# GENOTOXIC AND HISTOPATHOLOGICAL BIOMARKERS OF FOUR FISH SPECIES FROM THE MIDDLE SÃO FRANCISCO RIVER WITH DIFFERENT FEEDING HABITS

# BIOMARCADORES GENOTÓXICOS E HISTOPATOLÓGICOS DE QUATRO ESPÉCIES DE PEIXES DO MÉDIO RIO SÃO FRANCISCO COM DIFERENTES HÁBITOS ALIMENTARES

# BIOMARCADORES GENOTÓXICOS E HISTOPATOLÓGICOS DE CUATRO ESPECIES DE PECES DEL MEDIO RÍO SÃO FRANCISCO CON DIFERENTES HÁBITOS ALIMENTARIOS

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**ABSTRACT**

We compared variations in the occurrence of micronuclei (MN), morphonuclear alterations (AMN), and the presence of various liver damages among four fish species, *Salminus franciscanus* (*carnivorous*), *Leporinus obtusidens* (*omnivorous*), *Myleus micans* (*herbivorous*), and *Prochilodus argenteus* (*iliophagous-detritivorous*), occupying different trophic levels in an area of the middle São Francisco River, Januária, Minas Gerais. We hypothesized that (i) fish from different trophic levels present varying amounts of MN and AMN, as these damages are related to the presence of environmental contaminants, and (ii) fish from different trophic levels show multiple liver damages, since the liver is an important organ for the purification of organisms, reflecting the presence of aquatic contaminants. Cytogenetic analyses were performed using the MN test and histopathological analyses with photomicrographs of liver tissues. Species were ranked using Principal Component Analysis (PCA) based on genetic alterations and liver damage. Principal axis scores were compared using Generalized Linear Models (GLM) with Gaussian distribution. Significance was verified with ANOVA (F-test), followed by contrast analyses to identify statistical variations between groups. Our results indicated that P. argenteus presented the highest number of MNs, while M. micans and S. franciscanus had the highest quantities of AMNs. S. franciscanus and L. obtusidens were the most affected by degenerative liver lesions. Necrosis, a cell death lesion, had the highest score in P. argenteus. These findings indicate a strong correlation between cytogenetic and histopathological damage indices and the trophic levels occupied by the evaluated fish species.

**Keywords:** Morphonuclear Alterations. Liver Histopathology. Micronuclei. Trophic Levels.

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**RESUMO**
Comparamos as variações na ocorrência de micronúcleos (MN), alterações morfonucleares (AMN) e a presença de diversos danos hepáticos entre quatro espécies de peixes, *Salminus franciscanus* (carnívoro), *Leporinus obtusidens* (onívoro), *Myleus micans* (herbívoro) e *Prochilodus argenteus* (iliófago-detritívoro), que ocupam diferentes níveis tróficos em uma área do médio rio São Francisco, Januária, Minas Gerais. Hipotetizamos que (i) peixes de níveis tróficos distintos apresentam variadas quantidades de MN e AMN, pois estes danos estão relacionados à presença de contaminantes ambientais, e (ii) peixes de níveis tróficos diferentes evidenciam múltiplos danos hepáticos, visto que o fígado é um importante órgão de depuração dos organismos, refletindo a presença de contaminantes aquáticos. Foram realizadas análises citogenéticas através do teste MN e histopatológicas com fotomicrografias dos tecidos do fígado. As espécies foram ordenadas por Análise de Componentes Principais (PCA) com base nas alterações genéticas e danos hepáticos. Os scores dos eixos principais foram comparados por meio de Modelos Lineares Generalizados (GLM) com distribuição gaussiana. A significância foi verificada com ANOVA (teste F), seguida de análises de contrastes para identificar variações estatísticas entre os grupos. Nossos resultados indicaram que P. argenteus apresentou o maior número de MN, enquanto M. micans e S. franciscanus, os maiores quantitativos de AMN. Os mais afetados pelas lesões degenerativas do fígado foram S. franciscanus e L. obtusidens. A necrose, lesão de morte celular, teve o maior score em P. argenteus. Tais achados indicam forte correlação entre os índices de danos citogenéticos e histopatológicos com os níveis tróficos ocupados pelas espécies ícticas avaliadas.

