



ISSN INTERNATIONAL
STANDARD
SERIAL
NUMBER

eISSN: 2789-858X

JSFSU

SCIENTIFIC JOURNAL FOR THE FACULTY OF SCIENCE - SIRTE UNIVERSITY

DOI: 10.37375/issn.2789-858X - Indexed by Crossref, USA



VOLUME 3 ISSUE 2 OCTOBER 2023

**Bi-annual, Peer- Reviewed, Indexed, and
Open Accessed e-Journal**

**Legal Deposit Number@National Library
(Benghazi): 990/2021**



1.02/2022



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Isolated Some Fish Parasites from *Sarpa salpa* (Linnaeus, 1758) in Misurata City Coast, Libya

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DOI: <https://doi.org/10.37375/sjfssu.v3i2.1622>

ABSTRACT

ARTICLE INFO:

Received: 30 August 2023

Accepted: 02 October 2023

Published: 26 October 2023

Keywords: *Sarpa salpa*, helminths, Trematoda, Acanthocephala, Nematoda.

In this study, some parasitic infections were investigated in a type of herbivorous marine fish that is widely spread in the coastal water of Misurata city. The abdominal cavity, gills, stomach, intestines, liver, and spleen of ten *Sarpa salpa* fish were examined between February and April 2018. The results showed that the incidence of helminths varied among the study fish, with the highest infection rate recorded in the posterior and interior intestines, reaching 31-30% respectively, followed by the stomach at a rate of 22%. The liver had a parasitic infection rate of 17%, specifically Trematoda. Acanthocephalan was only observed in the posterior intestines. While the gills, abdominal cavity, spleen, and ectoparasites, their infection rates were 0%. Microscopic examination of the liver, gills, and intestines' tissues revealed the presence of various tissue diseases, including swelling, cysts, and degradation of most liver cells, as well as damage to the gill filaments.

1 Introduction

Fishes are considered one of the most important sources rich in protein, providing 16% of animal protein, vitamins, and essential minerals (Kassem and Bowashi, 2015). Therefore, fishery resources have become among the sources that many countries increasingly rely on to fill the food gap, and many studies have been conducted to identify the sources of pollution that affect fish in their natural environment (Bastawrows, 2003). Fishes are carriers of a large number of parasites, and thus the pathogenic effect of parasites that infect fish varies with each parasite, some of which cause little harm, while others can be severe and lead to death (Overstreet and Hawkins, 2017). Many parasites that infect fish cause the host to be deprived of part of its food, which affects its growth. Also, the various activities of fish are affected as a result of their activity within the host's tissues or secretion of some substances that may be toxic, reducing the nutritional value of fish. Fishes play

a carrier or transporter role for other pathogens, making some of them a source of disease for other fish or vertebrates, including humans, where they are transmitted by consuming raw or poorly cooked fish (Al-Alusi, 2011; Kassem and Bowashi, 2015). Parasitic diseases make up about 80% of all diseases that affect marine vertebrates, where warm-water fish with stable temperatures throughout the year are found, and this climate helps provide natural food in the water and the proliferation of intermediate hosts such as snails, copepods, crustaceans, Leeches and barnacles. Parasites are the primary source of most pandemic and epidemic diseases that enter as secondary infections like bacteria and fungi, which leads to significant economic losses due to high infection and mortality rates (Marcogliese, 2008).

Infection with helminths parasites such as Trematoda, Nematoda, and Acanthocephalan are among the most common parasites that affect fish, and the digestive

