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Histopathological and Morphometric Changes Caused by *Anisakis simplex*, Nematode Larvae in the Gut of *Lethrinus mahsena* from the Red Sea, Jeddah, Saudi Arabia

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ABSTRACT

Lethrinus mahsena, a species found throughout tropical and subtropical marine settings, is important to coastal ecosystems and has a high economic value. The impact of parasite infestations on these fish populations is widely recognized, but the precise histopathological responses to infestations requires further studies. This investigation aimed to investigate the histopathological changes in the intestines of *Lethrinus mahsena* caused by *Anisakis simplex* larvae. Over a year, 234 *Lethrinus mahsena* samples were monthly collected at the Rass Mehsen site on Saudi Arabia's Jeddah coast. The intestines of the examined fish were carefully checked for parasitic infestations. Based on the histopathological investigations of fish, considerable changes were recorded in the intestine. The mucosal layer was detected with the most obvious alterations, evidenced by significant desquamation and morphological abnormalities. The intestinal epithelium's structural integrity was disrupted, resulting in the loss of intestinal villi and the creation of inflammatory infiltrations. These findings reveal the severe impact of helminth nematodes infestations on the gut morphology of *Lethrinus mahsena*, emphasizing the importance of additional investigation into the mechanism driving these pathological changes.

INTRODUCTION

The Red Sea, known for its diverse marine life, is a home to a variety of fish species, including the emperor fish, *Lethrinus mahsena*. This species has substantial ecological and significance; however, it is susceptible to parasitic diseases, particularly from *Anisakis* nematodes previously identified in Saudi Arabia **(Hassan *et al*., 2013, Ibrahim *et al*., 2018, Mehanna *et al*., 2023)**. *Anisakis* nematodes, particularly those from the genus Anisakis, infect a variety of marine hosts, including fish, producing severe histopathological abnormalities in their gastrointestinal tracts **(Bušelić *et al*., 2018)**. These parasites have complex life cycles that involve numerous hosts, including crustaceans, fish, and marine mammals, with fish frequently acting as intermediate or paratenic hosts **(Klimpel & Palm 2011)**.

Fish infestations with *Anisakis* nematodes can cause significant pathological alterations in their gut, such as inflammation, necrosis, and granuloma development. These alterations may have an impact on the fish's development and survival rates by reducing their general health and digestive performance **(Audicana & Kennedy 2008)**. Commercially significant fish species that harbor these parasites also provide a health risk to humans since eating raw or undercooked contaminated fish can result in anisakiasis, a zoonotic illness that causes allergy and gastrointestinal symptoms **(Bušelić *et al.*, 2018)**.

According to **Lymbery and Cheah (2007)**, anisakids are typically found in large quantities in the gut cavity of hosts at different trophic levels of the food chain during the third larval stage. Fish ingest infected planktonic or semi-planktonic crustaceans or prey fish to acquire larval stages of *Anisakis* spp. The larva enters the visceral cavity of the new fish host by penetrating the intestinal wall. It then crawls around the organs, encysts on their surface, or migrates into the host muscle **(Mattiucci *et al.*, 2018)**. In fish musculature, larval migration can take place intravital **(Larsen *et al.*, 2002)** or post-mortem **(Cipriani *et al.*, 2016)** following host death. According to **Franke *et al.* (2014)**, the ability of helminths to influence and/or circumvent the host's immune system is crucial to the success of their infection.

Histopathological research has revealed that anisakids larvae can produce a variety of tissue reactions in fish, including eosinophilic granulomas, fibrosis, and necrosis **(Hochberg & Hamer, 2010)**. These reactions differ based on the severity of the infection and the kind of nematode implicated. *Anisakis simplex* and *Anisakis pegreffii*, for example, have been shown to cause distinct immunological responses in their host **(Umehara *et al.*, 2007)**.