**Palavras-chave:** Alterações Morfonucleares. Histopatologia Hepática. Micronúcleos. Níveis Tróficos.

**RESUMEN**
Comparamos las variaciones en la ocurrencia de micronúcleos (MN), alteraciones morfonucleares (AMN) y la presencia de diversos daños hepáticos entre cuatro especies de peces, *Salminus franciscanus* (carnívoro), *Leporinus obtusidens* (omnívoro), *Myleus micans* (herbívoro) y *Prochilodus argenteus* (*iliophagous-detritivorous*), que ocupan diferentes niveles tróficos en un área del río São Francisco medio, Januária, Minas Gerais. Planteamos la hipótesis de que (i) los peces de diferentes niveles tróficos presentan cantidades variables de MN y AMN, ya que estos daños están relacionados con la presencia de contaminantes ambientales, y (ii) los peces de diferentes niveles tróficos muestran múltiples daños hepáticos, ya que el hígado es un órgano importante para la purificación de organismos, lo que refleja la presencia de contaminantes acuáticos. Se realizaron análisis citogenéticos utilizando la prueba de MN y análisis histopatológicos con fotomicrografías de tejidos hepáticos. Las especies se clasificaron mediante Análisis de Componentes Principales (ACP) con base en alteraciones genéticas y daño hepático. Las puntuaciones del eje principal se compararon mediante Modelos Lineales Generalizados (MLG) con distribución gaussiana. La significancia se verificó mediante ANOVA (prueba F), seguido de análisis de contraste para identificar variaciones estadísticas entre los grupos. Nuestros resultados indicaron que P. argenteus presentó el mayor número de MN, mientras que M. micans y S. franciscanus presentaron las mayores cantidades de AMN. S. franciscanus y L. obtusidens fueron las más afectadas por lesiones hepáticas degenerativas. La necrosis, una lesión de muerte celular, presentó la puntuación más alta en P. argenteus. Estos hallazgos indican una fuerte correlación entre los índices de daño citogenético e histopatológico y los niveles tróficos ocupados por las especies de peces evaluadas.

**Palabras clave:** Alteraciones Morfonucleares. Histopatología Hepática. Micronúcleos. Niveles Tróficos.

## 1 INTRODUCTION

Brazil has the greatest biodiversity of freshwater fish in the world, corresponding to approximately 27.3% of the known species (Reis et al., 2016, Almeida, 2019). This diversity is probably related to its typically tropical geographic location, large territorial dimensions and the size of its hydrographic basins (Vieira; Alves; Pompeu, 2009). The São Francisco River is the largest watercourse entirely contained in the Brazilian territory. Its drainage area covers semi-arid to arid regions of the country, exerting a strong influence on its socioeconomic development, favoring irrigated agriculture, energy production, waterway transport, in addition to commercial fishing and the subsistence of riverside peoples (Pereira et al., 2007, Horodesky et al., 2020).

However, in recent decades, the scarcity of fish points to the significant impacts suffered by the ichthyofauna of the São Francisco River, resulting from aquatic pollution from waste from mining, domestic, industrial and agricultural sewage. In addition to these factors, the damming of its waters interferes with the migratory route of fish, increases the status of environmental vulnerability and the risk of extinction of several species (CBHSF, 2015, Souza-Shibatta et al., 2018, Gonçalves Júnior et al., 2019, Brandão et al., 2020, MMA, 2020).

It is known that contamination of the aquatic environment can lead to physiological, reproductive and behavioral disorders in aquatic fauna, contributing to the decrease of natural stocks (Barbosa et al., 2017, MMA, 2020). For this reason, the monitoring of the environmental quality of an ecosystem should go beyond the physicochemical analyses of environmental samples, also including assessments of possible adverse effects of environmental contaminants on its biota (Connon et al., 2012, Gupta, 2014).

From this perspective, fish are recognized as good indicators of environmental quality, as they accumulate toxic substances by direct exposure to contaminants dissolved in the water, or indirectly through trophic interactions (Ribeiro & Américo-Pinheiro, 2018, Angheben et al., 2019, Trolly, 2019, Qiu et al., 2020, Gabiatti, 2021, Brandts et al., 2022, Hamidian et al., 2023). In addition, they are sensitive to low concentrations of mutagens, teratogenic agents, and carcinogens (Grisolia, 2005, Deutschmann et al., 2016). Exposure to xenobiotics can trigger metabolic, cellular, molecular, histological, and behavioral disorders. These disorders can be measured and constitute biomarkers that can indicate the impacts of pollutants at different levels of biological organization, and are therefore of fundamental importance in monitoring the ecological integrity of an aquatic ecosystem (El-SiKaily & Shabaka, 2024).

Among the biomarkers used to evaluate the biological responses of aquatic fauna, the Micronucleus Test (NM) in fish erythrocytes is commonly recommended, as it consists of a simple, fast, and sensitive assay for the detection of structural and/or numerical chromosomal alterations (OECD, 2016, Lopes, 2017, Aguiar et al., 2018, Qiu et al., 2020, Córdoba-Tovar et al., 2023). Fish erythrocytes are normally mononucleated (Bueno et al., 2017). The presence of micronuclei (NM) indicates chromosomal fragments, or even entire chromosomes, not aggregated to the main nucleus of daughter cells during the process of cell division (Al-Sabti & Metcalfe, 1995). These NM are characterized by (i) being smaller than 1/3 of the main nucleus in size; (ii) not be refractive; (iii) have a color and intensity equivalent to the main nucleus and (iv) intracytoplasmic composition without touching the main nucleus (Grisolia, 2005, Oliveira et al., 2020). It is noteworthy that there may be spontaneous occurrence of MN among fish species, but this is considered low and uniform (Siu et al., 2004). However, other studies suggest a positive relationship between environmental pollution and MN formation in fish erythrocytes (Lopes, 2017, Pereira, 2019, Carmo et al., 2020, Oliveira et al., 2020, Gomes et al., 2021, Brandts et al., 2022, Caramello & Jorge, 2022, Noleto et al., 2022).

