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Histopathological Alterations in Gills of Neotropical Fish *Cheirodon interruptus* in an Environment Influenced by a Wood Treatment Plant

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Abstract: The gill epithelium has an important role in maintenance of ionic and osmotic balance of fish a getting as response to physiological and environmental factors. The aim of the present study was to evaluate the pathological alterations in the gills of the fish *Cheirodon interruptus* collected in the municipality of Triunfo – RS, in two consecutive years (2008 and 2009) from one point located inside the area of a wood treatment plant. The collected fish were fixed in formol 10% and preserved in 70% ethanol. The gills were removed and prepared using routine histological technique. The data demonstrated the occurrence of high frequency of moderate and severe changes in the two years of sampling in fishes. The Histopathological Alterations Index (HAI) demonstrated a small increase in 2009 as compared to 2008. The results suggest that individuals are suffering the action of stressors, but the gills may recover if there is an improvement of environmental conditions.

Keywords: Environmental quality, Neotropical fish, Histopathology, Wood treatment plant, Gill, *Cheirodon interruptus*

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Introduction

Environmental pollution and its effects on the health of aquatic ecosystems is one of the major problems that has been intensively studied in recent years (Fontaínhas-Fernandes, 2008; Bashir *et al*., 2020; Donat *et al*., 2020; Issac and Kandasubramanian, 2021). The aquatic ambient is fundamental in the composition of environments and, according to Silva *et al*. (2004), it has been considered one of the main environments affected by pollution and contamination due to the disposal of organic, inorganic, heavy metal and other xenobiotic effluents associated with the development of agricultural activities, mining and exploitation of fossil resources. Authman *et al*. (2015) commented that in aquatic ecosystem, heavy metals are considered the most important pollutants, since they are present throughout the ecosystem and are detectable in critical amounts. Aquatic environments have been increasingly affected by human activities, resulting in damage to biodiversity (Abdel Moneim and Abdel-Mohsen, 2010), mainly due to the exposure time and the type of toxic chemical pollutants to which these individuals are exposed.

Population growth in recent years, plus technological advances, and strong industrial production have led to an increase in aquatic ecosystem compounds level. This fact has contributed to the degradation of various environmental compartments (water, sediment, air and soil), as well as to an impairment in the health of the people who live in these ecosystems (Cajaraville *et al*., 2000). Jenkins (2004) have reported that physico-chemical analyses that assess water quality are of great importance, but the sole use of this tool can lead to misinformation about the ecological and biological conditions of an aquatic ecosystem, making it essential to use biological data as well.

Industrial, agricultural and domestic activities often discharge their waste, such as solvents, pesticides, oils, metals, fertilizers and solid particles into small rivers, lakes, and streams without proper treatment (Pandey *et al*., 2003). Some contaminants have a negative effect on water quality and cause many problems for the fish, such as structural alterations and diseases (Chang *et al*., 1998). There is an increasing trend to use pollutant behavior (bioavailability, bioaccumulation and biotransformation), as well as biological and biochemical effects induced by pollution on aquatic organisms to assess and predict the impact of chemicals in aquatic ecosystems (Van der Oost *et al*., 2003; Srivastav *et al*., 2019; Kawago *et al*., 2021).

Morado *et al*. (2017) stated that biomarkers are measurements within an organism that respond to environmental effects and are used as tools in bio-assessment programs, since they reflect physiological changes induced by exposure to pollutants. According to Adams (1990), pathological and physiological biomarkers have been extensively used in order to document and quantify both the exposure and the effects of environmental pollutants. Hinton and Laurén (1990) stated that the term environmental biomarker is used in relation to specific organs that suffer morphological alterations in cell or tissue structures as a result of exposure to a contaminant. Studies on fish biomarkers are of great significance as they can help to evaluate organ alterations, enzymatic responses, and histological changes when exposed to polluted aquatic environment (Bassey, 2019).

Fish are often exposed to highly contaminated water, which leads to different changes, ranging from biochemical alterations in single cells up to changes in the whole population (Bernet *et al*., 1999). Al-Sabti and Metcalfe (1995) reported that fish species are important vehicles for the transfer of contaminants to human populations and may indicate a potential exposure to pollutants. The use of fish in environmental monitoring studies, according to Jenkins (2004), has several advantages, as they are directly associated with sediments and water column, have poor mobility and exhibit some physiological mechanisms of biological response.

