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***Research Article***

# Ectoparasitic diversity, gill alterations in *Hoplerythrinus unitaeniatus* (Characiformes: Erythrinidae) and *Cichlasoma bimaculatum* (Perciformes: Cichlidae) and quality of fishing water in the Quilombola zone in Maranhão State, Brazil

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**ABSTRACT.** The current study aimed to investigate the ectoparasitic diversity and gill alterations in *Hoplerythrinus unitaeniatus* (Characiformes: Erythrinidae) and *Cichlasoma bimaculatum* (Perciformes: Cichlidae) and evaluate the microbiological and physicochemical quality of water samples deriving from a Quilombola zone in Maranhão State, Brazil. Water samples and 42 fish specimens, 21 *H. unitaeniatus* and 21 *C. bimaculatum*, were collected from a floodable environment. Water samples were subjected to physicochemical and microbiological analyses in the laboratory environment. Fish specimens were euthanized to collect and identify ectoparasites in animals' mucus, body surface, and gills, as well as to enable the histological analysis of the second right gill arch. The herein-identified ectoparasites have shown 30.95% prevalence and comprised three phyla: Platyhelminthes, Trematoda and Arthropoda. The herein-identified main histological changes comprised incomplete and complete fusion of several lamellae, lifting of respiratory epithelium, lamellar disorganization, lamellar epithelial hyperplasia, and blood sinus dilation. The herein calculated histological alteration index has shown that 23.80% of specimens presented mild-to-moderate tissue damage, 4.77% presented moderate-to-severe tissue changes, and 9.52% presented irreparable tissue damage. It was possible concluding that histological gill lesions identified in fish specimens analyzed may be adaptive responses to the affected environment and the incidence of ectoparasites.

**Keywords:** *Hoplerythrinus unitaeniatus*; *Cichlasoma bimaculatum*; ichthyoparasitology; histology; Monogenea; native fish

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## INTRODUCTION

Fish present the highest parasitic infection rates among all vertebrate animals due to aquatic environment peculiarities capable of intensifying parasites' reproduction, development, and survival strategies (Gonçalves et al. 2014, Acosta et al. 2016). Fish belonging to different trophic levels can be part of the life cycle of different parasites since they work as hosts to one, or more, parasitic species. Thus, these organisms are acknowledged for playing an essential role in the structure of ichthyological communities since they change hosts' physiology and behavior, as well as compromise their survival (Takemoto et al. 2009, Lagrue et al. 2011, Tavares-Dias et al. 2014, Alcântara & Tavares-Dias 2015).

Fish and parasites coexist in nature in a balanced manner; however, changes in ecological factors account for controlling parasitic diversity, infection, and infestation levels (Moreira et al. 2009, Tavares-Dias et al. 2014, Alcântara & Tavares-Dias 2015, Aquino et al. 2019). The health of fish living in impacted environments can be affected and lead to changes in parasite abundance or richness (Pavanelli et al. 2013). Therefore, using histological biomarkers in these environments can be a low-cost tool to help determine fish populations' health since it reflects the health of aquatic ecosystems (Pereira et al. 2014).

Despite the progress in research focused on investigating the dynamics of parasite-ichthyofauna relationships, Acosta et al. (2016) have emphasized the small number of studies about the life cycle of parasite groups found in Neotropical fish species. Given the ecological importance of parasite fauna and its association with fish, it is essential conducting investigations about parasites' life cycle, zoonotic potential, and effects on the health of hosts, such as species *Hoplerythrinus unitaeniatus* (Agassiz, 1829) (Characiformes: Erythrinidae), and *Cichlasoma bimaculatum* (Linnaeus, 1758) (Perciformes: Cichlidae), in order to establish control measures to avoid ecological imbalance and economic losses.

*H. unitaeniatus*, popularly known as jeju, is a benthopelagic species belonging to the family Erythrinidae; it is widely distributed in Central America and several South American countries. Although adult *H. unitaeniatus* are piscivorous, juveniles feed on plankton, crustaceans, insects, and seeds (Soares et al. 2008, Benigno et al. 2014, Alcântara & Tavares-Dias 2015). Erythrinids are sedentary animals living in the different river and lake environments, mainly in shallow water places close to submerged or marginal vegetation, which tend to show abundant prey.