In this study, we evaluated the variations in the occurrence of MN and AMN, as well as the presence of multiple liver damages, in fish occupying different trophic levels, collected from an area of the middle São Francisco River. Specifically, we expect that: (i) fish of different trophic levels present different amounts of MN and AMN of the blebbed, lobed, notched and eightshaped type, as these damages are related to the presence of environmental contaminants (Brandts et al., 2022, Nyholt et al., 2022, Viana et al., 2022, Cruz-Esquivel et al., 2023) and (ii) fish of different trophic levels present varied liver damage, such as hydropic degeneration, steatosis, hemosiderosis, hyperemia, inflammation, and necrosis, because the liver is an important organ for the purification of living organisms reflecting the presence of contaminants in the aquatic environment (Abalaka et al., 2020, Mladin et al., 2021, Jiang et al., 2022, Vieira et al., 2022, Hamidian et al., 2023).

## 2 THEORETICAL FRAMEWORK

MN are correlated with chromosomal instability, genome rearrangements, and mutagenesis (Krupina et al., 2021). They are biomarkers often associated with cancers, senescence, and genotoxic stress (Krupina et al., 2021). The NM nuclear envelope is commonly broken in integrity. The rupture of the micronuclear envelope, which is almost always irreversible, compromises mechanisms for repairing genetic material, especially the autophagic pathway, which consequently contributes to the localized accumulation of DNA damage (Terradas et al., 2016). In addition, they activate cGAS, a cellular immune response signaling enzyme, related to cancer progression (Krupina et al., 2021).

In addition to variations in the number of MN, other morphonuclear alterations (AMN) such as blebbed, lobed, notched and eightshaped, also resulting from errors in cell division, can be indicators of cytotoxicity, being important biomarkers of genotoxic damage in vivo (OECD, 2016, Lopes, 2017, Aguiar et al., 2018, Pereira, 2019, Carmo et al., 2020, Oliveira et al., 2020, Gomes et al., 2021, Brandts et al., 2022, Córdoba-Tovar et al., 2023, Cruz-Esquivel et al., 2023).

The assessment of histopathological damage has also been used as a biomarker for the verification of direct and indirect toxicological effects on fish tissues (Lopes, 2017, Aguiar et al., 2018, Pereira, 2019, Ferreira, 2020, Peixoto, 2020, Shahid et al., 2021, Brandts et al., 2022, Hamidian et al., 2023). The liver is an important organ to evaluate histological changes related to the presence of environmental contaminants, as it reflects the occurrence of aquatic pollutants in proportion to the levels found in the environment, performs numerous vital functions, and is directly related to detoxification mechanisms, through the biotransformation of toxic substances (Lopes, 2017, Abalaka et al., 2020, Shahid et al., 2021, Brandts et al., 2022, Hamidian et al., 2023). Several liver damages (e.g., loss of cell boundary and cytoplasmic integrity, nuclear deformation, cytoplasm vacuolization, reduction in cell density, melanomacrophage centers, fatty degeneration, inflammation, congestion, hepatitis, and necrosis) are related to fish exposure to environmental xenobiotics (Lopes, 2017, Pereira, 2019, Ferreira, 2020, Peixoto, 2020, Shahid et al., 2021).

Finally, the occurrence of these genotoxic and histopathological changes may be related to the trophic levels occupied by fish (Ribeiro & Américo-Pinheiro, 2018, Vreys et al., 2019, Peixoto, 2020, Viana et al., 2022, Córdoba-Tovar et al., 2023). It is assumed that fish species with different feeding habits can absorb variable amounts of environmental pollutants, which can occur through the respiratory, dermal or digestive route, being accumulated in the various tissues (Campos et al., 2018).

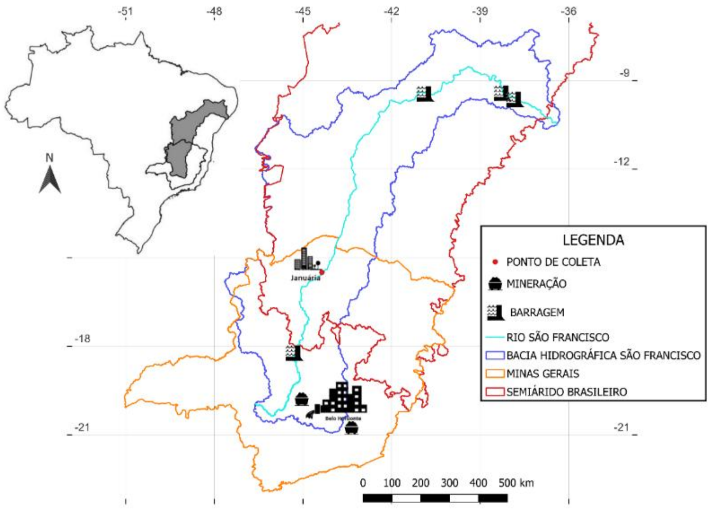
## 3 METHODOLOGY

### 3.1 FIELD OF STUDY

Fish were collected in the São Francisco River, near the urban area of Januária, northern Minas Gerais, Brazil. This municipality has a population of 67,958 inhabitants (IBGE, 2021) and is located in the middle São Francisco region, characterized by prolonged periods of drought and aridity (Castro & Pereira, 2019). The sampling site is impacted by erosion, siltation, irrigated agriculture, fishing, domestic sewage dumping, and pesticide and fertilizer contamination (Fig. 1) (CBHSF, 2015, IGAM, 2021).

