

Seasonal Feeding Dynamics and Parasitic Impacts on Immune and Histopathological Responses in *Brachirus Orientalis*

Hassan H.K. Al-Bayati^{1*}, Khelud A. Hassan², Israa A. Al-Atbee², Layla A. Aufy²

¹Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Tikrit, Tikrit, Iraq

²Department of Vertebrates, Marine Science Center, University of Basrah, Basrah, Iraq

*Corresponding Author: dr_patho80@tu.edu.iq

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ABSTRACT

With a focus on the related clinical and histological alterations, this study seeks to determine the connection between feeding practices and nematode infection in the flatfish *Brachirus orientalis*. The fish population under study showed significant seasonal change in feeding activity and food composition based on monthly investigation of gut contents. Over the course of the investigation, 87 fish were examined. For the majority of the months, the predominant food item was crustaceans. The amount of feeding varied significantly by month, with April showing the most activity and all of the fish under examination having completely swollen intestines. While higher percentages of empty bellies were noted in January and June, high feeding activity continued in May, July, August, and September. Fish overall length varied from 165 to 420mm; larger size ranges typically corresponded with times of increased eating activity. In January, two fish were infected with a total of three nematodes, corresponding to a prevalence of 22% and a mean intensity of 1.5 parasites per infected fish. A similar prevalence (22%) was recorded in May, although with a lower mean intensity. In September, infection prevalence reached 18.1% with a mean intensity of 1.5. Parasitic infections were observed during the remaining months. Immunological assessment showed a significant elevation in serum immunoglobulin levels in infected fish compared with controls. Serum IgT and IgE concentrations were significantly increased, indicating activation of the immune response following infection. Histopathological examination of infected livers revealed inflammatory alterations, including cellular infiltration, necrosis, congestion, and granulomatous lesions associated with parasite attachment, confirming tissue damage linked to nematode infection. The study demonstrates significant seasonal fluctuation in feeding activity and diet composition. Although nematode infections were infrequent, low in frequency and severity, they caused notable histopathological and immunological reactions.

INTRODUCTION

The consumption of fish has increased substantially due to their high nutritional and protein value (Colangelo *et al.*, 2009). Flatfishes are a significant class of commercial food fishes that live mostly on soft, muddy, and sandy bottoms. There, they serve as essential benthic predators and are crucial in the transfer of benthic energy to

higher trophic levels, including humans (Cooper, 2025). *B. Orientalis* is widely distributed in all regions of the Red Sea, Arabian Gulf, Indian Ocean, and Iraqi marine waters (Mohamed *et al.*, 2001). This type eats primarily on bottom-dwelling invertebrates like crustaceans (Mohammed *et al.*, 2008; Dogan, 2024). Such invertebrate's behavior increases exposure to a parasite, as fish commonly act as intermediate or definitive hosts for helminth parasites that may cause mechanical tissue damage, impair growth and reproduction, and alter host physiological functions (Madsen *et al.*, 2024). Some fish parasites or their larval can be zoonotic to humans through the consumption of affected fish (Hoffmann, 1999). Parasite effects host population dynamic and stability of ecosystem (Longshaw *et al.*, 2010). Several helminth species have previously been reported in *B. orientalis*, including *E. veli*, acanthocephalans such as *Neoechinorhynchida* and Serrasentis sagittifer, and trematodes like *Lasitotocus guptai* (Nahhas *et al.*, 2002; Abaza, 2024; Essa *et al.*, 2025). Despite the fact that these parasites are frequently limited to the colon, their harmful effects can spread throughout the body, causing tissue damage and inflammatory reactions in important organs like the liver (Jumaa & Alrudainy, 2016; Shields, 2022). The humoral immune response to infection, including IgT and other immunoglobulins, is necessary to provide protection (Hassan *et al.*, 2025). By inflicting damage, altering immunological responses, and producing pathological effects that reduce growth, fecundity, and survival, nematodes can negatively impact fish (Marcogliese, 2005; Iwanowicz, 2011). Despite this, limited information is available on the combined immunological and histopathological impacts of parasitic infections in *B. orientalis*. Thus, the goal of the current study is to examine intestinal parasite infections in *B. orientalis* and to assess the immunological reactions that are linked to these infections, namely changes in serum immunoglobulin levels and histopathological changes in impacted tissues. A thorough grasp of parasite-host interactions and their effects on fish health and ecological dynamics is provided by this integrated approach.

