HEMATOLOGY OF *Salminus hilarii* AND CONSIDERATIONS ON THE POLLUTION OF THE CERRADO RIVERS

Hematologia de *Salminus hilarii* e considerações sobre a poluição dos rios do Cerrado

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ABSTRACT

In this study, the health and well-being of fish species *Salminus hilarii*, in a stretch of a headwater river in the Araguaia River basin - Tocantins, Goiás State, were evaluated. Where the objective was to identify and measure the hematological parameters (erythrogram, thrombogram and leukogram of the referred species). The specimens (n = 50) were captured using fishing nets and cast nets. Blood samples were collected by caudal puncture. The hematological data were obtained according to the methodology described in the classic bibliography. Based on the results of the hematological analysis, the relationship between them and their collection area was obtained, as well as the analyzes by microscopy in the search for morphological (genotoxic) alterations, in the micronuclei. Changes or variations in these parameters can be used as indicators of environmental contamination since these animals are inserted in the environment. Thus, the immune responses presented by the analyzed specimens where the difference between the results of these parameters, as well as the presence of genotoxic markers, are indicative of changes in the studied river water quality.

Palavras-chave: Brazil, animal welfare, hematology, neotropical fish.

RESUMO

Neste estudo foram avaliadas as condições de saúde e bem-estar de peixes da espécie *Salminus hilarii*, em um trecho de um rio de cabeceira na bacia do rio Araguaia - Tocantins, Estado de Goiás. Onde se objetivou identificar e mensurar os parâmetros hematológicos (eritrograma, trombograma e leucograma da referida espécie. Os exemplares (n = 50) foram capturados através do uso de redes de pesca e tarrafas. As coletas das amostras de sangue foram efetuadas por punção caudal. Os dados hematológicos foram obtidos de acordo com metodologia descrita na bibliografia clássica. A partir dos resultados das análises hematológicas obtiveram-se a relação destes com sua área de coleta, assim como foram efetuadas as análises por microscopia na busca por alterações morfológicas (genotóxicas), os micronúcleos. Alterações ou variações nestes parâmetros, podem ser utilizadas como indicadores de contaminação ambiental, uma vez que estes animais estão inseridos ao meio. Assim, as respostas imunes apresentadas pelos exemplares analisados onde a diferença entre os resultados destes parâmetros, assim como a presença de marcadores genotóxicos, são indicativos de alterações na qualidade da água.

Keywords: Brasil, bem-estar animal, hematologia, peixes neotropicais

INTRODUCTION

The species *Salminus hilarii* Valenciennes, 1850, popularly known as tabarana, is a widely distributed fish, being recorded in the Amazon basin and other river basins in Brazil, such as: Paraná-Paraguay and São Francisco. Species morphologically and genetically close to *S. affinis* and *S. franciscanus*, being sympatric to the latter, in the São Francisco River basin. Recently, the genus *Salminus* Agassiz, 1829, was investigated by genetic and biogeographic studies, reaffirming the distribution of *S. hilarii* in several basins that drain the Brazilian Midwest, flowing to the South, North and Northeast (Machado et al., 2016, Ota et al., 2018).

This species is medium-sized, and in the food chain it is considered a predator that feeds on smaller species of fish. Its strategy is to remain on the lookout for its prey at the mouth of small bodies of water or runoff from marginal lakes (Andrade et al., 2004, Honji et al., 2019). It is a diurnal fish, attacking schools and small fish on the surface of its foraging area.

It is a widely studied species, mainly on its reproductive and physiological characteristics, for purposes in aquaculture, as well as in the conservation of the species (Honji et al., 2019). Of this, studies on its reproductive biology in the Paranapanema region (Honji et al., 2013, Moreira et al., 2015), feeding (Villares Júnior & Goiten 2016), parasitic ecology (Duarte et al., 2016), parasitic ecology (Duarte et al., 2016), stand out. as well as its systematics and aspects of taxonomy, more recently were investigated (Machado et al., 2016).

However, the knowledge about the histological and physiological reflexes of the interference of residues containing metals in *S. hilarii* is incipient. Because it is a predatory species, its eating habits directly reflect on the accumulation of metals. Therefore, changes in its physiology and histopathology (Gomes et al., 2021).

The study of the histology of organs, as well as fish musculature, may reflect the interference of environmental factors, punctual or of long duration, where such histological changes have the possibility of staying longer in the organism, depending on their severity, in addition to the permanence of the sources causing environmental changes (Alves et al., 2021, Koch et al., 2018, Lorenz et al., 2018, Lorenz et al., 2022). Indicating that it is not possible, in a natural environment, to infer when or for how long this organism was exposed to negative interference, due to the large number of environmental variables (Viana et al., 2021).

However, analyzes in blood tissue, as it has a higher rate of cell replacement than other tissues in the body, allow the deduction of the intensity of this exposure, as well as whether it has occurred recently (Moniz et al., 2019, Lizama et al., 2020, Maggi et al., 2019, Pereira et al., 2018, Rodrigues et al., 2019).

Thus, studies of hematological patterns are effective for the determination of pathological conditions in fish. However, the hematic composition also makes it possible to infer about the conditions of the environment where these organisms are inserted, allowing the verification of environmental conditions, such as: temperature, salinity, oxygen, among others, as they interfere in the physiological conditions of the hematological parameters (Del-Guercio et al., 2017, Lizama et al., 2020, Stozik et al., 2020, Tavares-Dias et al., 2018).

However, it is noteworthy that other factors also directly influence the conditions of hematological parameters, such as: malnutrition, age, size of this organism and seasonality, which can promote changes in the results of blood tests of these animals (Cicero et al., 2020, Tavares-Dias et al., 2018).

S. hilarii even being a relatively fast-growing and prolific fish like most representatives of the Bryconidae family and having migratory behavior during the reproductive period. Even so, it does not show this behavior during the first years of its growth, before sexual maturity (Moreira et al., 2015), having gregarious behavior, forming from small groups at this stage of its development, preferring lotic and dystrophic environments, with waters oxygenated near the mouth of small tributaries, or at the outlets of lakes and backwaters, not promoting large displacements (Bernardes et al., 2021, Portella, et al., 2021, Rosso et al., 2017), remaining in a restricted home range , being more exposed to local interference.