**Figure 1**

*Study area and sampling point in the region of the middle São Francisco River, municipality of Januária, Minas Gerais, Brazil. Factors of degradation of the watercourse (i.e., mining areas, dams and urban agglomerations such as Januária and Belo Horizonte) are evidenced*




Source: Prepared by the authors themselves.

3.2 SPECIES OF FISH ASSESSED

*Salminus franciscanus* (Lima & Britski, 2007) (golden) has a *carnivorous* feeding habit (Fig. 2A and 2E). On average, the specimens measure from 40 to 70 cm, weighing between 0.5 and 3.5 kg. The species is migratory and rheophilic. They preferentially inhabit running waters with a high concentration of oxygen. Golden fish are highly prized for consumption and ornamental breeding (Fig. 2A) (Lima & Britski, 2007, Flora et al., 2010, Campeche et al., 2011).

*Leporinus obtusidens* (Valenciennes, 1836) (piau) are omnivores (Fig. 2B and 2E), feeding on filamentous algae, leftovers from submerged crops, mollusks and insects. They can reach about 8 kg and measure around 22 to 40 cm in length. It has migratory behavior and periodic reproduction. It is a species highly appreciated by sport, extractive and commercial fishing due to the palatability of its meat (Campeche et al., 2011).

*Myleus micans* (Lütken, 1875) (pacu) has a preferential *herbivorous* feeding habit (Fig. 2C and 2E), feeding on aquatic macrophytes. They have a rheophilic behavior and can reach 50 cm in length and approximately 4 kg (Britski et al., 1988, Brasil-Sato & Santos, 2003, Da Luz et al., 2012, Andrade, 2013).

*Prochilodus argenteus* (Agassiz, 1829) (curimatã) is an iliophagous-detritivorous (Figs. 2D and 2E), ingests sediments from the bottom of rivers, including microorganisms and debris. Due to its feeding habit, it has a portion of the stomach adapted in the shape of a gizzard. The mouthparts have thick, protractile lips with denticles. Females reach greater weight than males, reaching 15 kg and 80 cm in length. They make an important contribution to aquatic environments because they cycle nutrients and purify watercourses (Campeche et al., 2011, Melo, 2011, Reis et al., 2018).

**Figure 2**

*Fish species evaluated in this study. In (A) Salminus franciscanus; (B) Leporinus obtusidens; (C) Myleus micans; (D) Prochilodus argenteus and (E) Trophic level occupied in the food chain by the species studied. 1) Detritivore/Iliophagous (P. argenteus), 2) Herbivore (M. micans), 3) Omnivore (L. obtusidens) and 4) Carnivore (S. franciscanus)*


Source: Prepared by the authors.

3.3 COLLECTION OF SAMPLES

Fish specimens of the species S. franciscanus, L. obtusidens, M. micans and P. argenteus were collected from the bed of the São Francisco River (-15º29.39'-044º21.31' Datum WGS 84 Lat and Long) with trawls in October/2021 (Fig. 1).

A total of 10 adult specimens of each fish species were sampled to evaluate genetic and hepatic alterations. After collection, all animals were placed in thermal boxes containing river water and then anesthetized with benzocaine through immersion (0.1 g of benzocaine in 1 ml of ethyl alcohol for every 100 ml of deionized water) remaining for 10 minutes after cessation of opercular movements and by the complementary method of euthanasia by spinal section.

### 3.4 MICRONUCLEUS (MN) TEST

The NM test was performed as described by Rivero (2007), with modification. Thus, the peripheral blood of the fish was collected through gill puncture with the aid of hypodermic, sterile and disposable 3 ml syringes with a needle measuring 0.80 mm x 32 mm.

The blood extension of each specimen was done at the collection site, with approximately 50 µl of blood, and three smears were prepared per animal. The slides were properly identified and exposed to drying at room temperature for 24 hours. After drying, they were stained using a kit for rapid differential staining in hematology, following the procedures as recommended by the manufacturer (ANVISA Registration 10097010105, code 620529, lot 90813007).

The cytological analysis was performed under a Physis® light optical microscope with immersion objective and 100x magnification, considering the count of 3,000 blood cells per animal. The records were made with the help of Lee Tools®analog statistical counters 680233. Total erythrocytes and those with micronuclei were quantified, as well as subgroups of four other distinct morphonuclear alterations, being blebbed, lobed, notched and eightshaped (Fig. 3) (Rivero, 2007, Azevedo et al., 2012, Lopes, 2017, Aguiar et al., 2018).

### 3.5 LIVER HISTOLOGY

The collected fish, after being euthanized, were dissected to remove a sample of liver tissues. Two portions of the liver measuring approximately 3.0 x 2.0 x 1.5 cm and weighing approximately 4 g were sectioned per specimen. These tissue fractions were washed in saline solution (0.9%) and fixed in buffered formalin solution (10%, pH 7.2) for 6 hours.

After fixation, the tissue fragments were transferred to a sodium phosphate buffer solution for 24 hours. Next, the material was dehydrated in an increasing series of alcohols (70%, 80%, 90% and 100%), diaphanization in xylol and inclusion in blocks with paraffin with a melting point between 56-62 ºC. Subsequently, the blocks were cut transversely with 4 µm thickness with a rotating microtome (Lopes, 2017). The slides were stained with hematoxylin-eosin (Paulete & Beçak, 1976). A permanent histological slide was made from each specimen.