The economic impact of *Anisakis* infections on fish is enormous. Consumers may reject infested fish due to apparent parasites or tissue damage, resulting in economic losses to fisheries **(Shamsi & Butcher, 2011)**. Furthermore, the possible health hazards connected with anisakiasis require strict monitoring and control methods in the seafood industry **(Sohn *et al*., 2015)**. Therefore, it is crucial to understand the histopathological effects of these parasites on fish. There are many factors as a multifactorial aspect caused by parasitic infestations to create efficient management plans. The objective of this work was to examine the histopathological alterations in the gut of *Lethrinus mahsena* from the Red Sea, Jeddah, brought on by anisakids nematode larvae. Moreover, the study aimed to look at the type and degree of tissue damage.

MATERIALS AND METHODS

1. Study area

Over the year, from 2023 to 2024, two hundred thirty-four *Lethrinus mahsena* fish samples were examined every month from the Rass Mehsen site along the Jeddah shore. Standard fishing procedures were used for the collection to assure an accurate representation of the population. This research was done *in vitro,* Department of Biological Sciences at King Abdulaziz University, Jeddah, Saudi Arabia.

2. Parasite identification

Every fish was placed in an ice box and taken to the laboratory for further examinations. Biological parameters were measured as weight and length to determine body condition. For dissecting the fish, a longitudinal incision was made along its ventral surface following the procedure outlined by **Madanire-Moyo and Barson (2010)**. The gut was gently extracted and immersed in a physiological saline solution to preserve tissue integrity during evaluation **(Mehanna *et al*., 2023)**.

The intestine was examined using a stereomicroscope to detect the presence of intestinal parasites. Intestinal samples from both infested and non-infested individuals were taken for histopathological examinations. Parasite identification and basic morphological characters as tooth, lips and anus were described according to the identification of **Yamaguti (1961)** and **Gibbons (2010)**.

3. Histopathological examination

Histopathological examinations were conducted on tissue samples that were obtained from the intestine of the infested and non-infested fish. To maintain tissue morphology, the intestinal samples were preserved for 24 hours in 10% neutral buffered formalin. After fixation, fixative was removed from the samples using a graded ethanol series (70, 80, 90, 95, and 100%). To assist thin sectioning, the dehydrated samples were subsequently embedded in paraffin wax after being cleared in xylene **(Bancroft & Stevens, 1996)**. Samples embedded in paraffin were cut with a microtome into slices that were 4µm thick. To distinguish between the various cellular components, the sections were placed on glass slides and stained with hematoxylin and eosin (HE). The samples were examined and photographed under a light microscope. Examination of stained sections was done by using the Olympus DP72 microscope in Al Borg Diagnostics Medicinal Research Center.

4. Morphometric analysis

The Leica Application Suite X (LAS X) Office software was used to perform morphometric analysis of the mucosa, submucosa, and muscular layers of the gut of *Lethrinus mahsena* fish infected with the nematode. Precise measurement (µm) of the thickness of the mucosa, submucosa, and muscular layers was applied using the LAS X Office software. Quantitative data on the degree of infection and tissue damage was obtained by comparing the measurements from infected fish with those from non-infested controls.

RESULTS

The morphological study showed that the nematodes, which infect fish intestines, were identified as *Anisakis simplex*. Third-stage larvae were found either free in host gastrointestinal (GI) tracts or encapsulated on the external surfaces of the host digestive tract and other visceral organs. A conspicuous boring tooth was always present at the anterior extremity of each worm with more developed lips (Fig. 1A, B).

