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CAROLINE DA SILVA MONTES

UTILIZAÇÃO DE PEIXES NATIVOS DA AMAZÔNIA COMO BIOMARCADORES NA AVALIAÇÃO DA QUALIDADE DA ÁGUA DE UMA ÁREA INDUSTRIAL NO RIO PARÁ - PA - BRASIL

Belém, PA

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Dissertação apresentada ao Programa de Pós-graduação em Ecologia Aquática e Pesca da Universidade Federal do Pará, como requisito parcial para a obtenção de grau de Mestre em Ecologia Aquática e Pesca.

Orientador (a): Rossineide Martins Rocha – ICB/ UFPA

Co–Orientador (a): Maria Auxiliadora P. Ferreira – ICB/ UFPA

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APOIO

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"Conhecimento é o único bem que se adquire por toda a eternidade"

(Benedito Nunes)

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SUMÁRIO

INTRODUÇÃO

A água é um recurso natural de extrema importância para os organismos vivos, entretanto tal recurso vem sofrendo grandes impactos em função em função de atividades antropogênicas poluidoras nas últimas décadas (ABEL, 1996). Este sistema é considerado o mais susceptível à contaminação, pois ele é o receptor final de centenas, talvez milhares de poluentes que afetam o ambiente aquático e cujos efeitos são preocupantes e a compreensão detalhada dos efeitos destes diferentes tipos de efluentes nos corpos d'água receptores é essencial para o controle da poluição (ADAMS e GREELEY, 2000). Dentre eles podemos destacar: hidrocarbonetos policíclicos aromáticos (PAHs), bifelinas policloradas (PCBs), compostos organoclorados (OCPs) e metais pesados, causando desestruturação do ambiente físico e químico, consequentemente queda acentuada na biodiversidade e alterações na dinâmica e estrutura das comunidades biológicas (VAN DER OOST et al., 2003).

A presença de composto xenobiótico no sistema aquático não significa necessariamente que este vem sofrendo efeitos nocivos, sendo necessáros o estabelecimento de conexões entre tempo de exposição, grau de contaminação e efeitos nas comunidades biológicas (JESUS e CARVALHO, 2008). Entretanto, ainda são insuficientes os métodos capazes de determinar a extensão e a severidade da contaminação (DE LA TORRE et al., 2005), tendo se intensificado a aplicação de programas de monitoramento ambiental em que pesquisadores estão cada vez mais preocupados em identificar novos compostos orgânicos e seus metabólitos e determinar seus impactos na vida aquática (ZAGATTO e BERTOLETTI, 2006).

Refletindo a crescente preocupação dos efeitos dos xenobióticos sobre os organismos, surgiu a ecotoxicologia, definida como estudo da ocorrência, natureza, incidência, mecanismos e fatores de risco dos efeitos deletérios de agentes químicos no meio ambiente (OGA et al., 2008). A ecotoxicologia baseia-se principalmente na resposta dos organismos aos agentes químicos, tendo como objetivo central verificar o comportamento e as transformações desses agentes no ecossistema, bem como seus efeitos sobre os organismos vivos, evidenciados pelas modificações estruturais, morfológicas, fisiológicas e bioquímicas (AZEVEDO e CHASIN, 2003).

O monitoramento da qualidade da água realizado por meio de organismos bioindicadores envolve o levantamento e avaliação de modificações na riqueza, diversidade e abundância de espécies resistentes; perda de espécies sensíveis; medidas de produtividade primária e sensibilidade a modificações abióticas ou a concentrações de substâncias tóxicas entre outros. (GOULART e CALLISTO, 2003; ARIAS et al., 2007). A utilização de peixes em programas de avaliação da qualidade dos ambientes aquáticos tem se destacado nos últimos anos (FLORES e MALABARBA, 2007). Estes animais são definidos como bons bioindicadores por terem a biologia e a ecologia bem conhecidas; possuírem mecanismos celulares de resposta ao estresse químico semelhantes aos dos mamíferos e estarem presentes em ambientes poluídos (SCHWAIGER, 2001).

A poluição aquática ocorre normalmente de forma crônica, com concentrações subletais de poluentes causando nos peixes efeitos deletérios:

mutagênicos, estruturais e funcionais em vez de mortalidade em massa dos organismos (POLEKSIC e MITROVIC-TUTUNDZIC, 1994). Essas alterações morfo-funcionais têm sido muito utilizadas como biomarcadores para indicar tanto a exposição quanto os efeitos de poluentes ambientais (MARTINEZ e SOUZA, 2002; ALMEIDA et al., 2005).

A avaliação da qualidade da água por biomarcadores tem sido aplicada há mais de 40 anos, uma vez que a verificação apenas por meio de parâmetros físicos e químicos é insuficiente (FLORES e MALABARBA, 2007).

Os biomarcadores são definidos como respostas biológicas adaptativas aos estressores, sendo que duas características importantes destacam-se: a) identificar as interações que ocorrem entre os contaminantes e os organismos vivos; b) verificar os efeitos sub-letais. Esta última permite por em prática ações remediadoras ou preventivas e incorporar esse tipo de análise em programas de avaliação da contaminação ambiental (TRIEBSKORN et al., 2008).

