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Health Insights from Nematode Larval Characterization in Greater Lizardfish, *Saurida tumbil* (Bloch, 1795) (Teleostei, Synodontidae)

Younes Ghahvei [1], Mohammad Mirzaei [1,*], Shahrzad Azizi [1], Shadi Hashemnia [2] and Shokoofeh Shamsi [3,*]

[1] Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman 7616914111, Iran; dr.younes2015@gmail.com (Y.G.); azizi.shahrzad@gmail.com (S.A.)
[2] Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman 7616914111, Iran; shadihashemnia@uk.ac.ir
[3] School of Agricultural, Environmental and Veterinary Sciences, Gulbali Institute, Charles Sturt University, Wagga Wagga, NSW 2678, Australia
* Correspondence: dr_mirzaie_mo@uk.ac.ir (M.M.); sshamsi@csu.edu.au (S.S.)

Abstract: Fish health is of significant ecological and economic importance. In response to public observations of parasite-like structures in a popular edible fish, this study aimed to characterize nematode larvae commonly found in the muscle and body cavity of *Saurida tumbil* (Bloch, 1795), a commercially important fish species inhabiting the Persian Gulf and Oman Sea. This fish, locally known as Hasoom, holds substantial culinary importance, being a staple in the diets of millions residing in countries around the Persian Gulf. A total of 458 *Saurida tumbil* specimens were obtained from fish markets between June 2022 and May 2023. Subsequent examination revealed the presence of a total of 6132 nematode larvae. Nematodes found in the body cavity were identified as belonging to the genus *Hysterothylacium* sp., family Raphidascarididae, while those in the muscle were identified as *Anisakis* sp. larval type, family Anisakidae. Histopathology results suggested that these parasites may have adverse health impacts on their fish host. Notably, both nematode genera were found in the third larval stage, which is known to be the infective stage for anisakidosis. Given the reported cases of anisakidosis among people living in the study region, it is strongly recommended that fish be properly cooked before consumption to mitigate health risks.

Keywords: fish health; marine environment; zoonoses; parasites; Nematoda

Key Contribution: This manuscript presents a significant finding regarding the health implications of consuming *Saurida tumbil* (Bloch, 1795) from the Persian Gulf and Oman Sea regions, as it reveals the presence of *Anisakis* sp. and *Hysterothylacium* sp. larval types, both in their infective stage. These findings underscore the potential risk of anisakidosis associated with consuming these fish, highlighting the importance of further research and awareness of the region's fish and public health strategies.

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1. Introduction

Fish health is important for several reasons, spanning ecological, economic, and public health perspectives [1,2]. Fish play a crucial role in aquatic ecosystems, serving as both predators and prey [3,4]. Robust fish populations are essential for preserving ecosystem equilibrium through regulating prey populations and facilitating nutrient cycling processes, thus safeguarding the overall health and resilience of aquatic habitats [5,6]. When fish populations are affected by diseases, it can disrupt these ecological processes, leading to imbalances in the ecosystem [7,8]. Fisheries and aquaculture industries contribute significantly to global food security and economic prosperity [9,10]. Healthy fish stocks support the livelihoods of millions worldwide, including fishermen, fish farmers, and those involved in related industries such as processing, transportation, and marketing [11–14].

Diseases in fish populations can result in reduced yields, economic losses, and even the collapse of fisheries, impacting food availability and economic stability [15,16].

The waters of the Persian Gulf and the Oman Sea are known for their richness and biodiversity, hosting thousands of species of fish, 20 species of whales and dolphins, and nearly 200 species of corals [17,18]. They also serve as vital sources of seafood for the countries surrounding them [9,19], sustaining millions of people who rely on marine fish to meet their population's seafood needs. Seafood harvested from the Persian Gulf and the Oman Sea is not only consumed domestically but is also exported to various parts of the world, contributing to the seafood supply in distant regions [9,20]. Among the fish living in these waters, the greater lizardfish, scientifically identified as *Saurida tumbil* (Bloch, 1795), stands out [21,22]. This marine fish belongs to the family Synodontidae and holds substantial culinary importance, being a staple in the diets of millions in countries around the Persian Gulf. For example, in the Sistan and Baluchestan Province in Iran, the annual catch of *Saurida tumbil* surpasses 3000 tons [23], which shows a reliance on this particular fish species within the region.

Commonly referred to as Hasoom locally, this marine species is primarily found in oceanic environments and is seldom encountered in brackish waters. Its habitat spans the Pacific, Atlantic, and Indian Oceans, with additional presence noted in the southern regions of Iran, including the Persian Gulf and the Sea of Oman, where it is commercially viable [24–27]. Hasoom typically thrives in depths ranging from 10 to 60 m, predominantly in tropical areas. Exhibiting a common trait among fish, it displays a darker dorsal side and lighter ventral or belly side, showcasing hues of brown and gray in markets, each color garnering its own following [28]. Under optimal conditions, this species boasts an average lifespan of up to 7 years [29,30].