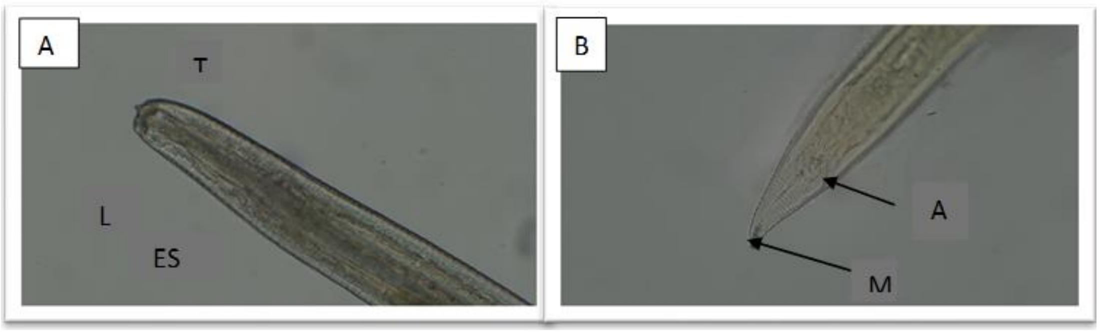

Fig. 1. Third stage larva of male of *Anisakis simplex* in *Lethrinus mahsena* A&B: The anterior end showing tooth (T), lips (L) and esophagus (ES): The posterior end showing mucron (M), anus (A). (A: 20x,50µm and B: 4x,200µm)

The cross-sections demonstrated the remarkable histopathological changes associated with the helminth infection. Normally, the intestine has several layers including mucosa and submucosa as well as a circular and longitudinal muscle system. Villi were folded structures that line the anterior surface of the intestine. (Fig. 2A, B, C, D).

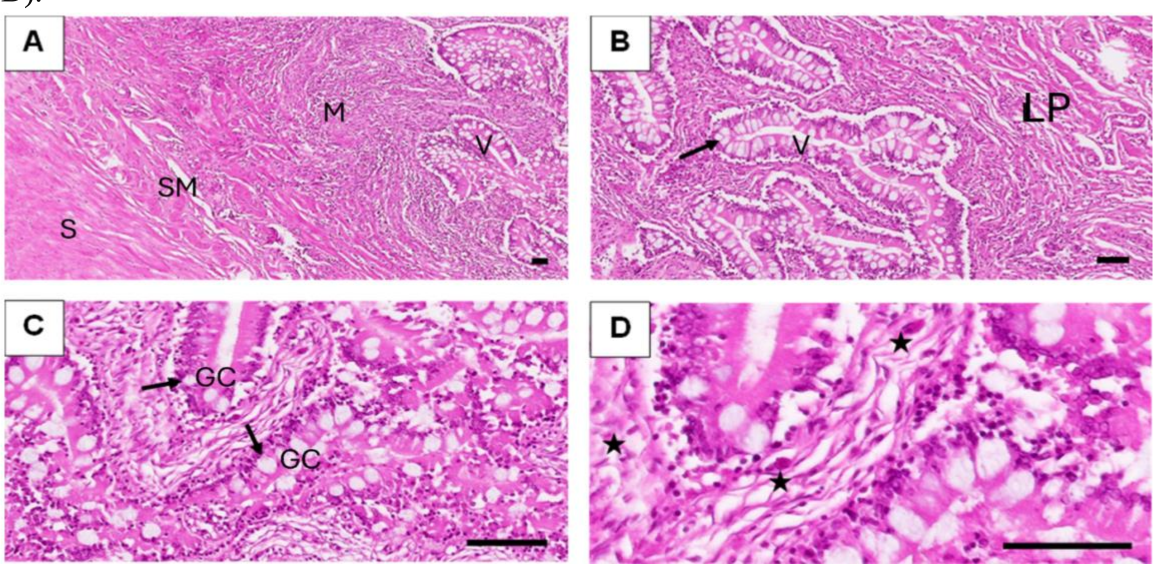

Fig. 2. (A-D). Cross- section in the midgut intestine of non-infested fish *Lethrinus mahsena* revealing different intestine layers of: Serosa layer (S), Tunica submucosa, (TSM) showing clear

connective tissue fibers (stars) (D). Muscular layer (MSL) is formed of internal circular muscular layer (IML); outer longitudinal muscle layer (OML), Tunica mucosa (TM) showed goblet cells (GC) (arrows) (C). Folded villi (VI), Lamina propria (LP). Magnifications: A, X50 – B, X100 – C, X200 – D, X400. Stain: H&E. Bars: 50µm

Infected intestine with *Anisakis simplex* showed pathological signs, including atrophy and distortion of villi and glands, infiltration of lymphocytes, and desquamation of the lining epithelium inside the lumen with erosion (Fig. 3A, D). As a result, the integrity of the typical intestinal structure was lost. Moreover, the wall of the blood vessels was slightly thickened and congested (Fig. 3E, F).

