Piscine micronucleus assay and the evidence of environmental degradation: the case of catfish from Brazilian tropical estuaries

Bioensaimo de micronúcleo em peixes e evidências de degradação ambiental: o caso dos bagres de estuários tropicais brasileiros

DOI: 10.34188/bjaerv3n4-048

Recebimento dos originais: 20/08/2020
Aceitação para publicação: 20/09/2020

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ABSTRACT
Assessing micronuclei (MN) rates in fish blood cells is a useful, fast and cheap tool for monitoring environmental quality, which can identify DNA damage in animals as an early warning of pollution irreversible effects. This technique was used to estimate genotoxicity in catfish collected at three estuaries of Ceará coast, Brazil, with different contamination status, in order to relate their respective damage rates with the contamination levels in each environment, under natural seasonality (dry and rainy season). Despite the estuaries degradation, all catfish presented extremely low rates of MN and other nuclear abnormalities (NA), contradicting the expected results. No statistical differences in the frequencies of both DNA damage endpoints were observed between seasons. Although literature confirms the presence of contaminants in these estuaries and the local biota exhibits biochemical impairment, our results indicate adaptive responses; the catfish seem to deal with contamination and do not exhibit chromosome damage. Besides, as all fish caught were juveniles, the low genotoxicity rates would be explained by younger greater efficiency to repair DNA damages, associated with low contaminants exposure due to the shorter lifetime or the low contaminants bioavailability under estuarine dynamics. Further studies are required to understand the genotoxic effects in catfish from Ceará and tropical estuaries.
Keywords: Environmental monitoring, Genotoxicity, *Sciades parkeri, Sciades proops, Sciades herzbergii*

RESUMO
A avaliação de micronúcleos (MN) é uma ferramenta útil, rápida e barata para monitorar a qualidade ambiental, capaz de identificar danos no DNA de animais como alerta precoce de efeitos irreversíveis da poluição. Essa técnica foi utilizada para estimar a genotoxicidade em eritrócitos de bagres coletados em três estuários do litoral do Ceará, Brasil, relacionando seus danos com os distintos níveis de contaminação em cada ambiente sob sazonalidade natural (seca e chuvosa). Apesar da degradação dos estuários, os bagres de todos os locais apresentaram taxas extremamente baixas de MN e outras anormalidades nucleares (NA) contrariando os resultados esperados. Nenhuma diferença estatística nas frequências de danos foi observada entre as estações do ano. Apesar da presença confirmada de contaminantes na costa do Ceará e dos bioquímicos danos relatados na biota local, nossos resultados indicam respostas adaptativas, com os peixes lidando com a contaminação e não apresentando danos cromossômicos. Além disso, como todos os bagres capturados eram juvenis, as baixas taxas de genotoxicidade seriam explicadas pela maior eficiência dos jovens em reparar os danos no DNA, associado à baixa exposição dos contaminantes devido ao menor tempo de vida ou à baixa biodisponibilidade dos contaminantes dada pela dinâmica estuarina. Mais estudos são necessários para entender os efeitos genotóxicos em peixes dos estuários tropicais e do Ceará.

Palavras-chave: Monitoramento ambiental, Genotoxicidade, *Sciades parkeri, Sciades proops, Sciades herzbergii*

1 INTRODUCTION
Coastal pollution derived from anthropogenic activities is a global concern. Despite the international agreements, e.g. the London Convention and the Law of the Sea Convention, have banned the release of toxic contaminants into the sea and established local regulations to prevent and control marine pollution, the efforts for coastal and estuarine conservation so far failed to restore former ecosystem structure and function, resulting in extinction and depletion of species, habitat destruction, water quality degradation, and decline of these ecosystems (Lotze et al. 2006). Studies confirm the detection of many different chemicals with trace metals, Polycyclic Aromatic Hydrocarbons (PAHs), Persistent Organic Pollutants (POPs), Pharmaceuticals and Personal Care Products (PPCPs), and Endocrine Disrupters Compounds (EDCs) in estuarine compartments (water, sediments and biota) (Bayen et al. 2012) including in Marine Protected Areas (MPAs) worldwide (Abessa et al. 2018).

Estuarine tropical ecosystems are particularly vulnerable to the contamination due to their natural complex dynamics under different river and tidal fluxes which favors the incorporation of contaminants into bottom sediments (Duaví et al. 2015, Oliveira et al. 2016, Gama et al. 2017), so sediments of these areas can become a sink for hydrophobic environmental mutagens and secondary sources of xenobiotics into aquatic ecosystems, producing risks of lethal and sublethal effects in biota,
especially the benthic fauna (Chen & White 2004, Burton & Johnston 2010). The chronic effects represent the great impairment to the biota of polluted estuaries, therefore, in the last decades, environmental biomonitoring programs have included the evaluation of the health of organisms and/or communities to establish the environmental quality and possible ecological risks, since damage can occur at all levels of biological organization (Cajaraville et al. 2000, Van der Oost et al. 2003, Fent 2004, Katsumiti et al. 2009).

