Ciências Agrárias: Campo Promissor em Pesquisa 3

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(Organizadores)

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APRESENTAÇÃO

A obra "*Ciências Agrárias Campo Promissor em Pesquisa*" aborda uma publicação da Atena Editora, apresenta seu volumem 3, em seus 23 capítulos, conhecimentos aplicados as Ciências Veterinárias.

A produção de alimentos nos dias de hoje enfrenta vários desafios e a quebra de paradigmas é uma necessidade constante. A produção sustentável de alimentos vem a ser um apelo da sociedade e do meio acadêmico, na procura de métodos, protocolos e pesquisas que contribuam no uso eficiente dos recursos naturais disponíveis e a diminuição de produtos químicos que podem gerar danos ao homem e animais. Este volume traz uma variedade de artigos alinhados com a produção de conhecimento na área de veterinária, ao tratar de temas como manejo nutricional de caprinos, peixes, cães, gatos, aves, avelhas, entre outros. São abordados temas inovadores relacionados com sistemas de produção e manejo, melhora da cadeia produtiva, qualidade e bem-estar animal. Os resultados destas pesquisas vêm a contribuir no aumento da disponibilidade de conhecimentos úteis a sociedade.

Aos autores dos diversos capítulos, pela dedicação e esforços, que viabilizaram esta obra que retrata os recentes avanços científicos e tecnológicos nas Ciências Veterinárias, os agradecimentos dos Organizadores e da Atena Editora.

Por fim, esperamos que este livro possa colaborar e instigar mais estudantes e pesquisadores na constante busca de novas tecnologias para a área da Agronomia e, assim, contribuir na procura de novas pesquisas e tecnologias que possam solucionar os problemas que enfrentamos no dia a dia.

Jorge González Aguilera
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SUMÁRIO

CAPÍTULO 9

HISTOLOGICAL CHANGES CAUSED BY LIGOPHORUS URUGUAYENSE (*Monogenoidea*) IN REARED MULLET MUGIL LIZA

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ABSTRACT: Monogenoidea pathogenic activity can elicit various histological responses in fish. Species of *Ligophorus* are specific parasites of mullets, and his relationship with host fish may result in a moderate pathogenic action. In order to ascertain this relationship, estuarine mullets (*Mugil liza*) were collected in an estuary, reared in laboratory, for three weeks, and forwarded for histological and parasitological analyses. *Ligophorus uruguayense* (Monogenoidea) infestation in the gills of the mullets was identified. The severe infestation by only one species of Monogenoidea may result from the specificity of these parasites to mullets. Mullets submitted to histological analysis exhibited respiratory epithelium detachment; mild, moderate and severe hyperplasia of the respiratory epithelium; atrophy; and telangiectasia of the gills. This is the first study reporting that mullets highly infested by Monogenoidea can show mild (100%) to severe (20%) gill changes with a distinct frequency of occurrence. By the high prevalence of mild alterations observed, it is possible to accept that *L. uruguayense* is moderately pathogenic to *M. liza*, even during high prevalence and intensity of infestation, as a result of its specificity. The hypotheses regarding the cause, histological damage frequency and implication of the host–parasite relationship in mullet rearing systems are discussed.

KEYWORDS: Aquaculture; histological changes; Monogenoidea; Mugilidae; parasitosis.

1 | INTRODUCTION

Species of *Ligophorus* Euzet and Suriano, 1977 (Ancyrocephalidae, Monogenoidea) parasitize marine fish and are highly specific for mullet species. The *Ligophorus*/Mugilidae specificity suggests that this is an association

that results from a long coevolutionary process (Euzet and Suriano 1977, Marchiori et al. 2015). In addition, studies have shown that long coevolutionary relationships can influence pathogenicity of the parasite, which certainly reflects selection processes of the host populations (Poulin et al. 2000, Little and Ebert 2004). Other authors showed this phenomena even in high prevalence and intensity of infestation by *Ligophorus* spp. of wild mullet, apparently without causing severe damage to the host (Merella and Garippa 2001).

