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Fish Gills Alterations as Potential Biomarkers of Environmental Quality in a Eutrophized Tropical River in South-Eastern Brazil

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With 1 figure and 2 tables

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Summary

Gill anomalies in three common fish species of different taxonomic order, habitat dwelling and feeding habits (one Characiformes, *Oligosarcus hepsetus*; one Siluriformes, *Hypostomus auroguttatus*; and one Perciformes, *Geophagus brasiliensis*) from a eutrophized tropical river in south-eastern in Brazil were compared. The aim of this study was to search for sentinel species that could be used as potential biomarkers of environmental quality. Most fish had gills with histological changes, namely epithelial lifting, interstitial oedema, leucocyte infiltration, hyperplasia of the epithelial cells, lamellar fusion, vasodilatation and necrosis. On the other hand, lamellar blood congestion and lamellar aneurysm, which are more serious and often irreversible changes, were recorded for the water column carnivorous *O. hepsetus* and, to a lesser extent, for the bottom-dwelling detritivorous *H. auroguttatus.* A histopathological alteration index (HAI) based on the occurrence and severity of gills anomalies indicated that *O. hepsetus* (mean score = 11.4) had significantly higher values (Kruskall–Wallis $H_{2,41} = 15.95$, $P = 0.0003$) compared with *G. brasiliensis* (mean score = 7.0). Overall, the omnivorous *G. brasiliensis* had comparatively lesser occurrence of most gill anomalies compared with other two species, being less suitable as biomarker of environmental quality. In contrast, the water column–dweller *O. hepsetus* (water column) and the bottom-dweller *H. auroguttatus* had gills most susceptible to changes, making them more suitable for using as histological biomarkers of the environmental quality in entrophized tropical rivers.

Introduction

The importance of the gills in respiration and ionic regulation of fish has prompted many investigations of the effects caused to this organ by changes in environmental factors (De La Torre et al., 2005; Nigro et al., 2006; Nogueira et al., 2008). The gills are efficient tools for biomonitoring potential impacts (Oliveira Ribeiro et al., 2005) because of their large area in contact with the water and high permeability (Arellano et al., 2004; Evans et al., 2005; Vigliano et al., 2006), and environmental impact caused by pollutants may affect fish gills tissues (Zeeman and Brindley, 1981; Schwaiger et al., 1997; Teh et al., 1997).

Pollutants can directly cause degeneration or necrosis of the gill tissues (Camargo and Martinez, 2007; Ayandiran et al., 2009), but fish can develop mechanisms to react to pollutants that can result in cell hyperplasia, with increased density of the cells of the secondary lamellae, as reported by Hughes and Perry (1976) and Tietge et al. (1988). Most of the gill injuries caused by sublethal exposure to pollutants affect the lamellar epithelium (Hinton and Laurén, 1990); however, some alteration in the blood vessels can occur when fish are under severe stress (Camargo and Martinez, 2007).

The Paraíba do Sul River (PSR) drains a major industrialized area in Brazil and suffers several impacts along its extension, with the heaviest alteration occurring in the middle reaches (Pfeiffer et al., 1986; Linde-Arias et al., 2008a,b), where there is generalized pollution from organic and industrial effluents from poorly planned municipal sewage systems (Carvalho and Torres, 2002). Sewage, toxic chemicals and excessive phosphorus cause the primary water quality problems. This middle reaches of the Paraíba do Sul River has poor water quality as indicated by dissolved oxygen between 3.5 and 6.9 mg/l, conductivity between 91 and 107 μS/cm, total phosphorous between 0.068 and 0.095 μg/g and ammoniacal nitrogen between 0.08 and 0.20 μg/g (Araújo et al., 2009).

Three common non-migratory fish species are abundant in the Paraíba do Sul River (Teixeira et al., 2005; Araújo et al., 2009; Santos et al., 2010): one Characiformes, *Oligosarcus hepsetus* (Cuvier 1829); one Siluriformes, *Hypostomus auroguttatus* Kner 1854; and one Perciformes, *Geophagus brasiliensis* (Quoy and Gaimard 1824).

