



**INFESTATION AND EFFECTS OF ANISAKID NEMATODE PARASITES ON THE
NILE TILAPIA (*Oreochromis niloticus* LINNAEUS, 1758) IN LAKE HAWASSA,
ETHIOPIA**

M.Sc. THESIS

BY

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HAWASSA UNIVERSITY, HAWASSA, ETHIOPIA

OCTOBER, 2024

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ETHIOPIA**

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A THESIS SUBMITTED TO

**THE DEPARTMENT OF AQUATIC SCIENCES, FISHERIES AND AQUACULTURE,
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**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
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OCTOBER, 2024

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This is to certify that the thesis entitled “**Infestation and Effects of Anisakid Nematode Parasites on the Nile Tilapia (*Oreochromis niloticus* Linnaeus, 1758) in Lake Hawassa, Ethiopia**” submitted in partial fulfillment of the requirements for the Degree of Master of Science with specialization in Aquaculture and Fishery Management, has been carried out by Ribka Menta Balcha, ID. No. GpFiAqR/0004/15, under my supervision. Therefore, I recommend that the student has fulfilled the requirements and hence she can submit the thesis to the Department of Aquatic Sciences, Fisheries and Aquaculture (ASFA). Hawassa University, Hawassa.

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Name of Advisor

Signature

Date

EXAMINER’S APPROVAL SHEET-I
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We, the undersigned, Members of the Boards of Examiners of the final Open Defense have read and evaluated her thesis entitled “**Infestation and Effects of Anisakid Nematode Parasites on the Nile Tilapia (*Oreochromis niloticus* Linnaeus, 1758) in LAKE Hawassa, Ethiopia**” This is therefore, to certify that, the thesis has been accepted in partial fulfillment of the requirement for the Degree of Master of Science in Aquaculture and Fishery Management.

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DECLARATION

I hereby declare that this M.Sc. thesis entitled “*Infestation and Effects of Anisakid Nematode Parasites on Nile Tilapia (Oreochromis niloticus Linnaeus, 1758) in Lake Hawassa, Ethiopia*”, is my original work, and it has not been presented for a degree in any other University, and that all sources of materials used for this thesis study have been accordingly acknowledged.

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DEDICATION

I dedicate this thesis to my beloved husband, Million Orsango who are largely responsible for my current state of being. Additionally, I dedicate it to my parents, especially my father Menta Balcha and eldest brother Ayele, who has been a guiding force for me throughout my studies and research work.

BIOGRAPHICAL SKETCH

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TABLE OF CONTENTS

CONTENTS	PAGE
DECLARATION	v
ACKNOWLEDGEMENTS	vi
DEDICATION	vii
BIOGRAPHICAL SKETCH	viii
TABLE OF CONTENTS	ix
LIST OF FIGURES	xiii
LIST OF ABBREVIATIONS /ACRONYMS	xiv
ABSTRACT	xv
1. INTRODUCTION	1
1.1 Background of the study	1
1.2 Statement of the Problem	3
1.3. Objectives	3
1.3.1. General Objective	3
1.3.2. Specific objectives	3
1.4 Research Questions	3
1.5 Scope of the study	4
1.6 Significance of the study	4
1.7 Limitation	4
2 LITERATURE REVIEW	5
2.1 Fish Parasitism	5
2.2 Nematode parasites	6
2.3 Anisakid	7
2.3.1 Infestation of anisakid nematode	10

2.3.2 Effect of Anisakid on fish	10
2.3.2.1 Effect of Anisakid parasites on heamatology	10
2.3.2.2 Effect on biochemical composition	11
2.4 Effect of water quality on fish parasites	11
2.4.1 Dissolved oxygen (DO)	12
2.4.2 Temperature	12
2.4.3 Hydrogen ion concentration (pH)	13
2.4.4 Nitrogen compound (ammonia, nitrate and nitrite)	14
2.7 Fish borne zoonotic parasites	14
2.5 Economic importance of Anisakid nematodes	15
CHAPTER THREE:	17
3. MATERIALS AND METHODES	17
3.1 Description of the Study area.....	17
3.2. Study populations.....	18
3.3. Sample size determination and sampling method.....	19
3.4. Sample Collection and study methodology	20
3.5 Collection and Preservation of <i>Contracaecum</i> species.....	20
3.7 Biochemical composition of fish tissue	22
3.9 Heamatology.....	24
3.9.1 Data Analysis	27
4. RESULTS	28
4.1 Infestation of <i>Contracaecum</i> spp. on <i>O. niloticus</i> in Lake Hawassa	28
4.2 Prevalence and intensity of infestation relation to fish length	29
4. 3 Prevalence and intensity of infestation in relation to fish weight	30
4.4 Prevalence and intensity of infestation in relation to fish sex	31

4.5. The prevalence of <i>Contraceacum</i> spp. was evaluated based on different body organs. ...	32
4.6 Physicochemical parameters of Lake Hawassa	33
4.7 Heamatological effects of <i>Contraceacum</i> spp. infestation on <i>O. niloticus</i>	35
Table 6: Heamatological parameters of infested and uninfested <i>O. niloticus</i> by <i>Contraceacum</i> spp.	36
4.8 Effect of infestation of <i>Contraceacum</i> spp. on biochemical composition of <i>O. niloticus</i> .	36
Table 7: Biochemical parameters value of infested and uninfested <i>O.niloticus</i> by <i>Contraceacum</i> spp.	37
CHAPTER FIVE	38
5. DISCUSSION	38
5.1. Prevalence and intensity of infestation of <i>Contraceacum</i> spp. on <i>O. niloticus</i> in Lake Hawassa in relation to Different parameters.....	38
5.2 The prevalence of anisakids nematode and body organs.....	40
5.4 Association of physicochemical parameters and prevalence of Anisakid nematode	41
5.5 Heamatological effects of <i>Contraceacum</i> spp.on <i>O. niloticus</i>	43
5.5 Effects of <i>Contraceacum</i> spp. on biochemical composition of <i>O. niloticus</i>	45
REFERANCES	50
APPENDICES	68

LIST OF TABLES

Table 1: Prevalence and intensity of infestation of <i>Contraceacum</i> on <i>O. niloticus</i> at Lake Hawassa (n = 180)	29
Table 2: Prevalence and intensity of <i>Contraceacum</i> spp. in relation to length of <i>O. niloticus</i> ...	30
Table 3: Prevalence and intensity of infestation in relation to fish weight of <i>O. niloticus</i>	31
Table 4: Prevalence and intensity of <i>Contraceacum</i> spp. based on sex of <i>O. niloticus</i> (n=180) .	32
Table5: Association of physicochemical parameters among them and prevalence of <i>Contraceacum</i> spp.	35
Table 6: Heamatological parameters of infested and uninfested <i>O. niloticus</i> by <i>Contraceacum</i> spp.	36
Table 7: Biochemical parameters of infested and uninfested <i>O. niloticus</i> by <i>Contraceacum</i> spp.	37
Table 8: Physicochemical parameters of Lake Hawassa	68

LIST OF FIGURES

Figure 1: Study area map of Lake Hawassa (Sources: ArcMap).....	18
Figure 2: Photograph indicating sampling site (Lake Hawassa)	20
Figure 3: Photograph indicating examination of <i>O. niloticus</i> from different organs for larval stage and adult stage of <i>Contraceacum</i> spp. by using naked eye and stereo microscope.....	21
Figure 4: Photograph indicating for the biochemical composition of infested and uninfested fish by <i>Contraceacum</i> spp.	22
Figure 5: Photograph indicating different organs of <i>O. niloticus</i> infested by <i>Contraceacum</i> spp.	32
Figure 6: Distribution of <i>Contraceacum</i> spp. in relation to different organs of <i>O. niloticus</i>	33
Figure 7: Photograph indicating samples of <i>O. niloticus</i> for <i>Contraceacum</i> sp. examination	68
Figure 8: Photograph indicating examination of <i>O. niloticus</i> of different organs for larval stage of <i>Contraceacum</i> spp. by using naked eye and stereo microscope	69
Figure 9: Photograph indicating <i>Contraceacum</i> spp. from <i>O. niloticus</i> body	69
Figure 10: Photographs showing blood sample collection for heamatology study from the infested and uninfested <i>O. niloticus</i> by <i>Contraceacum</i> spp.....	70

LIST OF ABBREVIATIONS /ACRONYMS

CARE	Center for Aquaculture Research and Education
DO	Dissolved Oxygen
EDTA	Ethylenediaminetetraacetic acid
MI	Mean intensity
MCV	Mean cell volume
PUFAs	Poly Unsaturated Fatty Acids
RASFF	Rapid Alert System for Food and Feed

ABSTRACT

Contracaecum species that is genus of Anisakid parasites is the most economically important internal fish parasite, that poses serious problem in culture and capture fisheries globally, as well as consumers. This investigation aimed to evaluate the infestations and certain effects of *Contracaecum* spp. on *O. niloticus* L. 1758) in Lake Hawassa. The study used a cross-sectional design from December 2023 to May 2024. A total of 180 *O. niloticus* were taken from the landing site. To assess the water quality, samples of water were taken from Amora Gedel. Infestation peaked in February and April each with 56.6% and lowest in December (40.0%). However, highest intensity of *Contracaecum* spp. was in May (9.66%) and lowest in January with (4.20%). Prevalence of *Contracaecum* with sex of *O. niloticus* were statistically not significant. The size of *O. niloticus* and the presence of *Contracaecum* were positively correlated. Hematological effects of *Contracaecum* on *O. niloticus* between infested and non-infested group were significant ($p < 0.05$) except for neutrophil count and basophil ($p > 0.05$). The results of the physicochemical parameters showed statistically significant difference with all parameters except ammonia that showed variation, but not statistically significant (p value = 0.294). There were positive correlation between physicochemical parameters and the prevalence of *Contracaecum*, however, negative correlation with dissolved oxygen ($r = -0.002$). In conclusion, the temperature of the water influences whether the *Contracaecum* infestation is high or low, depending on the sampled months. *Contracaecum* parasite prevalence is also influenced by variations in physicochemical parameters. The high prevalence (37.3%) of *Contracaecum* spp. in muscles of *O. niloticus* as compared to varied various body organs indicates that consuming raw fish poses a significant health risk to humans. Therefore, it is crucial to eat the fish only well cooked form, as the encysted larvae present in fish muscle can be transmitted to human hosts.

Key words: Anisakid, *Contracaecum*, Infestation, *O. niloticus*, Zoonosis

1. INTRODUCTION

1.1 Background of the study

Aquaculture and fisheries are important food production sectors that are gaining special significance all over the world as a source of additional food production to meet the food demand of the growing human population, especially in developing nations. Aquaculture and fisheries production reached a record of 214 million tonnes, with 178 million tonnes of aquatic animals' 36 million tonnes of algae in 2020. This increase was primarily attributed to the expansion of aquaculture, particularly in Asia (FAO, 2022). It has been reported that, the human population is continuously increasing from the present level of more than 7.3 billion to the expected level of 8.5 billion in 2030 to 9.7 billion in 2050 (DESA UN, 2015). It is seen that as the present human population of Ethiopia is about 120.5 million, which is predicted to reach 145 million by 2030 and 205 million by the year 2050 (Worldometer, 2022), there is an imperative need to concentrate more on increased fish production through capture and culture fisheries.