The analysis of the variation of hematological parameters can be efficient tools in the measurement and identification of specific environmental changes with quick reflexes of environmental stress conditions (Nogueira et al., 2020). However, these contributions to the inferences about environmental contamination represent good effectiveness for short-term impacts (Nascimento et al., 2020).

Thus, the present study aimed to evaluate environmental health, based on the presence of biomarkers resulting from genotoxicity in *S. hilarii* hematic cells in a stretch of river, in the headwaters of the Araguaia-Tocantins, under the influence of anthropic exploitation for agriculture, livestock and mineral deposits.

METHODOLOGY

DESCRIPTION OF THE STUDY AREA

The study was carried out in a stretch of river, headwaters region of the Araguaia-Tocantins basin, in the state of Goiás, Brazil. Being in the northeast part of the state, with floristic characteristics belonging to the Brazilian Cerrado ecosystem (Ratter et al., 1978), in this area five sampling points were pre-selected where the collection of *S. hilarii* specimens used for the record of data and analysis (Table 1 and Figure 1).

Table 1. Geographic UTM coordinates of collection points within the watershed explored for this study.

Collection points	Longitude 22M	Latitude
P1	680205.00 m E	8422958.00 m S
P2	673539.00 m E	8423221.00 m S
P3	669530.00 m E	8423224.00 m S
P4	661869.00 m E	8426228.00 m S
P5	656655.00 m E	8434760.00 m S

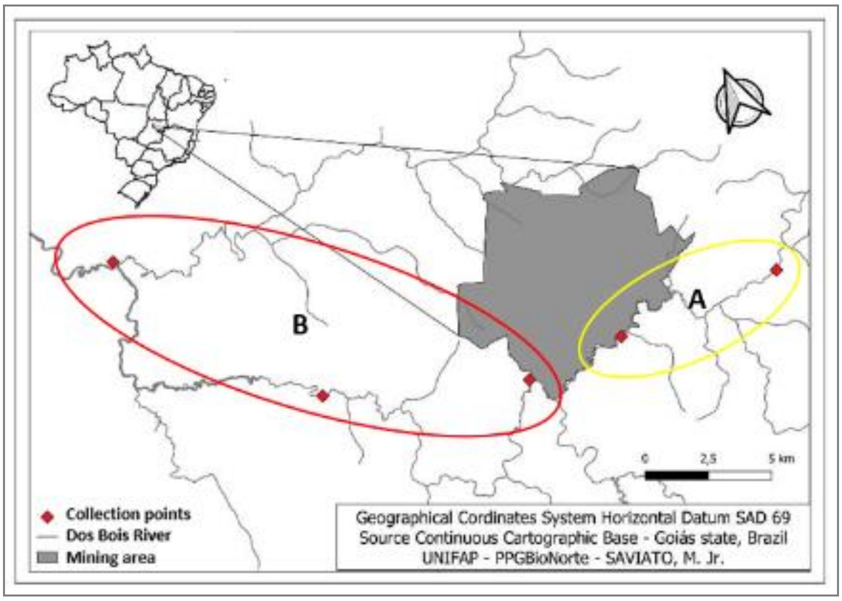

Figure 1. Location of the study area, located in the studied stretch, northeast of GO, being subdivided into 2 sample areas: A) Control Area, composed of points P1 and P2 - highlighted in yellow and B) Interfered Area, composed of points P3, P4 and P5 - in red.

On the banks of the studied river, there is still the presence of riparian forest (Figure 2). However, this area has been used mainly for agriculture, livestock and mineral exploration (Figure 3) (Ulrich et al., 2021), where the main channel of the studied river is a body that receives discharges from mineral exploration (Figure 4).

SAMPLE COLLECTION

The captures of *S. hilarii* specimens (Figure 5) for the extraction of samples were carried out along a stretch of river from the headwaters, which make up the Araguaia-Tocantins basin (see Table 1 and Figure 1), located in the northwest of the state of Goiás, Brazil. At each point, 10 specimens were captured, 5 in the rainy season (November 2021) and 5 in the dry season (July 2021). The samples were subdivided into Control Area (P1 and P2), and Interfered Area (P3, P4 and P5).

Figure 2. Headwaters of the studied river (P1), with the presence of riparian forests on the banks, being the most upstream of the Control Area (Personal archive).

Figure 3. Examples of activities carried out along the studied area. 3a) mechanized agriculture and 3b) mining of gold and copper (Personal archive).

Figure 4. Mineral exploration area from where the leachate flows at Point 03, the furthest upstream to the Interference Sample Area (Personal archive).

These fish are collected using fishing methodologies, such as: cast net (2m in Ø and 5mm mesh); and hammocks (1.5 x 10m and meshes of 1.0, 3.0 and 6.0cm), more effective methodologies for the location (Figure 6). The animals of interest for this study, which were collected, then underwent the process of blood extraction by puncture of the caudal blood vessels and were later anesthetized and euthanized with Eugenol solution (50mg L-1) and spinal cord section.

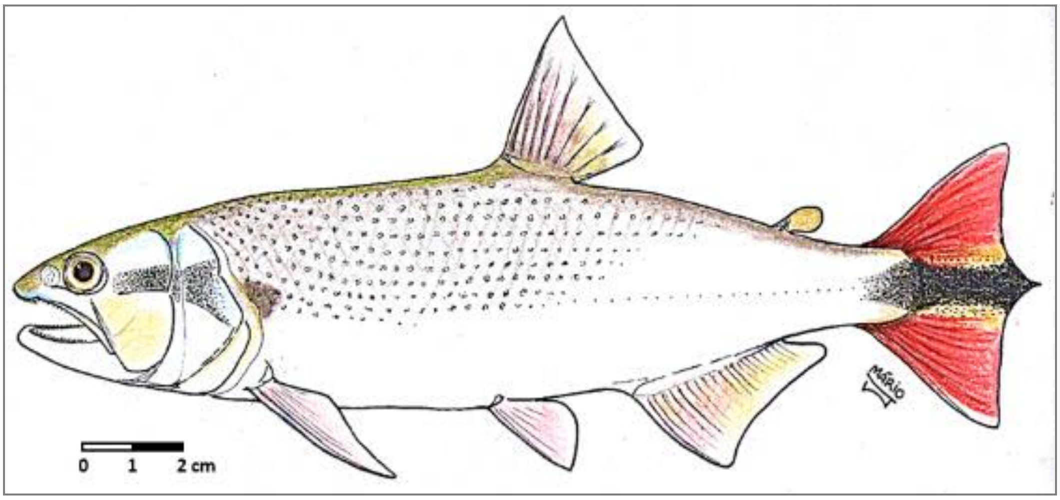

Figure 5. *Salminus hilarii* Valenciennes, 1850, based on juveniles of the species collected in the studied stretch.