The fish micronucleus assay rate in peripheral blood erythrocytes has been used as a pollution monitoring biomarker since the 1970s decade (Schmidt 1975). Micronuclei (MN) may form from chromosomal fragmentation or separation of entire chromosomes during the anaphase (Al-Sabti & Metcalfe 1995); they also can be induced by apoptosis or actions of physical agents and pollutants that interact with DNA (Bombail et al. 2001). High frequency of MN in organisms from polluted sites has been reported by several authors and it has been described as sensitive biodetector of genotoxicants, in studies involving chronic exposure to different types of environmental pollutants with clastogenic and aneugenic properties, providing early warning of eco-damage and stress on the health of organisms and ecosystems (Rodriguez-Cea et al. 2003, Udroiu 2006, Ergene et al. 2007, Monserrat et al. 2007, Ahmed et al. 2013, Arslan et al. 2015, Perez-Coyotl et al. 2017, Farag & Alagawany 2018, Hussain et al. 2018). Other non-specific Nuclear Abnormalities (NA) tend to occur prior the MN formation, and although NA is not totally considered as a genotoxic or mutagenic damage as their origin is not well described as MN, it is still used as a complement to MN frequency, then both together can indicate citogenotoxicity (Ayllon & Garcia-Vazquez 2000, Kirschbaum et al. 2009, Seriani et al. 2011).

Fish are ideal organisms to assess environmental problems since they are suitable bioindicators due to their high sensitivity to toxicants (AnvariFar et al. 2018; Pinto et al., 2020). Catfish (Siluriformes: Ariidae) species are considered appropriate to biomonitoring studies because of epibenthic habit, which makes them to be exposed to contaminants by dermal contact through skin and gills, and by ingestion of contaminated sediments and small benthic preys. Different species of catfish have already demonstrated MN e NA occurrence due to the exposure to pesticides (Ateeq et al., 2002, Harabawy & Ibrahim 2014, Nwani et al. 2014), metals (Ahmed et al. 2013, Fatima et al. 2015), Nonylphenol ethoxylate compound (Mekkawy et al. 2011), and in pollution monitoring (Andrade et al. 2004, Katsumiti et al. 2009, Azevedo et al. 2012, Angeli et al. 2013, Authman et al. 2013, Seriani et al. 2013, Gusso-Choueri et al. 2016).

In the Ceará state, northeastern Brazil, a set of compounds derived from anthropogenic are found in the estuaries and coastline, including urban runoff, effluents from shrimp farms, fertilizers
and pesticides from agriculture, domestic sewage, and industrial effluents (Cavalcante et al. 2009, Nilin et al. 2013, Santana et al. 2015). Although the presence of such contamination sources on Ceará coast, there are only few studies regarding the effects of contaminants to the local environments, including to the local biota (Santana et al. 2015). Thus the environmental quality in most of these areas is unknown and the existing information is insufficient to diagnose the threats to biodiversity or to support further actions (Abessa et al. 2018). Therefore, this lack of information represents a knowledge gap for biodiversity conservation and ecosystem management.

This investigation aimed to evaluate the rates of MN and NA in catfish collected in three tropical estuaries from the Ceará coast, to verify if the occurrence of genotoxic damage is related to the contamination levels in each area, and if it is influenced by natural seasonality (dry and rainy season).

2 MATERIALS AND METHODS

Study areas

Ceará state presents semiarid climate, with small variation of average annual temperatures (22 to 31°C). Seasonality is determined by rainfall regime: a rainy season (R), usually between January and May, and a marked dry season (D) from July to December. Sampling surveys were conducted along 2011 and 2012 at the estuaries of Ceará (ECR), Pacoti (EPR) and Jaguaribe (EJR) rivers, comprising both seasons.

These estuaries are strongly affected by marine influence and are impacted by diverse anthropogenic activities (Fig. 1). ECR (03°42’06.99”S; 038°35’43.00”W) and EPR (03°49’59.99”S; 038°25’14.00”W) are located at Fortaleza Metropolitan Region (FMR). The first one receives discharges of untreated domestic sewage and industrial effluents. Part of its basin is considered a protected area/MPA; however, since the 1960s the increasingly urbanization progress has been responsible for the decreasing of environmental quality and this is one of the most polluted river of the state (Santana et al. 2015). Marins et al. (2002) pointed out the degradation at ECR by low levels of oxygen dissolved in water column, high concentrations of nutrients and suspended solids, and moderate levels of Hg (28.1 ng L⁻¹). High fecal contamination was observed, especially during the R season (Farias et al. 2010), confirming domestic sewage as a relevant pollution source. Recent studies showed worse conditions, as ECR sediment contamination by the metals: Al (753.87 – 22,902.06 µg g⁻¹), Cu (0.63 – 35.38 µg g⁻¹), Cr (5.12 – 76.06 µg g⁻¹), and Zn (5.47 – 110.10 µg g⁻¹), due to industrial effluents and urban runoff (Nilin et al. 2013). PAHs (3.34 – 1,859.21 mg kg⁻¹) from urbanization and pyrogenic sources (Cavalcante et al. 2008, 2009) and the pesticides cypermethrin (45.7 – 134.6 ng g⁻¹)
1) and malathion (0.08 – 0.14 ng g⁻¹) were also found in ECR (Duaví et al. 2015). Moreover, ECR water and sediment were reported to be toxic (Nilin et al. 2007, 2013), and biochemical disturbances and genotoxic responses were found in local crabs (Davano et al. 2013).

