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Existem diferenças na frequência de micronúcleos em *Astyanax lacustris* em relação ao sexo, massa e comprimento?

Are there differences in the frequency of micronuclei in *Astyanax lacustris* in relation to sex, mass and length?

Existen diferencias en la frecuencia de micronúcleos en *Astyanax lacustris* en relación con el sexo, la masa y la longitud?

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Resumo

O objetivo deste estudo foi analisar os efeitos genotóxicos em *Astyanax lacustris* e testar a existência de diferenças na frequência de micronúcleos em relação ao sexo, massa e comprimento dos indivíduos. As amostras foram coletadas na Microbacia de Tarumã (Alto Rio Paraná, Mato Grosso do Sul, Brasil). Neste estudo, relatamos que essa espécie apresentou a mesma frequência relativa de micronúcleos para ambos os sexos e para indivíduos de diferentes massas e comprimentos.

Palavras-chave: Ambiente aquático; Contaminação; Genotoxicidade; Peixe; Biometria.

Abstract

The aim of this study was to analyze genotoxic effects in *Astyanax lacustris* and to test whether there are differences in the frequency of micronuclei in relation to sex, mass and length of individuals. The samples were carried out in the Tarumã Microbasin (Upper Paraná River, State of Mato Grosso do Sul, Brazil). In this study, we described this species presented the same relative frequency of micronuclei for both sexes, and for individuals of different masses and lengths.

Keywords. Aquatic environment; Contamination; Genotoxicity; Fish; Biometry.

Resumen

El objetivo de este estudio fue analizar los efectos genotóxicos en *Astyanax lacustris* y probar si existen diferencias en la frecuencia de micronúcleos en relación con el sexo, la masa y la longitud de los individuos. Las muestras se llevaron a cabo en la microcuenca Tarumã (río Alto Paraná, estado de Mato Grosso do Sul, Brasil). En este estudio, encontramos que la especie presentaba la misma frecuencia relativa de micronúcleos para ambos sexos y para individuos de diferentes masas y longitudes.

Palabras clave: Ambiente acuático; Contaminación; Genotoxicidad; Pez, Biometria.

1. Introduction

Freshwater ecosystems are under great anthropogenic pressures, mainly due to changes in their surroundings, with the decrease in native vegetation cover replaced by large agricultural areas (Lima et al., 2018; Pandey et al., 2018). Each year, agricultural boundaries expand and the scenery near water bodies becomes a fragmented vegetation presenting only mosaics or stretches of vegetation, causing several damages to the aquatic environment, such as fragile slopes, erosions, siltation and consequently habitat degradation and ecological imbalance (Dar et al., 2016; Lima et al., 2018). Thus, several contaminants are carried into the aquatic environment which can compromise survival, physiology of organisms and induce genetic damage, changes in cell cycle, chromosomal breakage and loss, and cell spindle malfunction, and compromising changes in DNA content (Darzynkiewicz et al., 2017; Corduk et al., 2018; Monteiro et al., 2018).

The environmental health assessment of aquatic ecosystems using fish as biomarkers (DNA damage) has been increasingly recommended due to responses to the effects of contamination on the aquatic environment by genotoxic agents (Viana et al., 2017; Lima et

al., 2018; Hussain et al., 2019). The micronucleus test, for example, detects aneugenic and clastogenic effects and has the ability to identify genotoxicity of a wide range of contaminants, which can lead to reduced local aquatic diversity, such as endemic species (Hariri et al., 2018; Hussain et al., 2019).

Astyanax lacustris (Lutken, 1875) (Characiformes, Characidae) is a species that have been widely used as an environmental bioindicator in both *in situ* and *ex situ* studies in the assessment of genotoxic effects (Trujillo-Jiménez et al., 2011; Chehade et al., 2014; Dourado et al., 2016; Viana et al., 2017; 2018; Milanin et al., 2018; Sposito et al., 2019). And in this context, it is important to note that recently Lucena and Soares (2016) proposed the unification of the species *A. asuncionensis*, *A. altiparanae* and *A. lacustris*, considered synonymous, under the name *Astyanax lacustris* (Lutken, 1875).