Oligosarcus hepsetus is a small- to middle-sized carnivorous species feeding mainly on fishes and insects using shallow marginal waters near to river margins (Araújo et al., 2005, 2009). Fishes with a carnivorous feeding habit increase the stability of an ecosystem, by regulating the abundance of prey species in communities controlled by mechanism of the type top-down (Nikolsky, 1963; Popova, 1978).

Hypostomus auroguttatus is a detritivorous species widely distributed across the Paraiba do Sul River basin. Lowe McConnell (1963) identified many species of *Hypostomus* as common species dwelling in rivers and small tributary environments, having rocky bottoms. Several species of *Hypostomus* are known for a closer association with fast flowing environments where they display a bottom-dwelling behaviour, feeding on the attached algae on rocky substratum (Buck and Sazima, 1995; Garavello and Garavello, 2004). Furthermore, Mazzoni et al. (2010) reported that species of *Hypostomus* are normally classified as grazer feeders, frequently inhabiting fast flowing streams in benthic areas close to rock substrate and/or submerse wood.

Geophagus brasiliensis has diurnal habits because it uses vision as the main sense for searching and pursuing its preys. It is widely distributed in Neotropical aquatic systems, ranking among the most abundant species with high trophic plasticity (Abelha and Goulart, 2004). It presents solitary behaviour, swimming in stocking water or close to the bottom of the standing water, always during daylight (Uieda, 1984; Costa, 1987; Sabino and Castro, 1990).

The present study describe gills histological analyses for these three common fish species that have different feeding habits, habitat dwelling and are abundant in the middle reaches of the Paraíba do Sul River. We aimed to assess the use of these fishes as biomarkers of environmental quality in rivers because environmental impact caused by pollutants may affect fish gills tissues. Therefore, the identification of species that may be particularly sensitive to pollution is important to detect environmental impacts.

Materials and Methods

Sample collection

Fish samples were caught in five occasions between February and May 2008 along the middle-lower reaches of the Paraíba do Sul River (22°31′-22°35′ S; 44°41′-44°55′ W), downriver from several highly developed municipalities, in south-eastern Brazil. Fishes were collected on the following dates: 26 February, 18 March, 2 April, 30 April and 15 May by using three gill nets (30 ×3 m, 40–70 mm mesh size). The sampled area encompassed the 30 km stretch of the river where five sampling sites were established covering the whole river extent. Samples were collected in late summer because in this period the river receives the most organic and industrial loads carried out by runoff. Overall, all species were distributed throughout the whole studied area. A total of 41 adult specimens were collected (19 *O. hepsetus*, 11 *H. auroguttatus* and 11 *G. brasiliensis*), because their size was above the size at first maturation (Mazzoni and Caramaschi, 1995; Mazzoni and Iglesisas-Rios, 2002; Gomiero et al., 2008) (Table 1).

Table 1. Means (±standard error) for total length and total body mass, dwelling and feeding habits (with references) of three fish species in Paraíba do Sul River. Number of examined individuals in brackets

Species	Habitat dwelling	Feeding habits and references	Total length (cm)	Total body mass (g)
Oligosarcus hepsetus (19)	Water column	Carnivore; Araújo et al. (2009)	24.3 ± 1.9	158.8 ± 19.8
Hypostomus auroguttatus (11)	Bottom	Detritivore; Araújo et al. (2009)	29.1 ± 2.6	229.8 ± 12.2
Geophagus brasiliensis (11)	Margins and shoreline	Omnivore; Sabino and Castro (1990)	19.5 ± 1.6	137.5 ± 12.8

Histological procedures

Immediately after collection, fishes were anesthetized in benzocaine hydrochloride (50 mg/l) and then killed rapidly by hipothermy. Then, fishes were identified according to species, measured and weighted. The fish were then dissected, and the first gill arch of the left side of each fish was excised keeping the filaments intacts, and fixed for 8 h in Bouin's fluid. The gills were subject to histological techniques and embedded in paraffin. Sagittal sections (5 μm of thickness) were cut, mounted on glass slides and stained in haematoxylin and eosin (HE). Three sections from each fish gills collected in different regions of the tissue were examined and photographed using an Olympus (Tokyo, Japan) B $\times$ 41 microscope fitted with photographic attachment, a Nikon Coolpix 4300 digital camera. The presence of histological alterations was examined and recorded for each three sections that encompassed the entire gill.

