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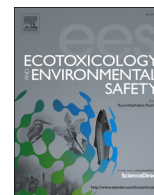

Investigation into the cytotoxicity and mutagenicity of the Marajó Archipelago waters using *Plagioscion squamosissimus* (Perciformes: Sciaenidae) as a bioindicator

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ABSTRACT

Maintaining water quality within tolerable limits is a basic need of the riverside communities in the Amazon. Using endemic aquatic organisms as biological models is useful for monitoring the environment. In this study, potential cytotoxic and genotoxic damages in *Plagioscion squamosissimus* (commonly known as silver croaker) from the Marajó Archipelago were evaluated using a flow cytometry assay and a survey of micronuclei (MN) frequency as well as other nuclear abnormalities (NA). *P. squamosissimus* specimens were collected at four locations in the Marajó Archipelago. Blood samples from these fish were used in the flow cytometry assay and piscine micronucleus test, and the resulting data were analyzed using analysis of variance (ANOVA). We did not observe a difference in the erythrocyte cell cycle distribution among the samples ($P=0.9992$), which suggests the absence of cytotoxic agent-induced apoptosis. The piscine micronucleus test exhibited differences in the samples from São Sebastião da Boa Vista (SSBV), and those from Anajás produced the highest mutagenicity indices. The MN frequencies were low for all groups, but the groups exhibited significantly different frequencies ($P=0.0033$). Reniform nuclei, nuclei with extensions, and lobed nuclei were combined and considered NA. The frequency differences for these NA were significant among sampling sites ($P < 0.0001$). This report is the first to use flow cytometry in fish to evaluate cytotoxic agent-induced apoptosis. The micronucleus test results indicate the presence of pollutants that can change the genetic material of the fish studied. We also demonstrate that the Amazonian fish *P. squamosissimus* is important not only as a comestible species but also as an adequate model for biomonitoring in aquatic environments.

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1. Introduction

Aquatic environments accumulate many contaminants that can cause damage at the molecular, biochemical, cellular, and physiological levels (Kirschbaum et al., 2009). In fact, among different ecosystems, aquatic ecosystems, either by direct or indirect release (loading), are the final repository for most xenobiotics discharged therein. Fish have successfully been used in cytogenetic analyses because they are easy to handle and maintain in a laboratory, and they are relatively inexpensive to analyze (Hayashi et al., 1998). Using fish red blood cells provides rapid results with little suffering by the organisms used in biomonitoring (Minissi et al., 1996). These tests can detect clastogenic (induced chromosome breaks) and aneugenic (induced aneuploidies or abnormal chromosome

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segregation) agents and have been validated *in vivo* and *in vitro* (Al-Sabti and Metcalfe, 1995). Many substances have been tested in various organisms, such as mollusca, fish, amphibians, reptiles, birds, and mammals (Zúñiga-González et al., 2000; Venier and Zampieron, 2005).

Measuring the rates of apoptosis using flow cytometry facilitates a more complete quantitation of these rates. Using this technique, programmed cell death can be detected using nuclear staining and quantified by identifying cells with fragmented DNA (Telford et al., 1992) or externalized phosphatidylserine at the cell membrane.

The micronucleus test, developed by Schmid in 1975 using bone marrow cells from mammals, has been extensively used to test chemical compound genotoxicity (Campana et al., 2003). Micronuclei (MN) are small corpuscles composed of chromosomal material. After chromatid separation during mitosis, two nuclei are reconstituted, one at each pole. The nuclear membrane is reconstructed around these two sets of chromosomes. However, if an entire chromosome or acentric chromosome fragment does not integrate into the new nucleus (because it is not attached to the mitotic spindle), this chromosome is also considered a small individual nucleus that is referred to as a micronucleus (Villela et al., 2003). Compared with other techniques that detect DNA damage, the micronucleus test features certain advantages: (a) it can be performed rapidly; (b) it is simple; (c) it is inexpensive; and (d) preparation and analysis are easier and faster compared with chromosome abnormality studies (Rocha et al., 2009).

The Archipelago of Marajó, fully located in the State of Pará in the Brazilian Amazon, constitutes one of the richest regions of Brazil in terms of water and biological resources. The Archipelago is formed by a group of islands, which as a whole, is the largest fluvial-maritime island in the world, with 49,606 square kilometers (BRASIL, 2007), extending from the mouth of the Amazon River. It is located between the equator and the parallel 1,55°S latitude and between 48° W and 51°W meridian of longitude.