*C. bimaculatum* (black acará) is a benthopelagic species belonging to the family Cichlidae; it is widely distributed in South America (Ottoni 2011, Froese & Pauly 2016). These omnivorous fish are often found in channels and floodplains; they can tolerate low oxygen levels in the environment and feed on crustaceans, seeds, algae, and insects (Gurgel et al. 1994, Froese & Pauly 2016, Tavares-Dias et al. 2017).

Species *H. unitaeniatus* and *C. bimaculatum* are commonly found in a region known as Baixada Occidental Maranhense (Western Maranhão State Lowland), whose hydrographic system comprises rivers, lakes, and floodplains. Baixada Maranhense comprises 21 municipalities; most rely on fishing as an important source of food and income for thousands of families (Almeida 2006). Its territorial extension covers 1,775,035.9 ha, making it the largest lake basin in the northeastern region (Pereira et al. 2016).

It is essential to investigate and understand the parasite fauna affecting native fish living in the Quilombola zone. The diversity of ectoparasites found in species *H. unitaeniatus* and *C. bimaculatum* is considered the dietary basis of traditional communities living in Maranhão State. Therefore, the current study aimed to investigate the ectoparasitic diversity and gill alterations in *H. unitaeniatus* and *C. bimaculatum*, and evaluate the microbiological and physicochemical quality of water samples deriving from a Quilombola zone in Maranhão State, Brazil.

## MATERIALS AND METHODS

### Research ethics

The present research was approved by the Ethics Committee on Animal Experimentation (CEEA - Comitê de Ética e Experimentação Animal) of Maranhão State University (UEMA - Universidade Estadual do Maranhão), under protocol N°08/2021. It complied with Federal Veterinary Medicine Council (CFMV - Conselho Federal de Medicina Veterinária) Resolutions N°879/2008 and 1000/2012, as well as with Federal Law N°11794/2008, which provide on ethical procedures in animal experimentation.

### Study site

The study site comprised Ponta Bonita Quilombola Community, which is located in the rural area of Anajatuba County, Maranhão State, Brazil. This county belongs to the Baixada Maranhense region (03º15'50''S, 44º37'12''W).

### Environmental variables and collection of water and fish samples

Environmental variables were measured, and water and fish samples were collected, in a natural floodable field, during two sampling campaigns carried out in 2021: the first in February (early rainy season) and the second in August (early dry season).

The following environmental variables were initially measured with the aid of a multiparameter instrument: temperature (ºC), hydrogenionic potential (pH), dissolved oxygen (mg $L^{-1}$), conductivity (μS $cm^{-1}$), and salinity. Subsequently, four water samples were collected (two in each campaign in collection points A01 and A02) in sterile borosilicate glass flasks (500 mL capacity) and protected from sunlight in isothermal boxes filled with recyclable ice.

Forty-two fish specimens were captured; 21 belonged to *H. unitaeniatus* and 21 to *C. bimaculatum*, with the aid of a 4-mesh (20 mm) gillnet, which was actively operated (trawlers/enclosures). Subsequently, they were placed and transported (alive) in a Styrofoam box (100 L capacity) - filled with water collected in the capture environment - to the Aquatic Resources-Reproduction Laboratory (LARAQUA - Laboratório de Reprodução de Recursos Aquáticos) at UEMA. Upon arriving at the laboratory, they were placed in a tank filled with water and subjected to constant oxygenation for 12 h until further biological material analysis and processing in the Parasitology Laboratory of Agrarian Sciences Center.

### Biological material processing: biometry and ectoparasite collection

Fish specimens were euthanized by perforating the upper part of their head with a pointy instrument (scalpel blade). All procedures were performed in compliance with ethical principles. Subsequently, with the aid of an analytical digital scale and millimeter ruler, the following biometric variables were measured in each individual: total weight (Wt; g), standard length (Ls; cm) measured from the mouth to the last vertebra of the spine (beginning of the caudal fin) and total length (Lt; cm) measured from the mouth to the end of the caudal fin.

