Acta Scientiarum. Biological Sciences
ISSN: 1679-9283
eduem@uem.br
Universidade Estadual de Maringá
Brasil

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Acta Scientiarum. Biological Sciences, vol. 32, núm. 1, 2010, pp. 93-99
Universidade Estadual de Maringá
.png, Brasil

DOI: 10.4025/actascibiolsci.v32i1.4800

Branchial histopathological study of *Brachyplatystoma rousseauxii* (Castelnau, 1855) in the Guajará bay, Belém, Pará State, Brazil

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ABSTRACT. This work analyzes the morphological alterations in *Brachyplatystoma rousseauxii* gills and evaluates whether this species can be considered an environmental monitoring biomarker of Guajará Bay. Sampling was carried out in four areas around Belém, Brazil, in four annual periods: dry/wet season, wet season, wet/dry season and dry season. Water pH, temperature and suspended material were evaluated. A total of 36 specimens were collected. The second right gill arch of each animal was removed and immediately fixed and processed for histopathology analysis with light microscopy. The physicochemical analysis of the water during the study period showed slight acidity, temperature and material in suspension within normal levels. Histopathological analysis of the gills from 14 individuals from area I presented no alterations, and only 2 individuals from this area presented some significant type of alteration. In contrast, all individuals captured in areas II, III, and IV presented at least one of the following alterations: aneurism-like alterations, epithelial elevation, infiltration, cell proliferation and cell hypertrophy. Based on the gill histopathological analysis, this organ is considered a good biomarker and the native species *B. rousseauxii* could be used as a bioindicator for environmental monitoring.

Key words: biomarkers, environmental monitoring, histological alterations, fish.

RESUMO. Estudo histopatológico da brânquia de *Brachyplatystoma rousseauxii* na baía do Guajará, Belém, Estado do Pará, Brasil. O objetivo deste trabalho foi analisar as alterações morfológicas branquiais em *Brachyplatystoma rousseauxii* e verificar se esta espécie pode ser considerada como indicador biológico para o monitoramento ambiental da baía do Guajará. As coletas aconteceram em quatro áreas ao redor da cidade de Belém: I (controle), II, III e IV (forte influência antrópica), nos quatro períodos anuais: estação seco/chuvoso, estação chuvoso, estação chuvoso/seco e estação seco. Nesses períodos foram medidos pH, temperatura e material em suspensão. Foram capturados 36 exemplares. O segundo arco branquial direito de cada animal foi retirado, imediatamente fixado e processado para análise histopatológica. A análise físico-química da água revelou pH levemente ácido, a temperatura e o material em suspensão se encontravam nos parâmetros de normalidade. As análises histopatológicas das brânquias de 14 animais da área I não apresentaram alterações branquiais e somente dois revelaram algum tipo de alteração. Todos os indivíduos capturados nas áreas II, III e IV apresentaram pelo menos uma das seguintes alterações: aneurisma, elevação epitelial, infiltração, proliferação celular, fusão lamelar e hipertrofia celular. De acordo com as análises histopatológicas branquiais, este órgão é considerado como um bom biomarcador e a espécie nativa *B. rousseauxii* pode ser utilizada como bioindicador no monitoramento ambiental.

Palavras-chave: biomarcadores, monitoramento ambiental, alterações histológicas, peixe.

Introduction

The aquatic environment is currently under threat by human activities. The release of chemicals known as "xenobiotics" triggers pollution, jeopardizes environmental health and endangers the human population (LIVINGSTONE, 1998; MORAES; JORDÃO, 2002).

Fish are usually considered good bioindicators for assessing the effects of environmental pollution, as they are at the top of the aquatic food chain and because they accumulate toxic substances (GERNHOFER et al., 2001). According to FAO (1993), an organism is a good indicator of pollutants if it has a wide geographical distribution and is easily caught.

The gills are among the fish organs that undergo changes because they have a large surface area that is in permanent contact with the external environment (POLEKSIC; MITROVIC-

TUTUNDZIC, 1994; KAMMENGA et al., 2000). They perform vital functions such as respiration, osmoregulation and excretion, and are also the contaminant depuration site, where the detoxification and metabolism of toxic agents can take place (PACHECO, SANTOS, 2002; MELETTI et al., 2003).

