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Original Article

Aluminum Toxicity in the Flag Tetra *Hyphessobrycon heterorhabdus* (Teleostei: Characidae) at Environmentally Relevant Concentrations in the Amazon Region

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Abstract

Aluminum (Al) is a metal that occurs naturally in the Amazon region in high concentrations, which may increase due to the effects of mining. As it is a pro-oxidant and generates oxidative stress, we investigated biochemical biomarkers in the gills, liver and muscle of the native fish Hyphessobrycon heterorhabdus after exposure to different concentrations of Al (0.5 mg/L, 1 mg/L, and 10 mg/L) in an acidic medium (pH = 5) for 96 hours. Biomarkers such as total antioxidant capacity and glutathione S-transferase activity showed no significant differences in the organs studied. The liver showed a uniform response, with no change in lipid peroxidation levels (LPO). In the gills, fish exposed to 1 mg/L showed a decrease in LPO and a subsequent increase at 10 mg/L, indicating metal toxicity at the highest concentration and an induction of antioxidant defenses at 1 mg/L. In muscle, exposure to 1 mg/L induced an increase in LPO. However, in the group exposed to 10 mg/L, the fish stabilized the damage level. We conclude that *H. heterorhabdus* is tolerant to acidity and the environmental concentration (0.5 mg/L) and suffers oxidative stress at higher concentrations, but with different responses for each tissue analyzed.

Keywords: Biomarkers; Ecotoxicology; Lipid peroxidation; Oxidative stress.

INTRODUCTION

In Brazil, the eastern Amazon region is an important location for numerous industrial companies that focus on the extraction and refinement of minerals (da Silva *et al*., 2014; Coelho *et al*., 2022). The soil of the Amazon region is rich in metals such as iron, manganese, and especially aluminum (Al), which is extracted from bauxite ore (de Oliveira *et al.*, 2016). The richness and abundance of this element are recognized worldwide, so much so that the region between the states of Pará and Maranhão is considered the third-largest bauxite ore deposit in the world (Santini *et al.*, 2015).

Artificial tillage of the soil favors the dissociation of Al from the sediment and triggers the release of this and other metals into the aquatic

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environment through direct human or water impact (e.g., rainfall) (Mayes *et al.*, 2016), leading to an increase in their concentration. In addition, Amazonian rivers have acidic water (pH between 4.0 and 6.0) (Walker, 1987), which favors the bioavailability of Al (Magalhães *et al.*, 2015). The acidity dissolves this metal and transforms it into a reactive chemical species (Al^{3+}) for biological molecules (Exley and Birchall, 1992). It is important to note that freshwater environments in the Amazon region naturally have Al concentrations above the limits allowed by Brazilian legislation (> 0.1 mg/L), so organisms are constantly exposed to these concentrations and their seasonal variations (Cantanhêde *et al*., 2023). However, the effects of Al on the resident biota are still poorly understood.

In fish, Al can damage various organs and tissues. It is capable of causing structural disorders in muscle tissue (Ferrandino *et al*., 2022), excessive proliferation of mucus cells in the gills, which impedes the diffusion of respiratory gases (Exley *et al.*, 1991), and in the liver, Al can cause excessive histopathological effects (Slaninova *et al*., 2014). Such damage can result from imbalances at the subcellular level, such as oxidative stress, in which there is excessive production of reactive oxygen species (ROS) and also inhibition of antioxidant enzymes affected by Al exposure (Ramírez-Duarte *et al*., 2017; Abdalla *et al*., 2019), leading to oxidation of DNA, proteins, and membrane lipids (Capriello *et al.*, 2021). These biochemical changes (antioxidant enzymes or molecules and by-products of oxidative damage) can serve as biomarkers and provide valuable information on the exposure and effect of xenobiotics or environmental conditions in fish (Birnie-Gauvin *et al*., 2017).

