



Short Communication

Profile of micronucleus frequencies and nuclear abnormalities in different species of electric fishes (Gymnotiformes) from the Eastern Amazon

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Abstract

The frequency of spontaneous micronucleus (MN) formation in fish species needs to be determined to evaluate their usefulness for genotoxic biomonitoring. The definition of a good bioindicator takes into account the current knowledge of its metabolic traits as well as other factors including its feeding behavior and relationship to the environment. In this study, we compared the basal frequencies of micronucleated erythrocytes and nuclear abnormalities (NA) among different species of the fish Order Gymnotiformes (*Rhamphichthys marmoratus*, *Steatogenys elegans*, *Sternopygus macrurus*, *Parapteronotus hasemani*, *Gymnotus mamiraua*, *Gymnotus arapaima*, *Brachyhyppopomus beebei*, *Brachyhyppopomus* n. sp. BENN) sampled in several localities of the Eastern Amazon. A baseline of MN and NA frequency in these fish was determined, enabling the identification of potentially useful species as models for genotoxicity studies. Only one impacted sample collected at a site in the River Caripetuba showed a significant number of NAs, which may be due to the release of wastewater by neighbouring mining industries and by the burnt fuel released by the small boats used by a local community. Our results may provide support for further studies in areas of the Eastern Amazon affected by mining, deforestation and other anthropogenic activities.

Keywords: biomonitoring, genotoxicity, Gymnotiformes, Amazon region, fish erythrocytes.

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The micronucleus assay (MN) has been widely used to detect clastogenic and aneugenic compounds in water. Micronucleus in peripheral erythrocytes of fishes are a suitable biomarker for genotoxic studies and for testing the pollution of aquatic ecosystems, due to easy sampling and high sensitivity (Carrasco *et al.*, 1990, Fenech, 2000, Bolognesi and Hayashi, 2011). In fish, erythrocyte MN and Nuclear Abnormalities (NAs) also appear spontaneously and their frequencies may be season-dependent (Bolognesi *et al.*, 2006). Large-scale biomonitoring programs for genotoxic compounds in aquatic environments require biomarkers that are technically easier and quicker to analyze than cytogenetic analysis or DNA damage assays. Thus, the evaluation of MN baseline frequencies can provide information on the level of MN caused by environmental and genetic factors and may be helpful to monitor degradation

processes in water bodies caused by anthropogenic activities (De Lemos *et al.*, 2001; Surowy *et al.*, 2011).

In addition to the formation of MN and NAs, spontaneous breaks in DNA in various organisms may result from intrinsic errors during DNA metabolism, without the influence of external agents. This damage may be enhanced by the exposure to mutagenic and genotoxic agents, which increase breaks or genetic recombination, giving rise to mutations and potential neoplasms (Ribeiro *et al.*, 2003). Fish are suitable organisms for the *in situ* investigation of aquatic pollutants, as well as for biomonitoring. Among their advantages as bioindicators are that they respond to the presence of pollutants in ways similar to other vertebrates, including humans, in terms of induction of damage to genetic material. They also play different roles in the trophic web, activating xenobiotics through the P450-dependent oxidative metabolism, as do mammals (Goksoyr *et al.*, 1991; Al-sabti and Metcalfe, 1995).

Fish of the Order Gymnotiformes are widely distributed in the Neotropical region (Albert and Sand-Crampton, 2005). They are known as electric fishes because they have

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electrical receptors and transmitters involved in intra-specific communication (Kramer, 1996). The frequency of their electric discharges is very stable in constant environmental conditions, but significant changes in discharge occur in response to changes in water quality (Thomas *et al.*, 1996), which makes them useful as bioindicators. Few studies have investigated mutagenic and genotoxic damage in Gymnotiformes. In this work we analyzed the frequency of spontaneous MN and NA formation in fishes of the Order Gymnotiformes aiming to evaluate their sensitivity as bioindicators and usefulness for further genotoxicity and mutagenicity studies and biomonitoring programs.