The gills are formed by branchial arches, constituting the gill filaments, which in turn contain primary and secondary gill lamellas. These are lined by mucus, epithelial, mucous, pillars and chloride cells (HIBIYA, 1982). These organs, sensitive to the exposure of pollutants, show varied and substantial biological responses (morphological and physiological) and have proven to be excellent biomarkers for monitoring the health of fish and their environment (BRAUNBECK, 1998; PACHECO; SANTOS, 2002; VEIGA et al., 2002; ADAMS, 2003; FLORES-LOPES; MALABARBA, 2007; MIRON et al., 2008).

In Pará (Brazil) the species *Brachyplatystoma rousseauxii* (Castelnau, 1855), commonly known as *dourada*, is one of the most important natural resources. It is considered one of the main export items, significantly contributing to the local economy (RUFFINO; ISAAC, 2000; PETRERE JR. et al., 2004). Owing to the fact that it is one of the predominant species in the bay that skirts the city of Belém, this species was chosen for monitoring. Therefore, the objective of this study was to analyze the branchial morphological changes in *B. rousseauxii* and to check whether this native species can be used as a biological indicator for the environmental monitoring of Guajará bay.

Material and methods

Study area

The collections were carried out in Guajará bay and the Guamá river that skirt the city of Belém (State of Pará, Brazil), in four areas: I - control, away from pollution sources; II - characterized by waste from the fishing industry and domestic sewage, III – by the presence of domestic waste; IV - loading and unloading waste from medium size vessels, unloading of fish and domestic waste. The collections took place in four annual periods: dry/wet season (December, 2004), wet season (March, 2005), wet/dry season (June, 2005) and dry season (September, 2005), with one collection per period.

The physico-chemical variables were obtainedduring the study: pH, temperature and concentration of total solids (material in suspension). The pH was measured using an Orion pH-meter, model 210; the temperature, using a mercury thermometer; and to determine the concentration of total dissolved solids, an Orion model 115 was used.

Sample obtainment

Thirty-six juvenile specimens were caught using nets with different mesh sizes (25, 40 and 50 mm). Sixteen specimens were from area I, thirteen individuals from area II, six individuals from area III and only one specimen from area IV. After the capture, the fish were packed in plastic bags, identified, appropriately refrigerated in isothermal boxes and transported to the laboratory to conduct the biometry (weight and length). Next, the second right gill arch was dissected, washed in saline buffer and immediately fixed in Bouin solution for 24h. After fixation, the samples were dehydrated in increasing ethanol concentrations, diaphanized in xylene, infiltrated and embedded in paraffin. 5 μm-thick cuts were obtained and stained with Hematoxylin-Eosin (H/E), analyzed and photographed using light microscopy (Olympus CH30).

The histopathological analysis of the gills were modified according to Schwaiger et al. (1997), and assigned four levels of change: Grade I = cell proliferation; Grade II = cell proliferation and hypertrophy; Grade III = cell proliferation, hypertrophy and infiltration or epithelial lifting; Grade IV = cell proliferation, epithelial lifting, infiltration, hypertrophy and aneurysm.

Statistical analysis

The statistical analysis was performed using the analysis of variance and the Bonferroni test assisted by the Bioestat 5.0 *software* (AYRES et al., 2007).