To understand the effects of Al on representative organisms of the Amazon, the native fish *Hyphessobrycon heterorhabdus* (Ulrey, 1894) was chosen here as a biological model. *H. heterorhabdus*, popularly known as the flag tetra, belongs to the Characidae family and is a neotropical nektonic species that feeds omnivorously and is widely distributed in the rivers of the Amazon basin (Moreira *et al.*, 2002; García-Alzate *et al.*, 2008). This animal has a sequenced genome and an established cell culture obtained from its caudal fin (HHEcf), which contributes to its use as a model in various scientific areas, including ecotoxicology (Cardoso *et al.*, 2025). Given the prooxidant potential of this metal, our study aims to assess the acute toxicity of aluminum using biomarkers of oxidative stress in the gill, liver, and muscle of *H. heterorhabdus* in a realistic perspective for the Amazonian biome.

MATERIAL AND METHODS

Fish collection and acclimatization

Adult fish (N = 125) were collected under the SISBIO collection license (number 63470-2) in a stream in Gunma Ecological Park (1°12'09.1" S 48°18'03.7" W) in the municipality of Santa Bárbara, Pará, northern Brazil; considered a reference site in this study due to low anthropogenic disturbance and distance from any large development such as mining. The animals were captured with a hand net and transported in gallons (5.0 L) of river water for acclimatization in the laboratory. They were kept for one month in glass aquaria (35 x 30 x 35 cm; 35 L; 1 g fish/L) with dechlorinated water (28 ºC, pH = 7.0), a biological filtration system, constant aeration, a 14L:10D photoperiod, and fed twice daily with commercial food (32% protein). Every two days, a partial water change (30%) took place to remove excreta and food residues. The experiment was conducted with the authorization of the Ethics Committee for the Use of Animals of the Universidade Federal do Pará (CEUA-UFPA, protocol number 6211260521). There was no mortality during the experiment.

Chemical substance

The Al stock solution was prepared with aluminum sulfate P.A. [$Al_2(SO_4)_3$] (DINÂMICA®, Brazil) diluted in reverse osmosis water. The quantification of Al in the stock solution was carried out using atomic absorption spectrometry by flame atomization in a graphite furnace (NOVAA 300 and Analytik Jena Zenit 600) according to the method of Welz and Sperling (1999). The stock solution had a concentration of 1 g/L Al and was used to produce the nominal concentrations for the experiment. All materials and glassware used were previously acidified in nitric acid (HNO_3 1%) for 24 hours to avoid unwanted metal chelation or contamination. The nominal concentrations were calculated and prepared based on the stock solution, using osmosis water reconstituted with salts ($CaSO_4$ 0.03 g/L; KCl, 0.002 g/L; $NaHCO_3$, 0.0048 g/L; $MgSO_4$, 0.061 g/L), with a low hardness (40 mg $CaCO_3$/L) to minimize ionic competition between the metal and the dissolved salts.

Experimental design

For the experiment, the animals were divided into five groups: a neutral control group (pH = 7.0), an acidic control group (pH = 5.0) and three groups exposed to aluminum only at acidic pH, at increasing concentrations of 0.5 mg/L, 1 mg/L, and 10 mg/L. Al is insoluble and not bioavailable at neutral pH (Gensemer and Playle, 1999); therefore, we did not consider an Al exposure group at neutral pH. Each group consisted of five replicates represented by five glass aquaria (3 L experimental medium) with five fish each, i.e., a total of 25 animals per group.

In Brazil, Resolution 357 of the National Environmental Council (CONAMA, 2005) establishes 0.1 mg/L as the permissible concentration of dissolved Al in freshwater. However, this concentration does not cover the concentrations found in the Amazon region (Dos Santos *et al*., 2023). Thus, the lowest concentration (0.5 mg/L) was determined from chemical analysis performed in the same stream where the fish were collected in two different hydrological periods (unpublished data), while Cantanhêde *et al.* (2023) found similar values in environments directly and indirectly influenced by mining activities, where Al concentrations around 0.3 mg/L were observed. The other concentrations were chosen to observe the potential toxic effects of this metal at two- and 20-times higher concentrations, respectively.

Exposure took place over 96 hours, with feeding suspended 24 hours beforehand, according to the recommendations of the Brazilian standards for acute toxicity tests in fish (ABNT, 2022).