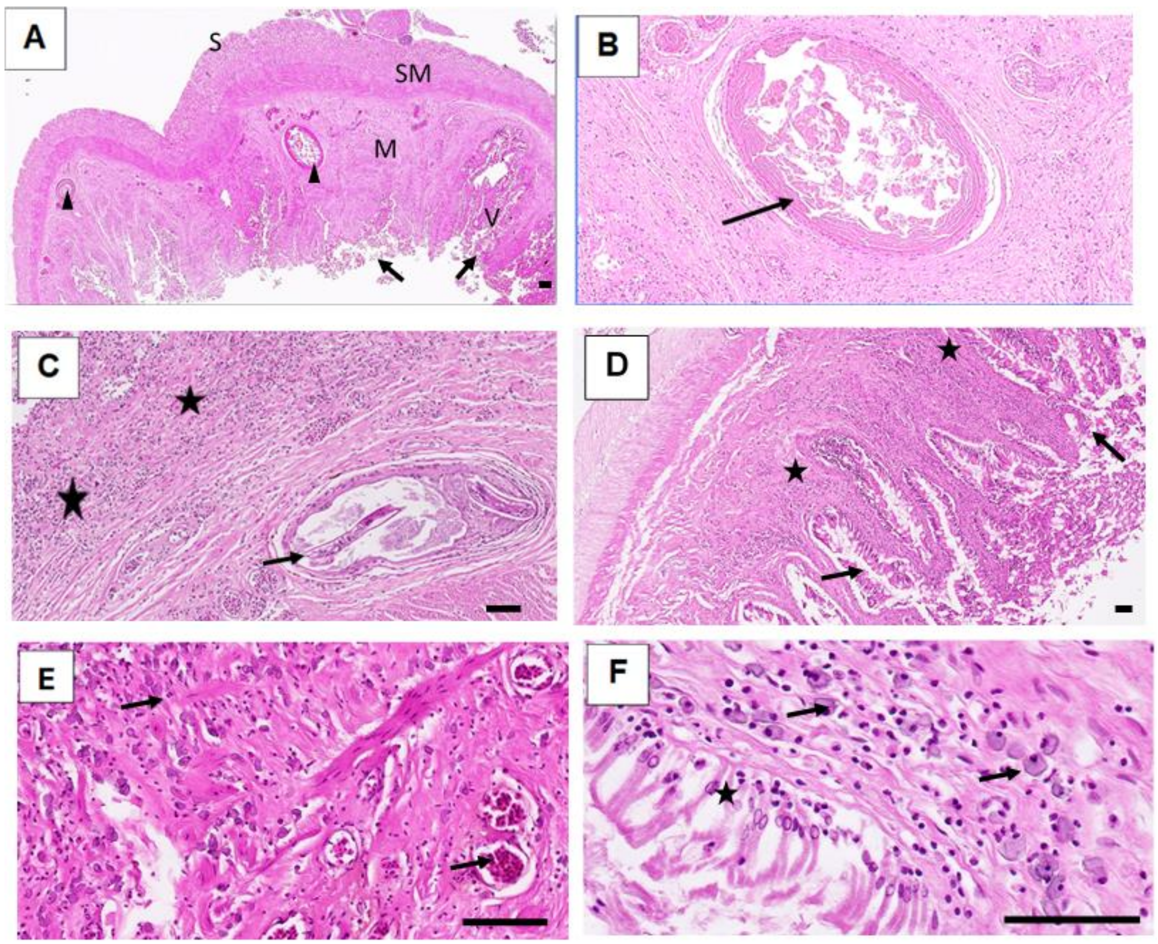

Fig. 3. (A - F). Cross-section in the midgut intestine of infected fish *Lethrinus mahsena* revealing different intestine layers and showing: Serosa layer (S), muscular layer (MSL), mucosa layer (M), distortion of villi (VL) with erosion and infiltration of inflammatory lymphocytic cells (IF), harbored encapsulated larvae of *Anisakis simplex* nematode (arrow) (B) and it is visible in the muscular layer (arrow) (C). Intense host reaction was encountered by the *A. simplex* larvae, three concentric layers were recognized, an inner mostly comprising electron-dense epithelioid cells and an outside layer of fibrous connective tissue and thin elongated fibroblasts, a middle layer of MCs

imprisoned in a light fibroblastic connective mesh and an interior layer of less electron-dense epithelioid cells (C, B). Blood vessels congestion and extensive hemorrhaging (arrow) (E, F). Magnifications; A, X10 –D X50 –B,C, X100 – E X200 – F X400. Stain: H&E. Bars: 50µm

