INFLUENCE OF WATER QUALITY ON PARASITE PREVALENCE IN NILE TILAPIA (*Oreochromis niloticus* Linnaeus, 1758) IN CULTURE SYSTEMS IN SIDAMA REGION, ETHIOPIA

M.Sc. THESIS

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OCTOBER, 2024

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A THESIS SUBMITTED TO THE

DEPARTMENT OF AQUATIC SCIENCES, FISHERIES AND AQUACULTURE,

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES,

SCHOOL OF GRADUATE STUDIES, HAWASSA UNIVERSITY, HAWASSA, ETHIOPIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN AQUATIC SCIENCES, FISHERIES AND AQUACULTURE

(SPECIALIZATION: AQUACULTURE AND FISHERIES MANAGEMENT)

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OCTOBER, 2024

HAWASSA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

ADVISOR APPROVAL SHEET

(Submission Sheet- 1)

This is to certify that the thesis entitled **"Influence of water quality on parasite prevalence in Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) in culture systems in Sidama Region, Ethiopia"** submitted in partial fulfillment of the requirements for the Degree of Master of Science (**Specialization in Aquaculture and Fisheries Management**), the Graduate Program of the Department of Aquatic Sciences, Fisheries and Aquaculture has been carried out by **Berhanesh Arficho** under my supervision. I recommend that the thesis be accepted as fulfilling the requirements for the Degree of Master of Science in **Aquaculture and Fisheries Management** and hence hereby she can submit the thesis to the department.

Natarajan Pavanasam (Prof.) _____ _____

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(Submission Sheet-2)

We, the undersigned, the Members of the Board of Examiners of the final open defense by **Berhanesh Arficho Bokoro**, have read and evaluated her thesis entitled **"Influence of water quality on parasite prevalence in Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) in culture systems in Sidama Region, Ethiopia"**. This is, therefore, to certify that, the thesis has been accepted in partial fulfillment of the requirements for the Degree of Master of Science (**Specialization in Aquaculture and Fisheries Management).**

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DECLARATION

I hereby declare that this thesis entitled "Influence of water quality on parasite prevalence in Nile tilapia (*Oreochromis niloticus* Linnaeus,1758) in culture systems in Sidama Region, Ethiopia**"** submitted to the Aquatic Sciences, Fisheries and Aquaculture Department, Collage of Natural and Computational Sciences, Hawassa University. It is being submitted in partial fulfillment of the requirements for the qualification of Master of Science in Aquaculture and Fisheries Management.

I confirm that this is my original research work and has not been submitted for any other Degree or Diploma at any other University. I further acknowledge that all sources of information and data used in this thesis have been properly acknowledged and referenced.

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ACKNOWLEDGMENTS

I am eternally grateful to the God Almighty for having kept my health in good condition, granting me the strength to navigate challenging paths and reach this significant milestone in my life. Without His mercy and guidance, I would not have accomplished it on my own. I extend my sincerest praises and appreciation.

I am indeed grateful to Professor Natarajan Pavanasam for having supervised my research work. I owe a lot for his time, dedication, support, guidance, priceless comments, and scientific discussions from the time of the proposal writing until the completion of this thesis.

I would like to express my sincere gratitude to Hawassa University for supporting my studies and research, which has significantly increased my knowledge and abilities in the field of Aquatic Sciences, Fisheries and Aquaculture. I would like to thank Head of the Department of Aquatic Sciences, Fisheries and Aquaculture Mr. Teklewoyni Asayehegn for his sincere support and encouragement. Further, I deeply express my gratitude to Mr. Sileshi Negash for introducing me to the Kokeb Fish Farmer to provide me with samples for my research study.

I also extend my thanks to the Director of CARE Ms. Feven Zerihun, the current Ph.D. student Mr. Teshome Belay and the former head Mr. Tiruken Aziz for their continuous support and encouragement. I wish to express my sincere gratitude to my classmates Ms. Ribka Menta, Mr. Ermias Shirko and Mr. Bereket Haji for their sincere support. My greatest gratitude is extended to Hawassa University Environmental Engineering lab assistant Mr. Tibebu Desalegn and my friend Ms. Aberash Bekena for their assistance with the laboratory work, fish sampling from the culture pond, and other assistance. Furthermore, I sincerely thank Werabe University for granting me permission to continue my M.Sc. Degree in Aquatic Sciences, Fisheries and Aquaculture in Hawassa University.

