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Salminus hilarii, as a biological indicator in the Brazilian Cerrado

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Abstract

This study was conducted in dos Bois River, Araguaia-Tocantins basin, investigating the connections between physical and chemical conditions, fish diversity and well-being of *Salminus hilarii* in the Cerrado of northwestern Goiás. The research covered five sampling points, from upstream, before the waste from a mining company, to downstream, where it meets a tributary, in two stages: dry and rainy seasons. Dissolved oxygen, electrical conductivity, water and air temperatures, pH, BOD, COD, nitrogen series, phosphate series, fecal coliforms, chlorophyll-α and heavy metals were analyzed, according to Conama Resolution 357. The results show a relationship between the parameters analyzed and the effects on the organisms studied, with distinct data between upstream and downstream, linked to the proximity of agricultural and industrial areas. *S. hilarii*, through its morphological changes, evidences the intense exploitation of the Cerrado. The results suggest the need for priority actions to preserve biological diversity in the region.

Keywords: histology, hematology, freshwater fish, amazon basin, bioindicators.

Resumo - *Salminus hilarii*, como indicador biológico no Cerrado brasileiro

Este estudo foi realizado no Rio dos Bois, bacia Araguaia-Tocantins, investigando as conexões entre condições físico-químicas, diversidade piscícola e bem-estar de *Salminus hilarii* no Cerrado do Noroeste goiano. A pesquisa abrangeu cinco pontos amostrais, desde a montante, antes dos despejos de uma mineradora, até a jusante, onde há o encontro com um tributário, em duas etapas: estação seca e chuvosa. Foi analisado oxigênio dissolvido, condutividade elétrica, temperaturas da água e do ar, pH, DBO, DQO, série nitrogenada, série fosfatada, coliformes fecais, clorofila-α e metais pesados, conforme a Resolução Conama 357. Os resultados mostram uma relação entre os parâmetros analisados e os efeitos sobre os organismos estudados, com dados distintos entre montante e jusante, ligados às proximidades com áreas agrícolas e industriais. *S. hilarii*, através de suas alterações morfológicas, evidencia a exploração intensa do Cerrado. Os resultados sugerem a necessidade de ações prioritárias para preservar a diversidade biológica na região.

Palavras-chave: histologia, hematologia, peixes de água doce, bacia amazônica, bioindicadores.

Resumen - *Salminus hilarii*, como indicador biológico en el Cerrado brasileño

Este estudio se realizó en el río dos Bois, cuenca Araguaia-Tocantins, investigando las conexiones entre las condiciones físicas y químicas, la diversidad de peces y el bienestar de *Salminus hilarii* en el Cerrado del noroeste de Goiás. La investigación abarcó cinco puntos de muestreo, desde aguas arriba, antes de los desechos de una empresa minera, hasta aguas abajo, donde se encuentra con un afluente, en dos etapas: estación seca y lluviosa. Se analizaron oxígeno disuelto, conductividad eléctrica, temperaturas del agua y del aire, pH, DBO, DQO, serie de nitrógeno, serie de fosfato, coliformes fecales, clorofila-α y metales pesados, de acuerdo con la Resolución Conama 357. Los resultados muestran una relación entre los parámetros analizados y los efectos sobre los organismos estudiados, con datos distintos entre aguas arriba y aguas abajo, vinculados a la proximidad de áreas agrícolas e industriales. S. hilarii, a través de sus cambios morfológicos, evidencia la intensa explotación del Cerrado. Los resultados sugieren la necesidad de acciones prioritarias para preservar la diversidad biológica en la región.

Palabras clave: histología, hematología, peces de agua dulce, cuenca amazónica, bioindicadores.

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Introduction

In the Brazilian Central-West region, the existence of an ecotone between ecosystems that border the Amazon biome, with its rich fauna and flora, calls for more in-depth studies (Tisott, Schmidt, 2021). However, human interference, notably livestock farming, mineral exploration, deforestation and sewage dumping, have a strong impact on the aquatic environment, imposing structural and dynamic transformations on the beings there (Saviato *et al.*, 2021a).