system and its accessories are considered among the organs susceptible to infection by these parasites, by ingestion of food contaminated by Infective stages or ingestion of animal tissues representing Intermediate hosts for some helminths (Al-Saadi *et al.*, 2016). Some fishes are considered intermediate hosts for Trematoda such as *Heterophyes heterophyes*, which cause some intestinal disorders in humans and stunted growth in children, and are considered one of the most dangerous helminthes for humans (Bastawrows, 2003). Acanthocephalan are common intestinal parasites, especially in fish intestines, and have an intermediate host that is represented by arthropods such as crustaceans (Taher *et al.*, 2017). Trematoda has a complex life cycle that requires more than one host, and most of them are hermaphroditic. Trematoda is a common helminths all classes of vertebrates, especially marine fish, and ranks second after Nematoda in their distribution, where the mature parasite produces eggs that pass through the final host's feces. Thousands of species of Trematoda have been described in different types of fish all over the world. The clinical picture of infection with these parasites depends on the size and number of helminths present in the host, organs, or affected tissues. The infection may be local or systemic, usually both, resulting in tissue ulceration and abscess formation. In fish, adult Trematoda are mostly found in the intestines and stomach, and some have been found in the liver, bile ducts, and other organs. Fish can act as final, intermediate, or carrier hosts or reservoirs for different species of Trematoda. It is also known that some types of animal infections caused by Trematoda affect humans and cause diseases, and many species cause economic losses to society (Shaukat., 2008).

A study found the presence of Trematoda infection in two species of marine fish, *Abudefduf luridus* and *Chromis limbata*, while no infection was recorded in *Boops boops* (Costa and Biscoito, 2003). Another study conducted on *Selene setapinnis* fish showed their infection with different types of Trematoda (Cordeiro and Luque, 2004).

As indicated by Parveen *et al.*, (2018) in their study on a type of marine fish, *Plectorhinchus cinctus*, infections with the parasite Trematoda were recorded in the stomach, leading to tissue damage. Similarly, Pardeshi *et al.*, (2012) explained in their study on tissue infections in a type of marine fish that the infections were due to the parasite Trematoda, resulting in abscesses, changes in the overall shape of the liver, and tissue damage. In a study by Abdul-Ameer (2010), two types of Trematoda were recorded in the gills of *Aspius vorax* and *Barbus sharpeyi*. As mentioned by

Heckmann (2001) in his study on fish species, the parasite Acanthocephalan was found in many organs of the study fish, including the liver, spleen, and muscles. AL-Zubaidy *et al.*, (2012) isolated five species of the parasite Acanthocephalan from 11 species of marine fish, where the study indicated the spread of adult Acanthocephala parasites in the intestinal cavity, while the larval stage was present in the body cavity and some organs such as the liver and spleen. Also, the study conducted by Alabbar (2014) on *Muraena helena*. showed the presence of three types of helminths on the internal parts of the fish (*Anisakis* sp., *Cucullanus* sp., *Nematodes* sp.), with infection rates of 96.49%, 57.02%, and 26.32% respectively.

Improving fish production in natural water and increasing it requires conducting many studies and research related to the aspects that negatively and positively affect fish productivity. Due to the importance of this topic, the current study was conducted and it was found necessary to provide effective solutions against helminths that infect some species of economically important fish on the coast of Misurata city and surrounding areas. This is a new addition to previous studies and research on fish parasites on the Libyan coast.

2 Materials and Methods

The targeted fish for this study were collected from the fishing port in Qasr Ahmed in the city of Misurata, Libya as shown in Figure (1), during the period between February and April 2018. The collection included 10 herbivorous fish of the *Sarpa salpa* species (Figure 2), with 4 collections per month during the study period, using trawl nets for fishing.



Figure (1): The location of the collection of study samples.



Figure (2): External feature of *S. salpa*. (TL): Total length.

The fish were transferred directly to the laboratory at the faculty of science at Misurata University in individual bags. External morphometric measurements were taken for the fish, where the average total length of *S. salpa* fish ranged between 30.04 ± 0.75 and the average weight ranged between 343.80 ± 9.27 . The fish were examined directly (With the naked eye) to detect any external observations or changes that may indicate infection with parasites, noting any changes in the color of the fish or eye color. The fish were dissected and then the abdominal cavity and digestive tract were examined after a longitudinal incision was made at the median abdominal line, in addition to examining the gills after removing the fish's operculum. The digestive tract was separated from the body, and the intestines were opened along their length inside a Petri dish containing a physiological solution and examined visually for helminths inside them. Similarly, the gills, stomach, liver, and spleen were examined, according to what DE Giusti (1949) mentioned.