Twenty photomicrographs were recorded per specimen, considering random fields of liver tissues. For this, a Zeizz® optical microscope model Primo Star, AxiocamERc5s® coupled camera, resolution 2560(H) x 1920(V) and Zen 2.5 lite® software, magnification of 40x, were used.

For morphometric analysis of the images, the ImageJ software (version 1.32j) was used. Photomicrographs were viewed and analyzed for lesions with a quantitative or semiquantitative approach according to the nature of the damage. The lesions evaluated quantitatively were inflammation and necrosis (Fig. 4, E, F), and the inflammatory Kupffer cells were recorded numerically, the areas of necrosis measured in square micrometers and later converted into square millimeters.

Lesions, hydropic degeneration, hemosiderosis, hyperemia, microsteatosis, macrosteatosis (Fig. 4, A, D, B, C) and total steatosis were measured semi-quantitatively following an adapted protocol (Brunt et al., 1999). The quadrants with positive fields for the respective lesions were added and, considering the calculation of the median, degrees of severity were assigned for each specimen sampled. In the absence of liver damage, grade 0, for the intervals of 0-3 (grade 1), 4-6 (grade 2), 7-10 (grade 3). However, grade 1 was conferred when the median was obtained.

## 3.6 STATISTICAL ANALYSIS

The different fish species were ordered based on genetic alterations (i.e., number of micronuclei, blebbed, lobed, notched and eightshaped) and liver damage (i.e., hydropic degeneration, steatosis, hemosiderosis, hyperemia, inflammation and necrosis) using different Principal Component Analysis (PCA). Thus, two biplot plots were generated to show the projection of the original axes on a scatter plot. These analyses were performed using the Past (Paleontological Statistics Software) software, version 4.03 (Hammer, 2001).

Finally, the scores of the two main axes of these PCAs were extracted and compared among the four fish species. These comparisons were made using Generalized Linear Models (GLM). In the construction of these models, fish species were the explanatory variables, and PCA scores, referring to genetic alterations or liver damage of these species, were the response variables, following the Gaussian distribution. Analysis of Variance (ANOVA), with F-test, was used to test the significance of the models. Contrast analyses were used to group statistically equal levels and separate those that were statistically different. All these statistical procedures were performed in the R software (R Core Team, 2023).

## 3.7 LEGAL ASPECTS OF RESEARCH

This research has environmental licensing issued by the Ministry of the Environment – MMA, Chico Mendes Institute for Biodiversity Conservation – ICMBio, through the Biodiversity Authorization and Information System – SISBIO No. 79384-1, qualification certificate by the National System for the Management of Genetic Heritage and Associated Traditional Knowledge – SisGen No. AF728DB and permission from the Ethics Council in

Experimentation and Animal Welfare of the State University of Montes Claros – CEEBEA/Unimontes protocol No. 233.

## 4 RESULTS AND DISCUSSIONS

The length and weight of the collected fish were measured, and the values were used to calculate the morphometric means and standard error of each parameter evaluated (Table 1). The photomicrographs analyzed revealed, in the erythrocytes of the specimens, the occurrence of MN and AMN with morphological patterns of the blebbed, lobed, notched and eightshaped types (Fig. 3), as well as, in the hepatic tissues, the presence of lesions compatible with hydropic degeneration, steatosis, hemosiderosis, hyperemia, inflammatory processes and necrosis (Fig. 4).

**Table 1**

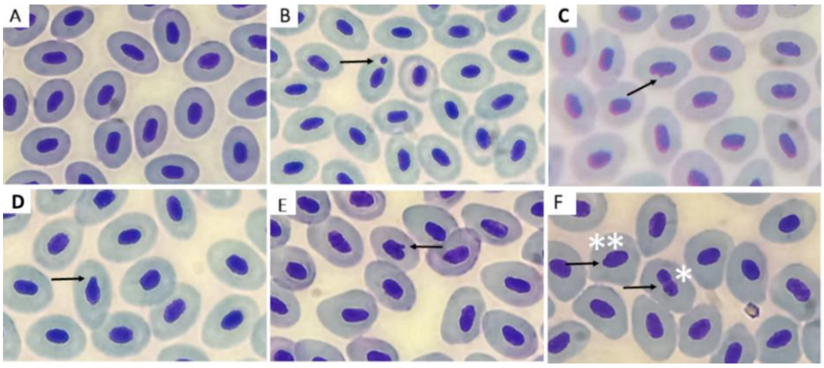
*Biometric data of the fish species studied*

| Species | Average Length (cm) | Standard Error Length | Average Weight (g) | Standard Error Weight |
|---|---|---|---|---|
| *Salminus franciscanus* | 55,0 | ±0.9 | 1680,0 | ±81.8 |
| *Leporinus obtusidens* | 43,0 | ±1.9 | 1210,0 | ±147.0 |
| *Myleus micans* | 21,0 | ±0.7 | 260,0 | ±23.1 |
| *Prochilodus argenteus* | 37,5 | ±1.1 | 630,0 | ±67.5 |

Source: Prepared by the authors.