The Goiás region, formed mainly by the Cerrado biome, with its vast hydrographic basins, has the intersection of other phytophysiognomic configurations, such as the ecotone with the Amazon biome (Gomes *et al.*, 2021). However, the Cerrado is a collection of unique ecosystems, which faces the imminent threat of degradation and loss of character, imposed by the hands of agriculture, livestock and industry (Rost *et al.*, 2021).

In this way, such hydrographic basins, which flow from the depths of this biome, experience the impact of these anthropic actions, expressed as the reduction of biological diversity, environmental changes and the possible impoverishment of fauna and flora (Barbosa *et al.*, 2021). And thus, urbanization, with its most impactful repercussions, evictions and disorderly occupation, emerges as a disruptive figure, modifying the compositions of species and their synergistic harmonies with the environment (Saviato *et al.*, 2021b).

However, there are still other threats to these freshwater environments of the Cerrado, with the possibility of environmental contamination and trophic magnification, caused by pollutants from agro-industries, urbanization and industries in general (Ulrich *et al.*, 2021). Thus, it is necessary to identify the synergies between environmental parameters and the degradation of these environments, as well as their impact on organisms and their well-being (Saviato *et al.*, 2023).

Where ecosystem services, essential for the dynamic balance of the ecosystem, are significantly transformed by mineral extraction and its environmental mutations. Since the Cerrado and its river basins are important places for freshwater fish fauna and their replenishment in fishing stocks in the North region, these changes have an imperative impact on the entire Amazon region (Lima *et al.*, 2021). The Araguaia-Tocantins basin, due to its unparalleled diversity, is under imminent threat, caused by agricultural activities, urban expansion, deforestation and the installation of hydroelectric dams (Saviato *et al.*, 2020). The exploitation of the Cerrado biome, with its decharacterization, reveals the causes and consequences of the loss of regional biodiversity (Saviato *et al.*, 2022a). The impacts triggered by human tricks are transformed into physical and chemical changes in the rivers, redesigning the physiognomy of these areas, affecting the ecosystem (Soares *et al.*, 2020). The residues of these activities carry contaminants and heavy metals, reverberating in the biota, especially in fish, resulting in physiological, morphological and histopathological changes, sometimes deleterious (Moron *et al.*, 2019).

Such environmental metamorphoses cast a veil of uncertainty over the quality of life and well-being of aquatic organisms, even transmuting and modulating regional microclimates. These transformations, due to the influence of human activities in aquatic and terrestrial ecosystems, are the cause of many losses in diversity (Saviato *et al.*, 2022b).

Thus, *Salminus hilarii* Valenciennes 1850, the tabarana, a fish found in Brazilian river basins, is well distributed in the Araguaia-Tocantins basin (Machado *et al.*, 2016), despite human interference. As a predator, its geographic distribution encompasses several river basins, exposing it to the impacts generated in its trophic group and beyond (Lorenz *et al.*, 2022). This species, studied mainly for its reproductive characteristics, remains little known regarding the histopathological and physiological effects under the influence of residues loaded with metals and other pollutants (Viana *et al.*, 2021). Therefore, the effectiveness of studies on hematological patterns and in deciphering the pathological conditions of these fish, at the mercy of environmental and biological influences, promises to reveal the true picture of impacts and their resonances in the delicate balance of the aquatic environment (Saviato *et al.*, 2023).

Materials and Methods

The research was conducted in the northeast of Goiás, in the town of Alto Horizonte. The climate in this region is identical to that of the state. The hydrography of the region, in turn, is delineated by the Dos Bois River, a sub-basin of the Crixas-Açu river, whose waters converge, as a tributary, to the Araguaia River (UTM 22M: pont 1 - 680205.00 m E, 8422958.00 m S; point 2 - 673539.00 m E, 8423221.00 m S; point 3 - 669530.00 m E, 8423224.00 m S; point 4 -661869.00 m E, 8426228.00 m S; e point 5 - 656655.00 m E, 8434760.00 m S).