Renowned for its flavor and texture among local populations, the Hasoom fish is a delicacy. Its relatively delicate fins soften upon frying, contributing to dietary calcium intake. Abundant in protein, omega-3 fatty acids, and various essential nutrients, the Hasoom fish offers a nutritious option for consumption, providing essential minerals and vitamins for bodily functions [31,32]. Thus, its inclusion in dietary regimes is strongly advised, owing to its high protein content and nutritional profile.

The surge in affordable Japanese restaurants in the Middle East, including in the countries surrounding the Persian Gulf and the Oman Sea, where this fish is popular, raises concerns about food safety. Given that many Japanese dishes incorporate raw and undercooked seafood, there's an inherent risk of parasitic infection among consumers.

In terms of its dietary habits, the greater lizardfish primarily feeds on fish, crustaceans, and squid [22,24]. It is adapted to preying on larger and faster prey, including finfishes and squids, and is known to target a wide range of prey groups [33]. Consequently, given its feeding behavior, it is conceivable that this fish species can host a variety of parasites [34,35].

Despite its significant role in the diets of people in the region, our knowledge of the parasites associated with the greater lizardfish and the safety aspects of consuming this fish is limited. Further research and investigation in this area would be valuable for a comprehensive understanding of the health and safety considerations related to the consumption of *S. tumbil*. The current research focuses on determining the prevalence and abundance of parasites commonly found in *Saurida tumbil* fish, sold in fish markets for human consumption, followed by characterizing of nematode larvae to determine their health significance for fish and consumers.

2. Materials and Methods

Following reports by members of the public about the presence of parasite-like organisms in greater lizard fish sold in fish markets, a total of 458 greater lizardfish (*Saurida tumbil*) were randomly sampled from various fish markets in Minab, Hormozgan Province, Iran (Figure 1) from June 2022 to May 2023. These fish were promptly placed on ice and transported to the laboratory for immediate examination after being weighed using a digital balance and measured using a ruler.

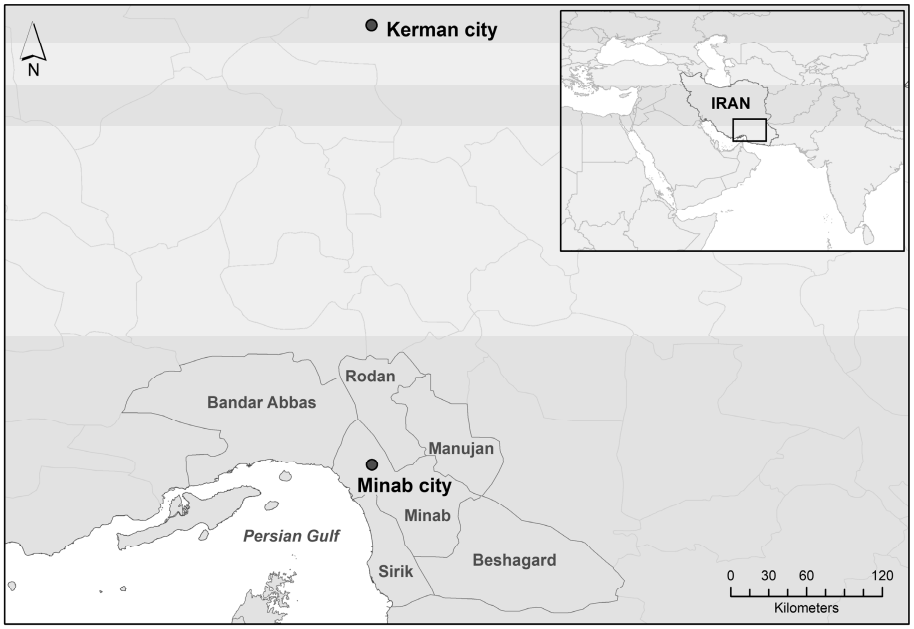

Figure 1. The geographical location of the study area.

The fish species were identified using the available literature [36]. Total lengths (±0.01 cm) of the fish were taken from the tip of the snout (mouth closed) to the extended tip of the caudal fin using a measuring board. The body weights of the fish were measured with a top-loading Mettler balance and recorded to the nearest gram (±0.01). The fish were grouped into two distinct weight ranges (Supplementary Table S1): those weighing 470–800 g and those weighing 800–1030 g. Fish ranging from 30–40 cm in length were classified as small, while those measuring 41–50 cm were categorized as large. The prevalence of infection was analyzed with respect to body weight and fish length.

The entire digestive system of each fish, including the stomach, intestine, celomic cavity, and muscular tissues, was dissected to collect nematodes. This followed standard protocols previously published [37,38]. The isolated larvae were carefully quantified then rinsed with a physiological saline solution before being preserved in 70% ethanol. For morphological identification, the fixed nematodes were clarified in lactophenol, mounted on slides, and then observed under an optical microscope. Identification was based on morphological characteristics of the esophageal ventriculus, ventricular appendix, labia, the position of the excretory pore, and the tail [39–42]. Up to five representative larvae from each morphotype were then subjected to further molecular analysis using polymerase chain reaction (PCR)to identify the specific species.

