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Histological Biomarker as Diagnostic Tool for Evaluating the Environmental Quality of Guajará Bay – PA - Brazil

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1. Introduction

It has been reported that in recent decades the level of foreign compounds known as xenobiotics in aquatic ecosystems has increased alarmingly as a result of domestic, industrial and agricultural effluents. In the 20th century, many thousands of organic trace pollutants, such as polychlorinated biphenyls (PCBs),organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), and dybenzon – p – dioxins (PCDDs) have been produced and in part, released into the environment (van der Oost et al., 2003). This has led to substantial reduction in environmental quality, adding to the deterioration of human health and living organisms that depend on these ecosystems (Cajaravlle et al., 2000). However, the presence of a foreign compound in a segment of an aquatic ecosystem does not, by it self, indicate injurious effects. Connections must be established between external levels of exposure, internal levels of tissue contamination and early adverse effects and determining the extent and severity of such contamination only by the results of water chemical analysis is insufficient and often overestimates the proportion and duration of exposure to the toxic agent (van der Oost et al., 2003 & Giari et al., 2008). Thus, studies using biomarkers are essential to complement such environmental monitoring, given that in order to control pollution effects of effluents on the animals that inhabit the water bodies must be understood (Martinez & Colus, 2002; Camargo & Martinez, 2006). Biomarkers are defined as responses to any exposure evidenced in histological, physiological, biochemistry, genetic and behavioral modification (Leonzio & Fossi, 1993). More recent, van der Oost et al. 2003 defined biomark as a biological indicator from an expousure to a stressor responding in various ways such a response can be seen and adaptation as a defense. Some authors note that biomarkers are used as a warning sign to emerging environmental problems (Au, 2004). In this type of environmental assessment, the health of an ecosystem can be measured by the health of its individual components (Hugget et al., 1992). It is essential to this study, as there is a variety of responses that can be used as tools to assess the health of animals exposed to certain chemicals, to provide information on spatial and temporal changes in pollutant concentrations and indicate the occurrence of environmental quality or adverse ecological consequences (Kammenga et al., 2000). In Brazil there are few studies about impact of contaminants on tropical ecosystems, therefore tropical ecotoxicology needs further studies on the effect of pollution on native aquatic organisms (Monserrat et al., 2007). The biological communities of Amazonian aquatic environments are poorly known, despite its economic and ecological importance. Belém and its surrounding areas are part of the Amazon estuary in northern Brazil. The Combú Island, near Belém, is included on Combú Environmental Protection Area (Law 6.083 of 11.13.1997) and corresponds to a lowland environment region, according to the daily tidal flooding, especially during the lunar cycles and rainy season (Ribeiro, 2004). The island's population depends on aquatic resources (fish and shrimp) as a source of food and income, and poses an imminent threat to the conservation of natural resources. The species *Plagioscion squamosissimus*, *Hypophthalmus marginatus* and *Lithodoras dorsalis* are economically important to the Amazon region, since in some areas this represents the main protein source for families. These animals occur in different types of environments, suggesting they are tolerant of a wide range of physico-chemical variables (de La Torre et al., 2005). Thus, they are suitable for environmental monitoring. The objective of this study was to evaluate the histological alterations in gills and liver of the species *P. squamosissimus, H. marginatu* and *L. dorsalis*, as well as assess the environmental influence on fish health from amazon estuary, Guajará bay.

2. Material and methods

2.1 Study area

The study area is situated around the island of Combú, near Belém-PA-Brazil, located between the coordinates 01 ° 25 'S and 48 ° 25' W. This island is inserted in the Area of Environmental Protection Combú (Law 6.083 of 11.13.1997). This area undergoes severe impacts that modify water quality due to increased population and its proximity to the metropolitan area of Belém-PA-Brazil. A total of ninety-one (91) specimens were captured in Guajará Bay and Guamá river during the dry period (July 2009). Samples were collected in three areas (Figure 1): Area A – away from pollution sources; Area B and C – considered impacted by the presence of domestic sewage and urban influence.

2.2 Biotic and abiotics data

During the study the physicochemical variables such as: pH, temperature, Dissolved oxygen (DO), nitrite, nitrate and phosphate were obtained. The pH and temperature were measured *in situ* using an Orion pH-meter, model 210 and a mercury thermometer. To determine the other variables, water samples were collected at the surface layer using a Van Dorn-type bottle. They were later processed (filtered and cooled) and taken to laboratory for analysis. We used three fish species of interest to the local population, *P. squamosissimus*, *L. dorsalis* and *H. marginatus*. These were caught by artisanal fishing, using gill nets with different mesh sizes (25 mm, 40 mm and 50 mm). After captured, the fish were placed in plastic bags, appropriately refrigerated in isothermal boxes and transported to the laboratory. The fish were then examined internally and externally for gross lesions, removing a fragment of the gills and liver. The tissue samples were fixed in Bouin's solution. After fixation, the tissues were dehydrated in increasing concentrations of alcohol, cleared in xylene and embedded in paraffin, obtained from 5mm thick sections and stained with HE (hematoxylin and eosin solution). The sections were examined and photographed using Carl Zeiss optical microscope (Axiostar Plus1169-151).

