Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

# Histological biomarkers and biometric data on trahira *Hoplias malabaricus* (Pisces, Characiformes, Erythrinidae): a bioindicator species in the Mearim river, Brazilian Amazon

## Biomarcadores histológicos e dados biométricos da traíra *Hoplias malabaricus* (Pisces, Characiformes, Erythrinidae): uma espécie bioindicadora no rio Mearim, Amazônia Brasileira

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**Abstract**

The objective of this study was to evaluate the levels of environmental contamination in a protected area in the Brazilian Amazon. For this, two areas were chosen along the Mearim River: the reference area (A1) and the potentially contaminated area (A2), where water samples were collected, for physicochemical and microbiological analyses, as well as specimens of *Hoplias malabaricus*, for the evaluation of biometric data and incidence of branchial lesions. The physicochemical analyzes of the water from both areas showed contamination (low levels of dissolved oxygen, tubidity and high iron concentrations, especially in A2). The microbiological analyzes showed that all water samples showed total coliform values higher than those acceptable by CONAMA and WHO (with higher values in A2), in addition to *E. coli* values higher than those allowed by legislation in A2. Regarding biometric data, male and female fishes were significantly longer and heavier in A1 during the dry and rain seasons and the gonadosomatic index also showed higher values in A1 than in A2 in both seasons. *H. malabaricus* showed gill lesions of minimal to moderate pathological importance in A1 and A2, indicating that specimens from both areas of the Mearim River showed biological responses to contamination. The observed changes in the water quality, bimetic parameters and the histological analyzes of the specimens of *H. malabaricus* directly reflect on the quality and health of the fishes in the Mearim River, and point to the urgent need for prevention and remediation of contamination in these ecosystems.

**Keywords:** aquatic biomonitoring, branchial lesions, river contamination, international protected areas.

**Resumo**

O objetivo deste estudo foi avaliar os níveis de contaminação ambiental em uma área protegida na Amazônia brasileira. Para isso, foram escolhidas duas áreas ao longo do rio Mearim: a área de referência (A1) e a área potencialmente contaminada (A2), onde foram coletadas amostras de água, para análises físico-químicas e microbiológicas, além de espécimes de *Hoplias malabaricus*, para avaliação de dados biométricos e incidência de lesões branquiais. As análises físico-químicas da água de ambas as áreas mostraram contaminação (baixos níveis de oxigênio dissolvido, turbidez e altas concentrações de ferro, principalmente em A2). As análises microbiológicas mostraram que todas as amostras de água apresentaram valores de coliformes totais superiores aos aceitáveis pelo CONAMA e OMS (com valores superiores em A2), além de valores de *E. coli* superiores aos permitidos pela legislação em A2. Em relação aos dados biométricos, os peixes machos e fêmeas foram significativamente maiores e mais pesados em A1 durante as estações seca e chuvosa e o índice gonadossomático também apresentou valores maiores em A1 do que em A2 em ambas as estações. *H. malabaricus* apresentaram lesões branquiais de importância patológica mínima a moderada em A1 e A2, indicando que espécimes de ambas as áreas do rio Mearim apresentaram respostas biológicas à contaminação. As mudanças observadas na qualidade da água, nos parâmetros biométricos e nas análises histológicas dos espécimes de *H. malabaricus* refletem diretamente na qualidade e saúde dos peixes do Rio Mearim, e apontam para a necessidade urgente de prevenção e remediação da contaminação desses ecossistemas.

**Palavras-chave:** biomonitoramento aquático, lesões branquiais, contaminação de rios, áreas de proteção internacional.

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Received: April 12, 2022 – Accepted: June 27, 2022

## 1. Introduction

The effect of human activities on natural ecosystems has increased the loss of biodiversity in terrestrial and aquatic environments (Lutinski et al., 2023). However, the reduction of the biological diversity has been accompanied by the search for environmental conservation strategies, including the establishment and management of protected areas, which aim to preserve biodiversity in a long-term (Nabout et al., 2022).

Among the several protected areas of international interest, the Baixada Maranhense Environmental Protection Area stands out as part of the Legal Amazon, since it has a vast and important aquatic and terrestrial diversity (Araujo et al., 2020). The region is a wetland of interest designated by the RAMSAR Convention, ranking 28th in terms of size, comprising a complex ecological set of gallery forests, mangroves, various species of migratory birds and several species of fishes (Cardoso et al., 2019). In addition to all the biological diversity described, the Baixada Maranhense Environmental Protection Area is also a territory of elevated productivity and high economic and social importance for the local population (Ribeiro et al., 2020).

