Genotoxicity assessment in two Amazonian estuaries using the *Plagioscion squamosissimus* as a biomonitor

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Abstract

Genotoxicity studies in coastal ecosystems have been a priority in Environmental Risk Assessment (ERA). This research aimed to study the genotoxicity by the micronucleus test and comet assay in two Brazilian Amazon estuaries (anthropized and control) using *Plagioscion squamosissimus* as a biomonitor. Blood samples were collected from 54 specimens. No significant genotoxic effects were detected in the cells analyzed, although the highest occurrence (MN and DNA damages) was observed in anthropized site. The percentage of genomic damage differed between the sites studied, being always higher in anthropized site as well. Of the nucleoids analyzed in this site, on average, $28 \pm 14.42\%$ of the cells were classified in the highest damage class. The fish analyzed in the present study are directly influenced by xenobiotic agents capable of producing damage to the genetic material of aquatic organisms in both sites and, consequently, may bring consequences still little reported in studies of morphophysiological alterations in humans.

Keywords Micronuclei · Brazilian Amazon estuaries · Comet assay · Xenobiotic agents · Genotoxicity · Environmental toxicology

Introduction

The hematological analysis of fish exposed to contaminants has been used increasingly for the monitoring of environmental quality, considering that these organisms respond to

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toxic compounds in a similar manner to other higher vertebrates, and may thus be used as biomonitor for the evaluation of chemical substances that are potentially teratogenic or carcinogenic in humans (Rivero 2007; Ranzani-Paiva et al. 2013; Oliveira and Valdes 2019; Correia Jorge et al. 2017; Bianchi et al. 2015). A number of different analytical tools have been developed for the detection of these alterations, including the comet assay, also known as single cell gel electrophoresis (SCGE), which has been widely used to test for the effects of the genotoxic agents found in industrial, domestic, and agricultural waste, which may damage the DNA or induce repairs to this material in a given environment, as well as being employed in cancer research (Hartmann et al. 2003; White and Rasmussen 1998; Lin et al. 2020). This type of assay was proposed by Ostling and Johanson (1984), and can be used in a range of different applications, including toxicological genetics, ecotoxicology, and studies of DNA repair and apoptosis (Lin et al. 2020). The technique was modified and perfected by Singh et al. (1988), through the introduction of alkaline conditions, and the development of single cell electrophoresis, which has been adopted by the majority of the laboratories that use this approach in tests of genetic toxicology (Moller 2005; Rivero 2007; Yang et al. 2021; Gomes et al. 2016).

The micronucleus test (MN), which is used as an experimental model for the detection of the genotoxic effects provoked by a range of physical and chemical agents, is a second widely used method in studies of environmental genotoxicity (Ranzani-Paiva et al. 2013; Hussain et al. 2018; Lin et al. 2020). These procedures evaluate the effects of pollutants on the biota of contaminated aquatic environments, to which humans may also be exposed (Matsumoto et al. 2006; Martins et al. 2010; Ranzani-Paiva et al. 2013; Mahurpawar 2015; Zahra Naseem Kalim Imran 2017). The MN test was developed by Schmid (1975), who initially focused on mammalian bone marrow cells, and has been used extensively to test the genotoxicity of chemical compounds in invertebrates, fish, and amphibians, with excellent results for the monitoring of contaminated areas (Çavas and Ergene-Gözükara 2005). The frequency of MN observed at any given moment can be considered to reflect the complex interaction between the genotoxic activity and the physiological mechanism of the test organism. In contrast with mammals, MN are found in the peripheral blood of fish, which avoid the need to extract the bone marrow (Mersch et al. 1996; Campana et al. 2003; Hamada et al. 2015; Hayashi 2016).

Considering the ecological and economic importance of the Amazonian estuaries that were the focus of the present study, the identification of the possible impacts caused by local bauxite smelting operations would provide important insights for the implementation of measures designed to minimize the deleterious effects of these impacts on the local biota and, ultimately, the human populations in the

area. Given this, the present study investigated environmental toxicity in the Murucupi River, an environment impacted by an industrial complex, and Furo da Laura, a control area, using the South American silver croaker, *Plagioscion squamosissimus* (Heckel 1840) as a biomonitor of environmental quality. This species is an important fishery resource in both study areas, where it is consumed by the local population, and is also exported as a fishery product (Viana et al. 2013; Furtado-Júnior et al. 2015; Rocha et al. 2016).