I am immensely grateful to my parents and family members for their help, support and encouragement throughout my studies. Finally, I would like to extend my sincere thanks to all those who helped me throughout my study period.

DEDICATION

I dedicate this thesis to my parents, Arficho Bokoro and Itenesh Amdeto, who were largely responsible for my current state of being. Additionally, I dedicate it to my cherished siblings, especially my eldest brother Gizachew Arficho, who has been a guiding force for me throughout my studies and research.

BIOGRAPHICAL SKETCH

I, Berhanesh Arficho Bokoro, was born in Lenchicho Kebele, Misha Woreda, Hadiya Zone, Central Ethiopia Regional State, on September 9, 2000, GC. I attended my primary education at Wasgebeta Primary School. After completing my primary school, I attended my secondary and preparatory education at Wachemo Mesenado Secondary and Preparatory School. After completion of my preparatory education, I joined Werabe University, Collage of Natural and Computational Science in 2019 and graduated with a B.Sc. Degree in Applied Biology in August 2021. After successfully completing my first-degree education, I was employed by the Werabe University and worked as a graduate assistant for one year in the Biology Department. Then I joined the School of Graduate Studies at Hawassa University in October 2022 to pursue my M.Sc. Degree in Aquaculture and Fisheries Management under the regular program.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

APPENDICES

LIST OF ACRONYMS AND ABBREVIATOINS

ANOVA:	Analysis of Variance
CARE:	Center for Aquaculture Research and Education
cm:	Centimeter
dL:	Deciliter
EDTA:	Ethylene diamine Tetra acetic Acids
FAO:	Food and Agricultural Organization
g:	Gram
K:	Condition factor
Km:	Kilometer
LR:	Lactated Ringer's solution
MA:	Mean Abundance
MI:	Mean Intensity
ml:	Milliliter
NTU:	Nephelometric Turbidity Unit
^{o}C:	Degree Celsius
pH:	Power of Hydrogen
RBCs:	Red Blood Cells
SE:	Standard Error
SFCS:	Sebeta Fish Culture Station
SPSS:	Statistical Package for Social Science
WBCs:	White Blood Cells

ABSTRACT

Parasitic infestation can lead to severe retarded growth and mortalities in high intensities in fish under culture systems. Importantly, the quality of water in the culture facility may influence the parasitic infestation. This study aimed to evaluate the influence of water quality on parasite prevalence in Nile tilapia, Oreochromis niloticus L,1758 in culture systems in Sidama Region. The study used a cross-sectional design from December 2023 to May 2024. Water physico-chemical parameters such as temperature, dissolved oxygen, pH and turbidity were measured in situ by following standard procedures. Water samples for nutrient contents such as ammonia, nitrate and nitrite were analyzed. A total of 240 O. niloticus (120 from Kokeb fish farm and 120 from CARE fish farm) were taken and examined in the laboratory for ecto and endo parasites by using stereo zoom dissection microscope. The parasitological aspects such as prevalence, mean intensity and mean abundance were calculated based on standard formulae. The results indicated that, the water quality parameters were significantly different ($p<0.05$) between both fish farms. The total number of parasite species recorded during the study was 11 species. While Kokeb fish farm showed 11 parasite species, there were only six species in CARE farm. Out of 240 fish sampled, 109 fish were infested representing an overall prevalence of 45.41%, mean intensity of 6.30 and mean abundance of 2.86. The prevalence, mean intensity and mean abundance in Kokeb farm were in the order of 59.16%, 7.46 and 4.41. In CARE farm, it was 31.66%, 4.48 and 1.30 respectively. The infestation showed a statistical difference ($p<0.05$) between the fish farms. There was a positive correlation between water quality and prevalence of parasite species, except dissolved oxygen. The size of O. niloticus and prevalence of parasite species was positively correlated ($r=0.978$). Prevalence of parasite species with sex of O. niloticus was statistically significant ($x^2=2.776$, $p<0.05$). The Fulton's condition factor of parasitized and non-parasitized O. niloticus showed no significant difference ($p>0.05$). Hematological effects of parasite species on O. niloticus between infested and non-infested groups were significant ($p<0.05$) except for neutrophil counts ($p>0.05$). The prevalence of parasite species was influenced by poor water quality. The study recommends that, to reduce or control parasite infestation in culture systems, there is an imperative need to undertake regular monitoring and management of water quality parameters in fishponds.

