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Can fish gill anomalies be used to assess water quality in freshwater Neotropical systems?

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Abstract Gill anomalies in two fish species (*Geophagus brasiliensis* and *Astyanax bimaculatus*) were compared among three freshwater systems with different water quality: one eutrophic river, one eutrophic reservoir, and one oligotrophic reservoir. The raised hypotheses are that reservoirs with low water quality (eutrophic) have fish with more gills anomalies compared with reservoirs with high water quality (oligotrophic), and that the more stable environmental conditions of eutrophic rivers have fish with better healthy conditions than eutrophic reservoirs that have lesser stable environmental conditions. Gills of 36 adult individuals of *G. brasiliensis* and 23 of *A. bimaculatus* collected during the winter 2008 and winter 2009 were examined, and the proportions of occurrence of nine histological alterations were compared for the two species among the three systems using a binomial *t* test for independent samples. Histological changes in fish gills that are reversible and unspecific, such as epithelial lifting, interstitial edema, leukocyte infiltration, hyperplasia of the epithelial cells, lamellar fusion, and vasodilatation were common in both fish species in the three systems. However, lamellar aneurism, which is a more serious and often irreversible anomaly, and lamellar blood congestion occurred only in fish from the two reservoirs. Alternatively, necrosis occurred more in fish from the river. Fish gill anomalies in both species did not differ between the two reservoirs, despite having different water quality. We rejected the hypothesis that reservoirs with lower water quality have fish with more gill injuries compared with high water quality reservoirs. Moreover, the eutrophic river seems to affect differently the healthy condition of fish species, compared with the eutrophic reservoir.

Keywords Biomonitoring · Freshwater fishes · Fish gills · Rivers · Reservoirs · Aquatic ecosystems

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Introduction

It has been widely reported that fish gills are sensitive to water quality due to their anatomic location within the fish body and direct contact with the environment. Gills are also an important indicator of pollution in aquatic systems, and histopathological changes have been used as biomarkers in fish exposed to contaminants (Abel 1976; Mazon et al. 2002; Garmendia et al. 2010). Alterations in the gill epithelium are a result of a variety of contaminant exposures, with the severity of changes depending on the pollutant concentration and exposure period (Franchini et al. 1994; Evans et al. 2005).

Pollutants can cause degeneration or necrosis of gill tissues; however, fish can develop mechanisms to deal with pollutants (Hughes and Perry 1976; 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. Despite the large amount of information on gill anomalies associated to pollutants (Abel 1976; Arellano et al. 1999; Mazon et al. 2002; Reis et al. 2009), most of these studies have been conducted in the laboratory and under controlled conditions, with few studies based on observational field conditions.

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), where there is generalized pollution from organic and industrial effluents from poorly planned sewage municipal sewage systems (Carvalho and Torres 2002). Sewage, toxic chemicals, and excessive phosphorus cause the primary water quality problems. The Funil Reservoir, which is a eutrophic impoundment of the PSR, is located downriver of excessively polluted reaches and receives large pollutants loads from the main river. Alternatively, the Lajes Reservoir has high water quality, with oligotrophic conditions, and is located in the upper slopes of the Sea Mountains, and is surrounded by well-preserved stretches of the Atlantic Forest. Both reservoirs were built for hydroelectric purposes but also serve as the water supply for Rio de Janeiro and adjacent areas.

Rivers differed from reservoirs in several aspects, among them the stability of environmental variables. One of the most commonly river disturbances is the rapid increase in discharge-associated bed movement (Scrimgeour et al. 1988; Matthaei et al. 1997). According to Vannote et al. (1980), the physical variables within a river system present a continuous gradient of physical conditions. Consequently, rivers usually have stable dissolved oxygen, pH, temperature, and other environmental variables. In contrast, such factors are very changeable in reservoirs, depending on the trophic state, depth, residence time, geomorphology, and winds (Tundisi et al. 2010). The interaction of solar radiation, water, and wind regimes influence the cycles of events in the vertical structure of reservoir ecosystems (Talling and Lemoalle 1998). The impact of precipitation during the cold fronts cannot also be disregarded. Precipitation can discharge nutrients by drainage introducing the "new nutrient component" to the reservoirs, therefore increasing the pool of nutrients available to phytoplankton growth (Tundisi et al. 2010). Moreover, water level changes in reservoir, especially those built for hydroelectric purpose, are also a considerable disturbance in these systems.