As capture fisheries production is mostly stagnating and that there is no possibility of producing more fish through capture fisheries in the near future due to several factors, the alternative sector of aquaculture has to be given importance (Manlosa *et al.*, 2019). Although aquaculture production is steadily increasing and that it has contributed significantly to food security, like any other food production sectors, aquaculture is also facing several problems of which one of the major problems that affect production is parasites and diseases in cultivable fishes (Subasinghe *et al.*, 2009).

Fish constitute one of the most important protein sources of food for humans all over the world, especially developing nations. Besides fish being a source of rich protein, fisheries play a significant role in improving rural livelihoods by generating income and employment opportunities (Béné *et al.*, 2016). However, a number of factors have been reported to affect fish production both in capture and culture fisheries of which the most important one is the onset of

infection in fish by parasites and diseases. Widespread parasite incidences in fish populations continue to be a significant public health issue and a barrier to economic development. Fish parasites are known to cause damage to fish tissues thereby reducing their marketability and consumer preference (EFSA, 2010). Among metazoan parasites, the parasites coming under Nematodes are important as they are widely affecting many of the cultivable fishes. It is seen from the literature that, among nematode parasites, the parasite species coming under the group Anisakid are most important as they cause not only mortality to fish populations, but also affect human health (Casti *et al.*, 2017). The etiologic agents of nematode parasites belong to the genera *Anisakids*, *Pseudoterranova* and *Contracaecum*. It is also reported that some of the nematode parasites are of zoonotic significance causing human health hazards (EFSA, 2010). Human infection occurs when raw or undercooked fish, with larvae are eaten (Buchmann and Mehrdana, 2016). Anisakid nematodes are economically important food borne parasites with unique characteristics. They frequently occur within the body cavity and viscera of fish and cause damage by feeding and attachment. All of the worms are associated with sociocultural and behavioral factors, especially the habit of eating raw fish (Macpherson, 2005). Besides zoonotic significance, Anisakid nematodes are also important in fisheries as they are widely used as biological tags for understanding fish migration (Aco Albuquerque *et al.*, 2020).

Anisakidosis has gained significance in recent years as the disease produce gastric, intestinal and allergic reactions in people who eat raw fish (Aibinu *et al.*, 2019). Anisakid nematode parasites have been found in catfish and tilapia in many African nations. According to Euaem Abebe *et al.*, 2006, fish from Ethiopia have been found to contain larval *Contracaecum*. Until recently most of the research work on nematode parasites are highly concerned with occurrence on marine fish, however research work relating to freshwater fishes are relatively less (Euaem Abebe *et al.*, 2006). Therefore, this study was conducted to determine the host parasite relationships between Anisakid nematodes and *O. niloticus* which is the predominant food fish in Ethiopia.

1.2 Statement of the Problem

Fish pathogens are important as they seriously harm and even kill fish (Stryński, 2022). It has been widely recognized that, occurrence of parasites and diseases of fish often leads to fish mortality producing great economic loss to fishing and farming industry (Behringer *et al.*, 2020). From available literature, it is seen that parasites of zoonotic significance has been dealt in detail in other countries (Shamsi, 2019). However, in Ethiopia, studies relating to parasites of zoonotic significance in fish species is not that much available rather than infestation of parasites (Fasil, *et al.*, 2017; Zekarias and Yimer, 2007) and hence the present study is needed. The present study attempted to investigate in detail their effects on the hematology and biochemical composition; the influences of water quality on the infestation of Anisakid nematodes on *O. niloticus* in Lake Hawassa, Ethiopia.

1.3. Objectives

1.3.1. General Objective

To determine the prevalence and effects of *Contracaecum* species parasites on *O. niloticus*

1.3.2. Specific objectives

To assess the prevalence and intensity of infestation of Anisakid nematodes on *O. niloticus*

To compare the influence of water quality on the infestation of Anisakid nematodes on *O. niloticus*

To examine the effects of Anisakid nematode on the hematology of *O. niloticus*.

To evaluate the biochemical composition of *O. niloticus* infested by Anisakid nematode

1.4 Research Questions

What is the infestation rate of Anisakid nematode parasites by *O. niloticus* in Lake Hawassa?

What is the influence of Lake Hawassa water quality on the infestation of Anisakid nematode in *O. niloticus*?

What is the effect of Anisakid nematode infestation on the hematology of *O. niloticus*?

How Anisakid nematode infestation reduces biochemical composition of *O. niloticus*?

1.5 Scope of the study

The scope of the study examined the occurrence of Anisakid nematode parasites on *O. niloticus* in Hawassa Lake. The study focusses on water quality parameters of Lake Hawassa, and the effects of nematode parasites on blood parameters and biochemical composition of the host fish.

1.6 Significance of the study

This study provide valuable insights into the potential threats of Anisakid nematode parasites on *O. niloticus* in Lake Hawassa. Understanding on the nature of water quality and occurrence of nematode parasites help to take appropriate measures to control infestation through better management of the Lake to reduce parasite load in fish species.

1.7 Limitation

- An important factors of fish parasites study such as condition factor and histopathology examination was not included under this study.
- The major constraint is laboratory equipments and budget for blood analyses and biochemical estimation.

2 LITERATURE REVIEW

2.1 Fish Parasitism

Parasitism is a symbiotic relationship in which two organisms live together in which the parasite either depends on and hurts the host or survives on it (Leung and Poulin, 2008). If swallowed in live form, fish parasites like roundworms and tapeworms can pose health risks to people. Anasakid worms are present in marine and freshwater species, and are coming under the group *Contracaecum*, *Pseudoterranova*, and they are of great importance since they cause serious problems to fish (Scanes and Toukhsat, 2018).

The occurrence and abundance of nematode parasites greatly depends on the feeding behavior of the host fish and the damage to host tissue is mainly due to their penetration and feeding nature of the parasite (Iyaji and Eyo, 2008). Besides, fish species generally act as intermediate or definite hosts for a number of parasites, and its occurrence and abundance depends greatly on feeding behavior of the fish. ectoparasites that are most frequently found in freshwater, but can also be found occasionally in brackish and marine waters. They infect teleosts, tadpoles of *Rana* species(Poly, 2003).

Fish endoparasites live inside the tissues, vital organs and body cavity of their host. Hosts provide habitat and a source of food for internal parasites. Endoparasites can cause various pathological disorders like internal bleeding, swelling, ulceration, obstruction and malnutrition in fish species. These diseases often produce a weakening of the hosts' immune system thereby increasing their vulnerability to other secondary infections (Eyo and Iyaji, 2014).

2.2 Nematode parasites

Nematode (roundworm) parasites are very common in freshwater, brackish water and marine fishes. Among metazoan parasites, nematodes constitute rich species diversity under diverse taxa (Luque and Poulin, 2007). They are mostly endo parasites of fishes. Nematodes are thread worms, the size of male ranges from 3-9 x 0.1-0.5mm and female from 7-21 x 0.18 mm. Body elongate, bilaterally symmetrical and unsegmented covered by a multilayered non-cellular thick cuticle, a thin cellular hypodermis and longitudinal muscle cells (Cheeseman, 2001).

They are dioecious with sexual dimorphism. Body consists of head with lipped mouth containing series of teeth. The digestive system is complete with buccal cavity, pharynx, esophagus, intestine and anus. Excretory system is with an excretory gland and the excretory products are discharged through an anterior ventromedial pore (Brewer and Greve, 2019). The parasite has no respiratory or circulatory system. The female reproductive organs consist of vulva, short vagina, and pair of uteri, oviducts and ovaries. The male reproductive system constitutes testes, seminal vesicle and vas deferens. The size and shape of buccal capsule, arrangements and morphometrics of the internal structures, alimentary tract and the orientation of the excretory pore, presence or absence of bursa, copulatory spicules in male reproductive organ are of taxonomic importance (Nguyen, 2010).

Nematodes have mostly indirect life cycle including mammals, fish, copepods and piscivorous birds depending on their species. They have four moults in the life cycle; eggs hatch to produce first stage larvae (L1), L1 moult to L2 then L3, L4 and finally the fourth stage larvae moult to become adults. The adult nematode parasite releases eggs in the aquatic environment through faeces of the host fish. Temperature plays a major role in the development of the parasite (Klimpel and Palm, 2011). In many cases moulting takes place within the egg and the eggs are hatched into free-swimming larva which on predation by the intermediate hosts (crustaceans and in paratenic hosts) develops in to larval stages. These larvae found in the body cavity, surfaces of

internal organs and muscle tissue. When entering the definitive host (vertebrate including fish), the advanced larvae moult into pre adult and then adults to reproduce. The females of *Camallanoidea* and *Dracunculoidae* are viviparous and release the young ones directly in to water (Williams and Bunkley-Williams, 2019). Among nematode parasites, anisakids are important as they are potent risk to humans and cause a disease known as anisakidosis.

2.3 Anisakid

Nematode parasites belonging to the family Anisakidae, are the most prevalent fish parasites, and are present in a wide variety of fish species. Nematodes of the genera *Contracaecum*, *Pseudoterranova* and *Hysterothylacium* are commonly found in the viscera and musculature of many species of teleost fish (Mattiucci *et al.*, 2017). Fish are intermediate host can infect humans causing significant clinical diseases to human beings. When fish ingest the larvae (usually through contaminated water or by consuming infected prey), the larvae migrate through the fish's digestive system and upon reaching the muscle tissue, they can encyst, forming a protective cyst around themselves. Anisakid are parasites of the alimentary tract and muscle of aquatic vertebrates show worldwide distribution (Zhou *et al.*, 2008). The taxonomy of this parasite are.

Kingdom: Animalia

Phylum: Nematoda

Class: Secernentea

Order: Ascaridida

Family: Anisakidae

Genus: *Contracaecum*

Anisakid have complex life cycle needs a number of hosts to complete the life cycle. Adult Anisakid worms lay eggs in the gut of many species of marine mammals, and then these eggs are excreted from the host through fecal materials in the water. Once these hatched larvae are in open water, they can be ingested by krill or other crustaceans. At this stage, the prevalence, or proportion of infected hosts, is rather low. The infected crustaceans can then be eaten by fish and cephalopods, where the parasite gets its development. Once the fish obtains the parasite, it then can reach the definitive host, or final host, through the consumption of the infested fish (Sitjà-Bobadilla, 2008). The prevalence of this parasite in humans is higher in places where people regularly consume raw fish.