Key words: *Fish farms, infestation, O. niloticus, parasite fauna, prevalence, water quality.*

1 INTRODUCTION

1.1 Background of the study

The growth of fisheries and aquaculture is important in the context of solving food insecurity which is prevalent in most of the developing nations (Bene *et al*., 2016). It is paramount that attention has to be focused on challenges that hinder aquaculture production. Aquaculture is the cultivating of aquatic organisms, especially fish in controlled environmental conditions (Abd El-Hack *et al*., 2022). For better survival and increased fish production, the culture system requires inputs such as fertilizer, nutrient rich source water, natural fish food organisms and supply of protein rich artificial feeds (Salin and Arome, 2018).

Aquaculture has been identified as an additional and fastest growing food production sector (Garlock *et al*., 2020). Its main objective is to provide food for growing human population especially in developing nations (Golden *et al*., 2021). FAO (2022) estimates that fisheries and aquaculture at present provide 17% of the world's total animal protein which is consumed by humans. The present aquaculture production is about 87.5 million tons (FAO, 2022). However, considering the human population rise, it is necessary that aquaculture production has to be increased to a considerable extent. At present the major contribution of aquaculture is by Asian countries, and China is the leader in aquaculture by producing more than 70% of the world aquaculture production (Zhang, 2020).

Aquaculture is getting importance in African continent considering its need for ensuring food and economic security. It is reported that many of the African countries are rich with water and fish species resources; however, they have not been fully tapped to increase food production (Msangi and Batka, 2015). Several reasons have been attributed for the slow development of aquaculture in

Africa which includes lack of capital and infrastructure, inadequate information, limited technical expertise, and poor governance, among other things (Hinrichsen *et al*., 2022).

Ethiopia's aquaculture development has begun in 1955 with the construction of some small aquaculture ponds with Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) as the cultured species. The Sebeta Fish Culture Station (SFCS) started in Sebeta in 1975 is actively involved in culturing fish species and producing seeds for moderate/large scale aquaculture (Mulugeta Wakjira *et al.*, 2013). However, aquaculture development in Ethiopia is still in its beginning stages (Chimdo Ayana, 2022; Kassaye Balkew and Bereket Haji, 2023). To satisfy the demands of Ethiopia's expanding human population which is expected to increase from the present population of 128.5million in 2024 to 149 million in 2030 and 215 million in 2050 (Worldometer, 2023). It is thus paramount that aquaculture production has to be expanded considerably in Ethiopia.

In this context, it is necessary that, the present setbacks of aquaculture in Ethiopia have to be properly understood, and action has to be initiated to overcome the challenges that hinder aquaculture production. One of the major factors identified was diseases in fish caused by parasites. Therefore, there is an imperative need to give emphasis on fish health of cultivable fishes (Chimdo Ayana, 2022). Fish suffers under stressful environment are subjected to infestation by diverse groups of parasites, and it is reported that, more than 80% of infestation is due to poor water quality (Keremah, 2013) which often causes losses and low output. Therefore, ideal water quality standards are necessary for successful aquaculture.

From available literature it is seen that, most of the studies relating to parasites of fishes in Ethiopia are largely concerned with the occurrence of diverse groups of parasites on commercially important groups of fishes such as *O. niloticus*, African catfish (*Clarias gariepinus*) and African Big Barb (*Labeobarbus intermedius*) from natural water bodies (Abreha Tesfaye *et al*., 2017; Hailekiros

Gebreegziabher *et al*., 2020). However, detailed studies relating water quality to host-parasite relationship in culture systems are very much limited. Therefore, this study was attempted to investigate the impacts of water quality on the prevalence of parasites, and their effects on *O. niloticus* in culture systems in Sidama Region, Ethiopia.

1.2 Statement of the problem

Fish ponds with poor water quality are more vulnerable to infestation by different kinds of ecto and endo parasites which reduce fish production and productivity in aquaculture (Nyambi *et al*., 2022). Fish parasites minimize profitability and cause great economic loss to farming industry (Outa *et al*., 2021). From a perusal of literature, it is seen that studies relating to fish parasites are mostly systematics. It is also evident that due to the importance of aquaculture to ensure food security, more studies are now focused on diseases in aquaculture in other parts of the world as diseases of fish greatly restrict fish production (Asche *et al*., 2021).