The large parasites were transferred to containers containing 5% formalin and tightly sealed, with the type of fish (Crites and Overstreet, 1991) written on them. Some of them were also placed in an ethanolic solution for ten minutes, spread on glass slides, and covered with a cover slide for examination under a light microscope, as reported by Al-Tae, and Zangana (2011).

Parts of different areas of fish samples, such as gills, digestive tract (1cm), and liver, were taken and placed in 10% formalin for histological examination after sections were taken using a microtome, according to Sheehan and Hrapchak (1980).

In addition to determining the density of infection for each parasite, some mathematical equations were used based on the study presented by Margolis *et al.*, (1982) to the American Society of Parasitologists regarding the adoption of infection standards according to infection terms, as reported by Al-Alusi, (2011) and Al-Saadi., *et al* (2016), as follows:

$$\text{Infection Rate} = \frac{\text{Number of infections in the month}}{\text{Total number of samples in the month}} \times 100$$

$$\text{Intensity of infection rate} = \frac{\text{Number of isolated worms}}{\text{Number of infected fish}}$$

2.1 Statistical Analysis

Statistical analysis was conducted using the statistical software SPSS (Version 25) to find the relationship between the number of parasites and their location in fish, as well as some descriptive measures including mean, standard error, and percentage of all samples.

3 Results

The results showed the presence of different parasitic infections with some helminths, where the total infection rate and the monthly infection rate in the *S. salpa* fish during the study months were shown in Table (1).

Table (1): shows the total infection rate and the monthly infection rate during the study months.

Study's duration	Number of samples per month	Overall infection rate %
February 2018	3	30%
March 2018	3	30%
April 2018	4	40%
Total	10	100%

The current study results show that no external helminths parasites were found during the external examination of *S. salpa* fish, as shown in Figure (3)

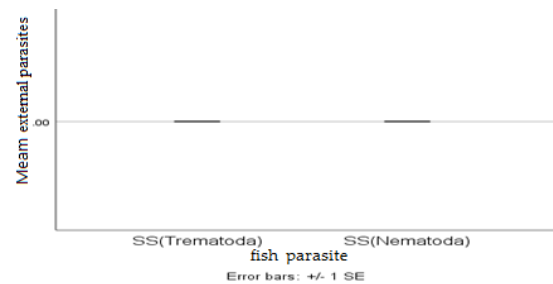


Figure (3): Ectoparasites of (SS): *S. salpa*.

Similarly, examination of the gills of the studied fish did not reveal any infection with Acanthocephalan or Trematoda (Figure 4). Microscopic examination of the abdominal cavity of *S. salpa* fish revealed no infection with any Acanthocephalan and Trematoda parasites, as shown in Figure (5).

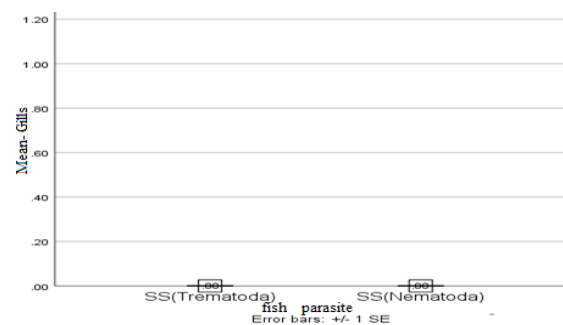


Figure (4) shows the average infection of the parasites Trematoda and Acanthocephalan in the gills of (SS): *S. salpa*.

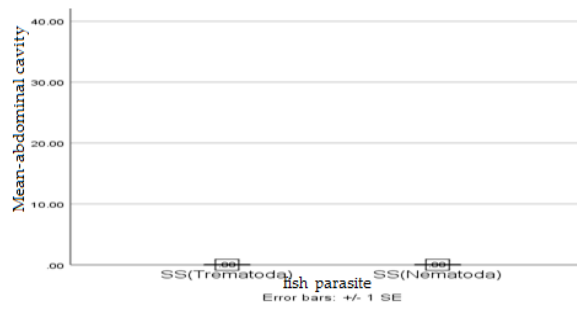


Figure (5): shows the average infection of parasites Trematoda and Acanthocephalan in the abdominal cavity of (SS): *S. salpa*.