Figure 1 Location of the estuaries of Ceará (ECR), Pacoti (EPR), and Jaguaribe (EJR) rivers on the coast of Ceará state, Brazil. Red dots indicate the fish sampling site. Yellow asterisks show areas of shrimp farming. Source: Google earth®

The EPR basin is on the edge of metropolitan expansion, where both installation of tourist facilities and real estate speculation have taken place. This estuary is a legally protected area/MPA, and till early 2000s this area was considered not polluted and a reference site. However, recent studies reported degradation by sewage, and some water parameters above national standards, as hardness, biochemical oxygen demand, quantities of thermotolerant coliforms and phosphate concentration (Fiuza et al. 2010). Pimentel et al. (2016) found contamination by estrogens in sediment associated with the occurrence of feminization (endocrine disruption) in the pufferfish Sphoeroides testudineus from EPR, showing impairment of their reproductive structure and function, which was attributed to sewage source. More recently, contributions from diffuse input related to urban and industrial activities, predominantly the discharge of treated or untreated sewage, vehicle traffic, and manufacture of red ceramics, were attributed as main sources of PAHs to the EPR (Lima et al. 2019).

The EJR (04°23’35.99”S; 037°49’40.00”W) belongs to the largest hydrographic basin of Ceará and contains the larger amount of shrimp farms of the state, which are responsible for environmental degradation due to mangrove deforestation and the release of untreated effluents (Queiroz et al. 2013). In EJR, levels of Cu (10 – 20 µg g⁻¹) and Hg (0.7 – 27.9 ng g⁻¹) were measured.
in sediments (Lacerda et al. 2009, Costa et al. 2013). Moreover, the EJR basin comprises the major 
agriculture zone of the state, where Gama et al. (2013) detected 201 types of highly toxic pesticides, 
with 151 active principles in estuarine region, as well as six herbicides (alachlor, bromacil, 
ethalfluralin, fluridone, tebuthiuron, and norflurazon – a banned compound) in levels ranging from 
111.43 to 419.35 ng·g⁻¹ (Gama et al. 2017). In another study, organochlorine pesticides were reported 
in levels ranging from 5.09 and 154.43 ng·g⁻¹ (Oliveira et al. 2016). Metals bioaccumulation within 
allowed limits for human consumption was observed in oysters and fish from EJR (Vaisman et al. 
2005, Costa et al. 2013).

Abiotic parameters from water column (salinity, temperature and pH) were measured in the 
low tide, by using a multipower probe. Rainfall data were obtained from the FUNCEME (Ceará 
Foundation of Meteorology and Water Resources) website, where recordings of the pluviometric 
stations near the estuaries of the rivers Ceará (Pici station, Fortaleza), Pacoti (Aquiraz station, 
Aquiraz) and Jaguaribe (Fortim station, Fortim) are available.

**Fish collection**

Catfish (n=96) were captured at each site (ICMBio license collection n° 28117-1) by local 
fishermen with artisanal fishing equipment (nets and traps) in the ECR (n=10), EPR (n=48) and EJR 
(n=34). Immediately after collection, animals were transported alive to the LABOMAR (ECR: 10.3 
Km; EPR: 19.8 Km; EJR: 140 Km) in thermal boxes (45 L maximum capacity) containing local water 
and constant aeration (air pump). To minimize the transportation stress, 500 g of ice were added 
previously to the boxes, reducing in 5°C the water temperature compared to the sampling moment 
(29 to 31°C) and keeping it constant lower (about 24°C) during transportation in order to decrease 
animals’ metabolism (Kubitza 1997). At the laboratory, fish were allowed to rest in the same boxes 
for 12h at 25°C under constant aeration before handling procedures. Prior to the blood collection, fish 
were anesthetized by immersion in clove oil (0.04%). The total body weight (BW) and total body 
length (TL) were measured. Species were identified according to Marceniuk (2005) and Eschmeyer 
et al. (2013).

**Micronuclei test**

Approximately 100µl of blood were pulled from the caudal vein by using heparinized 
syringes, then immediately a drop of blood was directly smeared on microscopy slides (Ishikawa et 
al. 2010), fixed in ethanol for 20 minutes and dried at room temperature. Slides were stained with 
May-Grunwald-Giensa (Rosenfeld 1947) for the total MN and NA counting. Two thousand cells were
analyzed per slide per specimen using optical microscopy (1000x), following Gusso-Choueri et al. (2016), Azevedo et al. (2012), Seriani et al. (2011), Katsumiti et al. (2009), and Udroiu (2006). The criteria for MN identification were round/ovoid-shaped non-refractory particles in the cytoplasm, with color texture and optical features resembling those of the nucleus, completely detached from the main nucleus and diameter of 1/3–1/20 of the main nucleus. Although MN is also a change in nuclear morphology, in this study the NAs were classified as any different aspect of main nucleus enough to distinguish them from the common nucleus in viable cells, most of all nuclear buds. The frequencies of MN, NA and sum of both (Σ MN+NA) were presented in absolute occurrence in 2000 cells and expressed in per 1000 cells (%).

Statistical analysis

Morphological parameters (BW and TL) and frequency of nuclear morphology alterations (MN, NA and Σ MN+NA) were verified for normality distribution, and then statistical differences between them in R and D seasons were verified by Analysis of Variance (ANOVA) followed by Tukey’s multiple comparison or Kruskall-Wallis test, using the SigmaPlot 11 software.