Os biomarcadores podem ser classificados como de exposição ou de efeito. Os de exposição são alterações biológicas mensuráveis que evidenciam a exposição dos organismos a um poluente específico. Os de efeito em geral não são específicos em relação aos estressores e não fornecem informações sobre a sua natureza (VAN DER OOST et al., 2003).

No Brasil os testes ecotoxicológicos para monitoramento e avaliação da qualidade da água, tem se tornado bastante comum nos últimos anos. A primeira iniciativa em termos metodológicos aconteceu em 1975, num programa internacional de padronização de testes de toxicidade aguda com peixes, desenvolvido pelo Comitê Técnico de Qualidade das Águas da International Organization for Standardization (ISO), com participação da Companhia de

Tecnologia de Saneamento Ambiental do Estado de São Paulo (CETESB) a convite da Associação Brasileira de Normas Técnicas (MAGALHÃES e FILHO, 2008).

As alterações histopatológicas em tecidos de peixes são biomarcadores de exposição aos estressores ambientais que sinalizam os efeitos resultantes da exposição a um ou mais agentes tóxicos (ADAMS, 2003). A microscopia de luz, a ultraestrutura e a imunohistoquímica tem sido ferramentas valiosas para ajudar na identificação da toxicidade de diversas substâncias em órgãos alvo e os mecanismos de ação dos contaminantes (KERR, 2002; SEPICI-DINÇEL et al., 2009). As alterações estruturais e imunohistoquímicas decorrentes da exposição ao xenobióticos podem ser utilizadas como biomarcadores no monitoramento ambiental (SCHWAIGER et al., 1997; WESTER et al., 2002).

Nos peixes as brânquias são extremamente importantes para a respiração, osmorregulação, equilíbrio ácido-básico e excreção de nitrogênio (MELETTI et al., 2003), a análise de sua morfologia é útil como parâmetro para o monitoramento ambiental (ZAGATTO e BERTOLETTI, 2006). Muitos estressores podem afetar, direta ou indiretamente, a estrutura branquial, afetando processos essenciais como as trocas gasosas e o balanço hidromineral (SCHWAIGER et al., 1997).

Segundo LAURENT e PERRY (1991), as alterações morfológicas das brânquias, em resposta a mudanças ambientais, podem representar estratégias adaptativas para conservação de algumas funções fisiológicas. Assim, os tipos de lesões histopatológicas podem indicar que os peixes estão respondendo aos agentes tóxicos presentes na água.

O município de Barcarena pertence à Mesorregião metropolitana de Belém. É limitado pela Baía de Marajó e influenciado por inúmeros rios, furos e igarapés, caracterizando-se como área de estuário (SOUZA e LISBOA, 2005). Nesta área ocorrem tanto empreendimentos industriais, com movimentação de bauxita, coque, alumina, alumínio primário, óleo combustível, soda cáustica, piche, fertilizantes agrícolas, manganês e caulim; quanto ação urbana. Este estudo tem como objetivo avaliar a saúde de duas espécies de peixes nativas da região Amazônica utilizando biomarcadores estruturais e imunohistoquímicos.

A dissertação foi elaborada no formato de artigo, intitulado de "Immunohistochemical and structural biomarkers in two fish species exposed to the industrial area in Amazon Estuary", submetido à revista Environmental Monitoring and Assessment, formatado segundo os padrões da revista.

OBJETIVOS

Objetivo Geral

Avaliar a qualidade da água de Barcarena – PA, utilizando diferentes biomarcadores de efeito em espécies de peixes nativos da Amazônia, *Plagioscion squamossissimus* e *Lithodoras dorsalis.*

Objetivos Específicos

- Determinar as principais variáveis físico-químicas da água.

- Identificar alterações morfológicas, histológicas, ultraestruturais e imunohistoquímicas das brânquias das espécies *Plagioscion squamossissimus* e *Lithodoras*, capturadas em diferentes áreas, uma considerada controle e duas consideradas impactadas localizadas na Baía do Marajó.

- Caracterizar ultra - estruturalmente as brânquias utilizando a técnica de microscopia eletrônica de varredura (MEV).
- Identificar corpos apoptóticos ultilizando a técnica de TUNEL.
- Atribuir um grau de severidade e um índice de alteração sob o ponto de vista de microscopia óptica em cada animal.

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Structural and immunohistochemical biomarkers in two fish species exposed to the industrial area in Amazon estuary

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Abstract: Indiscriminate dumping of toxic substances impairs and deteriorates the water quality. Fish gills have a large surface area; they remain in close contact with the external environment and are particularly sensitive to changes in water quality, for this reason they are quite relevant for environmental monitoring. This study analyzes the gill structure of the fish species *Plagioscion squamossissimus* and *Lithodoras dorsalis* from two different sites located an Amazon estuary. A total of 324 specimens were used, this total 176 were *P. squamossissimus* and 148 *L. dorsalis*, removing the second gill arch for histological, ultrastructural and immunohistochemical analyses. The histological changes were analysed semiquantitatively according to the histopathological evaluation. The sampled sites differ regarding the rate of occurrence of altered animals, only site A showed healthy animals, 84% of *L. dorsalis* and 77% of *P. squamossissimus* had normal gill structure, with the lamellae lined by simple squamous epithelial, composed of pillar, mucous and chloride cells. However, all the specimens collected in sites B revealed tissue changes in the gill lamellae such as: aneurysm; epithelial lifting; cell proliferation; lamellar fusion and cell hypertrophy, this result was also confirmed by scanning electron microscopy. TUNEL analysis indicated that only animals captured on

site B showed apoptotic cells. The presence of injury in gill tissues in the animals captured on site B emphasizes the need for more effective pollution control measures with regards to discarding pollutant loads as well as the need for urban planning in this region.

