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Bioaccumulation of metals and genotoxic effects in females of *Colomesus asellus* collected in an Amazon River estuary, Amapá, Brazil

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ABSTRACT

Bioaccumulation of metals and genotoxic effects in females of *Colomesus asellus* collected in an Amazon River estuary, Amapá, Brazil

Colomesus asellus (Amazonian Puffer) is endemic to the Amazon Basin. It uses channels and streams to reproduce, spawn and feed. In areas close to urban centers, these fish may be exposed to anthropogenic residues containing mixtures of metals that can be bioaccumulated, resulting in genetic alterations. Therefore, we aimed to determine the extent of nuclear alterations in erythrocytes of female *C. asellus* resulting from the bioaccumulation of metals in tissues and organs. Metal concentrations were analyzed by atomic absorption spectrophotometry. Tissue metal concentrations decreased in the following order: Fe > Zn > Cu > Mn > Hg > Pb > Ni > Cd > Cr. In fish tissues/organs, the concentration of metals followed, in decreasing order, liver > skin > gonads > musculature > bones. The most frequent nuclear alterations were nuclear invagination, nuclear budding and lobulated nucleus. Metal concentrations and resultant nuclear alterations observed can damage *C. asellus*, thus compromising the conservation of this species at the mouth of the Amazon River.

Key words: Brazilian Amazon, Amazonian Puffer, inorganic pollutants, genotoxicity biomarkers

RESUMO

Bioacumulação de metais e efeitos genotóxicos em fêmeas de* Colomesus asellus *coletadas no estuário do Rio Amazonas, Amapá, Brasil

Colomesus asellus *(Baiacu Amazônico) é endêmico da bacia amazônica. Ele usa canais e igarapé para reprodução, desova e alimentação. Em áreas próximas aos centros urbanos, esses peixes podem ser expostos a resíduos antropogênicos contendo misturas de metais que podem ser bioacumulados, resultando em alterações genéticas. Portanto, o nosso objetivo foi determinar a extensão das alterações nucleares em eritrócitos de fêmeas de* C. asellus *resultantes da bioacumulação de metais em tecidos e órgãos. As concentrações dos metais foram analisadas por espectrofotometria de absorção atômica. As concentrações de metais nos tecidos decresceram nessa ordem: Fe > Zn > Cu > Mn > Hg > Pb > Ni > Cd > Cr. Nos tecidos/órgãos dos peixes, a concentração de metais seguiu, em ordem decrescente, fígado > pele > gônadas > musculatura > ossos. As alterações nucleares mais frequentes foram invaginação nuclear, brotamento nuclear e núcleo lobulado. As concentrações de metais e as alterações nucleares observadas podem prejudicar o* C. asellus*, comprometendo a conservação desta espécie na foz do rio Amazonas.*

Palavras chave: *Amazônia brasileira, Baiacu Amazônico, contaminantes inorgânicos, biomarcadores de genotoxicidade*

INTRODUCTION

The Amazon Basin harbors the greatest biodiversity on the planet, and many fish species are considered restricted to the Brazilian Amazon (Dagosta & Pinna, 2019, Rico et al., 2022). Found within this endemic fish group is the species *Colomesus asellus* (Müller & Trochel, 1849), popularly known as "Amazonian Puffer". The Tetraodontiformes order is predominantly composed of marine fish species; however, the *Colomesus* is the only freshwater genus found in the Amazon Basin (Araujo-Lima et al., 1994, Viana et al., 2017). *C. asellus* is small, reaching 11 cm in length (Araujo-Lima et al., 1994, Amaral et al., 2013). It is considered sensitive to low concentrations of dissolved oxygen, and in dangerous and stressful situations, it has the ability to inflate its body. As a defense mechanism against predators, it produces the neurotoxin tetrodotoxin (Araujo-Lima et al., 1994, Camargo & Maia, 2008, Amaral et al., 2013). Despite the neurotoxin produced by pufferfish species, its muscle tissue is highly appreciated in oriental cuisine, especially in such countries as China, Korea, Japan, and Taiwan, owing to its high nutritional value (Karunanidhi et al., 2017).