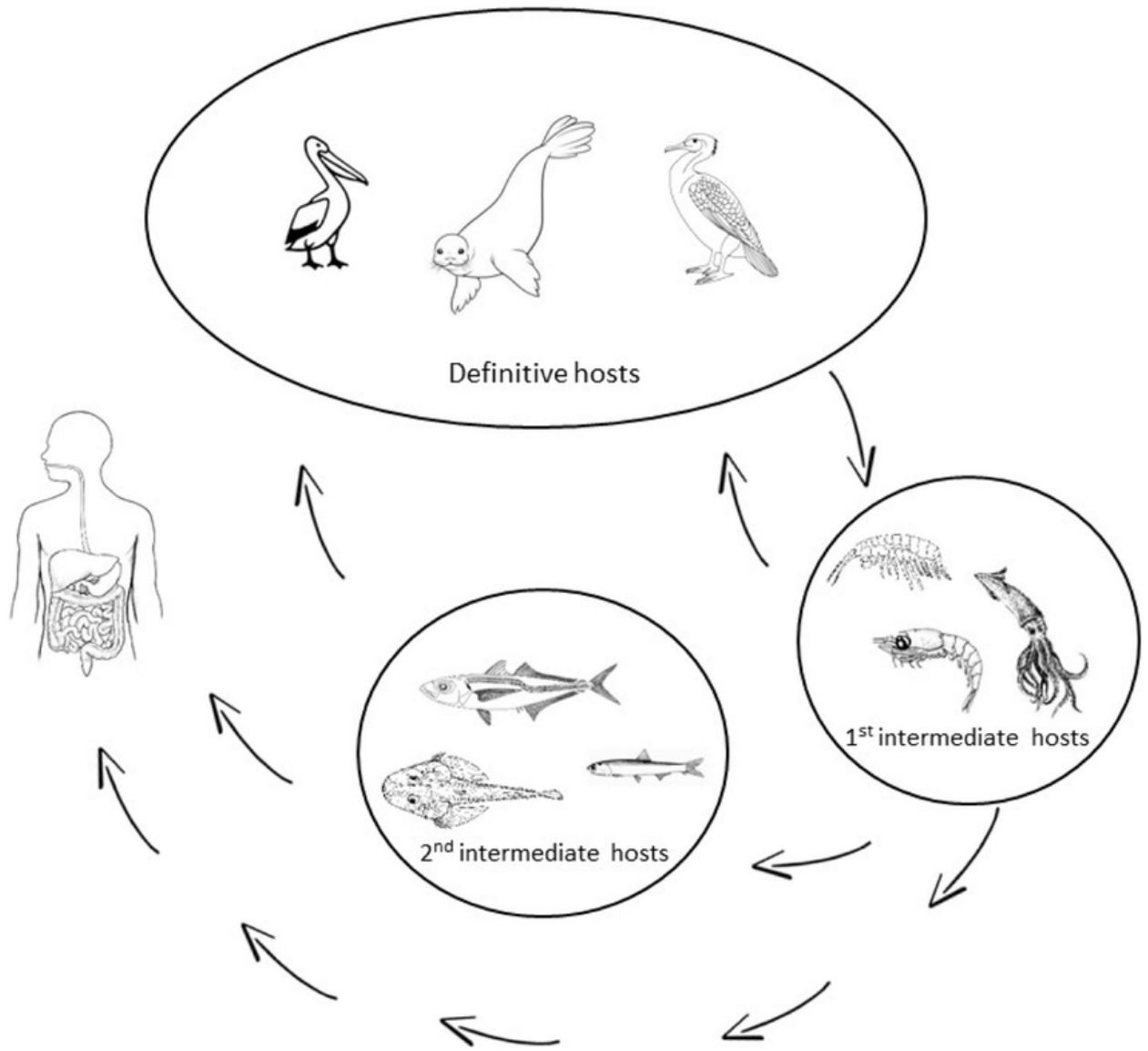


Figure 2. 1: Life cycle of *Contraceacum* species (Shams, 2019)

2.3.1 Infestation of anisakid nematode

Anisakid nematodes are prevalent in a wide range of fish species, especially pelagic fish. The prevalence may vary depending on the region, season, size, age and sex of fish. The prevalence of anisakid nematode larval infestation was 45.77% ranging from 1 to 60 per one fish (Mattiucci *et al.*, 2022).

Tesfaye Zinabu *et al.*, (2020), reported the over all prevalence of larval *Contracaecum* was 31.8% with higher prevalence in female (37.1%) than male (25.8%). In respect of weight category, it was more in class II category than I and III. The study conducted in Egypt revealed that, the overall prevalence of nematode parasite in *O. niloticus* was 65%.

Similar results were reported by Gulelat Yewubdar *et al.*, (2013) who documented a prevalence of 24.8% for *Contracaecum* followed by *Clinostomum* (27.4%) and 15 *Euclinostomum* (2.73%) in *O. niloticus* from Koka reservoir, Ethiopia and Amare *et al.*, (2014), who had also reported a prevalence of 42.6% for *Contracaecum* and 38.6% for *Clinostomum* in Lake Lugo, Ethiopia. Bekele Jossy and Hussein Daniel, (2015), also reported a prevalence of 62.50% from *Contracaecum* and 31.25% from *Clinostomum* from Lake Ziway, Ethiopia. Similar study conducted in another section of Ethiopia (Lake Lugo, North east Ethiopia) revealed that, out of the 412 sampled fish, 197 (47.8%) were found infested with internal parasites. Prevalence reported were 50.22%, 91.7% and 15.6% from *O. niloticus*, *C. gariepinus* and *C. carpio* species (Biu, 2014).

2.3.2 Effect of Anisakid on fish

2.3.2.1 Effect of Anisakid parasites on heamatology

Heamatological studies are crucial as they provide important information about health status of fish, their physiological status and the influence of various environmental factors, and serve as a tool for evaluating their immune system (Tavares-Dias and Moraes, 2007). According to Ruane

et al., 2000, parasitized fish may exhibit significant changes in their hematological, physiological, and developmental characteristics. Hematologic disorders are caused by anisaki species and their invasion causes stress-related behavioral and physiological responses in the host fish. According to Ali *et al.*, 2010, anemia in infected fish was characterized by a reduction in the number of red blood cells and hematocrit in the host blood because of the parasite feeding activity and a failure of the host osmotic regulation as a result of skin lesions.

Hematological parameters include hematocrit, hemoglobin concentration, erythrocyte and leukocyte counts, mean cell volume (MCV), mean cell hemoglobin, mean cell hemoglobin concentration, lymphocytes, monocytes and eosinophils. Significant difference is observed for all parameters between infected and uninfected fish (Fallah *et al.*, 2015).

2.3.2.2 Effect on biochemical composition

Fish is a source of rich protein with essential fatty acids such as eicosapentaenoic acid and docosahexaenoic acid. Several factors decide the level of proteins in fish and they are:- the state of environment; species, size, sex of fish, location of parasite, degree of infestation and immune response of host fish (Wilson, 2003). Biochemical composition of fish tissues is important in understanding the nutritive value of fish and human nutrition. Studies carried out indicate that parasitic infestations deplete nutritive value of fish and it reduces the amount of carbohydrates and calcium content in fishes. Anisakid nematodes are reported to affect fatty acid composition particularly polyunsaturated fatty acid (PUFAs) and it reduce protein content that are needed for human health (Jouini *et al.*, 2023).

2.4 Effect of water quality on fish parasites

Water quality determines the infestation of parasites in fish. The major water quality parameters such as temperature, dissolved oxygen, pH, ammonia, nitrite and nitrate will have greater influence on onset of infestation by diverse group of parasites in fishes and cause fish organs to function less physiologically (Boyd, 2009). Conte, 2004,, asserts that water with dissolved

oxygen level below or above optimal levels stresses fish and increase the risk of parasites growth. Lower levels of dissolved oxygen, brought in by the build-up of organic matter create an ideal environment for the growth of parasites and the appearance of pathogenic agents, particularly parasites that live in the organ surface of fish, such as endoparasites of helminths (Garcia *et al.*, 2009). Parasitic infestations could become more detrimental to host fish due to the water temperature's change. Water quality and the environment greatly influence the existence and proliferation of parasites in the water and consequently, fish (Woruiru, 2020). The quality of water in the aquatic ecosystem is a very sensitive issue and is controlled by many physical and chemical factors. The deterioration of water quality has variable effect on parasitic population and their rate of infestation and consequently the negative impact can affects fish viability and productivity (Mahmoud *et al.*, 2019).

2.4.1 Dissolved oxygen (DO)

Many aquatic animals can survive at dissolved oxygen levels between 4 and 7 mg/L. According to Omondi *et al.*, 2011, aquatic organisms exposed to low levels of dissolved oxygen may be more vulnerable to the negative effects of other stressors, such as disease and toxins. In order to prevent fish poisoning, it is crucial to maintain dissolved oxygen at the necessary level. *O. niloticus* can survive at DO levels much lower than 3 mg/L. Lower levels of dissolved oxygen, brought in by the build-up of organic matter, create an ideal environment for the growth of parasites and appearance of pathogenic agents, particularly parasites that live in the body of fish, such as endo helminths (Garcia *et al.*, 2009).

2.4.2 Temperature

The temperature of the aquatic environment affects a wide variety of aquatic organism, particularly fish (Ngaira, 2006). A preferred water temperature exists for each species of fish. Temperature between 20°C and 30°C are comfortable for freshwater fish. Fish are compelled to move to different areas when the temperature exceeds their ideal range because they have very

sensitive thermoregulation. Fish experience negative effects if the water temperature deviates too much from its ideal level. Although the rate of change in water temperature is slower than that of the air, it has serious negative effects on fish in tropical regions when it rises above the inlet temperature (Arle, 2002). Temperature has greater influence on the rate of egg incubation, development of the eggs and hatching out as naupliar larvae of anisakids nematodes. According to Sahoo *et al.*, 2013, the eggs of anisakid species incubated at 32°C hatched out to naupliar larvae in a period of 10 days with a hatching of 60%. The length of time needed for hatching gradually gets shorter as incubation temperature rises, demonstrating the temperature dependence of development. The temperature of 28°C was found to be ideal for development in to the infective naupliar stage and hatching. A very low rate of naupliar larvae hatching occurred as result of low temperature, which also slowed down development. According to Taylor *et al.*, 2009, lakes with water temperature below 10°C do not have anisakid parasites. The warmer month of the year influences the prevalence of anisakid species. Due to the fact that development is slowed down at lower temperature and hatching only happens when ambient temperature is present (Mikheev *et al.*, 2001).

2.4.3 Hydrogen ion concentration (pH)

According to Oduor, 2000, the addition of contaminant from various sources has a significant impact on the pH level in water bodies, which in turn has a greater impact on the life forms in water bodies. They weaken fish species defense mechanism, making them more susceptible to infestation from various parasites and pathogen species. Fish welfare is impacted by water pH levels that are either below or above normal. To be considered safe, water must have a pH of 6.5 to 8.5. For water quality, pH values below 6.5 become highly acidic and those above 8.5 become highly alkaline. Low water quality and problems for fish species result from high acidity or alkalinity (Oduor, 2000). Fluctuation in temperature below or above optimum levels can affect water pH and lead to the growth of parasites (Gaffar, 2007). Different types of fish parasites have different responses and respond differently to changes in pH of the water in which they live.