Key words: aquatic pollution; biomonitoring; histology; gills.

Introduction

The aquatic ecosystem remains subject to toxic contaminants as a result of industrial, agricultural and domestic activities (Abel, 1996). The main causes of water resource degradation that promoting physical, chemical and biological changes are: sedimentation, discharges of pesticides, domestic, hospital and industrial wastes (Adams e Greeley, 2000). These environmental modifications can demonstrate their effects on organisms at various organizational levels (Ferreira et al. 2005; Van der Oost et al. 2003). Consequently, many biomarkers have been proposed in environmental monitoring programs to assess the effect of toxic substances on animals (Eason and O'Halloran 2002), evidenced at histopathological, ultraestructural and immunohistochemical levels (Hill et al. 2000, Elahee and Bhagwant 2007, Fernandes et al. 2009, Montes et al. 2010). Moreover, these effects may link the bioavailability of the compounds of interest with their concentration at target organs and intrinsic toxicity (Burger et al., 2007). In the case of fish, the gill is the main organ examined as it is responsible for the vital functions, namely breathing, acid-base balance, nitrogen excretion and hormonal metabolism (Wilson and Laurant 2002). The purpose of this study was to investigate two species of fish as biomarkers; *Plagioscion squamosissimus* (Heckel, 1840) and *Lithodoras dorsalis* (Valenciennes, 1840). These two selected species are abundant in all sample sites, they occupy different niches and are economically important for the communities in the region. This study aimed to investigate the health status of two commercial fishes caugth from industrial area in Amazon estuary, evaluating the occurrence, type and intensity of histological, ultrastructural and apoptotic changes observed in the gill tissues of the fish species *P. squamossissimus* and *L. dorsalis*

Material and methods

Study area

The study area is influenced by the port of Vila do Conde, located on right bank of the estuary of Para River Barcarena. This sector is scope of transporting minerals and unloading of fuel oil, caustic soda and fertlizer industry to assist internacionl companies (Boulhosa and Mendes, 2009). Accordingly, the collection of abiotic and biotic material was conducted in two different areas (Figure 1): A - Reference (1º 26' 21.26'' S and 48º 32' 29.52" W) – Away from the pollution sources and B - Terminal of Vila do Conde (1º 34' 28'' S and 48º 47'13.9" W) – hugh industrial influence. The collections were carried out in two annual periods: rainy season (December 2009 and March 2010) and dry season (June and September 2010).

Water analysis

During the study were obtained the physicochemical variables pH, temperature, Dissolved oxygen (DO), nitrite, nitrate, alkalinity, hardness and phosphate. The pH and temperature were measured *in situ* using an Orion pH-meter, model 210 and a mercury thermometer. Dissolved oxygen levels were determined in the field using the Winkler method. 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.

Animals and biological samples

Two fish species were caught, *P. squamossissimus*, carnivorous and *L. dorsalis*, detritivore. After capture the fishes were packed in plastic bags, identified, appropriately refrigerated in isothermal boxes and transported to the laboratory to conduct the biometry (weight and length). Then they were examined internally and externally for gross lesions, immediatly extracting the second gill arch on the right side and fixed.

Microscopic analysis

Gill fragments were dissected, washed in saline buffer and immediately fixed in Bouin, paraformaldehyde 4% and Karnovsky solutions for 24h. The samples fixed in Bouin were embedded in paraffin, stained with HE (haematoxylin and eosin) analyzed and photographed using light microscopy (Carl Zeiss - Axiostar Plus 1169-151). The gill fragments fixed in Karnovsky Solution were post-fixed with 1% osmium tetroxide, dehydrated in ethanol gradient, submitted to the critical CO_2 drying point and then coated with gold a 10nm thick for analysis in a scanning electron microscope (SEM) LEO 910.

Gill tissues fixed in 4% paraformaldehyde were processed by routine histological techniques and embedded in paraffin. The slides were processed by TUNEL technique using the detection Kit (Basic TACS.XL - "Apoptosis detection"), following the manufacturer's instructions. The tissues were treated in proteinase K, immersed in 3% hydrogen peroxide and incubated with TdT - enzyme in a wet chamber at 37 ° C. The samples were immersed in blocking buffer Stop – solution, anti-BrdU and strep-HRP at 37 °C, then incubated in DAB solution. Next, the samples were stained with hematoxylin and eosin (HE) and photographed using optical microscopy (Carl Zeiss - Axiostar Plus 1169-151).