The experimental solutions were partially renewed every 24 hours (50) to keep the concentrations constant so that they could be absorbed by the animals. The pH of the experimental media was maintained at 7.0 (neutral control) or 5.0 (acidic control and other exposure groups) by adding HCl (10%) or NaOH (6M) solutions beforehand as required. At the end of the exposure period, the fish were anesthetized and euthanized on ice to avoid any influence of the anesthetics on the biochemical parameters. We measured the length (mm) and weight (g) of the animals and dissected them to remove the gills, liver, and muscle.

Sample preparation and biochemical analysis

Due to the small size of the fish (28.89 ± 2.19 mm; 0.47 ± 0.11 g), we pooled five individuals per sample to achieve the required mass (~ 250 mg) for the biochemical analyses. Samples were homogenized with ice-cold buffer (Tris-HCl 100 mM, EDTA 2 mM, $MgCl_{2.6}H_2O$ 5 mM, pH 8.0) at a weight/volume ratio of 1:10 (liver and gills) and 1:4 (muscle) according to Bainy *et al*. (1996), taking into account the amount of sample obtained per tissue. The extracts obtained were centrifuged for 20 minutes at 10,000 × g and 4 °C. The supernatant was then removed and stored at -80 ºC until further analysis.

The concentrations of total protein were determined spectrophotometrically according to Bradford (1976) using the Victor X3 multimodal microplate reader (Perkin Elmer) and bovine serum albumin as a standard. The biomarkers analyzed in all tissues were: the total antioxidant capacity against peroxyl radicals (ACAP), the activity of the enzyme glutathione S-transferase (GST), and the degree of lipid peroxidation (LPO).

We analyzed ACAP using the method of Amado *et al*. (2009), which determines the antioxidant capacity of samples with and without exposure to a radical generator, 2'2'-azobis-2-methylpropiamidine dihydrochloride (ABAP, 4 mM), using 2′,7′-dichlorofluorescein diacetate (H2DCF-DA) as a substrate. Peroxyl radicals are generated by thermal decomposition (37 °C) of ABAP. If the antioxidant defenses of the sample do not scavenge them, the ABAP-derived radicals oxidize H2DCF-DA, which emits fluorescence at excitation and emission wavelengths of 488 and 525 nm, respectively. The principle of this method is that samples with a higher antioxidant capacity scavenge a larger amount of ABAP-derived radicals, resulting in lower fluorescence emission. The total fluorescence generated is calculated by integrating the fluorescence units (FU) over the measurement time , after fitting the FU data to a second-order polynomial function. The results are expressed as the difference of the area FU/min in the same sample with and without ABAP and relativized by the area without ABAP. The reciprocal of the relative area difference with and without ABAP is the measure of the total antioxidant capacity.

GST activity was determined according to the method described by Habig and Jakoby (1981), which evaluates the conjugation of reduced glutathione (GSH, 1 μM) with the substrate 1-chloro-2,4-dinitrobenzene (CDNB, 1 μM), a process catalyzed by the GST enzyme in the sample. The complex formed has a maximum absorbance of 340 nm. 0.1 M phosphate buffer, pH 7.0, was used as the reaction medium. The results were expressed as U GST/mg protein, which corresponds to the amount of GST required to catalyze the conjugation of 1 μMol CDNB/min/mg protein.

LPO values were determined using the fluorimetric method for reactive thiobarbituric acid substances Kraak, as described by Oakes and Van der Kraak (2003). This method quantifies a by-product of lipid oxidation, malondialdehyde (MDA). In the test, MDA reacts with thiobarbituric acid (TBA, 0.8%) in an acidic medium (20% acetic acid) at a temperature of 95 °C and forms a complex that is detected with a fluorimeter at wavelengths of 515 and 553 nm for emission and excitation, respectively. Butylated hydroxytoluene (BHT) was used as an antioxidant for the samples, and 1,1,3,3-tetramethoxypropane (TMP) as a standard. Sodium dodecyl sulfate (SDS, 8.1%) served as a surfactant, and n-butanol was used to separate the organic from the inorganic phase. The results were expressed as nmol MDA/g wet tissue.

