Histopathologic alterations observed in fish gills as a tool in environmental monitoring

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(With 5 figures)

Abstract

The gills of fish have a great external contact surface and are particularly sensitive to chemical and physical changes in the aquatic environment. The aim of this study was to examine the histopathologic alterations in the gills of *Astyanax fasciatus* and *Cyanocharax alburnus* and to determine if there is a correlation between the severity of the alterations and environmental degradation and if this biological system can be used as a tool for evaluating water quality in monitoring programmes. The gills of 107 specimens of *Astyanax fasciatus* and 116 of *Cyanocharax alburnus* were collected seasonally and processed using routine histologic techniques for fixing and embedding in paraffin and staining of sections with haematoxylin and eosin. The main alterations observed in both species were alteration of the structure of the epithelium, vacuolisation, hyperplasia of the epithelium of the primary lamella, epithelial lifting, and alteration of the structure and occurrence of aneurysms in the secondary lamella. The locations Gasometer and F. do Celupa were the ones that showed the highest frequencies of moderate and severe alterations as the highest "histopathologic alterations index" means. The most severe alterations were found to be related to the most impacted environment, indicating the presence of stressors in the water.

Keywords: gills, *Astyanax fasciatus*, *Cyanocharax alburnus*, alterations.

Alterações histopatológicas observadas nas brânquias de peixes como instrumento no monitoramento ambiental

Resumo

As brânquias de peixes apresentam uma grande superfície de contato com o meio externo e são particularmente sensíveis às mudanças químicas e físicas do ambiente aquático. Este trabalho descreve as alterações histopatológicas observadas nas brânquias das espécies *Astyanax fasciatus* e *Cyanocharax alburnus*, testando-se a presença de correlação entre o grau de severidade das alterações e degradação ambiental. Pode ser utilizada na avaliação da qualidade da água em programas de monitoramento. Brânquias de 107 exemplares de *Astyanax fasciatus* e 116 de *Cyanocharax alburnus*, coletados sazonalmente, foram preparadas com a técnica histológica de rotina para inclusão e impregnação em parafina e coradas com H&E. As principais alterações observadas em ambas as espécies foram alteração da estrutura do epitélio, vacuolização, hiperplasia das células do epitélio da lamela primária, elevação do epitélio, alteração da estrutura e ocorrência de aneurismas nas lamelas secundárias. Os pontos Gasômetro e F. do Celupa foram os que apresentaram as maiores frequências de intensidade de alterações moderadas e severas como as médias mais elevadas de IAH. Foi verificado que as alterações com maior grau de severidade estavam relacionadas aos ambientes mais degradados, indicando a presença de agentes estressores na água.

Palavras-chave: brânquias, *Astyanax fasciatus*, *Cyanocharax alburnus*, alterações.

1. Introduction

The increased contamination of aquatic ecosystems causes severe morphologic and physiologic alterations in aquatic organisms (Mazon et al., 1999). In a degraded envrionment, particularly where pollutants occur in chronic and sublethal concentrations, changes in the structure and function of aquatic organisms are more frequent than mass mortality. One of the possible methods of evaluating the effects of pollutants in fish is to examine their organs for morphologic changes (Poleksic and Mitrovic-Tutundzic, 1994).

The gills of fish are a sensitive organ which is easily damaged by numerous pollutants, even at low concentrations (Karlsson, 1983). Since the gills perform various vital functions (respiration, osmoregulation and excretion) and have a large surface area in contact with the external environment, they are particularly sensitive to chemical and physical changes of the aquatic envrionment, thereby being the target organ in fish for pollutants carried by water (Mallatt, 1985; Mazon et al., 1999; Cerqueira and Fernandes, 2002). Cerqueira and Fernandes (2002) pointed out moreover that because the gills are the principal site for gas exchange and other important functions such as ionic and osmotic regulation in addition to acid-base balance, histopathologic changes in the structure of these organs involve respiratory disturbances and electrolyte imbalance.

Histopathologic studies have been used to evaluate the effects of contaminants on the health of fish in the envrionment and to help establish a causal relation between exposure to toxic substances and the various biological responses (Schwaiger et al., 1997). The incidence of diseases and pathological conditions in fish, with a variety of etiologies, has been growing, where they are used as indicators of environmental stress since they provide a definitive biological endpoint of the history of exposure to a pollutant (Matthiessen et al., 1993). Histopathologic alterations of specific organs express conditions and represent endogenous and exogenous impacts on organisms that originate from alterations in lesser levels of biological organisation (Stebbing, 1985). Currently, physiologic and histopathologic biomarkers are utilised extensively for documenting and quantifying exposure to as well as effects of environmental pollutants. As monitors of exposure, these biomarkers have the advantage of quantifying only pollutants that are biologically available. As monitors of effects, biomarkers can integrate the effects of multiple stressors and help in the elucidation of mechanisms of action (Adams, 1990).