Fish specimens had their body surface and gills thoroughly evaluated before ectoparasite collection, carried out through mucus scrape. After the macroscopic inspection, ectoparasites were removed and fixed based on the protocol by Jerônimo et al. (2012). The second-gill arch was removed from each specimen with tweezers and scissors and fixed in 10% formalin for histological analysis.

### Laboratory procedures

Based on the manufacturer's instructions, the enzyme-chromogenic system (Colilert, Idexx, USA) was used to measure total coliforms and *Escherichia coli*. The aliquot of 10 mL of each collected water sample was diluted in 90 mL of sterile distilled water and poured into sterilized flasks filled with the substrate at UEMA's Food and Water Microbiology Laboratory. Then, the solution was incubated in an oven at 35 ± 0.5°C for 24 h. Total coliforms were confirmed when the water sample color changed from colorless to yellow. Meanwhile, *E.coli* was confirmed by blue fluorescence emission from samples exposed to ultraviolet light at a wavelength of 365 nm (IDEXX Laboratories Inc.). Complementary physicochemical analyses were applied to the following parameters: alkalinity in $HCO^{3-}$, total alkalinity, total chloride, total dissolved solids (TDS), nitrate, iron nitrite, and turbidity. The adopted methods herein are described in the Analytical Standards of Adolfo Lutz Institute (Zenebom & Pascuet 2005).

Collected parasites were mounted on slides added with Hoyer solution (Putz & Hoffman 1963, Kritsky et al. 1995, Eiras et al. 2006), clarified to enable investigation of their structures and later identified based on the following references: Rushton-Mellor (1994), Dzika et al. (2009), Soes et al. (2010), Mousavi et al. (2011), Sujan & Shameem (2015). The incidence of ectoparasites in fish was measured based on Jerônimo et al. (2012), whereas parasitological prevalence and mean intensity indices were calculated based on Bush et al. (1997).

Histological analyses were performed at the Microscopy Laboratory, which is part of the Postgraduate Multiuser Laboratory (LAMP - Laboratório Multiusuários da Pós-Graduação) at UEMA, based on Caputo et al. (2010). Slides were read in an optical microscope at 10 and 40x magnification; the identified lesions were micro-photographed with an Axioskop-Zeis photomicroscope.

Histological changes in specimens' gills were evaluated in a semi-quantitative way by calculating the histopathological alteration index (HAI), adapted from Poleksic & Mitrovic-Tutundzic (1994), based on the severity of each lesion, as follows: stage I) alterations that do not compromise organs' functioning; stage II) alterations comprising more severe lesions capable of impairing the normal functioning of individuals organs, and stage III) alterations comprising signi-ficantly severe and irreversible lesions.

The HAI value was calculated for each fish based on the following formula: HAI = 1 × ΣI + 10 × ΣII + 100 × ΣIII, wherein I, II, and III referred to the number corresponding to stage alterations, respectively. HAI value was based on five categories established by de Poleksic & Mitrovic-Tutundzic (1994): 0-10 = normal tissue functioning; 11-20 = mild-to-moderate tissue damage; 21-50 = moderate-to-severe tissue alteration; 51-100 = severe tissue alteration; higher than 100 = irreparable tissue damage.

**Statistical analysis of collected data**

Tukey test was carried out in GraphPad InStat free software, version 3.1, to investigate whether there were significant differences between biometric variables and ectoparasites' prevalence per evaluated period at a 5% significance level.

## RESULTS

Table 1 presents results recorded for the physico-chemical and microbiological parameters of the analyzed water samples. Based on the comparison between these data and the maximum allowed values, it was possible seeing that temperature, salinity, total chloride, nitrite, and nitrate values complied with the established limits. On the other hand, dissolved oxygen, total dissolved solids, iron, and turbidity recorded values higher than those set by the legislation. Small variations were observed for pH; the most acidic values were recorded in the dry season at both collection points (A01 and A02). Based on the microbiological analysis, collection point A01 was contaminated with *Escherichia coli* in the rainy season.

