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Mutagenic effects of mercury pollution as revealed by micronucleus test on three Amazonian fish species[☆]

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Abstract

Genotoxic effect of mercury pollution over Amazonian fish species was evaluated by using the micronucleus test (MNT). Distinct mean frequencies of micronuclei (MN) were observed in three trophically distinct characin fish species collected in two riverine environments in the Amazon Basin: the Madeira (polluted area) and the Solimões (unpolluted area) rivers. Mean frequencies of MN observed in *Prochilodus nigricans* (detritivore), *Mylossoma duriventris* (omnivore), and *Hoplias malabaricus* (piscivore) from the Madeira River were significantly higher compared to the frequencies from the same species from the Solimões River. In addition, mean frequencies of MN from piscivore species were almost fivefold higher than the detritivore and/or omnivore species. We conclude that MNT in fish erythrocytes may be useful for indicating genotoxicity of mercury in Amazon rivers.

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1. Introduction

Mercury is considered one of the most dangerous of the heavy metals because of its high toxicity, bioaccumulative properties, and other deleterious effects on biota including genetic alteration or mutagenesis (WHO, 1990). Mercury contamination in Amazon rivers is generally associated with gold mining, since almost all gold produced in the Amazon is obtained by a process where mercury is used to amalgamate and concentrate gold (Lacerda et al., 1987). If we consider only anthropogenic sources, compared to gold-mining activity, neither industrial nor municipal wastewater has been considered relevant for heavy metal pollution in the major Amazonian rivers. However, soil erosion and

forest fires also have been advocated as other sources of mercury pollution in the region (Roulet et al., 1999). Key variables such as dissolved organic carbon and pH are thought to influence mercury levels in freshwater biota (Silva-Forsberg et al., 1999).

A huge concern about mercury pollution in Amazon rivers has been demonstrated in several papers and the incorporation and bioaccumulation of mercury in the aquatic food chain or in the riverside population has been investigated in different places in the Amazon Basin such as Amapá (Fostier et al., 2000), Gurupi (Palheta and Taylor, 1995), Madeira (Barbosa et al., 1995; Boischio and Henshel, 1996), Negro (Forsberg et al., 1995; Silva-Forsberg et al., 1999), Tapajós (Akagi et al., 1994), Tocantins (Aula et al., 1994), Uatumã (Kehrig et al., 1998), and Xingu (Barbosa et al., 1995) river drainages. Of these, Tapajós and Madeira are probably the best-studied sites given the historic gold-mining activities.

Fish are important indicators of water pollution and the piscine micronucleus test (MNT) is one method used

[☆]The care and treatment of the animals utilized in our study as well as all fish handling in the wild were in accordance with international guidelines and received institutional approval from INPA (Instituto Nacional de Pesquisas da Amazônia).

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to investigate the action of some classes of mutagenic compounds and chemical contaminants on fish (Hooftman and Raat, 1982). Micronuclei (MN) are thought to be whole chromosomes or fragments, which lag during cell division (anaphase) because of an aneuploidic or clastogenic event, and after telophase give rise to daughter cells as secondary nuclei that are, as a rule, refringent and smaller than the main nucleus (Evans et al., 1959; Heddle, 1973; Schmid, 1975).

Several studies have used MNT to screen for effects of exposure to carcinogenic and/or mutagenic chemicals in fish (Hooftman and Raat, 1982; Al-Sabti, 1986; Manna et al., 1985; Manna and Sadhukhan, 1986; Metcalf, 1988). The technique has also been used as in situ biological indicator in wild fish (Hose et al., 1987; Carrasco et al., 1990; Minissi et al., 1996; Sanchez-Galán et al., 2001; Rodriguez-Cea et al., 2003). In Brazil, there are few reports on the use of MNTs to assess mutagenic effects of mercury and clastogens agents in fish (Nepomuceno et al., 1997; Grisolia and Cordeiro, 2000; Matsumoto and Cólus, 2000) and none of these evaluated the genotoxic effects on distribution of MN either in environment or in fish at different trophic levels, a critical factor in assessment of exposure risks.

The main objective of this study was to assess the impact of mercury contamination on the Madeira River by examining the incidence of MN in circulating erythrocytes on three Amazonian characin fishes belonging to different trophic levels.