Various authors such as Karlsson-Norrgren et al. (1985), Mazon et al. (2002), Cerqueira and Fernandes (2002), Oliveira-Ribeiro et al. (2002) and Thophon et al. (2003) can be cited in regard to utilising the gills of fish as a tool for determining the toxicity of various pollutants in laboratory tests. Authors such as Jagoe and Haines (1997), Teh et al. (1997), Stentiford et al. (2003) and Flores-Lopes et al. (2005) have already utilised the gills of fish as a method of evaluating the presence of pollutants in natural environments. Field studies are important components for the evaluation and understanding of the biological and/or ecologic effects of chemical agents under natural conditions. The main advantages of such studies are the use of realistic exposures which will determine directly the effects observed, and the utilisation of natural environments which preclude the necessity of extrapolation of the results for the ecosystem (Graney et al., 1995).

The objective of this study was to describe the histopathologic alterations observed in the gills of specimens of the species *Astyanax fasciatus* (Cuvier, 1819) and *Cyanocharax alburnus* (Hensel, 1870), to determine if there is a relation between the severity of the alterations and environmental degradation and to test if this type of alteration can be utilised as a tool for the evaluation of water quality in monitoring programmes.

2. Material and Methods

The samplings were carried out using a seine net (15 m x 1.5 m x 0.5 cm) (Malabarba and Reis, 1987), at eleven sites in the hydrographic basin of lago Guaiba, where six sites were in lago Guaiba (1 – Gasometer, municipality of Porto Alegre; 2 – Saco da Alemoa, municipality of Eldorado do Sul; 3 – Foz do arroio Celupa, municipality of Guaiba; 4 – Praia da Alegria, municipality of Guaiba; 5 – Barra do Ribeiro, municipality of Barra do Ribeiro and 6 – Praia de Ipanema, municipality of Porto Alegre) and five sites in three tributary rivers. Of these five sites, two were in Rio Caí (7 – next to the bridge with highways BR 386 and 8 – locality of Morretes, close to the mouth of Rio Jacuí, both in the municipality of Nova Santa Rita), one in Rio dos Sinos (9 – next to the bridge with highway BR 386), two in Rio Gravatai (10 – close to highways RS 118 and 11 – locality of Passo das Canoas, both in the municipality of Gravatai) (Figure 1). The samplings were carried out seasonally over a period of two years, from December 2002 to October 2004. The first year corresponds to the period from December 2002 to October 2003 in lago Guaiba and from March 2003 to December 2003 in the tributary rivers, and the second year of sampling corresponds to January 2004 to October 2004 in lago Guaiba and from March 2004 to December 2004 in the tributary rivers.

At each collection site, standardised samplings were performed with 4 draggings along the bank. The specimens were fixed in the field in 10% formalin for a period of one week. In the laboratory, the material collected was sorted, identified to the species level and preserved in 70% alcohol. Voucher specimens were deposited in the scientific collection of the Department of Zoology of the Federal University of Rio Grande do Sul (UFRGS).

Twelve specimens, when possible, of the species *Astyanax fasciatus* and *Cyanocharax alburnus* were selected randomly from each sampling for each sampling site. The gills of ten specimens of each species from the locality of Praia das Pombas (Itapua) were utilised as reference material. Recorded for each individual were