Figure (6) indicates the average infection of Acanthocephalan and Trematoda parasites in the stomach of *S. salpa* fish, where the average infection of Trematoda was recorded as 2.00 ± 0.25 , while there were no recorded infections of acanthocephalan. Figure (7) examination of the anterior intestines of *S. salpa* fish showed that the average infection with Trematoda parasites was 2.70 ± 0.30 , while the infection with Acanthocephalan was absent. While, examination of the posterior intestines of *S. salpa* fish showed that the average infection of Trematoda parasites was 4.00 ± 0.47 , while the infection of Acanthocephalan was recorded at an average of 1.20 ± 0.13 (Figure 8)

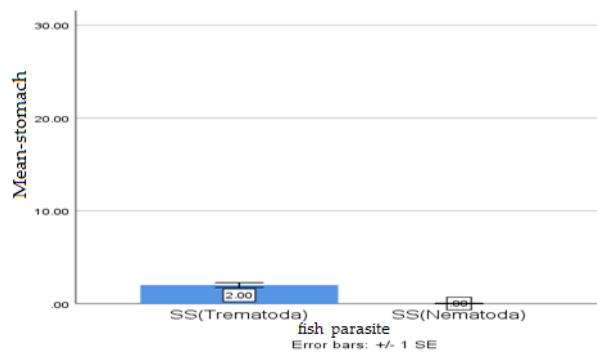


Figure (6): Average infection with the parasites Acanthocephalan and Trematoda in the stomach of *S. salpa*.

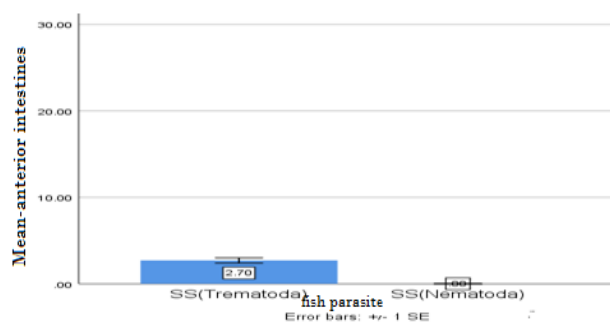


Figure (7): Average infestation of parasites Acanthocephalan and Trematoda in the anterior intestines of (SS): *S. salpa*.

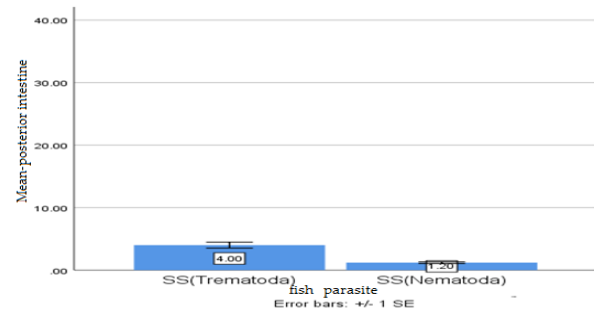


Figure (8): Average infection with parasites Acanthocephalan, and Trematoda in the posterior intestine of *S. salpa*.

By examining the liver of *S. salpa* fish, Figure (9) showed that the average infection with Trematoda parasites in the liver was 0.16 ± 1.60 , while the infection with acanthocephalan parasites was absent. Through microscopic examination of the spleen (Figure 10), it was found that there was no infection with any type of helminths parasites, whether Acanthocephalan or Trematoda, in *S. salpa* fish

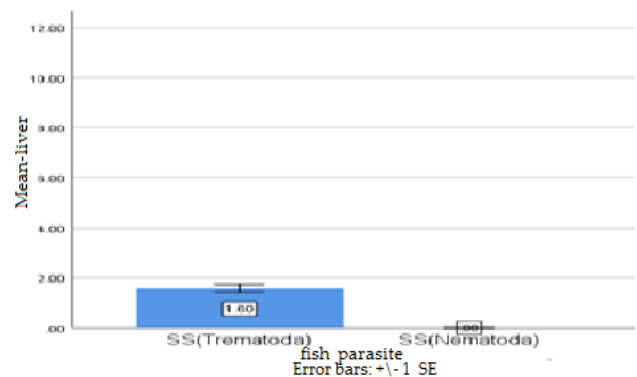


Figure (9): Average infection with parasites Acanthocephalan, Trematoda in the liver of *S. salpa*.