Material and methods

Study areas

The present study was based on the analysis of samples of the peripheral blood of *P. squamosissimus* collected in two estuarine environments, the Murucupi River, an environment that suffers anthropogenic impacts, and Furo da Laura, which was used as the control area (Fig. 1). The distance between the sampling sites was approximately 180 km, no water connectivity between them. The samples were collected during the dry season month of August, 2019, and the rainy season month of March, 2020. These areas were selected due to their proximity to the Pará state capital, Belém, the city in which the laboratories for the toxicological analyses are located. In addition, the population quantified by the latest demographic census reinforces the direct anthropic changes in each of the cities.

The Murucupi River, which is the principal watercourse of the Murucupi hydrographic basin, is located in the municipality of Barcarena, in northeastern Pará state, in northern Brazil. This municipality is part of the metropolitan region of Belém, the Pará state capital, which population according the last census (IBGE 2021) were about 129, 000 people, which is located within the Lower Tocantins microregion. This river was selected for the present study due to the high level of contamination of the water, derived from the effluents discharged by local residences and industrial installations (Hazeu 2015; Piratoba et al 2017). The river is approximately 8 km long, between its source in the area of environmental preservation near a tailing pond for the red mud produced by the Hydro/Alunorte bauxite smelting plant, and the Furo do Arrozal, a tributary of the Pará River, while passing through the principal urban centers of the municipality of Barcarena (Junior et al. 2019; Lemos and Pimentel 2021). The region has a rainy equatorial climate, with mean annual precipitation of 2587.7 mm, and rainfall of more than 60 mm in most months (Cunha 2018).

The Furo da Laura estuary is the principal hydrographic feature of the municipality de Vigia, in northeastern Pará, in the state's Salgado microregion, with population of about 54, 000 people according to the last census (IBGE 2021). This

Fig. 1 Location of the sampling point where the *P. squamosissimus* specimens were collected in two estuaries in eastern Brazilian Amazonia (A) Brazil; (B) Pará state; (C) map legend; (D) Furo da Laura estuary in Vigia; (E) Murucupi River in Barcarena

ample estuary has a heavy daily traffic of both artisanal and industrial fishing vessels (Lima et al. 2015a, b). The margins of the estuary are dominated by Holocene salt marshes and mangroves, with the vegetation cover being formed primarily by a mixture of mangrove forest and typical terra firme vegetation (Lima et al. 2015a, b). The local climate is typical of the equatorial Amazon, being super-humid with relatively high temperatures, i.e., 26–30 °C (Santos et al. 2020b), with a marked division into rainy and dry (or less rainy) seasons, with monsoon-type rains during the six first months of the year (January through June), that is, the rainy season, and much drier conditions during the second half of the year (July through December), which is defined as the dry season (Silva et al. 2011).

Collection and preparation of the samples

The specimens were collected with the assistance of local fishers, who captured the animals in fish weirs of varying

sizes (young and adults, considering Lima et al. (2019) to the estimative of the first maturation size) which contributed to the minimization of any selective effects in terms of specimen size, with stress being reduced using the approach of Ishikawa et al. (2010). Abiotic water variables were measured using a multiparameter probe (Hanna model HI98194). Considering the pandemic scenario of COVID-19 in 2020, the rainy season samples were not performed in Barcarena.

The fish were placed immediately in a bucket containing water and 2% benzocaine (190 mg/L) for approximately 3 min prior to biometry and the collection of blood samples. Blood samples were collected from blood vessels in the caudal portion of each fish using 3-mL syringes containing the anticoagulant EDTA (ethylenediaminetetraacetic acid), following the method proposed by Ranzani-Paiva et al. (2013) and Ishikawa et al. (2010). Once the samples were collected, everyone was weighed (in grams) with a semi-analytic balance, and its total length (TL, in cm) was measured using an ichthyometer. All individuals were then released back

into the water. The blood samples were stored in Eppendorf type micro-centrifugation tubes, which were kept on ice in a cooler at a temperature of 5–7 °C (Ishikawa et al. 2010) for transportation to the laboratory.