Samples were sent to Ferdowsi University of Mashhad for scanning electron microscopy (SEM). SEM micrographs were taken from both the anterior and posterior ends of the worms. For the primary and secondary fixation, they were placed in glutaraldehyde and osmium tetroxide, respectively. Dehydration was carried out using a graded series of increasing ethanol concentrations, with each grade taking 15 min. The specimens were then mounted on copper stubs with double-sided adhesive tape and sputter coated with gold using an SC7620 fine coater. Following this preparation process, the stubs were examined using a LEO1450VP scanning electron microscope at a voltage of 20 kV, with the micrographs captured digitally.

Deoxyribonucleic Acid (DNA) extraction was carried out using a commercial DNA extraction tissue kit (Roche, Germany) following the manufacturer's instructions. Polymerase chain reaction (PCR) was used to amplify the first and second internal transcribed spacers (ITS-1 and ITS-2, respectively) of ribosomal DNA (rDNA) regions, using primer sets, SS1 forward (5′-GTTTCCGTAGGTGAACCTGCG-3′) and NC13R reverse (5′-GCTGCGTTCTTCATCGAT-3′), respectively [43]. The PCR reaction was set up with a

total volume of 20 µL, consisting of 4 µL of DNA template, 1.5 µL of each primer, 10 µL of the master mix, and 3 µL of distilled water, with the distilled water serving as the negative control. The PCR amplification process consisted of the following steps: initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 45 s, extension at 72 °C for 90 s, and a final extension at 72 °C for 10 min. The resulting PCR products, representing the desired DNA fragments, were electrophoresed on a 1.5% agarose gel containing DNA Green Viewer with fluorescence dye and visualized and photographed under ultra violet (UV) light in a TBE buffer.

Following PCR amplification, the amplicons were submitted to Pishgam Biotechnology Company for further processing. The sequences were subjected to purification and bidirectional sequencing, using the same primers that were initially used for the amplification of the ITS-1 and ITS-2 genes. The obtained sequences were compared and aligned with previously documented sequences available in the GenBank database, hosted by the National Center for Biotechnology Information (NCBI). This alignment was performed using the Clustal method [44], to identify similarities and differences between the obtained sequences and existing genetic data.

The statistical analysis of the collected data was carried out using SPSS 18 software. Two commonly used statistical tests, the Chi-squared test and Fisher's exact test, were used to evaluate the statistical significance of the results. A 95% confidence interval and a p-value less than 0.05 were considered statistically significant. The prevalence and mean intensity of parasites were calculated in accordance with Bush et al. [45].

Tissue samples from the infected organs in 1 cm^3 dimensions associated with attached larvae were taken and fixed in 10% neutral buffered formalin for histopathological investigation. The samples were dehydrated in graded ethanol and embedded in paraffin wax. Then, under an optical microscope, sections of 5 µm thicknesses were routinely stained with hematoxylin–eosin for histopathological and parasitological examinations.

3. Results

Nematode larvae were found in the body cavities of most fish and attached to various organs, including the liver, stomach, intestine, and beneath the visceral serosa and perintestinal fat. Fewer fish were infected with larvae in the muscle of fish (Figure 2). Larvae in the body cavity presented as white structures with lengths that varied from 10 to 40 mm. They were found in free forms, coiled and uncoiled, on the serosal surface of the viscera within the celomic cavity. Some of these larvae were tightly encapsulated within the peritoneum, mesentery, and inner muscular layers of the fish's body wall, forming white nodules ranging in size from 3 to 5 mm. These larvae were identified as *Hysterothylacium* type XV, belonging to the family Raphidascarididae, based on the excretory pore being localized just beneath the nerve ring and the presence of both an intestinal cecum and ventral appendix. Sequences of the ITS-1 and ITS-2 regions obtained from the present study were identical to those in GenBank (accession numbers: LT576348 and LT576357) and demonstrated the presence of *Hysterothylacium amoyense*. Nematode larvae found in the muscle of the fish were identified morphologically as *Anisakis* sp. larval type I (Figure 3). However, we could not obtain a high-quality sequence for species identification.

The analysis of the biometric data indicated that the highest prevalence of infection was observed in fish 30–40 cm long (95.7%) and 800–1030 g (94.41%) (Supplementary Table S1). However, no statistically significant correlation was found between the prevalence of infection and the length and weight of fish ($p > 0.05$) within each season. When comparing prevalence between different fish groups across seasons, it was consistently lower in autumn compared to other seasons among various fish groups (Figure 4). However, as indicated in Supplementary Table S1, the mean intensity of parasites in autumn was significantly higher ($p < 0.05$) than that in other seasons.

