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Histopathological effects of *Amyloodinium ocellatum* (E.-M.Brown) E.-M.Brown & Hovasse, 1946 (Alveolata: Dinophyseae) on the gills of *Mugil liza* (Valenciennes, 1836)

Efectos histopatologicos de *Amyloodinium ocellatum* (E.-M.Brown) E.-M.Brown & Hovasse, 1946 (Alveolata: Dinophyseae) en las branquias de *Mugil liza* (Valenciennes, 1836)

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ABSTRACT | In this work, we describe lesions produced by the dinoflagellate protozoan *Amyloodinium ocellatum* on the gills of *Mugil liza*, which causes the velvet disease in several fish species. These parasites produce severe lesions in the gills, with hyperplasia of primary and secondary gill lamellae rupture of pillar cells, and hyperplasia of chloride cells (ionocytes). The gill structure is totally affected, which presumably impairs normal hematosis and electrolyte regulation and seriously compromises the fish's health. The lesions caused by *A. ocellatum* are responsible for high mortality rates in fish farms and economic losses in the industry. In the present study, we found cellular hyperplasia of the respiratory epithelium, epithelial disruption, the collapse of secondary lamellae, and various degenerative changes that seriously compromise the gill functioning.

Palabras clave:
Lesión, histopatología, múJol, parásito

RESUMEN | En este trabajo, describimos lesiones producidas por el protozoario dinoflagelado *Amyloodinium ocellatum* en las branquias de *Mugil liza*, causante de la enfermedad del terciopelo en varias especies de peces. Estos parásitos producen lesiones severas en las branquias, con hiperplasia de las células lamelares primarias y secundaria, ruptura de las células pilares e hiperplasia de las células de cloruro (ionocitos). La estructura branquial queda totalmente afectada, lo que impide una hematosis normal y altera la actividad osmoreguladora comprometiendo la salud de los peces. Las lesiones producidas por *A. ocellatum* son responsable por las altas tasas de mortalidad en los cultivos de peces generando pérdidas económicas en el sector productivo. En el presente trabajo, encontramos hiperplasia de células lamelares del epitelio respiratorio, disrupción epitelial, colapso de las laminillas secundarias y otras alteraciones que comprometen seriamente la función branquial.

The Mugilidae Family reached a production of 291.2 thousand tons in the year 2022, being considered the third-largest production of marine resources in the world (FAO, 2022). On the Brazilian coast, the species from this family that stands out is *Mugil liza* (Valenciennes, 1836), which holds significance in the country's fishing activity and is considered one of the most traditional fishing resources in the southeast and southern regions of the country (Lemos, 2015).

Due to its overexploitation, studies have been conducted on its production under captivity (Sampaio *et al.*, 2002; Poersch *et al*., 2007; Lisboa *et al.*, 2015; Meira-Filho *et al.*, 2017). *Mugil liza* possesses various attributes that make it suitable for aquaculture, such as hardiness and ease of feeding, as it readily accepts feed and shows broad tolerance to salinity and temperature variations (Miranda Filho *et al*., 2010).

Amyloodinium ocellatum (Alveolata: Dinophyceae) is an ectoparasite endemic to temperate and tropical zones worldwide and is considered a major parasite of marine teleosts and some elasmobranch species (Lawler, 1980; Paperna, 1984; Keller, 2006; Noga & Levy, 2006; Alvarez-Pellitero, 2008).

In general, its optimum temperature is between 23 and 27°C and salinity between 30 and 35 ppt. With high pathogenicity, *A. ocellatum* can adapt to a diversity of aquatic environments, which makes its control difficult.

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It is a nonspecific dinoflagellate protozoan with three-phase life cycle. The cycle comprises the infectious stage or Dinospore which is a free swimming spore with two flagella, one longitudinal, the other transverse; the parasitic stage or Trophont, which adheres and feeds on the host; the non-parasitic reproductive stage or Tomont, responsible for successive cell divisions, which will form 256 dinospores (Brown, 1931; Noga & Levy, 2006).

The gills and skin are the first and main organs of infection by the parasite (Carvalho-Varela, 2005). Thus, in *A. ocellatum* infection, clinical signs are loss of appetite, rapid breathing, erratic swimming, crowding on the water surface, and rubbing against the tank wall (Paperna, 1980; Abreu *et al*., 2005; Francis-Floyd & Floyd, 2011; Guerra-Santos *et al*., 2012). With the evolution of Amyloodinioniosis, depigmentation, congestion and erosion of the fins, loss of scales, abdominal dilation, and hypersecretion of mucus are observed (Reed & Francis-Floyd, 1994; Carvalho-Varela, 2005; Moreira *et al*., 2017).

In this work, we describe the gill lesions produced by *A. ocellatum* in *Mugil Liza* mullet, analyzed by optical microscope.

