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Exposure and effect biomarkers indicate health alterations in *Aequidens pallidus* (Cichliformes, Cichlidae) exposed *in situ* in degraded Amazonian urban streams

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ABSTRACT

In the Amazon, the rapid and disorderly occupation of large urban centers resulted in severe degradation of small rivers and streams, resulting in the reduction in abundance of native fish species. This study aimed to evaluate, through an *in situ* experiment, the effects of water from three differentially degraded urban streams on the health of the widely distributed cichlid *Aequidens pallidus*. Fish were exposed for seven days to the water of a stream with low anthropic impact signs (A) and two moderately degraded streams (B and C). After exposure, welfare indicators, and genotoxic, biochemical and histopathological biomarkers were analyzed [condition factor (K), hepatosomatic index (HI), erythrocytic nuclear abnormalities (ENA), micronuclei (MN), metallothionein (MT) concentration, and morphological changes to gills and olfactory organ]. Significant reductions were observed in K in fish exposed to B and C, and HI in fish from C. ENA and MN were observed in the fish erythrocytes from the three streams. Reniform nuclei increased significantly in fish from C, as well as MN in fish from B compared to A. MT concentration increased in fish from C compared to A. Lesions in the gills were aneurysms, epithelial detachment, and lamellar fusion. The olfactory organ showed vacuole formation, loss of cilia, and necrosis. Gill and olfactory organ lesion indices were significantly higher in fish from B and C compared to A. The results suggest a high sensitivity of the species to moderately degraded environments and reinforce the need for measures aimed at improving the environmental quality of urban streams.

KEYWORDS: ecotoxicology, urban streams, fish, genotoxicity, histopathology, metallothioneins

Biomarcadores de exposição e efeito indicam alterações de saúde em *Aequidens pallidus* (Cichliformes, Cichlidae) expostos *in situ* em igarapés amazônicos urbanos degradados

RESUMO

Na Amazônia, a ocupação rápida e desordenada de grandes cidades resultou na severa degradação de pequenos rios e igarapés, levando à redução da abundância de espécies nativas de peixes. Este estudo avaliou, por meio de um experimento *in situ*, os efeitos da água de três igarapés urbanos diferentemente degradados sobre a saúde do ciclídeo amplamente distribuído *Aequidens pallidus*. Os peixes foram expostos por sete dias à água de um igarapé com baixos sinais de impacto antrópico (A) e dois igarapés moderadamente degradados (B e C). Após a exposição, foram analisados indicadores de bem-estar e os biomarcadores genotóxicos, bioquímico e histopatológicos [fator de condição (K), índice hepatossomático (HI), anormalidades nucleares eritrocitárias (ENA), micronúcleos (MN), concentração de metalotioneínas (MT), e alterações morfológicas nas brânquias e órgão olfatório]. Houve redução significativa no K em peixes expostos a B e C, e no HI em peixes de C. MN foram mais elevados em peixes de B em comparação a A. Núcleos reniformes aumentaram significativamente em peixes de C. A concentração de MT aumentou em peixes de C comparados a A. Nas brânquias, observou-se aneurismas, descolamento epitelial e fusão lamelar. O órgão olfatório apresentou formação de vacúolos, perda de cílios e necrose. Os índices de lesão das brânquias e órgão olfatório foram significativamente maiores em peixes de B e C em comparação a A. Os resultados sugerem uma alta sensibilidade da espécie a ambientes moderadamente degradados e reforçam a necessidade de medidas para melhorar a qualidade ambiental de igarapés urbanos.

PALAVRAS-CHAVE: ecotoxicologia, riachos urbanos, peixe, genotoxicidade, histopatologia, metalotioneínas.

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INTRODUCTION

Urbanization has occurred intensely in some areas of the Amazon region, with many cities growing quickly and disorderly (Richards and VanWey 2015). Manaus, the capital city of Amazonas state (Brazil) is one of the largest urban centers in the region, with a current population of over 2 million inhabitants (IBGE 2023). Streams and small rivers, known regionally as *igarapés*, are among the most impacted natural environments in Manaus (Ferreira *et al*. 2021). Over the last decade, there has been a significant reduction in the native fish fauna in impacted urban streams in Manaus (Guarido 2014; Beltrão *et al*. 2018; Anjos 2022).

The native cichlid *Aequidens pallidus* Heckel 1840 (Cichliformes: Cichlidae) is a small fish (total length 14 cm) that inhabits clear and black water lotic environments with a wide distribution in the Amazon River basin, in the middle and low Negro, Uatumã, Preto da Eva and Puraquequara rivers, feeding mainly on fish and detritus (Kullander and Ferreira 1990). In Manaus, it is largely restricted to streams with a high degree of environmental integrity (Anjos 2007; Guarido 2014; Beltrão *et al*. 2018), and, with the growing degradation of streams, it may soon become locally extinct (Anjos 2007; Beltrão *et al*. 2018). Therefore, it is important to identify the sublethal effects on fish in streams under moderate anthropic impact (Perkins 1979; Robinet and Feunteun 2002).

Biomarkers are measurable biochemical, cellular or physiological variations in tissues or body fluid samples that provide evidence of exposure and/or effect of one or more pollutants (Depledge 1994). Exposure biomarkers determine the internal dose and/or biologically active concentration of a pollutant, and effect biomarkers indicate the adverse effects of pollutants on the organism (Schlenk *et al*. 2008). Metallothioneins comprise a group of low molecular weight cytosolic proteins that increase upon exposure to metal-group contaminants, and are therefore widely used as an exposure biomarker in animal tissues (Roesijadi 1992; Samuel *et al*. 2021). Genotoxic alterations, such as the formation of micronuclei and erythrocytic nuclear abnormalities (Carrasco *et al*. 1990; Corredor-Santamaría *et al*. 2016), and histopathological damage in specific organs, such as the gills and the olfactory organ, are effect biomarkers. In fish, the allometric condition factor and the hepatosomatic index are also used to assess the effect of contaminants (Valdez-Domingos *et al*. 2009; Liebel *et al*. 2013; De Oliveira *et al*. 2019).