According to Bague *et al.*, 2004, the composition and frequency of the parasites communities in various fish species depend on the pH value of water. A study by Halmetoja *et al.*, 2000 showed a decrease in the number of metazoan parasites species and their abundance in acidified water, for the response of perch parasites to acidified water in two artificial lakes in Finland. However, greater biodiversity was observed in high alkaline water compared to near neutral pH of the lakes.

3.4.4 Nitrogen compound (ammonia, nitrate and nitrite)

The amount of ammonia that is ionized and unionized depends on the temperature and pH of the water. The most prevalent form of ammonia when pH is high is unionized ammonia (NH₃), which is extremely toxic to fish. In water with a low pH, ionized ammonia (NH₄) predominates and is nontoxic unless present in extremely high concentrations. According to Santhosh and Singh, 2007, the maximum allowable concentration of ammonia for aquatic organisms is 0.1mg/L and thus aquaculture should use ammonia levels of less at this level according to Bhatnagar and Singh, (2010). The ideal range of ammonia for fish farming is 0.1mg/L, according to Stone, (2013). The more the toxic ammonia (NH₃) that is present in the water, the higher the water temperature and lower the pH. According to Mphuthi, 2021, the acceptable limits for ammonia-free nitrogen, nitrate and nitrite are 0-0.2 mg/L, 200 mg/L and 0-0.2mg/L, respectively.

2.7 Fish borne zoonotic parasites

Fish-borne zoonotic parasites are a major socioeconomic and public health risk (Chai *et al.*, 2017). Many fish-borne zoonoses are caused by aquatic helminths, such as nematodes, trematodes and cestodes (Bao *et al.*, 2019). The third-stage larvae of Anisakid nematodes found in fish can infect humans if ingested with raw or slightly cured fish. The most common cause of human infestation is through anisakid larvae, although *Contraceacum* larvae have also been implicated. The larvae of the parasitic nematode anisakid have been increasingly linked to adverse allergic reactions on ingestion of contaminated fish (Arizono *et al.*, 2011). Infectious

stage larvae (L3) found within fish may be accidentally ingested by humans. If ingested live, as a result of consumption of raw or undercooked fish the L3 larvae of *Contraceacum* can cause the zoonotic disease. Within hours of being ingested, *Contraceacum* L3 penetrates the mucosal layers of the gastrointestinal tract, causing direct tissue damage that may lead to abdominal pain, nausea, diarrhea and/or allergy (Pozio, 2018).

2.5 Economic importance of Anisakid nematodes

Anisakid nematodes can infect the stomach or intestine of humans when uncooked fish are eaten by human. The presence of anisakid in fishery products can reduce their marketability and commercial value due to food safety and quality implications (Fuentes, 2022). This can lead to a provoke economic losses to the fishing industry. According to experts from FAO/WHO, Anisakid are ranked 4th out of 24 food-borne zoonotic parasites in terms of relevance to international trade. In the European Union, the Rapid Alert System for Food and Feed (RASFF) reported total of 289 are RASFF notifications of parasitic infestation in fish and fishery products between 2010 and 2016. Of these, 234 (81%) were suspected to be Anisakid (Bao *et al.*, 2019).

In fact, the presence of the parasite in the fish's viscera and muscle can lead to fish sellers and customers rejecting fisheries products, significant detrimental impacts on fish marketability, and a growing lack of trust among consumers in fisheries products (EFSA, (2010). In addition to increasing the risk of anisakidosis for consumers, the high prevalence of anisakid nematode larvae in some commercially available fish species has negative impact on fish quality and appearance, which leads to consumers rejecting fish that has been parasitized. Consequently, the fishing industry suffered large losses. The effects of these diseases result to nutritive devaluation of fish and fish loss. Parasites also result to fish's declining swimming ability, decrease in growth rate and increase in mortalities (Osimen and Anagha, 2020).

Fishery products derived from many commercially important wild marine fish appear to be the only industrially processed food with a high risk of containing parasites when put on the market.

Inspection procedures to control and remove visible parasites carried out by the industry evidently introduce additional costs to commercial processing (Bao *et al.*, 2019). A survey-based contingent valuation study was recently performed to investigate the attitudes and opinions of fish consumers in Spain about the presence of Anisakid in fish, associated diseases (i.e. anisakidosis and allergy), and how much they would value the removal of Anisakid from fishery products (Matson *et al.*, 2019).

CHAPTER THREE:

3. MATERIALS AND METHODES

3.1 Description of the Study area

The study was conducted in Lake Hawassa, Sidama Region, Ethiopia. The Lake is situated 275 km south of Addis Ababa the capital city of Ethiopia. Geographically, the Lake lies between 6°33'–7°33' N and 38°22'–38°29' E. The altitude of the Lake is 1,680 m above mean sea level (Yitayal Alemu *et al.*, 2017) and it is one of the Ethiopian Rift Valley Lakes. Its surface area is 90 km² (Girma Tilahun and Ahlgren, 2009) and the maximum and mean depth of the Lake is 22 m and 11 m respectively (Elias Dadebo, 2000). Its annual rainfall is 969 mm (Halcrow, 2008) and Temperature ranges between 20 and 27° C. Cattle grazing, aquatic weed infestation, wetland cultivation, tourism, domestic and industrial discharges are the main sources of pollution in the Lake (Fentaw,Getachew *et al.*, 2022). *O. niloticus*, African Catfish *C. garipinus* and African barb *Labeobarbus* are some of the important food fishes of the Lake. *O. niloticus* contrinutes to about 85% of the fish catch (Dsikowitzky *et al.*, 2013).

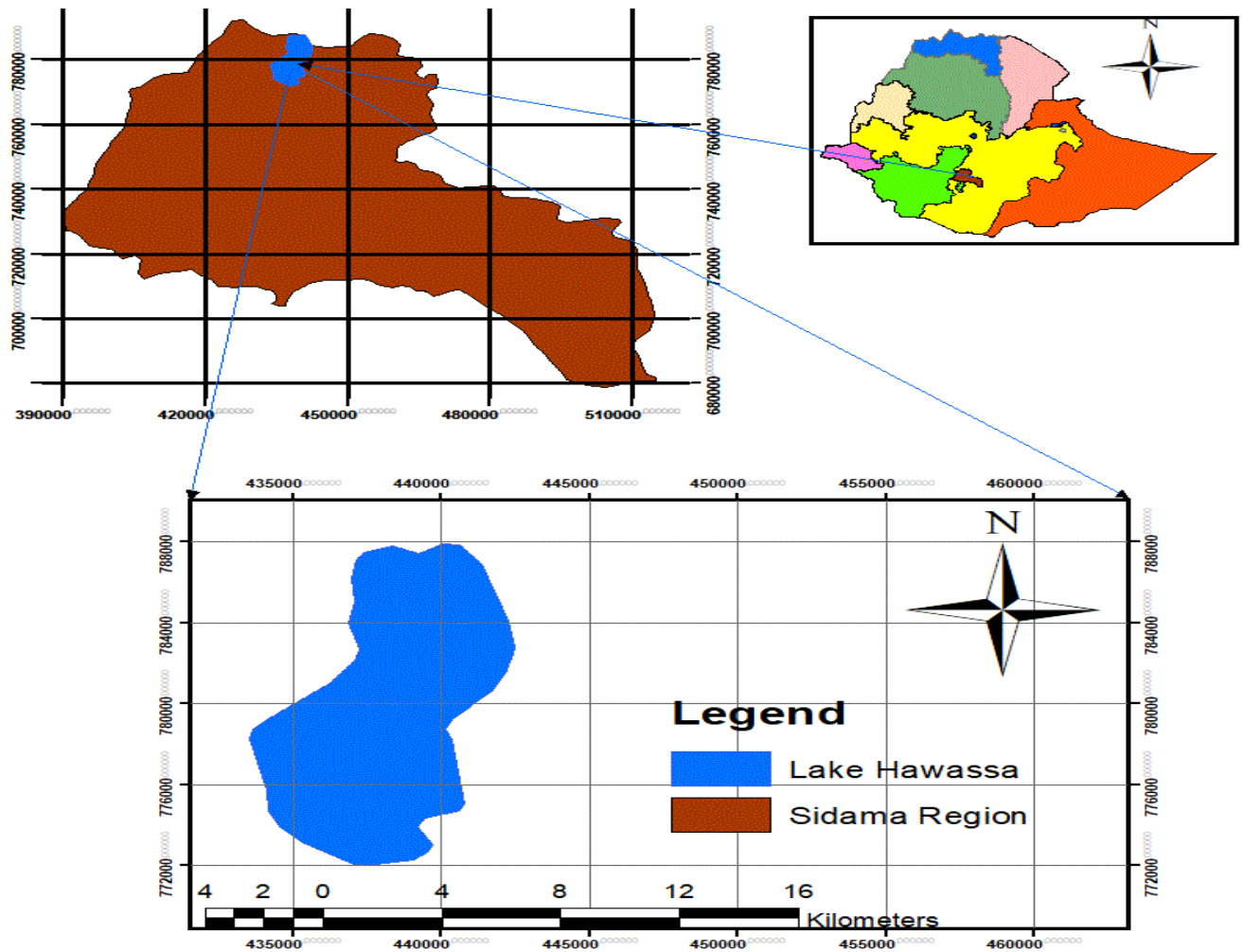


Figure 1: Study area map of Lake Hawassa (Sources: ArcMap)

3.2. Study populations

The sample for the study was *O. niloticus* of different weight, length and sex collected from Lake Hawassa.

3.3. Sample size determination and sampling method

A cross-sectional study was conducted from December 2023 to May 2024 at Lake, Hawassa to determine the infestation on the *O. niloticus* of different weight, length and sex from Lake Hawassa. Effects of *Contraceacum* spp. on *O. niloticus*. The study also included the influence of water quality on *Contraceacum* spp. on host fish. *O. niloticus* is selected for the study as it is the most common commercially important food fish and a good fish species for culture. According to Thrusfield (2005), the sample size for *O. niloticus* was calculated with a 95% confidence interval and a 5% absolute precision as shown in equation 1.

$$n = \frac{1.96^2 P (1 - P)}{d^2} \quad (1)$$

$$= \frac{1.96^2 * 0.5 (1-0.5)}{0.05^2}$$

$$= \frac{0.4704}{0.0025}$$

$$= 180.16 \sim 180$$

Where P is the expected prevalence, 1.96 is the standard deviation unit at a 95% confidence level, n is the necessary sample size, and d is the desired absolute precision. The sample size was calculated using a desired absolute precision of 5% and a prevalence of 90%. The study by Florio *et al.*, 2009, on the prevalence of parasites in wild fish in Eastern Africa estimated the prevalence at 95%. Therefore, a total sample size of 180 fish was obtained for this study based on the calculation made using the above formula in equation 1.



Figure 2: Photograph of sampling site of amora gedel (Lake Hawassa)

3.4. Sample Collection and study methodology

Fish species collected through suitable gill nets were immediately transported in icebox to the Centre for Aquaculture Research and Education (CARE), Hawassa University. Collected fish were kept in holding tanks in the laboratory. Fish were then taken from the holding tanks and were measured for length and weight. Total length of the fish was measured from the tip of the snout to the end of caudal fin using a measuring board and weight by digital balance to the nearest gram (g) (Elias Dadebo *et al.*, 2005) and sex of the fish was also differentiated. Data was entered in data register for further studies.