3 RESULTS

The biometric parameters of the catfish collected at the three tropical estuaries of Ceará coast are presented in Figure 2. Three species of the same gender were identified: *Sciades parkeri* (Traill, 1832) and *Sciades proops* (Valenciennes, 1840) in ECR, and *Sciades herzbergii* (Bloch, 1794) in EPR and EJR. In general, relatively larger and heavier fish were observed in the D period (Fig. 2).

In ECR, *S. parkeri* (n=5; BW=271.2 ± 79.8 g; TL=326.0 ± 26.8 mm) was captured in July 2011, and *S. proops* (n=5; BW= 6.5 ± 2.3 g; TL= 98.9 ± 12.2 mm) in January 2012. *S. parkeri* were the largest fish collect and *S. proops* were the smallest ones, with BW and TL significantly different from all others (P<0.001). In EPR, fish sizes and weights were similar between sampling surveys (BW= 67.8 ± 38.7 to 120.5 ± 50.7 g; TL= 220.4 ± 56.0 to 245.3 ± 45.7 mm), except in February 2012 (n=13; BW= 11.6 ± 4.4 g; TL= 115.2 ± 14.5 mm), when animals were significantly smaller (P < 0.001). *S. herzbergii* from EJR ranged from BW= 61.7 ± 14.9 g and TL= 190.9 ± 14.8 mm (n=15) in April 2012 to BW= 190.8 ± 84.7 g and TL= 280.0 ± 48.9 mm (n=12) in December 2011 (Fig. 2). Although the variations observed between catfish sizes, all the collected animals were juveniles, reinforcing the relevance of estuaries as nursery and feeding areas.
Figure 1 Biometric parameters of catfish collected in the estuaries of Ceará (ECR), Pacoti (EPR), and Jaguaribe (EJR) rivers, Ceará, Brazil, from June 2011 to November 2012. BW (Total body weight). TL (Total body length).

The frequency of MN, NA and Σ MN+NA found in the catfish blood from the three studied estuaries are presented in Table I and Figure 3. Despite the size variations observed (Fig. 2), the three species analyzed presented very low frequencies of nuclear morphological changes, above 0.7‰ (Table I), with no statistical differences between sites and/or periods and even between species (Fig. 3).

None of the analyzed nuclear morphological alterations were observed in *S. parkeri* of July 2011 in ECR and in *S. herzbergii* of June 2011 in EPR. *S. herzbergii* collected in September 2011 and February 2012 in EPR and in April 2012 in EJR presented only NA and no MN (Fig. 3).
Table I Sampling site parameters and rate of micronuclei (MN) and nuclear abnormalities (NA) in 2000 cells from peripheral blood of catfish collected in the estuaries of Ceará (ECR), Pacoti (EPR), and Jaguaribe (EJR) rivers, CE, Brazil, from June 2011 to November 2012. (Mean ± Standard Deviation). D (Dry season). R (Rainy season)

| Local | Month | Season | Total rainfall (mm) | Salinity | Temperature (°C) | pH | Species   | n   | MN       | %  | NA       | %  | Σ MN+NA  | %  |
|-------|-------|--------|---------------------|----------|------------------|----|-----------|-----|----------|----|----------|----|----------|----|
| ECR   | Jul11 | D      | 130.7               | 20.5     | 30               | 7.8| S. parkeri | 5   | 0 ± 0    | 0.00| 0 ± 0    | 0.00| 0 ± 0    | 0.00|
|       | Jan12 | R      | 48.1                | 23       | 29.5             | 8.0| S. proops  | 5   | 1 ± 1.73 | 0.50| 0 ± 0    | 0.00| 1 ± 1.73 | 0.50|
|       | Jun11 | D      | 2                   | 24       | 30.5             | 8.0| S. proops  | 3   | 0 ± 0    | 0.00| 0 ± 0    | 0.00| 0 ± 0    | 0.00|
| EPR   | Sep11 | D      | 33                  | 30       | 8.0              |   | S. herzbergii | 7   | 0 ± 0    | 0.00| 0.29 ± 0.49 | 0.10| 0.29 ± 0.49 | 0.10|
|       | Feb12 | R      | 231                 | 35       | 30               | 8.0| S. herzbergii | 13  | 0 ± 0    | 0.00| 0.33 ± 0.65 | 0.20| 0.33 ± 0.65 | 0.20|
|       | May12 | D      | 50                  | 35.6     | 29.5             | 7.9| S. herzbergii | 14  | 0.07 ± 0.27 | 0.00| 0.07 ± 0.27 | 0.00| 0.14 ± 0.36 | 0.10|
|       | Sep12 | D      | 0                   | 37.3     | 29               | 7.7| S. herzbergii | 11  | 0.91 ± 1.64 | 0.50| 0.45 ± 1.51 | 0.20| 1.36 ± 2.46 | 0.70|
|       | Total | -      | -                   | -        | -                |    | S. herzbergii | 48  | 0.23 ± 0.86 | 0.10| 0.25 ± 0.81 | 0.10| 0.48 ± 1.3 | 0.20|
| EJR   | Dec11 | D      | 10.4                | 17       | 31               | 8.0| S. herzbergii | 12  | 0.25 ± 0.87 | 0.10| 0 ± 0    | 0.00| 0.25 ± 0.87 | 0.10|
|       | Apr12 | R      | 55.6                | 21       | 30               | 7.9| S. herzbergii | 15  | 0 ± 0    | 0.00| 0.13 ± 0.35 | 0.10| 0.13 ± 0.35 | 0.10|
|       | Nov12 | D      | 0                   | 30.5     | 31               | 8.0| S. herzbergii | 7   | 0.43 ± 0.79 | 0.20| 0.14 ± 0.38 | 0.10| 0.57 ± 0.79 | 0.30|
|       | Total | -      | -                   | -        | -                |    | S. herzbergii | 34  | 0.18 ± 0.63 | 0.10| 0.09 ± 0.29 | 0.00| 0.26 ± 0.67 | 0.10|