This species, which exhibits benthopelagic behavior and a carnivorous feeding habit, is characterized by its four dental plates capable of crushing the rigid carapaces of its prey, allowing it to feed mainly on mollusks and other aquatic invertebrates (Oliveira et al., 2006). Owing to its behavior and yellowish-gray color patterns (Fig. 1), with several dark and irregular transversal bands on the back of the head and trunk, *C. asellus* is appreciated as an ornamental fish and exported to several countries (Bartolette et al., 2018). It is important to emphasize that *C. asellus* uses areas of channels and streams for reproducing, spawning, and feeding (Araujo-Lima et al., 1994). Along with urban and industrial expansion comes an increase of metals carried by effluent that flows into river water, directly harming this fish species, as well as security of the entire food chain (Paschoalini & Bazzoli, 2021), and posing a risk to the conservation of freshwater species.

Macapá, capital of the Brazilian state of Amapá, has an estimated population of 522 357 inhabitants and a land area of 6 563 849 km^2 (IBGE, 2021). Part of this area is bounded by the left bank of the Amazon River. The waters of the Amazon River are used for public supply, as well as a source of fish for the local fishing industry, fishing families, riverine and indigenous populations (Sá-Oliveira et al., 2016, Albuquerque et al., 2020). This segment of the Amazon River is also used as the main route for various types of boats that transport various products for sale along the riverbanks (Dos Santos Rodrigues et al., 2018). Despite the importance of its water, urban, industrial, and domestic waste generated in Macapá is discharged directly into the Amazon River (Sá-Oliveira et al., 2016).

Thus, Amazonian fish species are increasingly vulnerable to the exposure of different types of pollutants, especially toxic metals. Increased release of toxic metals in water bodies is mainly related to the expansion of agricultural and livestock areas, installation of hydroelectric plants, and mineral extraction (Viana et al., 2020, Araújo-Flores et al., 2021, Souza-Araujo et al., 2022, Nascimento et al., 2022). In addition, many agrochemicals have toxic metals in their composition, either as components or as contaminants, and these toxic residues do reach water resources (Ribeiro et al., 2017). Such exposure is known to alter physiological and biochemical functions that affect growth and reproduction (Jezierska et al., 2009, El-Shenawy et al., 2021) which calls for the urgent implementation of conservation strategies to preserve native and endemic species of Amazonian fish (Araújo-Flores et al., 2021, Nascimento et al., 2022). However, studies reporting on the impact of metals on freshwater fish species endemic to the Neotropics are still relatively scarce (Paschoalini & Bazzoli, 2021).

The Araguari River is one of the tributaries of the Amazon River and one of the most important rivers in the State of Amapá. Viana et al. (2020) reported high concentrations of Cd, Pb, Fe, Hg, Cu and Zn in water samples from the Araguari River. These metals, in bioavailable fraction, can bioaccumulate in different tissues and organs of fish (Karunanidhi et al., 2017). Hacon et al. (2020) found high concentrations of Hg in several freshwater species of Amazonian fish. For example, in 47 species of marine fish sampled on the Amazon Coast, high concentrations of Hg, Pb, Cd and As were observed (Souza-Araujo et al., 2022). Particularly, As was present in worrying concentrations in 63.8 % of the sampled species (Souza-Araujo et al., 2022). Together, these results indicate the need to evaluate bioaccumulated metals in tissues of endemic fish from the Amazon Basin since high concentrations of bioaccumulated metals can cause genotoxic alterations (Viana et al., 2020, Souza-Araujo et al., 2022).

Genotoxicity from the presence of bioaccumulated metals in fish can lead to carcinogenesis, loss of local biodiversity, and ecological imbalance (Gerolin et al., 2020). In addition, metals can induce genotoxic damage in several cell types, including erythrocytes, leading to interference in cell division, chromosomal breaks, or losses, and, in the long term, loss of entire native and endemic fish species (Maceda-Veiga et al., 2015). In this sense, the evaluation of genotoxicity biomarkers that do not require the sacrifice of fish, such as nuclear alterations, provides early signs of genetic damage and contributes to both environmental monitoring and fish conservation (Guilherme et al., 2012). Therefore, we aimed to identify genotoxic biomarkers in erythrocytes of female *C. asellus* as evidence of the bioaccumulation of such metals as Cu, Cd, Cr, Fe, Mn, Ni, Pb, Zn, and Hg in tissues and organs.