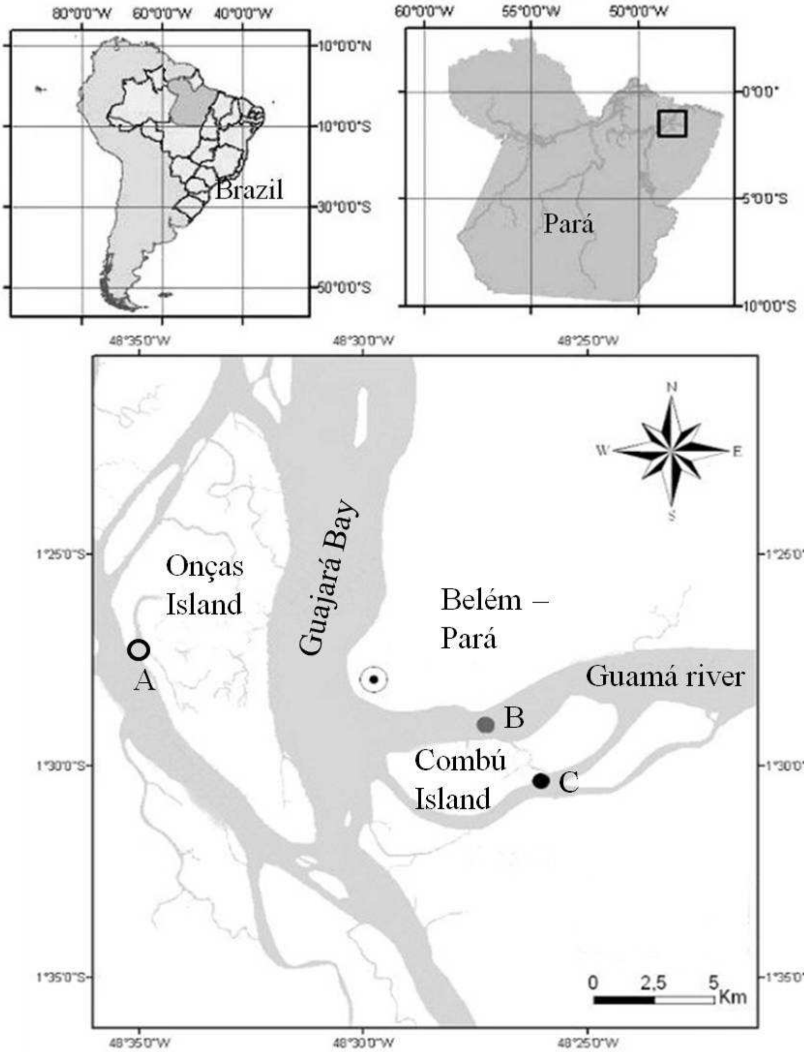

Fig. 1. Map of study area and collection points. A (away from sources polution); B and C (impacted).

2.3 Diagnostic histopathology

The histopathological changes were evaluated semi-quantitatively in two ways: The first one was modified according to Schwaiger et al. (1997), which assigned a numerical value to each animal according a degree of change: 1 (initial stage of change in some points with a chance of recovery), 2 (occasional occurrence of localized lesions with little chance of recovery) and 3 (widely distributed lesions in the body without chance of recovery). The second one was adapted from Poleksic & Mitrovic - Tutundzic (1994) that examines the calculation of the histopathological alteration index (HAI). For this, the changes were classified as progressive stages for the deterioration of organ functions: I (do not compromise the functioning of the organ) II (severe, affecting normal body functions) and III (very severe and irreversible) table 1. A value of HAI was calculated for each animal using the formula.

$$HAI = 10^0 \sum I + 10^1 \sum II + 10^2 \sum III \quad (1)$$

Since I, II, III correspond to the number of stages of change, the mean HAI was divided into five categories: 0-10 = normal tissue; 10-30 = mild to moderate damage to the tissue, 31-60 = moderate to severe damage to the tissue, 61-100 = severe damage to the tissue , greater than 100 = irreparable damage to the tissue.