Morphological alterations in the gills, kidneys and liver can be used in investigations concerning the toxicity of specific chemicals and the monitoring of acute and chronic effects in polluted or contaminated aquatic environments. According to Schwaiger *et al*. (1997), histopathological studies have been performed to assess the effects of contaminants on fish health and on the environment as a way to help establish a causal link between exposure to toxic substances and the various biological responses.

Histology is a useful technique towards the assessment of toxic substances in aquatic organisms from polluted ecosystems (Bassey, 2019). Histopathological characteristics of a target organ can express specific environmental conditions and represent the exposure time to which organisms are subjected (Schmalz *et al*., 2002). Thus, the pathological alterations can be identified as sublethal responses to stress, providing a fast method to evaluate the impairment of tissues and organs of specimens exposed to chemical stressors.

In this study, observational area is located on the left bank of Taquari River, 8.5 km away from the confluence with Jacuí River, in Rio Grande do Sul state, Brazil. It housed a wood treatment plant that operated from 1960 to 2005 and used a variety of processes and substances including creosote, penta-chlorophenol (PCP) and chromated copper arsenate (CCA) (Coronas *et al.*, 2013). Initial chemical analysis showed evidence of groundwater and soil contamination by PAHs, Cr, Cu, As, PCF, dioxins and furans (Fepam, 2010a, b). Samples in a small creek draining a small portion of this wood treatment plant area revealed the presence of a natural population of *Cheirodon interruptus* (Jenyns, 1842). This species belongs to the order Characiformes, family Characidae, subfamily Cheirodontinae (Malabarba, 1998), and occurs naturally in the southern drainages of South American in Brazil, Argentina and Uruguay (Malabarba, 2003).

The present study aimed to evaluate the impact of residues of different chemical compounds on the pathological alterations in the gills of a fish, *Cheirodon interruptus* (Jenyns, 1842) collected from the left bank of Taquari River under the indirect influence of substances generated by the wood treatment plant.

Materials and Methods

The Taquari-Antas basin is situated in the northeast of Rio Grande do Sul state (28° 10' and 29° 57' South latitude and 49° 56' and 52° 38' West longitude) and covers the geomorphic provinces of the Southern Plateau and Central Depression. It has an area of 26491.82 km^2, covering cities such as Antonio Prado, Bento Gonçalves, Veranópolis, Cambará do Sul, Carlos Barbosa, Caxias do Sul, Estrela, and Triunfo, with an estimated population of 1,207,640 inhabitants.

The rivers Antas, Tainhas, Lageado Grande, Humata, Carreiro, Guaporé, Forqueta, Forquetinha and Taquari are the main watercourses. The Taquari-Antas river source is located in São José dos Ausentes with the estuary in the Jacuí River. The water catchment basin is designed for irrigation, public supply, agribusiness and livestock usage. The Taquari-Antas basin covers part of the upper mountain ranges and the Taquari River Valley region, with a predominance of agriculture and the colonial region of Serra Gaúcha, characterized by intense industrial activity (Santos *et al*., 2008) (Fig. 1).

Cheirodon interruptus samples (UFRGS 15461) were collected at a single point (29° 52' 28.1" S and 51° 43' 16. 9" W) in a creek near the confluence of the Taquari and Jacuí rivers (ICMBio - Permanent License for Zoo material, nº 21408-1). This point is located inside the area of a wood treatment plant located on the left bank of the river Taquari. Local geology is characterized by sedimentary rocks, fine to very fine sand stones with intercalations of laminates (argillites and shales). In the surroundings of the plant there is a small residential neighborhood (with local shops and crops that are cultivated for subsistence). On the right bank of the river, the predominant use is agricultural (Fepam, 2010a, b; Coronas *et al*., 2013).

The samples were collected in the Creek of the Petrochemical Complex of Rio Grande do Sul, with dip nets and seine nets (Malabarba and Reis, 1987) once a year in 2008 and 2009. In the field, the specimens were anesthetized with MS222 (methanesulfonate tricaine), and then fixed in 10% formalin formaldehyde. In the laboratory, the specimens were arranged, identified by species, preserved in 70% ethanol and deposited in the Ichthyology collection at the Universidade Federal do Rio Grande do Sul (UFRGS). Gill was extirpated from specimens and processed by routine histological techniques, embedded in paraffin, and sectioned at 5 - 7 µm. Hematoxylin and eosin (HE) staining was used for a general viewing of the affected areas in the gill (Michalany, 1980).