Figure 6. Waiting net methodology used to collect specimens of *Salminus hilarii* used in this study, being installed perpendicularly to the river flow (Personal archive).

BLOOD COLLECTION

The specimens of *S. hilarii* (tabarana), immediately after capture, had blood collected by puncture of the caudal vessels using a 1mL syringe, containing 0.05ml of 10% EDTA as anticoagulant (Figure 7). Subsequently, hematological analyzes and blood smear slides were performed.

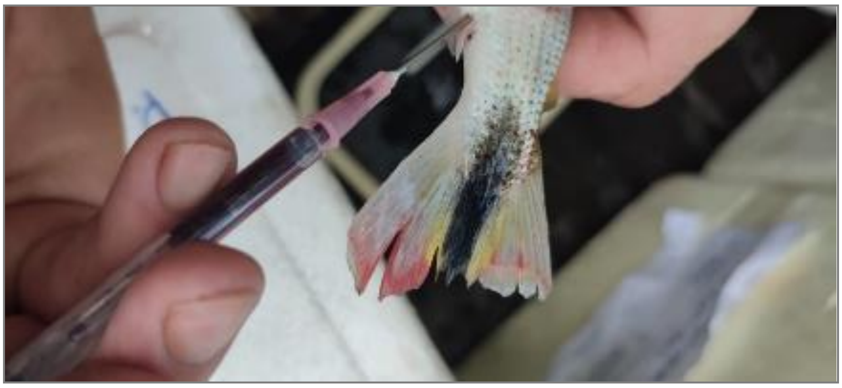

Figure 7. Blood extraction by caudal puncture in a specimen of *Salminus hilarii* collected in the studied area (Personal archive).

BLOOD ANALYSIS METHODS

Hematological analyzes of blood collected by caudal puncture were performed at the Osvaldo Cruz Clinical Analysis Laboratory, Campinorte - GO. During the procedures for hematological analyses, total hemocyte counts were performed using the Neubauer hemocytometer and the hemoglobin concentration.
Absolute RBC rates of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were also obtained. Blood smear slides were stained with Giemsa and May Grünwald (Rosenfeld 1947) (Figure 8).

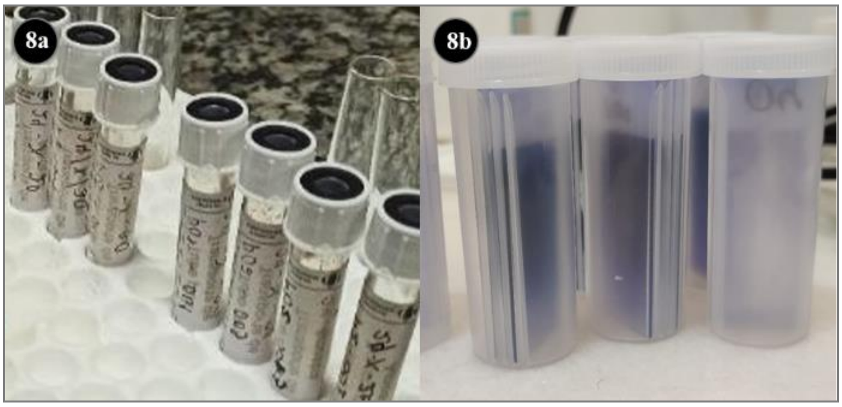

Figure 8. 8a) Blood samples delivered to the laboratory for complete blood counts; 8b) Prepared, dried and stored blood extension slides (Personal archive).

DATA ANALYSIS

Hematological data were submitted to Bartlett's analysis, in order to verify the homogeneity of variance, ANOVA One-way, and the differences between treatments were then analyzed using the Student-Newman-Keuls (SNK) test. Thus, the data on leukocyte, lymphocyte and monocyte counts were also submitted to regression analysis, as well as the coefficients evaluated for significance were evaluated by the t test. All tests were used at a 95% reliability level.

RESULTS

BIOMETRIC PARAMETERS

Fifty specimens of *Salminus hilarii* were collected, all of which were juveniles with similar body size and weight (P = 0.9308) (Body Length - Average = 20.26±1.93 cm; Max = 20.2 cm, Min = 17.5 cm; Weight – Average = 110.2±25.4g, Max = 193.0 g, Min = 57.17 g) (Figure 9). Due to the gillnet collection system, with fixed meshes, they limited the capture of animals of discrepant sizes, consequently of different ages and degrees of maturity. In this way, equalizing the sample differences that could possibly occur within a natural heterogeneous population.

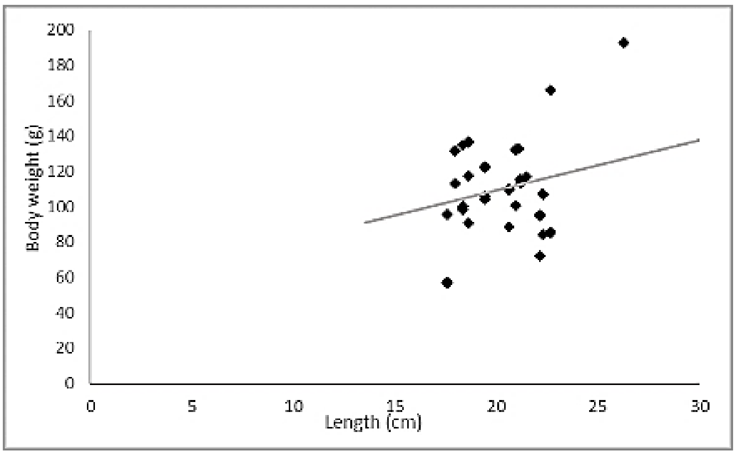

Figure 9. Relationship between body size and their respective lengths for the animals captured for this study.