Integrated Biomarker Response (IBR)

The IBR index was applied to identify the most sensitive biomarker (ACAP, GST, or LPO) of each experimental group in *H. heterorhabdus* tissues. Here, we

used the index proposed by Beliaeff and Burgeot (2002) and reviewed by Devin *et al.* (2014), on the digital interface CALIBRI (https://liec-univ-lorraine.shinyapps.io/calibri/), provided free of charge by the Laboratory of Continental Environments (University of Lorraine, France) (Devin *et al.*, 2023).

Briefly, biomarker values were standardized by the equation Y = (X - m) / s, i.e. X corresponds to the mean value of the biomarker in each experimental group; s is the standard deviation of these values, and m represents the mean of all biomarker values for all groups. An S value is obtained from the minimum value recorded (|Min|) of each biomarker per experimental group, using the calculation S = Z + |Min|, and Z is a coefficient calculated as Y (activation) or -Y (inhibition), depending on the expected activity of the biomarker in the study context. We considered Z = Y for all biomarkers in our study, which means that they may be activated as a result of Al exposure. Finally, a radar plot with standardized biomarker values, in the same order for all tissues, is generated. IBR is equivalent to the total area of the graph: IBR = ΣAi , where Ai represents the area of the triangle formed by two adjacent biomarkers, calculated by the formula Ai = Si × Si + 1 × sin (2 π) / 2, where Si is the standardized value.

Statistical analysis

The assumptions of normality (Shapiro-Wilks test) and homoscedasticity (Levene test) were tested for the biomarker data. Analysis of variance (ANOVA) with Tukey's post hoc test was then applied to test for statistical differences between the means of the experimental groups, and values were expressed as mean ± standard error. In cases where the assumptions of ANOVA were not met, the non-parametric Kruskal-Wallis test was applied, followed by multiple comparisons of means, and results were reported as median ± first quartile. In all cases, we assumed a significance level of $p < 0.05$ (Zar 1984).

RESULTS AND DISCUSSION

The geological formation rich in Al, combined with the naturally acidic water of the Amazon, are unique conditions that favor the bioavailability of Al in the aquatic environment. In our study, three biochemical parameters of antioxidant protection and oxidative damage were investigated in different organs of the native fish species *Hyphessobrycon heterorhabdus* when exposed to environmentally relevant concentrations of Al at acidic pH, simulating scenarios of possible contamination by mining activities.

Biomarkers

No changes in ACAP were observed in any of the tissues examined ($p > 0.05$) (Figure 1A, D, and G). This biomarker provides a general response to the body's ability to scavenge ROS, not distinguishing between enzymatic (superoxide dismutase [SOD], catalase [CAT], etc.) and non-enzymatic (ascorbic acid, glutathione [GSH], etc.) components (Amado *et al.*, 2009). On this basis, aluminum exposure could not induce a global antioxidant defense response in *H. heterorhabdus*. However, it is possible that specific antioxidant defense elements are activated and/or inhibited to an extent that does not significantly affect the overall response obtained from the ACAP analysis.

This assumption is supported by analyzing the trend of numerical decrease in the levels of this biomarker in the organs studied between exposure groups (see Table S1). Al, which is dissolved by low pH, is known to be a pro-oxidant (Capriello *et al*., 2021), which can inhibit the activity of enzymes such as SOD and CAT (Slaninova *et al.*, 2014). Despite these factors, the effect of metal exposure can be counterbalanced by the activation of additional antioxidant enzymes such as glutathione peroxidase (Ighodaro and Akinloye, 2017; Abdalla *et al*., 2019) and the expression of metallothioneins (Bakiu *et al*., 2022), attempting to balance the body's defenses under stress without altering the average result of the ACAP analysis.