The collection sites were distributed across two experimental zones: Control Area (points 1 and 2, upstream) and Interference Area (points 3, 4 and 5, downstream). Collections took place in July and November 2021, using a cast net, gill net, kick net, sieve and, in some stretches, a trawl net. The target species was chosen because it is a predator that feeds on smaller fish and does not migrate during the juvenile phase.

Specimens of *S. hilarii* were collected (SisBio/ICMBio Authorization, No. 75.706-2) in two campaigns in the dry and rainy seasons at 5 points along the dos Bois River. To minimize variations, animals of similar sizes were preferred. After collections, blood and tissue analyses were performed, in addition to water samples to correlate physical-chemical data with fish welfare.

The physical and chemical parameters of the water were measured at the same points and dates as the fish collections. Samples were stored at 4ºC and taken to the laboratory for analysis of BOD, nitrogen series, phosphate series, total solids, fecal coliforms and chlorophyll-α, using standard methods. Five individuals per sampling point and station were collected, totaling 50 blood samples. Blood was analyzed for cell count, hematocrit, hemoglobin and hematimetric indices. Blood samples were used to evaluate genotoxic alterations and erythrocyte nuclear anomalies. The methodologies were approved by the Ethics Committee on the Use of Animals of the Federal University of Amapá (Ceua/Unifap, protocol 15/2022). Fish were euthanized for extraction of gills and liver, with samples fixed in Bouin solution and prepared for histological analysis. Data were analyzed statistically, with significant differences considered at the 5% level. Analyses were performed using Anova and Tukey or Kruskal-Wallis tests followed by Dunn, according to the data distribution.

Results and Discussion

Water quality in the dos Bois River

The results on the physical-chemical parameters of the water, collected at five different sampling points, from higher areas to lower regions, during the dry and rainy seasons, show significant variations (Saviato *et al.*, 2022a).

According to Saviato *et al.* (2022a), there are indications of changes in the water quality in the dos Bois River, a tributary of the Araguaia-Tocantins basin, influenced by agricultural practices. Metals such as aluminum, copper, lead and iron showed variations outside the expected standard, mainly downstream of the mining effluent discharge point, harming environmental health (Poersch, Sebastien, 2021). The increase in temperature in the river affects the concentration of dissolved oxygen, while the absence of riparian vegetation and the release of effluents contribute to changes in aquatic parameters (Guarda *et al.*, 2020a).

River self-purification, essential for nitrogen cycling, is affected by agricultural and livestock activities, resulting in an increase in nitrogen compounds. Phosphate compounds and Chlorophyll α decrease along the course, as depicted by Serbeto *et al.* (2021). Water quality is only suitable for recreation and secondary uses. The study highlights the critical reality of Cerrado rivers (Saviato *et al.*, 2022a). The water quality of the dos Bois River presents discrepancies with legal regulations (Guarda *et al.*, 2020b), with high values of metals such as aluminum, copper, lead, iron and zinc, in non-compliance with Conama Resolution 357, especially downstream of point 2, at points 3 to 5 (Melo, Queiroz, 2021). Figure 4 shows the oscillation of these metals, which remained high during both climatic seasons (dry and rainy) (Saviato *et al.*, 2022a).

The local ichthyocenosis

The results described by Saviato *et al.* (2022b) reveal a rich diversity of fish in the Dos Bois River, with 46 species distributed in 7 orders and 19 families. The order Characiformes stands out with 26 species. Although none are on the lists of endangered species, the presence of migratory species such as *Salminus hilarii* and Prochilodus nigricans highlights the need to conserve this habitat.

S. hilarii stands out for being present in all areas along the river and for being important in the Amazonian aquatic ecosystem. Characiformes represent 92.24% of the fish collected (Agostinho *et al.*, 2018). These organisms have feeding habits divided into seven distinct groups, offering different ecosystem services (Zavala-Camin, 1996). Omnivores and invertivores are predominant, linked to the abundance of regional resources (Corrêa, Smith, 2019).

In the rainy season, other guilds emerge, such as detritivores and piscivores, in response to the increase in migratory species and their fry. The diversity, abundance and distribution indices of the ichthyofauna in the Dos Bois River show a considerable sampling amplitude, even with anthropic pressures (Souza *et al.*, 2020).