Two fish species are widespread in these three systems, the Perciformes *Geophagus brasiliensis* and the Characiformes *Astyanax bimaculatus*. *G. brasiliensis* and *A. bimaculatus* are omnivorous–opportunistic species that have successfully adapted to the PSR, and the Funil and Lajes Reservoirs. The main aim of this work was to assess water quality by comparing changes in the gill epithelium for these two fish species in each of the three systems. We hypothesized that lower water quality reservoirs would have fish with more gill anomalies compared with higher water quality. We also expected that the more stable environmental conditions in the rivers would enable healthier fish compared with the fish surrounded by environmental stressors in the reservoirs.

Specifically, it is expected that the river and its associated system (Funil Reservoir) will have fish with more gill injuries when compared with the fish from the Lajes Reservoir. It is also expected that the eutrophic river will have fish in better condition than fish from the eutrophic reservoir (Funil Reservoir), where general environmental variables are less stable compared with the river environmental variables.

Materials and methods

Study area

The main features of the Funil Reservoir (22°30′S, 44°45′W, altitude 440 m, Cwa in the Köppen system) and the Lajes Reservoir (22°43′S, 44°46′W, altitude 410 m, Aw in the Köppen system) are summarized in Table 1. Although both reservoirs are similar in size (Fig. 1), they differ in the outflow rate and water residence time (Table 1) because Funil Reservoir dams a large river (PSR), while Lajes Reservoir only receives water from small streams. Both reservoirs experience high water level fluctuations dictated by rainfall and hydroelectric demands. Overall, the annual water level fluctuation averages nearly 3 m, but extreme differences between high and low water levels can reach up to 10 m in both reservoirs (Santos et al. 2004).

Water quality is a major differential between the two reservoirs, with the Funil Reservoir being eutrophic and of low water quality compared with the Lajes Reservoir, which is an oligotrophic system with high water quality (Gomes et al. 2008). The Funil Reservoir has comparatively higher conductivity, total phosphorous, nitrogen, and chlorophyll-*a* compared with the Lajes Reservoir (Table 2).

The middle reaches of the Paraíba do Sul River (22°31′-22°35′ S; 44°41′-44°55′ W), located upriver of the Funil Reservoir, drains several highly developed municipalities. This river extent 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; Cetesb 2009).

Data collection and treatment

Thirty-six adult individuals of *G. brasiliensis* (21 from PSR; nine from Funil Reservoir; six from Lajes Reservoir) and 23 adult of *A. bimaculatus* (12 from PSR; six from Funil Reservoir; five from Lajes Reservoir) were examined (Table 3). *A. bimaculatus* was caught in all sampling sites of the three systems, and their average sizes (lengths) and weights ranged from 7.8 to 15.5 cm, and 20.5 to 50.8 g, respectively. *G. brasiliensis* was also captured at all sites; their average sizes (length) and body weights ranged from 11.2 to 29.5 cm and 40.3 to 345.9 g, respectively. Although body size for *G. brasiliensis* had wider range of variation compared with *A. bimaculatus*, which is a comparatively small-sized species, all individuals were adults and sex differentiation was not considered.

Fish were collected using gill nets at five sites in the middle reaches of the PSR (Fig. 1) in winter 2008 and at three sites in Funil and three sites in Lajes Reservoirs in winter 2009. The sites were distributed along the extent of the three systems aiming to encompass the whole studied areas. We sampled in winter (dry season) because the water environmental conditions are more stable. Overall, both species were distributed throughout all studied area, which were searched in four occasions in each winter.