Additionally, *Anisakis simplex's* trunk was in contact with mucosal folds, causing damage to villi, as evidenced by epithelium detachment into the intestinal lumen (Fig. 3B, C). The total number of mucous cells was significantly higher in the infested fish (Fig. 4C, D), as compared to the non-infested conspecifics (Fig. 4A, B).

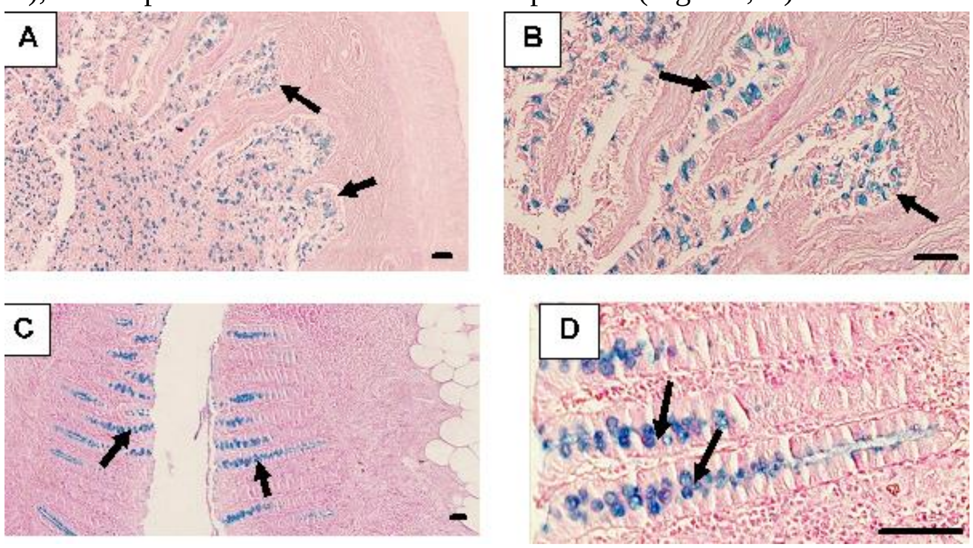

Fig. 4. (A) Cross-section of gut non-infested *Lethrinus mahsena*. (B) High magnification of the intestine epithelium with a low number of mucous cells containing acid (arrows). Magnifications: –A X50 – BX400. Stain: PAS & AB. Bars: 50µm. (C) Cross-section of gut infested *Lethrinus mahsena* fish near the attachment site of the larvae *Anisakis* sp, with a large number of mucous cells (arrows) in the epithelium. (D) Histological intestinal section of an infected *Lethrinus mahsena* fish with numerous epithelial mucous cells containing mainly acid (arrows). Magnifications: –A X50 – BX200 –C X400. Stain: PAS & AB. Bars: 50µm

The AB/PAS sequence (violet stain) indicated the mixed presence of acid and neutral mucins (Fig. 4A, B, C, D). Moreover, in the current study, measurements of the intestinal wall in non-infested (control) and infested groups were evaluated in micrometers (µm). The tunica muscularis and mucosa including its submucosa were measured. In general, parasitic infestation adversely affects the intestinal wall with significant values. In the midgut, clear degenerative effects were recorded. Tunica muscularis of the non-infested midgut measured about 578µm higher than in single infestations, while nematodes were about 262µm (Fig. 5). Tunica mucosa and submucosa of the midgut showed the strongest degenerative effects on both histological and morphometric levels, and they lost their epithelium in infested midgut (Fig. 6). Tunica mucosa and submucosa of uninfected midgut were about 560µm and showed the strongest harmful effect in nematodes (460µm).

