Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9283
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v35i3.18032

Fish histopathology and catalase activity as biomarkers of the environmental quality of the industrial district on the Amazon estuary, Brazil

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ABSTRACT. The environment quality of an industrial district on the river Pará, Amazon estuary, Brazil, based on the assessment of histological alterations and on the determination of catalase activity of the hepatic tissue of two fish species, *Plagioscion squamossissimus* and *Lithodoras dorsalis*, is provided. Histopathological changes were evaluated semi-quantitatively and qualitatively. Mean Assessment Values (MAV) and Histological Alteration Index (HAI) of organ lesions were calculated for each zone under analysis, with different impact levels: Zone 1 (industrial district, with high contamination risk); Zone 2 (medium risk) and Zone 3 (minimum risk). Strong positive catalase activity and histopathological changes were reported in Zone 1. None of the specimens of either species captured in Zones 1 and 2 was healthy, whereas more than 60% of the specimens from Zone 3 presented healthy hepatic tissue. The principal alterations observed in the tissue of the two species included an increase in the number of Melanomacrophagous centers, fatty degeneration, inflammation, congestion, hepatitis and focal necrosis. The carnivorous *P. squamosissimus* presented higher levels of alteration than the herbivorous *L. dorsalis*. Results showed that local anthropogenic impacts were affecting the health of the two fish species under analysis.

Keywords: biomonitoring, liver, industrialization, northern Brazil.

Histopatologia e atividade catalase em peixes como biomarcadores da qualidade ambiental no distrito industrial, estuário Amazônico, Brasil

RESUMO. Com o presente estudo foi avaliada a qualidade ambiental em um distrito industrial no rio Pará, estuário Amazônico, Brasil, baseado nas alterações histológicas e na determinação da atividade da catalase no fígado para duas espécies de peixes: *Plagioscion squamossissimus* e *Lithodoras dorsalis*. As mudanças histopatológicas foram avaliadas semiquantitativamente e qualitativamente. Os Valores Médios de Avaliação (MAV) e o índice de alteração histológica (IAH) das lesões nos órgãos foram calculados para cada zona com diferentes níveis de impacto: Zona 1 (distrito industrial, com risco alto de contaminação); Zona 2 (risco médio) e Zona 3 (risco mínimo). Na Zona 1 foi observada, para as duas espécies, forte atividade catalase positiva e algum tipo de alteração histopatológica. Não foram observados indivíduos saudáveis nas Zonas 1 e 2. Na zona 3, mais de 60% dos espécimes estudados apresentaram tecido hepático saudável. As principais alterações observadas no tecido hepático para as duas espécies foram: aumento dos centros melanocacrófagos, degeneração gordurosa, inflamação, congestão, hepatite e necrose focal. A espécie carnívora *P. squamosissimus* apresentou níveis mais elevados de alteração que o herbívoro *L. dorsalis*. Estes resultados mostram que as alterações da qualidade ambiental no entorno do distrito industrial está afetando a saúde destas espécies de peixes.

Palavras-chave: biomonitoramento, fígado, industrialização, norte do Brasil.

Introduction

Ever since aquatic ecosystems have been considered potential sources of contamination, fish have been used as bioindicators and as biomonitor organisms for the monitoring of environments (DE LA TORRE et al., 2005; OOST et al., 2003; SCHLACHER et al., 2007). The use of biomarkers in environmental monitoring provides information on not only the intensity, tolerance limits and effects of pollutants on the organisms but also on the process of transfer of these substances within the trophic level (OOST et al., 2003). In fact, these markers serve as a 'warning sign' of environmental integrity. They may be used to guide the development of effective bioremediation measures before the environment would undergo irreversible damage (DE LA TORRE et al., 2005).

Histopathological and biochemical analysis is widely used in environmental monitoring research. The organs targeted in this approach respond incisively to exposure to xenobiotic and other toxic substances. In fact, such alterations may be identified relatively easily (LIVINGSTONE, 1992). Fish liver is an excellent organ for the study of environmental quality biomarkers, due to its role in the specimen's metabolism, which include the production of proteins, the oxidation, conjugation, methylation, inactivation or detoxification of substances, or rather, the excretion of pollutants (BRUSLÉ; ANADON, 1996; CARROLA et al., 2009; ROBERTS, 2000).

