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Short Communication

Micronucleus test in fishes of the Rio Uruguay using epifluorescence

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ABSTRACT

Pollution of water resources by toxic urban, industrial and agrochemical pollutants is a growing problem all around the world. In a river shared between two countries, such as the Rio Uruguay (geographical limit between Uruguay and Argentina), the problem seems to be worse. We used the micronuclei assay (MN) to evaluate the exposure of *Salminus brasiliensis* and *Leporinus obtusidens* to genotoxic agents in the lower regions of this river. *S. brasiliensis* showed higher percentages of micronuclei with respect to *L. obtusidens*, probably due to bioaccumulation. However, both species exhibit a chronic exposure to genotoxic agents according to the MN test.

KEYWORDS: micronuclei, Rio Uruguay, *S. brasiliensis*, *L. obtusidens*

INTRODUCTION

The aquatic environment is the ultimate destination for almost all urban, industrial and agricultural wastes and for this reason pollution of water resources is a serious problem worldwide [1]. Despite the existence of relevant legislations, pollution of the aquatic environment by toxic chemical and microbiological pollutants is still growing [2]. The Rio Uruguay is a geographic limit between two countries of South America, Uruguay and Argentina. The river supports artisanal fishing that is controlled in the mentioned region by a bi-national commission integrated by both countries (in Spanish CARU) [3]. Of course, in a river which is a shared resource between two different countries, the discharge of effluents is a major problem. However and although it is hard to believe, there are no recent technical or scientific publications related to this issue available, at least in Uruguay.

The average flow of the Rio Uruguay is of 5000 m^3/s. According to [4], the Rio Uruguay carries heavy metals, hydrocarbons, organic matter, chemicals and solid wastes. The impact of these pollutants on the biota and on the Rio de la Plata, where the Rio Uruguay discharges, is determined by variations in its flow. Although recent values on contaminants in specific sites and seasons in the Rio Uruguay have not been published, the estimated discharge of metals made into the Rio de la Plata, are 2 t/d of copper (in tons per day), 1.6 t/d of lead, 1.6 t/d of chrome, 15.1 t/d of zinc (total concentration of metals = 20.3 t/d). The contribution of hydrocarbons is 25.1 t/d, while the ammonium and nitrate contributions are 13.2 t/d and 135.5 t/d, respectively, and the chemical oxygen demand (COD) is 9840 t/d [4]. Additionally, in Uruguay, the 2006/07 harvest of transgenic soybean cultivation that involves the use of glyphosate showed an increase of 360% over a period of 4 years, it represents 44% of the total area with field crops and is concentrated in the Rio Uruguay coast (west margin of the country) [5]. Due to the increasing environmental exposure to all these contaminant agents, especially in water

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streams where fishes are consumed by people, it is imperative to establish aquatic ecosystem monitoring plans [6]. Genotoxic pollution of aquatic ecosystem describes the introduction of contaminants with mutagenic potential [7]; in this sense genotoxins are a broader category of substances that induce changes to the structure or number of genes via chemical interactions with DNA [8]. The study of these changes can be made by biomarkers that are biological responses to environmental pollutants, demonstrating departure from normal status and may occur at the molecular, cellular or whole organism level [9], and the alterations that affect germ cells could generate genetic changes in descendants [10]. There are two kinds of biomarkers; biomarkers of exposure and biomarkers of effect, the latter being those which detect sequential exposures over time [11]. Fishes have become important models for determining the toxic effects of aquatic contaminants, with *in vivo* techniques such as the micronucleus test being efficient not only for assessing genotoxic potential but also for water quality monitoring [2]. Several studies have shown a high incidence of micronuclei in fish peripheral erythrocytes after exposure to different pollutants under both field and laboratory conditions. For this reason several species have been used as environmental sentinels [11].

Micronuclei (MN) are biomarkers of genetic damage; they are cytoplasmic chromatin-containing bodies formed when acentric chromosome fragments or entire chromosomes that fail to become incorporated into daughter cell nuclei during mitosis [12]. They are the product of genetic damage that arises as a result of chromosome breaks or spindle abnormalities [13].

