A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF ÇANKIRI KARATEKİN UNIVERSITY

THE EFFECT OF SOME PARASITIC WORMS ON PHYSIOLOGICAL AND HISTOLOGICAL CHANGES IN A SPECIES OF ECONOMIC FISH IN THE CITY OF MISURATA, LİBYA, USING STATISTICAL ANALYSIS

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
BIOLOGY

BY

EHAB Y. IBRAHIM ALORAFI

ÇANKIRI

2024

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June 2024

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ABSTRACT

THE EFFECT OF SOME PARASITIC WORMS ON PHYSIOLOGICAL AND HISTOLOGICAL CHANGES IN A SPECIES OF ECONOMIC FISH IN THE CITY OF MISURATA, LIBYA, USING STATISTICAL ANALYSIS

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Master of Science in Biology

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June 2024

The significance of helminthology and haematology features as critical instruments in fish health evaluations has been acknowledged. Blood parameter measurement has a long history of application in fish health monitoring. Liver and kidney function tests, lipase and amylase levels, creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) were among the blood indicators examined in this study to determine the impact of an invading parasite. The present investigation set out to examine the parasitic infection in a hundred fish specimens. The sample included fifty individuals of *Euthynnus alletteratus* and fifty individuals of Scombrus Scombrus. The seas around the Libyan city of Misrata are the source of this fish. Both *Euthynnus alletteratus* and *Scomber scombrus* showed statistically significant variances across multiple parameters. All of *Scomber scombrus*'s internal organs, with the exception of the outside body, showed a very positive connection with urea levels. Except for the exterior body and nose, urea levels were positively associated with many organs in *Euthynnus alletteratus*. Excluding tissues on the outside of the organism, the results of the research indicate a positive relationship between creatinine levels and several internal organs in *Euthynnus alletteratus*. With the exception of the stomach and internal organs, *Scomber scombrus* showed a positive correlation between sodium levels and a number of organs. Several organs of both *Scomber scombrus* and *Euthynnus alletteratus* showed a direct association between total protein levels, CPK, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lipase, amylase, and potassium. With the exception of *Scomber scombrus*'s

exterior body, a robust correlation between LDH levels and many organs was seen in both *Euthynnus alletteratus* and *Scomber scombrus*. Nostril, internal organ, and anterior intestine LDH levels were positively correlated in *Euthynnus alletteratus*. Examining the razam and kawali parasites' distribution throughout several organs is the primary objective of the current investigation. Parasite infection rates varied throughout fish organs, as seen in the research. Among the histological findings in the stomachs of Kawali species were fibroblasts, lymphocytes, macrophages, and eosinophilic granulocytes. Histopathological analysis of razam species mostly showed autolysis and hyperplasia in the stomach's submucosa and mucous membranes. Hypertrophy, systemic inflammation, and multifocal myocyte necrosis characterise this cardiac condition. Multifocal necrosis and congestion of interlobular veins are hallmarks of liver tissue deterioration, which occurs with substantial vacuole degradation and fatty hepatocytes. Alterations to the gut wall happen autolytically. Autolytic changes occur in the pancreas and liver.

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Keywords: Hematological evaluation, Fish parasite, *Euthynnus alletteratus*, *Scomber scombrus*, Liver function, Kidney function tests, Histopathological, Misrata

ÖZET

BAZI PARAZİT SOLUCANLARIN LİBYA'NIN MİSURATA ŞEHRİNDE EKONOMİK BİR BALIK TÜRÜNDE İSTATİSTİKSEL ANALİZ KULLANILARAK FİZYOLOJİK VE HİSTOLOJİK DEĞİŞİKLİKLERE ETKİSİ

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Haziran 2024

Balık sağlığı değerlendirmelerinde kritik araçlar olarak helmintoloji ve hematolojik özelliklerin önemi kabul edilmektedir. Kan parametresi ölçümünün balık sağlığının izlenmesinde uzun bir uygulama geçmişi vardır. İstilacı parazitlerin etkisini belirlemek için bu çalışmada incelenen kan göstergeleri arasında karaciğer ve böbrek fonksiyon testleri, lipaz ve amilaz düzeyleri, kreatin fosfokinaz (CPK) ve laktat dehidrojenaz (LDH) yer almaktadır. Bu çalışmada, yüz balık örneğindeki parazit enfeksiyonu incelenmiştir. Örneklemde, *Euthynnus alletteratus*'un elli bireyi ve Scombrus Scombrus'un elli bireyi yer almaktadır. Libya'nın Misrata kenti çevresindeki denizler bu balığın kaynağı olmakla birlikte hem *Euthynnus alletteratus* hem de *Scomber scombrus*, birden fazla parametre arasında istatistiksel olarak anlamlı farklılıklar göstermiştir. *Scomber scombrus*'un dış gövdesi dışındaki tüm iç organları, üre seviyeleriyle çok olumlu bir ilişki göstermiştir. *Euthynnus alletteratus*'ta dış gövde ve burun dışında birçok organla üre düzeyleri pozitif yönde ilişkili çıkmıştır. Organizmanın dışındaki dokular hariç tutulan araştırmanın sonuçları, *Euthynnus alletteratus*'ta kreatinin düzeyleri ile çeşitli iç organlar arasında pozitif bir ilişki olduğunu göstermektedir. *Scomber scombrus*, mide ve iç organlar haricinde, sodyum seviyeleri ile bazı organlar arasında pozitif bir korelasyon göstermiştir. Hem *Scomber scombrus* hem de *Euthynnus alletteratus*'un çeşitli organları, toplam protein seviyeleri, CPK, aspartat aminotransferaz (AST), alanin aminotransferaz (ALT), alkalin fosfataz (ALP), lipaz, amilaz ve potasyum arasında doğrudan bir ilişki göstermiştir. *Scomber scombrus*'un dış gövdesi dışında, hem *Euthynnus alletteratus* hem

de *Scomber scombrus*'ta LDH seviyeleri ile birçok organ arasında güçlü bir korelasyon gözlemlenmiştir. Burun deliği, iç organ ve ön bağırsak LDH düzeyleri *Euthynnus alletteratus*'ta pozitif korelasyon göstermiştir. Razam ve kawali parazitlerinin çeşitli organlardaki dağılımının incelenmesi mevcut araştırmanın temel amacıdır. Araştırmada görüldüğü gibi parazit enfeksiyonu oranları balık organları arasında farklılık göstermiştir. Kawali türlerinin midelerindeki histolojik bulgular arasında fibroblastlar, lenfositler, makrofajlar ve eozinofilik granülositler yer almaktadır. Razam türlerinin histopatolojik analizinde çoğunlukla mide submukozası ve mukozalarında otoliz ve hiperplazi görülmüştür. Hipertrofi, sistemik inflamasyon ve multifokal miyosit nekrozu bu kalp rahatsızlığını karakterize etmektedir. Multifokal nekroz ve interlobüler damarların tıkanması, önemli vakuol bozulması ve yağlı hepatositlerle ortaya çıkan karaciğer dokusunun bozulmasının belirtileridir. Bağırsak duvarındaki değişiklikler otolitik olarak gerçekleşmiştir. Pankreas ve karaciğerde otolitik değişiklikler meydana gelmiştir.

2024, 76 sayfa

Anahtar Kelimeler: Hematolojik, Balık paraziti, *Euthynnus alletteratus*, *Scomber scombrus*, Karaciğer fonksiyonu, Böbrek fonksiyon testleri, Histopatolojik, Misrata

PREFACE AND ACKNOWLEDGEMENTS

I can only extend my sincere thanks to God the Creator, Almighty, who gave me the wisdom to do this work. I can also extend my sincere thanks and gratitude to the supervisors Assoc. Prof. Dr. Efehan ULAŞ and Asst. Prof. Dr. Kamal ABUBAKER. They have been an inspiration to me for their constant patience and assistance in completing this thesis, and I am grateful to them for their guidance and encouragement throughout this research journey. I would also like to thank myself for the perseverance and endurance you have shown throughout the preparation of this letter. There were many challenges and difficulties in the practical aspect and writing the thesis, but thanks to dedication and determination I was able to overcome them and achieve success. Of course, I cannot forget (the spirit of my father and mother), who were the shining lamp on my life's path. Special thanks to (Soulmate) for its continuous encouragement and support, which had a great impact on achieving this achievement. And my beautiful little girl, you were part of this amazing support. I am grateful to you for all the love and happiness you bring into my life. Just as I cannot forget my family, sisters and brothers, who supported me and stood by me all the time. And all my friends who have given me invaluable support throughout this period. Finally, I would like to express my thanks and gratitude to all the people who have contributed to my academic journey in different ways. I am grateful to all of you and appreciate your support and encouragement in achieving my academic goals.

Ehab Y. Ibrahim ALORAFI

Çankırı-2024

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LIST OF SYMBOLS

cm	Centimeter
℃	Degree centigrade
ft	Feet
g	Gram
in	Inch
kg	Kilogram
lb	Paund
%	Percentage
±	Plus, minus

LIST OF ABBREVIATIONS

ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
AST	Aspartate aminotransferase
CPK	Creatine phosphokinase
LDH	Lactate dehydrogenase

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1. INTRODUCTION

Libya possesses a lengthy coastline and the second largest continental shelf in the Mediterranean Sea, spanning over 65.000 square kilometers. Despite having abundant fishing grounds, these resources have remained untapped thus far. This may be attributed to the fact that fishing is not a typical practice among the Libyan population, particularly among the fishing sector (Filogh 2019).

The abundance of fish species observed in the Sparidae and Serranidae families can likely be attributed to the presence of a favorable seabed along the Libyan shores, which is preferred by members of these two families. The stretch of land from Misrata to the Libyan-Tunisian border is widely regarded as the most optimal region for trawling (Filogh 2019)

According to Elmagalfta (2014), the city of Misrata is located on the coast of Libya at the coordinates 32.8774 degrees North latitude and 13.1848 degrees East longitude. It is the hub of the fishing activity in the area. Ghosh *et al.* (2022) noted that the abundance of marine fish around the city is a valuable resource for coastal communities and city dwellers alike. Maintaining a delicate balance between human exploitation and the preservation of marine ecosystem is crucial to the sustainable practices of the fisheries stated earlier (Maitieg *et al.* 2018).

Still, confined fish farming increases the risk of parasite infections due to several stresses including overcrowding, oxygen deprivation, aggressiveness, and nutritional deficiencies. Because ectoparasite infestations are so common in aquaculture across the world, disease management measures are crucial to the industry's continued expansion. Ectoparasite infections may cause major losses in fish farming when they occur (Hoai 2020).

Parasites are highly successful organisms that have evolved independently in several phyla. Microorganisms are found everywhere in biological systems and make up a large

amount of the world's biodiversity (Klimpel *et al.* 2019). They also have a considerable role in the biomass, abundance, and productivity of some ecosystems.

Parasites place significant energy requirements on individual hosts and can impact several aspects of their physical characteristics, ability to reproduce, behavior, and overall survival. Parasites have the ability to control the size of host populations, organize communities of organisms that are not dependent on each other, and change how ecosystems function (Poulin 2021).

Parasite ecologists recognize the significance of parasitic organisms in nature and their ability to offer valuable ecological insights. However, ecologists who focus on studying free-living organisms often overlook parasitism in their research. They tend to assume that their systems are parasite-free or that parasites are insignificant within the system (Carlson *et al.* 2020).

Fish biologists, like others in their field, often overlook parasites in their studies, despite the fact that parasites can significantly impact variables commonly used in fish ecology and fisheries. Furthermore, parasites can interact with host parameters that are typically used as indicators of physiological or reproductive status, either in an antagonistic or synergistic manner. Parasites can cause alterations in the behavior of their hosts, leading to changes in host distribution patterns, habitat preferences, diet composition, sexual behavior, and other factors. These changes have significant ramifications for the ecological dynamics of fish, as well as their predators and prey (Renick *et al.* 2016).

Parasitic infections account for 80% of fish diseases. Parasitic infections in fish and contaminants in the aquatic habitat are interconnected, making parasites a useful bioindicator of water pollution. This link is complex and essentially comprises a phenomenon with two opposing effects. On one hand, parasitization might make the host more vulnerable to hazardous pollutants. On the other hand, pollutants can either enhance or decrease the occurrence of particular parasites. The presence of parasites induces stress in fish, hence diminishing their resistance to bacterial co-infections. Additionally,

parasites create a gateway for secondary invaders to enter the fish's body (Islam *et al.* 2024).

Histological examination is employed to identify disorders associated with abnormal anatomy and assess the impact of exposure to harmful substances. For this reason, specific organs - the liver, kidney, and gills - play crucial roles in processes like breathing, waste elimination, and the processing of foreign substances in fish (Islam *et al.* 2024, Shah *et al.* 2015). Parasites infest various parts of the body such as the skin, gills, fins, gut, bodily cavities, and muscle (Faruk 2018).

Parasites encompass a wide array of creatures that exhibit considerable variation in their life history and strategies for exploiting hosts (Stella *et al.* 2017). Parasites play a role in multiple food chains and are present in the majority of ecosystems at all levels of the food chain. Multiple parasite species can infect many vertebrate species, acting as hosts for these parasites (Giari *et al.* 2020).

The appropriate method for assessing the environmental health condition involved analyzing the ratio of ectoparasites to endoparasites and the diversity of endoparasites (Bellay *et al.* 2015).

Understanding host responses to parasite infection is crucial as they serve as the foundation for the population's reaction (Costain *et al.* 2022). Within co-evolved host: parasite relationships, infections typically have a detrimental effect on the overall health and reproductive success of the host. These infections also influence the population dynamics of the host species and indirectly affect other species by altering the intensity of competitive interactions between different species (O'Dwyer *et al.* 2020).

Hosts offset the expenses of infection by building immune systems as a defense against infections and by adapting their life-history features, especially during the period before reproduction, to increase their tolerance to infections. Consequently, this has an effect on the amount of energy individuals put into reproduction compared to growth and survival,

resulting in changes in reproductive effort, and body size. This is done to guarantee that reproduction occurs before resources are exhausted, or before individuals become unable to reproduce or die. The response of hosts to introduced infections in the absence of co-evolution is not well understood (Valenzuela-Sánchez *et al.* 2021).

The likelihood of fish parasites being transported between regions in freshwater ecosystems is significant due to the increased rate of introduction of non-native fishes, which has doubled in the past three decades. This rise can be attributed mostly to the globalization of aquaculture (Kovalenko *et al.* 2021). The introduction of diseases caused by fish movements in aquaculture has already had considerable effects on certain fish species.

Research that specifically investigates the functional dynamics of organs in aquatic animals, especially fish, which are vital to marine ecosystems, is becoming more important due to rising environmental concerns. Vital metabolic activities in these species are regulated by enzymatic markers such as Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Aspartate Phosphatase (ALP) (Fathy *et al.* 2012). Recognising biochemical and physiological changes caused by environmental pollutants requires the evaluation they provide (Atli *et al.* 2015).

1.1 Significance of the Study

Research on the influence of introduced parasites on host populations has mostly focused on parasites that induce high mortality rates, leading to direct effects on population dynamics. Nevertheless, the relatively minor impacts of parasites, such as alterations in host behavior and growth rates, have been mostly disregarded, despite their capacity to have substantial repercussions for natural populations. The aim of this study was to assess the pathogenic impact, rate of growth, rate of feeding, and trophic consequences of infection in a host-parasite relationship that does not involve co-evolution. The objectives were to investigate the occurrence and quantity of parasites in both infected and uninfected fish from a natural population. The study sought to ascertain the influence of

the parasite on the well-being of the infected hosts, as well as its impact on their eating behavior and general life cycle characteristics.

The thesis is of great importance in the field of biology, physiology, histology, as well as parasitology, specifically in understanding the physiological and histological effects of helminths on fish.

- The thesis contributes to clarifying the physiological and histological effects of helminth infection on fish, which contributes to increasing scientific knowledge on this important and challenging topic in the field of fish resource management.
- By understanding the physiological and histological effects of parasitic worms on fish, factors affecting fish health can be identified and any negative effect that helminths may have on the general health of fish can be remedied.
- Understanding the physiological and histological effects of helminths on fish is vital for effective fish resource management. Through the thesis, preventive and curative measures can be identified to reduce the spread of worms and maintain the balance of the ecosystem.
- The results of the dissertation can be used in setting policies and legislation related to the management of fish resources in the city of Misurata and other marine areas.
- The thesis is considered an important tool for making effective preventive decisions to reduce the impact of parasitic worms on fish and to improve the environmental and economic sustainability of the fishing industry in marine areas.

1.2 Aim of the Study

The purpose of the thesis is to conduct a comprehensive and specialized study on the physiological and histological changes caused by the prevalence and effect of helminths on specific economic fish species in the city of Misurata. Understand the effect of parasitic worms on the health and functions of infected fish and how they affect the fish's vital organ systems, such as the liver, kidneys and heart. Using appropriate statistical analysis, the relationship between worm prevalence and associated physiological and

histopathological effects were studied. And provide an expanded scientific vision on the impact of parasitic worms and determine the effects that result from these infections. The expected results contribute to a better understanding of the physiological and histological changes and effects related to worm infection. This study enables researchers and those interested to take advantage of the new knowledge to develop better strategies for managing and monitoring physiological and histological changes and trying to develop plans to reduce them.

2. LITERATURE REVIEW

The Atlantic black skipjack, Al razam (*Euthynnus alletteratus*) is a prominent species of tuna in the Scombridae family. The small tunny has certain anatomical changes in comparison to other species of *Euthynnus alletteratus,* similar to the majority of other tuna species, does not possess a swim bladder, necessitating continuous movement in order to remain buoyant. The pectoral fins play a vital role for the small tunny in maintaining its position within the water column. The liver exhibits significant disproportionality, with the right lobe being considerably longer than the left or middle lobes. The gastrointestinal tract of the small tunny is an elongated sac that extends nearly the whole length of its body. The intestinal tract is rather brief, originating from the left and right sides of the stomach, and continuing linearly throughout the length of the body without any loops. Each part is distinguished by its specific diameter and color. The small tunny possesses a distinctive lattice-like structure in its ventral vertebral column, which holds significance within its Scombridae family. The divided haemapophyses, which are components of the vertebrate structure that create a lengthy canal, surround the substantial ventral blood vessel (Godsil 1954).

Research that specifically investigates the functional dynamics of organs in aquatic animals, especially fish, which are vital to marine ecosystems, is becoming more important due to rising environmental concerns. Vital metabolic activities in these species are regulated by enzymatic markers such as Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Aspartate Phosphatase (ALP) (Fathy *et al.* 2012). Recognising biochemical and physiological changes caused by environmental pollutants requires the evaluation they provide (Figure 2.1).

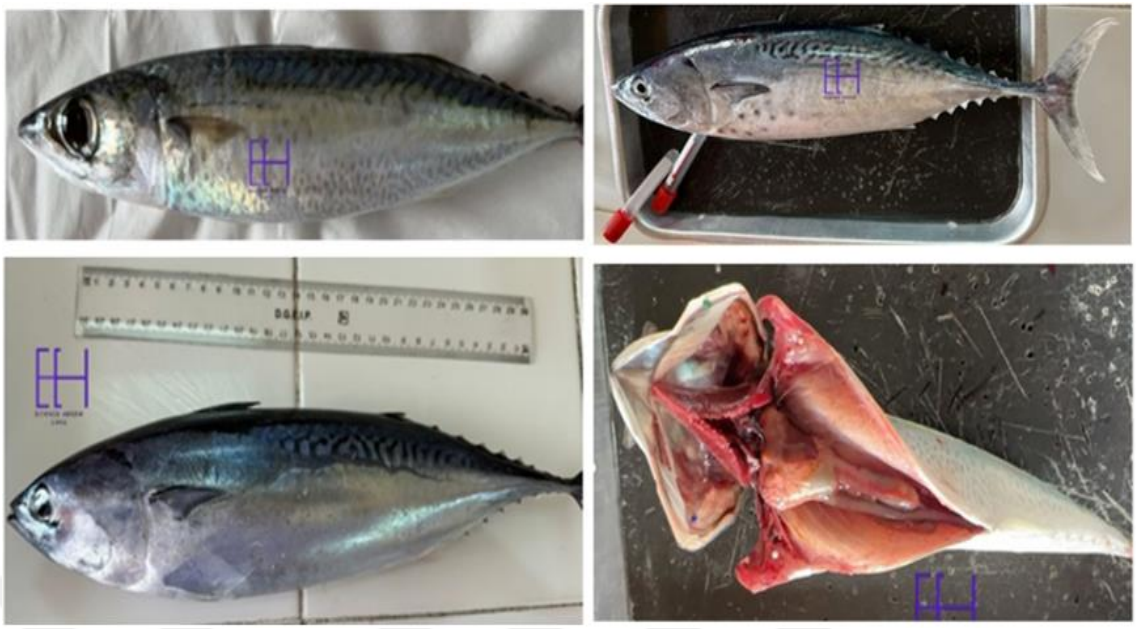


Figure 2.1 *Scomber scombrus* koala fish and *Euthynnus alletteratus* Razam fish

The Atlantic mackerel has an elongated body that is steel-blue in color and patterned with wavy black lines on its back, while its underside is silvery-white. It also has a long and pointed snout. The organism has two dorsal fins that are widely separated, two pectoral fins, and small caudal and anal fins that are likewise widely apart. This species typically possesses 4-6 dorsal finlets and 5 anal finlets. The fish's body gradually narrows towards its length, culminating in a substantial tail fin. The average size of a fully grown fish is 30 cm (0.98 ft), although some individuals have been captured measuring up to 60 cm (2.0 ft). The upper limit of the weight that has been officially documented is 3.4 kg (7.5 lb) (Nesbö *et al.* 2000). The Atlantic mackerel possesses a streamlined body shape that is elongated and tapered, featuring a lengthy and pointed snout. The eyes possess a substantial size and are concealed by a fatty eyelid, whilst the teeth are diminutive, pointed, and cone-shaped. The scales are generally tiny, save for those located directly behind the head and surrounding the pectoral fins. The Atlantic mackerel possesses tiny scales that impart a velvety texture (Sette 1947). The pair of dorsal fins are substantial in size and positioned at a considerable distance from each other. The presence of the second dorsal fin is typically accompanied by five dorsal finlets, although it may alternatively have either four or six. The anal fin, located immediately posterior to the second dorsal fin, exhibits similar dimensions and morphology, and is succeeded by a series of five finlets. The fish's body tapers to a slender caudal peduncle, which is the region where the

small but wide tail fin is attached. The fish exhibits a steel-blue hue on its dorsal side, accompanied by sinuous black stripes that traverse its body at a right angle to its longitudinal axis. The rest of its body is distinguished by a silvery-white to yellow color, which may have darker spots (Abdussamad *et al.* 2016). The organism can attain a maximum length of 60 cm (24 in), but its usual size is approximately 30 cm (12 in). The largest documented weight of the subject is 3.4 kg (7.5 lb) (Abdussamad *et al.* 2016).