Biomarkers are changes at the molecular, biochemical, cellular, physiological, morphological or behavioral level that reveal current or past exposure of an organism to at least one pollutant (Ahmad et al., 2023; Jesus et al., 2021). In other words, biomarkers are able to indicate the presence of contaminants in the environment, enabling the assessment and monitoring of ecosystems (Altenburger et al., 2019; Milinkovitch et al., 2019).

Gill lesions are commonly used as histological biomarkers since this organ has direct and permanent contact with the aquatic environment and is fundamental for respiration, osmoregulation, excretion and other vital functions in fishes (Kumar et al., 2017). These biomarkers are suitable to assessing the effects of human impacts on fishes' species, especially carnivorous predators (Kroon et al., 2017). On the other hand, biometric data are also very useful in the assessment of aquatic organisms, given that changes in somatic indices are directly linked to the interference of pollutants in fishes' metabolism and can be used in biomonitoring programs (Pinheiro-Sousa et al., 2021).

*Hoplias malabaricus* (trahira) is a neotropical fish that is distributed from Panamá to south of Argentina and is widely used in the diet of the riverside Amazon population (Silva et al., 2019). The species is essentially carnivorous, which facilitates the process of biomagnification of contaminants through the food chain (Faria et al., 2019). The characteristics of this species and its sensitivity to pollutants indicate that this aquatic predator is an effective bioindicator of water quality and biomagnification (Jesus et al., 2014; Tesser et al., 2021).

In addition to the importance of *H. malabaricus* for environmental monitoring described above, the analysis of this fish is necessary, since the human consumption of contaminated fishes promotes an important route of contaminant absorption (Vicari et al., 2012). Despite the undeniable importance of the topic, environmental monitoring studies with trahira in the Baixada Maranhense Environmental Protection Area are quite scarce. In this context, the aim of this study was to evaluate gill lesions and biometric data of *Hoplias malabaricus*, a bioindicator species, in a protected area in Brazilian Amazon.

## 2. Materials and Methods

### *2.1. Study area and sampling*

The research was authorized by the State Department of Natural Research and Environment (process n°18208/2014, SEMA). The protocol was approved by the Ethics Committee of Maranhão State University (13/2017 CRMV-MA) and met the guidelines of the Brazilian Society of Laboratory Animal Science (https://www.sbcal.org.br).

Two stretches of the Mearim River were selected for sampling (Figure 1), both located in the Baixada Maranhense Environmental Protection Area. The first stretch is the reference area (A1), located in Vitória do Mearim, in the Engenho Grande village (03°7'64.1"S, 44°46'79.7"W), located at 188.9 kilometers from the capital São Luís. This site still has native flora and fauna, with minimal urbanization and anthropic activities. The second stretch is the potentially contaminated area (A2), located in Arari, in the Curral da Igreja village (03°27'64.1"S, 44°46'79.7"W). Arari is a city with intense urbanization and various anthropic activities, located at 175.4 kilometers from São Luís. Arari's economy is predominantly related to fishing and agricultural activities (such as planting watermelon and rice on the banks of the river), hence the importance of Mearim River for the local population. At these sampling sites, water samples and specimens of *H. malabaricus* were collected in 2016 (August and November = dry season) and in 2017 (May and June = rainy season) at 12:00 pm. The collection sites were equally distant from urban centers. In total, 91 specimens of trahira were collected, 54 in the dry season and 37 in the rainy season.

### *2.2. Physicochemical and microbiological analyzes of water samples*

The physicochemical parameters of the water were measured in triplicate at each site during each study season using a multiparameter HI 9829 (Hanna Instruments, Woonsocket, RI, USA) 12:00 pm. Water samples were also collected and preserved in nitric acid, for further quantification of contaminants by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES Optima 8300, Perkin Elmer). Microbiological analyzes were performed using the Colilert®/18 system method (IDEXX Laboratories, Inc., Westbrook, ME, USA), focusing on the detection and quantification of total coliforms and *Escherichia coli* in the samples.

