Lat. Am. J. Aquat. Res., 41(2): 217-225, 2013
"Proceedings of the 3rd Brazilian Congress of Marine Biology"
A.C. Marques, L.V.C. Lotufo, P.C. Paiva, P.T.C. Chaves & S.N. Leitão (Guest Editors)
DOI: 10.3856/vol41-issue2-fulltext-2

Research Article

Glutathione S-Transferase as biomarker in *Sciades herzbergii* (Siluriformes: Ariidae) for environmental monitoring: the case study of São Marcos Bay, Maranhão, Brazil

Raimunda N.F. Carvalho-Neta[1] & Ana L. Abreu-Silva[2]
[1]Departamento de Química e Biologia, Laboratório de Pesca, Biodiversidade e Dinâmica Populacional de Peixes, Universidade Estadual do Maranhão, Campus Paulo VI, Tirirical São Luís-MA, 65000-000, Brazil
[2]Departamento de Patologia, Laboratório de Anatomopatologia
Programa de Pós-Graduação em Biotecnologia-Rede Nordeste de Biotecnologia (RENORBIO)
Universidade Estadual do Maranhão, Campus Paulo VI, Tirirical, São Luís-MA, 65000-000, Brazil

ABSTRACT. The Glutathione S-Transferase (GST) activity has been proposed as a biomarker of susceptibility to the presence of potentially damaging xenobiotics in aquatic organisms. The aim of this work was to measure GST activity in the liver of *Sciades herzbergii* (catfish) in order to evaluate the biochemical effects of pollutants. The catfish samples were collected along known pollution gradients areas (A1) and from areas regarded as relatively free of anthropogenic input (A2), in São Marcos Bay, São Luis de Maranhão, Brazil. The variables analyzed in fish were: length, weight, gonadal stages, gonadosomatic index and GST activity. The databases from this analysis were compiled, and generalized linear models were used to analyze the dependence of enzyme activity on the areas of sampling and on selected biological parameters of fish. A significant difference was observed in GST activity in the liver of *S. herzbergii* in the comparison between fish from the contaminated site and those from the reference site ($P < 0.05$). Morphometric (length and weight) parameters and gonadosomatic index of collected fish were significant in the linear model of GST activity only in the reference site. These results may be due to the activity pattern of the enzyme, which increases with the sexual maturity of the animals in healthy environments. In the contaminated area (A1) these correlations do not exist, probably as a result of the energy used in the biotransformation of the various contaminants.

Keywords: biomonitoring, gonadosomatic index, catfish, São Marcos Bay, Brazil.

Glutatión S-Transferasa como biomarcador en *Sciades herzbergii* (Siluriformes: Ariidae) para el monitoreo ambiental: el caso de estudio de la bahía de São Marcos, Maranhão, Brasil

RESUMEN. La actividad de Glutatión S-Transferasa (GST) ha sido propuesta como un biomarcador de susceptibilidad a la presencia de xenobióticos potencialmente perjudiciales en los organismos acuáticos. El objetivo de este trabajo fue medir la actividad de GST en el hígado del bagre *Sciades herzbergii* para evaluar los efectos bioquímicos de los contaminantes. Las muestras de bagres fueron colectadas a lo largo de aéreas con gradientes de contaminación conocidas (A1) y en áreas consideradas relativamente libres de intervención antropogénica (A2), en la bahía de São Marcos, São Luis de Maranhão, Brasil. Las variables analizadas en los peces fueron: longitud, peso, estado gonadal (madurez sexual), índice gonodosomático y actividad de la enzima GST. Las bases de datos de los análisis fueron compiladas y se utilizaron modelos generales lineales y no lineales para analizar la dependencia entre la actividad de la enzima en peces colectados en las aéreas de muestreo A1 y A2. Una diferencia significativa en la actividad de la GST fue observada en peces colectados en el área contaminada (A1) comparada con la actividad enzimática de aquellos colectados en el sitio de referencia (A2) ($P < 0,05$). El peso, longitud, estados de madurez sexual y el índice gonodosomático fueron significativos en el modelo lineal de actividad de la GST en A2, pero no en A1. Estos resultados pueden ser debidos a los patrones de actividad enzimática que aumenta con la madurez sexual de los peces en ambientes

saludables (A2). En el área contaminada (A1) estas correlaciones no existen, probablemente como resultado de la energía usada en la biotransformación de los contaminantes.