This study aimed to evaluate the sublethal effects of water from moderately degraded urban streams on the modulation of metal regulatory proteins, genotoxic, histological, and ultrastructural parameters, and welfare indicators in *A. pallidus* through *in situ* exposure. Our hypothesis was that the biomarkers will reflect significantly greater damage to the health of fish exposed to more impacted streams than to less impacted streams.

MATERIAL AND METHODS

Fish collection and acclimatization

Forty specimens of *Aequidens pallidus* (weight: 30.26 ± 18.50 g; standard length: 8.64 ± 1.82 cm) were collected using small fine-meshed seine nets and hand nets in a well preserved first-order stream (3º05'58.5"S, 59º57'34.3"W) in an urban fragment of primary rainforest that is a permanent reserve of the Federal University of Amazonas (UFAM) (Table 1). The fish were transported to the laboratory and acclimatized for 30 days in two outdoor 500-liter tanks (20 fish per tank) with daily 50% water renewal and fed once daily with commercial food pellets containing 45% crude protein. Temperature, pH, dissolved oxygen and electrical conductivity were measured daily in each tank (Table 1). The physicochemical parameters observed in the acclimatization tanks were within the expected limits. After acclimatization, five fish were removed from each tank (total = 10 fish) and euthanized to evaluate the health status of fish before the *in situ* exposure using the biomarkers

Table 1. Limnological parameters in the collection stream, acclimatization tanks and in streams A, B, and C where the *in situ* exposure experiment with *Aequidens pallidus* was carried out. Values are the mean ± standard deviation of two replicates (on the first and last day of the experiment) for *in situ* measurements, and of 30 replicates for acclimatization. Chemical compounds were measured only once. Nd = not detected, nm = not measured.

Parameter	Collection stream	Acclimatization	Stream A	Stream B	Stream C
Dissolved oxygen (mg L^{-1})	5.60	6.95 ± 0.21	5.91 ± 0.41	6.40 ± 0.08	4.41 ± 0.16
pH	5.57	5.10 ± 0.14	5.49 ± 0.78	6.91 ± 1.79	7.48 ± 1.76
Temperature (ºC)	25.40	25.20 ± 0.28	25.20 ± 0	25.41 ± 0.16	26.93 ± 0.81
Conductivity (µS cm^{-1})	14.20	16.50 ± 4.95	12.15 ± 2.62	60.50 ± 12.02	88.80 ± 3.11
Ammonia (mg L^{-1})	nm	nm	0.09	0.32	0.50
Nitrite (mg L^{-1})	nm	nm	0.01	0.02	0.03
Nitrate (mg L^{-1})	nm	nm	nd	0.01	0.25
Total phosphorus (mg L^{-1})	nm	nm	nd	0.01	0.05

as described below. The thirty remaining fish were transported in plastic bags with aeration for *in situ* exposure.

In situ experiment

The fish were distributed in three streams. Similarly to the stream where fish were collected, stream A (3º05'59.9"S, 59º57'31.4"W) is a well preserved first-order also located in the UFAM Reserve. Streams B and C are located near the UFAM Reserve and were characterized as moderately impacted by anthropic activities (Table 1). Stream B (3º05'29.5"S, 59º57'19.2"W) receives water from UFAM's sewage treatment station, which is still turbid and smelly when it reaches the stream. Stream C (3°05'29.0"S, 59°59'40.1"W) receives a high load of untreated effluent from domestic units and an experimental fish farm, and its water is also turbid and smelly. Solid waste is also carried to stream C by rain. All streams are located in the Mindu microbasin, which drains into the Negro River.

Dissolved oxygen, pH, electrical conductivity, and temperature were measured with a multiparameter equipment (YSI 556 MPS) in streams A, B, and C at the beginning and end of the experiment. A water sample was collected from each stream to determine the level of ammonia, nitrite, nitrate, and total phosphorus, according to the Standard Methods for the Examination of Water and Wastewater (Baird *et al.* 2005).

Streams B and C had pH above 6 and electrical conductivity above 60 µS cm^{-1}, which characterize moderately impacted streams in the Manaus region (Couceiro *et al.* 2012; Ferreira *et al.* 2012; Monteiro-Júnior *et al.* 2014; Ferreira *et al.* 2021). In contrast to moderately impacted streams, the pristine streams of Manaus present conductivity values around 10 µS cm^{-1}, while the severely impacted ones range between 100 and 200 µS cm^{-1} (Couceiro *et al.* 2012; Ferreira *et al.* 2012). In addition, the dissolved oxygen values in streams B and C ranged from 4 to 6 mg L^{-1}, while the severely impacted streams show levels even lower than 2 mg L^{-1} (Couceiro *et al.* 2012; Monteiro-Júnior *et al.* 2014). The phosphorus concentrations observed in streams B and C are lower than those found in severely impacted streams, which can reach up to 0.2 mg L^{-1} (Couceiro *et al.* 2012). The phosphorus concentrations above the limit of 0.025 mg L^{-1} established by CONAMA (2005) indicates eutrophication.

Ten fish were allocated to each stream placed individually in plastic cages (30x20x12 cm, with the lateral mesh opening measuring 1.5 cm and the bottom of the cage without openings) exposed to the natural water flow for seven consecutive days during May and June of 2019. During the experiment, the fish received no artificial feed. Although the bottom of the cages had no openings, they were placed directly on the sediment and secured to the streambed, allowing the water current to carry small invertebrates and detritus into the cages through the lateral openings, enabling the fish to feed on this material.

Tissue sampling

After exposure, the live fish were transported to the Laboratory of Aquatic Ecotoxicology in the Amazon at Instituto Nacional de Pesquisas da Amazônia - INPA. In the laboratory, the fish were anesthetized with Eugenol (50 mg L^{-1}), then blood was collected using heparinized syringes and blood smears were immediately prepared. Subsequently, the specimens were measured (total and standard length), weighed and euthanized by sectioning the cervical spine to remove the liver, gills and olfactory organ.