### *2.3. Biometric data*

*H. malabaricus* were stored in isothermal boxes with ice and transported to the Laboratory of Biomarkers in Aquatic Organisms (LABOAq) at the State University of Maranhão (UEMA). The fishes were anesthetized with

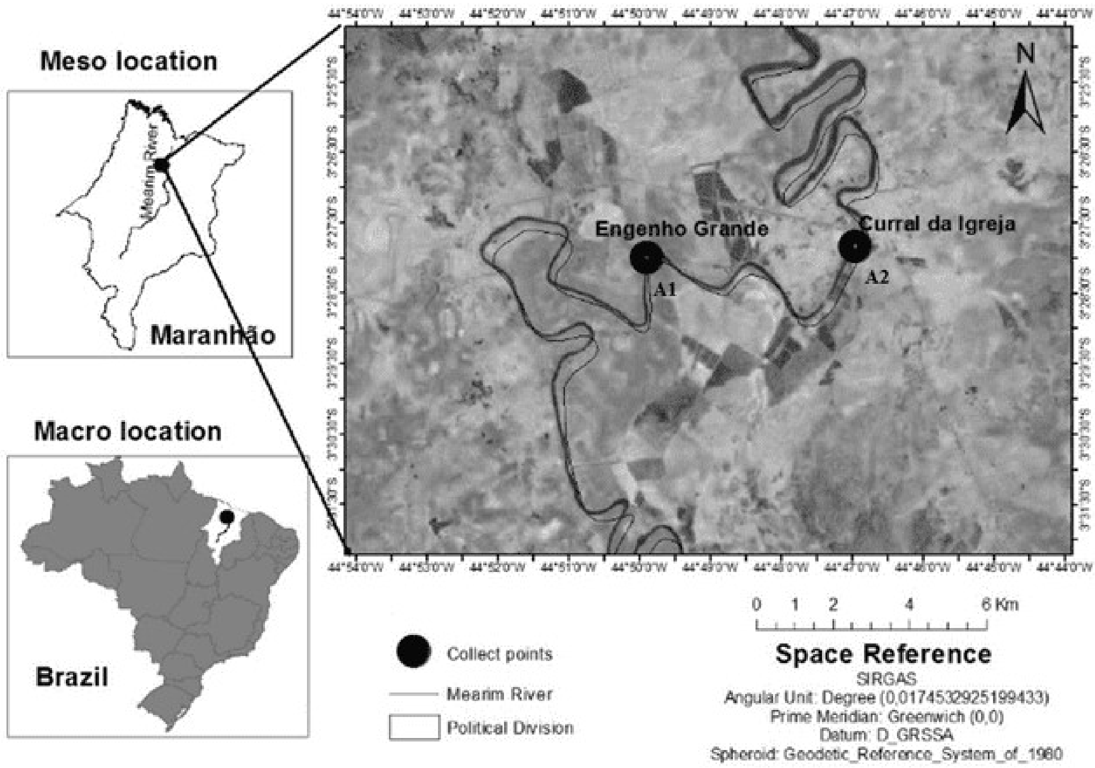


**Figure 1.** Location of the Mearim River stretches in the Baixada Maranhense Environmental Protection Area: Engenho Grande village (A1) and Curral da Igreja village (A2).

0.1% benzocaine and euthanized. Medullary sections were submitted to biometry and opened via a vertical incision for observation and macroscopic classification of the gonads (males and females). The following biometric data were evaluated for each specimen: total length (TL), standard length (SL), total weight (TW) and gonadal weight (GW). In addition, we calculated the gonadosomatic index (GSI) according to Vazzoler (1996): GSI = 100 x Wg/Wt, where Wg is gonad weight and Wt is total weight.

### *2.4. Histological analyzes*

The second right gill arch of each fish was removed, placed in 10% formaldehyde for 24 hours, placed in 10% nitric acid for 4 hours and processed according to the usual histological protocol (alcohol dehydration, xylol clearing and paraffin inclusion). Section (3-5 mm) were stained in hematoxylin/eosin and observed under a Zeiss light microscope (Carl Zeiss, Oberkochen, Germany) and photomicrographed using an OlympusBX51 microscope. Histological alterations were counted in each individual to obtain the histological index.

The first methodology (Bernet et al., 1999) was based on the pathological relevance of the histological lesion (w) and the extent of the observed lesions (a) calculated using the formula: $Iorg\ \Sigma rp = \Sigma alt\ (a.\ w)$, where "rp" is the reaction pattern, "alt" is the number of alterations found, "a" is the score value and "w" is the importance factor of the lesion. The second methodology (Poleksic and Mitrovic-Tutundzic, 1994) classified branchial lesions in three stages according to the damage to the functions of the analyzed organ to calculate the histopathological alteration index (HAI) using the formula HAI: $1 \times \Sigma I + 10 \times \Sigma II + 100 \times \Sigma III$, where "I", "II" and "III" indicate the degree of severity of the lesions observed.