2. Materials and methods

Archived slides containing blood smears of three commercially important Amazonian characin fish species (*Prochilodus nigricans*, Curimatã; *Mylossoma duriventris*, Pacu branco; and *Hoplias malabaricus*, Traira) were examined for the presence of MN in the circulating erythrocytes. To avoid intraspecific differences related to fish size for each species, only adult specimens with similar sizes were sampled. Blood samples were obtained during a field trip in September of 1991 (Madeira River) and October of 1991 (Solimões River), just after an intense period of gold-mining activity and high levels of mercury contamination in the Madeira River. The species sampled occupy different trophic levels in the freshwater food web: *P. nigricans* is a detritivore, *M. duriventris* is an omnivore, and *H. malabaricus* is a piscivore. The detritivore and omnivore species were collected at Teotonio waterfall, near the town of Porto Velho (64°05'W, 8°50'S), and the piscivore in the Igarapé Lisboa, a small stream near the city of Abunã (65°20'W, 9°40'S), 190 km upstream from Teotonio waterfall. For comparison, the same fish species were

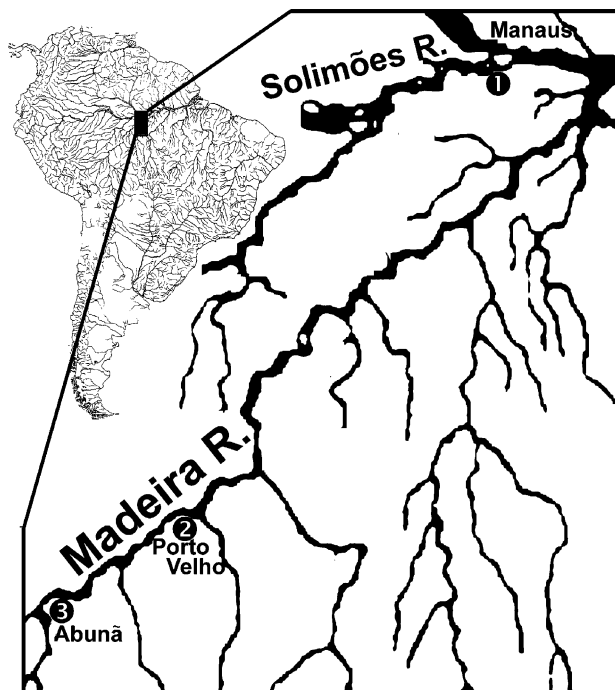


Fig. 1. Sampling site locations in the Solimões River (1), Teotonio waterfall (2), and Lisboa stream (3).

collected in the Solimões River, near Marchantaria Island (60°00'W, 3°14'S), 900 km downstream from Teotonio waterfall. Solimões River was considered a reference site for control since there was no gold-mining influence in the area and is considered free from any influence of municipal wastewater (Fig. 1).

Blood samples were obtained by caudal vein puncture using a heparinized syringe. Blood was smeared immediately onto two clean glass slides, air dried, and then fixed in absolute ethanol for 20 min or in absolute methanol for 10 min. Blood was drawn from 55 fish. In the lab, each slide was stained with 5% Giemsa solution for 10 min. MN were identified and scored microscopically under 100× in an Olympus microscope. Five thousand erythrocytes were scored for each specimen (2500/slide) to determine the frequency of micronucleated erythrocytes. Slides were scored by a single observer using blind review. For MN scoring purpose, only nonrefractive small nuclei (>1/3 of the main nucleus) located close to the main oval nucleus of round erythrocytes with intact cytoplasm were considered.

Mean MN frequencies and standard deviation, expressed as number of MN per 5000 erythrocytes, were calculated for each group. Data were analyzed for normality and MN frequencies were compared between species and between sampling sites by means of the Mann–Whitney *U* test ($\alpha = 0.01$). All differences were tested with ANOVA.

3. Results

Table 1 summarizes all results, including statistics. A total of 280,000 erythrocytes from 55 fish collected in the Madeira and Solimões Rivers were analyzed. Mean MN frequencies of *P. nigricans*, *M. duriventris*, and *H. malabaricus* collected in the Madeira River were 0.038%, 0.037%, and 0.18%, respectively. Data analysis of MN frequencies with respect to species collected in the polluted site yielded no significant differences among *P. nigricans* and *M. duriventris* (*U* test $P > 0.01$), but both species differed significantly from *H. malabaricus* (*U* test $P < 0.01$). Among fish collected in the Solimões River, the mean MN frequencies of *P. nigricans*, *M. duriventris*, and *H. malabaricus* were 0.01%, 0.01%, and 0.006%, respectively (Table 1). These frequencies did not differ among species (*U* test $P > 0.01$).