Concerning the captured specimens' biometry's minimum, mean and maximum values recorded for the analyzed variables are shown in Table 2. Tukey's test showed no significant seasonal difference ($P < 0.05$) in mean values recorded for Lt, Ls, and Wt in *C. bimaculatum* specimens. *H. unitaeniatus* specimens only presented a significant seasonal difference ($P = 0.03$) in the mean value recorded for Lt.

In total, 33.34% of *H. unitaeniatus* (n = 07/21) and 28.57% of *C. bimaculatum* (n = 06/21) were infested with ectoparasites (n = 13/42) among all 42 evaluated fish specimens, as shown in Table 3. Ectoparasites belonging to class Monogenea presented the highest mean intensity (MI) in *C. bimaculatum*, whereas those belonging to class Crustacea presented the highest MI in *H. unitaeniatus* (Table 3). No statistically significant difference ($P > 0.05$) in ectoparasites' prevalence was between the evaluated seasons per sampled species.

The following ectoparasites were identified in the analyzed fish, regardless of the evaluated species, in descending order: i) *Gyrodactylus* sp. class Monogenea (n = 8/42; 19.04%) - identified in specimens' gills and mucus, ii) *Argulus* sp. class Crustacea (n = 05/42; 11.90%) and *Dolops* sp. class Crustacea (n = 1/42; 2.38%) identified on specimens' body surface and mucus iii) *Diplostomum* sp. belonging to subclass Digenea (n = 1/42; 2.38%) identified in specimens' pectoral fin; and, Arthropod (n = 1/42; 2.38%), species not identified on specimens' body surface.

Three of the 13 parasitized fish have shown more than one ectoparasite species. Parasite richness ranged from 1 to 3 parasites (polyparasitism) per evaluated specimen; their mean distribution reached 2.1 parasites fish$^{-1}$. Parasitic associations observed in the current study comprised Monogenea, Digenea, and Crustacea; Monogenea and Crustacea; and Monogenea and Arthropod.

Given the similarity of alterations observed for both evaluated species, results were presented and discussed altogether (Table 4) -histopathological alterations at stages I, II, and III comprised gill alterations. Stage I lesions were often observed in the evaluated samples; they were mostly represented by incomplete and complete fusion of several lamellae (Fig. 1a), lifting of respiratory epithelium, lamellar disorganization, lamellar epithelium hyperplasia (Fig. 1b), and blood sinus dilation. Respiratory epithelium detachment was often observed in gill lamellae, likely due to previous edematous alteration. Respiratory epithelium hyper-plasia followed by lamellar fusion may have happened in response to parasitic action, as shown in Figure 1c. Stage II lesions were less often observed in the evaluated specimens; they were mainly represented by lamellar epithelium bleeding and rupture (14.28%) (Fig. 1d), complete fusion of all lamellae (9.52%) and uncontrolled proliferative tissue thickening (9.52%). Finally, stage III lesions, such as cell necrosis and degeneration, were identified in 9.52% of the evaluated specimens in the rainy season.

Based on HAI calculations, 38.09% (n = 16/42) of fish presented some histological gill alteration type; they were stratified as follows: 23.80% (n = 10/42) of fish presented mild-to-moderate tissue damage; 4.77% (n = 2/42) presented moderate-to-severe tissue alterations, and 9.52% (n = 4/42) of them presented irreparable tissue damage.

**Table 1.** Physicochemical and microbiological results recorded for four water samples deriving from a floodable environment in the Quilombola zone of Maranhão State, Brazil. Wherein: A01: collected sample 01; A02: collected sample 02; MAV: maximum allowed value; NI: not included. NTU: nephelometric turbidity unit; MPN: most likely number. Reference: CONAMA Resolution n. 357/2005.