Despite the diversity found, it is in line with regional expectations, indicating that this group of fish corresponds to the ichthyocenosis outlined for the Araguaia. The constant presence of *S. hilarii* along the river suggests that this species may reflect the different pressures at the sampling points (Saviato *et al.*, 2020, 2022b).

Histological and hematological parameters in *Salminus hilarii*

From specimens of the species *S. hilarii* (n = 50 individuals), 20 from the control area and 30 from the area affected by the gold mining discharge in the Dos Bois River region, Goiás (Saviato *et al.*, 2022c). Thus, the results identified histopathological alterations in this organism, which indicates negative interference in the tissue formation of the specimens collected in the sites further downstream of the discharge. These presented histological anomalies in the liver and gills of these animals (Saviato *et al.*, 2022b).

Where each specimen was analyzed, from body size to blood cell morphology, in the same way recommended by some authors such as Tavares-Dias *et al.* (2021). Some of these specimens exhibited no morphological alterations or complete impairment of the organism's homeostasis. Thus, histological analyses of the liver showed a lower degree of alterations in the rainy season (Sultana *et al.*, 2019), while samples from the dry season were more aggravated (Kapepa, 2020; Latif *et al.*, 2022a).

Thus, such inferences revealed differences between the climatic seasons and an established gradient from upstream to downstream points (Barbieri *et al.*, 2020), where an increasing appearance of histological alterations was identified along the studied section in accordance with the existing physicochemical changes (Mariano *et al.*, 2021) (Figure 1).