Fisheries play a vital role in guaranteeing food security and generating wealth and social advancement in underdeveloped countries. The production of fish is vital not only for generating economic gains, but also for guaranteeing food security and fostering social development in several countries. Hence, it is crucial to confront the prevalent occurrence of diseases that result from the interplay of pathogens, hosts, and the environment in order to efficiently manage this matter (Muktar *et al.* 2016).

Finfish are commonly regarded as hosts for a wide range of ecto- and endoparasites, which are integral components of all ecosystems and have an impact on the health of fish (Lieke *et al.* 2020). More than 50% of the decline in output in aquaculture can be ascribed to illnesses. High stocking numbers and low water quality provide optimal conditions for the infestation and proliferation of parasites and other pathogenic organisms. Unsanitary transportation of fish and equipment leads to the dissemination of infectious diseases (Assefa and Abunna 2018).

Fish infections continue to pose a significant global challenge, impacting both productivity and marketability. Additionally, the consumption of raw and uncooked fish meals in various regions of the world contributes to the spread of human diseases. Fish can be infested by different families of helminthic parasites, leading to harmful effects on their hosts. Nematode worms, primarily found in marine fish, constitute the biggest observed category. They have the ability to infest fish at various stages of their life cycle, either as adults or larvae, causing harm (Ţoţoiu *et al.* 2013).

Endoparasitic infection results in a range of complications within the host's organs and tissues. The tissues respond by surrounding the parasites with an excessive growth of

cells, leading to the formation of nodules that can be easily recognized visually. The parasite-host connection plays a crucial role in maintaining environmental equilibrium and is influenced by various aspects, including the parasite species, the targeted fish species, the severity of the illness, and the specific organ affected (Tavares-Dias and Moraes 2007).

Trematodes are non-segmented flatworms belonging to the Phylum Platyhelminthes, Class Digenea, and Family Heterophyidae. Trematodes encompass a diverse array of species, which are frequently observed as "black spots" on the external surface of fish. Additionally, these parasites can manifest as microscopic white, yellow, or black spots on the skin, fins, and flesh of fish. The spots represent the metacercaria, which is the juvenile form of the parasite. In order to reach adulthood as trematodes, they need to be consumed by a bird. Adult trematodes in the bird excrete eggs that are released into the surrounding environment. The eggs undergo hatching and give rise to a swimming miricidium stage that parasitizes the third host, which is a snail. Inside the snail, the trematodes undergo multiplication and transformation into cercariae, which then swim away from the snail in search of a fish host. The cercariae, depending on the kind of trematode, can either infect fish and develop into adult worms or become encysted as metacercariae (Scholz *et al.* 2016).

Fish typically remain unaffected by grubs until they experience a significant infestation. Adult worms often do not inflict injury upon the host and can be located in the gut, stomach, blood, gall bladder, and urine bladder. Metacercariae, which are parasitic larvae, are the primary cause of disease and can be located in the skin, gills, fins, muscles, and internal organs. The species commonly encountered are Neascus sp., Apophallus brevis, and Cryptocotyle lingua. Consuming diseased fish does not pose a risk to human health, provided that the fish is properly prepared. Swimmer's itch can be experienced by humans when they swim in water that contains a large number of infected snails. This skin irritation is caused by the trematode cercaria (Chi *et al.* 2008).

Cestodes, also referred to as tapeworms, are flat and segmented worms that are classified under the Phylum Platyhelminthes and Class Cestoda. Their life cycles are complex and

involve three hosts: the first intermediate host is copepods, the second intermediate host is fish, and the final host is a fish-eating bird, mammal, or another fish. Metacestodes, which are the juvenile stages of cestodes, are located in the internal organs or muscle of fish, whereas the mature stages are found in the intestine. Cestodes infrequently result in significant death, but they can hinder growth and produce adhesions that impede host metabolism and decrease reproductive capacity (Arnott *et al.* 2000).

Acanthocephalans are internal parasites that possess a retractable proboscis equipped with rows of hooks, which they employ to attach themselves to the intestines of fish. Several taxa have been detected in adult fish intestines, while several larval forms have also been found in the viscera.

Acanthocephalans necessitate a vertebrate animal as their definitive host and arthropods as their intermediate host. Fish often serve as the ultimate host for aquatic acanthocephalans, while microcrustaceans such as amphipods, copepods, isopods, or ostracods commonly act as the intermediate host. Intermediate hosts become infected by ingesting eggs excreted in the feces of fish that are parasitized. An egg will hatch inside the intermediate host, releasing an acanthor that will infiltrate the gut and mature into an acanthella or cystocanth. The life cycle reaches its culmination when a fish consumes a microcrustacean that is infected with parasites, leading to the maturation of the adult worm within the digestive system of the fish host. Fish can serve as both the second intermediate host and the final host in some situations (Nabi *et al.* 2015).

Nashaat and Maghawri (2022), declared that a total of 190 freshwater fish were investigated, with an average weight of 26±0.35 g and an average length of 11±0.25 cm. The hematological and biochemical characteristics were documented in the fish that were infested. Furthermore, a histological analysis was conducted on various organs of the afflicted fish. Fish that were naturally infested displayed distinctive clinical symptoms, including excessive mucus discharges, mouth opening, and in some instances, exophthalmia. An autopsy indicated an atrophic stomach and pallor of the internal organs. The overall prevalence of Capillariasis infestation was 41.1%. The prevalence of infestations was higher in males (65.38%) compared to females (34.62%). The study

results revealed a substantial disparity ($P \leq 0.05$) in hematological and biochemical parameters between infested and non-infested fish. The histopathological analysis of the liver, spleen, and gut of the fish revealed the presence of mild abnormalities caused by infestations.

Another paper details the pathological characteristics of the acanthocephalan, Tenuiproboscis sp., which is a highly prized food fish found around the southwest coast of India. Severe infestations of parasites were discovered in the back part of the gut, nearly completely obstructing the passageway. The area where the parasite attached showed an increase in thickness on the surface of the intestine, and the protective layer of cells lining the intestine seemed compressed and damaged. The intestinal folds experienced erosion, accompanied by thickening of the lamina propria. Although the worms caused significant harm to the structure of the intestinal tissues, there were no evident adverse impacts on the overall health of the fish (Sanil *et al.* 2011).

Rohlenová *et al.* (2011) aimed to examine the potential correlations between the physiological characteristics and immunocompetence of common carp (*Cyprinus carpio*) collected at five distinct time points within a certain year. The sampling we conducted encompassed periods characterized by temporal fluctuation, resulting in varying levels of exposure to parasites. Authors conducted an analysis to determine if seasonality or parasitism had a more pronounced influence on alterations in fish physiology and immunity. The prevalence of cestode infections appears to stimulate the activity of phagocytes. There was a tenuous correlation observed between the size of the spleen and the prevalence of trematodes, while considering fluctuations in different seasons. It is proposed that various parasite life tactics have an impact on different aspects of host physiology and trigger distinct immune pathways.

A study was done to examine the impact of the introduced cestode fish parasite Bothriocephalus acheilognathi on a group of young common carp Cyprinus carpio that had not evolved alongside the parasite. The incidence of parasites throughout the sample was 42%, and the amount of parasites in each individual ranged up to 12% of their body weight. The carp that were parasitized showed pathological changes in their digestive

tract, such as an increase in the size of the gut wall, compression and degradation of the epithelial layer, necrosis due to pressure, and various inflammatory reactions. Nevertheless, the growth effects persisted, as parasitized fish exhibited a considerably slower growth rate compared to non-parasitized fish. Additionally, their feeding rate, measured in items per second, was also much lower. Consequently, the introduction of a parasite to a host without prior exposure resulted in various detrimental effects on the host's health, ecological dynamics, and feeding relationships (Britton *et al.* 2011).

A study found that the presence of ectoparasites, leeches, and endoparasites in hosts varied among contaminated areas. This indicates that environmental degradation has a negative impact on fish health and increases the prevalence of various parasites, further exacerbating the health issues in fish. Postmortem analysis of diseased fishes with various parasitic infestations revealed noticeable characteristics, including liver paleness accompanied by areas of bleeding, as well as gut obstruction accompanied by hemorrhage (Nissa *et al.* 2022). Other research conducted on fish reported similar findings (Eissa *et al.* 2010).

Multiple studies have demonstrated a strong correlation between environmental circumstances and parasitism, particularly in aquatic populations. Fish parasites have been acknowledged as significant sentinel species capable of detecting alterations in environmental conditions. Parasitic infection tends to rise in eutrophic water conditions. However, the parasite load may decrease under highly eutrophic conditions, indicating the detrimental impact of greater eutrophication on the parasite load (Gilbert and Avenant-Oldewage 2017).

The effects of three kinds of monogenean parasites on the gills of farmed Colossoma macropomum were studied histologically. The parasites in question were Anacanthorus spathulatus, Notozothecium janauachensis, and Mymarothecium boegeri. A mean abundance of 81.3 ± 11.8 parasites was found in the parasitological indices, which showed a parasite prevalence of 100%. After the infection, the gill epithelium shifted, epithelial cells overgrowd in certain areas, gill lamellae merged, congestion developed, secondary lamellae shrank, and finally fused together. Although these changes were

localised and transient, they may not have an impact on the gills' ability to function normally. The detrimental effects of monogenean parasites on infected fish gills were also shown in this investigation. To encourage the growth of C. macropomum fish farms, it is crucial to establish hygienic procedures (Tavares-Dias *et al.* 2021).

Younis *et al.* (2020) did a research to evaluate the toxicity of several heavy metals and the presence of parasite infection in fish. The researchers are studying the immune response of these fish to various parasites. A thorough investigation was carried out on 360 Nile tilapia (Oreochromis niloticus) between January 2018 and January 2019 to analyse external and internal parasites. The investigation was conducted in Giza, including four farms and the Nile River. Quantitative real-time polymerase chain reaction was used to analyse samples for two distinct genes. Samples were collected from muscles and water to perform a toxicological investigation on various heavy metals. The average TNF-α levels in the skin were 18.00 ± 0.67 for monogenea parasites and 13.65 ± 0.54 for mixed protozoan parasites (Trichodina and Myxobolus spp.). The average TNF-α concentrations in the gills were 20.45 ± 0.74 for monogenea, 23.00 ± 0.74 for Centrocestus formosanus, and 25.78 ± 0.74 for encysted metacercaria (EMC) with monogenea group averages. The average TNF-α concentration in muscles impacted by EMC was 14.67 ± 0.70. The average TNF-α levels in livers affected by EMC was 26.78 ± 0.70. The average IL-1β concentration was substantially different between monogenea and protozoan parasites (Trichodina and Myxobolus spp.) in the skin, with values of 25.00 ± 0.69 and 15.00 ± 0.43, respectively. The monogenean group had an average IL-1β level of 21.00 ± 0.79 in the gills. C. formosanus obtained the highest value of 27.00 ± 0.74, which was considerably higher. The average IL-1β concentration in the EMC group with the monogenea subgroup was 24.00 ± 1.54. After histological evaluation, several tissues showed significant pathological changes. The fish that were studied were shown to be infected with monogenetic trematodes, leading to the hyperplasia and fusion of the secondary gill lamellae. Electron microscopy was often used to visually inspect the presence of digenetic trematodes in the gills and cartilage rod. A parasite infection may stimulate the generation of immune cytokines to varying degrees, depending on the particular kind of infection.

Suliman *et al.* (2021) conducted a study on the effects of ectoparasites on the gills and skin of Oreochromis niloticus. Specimens of fish and water were dispatched to the laboratory from a tilapia fish farm situated in the Riyadh region of Saudi Arabia. Over the span of one year, a total of 120 fish specimens were collected at consistent monthly intervals. The laboratory conducted monthly analysis of water samples to determine their physicochemical characteristics, using a volume of 1 liter. Gills and skin samples were prepared for investigation using histopathology and light and scanning electron microscopy techniques. The examination of water parameters revealed a substantial deterioration in water quality. The average value ± standard deviation shows that there are low oxygen levels ranging from 2.18 ± 0.08 to 2.85 ± 0.14 mg/l, high ammonia-N levels ranging from 2.01 ± 0.45 to 2.92 ± 0.66 mg/l, and high nitrite-N levels ranging from 0.82 ± 0.49 to 1.61 ± 0.66 mg/l. Trichodina, Monogenea, and Ambiphyra, which are external parasites, were found on the afflicted tissues. The histological examinations showed notable gill abnormalities, such as blood congestion, blood hemorrhage, fusion of secondary lamella, and raised epithelial tissue. Several fish displayed gill erosion and discharged mucus. The sickness has worsened the health of the fish, leading to the death of many individuals. The histological changes induced by ectoparasites on the gills and skin of fish can be used to evaluate the severity of ectoparasite infection. This allows for the development of effective strategies to enhance fish farm management and produce healthy fish that are safe for human consumption.

Brazilian aquaculture cultivates the alien species Nile tilapia. The aim of the study was to establish a pathological diagnosis in Oreochromis sp. fish species that are predominantly found in a river situated in southern Brazil. In addition, the study sought to identify the origin of these fish in their native environment, which is marked by unsatisfactory sanitary conditions caused by the release of industrial waste. A total of thirty fish were collected during three sample periods. The fish's health was assessed by conducting a survey of parasite fauna, analyzing parasitological markers, and examining the gills, liver, spleen, and kidney using histological procedures. The study identified five species of monogenea that were discovered infesting the gills and stomach. These species include Cichlidogyrus sclerosus, C. halli, C. thurstonae, Scutogyrus longicornis, and Enterogyrus cichlidarum. In addition, the researchers discovered four species of

trichodinidae that were infecting both the body surface and gills of the organism. These species include *Trichodina magna*, *T. compacta*, *T. centrostrigeata*, and *Paratrichodina africana*. The results showed differences depending on the seasonal pattern of the collection, with a higher quantity of parasites and more notable tissue alterations detected during the summer season. The histological examination showed different levels of severity, with necrosis being the most prevalent in all organs and fish. This suggests that the fish were not in an optimal condition of health (Tavares-Dias *et al.* 2021).

A study aimed to document the presence of myxosporean parasites on the gills and provide a detailed description of the resulting pathological changes. Changes in the cellular structure of the gills, mucous-producing cells, cells rich in mitochondria, levels of Heat Shock Protein 70 (Hsp70), and ATPase activity were seen in the gill tissue. The fish that were infected showed a significant amount of plasmodia of different sizes, which was detected by examining changes in their gills using histological methods. Plasmodia were found inside white ovoid-shaped structures situated on the gill filaments. Plasmodia were discovered on the main filament, causing considerable changes to the histological composition of the gill tissue. The research discovered an increase in both the concentration and dimensions of mucocytes in the affected areas of the gill tissue, whereas the quantity of cells rich in mitochondria dropped. No substantial disparity in the levels of Hsp70, a stress biomarker, was seen between the healthy fish and the infected fish (Lehmann *et al.* 2020).

3. MATERIALS AND METHODS

3.1 Fish Sample

A grand total of 100 fish were collected from various areas, such as the Misurata Fish Market and the sea fishing port. The fish in question are of two separate species and were caught from various areas in the coastal seas of Misrata, Libya. Consult APPENDIX 1 for additional information. The fish were harvested during the months of August and December. Their examination was performed to extract parasite helminths. Following the collection, the samples were brought to the laboratory of the Faculty of Science at Misurata University. Specifically, they were taken to the Department of Biology, Division of Zoology, in a container that maintained a cool temperature (APPENDIX 5) (APPENDIX 6) (APPENDIX 7).

The fish chosen for examination were acquired from the Qasr Ahmed sea fishing port and the Misurata Fish Market in Libya, during the period of August to December 2023. The study incorporated 50 predatory fish, specifically *Euthynnus alletteratus*, four times every month throughout the study period. Trawl nets are used to catch fish. 50 specimens of *Scomber scombrus*, also known as koala fish, were captured using the same manner and then examined to remove parasite worms.

3.2 Tools and Materials Required

The tools and materials need to used in the analysis are given as follow.

- Tools for sample collection: sterile, sharp blades, forceps, scissors, and a clean cutting board.
- Specimen receptacles: Petri tubes or dishes utilized for the storage of samples.
- Fixation solutions: for example, formalin maintained in water (4%) or pure alcohol.
- Ice is used to rapidly cool the samples.
- Safety equipment: gloves, goggles, lab coat.

3.3 Laboratory Steps

Collection and preparation in order to continue the research are given below.

- Collect fish ethically according to environmental standards.
- Preparing fish for blood draws and dissection of target organs

3.4 Samples

The samples used in this thesis are below

- Draw blood samples using safe and effective methods.
- Extracting the target organs (liver, stomach, heart).

3.5 Sample Preparation

During a comprehensive tissue study, the bulk of the fish specimens were examined while they were still in a fresh state, and in certain instances, while they were still alive. The technique commenced by precisely identifying the different fish species, followed by meticulously documenting crucial facts such as the inspection date, weight, and length. Visual inspection of the fish's external features without the aid of any instruments was an essential part of the procedure. The fish underwent a comprehensive examination to detect any evident signs of dermal alterations or alterations in ocular pigmentation indicative of a parasitic infestation.

As part of the inquiry, a dissection of the fish was performed. An incision was made along the ventral midline to inspect the fish's abdominal cavity and gastrointestinal tract. Moreover, after the operculum was extracted, a meticulous examination of the gills was conducted.

The experiments entailed the separation of the gastrointestinal tract and the longitudinal incision of the intestines within a Petri dish containing a physiological solution, in order to examine for potential parasitic worms. The worms were transferred meticulously to a 0.7% saline solution and stirred continuously for 15 minutes to ensure equilibrium. Subsequently, they were seen using a dissecting microscope and a magnifying glass. The worms were gathered using minuscule pipettes and meticulously rinsed with saline solution to eliminate any mucus or residual substances prior to being arranged in Petri dishes. In cases where parasites were securely adhered to the host tissues, samples were treated by subjecting them to a chilling process for several hours. This facilitated the natural detachment of the parasites, so simplifying the process of isolating them. In order to ascertain the presence of any possible parasites and assess their overall impact on the fish's well-being, analogous examinations were also conducted on the gills, stomach, liver, and spleen (APPENDIX 2).

The severity of parasitic infections in the study was assessed using a systematic and scientific approach, which involved estimating the density of each parasite infestation per host by mathematical calculations. This approach was established based on the pioneering research conducted by Margolis *et al.* (1982) and presented to the American Society of Parasitologists. The research aims to advocate for consistent infection criteria by adhering to exact terminological standards. The stated criteria formed the basis for the analytical framework of the inquiry, facilitating a comprehensive and intricate understanding of the parasite load and distribution in the analyzed specimens. The utilization of this empirical approach promoted uniformity and coherence in the analysis of parasitological information across diverse research settings, while also providing a detailed understanding of the distribution of infections.

The scientific equations used to calculate metrics for parasitic infection are as follows:

- The prevalence of infection is determined by dividing the total number of infections by the population at risk of the disease.

- This parameter quantifies the percentage of the fish population that has been impacted by the parasite at a certain moment, offering valuable information about the degree of parasitic dissemination among the group under investigation.
- The intensity of infection is calculated using the following equation

The infection intensity can be calculated by dividing the number of isolated worms by the number of infected fish. The infection intensity is calculated by dividing the number of infected fish by the number of isolated worms. This value represents the mean number of parasites detected in hosts that are afflicted, providing a metric for assessing the intensity of parasitic infestations among the impacted fish populations. These formulas are crucial for standardizing the evaluation of parasitic illnesses, therefore allowing for precise comparisons and analyses within and between various parasitological research. They offer a numerical basis for comprehending the epidemiology of the specific parasite illnesses (Figure 3.1) (Figure 3.2) (APPENDIX 3) (APPENDIX 4).