Parasites belonging to this genus are oviparous and monoxenous and, therefore, may proliferate rapidly under intensive farming systems. Six species of *Ligophorus* are so far known on the Atlantic coast of South America, all parasites of the same host species *Mugil liza* Valenciennes 1836: *L. uruguayense* Failla Siquier and Ostrowski de Núñez 2009 from Laguna de Rocha, Uruguay; *L. saladensis* Marcotegui and Martorelli 2009 from Samborombón Bay, Argentina; and four other species from the Guandu River, state of Rio de Janeiro, Brazil, namely *L. brasiliensis*, *L. guanduensis*, *L. lizae* and *L. tainhae*, all described by Abdallah et al. (2009). In addition, *L. uruguayense* was reported recently for the first time on the Brazilian coast by Pahor–Filho et al. (2012).

Distinguishing among closely related species of *Ligophorus* is not always easy to do due to the small size of the main diagnostic structures associated with the genus and their close resemblance under an optical microscope (Sarabeev et al. 2005). Nevertheless, Marchiori et al. (2015) have confirmed recently the validity of two closely related species, *L. uruguayense* and *L. saladensis*, through a combined morphological and molecular approach.

Studies have shown the potential damage caused by Monogenoidea species to their hosts (Kristmundsson et al. 2006, Hutson et al. 2007, Jorgensen et al. 2009). These parasites frequently decrease body weight (Ranzani–Paiva and Silva–Souza 2004) or even cause death of the host (Montero et al. 2004, Mansell et al. 2005, Dezfuli et al. 2007). In the gills, Monogenoidea can cause diverse responses that depend on the pathogenicity of the parasite species, such as mucus release, leucocytes infiltration (Arafa et al. 2009), telangiectasia, necrosis (Schalch et al. 2006), lamellar fusion (Campos et al. 2011), and reduction in the number of chloride cells (Dezfuli et al. 2007). Therefore, the understanding of the relationship among mullets and their parasites contributes to the development of new prophylactic management strategies in farming systems.

This study aimed to analyse the parasitological indexes and the frequency of histological changes caused by infestation of the estuarine mullets by *L. uruguayense*, Monogenoidea. Here, we report that estuarine mullets highly infested by Monogenoidea exhibited mild to severe gill changes with a distinct frequency of occurrence. The advances found in this study can contribute positively to the development of prophylactic techniques in the management of mullet rearing systems.

2 I MATERIAL AND METHODS

The experimental procedures were approved by the Ethics Committee Approval – 002234/15/CEUA/UNESP) and conducted according to the guidelines of ethical principles in animal experimentation, adapted by the Colégio Brasileiro de Experimentação Animal (COBEA).

2.1 Fish and acclimation in laboratory

A total of 60 juvenile mullets (1.2 ± 0.29 g, 4.5 ± 0.5 cm) were captured from a stream that flows into the Cassino beach, Rio Grande-RS, Brazil (32° 11' 55" S, 52° 11' 14" W) with the aid of a trawl net (3 m x 1.5 m x 5 mm) during winter. The fish were transported alive to the laboratory and acclimated for 3 weeks in a 1000-L fiber tank to simulate a farming condition of mullets. A 20% daily renewal rate of tank volume was performed and maintained the same original estuarine conditions: salinity (10 mg L^{-1}), constant aeration, photoperiod (12 h light), pH (7.5), dissolved oxygen (6.22 mg L^{-1}), temperature (22 °C) and total ammonia (0.26 mg L^{-1}). The parameter measurements were performed using an oximeter and a YSI 55/12 FT pH meter (USA) and Nessler's reagent for ammonia. During the acclimation period, a commercial diet INVE® with 28% crude protein was used to feed the fish, and the water quality parameters remained in an acceptable range for fish (Vinatea–Arana 2003). There was 100% survival of fish during the rearing period in laboratory.

2.2 Study design

After three weeks from fish rearing, ectoparasites in gills of mullets were identified, the parasitological indexes of 40 fish were calculated and the gill histological damage of 20 fish were described and quantified.