**Figure 3**

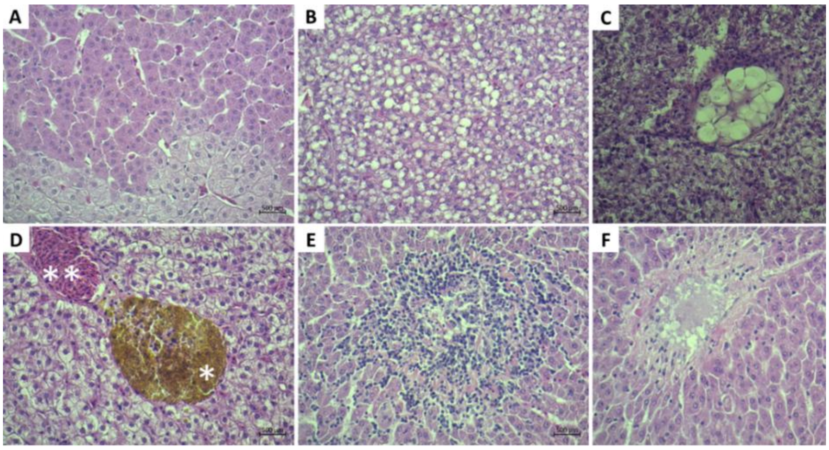
*Photomicrographs of fish erythrocytes, stained with panoptic rapid hematological use, 100X magnification; A) normal erythrocytes; B) micronucleated erythrocyte (NM); C) Blebbed AMN (bubble); D) lobed AMN; E) AMN of the notched type; F) *eightshaped brand AMN (eight) and **blebbed*




Source: Prepared by the authors.

**Figure 4**

*Photomicrographs of liver damage in fish, stained with hematoxylin-eosin, 40X magnification; A) hydropic degeneration; B) microsteatosis; C) macrosteatosis; D) *hemosiderosis and **hyperemia; E) inflammation (Kupffer cells); F) necrosis*



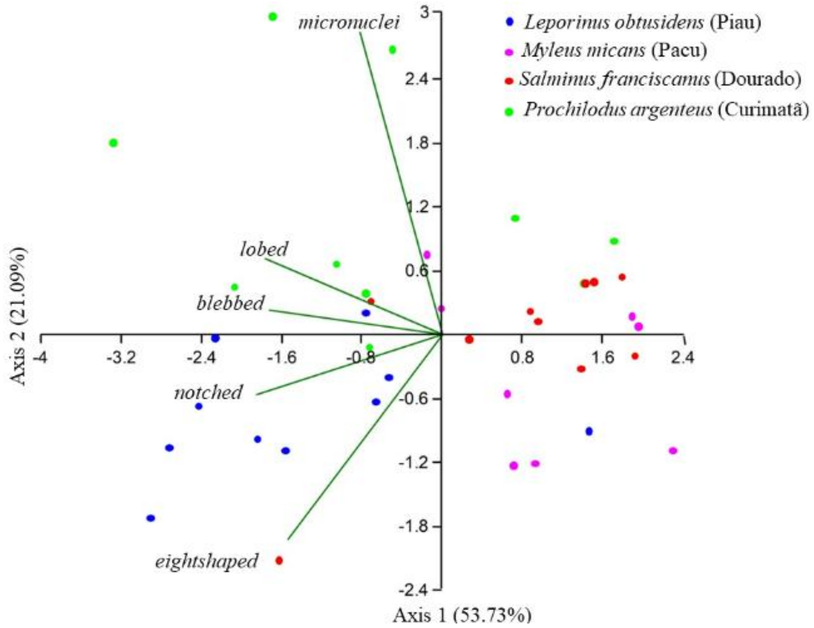

Source: Prepared by the authors.

4.1 GENETIC VARIATIONS

The first two axes of PCA together explain approximately 75% of all genetic alterations. Axis 1 showed a negative relationship with the blebbed, lobed, notched and eightshaped morphonuclear alterations. Axis 2 showed a positive relationship with micronuclei. Therefore, this distribution suggests that axis 1 represents morphonuclear changes (i.e., blebbed, lobed, notched and eightshaped) and axis 2 represents changes in the number of micronuclei (i.e., micronuclei) (Fig. 5).

**Figure 5**

*Principal component analysis biplot based on the occurrence of genetic alterations in the four fish species sampled. Axis 1 Lobed, blebbed, notched and eightshaped morphonuclear alterations. Axis 2 Variations in the number of micronuclei*

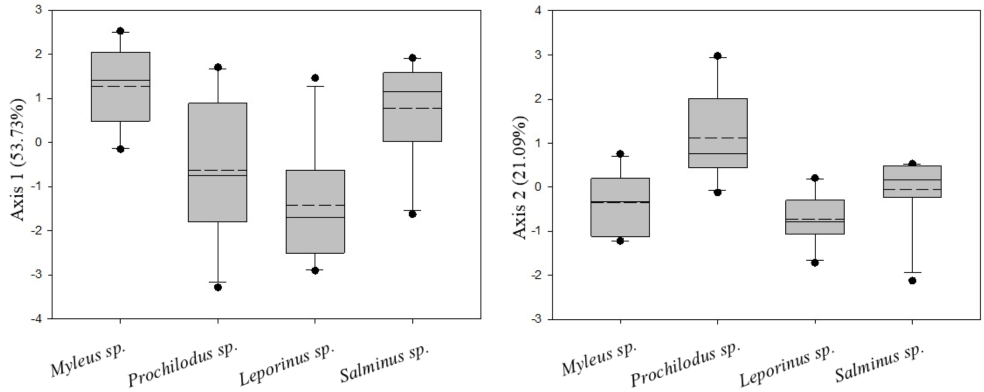



Source: Prepared by the authors.