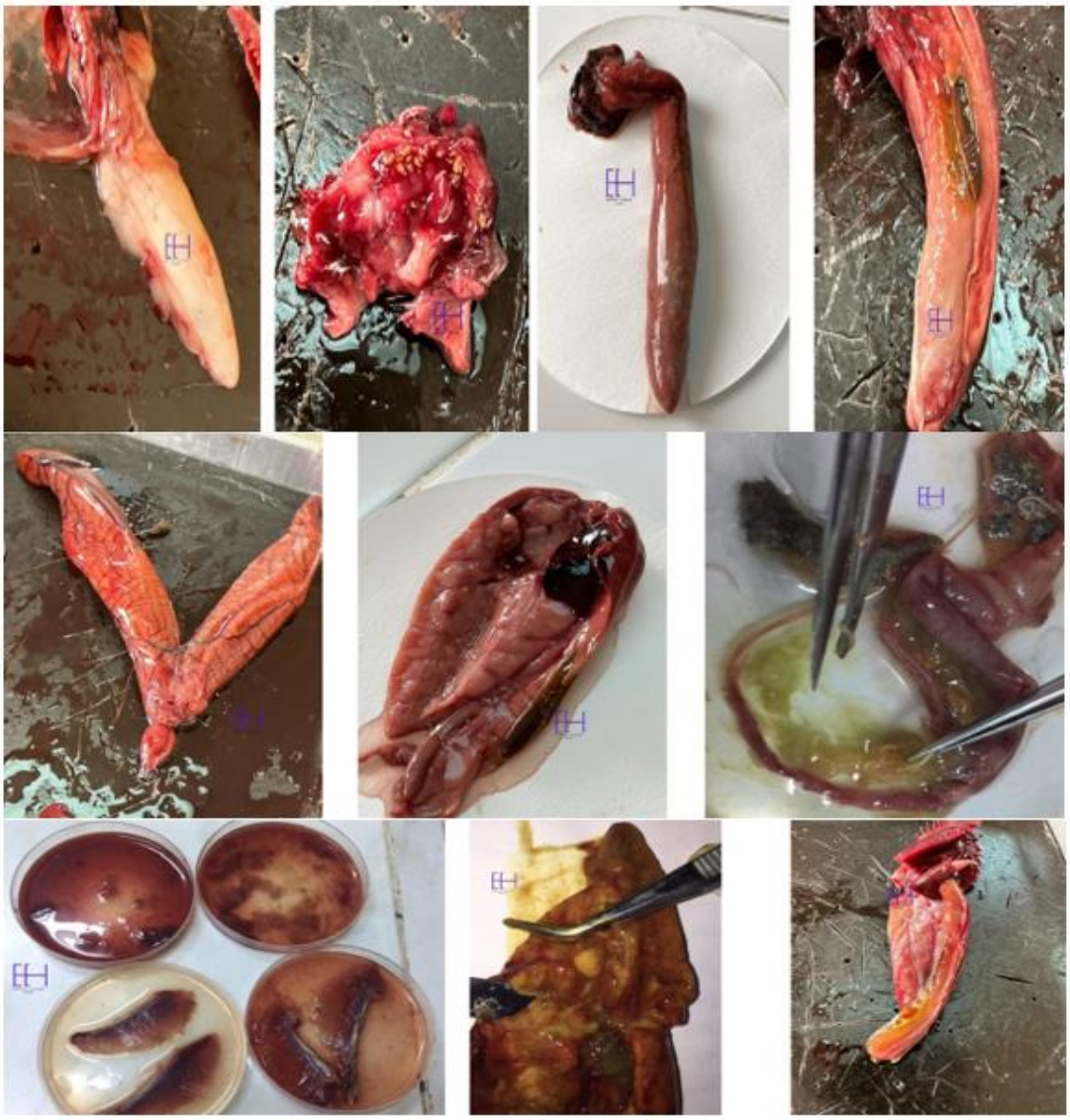


Figure 3.1 Fish tissue samples

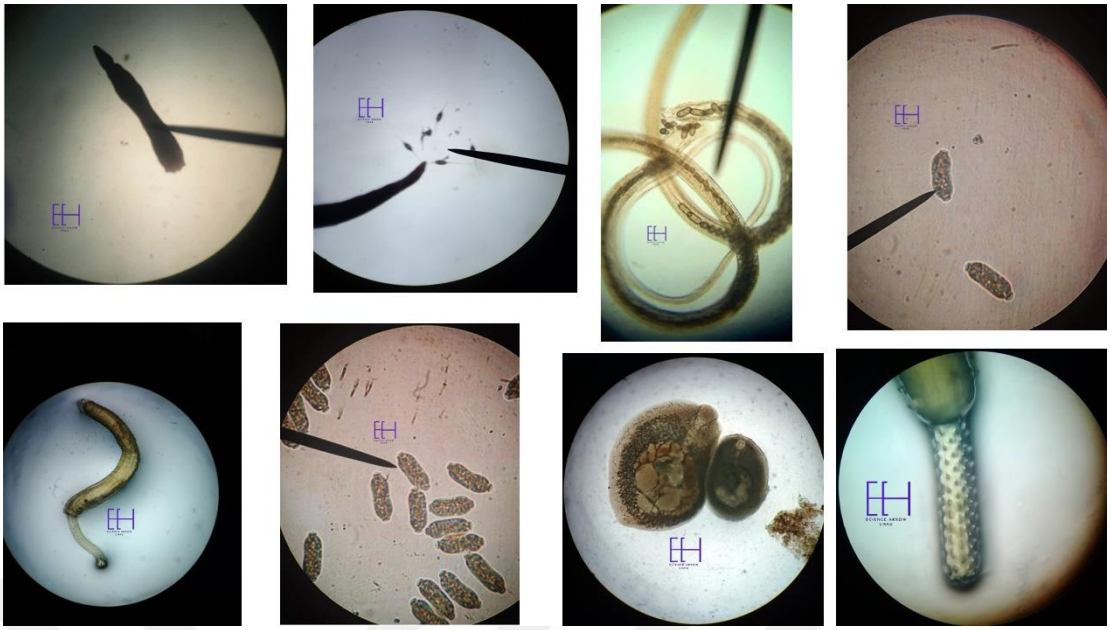


Figure 3.2 Parasitic finding under microscope

3.6 Histopathological Examination

- Overnight, tissues were rinsed in running water after being fixed for 48 hours in 10% neutral buffered formalin.
- The washed samples were dehydrated with ethyl alcohol at concentrations ranging from 70% to absolute alcohol.
- After 12 hours in each concentration, the samples were washed for 2 hours in xylol to remove any residual solvent.
- After the samples had been cleaned, they were placed in a sealed jar with 50% paraffin in xylol and incubated at 37°C for three hours.
- Afterward, the crucible was filled with melting paraffin and the oven was set to 48°C for another two hours.
- The tissue was subsequently blocked in hard paraffin and pieces of about 5 microns were cut from it.
- Histopathological investigation was performed by staining sections with HandE, mounting them with canada balsam.

3.7 Blood Sampling

To take blood samples from fish to study organ functions, below steps were followed;

- Preparation: All necessary equipment was brought, such as needles, gloves, and blood collection tubes.
- Determine the collection site: Blood is usually taken from the tail vein, but one of the blood samples is taken directly from the cardiac vein.
- Blood drawing: Using a suitable needle, blood is gently drawn from the selected vein.
- Sample storage: Blood samples were stored in special tubes and sent to the laboratory for analysis. After writing complete information on it.

As for the timing, blood was drawn from fish most of the time while they were alive and some of them were dead.

- Timing: Blood was drawn as soon as possible after the fish died to obtain accurate results. Because over time, changes in blood composition may occur due to post-mortem processes. This happened immediately after catching the fish, blood was drawn.
- Immediate analysis in the laboratory: The sample was analyzed as quickly as possible after collection to avoid negative effects of decomposition processes.

3.8 Measurements

The levels of urea, creatinine, sodium, potassium, ALT, AST, ALP, total protein, lipase, amylase, CPK, and LDH were measured using assay kits from Abcam (cat. No. ab83362/K375-100, ab204537, ab211096, ab252904, ab105134, ab105135, ab83369, ab219272, ab102524, ab102523, ab155901, ab102526) following the manufacturer's instructions.

3.9 Statistical Analysis

Statistical analyses were conducted for all measurements, which involved calculating the p-value using the Chi-Squares test. The significance of the measured data was assessed using the following methodology: In the realm of statistical analysis, a p-value that exceeds 0.05 is classified as non-significant (N.S), indicating insufficient evidence to reject the null hypothesis. Conversely, a p-value that is less than 0.05 is deemed significant, providing strong evidence to reject the null hypothesis.

4. RESULTS AND DISCUSSION

Table 4.1 displays the quantity of parasites found in each organ, together with the rate of infection. The numerals 0, 1, and 2 represent sample IDs, depending on the presentation. The subsequent column displays the quantity of parasites, whilst the final column denotes the rate of infection. Overall, these data emphasize the presence of two distinct categories of fish that were examined.

Table 4.1 The amount of parasites presents in each organ, and the infection rate

	Frequency	Percent	Valid Percent	Cumulative Percent
External body				
.00	57	60.0	60.0	60.0
1.00	24	25.3	25.3	85.3
2.00	12	12.6	12.6	97.9
3.00	2	2.1	2.1	100.0
Nostrils				
.00	44	46.3	46.3	46.3
1.00	31	32.6	32.6	78.9
2.00	10	10.5	10.5	89.5
3.00	5	5.3	5.3	94.7
4.00	3	3.2	3.2	97.9
5.00	2	2.1	2.1	100.0
Internal organs				
10 to 20	12	12.6	12.6	12.6
20 to 30	37	38.9	38.9	51.6
30 to 40	36	37.9	37.9	89.5
40 to 50	10	10.5	10.5	100.0
Stomach				
10 to 20	24	25.3	25.3	25.3
20 to 30	41	43.2	43.2	68.4
30 to 40	28	29.5	29.5	97.9
40 to 50	2	2.1	2.1	100.0
Anterior intestine				
10 to 20	50	52.6	52.6	52.6
20 to 30	26	27.4	27.4	80.0
30 to 40	18	18.9	18.9	98.9
40 to 50	1	1.1	1.1	100.0
Hind intestine				
10 to 20	15	15.8	15.8	15.8
20 to 30	49	51.6	51.6	67.4
30 to 40	27	28.4	28.4	95.8
40 to 50	4	4.2	4.2	100.0
Total	95	100.0	100.0	

The Figure 4.1 and Figure 4.2 depicts the quantity of parasites detected in each organ, along with the infection rate shown as a percentage. The numerals 0, 1, and 2 function as sample identifiers. The subsequent column denotes the number of parasites present, whereas the ultimate column signifies the percentage of infection. This data specifically offers insights into two discrete categories of fish that are being examined.

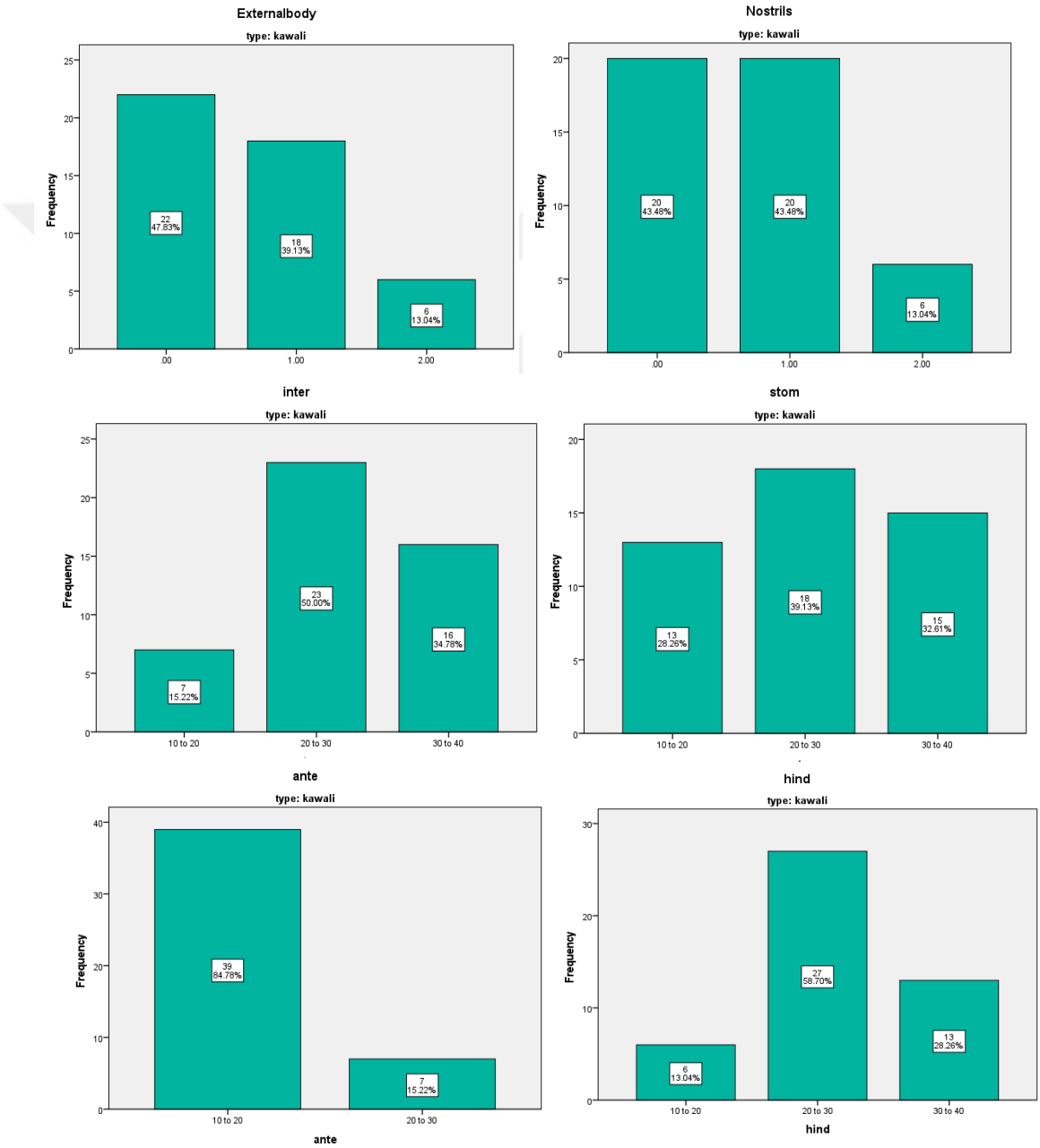


Figure 4.1 The quantity of parasites found in each organ and the rate of infection in Kawali

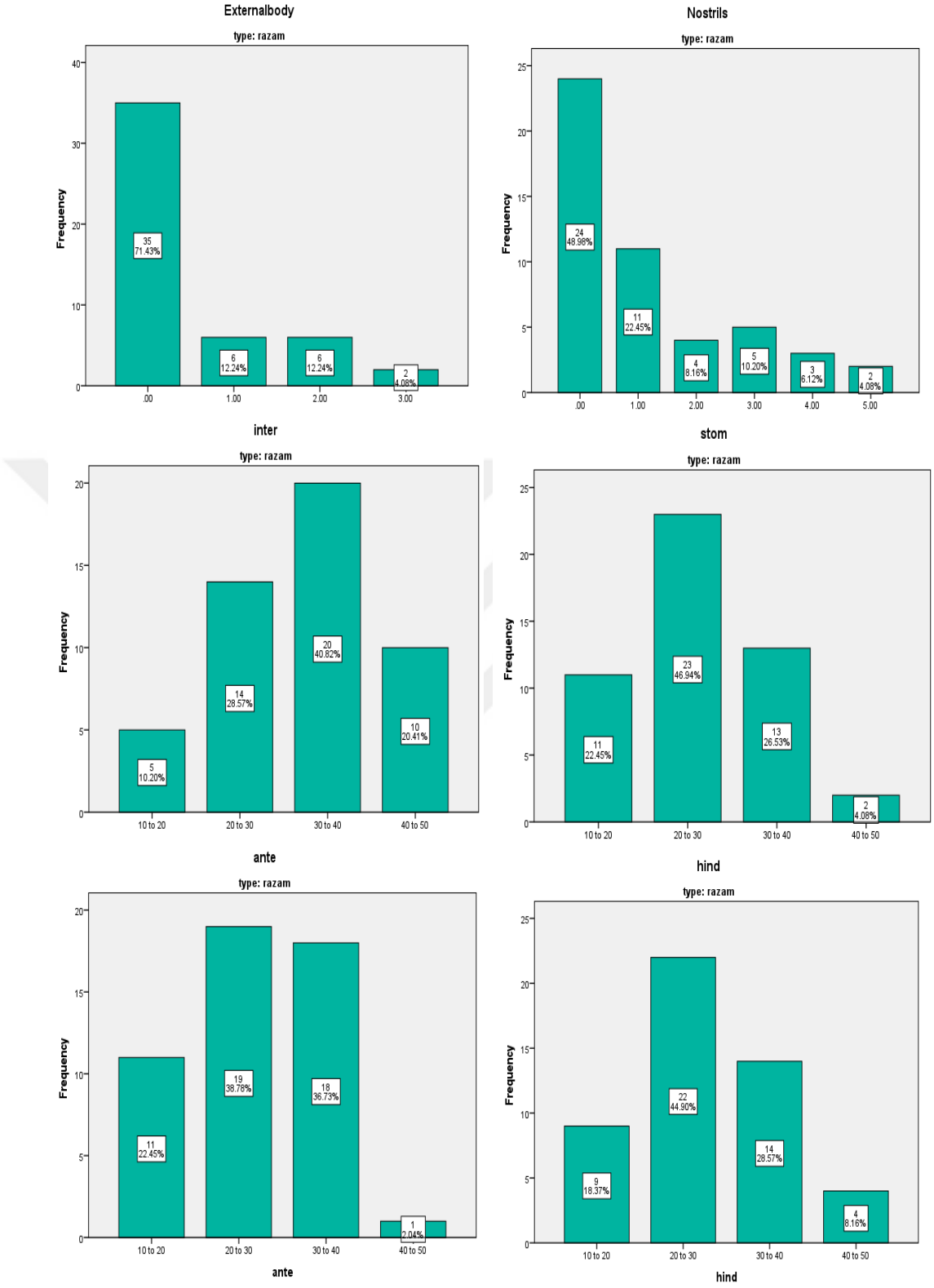


Figure 4.2 The quantity of parasites found in each organ and the rate of infection in Razam

Figure 4.3 displays the histopathological results in the Kawali species.

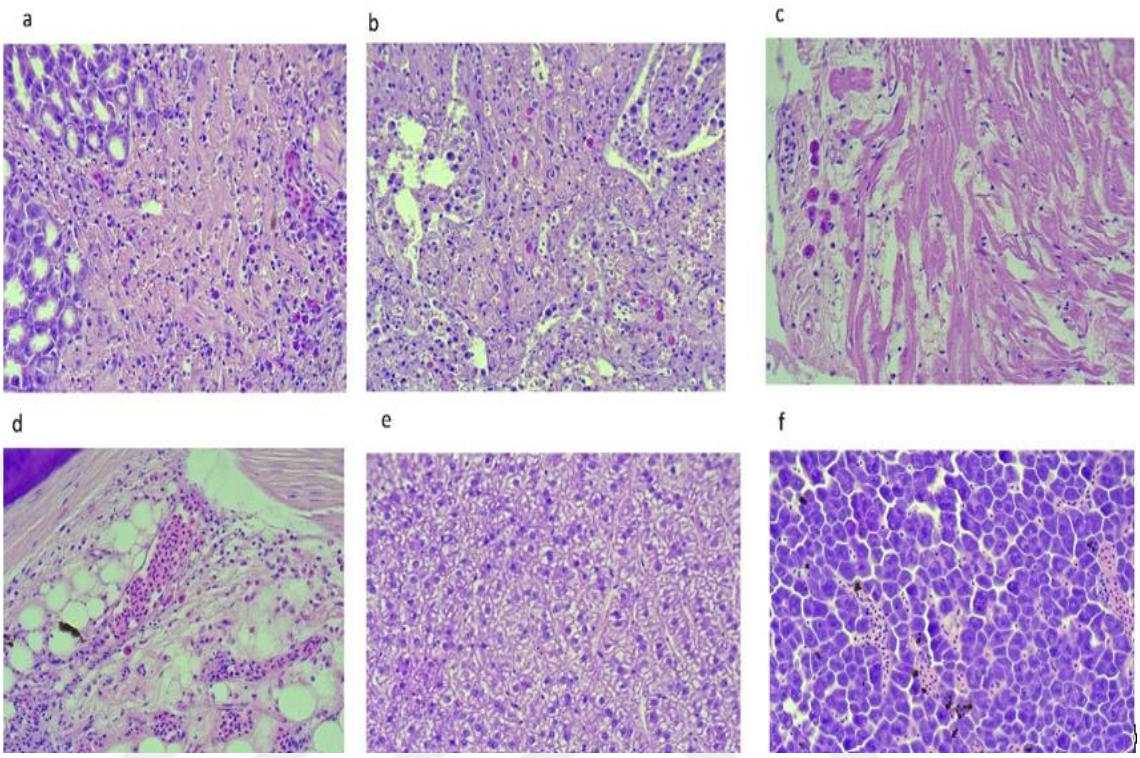


Figure 4.3 The histopathological results in the Kawali species

The stomach exhibits a predominance of fibroblasts, along with the presence of lymphocytes, macrophages, and eosinophilic granulocytes (a). In the gut, there is degradation of the mucosal lining and a tissue response characterized by the buildup of cellular debris at the interface between the tissue and the parasite. This is accompanied by the formation of a granulomatous reaction resembling a foreign body reaction, which is characterized by the presence of giant cells. The lamina propria exhibits significant and varying infiltration of eosinophils, as well as lymphocytes and macrophages with hemosiderin pigment (b). The myocardium, located in the heart, has significant pathological alterations characterized by widespread inflammation and multifocal necrosis of myocytes (c). Eosinophils, specifically, infiltrate the nostrils as part of an inflammatory response. The histological analysis of liver tissue revealed substantial congestion of blood vessels, infiltration of mononuclear cells in the portal triads, and hyperplasia of the bile duct in the liver. The hepatocytes exhibited degenerative alterations characterized by granular and vacuolar degeneration, as well as coagulative

necrosis (e). Hydropic degeneration and autolysis occur in the cells of the liver and pancreas.

Figure 4.4 displays the histopathological results in Razam species.