The South American silver croaker *Plagioscion squamosissimus* (Heckel, 1840), Sciaenidae, and the rock-bacu *Lithodoras dorsalis* (Valenciennes, 1840), Pimelodidae, play an important role in the local economy and in the subsistence of the communities within the area of the municipality of Barcarena, in the state of Pará, Brazil, on the Amazon estuary (PAZ et al., 2011). The two species are also relatively abundant throughout the study area. *P. squamosissimus* is a carnivorous, zoobenthic species, feeding on shrimp and fish (COSTA et al., 2009), while L. dorsalis is an opportunist species, feeding mainly on available plant material and seeds (SANTOS et al., 2006).

The selection of species with different feeding habits is important for current study since certain pollutant substances may accumulate at different rates at distinct trophic levels (TERRA et al., 2008). There may also be variations in the neutralization, modification or biotransformation of different substances, depending on their potential toxicity. Owing to their position at the top of the food chain, carnivorous fish are more affected by the accumulation of contaminants than herbivores and are more likely to affect humans through the consumption of contaminated fish (LAWRENCE; HEMINGWAY, 2003). Since the species analyzed are widely consumed in the local communities (ESPIRITO SANTO et al., 2005), the need for the assessment of possible contamination risks for the human population is reinforced.

The industrial processing of these minerals produces several residues, including fluorides, chlorides, sulfates and bicarbonates (RUBIO; TESSELE, 2002), which may provoke significant alterations in the quality of aquatic ecosystems. The assessment of environmental quality using biomarkers is extremely important, not only for the understanding of contamination effects on the ecological equilibrium of the area, but also for the social and economic implications vis-à-vis the local riverside communities that depend on these natural resources. Current study, which evaluates the environment quality of an industrial district in the river Pará (Amazon estuary), was based on the assessment of the occurrence, type and intensity of histological alterations and the determination of the catalase activity of the hepatic tissue of the fish species *Plagioscion squamossissimus* and *L. dorsalis*.

Material and methods

Study area and data collection

The area under analysis lies on the right bank of the river Pará in the Brazilian state of Pará, Amazon estuary, and consists of an estuarine environment with a considerable freshwater input, classified as a tidal freshwater estuary, according to Elliot and McLusky (2002). Current investigation was designed to test the effects of the probable environmental contamination in the area adjacent to industrial installations and cargo terminal on the estuary of the river Pará. The experimental design was organized in three distinct zones which represent different impact levels: Zone 1, located in the vicinity of the cargo terminal and industrial district (Figure 1), where the risk of contamination was highest; Zone 2, adjacent to Capim Island (Figure 1), distant 17 km from the cargo terminal and industrial district, classified as medium risk area due to its relative proximity to the Zone 1; Zone 3, located on Onças Island (Figure 1), distant 38.42 km from the cargo terminal and industrial district, classified as minimum risk due to its distance from Zone 1. Specimens were collected during the dry (September and December 2009) and rainy seasons (June 2009 and March 2010).

Immediately after capture, the specimens were weighed and their livers were removed and weighed. A sample of the liver was taken and fixed in Bouin solution for 48h and another sample was fixed with 'paraformaldehyde' solution (4%) for 24h.

Biological and histological parameters

Catalase (CAT) activity was evaluated by tissue dehydration in increasing concentrations of ethanol, washed in PBS tween 0.05% (tween 25564, SEM Hartfield, PA) for the rupture of membranes. Subsequently, it was incubated in primary antibody overnight antirabbit diluted 1:500 (PA 1-28564, Bioamerica Inc.), followed by a secondary antibody (Rabbit anticatalase, polyclonal, Chemical Int.) and finally incubated with diaminobenzidine (DAB - ImmPacttm, SK-4105).