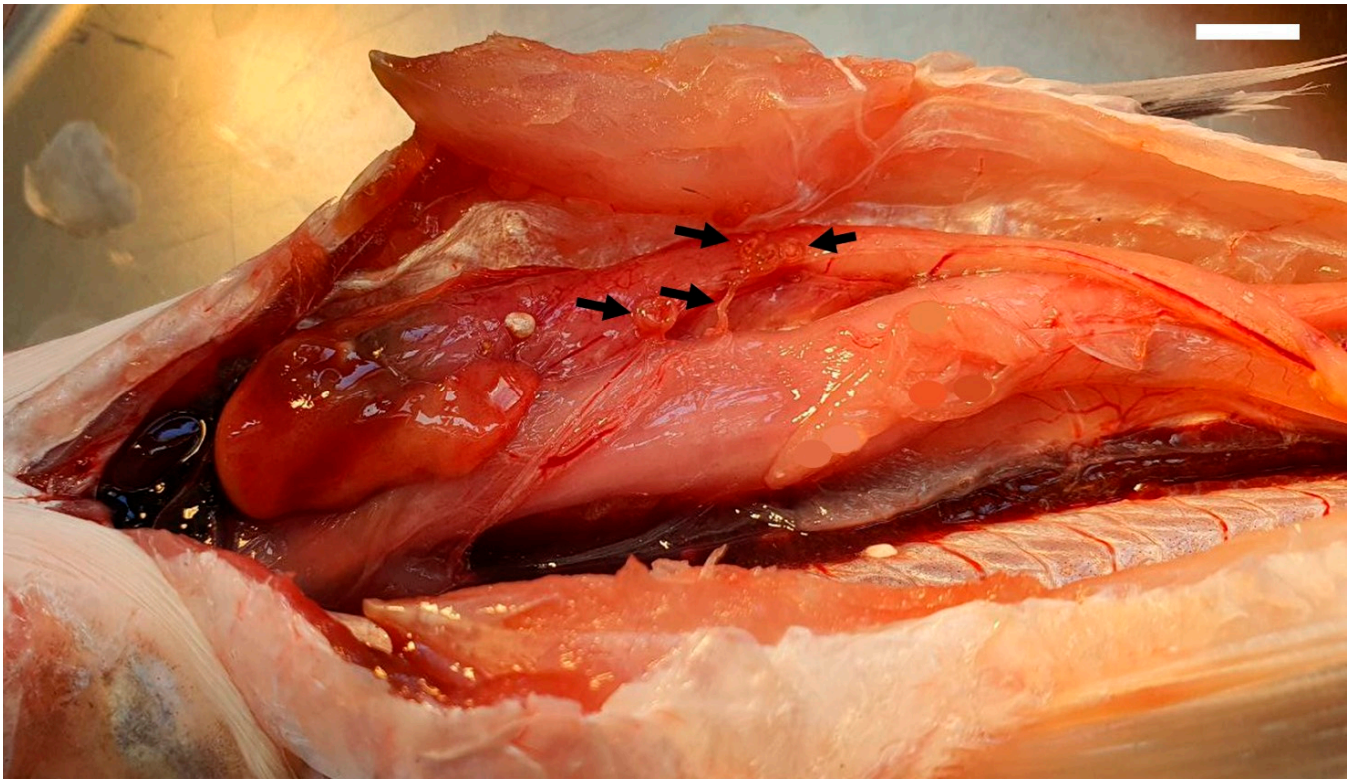

Figure 2. *Hysterothylacium* XV larvae in the celomic cavity (arrows). Scale bar: 1 mm.

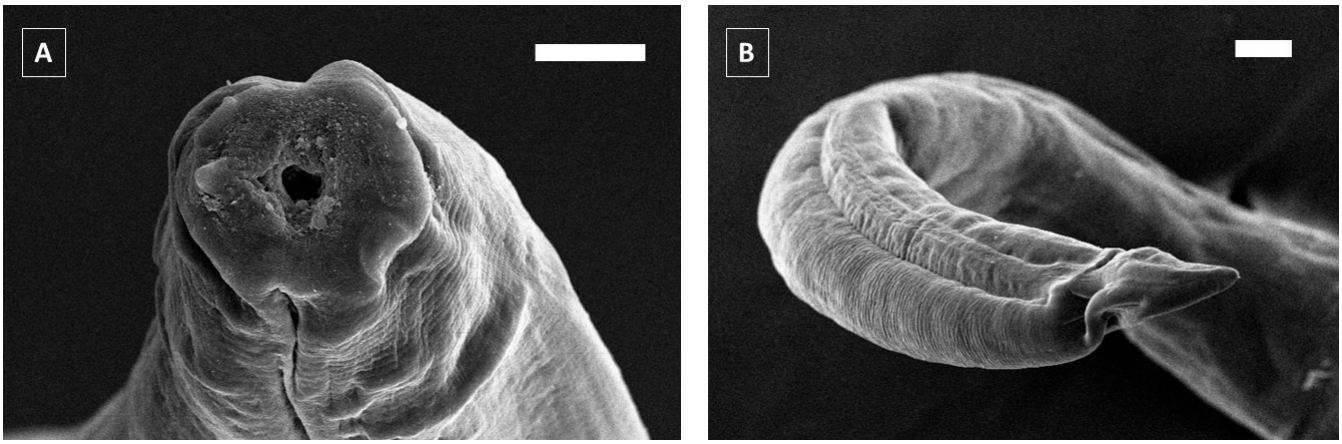

Figure 3. Scanning electron microscopy of the nematode larva found in the present study, showing the anterior end (**A**) and posterior end (**B**) of the parasite. Scale bars: 20 and 100 µm in image (**A**) and (**B**), respectively.