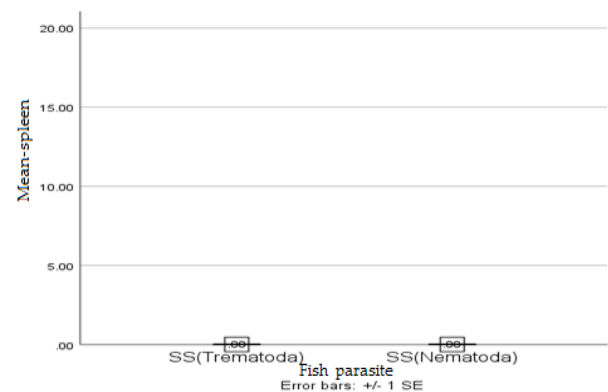


Figure (10): Average infection with parasites Acanthocephala, and Trematoda in the spleen of *S. salpa*.

Histological examination of samples of *S. salpa* fish showed parasitic infections with Trematoda worms, which led to damage and tearing of the secondary gill filaments (Figure 11, A). The same form indicates disintegration in the intestinal tissue and an increase in the size of some epithelial cell nuclei (Figure 11, B). Histological examination of liver samples showed significant tissue disintegration and clear fibrosis (Figure 11, C).

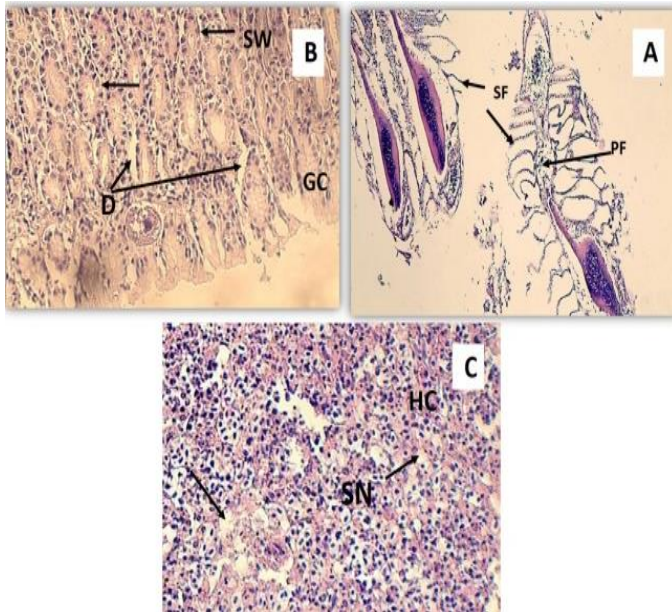


Figure (11): Parasitic infection of *S. salpa* (H&E): A- Gills,(PF): Primary gill filaments ,(SF): Secondary gill filaments:(↑), Laceration.(40X).B-Intestines,(GC):Goblet cells),SW):Cellular swelling(↑): Nuclei of epithelial cells. (400X). C- Liver), HC): Hepatic cells), SN): Venous sinusoids, (↑):Tissue decomposition. (200X).

4 Discussion

The results of this study showed the presence of various infections with helminths in the studied fish samples, where the overall infection rate for *S. salpa* was 100%. These results were consistent with what was found by Alabbar (2014), where the overall infection rate for parasites was 95.0% and 100% in species of bony fish (Herlyn *et al.*, 2003). Meanwhile, the monthly infection rate in the current study fish reached 30%, and this result is similar to the study by Shamsi (2018), where the monthly infection rate was 34.3%.