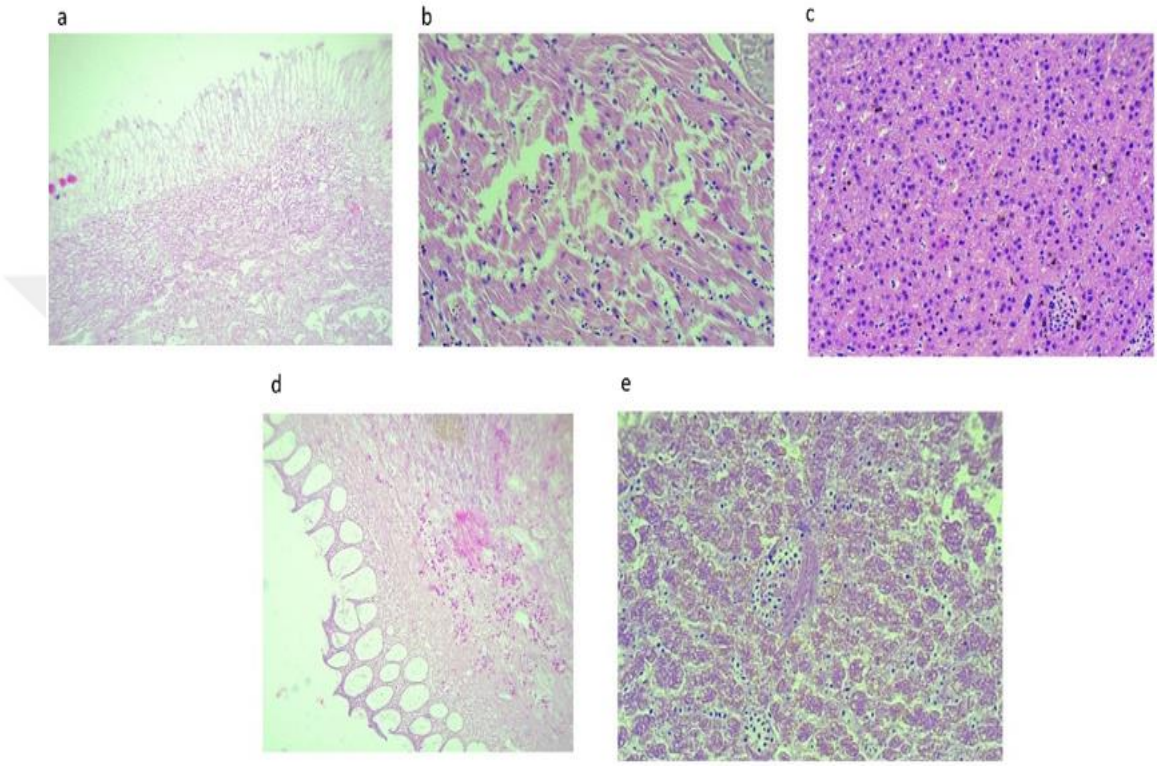


Figure 4.4 The histopathological results in Razam species

The stomach exhibits a predominance of hyperplasia in the mucous membranes and submucosa, accompanied by autolysis (a). The heart exhibits hypertrophy accompanied by widespread inflammation and multifocal necrosis of myocytes. The liver exhibits significant vacuole degradation and accumulation of fatty hepatocytes, accompanied by the loss of hepatic tissue structure characterized by multifocal necrosis. Additionally, there is congestion observed in the interlobular veins (c). In the intestine, there are autolytic changes occurring in the intestinal wall. In the liver and pancreas, there are autolytic alterations occurring in the liver.

The Table 4.2 show the amount of parasites (Cestoda, Trematode, Acanthocephalan) present in each organ, and the infection rate in both kawali and razam.

Table 4.2 The amount of each parasites presents in each organ, and the infection rate

Type	Dep. Variable	(I) MemName	(J) MemName	Mean Dif. (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
S. scomberus	Cestoda	External body	Nostrils	.02000	.09905	.840	-.1748	.2148
			Internal organs	-.90000*	.09905	.000	-1.0948	-.7052
			stomach	-.70000*	.09905	.000	-.8948	-.5052
			Anterior intestine	-.54000*	.09905	.000	-.7348	-.3452
			Hind intestine	-.50000*	.09905	.000	-.6948	-.3052
			liver	-.20000*	.09905	.044	-.3948	-.0052
		Nostrils	External body	-.02000	.09905	.840	-.2148	.1748
			Internal organs	-.92000*	.09905	.000	-1.1148	-.7252
			stomach	-.72000*	.09905	.000	-.9148	-.5252
			Anterior intestine	-.56000*	.09905	.000	-.7548	-.3652
			Hind intestine	-.52000*	.09905	.000	-.7148	-.3252
			liver	-.22000*	.09905	.027	-.4148	-.0252
		Internal organs	External body	.90000*	.09905	.000	.7052	1.0948
			Nostrils	.92000*	.09905	.000	.7252	1.1148
			stomach	.20000*	.09905	.044	.0052	.3948
			Anterior intestine	.36000*	.09905	.000	.1652	.5548
			Hind intestine	.40000*	.09905	.000	.2052	.5948
			liver	.70000*	.09905	.000	.5052	.8948
		stomach	External body	.70000*	.09905	.000	.5052	.8948
			Nostrils	.72000*	.09905	.000	.5252	.9148
			Internal organs	-.20000*	.09905	.044	-.3948	-.0052
			Anterior intestine	.16000	.09905	.107	-.0348	.3548
			Hind intestine	.20000*	.09905	.044	.0052	.3948
			liver	.50000*	.09905	.000	.3052	.6948
		Anterior intestine	External body	.54000*	.09905	.000	.3452	.7348
			Nostrils	.56000*	.09905	.000	.3652	.7548
			Internal organs	-.36000*	.09905	.000	-.5548	-.1652
			stomach	-.16000	.09905	.107	-.3548	.0348
			Hind intestine	.04000	.09905	.687	-.1548	.2348
			liver	.34000*	.09905	.001	.1452	.5348
		Hind intestine	External body	.50000*	.09905	.000	.3052	.6948
			Nostrils	.52000*	.09905	.000	.3252	.7148
			Internal organs	-.40000*	.09905	.000	-.5948	-.2052
			stomach	-.20000*	.09905	.044	-.3948	-.0052
			Anterior intestine	-.04000	.09905	.687	-.2348	.1548
			liver	.30000*	.09905	.003	.1052	.4948
		liver	External body	.20000*	.09905	.044	.0052	.3948
			Nostrils	.22000*	.09905	.027	.0252	.4148
			Internal organs	-.70000*	.09905	.000	-.8948	-.5052
			stomach	-.50000*	.09905	.000	-.6948	-.3052
			Anterior intestine	-.34000*	.09905	.001	-.5348	-.1452
			Hind intestine	-.30000*	.09905	.003	-.4948	-.1052
	Trematode	External body	Nostrils	.02000	.17474	.909	-.3237	.3637
			Internal organs	-1.36000*	.17474	.000	-1.7037	-1.0163
			stomach	-.98000*	.17474	.000	-1.3237	-.6363
			Anterior intestine	-.68000*	.17474	.000	-1.0237	-.3363
			Hind intestine	-.68000*	.17474	.000	-1.0237	-.3363
			liver	-.26000	.17474	.138	-.6037	.0837

Table 4.2 The amount of each parasites presents in each organ, and the infection rate (Continued)

Type	Dep. Variable	(I) MemName	(J) MemName	Mean Dif. (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Lower Bound
		Nostrils	External body	-.02000	.17474	.909	-.3637	.3237
			Internal organs	-1.38000*	.17474	.000	-1.7237	-1.0363
			stomach	-1.00000*	.17474	.000	-1.3437	-.6563
			Anterior intestine	-.70000*	.17474	.000	-1.0437	-.3563
			Hind intestine	-.70000*	.17474	.000	-1.0437	-.3563
			liver	-.28000	.17474	.110	-.6237	.0637
		Internal organs	External body	1.36000*	.17474	.000	1.0163	1.7037
			Nostrils	1.38000*	.17474	.000	1.0363	1.7237
			stomach	.38000*	.17474	.030	.0363	.7237
			Anterior intestine	.68000*	.17474	.000	.3363	1.0237
			Hind intestine	.68000*	.17474	.000	.3363	1.0237
			liver	1.10000*	.17474	.000	.7563	1.4437
		stomach	External body	.98000*	.17474	.000	.6363	1.3237
			Nostrils	1.00000*	.17474	.000	.6563	1.3437
			Internal organs	-.38000*	.17474	.030	-.7237	-.0363
			Anterior intestine	.30000	.17474	.087	-.0437	.6437
			Hind intestine	.30000	.17474	.087	-.0437	.6437
			liver	.72000*	.17474	.000	.3763	1.0637
		Anterior intestine	External body	.68000*	.17474	.000	.3363	1.0237
			Nostrils	.70000*	.17474	.000	.3563	1.0437
			Internal organs	-.68000*	.17474	.000	-1.0237	-.3363
			stomach	-.30000	.17474	.087	-.6437	.0437
			Hind intestine	.00000	.17474	1.000	-.3437	.3437
			liver	.42000*	.17474	.017	.0763	.7637
		Hind intestine	External body	.68000*	.17474	.000	.3363	1.0237
			Nostrils	.70000*	.17474	.000	.3563	1.0437
			Internal organs	-.68000*	.17474	.000	-1.0237	-.3363
			stomach	-.30000	.17474	.087	-.6437	.0437
			Anterior intestine	.00000	.17474	1.000	-.3437	.3437
			liver	.42000*	.17474	.017	.0763	.7637
		liver	External body	.26000	.17474	.138	-.0837	.6037
			Nostrils	.28000	.17474	.110	-.0637	.6237
			Internal organs	-1.10000*	.17474	.000	-1.4437	-.7563
			stomach	-.72000*	.17474	.000	-1.0637	-.3763
			Anterior intestine	-.42000*	.17474	.017	-.7637	-.0763
			Hind intestine	-.42000*	.17474	.017	-.7637	-.0763
	Acanthocephalan	External body	Nostrils	-.74000	1.24880	.554	-3.1963	1.7163
			Internal organs	-29.62000*	1.24880	.000	-32.0763	-27.1637
			stomach	-23.96000*	1.24880	.000	-26.4163	-21.5037
			Anterior intestine	-25.40000*	1.24880	.000	-27.8563	-22.9437
			Hind intestine	-26.66000*	1.24880	.000	-29.1163	-24.2037
			liver	-21.30000*	1.24880	.000	-23.7563	-18.8437
		Nostrils	External body	.74000	1.24880	.554	-1.7163	3.1963
			Internal organs	-28.88000*	1.24880	.000	-31.3363	-26.4237
			stomach	-23.22000*	1.24880	.000	-25.6763	-20.7637
			Anterior intestine	-24.66000*	1.24880	.000	-27.1163	-22.2037
			Hind intestine	-25.92000*	1.24880	.000	-28.3763	-23.4637
			liver	-20.56000*	1.24880	.000	-23.0163	-18.1037
		Internal organs	External body	29.62000*	1.24880	.000	27.1637	32.0763
			Nostrils	28.88000*	1.24880	.000	26.4237	31.3363
			stomach	5.66000*	1.24880	.000	3.2037	8.1163
			Anterior intestine	4.22000*	1.24880	.001	1.7637	6.6763
			Hind intestine	2.96000*	1.24880	.018	.5037	5.4163
			liver	8.32000*	1.24880	.000	5.8637	10.7763

Table 4.2 The amount of each parasites presents in each organ, and the infection rate (Continued)

Type	Dep. Variable	(I) MemName	(J) MemName	Mean Dif. (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Lower Bound
		stomach	External body	23.96000*	1.24880	.000	21.5037	26.4163
			Nostrils	23.22000*	1.24880	.000	20.7637	25.6763
			Internal organs	-5.66000*	1.24880	.000	-8.1163	-3.2037
			Anterior intestine	-1.44000	1.24880	.250	-3.8963	1.0163
			Hind intestine	-2.70000*	1.24880	.031	-5.1563	-.2437
			liver	2.66000*	1.24880	.034	.2037	5.1163
		Anterior intestine	External body	25.40000*	1.24880	.000	22.9437	27.8563
			Nostrils	24.66000*	1.24880	.000	22.2037	27.1163
			Internal organs	-4.22000*	1.24880	.001	-6.6763	-1.7637
			stomach	1.44000	1.24880	.250	-1.0163	3.8963
			Hind intestine	-1.26000	1.24880	.314	-3.7163	1.1963
			liver	4.10000*	1.24880	.001	1.6437	6.5563
		Hind intestine	External body	26.66000*	1.24880	.000	24.2037	29.1163
			Nostrils	25.92000*	1.24880	.000	23.4637	28.3763
			Internal organs	-2.96000*	1.24880	.018	-5.4163	-.5037
			stomach	2.70000*	1.24880	.031	.2437	5.1563
			Anterior intestine	1.26000	1.24880	.314	-1.1963	3.7163
			liver	5.36000*	1.24880	.000	2.9037	7.8163
		liver	External body	21.30000*	1.24880	.000	18.8437	23.7563
			Nostrils	20.56000*	1.24880	.000	18.1037	23.0163
			Internal organs	-8.32000*	1.24880	.000	-10.7763	-5.8637
			stomach	-2.66000*	1.24880	.034	-5.1163	-.2037
			Anterior intestine	-4.10000*	1.24880	.001	-6.5563	-1.6437
			Hind intestine	-5.36000*	1.24880	.000	-7.8163	-2.9037
E. alletteratus	Cestoda	External body	Nostrils	-.02000	.10319	.846	-.2230	.1830
			Internal organs	-1.06000*	.10319	.000	-1.2630	-.8570
			stomach	-.70000*	.10319	.000	-.9030	-.4970
			Anterior intestine	-.48000*	.10319	.000	-.6830	-.2770
			Hind intestine	-.50000*	.10319	.000	-.7030	-.2970
			liver	-.14000	.10319	.176	-.3430	.0630
		Nostrils	External body	.02000	.10319	.846	-.1830	.2230
			Internal organs	-1.04000*	.10319	.000	-1.2430	-.8370
			stomach	-.68000*	.10319	.000	-.8830	-.4770
			Anterior intestine	-.46000*	.10319	.000	-.6630	-.2570
			Hind intestine	-.48000*	.10319	.000	-.6830	-.2770
			liver	-.12000	.10319	.246	-.3230	.0830
		Internal organs	External body	1.06000*	.10319	.000	.8570	1.2630
			Nostrils	1.04000*	.10319	.000	.8370	1.2430
			stomach	.36000*	.10319	.001	.1570	.5630
			Anterior intestine	.58000*	.10319	.000	.3770	.7830
			Hind intestine	.56000*	.10319	.000	.3570	.7630
			liver	.92000*	.10319	.000	.7170	1.1230
		stomach	External body	.70000*	.10319	.000	.4970	.9030
			Nostrils	.68000*	.10319	.000	.4770	.8830
			Internal organs	-.36000*	.10319	.001	-.5630	-.1570
			Anterior intestine	.22000*	.10319	.034	.0170	.4230
			Hind intestine	.20000	.10319	.053	-.0030	.4030
			liver	.56000*	.10319	.000	.3570	.7630
		Anterior intestine	External body	.48000*	.10319	.000	.2770	.6830
			Nostrils	.46000*	.10319	.000	.2570	.6630
			Internal organs	-.58000*	.10319	.000	-.7830	-.3770
			stomach	-.22000*	.10319	.034	-.4230	-.0170
			Hind intestine	-.02000	.10319	.846	-.2230	.1830
			liver	.34000*	.10319	.001	.1370	.5430

Table 4.2 The amount of each parasites presents in each organ, and the infection rate (Continued)

Type	Dep. Variable	(I) MemName	(J) MemName	Mean Dif. (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Lower Bound
		Hind intestine	External body	.50000*	.10319	.000	.2970	.7030
			Nostrils	.48000*	.10319	.000	.2770	.6830
			Internal organs	-.56000*	.10319	.000	-.7630	-.3570
			stomach	-.20000	.10319	.053	-.4030	.0030
			Anterior intestine	.02000	.10319	.846	-.1830	.2230
			liver	.36000*	.10319	.001	.1570	.5630
		liver	External body	.14000	.10319	.176	-.0630	.3430
			Nostrils	.12000	.10319	.246	-.0830	.3230
			Internal organs	-.92000*	.10319	.000	-1.1230	-.7170
			stomach	-.56000*	.10319	.000	-.7630	-.3570
			Anterior intestine	-.34000*	.10319	.001	-.5430	-.1370
			Hind intestine	-.36000*	.10319	.001	-.5630	-.1570
	Trematode	External body	Nostrils	.00000	.25118	1.000	-.4940	.4940
			Internal organs	-1.42000*	.25118	.000	-1.9140	-.9260
			stomach	-.80000*	.25118	.002	-1.2940	-.3060
			Anterior intestine	-.56000*	.25118	.026	-1.0540	-.0660
			Hind intestine	-.60000*	.25118	.017	-1.0940	-.1060
			liver	-.10000	.25118	.691	-.5940	.3940
		Nostrils	External body	.00000	.25118	1.000	-.4940	.4940
			Internal organs	-1.42000*	.25118	.000	-1.9140	-.9260
			stomach	-.80000*	.25118	.002	-1.2940	-.3060
			Anterior intestine	-.56000*	.25118	.026	-1.0540	-.0660
			Hind intestine	-.60000*	.25118	.017	-1.0940	-.1060
			liver	-.10000	.25118	.691	-.5940	.3940
		Internal organs	External body	1.42000*	.25118	.000	.9260	1.9140
			Nostrils	1.42000*	.25118	.000	.9260	1.9140
			stomach	.62000*	.25118	.014	.1260	1.1140
			Anterior intestine	.86000*	.25118	.001	.3660	1.3540
			Hind intestine	.82000*	.25118	.001	.3260	1.3140
			liver	1.32000*	.25118	.000	.8260	1.8140
		stomach	External body	.80000*	.25118	.002	.3060	1.2940
			Nostrils	.80000*	.25118	.002	.3060	1.2940
			Internal organs	-.62000*	.25118	.014	-1.1140	-.1260
			Anterior intestine	.24000	.25118	.340	-.2540	.7340
			Hind intestine	.20000	.25118	.426	-.2940	.6940
			liver	.70000*	.25118	.006	.2060	1.1940
		Anterior intestine	External body	.56000*	.25118	.026	.0660	1.0540
			Nostrils	.56000*	.25118	.026	.0660	1.0540
			Internal organs	-.86000*	.25118	.001	-1.3540	-.3660
			stomach	-.24000	.25118	.340	-.7340	.2540
			Hind intestine	-.04000	.25118	.874	-.5340	.4540
			liver	.46000	.25118	.068	-.0340	.9540
		Hind intestine	External body	.60000*	.25118	.017	.1060	1.0940
			Nostrils	.60000*	.25118	.017	.1060	1.0940
			Internal organs	-.82000*	.25118	.001	-1.3140	-.3260
			stomach	-.20000	.25118	.426	-.6940	.2940
			Anterior intestine	.04000	.25118	.874	-.4540	.5340
			liver	.50000*	.25118	.047	.0060	.9940
		liver	External body	.10000	.25118	.691	-.3940	.5940
			Nostrils	.10000	.25118	.691	-.3940	.5940
			Internal organs	-1.32000*	.25118	.000	-1.8140	-.8260
			stomach	-.70000*	.25118	.006	-1.1940	-.2060
			Anterior intestine	-.46000	.25118	.068	-.9540	.0340
			Hind intestine	-.50000*	.25118	.047	-.9940	-.0060

Table 4.2 The amount of each parasites presents in each organ, and the infection rate (Continued)

Type	Dep. Variable	(I) MemName	(J) MemName	Mean Dif. (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Lower Bound
	Acanthocephalan	External body	Nostrils	1.06000	1.09294	.333	-1.0897	3.2097
			Internal organs	-21.72000*	1.09294	.000	-23.8697	-19.5703
			stomach	-23.50000*	1.09294	.000	-25.6497	-21.3503
			Anterior intestine	-13.54000*	1.09294	.000	-15.6897	-11.3903
			Hind intestine	-23.70000*	1.09294	.000	-25.8497	-21.5503
			liver	-13.42000*	1.09294	.000	-15.5697	-11.2703
		Nostrils	External body	-1.06000	1.09294	.333	-3.2097	1.0897
			Internal organs	-22.78000*	1.09294	.000	-24.9297	-20.6303
			stomach	-24.56000*	1.09294	.000	-26.7097	-22.4103
			Anterior intestine	-14.60000*	1.09294	.000	-16.7497	-12.4503
			Hind intestine	-24.76000*	1.09294	.000	-26.9097	-22.6103
			liver	-14.48000*	1.09294	.000	-16.6297	-12.3303
		Internal organs	External body	21.72000*	1.09294	.000	19.5703	23.8697
			Nostrils	22.78000*	1.09294	.000	20.6303	24.9297
			stomach	-1.78000	1.09294	.104	-3.9297	.3697
			Anterior intestine	8.18000*	1.09294	.000	6.0303	10.3297
			Hind intestine	-1.98000	1.09294	.071	-4.1297	.1697
			liver	8.30000*	1.09294	.000	6.1503	10.4497
		stomach	External body	23.50000*	1.09294	.000	21.3503	25.6497
			Nostrils	24.56000*	1.09294	.000	22.4103	26.7097
			Internal organs	1.78000	1.09294	.104	-.3697	3.9297
			Anterior intestine	9.96000*	1.09294	.000	7.8103	12.1097
			Hind intestine	-.20000	1.09294	.855	-2.3497	1.9497
			liver	10.08000*	1.09294	.000	7.9303	12.2297
		Anterior intestine	External body	13.54000*	1.09294	.000	11.3903	15.6897
			Nostrils	14.60000*	1.09294	.000	12.4503	16.7497
			Internal organs	-8.18000*	1.09294	.000	-10.3297	-6.0303
			stomach	-9.96000*	1.09294	.000	-12.1097	-7.8103
			Hind intestine	-10.16000*	1.09294	.000	-12.3097	-8.0103
			liver	.12000	1.09294	.913	-2.0297	2.2697
		Hind intestine	External body	23.70000*	1.09294	.000	21.5503	25.8497
			Nostrils	24.76000*	1.09294	.000	22.6103	26.9097
			Internal organs	1.98000	1.09294	.071	-.1697	4.1297
			stomach	.20000	1.09294	.855	-1.9497	2.3497
			Anterior intestine	10.16000*	1.09294	.000	8.0103	12.3097
			liver	10.28000*	1.09294	.000	8.1303	12.4297
		liver	External body	13.42000*	1.09294	.000	11.2703	15.5697
			Nostrils	14.48000*	1.09294	.000	12.3303	16.6297
			Internal organs	-8.30000*	1.09294	.000	-10.4497	-6.1503
			stomach	-10.08000*	1.09294	.000	-12.2297	-7.9303
			Anterior intestine	-.12000	1.09294	.913	-2.2697	2.0297
			Hind intestine	-10.28000*	1.09294	.000	-12.4297	-8.1303

The results of the Independent Samples Test offer information into the distribution of parasites throughout various organs and the infection rates linked to each parasite in Table 4.3. Levene's Test for Equality of Variances determines if the variances of parasite counts are the same across groups, while the t-test for Equality of Means evaluates if there are

significant variations in parasite counts between groups. In general, the findings indicate diverse patterns of parasite distribution and infection rates among different organs. Acanthocephalan parasites display notable variations in infection rates among different organs, with the anterior intestine, hind intestine, and liver showing higher infection rates compared to other organs. Nevertheless, Cestoda and Trematode parasites exhibit inconsistent patterns, since certain organs have notable variations in infection rates while others do not. Acanthocephalan parasites show significant variations in infection rates among different organs. Notably, there are statistically significant differences in the infection rates observed in the anterior intestine (mean difference=10.64, $p<0.001$), hind intestine (mean difference=10.64, $p<0.001$), and liver (mean difference=6.66, $p<0.001$) compared to other organs. Furthermore, there are notable variations in infection rates for Trematode parasites in the liver (mean difference=0.18, $p=0.075$) and Acanthocephalan parasites in the liver (mean difference=6.66, $p<0.001$). Nevertheless, Cestoda parasites do not exhibit any statistically significant variations in infection rates among different organs. These findings emphasize the distinct patterns of parasite distribution in different organs and emphasize the importance of developing customized treatment techniques that take into account the vulnerabilities of each organ. Additional study is necessary to clarify the underlying mechanisms that are causing these observed patterns and to understand their implications for attempts to control parasites and avoid diseases.