Palabras clave: biomonitoreo, índice gonodosomático, bagre, bahía de São Marcos, Brasil.

Corresponding author: Raimunda Carvalho-Neta (raifortes@gmail.com; raimundafortes@yahoo.com.br)

INTRODUCTION

The use of biomarkers has become relevant in (eco) toxicological assessments since it allows the early detection of overall effects of contaminants (Passino, 1984; Livingstone, 1993; Ahmad *et al.*, 2006; Amado *et al.*, 2006; Camargo & Martinez, 2006; Umbuzeiro *et al.*, 2006; Zanette *et al.*, 2006).

The International Council for the Exploration of the Sea (ICES) has proposed the evaluation of antioxidant and biotransformation enzymes as biomarkers of exposure to xenobiotics. The main antioxidant defence enzymes in aquatic organisms are the superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and the phase II biotransformation enzyme glutathione-S-Transferase (Pavlović *et al.*, 2004). Glutathione-S-Transferases (GSTs) are a multigene family of dimeric, mainly cytosolic enzymes, which play an important role in the biotransformation and detoxification of a number of electropholic compounds (Rao, 2006). The alterations in the GST activities directly reflect the metabolic disturbances and cell damage in specific organs of fish (Livingstone *et al.*, 2001).

Previous field studies using catfish (*Sciades herzbergii*) as a model on harbour water exposure under natural environmental condition showed that GST is a useful biomarker of pollution in São Marcos Bay, Brazil (Carvalho-Neta & Abreu-Silva, 2010). The *S. herzbergii* is a benthic euryhaline catfish widely distributed along the São Marcos Bay, an important fishing location and it has the most important port in the Northeastern Brazil. In the last decade, chemical contamination of this Bay due to sewage discharge, the nutrient runoff from pesticides, and industrial waste, have all induced environmental impact. The main contaminants that can be found in this area, such as heavy metals, derive from industrial activities through their respective marine terminals (Itaqui, VALE and ALUMAR/ALCOA) located in São Luís Island (Carvalho-Neta & Abreu-Silva, 2010). Other non-polluted areas are also found in São Marcos Bay. An example is the Caranguejos (Crab) Island, which is part of the Environmental Protected Area.

The aim of the present study was to investigate if the contaminants present in the marine terminals located in São Luís Island are generating biological responses by comparing catfishes from this site with those collected in the Caranguejos Island, a non-polluted site. To accomplish this objective, GST activity was measured in tissue samples from catfishes collected in these two regions, in rainy and dry periods.

MATERIALS AND METHODS

Study area

The studied areas are located along the Brazilian coast: São Luís Island and Caranguejos (Crab) Island (São Marcos Bay, Maranhão, Northeastern Brazil). São Marcos Bay is an estuarine system located in the Gulf of Maranhão (Fig. 1).

To evaluate the impact of human activities in the areas, two sampling sites were chosen in each studied region. The first site (A1) is located near the Itaqui port (2º42'18"S, 44º22'33"W) which receives agricultural, industrial and domestic sewage effluents, as well as harbor related residuals. The reference site (A2) was located in Caranguejos (Crab) Island, a protected natural reserve (3º2'18"S, 44º40'25"W).

Itaqui port is located to the west coast of São Luis Island. The infrastructure of this port has an extension 1,616 m of quaysides. Fuel, iron, ore, manganese, grains, fertilizers, petroleum, bauxite and alumina are shipped in this port (ANTAQ, 2010). The Caranguejos (Crab) Island, located at a distance of 30 km from the Itaqui port in São Marcos Bay is an Environmental Protection Area created by the government of the Maranhão. This region is uninhabited and occupies an area of 345.08 km^2 within a perimeter of 165.95 km, containing the largest continuous stretch of mangroves in Maranhão (Carvalho-Neta & Castro, 2008).

Sample collection

Specimens of *S. herzbergii* were collected from three streams, about a kilometer from each other at each site (A1 and A2). The capture of fish was made during the rainy period (June 2010) and the dry period (November 2010).