After the nets were retrieved, fish were identified to species, measured, and weighted. The fish were then dissected, and the first gill arch of the right side of each fish was excised keeping the filaments intact 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 hematoxylin and eosin. Three sections from each fish gill were examined and photographed using an Olympus B ×41 microscope fitted with photographic attachment, a Nikon Coolpix 4300 digital camera. The presence of histological alterations was examined and recorded for each of the 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 edema, lamellar fusion, lamellar blood congestion, vasodilatation, lamellar aneurism, and necrosis of the gill tissue) and two minor ones (hyperplasia of the lamellar

Table 1 Main physical features of Funil and Lajes reservoirs

Features	Funil reservoir	Lajes reservoir
Area, km^2	40.0	38.9
Volume, m^3	890×106	450×106
Maximum depth, m	70	40
Mean depth, m	22	15
Residence time, days	10–50	297
Outflow, mean (range)	318 m^3/s (109–950 m^3/s)	20 m^3/s (14–24 m^3/s)
Main tributary	Paraíba do Sul river	Pirai stream and other small streams

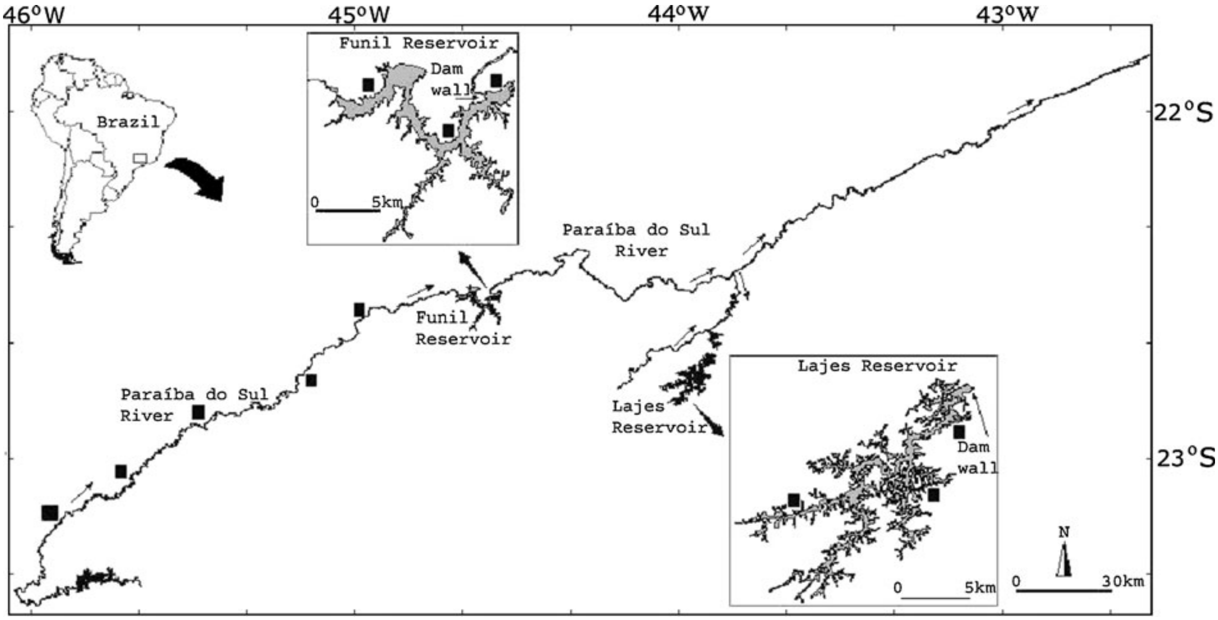

Fig. 1 Map of the study area: middle stretches of the Paraíba do Sul river and the two studied reservoirs (Funil and Lajes reservoirs) with indication of the sampling sites *black square*. The *arrows* show the direction of the water flow

epithelium and leukocyte infiltration) were considered. The percentage of occurrence of each anomaly in gill tissue of the two fish species from each system was calculated by dividing the number of fish with a given anomaly by the total number of examined fish. Differences in percentage of anomaly for each species between each two systems were compared using a binomial *t* test for independent samples with significance assigned for $\alpha=0.05$. Since the sample sizes are unequal, we used a more conservative approach to reject H_o, by considering the two-sided test, because the probability of incorrectly rejecting the null hypothesis is less likely. This statistical test was conducted using STATISTICA Version 7.1 (StatSoft, Inc. 2005).