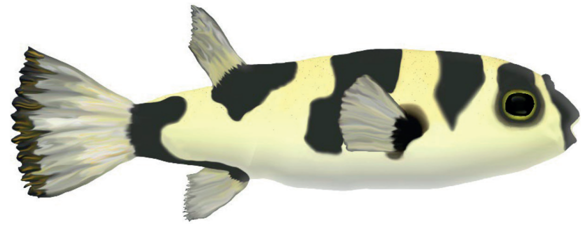

Figure 1. Image of *Colomesus asellus*, popularly known as "Amazonian Puffer". *Imagem de* Colomesus asellus*, popularmente conhecido como "baiacu amazônico".*

MATERIALS AND METHODS

Sampling site and fish sampling

Samples of fish were collected at the mouth of the "Pedrinhas Channel" on the banks of the Amazon River in the city of Macapá (Fig. 2). In total, 10 female *C. asellus* were sampled with standard length of 9.49 ± 0.97 (cm), weight of 48.90 ± 12.62 (g), and mature gonads in stage (C) of reproduction. These samples were taken during the daytime between March and June 2019, immediately after the daily tidal inflow of water from the Amazon River. *C. asellus* specimens were collected using casting nets and gillnets with mesh of various sizes (1.5 - 8.0 cm between adjacent nodes). After capture, the tail fin was punctured to obtain blood samples, followed by evaluation

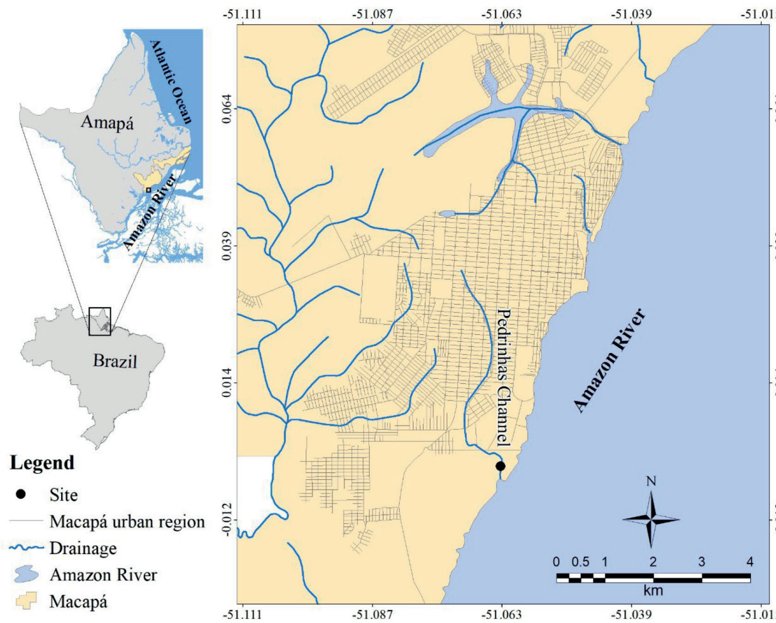

Figure 2. Location of the sampling site at the mouth of the "Pedrinhas Channel" on the bank of the Amazon River in the city of Macapá, state of Amapá, Brazil. *Localização do local de amostragem na foz do "Canal das Pedrinhas" às margens do Rio Amazonas na cidade de Macapá, Estado do Amapá, Brasil.*

for standard length (cm) and total weight (g). Fish specimens were immersed in ice water to reduce their activity, and then they were sacrificed by cervical transection to collect samples of muscle, liver, female gonads, bone, and skin.

Stages of gonadal maturation were recorded. Gonad stages were determined macroscopically, according to Vazzoler (1996), as A = immature; B = maturing; C = ripe; D = semi-spent; and E = spent. Taxonomic identification of the species was performed using specialized literature (Santos et al., 2004). This project was approved by the Committee on Ethics for the Use of Animals at the Federal University of Amapá (UNIFAP - 017/2019).