GILL/LIVER HISTOPATHOLOGY	STAGE
1. **Hypertrophy and hyperplasia of gill epithelium**	
Hypertrophy of respiratory epithelium	I
Lifting of respiratory epithelium	I
lamellar epithelial hyperplasia	I
lamellar disarray	I
Incomplete fusion of some lamellae	I
Complete fusion of all lamellae	II
Lamellar epithelium disruption	II
Uncontrolled proliferation of tissue	III
2. **Changes in blood vessels**	
Dilation of sinus blood	I
Constriction of sinus blood	I
Vascular congestion	II
Disruption of pillars cells	II
Lamellar aneurism	III
1. **Changes in hepatocytes**	
cell hypertrophy	I
cell atrophy	I
Melanomacrophage centers	II - III
Inflammation	II
Fatty degeneration	II
Necrosis	II - III
2. **Changes in blood vessels**	
Hepatitis	II
Vascular congestion	II

Table 1. Classification of histopathological changes of gill and liver in relation to the type, location and stage of lesions in which they operate. Modified Poleksić and Mitrovic - Tutundzic (1994).

2.4 Statistical analysis

The frequency of altered animals and the mean HAI for each fish caught at each site were calculated. The occurrence of histopathological lesions and HAI were compared between areas using the nonparametric Kruskal-Wallis tests. The differences were considered significant $p < 0.05$.

3. Results

Table 2. corresponds the total number of animals captured in the different study areas (A, B and C). The results of physico-chemical variables during the study are analyzed in Table 3. The temperature values observed are within the normal range for the tropics. Regarding pH, it was observed that this was slightly acid in areas B and C, while the DO was lower than what is recommended in all areas. The results of gill and liver changes are displayed in Tables 4 and 5 and Figures 2 - 8. The gills of the specimens were normal as described for teleosts, consisting of four arches, supported by partially calcified cartilaginous tissue, each gill arch has two rows of primary lamellae, which in turn support the secondary lamellae. The branchial lamellar epithelium is a mosaic of primary paviment cells, mucus-secreting cells and chloride cells. The chloride cells were less evident in light microscopy because of the color used. The secondary lamella formed by the epithelium has a single layer of paviment cells, supported by the basement membrane lining the pillar cells, which surround the space through which blood circulates (Figure 5). The liver tissue of teleost fish is composed of two lobes, the right lobe which is adjacent to the gallbladder and the left lobe near the spleen. The liver is composed of hepatocytes, epithelial cells of the bile ducts, macrophages, blood cells and endothelial cells. The hepatocytes are polyhedral cells with one or two large, spherical and centrally nuclei located with evident nucleolus, and granular cytoplasm and vacuolated appearance (Figure 7). Changes in these organizations were considered to be alterations. Several changes were observed in gill and liver that differed significantly from the animals caught in the impacted areas (B and C). The area A was the only one which had healthy animals, and fish with soft lesions of type I and II and no animals with severe lesions of type III (Table 4). It was also found that they had the lowest histopathological changes index (HAI) in the 0 to 10 range (Table 5). Unlike the fish collected in areas B and C, where they all had some kind of change, many were classified as degree 3 lesions, showing the most severe type III and the highest values of HAI ranging from 41 to 91, considered moderate to severe damage, such as lamellar aneurysm characterized by blood leakage inside the lamellae, causing disruption of pillar cells and consequent dilation of blood vessels; lifting epithelium which is the detachment of the lamellar epithelium; lamellar fusion, characterized by an increase in the number chloride cells between the secondary lamellae in the respiratory tract causing reduction in the gills (Figure 6). In liver were evident such diseases: cellular hypertrophy, necrosis, presence of centers of melanomacrophages, hepatitis and inflammation (Figure 8). Regarding the responses of different species, it was observed that the species *H. marginatus* showed the lowest values while the HAI *P. squamossissimus* presented the highest values. *L.dorsalis* and *P. squamossissimus* showed more type III lesions and were therefore classified as degree 3 (Figure 4-6).

Species	Number of fish caught		
	A	B	C
H. marginatus	10	6	10
L. dorsalis	14	15	9
P. squamossissimus	14	8	5

Table 2. Number of fish caught in different areas (A, B e C).