Results

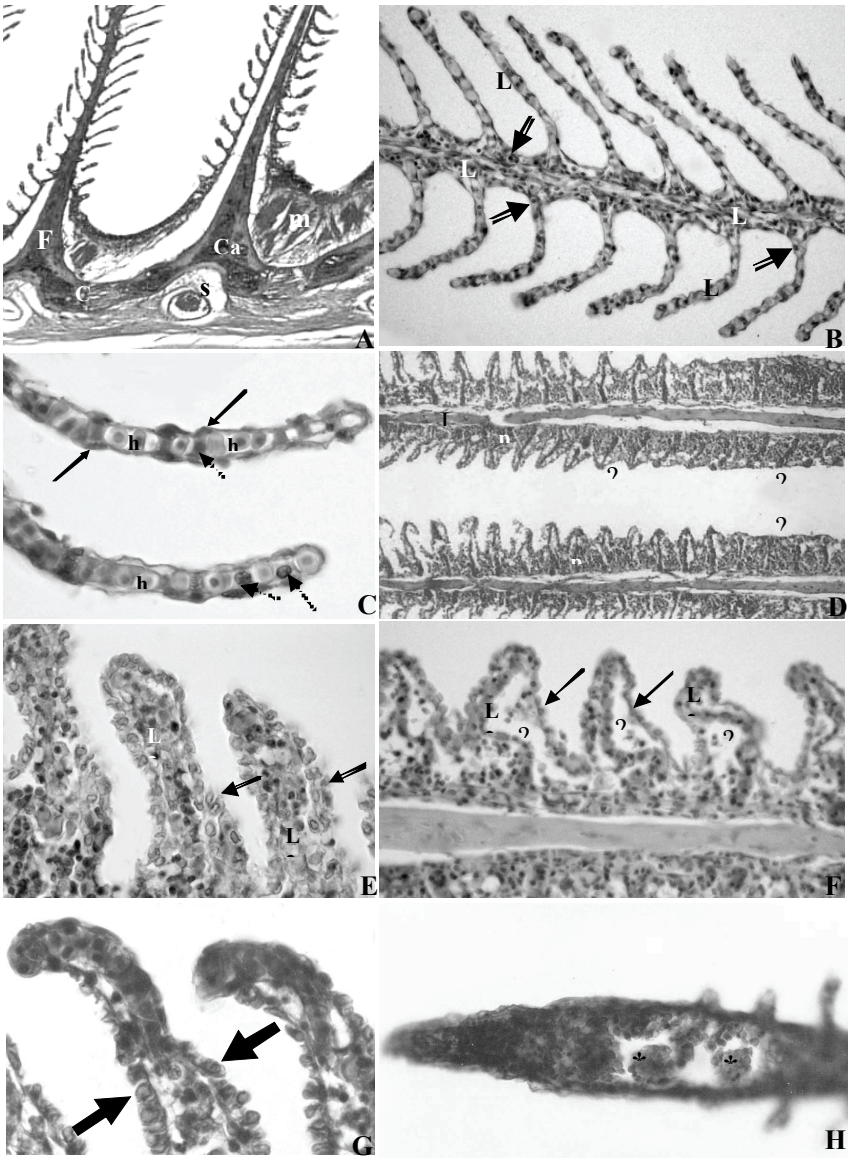
Table 1 shows the results of the physico-chemical parameters. The temperature and pH showed no significant variations during the four sampling seasons, showing approximately constant values in the four collection areas.

Macroscopically, no morphological change was observed in the fish specimens examined. Histologically, it was observed that 14 individuals caught in area I had unchanged branchial arches. In this case, each branchial arch was composed of a cartilaginous structure, vascular and muscle tissue supporting the branchial filaments (Figure 1).

Table 1. Physico-chemical values of the water during the study period.

Area/	AI			AII			AIII			AIV		
Period	T°C	pH	SM	T°C	pH	SM	T°C	pH	SM	T°C	pH	SM
A	31	6.08	3.71	31	5.78	4.29	31	5.93	4.29	31	5.93	3.95
B	31	5.31	80.33	32	5.10	58.44	32	5.78	36.17	33	5.4	10.76
C	31	5.67	24.50	32	5.20	23.83	32	5.42	11.83	32	5.67	33.8
D	32	5.70	39.00	32	5.70	52.25	31	5.39	36.83	31	5.30	54.13
Mean (SD)	31.2 ± 0.5	5.69 ± 0.31	36.88 ± 32.38	31.7 ± 0.5	5.44 ± 0.34	34.70 ± 25.26	31.5 ± 0.58	5.63 ± 0.26	22.28 ± 16.20	31.7 ± 0.96	5.57 ± 0.28	25.66 ± 22.87

AI = area I, AII = area II, AIII = area III, AIV = area IV, SM = suspended material (mg L^{-1}), SD = standard deviation, A = dry/wet season, B= wet season, C = wet/dry season, D= dry season.



Figures 1. Photomicrography of the gills of *B. rousseauxii*. **A** - Normal gill structure with gill filament (F), muscle (m), vascular cavity (vc) and cartilaginous structure (Ca), X40. **B** - Branchial filament consisting of primary lamella (L1), secondary lamella (L2) and mucous cells (double arrow- bold font), X400. **C** - Detail of secondary lamella with epithelial cells (thin arrow), pillar cells (dotted arrow) involving the capillary filled with red blood cells (erythrocytes) (h), X1000. **D** - Cell proliferation (p) in the primary lamella (L1) and secondary lamellar fusion (arrow head), X100. **E** - Detail of the secondary lamella (L2) with epithelial cell proliferation (thin double arrow), X1000. **F** - Epithelial elevation characterized by displacement of the epithelium (thin arrow) and empty spaces (●), X400. **G** - Secondary lamella with hypertrophy of the epithelial cells (thick arrow), X1000. **H** - Detail of a secondary lamella with aneurysm (⋆), X1000. H/E.