3.5 Collection and Preservation of *Contraceacum* species

For the collection of *Contraceacum* spp., the collected fish were dissected using scissors, needle, forceps and scalpel. An incision was made at the ventral region of the body and removed all the vital organs and placed in petridishes. The musculature and mesenteries were teased and removed parasites if any found. Before releasing the contents, care was taken to see the presence

of nematodes by observing their movement by naked eye. Parasites obtained were lifted through fine needles or through dropper by drawing parasites along with little water.

The collected parasites were cleared off from debris or any attached objects through needle and cleared in saturated Sodium bicarbonate solution. The parasites were then repeatedly washed in freshwater and preserved in 5% formalin for further identification (Natarajan, 1987).



Figure 3: Photograph of *O. niloticus* examination from different organs for larval stage and adult stage of *Contraceacum* spp. by using stereo microscope and naked eye

3.6 Prevalence and intensity of infestation

The prevalence and intensity of Anisakid nematode were calculated using the following methods; The ratio of the total number of infested hosts to the total number of hosts examined multiplied by 100 gives the prevalence (P), and expressed in percentage. Intensity (I) = Total number of parasites obtained /Total number of infested fish and expressed in number (Georgiadis *et al.*, 2001).

3.7 Biochemical composition of fish tissue

Required quantity of fish tissue was dissected out from the dorso-lateral side of fish aseptically and was homogenized manually. It was then weighed and dried in oven for 24 hrs at 65⁰C. After drying and taking the value of moisture content, the samples were stored in desiccators. The samples were then crushed using mortar and pestle for further biochemical analysis using standard methods. The biochemical composition such as moisture, protein (%), fat (%) and ash (%) were estimated using the standard method (AOAC, 2003).



Figure 4: Photograph for the biochemical composition of infested and uninfested fish by *Contraceacum* spp.

3.8 Water quality assessment

Water sample was collected from Lake Hawassa from one meter distance. Water quality parameters such as water temperature, dissolved oxygen (DO), and pH were measured in-situ using a portable multi-parameter probe (Model HQ40D). Ammonia, nitrate and nitrite were measured in Environmental toxicology laboratory in Hawassa University.

Water quality parameters of temperature and DO were measured by using a DO meter of DO-810 model to get accurate results. Then the pH was measured using pen type pH meter of model P301 to be more specific.

Water sample collection for ammonia, nitrate and nitrite analysis: One large composite bottle and three small sampler bottles were prepared. Before collecting the sample, both the

sampler and the composite bottles were rinsed by lake water for three times. At a relatively slow rate, sampler bottles were lowered vertically into the water until they reached a depth of one meters, at which point they were vertically pulled up. The water sample was poured to the composite bottle and mixed well. The water sample was put into a polyethylene bottle, and one ampule of sulfuric acid (1mL of H₂SO₄ for a 250 mL polyethylene bottle) was added to lower pH and stop reactions in the sample until it could be analysed. The polyethylene bottle was stored in insulated coolers containing ice in Hawassa University Environmental Toxicology Laboratory and kept in a refrigerator at 4 °C for analysis.

Analysis of water sample for Ammonia concentration: Using an acid-base titration technique and a pH electrode, the ammonia content of water was determined. A water sample was added after the analysis vessel had been cleaned. Sulfuric acid (H₂SO₄) was used to measure and correct the pH at its starting point. Sodium hydroxide (NaOH) was used to titrate the solution. Following the determination of the end point, the ammonia concentration was calculated.

Analysis of water sample for nitrate concentration: After being rinsed, sample water was poured into a test tube until it reached the 2.5 mL mark. The sample water was diluted with an acid-mixed reagent until 5 mL was reached. A screw cap was added, the mixture was mixed for one minute, and then two minutes were given. One level spoonful was added to the tube containing the sample along with one nitrate tablet, and 10 minutes were then allowed to pass. The Nitrate Nitrogen Comparator test kit was filled with the test tube. The sample color was blended with a reference color. The final result was recorded as mg/L of NO₃-N and converted to mg/L of NO₃ by multiplying by 4.4, i.e., NO₃-N * 4.4 = NO₃ (mg/L).

Analysis of water sample for Nitrite concentration: One Nitrite LR tablet was added directly from the foil to the 10 mL sample, and it was crushed with a stir rod. The flask was covered after allowing the tablet to completely dissolve, and 10 minutes passed before the reaction color was changed. A suitable solution was used to calibrate the Nitrite Nitrogen Test Kit. The "zero/test"

key was pressed after the sample solution had been added to the kit. Three seconds of the method symbol flashing preceded the display of the outcome. The meter reported a result in mg/L for nitrogen, and this nitrogen was multiplied by 3.3 to produce nitrite (mg/L), so $N \times 3.3 = NO_2$ (mg/L).

3.9 Hematology

Collection of Blood samples: If the fish are with *Contracaecum* species after examination it decided by referring infested and uninfested, and infested and non-infested *O. niloticus* were anesthetized with clove oil at a concentration of 1 mg/L. Blood was then collected from each anesthetized fish by using a 1ml Terumo medical syringe. The needle was inserted into the muscle along the lateral line behind the anal fin until it reached the spine. The plunger of the syringe was slowly withdrawn until the syringe is filled with blood. The collected blood was placed in a vacuum tube containing the anticoagulant (EDTA) and was then labeled. Blood samples were analysed for erythrocyte count, leukocyte count, hematocrit and hemoglobin content as well as for observation of leukocyte cell differentiation according to the methods of (Bojarski *et al.*, 2021).

Erythrocyte count: The number of erythrocytes were determined by aspirating blood into a special pipette for measuring the number of red blood cells, with red stirring grains in it until the scale of 1 mark was reached. The blood was then mixed with Hayem's solution to the 101 marks on the pipette. The pipette was shaken by making a figure of eight movements to ensure the contents were thoroughly mixed. The first two drops of the solution were discarded before transferring the liquid to a hemocytometer and covering it with a glass cover. Counts were performed under a binocular microscope with a magnification of 40X. The number of erythrocytes were counted in 10 small compartments in the hemocytometer and converted to obtain the number of blood cells per mm^3 . Erythrocyte was counted by using the following formula (Blaxhall and Daisley 1973).

$$\text{Number of red blood cells} = \frac{\text{No. cells counted} \times \text{dilution factor}}{\text{Area counted (mm}^2\text{)} \times \text{depth (mm)}} \quad (2)$$

Leukocyte count: The number of leukocytes were determined by aspirating blood into a special pipette until the 0.5 mark was reached. The blood was then mixed with Turk's solution to the 11 mark on the pipette. The pipette was shaken by making a figure of eight movements to ensure thorough mixing. The first two drops of the solution were discarded before transferring the liquid to a hemacytometer and covering it with a glass cover. The number of leukocytes were counted in 5 out of the 16 small compartments in the hemacytometer and converted to obtain the number of cells per mm³. Leukocyte was counted by using Blaxhall and Daisley (1973) formula:

$$\text{Number of White Blood Cells} = \frac{\text{No. cells counted} \times \text{dilution factor}}{\text{Area counted (mm}^2\text{)} \times \text{depth (mm)}} \quad (3)$$

Differential leukocyte observation: Two glass slides were used to make blood smears (slide A and slide B). A drop of blood was placed on slide A, and slide B was placed over the drop of blood at an angle of 45° to slide A. Slide B was then moved to the right and then to the left quickly and steadily to obtain a thin film of blood on each slide. The slides were then air-dried before being placed in metanol for 5 minutes, and then placed in Giemsa dye for 20 minutes. The slides were washed with running water for 5 minutes and dried. The dry preparations were observed using a microscope at 100X magnification. The leukocyte differential count was counted up to 100 cells and the percentage of each type (lymphocyte, monocyte, eosinophil, basophil, and neutrophil) were counted by using Blaxhall and Daisley, 1973, formula:

$$\text{Proportion of a leukocyte cell type} = \frac{\text{No. of the leukocyte cell type} \times 100\%}{\text{Total number of leukocytes}} \quad (4)$$

Heamatocrit: Micro heamatocrit method was employed. Corning glass capillary tubes measuring 15 cm in length and having a bore diameter of 1 mm were used. The tip of the

capillary tube was dipped in the thoroughly mixed oxalate blood sample, holding the capillary at a near horizontal position. When a blood column of 7.5 cm in length has risen in the capillary tube, the capillary was removed from the blood sample and its tip was wiped clean with a strip of filter paper and the length of the column was adjusted to exactly 7.5 cm. Holding the tube horizontally, the other end of the tube was sealed airtight with molten sealing wax. Extreme care was taken to avoid the blood column from getting heated up during sealing. The sealed capillary was carefully inserted into a Wintrobe tube filled with water and having a cotton pad on the bottom. The length of the capillary tube was always more than that of the Wintrobe tube, to avoid entry of water through the open end. The tube was centrifuged at 8,000 rpm for 15 minutes. After centrifugation, the length of the packed erythrocyte column and the plasma column was noted. Hematocrit was calculated as $L_1/L_2 \times 100$ (where, L_1 is length of the RBC column in mm and L_2 is total length of the blood column in mm) and was expressed by percentage.

Heamoglobin content: Sahli's method was employed for determining the heamoglobin content. This method is also known as the acid hematin method, where heamoglobin is converted to hematin by dilute hydrochloric acid. For this, 0.1 N HCl was added. The Sahli's pipette was filled with the oxalate blood to slightly beyond the 20 mark, the tip of the pipette was wiped with the filter paper strip to remove excess blood, and the volume was adjusted exactly to the 20 mark by drawing out the excess blood with a piece of filter paper. The blood was discharged into the graduated hemoglobin tube containing 0.1 N HCl up to 20 mark. The contents were mixed at first by gently drawing in and expelling the solution three to five times using the pipette, but avoiding froth formation. The contents were then thoroughly mixed using a glass stirrer and allowed to stand for 10 minutes and then were diluted with distilled water (was added drop by drop, was stirred and mixed thoroughly using the stirrer after the addition of each drop) so that the color of the solution matched with that of the color standard of the hemometer. The amount of the blood sample was directly read in gram per decilitre from the graduated hemoglobin tube.