HEMATOLOGICAL PARAMETERS

Blood analysis in *S. hilarii* showed parameters with a subtle discrepancy between the sampling points, however, this difference was highlighted with the grouping by sample area (n = 10 specimens per point). In the sample grouping, we have 20 animals for the sampling area called Control Area and 30 specimens for the downstream area called Interfered Area, totaling 50 specimens used for this study (Table 2 to Table 5).

Table 2. Distribution of hematological parameter values and their averages for each collection area, and standard deviation (X±DE), for the dry season (July/2021), segregated by sample area (Control and Interference). Caption: Hematological parameters - Leukocytes (GB)> Red blood cells (RB)> Hemoglobin (Hb)> Hematocrit (Ht)> Mean Corpuscular Volume (MCV)> Mean Corpuscular Hemoglobin (MCM)> Hemoglobin Concentration (HCCM)> Range of Distribution of Erythrocytes (ADVGVC) > Standard Deviation of Erythrocyte Distribution Range (ADVGSD) > Platelets (PLT) > Mean Platelet Volume (MPV) > Platelet Distribution Range (ADP) > Platelet Cells (PCT).

Parameters	Control			Interference		
	Average		X±DE	Average		X±DE
GB (10^3/µL)	142.03	±	13.17	162.58	±	12.67
GV (10^6/µL)	5.79	±	1.12	1.35	±	0.19
Hb (g/dL)	21.72	±	6.61	6.16	±	0.48
Ht (%)	10.14	±	1.51	95.25	±	58.09
VCM (fL)	77.59	±	16.97	167.56	±	71.55
HCM (pg)	41.80	±	7.78	44.67	±	6.34
CHCM (g/dL)	17.81	±	1.15	56.96	±	4.79
ADVGCV (%)	20.02	±	2.01	18.13	±	1.51
ADVGSD (fL)	108.71	±	11.93	164.65	±	40.62
PLT (10^3/µL)	108.19	±	24.61	150.19	±	81.35
VPM	2.32	±	0.52	9.39	±	2.90
ADP	10.42	±	2.97	7.99	±	0.68
PCT (%)	0.24	±	0.03	0.17	±	0.02

Table 3. Distribution of hematological parameter values and their averages for each collection area, and the standard deviation (X±DE), for the rainy season (November/2021), segregated by sample area (Control and Interference). Caption: Hematological parameters - Leukocytes (GB)> Red blood cells (RB)> Hemoglobin (Hb)> Hematocrit (Ht)> Mean Corpuscular Volume (MCV)> Mean Corpuscular Hemoglobin (MCM)> Hemoglobin Concentration (HCCM)> Range of Distribution of Erythrocytes (ADVGVC) > Standard Deviation of Erythrocyte Distribution Range (ADVGSD) > Platelets (PLT) > Mean Platelet Volume (MPV) > Platelet Distribution Range (ADP) > Platelet Cells (PCT).

Parameters	Control			Interference		
	Average		X±DE	Average		X±DE
GB (10^3/µL)	128.28	±	15.53	145.68	±	7.58
GV (10^6/µL)	2.72	±	0.47	4.32	±	1.26
Hb (g/dL)	10.11	±	3.00	12.07	±	1.09
Ht (%)	31.90	±	8.82	39.98	±	1.54
VCM (fL)	139.11	±	12.08	136.46	±	25.56
HCM (pg)	44.10	±	5.14	41.49	±	5.50
CHCM (g/dL)	33.14	±	0.76	33.86	±	1.58
ADVGCV (%)	17.91	±	1.03	22.67	±	1.00
ADVGSD (fL)	158.19	±	17.42	159.78	±	18.32
PLT (10^3/µL)	160.60	±	26.91	123.15	±	53.08
VPM	2.13	±	0.40	12.91	±	4.76
ADP	9.23	±	2.61	9.53	±	0.78
PCT (%)	0.22	±	0.01	0.20	±	0.02

Table 4. Distribution of hematological parameter values and their averages for each collection area, and the standard deviation (X±DE), for the two seasons (dry and rainy), compiled by sample area (Control and Interference).

Parameters	Control			Interference		
	Average		X±DE	Average		X±DE
GB ($10^3/\mu L$)	135.15	±	7.50	154.13	±	7.71
GV ($10^6/\mu L$)	4.25	±	0.69	2.83	±	0.61
Hb (g/dL)	15.91	±	4.65	9.11	±	0.72
Ht (%)	21.02	±	4.54	67.61	±	29.34
VCM (fL)	108.35	±	7.22	152.01	±	39.03
HCM (pg)	42.95	±	6.16	43.08	±	5.12
CHCM (g/dL)	25.48	±	0.75	45.41	±	2.55
ADVGCV (%)	18.97	±	1.49	20.40	±	1.00
ADVGSD (fL)	133.45	±	8.59	162.21	±	15.47
PLT ($10^3/\mu L$)	134.40	±	24.02	136.67	±	67.21
VPM	2.23	±	0.41	11.15	±	3.70
ADP	9.82	±	2.62	8.76	±	0.52
PCT (%)	0.23	±	0.02	0.19	±	0.02

Table 5. Distribution of hematological parameter values and their averages for all samples. Caption: Hematological parameters - Leukocytes (GB)> Red blood cells (RB)> Hemoglobin (Hb)> Hematocrit (Ht)> Mean Corpuscular Volume (MCV)> Mean Corpuscular Hemoglobin (MCM)> Hemoglobin Concentration (HCCM)> Range of Distribution of Erythrocytes (ADVGVC) > Standard Deviation of Erythrocyte Distribution Range (ADVGSD) > Platelets (PLT) > Mean Platelet Volume (MPV) > Platelet Distribution Range (ADP) > Platelet Cells (PCT).