Histopathological alterations were classified

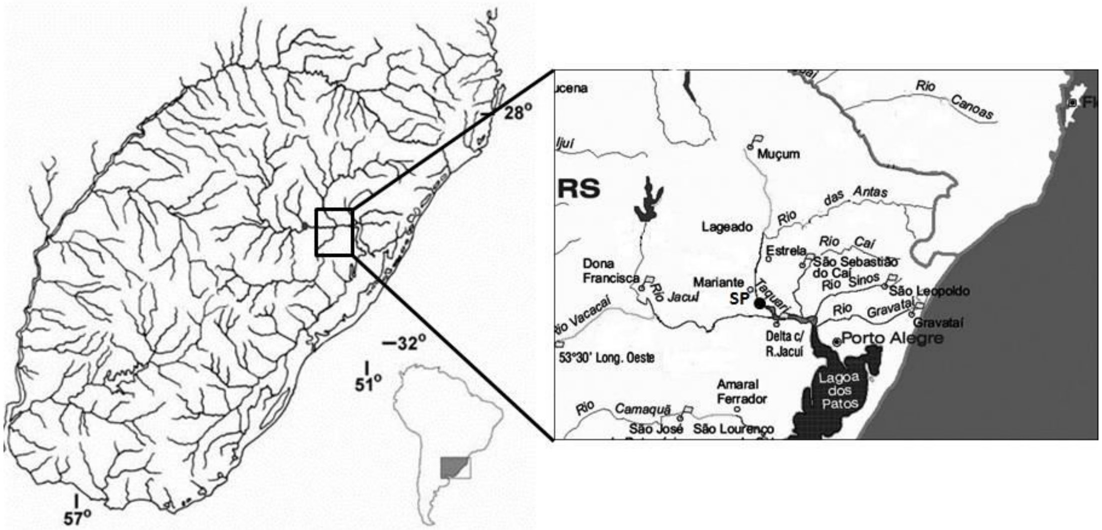

Fig. 1: Hydrological map of Rio Grande do Sul. In detail, the Taquari-Antas hydrological basin. ● = Sampling point.

according to Hose *et al*. (1996) on a scale from 0 to 3, in which 0 = no observed changes on tissues, 1 = light alteration, 2 = moderate alteration and 3 = severe alteration. Definitions of light, moderate and sever alterations followed as suggested by Flores-Lopes and Thomaz (2011) and were characterized as follows: (1) light alterations - alterations that do not damage the tissue of the gill in such a way that an improvement in environmental conditions would allow the restructuring and the resumption of normal gill operation. They are restricted to small parts of the gill, for example, light alterations in the epithelium of the primary lamella. (2) Moderate alterations - changes that are more severe and lead to effects in the tissues associated with organ function; these are repairable lesions, but if large areas of the organ are affected or kept in chronically polluted conditions, they could cause severe alterations. They occur on practically the entire organ surface. Example: elevation of the epithelium of the secondary lamella. (3) Severe change - recovery of the gill structure is not possible, even with an improvement in the quality of the water or with the termination of exposure to a toxic stimulus (i.e., aneurysm). This scale was used in order to determine the intensities of alterations for each sampling point.

Data of each individual were recorded regarding their standard lengths (SL) and total weights (TW). Subsequently, the outer gills, always from the left side of the fish, were removed and decalcified with EDTA (Ethyl 3-aminobenzoate methanesulfonate salt) for a period from three days to a week.

The presence of histopathological alterations for each organ was semi-qualitatively evaluated by the degree of tissue alteration (Histopathological Alteration Index - HAI), which is based on lesion severity (Table 1). For HAI calculation, modified from Poleksic and Mitrovic-Tutundzic (1994), the alterations were classified in progressive stages of tissue damage. An HAI value was calculated for each animal by the formula: HAI = (1 XSI) + (10 X SII) + (100 X SIII), where I, II and III correspond to modification stage number 1, 2 and 3. In turn, S denotes the sum of alteration in a particular stage.