¹Source: FUNCEME (Ceará Foundation of Meteorology and Water Resources)
The highest mean $\Sigma$ MN+NA frequencies occurred in *S. proops* from ECR (January 2012) and *S. herzbergii* from EPR (September 2012), but these rates were low and not significantly different (Table I). The total rainfall recorded in the sampling months was above historical levels expected for the rainy (R) season, and, regarding seasonal variations, fish from ECR showed relatively higher rates of MN and NA in R season, while fish from EJR and EPR exhibited relatively higher rates in the dry (D) season (Table I). Nevertheless, the data was not enough to support rainfall influence, due the low amount of rain and the reduced $n$ in some collections, despite the effort of other unsuccessful campaigns especially at ECR.

4 DISCUSSION

The catfishes from the estuaries of Ceará coast were practically not studied, so biological information regarding structure and dynamics of local populations of these fish are missing. The capture of different species was not intentional since the proper identification of catfish species requires the analysis of both external and internal morphological characteristics (Marceniuk 2005, Eschmeyer *et al.* 2013). *S. parkeri*, captured in ECR ($n = 5$), is of major concern, since its population is considered declining, and its status at the IUCN Red List of Threatened Species (International
Union for the Conservation of Nature and Natural Resources of Endangered Species) was altered from Near Threatened in 2010 to Vulnerable in 2015 (Betancur et al. 2015).

Like other Ariidae, tropical catfish reproduce in the rainy season, which strongly influences their migration within the estuary, due to the variation in salinity, temperature, dissolved oxygen and turbidity by the continental freshwater input in estuarine area that promotes greater food resource and protection against predators, ensuring the survival of juveniles (Dantas et al. 2010). Although the differences in size of the collected fish in this study, all catfish found in the Ceará estuaries were juveniles, similarly to that reported to other estuaries of NE Brazil (Gurgel et al. 2000, Dantas et al. 2010, Segura-Berttolini & Mendoza-Carranza 2013). This fact reinforces the importance preserving these ecosystems in order to guarantee the adequate development of recruits.

Due to the intensification of coastal pollution, organisms have been increasingly exposed to negative effects of toxic, carcinogenic, and mutagenic agents. In environmental monitoring assessment, the MN assaying has emerged as a simple, inexpensive and rapid method for detecting genotoxic effects as apparently the action of any chemical genotoxic agent may give rise to a gain in MN frequency (Salvagni et al. 2011).

Our results found out the Σ MN+NA frequency of 0.00 - 0.50 ‰ in ECR, 0.00 - 0.70 ‰ in EPR, and 0.10 - 0.30 ‰ in EJR (Tables I and II). As occurred in the present study (Table I and Fig. 3), a higher frequency of NA than MN was found in polluted monitoring studies (Kirschbaum et al. 2009, Osman et al., 2011, Azevedo et al. 2012, Fatima et al. 2015, Ghisi et al. 2016, Gusso-Choueri et al. 2016, Hussain et al. 2018) and elevated standard deviation in both absolute frequencies was observed in some research (Andrade et al. 2004, Gusso-Choueri et al. 2016). The levels of MN in the ECR and the EPR (this study) were similar to the observed in catfish collected in Cananéia–Iguape–Peruíbe/SP (Gusso-Choueri et al. 2016) and in Santos–São Vicente estuarine system/SP (Azevedo et al. 2012), but the Σ MN+NA in fish from both estuaries were higher than those reported in our study (Table II) evidencing the impact of human activities, such as industrial and domestic sewage disposal, in Brazilian estuaries. Our data was also similar to the findings in Tramandaí and Mampituba rivers/RS (Andrade et al. 2004) and very much lower than the levels exhibited in Pakistan, Egypt and Turkey fish (Table II).

The extremely low rates of nuclear morphologies alterations observed in this study were not expected because previous researches in the same estuaries revealed increasing environmental degradation (Santana et al. 2015) and other studies demonstrated that fish from polluted areas exhibit significantly higher rates of MN and NA than non-polluted sites (Bombail et al. 2001, Çavas & Ergene-Gözükara 2005, Amado et al. 2006, Kirschbaum et al. 2009, Rybakovas et al. 2009, Osman
et al. 2011, Arslan et al. 2015, Fatima et al. 2015). In Turkey, five species of fish collected at Aliaga Bay presented from 23 - 53.33 ‰ MN and 4 - 32.7 ‰ NA in the polluted area, compared to 11 - 18 ‰ MN and 1 - 5 ‰ NA in the clean area (Arslan et al. 2015). However, Carrasco et al. (1990) did not find significant differences between NA rates in fish from polluted and clean sites, as occurred in Ceará estuaries.
### Table II