The Gymnotiformes fishes were collected in several localities in the Eastern Amazon without anthropogenic impact: Mamirauá (a sustainable community 03°01'41.8" S; 064°51'16.6" W), the Municipality of Tefé - Amazon River (03°07'09.4" S; 064°47'24.3" W), Muaná - Marajó Island (01°15'38.2" S; 049°28'42.2" W) and Currálinho - Marajó Island (01°45'49.8" S; 049°43'53.4" W), and in an impacted area of the Caripetuba River - Abaetetuba (01°37'23.49" S; 048°55'33" W), that receives crude wastewater from mining. Eight species of fish from the Order Gymnotiformes were collected: *Rhamphichthys marmoratus* (n = 10), *Steatogenys elegans* (n = 6), *Sternopygus macrurus* (n = 12), *Parapteronotus hasemani* (n = 5), *Gymnotus mamiraua* (n = 9), *Gymnotus arapaima* (n = 7), *Brachyhyppopomus beebei* (n = 7), and *Brachyhyppopomus n. sp. BENN* (n = 5). Collections were performed with the authorization of the Instituto Brasileiro

do Meio Ambiente (IBAMA, permit 13248-1, registration 242396). Peripheral blood samples were smeared on glass slides, dried at room temperature, fixed in ethanol for 10 min, hydrolyzed in HCL (1N) for 5 min, and stained with Giemsa. 3000 erythrocytes per fish were scored at 1,000X magnification. The criteria for the identification of fish erythrocytes containing MN were as follows: (a) the MN had to be smaller than one-third of the main nuclei; (b) the MN could not touch the main nuclei; (c) the MN could not be refractive and should have the same color and staining intensity as the main nuclei. Nuclear abnormalities were considered as blebbed, lobed, notched, binucleated and vacuolized. MN and NAs were analyzed with the non-parametric test of Kruskal-Wallis, with significance set at 5%.

The mean frequencies of MN, NAs, standard deviation and sample size for each species of Gymnotiformes from Mamirauá are shown in Table 1. The obtained values for the baseline rate of MN and NA for each species collected at this site showed very low levels (Table 1), and no significant statistical differences were observed among them ($p > 0.05$). MN frequencies were also very low in other municipalities, but samples from Abaetetuba showed a higher frequency of NAs ($p < 0.05$) (Table 2). The Caripetuba River - Abaetetuba is under the influence of a bauxite mining region of many aluminum industries. The mining operation and its waste disposal into the Caripetuba River causes degradation of the water quality. As this region is also subject to the tidal regime, this may be

Table 1 - Frequencies of MNs and NAs in different species of the order Gymnotiformes sampled in the sustainable community of Mamirauá.

Species	Mean and standard deviation of MN	Mean and standard deviation of MN
<i>Rhamphichthys marmoratus</i> (n = 10)	0.013 ± 0.023	0.017 ± 0.023
<i>Steatogenys elegans</i> (n = 6)	0.006 ± 0.015	0
<i>Sternopygus macrurus</i> (n = 12)	0.019 ± 0.030	0.134 ± 0.181
<i>Parapteronotus hasemani</i> (n = 5)	0.033 ± 0.023	0.146 ± 0.132
<i>Gymnotus mamiraua</i> (n = 9)	0.007 ± 0.022	0.037 ± 0.045
<i>Gymnotus arapaima</i> (n = 7)	0.005 ± 0.012	0.058 ± 0.089
<i>Brachyhyppopomus beebei</i> (n = 7)	0.023 ± 0.030	0.083 ± 0.129
<i>Brachyhyppopomus n. sp. BENN</i> (n = 5)	0.164 ± 0.258	0.158 ± 0.142

Table 2 - Frequencies (mean and standard deviation) of MNs and NAs in *Sternopygus macrurus* from different localities of the Eastern Amazon.