The catfish were collected in their natural habitats using gill nets. Fish were anesthetized by placing them

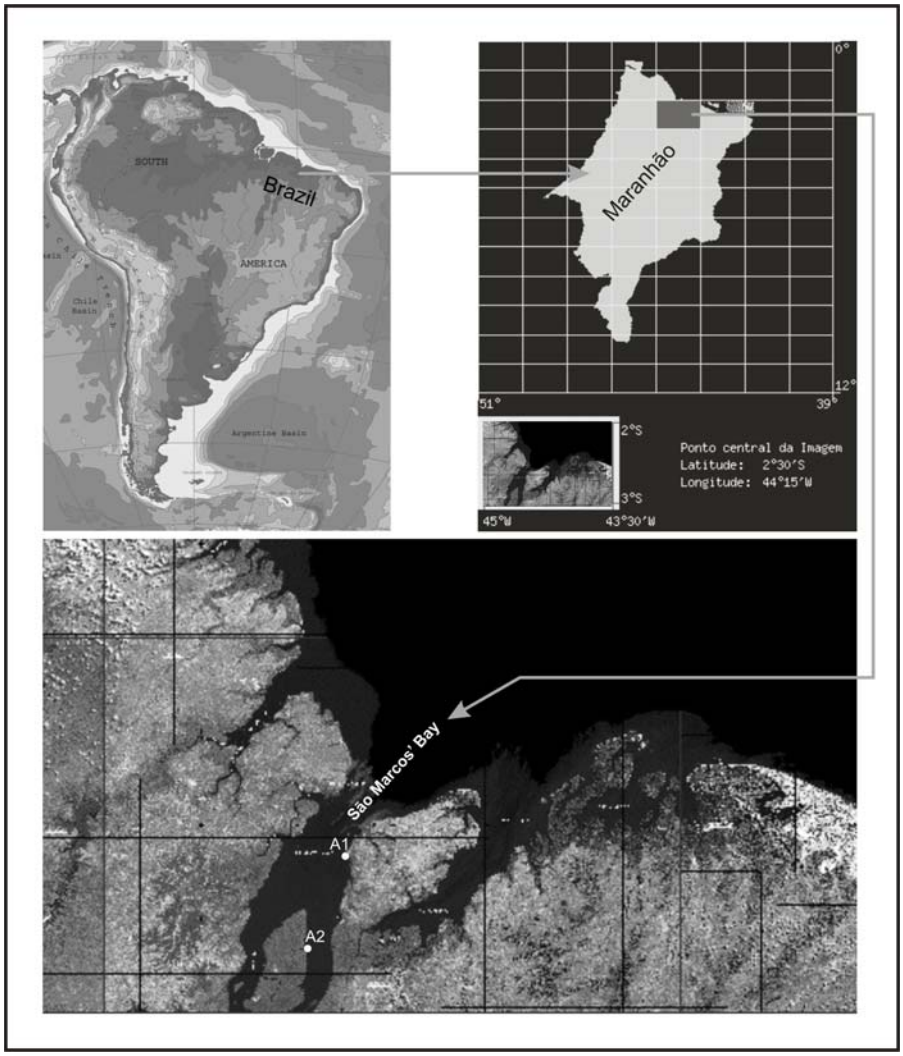

Figure 1. Map showing São Marcos Bay with details of its sampling sites (A1: contaminated, A2: reference).

for 10 min in benzocaine hydrochloride and then sacrificed by cervical section in order to remove the liver. Livers were frozen in liquid nitrogen and then stored at -80°C until GST activity assays.

At each sampling-site, water temperature, salinity, turbidity, dissolved oxygen (DO), conductivity and pH were measured. Water and sediment samples collected from sampling-sites along São Marcos Bay were analyzed for dissolved aluminum, total cadmium, dissolved iron, manganese, lead, total mercury, benzene, phenols, tributyltin and polychlorinated biphenyls. Metal determinations were performed by flame atomic absorption spectrophotometry (AAS), modified, with a nitrous oxide-acetylene flame reading (Abollino *et al.*, 1995). Organic analysis in water and sediment samples was performed by Electrochemical (EC) Methods.

