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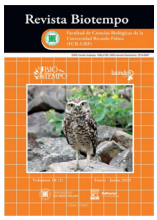

RESEARCH NOTE / NOTA CIENTÍFICA

CELLULAR MORPHOLOGICAL BIOMARKER IN *OREOCHROMIS NILOTICUS* (LINNAEUS, 1758) (PERCIFORMES: CICHLIDAE) OF WATER RESOURCES IN THE TOCANTINA REGION OF MARANHÃO, BRAZIL

BIOMARCADOR MORFOLÓGICO CELULAR EN *OREOCHROMIS NILOTICUS* (LINNAEUS, 1758) (PERCIFORMES: CICHLIDAE) DE RECURSOS HÍDRICOS EN LA REGIÓN TOCANTINA DE MARANHÃO, BRASIL

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ABSTRACT

The objective of this study was to evaluate a cellular morphological biomarker based on micronucleus count and nuclear abnormalities in the tilapia *Oreochromis niloticus* (Linnaeus, 1758). The tilapias were collected from a fish farm nursery in the city of Imperatriz, Maranhão, Brazil. *O. niloticus* blood was collected by caudal puncture, then micronucleus counting was performed, erythrocytic abnormalities were detected, and finally it was stained with Giemsa. The micronucleus count was determined from 2000 erythrocytes. Observations were made in three repetitions per fish (n=10). The frequency of nuclear morphological anomalies in the investigated fish presented an average of 27.39 ± 3 micronuclei per slide. Thus, the presence of micronuclei is an indicator to identify possible environmental contamination.

Keywords: micronucleus count – nuclear alteration – pollution

RESUMEN

El objetivo de este estudio fue evaluar un biomarcador morfológico celular basado en el recuento de micronúcleos y anomalías nucleares en la tilapia *Oreochromis niloticus* (Linnaeus, 1758). Las tilapias se recolectaron de un vivero de una

piscigranjas en la ciudad de Imperatriz, Maranhão, Brasil. La sangre de *O. niloticus* se recogió mediante punción caudal, luego se realizó el recuento de micronúcleos, se detectaron las anomalías eritrocíticas y finalmente se tiñó con Giemsa. El recuento de micronúcleos se determinó a partir de 2000 eritrocitos. Se realizaron las observaciones en tres repeticiones por pez (n=10). La frecuencia de anomalías morfológicas nucleares en los peces investigados, presentó un promedio de 27,39±3 micronúcleos por portaobjetos. Así, la presencia de micronúcleos es un indicador para identificar posibles contaminaciones ambientales.

Palabras clave: alteración nuclear – contaminación – recuento de micronúcleos

INTRODUCTION

The micronucleus test has been considered promising in investigations to detect the potential to cause gene mutations and chromosomal changes (Kus *et al.*, 2017). The micronucleus assay is described by the Economic Co-operation and Development (OECD) and is recommended by the International Conference on Harmonization (1997) among other regulatory agencies to investigate the genotoxic action of different agents (Davoren & Schiestl, 2018). The piscine micronucleus test (MNP) has already been investigated in different types of fish cells (Seriani *et al.*, 2015) and used in field monitoring studies covering several species including Nile tilapia (Nascimento-Monteiro *et al.*, 2018).

For several years around the world, aquatic ecosystems have been suffering from anthropic impacts, where the final destination of various pollutants are the rivers, tributaries and seas themselves (Liu *et al.*, 2021). The practice of fishing remains a simple activity performed in the region of the middle Rio Tocantins, Imperatriz, Maranhão, Brazil, as it is characterized by being a profitable and familiar practice represented by professional and/or subsistence fishermen who explore multispecific and multi-device fishing (Barbosa *et al.*, 2020).

In turn, Tilapia (*Oreochromis niloticus* (Linnaeus, 1758)), represents a species of fish intensively cultivated in fish farming worldwide and is among the most suitable species for intensive cultivation in tropical regions. This species has important characteristics for fish production, such as the short reproduction cycle and rapid growth (Vaseem & Banerjee, 2016). In addition, these fish are widely used in studies of toxicological genetics associated with environmental biomonitoring (Manzano *et al.*, 2015).

In view of the above, there is no information on the quality of the fish caught in the Tocantins River and in controlled environments such as psicultures. Thus, there is a need for environmental monitoring capable of detecting xenobian effects using, for example, biomarker detection techniques (Alimba *et al.*, 2019). In general, fish are the best bioindicators currently used by researchers to assess changes in the aquatic ecosystem, in addition to being considered capable of responding more accurately to adverse effects more appropriately (Cruz *et al.*, 2019).

For the analysis of biomarkers, the micronucleus test is the most used by researchers, as it is a simple, low-cost and accurate test and has been used as an experimental model for the evaluation of contamination and the genotoxic effects of pollutants in ecosystems aquatic (Al-Sabti & Metcalfe, 1995; Gomes *et al.*, 2019). Thus, the increase in the frequency of micronuclei is the result of DNA damage potentially caused by mutagens, such as metals and organic compounds (Fenech, 2000; Ribeiro *et al.*, 2003; Yokoi *et al.*, 2019).

This research is justified by the need to diagnose the health of Tilapia (*O. niloticus*) through the micronucleus test in peripheral erythrocytes and it is a research with the generation of partial and pioneering data for the Tocantina region of Maranhão, Brazil.

