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Baseline micronuclei and nuclear abnormalities frequencies in native fishes from the Paraná River (Argentina)

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(With 1 figure)

Abstract

This work aims to establish baseline frequencies of micronuclei (MN) and nuclear abnormalities (NA) in native fish species collected *in situ* from the Paraná River. For this purpose, the micronucleus test was applied in peripheral blood erythrocytes from specimens obtained from samplings collected at two localities (Posadas and Candelaria, Misiones, Argentina) during the period 2007-2010. The results were statistically analyzed using the Kruskal Wallis test. Data from nine fish species were obtained, among which *Steindachnerina brevipinna* (Characiformes) revealed the highest baseline frequency of MN and NA, showing statistically significant differences with regard to the other analyzed species. These results are the first report of baseline MN and NA frequencies for native fish species studied and could be useful for future comparisons with data of fishes belonging to other environments.

Keywords: biomonitoring, micronuclei, nuclear abnormalities, fishes, Paraná River.

Frequências basais de micronúcleos e anormalidades nucleares em peixes nativos do Rio Paraná (Argentina)

Resumo

O presente trabalho tem como objetivo estabelecer frequências basais de micronúcleos (MN) e anormalidades nucleares (AN) em espécies nativas de peixes obtidas *in situ* no Rio Paraná. Para este efeito, o teste do micronúcleo foi aplicado em eritrócitos de sangue periférico de espécimes provenientes de amostragens efetuadas em duas localidades (Posadas e Candelaria, Misiones, Argentina) durante o período 2007-2010. Os resultados foram analisados estatisticamente empregando o teste de Kruskal Wallis. Foram coletados dados de nove espécies e dentre estas *Steindachnerina brevipinna* (Characiformes) revelou a maior frequência basal de MN e AN, mostrando diferenças estatisticamente significativas com respeito às outras espécies analisadas. Estes resultados são o primeiro relatório de frequências basais de MN e AN para espécies nativas de peixes estudadas e poderiam ser úteis para futuras comparações com dados de peixes pertencentes a outros ambientes.

Palavras-chave: biomonitoramento, micronúcleos, anormalidades nucleares, peixes, Rio Paraná.

1. Introduction

Surface waters, such as lakes, rivers, and seas contain complex mixtures of pollutants as a result of anthropogenic action, including genotoxic compounds which cause adverse effects on public health and aquatic ecosystems (Ohe et al., 2004). The biomonitoring employing aquatic organisms such as fishes is essential to indicate quality and sustainability of the environment (Ramsdorf et al., 2012). In this regard, fishes are suitable as bioindicators of environmental conditions due to their position in the trophic chain and their sensitivity to low concentrations of genotoxic substances (van der Oost et al., 2003).

Any biological response to an environmental pollutant measured in an organism is described as a biomarker, comprising a broad range of parameters such as hematological, immunological, reproductive, endocrine, genotoxic, neuromuscular, physiological, histological and morphological (van der Oost et al., 2003). Within the variety of biomarkers for assessing aquatic environments, those which reflect effects on DNA are well-known due its ecological implications (Ramsdorf et al., 2012). The micronucleus test is one of the most employed cytogenetic techniques for genotoxicity assessment and has been widely applied in peripheral blood of teleostean fishes in studies of field (*in situ*) and bioassays (Al-Sabti and Metcalfe, 1995). Micronuclei (MN) arise from whole chromosomes or acentric fragments that are delayed during anaphase, either by the lack of centromeres or by damage to the mitotic spindle (Schmid, 1975). In addition, changes in the normal morphology of the nuclei are also considered indicators of genotoxic damage, described by Carrasco et al. (1990) as nuclear abnormalities (NA), and constitute a complementary analysis to the scoring of micronuclei.

MN frequencies spontaneously originated have been reported in different species of mammals, reptiles, and birds (Zuniga-Gonzalez et al., 2000). In this way, several studies of biomonitoring *in situ* in rivers and lakes were accomplished using the micronucleus test with diverse fish species bringing about interspecific variation in baseline frequencies of genetic damage (Grisolia et al., 2009; Rodriguez-Cea et al., 2003). However, researches employing biomarkers in tropical and neotropical freshwater fish from South America are scarce (Rabitto et al., 2005). In this part of the continent, the Paraná River is the second longest (4695 km) beginnig in south-central Brazil at the confluence of Paranaíba and Grande rivers and being the main tributary of the La Plata River (Agostinho et al., 2004). The province of Misiones is located at the northeastern of Argentina in the Mesopotamian region which is limited at the west by the Paraná River representing an area of considerable ichthyofaunistic diversity (López et al., 2005).