Micronucleated cells (MN) and nuclear abnormalities (NA) frequency in fish for environmental monitoring quality around the world. Minimum – maximum (n)

| Local | Species | Season | River course | MN (%) | Σ MN+NA (%) | Reference |
|-------|---------|--------|--------------|--------|-------------|-----------|
| Brazil |         |        |              |        |             |           |
| ECR/CE (MPA) | *S. parkeri* | D |              | 0.00 (5) | 0.00 (5) | This study |
|         | *S. proops* | R |              | 0.50 (5) | 0.50 (5) |           |
| EPR/CE (MPA) | *S. herzbergii* | D | E | 0.00 (3) - 0.50 (11) | 0.00 (3) - 0.70 (11) |           |
|         | R | 0.00 (13) | 0.20 (13) |           |
|         | AA | 0.10 (48) | 0.20 (48) |           |
| EJR/CE | *S. herzbergii* | D |              | 0.10 (12) - 0.20 (7) | 0.10 (12) - 0.30 (7) |           |
|         | R | 0.00 (15) | 0.10 (15) |           |
|         | AA | 0.10 (34) | 0.10 (34) |           |
| Cananéia–Iguape–Peruíbe/SP (MPA) | *Cathorops spixii* | D | E | 0.00 – 0.96 (10) | 6.34 – 16.44 (10) | Gusso-Choueri et al., 2016* |
|         | R | 0.00 - 0.47 (10) | 18.65 – 28.20 (10) |           |
| Santos–São Vicente estuarine system/SP | *Cathorops spixii* | W | E | 0.0 (60) | 1.20 (60) | Azevedo et al., 2012* |
|         | S | 0.0 (80) | 2.45 (80) |           |
| Pakistan | *Netuma sp.* | W | E | 0.33 (18) | - | Andrade et al., 2004* |
|         | S | 0.35 (22) | - |           |
|         | AA | 0.35 (81) | - |           |
| Mampituba river/RS | *Netuma sp.* | W | E | 0.30 (12) | - |           |
|         | S | 0.45 (23) | - |           |
|         | AA | 0.30 (80) | - |           |
| Control–Armazém lagoon/RS | *Netuma sp.* | W | E | 0.13 (20) | - |           |
|         | S | 0.30 (20) | - |           |
| E | 0.50 (26) | - |           |
| AA | 0.38 (100) | - |           |
| Mampituba river/RS | *Mugil sp.* | W | E | 0.30 (21) | - |           |
|         | S | 0.48 (23) | - |           |
|         | AA | 0.35 (76) | - |           |
| Control–Armazém lagoon/RS | *Mugil sp.* | AA |              | 0.15 (17) | - |           |
| Pakistan | *Labeo rohita* | U |              | 64.40 (35) | 214.40 (35) | Hussain et al. 2018 |
|         | E | 17.60 (35) | 98.40 (35) |           |
| Egypt | *Oreochromis niloticus* | U |              | 4.80 (35) | 45.20 (35) | Osman et al. 2011* |
|         | E | 5.25 - 12.33 | 21.67 – 53.37 |           |
|         | 21.33 - 23.88 | 127.69 – 142.92 |           |
| Location          | Species         | W         | S         | U            | E            | Control (laboratory recovery for 2 months) |
|-------------------|-----------------|-----------|-----------|--------------|--------------|------------------------------------------|
| Paradeniz Lake (saltwater) Clarias gariepinus * | 4.10 (50) | 3.10 (50) | 2.70 (50) | 4.10 – 9.00 | 3.10 – 6.43 | 2.70 – 6.15 |
|                   Mugil cephalus    |                |           |           |              |              |                                          |
|                   Alburnus orontis |                |           |           |              |              |                                          |
| Turkey            Akgol Lake (freshwater)  Clarias gariepinus * | 5.23 (50) | 3.86 (50) | 3.35 (50) | 5.23 – 9.98 | 3.86 – 8.06 | 3.35 – 7.50 |
|                   Mugil cephalus    |                |           |           |              |              |                                          |
|                   Alburnus orontis |                |           |           |              |              |                                          |
|                   Control (laboratory recovery for 2 months) Clarias gariepinus * | 1.10 (50) | 1.26 (50) | 0.68 (50) | 1.10 – 2.96 | 1.26 – 3.36 | 0.68 – 2.44 |
|                   Mugil cephalus    |                |           |           |              |              |                                          |
|                   Alburnus orontis |                |           |           |              |              |                                          |

MPA (Marine Protected Area). *Catfish. D (Dry). R (Rainy). W (Winter). S (Summer). AA (Annual average) U (Upstream). E (Estuary). * value converted into ‰. *n not available.
Four species of fish collected in dams from agricultural rural properties exhibited high and significant frequencies of 6.21 - 13.78 MN per 1,000 erythrocytes evaluated (Salvagni et al. 2011), differently from our finds in EJR, the most important agricultural area of Ceará state and where several biocides used in agriculture were already identified (Gama et al., 2013), such as carbofuran, chlorophenols, and other chemicals. Sublethal doses of the pesticide carbofuran induced changes in the hematological, blood biochemical parameters and genotoxic effects, including MN formation (0.2, 2.26, and 8.77 ‰ in control, 0.16 mgL⁻¹ and 0.49 mgL⁻¹ of carbofuran exposure respectively), which reflected physiological, cytological and genotoxic alterations in the African catfish Clarias gariepinus (Harabawy & Ibrahim 2014). Similar acute and chronic toxic effects on aquatic organisms are caused by chlorophenols, a ubiquitous contaminant in the environment released from agricultural and industrial wastewater (Ge et al. 2017). Acute effects to the exposure to chlorpyrifos-ethyl on haematological indices of African catfish were already described (Okechukwu et al. 2007).