HAI values between 0 and 10 indicate normal functioning of the organ, values between 11 and 20 indicate slight damage to the organ; between 21 and 50 indicate moderate changes in the organ,

Table 1: Histopathological alterations observed in the gills of *Cheirodon interruptus*. I, II and III – Stages of alteration severity (modified from Poleksic and Mitrovic-Tutundzic, 1994).

Stages	Histopathological alterations in the gills
I	Hypertrophy and hyperplasia of gill epithelium
	Sanguineous congestion
	Dilatation of marginal vascular channels
	Lifting of respiratory epithelium
	Fusion and disorganization 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

values between 50 and 100 indicate severe lesions and values above 100 indicate irreparable injuries to the organ (Poleksic and Mitrovic-Tutundizic, 1994).

The nonparametric Kruskal-Wallis Variance Analysis test for independent samples with $p < 0.05$ was used to compare the average evaluation values of alteration intensity in the gills. The differences between the locations of sampling for each parameter were tested through a non-parametric ANOVA. The results obtained were compared by the Tukey test. The degree of significance was 95% (Zar, 1999). Statistical analysis was performed using the PAST (version 1.11 software). Some cases were selected and photographed in an inverted light microscope with a digital camera.

Results

A total of 44 specimens of *Cheirodon interruptus* were analyzed, of which 24 were collected in 2008 and 20 in 2009. The standard length varied from 35.1 to 44.0 mm in 2008 and from 38.2 to 42.4 mm in 2009. The total weight varied from 1.06 to 2.30 g in 2008 and from 1.14 to 1.78 g in 2009.

The gill-arch structure and the gill lamellae in *Cheirodon interruptus* showed the same pattern as in other teleosts. The primary lamella is covered by a stratified epithelium in the interlamelar region. In this epithelium, there are epithelial cells, melanocytes, lymphocytes, macrophages, granulocytes and eosinophils. Chloride cells and mucous cells were also observed. The secondary lamella is covered by a squamous epithelium, whose thickness generally has one or two cell layers. Below the epithelium, lamellar blood sinuses are found and delimited by pillar cells, which have a contractile function. In the outermost region of the secondary lamella there is a blood vessel that is internally lined by endothelium.

The samples from 2008 showed a 54.17% light alterations frequency, 45.8% moderate alterations frequency, and no severe alterations. In 2009, it was observed 31.5% of light alterations, 47.3% of moderate and 20% of severe alteration (Fig. 2).

Histological observations of gill of *Cheirodon interruptus* had the same structure as the other fish species (Fig. 3A). The main changes observed were the proliferation of mucus (Figs. 3B, C), cell hyperplasia of the primary lamella (Fig. 3C), thickening of the primary lamella, fusion of some secondary lamellae (Fig. 3D), edema, epithelial hyperplasia of secondary lamellae, and elevation