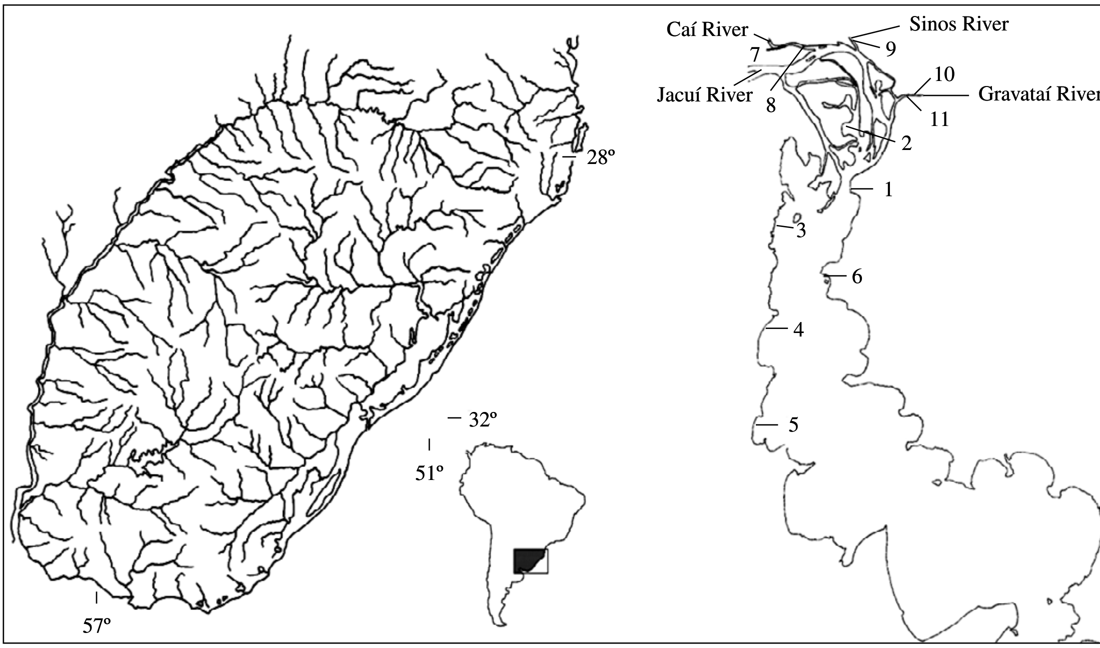

Figure 1. Map of the Lake Guaíba basin showing in detail the flowing rivers and sampling sites.

standard length (SL), total weight (TW) and sex. Next, the most external gill arches were removed, always from the left side of the fish. The integrated gills were decalcified with EDTA (tetrasodium ethylenediaminotetracetate) for a period of three days to a week. The specimens were prepared for histologic analysis using a routine technique of dehydration in ethanol and then impregnation and embedding in parrafin. Sections of 5-7 micrometer were made with microtome. The sections were stained with hematoxylin and eosin (H&E) for general visualisation of the affected tissues and organs (Michalany, 1980).

For better comprehension of the results, the histopathologic alterations were classified as scores of 0 to 3, where 0 = no alteration, 1 = slight alteration, 2 = moderate alteration and 3 = severe alteration (Hose et al., 1996). The definitions slight, moderate and severe alterations were modified from Poleksic and Mitrovic-Tutundzic (1994) and are characterised in the following manner. Slight alteration (1) involves changes that do not damage gill tissues such that restructuration and recovery of normal gill function can occur with improvement of the environmental conditions. These changes are limited to small parts of the gills or some filaments, for example, slight alteration of the epithelium of the primary lamella. Moderate alteration (2) involves changes that are more severe and 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. They occur on practically all the surface of the gills, for example, epithelial lifting of secondary lamella. Severe alteration (3) is where recovery of the gill structure is not possible, even with improvement in water quality or no further exposure to a toxic stimulus, for example, aneurysms. This scale was used to determine the mean values of the intensities of alterations for each sampling site.

The presence of histopathologic alterations for gills was determined semi-quantitatively by the degree of tissue alteration (histopathologic alterations index - HAI) which is based on the severity of the lesions. In the determination of HAI (modified from Poleksic and Mitrovic-Tutundzic, 1994), the alterations in each organ were classified in progressive stages of damage in the tissues (Table 1). The HAI value was calculated for each animal using the following formula: $HAI = (1X\ SI) + (10X\ SII) + (100X\ SIII)$, where *I*, *II* and *III* correspond to the number of stages of alterations 1, 2 and 3, and S represents the sum of the number of alterations for each particular stage.

Values of HAI between 0 and 10 indicate normal functioning of the organ; values 11 to 20 indicate slight damage to the organ; 21 to 50 indicate moderate changes in the organ; and 50 and 100 indicate severe lesions; and values over 100 indicate irreparable lesions of the organ (Poleksic and Mitrovic-Tutundzic, 1994). Some cases were selected and photographed with the use of a photomicroscope.

The non-parametric Mann-Whitney test for independent samples with $p < 0.005$ was utilised for comparison of means of intensity of anomalies in the two species. The differences between sampling sites, for each parameter, were tested using non-parametric ANOVA. The results obtained for each station were compared by Tukey's test. The degree of significance was 95%. Statistical analyses were carried out utilising the software Past version 1.11

The physico-chemical analysis of the water included the parameters temperature, pH, BOD_5, O_2 and levels of

Table 1. List of the histopathologic alterations observed in the gills of *Astyanax fasciatus* and *Cyanocharax alburnus* from the lago Guaiba basin. I, II and III – Stages of severity of the alterations (modified from Poleksic & Mitrovic-Tutundzic, 1994).

Stage	Histopathologic alterations in the gills
I	Hypertrophy and hyperplasia of gill epithelium
	Sanguineous congestion
	Dilation of marginal vascular channels
	Lifting of respiratory epithelium
	Fusion and disorganisation of secondary gill lamellae
	Shortening of secondary lamellae
	Leukocyte infiltration of gill epithelium
II	Hemorrhage and rupture of lamellar epithelium
	Hypertrophy and hyperplasia of mucous cells
	Empty mucous cells or their disappearance
	Hypertrophy and hyperplasia of chloride cells
III	Lamellar aneurysm
	Necrosis and cell degeneration
	Lamellar telangiectasis

fecal coliforms. The physico-chemical quality of the water was determined by inclusion of the mean obtained for each parameter within classes established by Resolution No. 357 of CONAMA (Brasil, 2005). A principal components analysis was utilised to determine correspondence and was carried out in order to identify which physico-chemical parameters influenced the quality of the water body. This analysis was performed with the software Multivariate Statistical Package v. 3.1. The physico-chemical data of the sites of lago Guaiba were supplied by DMAE – Municipal Department of Water and Waste. The data for the Rio dos Sinos were supplied by FEPAM – State Foundation for Environmental Protection. The data for the rivers Caí and Gravatai were provided by CORSAN – Rio Grande do Sul Sanitation Company.