The comet assay was based on the procedure of Singh et al. (1988), with minor modifications (Hartmann and Speit 1997). In the comet assay, cells with greater DNA damage exhibit greater migration of chromosomal DNA from the nucleus toward the anode. Such migration resembles the shape of a comet, when seen under a fluorescent microscope and hence the name of the test. The amount of DNA migration indicates the amount of DNA damage in the cell. Such damage can be scored by computer or visually, as performed in our experiments (Bajpayee et al. 2019). DNA damage was visualized at a magnification of 1000× under an epifluorescence microscope equipped with a 515–560-nm excitation filter and 590-nm barrier filter. For the comet assays, being allocated to one of the five damage classes defined by Collins et al. (2001). Using a cell counter, 100 nucleoids were counted per animal, that is, 50 on each slide, using the visual classification approach based on the migration of the DNA fragments, with the damage being assigned to one of five classes (Wachtel et al. 2019): 0 (no apparent damage), 1 (some damage), 2 (moderate damage), 3 (extensive damage), and 4 (maximum damage, apoptosis). These numerical values considered to be the ranks of the damage classes (0–4) for the statistical analyses. The DNA damage index (DI) was calculated according to the following formula: $DI (au): [(N1 * 1 + 2 * 2 + 3 * 3 + 4 * 4)] / 100$ (total number of analyzed cells), where *DI* is the DNA damage index, *au* is the arbitrary unit, and *N1–N4* are cells in classes 1, 2, 3, and 4. The micronucleus tests were conducted following Al-Sabti et al. (1995), using blood on clean, labeled slides. The frequency of micronuclei was determined based on the method proposed by Carrasco et al. (1990) and Oliveira et al. (2020). Once stained, the slides were visualized under an immersion optical microscope at a magnification of 1000×. A total of 2000 cells were analyzed per individual, to determine the number of micronuclei and morphonuclear alterations (MNA) present in each cell, using a Zeiss Primo Star optical microscope at a magnification of 1000×, with a manual blood cell counter. The data were recorded in Microsoft Excel® spreadsheets.

In addition to the presence of micronuclei, the nuclear abnormalities present in the cells were described, counted, and classified following Carrasco et al. (1990) as: (1) blebbed (nucleus with a minor evagination of the nuclear membrane with euchromatin or heterochromatin); (2) lobed (nucleus with larger and more ample evagination than the blebbed cells); (3) vacuolated (nucleus containing vacuoles); or (4) notched (nucleus well defined morphologically). The data on the quality of the water of the Murucupi River were obtained from the HIDROWEB site of the Brazilian

National Water Agency (ANA), as well as monitoring by the Chemical Environmental Laboratory (LQA) of the Federal Rural University of Amazonia (UFRA) in Belém.

Statistical analyses

The number of micronuclei and morphonuclear alterations were analyzed per specimen, considering the site (natural or anthropogenic), and local season (dry and rainy), as well as all possible combinations of these variables. Prior to the analyses, the data were tested for normality and the homoscedasticity of variances, to ensure the application of one-way and factorial analyses of variance (ANOVA), considering an error of 5%. The analyses were all run in the Statistica 10.0 software.

Finally, a redundancy analysis (RDA) of all the effects and factors analyzed in the study was run in CANOCO 4.5 (Software for Canonical Community Ordination), with the significance of the contribution of the independent variables (study areas and season) to the variability in the data being assessed by 9999 permutations (Monte Carlo Method). This analysis included only the significant ($p < 0.05$) variables identified in the analysis. The dependent variables (the percentage of micronuclei and the categories of DNA damage) were allocated to separate presence/absence matrices, in which each line represents an individual, with each matrix being related to a second (“treatment”) matrix, to which the independent variables were added one by one.

Results

A total of 54 specimens were collected during the present study, in the two estuaries. The fish collected in Vigia were significantly larger (TL) on average than those from Barcarena ($F = 17.69$; $p < 0.01$), although the specimens from Barcarena were heavier than those from Vigia (Table 1), but the difference was not significant ($F = 0.085$; $p > 0.05$).