Parameters	General		
	Average		X±DE
GB ($10^3/\mu L$)	146.54	±	12.58
GV ($10^6/\mu L$)	3.40	±	0.88
Hb (g/dL)	11.83	±	3.26
Ht (%)	48.97	±	31.93
VCM (fL)	134.55	±	36.41
HCM (pg)	43.03	±	5.54
CHCM (g/dL)	37.43	±	9.97
ADVGCV (%)	19.83	±	1.27
ADVGSD (fL)	150.71	±	18.10
PLT ($10^3/\mu L$)	135.76	±	50.34
VPM	7.58	±	4.66
ADP	9.19	±	1.43
PCT (%)	0.20	±	0.02

All statistical treatments are presented in the form of graphic comparison segregated by climatic season and sample area. Where we have data from the dry and rainy season, respectively, July/2021 and November/2021, as well as from the sample sections, Control Area and Intervention Area, concomitantly, upstream and downstream of the altered region (Figure 10).

To compare the two areas, all data were condensed for both climatic seasons, only segregated by sampling region. Where it was possible to identify that, the hematological changes follow the same pattern mentioned, evidencing the differences between the areas (Figure 11).

It is possible to observe that in the interfered area (see Figure 11b) there is a considerable increase in the amount of red blood cells (GB) and peaks in the amount of hematocrit (Ht), in relation to the control area (see Figure 11a), in the dry season.

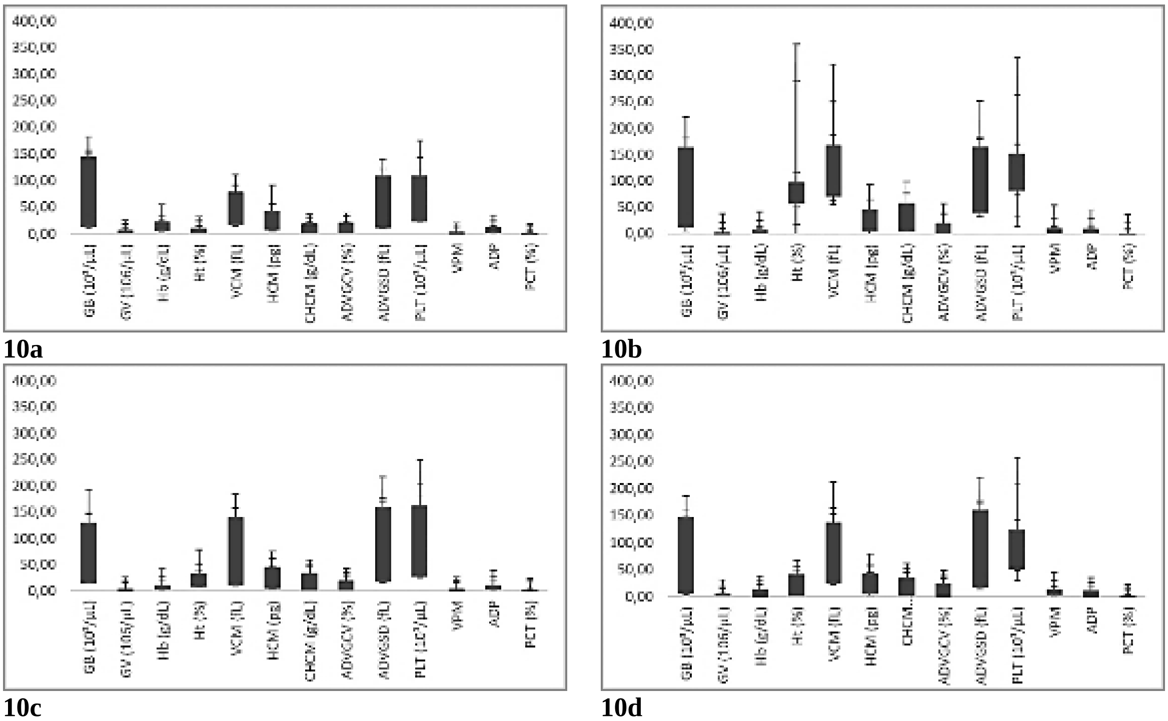

Figure 10. Variation of hematological parameters for all samples (±SD), and their respective standard deviations, segregated by climatic season: Dry (10a and 10b) and Rainy (10c and 10d); as well as, by sample area: Control (10a and 10c) and Interference (10b and 10d).

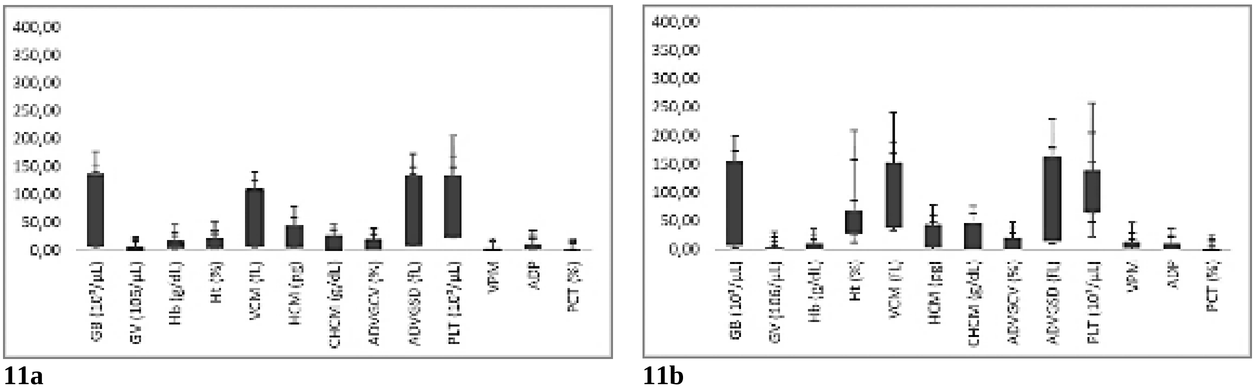

Figure 11. Variation of hematological data for all samples (±SD), and their respective standard deviations, segregated by sample area: Control (11a) and Interfered (11b).