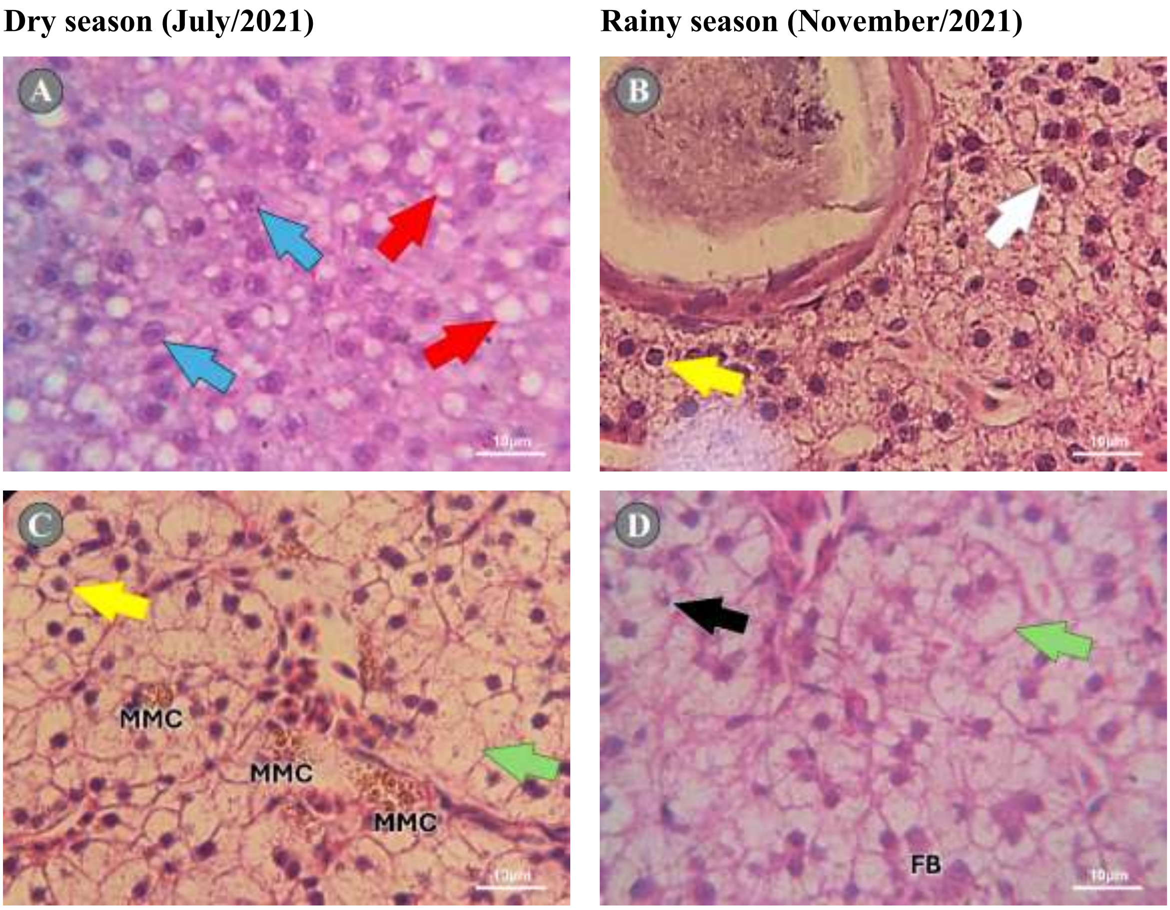

Figure 1. Histopathological changes in the liver of *Salminus hilarii*. Legend: hepatic sinusoids (circular area), peripheral nucleus (white arrows), nuclear vacuolation (yellow arrows), microvesicular steatosis (red arrows), macrovesicular steatosis (green arrows), nuclear hypertrophy (blue arrows), nuclear atrophy (black arrows), fibrosis (FB) and melanomacrophages (MMC). Staining: Hematoxylin and Eosin (Source: Saviato *et al.*, 2022c).

Likewise, it was identified that the presence of metals such as lead and copper were attributed as causes of direct interference in histological formation, with aluminum and zinc mainly promoting tissue degradation (Viana *et al.*, 2021). Likewise, the changes are related to the organism's life history and its exposure to a certain group of parameters (Ballotin, 2019) (Figure 2). From analyses of the correlation between metal concentrations and the severity of gill lesions, it was possible to identify correspondence for the lesions visualized (Savassi *et al.*, 2020), with the highest coefficients obtained for PEF (Latif *et al.*, 2022a; Almeida *et al.*, 2022).