Overall, mean MN frequencies were elevated almost four-fold in *P. nigricans* and *M. duriventris* and roughly 30-fold in *H. malabaricus* from the Madeira River, as compared to samples from the Solimões River (Table 1, Fig. 2). Indeed, data analyses of the MN frequencies

Table 1
Frequencies of micronuclei observed in the erythrocytes of *P. nigricans*, *M. duriventris*, and *H. malabaricus* from polluted (Madeira) and unpolluted (Solimões) sites

Samples	<i>n</i>	TMN	MN% frequency ± SD
Madeira river			
<i>P. nigricans</i>	10	19	0.038 ± 0.020*
<i>M. duriventris</i>	12	24	0.037 ± 0.028*
<i>H. malabaricus</i>	05	44	0.176 ± 0.091***
Solimões river			
<i>P. nigricans</i>	10	5	0.01 ± 0.011
<i>M. duriventris</i>	12	6	0.01 ± 0.018
<i>H. malabaricus</i>	06	2	0.006 ± 0.016

Note: *n*, number of analyzed specimens; TMN, total number of observed micronuclei; MN%, mean micronuclei frequency and the standard deviation.

U test, * $P < 0.01$ vs. control.

U test, ** $P < 0.01$ vs. *P. nigricans* and *M. duriventris* from the Madeira River.

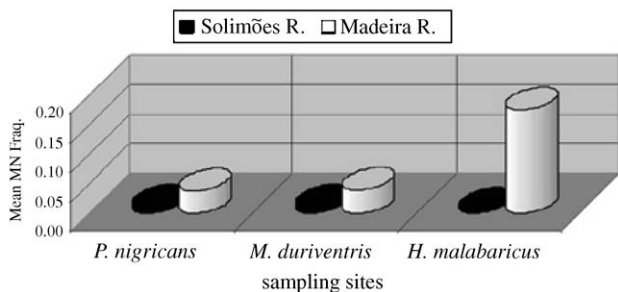


Fig. 2. Micronucleus-environment interaction for *H. malabaricus*, *M. duriventris*, and *P. nigricans* in the Solimões and Madeira Rivers.

with respect to sampling sites (polluted vs. reference sites) yielded significant differences in all three species (*U* test $P < 0.001$).

4. Discussion

The assessment of genotoxic effects of mercurial compounds in the erythrocytes of some fish species have been conducted by determining the induction of MN. Overall, the frequencies of MN were elevated in a dose-dependent manner in all cases when compared to the relevant controls (Babich et al., 1990; Al-Sabti, 1994; Nepomuceno et al., 1997; Sanchez-Galán et al., 2001; Ayllon and Garcia-Vazquez, 2000; Swartz et al., 2003).

It is well known that mercury compounds (methyl mercury and mercury chloride), but not inorganic mercury, interferes the regular chromosome segregation during cell division mainly by inhibition of polymerization of actin tubules, an essential structure of the mitotic spindle (Miura and Imura, 1987). Moreover, clastogenic effects on chromosomes of humans exposed to methyl mercury contamination, including Amazonian riverine people, have been reported (Betti et al., 1992; Ogura et al., 1996; Amorim et al., 2000). One may suppose that this could be the underlying mechanism in the origin of MN in the circulating erythrocytes of fishes from the Madeira River where considerable nuclear anomalies were observed.

Previous investigations have found that trophic level, ecosystem, body weight, and mobility can have a strong influence on the degree of bioaccumulation of mercury in fishes from the Madeira River. The level reached by *P. nigricans* from Madeira River range from 0 to 0.96 $\mu\text{g g}^{-1}$, *M. duriventris* 0 to 0.12 $\mu\text{g g}^{-1}$, and *H. malabaricus* 0.08 to 1.06 $\mu\text{g g}^{-1}$ (Lacerda et al., 1987, 1988; Pfeiffer et al., 1991; Malm et al., 1990; Padovani et al., 1995; Boischio and Henshel, 2000; Maurice-Bourgoin et al., 2000). Padovani et al. (1995) reported the total mercury concentration in *P. nigricans* and *M. duriventris*, based on a subset of our sampling, and in *H. malabaricus* based on sampling from the public market of Guajará-Mirim (considered a reference-unpolluted site). Taking into account their data, except for *H. malabaricus*, the average amount of mercury found could roughly reflect the amount of mercury of our analyzed fish specimens. They also measured the same fish species (but not same specimens analyzed by us) from the Solimões River (Forsberg, unpublished data) and the values found were significantly lower than those measured from the Madeira River.