The health status of fishes that inhabit the Río Uruguay as well as the environmental status of the river itself is a matter of debate among Argentina and Uruguay. The aim of this study was to analyze through the MN assay [11], blood samples obtained from two species of neotropical fishes- *Salminus brasiliensis* (Cuvier, 1816) (named “dorado” in Spanish) and *Leporinus obtusidens* (Valenciennes, 1836) (named “boga” in Spanish), collected in the winter of 2010 when a great mortality event of fishes was registered in the Rio Uruguay. Both species are very important resources because they are consumed in the internal market and are also exported. It is important to remark that we chose two species that occupy different trophic levels; *S. brasiliensis* is carnivorous and *L. obtusidens* is omnivorous. Although the bi-national commission (CARU) that regulates the Rio Uruguay and the Aquatic Resources National Direction (in Spanish DINARA) stated that this event may be explained by low temperatures registered in July and August (winter) in the lower regions of the Rio Uruguay, the studies developed by DINARA and CARU were limited to the analysis of agrochemicals in water samples (glyphosate, 2,4 D dichlorophenoxyacetic acid, alpha and beta endosulfan sulfate), that revealed levels under the permitted limits [14]; however, the great caudal of the Rio Uruguay could have diluted the agrochemical compounds making them undetectable. Information related to measurements of heavy metals, agrochemicals or other toxic compounds in fish tissues during the mortality event in the winter of 2010 are not available or have not been published. In this sense, the present work is the first study developed to analyze the possible causes of the great mortality of fishes registered.

MATERIALS AND METHODS

Ten adults (determined according to Baigún *et al*.) [15] of each species (*S. brasiliensis* and *L. obtusidens*) were collected by artisanal fishers from the lower region of the Río Uruguay, next to the outfall (Figure 1), during the period of July-August, 2010.

For each individual, two microscope slides were prepared by drawing a drop of peripheral fish blood over the slide to form a thin smear which was air-dried for 24 hours, shortly after they were fixed with absolute methanol (Dorwill) for an hour and stained with acridine orange (1 mg/ml). Two thousand erythrocytes per individual were examined under an immersion objective (1000x) magnification and scored for the presence of one or more micronuclei using an Olympus Flowview epifluorescence microscope with the appropriate filter (503-530/640) and the Image Pro Plus software (MediaCybernetics, USA) [16]. Percentages of erythrocytes with MN were tested for significant

Figure 1. Map showing sites of sample collection at the lower regions of the Rio Uruguay.

differences between species by non-parametric statistics (Mann-Whitney test; $p < 0.05$). We followed the test according to Hoftman and Ratt [17]. For scoring the micronuclei the following criteria were adopted [18]: a) The diameter of the MN should be less than one-third of the main nucleus, b) MN should be separated from or marginally overlap with the main nucleus as long as there is clear identification of the nuclear boundary and, c) MN should have similar staining as the main nucleus.

It is important to remark that there were no negative controls because this work was made *in situ* along a zone where mortality was reported by the local people. Besides, there are no pristine zones along the coast of the Rio Uruguay and the mortality events of fishes were registered all along Rio Uruguay.

RESULTS AND DISCUSSION

Some authors state that micronuclei are indicators of clastogenic and aneugenic effects provoked by xenobiotics [19]. MN test requires cell division and enables the simultaneous detection of chromosome and genome mutations, the discrimination between clastogen/aneugen events (using specific probes), the co-detection of apoptosis/necrosis and it is applicable on many cell types. For its simplicity, and for being a cheap technique that gives rapid results with statistical power, MN test has been applied in many living models ranging from cells to entire organisms [18, 19, 20]. In this work, for both species analyzed, the number of cells containing MN was the same as the number of micronuclei found. This means that we observed just one MN per cell (Figure 2). For each individual, the ratio among the total MN registered and 2000 erythrocytes were calculated. This procedure was done for all individuals of each species and the ratios were compared through non-parametric statistics (Mann-Whitney at $p < 0.05$). The values obtained for *S. brasiliensis* (0.08) were statistically significant with respect to *L. obtusidens* (0.03) (Figure 3). These differences