Dry season (July/2021) **Rainy season (November/2021)**

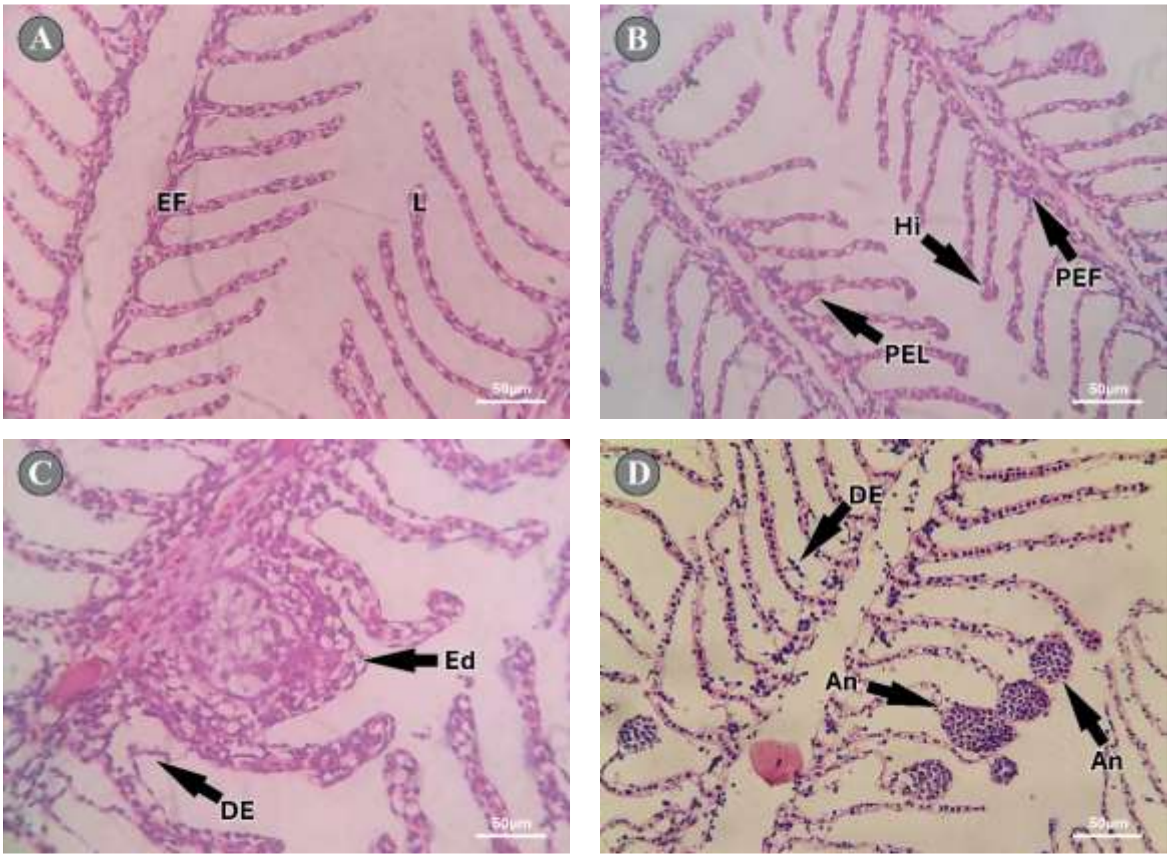

Figure 2. Microphotographs of histopathological changes occurring in *Salminus hilarii*, Dos Bois River - GO. Legend: Filamentary Epithelium (EF), Lamellar Epithelium (L), as well as tissues with hypertrophy (Hi), epithelial desquamation (DE), lamellar fusion (FL), edema (Ed), aneurysms (An), proliferation of filamentary epithelium (PEF), proliferation of the lamellar epithelium (PEL) and necrosis (Ne). Staining: Hematoxylin and Eosin (Source: Saviato *et al.*, 2022c).

Similarly, to the histological analyses of the liver, the organisms evaluated were segregated in the same sampling sites, and all animals studied here had similar size (age) (see Figure 4). The collection methodology used in this study, according to Tavares-Dias *et al.* (2021), was adopted to mitigate hematological discrepancies that may arise in the different growth phases of *S. hilarii*. Thus, hematological alterations were identified in two distinct areas: the Control Area and the Interfered Area and considering the climatic seasons (Dry and Rainy), according to Saviato *et al.* (2023) (Table 1).

Table 1. Distribution of hematological parameter values and their means for each collection area, and the standard deviation (X±DE), for the two seasons (dry and rainy), compiled by sampling area, Dos Bois River, State of Goiás.

Parameters	Control			Interference		
	Average		X±DE	Average		X±DE
Leukocyte - WBC ($10^3/\mu L$)	135,15	±	7,503	154,13	±	7,719
Red Blood Cell - RBC (106/µL)	4,25	±	0,690	2,83	±	0,613
Hemoglobin - Hb (g/dL)	15,91	±	4,652	9,11	±	0,728
Hematocrit - Ht (%)	21,02	±	4,540	67,61	±	29,346
Mean Corpuscular Volume - MCV (fL)	108,35	±	7,229	152,01	±	39,037
Mean Corpuscular Hemoglobin - MCH (pg)	42,95	±	6,169	43,08	±	5,121
Hemoglobin Concentration - MCHC (g/dL)	25,48	±	0,755	45,41	±	2,559
Red Blood Cell Distribution Width - ADVGCV (%)	18,97	±	1,497	20,40	±	1,000
Red Blood Cell Distribution Width - ADVGSD (fL)	133,45	±	8,590	162,21	±	15,479
Platelets - PLT ($10^3/\mu L$)	134,40	±	24,026	136,67	±	67,217
Mean Thrombocyte Volume - MPV	2,23	±	0,412	11,15	±	3,703
Platelet Distribution Width - ADP	9,82	±	2,621	8,76	±	0,525
Platelet Crit - PCT (%)	0,23	±	0,023	0,19	±	0,021

Source: Saviato *et al.* (2023).