After drying, the blood smears were fixed in absolute ethanol and stored in slide boxes until processing. Liver samples were deposited in microtubes and stored in a -80ºC freezer. For optical microscopy analyses, samples of gills and olfactory organ were fixed in ALFAC solution for 24 hours and stored in 70% ethanol. For scanning electron microscopy analyses, the samples were fixed in glutaraldehyde 3% and sodium cacodylate buffer 0.1M.

Condition factor and hepatosomatic index

The allometric condition factor (K) (Vazzoler 1996) was calculated according to the equation [1]. The hepatosomatic index (HI) was calculated according to equation [2].

$$K=Wt/Lt^b \qquad [1]$$

where Wt = total weight of the fish (g); Lt = total length (cm); and b = allometric coefficient of the weight-length relationship.

$$HI=Wf/Wt*100 \qquad [2]$$

where Wf = weight of the liver (g); and Wt = total weight of the fish (g).

Genotoxicity

Blood smears were stained with Giemsa 10% and analyzed under an optical microscope at 1000x magnification (Zeizz Axiophot 2/AxioCam MRc). The frequency of normal nuclei, micronuclei (MN), and erythrocytic nuclear abnormalities (ENA) were recorded. Two thousand cells were analyzed per individual and the result was expressed per 1000 cells (Carrasco *et al.* 1990).

Metallothionein concentration

Liver samples were homogenized in tris-HCl/sucrose buffer (20 mM, pH 6.8), and metallothioneins (MT) were isolated by fractionation with ethanol and chloroform in an acid medium (Viarengo *et al.* 1997). MT concentration was determined by reading absorbance at 412 nm. Total proteins were quantified according to Bradford (1976) in a spectrophotometer (SpectraMax M2, Molecular Devices) at 595 nm. The result was expressed in µg MT mg of $protein^{-1}$.

Histopathology

The stored olfactory organ samples were decalcified in formic acid 10% for 24 hours for dissection of the olfactory rosette under a stereomicroscope. The samples of gills and olfactory organ were dehydrated in increasing ethanol concentrations, diaphanized in xylol, embedded in Paraplast Plus, and sectioned at a thickness of 5μm. The sections were stained with hematoxylin-eosin (HE) and analyzed under a light microscope Axiophot 2 coupled with an AxioCam MRc Zeiss image capture system.

A lesion index (LI) was calculated for gills and the olfactory organ according to Bernet *et al*. (1999), using equation [3].

$$LI = \Sigma\ (S \times IF) \qquad [3]$$

where S = score value [0 = absent/normal (0 to 10% of tissue), 2 = slight occurrence (11 to 30% of tissue), 4 = moderate occurrence (31 to 70% of tissue), 6 = extensive occurrence (71 to 100% of tissue), depending on the extent of each lesion]; and IF = factor of pathological importance of the lesion, which can assume the values: (1) minimal pathological importance, (2) moderate pathological importance, or (3) high pathological importance.

We used the Van Dyk *et al*. (2009) methodology (adapted from Zimmerli *et al*. 2007, based on an index by Bernet *et al*. 1999) to interpret the LI of the gills. In gills, Van Dyk *et al*. (2009) consider that LI < 10 indicates normal tissue structure with slight histological alterations, LI = 10 to 25 indicates moderate histological alterations, and LI > 25 indicates alterations of high pathological importance. We used Zimmerli *et al*. 2007 to interpret the LI in the olfactory organ, where LI < 10 indicates normal and healthy tissue structure, LI = 11 to 20 indicates slight alterations, LI = 21 to 30 moderate alterations, LI = 31 to 40 pronounced alterations and LI > 40 severe alterations in the tissue architecture.

Scanning electron microscopy

The fixed samples of gills and olfactory organ were dehydrated in increasing concentrations of ethanol. The critical point was obtained with CO_2, and the samples were metalized with gold. The samples were analyzed under a scanning electron microscope (Tescan Vega 3 INCAx-act). The observed changes were qualitatively evaluated and photographed.

Statistical analysis

The values for each biomarker were expressed as mean, standard deviation, and median of 10 replicates. The response of each biomarker among the three streams was assessed with the non-parametric Kruskal-Wallis, recommended for small sample sizes, followed by Dunn's *post-hoc* test with Bonferroni correction (Weissgerber *et al*. 2015). The significance level adopted was $p \leq 0.05$. Statistical analyses were performed using R Studio software with the rstatix package.

Legal compliance

Fish collection was authorized by SISBIO license # 62882-1. Fish handling and experimental procedures were approved by the Ethics Committee on the Use of Animals in Research (CEUA/INPA) through protocol # 021/2013.

RESULTS

Fish euthanized after acclimatization showed responses closer to those observed in fish from stream A (Table 2). No mortality was recorded during *in situ* exposure. Post-exposure fish length and weight did not differ significantly among streams. Post-exposure K differed significantly among streams ($H = 25.806$, $df = 2$, $p < 0.001$) (Table 2), being significantly higher in stream A than in stream B and C (Dunn test, $p < 0.001$), and significantly higher in stream B than in stream C (Dunn test, $p < 0.001$) (Figure 1a). HI differed significantly among the streams ($H = 7.009$, $df = 2$, $p = 0.030$) (Table 2). Values in stream A were higher than in stream C (Dunn test, $p = 0.050$) (Figure 1b).

Post-exposure MT concentration varied significantly among streams ($H = 7.4426$, $df = 2$, $p = 0.024$) (Table 2), with values in stream C ($p = 0.050$) significantly higher than in stream A (Figure 2a). MN occurrence varied significantly among streams ($H = 7.5122$, $df = 2$, $p = 0.023$) (Table 2), with significantly higher values in stream B than in stream

Table 2. Welfare indicators, and exposure and effect biomarkers of *Aequidens pallidus* after acclimatization and after *in situ* exposure in three urban streams with different degree of anthropic impact. Values are the mean ± standard deviation of 10 replicates per group. Statistical comparisons were made only between the streams. Different letters indicate significant pairwise differences among the streams ($p \leq 0.05$). K = condition factor; HI = hepatosomatic index; MT = metallothionein concentration; MN = frequency of micronuclei; ENA = frequency of erythrocytic nuclear abnormalities; LI = lesion index.