Histopathological examinations of infected fish (Figure 5) showed multiple granulomas containing sections of larvae surrounded by a fibrous capsule. The observed inflammation was characterized by a mild to moderate infiltration of mononuclear inflammatory cells, predominantly lymphocytes, macrophages, and eosinophils. A distinguishing feature in all infected tissues was a translucent space between the parasite's cuticle and the host tissue. Additionally, some granulomas contained tunnels filled with eosinophilic and slightly basophilic materials, along with inflammatory reactions around the granuloma capsule. Some of these tunnels were empty, leaving only fibrotic capsules. Several solid nodules filled with homogenous eosinophilic substances and surrounded by inflammatory responses were located on the serosal surface of visceral organs and abdominal muscles.

The muscular fibers near these parasitic nodules exhibited Zenker necrosis, rhabdomyolysis, and severe inflammation.

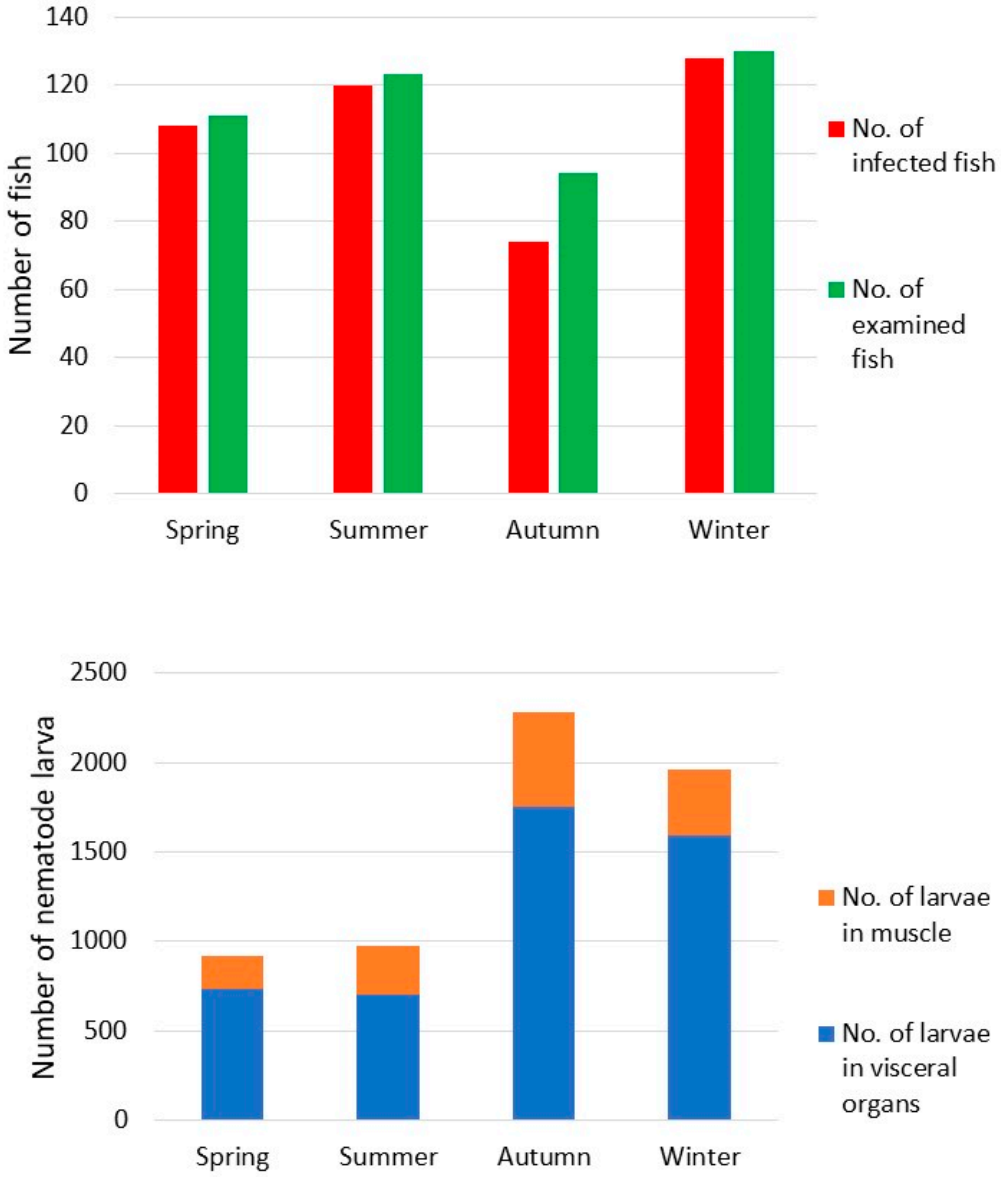

Figure 4. The graph at the top displays the number of infected and examined fish in the present study. The graph at the bottom illustrates the number of nematode larvae found in various fish body parts throughout different seasons in the present study.