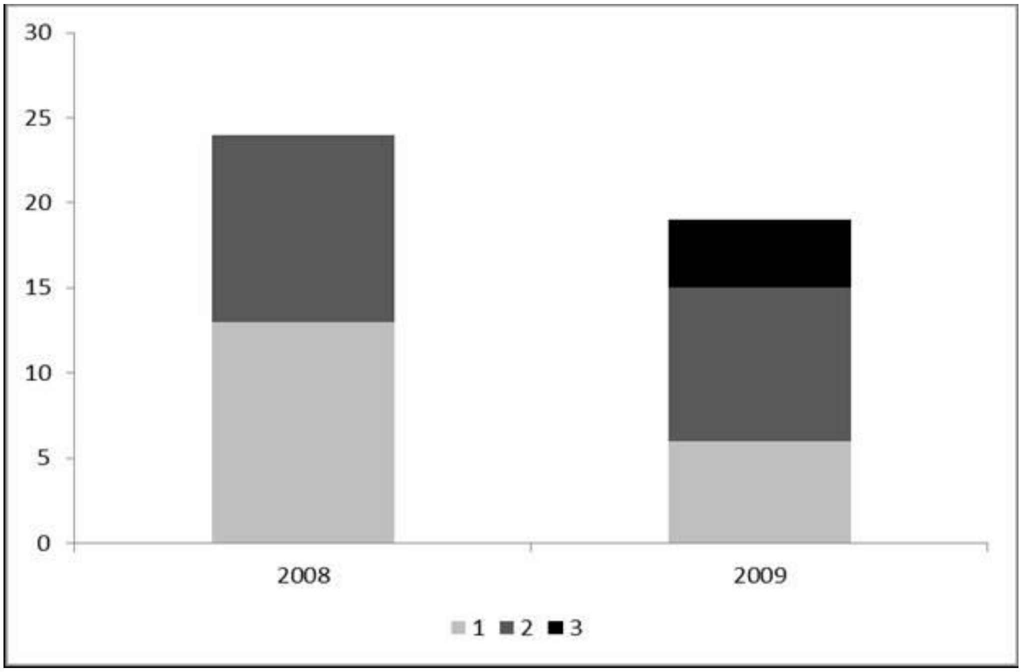

Fig. 2: Average relative frequency of histopathological alteration intensity observed in the gills of *Cheirodon interruptus* per year of sampling in a stream tributary of the Taquari-Antas. 1 = mild alteration, 2 = moderate alteration and 3 = severe alteration.

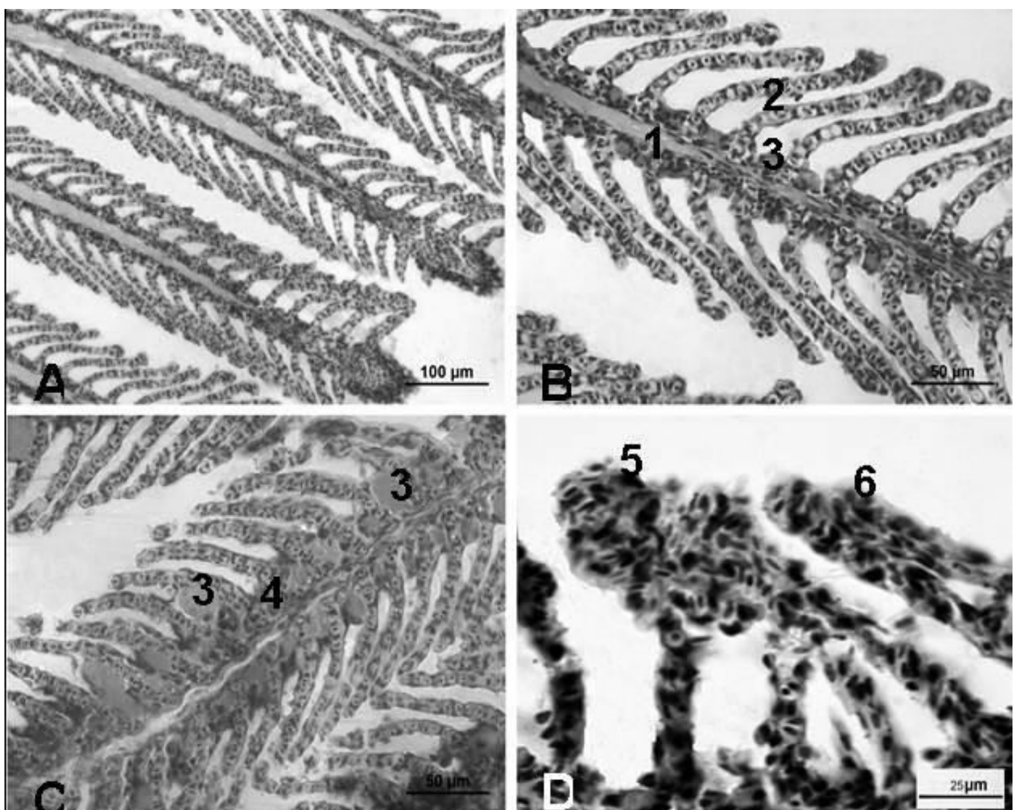

Fig. 3: Photos of normal histology and histopathological alterations in the gills of *Cheirodon interruptus*. A - Normal gill, H&E (400 x). B to D - Gills with histopathological alterations, H&E (400 x). 1- Primary lamella, 2 - Secondary lamella, 3 - Hypertrophy of the mucous cell, H&E, 4 - Hyperplasia of epithelial cells; 5 - aneurysm in initial stage, H&E (100 x); 6 -fusion of secondary filament.