Fish blood samples for analysis of genotoxicity biomarkers

Blood samples were obtained through caudal puncture with heparinized syringes. For each sample, two thin smears were prepared on slides using a single drop of blood; then the smears were dried in air for 15 min and fixed in ethanol. Smears were stained using Giemsa 10 % solution (De Jesus et al., 2016). Count of nuclear changes was performed under optical microscopy (Nikon Eclipse, E200) with ×1000 magnification. One thousand erythrocytes were counted on each of two slides, totaling 2000 erythrocytes per fish sample. The percentage of nuclear alterations was calculated by the ratio between the total number of altered erythrocytes and the total number of observed erythrocytes, followed by multiplying the product of this ratio by 100. To calculate the genotoxicity index, all nuclear changes identified in fish samples were grouped, followed by calculating the ratio between the total number of altered cells and the total number of cells observed and then multiplying this ratio by 100 (Viana et al., 2021).

Protocol for preparation of fish tissues and organs

Fish samples of muscle, liver, female gonads, bone, and skin were frozen for a maximum period of 30 days until analysis of metals. Briefly, af-

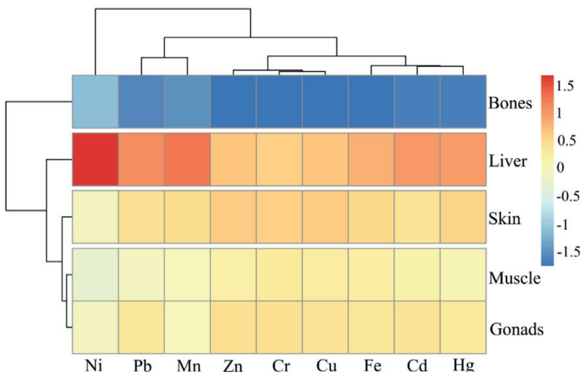

Figure 3. Hierarchical clustering of average concentration for each metal found in the pool of tissue samples collected from females of *C. asellus*. *Agrupamento hierárquico de concentração média para cada metal encontrado no pool de amostras de tecido coletadas de fêmeas de* C. asellus.

ter removal of these samples, they were weighed. Aliquots of 0.5 g of each tissue sample were placed directly in a digestion tube, followed by the addition of 10 mL sulfonitric mixture (HNO_3: H_2SO_4; 1:1 v/v) with 0.1 % (w/v) vanadium pentoxide and left to stand for 2 h. Afterwards, the mixture was heated in a water bath at 90 °C for 3 h. Then, 5 mL 7 % (w/v) $KMnO_4$ were added, and the mixture was heated in a water bath at 90 °C for another 3 h. Excess oxidant was reduced with 900 µL 20 % (w/v) hydroxylamine hydrochloride solution. Tube contents were transferred to a 50 mL volumetric flask, completing the volume with double-distilled water (DDW).

Chemical analysis of metals

Blank samples were prepared according to the protocols proposed by Vieira & Passarelli (1996) and Morgano et al. (2005). The digested samples from each tissue were submitted to analysis of Cd, Pb, Cr, Ni, Fe, Mn, Cu and Zn, in duplicate, using an Atomic Absorption Spectrophotometer (Shimadzu, model AA7000) with flame atomization. To quantify total Hg present in the digested samples, we used a hydride generator coupled with Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), according to the method proposed by Morgano et al. (2005). Each metal was measured according to its calibration curves with the following detection limits (µg/g): Cu = 0.06; Cd = 0.01; Cr = 0.01; Fe = 0.05; Mn = 0.03; Ni = 0.04; Pb = 0.06; Zn = 0.08; and Hg = 0.10.

Statistical analysis

Spectrophotometry is a method used to measure how much a chemical substance absorbs light by measuring the intensity of light. In this case, the similarity in intensities of metal concentrations in tissue samples from *C. asellus* females was visualized using a hierarchical grouping by tissue sample type. The intensity of metal concentrations by tissue was centered by the mean (zero) and standard deviation (1). Using a "heatmap" command in the "pheatmap" package, a specific color-coded grouping by tissue was generated when the intensity of metal concentration was represented by different colors. Color scale represents mean values where red squares indicate above-average values for a particular metal, and blue squares indicate below-average values. All analyses were performed using the R platform (R Development Core Team, 2021).