RBC MORPHOLOGY

In the morphological evaluations of the blood cells, the smear samples showed morphology and distribution of red and white cells in similar amounts and shape (Figure 12a, b). However, the samples from the interference area showed some alterations with the presence of a greater number of immature erythrocytes and the appearance of cells with micronucleation (Figure 12 c, d), a presentation inherent to the presence of genotoxic agents in the environment.

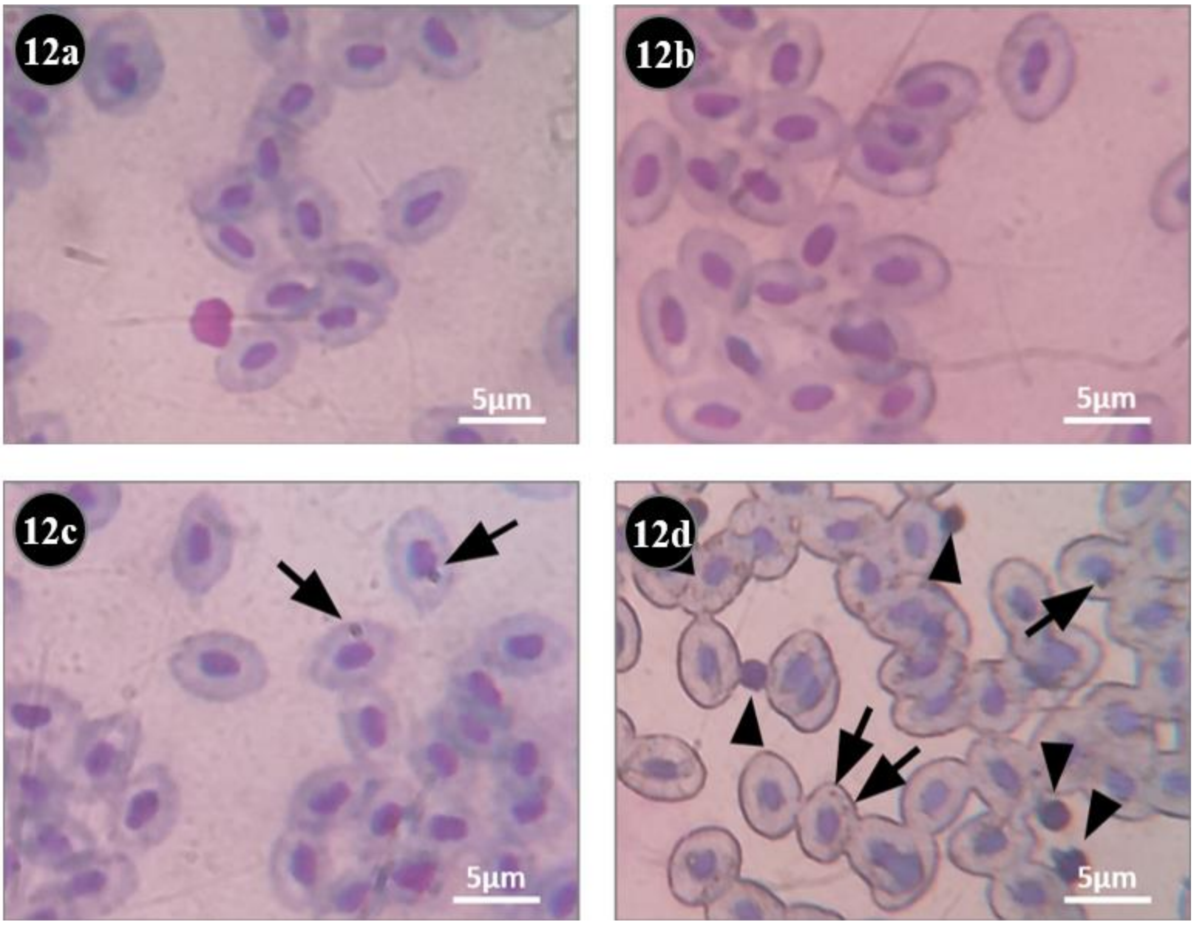

Figure 12. Changes in *Salminus hilarii* blood cells in the gradient of the collection sections, Control Area (12a and 12c), showing little or no visible change, and Interference Area (12b and 12d) fish exposed to water with intervention of the tailing's leachate from the mineral exploration. Caption: Immature erythrocyte (Ei) (arrowhead), Micronucleus (black arrow). Coloring: Giemsa and May Grünwald. Personal archive.

In general, in the evaluation of the images generated for these slides, as well as, after accounting for the changes seen, it was found that 12.99% of these images had some type of hematological variation. Where the highest concentration occurred in the interference area, more specifically at point 03, with a mean of 17.32 ± 8.01 cells with micronucleus (Mn) and a mean of 1.91 ± 1.70 immature erythrocytes (Ei) per image. The other points showed similar sample means, the same did not occur when we grouped the results by sample area, where the interference area showed higher means for the hematological variations evaluated (Table 6 and Table 7).

Table 6. Distribution of mean values for the count of immature erythrocytes (Ei) and micronucleus (Mn), by Collection points.

Collection 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

Table 7. Distribution of mean values for the count of immature erythrocytes (Ei) and micronucleus (Mn), segregated by sample group.

Sample groups	(Ei)			(Mn)		
	Average		X±DE	Average		X±DE
Control	1.05	±	0.45	0.76	±	0.63
Interference	1.34	±	0.76	8.28	±	6.02

DISCUSSION

Several studies that result in the investigation of hematological parameters in fish in the genus *Salminus* and their relationship with variations in environmental quality parameters have been carried out, most of which are based on experiments under controlled conditions (Lorenz et al., 2018, Lorenz et al., 2018, Lorenz et al., al., 2022, Pádua et al., 2009, Sabioni et al., 2022, Satake et al., 2009, Tavares-Dias et al., 2008). And few effectively promote these investigations, in the natural environment, specifically with S. hilarii (Garcia et al., 2007) and *S. maxilosus* (synonym of *S. brasiliensis*) (Paiva et al., 2001), however, in the latter, not there is a correlation with the environmental quality parameters.

