresia in fish have been

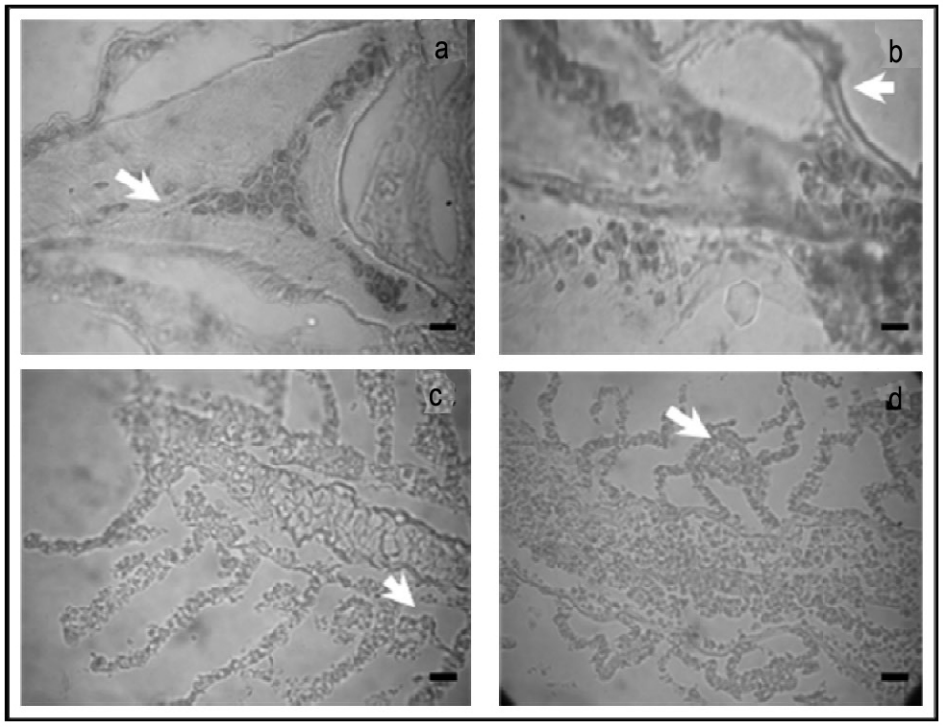

Figure 3. Photomicrographs of the gill of *Sciades herzbergii* caged in São Marcos' Bay with branchial lesions. a) Lamellar narrowing (arrow), b) epithelial lifting of the primary lamella (arrow), c) fusion of secondary lamellae (arrow), d) lamellar aneurysm (arrow). Scale bar = 20 µm.

Table 5. Occurrence of lesions in gills of *S. herzbergii* sampled during dry (August 2010) and rainy (April 2011) seasons from São Marcos' Bay.

Lesions	Reference		Potentially contamined	
	Dry season	Rainy season	Dry season	Rainy season
Lamellar narrowing	0	0	70%	62%
Epithelial lifting of the primary lamella	0	0	22%	25%
Fusion of secondary lamella	0	0	4%	7%
Lamellar aneurysm	0	0	4%	6%
Total	0	0	100%	100%

investigated in terms of chemical exposures (Blazer, 2002).

The gills of catfish collected from the reference site in São Marcos' Bay showed no histopathological changes. However, the animals collected in the potentially contaminated site showed severe histopathological changes, such as narrowing of the lamellae, epithelial lifting, fusion of lamellae and lamellar aneurism. Kim *et al.* (2001), has emphasized that histopathological changes in fish tissues has been important biomarkers of exposure to toxic substances, which reflect changes in biochemical functions. The histopathological examination performed in the gill epithelium of catfish could clearly differentiate the region of Ilha dos Caranguejos (reference area) and the harbor site (potentially contaminated). The great number of severe branchial lesions indicates that fish of the harbor are stressed by the pollutants. Branchial lesions like epithelial lifting, hypertrophy of the epithelial cells, and fusion of some secondary lamellae are examples of defense mechanisms (Camargo & Martinez, 2007; Fernandes & Mazon, 2003).

Histopathological lesions may occur earlier than reproductive changes and they are more responsive than the patterns of growth and reproduction of organisms. When used as an integrated parameter, this

biomarker allows a better assessment of health status of fish than a simple biochemical parameter (Fontaínhas-Fernandes, 2006). In São Marcos' Bay, the integrated analysis of branchial lesions and GSI was useful because gills alterations were detected only in fish of the potentially contaminated site. Furthermore, in that area GSI was very low and juvenile fish were not found. Carvalho-Neta *et al.* (2012), suggested more studies to validate the use of branchial lesions and enzymes correlated with GSI as biomarkers of aquatic contamination in the same region.

Those results reinforce the importance of using different methods of biomonitoring of the estuarine ecosystems. The use of biomarkers on only one level of biological organization (*e.g.*, branchial lesions = histological) would not accurately represent the impact to environment (Moore *et al.*, 2004). In this study, the method based on branchial lesions and GSI proved to be sensitive for the monitoring of the environmental impacts with relatively low cost and speed.

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