### *2.5. Data analysis*

Physicochemical and microbiological analyzes, biometric data, gonadosomatic indexes and histological change index were expressed as the mean ± standard deviation. Significant differences between groups were checked by a t-test ($p < 0.05$ accepted as significant). The analysis of similarity of the branchial lesions between the studied areas was conducted through cluster analysis based on the values in paired groups (similarity) measured by Bray-Curtis index. These analyses were performed with the statistics program PAST (Hammer et al., 2001).

## 3. Results

### *3.1. Physicochemical and microbiological parameters*

Several physicochemical parameters showed alterations in both A1 and A2 (Table 1), which suggests that both areas are contaminated, especially A2.

A worrying result was the low levels of dissolved oxygen in both areas. All the values found, with the exception of sample 2 collected in A1 and sample 2 collected in A2 in the rainy season, were below the limits established by Brazilian legislation (CONAMA resolution 357/2005) and by the World Health Organization for water quality monitoring (WHO, 2011). Dissolved oxygen is one of the most important components in the control of water quality, since the presence of oxygen in water is essential for the survival of biological life and the effect of wastewater discharge on rivers is largely determined by the oxygen balance of the system (Salmasi et al., 2021).

Another parameter that presented results outside the quality standards was turbidity. Sample 2 collected in A1 and samples 1 and 2 collected in A2 in the dry season showed turbidity values superior to those established by CONAMA and WHO. Turbidity is characterized by large amounts of organic and inorganic material suspended in water, which can originate from domestic and industrial sewage discharges into the aquatic environment (Salvinelli et al., 2016). High levels of turbidity greatly affect aquatic environments, as they block the entry of sunlight into the water, interfering with the photosynthesis of aquatic plants and, consequently, the supply of food for herbivorous fish, causing an imbalance in food chains (Alves et al., 2008). The high turbidity values are directly linked to the high incidence of total coliforms in the water (Robert et al., 2016), as found in our work.

Iron also presented values above those allowed in both areas and periods, with higher values in A2. Iron is a natural component of the region's soil as it occurs in the geological formation of the Mearim River Basin (Lima, 2013) and the acidic pH of the surface waters of Baixada Maranhense may favor the solubilization of this element (Marques et al., 2010). Globally, iron overloaded water sources are a problem due to natural sources of iron and increased human activities, which erode regions around water bodies and increase the concentration of the compound in aquatic ecosystems (Yadav et al., 2020).

Elevated environmental iron can cause several toxic effects that negatively impact fishes, such as oxidative stress and DNA damage (Tarifeño-Saldivia et al., 2018), as well as severe tissue damage to fishes' livers and gills (Singh et al., 2019). It has been documented that tissue injuries caused by excess iron decrease the functional capacity of the gills and cause respiratory dysfunction in fishes (Slaninova et al., 2014). The high values of iron found in all sampling sites, in all seasons of the year, may be one of the causes of histological damage to the fishes analyzed in this work.

Microbiological analyzes (Table 1) showed that all water samples presented total coliform values higher than those acceptable by CONAMA and WHO, with higher values in A2. The presence of total coliforms was higher in the rainy

**Table 1.** Physicochemical and microbiological analysis of the water collected at the Mearim River sampling points in the Baixada Maranhense Environmental Protection Area (Brazil).