The histological changes in the gill tissues were classified according to the system proposed by Monteiro et al. (2008). Seven major histological changes (epithelial lifting, interstitial oedema, lamellar fusion, lamellar blood congestion, vasodilatation, lamellar aneurysm and necrosis of the gill tissue) and two minor ones (hyperplasia of the lamellar epithelium and leucocyte infiltration) were considered. The percentage of occurrence of each anomaly in gill tissue of each fish species was calculated by dividing the number of fish with a given anomaly by the total number of examined fish.

Data treatment

Differences in percentage of anomaly for each species were tested to compare the proportions for significant differences in changes among the different feeding habits using a binomial *t*-test for independent samples. We used the Bonferroni correction to adjust *P*-values in order to minimize committing error Type I.

A lesion index of each studied fish was calculated after the histopathological analysis. A score value was attributed (1, 2 or 3) to the fish according to the stages of severity of the alterations. The definitions slight (1), moderate (2) and severe (3) alterations followed Flores-Lopes and Thomaz (2011). Slight alterations are reversible changes that allow gill tissues to restructure and recovery normal gill function when environmental conditions improve and include interstitial oedema, leucocyte infiltration, epithelial hyperplasia, lamellar fusion, vasodilatation and lamellar blood congestion. Moderate alterations are more profound changes that lead to effects in tissues associated with the functioning of the organ. These changes are reparable lesions, but if wide areas of the gills are affected or maintained in situations of chronic pollution, they can lead to severe alterations, for example epithelial lifting. Severe alterations are irreversible changes which implicate that gill structure will not recover, even when water quality improves or no further exposure to a toxic stimulus occurs, such as aneurysms and necrosis. The total score index was obtained multiplying the stages of severity of the alteration (1, 2 or 3) by the number of a given alteration for each individual. For each species, score index mean value was calculated. The non-parametric Kruskall–Wallis test for independent samples with $P < 0.005$ followed by a multiple comparison of mean ranks for all groups was utilized to compare the three fish species. All statistical tests were conducted using STATISTICA Version 7.1 (StatSoft, Inc., 2005).

Results

Histological changes observed in gill tissue of all three species included epithelial lifting, interstitial oedema, leucocyte infiltration, hyperplasia of the gill epithelium, lamellar fusion, vasodilatation and necrosis (Fig. 1). Besides these changes, in *O. hepsetus* and *H. auroguttatus* (Fig. 1c), there were blood congestion and lamellar telangectiases (aneurysms) (Table 2).

The distribution of the different histological changes was not uniform for the three species. High average frequencies of all changes were recorded for *O. hepsetus* which had at least 80% (78.9%) of the individuals with gill anomalies. This species had significantly higher prevalence of interstitial oedema, epithelial hyperplasia and aneurysm compared with the other two species (Table 2). Lamellar blood congestion was significantly higher in *O. hepsetus* and *H. auroguttatus* compared with *G. brasiliensis*, which did not present this malformation. Moreover, in this species, lamellar congestion and aneurysm were also not detected (Table 2).

Tissue alterations that suggest a general gill inflammation, such as epithelial lifting, characterized by elevation of the external layer of the lamellar epithelium (Fig. 1a,e), oedemas (Fig. 1e) formed between the epithelial layers (interstices) and leucocyte infiltration (Fig. 1d) were observed in all analysed species. Hyperplasia of the mucous cells (Fig. 1f), vasodilatation (Fig. 1a–d) and lamellar fusion (Fig. 1a,d,f) were observed. Although they considered as slight changes, they may have repercussions in gill function. The moderate change lamellar blood congestion (Fig. 1c) and the severe aneurysms (Fig. 1b) were not observed in *G. brasiliensis* but frequent in *O. hepsetus* and occurring in comparatively lesser extent in *H. auroguttatus*. Necrosis, the other severe change, was evenly found in all the three species.