BIOMETRIC PARAMETERS

For the genus Salminus, the hematological parameters discussed appear with relative similarity to the control groups, at an age considered juvenile. The weight-length relationship is a way of stipulating sample groups by relative age or sexual maturity in *S. hilarii* (Villares et al., 2007, Honji et al., 2009) and *S. brasiliensis* (Ferreira et al., 2020).

Thus, the application of the methodology of collection by gillnet, captured animals of similar sizes. Thus, the variation of hematological parameters by age, if under appropriate conditions, has little variation, which can be diluted by increasing the sample size (Cicero et al., 2020, Ferreira et al., 2020, Sabioni et al., 2022).

HEMATOLOGICAL PARAMETERS

For this study, hematological parameters were found with a significant difference in relation to seasonal variations, such as those presented for tropical fish, such as *Brycon amazonicus*, the matrinxã (Nascimento et al., 2020), *Piaractus mesopotamicus*, the pacú (Ventura et al., 2020), and *Oreochromis niloticus* the tilapia (Rodrigues et al., 2018, Marengoni et al., 2019), and to subtropical fish such as *Rhamdia quelem* the jundiá (Fredianelli et al., 2018).

One of the parameters listed here, the densification of blood cells, may be related to dehydration, which coincides with the results of other studies with different species (Lizama et al., 2020). Results relevant to the receipt of leachate from mineral exploration tailings, at the launch point that is prior to the interference area, providing an increase in the hardness of the water downstream of it (Cruz et al., 2019, Ulrich et. al., 2021).

There is, therefore, a relationship with climatic factors, since in the dry season there is a greater concentration of organic compounds from plant and animal remains, as well as in the rainy season these are more diluted due to rainfall in the period (Guarda et al., 2020a). Intrinsic characteristic of typical regions of the Cerrado, as verified for the Traíras river-GO (Guarda et al., 2020b, Silva et al., 2020), and for the basins of the Pantanal plain, in the Alto São Lourenço river (Cruz et al., 2019), as well as in the southern Amazonian Cerrado (Prado et al., 2021).

These variations were also contacted, including in cultured waters, with due restrictions of connection with the natural environment, in experimentation in the state of Rondônia (Lopes et al., 2019). As well, the same was observed in this study for the rainy season, but with a more subtle difference, only having significant differences for the increase in corpuscular volume (MCV) and peaks of increase for the number of platelets (PLT). The same presented by Portella et al., (2021) investigating the hematological characteristics of Neotropical Characiformes.

Data that may be related to increased respiratory effort, as a result of histological injuries to the gills or decreased oxygen available in the environment, as presented for *O. niloticus* reared in net cages (Rodrigues et al., 2018) and for *Colossoma macropomum* under Roundup® interference (Vieira et al., 2019). Where the decrease in respiratory capacity relative to the increase in leachate, both natural as a result of the rains at the beginning of the wet season, and the tailings of mineral exploration (Lima et al., 2019), causes negative reflexes on hematological parameters, similarly to effects of the concentration of pollutants in the water pointed out by Del-Guercio et al., (2017), with the use of fish as biological indicators in the evaluation of domestic sewage treatment, which can interfere with both the availability of oxygen by chemical and histopathological influence, causing hematic and tissue deformity in the body (Martelli et al., 2021).

However, the evident change in hematological parameters does not only fluctuate in relation to climatic events, but also reflects under the influence of anthropic changes in the region studied, in agreement with what was evidenced in the study carried out in Maranhão State (Aguiar et al., 2017) or in Santa Catarina State (Melo-Junior & Lorenzi 2022), as well as that found for the semiarid region, in the Ceará State (Braga & Matushima 2021).

It is evident that there is a discrepancy of values between the control area and the interference area, however when we segregate by sampling point, we can observe that point 03 has the highest values of these changes. This part of the interfered section is more exposed to the effects of leachate concentration in mineral exploration area, as shown by Braga et al., (2021) in São Francisco River.

As well, it is verified that there is a gradient of decrease in these incidences, which can be explained by the gradient of self-purification of the river (Thomé et. al. 2016). Taking into account the degradation of the leachate, as well as the precipitation and aggregation of metals at the bottom of the riverbed (Costa et al., 2021) not being available in the water and consequently reducing its action on the organisms existing there.

Given that some hematological changes, such as the increase in the amount of red blood cells or their concentration, are directly related to dehydration (Lizama et al., 2020), which in freshwater fish is due to an increase in the concentration of salts in the water. Relationship examined in other regions, as reported by Fredianelli et al., (2018) in the state of Paraná.

As verified for the region in question, the same implications were shown in material from mineral exploration areas (Costa et al., 2020, Oliveira et al., 2022), where tailings are produced in the form of copper salts, manganese, aluminum and zinc (Oliveira et al., 2018). This same finding was corroborated in studies with catfish showing histological alterations by metals (Barbieri et al., 2019) in the same way as studies in fish from the middle São Francisco River, where it links the encounter of micronucleus with the accumulation of heavy metals in these organisms (Braga et al., 2021). However, similar results were found for *O. niloticus*, under the effects of wastewater (Del-Guercio et al., 2017).

The relationship between water quality and the negative reflexes resulting from the changes promoted an increase in hematocrits, with a consequent increase in blood viscosity, the same verified by Flores et al., (2020), in *Astronotus ocellatus*, can promote physiological variations that lead the animal to experience respiratory difficulty, results similar to those verified as a result of parasitism in farmed fish (Tavares et al., 2021). As well, they can promote cumulative changes such as: increase in corpuscular volume (MCV), by implication of this low blood oxygenation, reflecting the reduction of respiratory capacity in *Colossoma macropomum*, the tambaqui (Vieira & Silva 2019).

Such changes promote depreciation in the health and well-being of organisms, as pointed out for farmed fish (Tavares-Dias et al., 2009), and more recently observed under the effects of agrochemicals on fish (Pinheiro & Mercado 2022), and even those cultivated with new technologies.