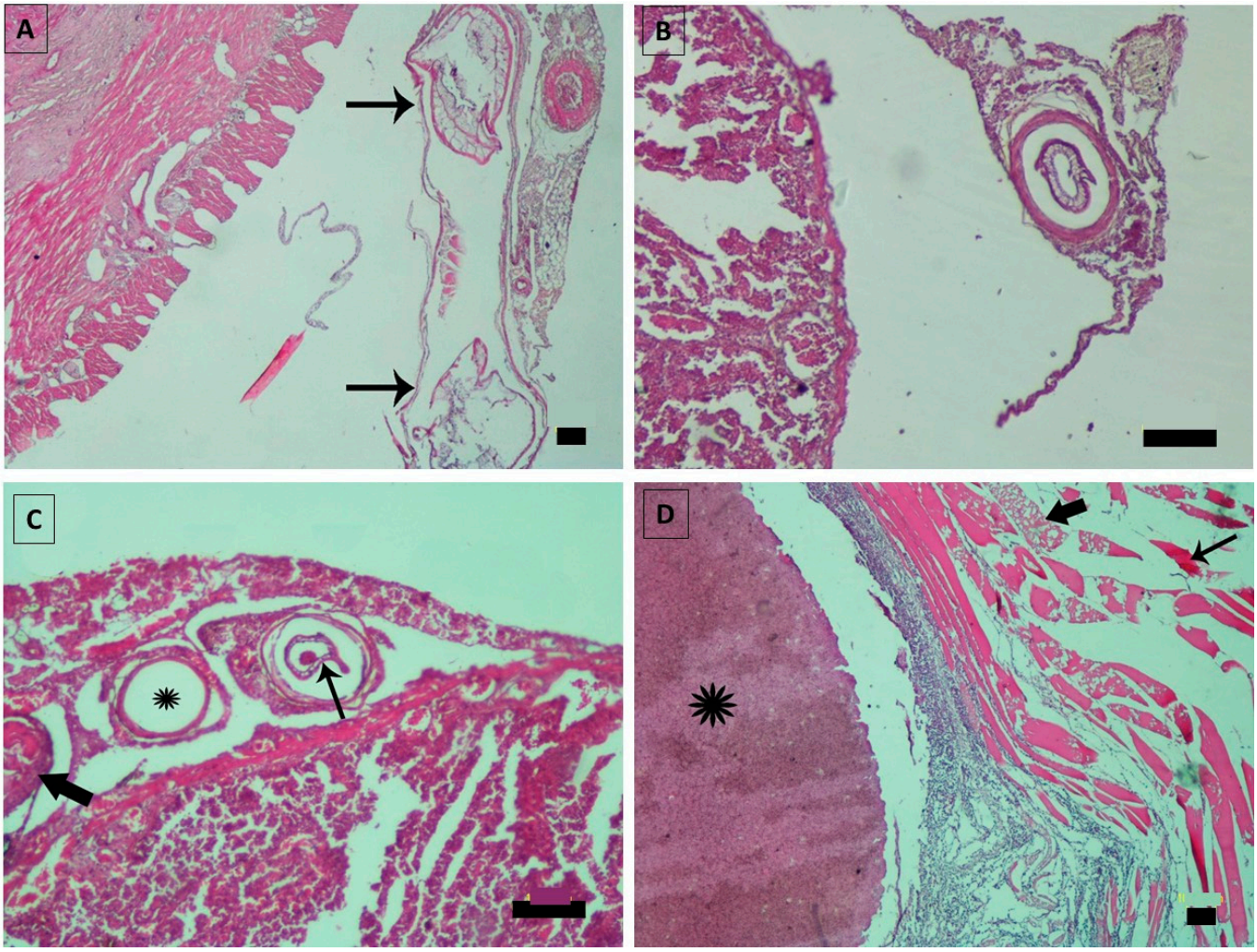

Figure 5. (**A**) Longitudinal section of *Hysterothylacium* sp. larvae in the serous surface of the intestine (arrows); (**B**) cross-section of *Hysterothylacium* sp. larvae surrounded by a capsule of connective tissue in the fish spleen; (**C**) multiple granulomas in spleen composed of *Hysterothylacium* larvae (thin arrow) surrounded by a connective tissue capsule and inflammation around the capsule, empty granuloma (asterisk), also filled with degenerated larvae (thick arrow); (**D**) parasitic nodule containing homogenous eosinophilic materials (asterisk) in the abdominal muscles of fish. Muscular fibers show Zenker necrosis (thin arrow), rhabdomyolysis (thick arrow), and severe inflammation. Scale bars: 100 µm.