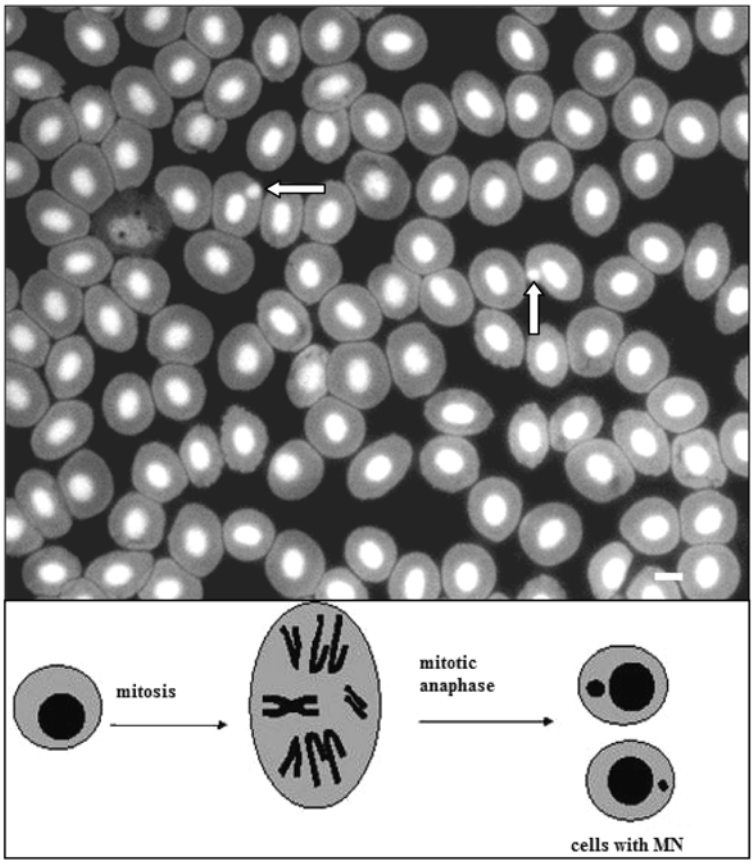

Figure 2. Arrows in the photograph indicate MN in fish erythrocytes. White bar at the bottom right corner of the figure indicates 5 µm magnification. Lower diagram shows the formation process of micronuclei.

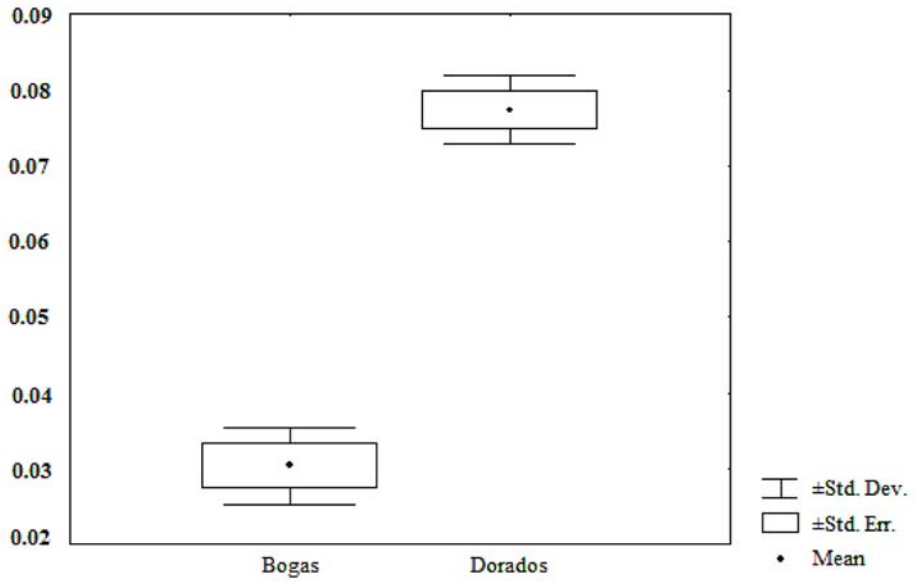

Figure 3. Graph showing significant differences between MN frequencies of *S. brasiliensis* (dorados) and *L. obtusidens* (bogas) at $p < 0.05$.

may be due to the trophic levels that each species occupies in the community. *S. brasiliensis* is a carnivorous species that consumes fishes of several sizes, while *L. obtusidens* is an omnivorous species that consumes mainly grains and invertebrates. *S. brasiliensis* could accumulate genotoxic compounds due to the fact that this species occupies a high trophic level. The MN frequencies are similar to negative controls (0.029) reported by Vanzella *et al*. [21] in laboratory experiments using hydrocarbons as positive controls for a detritivorous species. Pavan *et al*. [22] found higher levels of MN on exposure to microcystins (0.097-0.12) in a carnivorous species. The values found are higher than those reported by Grisolia *et al*. [23] in an eutrophic tropical lake for seven species of fishes (0.029-0.062). This may indicate that the species analyzed in this work could be exposed to genotoxic agents which might explain the high levels of mortality rates registered, especially if we consider that the MN test reveals chronic exposure to xenobiotic agents. However, it is important to remark that this work is a first look at this issue, and for that reason, we strongly state the necessity of conducting further studies in other sites, seasons and species using the MN test and other biomarkers of genetic damage.

CONCLUSION

The micronucleus test used as chronic genetic damage biomarker, showed exposure of two fish species to xenobiotics in the Rio Uruguay during the mortality event of fishes registered in the winter of 2010. *S. brasiliensis* exhibited higher levels of genetic damage compared to *L. obtusidens* probably, because they accumulate substances due to their food habits although they also could be more susceptible to genotoxic compounds than *L. obtusidens.*

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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