| Parameters | Reference (A1) | | | | Contaminated (A2) | | | | CONAMA/ WHO limits |
|---|---|---|---|---|---|---|---|---|---|
| | Dry Season | | Rainy Season | | Dry Season | | Rainy Season | | |
| | Sample 1 (August) | Sample 2 (November) | Sample 1 (May) | Sample 2 (June) | Sample 1 (August) | Sample 2 (November) | Sample 1 (May) | Sample 2 (June) | |
| Alkalinity total (mg/L $CaCO_3$) | 62 ± 0.6 | 32 ± 0.4 | 0 | 40 ± 0.3 | 90 ± 0.5 | 34 ± 0.7 | 0 | 38 ± 0.3 | 200 |
| Chlorides (mg/L Cl-) | 32.53 ± 0.2 | 31.9 ± 0.3 | 40.78 ± 0.5 | 6.9 ± 0.1 | 35.32 ± 0.3 | 51.9 ± 0.8 | 44.8 ± 0.6 | 8.9 ± 0.2 | 250 |
| Conductivity (µJ/cm) | 470 ± 0.8 | 74.6 ± 0.7 | 115.7 ± 0.9 | 160 ± 0.4 | 606 ± 0.8 | 104.9 ± 0.3 | 116 ± 0.2 | 298.2 ± 0.3 | 750 |
| Total dissolved solids (ppm) | 231± 0.7 | 37.4 ± 0.3 | 58 ± 0.6 | 209 ± 0.5 | 303 ± 0.3 | 52.6 ± 0.9 | 60 ± 0.5 | 310 ± 0.9 | 500 |
| NaCl (%) | 1 ± 0.03 | 0.2 ± 0.01 | 0.2 ± 0.02 | 0.8 ± 0.04 | 1.3 ± 0.08 | 0.2 ± 0.07 | 0.2 ±0.04 | 0.3 ± 0.06 | - |
| pH | 6.2 ± 0.2 | 5.21 ± 0.3[a] | 6.8 ± 0.8 | 6.2 ± 0.7 | 6 ± 0.3 | 5.67 ± 0.4[a] | 6.8 ± 0.7 | 6.18 ± 0.2 | 6.0 - 9.0 |
| Dissolved oxygen (mg/L $O_2$) | 0.7 ± 0.02[a] | 0 ± 0.03[a] | 0.3 ± 0.01[a] | 10.8 ± 0,09 | 0.6 ± 0,02[a] | 0 ± 0.01[a] | 0.3 ± 0.02[a] | 14.3 ± 0.09 | 5.0 |
| Turbidity (U.N.T) | 26.86 ± 0.2 | 369 ± 9.8[a] | 1.75 ± 0.6 | 0.51 ± 0.7 | 113 ± 1.6[a] | 376 ± 8.2[a] | 1.87 ± 0.6 | 0.73 ± 0.2 | 100 |
| Nitrate (mg/L N) | 4.43 ± 0.05 | 0 ± 0.02 | 2.21 ± 0.06 | 0 ± 0.07 | 4.43 ± 0.08 | 0 ± 0.04 | 2.21 ± 0.09 | 0 ± 0.03 | 10 |
| Iron (mg/L Fe) | 2.61 ± 0.08[a] | 1.66 ± 0.04[a] | 2.13 ± 0.06[a] | 0.68 ± 0.03[a] | 5.01 ± 0.09[a] | 2.53 ± 0.07[a] | 3.77 ± 0.04[a] | 4.12 ± 0.03[a] | 0.3 |
| T. coliforms (MNP [c]) | 3,410 ± 85[a] | 2,481 ± 62[a] | 24,196 ± 108[a] | 4,352 ± 61[a] | 5,940 ± 39[a] | 12,033 ± 76[a] | 24,196 ± 48[a] | 9,208 ± 22[a] | 1000 |
| *Escherichia coli* | 900 ± 32 | 175 ± 11 | 20 ± 2 | 20 ± 2 | 1,730 ± 28[a] | 816 ± 42 | 41 ± 2 | 108 ± 6 | 1000 |

N= 3. [a]Values not in accordance with Brazilian legislation (CONAMA resolution 357/2005)/World Health Organization (WHO) limits.

season. *Escherichia coli* was also more present in A2 than in A1, but it was more frequent in the dry season.

Total coliforms and *E. coli* are essential indicators of fecal pollution in aquatic environments, as they are commonly found in the large intestine of humans and other warm-blooded animals (Ma et al., 2022). Thus, they are abundant in water systems as a result of direct discharge containing feces or indirect discharge via untreated wastewater (Osińska et al., 2017).

The low levels of dissolved oxygen, high turbidity, high levels of iron and a large number of total coliforms and *E. coli* found in the water samples point to environmental contamination, mainly due to erosion of the river banks and the release of domestic and industrial sewage in diffuse sources along the Mearim River.

In this context, the low levels of oxygen, high turbidity, high levels of iron and a large number of total coliforms and *E. coli* found in the water samples point to environmental contamination, mainly due to erosion and discharge of domestic and industrial sewage into diffuse sources along the of the Mearim River.

Modifications in aquatic environmental conditions, especially chemical and microbiological modifications, influence the health of fishes, causing histological lesions, mainly in the gills, which affect the respiratory and osmoregulatory functioning of these organisms (Pinheiro-Sousa et al., 2019). In addition, these environmental alterations affect the growth and reproduction of fishes (Santos et al., 2016), as will be discussed in the next topic.