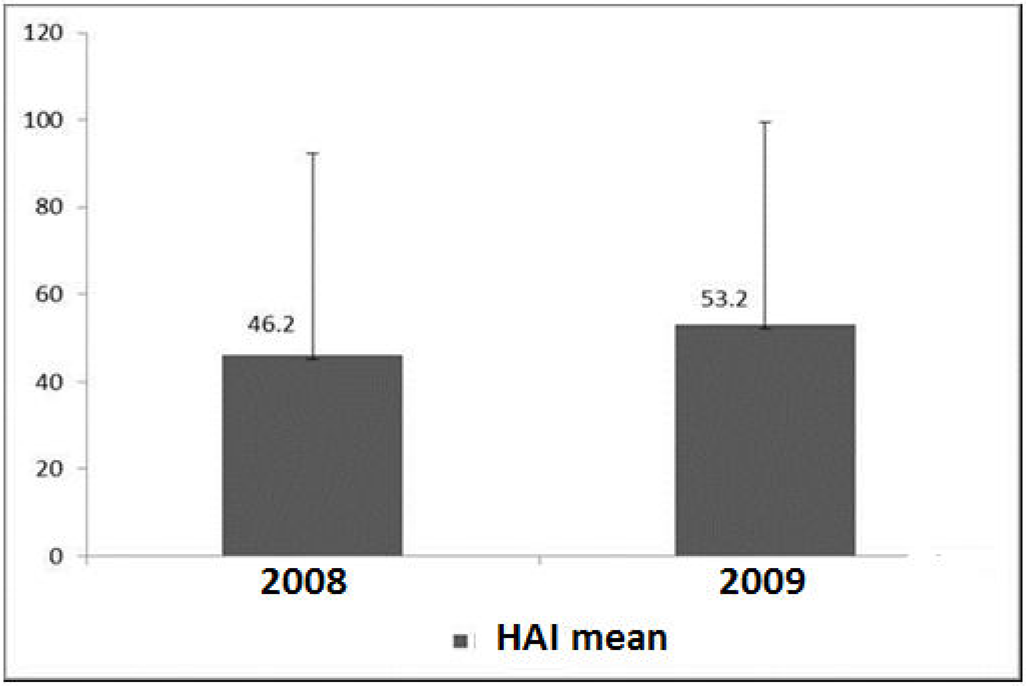

Fig. 4: Mean values of HAI for the gills of *Cheirodon interruptus*, per year of sampling, in a stream tributary of the Taquari-Antas. Vertical Line - Standard deviation.

of the secondary lamella epithelium. The most abundant changes were the proliferation and hypertrophy of secondary lamellae and secretion of mucus, mainly on the extremities of the cells (Figs. 3B, C). Aneurysms in early stage and lamellar telangiectasis were among the severe alterations observed (Fig. 3D). In this study, we observed an increase of mucus-secreting cells in the fish.

The Histopathological Alteration Index (HAI) (Fig. 4) showed an average of 46.2 in 2008 and 53.2 in 2009. In this way, the 2008 average was classified as "moderate alterations in the organ" (between 21 and 50), demonstrating that the individuals still showed an ability to repair their organs if environmental conditions improved. The mean for 2009 was classified as “serious body lesions on the organ”, indicating that organ repair was difficult, even if environmental conditions improved. Although the histopathological alterations intensity frequency and HAI were higher in 2009 when compared to 2008, the Kruskal-Wallis variance analysis showed no statistically significant difference ($p = 0.75$, $P \leq 0.05$) between the years. The non-parametric Mann-Whitney test also showed no statistically significant results when compared to average rating of alterations between 2008 and 2009.

Discussion

Aquatic environments are being increasingly affected by human activities, resulting in damage to biodiversity (Abdel-Moneim and Abdel-Mohsen, 2010), mainly due to the exposure time and the type of toxic chemical pollutants to which these individuals are exposed. According to Buss *et al.* (2003), it is critical to conduct environmental monitoring studies involving consistent and effective measures to ensure that the quality of rivers is preserved. Histopathological biomarkers, according to Adams (1990), are extensively used to record and quantify exposure to, and the effects of, environmental pollutants. Teh *et al*. (1997) and Bernet *et al*. (1999) stressed that fish are often exposed to highly contaminated water, which leads to different changes, ranging from biochemical alterations in single cells up to changes in the whole population.