The results showed that during the external examination of *S. salpa*, no presence of any external helminths. Meanwhile, the external infection rate of helminths was absent, and there were no external changes in the appearance of the fish. These results agreed with the results of the study done by Mohammed *et al.*, (2017),

during the external examination of the collected *Microine epinephelus*, no external parasites were found, unlike the results of the study done by Hussein (2017), where different external parasites were present in 122 fish samples with minor infections on the skin, gill covers, and fins. The reason for these discrepancies could be attributed to the differences in fish species, sample sizes, and study seasons. Additionally, the microscopic examination of the study's fish gills showed an absence of parasitic infections (0%), which contradicts the findings of Yousry *et al.*, (2019), where *Euthynnus alletteratus* fish had an infection rate of 0.67 ± 0.41 with Acanthocephalan and Trematoda parasites. Furthermore, the current study did not match the findings of Abdul-Ameer (2010), who recorded two types of Trematoda in the gills of *A. vorax* and *B. sharpeyi* fish.

The statistical analysis of the data indicated that the incidence of Trematoda and Acanthocephalan in the abdominal cavity of the studied fish was (0%). These results were consistent with the study conducted by Salman *et al.*, (2010) on Mugilidae fish to determine some species of Trematoda, where no helminth infection was recorded upon examination of the abdominal cavity. In contrast to what Yousry *et al.*, (2019) indicated, where the infection rate of the Acanthocephalan parasites was 32.2 ± 1.52 , while no infection of the Trematoda parasite was recorded in *E.alletteratus* fish. Hui-Shih (2004) also recorded helminth infections in the abdominal cavity in his study on fish *Trichiurus lepturus*. Similarly, Al-Tae, and Zangana (2011) in their study on marine fish species (Cyprinidae) recorded two types of Nematoda in the abdominal cavity, as Alabbar (2014) indicated a high incidence of helminths in the abdominal cavity of *M. helena* fish, at a rate of (47.28%). Many studies suggest that the density of infection is associated with seasonal changes, and therefore the density of infection may be higher in the summer and lower during the winter season based on the activity of the parasite (Kundu, *et al* 2015; Sures *et al.*, 2003; Hui-Shih, 2004).

figure (6) indicates the average infection of Acanthocephalan and Trematoda parasites in the stomach of *S. salpa* fish, where the average infection rate of Trematoda was recorded (2.00 ± 0.25), while no infection by Acanthocephalan was recorded. This is consistent with the study conducted by Parveen *et al.*, (2018) on a type of marine fish, *P. scinctus*, which was recorded as a Trematoda parasite infection in the stomach, leading to tissue damage. In contrast, Yousry *et al.*, (2019) reported in their study on *E. alletteratus* fish and their infection with Acanthocephala and

Trematoda parasites in the stomach, where the average infection rate of the first type was 25.40 ± 1.52 , while no infection by the second type of parasites was recorded.

It was observed that the infection rate of intestinal parasites in the anterior and posterior intestines of the fish in the current study was 30-31% respectively, these results were close to the study conducted by Al-Alusi, (2011), which included the intestines of (carp) fish, with an infection rate of 29.8% and 34.6%, respectively. The results were also somewhat consistent with those of Al-Tae, and Zangana (2011), who recorded a parasitic infection of Nematode in the intestines of *ALburnus capito* with a rate of 20%. In a study conducted by Kennedy (1976), the infection rate in the anterior part of the intestines of salmon was 14%, while the infection rate in the posterior part varied and reached 46%. Alabbar (2014) noted in his study on internal helminths that infect a type of marine fish that the infection rate in the anterior intestines was 13.95%, while it was 9.86% in the posterior intestines.

In examining the liver of *S. salpa* fish, the results showed that the percentage of parasitic infection in the liver was 17%, which corresponds to the study conducted by Alabbar (2014) that indicated the presence of parasitic infection in the liver of a type of marine fish at a rate of 16.33%. The results of this study also showed that the average infection rate with the Trematoda was 0.16 ± 1.60 , while the infection with the Acanthocephala parasite was absent. This is contrary to what was reached by Al-Tae, and Zangana (2011) who recorded an infection with Nematode in the liver of *A. capito* fish at a rate of 20%.