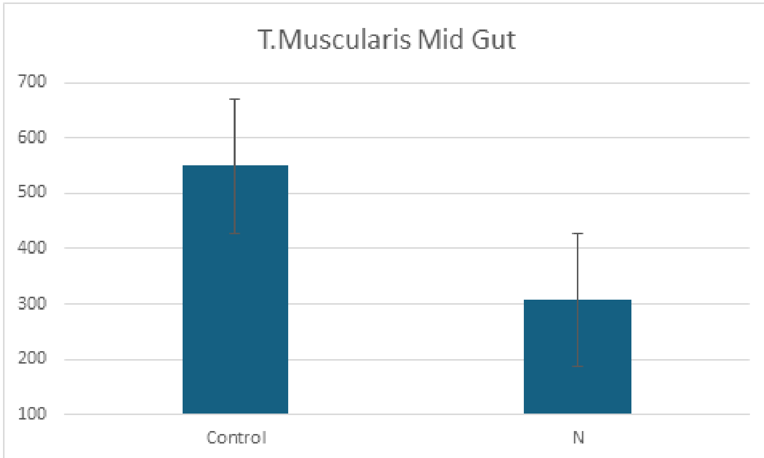

Fig. 5. Morphometric analysis showing the effect of *Anisakis simplex* infestation on the thickness of Tunica muscularis of the midgut of infested *Lethrinus mahsena* fishes. Data are expressed as mean ± SD. Control: Uninfected, N: Nematode infested*indicates statistical significance when compared with control, ($P<0.05$; one-way ANOVA and Bonferroni multiple comparison test)

DISCUSSION

The current study highlights the histopathological alterations in the gut of *Lethrinus mahsena* from the Red Sea, Jeddah, caused by *Anisakis* nematode larvae, with an emphasis on immunohistopathological responses. Significant changes in the gut of infested fish were found in this study. All internal organs were infested with the anisakis nematode's third larval stage given the severity of the gut infection. We focused on this organ to assess the degree of tissue destruction because the gut plays a crucial role in nutritional absorption and its damage resulted in malnutrition and decreased performance **(Bušelić *et a.,*. 2018)**.

Anisakis simplex larvae caused several chronic fibrosis, destruction of intestinal villi and necrosis and degenerative changes in mucosal epithelium adversely affect motility and absorptive efficiency of the fish intestine **(Dezfuli *et al*., 2015)**. A significant decrease in the thickness of muscularis, mucosa and submucosa midgut *Lethrinus mahsena* infected was observed clearly in the graphs of the whole thickness intestine layers compared to uninfected fish. However, on the histological and cellular level, *Anisakis simplex* larvae reduced the thickness of the intestine layers. Even more, the gut damage observed agrees with the previous findings described at the histological level and immune gene expression in the Atlantic salmon and Chinook salmon **(Smith *et al*., 2020, Brown *et al*., 2021; Anderson *et al*., 2024)**.

The non-infected *Lethrinus mahsena* group showed normal histological gut, which included a tunica muscularis made up of different layers of smooth muscle fibers, a submucosa rich in connective tissue fibers, blood vessels, and immune cells, and a well-organized mucosa with tall columnar epithelium and many goblet cells. The gut's healthy condition in the absence of parasite infection was highlighted by this baseline histology **(Brown *et al*., 2018; Smith *et al*., 2020)**.

In contrast, the intestines infested with nematodes showed significant degenerative alterations, especially in the mucosa and submucosa. Fish with the infestation had widespread epithelial desquamation, intestinal villi enlargement and deformities, as well as blood vessel degeneration and congestion in the midgut. Further exacerbating these states were parasite larvae nematodes found in the intestinal lumen and submucosa **(Williams *et al*., 2018; Jones *et al*., 2019; Taylor *et al*., 2022)**.