Tissue processing and biochemical measurements

To determine glutathione-S-transferase (GST) activity, liver samples were processed according to previously established protocols (Livingstone, 1988). They were homogenized with 1:4 vol. of buffer (Tris-HCl 50 mM, 0.15 M KCl, pH 7.4), and centrifuged at 9,000 g for 30 min at 4ºC. A fraction of the supernatant was centrifuged at 37,000 g for 60 min at 4ºC, to obtain the cytosolic fraction. This fraction was used to analyze the enzymatic activity of the GST. Protein concentration of the supernatant was determined in accordance with the modified method of Lowry *et al.* (Peterson, 1977), using bovine serum albumin as a standard.

GST activity was assessed following the method described by Habig & Jakoby (1981). This method is based on the conjugation of 1 mM glutathione (Sigma)

with 1 mM of 1-chloro-2,4-dinitro-benzene (CDNB; Sigma). Enzyme activity was measured as absorbance increments at 340 nm and it was expressed in GST units, where one unit is the amount of enzyme necessary to conjugate 1 µmol de CDNB/min/mg protein, at 25°C and pH 7.00. The GST activity is expressed as µmol min^{-1} mg^{-1} protein.

Biometric data and gonadosomatic index

For all the fish whose livers were removed biometric data were recorded: total length (Lt), standard length (Ls), furcal length (Lf), total weight (Wt) and gonad weight (Wg). The macroscopic classification of the gonadal stage (GS) was also undertaken: immature (GS1), maturing (GS2), mature (GS3) and spent (GS4), following the scale given by Vazoller (1996). The gonadosomatic index (GSI) was calculated as gonad wet weight expressed as a percentage of body wet weight (total weight).

Statistical analysis

Results were expressed as mean ± standard deviation. For each variable studied differences observed among the sampling-sites (on the potentially contaminated and the reference site) were tested for significance by single-factor ANOVA or the Kruskal-Wallis test, depending on whether the data followed a normal distribution and showed homogeneity of variance. Significant differences between groups (A1 and A2) were verified using the Student's t-test. Confidence level adopted was 95%. Linear correlation (Pearson's coefficient) between GST activity and biometric data (Lt, Ls, Lf, Wt, Wg and GSI) were calculated using the mean values observed for each parameter.

RESULTS

Water and sediment chemistry measured at the moment of fish collection is listed in Tables 1 and 2. The concentrations of Al, Cd, Pb, Cr, Fe, Hg, benzene, total phenols, tributyltin and polychlorinated biphenyls in potentially contaminated site were higher than the acceptable limit by national standards (CONAMA, 2005). In the sediment (A1: potentially contaminated) these values were even higher (30 to 100%), confirming the character of chemical contamination of the marine terminal (São Luís Island). The average water surface temperatures were constant during both the periods analyzed. Salinity was uniform in both areas sampled, being lower during the rainy season in both areas. Dissolved oxygen and the saturation of dissolved oxygen were always lower in the contaminated area. The values for pH and turbidity were constants for both areas, demonstrating the homogeneity of these abiotic factors in both areas studied.

Morphometric parameters (total length, standard length, furcal length, total weight and gonad weight) of collected fish are showed in Table 3. At the non-polluted site, catfishes were bigger ($P < 0.05$) than those from the polluted area. The gonadosomatic index (GSI) was also higher in the reference site than the one from the polluted site in both periods (Fig. 2). The GSI in fish from the contaminated site was significantly lower ($P < 0.05$) than in control fish during all the phases of the gonadal cycle.

In each period, differences (t-test; $P < 0.05$) in GST activity were found between catfishes from the polluted and non-polluted sites (Tables 4 and 5). When comparing GST values among females and males, activity did not appear to be sex-dependent ($P > 0.05$). In the contaminated area the greatest values for the GST activity (2.82 ± 0.33 µmol mg^{-1} of protein) were registered in juveniles females (GS1). However, in the reference area the values for enzymatic activity increased progressively with the gonads stages (GS1-GS2-GS3-GS4) in males and females (t-test; $P < 0.05$). On the other hand, seasonal variations were not observed in the GST activity of catfishes from the A1 and A2 ($P > 0.05$).

A significant linear correlation was established among GST activity and all the biometric data of the reference area, but not with those of the contaminated area in any of the periods analyzed (Tables 6 and 7).