We also evaluated GST activity, a biotransformation enzyme that catalyzes the conjugation of xenobiotics or reactive endogenous compounds to GSH for excretion by cells (Van der Oost *et al*., 2003). This enzyme indirectly contributes to the maintenance of redox balance, mainly through the elimination of lipid hydroperoxides from the LPO (Zhang *et al.*, 2021). In turn, this enzyme remained stable ($p > 0.05$), suggesting that Al exposure does not affect phase II detoxification pathways in the fish *H. heterorhabdus* in the tissues analyzed (Figure 1B, E, and H). Moreover, its stability in the gills and muscles could be related to the fact that this enzyme is mainly expressed in the liver (Akkemik *et al*., 2012). Paradoxically, no changes in GST activity were detected in this organ, and oxidative damage levels remained stable (Figure 1F), suggesting that basal levels of GST and other antioxidants in the liver are responsible for maintaining homeostasis in this organ after exposure to Al.

Corresponding reactions are found in the LPO values of the other organs examined in this study. In the gills (Figure 1C), the decreased LPO in the group exposed to 1 mg/L Al at a lower level than in the controls (Control pH 7: $p = 0.005$; Control pH 5: $p = 0.002$) and 0.5 mg/L Al ($p = 0.005$) suggests that this concentration may be sufficiently high to trigger physiological defense

mechanisms in the fish, such as increased mucus production, a mechanism known to occur in fish to prevent the uptake of metals in ionic form into the bloodstream (Kwong, 2024). In this scenario, Al is complexed with the organic matter present in the water, reducing its bioavailability (Walton, 2019) and potentially reducing oxidative damage to this organ.

Considering the increase in LPO levels in the group exposed to 10 mg/L compared to the 1 mg/L exposure group ($p = 0.001$), this concentration could be high enough to overcome the gill defense of the fish, resulting in higher Al absorption and causing toxicity in this organ. The differences between MDA levels in the gills could be related to a possible hormetic effect of Al exposure. Hormesis corresponds to a process in which low doses or concentrations of xenobiotics, including metals, can activate physiological and molecular defense mechanisms in exposed organisms, in contrast to higher concentrations of the stressor (Rix *et al.,* 2022). These defense mechanisms may be related to the activation of transcription factors such as Nrf2, which mediate the antioxidant response of cells (Calabrese and Kozumbo, 2021). Therefore, these mechanisms could contribute to the different MDA levels observed in fish exposed to 1 mg/L of Al compared to the control group and fish exposed to 10 mg/L of Al.

The opposite response was observed in muscle, as the group exposed to 1 mg/L Al showed a significant increase in LPO compared to the controls (Control pH 7: $p = 0.004$; Control pH 5: $p = 0.024$) and 0.5 mg/L ($p = 0.001$) (Figure 1I). The antioxidant defenses in this tissue may be less effective as the muscle is predominantly white, prefers anaerobic metabolism, and naturally produces little ROS (Halliwell and Gutteridge, 1999; de Almeida-Val *et al*., 2005). However, Ferrandino *et al.* (2022) observed that *Danio rerio* fish can modulate their antioxidant defenses in this tissue depending on the exposure time at a concentration of 11 mg/L Al, and over longer periods of time, such as 10 days, so that there is no increase in lipid peroxidation. In view of this, the muscle of *H. heterorhabdus* was unable to adapt to the 96-hour exposure, with metal toxicity at 1 mg/L. At 10 mg/L Al, the fish exhibited stable LPO values comparable to those of all other groups ($p > 0.05$), indicating a modulation of defenses that was not detected by ACAP and GST analysis. An in vitro Al toxicity assay in HHEcf cell cultures showed an increase in metallothionein gene expression in the treatment exposed to 10 mg/L (Cardoso *et al*., 2025), suggesting that at very high concentrations, metal scavenging takes precedence as a defense mechanism for these cells. These results thus confirm the findings of our study, which showed a stronger effect of Al toxicity at 1 mg/L in the muscle of *H. heterorhabdus* compared to the group exposed to 10 mg/L.

Similar results were found in the study by Cantanhêde *et al*. (2022), which was conducted with the native fish *Bryconops caudomaculatus.* There, no oxidative damage to the organs was found at low concentrations (0.3 mg/L Al), but other parameters, such as the heart rate of *B. caudomaculatus*, changed. Thus, Al may cause changes in other metabolic and physiological parameters in *H. heterorhabdus*, which requires further analysis for a clear understanding of the effects of this metal.