Locality	Mean ± SD of MN	Mean ± SD of NA
Sustainable Community of Mamirauá (n = 12)	0.019 ± 0.030	0.134 ± 0.181
Muaná - Marajó Island (n = 4)	0.017 ± 0.019	0.042 ± 0.042
Currálinho - Marajó Island (n = 9)	0.013 ± 0.030	0.151 ± 0.078
Tefé - Amazon River (n = 5)	0.077 ± 0.106	0.249 ± 0.370
Caripetuba River - Abaetetuba (n = 14)	0.048 ± 0.065	0.504 ± 0.241*

*Statistically different from Mamirauá (AM) and Muaná (Marajó Island-PA) (Kruskal-Wallis, $p < 0.05$).

responsible for the fluctuating levels of pollutants discharged and present in the aquatic environment. Furthermore, the Caripetuba River is characterized by an intense traffic of boats by a local community. The burnt fuel of these boats contains a series of compounds capable of inducing DNA damage, which may influence the formation of NA found in specimens collected in the river. This analysis was carried out only in *S. macrurus*, because this is the most abundant species and it was found at all sampled sites. The genotoxicity studies with fish in the literature use the baseline MN frequency in the control groups as reference values. Considering laboratory and field studies, the baseline MN frequency showed very low levels (Table 3). The same analysis was carried out for nuclear abnormalities (Table 3).

Some fish species are less sensitive than others as bioindicators of genotoxicity in the water. Many factors may be responsible for these differences, such as metabolism, DNA repair efficiency, defense mechanisms and trophic level in the food chain (Grisolia *et al.*, 2009, Bolognesi and Hayashi, 2011). Field studies have reported higher variability in baseline MN frequency compared with laboratory studies. Several factors may explain this variability, such as genetic heterogeneity in wild populations, variability in the water environment, seasonal effects, variations in physico-chemical parameters of the water, food availability, and other stressor agents (Bolognesi *et al.*, 2006; Bolognesi and Hayashi, 2011).

Colossoma macropomun, known as tambaqui, is the largest Characiform fish in the Amazon River. In laboratory conditions, it presented a basal MN frequency of 2.4% (Rocha *et al.*, 2011). In a study carried out by Grisolia *et al.* (2009) in a tropical eutrophic lake, the baseline MN frequencies and NAs were scored for different fish species according to their food habits (omnivorous, detritivorous, piscivorous, algivorous and herbivorous). Top-chain and detritivorous species presented a higher MN baseline frequency, probably due to the bioaccumulation and/or biomagnification of toxic contaminants present in that lake (Table 3). Porto *et al.* (2005) evaluated the MN frequencies in peripheral erythrocytes of three fish species from the Solimões River (central Amazon): *Prochilodus nigricans* (detritivorous), *Mylossoma duriventris* (omnivorous) and *Hoplias malabaricus* (piscivorous). The mean frequencies of MN observed were 1.0%, 1.0% and 0.6%, respectively. These values were considered very low, associated with the fact that there was not any kind of anthropogenic activities at the sampled sites. Compared with the Madeira River (southwestern Amazon), highly impacted by mercury due to gold-mining, the MN means for these three fish species were 3.8%, 3.7% and 17.6%, respectively, significantly higher than in the Solimões River. *H. malabaricus* is also a carnivore top-chain species and presented the highest level of MN. Table 3 shows native fish species commonly found in South American rivers, such as *P. nigricans*, *H. malabaricus*, *A. bimaculatus*, *M. duriventris*, *A.*

Table 3 - Baseline MN frequencies and nuclear abnormalities obtained from control groups in laboratory and field studies.