DISCUSSION

In the present study, GST activity showed a different pattern in catfishes from both polluted and non-polluted sites. In a similar study carried out by Carvalho-Neta & Abreu-Silva (2010), samples of *Sciades herzbergii* (Ariidae) caught at São Marcos Bay showed significantly higher levels of GST only in polluted areas (compared to Caranguejos Crab Island), which confirms that São Marcos Bay is a site with high exposure risks for some contaminants.

São Marcos Bay receives contaminant input from hundreds of industrial and domestic sources in the São Luís (Maranhão, Brazil) metropolitan area. Benzene, phenols, TBT and the PCB measured in the contaminated area of São Marcos Bay were considerably greater than the largest tolerable value prescribed by Brazilian Environmental legislation (Conselho Nacional de Meio Ambiente, 2005) and can affect the detoxifying responses and biometric data of many aquatic species, especially fish (Oruç *et al.*, 2004).

Table 1. Physical and chemical analysis of water in rainy and dry periods at the polluted and non-polluted sites of the São Marcos Bay (Brazil). Rainy period: June 2010, dry period: November 2010.

Parameters	Contaminated (A1)						Reference (A2)					
	Rainy period			Dry period			Rainy period			Dry period		
	Point 1	Point 2	Point 3	Point 1	Point 2	Point 3	Point 1	Point 2	Point 3	Point 1	Point 2	Point 3
Temperature (^{o}C)	29.0	29.0	29.0	29.1	29.1	29.1	29.0	29.0	29.0	29.0	29.0	29.0
Salinity (UPS)	14	14	14	15	15	15	10	10	10	15	15	15
pH	8.2	8.1	8.3	8.2	8.1	8.3	8.1	8.1	8.1	8.0	8.1	8.1
DO (mL D_2 L^{-1})	5.1	5.1	5.1	4.9	4.9	4.9	6.1	6.1	6.1	6.0	6.0	5.9
% satur. OD	86.3	86.7	86.4	86.2	86.3	86.1	88.9	88.8	88.7	88.6	88.7	88.6
Turbidity (NTU)	14.0	13.0	13.0	12.0	12.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0
Aluminum (mg L^{-1})	0.2	0.1	0.1	0.2	0.2	0.2	0.001	0.001	0.001	0.001	0.001	0.001
Cadmium (mg L^{-1})	0.007	0.007	0.007	0.007	0.007	0.007	0.003	0.003	0.003	0.003	0.003	0.003
Lead (mg L^{-1})	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01
Chromium (mg L^{-1})	0.08	0.08	0.08	0.08	0.08	0.08	0.03	0.03	0.03	0.03	0.03	0.03
Iron (mg L^{-1})	0.7	0.7	0.7	0.8	0.8	0.8	0.2	0.2	0.2	0.3	0.3	0.3
Mercury (mg L^{-1})	0.001	0.001	0.001	0.002	0.002	0.002	0.000	0.000	0.00	0.000	0.000	0.000
Benzene (µm L^{-1})	850	850	850	900	900	900	50	50	50	100	100	100
Total phenols (mg L^{-1})	0.004	0.004	0.004	0.004	0.005	0.005	0.001	0.001	0.001	0.001	0.001	0.001
TBT (µm L^{-1})	0.02	0.02	0.02	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Total PCB (µm L^{-1})	0.06	0.06	0.06	0.05	0.05	0.05	0.01	0.01	0.01	0.01	0.01	0.01

Table 2. Physical and chemical analysis of sediment in rainy and dry periods at the polluted and non-polluted sites of the São Marcos Bay (Brazil). Rainy period: June 2010, dry period: November 2010.