3. Results

A total of 107 individuals of *Astyanax fasciatus* were analysed, including 62 males, 42 females and three individuals where it was impossible to determine the sex. The standard length of the specimens of this species varied from 21.28 to 97 mm. A total of 116 individuals of *Cyanocharax alburnus* were analysed, including 65 females and 53 males. The standard length of the specimens of this species varied from 23.10 to 59 mm.

The structure of the gill arch and gill filaments of *Astyanax fasciatus* and *Cyanocharax alburnus* shows the same pattern as in other teleosts. The filament is covered by a stratified epithelium in the interlamellar region. In this epithelium, there are epithelial cells, melanocytes, lymphocytes, macrophages, granulocytes and eosinophils. Also present are chloride cells and mucosal cells. The secondary lamella is covered by a squamous epithelium which generally shows a thickness of one or two cell layers. Below the epithelium, there are lamellar blood spaces which are bordered by pillar cells which have a contractile function. In the outer most region of the secondary lamella, there is a blood vessel which has an internal covering of endothelium.

The histopathologic alterations observed in *Astyanax fasciatus* were alteration of the structure of the epithelium, vacuolisation and hyperplasia of the epithelium of the primary lamella. In the secondary lamella, there were hypertrophy of the secretory cells, epithelial lifting, alteration of the structure, pyknotic nucleus of the pillar cells, secretion of mucus in interlamellar space, infiltration of cells of the immune system, fusion and occurrence of aneurysms of various sizes in some secondary lamellae (Figure 2).

In *A. Fasciatus,* a specimen was found with hypertrophy and hyperplasia of the mucous-secreting cells in the epithelium next to the gill rakers, and another specimen with hyperplasia of the epithelial tissue in interlamellar space. It was also observed in this tissue that the cells maintained a normal pattern in their shape, along with a large amount of vessels with blood cells and immune cells, which characterises an irritated lesion.

The highest frequencies of the more severe alterations (2 and 3) in *A. fasciatus* were observed in Praia das Pombas and in lago Guaiba, with the exception of the sites Praia da Alegria and B. Ribeiro, than in the tributary rivers, which showed the highest frequencies of the least severe alterations (1) or no alterations in the gills (0). The sampling sites Sinos, Gravatai RS118 and Gravatai PC demonstrated low frequencies of alterations of degree 2 (Figure 3a). *A. fasciatus* displayed mean values of the intensity of alterations most elevated at Praia das Pombas and the sites Gasometer, S. Alemoa, F. Celupa and P. Ipanema. The highest means for the two species were found at F. Celupa. The non-parametric Mann-Whitney test, in comparing the mean values of the evaluation of alterations, demonstrated no statistically significant difference between the two species.

The histopathologic alterations observed in the species *Cyanocharax alburnus* were alteration of structure of the