Results and discussion

The three systems had high incidences for several of the histological changes in the gills of the two examined fish species, including the following: epithelial lifting (Fig. 2a), interstitial edema (Fig. 2a), epithelial hyperplasia (Fig. 2b), leukocyte infiltration (Fig. 2c), lamel-

Table 2 Median values and confidence intervals of limnological variables measured in Funil and Lajes reservoirs

Environmental variables	Funil reservoir		Lajes reservoir	
	Mean	Range	Mean	Range
Temperature, °C	26.0	20.0–31.6	25.3	17.1–30.3
Dissolved oxygen	92.1	10.2–185.7	94.8	71.0–117.7
pH	7.0	5.6–9.9	7.3	5.2–8.2
Conductivity, μS/cm	85.9	64.6–107.6	27.6	18.0–31.0
Euphotic zone, m	3.2	0.5–8.1	7.8	1.1–13.5
Total phosphorus, μM	3.2	0.8–25.6	1.0	0.2–2.5
Soluble reactive phosphorus, μM	1.2	0.1–12.3	0.6	0.1–3.0
Total nitrogen, μM	40.2	3.3–75.5	22.2	6.6–78.8
Dissolved inorganic nitrogen, μM	32.9	17.1–56.6	3.1	1.4–6.5
Chlorophyll-a, μg/L	5.2	1.1–168.9	2.1	0.2–16.6

Source: Soares et al. (2008)

Table 3 Total length (TL) and body weight (BW) of *G. brasiliensis* and *A. bimaculatus* sampled from the three aquatic systems

Aquatic systems	*G. brasiliensis*		*A. bimaculatus*	
	TL, mm	BW, g	TL, mm	BW, g
Paraíba do Sul River	16.5±0.9	83.8±11.5	13.5±0.4	43.2±4.6
Funil Reservoir	21.0±0.8	140.7±11.9	11.6±1.5	45.7±8.5
Lajes Reservoir	26.9±1.3	256.2±33.9	14.9±0.3	45.8±1.8

Values are expressed as mean±SE

lar fusion (Fig. 2c), and vasodilatation (Fig. 2c). These changes did not differ among the three studied systems for the two fish species. These are cases of reversible vascular changes if the water quality improves, or they can become progressively worse in cases of persistent exposure to contaminants, eventually compromising the organ's function (Albinati et al. 2009).

Lifting of the lamellar epithelium is one of the first changes seen in fish gills exposed to toxic substances, such as oils, detergents, ammonia, phenols, acids, and metals like mercury (Heath 1995). This change was present in at least 86% of the *G. brasiliensis* and 67% of *A. bimaculatus* in each studied system. Edema and leukocyte infiltra-