In Ethiopia, aquaculture is getting greater attention in recent years mainly to meet the existing food demand of the growing human population. Studies so far carried out in Ethiopia are towards the occurrence of parasites of some commercially important groups of fishes from the natural water bodies (Mekonen Hailu *et al*., 2023). But studies in culture systems are very much limited. Excepting a few studies on host parasite relationships (Marshet Adugna, 2017), not many studies are focused on host parasite relationships particularly the relationship between water quality parameters and parasite incidences in fish. It is in this context, studies relating to the influence of water quality on the prevalence and intensity of infestation and host-parasite relationships, especially the effects of parasites on the wellbeing of the host fish in culture systems are very important, and hence the present study.

1.3 Objectives of the study

1.3.1 General objective

- The general objective of this study was to investigate the influence of water quality on parasite infestation in *O. niloticus* in culture systems in Sidama Region, Ethiopia.

1.3.2 Specific objectives

The specific objectives of this study were:

- To assess the status of certain water quality parameters of selected fish culture ponds.
- To determine the influence of water quality on parasite prevalence in *O. niloticus.*
- To evaluate the prevalence, intensity and abundance of parasite species and their infestation in relation to size and sex of *O. niloticus.*
- To assess the effects of infestation on growth and condition of *O. niloticus*.
- To evaluate the hematological effects of parasites on *O. niloticus.*

1.4 Research questions

To achieve the stated objectives, the study was focused on the following questions.

- Is there any difference in water quality parameters between the culture systems?
- How do water quality parameters influence parasite infestation in culture systems?
- How do parasite species infestation relates to size and sex of *O. niloticus*?
- Do infestation levels determine the body condition and wellbeing of *O. niloticus*?
- Do infestation levels alter hematological characteristics of *O. niloticus*?

1.5 Significance of the study

It is widely recognized that fish reared in confined conditions are more vulnerable to infestation by different kinds of parasites and subject to illnesses (Paladini *et al*., 2017). Thus, an understanding of parasite species that affect host fishes, and a detailed study on host parasite relationships

between parasites and host fishes will help to understand the health status besides following appropriate measures to control parasitic infestation in culture systems. The results of the study will enable researchers to identify specific water quality parameters that contribute to the spread and severity of parasitic infestations. This knowledge can help fish farmers to maintain appropriate optimal water quality to control parasitic infestation in aquaculture systems.

Some fish parasites are of zoonotic importance, meaning that they can be transmitted from fish to humans. Therefore, the results of this study can also have implications for human health. By identifying and managing parasite infestations in farmed fish, the risk of zoonotic diseases can be reduced. The current study will also serve as a reference for future scholars to undertake detailed studies on host-parasite relationships and control methods to ensure increased production and productivity in fish culture.

1.6 Scope of the study

This study was conducted in two selected fish farms, one in Kokeb fish farm and the other in CARE fish farm, for 6 months from December 2023 to May 2024. The Study also dealt with water quality parameters, effects of water quality on infestation, prevalence, mean intensity and mean abundance of parasites, effects of infestation on growth and condition, and hematology of *O. niloticus*.

1.7 Limitations of the study

- ✓ The study did not include the whole area of Sidama Region, it was limited to only two selected fish farms (Kokeb and CARE fish farms).
- ✓ The present study did not address histopathological parameters and biochemical composition due to limited laboratory equipments and research budget.

1.8 Definitions of terms

Basophils: A type of white blood cell that works closely with the immune system to defend the body from allergens, pathogens, and parasites. It releases enzymes to improve blood flow and prevent blood clots. They are the largest type of granulocytes.

Ecto-parasites: Parasite that lives on body surface, branchial and buccal cavity of fish.

Endo-parasites: Parasite that lives inside its host.

Eosinophils: A type of disease fighting white blood cell. It is a variety of WBCs.

Hematocrit: The percentage by volume of red blood cells in the blood.

Infestation: The presence of parasites in an animal, may be external or internal.

Intensity: The number of parasites found in a single infected host.