| Physicochemical parameters | Rainy season | | Dry season | | MAV* |
|---|---|---|---|---|---|
| | A01 | A02 | A01 | A02 | |
| pH | 7.31 | 6.25 | 5.30 | 5.97 | 6.0 a 9.0 |
| Dissolved oxygen (mg $L^{-1}$) | 2.7 | 2.1 | 3.8 | 3.3 | $\geq$5 |
| Temperature (°C) | 28.3 | 28.1 | 27.4 | 28.9 | $\leq$40 |
| Conductivity (uS $cm^{-1}$) | 184.7 | 184.1 | 95.6 | 347 | NI |
| Salinity | 0.09 | 0.09 | 0.16 | 0.16 | <0.5 |
| Alkalinity in $HCO^{3-}$ (mg $L^{-1}$ $CaCO_3$) | 36 | 30 | 10 | 134 | NI |
| Total alkalinity (mg $L^{-1}$ $CaCO_3$) | 36 | 30 | 10 | 134 | NI |
| Total chlorides (mg $L^{-1}$ Cl ) | 63.98 | 47.98 | 9.99 | 237.92 | 250 |
| Total dissolved solids (ppm) | 1.475 | 1.473 | 1.417 | 2.078 | 500 |
| Nitrate (mg $L^{-1}$) | 3.24 | 2.75 | 10.00 | 9.16 | 10.0 |
| Nitrite (mg $L^{-1}$) | 0.025 | 0.025 | 1.00 | 0.45 | 1.0 |
| Iron (mg $L^{-1}$) | 5.01 | 4.77 | 4.85 | 3.04 | 0.3 |
| Turbidity (NTU) | 218.66 | 210 | 127 | 85.6 | $\leq$100 |
| **Microbiological parameters** | **Rainy season** | | **Dry season** | | **MAV*** |
| | A01 | A02 | A01 | A02 | |
| Total coliforms (MPN 100 $mL^{-1}$) | 17.329 | 8.164 | 241.960 | 8.664 | NI |
| *Escherichia coli* (MPN 100 $mL^{-1}$) | 1.607 | 909 | 370.3 | 336 | 1.000 |

**Table 2.** Biometric variables of *Hoplerythrinus unitaeniatus* and *Cichlasoma bimaculatum* specimens deriving from the floodable environment in the Quilombola zone of Maranhão State, Brazil. Wherein. N: number of fish, Lt: total length, Ls: standard length, Wt: total weight, Min: minimum value, Max: maximum value, SD: standard deviation.

| Season | n | *Hoplerythrinus unitaeniatus* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Lt (cm) | | | Ls (cm) | | | Wt (g) | | |
| | | Min. | Max. | Mean ± SD | Min. | Max. | Mean ± SD | Min. | Max. | Mean ± SD |
| Rainy | 03 | 17 | 23.4 | 19.63 ± 3.34 | 13.8 | 20 | 16.75 ± 3.10 | 88.42 | 146 | 113.90 ± 29.35 |
| Dry | 18 | 14 | 20.4 | 16.68 ± 1.70 | 12 | 16.7 | 13.86 ± 1.31 | 39.41 | 109 | 69.16 ± 22.02 |
| **Season** | **n** | ***Cichlasoma bimaculatum*** | | | | | | | | |
| | | Lt (cm) | | | Ls (cm) | | | Wt (g) | | |
| | | Min. | Max. | Mean ± SD | Min. | Max. | Mean ± SD | Min. | Max. | Mean ± SD |
| Rainy | 18 | 11 | 14.4 | 12.59 ± 1.05 | 8.5 | 11.5 | 9.72 ± 0.90 | 28.27 | 62.75 | 41.41 ± 10.52 |
| Dry | 03 | 10.5 | 11.5 | 11 ± 0.5 | 8.0 | 9.0 | 8.40 ± 0.52 | 25.9 | 43.93 | 34.93 ± 09.02 |

## DISCUSSION

Limnological parameters play an important role in maintaining balance in aquatic ecosystems. Therefore, changes in these parameters can compromise ichthyofaunal integrity (Esteves 2011). According to Ribas et al. (2017) and Rodrigues et al. (2017), these changes can lead to stress and reduce fish's immune resistance; moreover, it enables parasitic actions and disease development. Reduced dissolved oxygen rates and imbalance in organic matter levels (total dissolved solids) were herein observed at both collection points and investigated seasons.

According to Santos et al. (2018), disturbances in dissolved oxygen values may be associated with the accumulation of organic matter from waste and effluent release whenever these pollutants are not fully neutralized during the self-purification process. Organic matter deriving from waste and effluent release may have influenced the results recorded by these authors because it is a lentic environment.