As compared to *P. nigricans* and *M. duriventris*, the greater frequency of MN detected in the piscivore *H. malabaricus* suggests that in the polluted

environment the cell damage could be associated to mercury pollution. Three factors could contribute to this condition: (i) *H. malabaricus*, as a carnivore, is a high trophic level fish being subject to events of biomagnification; (ii) it usually inhabits small streams where low pH and conductivity favor production and bioaccumulation of methyl mercury; and (iii) it is a sedentary species that would continually uptake and accumulate mercury. In contrast, the lower frequency of MN encountered in the species *P. nigricans* and *M. duriventris* could reflect the inferior position on the food chain, the more basic pH in their aquatic environment, and finally their migratory behavior leading to different exposure on contaminated environments.

The correlation between higher frequencies of MN in fish from the mercury polluted site (Madeira River) as compared to the nonpolluted site (Solimões River) is comparable in order of magnitude to the results of Hose et al. (1987), in which they observed that MN frequencies in two marine fish species (*Genyonemus lineatus* and *Paralabrax clathratus*) from contaminated areas of Southern California were elevated relative to fishes from less contaminated sites. However, the increased MN frequency was related to previously determined environmental concentrations of chlorinated hydrocarbons (DDTs and PCBs) and polycyclic aromatic hydrocarbon metabolites and not mercury.

The assessment of the piscine MNT as in situ biological indicator of chemical contaminant effects has been evaluated by several studies (Hose et al., 1987; Carrasco et al., 1990; Minissi et al., 1996; Sanchez-Galán et al., 2001; Rodriguez-Cea et al., 2003). The test has not always been sensitive for detecting in situ pollution in freshwater ecosystems. Our results revealed that MNT was sensitive to detect different levels of nuclear response to mercury pollution in the wild fish and deserve some credit. However, the lack of additional species in each trophic level, additional sampling sites (mainly intermediary zone), and an effective control of direct tissue measurement of mercury certainly reduces robustness of our analysis.

It is noteworthy that the mercury that has been released in the Amazon Basin during events of gold mining, deforestation, damming of rivers, and when associated with natural pedogeochemical and atmospheric transformation processes has severely affected the Amazonian biota (Artaxo et al., 2000). The high mercury levels found in the Madeira River, including soils, sediments, water, and fish, are due not only to historic anthropogenic mercury inputs but also to natural sources and natural biogeochemical processes (Lechler et al., 2000; Maurice-Bourgoin et al., 2000). Given the evidences of mercury contamination in the Madeira River, but no other direct-acting mutagens, and the well known genotoxicity of mercury we considered that the differences in MN frequencies

between sites was due to differential exposure to mercury.

After having stopped gold mining for over a decade in the Madeira River Basin, complementary surveys are still being carried out in the city of Porto Velho and nearby areas to determine the amount of mercury that remains in the region (Bastos et al., 2004). At present, a monitoring of mercury level plus an MN survey in fish species is necessary in order to provide additional information about an expected decay on the levels of mercury pollution and frequency of MN in circulating erythrocytes, mainly in the piscivore *H. malabaricus* that seemed to be a good sentinel fish for biomonitoring.

5. Conclusion

Based on our results, the following contributions of this paper might be highlighted: (i) there was an erythrocytic nuclear response to mercury pollution in the fish community of the Madeira River during the gold mining rush era; (ii) the strongest genotoxic effect detected in the piscivore fish, which lives in acid streams and presents low vagility, probably reflects an interaction of environmental and biological factors leading to a greater biomagnification of mercury on them; (iii) the effect of mercury pollution can be assessed by using MNT, even in archived slides, providing a support for the effectiveness of the test for indicating genotoxicity in Amazon rivers.

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