Diagnostic Histopathology

The histopathological changes were evaluated semiquantitatively in two ways: The first modified according to Schwaiger et al. (1997), which was assigned a numerical value for each animal according to a degree of change: grade 1 –mild and focal changes; grade 2 - mild to moderate changes and grade 3 - severe and extensive pathological alterations. The second was adapted from Poleksic and 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: $\mathbf{HAI= 10^0 \sum I+10^1 \sum II+10^2 \sum III}$. Since I, II, III correspond to the number of stages of change. The histopathological alteration index is divided into five categories: 0-10 = normal tissue; 11-40 = mild to moderate damage to the tissue, 41-80 = moderate to severe damage to the tissue, 81-120 = severe damage to the tissue, greater than 120 = irreparable damage to the tissue.

Statistical analysis

With the individual data, the frequency of animals with different degrees of alteration and the mean HAI for each fish caught at each site were calculated. Biometrics, the occurrence of histopathological lesions and HAI were compared between areas using the nonparametric Kruskal-Wallis, where differences were considered significant when $p < 0{,}05$, using the program Bioestat 5.0 (Ayres et al. 2007).

Results and discussion

Water quality

Temperature, pH, phosphate, nitrite and conductivity are variables essential for determining water quality and they have influence on various biochemical reactions in the aquatic system (Hacioglu and Dulger 2009). The physicochemical variables analyzed are shown in Table 2, these showed no significant alteration in physicochemical parameters on two sites evaluated. However, on the site B pH was slightly acidic. The acidification of water can occur with the production of carbon dioxide released by bacteria and biodegradable substances (Suhett et al. 2006). The process of osmoregulation can be disrupted under acidic conditions, since the gills are in direct contact with the water (Copatti and Amaral 2009). Although no process of osmoregulation was evaluated in this study, the alterations in gill structure were evident in all animals from area B, close of the port Vila do Conde. Nitrite and total alkalinity were greater than the maximum permitted quantities (MPQ) allowed by the current regulatory statutes in the country, CONAMA n° 225/2005, early signal of pollution, since the presence of nitrite suggests recent pollution stage, while nitrato indicates an advanced stage and high values of nitrite and alkalinity, can cause serious damage to fish metabolism and may affect the development of fish (Rojas et al. 2001), this confirms the changes found in all fishes tissues from area B. According Melo Jr., 2002, this industrial area produce waste releasing fluoride, chloride, sulfate, bicarbonate, and other substances which in contact with water tend causing significant changes in their quality. However the physicochemical variables may not signal some type of environmental modification due to capacity to filter out pollutents (Berrêdo et al. 2001).

Area A showed lows values of DO below the levels allowed by the regulatory statutes, which may have contributed to the gill changes observed in these animals during the study, since exposure to low values of DO can causes stress and consequently damages the gills (Laurant and Perry 1991). Nevertheless, this area remains as reference, by the minimal impact undergone, representative of the natural conditions of the study area, because few animals showed changes in the gill tissue. Industrial waste water discharge represents a constant polluting source, has been extremely necessary the application of biomonitoring programs, to realize frequent assessments of water quality that in addition the use of biological data (Singh et al. 2004).

Animals and Histopathology

A total of 324 specimens were collected, 176 *P. squamossissimus* (69 from area A and 107 from B) and 148 *L. dorsalis* (78 from area A and 70 from B). The mean values and standard deviation for weight and length demonstrated that the animals examined were juveniles. The description of the fish gills follows the pattern as is observed in the majority of teleost fishes, consisting of four pairs of gill arches, supported by partially calcified cartilage tissue. The gill arches presented two rows of primary lamellae, which in turn support the secondary lamellae, which are lined by a simple squamous epithelial, composed of pillar, mucous and chloride cells. The chloride cells were not evident under light microscopy because of the color used (Figure 2). The modifications in this organization were considered as gill abnormalities.

As part of a research programme this study emphasizes the use of histopathological investigations for assessing the effects of an industrial area on the health of two fish species. The histopathological responses clearly differentiate fishes caught from area A from those individuals of B. The gill histopathology frequency of animals caught from site A differed significantly from those on B. Were verified 77% of *P. squamossissimus* and 84% of *L.dorsalis* with normal gill structure, while the remaining presented few and mild gill lesions, classified as stage I and II (Table 3). Therefore few animals were classified as degree 1 or 2 and (Figure 4 and 5), furthemore the animals showed low values of HAI (table 4). Similar result has been described for *P. Lineatus* in areas remote from human activities, the animals had lower rates of gill histopathology (Camargo and Martinez, 2007). While all animals captured on site B exhibited some type of alteration, the majority of stage III such as: lamellar aneurysm; epithelial lifting and lamellar fusion, causing a decrease in the respiratory tract (Figure 2). Mostly the animals were classified as degree 3 and high values of HAI, especially in the rainy period (Table 4). Different toxicants can damage the gill structure, which causes generalized stress on the animal and is not a specific toxic response (Simonato et al. 2008). Although no present study there is no evidence thar the water is polluted, however, significant differences of degrees of HAI were observed between area A (reference) and B (Terminal of Vila do Conde). Morphological changes of the gills in response to changes environmental, may represent adaptive strategies for conservation of some physiological functions. Thus, the types of histopathological lesions observed in this study indicate that fish are responding to the effects of toxic agents in water and sediment (Laurant and Perry, 1991). The species did not show significant differences regarding the degree and intensity of gill histopathology, since site B affect both species, independent of the niche they occupy. Result alredy verified in *Scarus ghobban*, *Epinephelus merra* and *Siganus sutor* from the presumably contaminated lagoon of Baindes Dames, Mauritius, which the difference lies in time and intensity of exposure to the pollutant and not the eating habits (Elahee and Bhagwant 2007)