In this way, the control group presented a smaller variation of results for the evaluated parameters, making it possible to identify that there is a negative interference downstream of this area, the same was verified by Saviato et la. (2021) when evaluating hematological conditions in *Astyanax novae* in a river under the influence of urbanization. The affected area showed marked variation for hematocrit (Ht), corpuscular volume (MCV) and platelet count (PLT), indicating that in this area the individuals collected underwent respiratory stress, as verified by Vieira et al., (2019) who evaluated the interference of Roundup® in the hematological conditions of *C. macropomum*.

Evidencing that the characteristics of an increase in the number of erythrocytes and hematocrits are related to the decrease in oxygenation (Rodrigues et al., 2018), as well as the increase in the number of platelets reflects greater effort made by the body (Vieira et al., 2019). Indicating that these hematimetric anomalies are related to the discharge area, as they were verified with greater incidence in the samples downstream of it.

The values listed in this study are in line with others performed with species of this genus and widely experienced, especially *S. brasiliensis* (Paiva et al., 2001, Lorenz et al., 2018, Koch et al., 2018), as well as the species *S. affinis* (Garcia et al., 2007), which is genetically and morphologically close to *S. hilarii*.

Such similarities attest that the control group, presented for this study, was exposed to conditions that had little negative effect on their health and homeostasis. The group collected in interference by the leachate from the mineral tailings, showed a negative evolution of hematic variations, as explained in studies with other species, which negatively had their hematological parameters altered by exposure to biopesticides (Mariano et al., 2019, Mariano et al., 2021), agrochemicals (Porto et al., 2021), and urban wastewater (Saviato et al., 2021).

The results in this study indicate that the negative reflexes of the acute or chronic pressures of the parameters of water quality, result in conditions of respiratory deficit for this region as a result of the increase of Ht and VCM, linked with other histological alterations related to Organs respiratory organs, which in general, it has its cause in the increase of dissolved metals in the medium, being these, mainly aluminum, copper, lead and zinc.

RBC MORPHOLOGY

The immune response to aquatic organisms can be quite varied, however some groups of pollutants can cause genotoxic reflexes in the tissues of these beings, evidenced in the study in *O. niloticus*, exposed to biopesticides (Simão et al., 2021). The study of genotoxicity in fish is presented by assays that aim to identify biological markers such as the presence of histopathological deformations, one of which is the presence of satellite nucleation in red blood cells, identified as micronuclei. Also evidenced in other basins and with other species, as presented for the middle São Francisco River (Braga et al., 2021), or with the species *Astyanax bimaculatus*, in the Paraná River (Oliveira et al., 2018),

The presence of these erythrocytic deformities evidences the exposure of the organism in an environment containing substances that act as generators of genotoxic events (Merey et al., 2021). Thus, even if in low quantity as presented for this study, with few erythrocytes containing micronucleation (17.32% ± 8.01), mainly in the region called P3, belonging to the interference area, we can infer that this river is the receiving body of loads that alter the environmental quality and that promote negative changes in the health and well-being of these organisms.

These data do not corroborate the data presented for another Amazon region (28% ± 14.42), also under anthropic interference and with the use of the species *Plagioscion squamosissimus*, also a predator (Oliveira et al., 2022). Even so, such values show that negative pressures occur in the mentioned place, which promote genotoxic effects.

In the same way that these changes are reversible if the polluting source ceases, since the rate of blood cell replacement is high, in relation to other tissues of the body. Evidencing that the eviction in question is either constant or was occurring during the process of collecting biological material along the studied section.

CONCLUSIONS

The analyzed data may indicate that the blood samples of *S. hilarii* present differences in their respective hematological parameters. These differences are grouped and symmetric for each sampling point. However, these differences become more evident when the grouping by sample area occurs, the Control Area, formed by points P1 and P2, and the Interfered Area formed by points P3, P4 and P5. Indicating that there are negative pressures that lead these animals to undergo physiological changes, reflected in the hematological analyses.

Considering the presence of leachate dumping sites from mineral exploration tailings, which alters the quality and quantity of dissolved solutes in the environment, aggravated by the decrease in the hydrological volume, presented for the dry season, it is possible that this sum of factors corroborates the concentration of pollutants or toxic agents, differently for each sample region.

Thus, it can be affirmed that these fish have different health conditions for each sampled environment. Being in conditions of homeostasis closer to the ideal for the species, in the upstream sample areas where there is also less anthropic interference, with the presence of better-preserved riparian forest.

These findings may be based on the dynamics of distribution of hematological variations for *S. hilarii* in the study area. Where the variations showed a negative influence of the environment on the health and well-being of these animals, providing a depreciation of the respiratory capacity, either by the increase in hardness, or by the genotoxicity causing the nuclear malformation evidenced by the presence of micronuclei, in the erythrocytes, mainly in P3, belonging to the sample area called Interference. Since *S. hilarii* is a predator, its unfavorable health implies inferring that the entire trophic chain of lower levels may be contaminated, as well as, this species participating in the diet of riverside communities, may be contaminating the human populations that feed on it.

Because this species (*S. hilarii*) has a wide distribution, both in the studied basin and in other Brazilian basins, and in its hematological analyzes they reflect sensitive disparities visibly influenced by the environmental discrepancy, as well as by the quality of the water, between the collection points is It is possible that this species may express the same relationships in other regions with similar environmental characteristics. Thus, the species *S. hilarii* presents intrinsic qualities of an organism of importance for environmental monitoring, making it a possible indicator of environmental quality in areas where the species occurs.

And in this way, the results presented in this study can be used as an indication of the use of the species and similar analyses, where the results help in decision making to mitigate the environmental impacts resulting from human pressures, especially in aquatic ecosystems. Facilitating the understanding of the effects of these in the natural environment, especially in the rivers that drain these areas subject to contamination, both in the Cerrado biome, as in other Brazilian biomes.

In view of the overuse of natural resources in the region, these activities have a negative impact on the environment, requiring measures to be taken to minimize their effects in the medium and long term. Since there is already a chronic problem, caused over time by the tailing's dumps, which were accumulated at the bottom of the bed. In this way, it is imperative to contain measures for these wastes, as well as better ways of disposal so that the environment has a self-cleaning capacity, making it locally with adequate conditions for the conservation of this ecosystem, as well as the conservation of resident species.

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