RESULTS AND DISCUSSION

Bioaccumulation of metals in different tissues of *C. asellus*

Metal concentrations decreased in tissue samples from *C. asellus* females in the following order: Fe > Zn > Cu > Mn > Hg > Pb > Ni > Cd > Cr (Table 1). Karunanidhi et al. (2017) emphasize that the bioaccumulation of metals in fish, mainly Pb, Cd and Hg, which are highly toxic metals, can cause damage to vital organs, blood cells and reproductive organs, in addition to causing changes in the behavior and development of organisms. Moreover, when metals reach high concentrations in fish tissues, even those considered essential, serious damage, such as nephrotoxicity, can occur (Karunanidhi et al., 2017).

By using the method of hierarchical grouping, differentiation in the concentration of bioaccumulated metals was revealed among the different tissue samples such that higher concentrations

Table 1. Average metal concentrations (µg/g) in each tissue sample type from *C. asellus* females. *Concentrações médias de metal (µg/g) em cada tipo de amostra de tecido de fêmeas de* C. asellus.

C. asellus	Average metal concentrations (µg/g)								
tissues	Fe	Zn	Cu	Mn	Hg	Ni	Pb	Cd	Cr
Bones	44.16	35.19	26.38	0.62	0.43	0.20	0.23	0.05	0.05
Liver	49.27	35.88	26.50	0.77	0.49	0.36	0.28	0.06	0.05
Skin	44.16	35.19	26.38	0.62	0.42	0.20	0.23	0.05	0.05
Muscle	39.22	29.43	22.09	0.50	0.35	0.18	0.18	0.05	0.05
Gonads	41.24	33.08	23.65	0.50	0.38	0.20	0.22	0.05	0.05

were evident across the spectrum in liver tissue, followed by skin, gonads, muscles, and the lowest concentrations in bones (Fig. 3). Studies on the accumulation of metals in different species of pufferfish are scarce. However, in the marine pufferfish species *Takifugu oblongus*, *Lagocephalus guentheri*, *Arothron hispidus*, *Chelodan patoca*, and *Arothron immaculatus*, Karunanidhi et al. (2017) observed relatively high concentrations of Cd (0.24 µg/g), Cu (1.80 µg/g), Pb (8.31 µg/g) and Zn (43.37 µg/g) in organs such as skin, gills, ovary, intestine, muscle, kidney, and liver. Karunanidhi et al. (2017) also found that Pb concentrations decreased in the following order: kidney > skin > intestine > liver > muscle > gills > ovary. In the marine species *Lagochepalus lunaris* (Banana Puffer fish), Nurjanah et al. (2015) found high concentrations of Zn (73.63 µg/g) in muscle tissue and skin (41.80 µg/g). In internal organs, Fe (167.03 µg/g), Pb (57 µg/g) and Cd (0.19 µg/g) presented high concentrations (Nurjanah et al., 2015). These findings support our results for the freshwater species *C. asellus* with respect to the bioaccumulation of metals in different organs. Such multi-organ-specific bioaccumulation of metals may be linked to benthopelagic foraging behavior and feeding habits (Karunanidhi et al., 2017). For instance, Nurjanah et al. (2015) also attributed metal accumulation to the feeding habits of *L. lunaris*.

Particularly, the greater accumulation of metals in liver tissue may be mainly related to organ functions that include metabolism, energy storage, and biotransformation of toxicants (Viana et al., 2020), in addition to being a highly vascularized organ that receives a high blood supply. The accumulation of metals in the liver tissue of *C. asellus* can lead to injury and disease, even death in extreme situations (Urien et al., 2018). Elevated concentrations of metals in the liver are also associated with increased vacuolization, hypertrophy, and necrosis of hepatocytes (Weber et al., 2020). The liver´s ability to accumulate metals makes the quantification of these contaminants in the liver tissue of fish relevant for assessing the contamination of aquatic environments (Viana et al., 2020). Metal concentrations in *C. asellus* liver tissue are similar to those previously found by Viana et al. (2020) in liver samples from four native fish species (*Anodus orinocensis*, *Hemiodus unimaculatus*, *Curimata vittata*, and *Plagioscion squamosissimus*) collected in the Araguari River lower section, also located in the state of Amapá. These results are also corroborated by the findings of Albuquerque et al. (2021) who determined the concentrations of inorganic contaminants in liver tissue of *Cichla temensis* (carnivorous species) and *Pterygoplichthys pardalis* (detritivorous species). Based on this finding, they recommended the use of this approach for biomonitoring the contamination of aquatic environments, such as that found in the lower Amazon River. It is important to note that the liver is also an organ that contributes to reproductive activity in female fish, mainly from the production of vitellogenin, which is essential for the normal development of embryos (Wolf & Wheeler, 2018, Vicentini et al., 2022). Thus, studies conducted in natural environments can provide a more realistic picture of the health status of fish exposed to metals present in aquatic environments. However, it is important to emphasize that these studies are not conducted under controlled conditions. As a result, important information, such as exposure time and possible synergistic or antagonistic influences from other classes of contaminants, is difficult to assess. Thus, in recent years, studies on the impacts of metals present in natural environments located in the Neotropical region mainly addressed the concentration/accumulation of different metals in muscles and other organs such as liver (Paschoalini & Bazzoli, 2021).