2.3 Parasitological analysis

The necropsies were performed using 40 gills, in which the minimum sample size was chosen according to Marques and Cabral (2007), to give reliability to the parasitological analysis. The hosts were euthanised using a section of the medullae to the head, as this does not compromise the estimation of parasitological indexes (Eiras et al. 2006). The collection, fixation and preparation of parasites for identification were performed according to Eiras et al. (2006). Monogenoidea were preserved in 5% formaldehyde and transferred to 70% ethanol after 24 h. Some specimens were stained with Masson trichrome or Semichon's carmine, dehydrated in 70–100% ethanol, clarified in Faia creosote and mounted in Canada balsam. Other specimens were mounted in Grey–Wess's medium to check the sclerotized pieces and copulatory apparatuses, both male and female.

2.4 Parasitological indexes

Parasitological indexes were determined using the calculation of the prevalence, intensity of infestation, mean intensity of infestation and mean abundance, according to Bush et al. (1997).

2.5 Histological analysis of the gills

For histological analysis, 20 fish were euthanized with benzocaine (100 mg L^{-1}). Gill tissue samples were fixed in Bouin solution during 4 h, and after this period, they were maintained in 70% ethyl alcohol to be processed histologically. The samples were embedded in Paraplast (Sigma-Brazil). Histological sections of 5 mm were stained with hematoxylin and eosin.

2.6 Identification and quantification of histological changes

The histopathologies were classified according to Randi et al. (1996) and Pahor–Filho et al. (2014), in which a red line in the photomicrograph shows the limit of the affected portion in gill filaments. Thus, it was considered 'mild' when hyperplasia affected less than half of the gill filament length, 'moderate' when it affected more than half of the gill filament length and 'severe' when it affected the entire gill filament length. Photomicrographs were taken with a Leica DM2500 microscope. The occurrence of histological changes was quantified in the 20 gills forwarded to histological analysis, on a scale between 0 to 100%.

2.7 Parasitological data analysis

The parasitological indexes were calculated using the appropriate parasitological statistical analysis protocol, quantitative parasitology (Reiczigel and Rózsa 2005).

3 I RESULTS

3.1 Parasitological indexes

In the gills of mullets, *L. uruguayense* Failla Siquer and Ostrowski de Núñez 2009 (Ancyrocephalidae, Monogenoidea) were identified. Parasitological indexes of juvenile mullets were prevalence (100%), intensity of infestation (2–125), mean intensity of infestation (25.2±5.1), and mean abundance (23.2±4.8).

3.2 Identification and quantification of histological changes

In the gills of mullets, respiratory epithelium detachment; mild, moderate and severe hyperplasia of the respiratory epithelium; atrophy; and telangiectasia were observed (Figure 1). Of a total of 20 fish analysed, 100% of juveniles exhibited mild hyperplasia; 40% exhibited telangiectasia; 30% moderate hyperplasia and respiratory epithelium detachment; and 20% severe hyperplasia and necrosis of the respiratory

epithelium.