The present study intends to establish the baseline frequencies of MN and NA in native fish species from the Paraná River (Argentina) in the sampling areas of Posadas and Candelaria towns, describing their interspecific variation. The data obtained will be useful since they will provide support for future comparative studies with fishes belonging to other species and environments.

2. Material and Methods

2.1 Samplings

Specimens were collected from the Paraná River at the old bathing sites of Posadas (Itacurubí beach and Pyrá Pytá yacht club) and Candelaria (El Anselmo) towns located at the province of Misiones (Argentina). With respect to the first town, the two areas sampled were authorised bathing sites since they satisfied the water quality guidelines for human recreation. The second place, El Anselmo, is an uninhabited coastal area near the town of Candelaria without direct anthropic influence. A total of twenty three samplings were realized during the period 2007-2010 comprising the months of February, March, April, June, August, October and November.

Fishes were collected using gillnets (sizes: 4, 5 and 6 cm) and a seine net. Each specimen was recorded in the database with a numerical code noting the species, date and place of collection, frequencies of MN and NA. Specimen catches and extraction of biological samples were authorized by the "Ministerio de Ecología y Recursos Naturales Renovables" of the province of Misiones.

2.2 Micronucleus test and cytological analysis

The MN test was performed according to the protocol developed by Hooftman and De Raat (1982), with modifications. Peripheral blood samples were obtained from the specimens collected by puncture in the caudal vein using syringes previously heparinized. For each individual, the samples were smeared in two slides and subsequently dried at room temperature overnight. The smears were fixed in methanol 100% for 15 minutes and stained with a 10% Giemsa solution for 10 minutes after drying. The slides were analyzed through an optical microscope under 100x magnification and 1000 cells were scored from each slide (a total of 2000 erythrocytes for each specimen) registering the MN and NA frequencies.

The following inclusion criteria were used for MN scoring: 1) separated and with the same staining of the main nucleus, 2) size smaller than 1/3 of the main nucleus, 3) nonrefractive.

The NA were distinguished in Notched, Lobed, and Blebbed nuclei, according to the classification proposed by Carrasco et al. (1990). Furthermore, two types of NA found in the studied individuals, which were denominated "Eightshaped" (nuclei presenting a constriction acquiring an eight shape) and "Bud" (buddings partially separated from the nucleus), were added.

2.3 Statistical analysis

First, the Shapiro-Wilks test was applied to check the normality of the data. Since data obtained from most of the studied species did not present a normal distribution for both frequencies (MN and NA), they were analyzed by the nonparametric Kruskal Wallis and Mann Whitney tests ($p \leq 0.05$) for comparison of differences between groups. The entire analysis was done with the statistical programme InfoStat version 2011 (Di Rienzo et al., 2011).

3. Results

In the present study, nine native species belonging to three different orders were assessed (see Table 1). Other species like *Catathyridium jenynsii* and *Pseudotylosurus angusticeps* were catched but since their capture frequency was very low they were not included in this study.

The global analysis of MN and NA showed the highest frequencies in *S. brevipinna*, while the species *S. nasutus*, *A. affinis* and *C. niederleinii* only presented alterations in the nuclear morphology (see Figure 1, Tables 2 and 3). The comparison of medians using the Kruskal Wallis test indicated that *S. brevipinna* presents significantly higher frequencies of MN ($P=3.2.10^{-6}$–0.02) and NA ($P=1.2.10^{-7}$–0.03 respectively) than the other analyzed species. Also, it was possible to observe that *A. asuncionensis* presents statistically higher frequencies of NA than *S. nasutus*, *A. affinis*, *C. niederleinii,* and *L. simillima* ($P=1.4.10^{-3}$–0.03).

In regard to the NA analysis, *S. brevipinna* showed the highest level and most of them were nuclear buds (see Table 3). However, for the other species, the most

Table 1. Fish species, number of individuals (N) and site of collection from the Paraná River (Posadas and Candelaria, Misiones).