A group of 100 specimens of *Mugil liza* that were in the vivarium of the Federal University of Rio Grande - FURG presented an outbreak of Amyloodiniosis with 70% prevalence, and 10 specimens still alive were taken to the Laboratory of Immunology and Pathology of Aquatic Organisms (LIPOA). Fish weighing 0.50 g to 1.3 g were euthanized with a benzocaine immersion bath (500 mg/L) and subsequently dissected. The gill tissue samples were fixed in 10% neutral buffered formol and then processed in an automatic processor (LEICA TP1020) and included in paraplast. The tissue sections were made on a semi-automatic microtome (LEICA RM2245). For histological analysis, the slides were stained with hematoxylin and eosin (Luna, 1968) and subsequently observed in a Zeiss Primo Star optical microscope equipped with an Axiocam ERC5s - AxionVision (LE) camera.

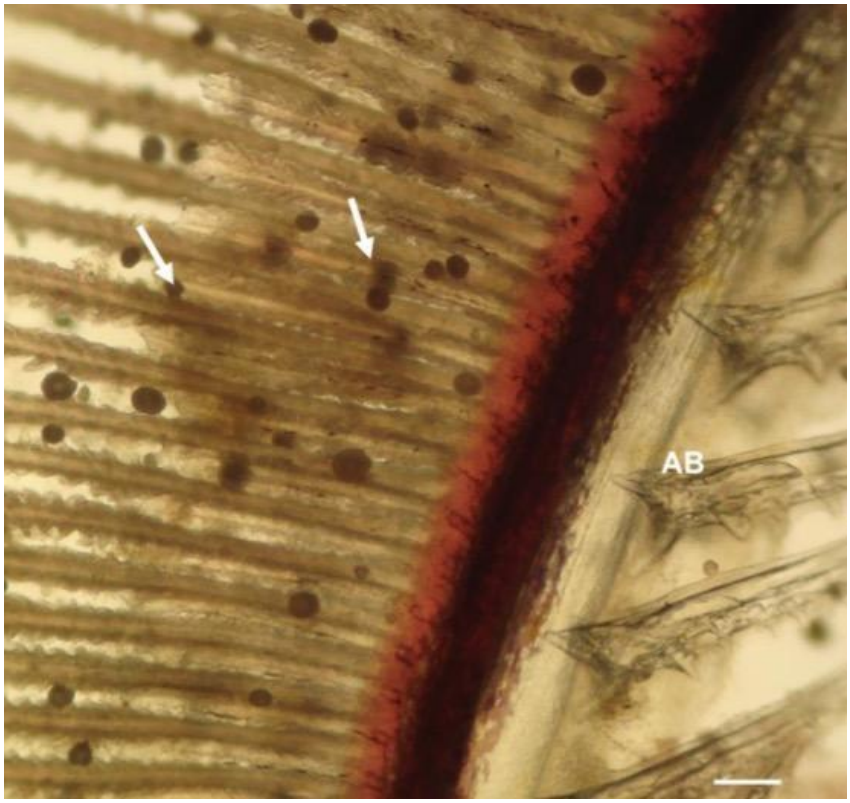

Figure 1. Trophonts of *Amyloodinium ocellatum* (arrows) observed on fresh examination in the gills of *Mugil liza* specimens affected by the parasite. Branchial arch (AB). BAR: 10 mm.

Macroscopically, we found the gills totally infiltrated by *A. ocellatum* (Fig.1 and 2-A). Microscopically, it is observed that the parasites adhered to the epithelium of the primary and secondary lamellae (Fig.2-B and 2-C) that

present marked hyperplasia. It is possible to observe the rupture of pillar cells with an agglomeration of erythrocytes and hyperplasia of chloride cells (ionocytes) (Fig.2-D, 3-A and 3-B)