PAHs also have been pointed as a cause for genotoxic effects in fish or fish cells detected with NA (Katsumiti et al. 2009). Despite PAHs occurrence in the ECR and in the FRM estuaries due to the urbanization development (Cavalcante et al. 2008, 2009, 2017, Lima et al. 2019), our Σ MN+NA frequency was low in both ECR and EPR estuaries.

As species-specific differences may be relevant in MN assay, the test can be performed in situ comparing the frequencies of animals caught in different sites but a positive control group, besides the exposed one and the negative controls, should be established (Udroiu 2006). For an environmental assessment, it is particularly important to define reference levels of biological effects (Rybakovas et al. 2009; Prestes & Vincenci, 2019). The EPR was chosen at first to be our reference site. However, along the research development it has been found that contamination in this area is already in progress, making this an inappropriate estuary to be classified as a reference site. This is the same case of Cananéia estuary, which is frequently used as reference site for environmental assessment studies, but which is clearly subject to toxic metal pollution (Azevedo et al. 2012). In fact, the Σ MN+NA frequency in the EPR was the higher observed (Tables I and II). Even so, the levels we found in the EPR (and also in the other two estuaries investigated in the Ceará coast) were similar or under the levels at reference/control reported in other researches (Table II), and are in agreement with Rybakovas et al. (2009), which accepted MN frequency lower than 0.05‰ as a reference level in the peripheral blood erythrocytes of flatfish from the Baltic Sea and lower than 0.1‰ in the North Sea. These authors assumed that higher water temperature in the offshore areas of the Baltic and the North Seas affects mitotic activity in the though baseline frequency of MN in fish (Rybakovas et al. 2009). Katsumiti et al. (2009) used a reference site located away from the influence of harbor activities in a
relatively well preserved area of the estuarine complex and observed high levels of genetic alterations in fish (> 0.5 to 2.0 ‰) - whereas in polluted site presented ≥ 3 to 4 ‰ - that may indicate chronic effects of contamination also in the reference site, or, alternatively, recent migration of fish from more contaminated sites to cleaner areas, where they may have been sampled.

As previously mentioned in this manuscript, catfish are particularly relevant for monitoring environmental pollution, since they are benthic and feeds of other organisms and detritus in the sediment, which raises exposure rate to toxic agents in both water and sediment (Andrade et al. 2004, Ahmed et al. 2013). Interspecies comparisons showed that the African catfish had the higher level of frequencies of MN and NA while the Bleak fish Alburnus orontis had the lowest frequencies in an environmental monitoring quality study (Ergene et al. 2007) as can be compared in Table II.

On the other hand, some authors discuss that low rates of MN and NA in fish from degraded environments may be due to an adaptation to such conditions (Bombail et al. 2001, Seriani et al. 2013) and factors like interspecies sensitivity, metabolic capacity, DNA repair, defense mechanisms (Rodriguez-Cea et al. 2003) and compensatory mechanisms due to chronic chemical disturbance by a complex mixture of deleterious chemicals present over a period of the organisms life in those environments (Katsumiti et al. 2009). Nile tilapia Oreochromis niloticus appeared to be a more suitable bioindicator species than African catfish in studying genotoxic chemical pollution in the river Nile, due to its higher sensitivity (Osman et al. 2011), which is in agreement with Fatima et al. (2015) who affirmed that catfish species are hardier to environmental contaminants, which can explain the low rates of genotoxic damages observed in the present study. Our results suggest that even catfish juveniles may be not so sensitive to genotoxic contaminants, contradicting Udroiu (2006) which affirms that the early developmental stages of fish is characterized by a greater sensitiveness to genotoxic agents. No significant correlations were obtained between MN or NA frequency and biometric parameters (length and weight) and between MN and NA levels and age (p > 0.05, n = 102) by Cuevas & Zorita (2018).

Many species show a very low frequency of spontaneous MN formation, which is considered normally very rare and nearly uniform among species (Siu et al. 2004, Udroiu 2006). Siu et al. (2004) suggested that the chronic exposure may lead to a greater genotoxic impact than acute exposure and the MN response may persist over relatively long exposure periods. MN frequencies increased along with a continuous addition of genotoxicants but did not decrease significantly under gradually decreasing concentrations or cessation of exposure. Moreover, a two-fold higher mean MN frequency was recorded in the chronically exposed organisms than in those acutely exposed, even when they had received the same nominal dose of genotoxicants. Besides, micronucleated erythrocytes from the
Peripheral circulation reflect events that occurred in a time equal to the lifespan of the circulating erythrocytes (Schlegel & MacGregor 1982). Therefore, the application of the MN test on peripheral blood samples is particularly indicated for conditions of chronic exposure, as the common case of species exposure in situ. Changes in hematopoiesis play a key role in the MN test, acting as a possible confounding factor. Several genotoxic agents, at certain concentrations, own cytotoxic properties, resulting in a stop of erythropoiesis. Therefore, not only the production of erythrocytes, but also the micronucleated erythrocytes ends, producing thus a false negative (Udroiu 2006).