4. Discussion

The nematode larvae identified in the current study belong to the genera *Anisakis* and *Hysterothylacium*. The genus *Anisakis* belongs to the family Anisakidae [46]. The family Anisakidae comprises over one hundred species, with the genus *Anisakis* alone consisting of at least nine species [47]. The main definitive hosts for *Anisakis* spp. are marine mammals [48,49]. A wide range of invertebrates, such as crustaceans, are their first intermediate hosts [50–52]. Second/paratenic intermediate hosts include fish, cephalopods, fish-eating birds, and sea snakes [53–56]. From a medical perspective, the presence of *Anisakis* sp. larvae in fish muscle holds significant importance, as they represent the infective stage of the parasite, capable of inducing a severe illness known as anisakiasis upon consumption of infected seafood by humans [57]. *Anisakis* spp. have been associated with adverse effects on the immune system, gastrointestinal symptoms, and various other diseases [58]. Consequently, numerous studies have been conducted on the medical implications of these parasites, leading to the establishment of seafood safety protocols and guidelines regarding

parasitic diseases transmitted through seafood in numerous countries [59]. However, these protocols and guidelines have not been established in Iran, despite the proven occurrence there of human infection [60]. Additionally, *Anisakis* spp. have veterinary significance due to their adverse effects on the health of their hosts. Examples include the induction of red vent syndrome in Atlantic salmon [61], gut hemorrhages in gilthead sea bream [62], hemorrhages and irregular neoformations within the coelomic cavity of European sea bass [63], and ulcerative lesions in cetaceans [64,65]. In yellowmouth barracuda (*Sphyraena viridensis*, Cuvier, 1829) granulomatous reactions composed of macrophages, epithelioid cells, some lymphocytes, and an external connective sheet were found to surround *A. pegreffii* [66].

Hysterothylacium spp. were previously categorized within the Anisakidae family, but they are now recognized as part of the Raphidascarididae family [67,68]. The life cycle of *Hysterothylacium* spp. includes invertebrates as their first intermediate hosts and various species of fish as their intermediate/paratenic/definitive hosts [43,69–72]. While there is a consensus among scientists regarding the zoonotic potential of *Anisakis pegreffii* and *A. simplex*, debates persist concerning the zoonotic potential of *Hysterothylacium* spp. and other *Anisakis* spp. [58,73]. Debates and conflicting findings have arisen among researchers regarding the pathogenicity and zoonotic significance of *Hysterothylacium* nematodes [73]. These have typically emerged from research based on animal models. Some authors proposed that *Hysterothylacium aduncum* larvae might not undergo evolution in homeothermic animals and, therefore, not in humans [74]. However, experimentally *Hysterothylacium* sp. larvae penetrated the stomach wall of a rhesus monkey (*Macaca mulatta*) and caused hemorrhage and attracted eosinophils [75]. There is also a report of naturally acquired human infection with *Hysterothylacium* sp. [76]. It is crucial to emphasize that many reports of human cases of anisakiasis/anisakidosis are based on the assumption that the nematode involved is *Anisakis* sp. larvae [58]. In their review, Shamsi and Barton [58] showed that, in over 90% of reported anisakidosis cases worldwide, there was a lack of concrete evidence identifying the causative agents. They suggested that a more thorough identification of anisakis species in humans could be achieved if healthcare professionals took appropriate steps in pathogen identification. Therefore, accurate identification of the parasite in human cases is essential for informing policies related to fisheries, food safety guidelines, and other relevant disciplines [77,78].

Although both nematode larval types found in the present study are known for their potential to cause infections in humans [58,73,76,79,80], there is less information available about their adverse health impact on fish hosts. Infection of fish with these nematodes is significant, not only due to the potential risk of anisakidosis in humans, but also because of the impact they have on the infected fish. These larvae can induce disease in fish, with symptoms and severity varying depending on factors such as the fish species, the species and intensity of the infecting parasite, and the specific organ invaded [81–83]. The disease is most severe when these larvae infect the liver, leading to fibrosis and atrophy of the organ, which results in a significant loss of body weight. Other symptoms may include granulomatous inflammation and necrosis of the muscularis externa of the pyloric caeca, gallbladder, intestine, and body cavity, potentially causing substantial mortality in fish [81].

Hence, the larvae of both *Hysterothylacium* sp. and *Anisakis* sp. found in this study may have zoonotic potential for consumers. Notably, both larvae were identified in the third stage of development, a known infectious stage for these parasites in humans. This shows the importance of considering the potential health risks associated with consuming seafood harboring these nematode larvae, necessitating vigilance and proper measures in food safety protocols to safeguard consumers from potential zoonotic infections [84].