Variables	A	B	C	Recommended
T (°)	30	30	31	-
pH	6.1	5.8	5.9	6.0 – 9.0
DO (mg/L)	4	4.2	4.5	> 5 (mg/L)
Phosphate (mg/L)	0.01	0.01	0	0.01
Nitrite (mg/L)	0.001	0.001	0.001	0.001
Nitrate (mg/L)	1.3	1.2	1	>1

Table 3. Physico-chemical variables observed in different study areas and the value recommended.

species	Types	Gill			Liver		
		A	B	C	A	B	C
H. marginatus	I	12	17	29	12	18	24
	II	3 [a, b]	11	13	3 [a]	12	9
	III	-	2	4	-	2	3
L. dorsalis	I	13 [a]	49	33	13 [a]	47	27
	II	5 [a, b]	23	20	5 [a, b]	27	14
	III	-	7	4	-	7	4
P. squamossissimus	I	4 [a, b]	26	14	13	28	16
	II	1 [a, b]	15	10	5 [a]	20	11
	III	-	5	3	-	5	3

Table 4. Total number of different types of histopathological lesions in gill and liver from three fish species in study areas.
Note: Significant difference (p<0,05): [a] between A and B ; [b] between A and C.

	Gill			Liver		
Species	A	B	C	A	B	C
H. marginatus	4.2 ± [a, b] 0.3	54.5 ± 9.6	55.9 ± 8.3	4.2 ± [a, b] 1.3	56.33± 8.7	41.4 ± 5.5
L. dorsalis	4.5 ± [a, b] 2.1	65.27 ± 7.8	70.33 ± 10.6	3.94 ± [a, b] 2.1	67.8 ± 14.4	63 ± 6.5
P. squamossissimus	1 ± [a, b] 1.1	84.5 ± 16.5	82.8 ± 15.7	4.2 ± [a, b] 0.5	91 ± 19.9	85.2 ± 24.5

Table 5. Mean and standard deviation of HAI calculated from histological alterations in gill and liver tissue from three fish species in study areas.
Note: Significant difference (p<0,05): [a] between A and B ; [b] between A and C.

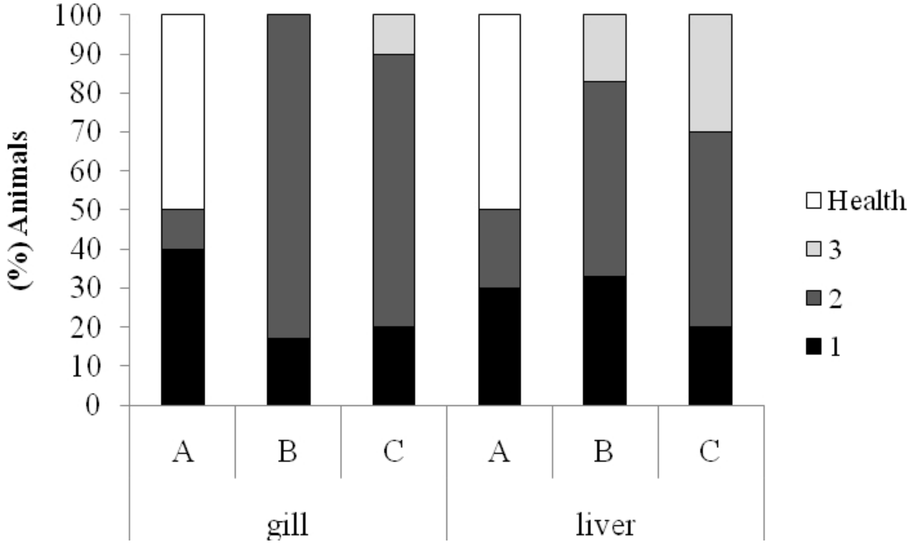

Fig. 2. Percentage of the species *H. marginatus* with gill and liver changes captured in the study areas (A, B and C). 1, 2 and 3 correspond to the different degrees of alteration of animals and health corresponds to those with no alteration.