Values observed for parameters such as temperature, salinity, nitrate, and nitrite in samples analyzed in both seasons complied with Class 2 values determined by National Environment Council (CONAMA) Resolution N°357/2005 (Brasil 2005). The range of pH

**Table 3.** Ectoparasite prevalence and mean intensity in *Hoplerythrinus unitaeniatus* and *Cichlasoma bimaculatum* deriving from the floodable environment in a Quilombola zone in Maranhão State, Brazil. Wherein. IF: infected fish, TNP: total number of collected parasites, P: prevalence, MI: Mean intensity.

| Seasons | n | IF | TNP | *Hoplerythrinus unitaeniatus* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Monogenea | | Digenea | | Crustacea | | Arthropod | |
| | | | | P | MI | P | MI | P | MI | P | MI |
| Rainy | 03 | 01 | 04 | 33.34 | 3.00 | 33.34 | 1.00 | 00 | 00 | 00 | 00 |
| Dry | 18 | 06 | 07 | 5.55 | 1.00 | 00 | 00 | 27.78 | 1.20 | 00 | 00 |
| Total | 21 | 07 | 11 | 9.52 | 2.00 | 4.77 | 1.00 | 23.80 | 1.20 | 00 | 00 |
| Seasons | n | IF | TNP | *Cichlasoma bimaculatum* | | | | | | | |
| | | | | Monogenea | | Digenea | | Crustacea | | Arthropod | |
| | | | | P | MI | P | MI | P | MI | P | MI |
| Rainy | 18 | 05 | 08 | 27.78 | 1.60 | 00 | 00 | 00 | 00 | 5.55 | 1.00 |
| Dry | 03 | 01 | 01 | 33.34 | 1.00 | 00 | 00 | 00 | 00 | 00 | 00 |
| Total | 21 | 06 | 09 | 28.57 | 1.50 | 00 | 00 | 00 | 00 | 4.76 | 1.00 |

**Table 4.** Frequency of gill lesions in *Hoplerythrinus unitaeniatus* and *Cichlasoma bimaculatum* specimens deriving from the floodable environment in a Quilombola zone in Maranhão State, Brazil. n: number of individuals.

| Stage | Histological changes in gills | n | Frequency (%) |
|---|---|---|---|
| I | Congested blood vessels | 32 | 76.19 |
| | Lifting of respiratory epithelium | 34 | 80.95 |
| | Lamellar disorganization | 32 | 76.19 |
| | Lamellar epithelial hyperplasia | 32 | 76.19 |
| | Incomplete fusion of several lamellae | 36 | 85.71 |
| | Complete fusion of multiple lamellae | 36 | 85.71 |
| | Blood sinus dilation | 28 | 66.67 |
| | Respiratory epithelium hypertrophy | 06 | 14.28 |
| II | Lamellar epithelium bleeding and rupture | 06 | 14.28 |
| | Mucus cell hyperplasia and hypertrophy | 02 | 4.76 |
| | Chlorine cell hyperplasia and hypertrophy | 02 | 4.76 |
| | Complete fusion of all lamellae | 04 | 9.52 |
| | Uncontrolled proliferative tissue thickening | 04 | 9.52 |
| | Pillar cell rupture | 02 | 4.76 |
| III | Necrosis and cellular degeneration | 04 | 9.52 |

variation in the rainy and dry seasons was 6.78 ± 0.37 (in compliance) and 5.97 ± 0.47 (noncompliant), respectively. Small variations observed in this chemical parameter between the analyzed seasons may have a natural or anthropogenic origin.

According to CONAMA Resolution n. 357/2005, the nephelometric turbidity unit value must not exceed 100 uT to fit Class 2 water bodies; therefore, the variation range herein observed for this parameter in the rainy (214.33 ± 6.12 uT) and dry (106.30 ± 29.27 uT) seasons were above the limit established in the resolution mentioned above. These results may be associated with the incidence of suspended particles, colloids, and microscopic organisms (Brasil 2005).

Large total populations of coliforms were found in samples analyzed in both seasons. However, the current legislation does not determine maximum allowed value (MAV) for this microorganism group. According to Liuson (2003), total coliforms indicate hygiene quality and provide information about the microbial pollution degree fish are exposed to along the production chain.