Samples for histopathological analyses were embedded in paraffin, stained with HE (haematoxylin and eosin), analyzed and photographed by light microscopy. The histopathological changes were evaluated in semi-quantitative mode by ranking the severity of the tissue lesions, following Schwaiger et al. (1997) with modifications. A numerical value was assigned for each animal according to change degree:

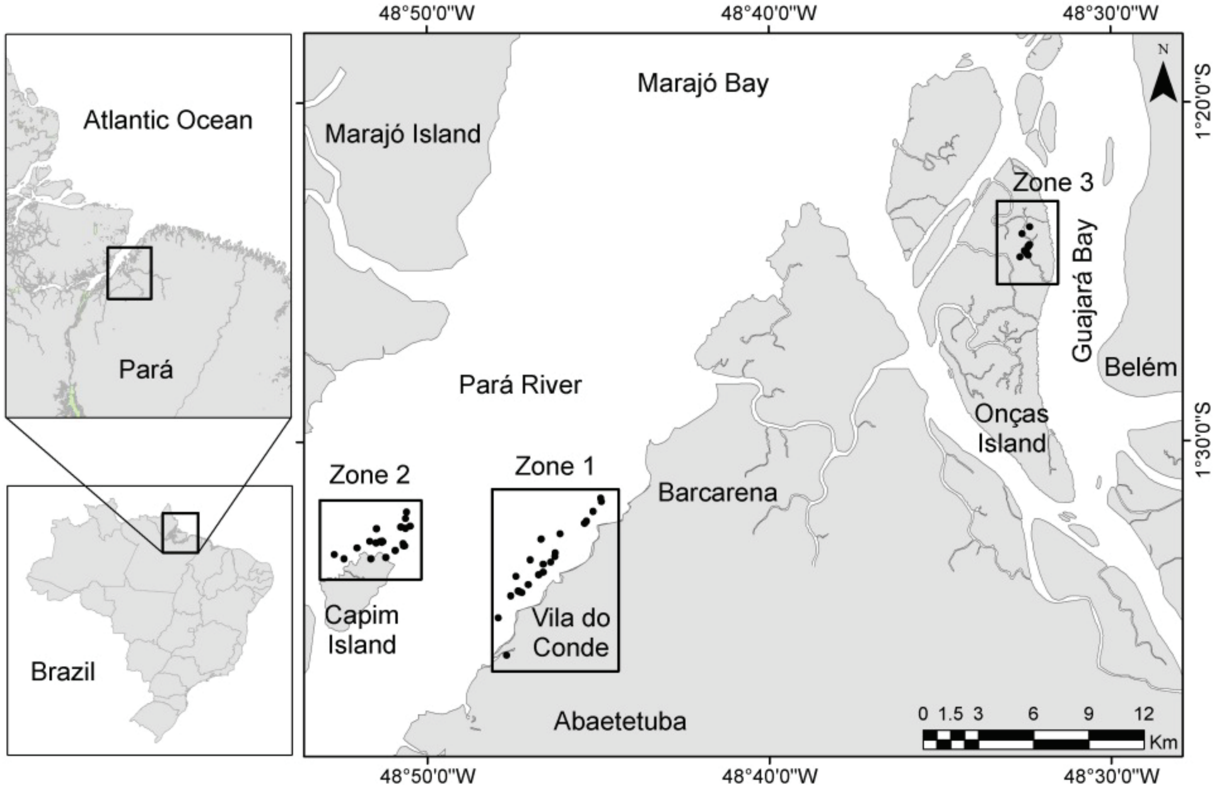

Figure 1. Study area on the estuary of the river Pará (Amazon estuary), with the sampling points within each zone. Zone 1 (maximum impact), Zone 2 (medium risk) and Zone 3 (minimum impact).

Grade 1 – mild focal changes; Grade 2 - mild to moderate focal changes; Grade 3 - severe and extensive pathological alterations. Ranking established an overall assessment rate of the histopathological lesions for each fish. Mean Assessment Values (MAVs) of organ lesions were calculated for each study area on the above data basis.

The severity of the tissue lesions was assessed by a modified ranking scheme by Poleksic and Mitrovic-Tutundzic (1994): Stage I – alterations which do not affect the normal functioning of the tissue; Stage II – severe alterations which impair normal functioning of the tissue; Stage III – severe alterations that cause irreparable damage (Table 1). These data were used to estimate the Histological Alteration Index (HAI) for each animal: HAI= $10^1 \sum I + 10^2 \sum II + 10^3 \sum III$. Mean HAI rates assessed the severity of the functional impairment of the liver and its capacity for regeneration of each species. Rates 0 - 10 indicate normal functioning of the organ; rates 11-20 represent slight damage; rates 21 - 50 moderate changes; 51-100 severe lesions; rates over 100 indicate irreversible damage to the organ.