The present study revealed the presence of *Anisakis* sp. larval types in the muscle of the examined fish, indicating an elevated risk. While *Hysterothylacium* sp. larvae were not detected in the fish muscle, it is noteworthy that they can migrate from internal organs and body cavities, posing a risk of contaminating the edible portions of the fish. For example, *H. incurvum* L3 larvae were found to migrate from the coelomic cavity of their fish host to the bloodstream, with consequent development to the adult stage within the heart [85].

The European Food Safety Authority [86] states that *Anisakis* sp. larvae found in fish muscle pose a potential risk of eliciting allergic reactions, including gastroenteritis and rheumatological and dermatological symptoms in consumers. It acknowledges that, based on current knowledge, no sea fishing areas for wild-caught fish can be considered entirely free of *Anisakis* sp. larvae. Therefore, prevention methods such as freezing at −15 °C for no less than 96 h, or −20 °C for 24 h or −35 °C for 15 h, and heating at more than 60 °C for at least 1 min, have been recommended.

The traditional cuisines in the study area and the countries surrounding the Persian Gulf and Oman Sea often involve the prolonged cooking of fish [87], a practice deeply embedded in the culinary heritage. However, in alignment with global trends [88], the consumption of raw seafood, exemplified by dishes like sushi and sashimi, is on the rise in these countries [89–91], as well as in regions of the world away from the sea [92]. A recent study conducted in Bushehr, located on the northern coast of the Persian Gulf, showed previously undisclosed cases of anisakiasis, a parasitic infection caused by Anisakis larvae [60]. The identification of three positive DNA cases of Anisakidae family parasites in gastric tissue biopsies of hospitalized patients serves as a stark indication of the potential health risks for residents in coastal areas of the Persian Gulf, where the consumption of infected fish is prevalent. As suggested by these authors, it is imperative to initiate a comprehensive population study in the region, focusing on the investigation of human sera for seropositive cases, and considering the allergic aspects of infection, which are common in regions where anisakiasis is prevalent. This underscores the need for increased awareness among physicians and researchers, emphasizing the potential health implications associated with consuming raw seafood in the region [60].

Our study indicates a considerable increase in the infection rate of this fish compared to previous research. In a study in the same region [93], out of 120 fish examined, only 5 were found to be infected with *Anisakis* (one larva per fish), and 30 were infected with *Hysterothylacium* larval type XV infections, ranging from one to fifteen larvae per fish. In contrast, our study revealed a substantial rise in infection rates, with 430 out of 458 fish (93.9%) found to be infected with the same nematode larvae. The Persian Gulf, recognized as the warmest sea globally, is experiencing elevated temperatures attributed to climate change. Consequently, it is undergoing increased temperatures, heightened salinity, sea level rise, and decreased oxygen levels [94,95], factors known to positively influence the abundance and prevalence of anisakis nematodes. The hatching duration of these nematodes inversely correlates with temperature, while light exposure diminishes hatching time. Moreover, the viability of newly hatched larvae is enhanced under higher salinity levels [96]. These factors may account for the disparities observed between our current study and the previous investigation conducted approximately a decade ago.

In our study, we observed that heavier fish displayed more parasitic infections. It is noteworthy to consider that this might be attributed to the weight of the parasite itself, rather than establishing a direct link between the size of the fish and susceptibility to nematode larvae infestation [97]. The notable increase in mean intensity during autumn warrants further investigation to gain a deeper understanding of the biology of these parasites. It is plausible that the warmer months during late spring and summer contribute to a higher number of egg and larval stage productions, resulting in the observed high intensity during autumn. Additionally, while statistically insignificant, smaller fish exhibited a greater prevalence of parasites than larger fish. This phenomenon could be attributed to the substantial health impact of parasites on fish, potentially hindering the growth of infected individuals [98,99], supporting the histopathological changes observed in the present study. The formation of a connective tissue capsule, the inflammation, and the sequestration of the parasite is one of the host's reactions to the presence of the parasite [100].

5. Conclusions

In conclusion, this study documents a heightened occurrence of potentially zoonotic nematodes in commonly consumed fish within the Persian Gulf region, with no statistically significant correlation between the prevalence of infection and the length and weight of fish within each season. It was found that, in autumn, the prevalence of infection was lower, but the mean intensity of parasites was significantly higher. A limitation of the study pertains to the unknown provenance of the samples, which hinders the precise determination of the true infection status in the studied area.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes9040143/s1, Table S1: Correlation between the morphology of *Saurida tumbil* and the rate of *Hysterothylacium* sp. infection in different seasons; mean intensity was calculated for total number of larvae in infected fish.

Author Contributions: Conceptualization, Y.G., M.M. and S.S.; methodology, Y.G., S.A. and S.H.; validation, S.S.; formal analysis, Y.G., S.A. and S.H.; investigation, Y.G., S.A. and S.H.; resources, M.M.; data curation, Y.G., S.A. and S.H.; writing—original draft preparation, Y.G., S.A. and S.H.; writing—review and editing, S.S.; visualization, S.S.; supervision, M.M. and S.S.; project administration, M.M.; funding acquisition, M.M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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