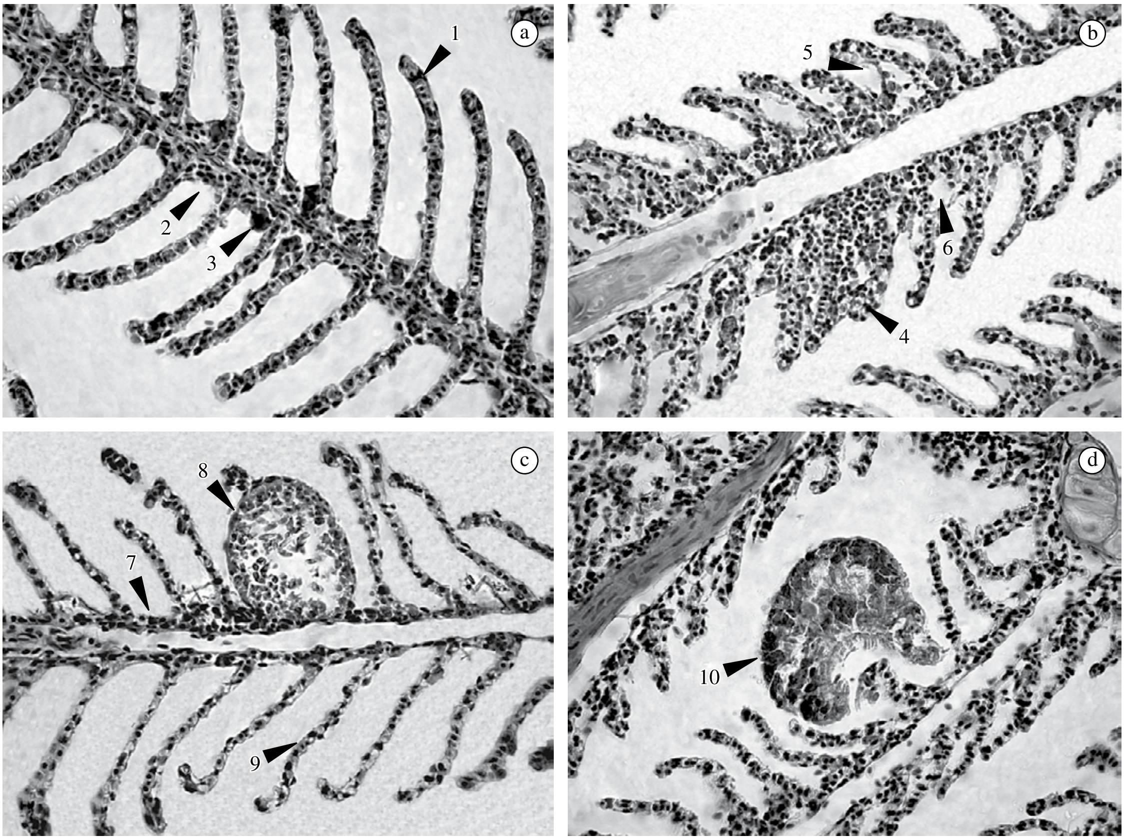

Figure 2. Histologic sections of gills of specimens of *Astyanax fasciatus*. a) Normal gill (1 – secondary lamella; 2 – primary lamella; 3 – chloride cells; 400X); b-d) gills with alterations; b) 4 – fusion of secondary lamella; 5 – detachment of epithelium; 6 – vacuolization; 400X; c) 7 – alteration of normal structure of primary filament; 8 – cyst with parasitic protozoans; 9 – alteration of normal structure of secondary lamella; 400X; and d) 10 – monogenetic parasite; 400X.

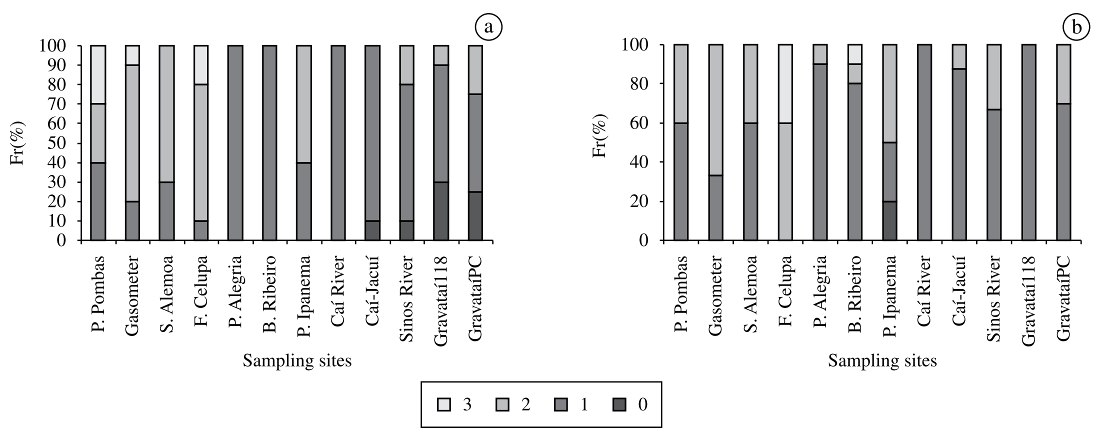

Figure 3. Frequency of intensity of alterations in gills, per sampling point, during the study period in the hydrographic basin of lago Guaiba. a) *Astyanax fasciatus*; b) *Cyanocharax alburnus*; 0 – without alterations; 1 – slight alteration; 2 – moderate alteration; and 3 – severe alteration.

epithelium, vacuolisation of the cells and hyperplasia of the epithelium of the primary lamella. The secondary lamellae showed hypertrophy of the secretory cells, epithelial lifting, alteration of the structure, pyknotic nucleus of the pillar cells, secretion of mucus in interlamellar space, infiltration of cells of the immune system and fusion and occurrence of aneurysms of various sizes in some secondary lamellae (Figure 4). Higher frequencies of the most severe alterations