### *3.2. Biometric data*

Male and female fishes were longer and heavier in A1 during the dry and wet seasons (Table 2), what is probably related to a greater retention of pollution in A2. Biometric data are an excellent tool for assessing contamination in biomonitoring studies, since pollutants induce changes in growth rates because energy is reallocated to detoxification mechanisms, which deplete reserves originally intended for growth (Pinheiro-Sousa et al., 2017; Domingos et al., 2009).

The gonadosomatic index (GSI) also showed higher values in A1 than in A2 in both dry and rainy seasons (Table 2). Several studies have shown a decrease in the gonadosomatic index of fishes in a contaminated site, considering that xenobiotics cause problems in the endocrine and reproductive systems of fishes, which directly affect the development of gametes and their viability (Mayon et al., 2006; Hansson et al., 2014; Pinheiro-Sousa et al., 2021). It is also important to note that the GSI was higher in the dry season for both male and female fishes caught in A1, whereas it was not in A2. These data point to a superior maturational development of the fishes found in A1 than A2. Typically, tropical river fishes accumulate reserves in the dry season and spawn in the rainy season, when the supply of food and habitats is more suitable for larvae and juveniles (Alkins-Koo, 2000). A reduced GSI may indicate that the fishes are not accumulating reserves for reproduction and are investing energy to biotransform pollutants.

### *3.3. Histological analyzes*

The histological alterations in the gills (Figure 2), found using methodology 1, showed that both in the fishes collected in A1 and A2, the changes in the structure of the branchial epithelium were the most significant lesions and were included in the regressive changes group (Table 3).

**Table 2.** Mean and standard deviation of biometric data for the *H. malabaricus* specimens collected from the Mearim River during the dry and rainy seasons.

| Parameters | Dry Season | | | |
|---|---|---|---|---|
| | A1 (mean ± SD) | | A2 (mean ± SD) | |
| | Females | Males | Females | Males |
| TL (cm) | 25.18 ± 3.87 | 26.31 ± 3.91 | 23.32 ± 3.04* | 19.96 ± 3.34* |
| SL (cm) | 21.02 ± 2.73 | 20.81 ± 2.90 | 18.36 ± 2.31* | 16.10 ± 2.88* |
| TW (g) | 162.12 ± 41.20 | 158.42 ± 70.87 | 131.6 ± 32.83* | 121.46 ± 23.12* |
| GW (g) | 5.98 ± 1.86 | 0.98 ± 0.39 | 3.06 ±1.07* | 0.46 ± 0.11* |
| GSI | 3.68 ± 0.69 | 0.62 ± 0.29 | 2.32 ± 0.32* | 0.37 ± 0.17* |
| **Parameters** | **Rainy Season** | | | |
| | **A1 (mean ± SD)** | | **A2 (mean ± SD)** | |
| | **Females** | **Males** | **Females** | **Males** |
| TL (cm) | 23.6 ± 1.86 | 21.46 ± 2.23 | 16.4 ± 1.32* | 16.07 ± 0.87* |
| SL (cm) | 18.42 ± 1.53 | 17.56 ± 1.66 | 12.3 ± 1.24* | 12.87 ± 0.99* |
| TW (g) | 140.46 ± 32.76 | 121.43 ± 28.71 | 88 ± 8.23* | 87.75 ± 7.97* |
| GW (g) | 4.86 ± 1.03 | 0.67 ± 0.69 | 2.41 ± 0.93* | 0.35 ± 0.14* |
| GSI | 3.46 ± 0.32 | 0.55 ± 0.25 | 2.43 ± 0.71* | 0.39 ± 0.21* |

*Indicates a significant difference in relation to the reference area ($p < 0.05$). TL: total length; SL: standard length; TW: total weight; GW: gonad weight; GSI: gonadosomatic index; cm: centimeters; and g: grams. Dry season = 54 fish, and rainy season = 37.

When methodology 2 was applied, the epithelial displacement, congestion and aneurysm were the lesions with highest percentages, with similar values in fishes captured in A1 and A2 (except for congestion, which had a significantly higher percentage in A1) (Table 3). Other types of injury were also found in both areas, such as: hyperplasia, incomplete and complete lamellar fusion, lamellar disorganization, mucus cell proliferation and dilation of venous sinus (Table 3).

The presence of gill lesions in fishes collected in A1 and A2 indicates that specimens from both areas of the Mearim River showed biological responses to contamination. As previously described, impacts on the Mearim River caused by agricultural, domestic and industrial activities can affect the conditions of the aquatic ecosystem, affecting the health of resident fishes. In a study that used the same species for analysis of aquatic contamination in the Maracanã Environmental Protection Area in São Luís - MA, the authors recorded the same gill lesions that are described in the present work: lamellar fusion, epithelial displacement, epithelial dilation, proliferation of mucous cells and aneurysms (Castro et al., 2014), which confirms the applicability of *H. malabaricus* as a bioindicator species of environmental contamination.