Micronuclei are formed during cell division, and the mitotic activity of poikilothermic organisms very largely depends on a variety of biotic and abiotic factors, especially on water temperature (Brunetti et al. 1988, Rybakovas et al. 2009). In Solea solea, NA frequencies were positively correlated with water temperature ($R = 0.815$, $p < 0.01$, $n = 11$) and salinity ($R = 0.909$, $p < 0.01$, $n = 11$) but no significant correlations were found between MN frequency and temperature and salinity (Cuevas & Zorita 2018). Seasonal differences in MN and NA rates of fish erythrocytes were observed by some authors. Higher frequencies of MN and nearly all detected NA in Nile tilapia and African catfish were observed in the blood of fish collected during summer followed by winter (Osman et al. 2011) (Table II). Minissi et al. (1996) and Çavas & Ergene-Gözükara (2005) also found higher rates of MN genotoxic responses in the summer, while Hayashi et al. (1998) observed higher MN rates in spring and summer, to different species. Authors attempted to explain these variations and stated that as fish are heterothermic, the higher rates of MN would be expected in warmest seasons as a result of metabolism activation (Hayashi et al. 1998). Kirschbaum et al. (2009) also found higher MN rates in fish from São Vicente collected in summer but without significant seasonal difference and an increase in NA frequency in the organisms from Cananéia in summer, but this was considered as a natural variation from a non-polluted tropical estuary.

In our study sites, however, temperature variations are very small, and rainstorm rates are the main climatic changes along year (Table I). Thus, the trends of higher MN rates in fish from ECR in R season could be related to the rainfall precipitation volume, which could increase the inputs of pollutants dragged by stormwater runoff. On the other hand, MN levels in EPR and EJR were higher in D (Table I), suggesting an agreement with Hayashi et al. (1998), of a result of metabolism activation in warmest period. Nevertheless, since no differences were observed in genotoxicity between areas, further studies are required to improve sample data amount and results. Along the Indian coast at Goa state, the catfish Arius arius from a place polluted by metals (Fe, Mn, Cu and Cd) and total petroleum hydrocarbons had MN frequencies around 0.6%, whereas fish from the unpolluted area exhibited frequencies around 0.1% of MN (D’Costa et al. 2017). Yet, there was no seasonal
influence on MN frequencies between the pre-monsoon, monsoon and post-monsoon periods, characterized by intense rainfall amount, in the same study (D’Costa et al. 2017), similar condition to the seasonality in our study areas.

Santana et al. (2015) reviewed the contamination levels and the effects to biota in the same three studied sites, highlighting that estuarine depreciation has been intensified in the last decade, which is evidenced by the increasing levels of contaminants in water and/or sediment, and negative effects on the native biota. Thus, the contamination data at these estuaries demonstrate high potential of occurrence of sublethal effects in biota, including genotoxicity. Santana (2014) classified the level of contamination as moderate to severe at ECR, and moderate at EPR and EJR, based on a review of contaminants described for the sites and on ecotoxicological responses, including histopathological and biochemical biomarkers, and bioaccumulation of Hg in catfish muscle tissues. Despite the low rates of MN and NA in this study, severe histological alterations, especially in the kidneys of ECR catfish, indicate chronic damages in these animals (Santana 2014), suggesting that contaminants are bioavailable but are not causing genotoxic effects to the catfish blood cells. This is in agreement with Ghisi et al. (2016) whose results showed the necessity of a multivariate analysis, evaluating several biological parameters to obtain an integrated response to the effects of the environmental pollutants on the organisms, in order to give more precise diagnosis, more valid results and higher social credibility. Furthermore, high frequencies of MN (74.4 ‰) and NA (150.00 ‰) induction (Table II) were found to be the cause of reduction of 96% of the population of fish Labeo rohita in the River Chenab, Pakistan (Hussain et al. 2018). So, the catfishes of Ceará estuaries require more attention, especially the vulnerable species S. parkeri founded in ECR.

Therefore, despite sampling design failures, the results raised here are important especially because this is the only study that evaluated catfish genotoxicity at Ceará coast, where if the legal actions for the environmental protection and management continues to fail these species may become extinct. The ECR and EPR are MPAs, but this mere label designation is not sufficient to guarantee effective protection from contaminants.

5 CONCLUSION

According to the results of the present study, genetic damages found in red blood cells of S. parkeri, S. proops and S. herzbergii from ECR, EPR and EJR, were low and not significant, so, in this case, environmental contamination posed no threat to the chromosomal structures of these species. Nevertheless, further ecotoxicological effects may be occurring at other levels of biological organization, as shown by the literature, and they may impair animals’ fitness in long-term. The
integration to other biomarker responses is fundamental for an adequate diagnosis of estuaries quality and to understand fully why fish from these three estuaries do not exhibit chromosomal fragility.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

ACKNOWLEDGMENTS
Lígia M. B. M. Santana thanks CAPES-DS for the doctoral scholarship. Denis M. S. Abessa thanks CNPq for the financial support and PQ fellowship. We are also thankful to Robson Seriani for his assistance and to the local fishermen for the support during the fish sampling.
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