MATERIALS AND METHODS

Study Area and Fish Collection

Fish samples were collected monthly from Iraqi marine waters near the Food and Agriculture Organization (FAO) station. Specimens were captured using commercial fishing nets. Immediately after collection, fish were placed in insulated containers with ice packs and transported to the laboratory for further examination. Eighty-eight fish samples (*Brachirus orientalis*) with a mean total length of 165- 420mm and a mean total weight of 30±38gm were taken from Iraqi coastal water in the Arabin Gulf between June 2024 and September 2025. The fish that were gathered displayed symptoms of illness, including aberrant swimming patterns, gasping, bleeding, sores, attached parasites, and unusual coloring. The fish samples were housed in an aquarium filled with 30m³ of aerated water after being brought alive in polyethylene bags that contain two-thirds air

and one-third aquarium water. The sampling site, species name, sample number, total length, weight, and sampling date were all noted and tallied.

Study Area and Fish Collection

Samples of *B. orientalis* fish were collected monthly from the Iraqi marine waters near the Al-Faw area. Commercial fishing nets were used to catch the samples. Immediately after collection, the fish were placed in insulated containers with ice packs and transported to the laboratory. A total of 88 samples were collected (length: 165-420mm; weight: 30±38g) during the period from June 2024 to September 2025. Some fish showed signs of disease, including ulcers and color changes. Live fish were transferred in polyethylene bags containing two-thirds air and one-third aquarium water, then placed in tanks containing 30 cubic meters of aerated water. Sampling details were recorded (location, species, number, length, weight, and date).

Examinations, Both Clinical and Postmortem

The fish's internal organs and body surface were examined externally, and the method outlined by **Noga (2010)** was used to record aberrant macroscopic observations. To find any parasite and the lesions that go along with it, such as erosion, ulcers, cyst formation, bleeding, and other changes, extra attention was paid. Every large lesion was captured via camera.

Clinical and Anatomical Examination

External and internal examinations were conducted according to the method of **Nouga (2010)** and **Jumaa (2024)**. Abnormal macroscopic changes were documented, and the main parasites were photographed for documentation purposes.

Biometric Measurements

In the laboratory, each fish specimen was measured for total length to the nearest millimeter (mm) and weighed to the nearest 0.10g using a digital balance. The total length of each sample (to the nearest millimeter) and its weight were measured using a digital scale (to the nearest 0.10 grams).

Anatomy and Analysis of Stomach Contents

The fish were dissected using standard parasitic procedures. The viscera were removed, and the stomach and intestines were separated. The viscera were preserved in a 10% diluted neutral formalin solution. The contents of the stomach were examined under an anatomical microscope to determine the nutritional components.

Dissection and Stomach Content Analysis

Fish were dissected using standard parasitological procedures. The visceral organs were carefully removed, and the stomach and intestinal tract were separated. The viscera were preserved in 10% neutral buffered formalin for detailed examination. Stomach contents were emptied into Petri dishes and examined under a dissecting (anatomical) microscope to identify dietary components.

Parasite Identification

Giemsa stain was used to stain protozoan parasites in accordance with the technique of **Klein (1958)** and determined by the **Basson and van As (2006)** technique. Platyhelminths were preserved in 5% formalin, dyed with Semichon's acetocarmine, and identified using the methods of **Ogawa and Egusa (1979)** and **Mehrdana *et al.* (2014)**. Crustacea were cleaned in lactophenol, mounted in glycerine-gelatin, and identified. In accordance with **Bell and Beverley (2006)**, nematodes were kept in 10% buffered formalin and 70% ethanol (**Mousavi *et al.*, 2011**).

Photomicrography

Representative histological sections and parasite specimens were photographed using a digital camera attached to the light microscope to document pathological and morphological findings.

Estimation of IgT and IgE

Serum levels were assayed using the sandwich ELISA kit (IgT and IgE) from SunLong Biotech Company, LTD, in accordance with the manufacturer's methodology and guidelines. An anti-serum kit antibody has been pre-coated on the Micro ELISA strip plate in this kit. Samples or standards were connected to the particular antibody and were put in the proper Micro ELISA strip plate wells. The kit-specific Horseradish Peroxidase (HRP)-conjugated antibody was then applied to each Micro ELISA strip plate well and incubated. The unnecessary parts were removed with a rinse. Each well was treated with TMB, a chromogenic substrate solution. Only the wells with the serum kit and HRP-conjugated kit antibodies were initially blue; after the stop solution was added, they turned yellow.