The species M. micans and S. franciscanus were positively correlated with axis 1, while L. obtusidens and P. argenteus showed a negative correlation with this same axis (Fig. 5). In addition, the scores derived from P. argenteus and L. obtusidens are lower than the scores derived from M. micans and S. franciscanus (Fig. 6), suggesting that the latter two species have a greater number of morphonuclear alterations. Axis 2 shows a positive correlation with the species P. argenteus, which has the highest micronucleus scores compared to the other species (Fig. 6).

**Figure 6**

*Variation in the scores of the first (A) and second (B) axis of Principal Component Analysis (PCA) based on the morphonuclear variations of the species M. micans, P. argenteus, L. obtusidens, S. franciscanus, collected in the São Francisco River, Januária, Minas Gerais*



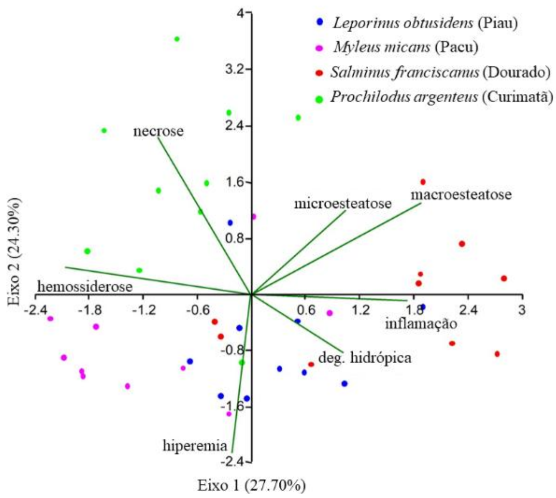

Source: Prepared by the authors.

## 4.2 LIVER CHANGES

The first two axes of PDA together explain approximately 52% of all liver alterations. Axis 1 showed a positive relationship, especially with macrosteatosis and inflammation, a negative relationship with hemosiderosis. Axis 2 showed a positive relationship with necrosis and a negative relationship with hyperemia. Therefore, axis 1 should represent degenerative lesions (i.e., hydropic degeneration, steatosis, hemosiderosis, hyperemia, inflammation) and axis 2, relationship with cell death lesion (i.e., necrosis) (Fig. 7).

**Figure 7**

*Biplot of principal component analysis based on the occurrence of liver damage in the four fish species sampled. Axis 1 Degenerative lesions (hydropic degeneration, steatosis, hemosiderosis, hyperemia, inflammation). Axis 2: Cell death lesion (necrosis)*

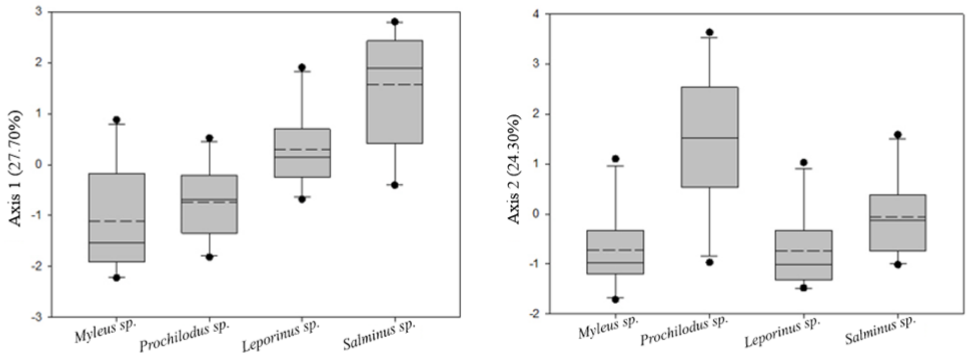



Source: Prepared by the authors.

Axis 1 showed a positive correlation with the species S. franciscanus and L. obtusidens and a negative correlation with the species M. micans (Figure 7). The scores derived from M. micans were lower than the scores derived from S. franciscanus (Fig. 8), suggesting that the latter species has higher rates of hydropic degeneration, steatosis and inflammation. Axis 2 shows a positive correlation with P. argenteus, which has the highest necrosis scores compared to the other species (Fig. 8).

**Figure 8**

*Variation in the scores of the first (A) and second (B) axis of Principal Component Analysis (PCA) based on the liver lesions of the species M. micans, P. argenteus, L. obtusidens, S. franciscanus, collected in the São Francisco River, Januária, Minas Gerais*




Source: Prepared by the authors.