The bioaccumulation of metals in the skin of *C. asellus* is related to direct contact of the fish with contaminated water. This is a remarkable finding in that this tissue is considered an important barrier against the entry of this type of contaminant (Uysal et al., 2008). Owing to the exchange reaction with calcium ions for metals, bone development can be retarded in *C. asellus* (Rezk et al., 2018). According to the literature, metals are accumulated and stored in bone structure with resultant loss of Ca levels (Hansson et al., 2020). The concentrations of Cd and Hg found in the muscle tissue of *C. asellus* were similar to those found by Da Silva Costa et al. (2022) in the muscle tissue of the fish species *Plagioscion squamosissimus* (Cd ~ 0.05 μg/g and Hg ~ 0.31 μg/g), which was collected in the Araguari River middle section, also located in the State of Amapá.

The accumulation of metals in the gonads of *C. asellus* females can result in reproductive impairment, damage to reproductive germ cells, delay in embryonic development, as well as an increase in the incidence of deformities and mortality rate. As a result, offspring with lower vitality will be produced, and the rate of survival will be reduced (Cazan & Klerks, 2015, Urien et al., 2018). Lopes et al. (2020) reported that the sources of contamination by metals in the Amazon Basin are related to the sum of various human activities in its surroundings, placing emphasis on urbanization, mineral extraction, deforestation, and forest burning. All these factors contribute to the transport of waste contaminated with mixtures of toxicants, including metals, into the waters of the Amazon River. Vicentini et al. (2022) highlighted that some metals, especially Cd, are considered endocrine disruptors in different fish species, which can affect their reproductive parameters. Therefore, assessment of the damage caused by inorganic contaminants in female fish is essential since these toxicants can be transferred to offspring or even affect the early stage of embryos (Bila & Dezotti, 2007, Vicentini et al., 2022). Vicentini et al. (2022) also reported that environmentally relevant concentrations of Cd (0.1 and 100 μg/L) could alter the reproductive aspects of the native fish species *Rhamdia quelen*.

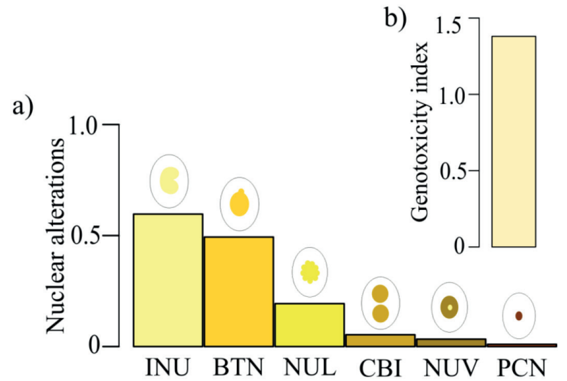

Figure 4. a) Average values of nuclear invagination frequency (INU); nuclear budding (BTN); lobulated nucleus (NUL); binucleated cell (CBI); vacuolated nucleus (NIV); and pyknosis (PCN) in *C. asellus* erythrocytes. b) Genotoxicity index. *a) Valores médios de frequência de invaginação nuclear (INU); brotamento nuclear (BTN); núcleo lobulado (NUL); célula binucleada (CBI); núcleo vacuolado (NIV); e picnose (PCN) em eritrócitos de* C. asellus*. b) Índice de genotoxicidade.*

Genotoxicity biomarkers

To evaluate the genotoxic effects on *C. asellus* erythrocytes, six types of nuclear alterations were investigated. Nuclear invagination was the most frequent alteration, followed by nuclear budding, lobulated nucleus, binucleated cell, vacuolated nucleus and pyknosis (Fig. 4a). Nuclear alterations that most contributed to genotoxicity index increases were nuclear invagination, nuclear budding and vacuolated nucleus with means of ~ 0.63, ~ 0.37, and ~ 0.22, respectively (Fig. 4b).