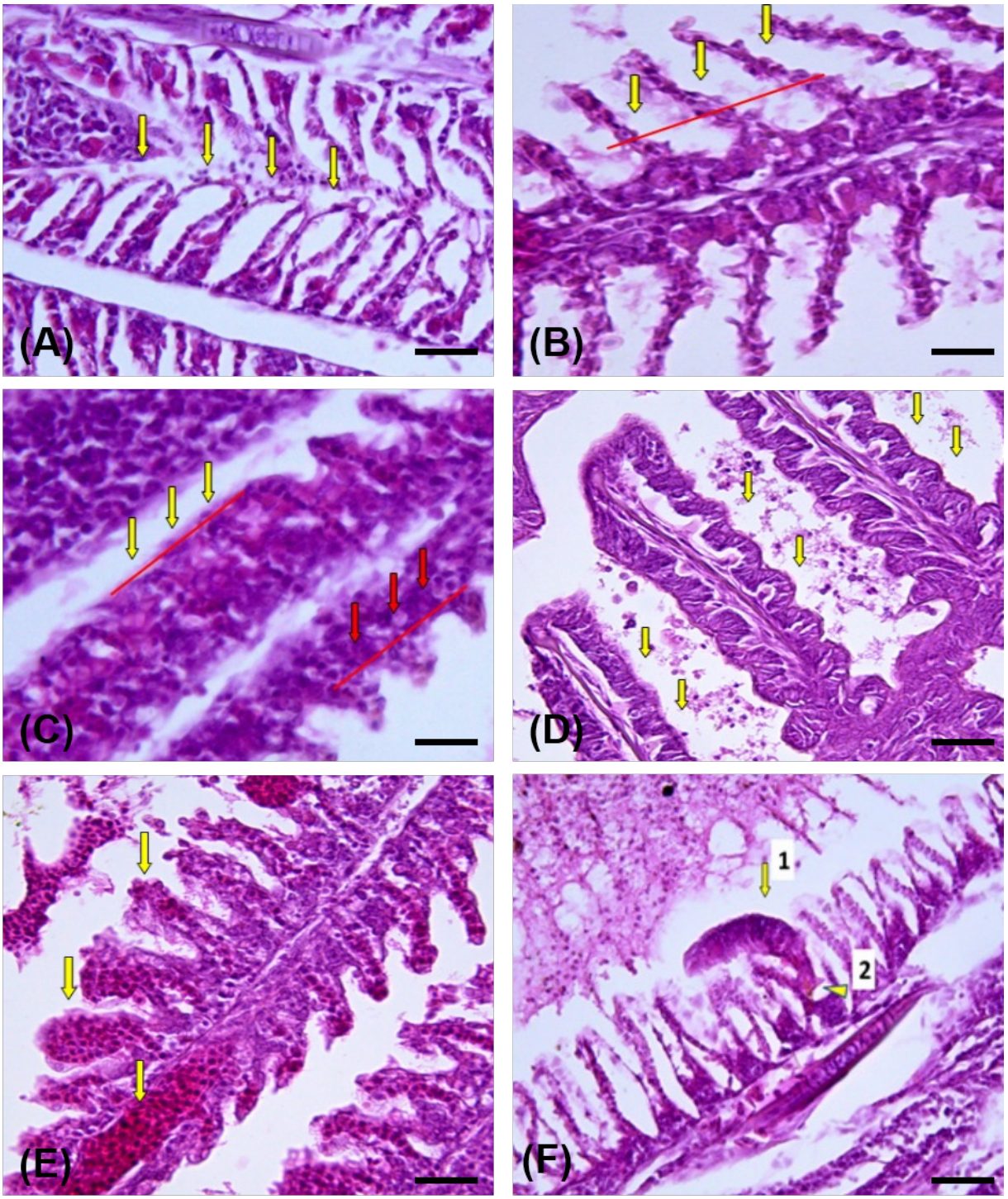

Figure 1. Longitudinal section of gills of juvenile mullet *Mugil liza* with histological alterations caused by infestation of *Ligophorus uruguayensis*. (A) respiratory epithelium detachment (yellow arrow). (B) mild hyperplasia (yellow arrow). Red line shows that hyperplasia affects less than half of the gill filament length. (C) moderate hyperplasia (red arrow). Red line shows hyperplasia affects half the length of the filament. Severe hyperplasia (yellow arrow). Red line shows that hyperplasia affects all the gill filament length. (D) necrosis of the respiratory epithelium (yellow arrow). (E) telangectasia (yellow arrow). (F.1) *Ligophorus uruguayensis* fixed on the gill filament (yellow arrow). (F.2) Haptor (yellow arrow). (n = twenty fish). All hematoxylin eosin staining, Scale bar = 200 μm.

4 I DISCUSSION

A high index of *Ligophorus* spp. infestation in *Mugil* species has been reported

by other researchers (Sarabeev and Balbuena 2004, Sarabeev et al. 2005, Failla Siquier and Ostrowski de Núñez 2009). The severe infestation by only one species of Monogenoidea found in juvenile mullets may result from the specificity of these parasites to mullets (Euzet and Suriano 1977, Marchiori et al. 2015) and by the life cycle of mullets, since the mullets form shoals and stay in estuaries during development of their juvenile phase (Godinho 2004). These sites contain high concentrations of organic matter and high densities of fish of the same species, which increase the possibility of transmission of these parasites (Buchmann and Lindenstrom 2002).