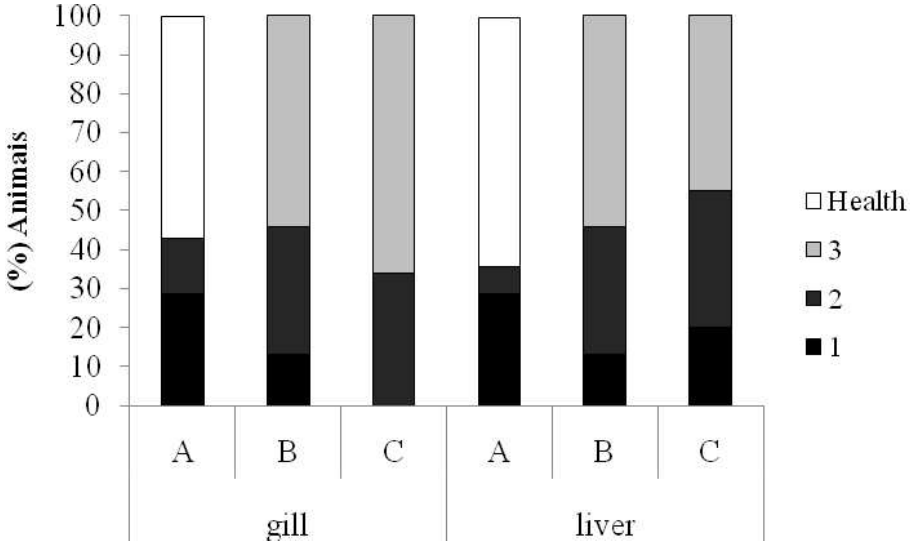

Fig. 3. Percentage of the species *L. dorsalis* with gill and liver changes captured in the study areas (A, B and C). 1, 2 and 3 correspond to different degrees of alteration of animals and health corresponds those with no alteration.

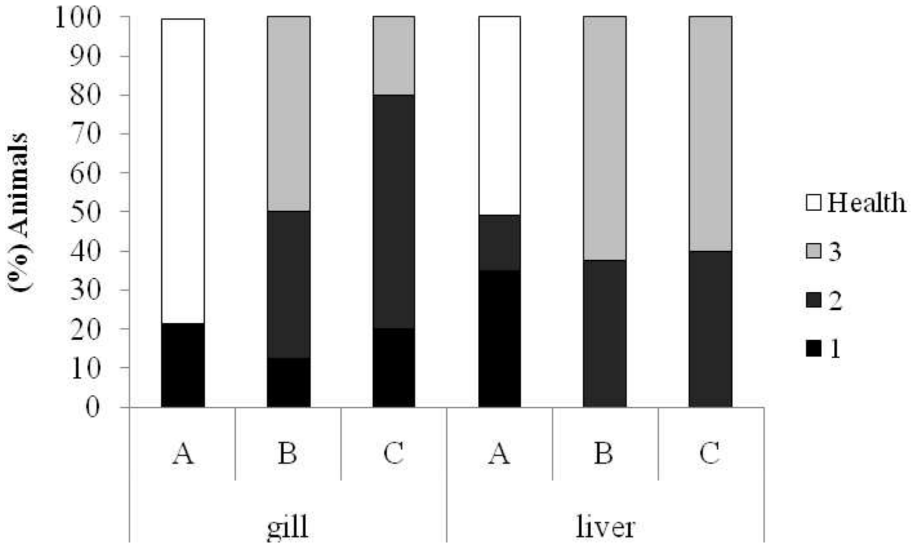

Fig. 4. Percentage of the species *P. Squamossissimus* with gill and liver changes captured in the study areas (A, B and C). 1, 2 and 3 correspond to different degrees of alteration of animals and health corresponds to those with no alteration.

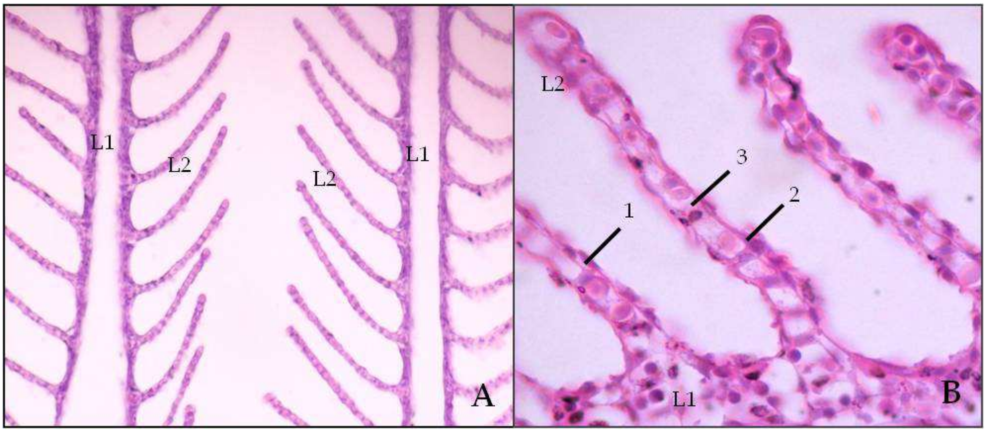

Fig. 5. Photomicrography of the gills tissue of animals captured in area. A – Normal gill structure with primary lamella (L1) and secondary (L2) with a single layer of pavement cells of slender appearance. 400X. B - Detail of a normal secondary lamella showingall cell types, 1 - squamous cell, 2 - interlamellar cells and 3 – pillars cells 1000X. HE.