Parameter	Acclimatization	Stream A	Stream B	Stream C
K	0.021 ± 0.002	0.031 ± 0.002a	0.022 ± 0.002b	0.018 ± 0.001c
HI	2.53 ± 0.24	1.63 ± 0.53a	1.62 ± 0.51ab	1.14 ± 0.28b
MT (μg mg of protein^{-1})	6.35 ± 6.35	6.01 ± 1.34a	10.54 ± 4.44ab	14.48 ± 13.97b
MN (‰)	2.30 ± 0.82	2.15 ± 0.88a	4.00 ± 1.53b	4.40 ± 2.91b
ENA (‰)	2.90 ± 1.78	3.00 ± 1.33a	3.80 ± 1.36a	4.50 ± 1.83a
LI gills	5.60 ± 2.46	7.40 ± 1.89a	11.00 ± 2.50b	11.90 ± 3.54b
LI olfactory organ	4.00 ± 2.83	6.30 ± 3.40a	14.60 ± 4.22b	11.00 ± 3.30b

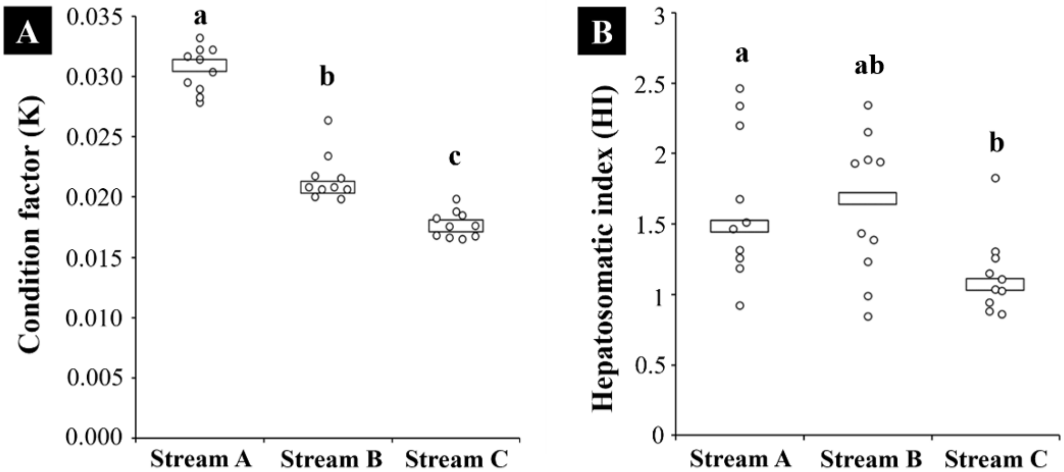

Figure 1. Condition factor (K) and hepatosomatic index (HI) of *Aequidens pallidus* submitted to *in situ* exposure in differentialy anthropically impacted urban streams (n = 10 per stream). White circles are the individual values, white bars the median. Different letters above the scatterplots in each graph indicate significant pairwise differences among the streams (p ≤ 0.05).

A (Dunn test, p = 0.041) (Figure 2b). ENA frequency did not difer significantly among the streams (Figure 2c), except for reniform nuclei (H = 5.9429, df = 2, p = 0.051), which were significantly more frequent in stream C than in stream A (Dunn test, p = 0.044). Erythrocytes with an elliptically-shaped nucleus, considered normal in fish (Carrasco *et al.* 1990), were predominant in all analyzed fish. Other forms of MN and ENA observed were reniform, lobed, vacuolated, and segmented (Figure 3).

The changes observed in the gills in streams B and C can be considered moderate (Table 2). LI of the gills varied significantly among streams (H = 11.356, df = 2, p = 0.003), being significantly higher in streams B and C than in stream A (Dunn test, p = 0.020 and 0.006, respectively) (Figure 2d). In all streams, histological changes were observed in the gill tissue relative to normal gill morphology (Figure 4a), such as circulatory disorders (aneurysm and blood congestion), progressive changes (epithelial detachment, hyperplasia, hypertrophy, partial and total lamellar fusion), and regressive change (necrosis) (Figures 4b-e). SEM images of gills from stream A showed an overall normal structure, with well-defined filaments and lamellae, and the presence of microridges on the surface of the pavement cells (Figures 5a,b). In streams B and C, gills showed lamellar fusion, hyperplasia in the filament and lamellae, reduction or loss of microridges of pavement cells, and aneurysms (Figures 6c-f).

The changes observed in the olfactory organ in streams B and C can be considered slight (Table 2). LI of the olfactory organ varied significantly among streams (H = 13.67, df = 2, p = 0.001), with higher values in streams B and C than in stream A (Dunn test, p < 0.001 and p = 0.031, respectively) (Figure 2e). Histopathological changes in the olfactory organ were observed in all streams. Progressive changes included cell hyperplasia and hypertrophy, sometimes resulting in lamellae deformities with loss of lateral rectilinear shape and formation of projections, while regressive changes included vacuole formation, loss of apical surface cilia, and necrosis (Figures 6c-e).

The SEM images showed that overall the olfactory organ retained its normal structure in stream A, with 18-20 lamellae extending from the median raphe, narrow at the insertion in the raphe, and thickening towards the periphery of the nostril (Figure 7a), and organized cilia on the surface of the lamellar epithelium in the region distal to the raphe (Figure 7b). In these fish, small regions with no cilia were observed on the surface of the olfactory epithelium. In streams B and C, the organ showed holes on the epithelial surface, as well as loss and disorganization of cilia on the epithelial surface (Figures 7c,d).