Species	Food habits	Baseline MN frequencies (%)	Baseline nuclear abnormalities (%)	References
<i>Prochilodus nigricans</i>	Detritivorous	0.01	-	Porto <i>et al.</i> (2005)
<i>Mylossoma duriventris</i>	Omnivorous	0.01	-	Porto <i>et al.</i> (2005)
<i>Hoplias malabaricus</i>	Piscivorous	0.006	-	Porto <i>et al.</i> (2005)
<i>Astyanax bimaculatus</i>	Omnivorous	0.013	-	Pantaleão <i>et al.</i> (2006)
<i>Hopliasmalabaricus</i>	Piscivorous	0.033	-	Pantaleão <i>et al.</i> (2006)
<i>Astyanax jacuhiensis</i>	Omnivorous	0.021 0.019	--	De Lemos <i>et al.</i> (2008)
<i>Oreochromis niloticus</i>	Omnivorous/ Detritivorous	0.05	-	Rocha <i>et al.</i> (2009)
<i>Steindachnerina insculpta</i>	Omnivorous/ Detritivorous	0.70	3.27	Grisolia <i>et al.</i> (2009)
<i>Geophagus brasiliensis</i>	Omnivorous	0.86	5.86	Grisolia <i>et al.</i> (2009)
<i>Oreochromis niloticus</i>	Omnivorous/ Detritivorous	0.87	3.29	Grisolia <i>et al.</i> (2009)
<i>Cyprinus carpio</i>	Algivorous	0.94	5.51	Grisolia <i>et al.</i> (2009)
<i>Astyanax bimaculatus</i>	Omnivorous	1.13	12.2	Grisolia <i>et al.</i> (2009)
<i>Hoplia smalabaricus</i>	Piscivorous	1.80	1.89	Grisolia <i>et al.</i> (2009)
<i>Cichla temensis</i>	Piscivorous	1.86	9.15	Grisolia <i>et al.</i> (2009)
<i>Oreochromis niloticus</i>	Onnivorous/ Detritivorous	0.65	2.20	Souza and Fontanetti (2006)
<i>Cyprinus carpio</i>	Algivorous	0.40	-	Buschini <i>et al.</i> (2004)
<i>Prochilodus lineatus</i>	Detritivorous	0.00	-	Cavalcante <i>et al.</i> (2008)
<i>Oncorhynchus mykiss</i>	Omnivorous	0.18	-	Ayllón and Garcia-Vasquez (2001)
<i>Barbus plebejus</i>	Benthic feeder	0.50	-	Minissi and Rizzoni (1996)

jacuhiensis, *S. sculpita*, *G. brasiliensis*, *C. temensis* and *P. lineatus*. The basal MN interspecies variability ranged from 0% to 1.86%. Non-native species, such as *C. carpio* (common carp), *O. niloticus* (tilapia), *B. plebejus* and *O. mykiss* (rainbow trout) are widely used around the world for laboratory MN testing. Among these four non-native species the MN variability ranged from 0.05% to 0.94%. On the other hand, this MN baseline frequency may show different values depending on laboratory scoring criteria and biotic factors, such as age, sex, feed, genetic make-up, etc (Bolognesi and Hayashi, 2011). Zúñiga-González et al. (2000) determined the spontaneous MN in peripheral blood erythrocytes from 54 species including mammals, reptiles and birds. The authors found that some groups of species had more spontaneous MN than others. NAs are considered by different authors to result from cytotoxicity or from genotoxicity. Nevertheless, Ayllón and Garcia-Vasquez (2001) demonstrated a direct relationship between MN induction and an increased level of NAs in rainbow trout after exposure to different clastogens, and recommended that NAs should be considered also as indicators of genotoxicity, even though their formation mechanisms are not totally understood.

This study showed the importance of establishing the basal MN frequency and the nuclear abnormalities in different native fish species in order to provide *in situ* biological indicators for biomonitoring anthropogenic impacts on the Amazon Basin. Our results, obtained from different species of the Order Gymnotiformes, may provide support for further studies in the eastern Amazon in regions affected by mining, deforestation and other anthropogenic activities.

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