Order	Species	Site	N
	Steindachnerina brevipinna (Eigenmann & Eigenmann, 1889)	Posadas	17
	Astyanax asuncionensis Géry, 1972	Posadas	38
Characiformes	*Astyanax schubarti* Britski, 1964	Posadas	8
	Leporinus obtusidens (Valenciennes, 1837)	Posadas	4
	Schizodon nasutus Kner, 1858	Posadas	9
	Apareiodon affinis (Steindachner, 1879)	Posadas	8
Perciformes	*Crenicichla niederleinii* (Holmberg, 1891)	Posadas	8
Siluriformes	*Pimelodella cf griffini* Eigenmann, 1917	Candelaria	8
	Loricaria simillima Regan, 1904	Candelaria	10

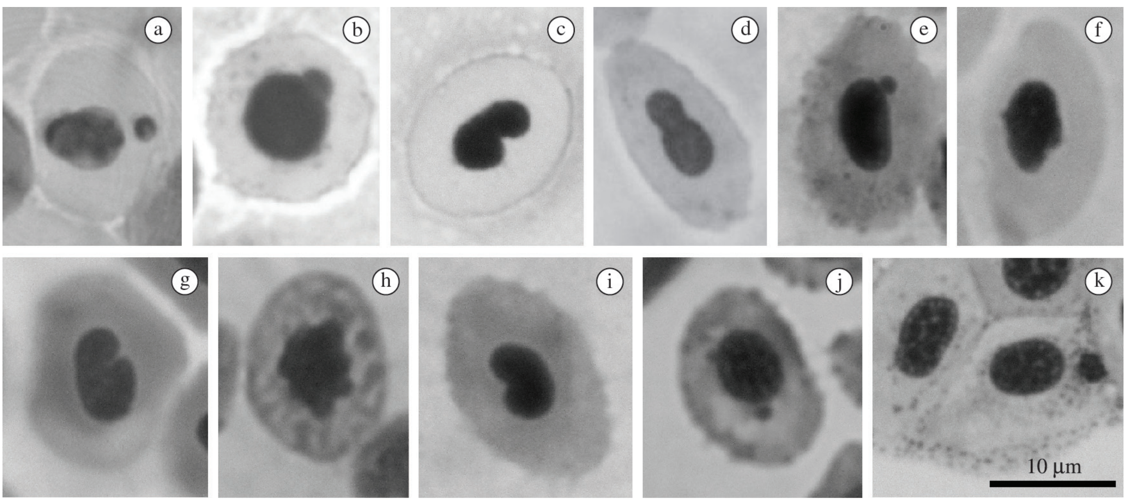

Figure 1. Photomicrographs of MN and NA in erythrocytes from the analyzed species. a) MN, *S. brevipinna*. b) Bud, *S. brevipinna*. c) Notched, *A. asuncionensis*. d) Eightshaped, *A. asuncionensis*. e) MN, *A. schubarti*. f) Blebbed, *L. obtusidens*. g) Notched, *S. nasutus*. h) Lobed, *A. affinis*. i) Notched, *C. niederleinii*. j) MN, *P. cf griffini*. k) MN, *L. simillima*.

Table 2. Baseline micronuclei (MN) frequencies[a] (Fr) and means ± standard deviations ($\overline{X}$ ± SD) for the nine studied species sampled from the Paraná River.

Species	Fr	$\overline{X}$ ± SD
S. brevipinna	0.0028*	5.65 ± 3.9
A. asuncionensis	$6.7.10^{-4}$	1.34 ± 2.4
A. schubarti	$4.3.10^{-4}$	0.88 ± 0.8
L. obtusidens	$3.7.10^{-4}$	0.75 ± 1.5
S. nasutus	0	0
A. affinis	0	0
C. niederleinii	0	0
P. cf griffini	$6.2.10^{-5}$	0.13 ± 0.3
L. simillima	5.10^{-5}	0.1 ± 0.3

[a] Frequencies calculated per 2000 cells. * Significative $p \leq 0.05$ – Kruskal Wallis.

frequently observed NA were blebbed (*A. asuncionensis*, *L. obtusidens*, *A. affinis*, *P. cf griffini*) and notched nuclei (*A. schubarti*, *S. nasutus*, *C. niederleinii*, *L. simillima*, *P. cf griffini*).

According to the fact that *S. brevipinna* and *A. asuncionensis* were the most frequently captured species in different periods, was possible to evaluate their baseline frequencies through time. The first species was collected in two periods during 2007 not showing temporal variations in the MN and NA frequencies since they were not statistically significant using the Mann Whitney test. The other species was catched in three periods, two of them in 2007 and the other one in 2009. For the first year was observed a significant decrease (P=0.01; $5.9.10^{-5}$) through time in the baseline frequencies of MN and NA keeping the lower values of the last period even 2009.