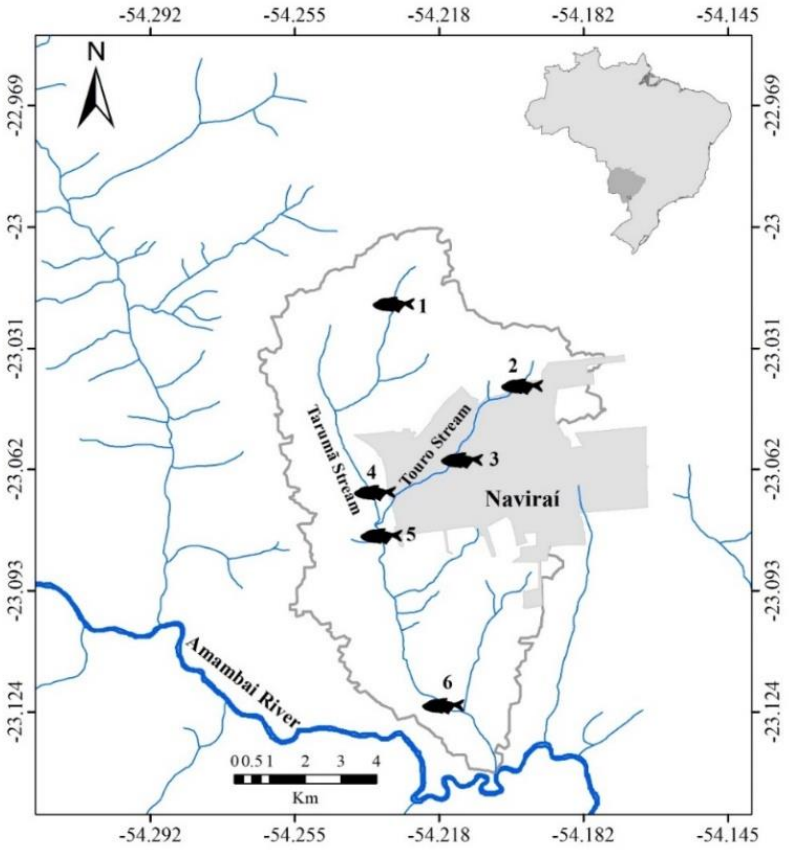
A. lacustris, known as 'yellow tail lambari', has a great ecological importance for the conservation of riparian forest, acting as a seed disperser helping to contribute to the balance in Brazilian hydrographic basins (Lima et al., 2011; Chehade et al., 2014; Siqueira-Silva, 2015; Milanin et al., 2018; Pinheiro et al., 2019; Abdalla et al., 2019). Besides this, shows characteristics that make it particularly useful as a bioindicator species, because it is an omnivorous, opportunistic species, and also has high plasticity for different environmental conditions that is abundant has a wide distribution in the Neotropical region (Siqueira-Silva, 2015; Milanin et al., 2018). Despite this, there is no information in the literature on the variation in genotoxic responses due to variables such as sex and size of individuals. In this background, the study aimed to assess genotoxic effects in the *Astyanax lacustris* and to test whether there are differences in the frequency of micronuclei in relation to sex, mass and length of individuals.

2. Methodology

Study area

The samples were carried out in the Tarumã Microbasin (Upper Paraná River, State of Mato Grosso do Sul, Brazil), in six collection sites, between July 2014 and December 2015 (see Figure 1). The study was field and laboratory, with a quantitative response (Pereira et al., 2018).

Figure 1. Location of sampling sites in the Tarumã microbasin, Upper Paraná River, Brazil.



Source: Viana et al. (2018).

Figure 1 shows the location of the study points in the Tarumã microbasin.

Data collection and analysis

The specimens of *A. lacustris* were sampled in the diurnal period with a rectangular metal sieve measuring 0.8 x 1.2 m, with a mesh of 2 mm. Soon after capture, the fish were immersed in cold water to reduce their activity, to obtain blood samples through caudal puncture, with heparinized syringes. For each fish sample, a drop of blood was placed on two slides, to form a thin layer. The smears were dried in air for 15 min, fixed in absolute alcohol for 10 min and then stained with Giemsa solution at 10% for 20 min (Schmid, 1975; Jesus et al., 2016). 2000 blood cells per slide were analyzed, resulting in a total of 4000 cells for everyone, using an optical microscope with magnification of 1000x. The micronuclei were identified following the criteria proposed by Fenech et al. (2003). The collection procedures were approved by the Ethics Committee on the Use of Animals at UEMS (011/2014) and

authorized by IBAMA (SISBIO 11156-1).

In the laboratory, some biometric data were obtained for everyone: standard length (mm), total length (mm), total weight (g) and sex.

For statistical analyses of total weight, total length and micronuclei frequency in relation to sex we used a t-test (α=0.05), after to verify the assumptions of normality and homoscedasticity. To assess the micronuclei frequency in relation to mass and length of the individuals we calculated the correlation by Spearman's coefficient. All tests were performed using the R software (R Development Core Team, 2019).