Leukocyte: A type of blood cell that is made in the bone marrow and found in the blood and lymph tissue. Leukocytes are part of the body immune system. It is also called white blood cells (WBCs).

Lymphocyte: A type of immune cell that is made in the bone marrow and is found in the blood and in lymph tissue.

Mean Abundance: The total number of parasites in a sample of a particular host species divided by the total number of hosts of that species examined (both infected and uninfected).

Mean Intensity: The total number of parasites in a single infested host (number of parasites divided by number of positive hosts).

Monocyte: A type of leukocyte or white blood cell. They are the largest type of leukocyte in blood and can differentiate into macrophages and monocyte derived dendritic cells.

Neutrophils: A type of white blood cell that is an important part of the immune system and helps the body fight infection. When microorganisms, such as bacteria or viruses, enter the body, neutrophils are one of the first immune cells to respond.

Prevalence: The number of hosts infected with 1 or more individuals of a particular parasite species divided by the number of hosts examined for that parasite species (expressed as percentage).

Zoonosis: Any disease or infection that is naturally transmissible from animals to humans and vice versa.

1.9 Organization of the thesis

The thesis is presented in six chapters:

Chapter one deals with the introduction, statement of the problem, objectives, research questions, significance, scope and limitations of the study.

Chapter two is a literature review, which describes the work carried out on water quality parameters and different aspects of parasite species by different authors.

Chapter three deals with materials and methods, which elaborates the study design and methods of collection, examination, assessment of water quality and parasite infestation.

Chapter four describes the results of the study with tables and illustrations.

Chapter five discusses the results of the study with the earlier findings of similar studies.

Chapter six states the conclusions and recommendations based on the results and discussion of the study.

2 LITERATURE REVIEW

2.1 *O. niloticus* aquaculture

Among tilapia spp, *O. niloticus* is widely cultured worldwide as it possesses the desirable cultivable characteristics such as fast growth, hardy, greater adjustability, good taste, marketability, and accessibility (Prabu *et al*., 2019). Its preferred habitat is shallow water bodies with sufficient vegetation. It grows to a maximum size of about 62 cm (Lamtane *et al*., 2008). It is an omnivore, feeds mainly on phytoplankton, periphyton, aquatic plants (macrophytes), invertebrates and detritus. Adult *O. niloticus* are filter feeders, whereas juveniles feed primarily on phytoplankton, zooplankton, and detritus (Ng'wala, 2014). As *O. niloticus* feeds on natural fish food organisms, under culture, it also accepts supplementary and artificial feeds.

O. niloticus has the ability to survive under low water quality, and is suitable for culture under extensive, semi-intensive, intensive, or super intensive systems of culture (Martins *et al*., 2019). *O. niloticus* is a suitable species for integrated and cage fish farming. It is the most frequently produced species in Ethiopia, has got good consumer demand. Aquaculture production of *O. niloticus* has increased from 2.8 million metric tons in 2008 to 8.89 million metric tons in 2020 (Sriwongpuk, 2020).

Figure 2.1: *O. niloticus*

2.2 Water quality parameters

2.2.1 Temperature

Temperature refers to the level of heat or coldness present in the body of a living organism, whether it is in water or on land. Since many biological and chemical processes are temperature dependent, it is a crucial physical characteristic in an aquatic environment. Water temperature is an important factor for the growth and metabolism of fish in aquaculture. The physiology of fish is significantly impacted by the water temperature in which they live. Fish are exothermic animals; directly affected by the rearing water temperature because their body temperature fluctuates with external environment. While cold water slows metabolism, warm water increases metabolic rate of fishes (Hossain *et al*., 2007).

According to Lefébure (2012), metabolic rate of fish increases in response to increasing levels of temperature, which in turn increases the demand of food for the fish. Fish particularly under culture system are sensitive to temperature fluctuations (Hossain *et al*., 2007). Several fish species suffered when exposed to temperatures below 12^0C and warm-water fish species exhibited optimal survival and growth when the water temperature exceeded 18^0C. Since most fish prefer optimal temperatures for growth and survival. The preferred temperature for aquaculture of *O. niloticus* ranges between 20^0C and 30^0C (El-Sayed and Kawanna, 2008).