3.9.1 Data Analysis

The collected raw data was entered in to Microsoft excel-13 data sheets, managed and analysed by using SPSS-25 statistical software. Prevalence and intensity of infestation were calculated by descriptive statistics, percentages and 95% confidence intervals were used to summarize the proportion of infested fish and analysed by the method of Bush *et al.*(2001). The association of *Contraceacum* spp. prevalence and water quality parameters was checked by Pearson correlation. Infestation on weight and length difference of *O. niloticus* was analysed by also Pearson correlation test. Infestation on sex difference of *Oreochromis niloticus* was analysed by chi-square (χ^2) test. One-way analysis of variance (ANOVA) were used to test the difference of physicochemical parameters among them. T-test at 95% confidence level ($p < 0.05$) was used to evaluate the significance of differences in hematological and biochemical composition of infested and non-infested *O. niloticus*

CHAPTER FOUR

4. RESULTS

4.1 Infestation of *Contracaecum* spp. on *O. niloticus* in Lake Hawassa

The Anisakid nematode collected from the musculature, mesenteries and vital organs of *O. niloticus* were identified as *Contracaecum* spp. A total of one hundred and eighty (180) *O. niloticus* selected randomly from December 2023 to May 2024 from the study sites were examined for the presence of Anisakid nematode parasites out of the 180 *O. niloticus* examined, 91 (50.55%) *O. niloticus* were infested by *Contracaecum* spp. The prevalence of *Contracaecum* spp. was highest in February and April each with 56.6% and lowest in December 40.0%. The highest intensity of *Contracaecum* spp. was recorded in May 9.66 and lowest in January with 4.20. It is seen from the table 1 below, that the prevalence in relation to different months did not show any definite pattern of infestation. The intensity however showed a definite pattern of increase from January to May 2024.

Table 1: Prevalence and intensity of infestation of *Contraceacum* spp. on *O. niloticus* at Lake Hawassa (n = 180)

Month and Year	N _o of fish Examined	N _o of fish infested	Prevalence (%)	N _o of <i>Contraceacum</i>	Intensity
Dec. 2023	30	12	40	56	4.66
Jan. 2024	30	14	46.6	59	4.20
Feb. 2024	30	17	56.6	74	4.35
Mar. 2024	30	16	53.3	77	4.81
Apr.2024	30	17	56.6	117	6.88
May. 2024	30	15	50	145	9.66
Total	180	91	50.55	528	5.76

4.2 Prevalence and intensity of infestation relation to fish length

In terms of fish size, fish length >23 cm showed a parasite prevalence of 66.66% which is higher than the other length groups. The length group 8-13 cm did not harbor any parasites, while the length groups 13-18 cm and 18-23 cm showed the prevalence of 35.70% and 56.12% respectively. The length group > 23 cm had the highest intensity of parasites(12.5), followed by

the length group 18-23 cm and 13-18 with 6.96 and 4.6 respectively. There was a strong positive correlation between the length of *O. niloticus* and the prevalence and intensity of infestation ($r = 0.969$).

Table 2: Prevalence and intensity of *Contracaecum* spp. in relation to length of *O. niloticus*

Length group(cm)	No of Fish Examined	No of fish infested	Prevalence (%)	Intensity
8-13	9	0	0	0
13-18	70	29	35.7	115 (4.6)
18-23	98	60	56.12	383 (6.96)
>23	3	2	66.66	25 (12.5)
Total	180	91	45.55	6.37

4.3 Prevalence and intensity of infestation in relation to fish weight

The results of infestation in relation to fish weight is presented in Table 4. Fish weighing 170-230 (g) were highly infested with the prevalence of 85.71% followed by the weight group 230-290 cm with 75% prevalence. The lowest prevalence (33.73%) was exhibited by the lowest weight group (50-110cm). Thus it is clear that, the prevalence in relation to different weight groups did not follow any definite pattern. The intensity however showed an increasing trend up to the length group 170 -230 cm and then there is a drop in the highest weight group. There was a strong positive correlation between the weight of *O. niloticus* with the prevalence of *Contracaecum* spp. ($r = 0.955$).

Table 3: Prevalence and intensity of infestation in relation to fish weight of *O. niloticus*

Weight (gm)	No of Fish Examined	No of fish infested	Prevalence (%)	Intensity
50-110	83	30	33.73	122 (4.35)
110-170	75	36	48.00	218 (6.05)
170-230	14	14	85.71	123 (10.25)
230-290	8	11	75.00	54 (6.75)
Total	180	91	45.55	6.37

4.4 Prevalence and intensity of infestation in relation to fish sex

The overall prevalence and intensity in male and female *O. niloticus* were assessed to observe their influence on sex of fish, and the results are presented in Table 4. While analyzing the infestation rate it is seen that out of the eighty-seven male fish examined, forty fish were infested by *Contraceacum* spp. (45.97%). On the other hand, out of 93 females examined 44 were infested with the prevalence of 47.31%. Hence, female fishes were more infested than males. The statistical significance of infestation between male and female was $\chi^2 = 0.176$, $p < 0.05$ that is (P value= 0.021), which was less than 3.841 ($\chi^2_{\text{calculated}} < \chi^2_{\text{tabulated}}$, $0.176 < 3.841$). There was significant difference of infestation between male and female fish. Intensity of infestation on sex of fish was 5.26 and 7.42 for males and females respectively.

Table 4: Prevalence and intensity of *Contraceacum* spp. Based on sex of *O. niloticus* (n=180)

Sex	No. of fish		Prevalence (%)	No. of parasites	Intensity
	Examined	Infested			
Male	87	40	45.97	216	5.26
Female	93	44	47.31	312	7.42
Total	180	91	45.55	528	6.37

4.5. The prevalence of *Contraceacum* spp. was evaluated based on different body organs.

The prevalence of *Contraceacum* spp. in relation to different organs is presented in figure 5. The prevalence was maximum in muscle 197(37.31%), followed by intestine 151(28.59), gonad 87(16.47%), stomach 57(10.79%), liver 22 (4.16%) and heart.14(2.65%).



a) Heart



b) muscle



c) intestine

Figure 5: Photograph indicating different organs of *O. niloticus* infested by *Contraceacum* spp.

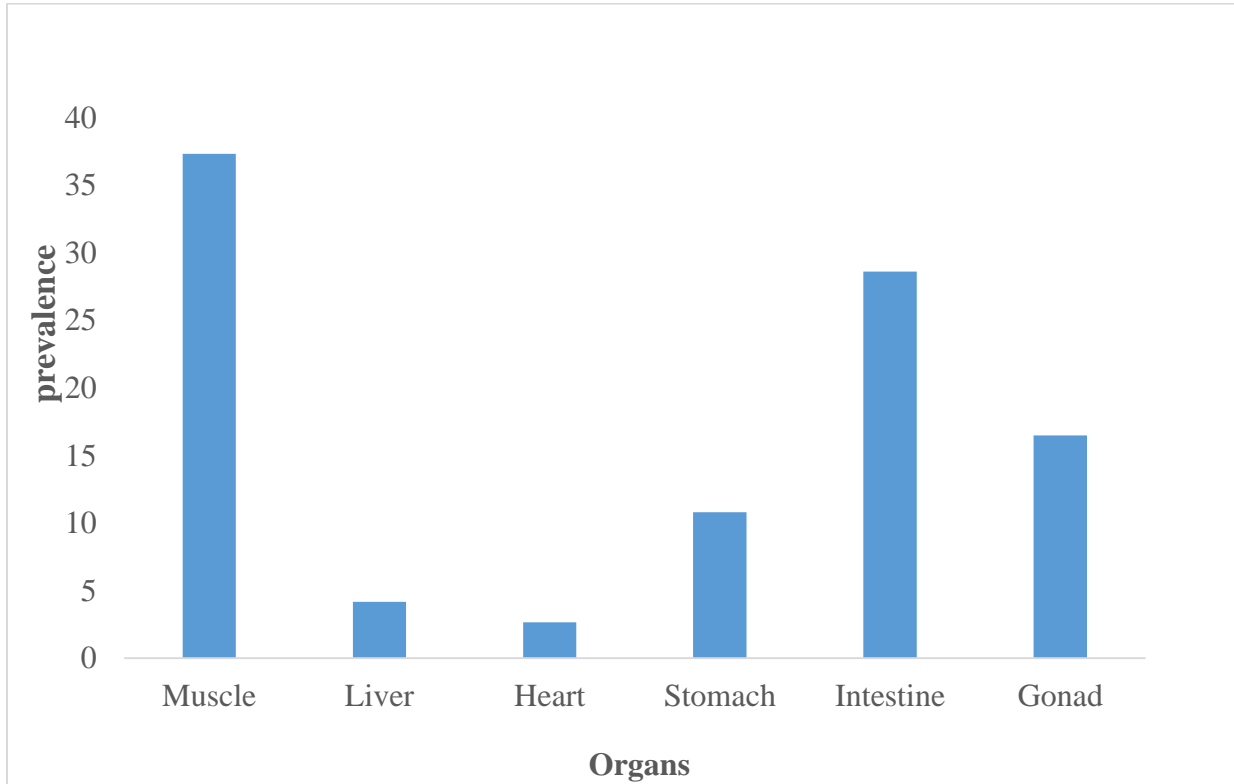


Figure 6: Distribution of *Contraceacum* spp. in relation to different organs of *O. niloticus*

4.6 Physicochemical parameters of Lake Hawassa

The physico-chemical parameters of Lake water is presented in Apendixs Table 1. The results show that, the water temperature ranged from 20.20°C to 27.30°C, the pH was between 6.09 and 9.02 and the dissolved oxygen (DO) levels were between 3.20 and 6.00 mg/L. The ammonia levels were between 0.09 and 1.93 mg/L, while nitrite levels were between 0.03 and 0.15 mg/L.

Nitrate levels varied between 3.00 and 3.90 mg/L. The relationship between physico-chemical parameters of Lake water and infestation of *Contraceacum* spp. is presented in Table 5. The prevalence of *Contraceacum* spp. has a significant positive relationship with temperature, DO, ammonia, nitrate, and nitrite (p-values of 0.043, 0.035, 0.045, 0.022, and 0.043, respectively), but insignificant relationship with pH (p-value of 0.294).

Table 5: Association of physicochemical parameters among them and prevalence of *Contraceacum* spp.

parameters	Temperature	DO	PH	Ammonia	Nitrite	Nitrate	Prevalence	p-value	R-value
Temperature	1	0.008	0.038	0.36	0.045	0.056	40	0.043	0.76
DO	0.008	1	0.041	0.155	0.024	0.108	46.6	0.035	- 0.22
pH	0.038	0.041	1	0.323	0.212	0.307	56.6	0.294	0.80
Ammonia	0.350	0.155	0.323	1	0.055	0.198	53.3	0.045	0.65
Nitrite	0.045	0.024	0.212	0.055	1	0.027	56.6	0.022	0.75
Nitrate	0.056	0.108	0.307	0.198	0.027	1	50	0.043	0.67
prevalence	40	46.6	56.6	53.3	56.6	50	1	0.813	

4.7 Heamatological effects of *Contraceacum* spp. infestation on *O. niloticus*

Heamatological parameters of infested and non infested fish is presented in Table 6. The results show that except erythrocyte counts all other blood values were more in infested fish. The blood values of infested and uninfested groups demonstrated a significant difference in heamatological parameters ($p < 0.05$) between the two groups of fish. The total number of leukocyte cell counts, percentage of lymphocytes, monocytes, eosinophils, heamatocrit and heamoglobin in the parasitized *O. niloticus* increased significantly ($p < 0.05$) than the uninfested.

However, the proportion of neutrophils and basophils between infested and uninfested fish was not statistically significant ($p>0.05$).

Table 6: Hematological parameters of infested and uninfested *O. niloticus* by *Contraceacum* spp.