No significant variation was found in the pH, although the dissolved oxygen concentrations were lower in the Murucupi River than Furo da Laura. The highest nitrate concentrations were recorded in Barcarena in both seasons (Table 2).

Comet assays

The DNA damage index varied significantly between the study sites ($F = 64.96$, $p < 0.01$) and was higher in Barcarena than in Vigia (Fig. 2). Table 3 shows the distribution of the different nucleoid classes observed in the samples from the two study sites (Fig. 3). On average, $28.25 \pm 14.4\%$ of the nucleoids analyzed in the samples from Barcarena were assigned to the highest damage class (4), whereas only 1.8%

Table 1 Parameters of total length (cm) and weight (g) of the *P. squamosissimus* specimens collected in Barcarena and Vigia, in eastern Brazilian Amazonia

Study area	Season	N	TL (cm)				Weight (g)			
			Max	Mean	Min	SD	Max	Mean	Min	SD
River Murucupi	Dry	16	35.5	31.91	28	2.70	905	646.19	239	195.05
	Rainy	24	29.5	21.06	17	3.21	240	98.46	27	51.85
Furo da Laura estuary	Rainy	14	40.2	32.84	23.6	4.44	474	293.5	114	107.32

Table 2 Abiotic variables (Temp. temperature, DO dissolved oxygen) recorded in the rainy and dry seasons in Barcarena and Vigia, in eastern Brazilian Amazonia *

	Murucupi River		Furo da Laura estuary		Acceptable values*
	Dry	Rainy	Dry	Rainy	
Temp. °C	30.36	29.39	28.94	29.16	-
Ph	6.05	5.82	6.86	6.37	6–9
DO (mg.L ⁻¹)	5.60	5.16	8.48	7.28	>5
NO3 (mg.L ⁻¹)	2.70	5.00	0.85	0.49	<0.7
PO ₄ ³⁻	0.078	0.026	0.29	0.04	<0.093

Resolution by N°. 357/2005—Brazilian National Environment Council (CONAMA), for class II fresh water

Source: HidroWeb-ANA (2020)

of the cells in the samples from Vigia were assigned to this class (Table 3).

Micronuclei and morphonuclear alterations

A total of 107,990 erythrocytes were analyzed in the present study. Overall, the largest number of abnormal cells was observed in the specimens from Barcarena (Table 4; Fig. 4). All the nuclei observed with abnormalities were of the evagination type.

Uni- and multivariate analyses

The fish collected in the municipality of Vigia were larger (TL), on average, than those collected in Barcarena, although the latter were heavier. Significant differences were recorded

Fig. 2 Mean and standard deviation (sd) of the DNA damage index in *P. squamosissimus* collected of two estuaries in eastern Brazilian Amazonia **Table 3** Mean number (and standard deviation) of *P. squamosissimus* cells, from specimens collected in Barcarena and Vigia, which were assigned to each damage class (0 = no damage; 1 = some damage; 2 = moderate; 3 = extensive; 4 = maximum damage) in the comet assays

Study area	Comet assay cell damage (mean ± sd) %				
	0	1	2	3	4
Murucupi River	11.69 ± 10.70	24.13 ± 16.34	16.44 ± 5.1	20.13 ± 13.79	28.25 ± 14.43
Furo da Laura estuary	59.42 ± 16.78	22.92 ± 8.69	7.67 ± 7.70	8.5 ± 8.28	1.8 ± 2.20
					Total
					1610
					1200

Fig. 3 DNA damage classes visualized by optical microscope (1000×magnification) of *P. squamosissimus* specimens collected of two estuaries in eastern Brazilian Amazonia

Table 4 Frequency of nuclear abnormalities and micronuclei observed in 2000 *P. squamosissimus* erythrocytes from each of the specimens collected in Barcarena and Vigia in the dry and rainy seasons. NC normal cells; NA nuclear abnormality (evaginated cell); MN micronuclei