Based on the hematological results, it was identified that, in the Interfered Area during the Dry Season, red blood cells increase in number, reaching important peaks for hematocrit, in contrast to the Control Area, as observed by Sabioni *et al.* (2022). Similarly, when observing blood cells, changes were observed in the Interfered Area, where immature erythrocytes proliferate and cells with micronucleation appear, indicative of genotoxicity at the site, as pointed out by Nascimento *et al.* (2020).

Reports on hematological parameters in fish of the genus *Salminus* show an intrinsic connection between fluctuations in environmental quality, according to Marengoni *et al.* (2019). However, this study focuses on hematological variations in a natural habitat with *S. hilarii*, comparing distinctly interfered areas and climatic seasons, following Ulrich *et al.* (2021).

Recent studies, such as those by Martelli *et al.* (2021), reveal that the genotoxic effects of pollutants are reflected in the immune response of fish, varying according to the nature of the pollutants, according to Braga, Matushima (2021). In *Oreochromis niloticus*, biopesticides cause histopathological deformations, such as micronuclei in red blood cells, reported by Rodrigues *et al.* (2018). These erythrocytic deformities are biological markers of exposure to pollutants, according to Oliveira *et al.* (2022).

Micronucleation, indicated by Pinheiro, Mercado (2022), although rare, suggests that the studied water body receives pollutant loads that disturb environmental stability, affecting fish health, according to Machado *et al.* (2021), as illustrated in Figure 3.