Integrative response

A tool for understanding complex biomarker results is the IBR index. This index can be used to graphically assess the level of induction of biological responses in field and laboratory studies under different stress conditions (Sedrati *et al*., 2025). The integrative analysis of biomarkers in *H. heterorhabdus* showed distinct patterns for each tissue. Despite the few significant differences found, the IBR demonstrates that all biomarkers responded differently in each experimental group.

In gills, a slight increase in LPO and ACAP was already observed in the control group at pH 5 compared to the control at pH 7 (Figure 2A), which suggests that water acidity can induce moderate oxidative responses, probably as part of physiological adaptation to naturally acidic environments in the Amazon (Horbe & Oliveira, 2008). In the Al-exposed groups, the contribution of biomarkers increased progressively as the concentration rose. At concentrations of 0.5 and 1 mg/L, GST plays a significant role, indicating the early activation of the lipid peroxide detoxification pathway. This is consistent with the reduction in LPO observed in 1 mg/L. However, at 10 mg/L, there is a concurrent increase in LPO, ACAP, and GST contributions, increasing IBR (see Table S2), which suggests that this concentration surpasses the tissue's baseline defense capacity. As a result, oxidative damage accumulates, a pattern that aligns with the metal toxicity model in other organisms (Abdall *et al.*, 2019).

The liver exhibited minimal variation in biomarkers among the control groups (Figure 2B), which emphasizes its homeostatic function and its natural capacity to manage moderate environmental fluctuations (Bainy *et al.*, 1996). In the groups exposed to Al, there was a more pronounced increase in the IBR at concentrations of 1 and 10 mg/L. Notably, the contributions from GST and LPO were expressive, indicating that although the liver did not show clear oxidative damage in isolated analyses (Figure 1F), it activates response mechanisms to counter the rise in ROS. This combined response, involving detoxification and damage control, underscores the liver's vital role in the systemic antioxidant defense of tropical fish (Sales *et al*., 2025).