The differences between zones and seasons were tested by one-way ANOVA, followed by Tukey's *post hoc* test or the Kruskal-Wallis non-parametric analysis of variances, with a ranked means multiple comparison test (ZAR, 1996). Multivariate Multidimensional Scaling (MDS) was used to compare the occurrence of histopathological alterations by zone and season. This analysis was based on Euclidean distance coefficients for presence/absence data for the full set of 19 histopathological indicators in the two species. Data from Zone 2 were excluded from this analysis, given the reduced number of specimens collected for both species. The significance of all the groups defined by this approach was tested by two-way nested ANOSIM.

Table 1. Classification of histopathological alterations of the liver, modified from Poleksić and Mitrovic-Tutundzic (1994).

Type of alteration	Standard reaction	Stage
1 Alteration of the Hepatocytes	Deformation of the cell contour	I
	Cellular hypertrophy	I
	Cellular atrophy	I
	Melanomacrophagous centers	I, II and III
	Vacuolization of the cytoplasm	I
	Degeneration of the cytoplasm	II
	Fatty degeneration	II
	Cell rupture	II
	Inflammation	II and III
	Congestion	II
2 Alteration of the blood vessels	Congestion	II
	Hepatitis	II and III
3 Necrosis	Focal necrosis	III

Results

The catalase activity in hepatocytes of fish caught in Zone 3 was low (negative staining for the enzyme). However, positive staining was observed in hepatocytes of animals captured in Zones 1 and 2 (Figure 2).

Some form of pathology occurred in 68.82% of the 385 histologically analyzed specimens. All specimens captured in Zones 1 and 2 had pathological alterations. Contrastingly, in Zone 3, based on the absence of pathological alterations of the liver, approximately 60% of *P. squamosissimus* specimens and 75% of *L. dorsalis* were considered healthy.

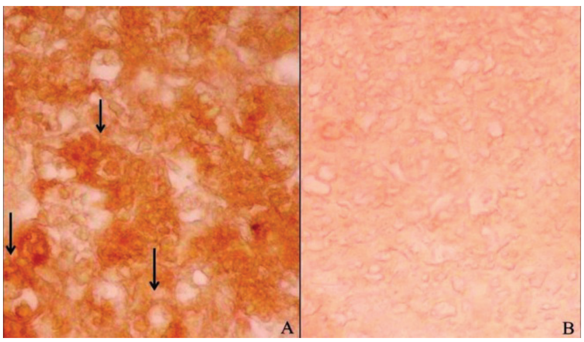

Figure 2. Photomicrograph of the catalase activity in the hepatic tissue of *Plagioscion squamosissimus* and *Lithodoras dorsalis*. A – Hepatocyte with dark deposit showing positive staining for catalase. 400X. B – Hepatocyte without marking the activity of catalase. 400 X.

Mean Assessment Values (MAV) of organ lesions calculated for each species were significantly lower in Zone 3 when compared to those in other zones (Kruskal-Wallis, d.f. = 2, $p < 0.001$) although no seasonal effect was observed (Kruskal-Wallis, d.f. = 1; $p > 0.05$, Table 2). Since no *L. dorsalis* specimen from Zone 2 was captured during the rainy season, none was examined histologically and no MAV occurs for this period.

Table 2. Mean Assessment Values – MAV (%) for *Plagioscion squamosissimus* and *Lithodoras dorsalis* by zone (Z): Zone 1 (maximum impact); Zone 2 (medium risk) and Zone 3 (minimum impact). N/A = no alteration; Grade 1 = mild focal changes; Grade 2 = mild to moderate focal changes; Grade 3 = severe and extensive pathological alterations. n – number of specimens.