In each branchial filament the primary lamella and secondary lamella lined by simple squamous epithelium were observed, formed by epithelial, columns and mucous cells. This epithelium lined a thin capillary network (Figures 1A and C). However, two specimens caught in area I and all individuals collected in areas II, III and IV had some type of tissue changes, which were diagnosed as: proliferation of epithelial cells, with thickness increase of the primary and secondary lamellae and secondary lamellar fusion, with a consequent reduction in the respiratory area (Figures 1D and E); epithelial elevations, causing the displacement of the epithelium lining, hence the appearance of spaces within the secondary lamella and promoting the development of edemas (Figure 1F); hypertrophy of the epithelial cells, characterized by the increase of cell volume with a rounded shape and inflammatory infiltrate in the lamella (Figure 1G); and aneurysm, characterized by the extravasation of blood in the secondary lamella, with rupturing of the pillar cells, thereby causing dilation of the blood vessels (Figure 1H).

For most of the specimens that showed branchial changes, there was a higher incidence of cell proliferation associated with other changes (Table 2).

Table 2. Degree of branchial change and number of individuals caught in the study areas that showed branchial changes with regards to the histopathological analysis of the gill.

Degree of branchial change	Study area / collect of period															
	AI				AII				AIII				AIV			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Degree I	-	-	-	-	1	-	4	-	-	1	-	-	-	-	-	-
Degree II	-	1	-	-	2	-	-	-	1	1	-	1	-	-	-	-
Degree III	-	-	1	-	1	-	-	-	1	-	-	-	-	-	-	-
Degree IV	-	-	-	-	3	-	2	-	-	-	-	1	-	-	1	-
n	2				13				6				1			

AI = area I, AII = area II, AIII = area III, AIV = area IV; n = number of individuals that showed branchial changes per study area; A = dry/wet season, B = wet season, C = wet/dry season, D = dry season. (-) no individuals in the period. Degree I = cell proliferation, Degree II = cell proliferation + hypertrophy, Degree III = cell proliferation + hypertrophy + infiltration or epithelial elevation, Degree IV = cell proliferation + epithelial elevation + infiltration + hypertrophy + aneurism.

Table 3 shows the total weight and total length analysis of the specimens in the study areas. Area IV was not considered due to the capture of only one sample, which was 38 cm in length and 308 g weight.

Table 3. Averages (±DP) of total length (cm) and total weight (g) of *B. gill rousseauxii* with normal and modified gill in three study areas of the Guajará bay.

Study area/ Biometry	AI N n= 14	AI A n= 2	AII n= 13	AIII n= 6
total length	30.9 (± 6.23)[a]	35.3 (± 0.0)[a]	34.05 (± 7.36)[a]	23.85 (± 3.93)[b]
total weight	178.67 (± 137.09)[a]	161.55 (± 2.17)[a]	242.72 (± 128.43)[a]	67.02 (± 36.73)[b]

*Different inscriptions on the same line indicate statistically significant differences ($p < 0.05$); n = number of individuals caught per area; AI N = area I, with control individuals with no branchial change; AI A = area I with the control individuals with branchial change; AII = area II; AIII = area III.

Discussion

In the study herein, the temperature values were considered normal and the pH values slightly acidic. There is a close relationship between pH and carbon dioxide levels in the water. The sewage effluents discharged into the rivers alters the water quality, which produces contamination by pathogenic bacteria and by degradable organic substances. The decomposition of these micro-organisms produces the release of carbon dioxide and the consequent acidity increase in the water (FANTA, 1991; LIVINGSTONE, 1998). A similar situation may have occurred in areas II, III and IV, since the concentration of total and fecal coliforms is above the limit established by the Conama Ordinance No 357/2005 (BRASIL, 2005) in the areas that border the city of Belém, Brazil (SILVA, 2006). These areas in the city of Belém showed an accelerated process of population and industrial occupation, with environmental changes observed, resulting in the reduction of the number of aquatic species, and also the presence of domestic and industrial waste (COHAB, 1997). The pH of area I, control, free of human action, had a slight increase that can be explained by the increase in the rate of photosynthesis of phytoplankton and other aquatic plants. According to Esteves (1988), the increase in the pH and dissolved oxygen is directly involved with the photosynthesis process, which in turn is related to the photoperiod, intense light and temperature. However, in this investigation the dissolved oxygen was not measured.