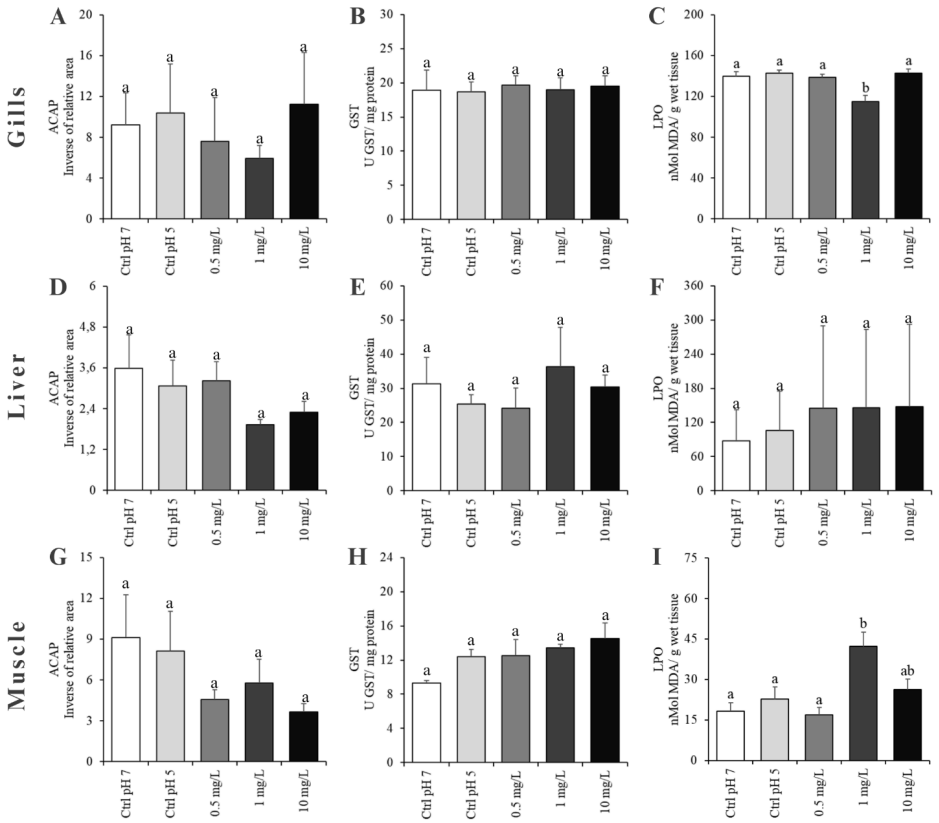

Figure 1. Oxidative stress biomarkers in gills, liver and muscle of *Hyphessobrycon heterorhabdus* (n = 25) after acute exposure to Aluminum. (A; D; G) Total antioxidant capacity against peroxyl radicals. (B; E; H) Glutathione S-transferase activity. (C; F; I) Lipid peroxidation levels. Different letters indicate a statistically significant difference ($p < 0.05$) between the experimental groups. Values expressed as mean ± standard error (A-E; G-I) and median ± first quartile (F). Note the difference in the graph scales.

Table S1. Biomarkers in gills, liver and muscle of *Hyphessobrycon heterorhabdus* after Aluminum exposure.

Biomarker/ Group		Ctrl pH 7	Ctrl pH 5	0.5 mg/L	1 mg/L	10 mg/L
Gills	**ACAP**	9.21 ± 3.11^{a}	10.38 ± 4.80^{a}	7.58 ± 4.32^{a}	5.95 ± 1.25^{a}	11.23 ± 5.04^{a}
	GST	18.87 ± 2.95^{a}	18.70 ± 1.42^{a}	19.66 ± 1.39^{a}	18.99 ± 1.76^{a}	19.51 ± 1.55^{a}
	LPO	139. 93 ± 4.17^{a}	142.50 ± 3.38^{a}	138.79 ± 3.12^{a}	114.79 ± 6.00^{b}	142.87 ± 3.77^{a}
Liver	**ACAP**	3.58 ± 0.98^{a}	3.07 ± 0.74^{a}	3.21 ± 0.13^{a}	1.93 ± 0.13^{a}	2.28 ± 0.32^{a}
	GST	31.27 ± 7.81^{a}	25.34 ± 2.76^{a}	24.10 ± 5.95^{a}	36.34 ± 11.45^{a}	30.32 ± 3.59^{a}
	LPO	87.52 ± 54.87^{a}	106.14 ± 69.09^{a}	145.32 ± 137.84^{a}	145.75 ± 137.84^{a}	147.75 ± 144.47^{a}
Muscle	**ACAP**	9.12 ± 3.15^{a}	8.11 ± 2.93^{a}	4.57 ± 0.69^{a}	5.76 ± 1.74^{a}	3.64 ± 0.62^{a}
	GST	9.30 ± 0.27^{a}	12.36 ± 0.85^{a}	12.54 ± 1.85^{a}	13.43 ± 0.39^{a}	14.49 ± 1.85^{a}
	LPO	18.21 ± 3.24^{a}	22.73 ± 4.52^{a}	16.92 ± 2.60^{a}	42.28 ± 5.13^{b}	26.33 ± 3.84^{ab}

Note: Values expressed in mean ± standard error, except for the LPO in liver, which were expressed in median ± first quartile. Different letters indicate statistical difference ($p<0.05$) between the groups.