Study area	Season	Micronuclei test (mean $\pm$ sd)			
		NC	NA	MN	Total
Murucupi River	Dry	1.93 $\pm$ 0.25	7.44 $\pm$ 11.97	4.25 $\pm$ 6.76	79,990
	Rainy	2.00 $\pm$ 0.05	3.54 $\pm$ 4.29	0.75 $\pm$ 1.15	
Furo da Laura estuary	Rainy	1.99 $\pm$ 0.01	7.21 $\pm$ 7.67	0.69 $\pm$ 0.95	28,000

Fig. 4 Micronuclei (A) and morphonuclear alteration (B) visualized by optical microscope (1000×magnification) of the *P. squamosissimus* collected of two estuaries in eastern Brazilian Amazonia. Fast Panoptic

between the Vigia and Barcarena samples in the mean number of cells in damage classes 0, 2, 3, and 4, with the largest numbers in all classes except 0 being recorded at Barcarena, peaking in class 4 (in absolute and relative terms), with a mean of 28.25 ± 14.43 in comparison with 1.8 ± 2.20 at Vigia (Table 5; Fig. 2). The difference between estuaries in class 1 was not significant. The morphonuclear alterations recorded at Vigia were not significantly different from those recorded at Barcarena, although the presence of micronuclei at Barcarena was much greater than that recorded at Vigia (Table 5; Fig. 2).

In the redundancy analysis, the highest nitrate concentrations were recorded in quadrants 1 and 2 (Fig. 5), which were associated with the impacted area (Barcarena), while the highest pH and dissolved oxygen concentrations were associated with Furo da Laura, where the lowest levels of genomic damage and morphonuclear alterations were recorded, and the lowest number of erythrocytes with micronuclei. Overall, then, the cellular effects analyzed in the present study are associated with the quality of the water in the

two study estuaries, given that the factors tested accounted for 76.31% of the variability in the data (Table 6).

Discussion

Fish have been used as environmental biomonitor in a number of genotoxic studies (Grisólia and Cordeiro 2000; Souto 2004; Arias et al. 2007; Costa et al. 2008; Delunardo et al. 2013; Carrola et al. 2014; Lima et al. 2015a, b; Rocha et al. 2016; Bueno et al. 2017; Hussain et al. 2018; AnvariFar et al. 2018; Delunardo et al. 2020; Santana et al. 2020). These vertebrates are appropriate models for this type of research due to their capacity for the metabolism and accumulation of pollutants that also pose a risk to humans (Ranzani-Paiva et al. 2013; Ventura et al. 2015; Lima et al. 2015a, b). *P. squamosissimus* is an important fishery resource in the Amazon region, providing subsistence and a source of income for many local populations (Chao et al. 2015; Barbosa et al. 2021), although this species is also an important

Table 5 Results of the one- and two-way analyses of variance of the variation in the length, weight, and cellular abnormalities recorded in the *P. squamosissimus* specimens collected from two Amazonian estuaries. CA comet assay; 0–4 the level of genomic damage; AN morphonuclear alterations; MN micronuclei; VIG Furo da Laura, municipality of Vigia; BAR Murucupi River, municipality of Barcarena; P probability; Df degrees of freedom; MS mean squares; SS sum of the squares

Dependent variable	Factor	MS	SS	Df	Fisher's test	p	Pattern
Total length (cm)	Area	574.1	574.1	1	17.69	<0.01*	VIG > BAR
	Season	477.1	477.1		13.91	<0.01*	Dry > Rainy
Weight (g)	Area	5998	5998		0.085	>0.05	BAR ≥ VIG
	Season	2.55E+06	2.55E+06		118.5	<0.01*	Dry > Rainy
Kr	Area	3.568	3.568		19.19	<0.01*	BAR > VIG
	Season	8.351	8.351		88.9	<0.01*	Dry > Rainy
CA0	Area	1.56E+04	1.56E+04		84.33	<0.01*	VIG > BAR
CA1		10.01	10.01		0.054	>0.05	BAR ≥ VIG
CA2		527.5	527.5		13.08	<0.01*	BAR > VIG
CA3		926.7	926.7		6.68	<0.05*	BAR > VIG
CA4		4305	4305		32.63	<0.01*	BAR > VIG
NC		0	0		0.094	>0.05	BAR ≥ VIG
AN		46.36	46.36		0.692	>0.05	VIG > BAR
MN		20.85	20.85		1.26	>0.05	BAR > VIG