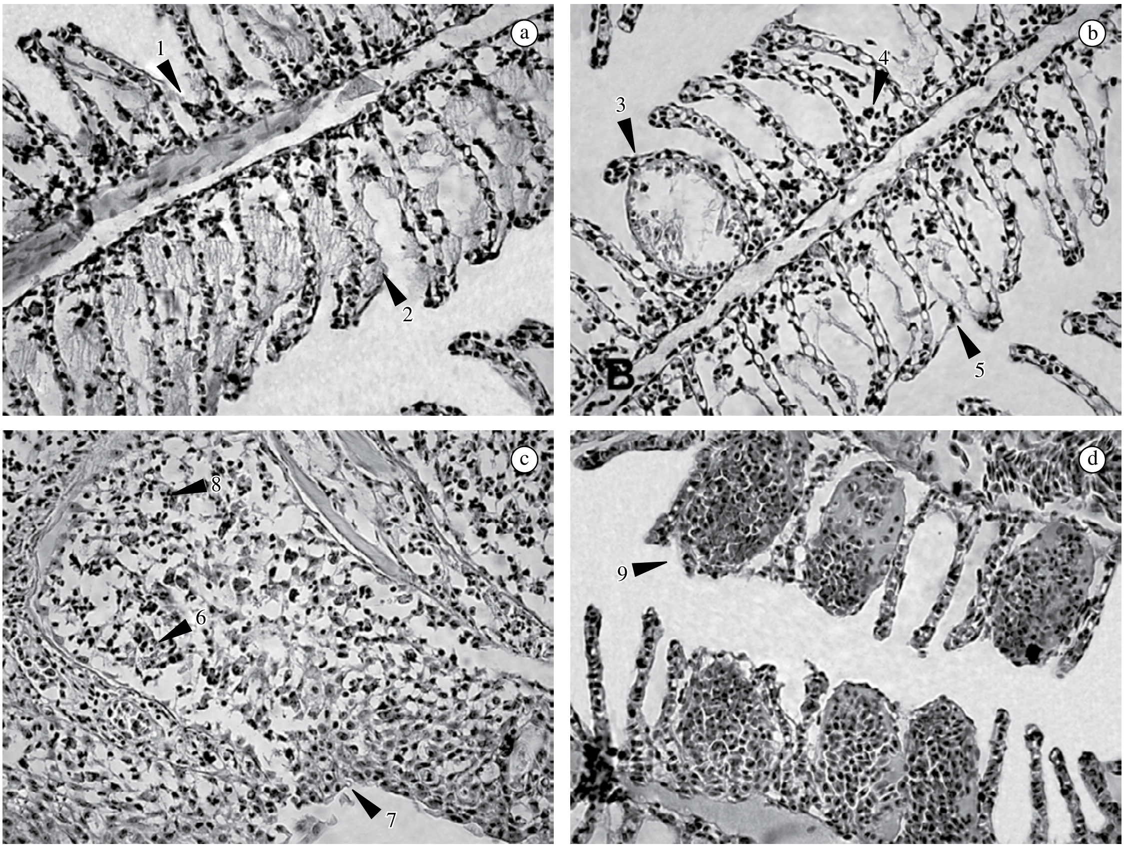

Figure 4. Histologic sections of gills of specimens of *Cyanocharax alburnus*. a) 1 – alteration of normal structure of primary lamella; 2 – secretion of mucus; 400X; b) 3 – cyst with parasitic protozoans; 4 – concentration epithelial cells; 5 – epithelial lifting of secondary lamella; 400X; c) 6 – blood vessel; 7 – hyperplasia of epithelial cells; 8 – cells of immune system; 400X; and d) 9 – aneurysm of secondary lamella; 400X.

(2 and 3) were observed in Praia das Pombas and in lago Guaiba than in the tributary rivers. At the site F. Celupa, only alterations of degrees 2 and 3 were observed. In the tributary rivers, only slight alterations (1) were found at sites 7 and 10 (Figure 3b). *C. alburnus* showed the highest mean values of intensity of alterations in lago Guaiba, more specifically at S. Alemoa and F. Celupa.

The histopathologic alteration index (HAI) for the gills of the species *Astyanax fasciatus* and C*yanocharax alburnus* varied from 1 to 114 and from 1 to 115, respectively, with a mean of 41.67 for *A. fasciatus* and 35.69 for *C. alburnus*, demonstrating that the gills of the individuals of these species showed moderate alterations. The results obtained by ANOVA for these species were considered statistically significant ($p < 0.005$). Tukey's test demonstrated that the site Foz do Celupa differed significantly from the other sampling sites for both species, as it had higher HAI values and means (over 100 for *C. alburnus*) (Figure 5). These results demonstrate that based on this method this site can be considered as having the worst environmental quality in the hydrographic basin studied. Elevated means of HAI were observed for *A. fasciatus* (63.6) and *C. alburnus* (65.11) at the site Gasometer, which characterises the occurrence of severe alterations in the gills of these species, demonstrating that the species present in at this location are also affected by stressors. Severe alterations were also seen in individuals of *A. fasciatus* at the sites

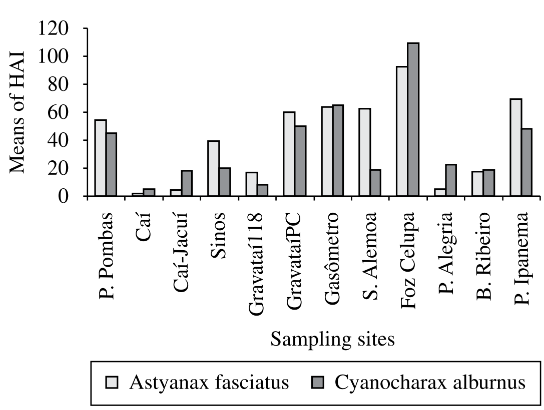

Figure 5. Means of HAI for the species *Astyanax fasciatus* and *Cyanocharax alburnus* at the sites sampled during the study period.