	P. squamosissimus			*L. dorsalis*		
	%MAV			%MAV		
Degree of change	Zone 1 (n:83)	Zone 2 (n:51)	Zone 3 (n:91)	Zone 1 (n:72)	Zone 2 (n:8)	Zone 3 (n:80)
N/A	0	0	69,23	0	0	66,25
1	0	0	19,78	0	0	16,25
2	8,43	33,33	10,99	23,61	25	16,25
3	91,57	66,67	0	76,39	75	1,25

The absence of liver pathological alterations was obtained only from Zone 3. The organs had a homogeneous hepatic parenchyma with hepatocyte cords and sinusoidal capillaries surrounded by the portal vein (Pv) (Figure 3A and B). Alterations in the two species, comprising an increase in the number of MMCs, fatty degeneration, inflammation, congestion, hepatitis and focal necrosis, were reported in other zones (Figure 3 C, D, E and F). Table 1 shows other alterations.

Specimens from Zones 1 and 2 also presented high frequencies of alterations at all stages of severity (678 alterations for *L. dorsalis* and 1.162 for *P. squamosissimus*) when compared to those in Zone 3 (72 alterations for each species) and no alteration in stage III. While the two species presented similar patterns of alteration, those observed in *P. squamosissimus* were generally more severe than those in *L. dorsalis*.

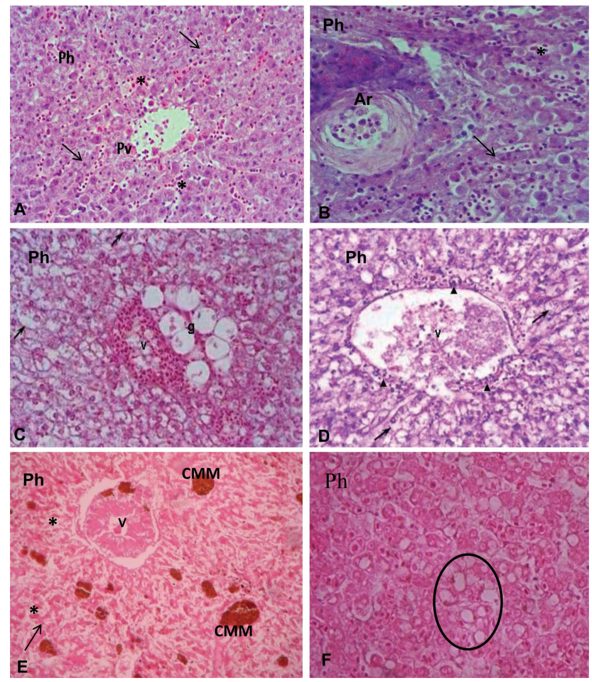

Figure 3. Photomicrograph hepatic tissue of *Plagioscion squamosissimus* and *Lithodoras dorsalis*. A) and B) Normal hepatic tissue: Ph – Hepatic parenchyma with blood vessel, detail of the portal vein (Pv) and hepatic artery (Ar), hepatic cords (fine arrow) and sinusoidal capillaries(*). A 100x, B 400x.HE. C) inflammation of hepatic tissue: hepatic parenchyma (ph) with spongy appearance, vacuolized hepatocyte (fine arrow) and lipid deposit (g) 100 x; D) hepatic tissue altered by hepatitis: dilated veins (v), hypertrophied hepatocytes (fine arrow) and agglomeration of blood cells (arrow head) 400 x; E) Hepatic tissue with concentration of melanomacrophagous centers (MMCs) and congested vein (v), sinusoidal capillaries(*) and hepatocyte cords (fine arrow), 100x; F) necrotic tissue with intense cell death (circle), 100x. HE.

The Histological Alteration Index (HAI) varied significantly among the zones for both *P. squamosissimus* (Kruskal-Wallis, d.f. = 2, $p < 0.001$) and *L. dorsalis* (Kruskal-Wallis, d.f. = 2, $p < 0.001$). Results for Zone 3 indicate that the specimens had relatively healthy livers, with HAI rates of less than 10 (Figure 4), with mean rates 2.52 (S.D. ± 5.1) for *P. squamosissimus* and 2.70 (S.D. ± 5.33) for *L. dorsalis*. Contrastingly, in Zones 1 and 2, all the livers analyzed presented some form of function-impairing alteration, with HAI values above 100 in the two areas (Figure 4). HAI rates for *P. squamosissimus* were also significantly higher than those recorded for *L. dorsalis* (Kruskal-Wallis, d.f. = 1, $p < 0.05$), with mean rate 115.84 and 90.25, respectively. No significant difference was recorded between the rainy and dry seasons (Kruskal-Wallis, d.f. = 1, $p > 0.05$).