*Significant difference

Table 6 Results of the redundancy analysis (RDA) of the effects of the different variables (Fig. 3) on the *P. squamosissimus* specimens collected during the present study from two estuaries in the Brazilian Amazon basin

Parameter	Axis 1	Axis 2
Eigenvalue	0.7631	0.0810
Variation explained (cumulative)	76.31	84.41
Pseudo-canonical correlation	0.9941	0
Adjustment explained (cumulative)	100	-

animal model for the biomonitoring of aquatic environments (Rocha et al. 2016), given its sensitivity to toxic substances, as well as its relative abundance in most areas (Jonsson and Castro 2005; Santos et al. 2020a, b). In the present study, significantly higher levels of damage were recorded in the *P. squamosissimus* treatment (contaminated) group in comparison with the control in both the comet assays and the micronucleus test.

In the present study, the fish collected in the Barcarena were shorter (TL), on average, than those from Vigia, but heavier, with higher frequencies of micronuclei, nuclear alterations, and indices of comet damage in comparison with Vigia, which point to the influence of the differences in the quality of the water in the two estuaries. In particular, while the maximum damage class (4) was the most frequent in the Barcarena samples, with 28% of the nucleoids analyzed, it was the least frequent class (1.8%) in the Vigia samples, where a majority of the nucleoids (59%) were undamaged (class 0). The results of the micronucleus test were similar to the findings of Santos et al. (2015), who analyzed the weight-length ratio and the frequency of micronuclei in the tambaqui, *Colossoma macropomum*, exposed to agricultural toxins and herbicides in northern Brazil. As in the present

Fig. 5 Ordination diagram of the first two axes of the redundancy analysis of some abiotic factors (pH – hydrogen potential; DO – dissolved oxygen; FOF – phosphorous index) with the level of damage recorded in the comet assay (C0–C4), body weight (W-g), total and standard length (TL and SL, cm), morphological nuclear alterations (MNA), cells with micronuclei (MN), and normal cells (CN) in the *Plagioscion squamosissimus* specimens collected from two estuaries in eastern Brazilian Amazonia. RN=rainy season; VR=Vigia Rainy; BD=Barcarena Dry; BAR=Barcarena (Murucupi river); VIG=Vigia (Furo da Laura estuary)

study, these fish had gained relatively more weight than length, and the correlation between the weight-length ratio and the micronucleus test may be a useful parameter for the biomonitoring of contaminated environments (Santos et al. 2015), as well as providing important insights into the physiological condition of fish exposed to pollution, given that animals from more impacted environments tend to have a

lower condition factor (K) than those from unpolluted areas (Oliveira-Ribeiro et al. 2013).

As in the present study, descriptive indicators of size classes have been used in several studies to evaluate the seasonal and spatial variation in fish communities, which may be related to shifts in the behavior of the species and their physiological response to pollution (Arias et al. 2007; Costa et al. 2008; Copatti and Copatti 2011; Santos et al. 2015). In Senegal, for example, a reduction of the maximum length of the fish of a community was observed after 20 years of anthropogenic impact (Ecoutin et al. 2010). However, the fish collected from the Murucupi River in the present study were similar in size to those collected from this same river by Oliveira et al. (2019).

This study showed that the physical and chemical parameters of the water are complemented by the toxicity tests, given that these tests evaluate the potential effects of the substances found in the water on the local biological system. In the present study, the dissolved oxygen concentrations recorded in the Murucupi River were lower than those found in the control area, but higher than those recorded by Pereira et al. (2007), that is, 2.6 mg/L, which is well below the concentration recommended in resolution 357 of the Brazilian National Environment Council, CONAMA (DO > 5.0 mg/L). These authors associated the low dissolved oxygen concentration recorded in the Murucupi River with a significant increase, above natural levels, in the organic matter in the water, which was derived from the effluents discharged into the river. The relatively acid pH recorded in both study areas in both seasons is typical of Amazonian watercourses, which tend to be relatively rich in kaolinite and humic acid derived from the decomposition of plant material (Pereira et al. 2007; Medeiros Faial et al. 2017).