CONAMA Resolution N°357/2005 has established that 80%, or more, of at least six samples collected throughout one year, must not exceed the limit of 1,000 MAV 100 $mL^{-1}$ for *E. coli*. Although the sampling plan established in this normative act was not followed, the observed microbiological results have shown mean *E. coli* values above the limit set for the rainy season

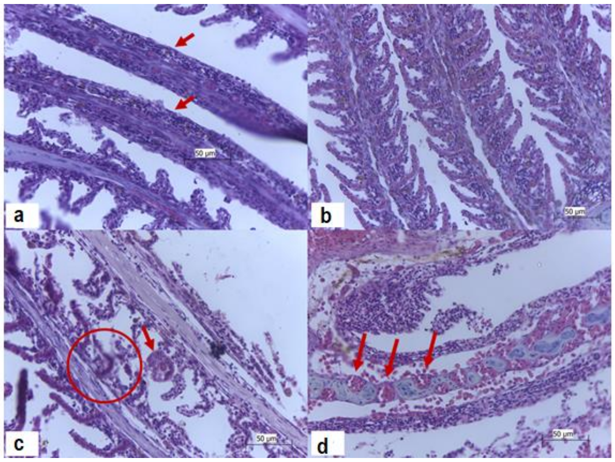


**Figure 1.** Photomicrographs of gill alterations observed in a-b) *Hoplerythrinus unitaeniatus* and c-d) *Cichlasoma bimaculatum* specimens deriving from the floodable environment in a Quilombola zone in Maranhão State. a) Complete fusion of several lamellae (arrows), b) hyperplasia and disseminated focal lamellar fusion, c) respiratory epithelium detachment (circle) and parasite attached to the apical region of hyperplastic and fused lamellae (arrow), d) gill bleeding (arrows). HE, 40x objective lens.

(1,258 ± 493.56). *E. coli* populations ranging from 336 to 370 MAV 100 mL$^{-1}$ were observed in the dry season; they were below the limit established by Brazilian legislation.

Seasonality is the main variable influencing the degradation of microbiological quality in surface waters since surface runoff intensification increases the number of particles contaminated by the fecal material transported to water bodies. Melo et al. (2015) studied the lagoons of Rio Doce State Park and Minas Gerais State and observed larger *E. coli* populations in months recording the highest rainfall rates.

Scorsafava et al. (2010) have highlighted that turbidity (water transparency measurement) is often used to indicate the presence of dissolved solids in suspension or material in a colloidal state, either organic or inorganic. It also indicates the risk of microbiological contamination and treatment effectiveness, which may be associated with high iron concentrations. Based on the comparative analysis between the physical parameter "turbidity" and microbiological parameters "total coliforms" and "*E. coli*", it appears that the quantification of these microorganism groups may have influenced the turbidity value observed for the rainy season.

Concerning biometric data, maximum Lt values recorded for *C. bimaculatum* ranged from 11.5 to 14.4 cm in the dry and rainy seasons, respectively. These values complied with those recorded by Gurgel et al. (1994), who observed a maximum length of 35 cm for this species. The maximum total length observed for *H. unitaeniatus* ranged from 23.4 cm (rainy season) to 20.4 cm (dry season); these values complied with those observed by Sato (1999), who recorded a maximum total length of 40 cm for this species.

Concerning ectoparasites, different studies carried out in Maranhão State, as well as in other Brazilian states, have shown that the incidence of these organisms in fish is a common issue, whether in fish farms or the natural environment (Cunha 2015, Rodrigues et al. 2017, Fujimoto et al. 2019, Leite et al. 2020). It is worth emphasizing that parasitism in fish is a challenge, regardless of the season of the year, due to the complexity of factors involved in it, such as the ones inherent to hosts (age, immunological status, intercurrent diseases, among others), parasitic agents (species and pathogenicity) or the environment (climate, local hygiene, biosecurity, reservoirs, among others).