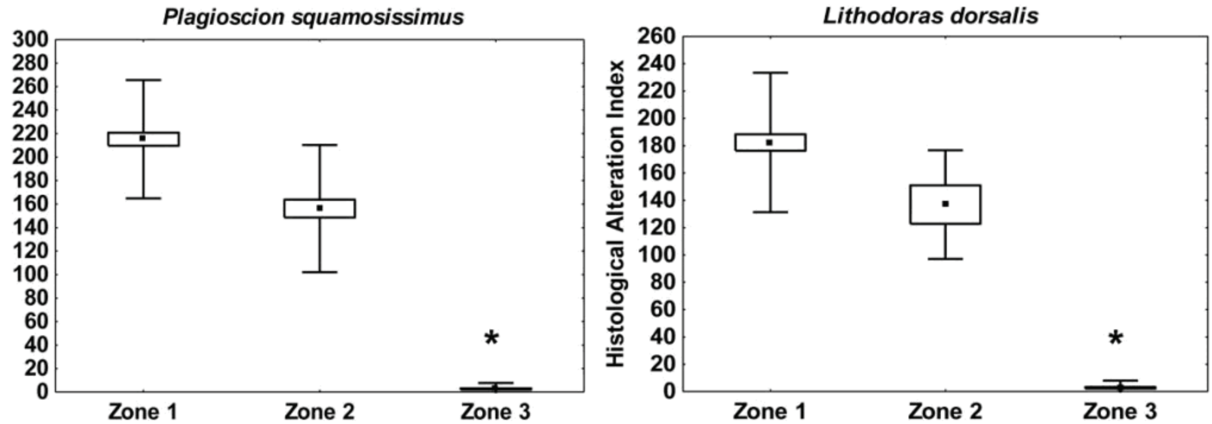

Figure 4. The Histological Alteration Index (HAI), with mean rates of specimens of *Plagioscion squamosissimus* and *Lithodoras dorsalis* by Zone (Z): Zone 1 (maximum impact); Zone 2 (medium risk); Zone 3 (minimum impact). (*): significant at $p < 0.001$.

The multivariate analysis of the histopathological indicator set revealed significant differences among zones for the two species (ANOSIM, d.f. = 3, 19, $R = 0.95$, $p < 0.01$) (Figure 5). MDS analysis indicated a clear separation between Zones 1 and 3, primarily due to the much lower frequency of histopathological alterations in Zone 3.

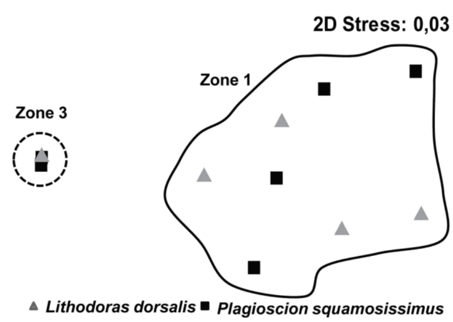

Figure 5. Multivariate multidimensional scaling (MDS) based on the presence/absence of histopathologies for *Plagioscion squamosissimus* and *Lithodoras dorsalis* by Zone (Z): Zone 1 (maximum impact), Zone 2 (medium risk) and Zone 3 (minimum impact).

Discussion

The use of histopathological and biochemical biomarkers is one of the best procedures for the evaluation of the effects of pollutants on aquatic ecosystems. The liver is often used for such studies since it produces reliable results for the evaluation of environmental quality (CARROLA et al., 2009; SCHWAIGER et al., 1997; VASYLKIV et al., 2011).

Catalase analysis showed strong CAT activity in the hepatocyte of the two species caught close to the cargo terminal and the industrial district (Zones 1 and 2).

CAT activity is often enhanced in high oxidative stress as an adaptive response to help detoxify oxygen-free radicals and limit or prevent damage to macromolecules (KERAMATI et al., 2010; ORBEA et al., 1999, ORTIZ-ORDOÑEZ et al., 2011, VASYLKIV et al., 2011). The histopathological analyses emphatically foregrounded the conclusion that local anthropogenic impacts were affecting the health of *P. squamosissimus* and *L. dorsalis*. MAV and HAI rates and MDS analysis indicated clear differences between the areas surveyed. Alterations were more severe (in some cases, irreversible) in Zone 1, which was closest to the cargo terminal and the industrial district. In their experimental study, Schwaiger et al. (1997) recorded high MAVs for the livers of *Salmo trutta fario* and *Barbatula barbatula* exposed to contaminants, which reinforces the effectiveness of histopathological alteration in this organ.