DISCUSSION

Regarding the higher values of K observed in B compared to C, it is possible that greater vegetation cover in stream B provided a greater supply of fallen leaves in the stream bed, creating microenvironments for potential prey and increasing the food supply for fish in this stream. It is also important to consider that the anthropogenic impact occurring in streams B and C influences the reduction in prey abundance in these environments and consequently the K of fish. The reduction of the condition factor in fish is often reported upon exposure to different categories of contaminants (Liebel *et al.* 2013; De Oliveira *et al.* 2019). In our study, the significant variation in K among the streams can be attributed to the reallocation of energy to detoxification mechanisms in streams B and C, which leads to the depletion of reserves destined for somatic growth (Liebel *et al.* 2013).

Low values of HI result from a high demand for metabolic energy from exposure to contaminants, causing

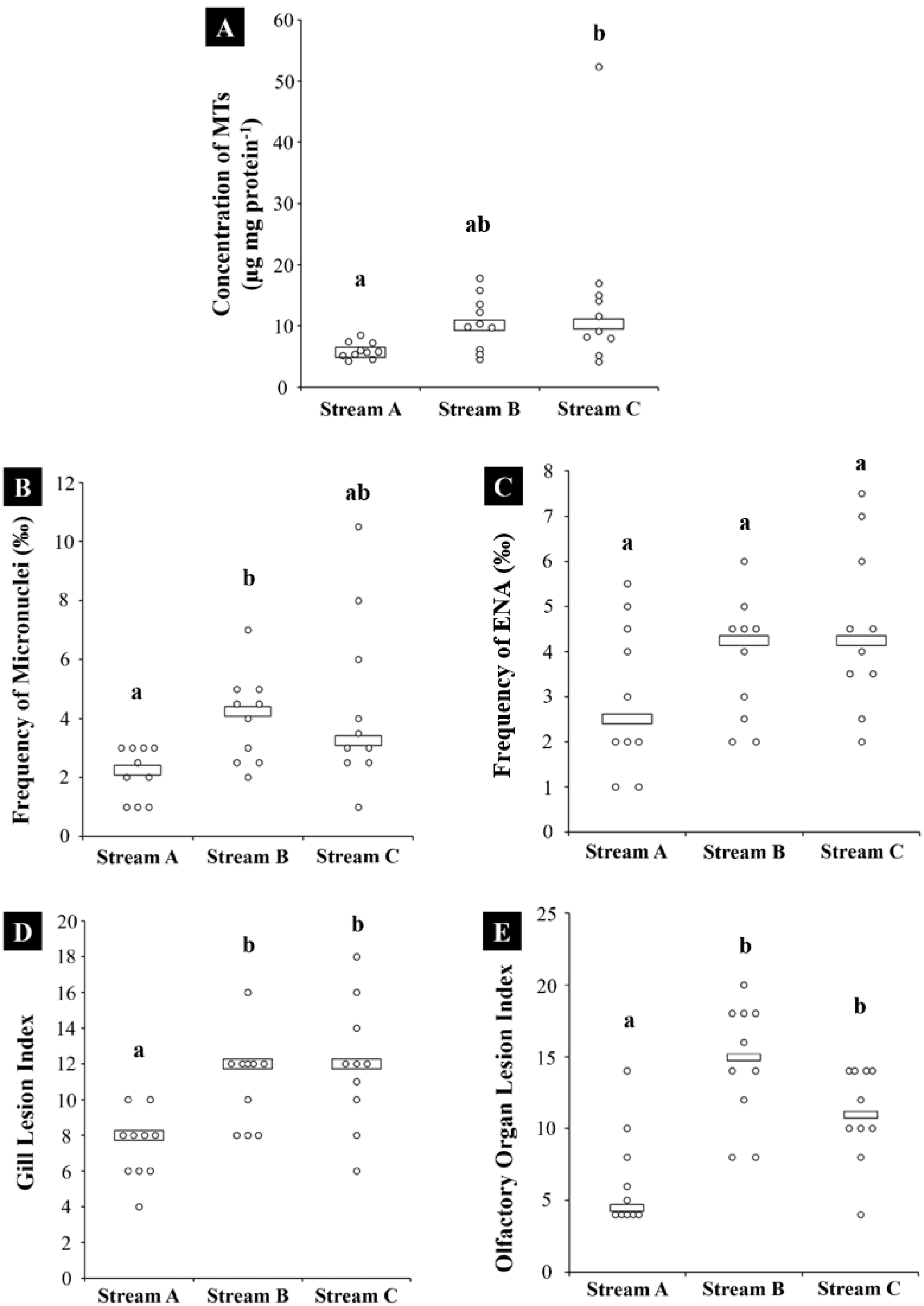

Figure 2. Concentration of metallothioneins (**A**); micronucleus frequency (**B**); frequency of ENA (**C**); gill lesion index (**D**); and olfactory-organ lesion index (**E**) in *Aequidens pallidus* after *in situ* exposure in three urban streams with different anthropic impact (n=10 fish per stream). White circles are the individual values, white bars the median. Different letters above the scatterplots in each graph indicate significant pairwise differences among the streams ($p \leq 0.05$).