Table 4.3 The amount of each parasites presents in each organ, and the infection rate by Independent Samples Test

		Levene's Test for Equality of Variances		T-test for Equality of Means						
									95% Confidence Interval of the Diff.	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Diff.	Std. Error Diff.	Lower	Upper
External body	Cestoda	4.168	.044	1.000	98	.320	.02000	.02000	-.01969	.05969
				1.000	49.000	.322	.02000	.02000	-.02019	.06019
	Trematode	4.168	.044	1.000	98	.320	.02000	.02000	-.01969	.05969
				1.000	49.000	.322	.02000	.02000	-.02019	.06019
	Acanthocephalan	5.742	.018	-2.209	98	.030	-1.22000	.55229	-2.31599	-.12401
				-2.209	53.151	.032	-1.22000	.55229	-2.32767	-.11233
Nostrils	Cestoda	4.168	.044	-1.000	98	.320	-.02000	.02000	-.05969	.01969
				-1.000	49.000	.322	-.02000	.02000	-.06019	.02019
	Acanthocephalan	20.611	.000	2.428	98	.017	.58000	.23886	.10599	1.05401
				2.428	70.084	.018	.58000	.23886	.10362	1.05638
Internal organs	Cestoda	.818	.368	-1.016	98	.312	-.14000	.13777	-.41339	.13339
				-1.016	95.743	.312	-.14000	.13777	-.41347	.13347
	Trematode	.224	.637	-.083	98	.934	-.04000	.48063	-.99379	.91379
				-.083	69.057	.934	-.04000	.48063	-.99881	.91881
	Acanthocephalan	.359	.550	3.962	98	.000	6.68000	1.68592	3.33435	10.02565
				3.962	97.685	.000	6.68000	1.68592	3.33422	10.02578
stomach	Cestoda	.034	.855	.152	98	.880	.02000	.13183	-.24162	.28162
				.152	97.865	.880	.02000	.13183	-.24162	.28162
	Trematode	1.517	.221	1.136	98	.259	.20000	.17613	-.14952	.54952
				1.136	89.113	.259	.20000	.17613	-.14995	.54995
	Acanthocephalan	.035	.853	-.533	98	.596	-.76000	1.42708	-3.59199	2.07199
				-.533	97.941	.596	-.76000	1.42708	-3.59201	2.07201
Anterior intestine	Cestoda	.000	1.000	.691	98	.491	.08000	.11571	-.14961	.30961
				.691	97.998	.491	.08000	.11571	-.14961	.30961
	Trematode	2.206	.141	.919	98	.360	.14000	.15229	-.16221	.44221
				.919	90.649	.360	.14000	.15229	-.16252	.44252
	Acanthocephalan	13.327	.000	8.400	98	.000	10.64000	1.26662	8.12643	13.15357
				8.400	83.748	.000	10.64000	1.26662	8.12108	13.15892
Hind intestine	Cestoda	1.037	.311	.172	98	.863	.02000	.11602	-.21024	.25024
				.172	96.565	.863	.02000	.11602	-.21029	.25029
	Trematode	.681	.411	.559	98	.578	.10000	.17900	-.25522	.45522
				.559	85.146	.578	.10000	.17900	-.25589	.45589

Table 4.3 The amount of each parasites presents in each organ, and the infection rate by Independent Samples Test (Continued)

		Levene's Test for Equality of Variances		T-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Diff.	Std. Error Diff.	95% Confidence Interval of the Diff.	
									Lower	Upper
	Acanthocephalan	4.126	.045	1.392	98	.167	1.74000	1.24983	-.74024	4.22024
				1.392	91.836	.167	1.74000	1.24983	-.74232	4.22232
liver	Cestoda	2.916	.091	.918	98	.361	.08000	.08713	-.09291	.25291
				.918	96.178	.361	.08000	.08713	-.09295	.25295
	Trematode	13.615	.000	1.797	98	.075	.18000	.10016	-.01877	.37877
				1.797	69.912	.077	.18000	.10016	-.01977	.37977
	Acanthocephalan	1.224	.271	6.001	98	.000	6.66000	1.10981	4.45762	8.86238
				6.001	94.313	.000	6.66000	1.10981	4.45654	8.86346

The Figure 4.5 and Figure 4.6 shows the amount of Cestoda were high in internal organs, stomach, anterior intestine, hind intestine, and liver in both *Scomber scombrus* and *Euthynnus alletteratus*.

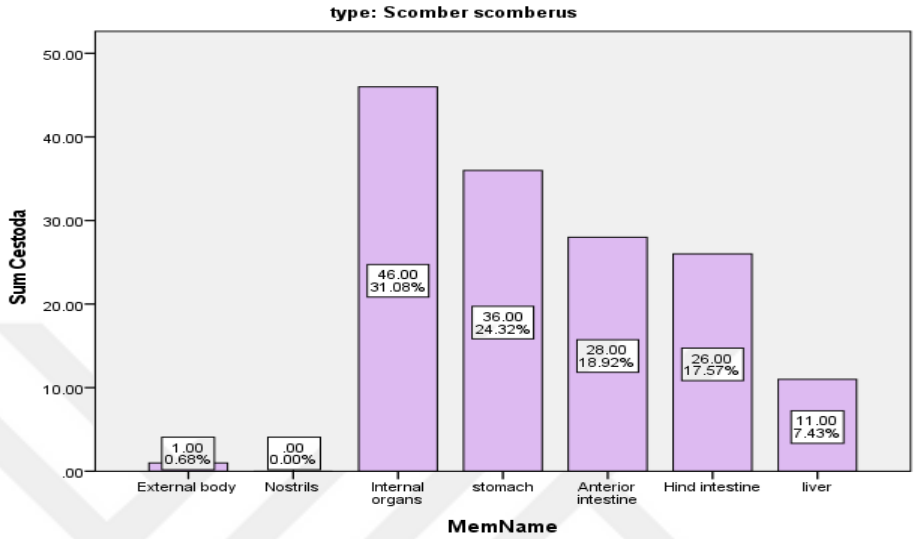


Figure 4.5 The quantity of cestoda found in each organ and the rate of infection in *Scomber scombrus*

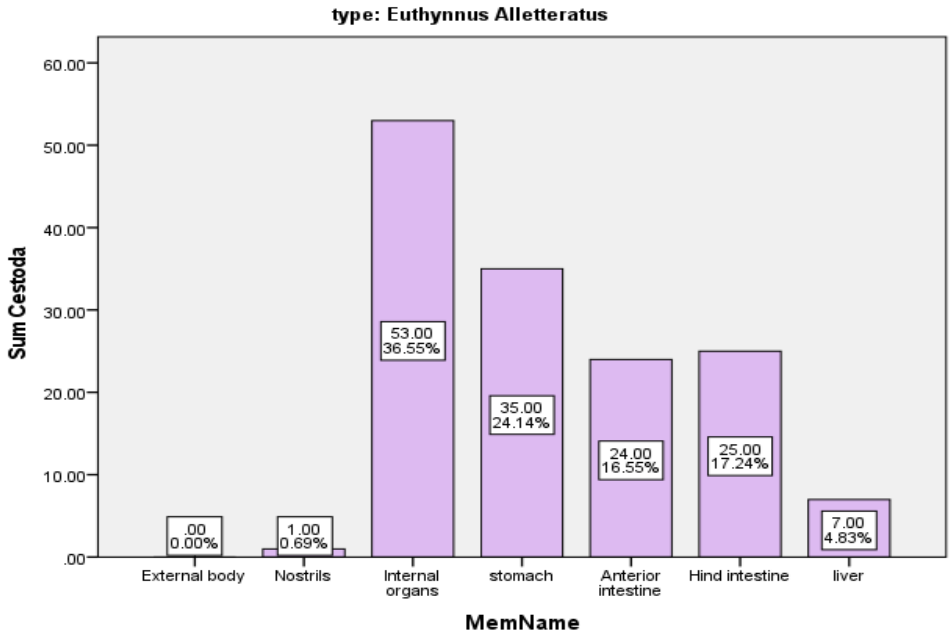


Figure 4.6 The quantity of cestoda found in each organ and the rate of infection in and *Euthynnus alletteratus*

The following pictures show the percentage of the cestoda parasite in the two types of fish in each organ (Figure 4.7) (Figure 4.8) (Figure 4.9) (Figure 4.10) (Figure 4.11) (Figure 4.12) (Figure 4.13).

Figure 4.7 The quantity of cestoda found in extranal organs and the rate of infection in both types of fish

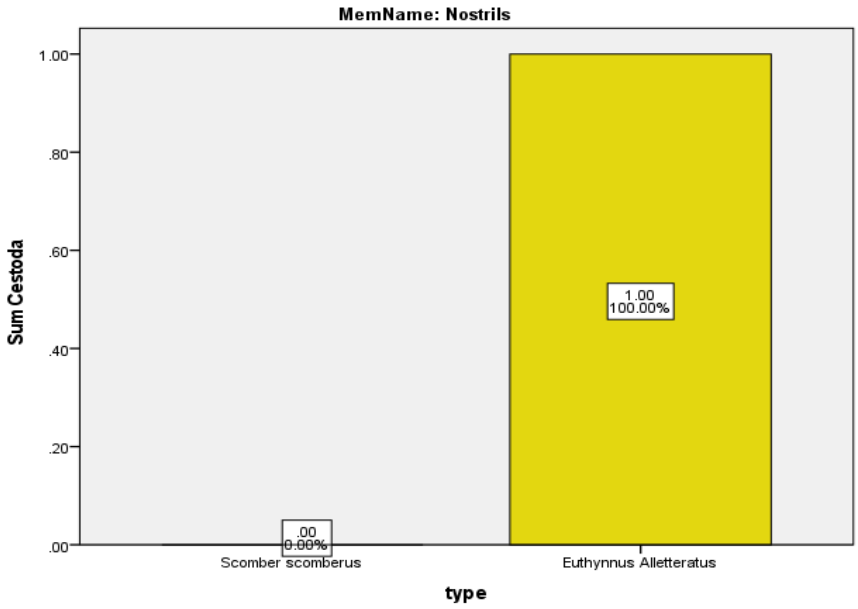


Figure 4.8 The quantity of cestoda found in nostrils and the rate of infection in both types of fish

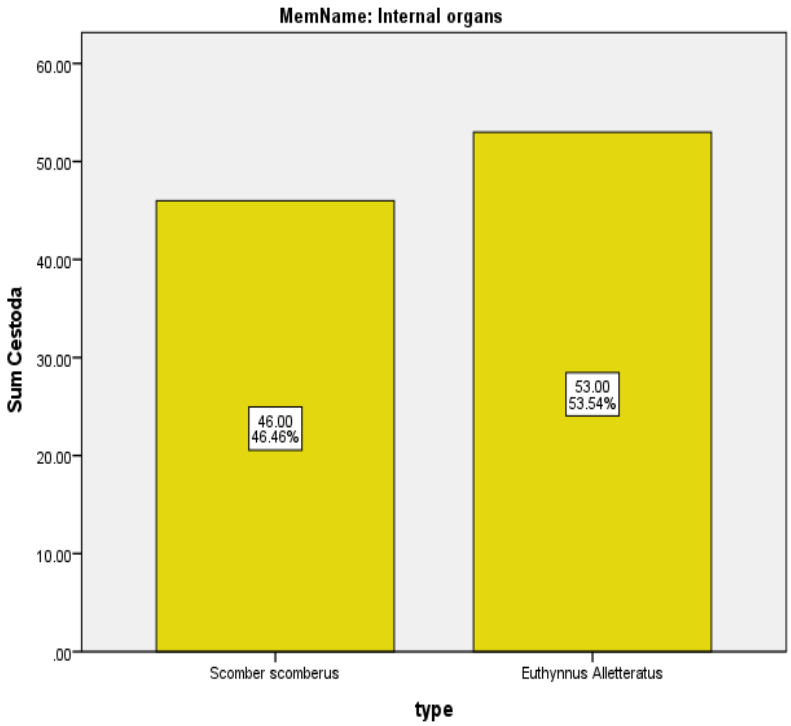


Figure 4.9 The quantity of cestoda found in internal organs and the rate of infection in both types of fish

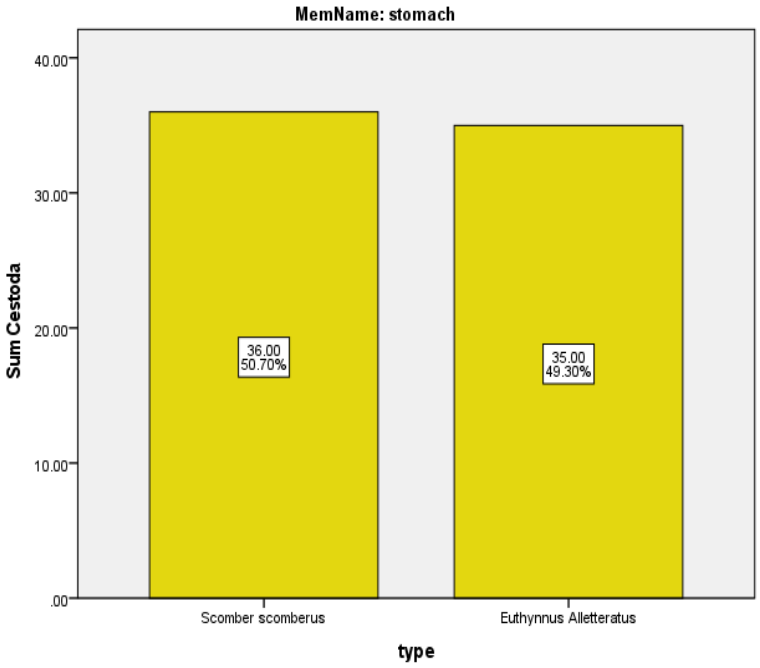


Figure 4.10 The quantity of stomach found in internal organs and the rate of infection in both types of fish

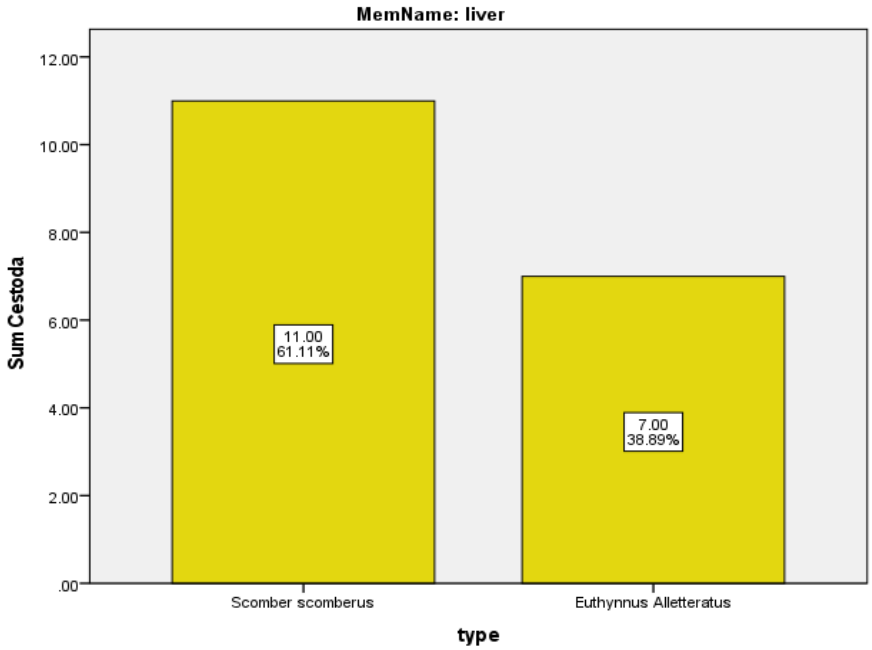


Figure 4.11 The quantity of cestoda found in liver and the rate of infection in both types of fish

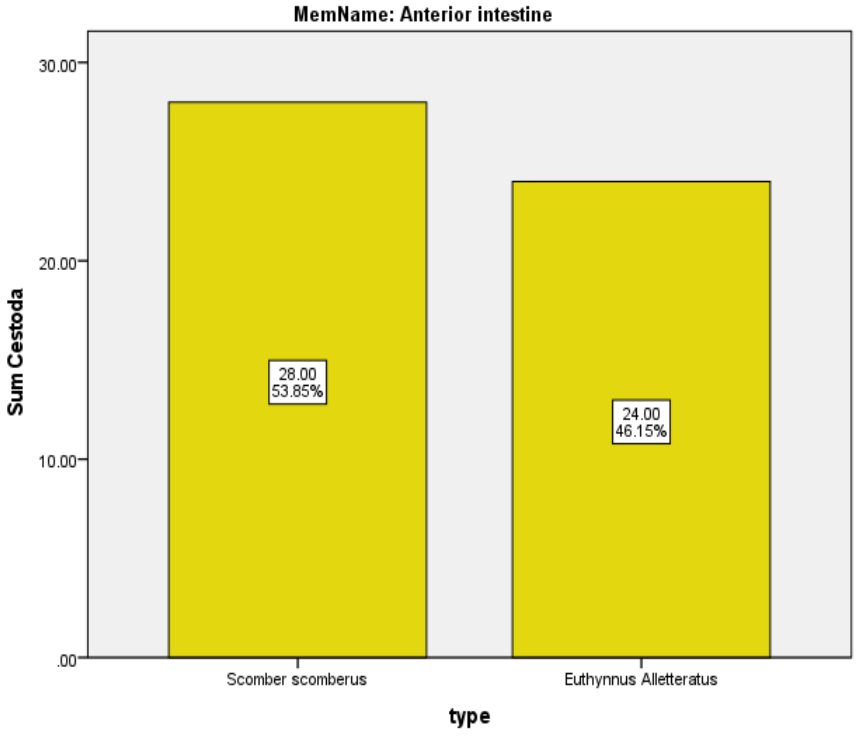


Figure 4.12 The quantity of cestoda found in anterior intestine and the rate of infection in both types of fish

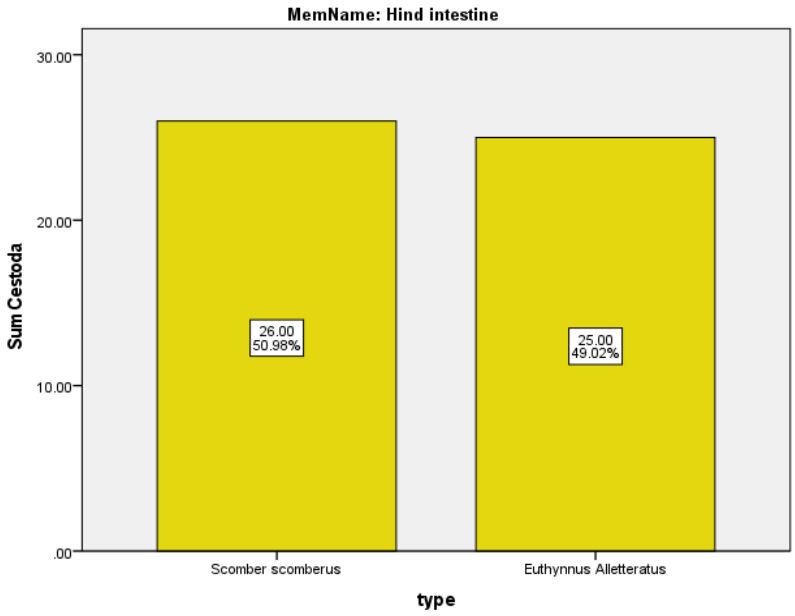


Figure 4.13 The quantity of cestoda found in hind intestine and the rate of infection in both types of fish

Figure 4.14 and Figure 4.15 shows the amount of trematode were high in internal organs, stomach, anterior intestine, hind intestine, and liver in both *Scomber scombrus* and *Euthynnus alletteratus*