In Zones 1 and 2, the most significant alterations in the two species comprised an increase in the number of melanomacrophagous centers (MMCs), fatty degeneration, inflammation, hepatitis, congestion of the blood vessels and focal necrosis. Similar results have been obtained for contaminated natural environments with adequate water quality in studies involving *Pleuronectes vetulus* (STEHR et al., 2003), some cyprinids species (GÜL et al., 2004) and *Salmo trutta f. fario* (CARROLA et al., 2009).

Fatty degeneration for the two species was confirmed by isolated or group fatty globules close to the hepatic vein. Marchand et al. (2008) reported histopathological alterations (MMCs and fatty degeneration) in *Clarias gariepinus* obtained from polluted aquatic environments. Both were associated with contamination by metals. A similar situation was also likely in current study area due to the evidence of contamination by heavy metals (BERRÊDO et al., 2001; LIMA et al., 2011). Hepatitis is a degenerative lesion of the liver, associated with a process of cellular inflammation, while congestion is an accumulation of blood in the sinusoids (HIBIYA, 1982), observed in the two species analyzed in current study.

Focal necrosis is associated with the inflammatory process. Nero et al. (2006) found a strong correlation between petroleum concentrations and focal necrosis in the liver of *Perca flavescens* and *Carassius auratus*. While this condition is irreversible, it does not disable the liver completely since the associated release of chemical signals induces the proliferation of cells that substitute the necrotic material and thus maintains the liver's structure and functions (MELO et al., 2008).

Hepatic alterations in current study were generally more intense in the carnivorous *P. squamosissimus*. Carnivorous fish tend to ingest and accumulate more toxins than organisms that occupy the lower trophic levels (TERRA et al., 2008). This is due to the fact that they normally feed on already contaminated organisms and is especially true when the prey species are benthic organisms (ASUQUO et al., 2004; TERRA et al., 2008).

Previous studies of the sediments of Zone 1 have recorded levels of heavy metals (Cadmium, Cooper, Chromium, Iron, Zinc, Aluminum, and Sodium) above the limits permitted by Brazilian legislation (BERRÊDO et al., 2001; LIMA et al., 2011). This situation appears to be related primarily to the local processing of bauxite and to the industrial accidents that have occurred within the study area. High levels of heavy metals may be extremely damaging to the aquatic biota, due to their accumulation in both sediments and in the trophic web by transference. This appears to be confirmed by the hepatic alterations and biochemical observed in the fish specimens analyzed in current study. Furthermore, the above-mentioned impacts were also evident in this area when more sophisticated methods, such as selection of fish based on multimetric indexes of ecosystem integrity, were applied (VIANA et al., 2012). These methods showed that all biological indexes used were excellent indicators of ecological integrity and were especially effective for the demonstration of the critical alterations in fish communities.

Current analysis was the first study using histopathology and catalase activity of fish liver as a biomarker of the environmental quality in the Amazon estuary. Data on the histopathology and enzymatic alteration of *P. squamosissimus* and *L. dorsalis* may provide environmental managers with important insights to monitor needs and the potential recovery of the impacted area. The above is especially relevant due to the prominence of the two fish species in the diet of the local communities.

Conclusion

Results showed that local anthropogenic impacts were affecting the health of *Plagioscion squamossissimus* and *Lithodoras dorsalis*. The strong positive catalase activity and histopathological changes are evidence of the environmental alteration. Additionally, the carnivorous *P. squamosissimus* presented higher levels of alteration than the herbivorous *L. dorsalis*.

Acknowledgements

Current study was funded by the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) through a student grant to the first author and through a research grant to the second author (308421/2010-8) and by the International Institute of Education of Brazil and Moore Foundation, through financial assistance during the development of the project. The authors would also like to thank technician Lia Sogabe Priante for her help with the histological samples.

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Received on July 24, 2012.
Accepted on November 19, 2012.