## 5 CONCLUSION

We compared the variations in the occurrence of NM, AMN and hepatic histopathological alterations among four fish species with different feeding habits from the same area of the middle São Francisco River. Our results indicated that the species most affected by MN was P. argenteus, and by AMN were M. micans and S. franciscanus. These results corroborate our initial hypothesis that fish of different trophic levels tend to present different amounts of genetic damage, due to feeding habits, since the species P. argenteus, M. micans and S. franciscanus have detritivorous, *herbivorous* and *carnivorous* feeding habits, respectively.

The species P. argenteus, being iliophagous-detritivorous, forages the river substrate, where it ingests sediments, microorganisms and debris, playing a relevant role in the cycling of nutrients and purification of watercourses (Campeche et al., 2011, Reis et al., 2018). Sediments are the main sources of contamination of aquatic organisms and can induce an increase in MN and AMN (Córdoba-Tovar et al., 2023). Detritivorous fish tend to accumulate higher amounts of contaminants, because they directly concentrate, throughout the life cycle, greater amounts of xenobiotics in their body due to feeding habits (Viana et al., 2022). In a study previously carried out by this research group, the gill and intestinal metagenome of P. argenteus, a detritivorous iliophagous species, endemic to the São Francisco River, was characterized for the first time, also evaluated in this work. A significant number of bacterial taxa resistant to heavy metals were noted in these microbiomes, as iliophagous fish feed on the sediment and are probably more subject to bioaccumulation (Damasceno et al., 2022).

In our study, AMN had the highest scores in M. micans and S. franciscanus, which are respectively *herbivorous* and *carnivorous* species. Other studies indicate that AMN are primary responses that precede the formation of micronuclei (Ayllon & Garcia-Vazquez, 2000, Kirschbaum et al., 2009). During the repair mechanisms of the genetic material, which may have been damaged due to contamination of the fish by environmental xenobiotics, the nuclear membrane would present imperfections that characterize AMN (Gomes et al., 2021). Another explanation would be oxidative stress, generated by the probable presence of pollutants in the aquatic environment, which alters the permeability and selectivity of membranes, making the nucleus more vulnerable, favoring the formation of AMN and MN respectively (Silva de Assis et al., 2013, Gomes et al., 2021). Thus, the AMN indices in M. micans can be explained by the possible presence of environmental contaminants, which were bioaccumulated and transferred through the food chain, to the specimens of this species.

The species S. franciscanus showed high AMN scores. This result reinforces the biomagnification process, indicating that fish at higher trophic levels tend to concentrate higher amounts of pollutants along the food chain, since they ingest numerous organisms already contaminated during their life cycle (Gomes et al., 2021, Jiang et al., 2022, Nyholt et al., 2022, Córdoba-Tovar et al., 2023).

Regarding the occurrence of liver damage, the comparison between fish species of different trophic levels showed that P. argenteus was also the most affected. These fish showed a high occurrence of necrosis, irreversible injury. As previously described, P. argenteus is more exposed to conditions of contamination with environmental residues due to its iliophagous-detritivorous feeding habit. The effect of trophic magnification is also corroborated by the higher occurrence of degenerative lesions (i.e., hydropic degeneration, steatosis and inflammation) found in S. franciscanus and L. obtusidens, respectively *carnivorous* and *omnivorous*, when compared to the other species in this study. Thus, species with *carnivorous* habits are generally more affected by liver damage than *omnivorous* and *herbivorous* species (Mladin et al., 2021) and for the same reason, *omnivorous* species have higher occurrences of liver damage than herbivores (Hamidian et al., 2023).

It is noteworthy that during the collection period and at the sampling site of this study, there was the presence of a large volume of dead mollusks, some of which were found in the stomach contents of certain fish species. The waters of the river, as well as the fish, exuded a foul odor, evidencing an evident environmental imbalance, probably due to processes of pollution and degradation of the ecosystem (Cavalcante, 2021). The quarterly bulletin of the Minas Gerais Institute of Water Management (IGAM), referring to the last quarter of 2021, identified non-conformities in the parameters Escherichia coli, total phenols, total phosphorus and dissolved copper in the same period and place of sampling of the fish in this study (COPAM, 2008, IGAM, 2021), suggesting that water quality may have contributed to the genetic alterations and liver damage observed in the organisms. Several studies have already established a positive correlation between aquatic environmental degradation and the physiological responses of fish species (Abalaka et al., 2020, Peixoto, 2020, Mladin et al., 2021, Shahid et al., 2021, Jiang et al., 2022, Viana et al., 2022, Córdoba-Tovar et al., 2023, Hamidian et al., 2023).

In summary, our results indicate that fish of different trophic levels have different rates of genetic damage and liver injury. The iliophagous-detritivorous species, P. argenteus, was the most affected by the occurrence of MN and necrosis, possibly because its habitat and diet expose it to higher concentrations of aquatic contaminants. We pointed out the possible effects of bioaccumulation along the food chain, since S. franciscanus (carnivore) and L. obtusidens (*omnivorous*) showed high rates of degenerative lesions of the liver, among them, hydropic degeneration, steatosis and inflammation.

We suggest that further studies correlate important variables, such as water seasonality and the identification of specific pollutants to the feeding habits of the species already evaluated by this work, as well as others that can be investigated, especially endemic ones. Such research is fundamental to fill the gaps in knowledge that still exist about the middle course of the São Francisco River.

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