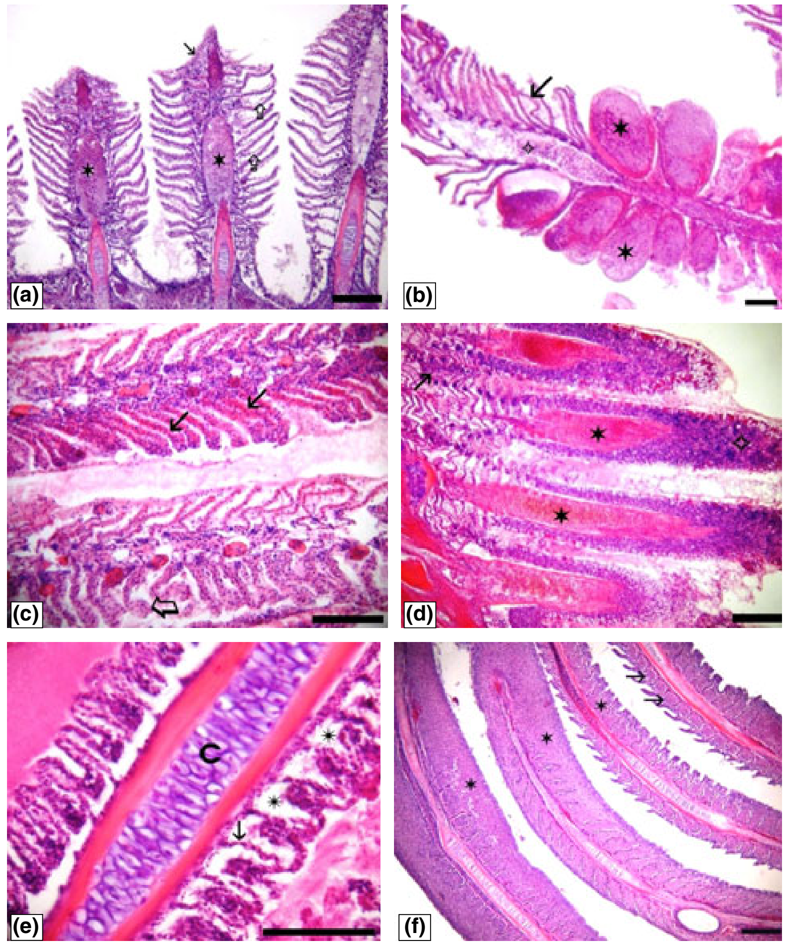

Fig. 1. Photomicrographs of the gill filament of: (a and b) *Oligosarcus hepsetus* – (a) Partial fusion at the top of the secondary lamellae (↘); vasodilatation of the central venous sinus (*) and raised lamellar epithelium (⇧). Scale bar = 50 μm. (b) Extremely deformed secondary lamellae (arrow), aneurysms (*) and vasodilatation (✧) of the central venous sinus. Scale bar = 50 μm. (c and d) *Hypostomus auroguttatus* – (c) Blood congestion in secondary lamellae (⇦) and vasodilatation of the lamellar vascular axis (↙). Scale bar = 50 μm. (d) Leucocyte infiltration in the gill epithelium (✧), fusion between lamellae of adjacent filaments (arrow) and vasodilatation of the central venous sinus (*). Scale bar = 50 μm. (e and f) *Geophagus brasiliensis* – (e) elastic cartilage of the filament (C), raised lamellar epithelium (arrow) and interstitial oedema (*). Scale bar = 50 μm. (f) Hyperplasia of epithelial cells at the base of the secondary lamellae (arrow) and different degrees of lamellar fusion (*). Scale bar = 100 μm.

Table 2. Frequency of occurrence (in bold) of histological changes in gill tissues in the three studied fish species. Number of species with changes (number of examined species)