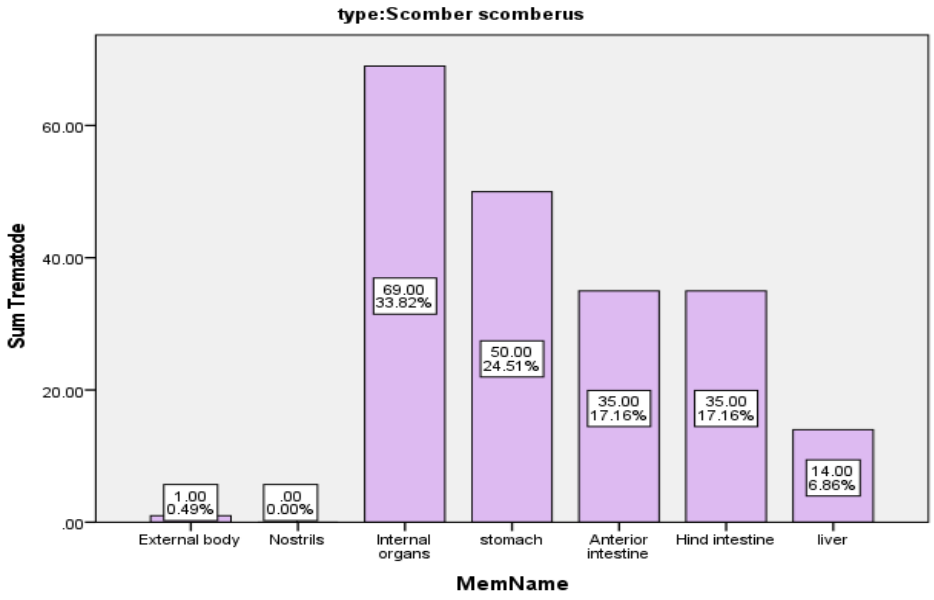


Figure 4.14 The quantity of trematode found in each organ and the rate of infection in *Scomber scomberus*

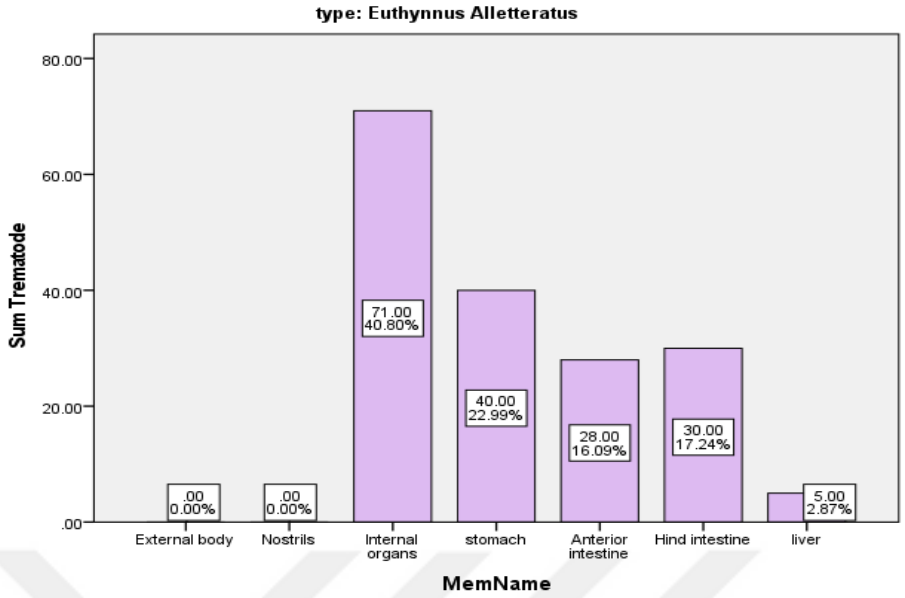


Figure 4.15 The quantity of trematode found in each organ and the rate of infection in and *Euthynnus alletteratus*

The following pictures show the percentage of the Trematode parasite in the two types of fish in each organ (Figure 4.16) (Figure 4.17) (Figure 4.18) (Figure 4.19) (Figure 4.20) (Figure 4.21) (Figure 4.22).

Figure 4.16 The quantity of trematode found in external body and the rate of infection in both types of fish

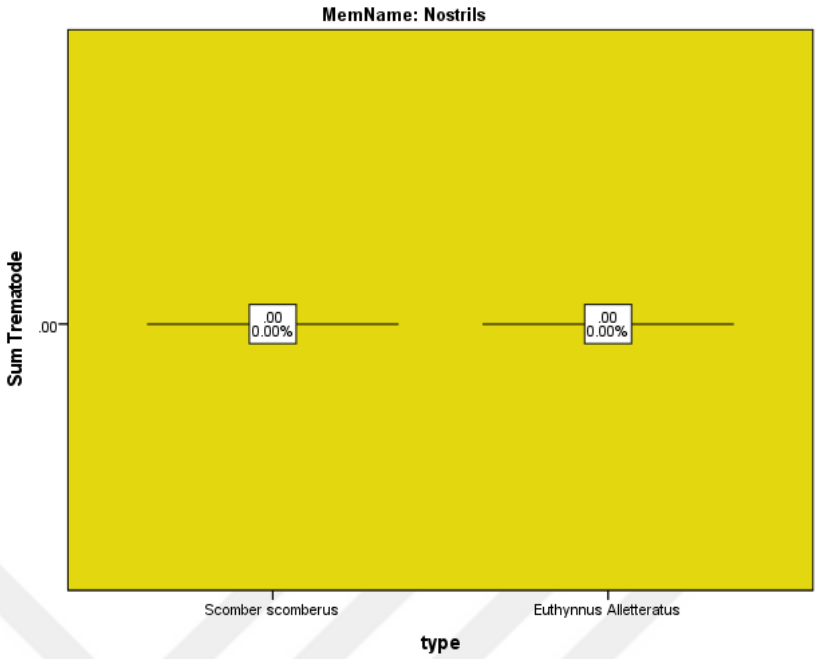


Figure 4.17 The quantity of trematode found in nostrils and the rate of infection in both types of fish

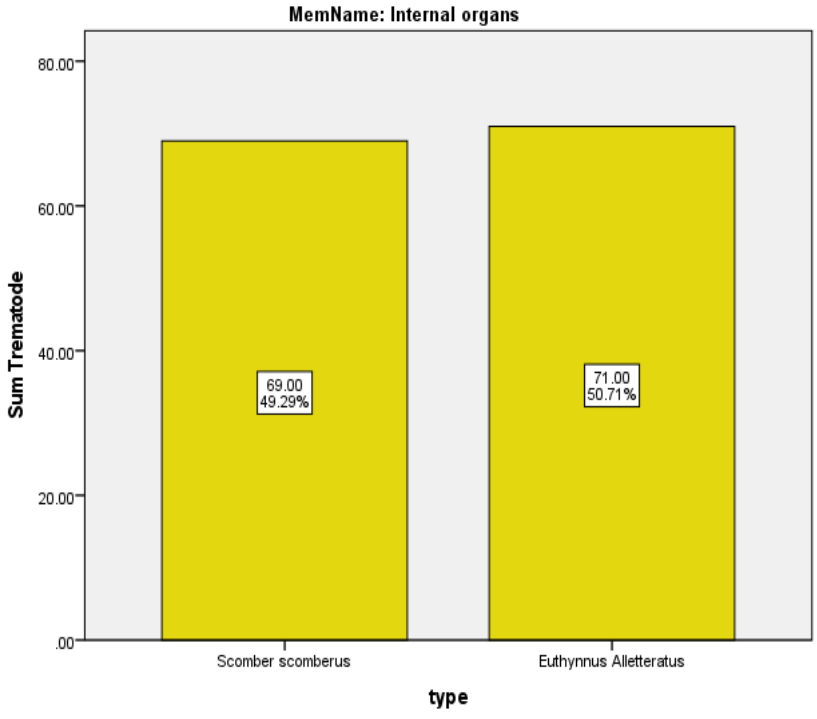


Figure 4.18 The quantity of trematode found in internal organs and the rate of infection in both types of fish

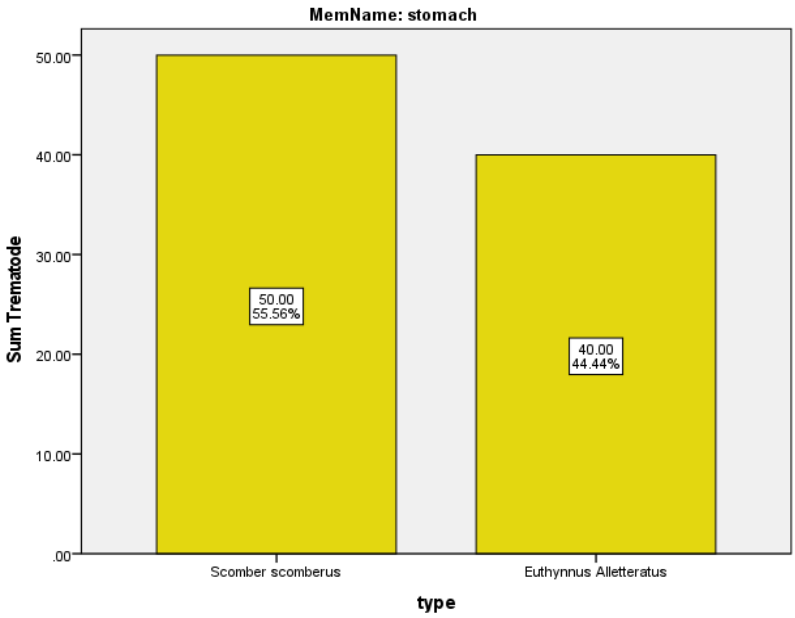


Figure 4.19 The quantity of trematode found in stomach and the rate of infection in both types of fish

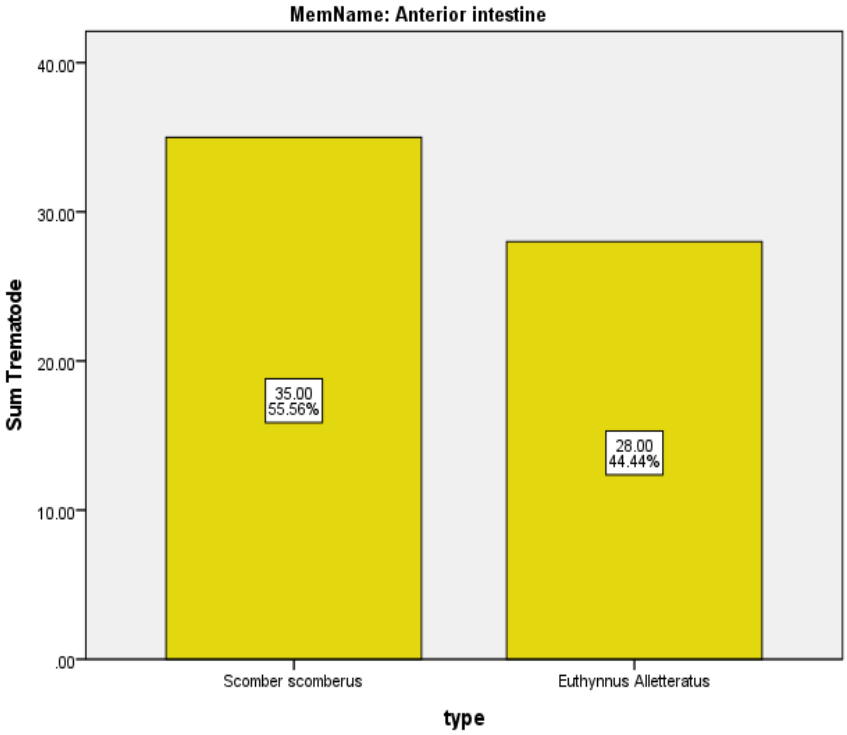


Figure 4.20 The quantity of trematode found in anterior intestine and the rate of infection in both types of fish

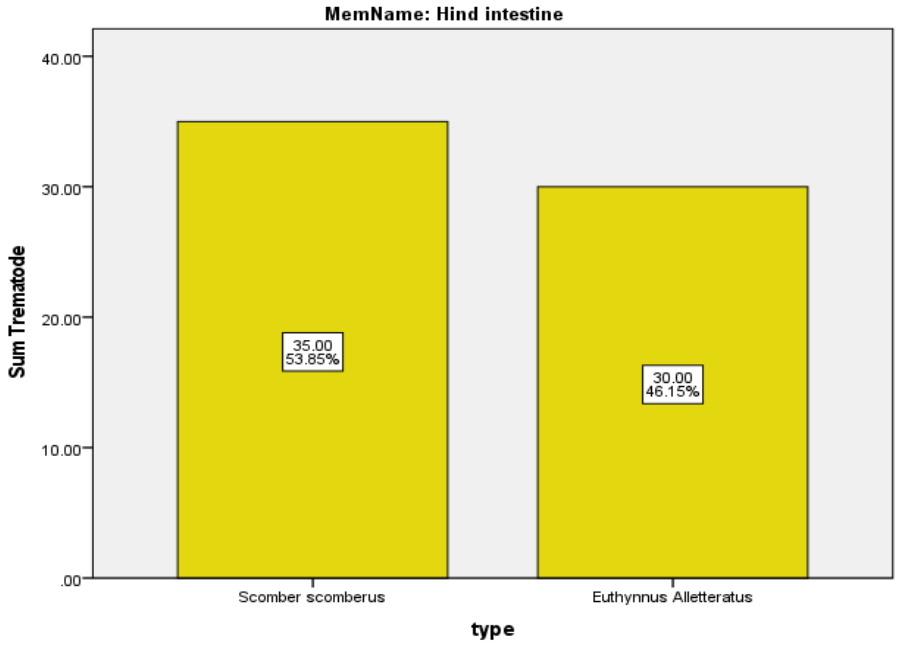


Figure 4.21 The quantity of trematode found in hind intestine and the rate of infection in both types of fish

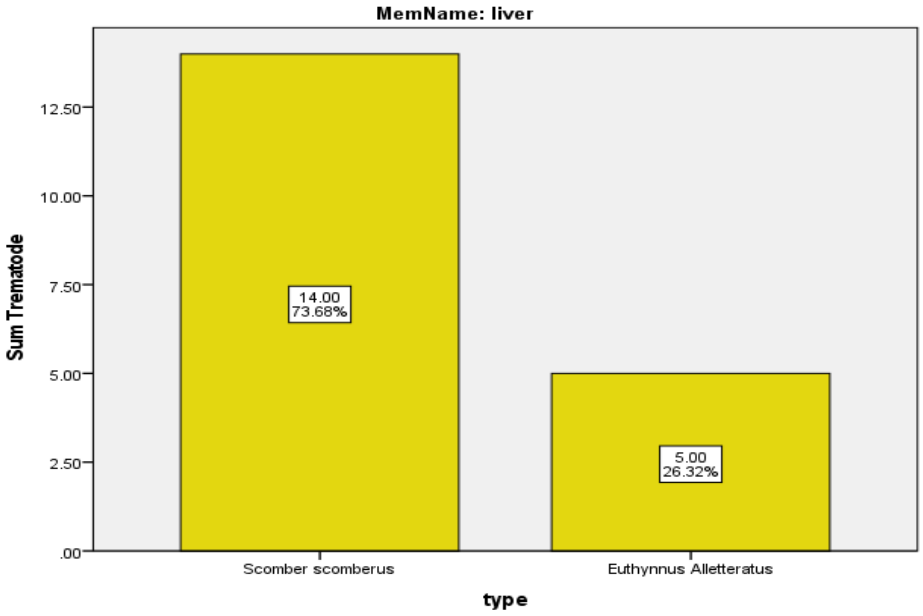


Figure 4.22 The quantity of trematode found in liver and the rate of infection in both types of fish

Figure 4.23 and Figure 4.24 shows the amount of Acanthocephalan were high in internal organs, stomach, anterior intestine, hind intestine, and liver in both *Scomber scombrus* and and *Euthynnus alletteratus*.

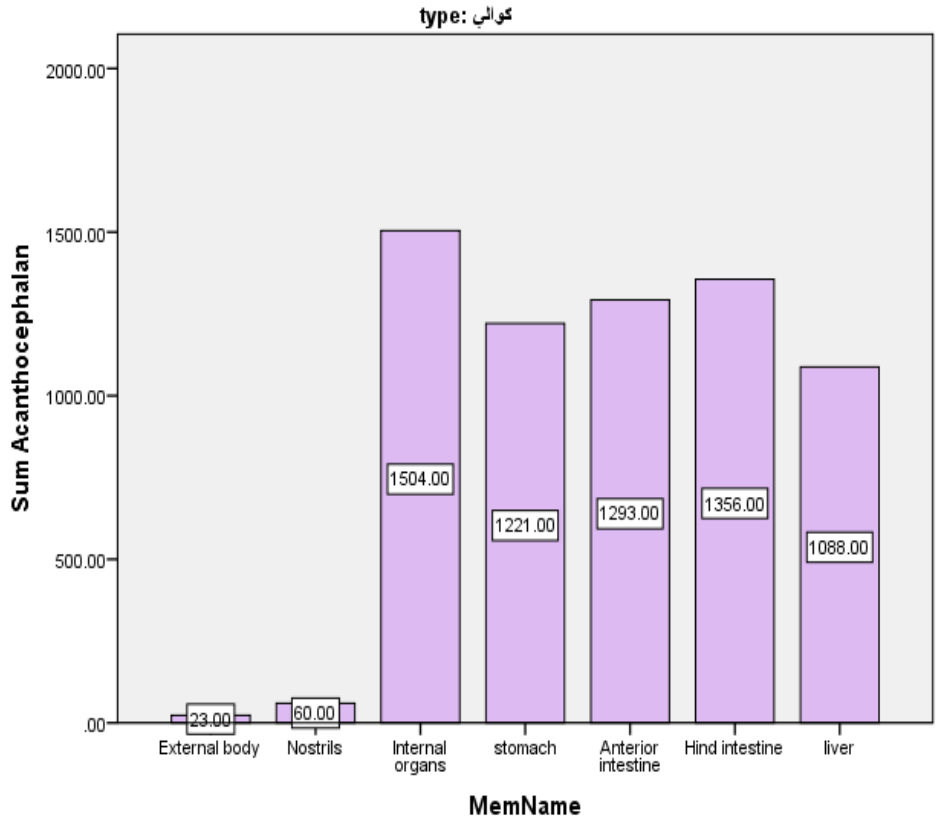


Figure 4.23 The quantity of Acanthocephalan found in each organ and the rate of infection in *Scomber scombrus*

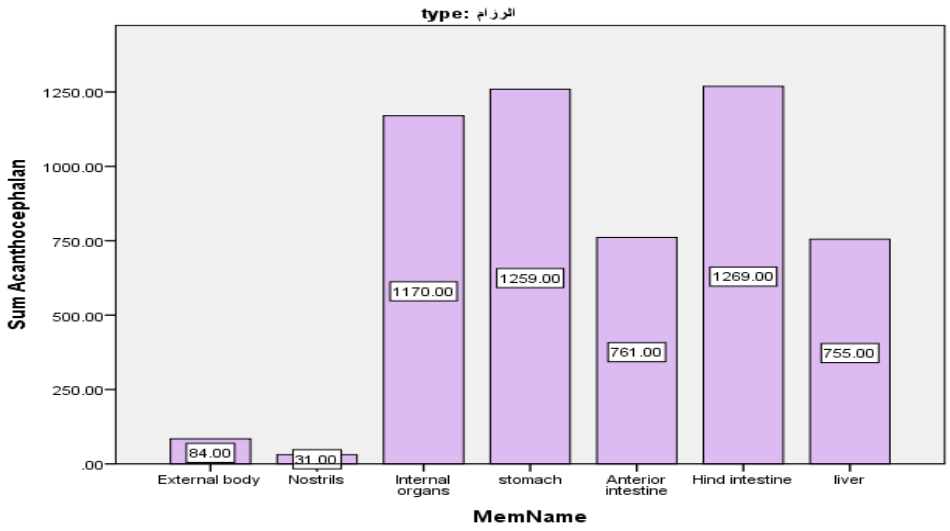


Figure 4.24 The quantity of Acanthocephalan found in each organ and the rate of infection in *Euthynnus alletteratus*

The following pictures show the percentage of the Acanthocephalan parasite in the two types of fish in each organ (Figure 4.25) (Figure 4.26) (Figure 4.27) (Figure 4.28) (Figure 4.29) (Figure 4.30) (Figure 4.31).