Ultraestructure and TUNEL

The evaluated by scanning electron microscopy (SEM), confirmed that fishes captured only on the port of Vila do Conde (B) had the worst alterations, differently from those captured on the area A (reference) Figure 3. Thus the proliferation of chloride cells and edema in the secondary lamellae was similar found in *Poecilia vivipara* exposed to increasing concentrations of chemical pollutants (Araújo et al. 2001). Immunohistochemical biomarkers can also be an excellent tool to detect and characterize the biological endpoint of toxic substances in the aquatic environment (Moore and Simpson 1992). Apoptosis is characterized by several of events or morphological and biochemical changes that cause the activation of the physiological cell death mechanisms (Kerr 2002). *P. squamossissimus* and *L. dorsalis* from site B had gill cells with apoptosis. This results is similar to the one found in *Oreochromis mossambicus* exposed to degraded environments (Li et al. 1998) and in *O. kisutch*, which showed a high number of tunel cells - positive, indicating that these cells were directly related to the damaged environment. (Hill et al. 2000). This study confirms that site B has undergone a process of pollution due to the gill changes evidenced in all the animals from these areas.

Conclusion

The animals that inhabit the region close to the industries undergo anthropogenic pressures and thus show changes in gill structure as a way to adapt to this environment. The species *Plagioscium squamossissimus* and *Lithodoras dorsalis* independent of trophic level, they react similarly to the same intensity of pollution, since there was no difference between species, with respect histopathological alteration index and rate of change found. The different biomarkers used in this study were effective and decisive in the final diagnosis and the presence of injury in gill tissues in the animals captured on polluted site emphasizes the need for more effective pollution control measures with regards to discarding pollutant loads as well as the need for urban planning in this region.

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Table 1. Classification of the type, location and stage of gill histopathological changes. Modified Poleksić and Mitrovic - Tutundzic (1994).

GILL HISTOPATHOLOGICAL CHANGES	STAGE
1. Hypertrofy and hyperplasia of gill	
Hypertrophy of respiratory epithelium	I
Incomplete fusion of several lamellae	I
Lamellar epithelial hyperplasia	I
Lamellar disarray	I
Lamellar lifting	II
Complete fusion of several lamellae	II
Complete fusion of all lamellae	III
Rupture of the lamellar epithelium	III
Uncontrolled proliferation of tissue thickening	III
2. Changes in blood vessesls	
Dilation of the blood sinus	I
Constriction of the blood sinus	I
Vascular congestion	II
Disruption of the pillar cell system	II
Lamellar aneurism	III

Table 3. Total number of different types of histopathological lesions observed in the species *P. squamossissimus* and *L. dorsalis* in the sites in both periods during the study. *Note:* Significant difference (p<0,05). [a] Between A and B.

Species	**Type of alteration**	**Dry**		**Wet**	
		A [a]	**B**	**A** [a]	**B**
L. dorsalis	I	15	139	20	136
	II	7	65	3	58
	III	0	47	0	49
P. squamossissimus	I	17	177	18	255
	II	6	75	4	124
	III	0	60	0	95

Tabela 2. Physical and chemicals variables during the study.

Periods / Variables	Dry season		Rainy season		MPQ
	A	**B**	**A**	**B**	
Temperature (°C)	27	29	28	29	27 -29
pH	6,52	5,59	6,7	6,0	6 -9
Dissolved Oxygen (mg/l)	3 [a]	5,4	3 [a]	5,8	> 5
Nitrato	0,2	1,3	0,85	3,12	Until 10,0
Nitrite	0,55	1,38	0,04	1,05	Until 1,0
Total Alkalinity (mg/l)	10,01	17,85	7,25	34,5	Until 10
Total Hardness (mg/l)	20,1	44,8	32,7	18,7	Until 500

MPQ (Maximum permitted quantities). *Note:* Significant difference (p<0,05). [a] Between A and B.

Table 4. Mean and standard deviation values of HAI (histopathological alteration index) calculated during the study.

Specie	**Dry**		**Wet**	
	A [a]	**B**	**A** [a]	**B**
L. dorsalis	2,23±4,7	154,7±54,0	1,45±2,9	177,5±55,14
P. squamossissimus	2,6 ± 3,57	157,43 ± 55,63	1,45±3,74	174,5±54,63

Note: Significant difference (p<0,05). [a] Between A and B.