MATERIALS AND METHODS

The research was conducted captive environment Imperatriz, Maranhão, Brasil. During the collection, ten samples were total, four from the Fish Farm collected in May 2017 and six in November of that year belonging to the Tilapia species (*O. niloticus*). The fish was captured and transported alive in a plastic container (bucket) to the anatomy laboratory of the State University in the Tocantina Region of Maranhão (UEMASUL), Brazil.

Blood samples were collected via caudal puncture with heparinized and disposable syringes. A drop of blood from each specimen was used to make the smear. After the slides were dried for about 30 minutes, the staining was continued through the giemsa for six minutes and then washed with distilled water for four minutes and allowed to dry at room temperature. In each blade, a total of 2000 cells were analyzed (Dittmar *et al.,* 2010). The analysis of fish erythrocytes was performed according to Ditmar *et al.* (2010) for determining the frequency of micronuclei and nuclear morphological changes (AMN). After making the blades, they were taken to the microscope for viewing in a 100-degree objective, using emersion oil for better visibility of the cells. Carrasco *et al.* (1990) described and photographed the morphological changes found in fish erythrocyte nuclei, in addition to micronuclei, and classified as: bebbled; lobed; notched and vacuolated. Fenech (2000), added the technique describing binucleus as another morphological alteration that detects the presence of xenobiotics in the environment. All of these characteristics were taken into account when viewing the erythrocytes from blood smears performed on specimens captured for the current research. The data were analyzed using mean and standard deviation.

Ethic aspects

The research was approved with the Ethics Committee on Animal Experimentation at the State University of Maranhão (UEMA / CCA), being in accordance with by the ethical principles of animal experimentation, adopted by the mentioned protocol committee N° 21/2017.

RESULTS

For the species of fish studied, alterations of the blebbed, lobed, binucleus and vacuolated type were not observed in any of the slides analyzed (Table 1). The analysis using the micronucleus test showed an average of 27.39±3.

Table 1. Number of micronuclei (NM) and nuclear abnormalities (NA) in peripheral erythrocytes from specimens (N=10) collected in the local fish farm of Imperatriz, Maranhão, Brasil.

Study	Specimens	Total Cells	Total MN	Total NA
Fish farm	*Oreochromis niloticus*	2000	73	0

DISCUSSION

According to Cantanhêde *et al.* (2016), the micronucleus formation process depends on the event of cell division after exposure to the genotoxic agent. In addition, the time required for cell segmentation depends on the type of tissue, species and environmental conditions (Hader & Erzinger, 2017). Different aquatic environments have different dissolved compounds, these components have been associated with impacts on biota, however studying their combined effects and determining the risk of exposure is still a challenge (Shiroma *et al.*, 2019).

Taking into account the importance of blood in the systemic circulation in fish, changes in hematological indices in response to water contamination are considered a biomarker sensitive to the health of these organisms (Seriani *et al.*, 2015). In general, changes in fish hematological indexes occur before any morphological and degenerative damage begins (Mazon *et al.*, 2002). In addition, the significant increase in NM frequencies is associated with genome instability and is strongly correlated with different pathogens, reproductive disorders and cancer formation in fish (Alimba *et al.*, 2015; Yamamoto *et al.*, 2017). According to Fenech (2007), the presence of NM serves as a marker of genomic instability, being a typical characteristic of cancer. A study by Yokoi *et al.* (2019) demonstrated that micronuclei are more prevalent in cancer cells than in healthy cells suggesting that cells with a higher NM index have high instability.

The elevated levels of micronuclei and nuclear abnormalities were observed in the erythrocytes of the genetic model, the Nile tilapia (*O. niloticus*) exposed to water samples from the two sources tested when compared to a control group indicating the presence of genotoxic and hazardous pollutants in the water bodies of Estero de Paco and Estero de Vitas, Philippines (Alam *et al.*, 2019). Manganese and iron were found at high concentrations (3.61 $mg \cdot L^{-1}$ and 19.8 $mg \cdot L^{-1}$, respectively) in the water of the Rio Doce after the dams of Fundão and Santarém broke in Mariana/MG (Brazil). These same metals were found in fish (*O. niloticus*) and crustacean muscle (15 $mg \cdot kg^{-1}$ and 8 $mg \cdot kg^{-1}$ wet weight, respectively) in the

specimens collected near the Rio Doce's outfall. These exposures caused significant erythrocyte micronucleus formation in the organisms exposed to the highest concentration, as well a significant increase in the DNA damage index of erythrocytes (Coppo *et al*., 2018).

According to Grisolia *et al*. (2009), one should be aware of the differential sensitivity of aquatic organisms to genotoxic agents and their responses to them and their relationships in the aquatic ecosystem. In view of these changes, other studies point to the influence of intraspecific factors in responses to the MNP test, these factors include: age, sex, diet, health, reproductive status and genetic lineage (Al-Sabti & Metcalfe 1995).

Matias & Flohr (2015) developed a mathematical model (Model WTox) to assess and classify environmental risk based on the results of the study of the relationship between the organism and complex mixtures, addressing parameters of global and specific toxicology including the micronucleus test. Toxicity is classified into levels ranging from A to E, ranging from extremely toxic to non-toxic, respectively. According to this criterion, the value described for the current study is highly toxic.

The age of fish is another possible variable, since it has been reported in studies with mammals that older individuals tend to be more sensitive to cytogenetic damage and in fish in the early stages of development than in the adult stage due to exposure to genotoxic agents (Christie & Costa, 1983). Diet is another factor that can influence the metabolic activation of cell pathways by genotoxic chemicals (De Flora *et al*., 1993). Thus, in fish, studies are needed to better delimit the responses to the micronucleus test according to sex, environment, diet and specific-species.

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