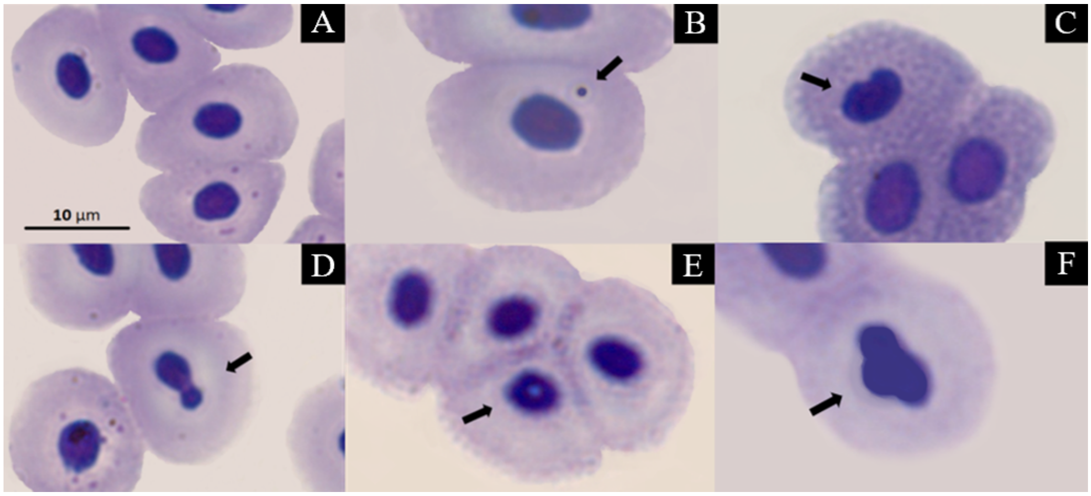

Figure 3. Erythrocyte nuclei in blood samples of *Aequidens pallidus* after *in situ* exposure in three urban streams with different anthropic impact. **A** – normal nucleus; **B** – micronucleus; **C** – reniform shaped nucleus; **D** – segmented nucleus; **E** – vacuolated nucleus; **F** – lobed nucleus.

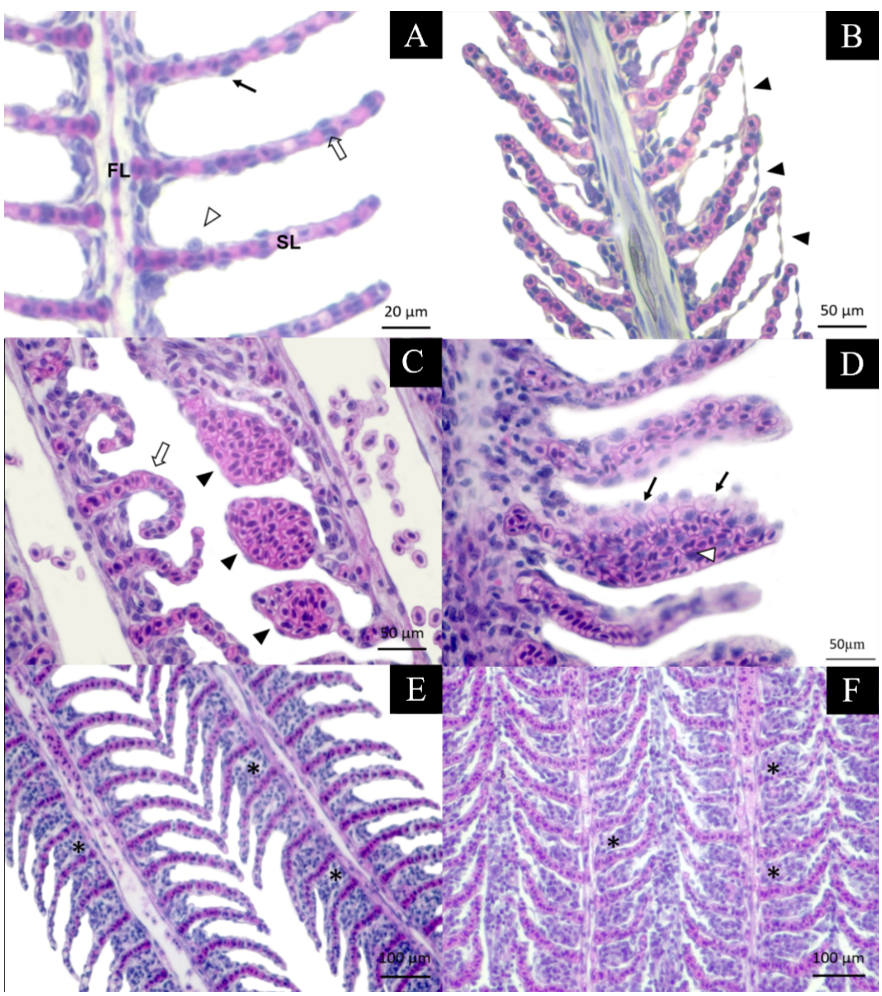

Figure 4. Gills of *Aequidens pallidus* after *in situ* exposure in urban streams with different anthropic impact. **A** – normal structure of the organ showing the gill filament (FL), secondary lamella (SL), pillar cell (white arrow), pavement cell (black arrow), mucous cell (white arrowhead). Histological changes: **B** – epithelial detachment; **C** – loss of structural conformation (white arrow) and aneurysms (black arrowheads); **D** – blood congestion (white arrowhead) and necrosis (black arrow); **E** – partial fusion of secondary lamellae (asterisks); **F** – total fusion of secondary lamellae (asterisks). Slides stained with HE.