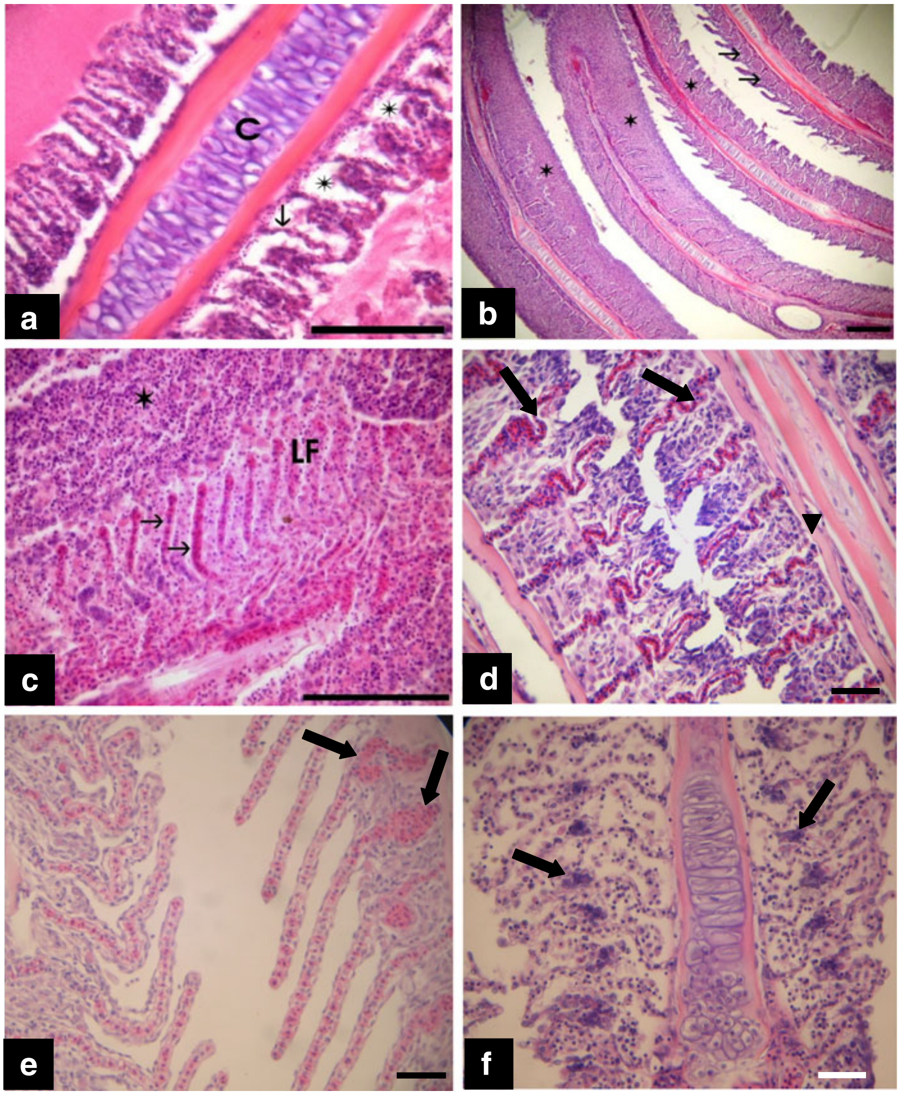

Fig. 2 Photomicrographs of the gill filament. **a** *G. brasiliensis* in Paraíba do Sul River. Elastic cartilage of the filament (*C*), epithelial lifting (*arrow*), and interstitial edema (*star*). *Scale bar*=50 µm. **b** *G. brasiliensis* in Paraíba do Sul River. Epithelial hyperplasia (*arrow*) and lamellar fusion (*star*). *Scale bar*=100 µm. **c** *G. brasiliensis* in Paraíba do Sul River. Lamellar fusion (*LF*), leukocyte infiltration (*star*), and vasodilatation (*arrow*). *Scale bar*=50 µm. **d** *G. brasiliensis* in Lajes Reservoir. Vasodilatation (*arrow*) and lamellar fusion (*triangle*). *Scale bar*=50 µm. **e** *G. brasiliensis* in Lajes Reservoir. Aneurism (*arrow*). *Scale bar*=100 µm. **f** *A. bimaculatus* in Lajes Reservoir. Necrosis (*arrow*). *Scale bar*=100 µm

tion were also very common in the two studied species and have been interpreted as defence responses to toxic agents, as described by Heath (1995). In this study, edema occurred in at least 78% of *G. brasiliensis* and 50% of *A. bimaculatus* in each studied system, while leukocyte infiltration occurred in at least 89% and 67% of *G. brasiliensis* and *A. bimaculatus*, respectively.