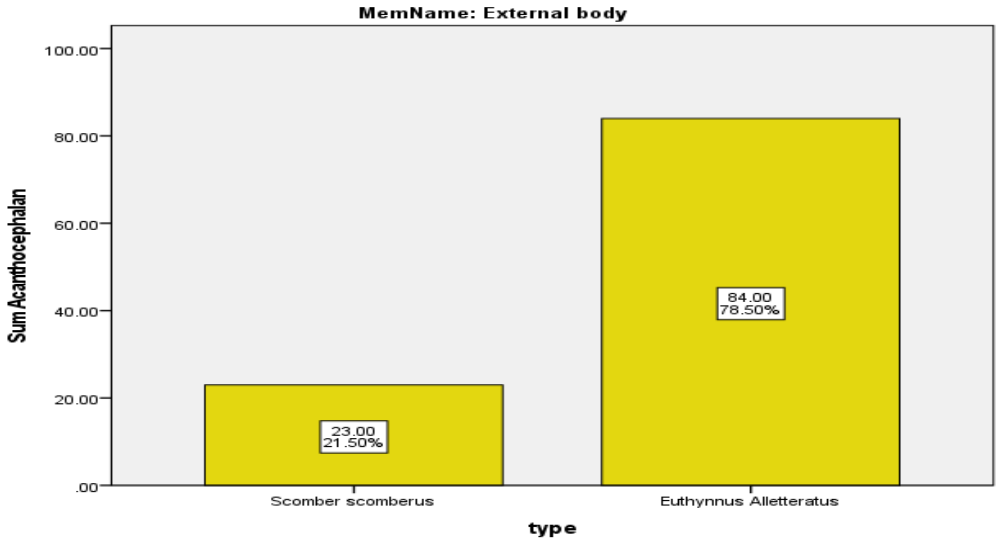


Figure 4.25 The quantity of Acanthocephalan found in external body and the rate of infection in both types of fish

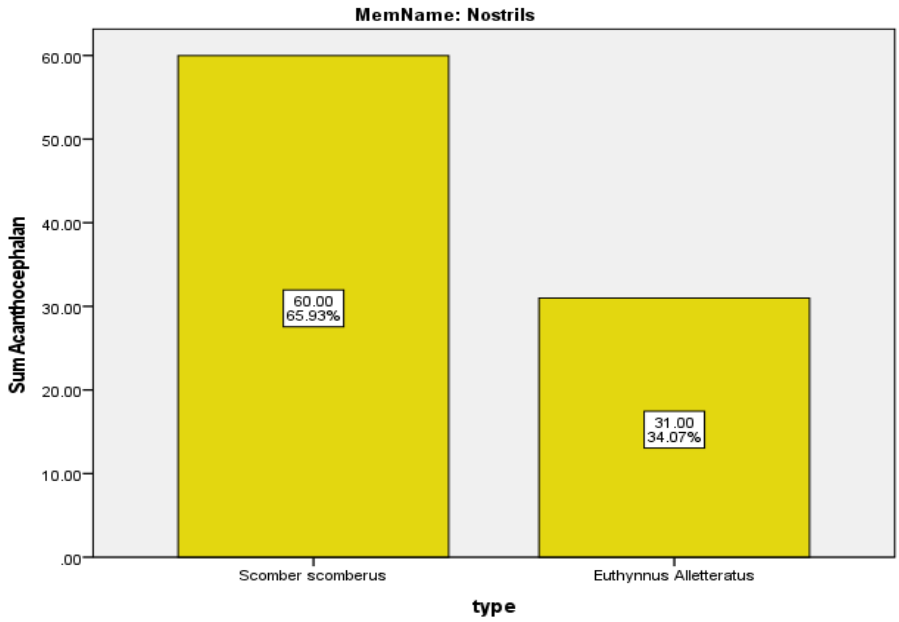


Figure 4.26 The quantity of Acanthocephalan found in nostrils and the rate of infection in both types of fish

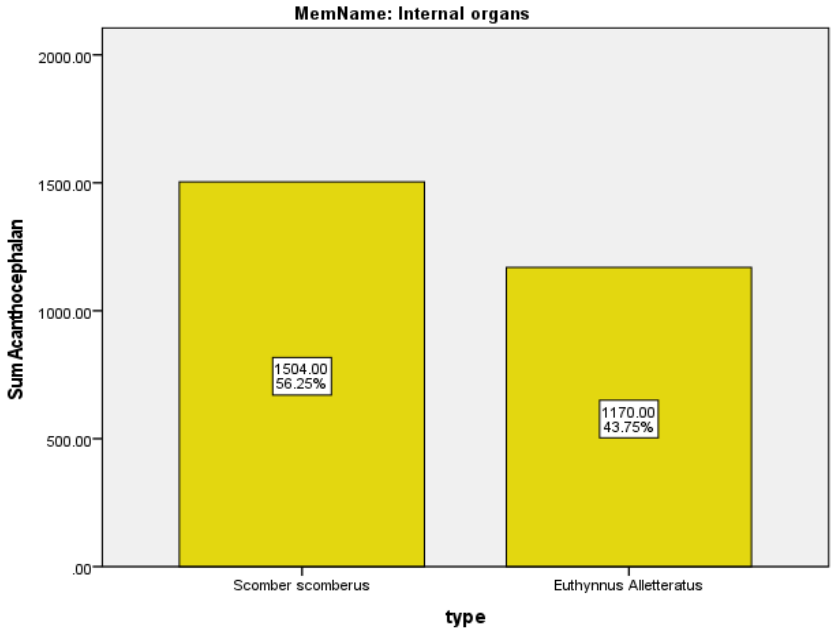


Figure 4.27 The quantity of Acanthocephalan found in internal organ and the rate of infection in both types of fish

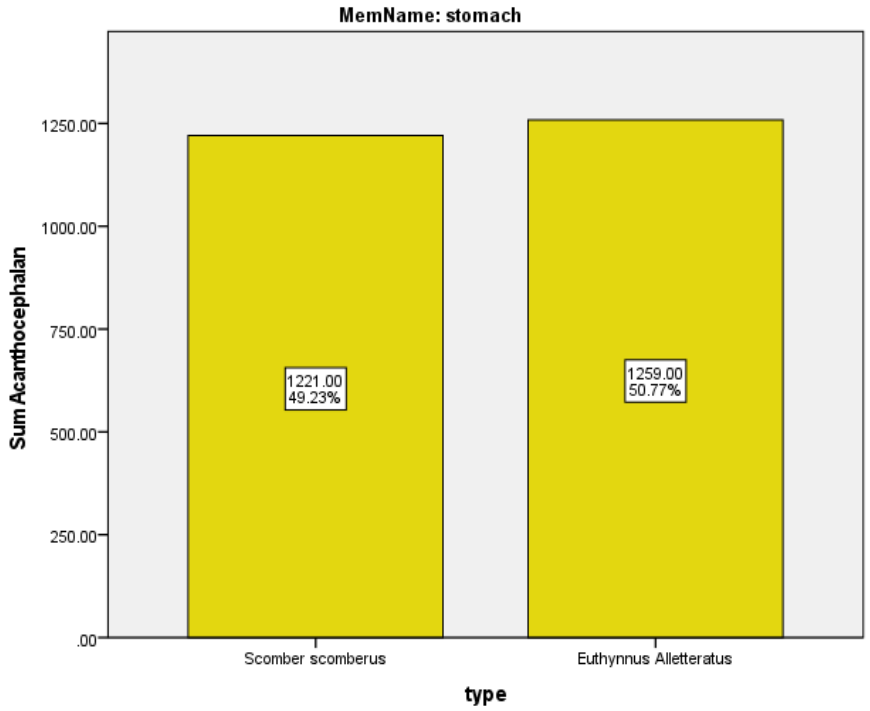


Figure 4.28 The quantity of Acanthocephalan found in stomach and the rate of infection in both types of fish

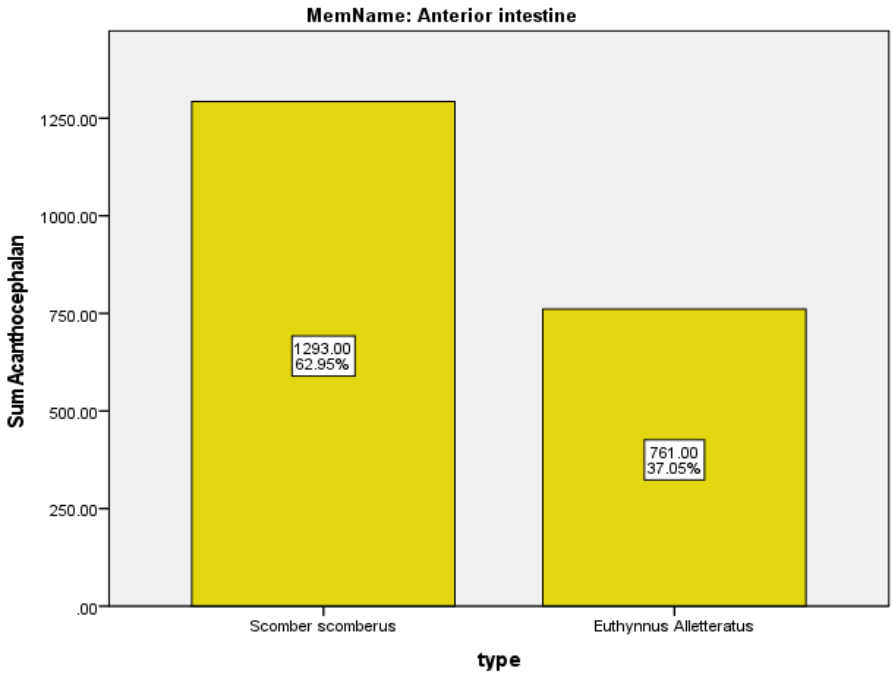


Figure 4.29 The quantity of Acanthocephalan found in anterior intestine and the rate of infection in both types of fish

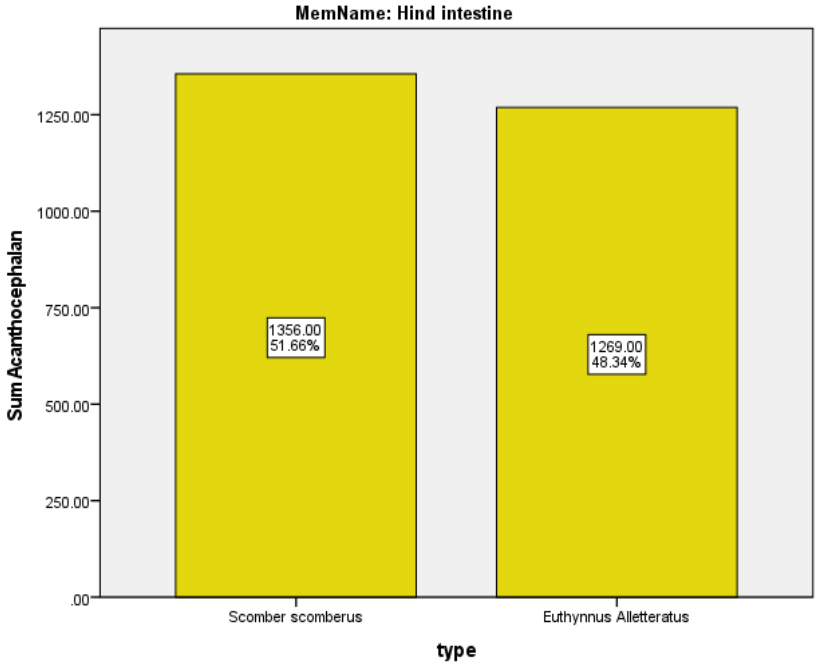


Figure 4.30 The quantity of Acanthocephalan found in hind intestine and the rate of infection in both types of fish

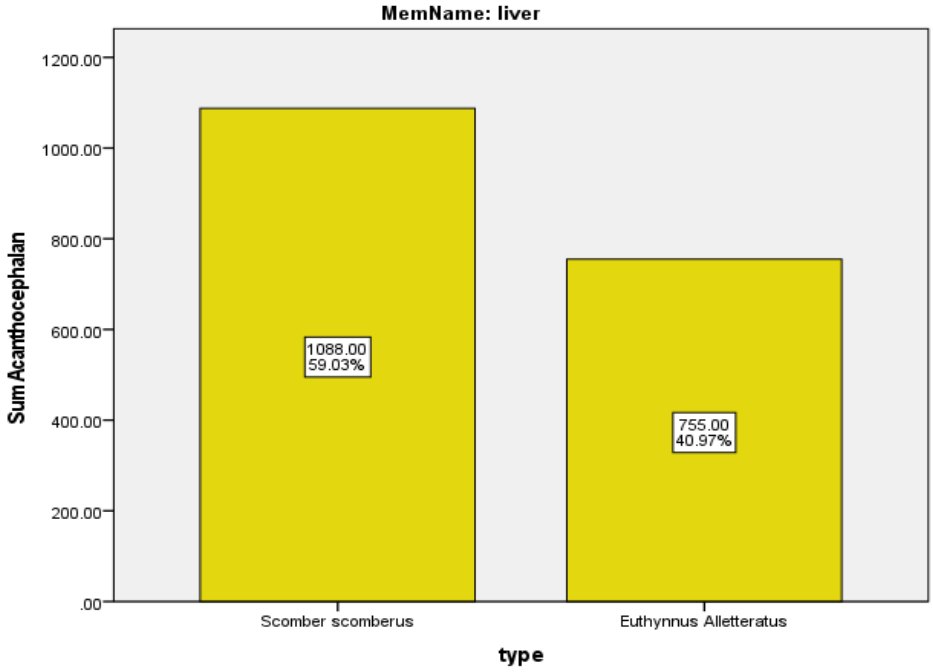


Figure 4.31 The quantity of Acanthocephalan found in liver and the rate of infection in both types of fish

Table 4.4 illustrate the amount of each parasites presents in each organ, and the infection rate in both types of fish.

Table 4.4 The amount of each parasites presents in each organ, and the infection rate in both types of fish

MemName		Type	N	Mean	Std. Deviation	Std. Error Mean
External body	Cestoda	Kawali	50	.0200	.14142	.02000
		razam	50	.0000	.00000	.00000
	Trematode	Kawali	50	.0200	.14142	.02000
		razam	50	.0000	.00000	.00000
	Acanthocephalan	Kawali	50	.4600	.78792	.11143
		razam	50	1.6800	3.82494	.54093
Nostrils	Cestoda	Kawali	50	.0000	.00000	.00000
		razam	50	.0200	.14142	.02000
	Trematode	Kawali	50	.0000	.00000[a]	.00000
		razam	50	.0000	.00000[a]	.00000
	Acanthocephalan	Kawali	50	1.2000	1.52530	.21571
		razam	50	.6200	.72534	.10258
Internal organs	Cestoda	Kawali	50	.9200	.63374	.08963
		razam	50	1.0600	.73983	.10463
	Trematode	Kawali	50	1.3800	1.42700	.20181
		razam	50	1.4200	3.08446	.43621
	Acanthocephalan	Kawali	50	30.0800	8.66577	1.22552
		razam	50	23.4000	8.18660	1.15776
stomach	Cestoda	Kawali	50	.7200	.67128	.09493
		razam	50	.7000	.64681	.09147
	Trematode	Kawali	50	1.0000	1.01015	.14286
		razam	50	.8000	.72843	.10302
	Acanthocephalan	Kawali	50	24.4200	7.22267	1.02144
		razam	50	25.1800	7.04704	.99660
Anterior intestine	Cestoda	Kawali	50	.5600	.57711	.08162
		razam	50	.4800	.57994	.08202
	Trematode	Kawali	50	.7000	.86307	.12206
		razam	50	.5600	.64397	.09107
	Acanthocephalan	Kawali	50	25.8600	7.52685	1.06446
		razam	50	15.2200	4.85416	.68648
Hind intestine	Cestoda	Kawali	50	.5200	.54361	.07688
		razam	50	.5000	.61445	.08690
	Trematode	Kawali	50	.7000	1.05463	.14915
		razam	50	.6000	.69985	.09897
	Acanthocephalan	Kawali	50	27.1200	7.01206	.99165
		razam	50	25.3800	5.37906	.76071
liver	Cestoda	Kawali	50	.2200	.46467	.06571
		razam	50	.1400	.40457	.05721
	Trematode	Kawali	50	.2800	.64015	.09053
		razam	50	.1000	.30305	.04286
	Acanthocephalan	Kawali	50	21.7600	6.07289	.85884
		razam	50	15.1000	4.97032	.70291

Younis *et al.* (2020) did a research to evaluate the toxicity of several heavy metals and the presence of parasite infection in fish. The researchers are studying the immune response of these fish to various parasites. A thorough investigation was carried out on 360 Nile tilapia (Oreochromis niloticus) between January 2018 and January 2019 to analyse external and internal parasites. The investigation was conducted in Giza, including four farms and the Nile River. Quantitative real-time polymerase chain reaction was used to analyse samples for two distinct genes. Samples were collected from muscles and water to perform a toxicological investigation on various heavy metals. The average TNF-α levels in the skin were 18.00 ± 0.67 for monogenea parasites and 13.65 ± 0.54 for mixed protozoan parasites (Trichodina and Myxobolus spp.). The average TNF-α concentrations in the gills were 20.45 ± 0.74 for monogenea, 23.00 ± 0.74 for Centrocestus formosanus, and 25.78 ± 0.74 for encysted metacercaria (EMC) with monogenea group averages. The average TNF-α concentration in muscles impacted by EMC was 14.67 ± 0.70. The average TNF-α levels in livers affected by EMC was 26.78 ± 0.70. The average IL-1β concentration was substantially different between monogenea and protozoan parasites (Trichodina and Myxobolus spp.) in the skin, with values of 25.00 ± 0.69 and 15.00 ± 0.43, respectively. The monogenean group had an average IL-1β level of 21.00 ± 0.79 in the gills. C. formosanus obtained the highest value of 27.00 ± 0.74, which was considerably higher. The average IL-1β concentration in the EMC group with the monogenea subgroup was 24.00 ± 1.54. After histological evaluation, several tissues showed significant pathological changes. The fish that were studied were shown to be infected with monogenetic trematodes, leading to the hyperplasia and fusion of the secondary gill lamellae. Electron microscopy was often used to visually inspect the presence of digenetic trematodes in the gills and cartilage rod. A parasite infection may stimulate the generation of immune cytokines to varying degrees, depending on the particular kind of infection (Younis *et al.* 2020) (Figure 4.32) (Figure 4.33) (Figure 4.34) (Figure 4.35).

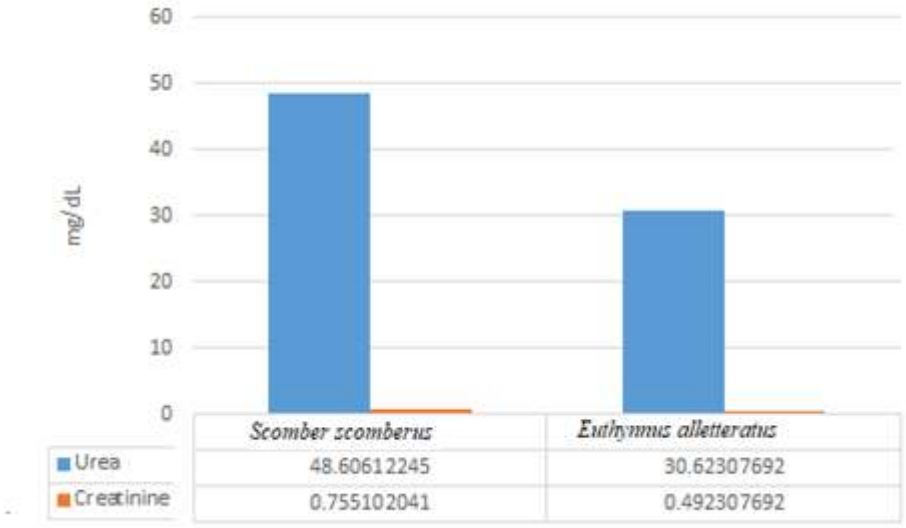


Figure 4.32 Urea and creatinine levels in *Scomber scombrus* koala fish and *Euthynnus alletteratus* Razam fish

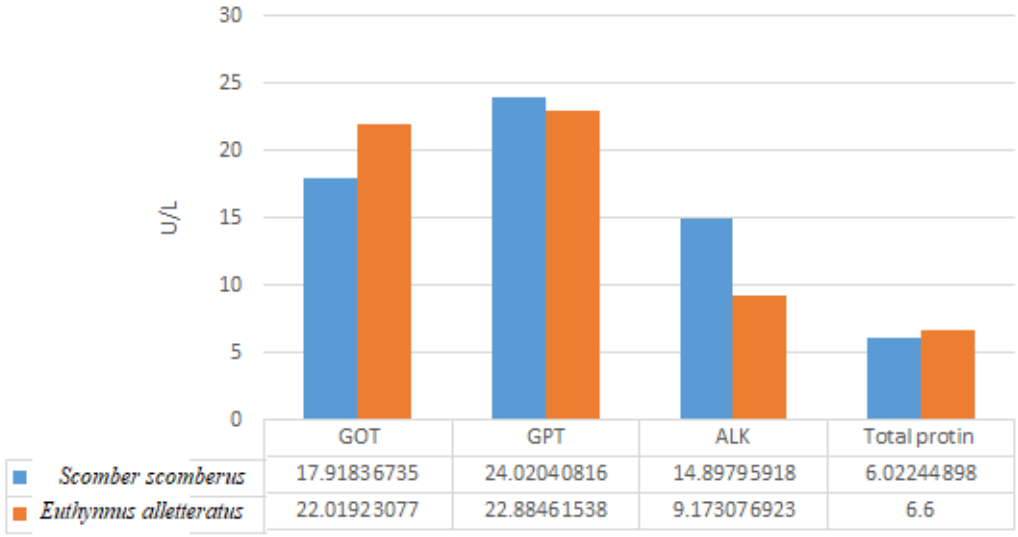
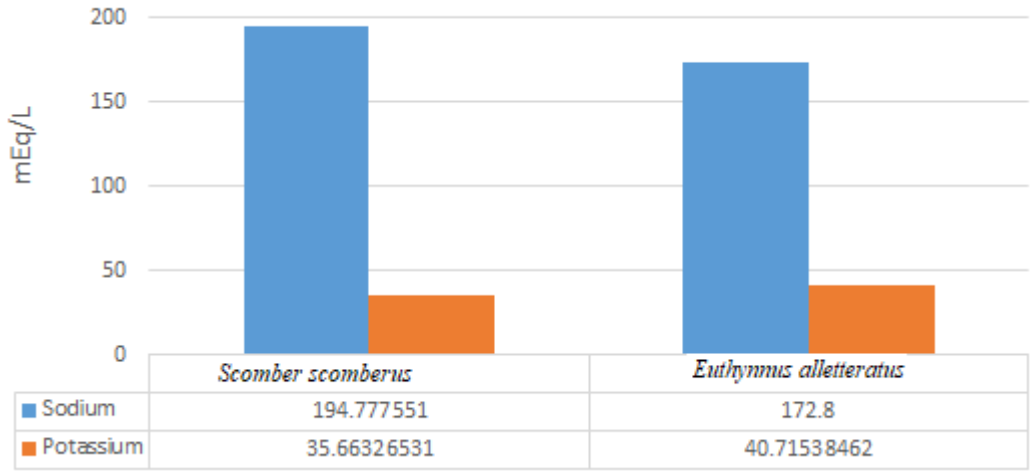
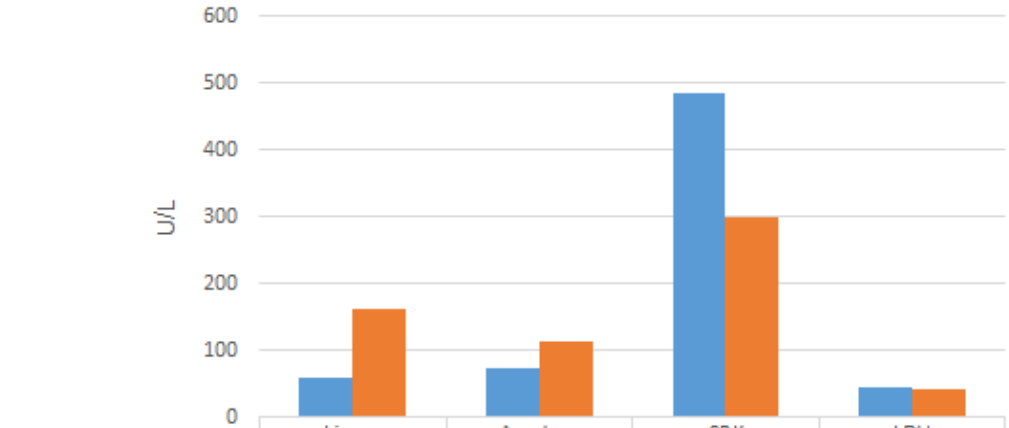


Figure 4.33 GOT, GPT, ALK, and total protein levels in *Scomber scombrus* koala fish and *Euthynnus alletteratus* Razam fish



	Scomber scomberus	*Euthynnus alletteratus*
Sodium	194.777551	172.8
Potassium	35.66326531	40.71538462

Figure 4.34 Sodium and potassium levels in *Scomber scombrus* koala fish and *Euthynnus alletteratus* Razam fish



	Lipase	Amylase	CPK	LDH
Scomber scomberus	57.63469388	74.49183673	483.9040816	44.32040816
Euthynnus alletteratus	160.7442308	112.4269231	299.5634615	40.321

Figure 4.35 Lipase, amylase, CPK, and LDH levels in *Scomber scombrus* koala fish and *Euthynnus alletteratus* Razam fish

Table 4.5 showed significant statistical differences in most variables for both *Euthynnus alletteratus* and *Scomber scombrus*. Urea levels in *Scomber scombrus* showed a high positive connection with several organs, excluding the external body. No connection was found in *Euthynnus alletteratus* between the external body and nostrils. Creatinine showed a favourable connection with several internal organs in *Euthynnus alletteratus*,

except for those situated externally. Sodium had a positive correlation with several organs in *Scomber scombrus*, with the exception of internal organs and the stomach. A correlation was observed between the concentrations of potassium, ALT, AST, lipase, amylase, CPK, and total protein in several organs of both *Euthynnus alletteratus* and *Scomber scombrus*. LDH levels were positively correlated with many organs in both *Euthynnus alletteratus* and *Scomber scombrus*, except for the external body in *Scomber scombrus*. Elevated LDH levels in *Euthynnus alletteratus* were positively associated with the nose, internal organs, and anterior intestine.