Praia das Pombas, Gravatai PC, Saco da Alemoa and Praia de Ipanema (Figure 5). These results demonstrated that the species *A. fasciatus* appears to be more sensitive than *C. alburnus* to the environmental changes occurring in these envrionments. The results obtained indicate that the site Caí can be considered the one with the best environmental quality, because of the low HAI means for both species (Figure 5).

The principal components analysis produced two significant axes which showed a cumulative percentage of 67.21% of the variations in the environmental parameters examined among the sampling sites. The study revealed that site B. Ribeiro showed the best water quality in all the hydrographic basin, due to the fact that this site had the highest mean O_2 level and lowest mean levels of fecal coliforms and BOD_5 (Table 2). This site was included in class I by the Resolution of CONAMA for all parameters analysed. The sampling sites S. Alemoa, F. Celupa, P. Alegria and P. Ipanema demonstrated a water quality inferior to that of B. Ribeiro, varying from good to reasonable during the sampling period. Both sites were included in class I for pH, O_2 and BOD_5. In relation to the presence of fecal coliforms, the site S. Alemoa was included in class IV, F. Celupa in class II and P. Alegria in class III.

The sampling sites F. Celupa and B. Ribeiro showed the greatest variations in temperature. The site P. Ipanema demonstrated a reasonable quality of its waters, which varied from good to reasonable during the sampling period. The sites Gasometer, Caí, Caí-Jacui and Sinos were those that showed waters with the worst quality. This is due to the fact that they had the lowest mean levels of O_2 and highest means of fecal coliforms and BOD_5, putting them in class IV for fecal coliforms and class I for the other parameters. At these sites, there was also little variation in temperature and a large variation in pH. The site Gasometer can be considered of poor quality because of its high mean level of fecal coliforms and low means of O_2 and BOD_5. The sampling sites Gravatai RS118 and Gravatai PC demonstrated a reasonable quality, being considered in class I for O_2, pH and BOD_5 and class II for fecal coliforms. At these sites, there was little variation in temperature observed.

4. Discussion

In the gills, only a few micrometers separate the blood from the water (Wood and Soivio, 1991), which not only facilitates the exchange of gases, but also allows the gill tissue to be exposed to variations in the environment. Consequently, the presence of toxic substances in the environment causes alterations in the vital functions carried out by the gills and alterations in the morphologic structure of these organs (Poleksic and Mitrovic-Tutundzic, 1994). Thus, the histopathologic analysis of fish gills has been used as a tool that is extremely important in the evaluation of the quality of aquatic ecosystems.

The results obtained for the species *Astyanax fasciatus* and *Cyanocharax alburnus* demonstrated the occurrence of alterations such as change in the structure of the epithelium, vacuolisation and hyperplasia of the epithelium of the primary lamella. The secondary lamellae showed hypertrophy of the secretory cells, epithelial lifting, alteration of structure, pyknotic nucleus of the pillar cells, secretion of mucus between the filaments, infiltration of cells of the immune system and fusion and occurrence of aneurysms of various sizes in some secondary lamellae.

According to Klontz (1972), fish are intimately associated with their aqueous environment and physical and chemical changes in this ecosystem are rapidly reflected as quantifiable physiologic measurements in the fish. In general, reacions

Table 2. Total values and means of HAI, per sampling site, for the species *Astyanax fasciatus* and *Cyanocharax alburnus*. n = number of individuals, Tot. = total, Xmed. = mean, * significant differences.

Site	*Astyanax fasciatus*			*Cyanocharax alburnus*		
	n	**Tot.**	**Xmed.**	**n**	**Tot.**	**Xmed.**
Praia Pombas	10	544	54.4	10	453	45.3
Cai	4	8	2	10	48	4.8
Cai-Jacui	10	41	4.1	8	146	18.25
Sinos	10	394	39.4	9	180	20
GravataiRS118	10	168	16.8	10	83	8.3
Gravatai PC	4	239	**59.75***	10	497	49.7
Gasometer	10	636	**63.6***	9	586	**65.11***
S. Alemoa	10	624	**62.4***	10	187	18.7
Foz do Celupa	10	924	**92.4***	10	1094	**109.4***
Praia Alegria	10	47	4.7	10	228	22.8
Barra Ribeiro	9	156	17.33	10	187	18.7
Praia Ipanema	10	695	**69.5***	8	383	47.87
Total	107	4459	41.67	116	4069	35.69

of the gills of fish due to an irritant includes inflammation, hyperplasia, lamellar fusion, excessive production of mucus, epithelial lifting, flattening of the secondary lamella and formation of aneurysms. Inflammation, hyperplasia, secretion of mucus and aneurysms were also observed in *A. fasciatus* and *C. alburnus*, demonstrating that the gills of these individuals had been affected by the action of various stressors. As a consequence of the epithelial lifting, there is an increase in the distance between the water and blood, impairing oxygen uptake. However, in these conditions, the fish increase their rate of respiration by compensating for the low entrance of oxygen (Fernandes and Mazon, 2003). According to Winkaler et al. (2001), these types of histopathologic lesions indicate that the fish respond to the effects of toxic agents present in the water and in the sediment.