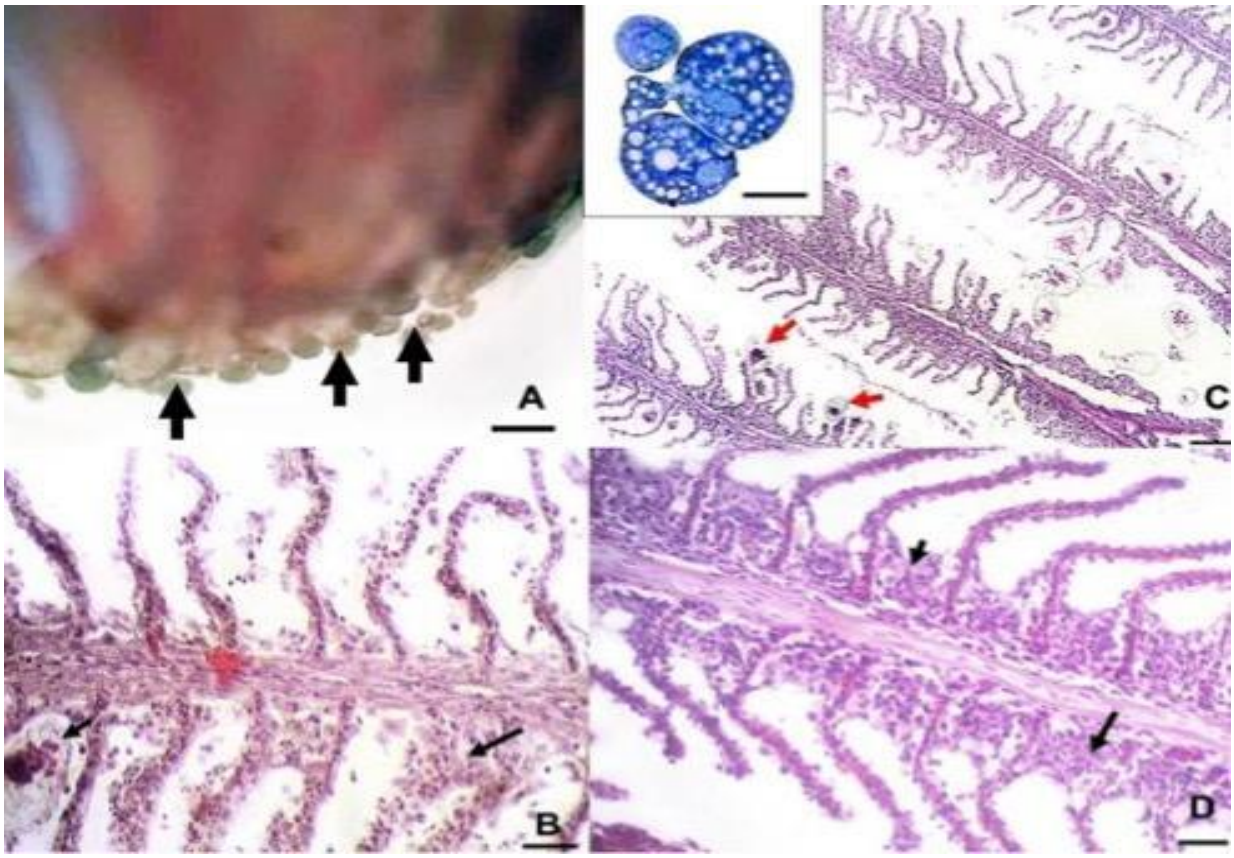

Figure 2. (A) *Mugil liza* gill with multiple *Amyloodinium ocellatum* adhered to the lamellae (arrows). BAR: 0.1 mm. **(B)** Gill where a Trophont is observed adhered to the secondary lamellae of the gill (short arrow). H.E. BAR: 100 µ. **(C)** Panoramic aspect of the gill with multiple parasites attached to the coverslips and released between the gill sheets. H.E. BAR: 200 M. Insert: Dinospore, the infective stage of *A. ocellatum*. Giemsa. BAR: 10 µ. **(D)** Gill with increased chloride cells (short arrow) and marked basal hyperplasia (long arrow). H.E. BAR: 100 µm.

The severe tissue damage caused in the gill by the parasite can induce hypoxia and osmoregulatory imbalance (Lawler, 1980, Noga, 2010), as well as provide conditions for other infectious agents to enter the tissues. Furthermore, the hyperplasia observed in the lamellae compromises the entire water circulation space, which further impairs the osmotic functions of the fish.

The hyperplasia of chloride cells (Fig. 2-D, 3-A and 3-B) observed in the histology, may be an indication of osmotic imbalance of the fish since the inflammatory and degenerative changes corroborate with the respiratory inability of the parasitized fish (Schalch *et al*., 2006).