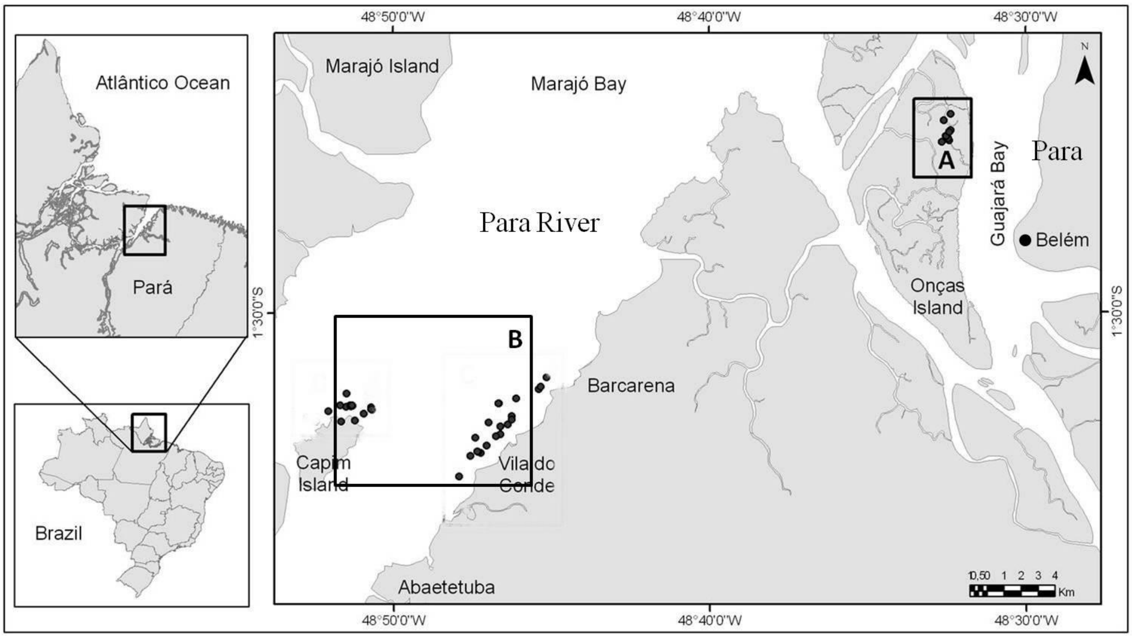

Figure 1. Map of the Amazon estuary, Para River - PA (Brazil), indicanting the points where the species were captured: A – Reference and B – Area next to the Terminal of Vila do Conde.

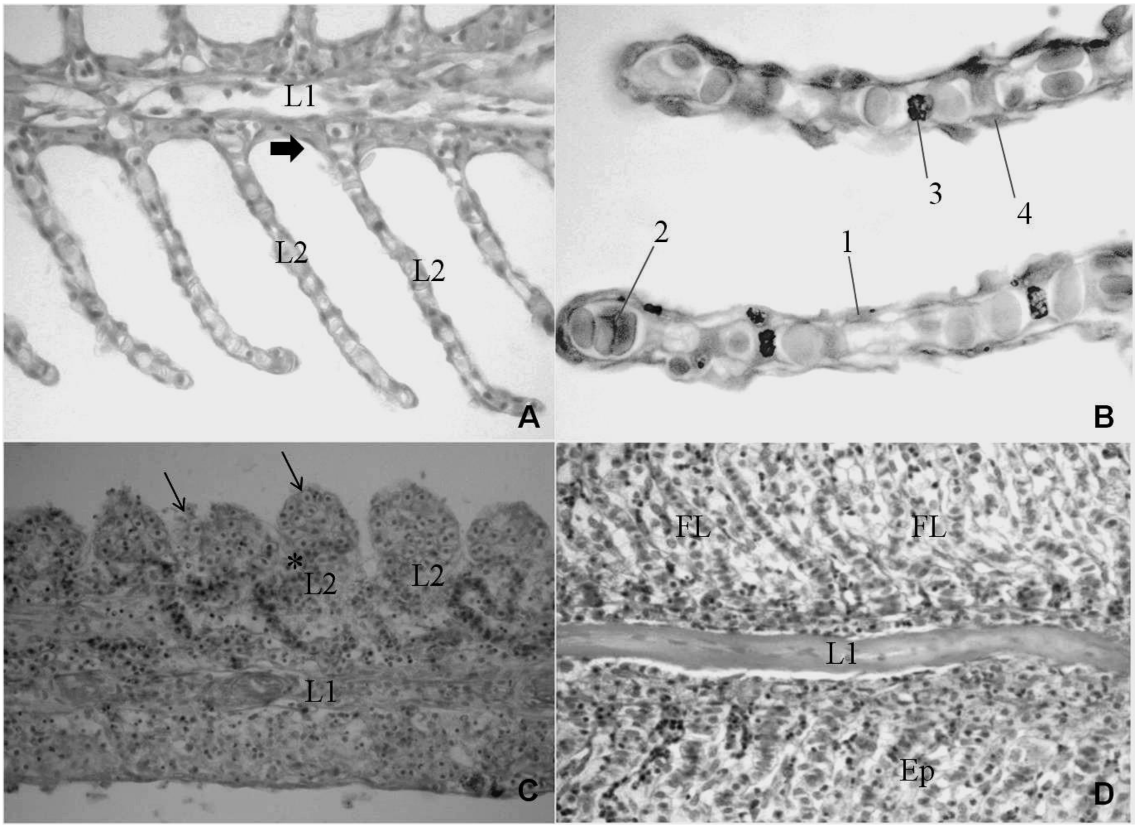

Figure 2. Photomicrography of the gills of *L.dorsalis* and *P.squamossissimus*. A - Normal gill structure with primary lamella (L1) and secondary (L2) with a single layer of pavement cells (thick arrow) of slender appearance. B - Detail of a normal secondary lamella showing all cell types, 1 - squamous cell, 2 - Erythrocytes, 3 - interlamellar cells and 4 - cells pillars 1000X. C - Changed gill tissue with hypertrophy (thin arrow) and early aneurysm (*) 200X. D – Changed gill tissue with hyperplasia causing severe lamellar fusion (LF) and the elevation of the epithelium (Ep) 400X.