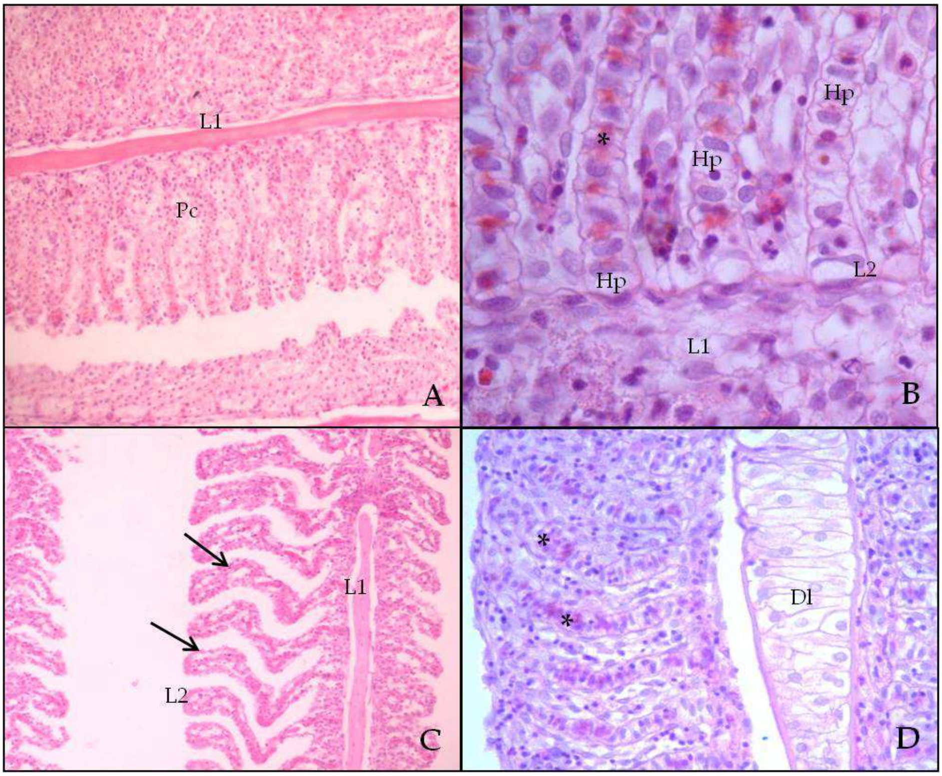

Fig. 6. Photomicrograph of branchial histopathology of animals captured in areas B and C. A – Changed gill tissue with intense celular proliferation (Pc) causing severe lamellar fusion 200X. B – hypertrophy (Hp) 1000X. C – Epithelium Lifting (Arrow) 400X. D – Dilation of sinus blood (Dl) and early aneurysm (*) 400X. HE.

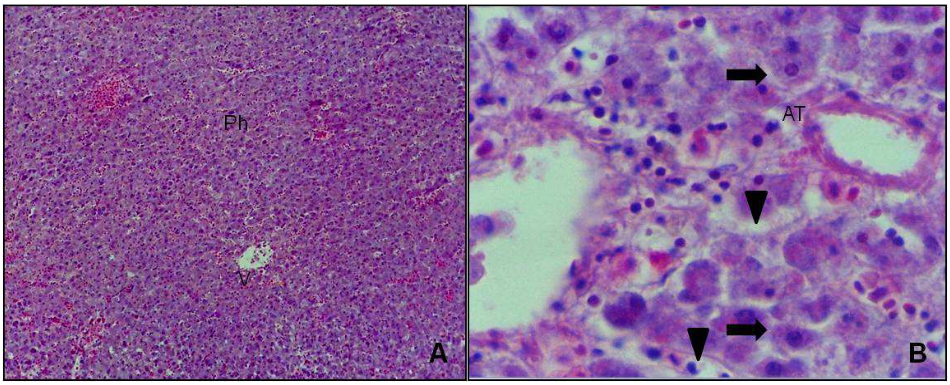

Fig. 7. Photomicrography of liver tissues of animals captured in control area – Normal liver structure with parenchyma (Ph) and veins (V) well defined 50X. B - Detail of a normal parenchyma, Hepatocytes (Thick Arrow) and sinusoids (head Arrow) 1000X. HE