The endogenous occurrence of micronuclei is well documented, although it rarely exceeds one micronucleus per thousand cells, i.e., 0.1% (Thomé et al. 2016). There is no strict consensus on the number of cells that need to be analyzed per animal to provide a reliable estimate of the frequency of micronuclei, with published studies being based on samples of between 1000 and 10,000 cells. Ghisi et al. (2010) investigated the optimum sample size with the aim of standardizing procedures, and compared counts of 1000, 2000, 3000, and 4000 cells, eventually reaching the conclusion that a sample of only 1000 cells provided satisfactory results. In the present study, the counts of 2000 erythrocytes per animal proved to be more than adequate for the identification of micronucleated cells and nuclear alterations in the erythrocytes of *P. squamosissimus* from the two study estuaries. The numbers of micronuclei and morphological nuclear alterations found in both Barcarena and Vigia were similar to the findings of Rocha et al. (2016), who also used *P. squamosissimus* as a bioindicator in the Marajó Archipelago of northern Brazil, although the values were much

higher than those recorded in the Nile tilapia, *Oreochromis niloticus* (Cichlidae), by Bueno et al. (2017) from a reservoir in southeastern Brazil during both the dry and rainy seasons. *P. squamosissimus* presented $2n = 48$ chromosomes (almost all acrocentric), which makes it easier to see the MNs (Feldberg et al. 1999).

The high genotoxic concentration observed in the water from the impacted area during the rainy season may be linked to the more intense rains, given that Silva et al. (2014) found that more intense rainfall provokes a greater lixiviation of the chemical substances into the river from the surrounding soil.

The comet assay is not used to detect mutations per se, but rather, genomic lesions that can, in fact, be corrected, and can thus also be used to study DNA repair mechanisms, providing important insights into the kinetics and the type of lesion that has undergone repair, although it cannot confirm the adequacy of the repair process itself (Albertini et al. 2000; Ghisi et al. 2010). The frequencies of the nucleoid classes analyzed in Barcarena were similar to those recorded by Rocha (2009) in the peripheral blood of *C. macropomum* and the saddle cichlid, *Aequidens tetramerus* (Cichlidae), exposed to different concentrations of methylmercury. At the same way, Xian et al. (2021) and De Castro et al. (2017) found similar results in China and Brazilian Northeast body waters.

This study showed that the damage levels detected by the comet assay were greater than those observed in the micronucleus test. A similar pattern to the results of the present study, which can be considered to be the normal pattern, given that the comet assay evaluates primary DNA damage, which is rarely passed down to future generations of cells.

The high percentages of micronuclei, nuclear alterations, and comet damage observed in the *P. squamosissimus* specimens from Barcarena may be related, in part, to the piscivorous feeding habits of this species. Porto et al. (2005) evaluated the genotoxic effects of mercury pollution in three fish species of the order Characiformes with distinct feeding adaptations, using the micronucleus test, and found that the mean frequency of micronuclei in the piscivorous species was approximately five times higher than those recorded in the detritivorous and omnivorous species. Hussain et al. (2018) also used the micronucleus test, nuclear alterations, and comet assay to investigate the effects of exposure to industrial and domestic effluents in the rohu, *Labeo rohita* (Cyprinidae), in the Chenab River in Faisalabad, Pakistan. This study recorded acute levels of toxicity and high contamination rates, which contributed to an increase in the mortality of the fish, which indicated that the water of the river should not be used even for irrigation.

The smelting plant in Barcarena belongs to one of the world's largest corporations in the aluminum sector. Over the past 20 years, a number of environmental accidents

related to the processing of bauxite have been recorded in the municipality, in particular the 2018 incident, when toxic red mud leaked from one of the tailing ponds, which was considered to have been one of the most significant environmental disasters in the history of the Amazon region (Steinbrenner et al. 2020). Following this incident, the company received an official warning, and was required to implement a number of mitigatory measures (Lemos and Pimentel 2021).