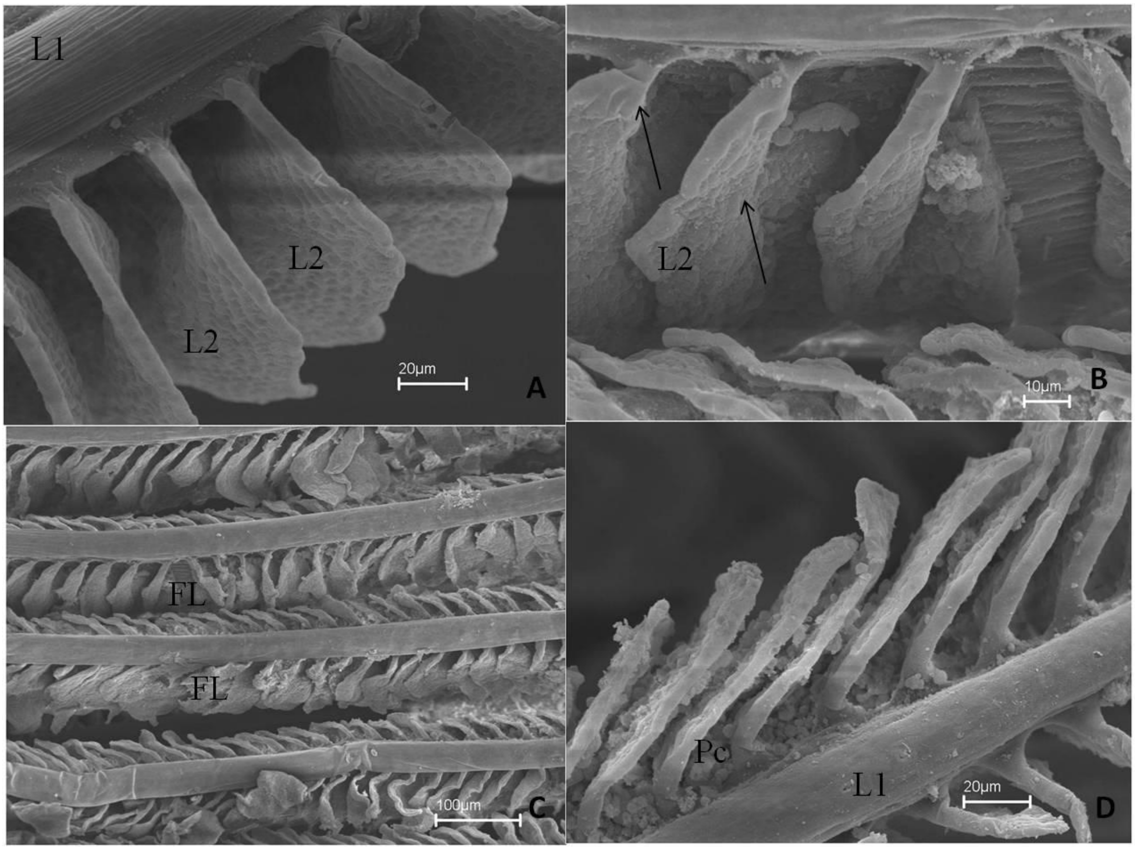

Figure 3. Photomicrography of the gills of *L.dorsalis* and *P.squamossissimus* using scanning electron microscopy (SEM). A – Normal gill structure with primary lamella (L1) and secondary (L2) with slender appearance (bar = 20µm). B – Changed Gill tissue with hypertrophy of secundary lamellas (thin arrow) (bar = 10µm). C – Changed Gill tissue with intense hyperplasia causing severe lamellar fusion (LF) (bar = 100µm). D – Detail of a gill filament showing the early changes of cell proliferation (Pc) (bar = 20µm).