Species/changes	*Oligosarcus hepsetus*		*Hypostomus auroguttatus*		*Geophagus brasiliensis*	
Epithelial lifting	17 (19)	**89.5%**	9 (11)	**81.8%**	10 (11)	**90.9%**
Interstitial oedema	18 (19)	**94.7%^{a}**	7 (11)	**63.65^{b}**	10 (11)	**90.9%**
Leucocyte infiltration	18 (19)	**94.7%**	9 (11)	**81.8%**	9 (11)	**81.8%**
Epithelial hyperplasia	18 (19)	**94.7%^{a}**	9 (11)	**81.8%**	7 (11)	**63.6%^{b}**
Lamellar fusion	17 (19)	**89.5%**	8 (11)	**72.7%**	7 (11)	**63.6%**
Vasodilatation	18 (19)	**94.7%**	11 (11)	**100%**	9 (11)	**81.8%**
Lamellar blood congestion	18 (19)	**94.7%^{a}**	8 (11)	**72.7%^{a}**	0 (11)	**0%^{b}**
Aneurysm	18 (19)	**94.7%^{a}**	4 (11)	**36.4%^{b}**	0 (11)	**0%^{c}**
Necrosis	15 (19)	**78.9%**	8 (11)	**72.7.%**	8 (11)	**72.7%**

Superscripts letters indicate differences highly significant according to *t*-test for difference of proportion.

The histopathological alteration index (HAI) for the gills ranged from 3 to 13 for *O. hepsetus*, 4 to 12 for *H. auroguttatus* and from 3 to 9 for *G. brasiliensis*. Highly significant differences were found between *O. hepsetus* (mean 11.4, SD 3.4) and *G. brasiliensis* (mean 7.0, SD 2.0) according to Kruskall–Walis test ($H_{2,41} = 15.95$, $P = 0.0003$). HAI for *H. auroguttatus* (mean 8.7, SD 2.8) did not differ significantly from the other two species.

The carnivorous fish species *O. hepsetus* was the most susceptible species to gill alteration, because it had all the recorded changes in great proportions compared with the other two species. The detritivorous species *H. auroguttatus* had comparatively greater frequency of gill anomalies compared with the omnivorous *G. brasiliensis* (Table 2).

Discussion

The gill tissue changes in the three examined species from the middle reaches of the Paraíba do Sul River indicate a substantial degradation of the water quality because of non-point source of pollution, mainly from industrial and agricultural activities. Gill changes were reported in the Siluriformes *Pimelodus maculatus* from a reservoir damming the Paraíba do Sul River ca. 10 km downriver the study area (Nogueira et al., 2008). Such changes were associated with anthropogenic activities that in the recent decades impact the watershed and lead the reservoir to intense process of eutrophization (Branco et al., 2002). Changes in fish gills that live in altered environments were also reported by Oliveira Ribeiro et al. (2005) and De La Torre et al. (2005). These alterations can be interpreted as resulting from the acute effects of xenobiotics (Zodrow et al., 2004).

Lifting of the lamellar epithelium is one of the first changes in fish gills under acute exposure to toxic substances, such as oils, detergents, ammonia, phenols, acids and metals like mercury (Müller and Lloyd, 1994; Heath, 1995). This change was present in more than 85% of the specimens of the three analysed species.

Oedema and leucocyte infiltration were also very common gill changes in all studied species and can be interpreted as defence responses to toxic agents, as described by Heath (1995). Arellano et al. (1999), studying the effects of copper compounds on the Pleuronectiformes *Solea senegalensis* Kaup 1858, Fanta et al. (2003), studying the effects of organophosphates on the Siluriformes *Corydoras paleatus* (Jenyns 1842), and Simonato et al. (2008) and studying the effects of diesel oil on the Characiformes *Prochilodus lineatus* (Valenciennes 1837), found the presence of oedema and leucocyte infiltration as sublethal alterations.

The increased number of mucous cells in the epithelium of the secondary lamellae (hyperplasia) is also very common (>85% in the examined fishes) and has been associated with an increase in the surfactant layer that protects the gill lamellae (Temmink et al., 1983). Hyperplasia has also been described for the Cyprinodontiformes *Poecilia vivipara* Bloch and Schneider 1801 (Motter et al., 2004) and for the Siluriformes *Steindachnerina brevipinna* (Eigenmann and Eigenmann 1889) (Lima et al., 2009).