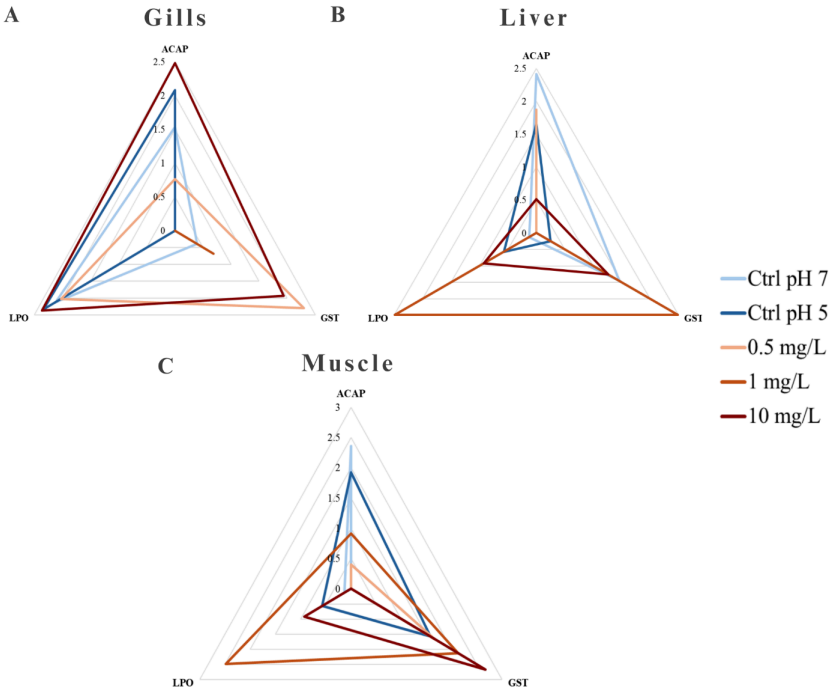

Figure 2. Radar plots of Integrated Biomarker Response (IBR) index in gills (A), liver (B) and muscle (C) of *Hyphessobrycon heterorhabdus* after acute exposure to Aluminum.

Table S2. Quantitative values of the integrated biomarker response index (IBR) in *Hyphessobrycon heterorhabdus* tissues after aluminum exposure.

IBR	Ctrl pH 7	Ctrl pH 5	0.5 mg/L	1 mg/L	10 mg/L
Gills	2.04	2.1	3.44	0*	6.61
Liver	1.52	0.75	1.74	2.15	1.96
Muscle	0.13	2.18	0.29	4.12	1.07

Note: * The zero result obtained indicates that a single biomarker contributed in radarplot, thus making it impossible to calculate the IBR.

In muscle tissue, the IBR displays a distinct pattern (Figure 2C). Control groups exhibit low levels of biomarker activation, whereas groups exposed to Al, particularly at 1 mg/L, demonstrate a significant contribution from LPO. This indicates that muscle tissue is highly susceptible to oxidative stress (Cantanhêde *et al*., 2023). Additionally, at a concentration of 10 mg/L, there is an increased involvement of GST, suggesting a cellular attempt to mitigate lipid peroxidation by removing hydroperoxides. However, this effort does not completely prevent damage.

CONCLUSION

The biochemical responses of *H. heterorhabdus* suggest that this species may be more tolerant and better adapted to the environmental concentrations of Al in the Amazon, which were 0.5 mg/L in our study, and show tissue-specific responses in the antioxidant defense system of fish exposed to Al at low pH. No changes in oxidative damage were observed in the group exposed to 0.5 mg/L, with controlled and discrete responses in antioxidant defenses. While the liver is robust in maintaining homeostasis, the gills may have strategies to prevent the uptake of Al, but are affected at the highest concentration, suggesting a hormetic effect of the metal. The muscle, on the other hand, showed signs of oxidative stress induced by exposure to 1 mg/L. This underlines the importance of analyzing biomarkers in different tissues to obtain a clearer assessment of the antioxidant apparatus in organisms exposed to Al. Finally, we emphasize the use of local species as biomonitor organisms to obtain results that show the local realism of toxic effects at eventual contaminations of these environments.

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DECLARAÇÃO DE CRÉDITO DO AUTOR

YJCN: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **DSM:** Methodology, Writing – original draft. **AJS:** Formal analysis, Methodology. **HMFN:** Formal analysis, Methodology. **EM:** Formal analysis, Methodology **LMC:** Resources, Methodology. **ISCC:** Formal analysis, Methodology. **LLA:** Conceptualization, Resources, Methodology, Supervision, Writing – review and editing, Project coordination.

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