Other studies show different intensities of gill hyperplasia and occurrences of other changes as host responses to Monogenoidea infestation (Montero et al. 2004, Schalch et al. 2006, Arafa et al. 2009). Mild effects, such as hyperplasia, caused by Monogenoidea were observed in kingfish (*Seriola lalandi*) heavily infested by *Zeuxapta seriolae* (Mansell et al. 2005) as well as tilapia (*Oreochromis niloticus*) infested by *Cichlidogyrus sclerosus*, according to Azevedo et al. (2006). Corroborating these results, in this study, even with high incidence of mild hyperplasia (100%) observed in the gills of juveniles infested by Monogenoidea, there was a moderate pathogenicity as result of *L. uruguayense* parasitosis in the mullets. However, these parasites attach to the gills by the haptor and feed on skin cells and blood (Buchmann and Lindenstrom 2002), and may until to cause immunosuppression (Chaves et al. 2006). Thus, it is possible to suggest that alterations caused in the gills of juvenile mullets may be due to moderate pathogenicity of the *L. uruguayense* by mullets. This would preserve the host–parasite relationship. According to Buchmann and Lindenstrom (2002), some mechanisms are responsible for the success of this relationship, such as the presence of parasite receptors that recognise the adequate host, the infestation site and the host's innate immune system that preserves the specific parasite.

On the other hand, the *L. uruguayense* infestation, in our study, can be associated to severe damage observed in the gills of juvenile mullets. However, a low incidence of severe hyperplasia and necrosis of the respiratory epithelium were observed in 20% of the hosts. These could be specimens not favored by selection processes, as suggested by Little and Ebert (2004). Moreover, these data confirm the hypothesis of Thatcher (1991), which supports that the parasites can cause severe injuries in the host, and only in some cases this is detected in nature, because these debilitated fish are preyed upon quickly. Other authors also reported severe changes caused by Monogenoidea in other fish, such as fusion of the secondary lamellae (Kristmundsson et al. 2006), atrophy of the respiratory epithelium, haemorrhage (Dezfuli et al. 2007), and epithelial necrosis (Arafa et al. 2009), suggesting that *L. uruguayense* also might cause or induce severe damage in debilitated juvenile mullets, captured by trawl net in the estuary.

In Brazil, the mullet *M. liza* has high demand for fishermen and local consumers (Godinho 2004). Pilot tests for breading of this fish have been successfully carried out in state of Santa Catarina, evidencing their productive potential to Brazilian

aquaculture (Carvalho et al. 2015). In this line, in our study, even the rearing system containing acceptable water quality parameters for marine fish, the mullets infested by *L. uruguayense* exhibited telangectasia (40%), moderate hyperplasia and respiratory epithelium detachment (30%), and severe hyperplasia and necrosis (20%). These results show the possibility of aggravation of these histological changes in cases of water quality fall, in which fish become more susceptible to parasitic infestations in fish farms.

Monogenoidea proliferate rapidly in breeding systems, and attacks the host by the haptor's abrasive activity. Therefore, the maintenance of water quality and the prophylaxis of the medium are fundamental factors to avoid the damage to the fish (Kristmundsson et al. 2006, Hutson et al. 2007, Jorgensen et al. 2009). A better understanding of the host–parasite relationship as well as the parasite species, intensity of infestation and histological damage to the host may contribute to the improvement of prophylactic techniques in the management of mullets in rearing systems. The preventive techniques for parasite control are aimed at interrupting or preventing development of the parasite life cycle. Thus, it is possible to reduce the intensity of parasitic infestation, increasing the possibility of success in breeding systems.

Here, we determined the method of parasitism in juvenile mullets and the damage observed in the gills of the host. However, by the high prevalence of mild alterations observed in the gills of mullets, it is possible to accept that *L. uruguayense* is moderately pathogenic to *M. liza*, even during high prevalence and intensity of infestation, as a result of its specificity. Further studies should investigate whether the high Monogenoidea infestation can cause mortality in other life stages, such as adults and breeding mullets, and whether the presence of these parasites may increase histological changes in the host.

Note from authors: All information in this E-book chapter belong to article "Moderate pathogenic effect of *Ligophorus uruguayense* (Monogenoidea, Ancyrocephalidae) in juvenile mullet *Mugil liza* (Actinopterygii, Mugilidae) from Brazil" published in Anais da Academia Brasileira de Ciências, 89: 2997-3003, 2017.

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SOBRE OS ORGANIZADORES

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