Parameters	Contaminated (A1)						Reference (A2)					
	Rainy period			Dry period			Rainy period			Dry period		
	Point 1	Point 2	Point 3	Point 1	Point 2	Point 3	Point 1	Point 2	Point 3	Point 1	Point 2	Point 3
Temperature (°C)	28.6	28.6	28.5	29.0	29.0	28.6	28.5	28.5	28.5	28.5	28.5	28.5
Salinity (UPS)	14	14	14	15	15	15	10	10	10	15	15	15
pH	5.8	5.7	5.7	5.8	5.8	5.8	5.5	5.5	5.5	5.5	5.5	5.5
DO (mg L^{-1} O_2)	2.9	3.8	3.7	2.8	2.9	2.8	3.2	3.2	3.2	3.2	3.2	3.2
Aluminum (mg L^{-1})	0.5	0.5	0.5	0.6	0.6	0.6	0.001	0.001	0.001	0.001	0.001	0.001
Cadmium (mg L^{-1})	0.009	0.009	0.009	0.009	0.009	0.009	0.003	0.003	0.003	0.003	0.003	0.003
Lead (mg L^{-1})	0.05	0.05	0.05	0.05	0.05	0.05	0.02	0.01	0.01	0.02	0.01	0.01
Chromium (mg L^{-1})	0.09	0.09	0.09	0.09	0.09	0.09	0.05	0.05	0.05	0.05	0.05	0.05
Iron (mg L^{-1})	0.16	0.16	0.16	0.16	0.16	0.16	0.4	0.4	0.4	0.4	0.4	0.4
Mercury (mg L^{-1})	0.003	0.003	0.003	0.003	0.003	0.003	0.000	0.000	0.00	0.000	0.000	0.000
Benzene (µm L^{-1})	1800	1800	1800	1800	1800	1800	100	100	100	150	150	150
Total phenols (mg L^{-1})	0.008	0.008	0.008	0.008	0.008	0.008	0.001	0.001	0.001	0.001	0.001	0.001
TBT (µm L^{-1})	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Total PCB (µm L^{-1})	0.15	0.15	0.15	0.15	0.15	0.15	0.02	0.02	0.02	0.02	0.02	0.02

Table 3. Morphometric data of catfish (*Sciades herzbergii)* as mean values (± standard deviation) collected in rainy and dry period in the polluted and non-polluted sites of the São Marcos Bay (Brazil).

Parameters	Contaminated (A1)		Reference (A2)	
	Females	Males	Females	Males
Total length (cm)	29.36 ± 5.52	28.28 ± 6.64	57.49 ± 1.64	58.78 ± 1.67
Furcal length (cm)	23.2 ± 4.5	22.26 ± 4.6	52.5 ± 1.70	51.50 ± 1.60
Total weight (g)	216.2 ± 35.1	217.39 ± 58.35	355.4 ± 18.1	353.7 ± 15.19
Gonad weight (g)	2.01 ± 0.84	1.09 ± 0.95	15.75 ± 0.19	7.58 ± 0.16

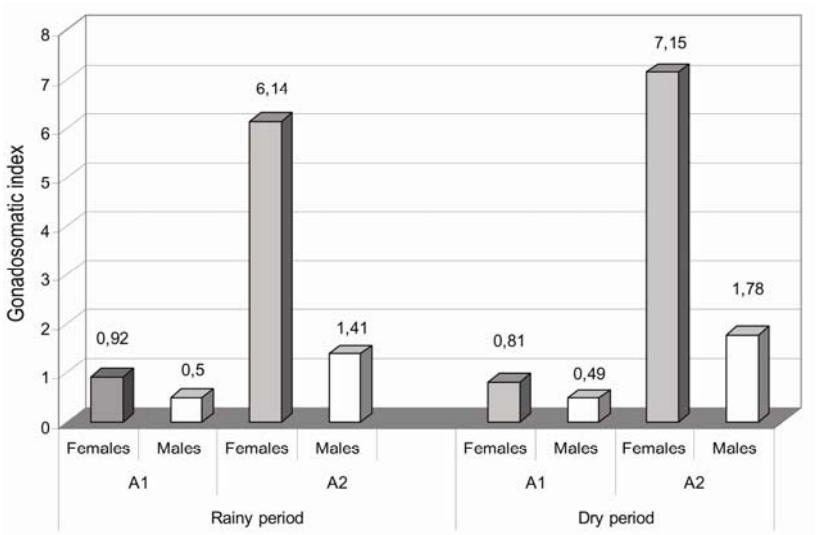

Figura 2. Gonadosomatic index of males and females of *Sciades herzbergii* collected in São Marcos Bay in the rainy (June 2010) and dry period (November 2010). (A1: contaminated, A2: reference).