Speare et al. (1991) apud Thiyagarajah et al. (1996) observed that alterations such as hyperplasia of the mucosal cells, hyperplasia of the chloride cells and proliferation of the epithelial cells occur as a consequence of infectious conditions or due to the presence of pollutants. Stentiford et al. (2003) found an increased frequency of aneurysms in specimens captured in contaminated areas. These authors observed that these lesions, which cause disturbances in blood flow in the gills of fish, can be associated with the presence of irritants in the water, and therefore, the presence of these alterations can be utilised as useful biomarkers of certain substances. Eller (1975), pointed out still that aneurysms can be a specific reaction of the tissue to the action of toxic substances.

Winkaler et al. (2001) also observed severe histopathologic alterations such as lamellar aneurysm and epithelial lifting in specimens of *Astyanax fasciatus* and *Astyanax altiparanae*. Similar results were observed by authors such as Cardoso et al. (1996), Thiyagarajah et al. (1996), Teh et al. (1997), Cerqueira and Fernandes (2002), Oliveira-Ribeiro et al. (2002), Mazon et al. (2002), Thophon et al. (2003) and Stentiford et al. (2003). However, the results obtained by Camargo and Martinez (2007) with gills of specimens of *Prochilodus lineatus* in an urban stream in the city of Londrina, Brazil differed a little from those obtained in this study, since the histopathologic alterations were found to be in stage I.

The results obtained for both species showed that the highest frequencies of alterations observed in the gills were found in lago Guaiba, compared to its affluents, with the exception of the sites P. Alegria and B. Ribeiro for the species *A. fasciatus*. The highest means of HAI were also observed in lago Guaiba, demonstrating this index to be associated with envrionments with major degradation of environmental quality, mainly chemical contamination. This is due to the fact that the sampling sites that showed the highest frequencies of alterations and most elevated HAI means were found to have the lowest levels of fecal coliforms, with the exception of Gasometer, where HAI levels were within the classes that varied from moderate to severe (II and IV). This finding demonstrates that other stressors that were not analysed affected the gills of these species.

There are various stressors that can be responsible for causing irritating reactions in fish. Generally, these types of alterations are associated with the presence of some toxic substance in the environment. In the species *Astyanax fasciatus,* a specimen was observed with hypertrophy and hyperplasia of the mucus-secreting cells in the epithelium next to the gill rakers and another with hyperplasia of the epithelial tissue between the secondary lamellae, where there was a large quantity of vessels with blood cells and cells of the immune system, which according to Thiyagarajah et al. (1996), Teh et al. (1997) e Stentiford et al. (2003), characterises a lesion caused by some type of stressor.

The waters of Guaiba, according to Bendati et al. (2003), Morandi and Bringhenti (1997), and Malabarba et al. (2004) receive a large load of pollutants which are cast into the tributary rivers of lago Guaiba and receive even high loads of domestic and industrial wastes which are discharged into lower parts of the rio dos Sinos (FEPAM, 1999) and in the region of Gasometer. This combination of toxic substances and organic matter that is discharged into lago Guaiba compromises water quality to some extent. The consequent increase in the degradation of the environmental quality of the hydrographic affects the organisms present there. The results obtained in this work demonstrate that the gills of specimens of the species *Astyanax fasciatus* and *Cyanocharax alburnus* are affected by stessors of different origins.

In this study, the site Gasometer was considered as being of low quality due to the finding of an elevated frequency of morphologic alterations, high frequency of moderate and severe alterations and an increased HAI mean, low mean level of dissolved oxygen and high mean of fecal coliforms, where the levels for dissolved oxygen were considered to be in class II and levels of fecal coliforms were found to be in class IV. Malabarba et al. (2004) also classified this site as being of low quality due to the finding of an elevated frequency of external morphologic alterations in specimens of fish. However, the sampling site F. do Celupa showed elevated frequencies of moderate and severe alterations and the highest means for HAI for both species, despite its waters being considered of good quality according to Resolution 357 of CONAMA, where it was included in class I. These results indicate that probably other factors or stressors besides those that were analysed in this study influenced water quality in these envrionments and affected the fish populations inhabiting these locations.

Thus, the results obtained in the present work reinforce the importance of the addition of histopathologic analysis in programs for the evaluation of water quality, as well the use of different methods of evaluating environmental alterations and degradations of anthropic origin that can compromise the quality of the ecosystems, all together serving as tools for a more precise evaluation of environmental quality of the region to be studied in programmes of environmental monitoring.

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