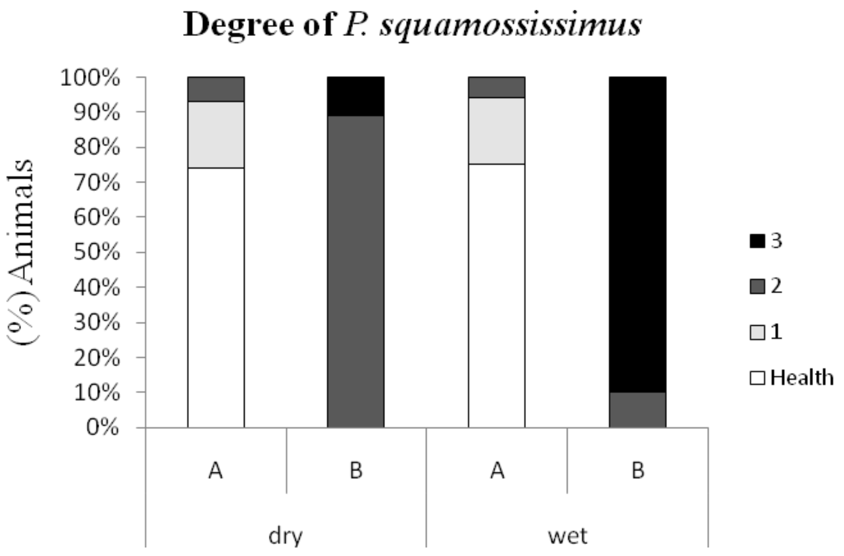

Fig 4. Percentage of altered animals from sites A and B in rainy and dry season. 1, 2 and 3 represent the differents degrees faixas of *P. squamossissimus*.

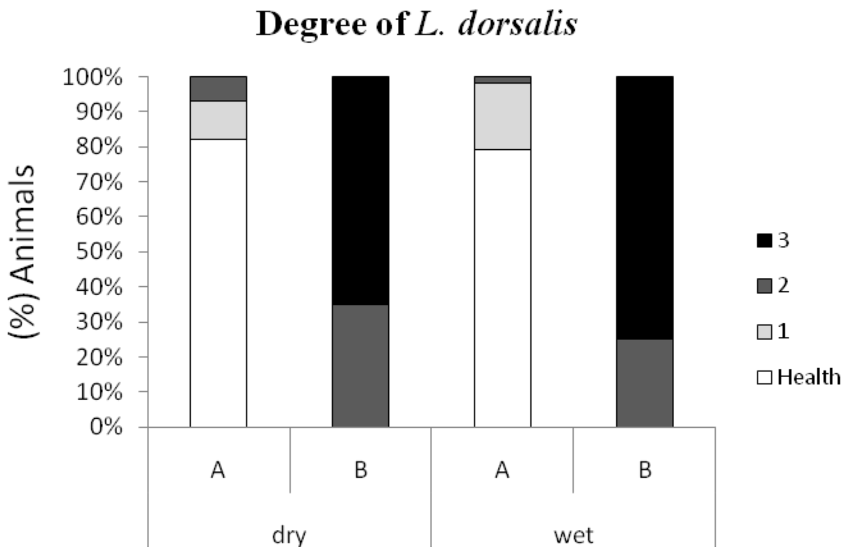

Fig 5. Percentage of altered animals from sites A and B in rainy and dry season. 1, 2 and 3 represent the differents degrees faixas of *L. dorsalis.*

CONCLUSÃO

Este estudo permitiu concluir que existe uma diferença significativa entre as duas áreas (A e B) no que se refere ao efeito da poluição nos organismos utilizados como bioindicadores, principalmente entre a area menos impactada com as demais áreas, dessa forma, os animais que habitam a região próximo às indústrias sofrem grandes pressões antropogênicas e com isso apresentam modificações na estrutura branquial como uma forma de se adaptar a este ambiente. Além disso, foi possível observar que os animais independente do nível trófico que ocupam, reagem de forma semelhante a uma mesma intensidade de poluição, uma vez que não houve diferença entre as especíes *Plagioscium squamossissimus* e *Lithodoras dorsalis* em relação ao grau e ao índice de alteração encontradas em ambas. Os diferentes biomarcadores utilizados neste estudo foram efetivos e determinantes no diagnóstico final.

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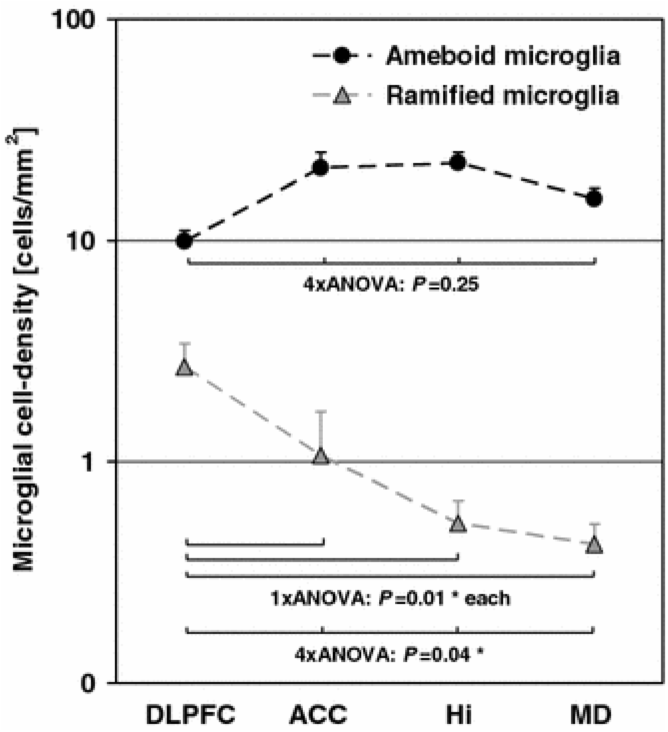

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