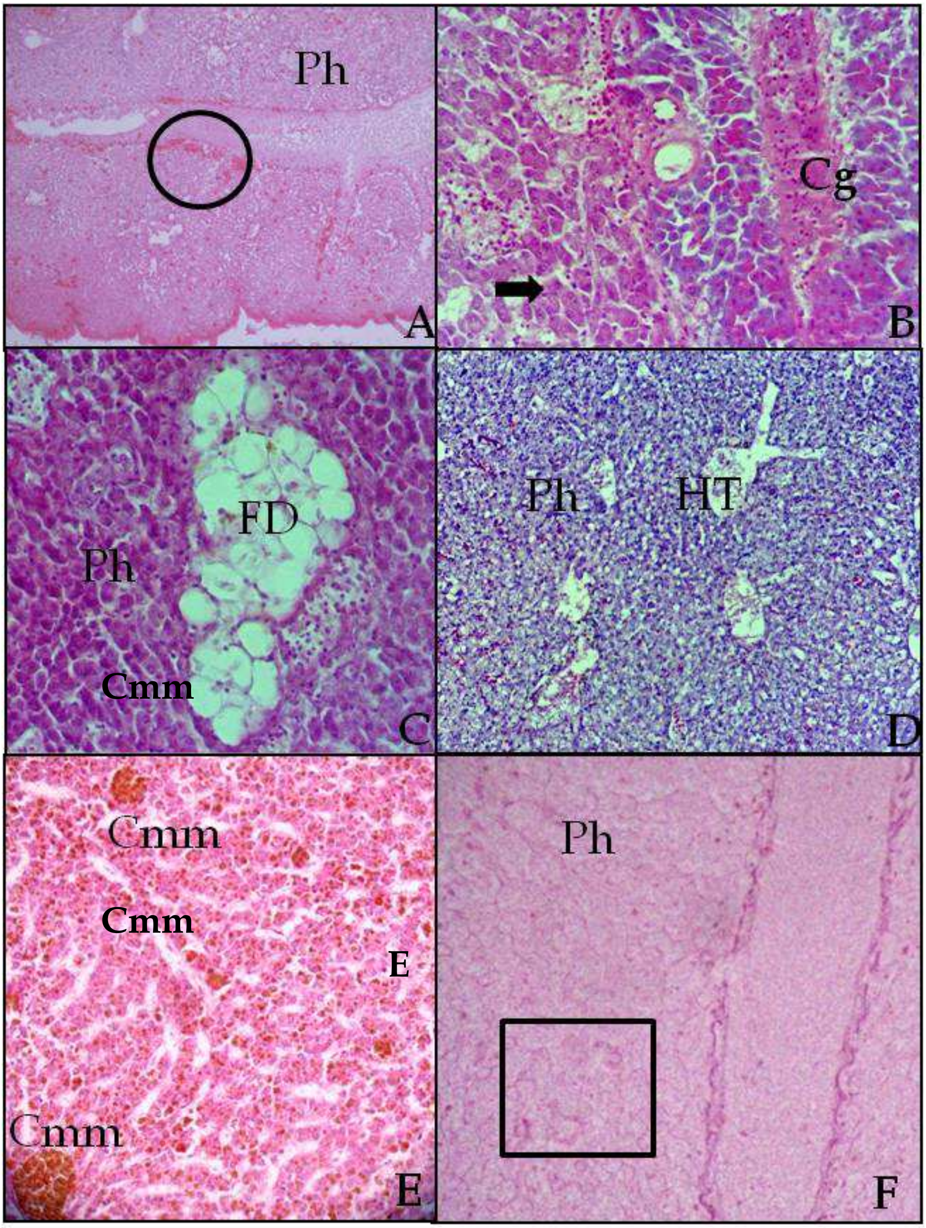

Fig. 8. Photomicrograph of hepatic histopathology of animals caught in areas B and C. A – Changed liver tissue with intense inflamation (circle) 200X. B – Congested vein (Cg) 400X. C – fatty degeneration (FD) 400X. D – Hepatites (HT). 200X. E – Intense melanomacrophages centers (Cmm). 400X. F – Detail of a hepatic parenchima with necrosis (□). 400X. HE