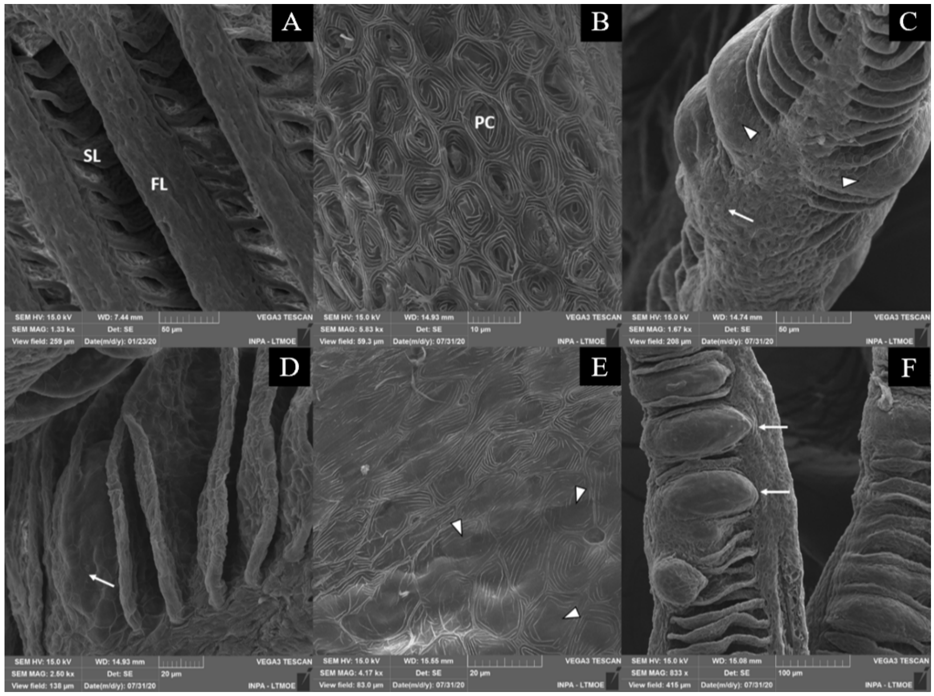

Figure 5. Gills of *Aequidens pallidus* in scanning electron microscopy. **A** – normal structure of the organ, showing well-defined filament (FL) and secondary lamellae (SL); **B** – detail of the gill filament region showing pavement cells (PC) with surface microridges. Histological changes: **C** – lamellar fusion (white arrowheads) and gill filament epithelial cell hyperplasia (white arrow); **D** – lamellar hyperplasia (white arrow); **E** – reduction and loss of pavement cell cilia (white arrowhead); **F** – aneurysms (white arrows).

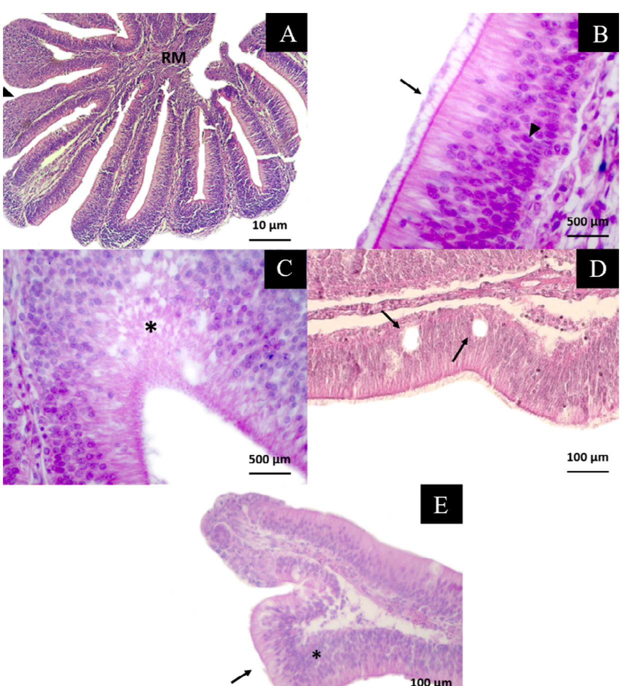

Figure 6. Olfactory epithelium of *Aequidens pallidus* after *in situ* exposure in three urban streams with different anthropic impact. **A** – olfactory rosette, formed by the median raphe (RM) and lamellae (black arrowhead); **B** – normal tissue composed of support cells with cilia (black arrow) and basal cells (black arrowhead). Histological changes: **C** – foci of necrosis; **D** – formation of vacuoles (black arrow) (note the reduction of cilia on the surface of the epithelium); **E** – lamella deformities (black arrow) and basal cell hyperplasia (*). Slides stained with HE.

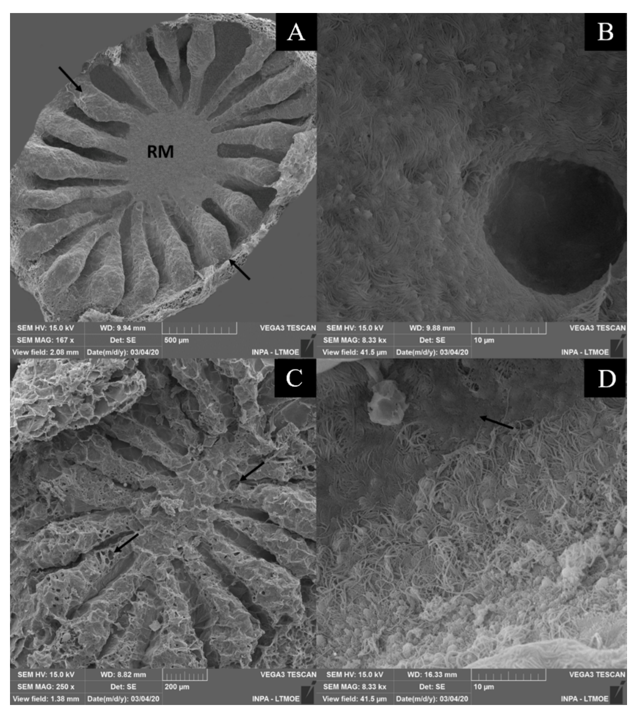

Figure 7. Olfactory rosette of *Aequidens pallidus* in scanning electron microscopy. **A** – normal organ morphology, with a median raphe (MR) and 19 lamellae (black arrow); **B** – detail of the lamella showing dense, organized cilia on the surface of the epithelium. Histological changes: **C** – olfactory rosette quite degenerate with holes; **D** – disorganization and loss of cilia on the surface of the epithelium.

the depletion of energy reserves stored as hepatic glycogen and the consequent reduction in the organ's weight (Maceda-Veiga *et al*. 2012), which can also affect K. *Aequidens metae* Eigenmann 1922 collected upstream, near, and downstream of a petroleum effluent discharge pipe, as well as at a reference site isolated from human activities, showed significantly higher K values at the upstream site and higher HI values near the discharge pipe during the dry season, the latter being attributed to hypertrophy of liver cells in response to the contaminant (Corredor-Santamaría *et al*. 2021).

Increased hepatic concentrations of MT, as observed in stream C, have been reported in other cichlid species exposed to contaminants from the metal group (Carvalho *et al.* 2012). Our results for MT suggest the presence of contaminants from the metal group in stream C.