On the other hand, similar studies have shown a higher incidence of parasites in fish during the dry season (Gomes 2021). Vital et al. (2011) associated this finding with the decreased water level in water bodies during the dry season, a fact that forces fish to migrate to more crowded regions and results in stress, as well as with decreased food availability, which compromises animals' immune system and increases their susceptibility to parasitic diseases.

Moravec et al. (2002) and Cunha (2015) have emphasized that the increased aquatic yield observed during the rainy season stimulates the seasonal cycles of potential intermediate hosts, and their parasites, by strengthening the relationship between parasite and host cycles, a fact that can lead to higher prevalence rates in this season. Moreover, the authors above have inferred that the increased water volume observed during the rainy season and the increased density of fish migrating from other rivers/lakes with endemic parasitism can lead to water/environmental contamination and enable parasitism to spread.

Parasite prevalence analysis has shown that parasites belonging to class Monogenea recorded high prevalence rates in both investigated species (33.34%). This finding was similar to that observed by Malacarne & Godoi (2012), Corrêa et al. (2013), Graça et al. (2013), Rodrigues et al. (2017), and Ferreira et al. (2018), who recorded parasite prevalence rates ranging from 33.30 to 98.75%.

The current study recorded the incidence of parasites fixed on *C. bimaculatum*'s gills. Parasites can hinder gas exchange processes by causing damage to fish's gills, such as gill circulation occlusion, necrosis, and the destruction of important areas of this organ. According to Pavanelli et al. (2008), fish can lose weight and change lipid levels and growth rate, as well as behavioral changes, depending on the intensity and severity of the parasitic action (Pereira et al. 2020).

Histological changes in fish gills are used as biomarkers of exposure to environmental stressors since they indicate effects from fish exposure to one or more harmful agents (Winkaler et al. 2001). Alterations herein observed for *C. bimaculatum* and *H. unitaeniatus* were compatible with lesions previously recorded by other scholars -such as Machado (1999), Lupi et al. (2007), and Pereira et al. (2014)- for fish deriving from contaminated environments.

Among the herein recorded stage I alterations, incomplete and complete fusions of several lamellae (85.71%) and lifting of respiratory epithelium (80.95%) were more often observed in the investigated fish. According to Poleksic & Mitrovic-Tutundzic (1994) and Cantanhêde et al. (2014), these lesions are considered mild since it is possible recovering gill tissues' structure and function when environmental conditions improve.

The lifting of lamellar epithelium is considered a primary sign of gill pathology; it consists of the elevation of the respiratory epithelium covering the secondary lamellae, a fact that increases the distance between the external environment and the blood (Thophon et al. 2003, Barni et al. 2016). Lamellar fusion is caused by hyperplasia of lamellar and gill filaments' epithelial cells. Lamellar fusion degree depends on hyperplasia intensity and site; it can be classified as incomplete or complete (Cantanhêde et al. 2014). According to Winkaler et al. (2001), these lesions are adaptive responses used to preserve some physiological functions when water quality in aquatic ecosystems is compromised. However, according to Castro et al. (2018), these lesions hinder the gas exchange process and compromise fish breathing and survival. Stage II and III gill alterations were less frequent in the current study.

Stage II lesions are seen as stage I lesions severity progression. Stage III lesions, on the other hand, lead to severe and irreversible impairment in gill structures since they cause irreparable damage to gill morphology and function, such as inefficiency in respiratory processes, in maintaining homeostasis, and in excreting different compounds (Poleksic & Mitrovic-Tutundzic 1994, Paulino et al. 2012, Pereira et al. 2020).

Severity ratings applied to the HAI indicated intense and severe structural damages to fish's gills. These damages can hinder gas exchange and osmoregulation processes, which play an essential role in fish's life, as Barni et al. (2016) and Pereira et al. (2020) mentioned.

Based on data collected in the current research, it was a possible conclusion that the floodable environment in the Ponta Bonita Quilombola community presents physicochemical and microbiological parameters in disagreement with standards set by the legislation. These changes, together with the observed ectoparasites, can lead to gill lesions such as the ones identified in the present study. Therefore, studies of this nature play an important role in helping to understand parasite-host relationships better since they can contribute to adopting management measures focused on avoiding the collapse of aquatic ecosystems.

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