4. Discussion

In this study the temperature values were considered normal and slightly acidic pH. According to Campagna (2005), there is a close relationship between pH and carbon dioxide levels in the water, because with the discharge of sewage into rivers the water quality is altered by pathogenic bacteria and degradable organic substances and the decomposition of microorganisms involves the release of carbon dioxide and a consequent increase in the acidity of water. A similar situation may have occurred in areas B and C, since these areas have anthropogenic interference. These areas around the island showed a rapid population occupancy process, evidenced by domestic and industrial waste (Pinheiro, 1987; COHAB, 1997), particularly worrying since this island is inserted in an environmental protection area. The low values of DO in area A it is a typical feature of the region due to high turbidity and low incidence of light, hence causing a decrease in DO. Histology is a sensitive tool for the diagnosis of direct and indirect toxic effects that affect animal tissues (Braunbeck & Volk, 1993; Heath, 1995;Ferreira et al., 2005). Therefore it is considered an excellent method for assessing environmental quality (Freire et al., 2008). Thus this study used the histological responses of gill and liver of fish native to the Amazon as biomarker tools. The tissue damage could clearly differentiate the areas compromised as well as the control of the area, because healthy animals or mild changes of type I and II were found only in area A, result totaly different from those observed in areas B and C, where several animals showed gill lesions and hepatic diseases classified as type III. Both gill and liver are extremely important organs because they serve for respiration and osmoregulation and for regulating ion concentrations (Hinton et al., 1992), hence they play a central role in metabolism (Arellano et al., 1999). Because the gills are in direct contact with water, toxic substances can easily interfere the morphophysiology of these organs, as for instance the use of organic pesticides (Laurent & Perry, 1991), detergents (Bolis & Rankin, 1980), acids (McDonald, 1983), salt (Fanta et al., 1995), industrial waste (Lindesjöö & Thulin, 1994), ammonia (Miron et al., 2008) and heavy metals (Oliveira Ribeiro et al., 1996). During the breathing process, to prevent secondary lamellae, solid agents cross the filaments during the inflow of water, however, high concentrations of irritants dissolved in water inevitably come into contact with the outer surface of the gill filaments and secondary lamellae of the current circulation, which can alter the normal gill morphology, causing cell proliferation, epithelial lifting, hypertrophy, infiltration, and aneurysm (Simonato et al., 2008). When fish are subjected to stress, the proliferation of epithelial cells is one of the earliest changes that occurs rapidly in order to eliminate toxic agents (Laurent & Perry., 1991). Similar results were observed in this study since cell proliferation was the most found in all animals from areas B and C and some from area A. The epithelial lifting, which is a more severe injury, is caused by the change in distance of the respiratory epithelium basement membrane, causing the inefficient absorption of oxygen (Hibiya, 1992;Nowak, 1992). Result observed by Montes et al., 2010. Berrêdo et al. (2000) in a study conducted in Guajará Bay, showed high lead and chromium concentrations, metals that can undermine the tissue structure in the exposed animals. Thus we can infer that the animals evaluated in this study were responding to the effects of toxic substances. The histological changes found in the liver were: congestion, inflamation, hepatites, hemorrhage and necrosis. Vacuolated hepatocytes were also observed. The first effects of the contaminants usually occur at the cellular or intracellular level (Stephan & Mount, 1973). The melanomacrophage, also know as pigmented cells are related with the first segmento of organism defense, therefore are responsible for storing foreign material by capturing and processing of exogenous antigens and products of cell degradation (Truslé et al., 1996; Bombonato et al., 2007), as a result the increase in the amount of pigment indicate the increasing expousure (Fernandes et al., 2009), result observed in this study since in impacted areas the number of pigments were greater. Baldisserotto (2002) developed a classification that relates the degree of changes in liver tissues according to the degree of pollution in aquatic systems. These authors consider that a compromised parenchyma with several melano-macrophages centers of already exposed tissue can be considered as highly polluted environments, the situation seen in the specimens collected in areas B and C. Necrosis is induced by high concentrations of toxic substances (Rocha et al., 2010), it is then considered as a type III alteration. Some animals in the study areas had such an injury, thus we can infer a certain degree of pollution to the area. There was no significant difference among the animals, and despite their similar feeding habits, they responded similar to the same degree of toxicity. The morphological changes observed in the gill and liver of juveniles evidences an early sign of contamination in the Guajará Bay, thus indicating that these species can be used in environmental monitoring programs. However more studies should be performed as this is a protected area.

5. Conclusion

This study presented significant results and was effective in showing that human action can be mischievous if not properly controlled and the proximity of the Combú island with the urban area may be affecting water quality. In addition both the results of the pathology and the species were excellent tools for diagnosing and determining and such data may be used by managers as a form of environmental monitoring and possible remediation of the impacted area as this island is a protected area.

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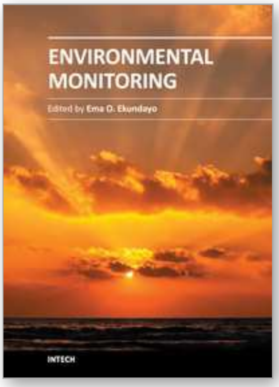

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"Environmental Monitoring" is a book designed by InTech - Open Access Publisher in collaboration with scientists and researchers from all over the world. The book is designed to present recent research advances and developments in the field of environmental monitoring to a global audience of scientists, researchers, environmental educators, administrators, managers, technicians, students, environmental enthusiasts and the general public. The book consists of a series of sections and chapters addressing topics like the monitoring of heavy metal contaminants in varied environments, biolgical monitoring/ecotoxicological studies; and the use of wireless sensor networks/Geosensor webs in environmental monitoring.

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