Hyperplasia was common in all three systems, being observed in at least 78% in *G. brasiliensis* and 60% in *A. bimaculatus* (Table 4). This type of response has also been described for the Cyprinodontiformes *Poecilia vivipara* (Motter et al. 2004) and for the Siluriformes *Steindachnerina brevipinna* (Lima et al. 2009). The occurrence of hyperplasia reduces the interlamellar space, causing fusion of the lamellae. Lamellar fusion occurred in at least 67% for both fish species (Table 4) and 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).

More serious anomalies, such as lamellar blood congestion (Fig. 2d), aneurism (Fig. 2e), and necrosis (Fig. 2f) occurred at lower frequencies compared with the other histological changes and differed significantly for each fish species among the studied systems (Table 4). Congestions and aneurisms impair the blood flow and compromise the gills' basic function, which is to perform gas exchange (Heath 1995). Besides impairing this important function, they also interfere with other functions, such as maintenance of the acid–base and osmotic balances (Garcia-Santos et al. 2007). Lamellar blood congestion was observed in species of the two reservoirs with no significant differences between these two systems (Fig. 3; Table 4). This change occurred in 56% of *G. brasiliensis* and 83% of *A. bimaculatus* in the Funil Reservoir, and in 83% of *G. brasiliensis* and 80% of *A. bimaculatus* in the Lajes Reservoir (Fig. 3; Table 4). Fish from the PSR were not recorded with lamellar blood congestion. This alteration can harm the gas exchange function of the gill structure and occurs mainly after vasodilatation, which occurred in at least 83% of *G. brasiliensis* and 58% of *A. bimaculatus* from the three studied systems.

Aneurisms (Fig. 2e) were not recorded for either species in the PSR, while it occurs in 67% of *G. brasiliensis* (Fig. 2e) and 83% of *A. bimaculatus* in the Funil Reservoir (Table 4). In Lajes Reservoir, aneurisms occurred in 67% of *G. brasiliensis* and in 80% of *A. bimaculatus*. No significant difference was found for this anomaly between the two reservoirs.

Table 4 Frequency of occurrence (percent in brackets) and *P* values from *t* test comparisons of histological changes in gill tissues of the two studied fish species between each pair of systems

Histological changes	*G. brasiliensis*			*A. bimaculatus*		
Number of fish	PSR=21; FR=9; LR=6			PSR=12; FR=6; LR=5		
(Comparisons) *P* values	(PSR–FR) *P* values	(PSR–LR) *P* values	(FR–LR) *P* values	(PSR–FR) *P* values	(PSR–LR) *P* values	(FR–LR) *P* values
Epithelial lifting	(86–100) 0.38	(86–100) 0.11	(100–100) 0.11	(67–83) 0.30	(67–100) 0.1	(83–100) 0.19
Interstitial oedema	(86–78) 0.15	(86–100) 0.46	(78–100) 0.19	(50–67) 0.36	(50–100) 0.04*	(67–100) 0.09
Leukocyte infiltration	(90–89) 0.90	(90–100) 0.06	(89–100) 0.41	(75–67) 0.2	(75–80) 0.5	(67–80) 0.32
Epithelial hyperplasia	(86–78) 0.15	(86–100) 0.46	(78–100) 0.19	(67–83) 0.30	(67–60) 0.28	(83–60) 0.19
Lamellar fusion	(86–78) 0.15	(86–100) 0.46	(78–100) 0.19	(67–83) 0.30	(67–80) 0.38	(83–80) 0.43
Vasodilatation	(90–100) 0.22	(90–83) 0.06	(100–83) 0.24	(58–100) 0.05	(58–80) 0.28	(100–80) 0.14
Lamellar blood congestion	(0–56) 0.002*	(0–83) 0.0002*	(56–83) 0. 18	(0–83) 0.002 *	(0–80) 0.003*	(83–80) 0.43
Aneurism	(0–67) 0.0007*	(0–67) 0.003*	(67–67) 0.31	(0–83) 0.002 *	(0–80) 0.003*	(83–80) 0.43
Necrosis	(86–33) 0.002*	(86–67) 0.008*	(33–67) 0.36	(83–33) 0.04*	(83–80) 0.36	(33–80) 0.16