Given the potential impact of the smelting plant, a number of studies have investigated the damage to the Murucupi River, in particular the concentrations of heavy metals present in the water and the local soils (Pereira et al. 2007; IEC 2009, 2018; Lima et al. 2015a, b; Medeiros et al. 2016 and 2017; Almeida-Junior et al. 2019), as well as the water-borne diseases transmitted to the local population (Marinho et al. 2016). Pereira et al. (2007) recorded aluminum concentrations 13.2 times higher than the recommended level in the water of the Murucupi River. In high concentrations, aluminum may provoke neurological disorders in human beings, such as Alzheimer's and Parkinson's diseases (SILVA JÚNIOR 2013). In fish, the aluminum dissolved in acidic water, which comes into contact with the gills, provokes an increase in the pH and the formation of insoluble $Al_2(OH)_3$, which damages the DNA (Muniz and Oliveira-Filho 2006; Sultana et al. 2020) and eventually suffocates the animal. This has raised a number of concerns in the population of Barcarena in terms of the quality of the fish available for human consumption in the region. Barros et al. (2010) and Foran (1990) concluded that the health risks of the consumption of contaminated fish are 20–40 times higher than those associated with the ingestion of contaminated water, given that aquatic organisms are capable of concentrating trace elements to up to 105 times the concentrations observed in the environment. Marinho et al. (2016) evaluated the profile of the morbidity of the residents of the municipality of Barcarena and the surrounding area, and concluded that a number of different infirmities caused by parasites, as well as infectious and respiratory diseases, may be maximized by the poor quality of the water and other natural resources used as a source of food by this population. This highlights the need for a more detailed quantitative and qualitative investigation of possible environmental contamination in the region.

The analyses employed in the present study permitted the application of a holistic approach to the investigation of the environmental variables that affect the biological parameters of *P. squamosissum*, providing, with multivariate analyses, a powerful tool for the interpretation of complex data on water quality. Shrestha and Kazama (2007) evaluated the efficiency of statistical models for the analysis of water quality data, based on the standard parameters most frequently employed, and concluded that the data on river discharge, temperature, the biochemical demand for oxygen,

pH, electrical conductivity, nitrate, and ammoniated nitrogen are the parameters that best correlate with the quality of the water of aquatic environments including estuaries, and should be employed in this type of analysis. In the present study, the temperature, pH, and dissolved oxygen were employed, with clear results in terms of the deleterious effects on the watercourses and one of the principal local fishery resources, i.e., *P. squamosissum*.

Considering the ecological and economic importance of the Amazonian estuaries that were the focus of the present study, the identification of the possible impacts caused by local bauxite smelting operations would provide important insights for the implementation of measures designed to minimize the deleterious effects of these impacts on the local biota and, ultimately, the human populations in the area. Adequate mitigatory measures should guarantee the productivity of these environments for future generations, especially considering the importance of their fishery resources as a source of income and subsistence for the traditional local populations. This is the first study of its type in this region, and it provides important insights into the physiological response of the species to the environmental alterations evaluated in the analyses. Direct and immediate action is necessary from the public authorities to ensure the highest level of care with the treatment and storage of the residues in the tailing ponds to avoid potentially major impacts on the local rivers. The present study also provides the first detailed data on genotoxicity in the fish populations of the study area.

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Author contribution All authors contributed to the study conception and design.

CACRO “collected and captured the animals in the field and analyzed the data of the comet and micronucleus assay in the laboratory”.

PSSS “performed the collection in the field”.

DCP “analyzed the micronucleus in the laboratory”.

MOB “interpreted the data from the comet assay”.

LAC “performed the comet test procedures”.

MLSS “performed the water collections and analysis”.

TNMR “assistance in writing and reviewing the manuscript”.

BB “assistance in writing and reviewing the manuscript”.

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Declarations

Ethics approval and consent to participate The collection of the fish specimens analyzed in the present study was authorized by the Ethics Committee for the Use of Animals in Research (CEUA) of the Federal University of Pará in Belém, Brazil, through license number 727721082 (Appendix 1). Specimen collection was also authorized by the federal Biodiversity Information and Authorization System (SIS-BIO), through license number 15080–9.

Consent for publication All authors are aware of the publication of data in this article.

Competing interest The authors declare no competing interests.

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