Table 4.5 Pearson correlation between different enzymes and each organ

	Scomber scomberus		***Euthynnus alletteratus***	
	Pearson Correlation	**P value**	**Pearson Correlation**	**P value**
Urea				
External body	-0.210	0.160	-0.176	0.226
Nostrils	0.036	0.041	-0.060	0.68
Internal organs	0.003	0.035	0.035	0.045
Stomach	0.232	0.012	0.040	0.02
Anterior intestine	0.055	0.07	0.224	0.012
Hind intestine	0.098	0.05	0.071	0.031
Creatinine				
External body	0.212	0.006	0.156	0.128
Nostrils	0.158	0.03	0.200	0.018
Internal organs	0.842	0.04	0.127	0.038
Stomach	0.654	0.026	0.135	0.031
Anterior intestine	0.540	0.012	0.108	0.042
Hind intestine	0.025	0.034	0.152	0.029
Sodium				
External body	0.173	0.020	0.276	0.05
Nostrils	0.101	0.005	0.105	0.047
Internal organs	-0.148	0.326	0.160	0.027
Stomach	-0.179	0.234	0.069	0.026
Anterior intestine	0.297	0.045	0.137	0.034
Hind intestine	0.037	0.008	0.179	0.021
Potassium				
External body	0.032	0.060	0.112	0.042
Nostrils	0.127	0.023	0.055	0.001
Internal organs	0.160	0.011	0.184	0.021
Stomach	0.088	0.001	0.154	0.035
Anterior intestine	0.031	0.03	0.113	0.048
Hind intestine	0.158	0.04	0.081	0.05
ALT				
External body	0.298	0.031	0.158	0.048
Nostrils	0.078	0.011	0.137	0.05
Internal organs	0.040	0.027	0.039	0.021
Stomach	0.072	0.02	0.069	0.049
Anterior intestine	0.129	0.009	0.004	0.003
Hind intestine	0.062	0.031	0.101	0.025

Table 4.5 Pearson correlation between different enzymes and each organ (Continued)

	Scomber scomberus		*Euthynnus alletteratus*	
	Pearson Correlation	**P value**	**Pearson Correlation**	**P value**
AST				
External body	0.028	0.005	0.176	0.025
Nostrils	0.026	0.061	0.225	0.018
Internal organs	0.013	0.051	0.045	0.04
Stomach	0.118	0.024	0.016	0.026
Anterior intestine	0.191	0.031	0.033	0.032
Hind intestine	0.109	0.009	0.081	0.038
ALP				
External body	0.289	0.110	0.121	0.024
Nostrils	0.017	0.14	0.175	0.01
Internal organs	0.256	0.02	0.216	0.02
Stomach	0.174	0.034	0.341	0.043
Anterior intestine	0.062	0.07	0.090	0.015
Hind intestine	0.150	0.09	0.013	0.039
Total protein				
External body	0.116	0.009	0.100	0.026
Nostrils	0.114	0.037	0.010	0.068
Internal organs	0.102	0.037	0.018	0.021
Stomach	0.049	0.007	0.086	0.024
Anterior intestine	0.239	0.013	0.102	0.042
Hind intestine	0.206	0.036	0.048	0.011
Lipase				
External body	0.279	0.05	0.017	0.030
Nostrils	0.061	0.034	0.072	0.012
Internal organs	0.057	0.031	0.106	0.042
Stomach	0.154	0.023	0.288	0.010
Anterior intestine	0.055	0.005	0.216	0.004
Hind intestine	0.110	0.008	0.088	0.006
Amylase				
External body	0.084	0.004	0.108	0.021
Nostrils	0.073	0.035	0.022	0.028
Internal organs	0.208	0.010	0.160	0.015
Stomach	0.074	0.018	0.036	0.012
Anterior intestine	0.149	0.047	0.193	0.002
Hind intestine	0.048	0.039	0.051	0.011
CPK				
External body	0.105	0.01	0.035	0.008
Nostrils	0.022	0.024	0.134	0.006
Internal organs	0.064	0.005	0.202	0.015
Stomach	0.255	0.002	0.054	0.031
Anterior intestine	0.065	0.006	0.074	0.042
Hind intestine	0.068	0.036	0.121	0.051
LDH				
External body	0.248	0.19	0.2786	0.001
Nostrils	0.118	0.011	0.067	0.08
Internal organs	0.222	0.025	0.433	0.06
Stomach	0.066	0.032	0.146	0.008
Anterior intestine	0.122	0.043	0.239	0.09
Hind intestine	0.073	0.032	0.041	0.034

Parasites are influenced by the environment of their hosts due to their specialised distribution and presence in distinct microhabitats. This research attempts to analyse the distribution of the razam and kawali parasites throughout many organs. The research identified the differing rates of parasite infection in several organs of fish. The investigation showed different parasite infestations in the razam and kawali species.

Yamaguti (1965) first created the genus Neonematobothrium to include the species N. kawakawa, which was discovered residing in the subcutaneous tissue near the opercular area of E. affinis along the Hawaiian coast. N. dorsale, the second species of the genus, was discovered inhabiting the subcutaneous layer of the dorsal body folds at the base of the dorsal fin in the same host from Hawaiian seas. Yamaguti (1970) reported this finding. Subsequently, reports emerged of the two species being discovered in the Bay of Bengal, particularly on the specified host (Madhavi and Ram 2000). Mele *et al.* (2016) discovered four different species/morphotypes of Didymozoidae in the head of E. alletteratus in the Mediterranean Sea, one of which is N. Kawakawa. The scientists state that each parasite detected has a capsule enclosing a few hermaphroditic individuals that share similarities and are surrounded by the host's tissues. The authors conducted a molecular study but just provided a brief description without any morphometric data. Based on the authors' morphological description, the specimens do not fit the classification of the Neonematobothrium genus. Yamaguti previously identified two species of this genus that are widely spread in the subcutaneous tissues and do not form a capsule inside the host tissue. Additionally, in both species of the genus, the testes extend beyond the genital connection, with the posterior testis reaching the rear end of the body. The species N. annakohnae also exhibits similar characteristics (Yamaguti 1965, Yamaguti 1970).

Previous research on Scomber japonicus and *Scomber scombrus* in the Western Mediterranean waters has identified A. simplex sensu stricto and its hybrids (Ferrantelli *et al.* 2014). Mackerels from two distinct locations along the Libyan coast were discovered to have A. pegreffii and hybrid forms (Eissa *et al.* 2018). Two other Anisakis species are Anisakis physeteris (Goffredo *et al.* 2019) and Anisakis typica (Farjallah *et al.* 2008). Varying distribution of anisakid species in the Mediterranean basin may aid in fisheries management, assessing food quality based on consumer behaviour, and

effectively tagging Scomber spp. fish populations with Anisakis larvae. Knowing the upper and lower thresholds of Anisakis infection levels might be advantageous for fisheries management. Previous findings of parasitic species in *Scomber scombrus* from Tunisian shores may be linked to mackerel populations migrating across the Gibraltar strait from the Atlantic Ocean. This phenomenon is frequently seen in parasite ecology and could have implications for managing organisms by affecting sample size assessment in routine monitoring and control programmes (Shvydka *et al.* 2018).

An associated study examining the zoonotic capacity of parasite infection obtained from Libya indicated that the limited incidence of human infections was likely attributable to the seldom ingestion of uncooked fish. Nevertheless, due to the limited understanding of parasite infection among Libyan doctors, it is possible that human infections may arise (Sharif and Negm-Eldin 2013).

A study revealed that persons who lacked the ability to utilize fish correctly had a higher likelihood of acquiring parasite infections. The results indicate that the improper use of latrines and the practice of open defecation lead to the pollution of water sources and the surrounding environment, hence playing a significant role in the transmission of parasitic infections (Adekeye *et al.* 2016).

Parasites are influenced by the environment of their hosts due to their specialised distribution and presence in distinct microhabitats. This research attempts to analyse the distribution of the razam and kawali parasites throughout many organs. The research identified the differing rates of parasite infection in several organs of fish. The investigation showed different parasite infestations in the razam and kawali species.

5. CONCLUSIONS AND RECOMMENDATION

The current research revealed a significant prevalence of parasite infection in the analyzed locale. Therefore, it is crucial to establish a thorough control program that includes enhancing personal cleanliness, improving environmental sanitation, and providing health education. The study results indicate a direct relationship between the levels of creatinine and several internal organs in *Euthynnus alletteratus*, with the exception of external body tissues. An affirmative correlation was observed between sodium levels and several organs in *Scomber scombrus*, with the exception of internal organs and the stomach. A positive correlation was seen between potassium, AST, ALT, ALP, lipase, amylase, CPK, and total protein levels in several organs of both *Euthynnus alletteratus* and *Scomber scombrus*. A significant correlation was seen between LDH levels and several organs in both *Euthynnus alletteratus* and *Scomber scombrus*, with the exception of the external body in *Scomber scombrus*. The LDH levels in *Euthynnus alletteratus* exhibited a direct relationship with the snout, internal organs, and anterior intestine.

The present study aims to analyze the distribution of the razam and kawali parasites throughout several organs. The study determined the varying prevalence rate of parasite infection in different organs of fish. The histopathological examination of Kawali species revealed a predominance of fibroblasts in the stomach, along with the presence of lymphocytes, macrophages, and eosinophilic granulocytes. Within the gut, there is damage to the mucosal lining and a response from the surrounding tissue, resulting in the buildup of cellular waste at the interface between the tissue and the parasite. This area is characterized by a granulomatous reaction similar to the response to foreign objects, which includes the formation of large cells. The lamina propria exhibits significant and varying infiltration of eosinophils, as well as lymphocytes and macrophages with hemosiderin pigment. The myocardium is the primary site of pathological abnormalities in the heart, characterized by widespread inflammation and multifocal necrosis of myocytes. In the nostrils, there is an influx of inflammatory cells, particularly eosinophils. The histological analysis of liver tissue revealed pronounced congestion of blood vessels, infiltration of mononuclear cells in the portal triads, and hyperplasia of the bile duct in the liver. The hepatocytes exhibited degenerative alterations characterized by granular

and vacuolar degeneration, as well as coagulative necrosis. Hydropic degeneration and autolysis occur in the cells of the liver and pancreas. The histopathological examination of razam species revealed a predominance of hyperplasia in the mucous membranes and submucosa of the stomach, accompanied by autolysis. The heart exhibits hypertrophy accompanied by widespread inflammation and multifocal necrosis of myocytes. The liver exhibits significant vacuole degeneration and fatty hepatocytes, accompanied by the loss of liver tissue structure characterized by multifocal necrosis and congestion of interlobular veins. Autolytic alterations occur in the gut wall. The liver and pancreas undergo autolytic alterations.

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APPENDICES

APPENDIX 1. Some steps of the practical section during the examination of the internal organs of fish

APPENDIX 2. Some of the types of parasites

APPENDIX 3. Tissue cutting device (Microtome)

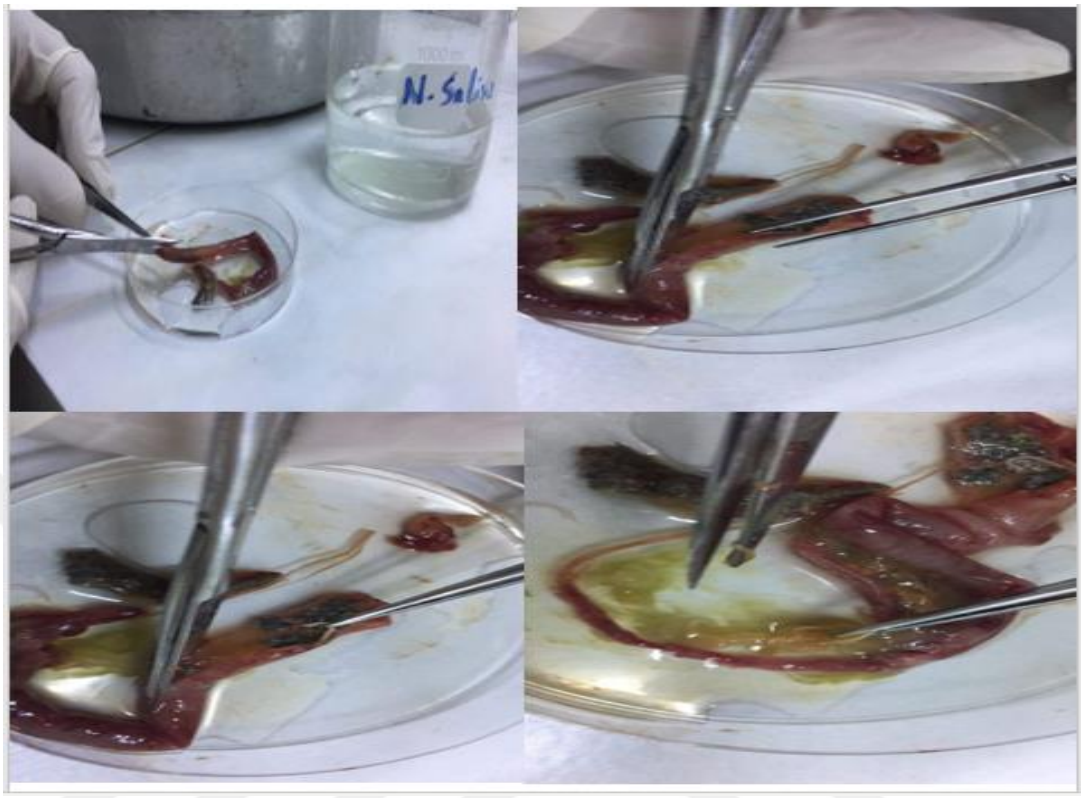
APPENDIX 4. Tissue slices from various fish organs

APPENDIX 5. Acceptance letter for work conducted in the laboratory of the Faculty of Science, Misurata University

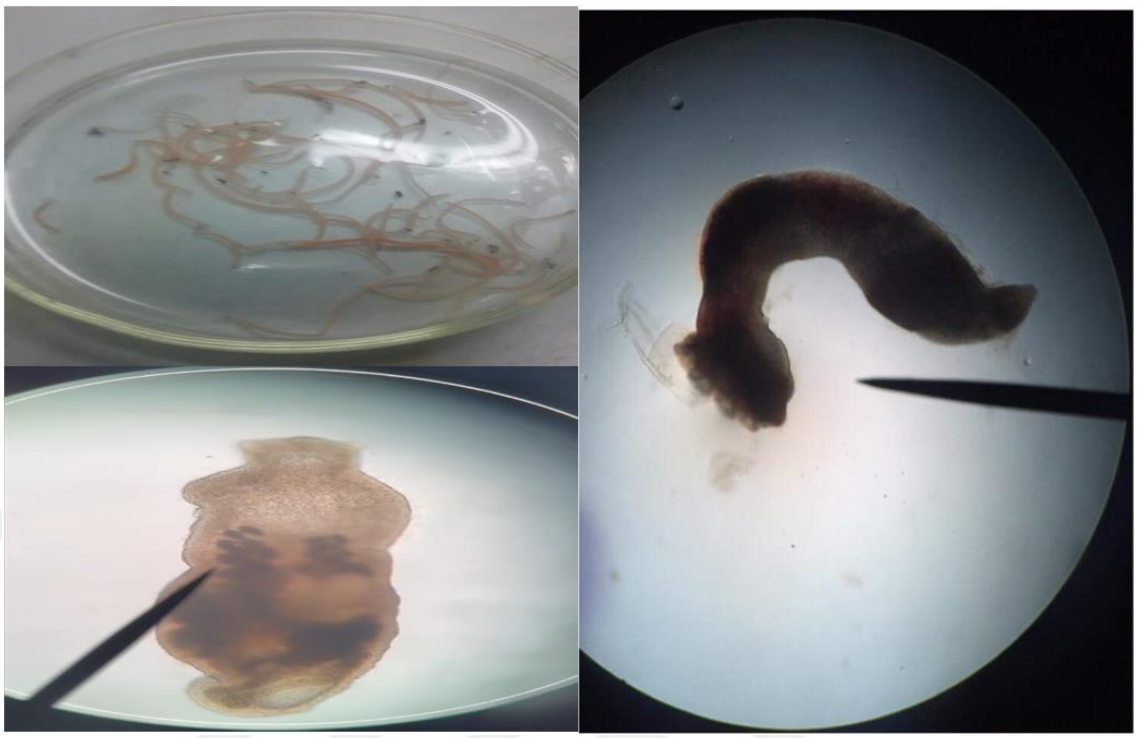
APPENDIX 6. Acceptance of the research paper for publication in the Journal of Advanced Zoology

APPENDIX 7. Acceptance letter for work conducted in the laboratory of the Faculty of Science, Misurata University

APPENDIX 1. Some steps of the practical section during the examination of the internal organs of fish

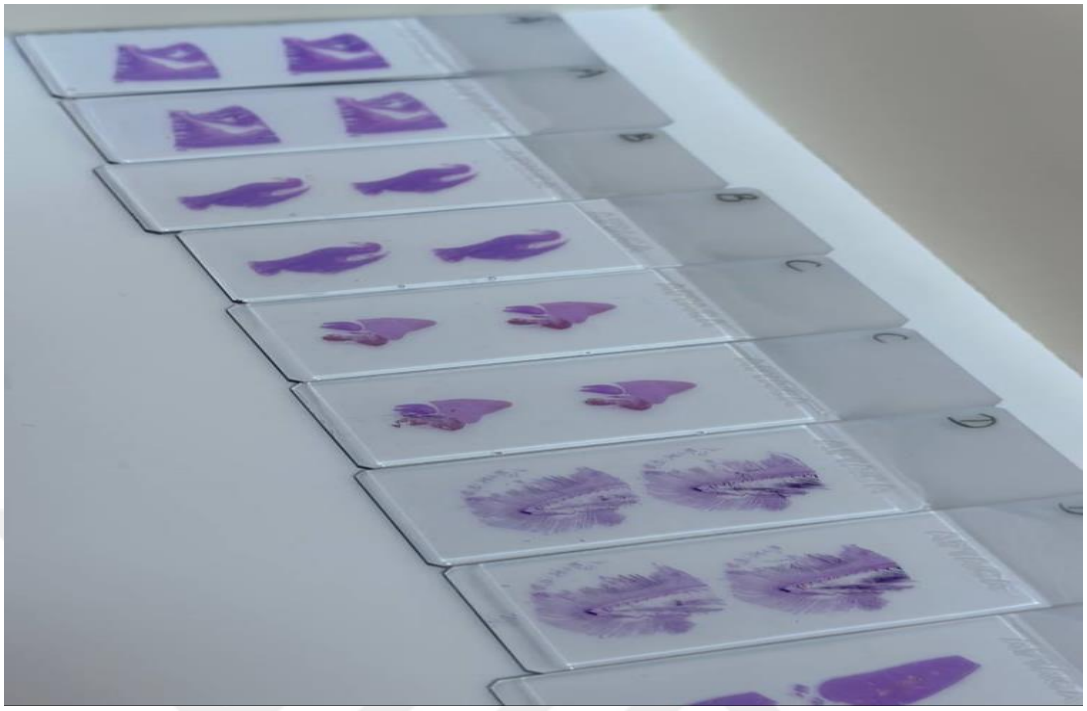


APPENDIX 2. Some of the types of parasites



APPENDIX 3. Tissue cutting device (Microtome)

APPENDIX 4. Tissue slices from various fish organs



APPENDIX 5. Acceptance letter for work conducted in the laboratory of the Faculty of Science, Misurata University

APPENDIX 6. Acceptance of the research paper for publication in the Journal of Advanced Zoology

APPENDIX 7. Research paper published in the International Journal of Chemical and Biochemical Sciences

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