Parameters	Infested	Non-infested	p-value
Erythrocyte ($\times 10^6$ cells/mm ³)	0.62	1.58	0.029 ^{sn}
Leukocyte ($\times 10^3$ cells/mm ³)	11.75	7.21	0.032 ^{sn}
Lymphocyte (%)	81.5	66.30	0.009 ^{sn}
Monocyte (%)	25.98	16.52	0.01 ^{sn}
Eosinophil (%)	6.58	4.40	0.002 ^{sn}
Basophil (%)	6.60	4.30	0.058 ^{isn}
Neutrophil (%)	8.90	6.40	0.084 ^{ins}
Heamoglobin (g/dL)	8.20	7.80	0.039 ^{sn}
Heamatocrit (%)	19.98	16.40	0.002 ^{sn}

sn = significant, ins = insignificant

4.8 Effect of infestation of *Contraceacum* spp. on biochemical composition of *O. niloticus*

A total of 20 fishes were examined for biochemical composition owing to the infestation by *Contraceacum* spp. Out of 20 fishes examined, eight fishes were infested by *Contraceacum* spp. and the remaining were uninfested. All the parameters presented in the below table are statistically significant with (p -value <0.05). It shows that real and measurable effect of the

infestation on the fish's composition, which could have implications for health, nutrition, fisheries management, and economic factors. It also suggests that the infestation is having a noticeable impact on the fish's physiological condition, which could affect its nutritional quality. So, generally parameters with p-value of protein (0.021), lipid (0.011), ash content (0.003) and moisture content (0.014).

Table 7: Biochemical parameters value of infested and uninfested *O.niloticus* by *Contraceacum* spp.

Parameters	Infested and	Non-infested
Protein (%)	14.52	16.62
Moisture (%)	62.50	67.44
Lipid (%)	5.88	6.48
Ash (%)	4.12	4.70

CHAPTER FIVE

5. DISCUSSION

5.1. Prevalence and intensity of infestation of *Contraceacum* spp. on *O. niloticus* in Lake Hawassa in relation to Different parameters.

The present study on the prevalence of *Contraceacum* spp. in *O. niloticus* collected from Hawassa Lake was 50.55 %. Similar prevalence of infestation ranging from 39 to more than 50 % has been reported by a number of authors. (Zekarias Tsige and Yimer Enyewu, 2008, reported 39.67% from Lake Hawassa; Busiso 2017, reported 43.8% from the lower Umguza dam; Ageze Necho and Menzir Awake, 2018, reported 49.5% in North East Ethiopia and Lake Tana, Bahidar). Higher prevalence of infestation has been reported by (Marshet Adugna *et al.*, 2018, reported 98.43% from the Gigel Gibe-I Dam, Jimma Zone, Ethiopia; Muluken Abiyu *et al.*, 2020, reported 54.4% in Lake Tana). The higher prevalence of infestation of nematode in *O. niloticus* in Hawassa Lake observed during the current study can be attributed to the presence of a large number of aquatic piscivorous birds which are final hosts for the anisakid nematodes (Yanong, 2002). The poor and unhygienic way of processing fish at shore site and indiscriminate disposal of fish remains along shore and water bodies can be another factor for the increased infestation of nematode parasites in fish in the lake. The present study agreed with the study of Gulelat Yewubdar *et al.* (2017), who reported that dumping of fish wastes in shoreline creates a favourable environment for wild fish to get infected by larval stages of anisakid larvae found in the gut of the host fish. It is also seen that, in some cases the prevalence of nematode parasites in fish species was much low as reported by Amare Abera *et al.* (2014), who observed 9.5% infestation from the Lake Lugo (Hayke) Northeast Ethiopia; and 8.46% infestation by (Hussien Daniel 2015), from the Lake Ziway, Ethiopia. The reason for low level of

infestation may be due to dry months where in the disposal of fish wastes through rain water in to the water bodies is reduced and the difference in sample sizes and methodologies may be the reason for the variation between this study and others.

The present study also reported that, the infestation was slightly higher in female fish than male fish. This observation is similar with the observation of (Akinsanya *et al.*, 2009; Qasim and Ayub 2012), who reported that increased infestation in females could be related to genetic predisposition and increased susceptibility of female fish. Further, they are of the opinion that gravid females do not feed actively during breeding month and hence they are more susceptible to infestation. However, this study contradicts with the study reports of (Aloo, 2002), who reported that male fishes are infested more than that of female fish.

The prevalence of Anisakid nematode parasites was evaluated based on different length and weight groups, and the results indicated that, the prevalence in relation to length groups was significant while the weight group was not significant. While the lowest length group did not show the prevalence of infestation, it was highest in the largest length group. The findings of higher infestation in larger length groups are almost similar with the reports of (Bichi *et al.*, 2010; Allumma *et al.*, 2011; Amaechi *et al.*, 2015; Muluken Abiyu *et al.*, 2020), were reported greater infestation in larger fish than smaller fish. The reason for the higher infestation in larger fish may be due to their larger body size and longer stay in the environment. Similar observation of higher infestation in larger fish has been reported by Amare Abera *et al.* (2014), who noted that, longer fish provide greater surface area for infestation than smaller fish. In addition, a higher prevalence and intensity in adult fish than juvenile fish may be due to the accumulation of worms over a longer period of time (Gichohi *et al.*, 2008; Tsige Zekarias and Yimer Enyewu 2008).

5.2 The prevalence of anisakids nematode and body organs

Contracaecum is a genus of anisakid nematodes that can infest fish, particularly marine species. When humans consume raw or undercooked fish containing the third-stage larvae (L3) of *Contracaecum*, they can develop a condition known as anisakiasis. They are distributed in different organs of the fish. The current study reported that although the parasite was distributed in different organs, it was found more in the muscle tissue followed by intestine. It is also seen that the larval stages are encysted more in the muscle tissue while the adult parasites were more in the intestine. The current findings support with the findings of Barson (2004), who observed that, muscle and intestine harboured the highest number of parasites from Lake Chivero, Zimbabwe. Omeji *et al.* (2022), who investigated the infestation of nematode parasites stated that, the distribution of nematode parasites was more in the intestine than other organs of wild and cultured *O. niloticus*. Ogaba *et al.* (2022), who also reported a higher helminth infestation in the intestine (56.37%) than other organs of attachment of *Bagrus bayad* from the lower River Benue Makurdi, Nigeria. The present study however, differs with the observation of both Omeji *et al.* (*loc.cit*) and Ogaba *et al.*(*loc.cit*) that, the distribution of larvae of nematode parasites was more in the muscle than the intestine. The reason attributed for increased infestation in intestine by the above authors was that it could probably be attributed to the presence of digested food present in the infested intestine or due to greater surface area present in the intestines. The reason for increased infestation in muscle tissue as observed in the current study may be due to the fact that internal parasites particularly at their larval stage encyst more in the muscle tissue than the vital organs of the fish as muscle tissue ensures encystment of the larvae with their connective tissue, provides a nutrient-rich environment that supports the growth and development of larval parasites, a lower immune response compared to other tissues, more accessible to parasites that enter the fish through the skin or gills and behavioral changes that make them more susceptible to predation by definitive hosts. That reach in to the muscle through different way such as ingestion, migration, heamatogenous, encystment, and direct penetration.

5.4 Association of physicochemical parameters and prevalence of Anisakid nematode

In the current study, the water temperature of Lake Hawassa varied between a low of 20.20 °C in December and a high of 27.30 °C in March with a mean value of 24.33 °C. This result is almost similar with those of Dessie Tibebe *et al.* (2022), who stated that Lake Hawassa water temperature ranged between 21.4 °C and 25.3 °C. The water temperature in the current study was higher than the report of Zemed Menberu *et al.* (2021), who observed water temperature of Lake Hawassa was between 19.5 and 21.7 °C with an average value of 20.8 °C. The study also clearly show that there is a strong positive correlation between temperature and prevalence of infestation indicating that increased temperature influenced the prevalence of *Contraceacum* spp. on *O. niloticus* with (r-value of 0.76). This finding is in line with that of Ojwala *et al.* (2018), who reported that there was a highly positive correlation between parasite prevalence and water temperature.

The present findings showed that there evident a slightly strong inverse relationship between dissolved oxygen concentration and prevalence of *Contraceacum* spp. on *O. niloticus* ($r = -0.002$). The present result is in agreement with those of Alsarakibi *et al.* (2014), who reported that dissolved oxygen has a negative correlation with the prevalence of the nematode *Capilaria*. The findings are also consistent with those of Yunikasari and Mahasri (2020), who reported a strong negative relationship between dissolved oxygen and the prevalence of the nematode parasite.

The invers relationship of dissolved oxygen with the temperature affects lifecycle and overall developmental stages of aquatic organism, and this situation is possible to parasite infestation, with the fact of cold water can hold more dissolved oxygen than hot water and dissolved oxygen increases as water temperature drops. Temperature has a negative effect on dissolved oxygen levels, according to Bhatnagar and Devi (2013). It is also clear that when temperature increases,

it leads to reduction in oxygen level which causes stress to fish resulting in increased infestation by parasites Löhmus and Björklund (2015).

The results on pH of the lake was between 6.09 and 9.02 and this observation is almost in agreement with the observation of Dessie Tibebe *et al.* (2022), who reported that the pH of Lake Hawassa Lake ranged between 7.1 and 9.3. The current study found that, there was a strong positive relationship between pH and prevalence of infestation ($r=0.824$), however, the results show that the relationship is not statistically significant with prevalence, p-value (>0.05). This finding is consistent with the findings of (Sahoo *et al.*, 2013); Ojwala *et al.*, 2018), who found a positive correlation between water pH and parasite prevalence. However, the current findings contradict with the results of Alsarakibi *et al.* (2014), who found a negative correlation between pH and parasite prevalence. The highest pH developed due to increased in inorganic ammonia, and dissociation of some heavy metals causes pollution stress to fish. The stressed fish are easily susceptible to parasitic infestation due to immune system suppression and such a stressful environment promotes favorable condition for parasite prevalence Rathor and Swain (2024).

There is strong positive correlation between ammonia and infestation of parasites in the current study with ($r=0.69$). This result is similar with the result of (Yunikasari and Mahasri 2020; Waruiru *et al.*, 2020), who observed a positive relation of ammonia concentration with the prevalence of parasites. The ammonia concentration in the Hawassa Lake observed during the present study ranged between 0.09 mg/L and 1.93 mg/L with the mean value of 1.09. The ammonia value observed for the same lake by Lencha Semaria *et al.* (2021) was 0.94 mg/L, which is lower than the present study. The reason for higher concentration of ammonia in the current study may be due to the accumulation of fish wastes originates from the industries and processing of fish along the shore line of the lake. This situation lowers the quality of the water and has a positive impact on the parasite infestation (Nadarajan and Sukumaran 2021). Acosta-

Pérez (2022) reported that, an accumulation of organic waste is harmful to fish because it contains toxic substances like nitrite and ammonia.