PSR Paraíba do Sul River, *FR* Funil Reservoir, *LR* Lajes Reservoir

*$P<0.05$ shows significant differences

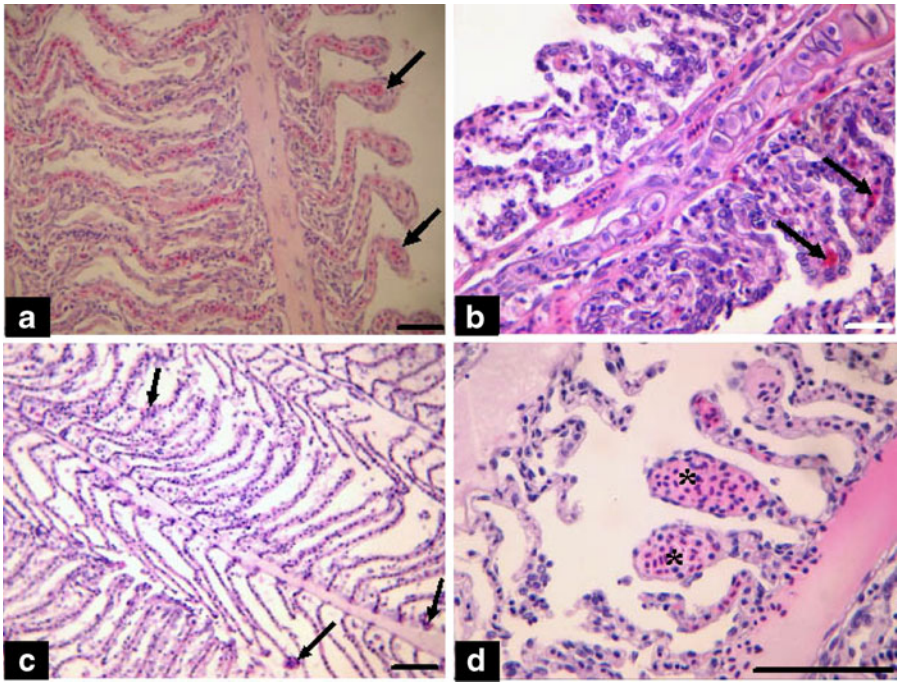

Fig. 3 Photomicrographs of the gill filament. Lamellar blood congestion (*arrow* and *star*). **a** *G. brasiliensis* in the Lajes Reservoir. *Scale bar*=100 μm. **b** *G. brasiliensis* in the Funil Reservoir. *Scale bar*=100 μm. **c** *A. bimaculatus* in the Lajes Reservoir. *Scale bar*=100 μm. **d** *A. bimaculatus* in the Funil Reservoir. *Scale bar*=50 μm

This anomaly is characterized by blood extravasations inside the lamellae and the rupture of the pillar cell system, which causes dilatation of the blood vessels. This anomaly is considered a more serious change, is often irreversible, and can originate from the appearance of vasodilatation, as described by Heath (1995). Lamellar aneurisms were also observed in the gills of the Perciformes *Oreochromis niloticus* exposed to cadmium, a heavy metal often used in experimental toxicological studies because of its significant build-up in the environment from the accumulation of industrial and household wastes (Garcia-Santos et al. 2007).

The reason for the lamellar aneurisms in the two examined species from the reservoirs may be associated with the variable environmental conditions in the reservoirs compared with the more stable conditions in the river; however, further studies should be performed to test this hypothesis. It is widely known that diel and seasonal changes in environmental variables, such as dissolved oxygen, pH, temperature, and conductivity have a wider range of variability in reservoirs compared with rivers. Additionally, biotic interaction is more intense in closed systems, like the Lajes Reservoir. These factors could be contributing to an increase in fish stress, thereby increasing the occurrence of these serious anomalies in fish from the reservoirs.