According to Bassey (2019) histological analysis is an efficient method to detect irritant effects, particularly some chronic effects in various tissues and organs and it is important as an indicator of exposure to contaminants and a great tool to assess the degree of pollution, particularly chronic and sublethal effects. Flores-Lopes (2000), Flores-Lopes *et al*. (2005, 2014, 2019, 2020), Flores-Lopes and Malabarba (2007), Flores-Lopes and Thomas, (2011) and Paulo *et al*. (2012, 2020) used histopathological analysis to demonstrate the meaning and importance of using the histopathological method as a form of environmental assessment.

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, these are particularly sensitive to chemical and physical changes of the aquatic environment, thereby being the main affected organ in fish for pollutants carried by water (Cerqueira and Fernandes, 2002). For these authors, the gills are the first organ in the body in contact with pollutants and, therefore, are the first organ to react to unfavorable environmental conditions due to both to its large surface area in direct contact with the external environment and the reduced distance between the external and internal environment.

According to Ayas *et al*. (2007), histopathological events are considered a fast and efficient method to detect acute and chronic adverse effects in fish. Fish communities can provide important information about the aquatic environment and, for this reason, are excellent indicators of environmental quality (Karr, 1981). Vasanthi *et al*. (2013) reported that the gills of fish collected showed aneurism or nodule formation in the secondary lamellae and hypertrophy is observed with the enlargement of the tissues. The ultra thin section of control fish gills showed a smooth surface topography and organized arrangements of primary and secondary lamellae with uniform interlamellar space.

The histopathological alterations more frequently observed in *Cheirodon interruptus* individuals were hyperplasia of epithelial tissue, inflammation, hyperplasia, hyperplasia of mucous cells, secretion of mucus and rupture of the branchial epithelium, mucus hypersecretion, aneurysms, lamellar edema and fusion. These alterations were also observed by Flores-Lopes and Thomaz (2011) in *Astyanax fasciatus* (Cuvier, 1819) and in *Cyanocharax alburnus* (Hensel, 1870) from the lake Guaíba.

Flores-Lopes *et al*. (2005) further observed the alterations in *Astyanax jacuhiensis* (Cope, 1894) collected in stabilization ponds of a Petrochemical plant waste treatment facilities. Similar results were also verified by Mallat (1985), for whom these changes are responses from the gills of fish exposed to toxic agents. The results of these studies indicated that the fish from the creek were exposed to environmental stressor agents.

Data analysis still revealed a high frequency of individuals with HAI values above 100, characterized by irreversible damage to the organ, which showed a decrease in the environmental quality from one year to the next. Morphological gill alterations in response to environmental changes may be an adaptive strategy for the conservation of some physiological functions (Laurent and Perry, 1991). According to Mallatt (1985), Flores-Lopes and Malabarba (2007), Flores-Lopes and Reuss-Strenzel (2011), Flores-Lopes and Thomaz (2011), Flores-Lopes *et al*. (2020) and Paulo *et al*. (2012, 2020) this type of change indicates the exposure of the fish to a stressor agent and they have reported that the high frequency of morphological alterations may indicate that these organs are subject to stress in the aquatic environment.

Flores-Lopes and Malabarba (2007) and Paulo *et al*. (2012) demonstrated that several types of alterations results from the continuous action of stressors on the body over a long period of time. The occurrence of this type of alteration demonstrates that the water quality of the environments are undergoing important changes in its constitution, being much influenced by the presence of several types of polluting substances, that will cause several types of alterations in the diverse species types (Flores-Lopes *et al*., 2019).

Working with bioassays, Fontaínhas-Fernandes *et al*. (2008) observed that the elevation in epithelial cell hypertrophy and epithelial hyperplasia, slight lamellae deformation and fusion of adjacent lamellae were more prevalent and pronounced in specimens exposed to sewage water than in the control group. They found that the lesions in the gills included edema, which is more evident after 24 h exposure, with an elevation of the filament and lamellar epithelium. The severity of the lesions also increased with exposure time. According to Figueiredo-Fernandes *et al*. (2007), probably histological changes in fish are normally associated with the time of exposure and the high concentration of heavy metals. Hadi and Alwan (2012) reported that exposure of fish to aluminum resulted in various forms of histopathological alterations, such as hypertrophy or cellular hyperplasia in the epithelial layer of primary filaments and secondary lamellae fusion, epithelial elevation, interstitial edema and blood congestion in the vascular axis of primary filaments. There was also some telangiectasia, cell degeneration and necrosis in branchial lamellae.