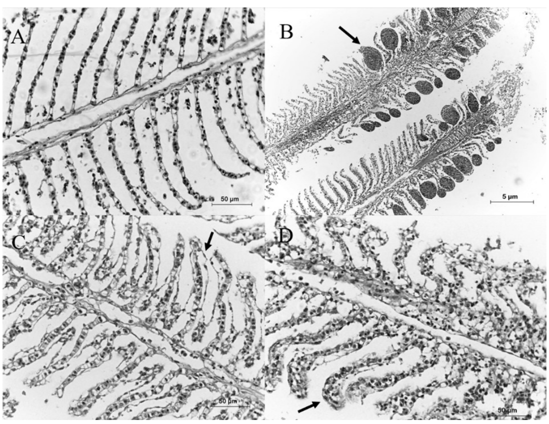


**Figure 2.** Histological lesions in *H. malabaricus*. (A) Normal gill tissue; (B) aneurysm (arrow); (C) epithelial displacement (arrow); and (D) congestion (arrow).

Displacement of the lining epithelium of the secondary lamellae is one of the first lesions found in fish and occurs when the respiratory epithelium is lifted, with the formation of edema (Santos et al., 2014). This lesion is related to the presence of chemical contaminants and causes a reduction in the surface of the gills, in addition to compromising the gas exchange process (Winkaler et al., 2001). In congestion, there is the formation of a cluster of erythrocytes, which will affect the pillar cells and the structural arrangement of the cells, which may result in a blood leakage (Stentiford et al., 2003). The aneurism can be much more serious, making it irreversible if the animal remains in a contaminated environment (Flores-Lopes and Thomaz, 2011). Hyperplasia and lamellar fusion are formed in the gill tissue in order to reduce the stress caused by a polluted environment, however these changes in the tissue structure directly affect respiration and osmoregulation mechanisms (Nogueira et al., 2011).

Dilation of the gill epithelium can be generated by irritation of blood vessel walls caused by chemical elements present in the environment (Fiuza et al., 2011). This can seriously compromise the respiratory system of fishes, as it affects vascular integrity and an epithelial rupture may occur (Cantanhêde et al., 2016).

Lesions such as mucosal cell proliferation and lamellar disorganization were also found in the fishes collected in both areas. Intense mucus production is influenced by environmental stress and is responsible for protecting lamellae against microorganisms, contaminants and other particles found in the water (Menezes et al., 2015). Cell disorganization is characterized by an imbalance in the structure of gill cells, affecting the normal functioning of the gills, mainly in terms of $O_2$ absorption by the fishes (Machado, 1999).

**Table 3.** Observed lesions and severity of the lesions relative to the methodologies used, as well as the occurrence (%) in each area.

| Methodology | Reaction pattern (groups) | Alterations | w | % (A1) | % (A2) |
|---|---|---|---|---|---|
| 1 | Circulatory disorders | Aneurysm | 1 | 17 | 16 |
| | Regressive changes | Structural alterations | 1 | 73 | 73 |
| | Progressive changes | Hyperplasia | 2 | 10 | 11 |
| 2 | Hypertrophy and hyperplasia of gill epithelia | Hyperplasia | 1 | 10 | 11 |
| | | Incomplete fusion | 1 | 8 | 10 |
| | | Complete fusion | 2 | 8 | 7 |
| | | Epithelial displacement | 1 | 26 | 28 |
| | | Lamellar disorganization | 1 | 2 | 7 |
| | Changes in mucous/chloride cells | Mucous cells proliferation | 2 | 1 | 1 |
| | Blood vessel changes | Dilation of venous sinus | 1 | 7 | 8 |
| | | Congestion | 1 | 21 | 12 |
| | | Aneurysm | 2 | 17 | 16 |

w: severity of the lesions; 1: Bernet et al. (1999); 2: Poleksic and Mitrovic-Tutundzic (1994).

These histological changes were also found by Ogbeide et al. (2019) in a study that associated characteristics of benthic and pelagic fishes and the effects of exposure to a river impacted by pesticides. It is important to highlight that in the studied areas (especially A2), agriculture occurs close to the banks of the Mearim River and the use of pesticides in these activities can contaminate aquatic ecosystems.