Gut diseases of fish might be induced by bacteria or viruses colonizing the mucosal epithelium, by parasites attaching to the intestinal wall, or by toxins released by these pathogens **(Manchanayake *et al*., 2023)**. It is known that mucins represent a dynamic component of mucosa and they are regulated by adaptive and innate immune systems. The secretion of intestinal mucins increased under pathological conditions, as demonstrated by the hyperplasia and hypertrophy of the mucous cells in several fish-helminth systems **(Dezfuli *et al*., 2015; Dezfuli *et al*., 2016)**. The significant increase in the number of mucous cells in parasitized fish was likely a response of the innate immune system to the parasite itself or its products. The investigation also reveals a noteworthy immunohistopathological reaction in the fish that were infected. The cells' entry into the submucosa indicates that the immune system is actively fighting the parasite invasion. Although the host's attempt to create a defense against the nematodes was demonstrated by this lymphocytic infiltration, the extent of the tissue damage seems to be compromising the effectiveness of this response **(Williams *et al*., 2018; Johnson *et al*., 2023; Anderson *et al*., 2024)**.

According to **Dezfuli *et al*. (2016)**, fibroblasts and macrophages may aid in tissue repair when nematode larvae cause serious tissue damage. In contaminated areas, phagocytes engage in pro- and anti-inflammatory responses by phagocytosis, a natural defense mechanism **(Grayfer *et al*., 2014)**. Mononuclear phagocytes (circulating monocytes and tissue macrophages) and granulocytes (especially neutrophils) have been identified as significant professional phagocyte types in fish **(Esteban *et al*., 2015)**. When neutrophils go from the bloodstream to an area of injury or parasite infection in the early phases of a pathogen challenge, they play a crucial role in the inflammatory response **(Havixbeck *et al*., 2016)**.

The submucosa and intestinal lumen contain parasite larvae, indicating that the nematodes not only directly harm tissue but also trigger a strong immune reaction. By aggravating tissue damage to eradicate the parasites, the immune response may contribute to the overall pathology, as evidenced by the observed intestinal villi hypertrophy and deformity as well as the villi core degeneration **(Smith *et al*., 2020; Brown *et al.*, 2021; Taylor *et al.*, 2022)**.

Fish macrophages were known as macrophage aggregates or melanomacrophage centers because they include pigments such as melanin, chromolipoids, and hemosiderin **(Wolke, 1992)**. Macrophage aggregated enclose parasites in tissue and were responsive to helminth infection **(Dezfuli *et al*., 2015)**. The inner layer of the capsule that protects anisakids nematode larvae was composed of epithelioid cells. In terms of appearance, these cells were similar to epithelial cells that arise after protracted inflammatory activation **(Gauthier *et al.*, 2004; Alowaidi *et al.*, 2022)**.

Our findings have crucial significance for understanding how infestations of parasites affect marine fish species. The considerable histopathological alterations and immunological responses reported in *Lethrinus mahsena* underline the importance of future investigation into the mechanisms underlying these pathological processes. Future research should concentrate on identifying the molecular and cellular pathways involved in the host's immune response to nematode infestation, as well as investigating potential interventions to mitigate the negative effects of such infections.

Finally, the histopathological alterations identified in the gut of *Lethrinus mahsena* infected with anisakis nematode larvae highlight the serious impact of parasitic diseases on fish health. These findings highlight our understanding of host-parasite interactions and lay the groundwork for future research aimed at developing effective parasitic infection management and control strategies in marine fish populations **(Smith *et al.*, 2020; Brown *et al*., 2021; Anderson *et al*., 2024)**.

CONCLUSION

In conclusion, *Lethrinus mahsena* can be susceptible to parasitic infections which lead to decline in the economic value of this type of fish. This study examines the effects of parasitic larvae on the gut of *Lethrinus mahsena* in the Red Sea. A severe histopathological change was observed, including epithelial damage, deformed intestinal villi, and blood vessel degeneration, resulting in impaired nutrient absorption. In addition to causing direct tissue damage, nematodes in the gut lumen and submucosa triggered an immune response that made the problem worse. Immune cells, such as macrophages and neutrophils, were involved in the inflammatory response. In order to prevent harmful effects on fish health, host-parasite interactions must be understood and control strategies developed.

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