The sampling point was subjected to the discharge of effluents rich in chemical compounds originating from the region of the Triunfo Petrochemical Complex– RS and within the influence area of a wood treatment plant that uses hazardous compounds generating substances which may contaminate soil, surface groundwater and air (Gallego *et al*., 2008). The wood treatment processes the chemicals used and contaminate the environmental compartment through vaporization, leakages and dripping. Over the years, the processes and substances used to treat wood changed due to the discovery of these chemical's toxicity. Coal tar creosote is a complex mixture that contains hundreds of compounds, among which aromatic hydrocarbons predominate, including polycyclic aromatic hydrocarbons (PAHs).

Costa *et al*. (2011) found an elevated concentration of pentachlorophenol in the stream sediment, which confirms the presence of contaminant runoff from the Taquari river area. These studies brought to light an elevated concentration of arsenic in the same area, suggesting a major incorporation of the metalloid into the stream sediments. Guerra and Junior (1998), found that erosion (leaching and/or erosion) of the rocks, and land use associated with flood water exert an important influence on the Taquari river water quality. They also observed that human factors (fecal coliforms, Ni, DBO, nitrites and phenols) had inconsistent variation over time and along the river, having better and poorer water quality periods during the study. From these data, the authors classified the Taquari as Class 2, according to the CONAMA/96 (Conselho Nacional do Meio Ambiente – Brazil) resolution.

According to the CONAMA 357 resolution, Larentis *et al*. (2008) demonstrated that there was little change in the quality of water resources from the Taquari/Antas basin when compared to previous data. However, these authors observed a different behavior for each variable of water quality and major changes in the concentrations in specific parts of the basin. Kazimirski *et al*. (2009) studied the Marau river, a tributary of the Taquari/Antas, and found that it showed signs of environmental disturbance, such as the lack of riparian vegetation. The main sources of pollution were pesticide contamination, tannery presence, release of organic waste (sewage) and trash.

Our results obtained from samples taken four years after the wood treatment plant stopped operation, show that contamination and residual effects continue impacting the fish fauna inhabiting the creek located close to this area. The analysis showed that, due to the severity of histopathological alterations observed *Cheirodon interruptus*, it is possible to infer that they were suffering the action of some stressor agent. These data, added to the previous studies by Hingston *et* *al*. (2001), Bhattacharya *et al*. (2002), Moghaddam and Mulligan (2008) show that the soil from wood treatment plant is contaminated by arsenic due to the use of CCA and that this substance treated wood leaches As, Cu and Cr. This suggests the indirect contamination of the water resources.

Although the histopathological results showed the occurrence of lesions due to the presence of several types of substances that are considered as pollutants, the HAI test showed that there was no significant difference among the samples collected. According to Vasanthi *et al*. (2013), contaminants usually appear in the environment as very complex mixtures that can cause interactive effects on the biota, which are impossible to evaluate only by means of chemical analysis. Vazzana *et al*. (2014) has reported that exposure to this metal affects the levels of circulating leukocytes in tissues and promotes the condensation of the leukocyte chromatin, resulting in apoptosis, specific fragmentation in the DNA and death of these cells in long term exposures. The presence of heavy metals, pesticides, other harmful chemicals, liquid wastes and even half burnt bodies are producing deleterious effect on the various biological systems of the organisms living in the water, specially the lymphatic system (Khan, 2016).

These results indicated a slight increase in the impact on the fish fauna in 2009 compared to the previous year. This is probably due to the fact that the longer exposure time for individuals of the *Cheirodon interruptus* species is exposed to the presence of stressors. The high occurrence of alterations is mainly due to the fact that the specimens were collected in the Petrochemical Complex – RS, which allows a higher presence of chemical substances in the point, due to the presence of the greater amount of industries in the region, which causes a high release of stressors.

Previous studies were done in the region by Reis and Malabarba (1987), Flores-Lopes *et al*. (2005), Guerra and Júnior (1998), Larentis *et al*. (2008) and Kazimirski *et al*. (2009) who demonstrated that the environmental quality of the Taquari/Antas river has been suffering changes over time.

Data from this study reinforce the importance of constant monitoring of natural environments as a strategy for conservation and the use of histopathological techniques as important tools of analysis in this type of water quality evaluation program. It is also of utmost importance not to pollute the environments so that the substances do not cause changes in the species or cause their extinction.

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