The Amazon exhibits several consequences from urban growth, including accelerated deforestation; natural resource degradation; pressure on urban infrastructure and equipment; an absence of adequate basic sanitation that has resulted in infectious disease proliferation and is directly linked to child mortality; pollution of rivers primarily due to poor sanitation; a lack of adequate locations for urban waste disposal, which is often deposited in the open or in waterways; and illegal occupation of the territory (Maniçoba, ,2006). In contrast, riverside communities would not exist without an organic relationship with the river and/or lake because their way of life is based on the principle of water-earthwork association (Canto et al., 2009). Thus, maintaining water quality within tolerable limits is a basic need for these populations.

The aim of the present study was to test *Plagioscion squamosissimus* erythrocytes from four municipalities in the Marajó Archipelago (São Sebastião da Boa Vista [SSBV], Anajás, Portel, and Chaves), PA, Brazil, for genotoxic and cytotoxic effects from the river water.

2. Materials and methods

The Marajó Archipelago includes sixteen municipalities that belong to the statistical entity called Mesoregion Marajó. Municipalities sampled in this study (SSBV, Anajás, Portel and Chaves) were indicated by the Association of Municipalities of the Archipelago of Marajó (AMAM) depending on the availability of infrastructure.

Table 1 summarizes the physicochemical parameters of the study area. The temperature is the parameter that experiences the least fluctuation throughout the year across the Archipelago. This pattern of fairly regular temperature distribution shows the typical thermal stability of low-latitude regions. Large changes in the other parameters are more seasonal than spatial, differing markedly between the rainy and less rainy periods.

Table 1.
Summary of physicochemical parameters of the river waters of the Marajó Archipelago. The minimum, maximum, and average of each parameter are displayed.

Parameters	Minimum	Maximum	Average
Transparency (cm)	40.00	118.89	68.12
Temperature (°C)	26.37	32.10	28.72
pH	3.30	7.88	6.14
Conductivity (μS.cm^{-1})	34	11002	1772.8
Total solids (mg.L^{-1})	10	5505	583.19
Dissolved oxygen(mg.L^{-1})	2.60	6.52	4.36
Total nitrogen (mg.L^{-1})	2.01	6.36	4.42
Total phosphorus (mg.L^{-1})	0.001	2.31	0.14

We used the fish species *Plagioscions quamosissimus* [Heckel, 1840] (South American silver croaker, corvina or pescada in Portuguese) as a bio-indicator. This species is a carnivorous freshwater fish restricted to South America and originally from the Amazon basin with a wide distribution in Brazil. In total, 68*P. squamosissimus* specimens were collected with a mean length of 19.22 ± 5.143 cm and a mean weight of 78.03 ± 36.603 *g*, including the control group and specimens from the four locations in the Marajó Archipelago. At each municipality included in the project in the Marajó archipelago, 15 silver croaker specimens were collected using cast nets and gill nets. The same fishing gear was used to collect eight specimens from a reference location (control) in the Baixo Rio Jarumã near Abaetetuba, PA, Brazil (coordinates: 1° 41′ 13.6″ S and 48° 52′ 48.8″ W). Blood samples were collected from each specimen using heparinized syringes. Blood samples were used for two analyses: (I) Approximately 2 mL of blood from each fish was removed and placed in opaque Eppendorf microcentrifuge tubes on ice and then transported to the Human Cytogenetics and Environmental Toxicology Laboratory (Laboratório de Citogenética Humana e Toxicologia Ambiental) at UFPA for flow cytometry analyses. (II) Blood smears were also prepared for each fish and transported to the Aquatic Biology, Reproduction and Larviculture Laboratory (Laboratório de Biologia Aquática, Reprodução e Larvicultura) at the IFPA for Piscine Micronucleus Analyses.

2.1. Flow cytometry assay

In this experiment, the cell cycle distribution was assessed following the procedure described by Nicoletti et al. (1991). Peripheral blood erythrocytes from the fish were incubated at 37° C for 3 h in a lysis solution containing 0.1% citrate, 0.1% triton X-100, and 50 µg/mL propidium iodide in the absence of light. Cellular fluorescence was determined using flow cytometry (*EasyCyteTM Mini System cytometer Guava Technologies Inc*®*., Hayward, CA,* USA) and *CytoSoft 4.1* software. Five thousand events per experiment were evaluated, and cellular debris was omitted from the analysis.

2.2. Piscine micronucleus test

In total, 136 slides were prepared (two slides per fish). The smears were fixed in absolute ethanol for 10 min at the collection sites. At the Aquatic Biology, Reproduction and Larviculture Laboratory (Laboratório de Biologia Aquática, Reprodução e Larvicultura) at the IFPA, the preparations were stained with 5% Giemsa diluted in phosphate buffer (pH 6.8) for 20 min and examined under a microscope at 1000X magnification. The number of normal erythrocytes without MN and the number of damaged cells

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