Water and sediment chemistry parameters (temperature, salinity and oxygen) showed no seasonal variations, being similar in both studied sites (A1 and A2). However, a heavy metal concentration (Al, Cd, Pb, Cr, Fe and Hg) in surface waters and in sediments has much higher values for the contaminated area than for the area of reference. The heavy metals, such as Cd, Ni, Cr, Pb and Hg are especially toxic to aquatic organisms due to their oxidative potential, whereas other metals such as Fe, Zn, Cu, Se and Mn are essential for their metabolism but, could become toxic in excessive concentrations (Chang *et al.*, 1996).

Our results have shown a decrease in the gonadosomatic index (GSI) in fish from the contaminated site (São Marcos Bay). Several in situ studies have demonstrated a decrease in the GSI in fish collected from polluted areas (Carvalho-Neta & Abreu-Silva, 2010; Noaksson *et al.*, 2001). This decrease in the index can result in abnormal gonadal development in the form of delayed maturation, high levels of atresia or intersexuality (Kime, 1998).

The specimens of *S. herzbergii* of the contaminated area of São Marcos Bay had a significantly higher level of GST activity than that found in the organisms of the reference area. Previous studies using *Sciades herzbergii* caught in São Marcos Bay on the Maranhão coast showed a possible susceptibility of the youngest and sexually immature fish to oxidative stress in the studied area (Carvalho-Neta & Abreu-Silva, 2010). Increased GST activity in fish liver has been demonstrated in various fish species as the result of exposure to PCBs (Gadagbui & Goksoyr, 2001), PAHs and pesticides (Bello *et al.*, 2001).

The GST activity has grown linearly proportional to the phase of maturity of *S. herzbergii* only in the reference area. The same type of response was found by Winston & Di Giulio (1991), who demonstrated that the activity of detoxifying enzymes differs

between the various organs and tissues of fish, depending on their stage of gonadal development in health environments. GST is an important enzyme responsible for the hepatic degradation of sex steroids and marked differences observed in GST could be connected with hormone alterations (Gallagher *et al.*, 2001). However, exposure to xenobiotics usually causes changes both in enzyme expression and in reproductive endocrine system in fishes, initiating degenerative processes (Vargas *et al.*, 2001). GST activity showed a similar pattern in *S. herbergii* from both rainy and dry periods. Amado *et al.* (2006) in their study about biomarkers in croakers *Micropogonias furnieri* (Sciaenidae) from polluted and non-polluted areas from the Patos Lagoon estuary (southern Brazil) indicated a clear seasonal variation pattern for GST activity (enzymatic activity was higher in the warmer season). The results suggest that the seasonal adjustments in the antioxidant defence of fish from northern Brazil are different from those of southern Brazil. In southern Brazil the climate is subtropical with a wide water temperature range (up to 32ºC in summer and below 9ºC in some winter), but in northern Brazil the climate is tropical (Pell *et al.*, 2007). The temperature influences a great variety of biological processes of fish species (Bicego *et al.*, 2007). Consequently, significant changes in water temperature may cause a serious challenge to the maintenance of physiological function in fish (Garcia *et al.*, 2008).

The linear correlation among the GST activity when related to all the biometric data of the catfish is significant in the reference area, but not in the contaminated area, probably due to the pattern of enzyme activity which increases with age and sexual maturity of the animals in environments free of contaminants. In the port area, this correlation does not exist, probably as a result of the energy used in the biotransformation of the various contaminants in the water and in the sediment. The fact that the polluted site is characterized by higher levels of metals like Al, Cd, Pb, Cr, Fe, Hg, that can generate oxidative stress in fish, gives support to our hypothesis. This is a significant result because the morphometric differences between two groups of the population of the same fish species indicate that these populations are subjected to different selection processes (Shibatta & Hoffmann, 2005).

Ecological and biological factors should be taken into account to explain variations in enzymatic biotransformation activities in fish (Mayon *et al.*, 2006), but the use of GST in this study enhances our understanding of the *Sciades herzbergii in situ* as demonstrating its great suitability in the monitoring of polluted and non-polluted areas.

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Received: 16 May 2011; Accepted: 22 October 2012