The present study showed that, there is a strong positive relationship between nitrate concentration and Anisakid prevalence with ($r=0.67$). This result suggest that, there is relationship between nitrate concentration and parasite prevalence. However, it varied significantly in the study months and the difference was ststistically significant with p-value <0.05 . This result supports the findings of Waruiru *et al.* (2020), who reported a positive relationship between parasite prevalence and nitrate concentration.

The nitrite concentration of the current result was 0.03 to 0.15 which is higher than the value (0.01) reported by Girum Tamire and Seyum Mengistou (2013) for Lake Ziway. The result of the present study showed a strong positive relation between nitrite concentration and parasite prevalence with ($r=0.75$), and the relationship is statistically significant with p-value <0.05 .

Generally, the prevalence of *Contracaecum* spp. was positively correlated with almost all the water quality parameters mentioned except for dissolved oxygen. Temperature, ammonia, nitrate, nitrite and pH have a significant correlation with the prevalence of *Contracaecum* spp. These results are consistent with those of Ojwala *et al.* (2018), who noted that temperature, pH, and ammonia were all strongly correlated with parasite prevalence.

5.5 Heamatological effects of *Contracaecum* spp.on *O. niloticus*

Contracaecum spp. parasite infestations can cause stress reactions with behavioral and physiological in host fish and fish parasites can also cause blood disorders such as anemia, leukocytosis, and thrombocytopenia (Rocha *et al.*, 2018). Parasite feeding activity results in a decrease in the number of erythrocytes in the host blood, and skin lesions cause the host osmoregulation to fail, leading to anemic conditions in infested fish Ali *et al.*, (2010). Blood samples from fish with and without *Contracaecum* spp. infestations on *O. niloticus* were

observed the differences between the two groups. The present findings investigated that the number of erythrocyte and hemoglobin cells count in not infested *O. niloticus* were significantly different ($p < 0.05$) and have a higher mean value. These results support the findings of Nashaat and Maghawri (2022), who reported that there was significant difference with lower values of erythrocyte and hemoglobin cells count in infested group of fish than uninfested by *Capillaria* sp. This finding is also corroborated with that of Azevedo *et al.* (2006), who reported that erythrocyte count was slightly decreasing in parasitized *O. niloticus*. *O. niloticus* parasite feeds on host blood cells that is why the RBCs in infested fish was lower than that of uninfested one.

Hemoglobin is one of the most important proteins found in the RBCs that carries and transport oxygen from the lungs/gills to rest part of the body. So the infested fish have low oxygen carrying capacity in case of the parasite withdrawing RBCs. However the present result conflicts with that of Jones and Grutter (2005), who reported that erythrocyte counts can also rise in *Contracaecum* - infested fish than non-infested, as a result of the release of blood cells from the spleen that have been stored there, the loss of plasma, or the swelling of red blood cells as a stress response in *Contracaecum*-infested.

Besides, it was reported that the parasitized *O. niloticus* had higher percentages of basophil, eosinophil, leukocyte and neutrophil cells compared to the non-infested *O. niloticus*, the percentage of monocytes showed a significant difference ($p < 0.05$), with higher values in the infested fish. This finding was consistent with that of Furtado *et al.* (2019), who observed an increase in monocyte count in *O. niloticus* that was parasitized by *Contracaecum* spp. In the present findings, neutrophils and basophils showed insignificant difference ($p > 0.05$) between the infested and non-infested *O. niloticus*. Which is confirmed with that of Amriana *et al.* (2021), who reported that there was no significant difference in the percentage of neutrophil and basophil between infested and non-infested *O. niloticus* by *Contracaecum* spp.. It also corroborated those of Nashaat and Maghawri (2022), who found no significant difference in neutrophil counts

between red tilapia (*Oreochromis*) infested and uninfested with nematode parasites (*Capillaria* sp.). Increasing leukocyte counts may be an indication of the body immune system defenses against parasites. Monocyte cells have also increased in *Contracaecum*-infested fish than non-infested (Tavares-Dias and Moraes, 2007). Increasing leukocyte counts monocyte may be an indication of the body immune system defenses against parasites. The primary phagocytes in fish are called monocytes, and during the process of fighting an infestation, monocytes move from blood vessels to inflammatory foci (Esteban *et al.*, 2015). Additionally, in the present finding lymphocyte cells were significantly higher in parasitized than uninfested *O. niloticus* ($p < 0.05$), this investigates that during infestation lymphocyte cells transported to infestation site and increase their number to overcome the condition. These finding are similar with that of Haond *et al.* (2003), who reported that number of lymphocyte cells higher in Rainbow trout (*Oncorhynchus mykiss*) that infested by *A.japonicus* due to the migration of lymphocytes out of the blood and into the epidermis. Generally monocytes, lymphocyte and neutrophils play a role in fish fighting against parasites. Furthermore, in the host-parasite relationship, the number of parasites may have an impact on the health changes of fish (Fazio, 2019) and hematological changes can be correlated with physiological traits, fish disease.

5.5 Effects of *Contracaecum* spp. on biochemical composition of *O. niloticus*.

Exposure of fishes to aquatic pollutants for long periods even if in very low concentration may stimulate morphological and biochemical changes in fish tissues, which may impress the quality of fish and marketability (Mohamed *et al.*, 2020). Moisture, protein, and fat are the principal nutrient constituent of fish muscle, while vitamins, carbohydrates and minerals are the minor components. So, the fish muscle includes all the nutrient components necessary for the maintenance of the human body (Begum *et al.*, 2012).

The results of present study clearly revealed that, the biochemical constituents of host fishes decreases depending on the types of metazoan parasites especially *Contracaecum* spp.. Cui and

Wootton (2011), had reported that biochemical composition of fish such as moisture, protein, fat and minerals varies widely from one species to another. The results of Cui and Wootton (2011), agree with the current results that, the nutritive value of fish may be degraded through the activities of helminth parasites and it lowers the quality of the fish meat due to a fall in the absorbed amino acids which is essential in protein synthesis. Amaal Hassan *et al.* (2015), reported that, helminth infection in some cases may cause increase in the total muscle protein. This finding is disagree with the present study that, the muscle protein decrease helminth infection of nematode.

The effects of parasites on muscle biochemical constituents of host fish depend on types of parasite infestation. This observation agrees with the previous findings of Amaal Hassan *et al.* (2015) in which nematodes have more effect on muscle protein compared to cestodes. According to Singh and Srivastava (2010), the nutritive value of fish may be degraded through the activities of helminth parasites and their cysts. Ali (2001) reported depletion of glycogen in fish infected with cysts as a function of stress since the worm obtains nourishment through cyst wall from the host liver. Gut parasites like digenea, nematoda, cestoda and acanthocphala which feed on gut contents of host fish may have a contribution for the decreasing of muscle protein and other biochemical constituents of host fish. This result is the same as that of the findings of (Habiballah *et al.* 2011); Amaal Hassan *et al.* 2015)

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

From the findings of the current study, the following conclusions have been drawn

- ❖ Poor water quality of Lake Hawassa influenced the infestation of *O. niloticus* by the nematode parasite *Contracaecum* spp. The evaluation of the impact of poor water quality on the infestation of *O. niloticus* by *Contracaecum* spp. involves understanding the interplay between environmental stressors, host health, and parasite dynamics.
- There is a positive relationship between *Contracaecum* spp. infestation and *O. niloticus* in Lake Hawassa, based on length, weight and sex of *O. niloticus*.
- Larger fish showed higher prevalence rate of *Contracaecum* spp. than smaller fish, however, small weight *O. niloticus* exposed to high intensity of *Contracaecum* sp.
- Prevalence of *Contracaecum* spp. has positive correlation with temperature, DO, ammonia, nitrate and nitrite. Prevalence of *Contracaecum* spp. has strong negative relationship with pH.
- Infestation in relation to sex of fish showed that female fishes were slightly infested than the male fish with *Contracecum* spp. The difference in the infestation rate between sex of fish could be related to genetic predisposition and differential susceptibility owing to the difference of their physiological condition.
- *Contraceacum* parasite have significant hematological effects on the *O. niloticus* blood parameters, especially leukocyte cells, percentage of monocyte and lymphocyte cells, which are high in the infected fish. Furthermore, neutrophil and basophil cells differences are statistically significant with infected *O. niloticus* by the *Contraceacum* spp.

- The ecto and endo-parasites accumulation affects biochemical composition of host fish by changing the behavior of host fish and makes the fish to be stress and reduce nutritious value such as protein, lipid, ash content and moisture to human consumption. Although zoonotic study has not been attempted, there is a possibility that human beings are likely to be affected if they consume live larvae in raw fish.

Recommendations

Based on the findings of this study, the following recommendations are forwarded:

- ❖ The most common nematode parasite encountered in the present study belonged to Anisakidae. As species belonged to the genus Anisakid are of zoonotic significance it is recommended that, as a precautionary measures, there is need to avoid eating uncooked or rawfish.
- ❖ As fish is one of the preferred food item among local communities, it is necessary to create awareness among local people the zoonotic significance of Anisakid nematodes, by emphasizing the potential dangers of parasitic infestations in fish muscle, particularly for those who consume raw fish, these recommendations aim to protect public health and promote safer fishing and consumption practices in the Lake Hawassa region.
- ❖ People are advised not to dump fish wastes into the lake, as fish wastes contain high protein content and its decomposition causes increased ammonia concentration in the lake which in turn influences increased infection by parasites.
- ❖ Control a buffer zone by planting trees to reduce the amount of pollution through agricultural and urban wastewater sources.
- ❖ Detailed studies should be focused on the effects of parasites especially internal parasites on the nutritive value of fish species.

- ❖ Establish and promote guidelines for safe fish consumption, particularly for those who consume raw fish and implement health monitoring programs for fishermen who frequently consume raw fish.
- ❖ Further research should be conducted for series zoonosis and correct identification of the *Contracaecum* spp. by morphological and molecular techniques that lead to anisakiasis, a condition caused by the ingestion of larval nematodes. These species are known to cause health problems, particularly in individuals who consume raw or undercooked fish containing the larvae.

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APPENDICES

Appendix Table

Table 8: Physicochemical parameters of Lake Hawassa

Months/Years	Temperature(°C)	pH	DO (mg/L)	NH ₃ (mg/L)	NO ₃ (mg/L)	NO ₂ (mg/L)
Dec-23	20.20	6.15	3.20	0.9	3.00	.030
Jun-24	24.10	7.03	4.01	0.11	3.70	.060
Feb-24	25.50	9.02	5.20	1.93	3.70	.110
Mar-24	27.30	8.04	6.00	1.67	3.90	.150
Apr-24	26.80	8.05	4.90	0.89	3.90	.130
May-24	22.10	6.09	3.90	1.76	3.80	.110



Figure 7: Photograph of samples *O. niloticus* for *Contraceacum* spp. examination



Figure 8: Photograph indicating examination of *O. niloticus* of different organs for larval stage and adult of *Contraceacum* spp. By using naked eye and stereo microscope



Figure 9: Photograph indicating *Contraceacum* spp. from *O. niloticus* body



Figure 10: Photograph of blood sample collection of infested and uninfested *O. niloticus* by *Contraceacum* spp.