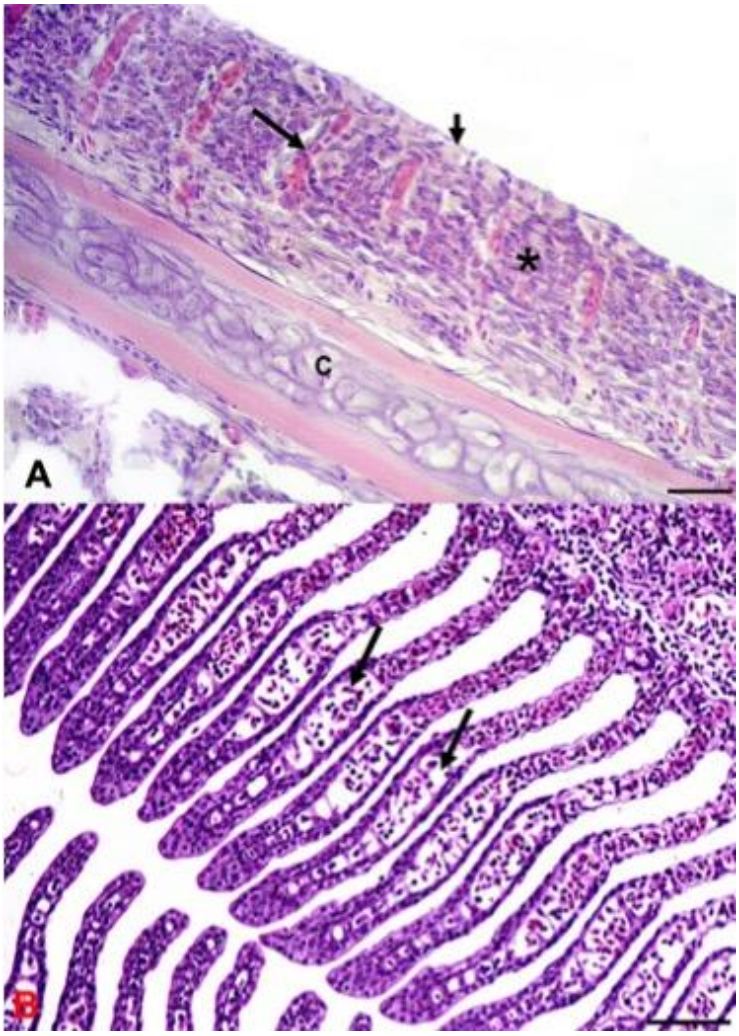

Figure 3. (A) *Mugil liza* gill lamella with marked hyperplasia (*). Erythrocytes circulating among the hyperplastic cells (long arrow) and also enlarged chloride cells (short arrow) can be seen, "C" being the gill cartilage. H.E. BAR: 50 µm. **(B)** Gill tissue with secondary lamella where rupture of pillar cells is observed (arrows). H.E. BAR: 100 µm.

The histopathological changes resulting from parasitosis by *A. ocellatum* were cellular hyperplasia of the respiratory epithelium, epithelial disruption, collapse of secondary lamella, and varied degenerative changes. They directly interfere with gill functionality and fish health. These changes were similar to those reported by Byadgi *et al.* (2019) and Marques *et al.* (2019) in *Dicentrarchus labrax*; Moreira *et al.* (2018) in *Sparus aurata*; Guerra-Santos *et al.* (2012) in *Rachycentron canadum* and Kumar *et al.* (2015) in *Trachinotus blochii*.

Similarly, Steckert *et al.* (2018) reported a gill infection caused by different parasites (*Trichodina spp.*, *Ichthyophthirius multifiliis*, *Cichlidogyrus sclerosus* and *Cichlidogyrus halli*) in *Oreochromis niloticus*, where the histopathological changes observed were similar to those found in the present study.

Although the proliferative increase of mucous cells in the gills is a common feature in fish infested by various ectoparasites (Rogers & Gaines, 1975), in all cases studied in our laboratory we did not find mucous cells. According to Paperna (1980), the reduction or absence of mucous cells represents a more specific feature of the histopathology of fish parasitized by *A. ocellatum.*

As reported in the literature, cellular hyperplasia of the respiratory epithelium is still expected with a fusion of the lamella and edema, detachment of the cells of the respiratory epithelium, as well as various degenerative changes, inflammation, hyperplasia, bleeding, necrosis, and depletion of mucous cells (Paperna, 1980; Johnson, 1990; Scott, 2000; Ramos & Oliveira, 2001, Pavanelli *et al.*, 2008). Thus, our study meets this information.

Because it is a parasite capable of such severe and pathogenic damage, *A. ocellatum* is a major threat to the aquaculture of marine animals worldwide. Although the parasite does not transmit directly from fish to fish (Francis-Floyd & Floyd, 2011), aquaculture production systems provide the density of animals, proximity between individuals, and ideal conditions for their development.

Moreover, as this parasite is characterized by low specificity for host species, it can infect several aquatic organisms, including crustaceans and bivalves (Moreira *et al.*, 2017) and cause moderate to intense tissue reactions (Aravindan *et al*., 2007; Francis-Floyd & Floyd, 2011).

In conclusion, the histopathological findings are consistent with that described in the literature, characterized by severe lesions in the gills, such as hyperplasia of primary and secondary lamellae cells, rupture of pillar cells, and hyperplasia of chloride cells (ionocytes). With the gill structure of *Mugil liza* mostly affected, normal hematosis and electrolyte regulation do not occur, which seriously compromises fish health. We did not find mucous cells in the gills of fish parasitized by *A. ocellatum*, as described by Paperna (1980). Considering the severity of the lesions caused by the parasite, we reaffirm the importance of control of this parasite in the different cultures of marine species, since this is a great threat to the aquaculture of marine animals worldwide.

Conflict of interest

The authors declare no conflict of interest.

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