Lamellar fusion observed in more than 63% of the analysed individuals has already been reported by other authors as a response to specific contaminants, such as copper (Arellano et al., 1999), sewage from a secondary treatment plant (Coutinho and Gokhale, 2000) and effluents from a bleached paper mill (Pacheco and Santos, 2002). Overall, histological alterations in response to environmental changes can represent adaptive strategies for conservation of some physiological functions in the face of a stressor. This can trigger a series of endocrine responses, together called stress response, a mechanism that permits the fish to preserve its health when faced with threats from stressors.

In the evaluated species, vasodilatation was found in 81% or more of the specimens which seems to indicate severe exposure to water pollutants. When fish are under more severe stress, vascular changes can occur in the lamellae and the response function can become dysfunctional and impair their physiology (Heath, 1995; Lima et al., 2006).

Necrosis of the gill epithelium observed in this study affected more than 72% of the individuals in each specie which reflects the direct effect of pollutants (Temmink et al., 1983; Garcia-Santos et al., 2007).This tissue alteration has been described as occurring under more toxic (Abel, 1976). Degeneration by necrosis and apoptosis of the gill epithelial cells was also mentioned in an ultrastructural study carried out with the Characiformes *Prochilodus scrofa* (Valenciennes 1837) submitted to pollutants (Mazon et al., 2002). Furthermore, filament epithelial thinning might be due to a decrease in the number of mucous cells (De Boeck et al., 2001) or to cell necrosis (Bury et al., 1998; Li et al., 1998), while epithelial thickening has been attributed to the appearance of macrophages and other leucocytes integrated in a compensatory response of tissue repair (Teh et al., 1997).

Lamellar blood congestion occurred only in *O. hepsetus* and *H. auroguttatus*. This alteration can harm the gas exchange function of the gill structure causing rupture of the pillar cell system, with loss of their support capacity and consequently structural disorder of the lamellae. These are cases of reversible vascular changes if the water quality improves. However, they can become progressively worse in cases of persistent exposure to contaminants, eventually compromising the organ's function (Poleksić and Mitrovic-Tutundzic, 1994; Albinati et al., 2009).

Lamellar aneurysm was also only recorded for *O. hepsetus* and *H. auroguttatus*. This anomaly is characterized by blood extravasations inside the lamellae and rupture of the pillar cell system, with consequent dilatation of the blood vessels. This is considered a more serious change, which is often irreversible. The reason for the occurrence of lamellar aneurysm in the carnivorous species, such as *O. hepsetus*, can be associated with its eating habits, as top carnivorous tend to accumulate pollutants from the trophic web. Conversely, occurrence of aneurysms in the detritivorous *H. auroguttatus* can be associated with it feeding habits to eat organic matter on the sediment where pollutants tend to accumulate (Stehr et al., 1998).

The results of this study support the hypothesis that there are heavy toxic organic and industrial loads in the waters of the Paraíba do Sul River, as would be expected owing to the intense pollution from the watershed (Carvalho and Torres, 2002; Linde-Arias et al., 2008a,b). The HAI for the gill of the three species demonstrated that most individuals showed moderate to severe gills alterations. This index confirmed that *O. hepsetus* had significantly more severe alterations compared with *G. brasiliensis*, although it did not detect significant differences between *H. auroguttatus* and the other two species. This stretch of the Paraiba do Sul River has been receiving diffused pollution for the past two decades from Municipalities with poor and unplanned sewage treatment which has been responsible for the poor environmental quality. The results obtained in this study seem to demonstrate that the species that live in this stretch of the river are affected by the stressors present in the water. These results also indicate that *O. hepsetus* may be more sensitive than the other species to the environmental changes because it presented higher incidence of gill anomalies compared with the other two species.

According to Klontz (1972), fish are intimately associated with their aqueous environment and physical and chemical changes in ecosystems are rapidly reflected as quantifiable physiological measurements in the fish. The carnivorous *O. hepsetus* and the detritivorous *H. auroguttatus* proved to be susceptible to environmental changes because they were more sensible as indicated by their gill alterations. We believe that these two species are suitable to be used as sentinel in water quality monitoring programmes in tropical lotic systems. Furthermore, these results reinforce the importance of the addition of histopathology analysis in programmes for the evaluation of water quality to assess the alteration of anthropogenic origin that can compromise the quality of the ecosystems, as tools for a more precise evaluation of environmental quality should be used.

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