An increase in the frequency of genotoxic marker was also recorded in other fish in environments impacted by urban effluents (Popovic *et al.* 2015; Gomes-Silva *et al.* 2020; Lehun *et al*. 2021), pesticides (Vieira *et al.* 2016; Vieira *et al.* 2017; De Oliveira *et al.* 2019), and metals (Porto *et al.* 2005; Maceda-Veiga *et al.* 2012; Cruz-Esquivel *et al.* 2023). The increase in the frequency of genotoxic indicators indicates damage to the genetic material as a result of exposure to carcinogenic or mutagenic agents (Al-Sabti and Metcalfe 1995; Crott and Fenech 2001). ENA indicate early stages of damage to genetic material (Shimizu *et al.* 1998; Strunjak-Perovic *et al*. 2009), and may be linked exclusively to oxidative stress, which promotes the production of reactive oxygen species that can induce damage in cell membranes and genetic material (Strunjak-Perovic *et al.* 2009; Gomes *et al.* 2015). Changes in the erythrocyte nucleus can lead to the inhibition of DNA replication, which leads to the initiation of the apoptotic process (Walia *et al.* 2013; Gomes *et al.* 2015). While the occurrence of MN indicates mutagenic effect caused by clastogenic or aneugenic agents (Carrasco *et al.* 1990).

Gill lesions similar to those observed in our study have been recorded in fish subjected to different classes of pollutants (Martinez *et al*. 2004; Katsumiti *et al*. 2009; Valdez-Domingos *et al*. 2009; De Oliveira *et al*. 2019; Chen *et al*. 2022). Blood congestion is a pathological condition characterized by well-defined and delineated dilatations of blood vessels (Bernet *et al*. 1999), while aneurysms are associated with the rupture of pillar cells and the consequent accumulation of blood in the lamellae (Bernet *et al*. 1999; Martinez *et al*. 2004). Progressive lesions, such as hypertrophy and hyperplasia, are indicators of dysfunctions that may lead to neoplastic lesions and can often result in the fusion of adjacent lamellae (Valdez-Domingos *et al*. 2009; Martinez *et al*. 2004). The detachment of epithelial cells and hyperplasia are considered defense mechanisms of the organism, as they increase the distance of diffusion of contaminants to the bloodstream (Martinez *et al*. 2004; Katsumiti *et al*. 2009). However, this reaction impairs gas exchange processes and causes disturbances in osmoregulation functions, which are essential mechanisms for fish adaptation to stress conditions (Valdez-Domingos *et al*. 2009). The loss of pavement cell microridges reduces the functional surface of the organ and impairs the anchoring of mucus to the surface of the epithelium (Wilson and Laurent 2002; Evans *et al*. 2005). Although the LI of gills in streams B and C were categorized as moderate, we emphasize that the pathologies observed are of great relevance, and resulted from an exposure of only seven days. It can be extrapolated that long-term exposure can compromise gill morphology and function as well as fish survival in these environments.

Lesions observed in the olfactory epithelium of *A. pallidus*, such as hyperplasia, hypertrophy, vacuole formation, loss of apical surface cilia, and necrosis, have been reported in the olfactory epithelium of different species of fish as a result of exposure to contaminants of the class of metals. Basal and mucous cell hyperplasia and vacuole formation occurred in the olfactory epithelium of *Channa punctatus* Bloch 1793 after exposure to $CdCl_2$ (Roy *et al*. 2013). Hyperplasia, epithelial detachment, vacuoles, and necrosis occurred in the olfactory epithelium of *Labeo rohita* Hamilton 1822 after exposure to different concentrations of mercuric chloride (Ghosh and Mandal 2013). Progressive lesions, such as hyperplasia and hypertrophy, were observed in the fish *Menidia beryllina* Cope 1867 and *Trinectes maculatus* Bloch and Schneider 1801 after exposure to soluble fractions of petroleum (Solangi and Overstreet 1982). These lesions can compromise the organ's sensory functions, as cell proliferation considerably reduces the sensory surface area exposed to the environment (Tierney *et al*. 2010). Basal cell hyperplasia is related to the regeneration of the olfactory epithelium. Cell death induced by the effects of contaminants stimulates mitotic activity, proliferation, and differentiation of basal cells to repair the damage (Ghosh and Mandal 2013). The loss of epithelial cell cilia can reduce the adhesion capacity of the mucus that protects the epithelium (Ghosh and Mandal 2013), and can impair the water flow over the organ, compromising the chemoreception mechanism in fish (Tierney *et al.* 2010). This directly implies the loss or reduction of function of an important sensory pathway in fish, as olfaction is involved in the behavior control of feeding, reproduction, parental care, and territory defense (Kasumyan 2004). Altered chemoreception can result in impaired recognition of prey, sexual partners, and alarm substances (Tierney *et al*. 2010). The results observed in fish exposed to streams B and C may also be associated with the presence of metals, or with other categories of contaminants, such as different classes of insecticides, herbicides, sterols, antibiotics, and other pharmaceutical products already reported in urban streams from Manaus and other cities of Amazon region (Melo *et al.* 2019; Rico *et al.* 2021; Rico *et al.* 2022).

CONCLUSIONS

Aequidens pallidus exposed to water from anthropically impacted streams showed significant biochemical, genotoxic and tissue changes in relation to fish exposed in a less impacted stream, indicating impairment of physiological functions and changes in genetic material. The results suggest a high sensitivity of the species to degraded environments, which may be associated with contaminants such as metals, which are commonly reported in urban streams. Our results reinforce the importance of using biomarkers besides water quality parameters to evaluate the impact of urban streams. This study reinforce the urgent need of public policies that increase environmental quality of impacted urban streams in the Amazon, otherwise more severe ecological consequences can occur.

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SILVA-BORGES, L.A.: Conceptualization, Data curation, Formal analysis, Methodology, Writing - original draft, Writing - review & editing.
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