The occurrence and increase in the frequency of genotoxic damage in fish erythrocytes are a function of the sum total of pollutants to which fish are exposed in the aquatic environment related to the sum of the action of pollutants existing in the aquatic environment (Azevedo-Silva et al., 2016, Calado et al., 2020). The nuclear alterations observed in the erythrocytes of *C. asellus* females constitute evidence of the existence of genotoxic contaminants in the waters of the “Pedrinhas Channel”, which is located at the mouth of the Amazon River (Viana et al., 2021). Gomes-Silva et al. (2020) pointed out that the presence of genotoxic contaminants in water impairs the interaction between DNA molecules and nuclear proteins during cell division, resulting in changes in the nuclear morphology of fish erythrocytes. Using the model fish *Danio rerio*, Viana et al. (2021) investigated toxic and genotoxic effects associated with metals present in water samples collected from the Araguari River, a tributary of the Amazon River. In comparison to the results obtained by Viana et al. (2021), the species *C. asellus* showed greater genetic damage in erythrocytes, mainly from nuclear invagination, nuclear budding and lobulated nucleus. The comparison also revealed higher genotoxicity index for *C. asellus*. It should be noted that various metals can bind to plasma proteins in fish blood and induce nuclear changes in erythrocytes (Vicentini et al., 2022).

Excess metals in the environment, even those essential to aquatic life, can have genotoxic effects. Current legislation correctly forbids such excess to protect aquatic life from damage to genetic material or, in some cases, apoptosis of affected cells, such as that which occurs with nonessential toxic metals (Palermo et al., 2015, Tuzuki et al., 2017; Murray & Carr, 2018, Guo et al., 2021). Palermo et al. (2015) observed damage to the genome of *Prochilodus lineatus* after exposure to Ni. Tuzuki et al. (2017) reported that Mn induced genotoxic effects, including DNA strand breaks, in fish erythrocytes. For nonessential metals, such as Pb, Hg, Cd and Cr, several studies have reported their genotoxic potential in fish, even when present in low concentrations in the aquatic environment (Pereira et al., 2016, Ali et al., 2020, Viana et al., 2020, Viana et al., 2021).

Sá-Oliveira et al. (2016) emphasized that the edge of the city of Macapá, bounded by banks of the Amazon River, is subject to different types of anthropogenic impacts and stress, especially the discharge of improperly treated urban waste. Thus, the Amazon River estuary acts as the main recipient of industrial and domestic effluents, often without the necessary treatment (Dos Santos Rodrigues et al., 2018). In this context, *C. asellus* is exposed to different sources of potentially bioaccumulated pollutants. In the long term, this could lead to the reduction and eventual disappearance of this endemic species. Therefore, contamination and accumulation of potentially toxic metals in fish is a major national and international concern (Hacon et al., 2020). In addition, capture for the aquarium trade contributes to the decrease in ornamental fish populations, including *C. asellus*, in the Amazon Basin (De Melo et al., 2019).

CONCLUSION

Females of *C. asellus* showed higher concentrations of metals in liver tissue, followed by skin, gonads, muscles, and bones, indicating contamination of their habitat by metals, and suggesting that this area is not suitable for the conservation of this endemic fish species. Such high metal concentrations are known to affect reproductive capacity. Moreover, sampled females showed nuclear alterations in their erythrocytes, particularly nuclear invagination, nuclear budding and lobulated nucleus. Thus, the occurrence of *C. asellus* in this region may be threatened. This calls for the development of specific programs for monitoring and environmental management of Amazon River water quality in order to guarantee the maintenance of the biodiversity of native species.

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