Histopathological Findings

Tissues (liver, spleen and small intestine) were preserved by 10% formol to keep structure. After sectioning they are subjected to tissue processing; dehydration in alcohol and clearing in xylene, followed by infiltration with molten paraffin wax. The tissues are embedded in paraffin blocks and sectioned on a microtome in very small pieces for mounting on slides. The slices are then stained with hematoxylin and eosin and the nuclei made blue, while the cytoplasm and extracellular matrix are dyed pink, ready to be microscopically studied and diagnosed.

Statistical Analysis

Parasitological indices, including prevalence, mean intensity, and abundance of infection, were calculated according to **Margolis *et al.* (1982)**. The results were summarized and presented as descriptive statistics. Differences between infected and non-infected fish were evaluated where applicable.

RESULTS AND DISCUSSION

The results of the current study, as shown in Tables (1, 2) and Fig. (1), indicate that the length of the studied fish ranged between 165 - 420mm. The lowest length was recorded in February, while the highest length was recorded in April. For fish with empty stomachs, the highest number was recorded in April and May, amounting to 10 fish. Regarding fish with full stomachs, the highest number was recorded in January, totaling 6 fish. Three groups appeared: mollusks + crustaceans + fish. Crustaceans were found in the fish in all months, while mollusks appeared in four months: February, April, May, and July. Fish were recorded in the three months of July, February, and September. Most previous studies indicated that soles are carnivorous fish. The same was confirmed in the current study, as the species fed mainly on mollusks (bivalves). For the empty stomachs, they had the lowest values in March, July, and August, with a value of 1, and their lowest values were in January, reaching 6. After examining the stomach contents, three groups appeared: mollusks + crustaceans + fish. Crustaceans were found in fish in all months, while mollusks appeared in four months: February, April, May, and July. Fish were recorded in three months: July, February, and September. Although crustaceans dominated the diet, the proportion of annelid worms was lower than that recorded by **Allam (1995)**. This may reflect a difference in the environment or season. **Allam (1995)** studied the food and nutrition of several species of sole (*S. vulgaris*, *S. impaire*, *S. aegyptiaca*, and *S. kleini*) in Abu Qir (southeastern Mediterranean, Egypt), and these species are carnivorous, relying on crustaceans and mollusks. Additionally, **Allam (1995)** studied the diet of juveniles and adults of two species of Soles in northern France and found that they were carnivorous and their diet consist mainly of soft bottom benthic invertebrates (Polychaetes, Shrimps and Mysids). Our data showed that January, May, and September are the months when nematode infections occur, while parasites disappear in the summer. This agrees with a study published in **Oecologia (2024)**, which indicated that seasonal changes affect the life cycle of parasites, but the relationship with temperature may be weak or vary depending on the species and environment. In temperate environments, parasites take advantage of the fish's weakened immunity in winter or during breeding seasons to increase their spread, which illustrates monthly variations in mean feeding activities and feeding intensities of *S. elongata* in the Shatt Al-Arab Estuary. It could be concluded that this species never cease feeding all over the year. Feeding activity values fluctuated from 50% in February to 85 % in June. The minimum value of feeding intensity (0.4 point/fish) was calculated in February, while

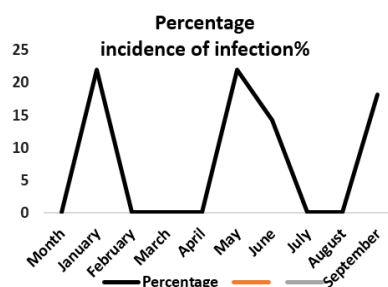
mean maximum intensity (3.2 point/fish) was encountered in June. It is clear that the trend of this criterion almost resembles that of the feeding activities. The analyses of the stomach contents of *S. elongata* revealed that this species fed exclusively on molluscs (bivalves, *Bithynia* sp.). Parasites can infect fish at different stages of their life, the different aquatic environmental conditions, which is a biological indicator of environmental pollution. Parasites interfere with the nutrition, metabolism, and secretory function of the digestive system. The food and feeding habits vary with reference to season (Mohammed & Hoda, 1993). The intensity of infection in our data was low (1–1.5), indicating that the parasites are present seasonally without reaching epidemic levels. This is consistent with the findings of Paterson *et al.* (2023), who postulated that the correlation between temperature and parasite density is weak and not consistent globally, and that other factors such as food and fish density play an important role. Additionally, accompanying factors such as food, when the intestines are full in the moderate months of April–May, it increases the chances of parasite ingestion through the food chain. As for the other factor, immunity, the decrease in immunity during winter increases the susceptibility of fish to infection (Bertaglia *et al.*, 2023). The data indicate that winter and spring are more favorable periods for the emergence of parasites due to the decreased immunity of the fish or the increased density of parasites in the environment. However, in summer, there is a noticeable decrease in infection rates, which may be attributed to the effect of temperature on the parasites' life cycle or the fish's ability to resist (Gopko *et al.*, 2020)