Through microscopic examination of the spleen, it was found that there was no infection with any type of helminths, including Acanthocephala and Trematoda, in *S. salpa* and that is consistent with the study conducted by Alabbar (2014). No cases of internal helminths were recorded in the spleen of *M. Helena* fish. However, this study did not agree with the study conducted by Blazer et al., (2010), where helminth infections were recorded in the spleen of (sunfish) at moderate to high rates.

The study conducted by Salman et al. (2010) on the Burian family fish (Mugilidae) to identify Trematoda infection, the study presented that the seasonal and monthly variations affect Trematoda's distribution and spread. They appeared in all seasons of the year, but with different rates, with the highest infection rate recorded during the summer and spring seasons, While the lowest rate of infection with these worms was recorded during the winter, indicating the clear effect of seasonal changes in temperature on the rate and severity of Trematoda infection. On the other hand, parasites are

essential components in animal communities, and they are usually more abundant than their hosts. In addition, completing the life cycle of many parasitic species requires various models of vertebrate and invertebrate animals that act as intermediate or final hosts. Mollusks are a common intermediate host for these parasites. Therefore, the change in the composition of parasitic communities reflects the change in the composition of marine animal species (Galli et al., 2001). In general, the differences that occur in the rate and percentage of infection are due to many reasons. The reason for the difference may be due to the nature of the nutrition of the host, as well as differences in feeding habits and not being in the same environmental conditions (Kennedy,1976), or the reason for the presence of differences in infection is due to the size of the sample and its inclusion for different lengths or studied over different months. Since both the proportion and severity of the injury increases with the length and size of the fish or with the age of the fish (Taher et al., 2017).

The histological examination of samples of *S. salpa* fish showed the presence of helminths, which led to damage and tearing of the secondary gill filaments (Figure 11, A). The same figure also indicates the breakdown of intestinal tissue and an increase in the size of some epithelial cell nuclei (Figure 11, B). The histological examination of liver samples from the fish studied showed significant breakdown of the liver's basic tissue and clear fibrosis of its tissues (Figure 11, C). These results are consistent with the study of Sures et al., (2003), which indicated tissue breakdown in (salmon) due to parasitic secretions and the fish's immune resistance to infection. helminths, when found in fish, migrate extensively, causing tissue changes in various organs, leading to direct tissue damage and the onset of hypersensitivity reactions. Feeding of threadworms on host tissues is considered an important cause of various diseases (Parveen et al., 2018). The current study agrees with the study conducted by Parveen et al., (2018) which revealed from a histological examination of the stomach of *P. cinctus* fish that it was infected with a number of helminths and a contagious tumor in the mucous membrane with complete destruction and damage to the mucous epithelial. In addition to these changes, the entire structure of the villi was completely destroyed and shrunk, causing a change in the morphology of the stomach. Pardeshi et al., 2012 indicated in their study of tissue injuries in a type of marine fish liver caused by the Trematoda parasite that the anatomical examination of the affected liver revealed the presence of abscesses associated with the serous membrane of the affected liver and the presence of a large cyst filled with eggs that caused disruptions in

the vital functions of the glands. These disruptions may directly affect the chemical nature of the affected tissues, thus damaging the liver tissues and changing their shape and size.

5 Conclusions

From this study, it can be concluded that there are infections of some helminths in *S. salpa* fish, and the rate of prevalence of Trematoda was higher compared to acanthocephalan. The highest infection rate was recorded in the posterior and anterior intestines, followed by the stomach, and then the liver. As for the spleen, gills, abdominal cavity, and ectoparasites, the infection rate was absent. Examining the tissue sections revealed the presence of various infections in fish organs (gills, liver, and intestines).

Acknowledgments

The researchers express their thanks and appreciation to Dr. Ibrahim Sulaiman Hanish for statistically analyzing the data.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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