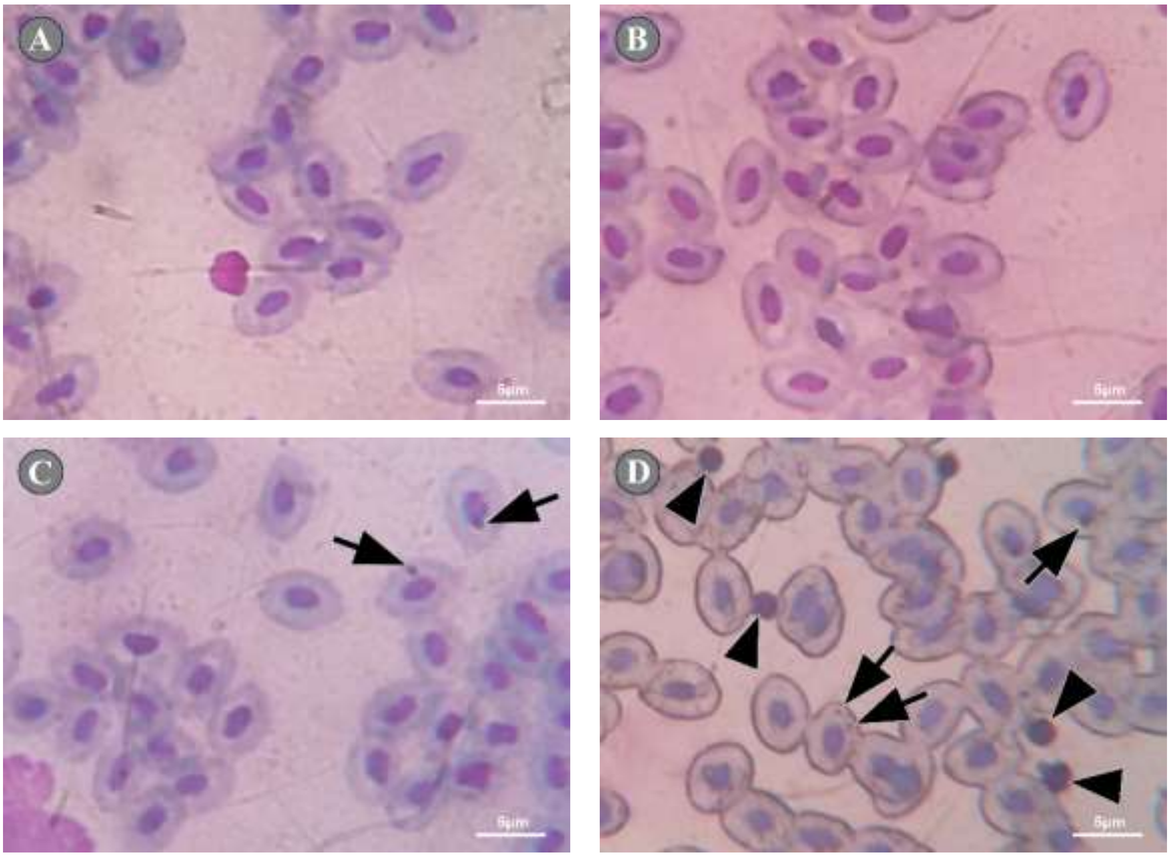

Figure 3. Changes in *Salminus hilarii* erythrocytes in the gradient of the collection sections, Control Area (A and C), showing little or no visible changes, and Interference Area (B and D) fish exposed to water with waste leachate intervention from mineral exploration. Legend: Immature erythrocyte (Ei) (arrowhead), Micronucleus (black arrow). Coloring: Giemsa and May Grünwald (Source: Saviato *et al.*, 2023).

These results vary depending on the region and the fish species studied, according to Lorenz *et al.* (2022). In the Amazon region, for the species *Plagioscion squamosissimus*, the number of red blood cells with micronucleation reached 28% ± 14.42, under environmental pressures that triggered genotoxic effects, as recorded by Porto *et al.* (2021). Furthermore, these results are similar in other geographic areas or for other fish species studied (Saviato *et al.*, 2021b). Although genotoxic changes can be reversible if the pollutant source is removed (Simão *et al.*, 2021), continuous exposure to these contaminants can produce more severe and chronic genotoxic effects (Oliveira *et al.*, 2022).

It is worth noting that, although the replacement of blood cells is accelerated compared to other tissues in the body (Mariano *et al.*, 2019), constant exposure to pollutants can lead to harm to the health and well-being of these aquatic organisms (Braga *et al.*, 2021), as shown in

Table 2. Distribution of mean values for the count of immature erythrocytes (Ei) and micronucleus (Mn), recorded in *Salminus hilarii* samples per sampling point.
Source: Saviato *et al.* (2023).

Sample points	(Ei)			(Mn)		
	Average		X±DE	Average		X±DE
P1	1,05	±	0,88	0,13	±	0,09
P2	1,05	±	1,70	1,39	±	0,05
P3	1,91	±	1,70	17,32	±	8,01
P4	1,91	±	0,88	6,80	±	3,88
P5	0,20	±	0,02	0,73	±	0,40

Conclusion

Data analysis revealed a correlation between changes in water quality and the health of *Salminus hilarii*, highlighting liver and gill lesions associated with aluminum, copper, lead and zinc. The gills showed epithelial desquamation and hypertrophy, related to aluminum and zinc, while the liver showed peripheral nucleus, steatosis and fibrosis, linked to copper and lead. Gill lesions compromise gas exchange and osmotic balance, while liver lesions affect homeostasis. As a predator, *S. hilarii* accumulates metals in its tissues due to trophic magnification, serving as a bioindicator. Water contamination by metals negatively impacts aquatic organisms and humans and is especially worrying after effluent discharge points. Histological analysis of gills and liver revealed significant damage, such as edema, aneurysms and necrosis, associated with metals. Similarly, hematological analyses indicated significant changes from upstream to downstream, suggesting the sensitivity of *S. hilarii* to pollution. This indicates that less invasive assessments, such as hematological analysis, can indeed reflect the organism's immune status in the face of environmental injuries. This provides a less costly alternative both for the organism that can be returned to the river and for the cost of the analyses and the speed of responses, which can be performed with complete blood counts and in less time than water analyses, which are more costly and time-consuming. This demonstrates that the species in question can be a good bioindicator and that the assessment of its physiological conditions is a very useful and accessible tool for environmental assessment. Furthermore, from an ecological perspective, it is recommended that regulatory measures be implemented for effluent discharge and continuous monitoring of water quality and the health of the organisms. These results can guide more responsible practices in mineral exploration.

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