3. Results and Discussion

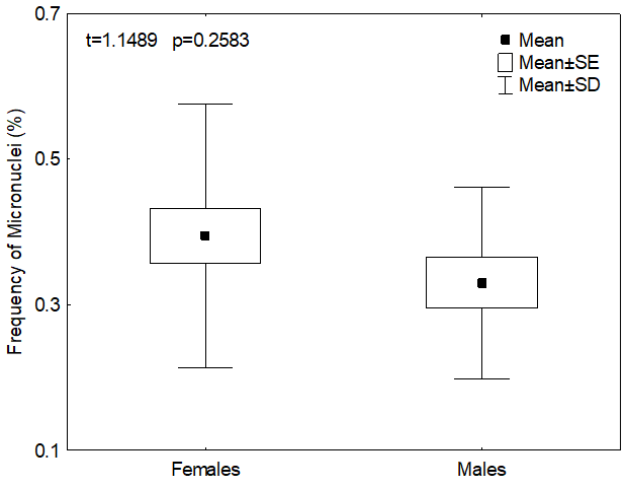
In total, 37 specimens of *A. lacustris* were collected: 23 females and 14 males (Table 1). The Figure 2 shows the frequency of micronucleus observed in males and females of *A. lacustris* and the Figure 3 shows the scatterplots representing the relationship between frequency of micronuclei and total weight (3A) and between frequency of micronuclei and total length (3B) of individuals.

Table 1. Biometric data (mean ± standard deviation) of *A. lacustris* in the Tarumã microbasin, Upper Paraná River, Brazil.

Group	N	Total length (mm)	Total weight (g)
Females	23	86.542±16.978	11.404±8.293
Males	14	89.864±21.942	8.805±3.519

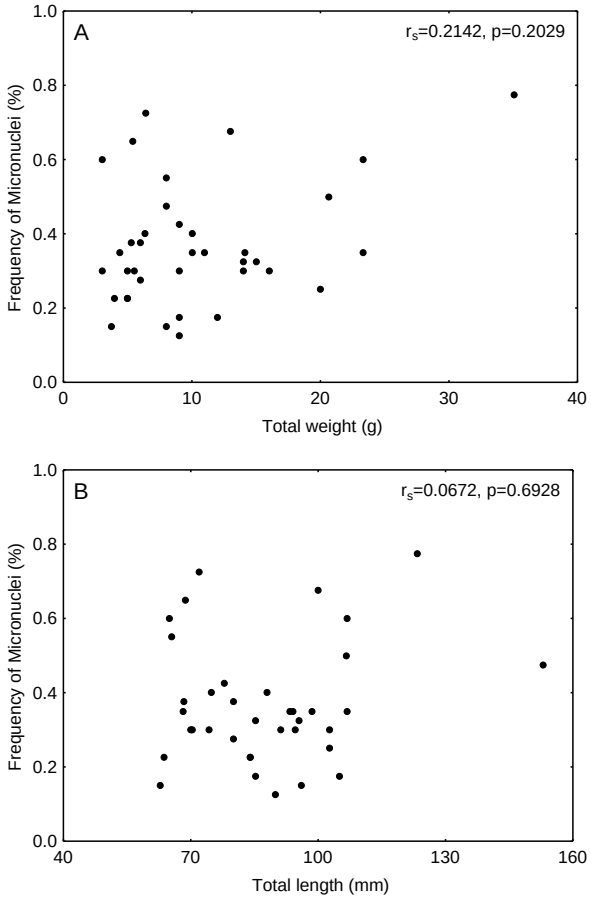
N = number of the individuals. Source: Authors.

Figure 2. Frequency of micronuclei (mean ± standard deviation) in males and females of *A. lacustris* in the Tarumã microbasin, Upper Paraná River, Brazil.



Source: Authors.

Figure 3. Frequency of micronuclei in relation to total weight (A) and total length (B) of the individuals of *A. lacustris* in the Tarumã microbasin, Upper Paraná River, Brazil



Source: Authors.

We found that there was no difference in the total weight (t=-0.5164, p=0.6088), total length (t=1.1082, p=0.2753) and micronuclei frequency between the sexes (t=1.1489, p=0.2583; Figure 2), and there was also no significant correlation between the micronuclei frequency and the total weight and total length of *A. lacustris* (r_s=0.2142, p=0.2029 and r_s=0.0672, p=0.6928, respectively; Figure 3).

Several studies in the literature report positive responses of the species *A. lacustris* (including its synonyms species) as an environmental model to assess mutagenic and genotoxic effects of various contaminants through bioassays (Dourado et al., 2016; Viana et al., 2018; Pinheiro et al., 2019), but there is no information on the effect of sex and size of individuals on this biomarker. Another species of Characiformes (*Prochilodus argenteus*), for example, shows differences in the response of histopathological biomarkers in relation to the sexes, since males showed greater foci of chronic inflammation in the gills compared to females (Procópio et al., 2014). This result reinforces the need to know whether individuals of different sexes or different sizes of the *A. lacustris* have different genotoxic responses when exposed to the same environmental conditions.

4. Final Considerations

In our study we found that the species showed the same relative frequency of micronuclei for both sexes, and for individuals of different masses and lengths. As this species has been widely used in recent years as an bioassays model for different mutagenicity and genotoxicity tests, our results contribute to these assessments, so we demonstrate that there is no need to control the variables of sex, length and weight when carrying out these bioassays. The results of this study answered the proposed objectives and in addition to showing that both males and females of individuals of the species *A. lacustris* can be used as animal models for *in situ* and *ex situ* studies.

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