The results of the indexes of the branchial lesions in the collected fishes showed a low and moderate degree of severity (Figure 3). However, gill's changes, even at low levels, have significant effects on the respiratory balance of the animal (Martins et al., 2016). The normal functioning of the respiratory and osmoregulatory systems of fishes can be affected by minimal structural changes in the gills, which may become irreversible for animal health and be fatal (Galat et al., 1985).

The rainy season showed higher histological changes indexes both in A1 and A2, but mainly in A2. During the rainy season, environmental changes are intensified, influencing the use of some defense mechanisms of fishes' organisms and causing histological alterations (Iida et al., 2011; Robert et al., 2016).

The cluster analysis performed for the histological lesions of the fishes presented differentiated groups for the two sampled areas (Figure 4). The most similar groups of gill lesions (100%) were complete lamellar fusion and incomplete lamellar fusion (A1) and complete lamellar fusion and lamellar disorganization (A2). Cluster analysis showed that the grouped histological lesions represented mild and moderate severity.

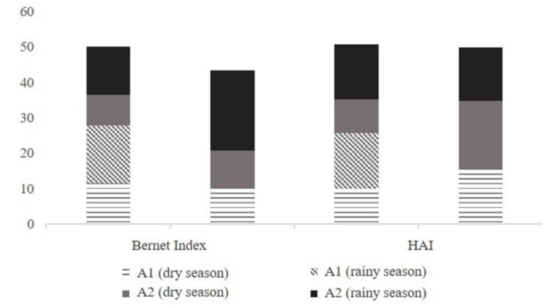


**Figure 3.** Values of Bernet et al. (1999) index and Poleksic and Mitrovic-Tutundzic (1994) (HAI), in the dry and rainy seasons.

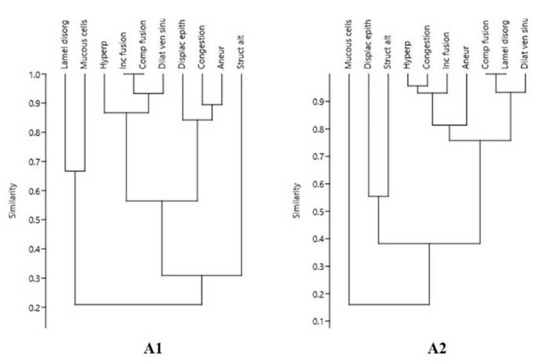


**Figure 4.** Tree of similarity of the branchial lesions observed in the specimens collected in the two studied areas (A1 and A2). Aneu: aneurysm; Hyperp: hyperplasia; Displac epith: displacement of the epithelium; Lamel disorg: lamellar disorganization; Dilat ven sin: dilation of venous sinus; Mucous cells: proliferation of mucus cells; Comp fusion: complete lamellar fusion; and Inc fusion: incomplete lamellar fusion.

The poor water quality of Mearim river reflected on the health of the analyzed fishes, which had many lesions on the gills. In this context, alterations in the gill tissues of *H. malabaricus* can be used to assess the effects of specific and non-specific pollutants on water bodies and predict future effects on the ichthyological communities of chronically contaminated rivers (Castro et al., 2018).

The branchial lesions of *H. malabaricus* indicate that the environment is under stress, probably due to inappropriate land uses that cause contaminants to enter the aquatic environments of the region. The use of native fish species as biomarkers is a fundamental approach for environmental monitoring in developing countries, as it is an easy and low-cost way to obtain information for environmental management.

## 4. Conclusions

Both studied areas showed signs of environmental contamination, since the data from the physicochemical and microbiological analyzes of the water samples, in addition to the biometric and histological data of *H. malabaricus* collected, showed results outside acceptable standards. The area with higher human activity (A2) still showed more contamination marks than the reference area (A1). The specimens of *H. malabaricus* presented gill lesions that differentiated the quality and health of the fishes. The results of the present research point to an urgent need for measures to prevent and remedy contamination along the Mearim River, in addition to show that the use of *H. malabaricus* can contribute to environmental monitoring actions in this region and other areas worldwide.

## Acknowledgements

The authors gratefully acknowledge the staff of the Laboratory of Biomarkers and Aquatic Organisms (LABOAq) and the Microscopy Group of the State University of Maranhão for providing laboratory facilities and technical assistance. Authors extend their thanks to the Coordination of Higher Education Personnel (CAPES) and the Foundation for Research and the Scientific and Technological Development of Maranhão (FAPEMA) for financial support in the form of grants and fellowship.

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