The material in suspension can cause serious disturbances in the aquatic communities, since it can stick to the surface of fish eggs and to the gills, impairing their normal function and preventing the exchange of oxygen and carbon dioxide (MALLATT, 1985, POWELL et al., 1992). In this work, this variable was within the parameters required by Conama No 357/2005, which is up to 100 mg L^{-1}. Although the observed values are different throughout the study, they may be associated with two factors: the seasonality, as during the wet period, considered winter in the Amazon region, there is a rising of the water levels that cause the lixiviation of the Guama river banks and the islands near the metropolitan region of Belém. Silva (2006) reported that in tropical regions the rates of rainfall variation alters the speed of the river currents, interfering with the entrainment of pollutants to the aquatic system, in addition to the tide flow that places a great deal of material in suspension on the water surface. Thus, the low concentrations obtained in the dry/wet season period might be caused by the tidal flow reduction, then, the deposition of material in

suspension occurs. Similar values were obtained by Ribeiro (2004) studying streams in the Combu Island, Pará State, Brazil.

As the gills play a role in the gaseous exchange and osmoregulation, the tissue transformations directly affect the morphophysiological mechanisms (HIBIYA, 1982; MEYERS; HENDRICKS, 1985). Because the gills are in direct contact with water, toxic substances can easily interfere in the morphophysiology of these organs, as observed in the use of organic pesticides (RAO; RAO, 1981; MALLAT, 1985; LAURENT; PERRY, 1991), of detergents (BOLIS; RANKIN, 1980) acids (McDONALD, 1983), salts (FANTA et al., 1995), industrial rejects (LINDESJÖÖ; THULIN, 1994), ammonia (SODERBERG et al., 1994; MIRON et al., 2008) and heavy metals (OLIVEIRA-RIBEIRO et al., 1996). In the respiratory process, the secondary lamellas prevent that solid agents cross the filaments during the water flow passage. However, the high concentration of irritant agents dissolved in water inevitably come into contact with the gill filaments through the external surface of the secondary lamella and the arterial circulation, possibly altering the normal morphology of the gills and causing cell proliferation, epithelial proliferation, hypertrophy, infiltration and aneurysm (SEPICI-DINÇEL et al., 2009). When animals are under stress, the proliferation of the epithelial cells is one of the first changes in order to quickly remove toxic agents (LAURENT, PERRY, 1991). The epithelial increase is caused by changes in the basal membrane, which distances from the respiratory epithelium, hence causing the oxygen absorption to be inefficient (NOWAK, 1992). Giari et al. (2008), analyzing the histologic response in the gills of *Dicentrarchus labrax* in view of the mercury exposure, found mainly diffuse edema, aneurysm and exfoliated epitheliums. In this study, it was not possible to determine the types of residues in the water, but the changes observed are similar to those described by the aforementioned authors. Thus, it can be inferred that the animals assessed in this study were responding to the effects of toxic substances and sediments.

The first effects of the contaminants usually occur at the cellular or intracellular level (STEPHAN; MOUNT, 1973). In this study, responses to branchial changes in *B. rousseauxii* enabled to differentiate animals from the areas undergoing effluent reactions (areas II, III and IV) from those in the area without effluent reactions (area I, control).

Mallat (1985) and Baldisserotto (2002) developed a classification that associates the degree of changes in gill tissues according to the degree of pollution in the aquatic system. These authors consider that an epithelial with cell proliferation and total fusion of two secondary lamellas can be considered tissue exposed to a highly polluted environment. The cell proliferation is induced by high concentrations of toxic substances, and the aneurysm is the accumulation of blood in the respiratory lamellas, which leads to the collapse of pillar cells and consequent structural breakdown of the lamellas (TEMMINK et al., 1989; HEATH, 1995). In this study, cell proliferation was the most observed change in areas II, III and IV.

The lack of samples during some periods in the areas examined may be related to the discharge of organic and chemical material, as area IV receives a larger amount of waste, which may have resulted in the reduced number of *B. rousseauxii*.

On the other hand, the number of samples may be partly responsible, given that only one collection was performed per period. The branchial morphological changes observed in juvenile individuals signal the beginning of contamination in the Guajará bay and indicate that the species can be used in the environmental monitoring program. However, further studies should be conducted, including the use of other fish species with different eating habits, in order to evaluate the effects of environmental contamination in fish populations.

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Received on August 22, 2008.
Accepted on January 30, 2009.