Table 1. Length of fish, empty and filled stomach ratios

Month	No. of fish	Food categories	Gut		Length Range, mm
			Full	Empty	
January	9	Crustacea	3	6	229-370
February	12	Mollusca+ Crustacea+fish	9	3	165-320
March	9	Crustacea	5	4	300-320
April	10	Mollusca+ Crustacea	10	0	210-420
May	14	Mollusca+ Crustacea	10	4	230-362
June	6	Crustacea	1	5	180-241
July	8	Mollusca+ Crustacea+fish	7	1	227-320
August	8	Crustacea	7	1	233-340
September	11	Crustacea+fish	9	2	240-345

Table 2. The severity and proportion of parasites

Month	Fish Infected	N-parasites	type parasite	Percentage incidence infection%	Intensity of infection
January	2	3	Nematode	22	1.5
February	0	0	-	0	0
March	0	0	-	0	0
April	0	0	-	0	0
May	2	2	Nematode	22	1
June	0	0	-	14.2	0
July	0	0	-	0	0
August	0	0	-	0	0
September	2	3	Nematode	18.1	1.5

**Fig. 1.** The severity and proportion of parasites

ELISA Assay for Serum IgT and IgE Level Determination

IgT and IgE levels rose significantly in the infected and non-infected groups, with the highest levels recorded in the infected group, and the elevated cytokine levels reflect a potent pro-inflammatory response induced after nematode infection, suggesting a more regulated immune activation. The data in Table (3) indicate high level of IgT and IgE. This fact is represented in the level of IgT in control group which was 5.07 ± 0.187 , compared to in infected groups (15.11 ± 0.464), while the IgE level in control groups was 6.82 ± 0.144 and was 17.22 ± 0.66 in infected group. Exposure to the parasite nematode produces an acute and self-limiting infection with gastrointestinal symptoms, also antibodies in fish serum like IgM, IgT and IgE, are essential components of the early immune response, contributing to the neutralization of pathogen, thereby helping to eliminate pathogens in the early stages of infection (Sakai *et al.*, 2021; Hassan *et al.*,

2025). The current results indicate that the vaccine promotes the organized production of IgT antibodies. Nematode infection strengthens the host's adaptive immunity and reduces cumulative mortality. This observation is consistent with previous studies that have demonstrated the excellent immunoprotected effects (Abdelhamed *et al.*, 2017). Dong *et al.* (2015) showed that the immunoglobulins are considered one of the most important indicators of humoral immunity, which rises early after exposure to antigens (parasite), as it is the first type of antibody to be produced in response to immunization (Zhang *et al.*, 2011; Noomi *et al.*, 2022). In addition, the increase in concentration of globulin after infection is important, as it plays a protective role against harmful effect of parasite (Harikrishnan *et al.*, 2011; Noomi *et al.*, 2025). The results of the current study are consistent with those reported by Al-Bayati *et al.* (2020), who indicated that the antigen can provide effective protection.

Table 3. Serum levels of IgT and IgE in *Brachirus orientalis* parasitic infection ($\mu\text{g/mL}$)

Serum level	Groups / Mean \pm S.E	
	Control	Infected
IgT	5.07 \pm 0.187 ^b	15.11 \pm 0.464 ^a
IgE	6.82 \pm 0.144 ^b	17.22 \pm 0.66 ^a

A *P*-value of less than 0.05 was significant.