Epithelial necrosis (Fig. 2f) was observed in the gills of both species, with comparatively higher prevalence in the Paraíba do Sul River (Table 4). The highest incidence of necrosis in the PSR (86% in *G. brasiliensis* and 83% in *A. bimaculatus*) differed significantly from the Funil (33% in *G. brasiliensis* and 33% in *A. bimaculatus*) and Lajes Reservoirs (67% in *G. brasiliensis* and 80% in *A. bimaculatus*). Necrosis was the only epithelial change to predominate in the river (Table 4). Reasons for these differences are not fully explained. According to Garcia-Santos et al. (2007), this change reflects the direct effect of pollutants and occurs under more toxic conditions (Abel 1976). Degeneration by necrosis and apoptosis of the gill epithelial cells were also mentioned in an ultrastructural study carried out with the Characiformes *Prochilodus scrofa* subjected to pollutants (Mazon et al. 2002) in experimental conditions.

Unexpectedly, fish from Lajes Reservoir had a large occurrence of gill anomalies despite having high water quality and oligotrophic conditions. This observation suggests that gill anomalies can be caused by stressors other than pollution, such as changes in environmental variables, biotic interactions, and limitations on feeding resources (Nogueira et al. 2008). Lajes Reservoir has low resource availability due to a low inflowing water rate from small tributaries, oligotrophic conditions, and a lack of habitat complexity (Santos et al. 2004). In this type of "lake" reservoir, changes in environmental variables are relatively wider than in the two other systems, which have lotic (PSR) and short residence time (Funil Reservoir) conditions. Fluctuations in dissolved oxygen, temperature, and pH are reflected in the behavior of the gill epithelium through variations in the lamellar area and the number of mucous cells (Reis et al. 2009). Furthermore, the use of biomarkers of exposure to stressors may follow osmotic challenges (Giari et al. 2006), seasonality (Leino 2001), and overcrowding (Poltronieri et al. 2009), among other factors. Distinctions between systems with different water quality could not be achieved in the present study. Therefore, it is reasonable to suppose that gill anomalies are non-efficient bio-monitoring tools to predict water quality when other factors are not controlled.

The expectations of injuries in fish gills from the PSR and Funil Reservoir were met because these two systems receive large organic loads from industrial and domestic areas in the watershed. The main difference between these two eutrophic systems was the flow rate, with the PSR being a typically lotic system, while the Funil Reservoir is a lentic system with a comparatively low residence time (10–50 days). The only available information on fish gill anomalies in these studied systems was lamellar fusion reported in the Siluriformes *Pimelodus maculatus* from the Funil Reservoir (Nogueira et al. 2008). These anomalies were associated with anthropogenic alterations, which lead to the eutrophication of the reservoir (Branco et al. 2002).

Conclusions

There were no significant differences in fish gill anomalies between the two reservoirs with differing water quality and trophic state. This indicates that other stressors (not water quality) can interfere with the occurrence of the anomalies. This result suggests the occurrence of harsh conditions in reservoirs, is probably associated with greater variability in environmental variables, intense biotic interactions, and limited environmental resources (e.g., feeding resources and shelter). Moreover, the eutrophic river seems to affect differently the healthy condition of fish species, compared with the eutrophic reservoir.

Our results support the hypothesis that not only heavy toxic loads in the waters of Paraíba do do Sul River along with other non-controlled variables can lead to gill anomalies in the examined fish. The hypothesis that fish in more polluted sites have more gill changes was rejected. Therefore, we should use caution when using fish species as an indicator of water quality in rivers and reservoirs. Some species can be susceptible to different anomalies, making them unsuitable for water quality monitoring programs in tropical systems.

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