4. Discussion

These results show interespecific variations in the baseline MN and NA frequencies (see Tables 2 and 3). Similar results were described in studies carried out with other fish species establishing *in situ* frequencies (Rodriguez-Cea et al., 2003; Grisolia et al., 2009). The highest baseline MN and NA frequencies were found in the species *S. brevipinna*. Since the localities sampled did

Table 3. Baseline nuclear alterations (NA) frequencies[a] (Fr) for each class of alteration and the total of them[b]. Means ± standard deviations ($\overline{X}$ ± SD) of total NA for the nine studied species sampled from the Paraná River.

Species	Fr of different classes of NA					Total NA Fr	Total NA $\overline{X}$ ± SD
	Lobed	Blebbed	Notched	Eightshaped	Bud		
S. brevipinna	0.0026	0.0049	0.0011	0.0023	0.0092	0.02*	40.5 ± 35.8
A. asuncionensis	0.0011	0.0025	0.001	$6.8.10^{-4}$	$4.4.10^{-4}$	0.0057*	11.4 ± 13.3
A. schubarti	$1.2.10^{-4}$	$8.1.10^{-4}$	0.0012	$1.8.10^{-4}$	0	0.0023	4.7 ± 4.5
L. obtusidens	$1.2.10^{-4}$	0.0013	0	0	$2.5.10^{-4}$	0.0017	3.5 ± 4.7
S. nasutus	$5.5.10^{-5}$	$2.2.10^{-4}$	0.0011	$2.7.10^{-4}$	$1.1.10^{-4}$	0.0018	3.6 ± 5.1
A. affinis	$1.2.10^{-4}$	$4.3.10^{-4}$	$6.2.10^{-5}$	$1.2.10^{-4}$	$3.1.10^{-4}$	0.001	2.1 ± 3.7
C. niederleinii	$6.2.10^{-5}$	$4.3.10^{-4}$	$6.2.10^{-4}$	0	$1.2.10^{-4}$	0.0012	2.5 ± 4.4
P. cf griffini	$2.5.10^{-4}$	$5.6.10^{-4}$	$5.6.10^{-4}$	$1.2.10^{-4}$	0	0.0015	3 ± 2.2
L. simillima	2.10^{-4}	$2.5.10^{-4}$	$4.5.10^{-4}$	$1.5.10^{-4}$	3.10^{-4}	0.0013	2.9 ± 6.7

[a] Frequencies calculated per 2000 cells; [b] Total NA = Σ Frequencies of Lobed, Blebbed, Notched, Eightshaped, Bud. * Significative p ≤ 0.05 – Kruskal Wallis.

not receive known direct discharges of pollutants and the collected fishes were rarely exposed to urban sewage the higher frequencies in this species could not be explain because of environmental substances in surface waters. In this case, *S. brevipinna* probably presents greater frequencies than the other analyzed species, due to its detritivore habit and its particular intestinal structure, providing a greater surface area for absorption of those lipophilic substances found in the sediment. Moreover, the same species did not show temporal variations in spontenous frequencies while *A. asuncionensis* showed a decline towards the last period in 2007 remaining this lower frequencies through the time.

All results reached in this work constitute a database of baseline MN and NA frequencies that enables comparisons between native studied species aiming to detect possible variations in response to the environmental conditions of the area of sampling. Furthermore, this paper provides additional information on similar records of spontaneous MN frequencies reported for fishes by other working groups (Grisolia et al., 2009). This record provides a base for the selection of species as suitable indicators in biomonitoring studies. The establishment of these frequencies for other species should be considered, since the differential sensitivity of genetic material may be influenced by the ecological habits. In this sense, the interspecific variations in the MN and NA baseline frequencies are probably related to the inherent ecological characteristics of the organism to efficiently ingest, accumulate, metabolize and excrete (Jha, 2004). Furthermore, other factors including the efficiency in DNA repair and the cell removal kinetics which allow the elimination of those damaged cells could be involved in the interspecific variation of spontaneous MN and NA frequencies (Bolognesi and Hayashi, 2011).

Thus, systematic monitoring would detect variations in the levels of spontaneous genetic damage, which could affect genetic diversity in natural populations that can be influenced by pollution-induced mutations (Handy et al., 2002).

In conclusion, this work offers an *in situ* database of baseline MN and NA frequencies for native fish species from the Paraná River (localities of Posadas and Candelaria) and constitutes the first report on the nine analyzed species in this area. The highest baseline MN and NA frequencies were found in the species *S. brevipinna.* This database could be useful for future comparisons with data of fishes belonging to other environments while the establishment of these frequencies for other species should be considered in future biomonitoring programmes.

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