Histopathological Observation

Under specific circumstances, nematodes, a common conditional parasite, can infect humans and a variety of animals like fish. The main pathological lesions in positive control groups (Fig. 2 A, B, C and D) were characterized by necrosis and inflammation in portal area of liver parenchyma with polymorphonuclear cell in filtration with edematous fluid with aggregation of inflammatory cell and congestion. These lesions were followed by multi granulomatous lesion due to parasite invasion of the liver, surrounded by fibrous connective tissue. The pathohistological findings that were typical of histopathology were in line with the findings of Vieira *et al.* (2010) and Oday *et al.* (2025). Nematode spp. affected all the internal organs; however, the extremely high intensity of nematode infection in the liver prompted us to concentrate on this organ in order to precisely evaluate the hepatic tissue damage (Sayyaf *et al.*, 2016). Neutrophils play a crucial part in the inflammatory process, particularly when there is an initial pathogen exposure (Havixbeck *et al.*, 2016), as was noted in the preceding section, neutrophils were found in the hepatic sinusoid lumen, parenchyma, and/or interstitial surrounding the capillary in the liver of an infected sample. Remarkably, no neutrophils were found near the parasite or inside the granuloma's thickness, also were not seen inside the capsule that surrounded

the pathogen (larva) that had infected the liver (**Dezfuli *et al.*, 2009**). Many larvae were discovered inside the granuloma wall's thickness, sporadically inside the inner layer, and in close proximity to the larval body. Although there are many neutrophils in the parasitized liver, there is no concrete proof that these phagocytes killed the larva (**Sayyaf *et al.*, 2016; Abdullah *et al.*, 2024**).

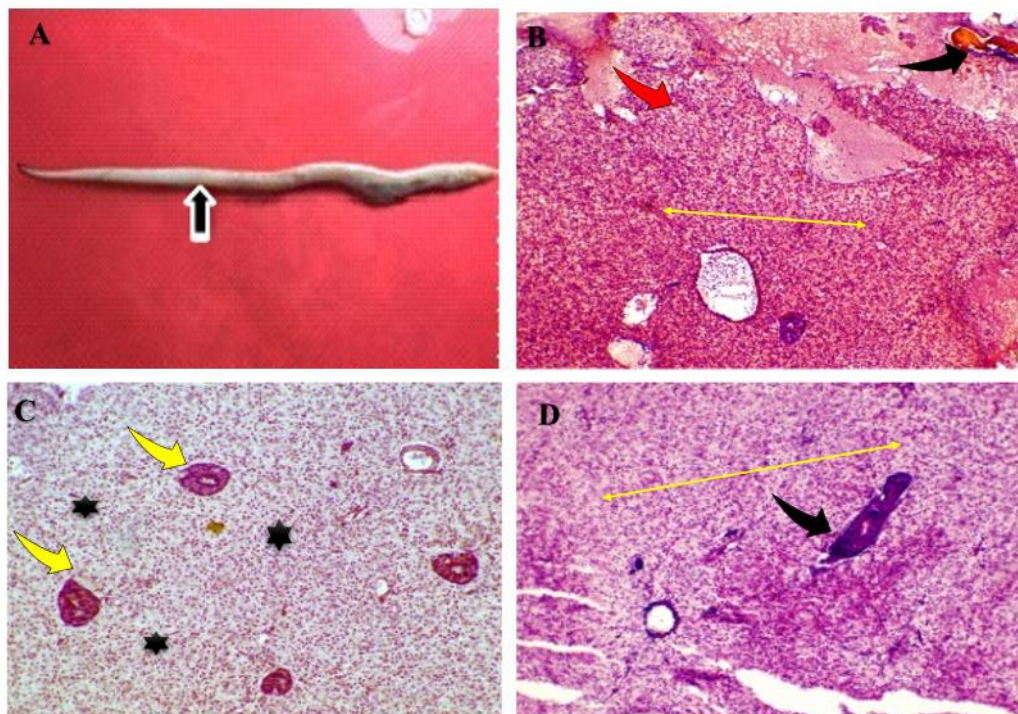


Fig. 2. Histopathological sections of the liver of (normal and infected groups) (H.E. stain 100×). **A.** Photograph during necropsy (Nematode) (arrow) isolated from liver, **B.** Histopathological sections (Infected groups) show necrosis in liver (red arrow), with congestion of the capillary in the portal area (black arrow), and infiltration of inflammatory cells (yellow arrow) with edematous fluid with cluster nematodes. third-stage larva in cysts (arrows). **C.** Histopathological sections of the liver (Infected groups) show hepatitis huge polymorphonuclear cell infiltration (star) (100×). **D.** The distinct granulomatous reactions visible with the granuloma in the section (black arrow) clearly shows the presence of necrotic material peeling from the inner layer

CONCLUSION

Brachirus orientalis shows seasonal variation in feeding, mainly consuming crustaceans and mollusks. Nematode infections were sporadic but triggered significant immune responses like IgT and IgE, and elevated IgT and IgE levels. Histopathological changes in the liver confirm tissue damage, highlighting the impact of parasites on fish health.

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