2.2.2 Dissolved oxygen

In terms of water quality, dissolved oxygen concentration is regarded as being the most crucial important factor in fish farming. It is essential to the survival (respiration) of fish. Adequate amount of dissolved oxygen is required for better survival of fish species, to sustain healthy fish, and bacteria that decompose the waste produced by the fish, and to meet the biological oxygen demand

(BOD) within the culture systems. Inadequate dissolved oxygen concentration affects fish breathing, feeding activity as well as ammonia and nitrite toxicity (Sriyasak *et al*., 2014).

Khater *et al*. (2021) mentioned that the dissolved oxygen levels for *O. niloticus* should be in the range between 5 and 7 mg/l. When dissolved oxygen levels are lower than the optimum range, the growth of the fish can be highly affected by an increase in stress, tissue hypoxia, a decrease in swimming activities, cannot utilize the food optimally, and a reduction in immunity to diseases (Khater *et al*., 2021). Growth and feed conversion of fish will be lowered when dissolved oxygen concentrations are low or high from minimal level. Sriyasak *et al*. (2014) suggested that utilizing mechanical mixing and aeration is important to reduce fish stress there by to avoid fish death from dissolved oxygen accumulation.

2.2.3 Hydrogen ion concentration (pH)

pH is an important factor in aquaculture as it decides the productivity of the pond and health status of fish in culture ponds. It quantifies the concentration of hydrogen ions in the water, which determines its level of acidity or alkalinity. The pH scale ranges from 0 to 14, with 7 representing a neutral state. A pH value below 7 indicates acidity, whereas a pH value above 7 indicates alkalinity. This pH measurement provides a standardized way to express the intensity of the acid or alkaline condition of a solution (Buck *et al*., 2002).

pH level between 6.5 and 8.5 is favorable for fish growth (El-Sherif and El-Feky, 2009). If pH level below 4 and above 11 causes acidic and alkaline death of fish species in culture ponds. According to Odor (2000), the pH levels of water can be changed by different factors including effluent discharges from quarries, industries, storm water, and wastewater treatment facilities which will affect the pH of the water. Excessive levels of acidity or alkalinity harm the quality of water and as a result it leads to infestation by parasites.

2.2.4 Turbidity

The term turbidity refers to the degree of clearness in water that is impacted by the presence of contaminants, tiny particles, sediments, or solids. Increased turbidity can be caused by a variety of factors, such as excessive algal growth, stream erosion, urban runoff, wastewater discharge, and eroding banks (Masaba, 2020). Turbidity reduces water visibility and makes water harder for fish. This can be particularly beneficial for free-living stages of parasites like protozoan cysts or helminth eggs and some parasites like ciliates which feed on suspended organic matter (Davies Colley and Smith, 2001). Higher turbidity often indicates more organic particles in the water, potentially providing a plentiful food source for these parasites. According to Omondi *et al*. (2011), suspended materials in water permit less light penetration, which raises the water temperature due to suspended particles carrying more heat. Therefore, suspended particles may clog fish gills, affecting the development of fish eggs and larvae as well as growth rates and making fish more susceptible to diseases.

2.2.5 Nitrogen compounds (ammonia, nitrate, and nitrite)

Fish waste products, dead phytoplankton, leftover protein feed, and the breakdown of organic materials all contribute to the production of ammonia in fishponds (Wurts, 2003). Ammonia is released from fish through their gills after they ingest protein. The quantity of food that is added to the ponds that contain protein affects fish. The bacterial breakdown of organic materials, including uneaten feed, dead algae, and aquatic plants, releases ammonia into the ponds as well (Wurts, 2003). According to Hargreaves and Tucker (2004), fish become toxic if ammonia builds up in fish ponds. Regular monitoring of ammonia levels and keeping it at low levels at ideally below 0.05mg/l is essential for fish health (Ip *et al*., 2001).

High concentrations of ammonia affect fish species in different ways such as decreased development, poor feed conversion, and decreased disease resistance (Hargreaves and Tucker, 2004). The pH of the water has the biggest impact on the relative amounts of the two types of ammonia in it. In a high pH environment, ammonia is dominant, but in a low pH environment, ammonium ions are comparatively non-toxic. Similarly, as the water temperature increases, the amount of unionized ammonia (NH_3) increases (Girma Tilahun and Ahlgren Gunnel 2010). In general, both temperature and pH influence unionized (NH_3) and ionized (NH_4^+) ammonia in water. Nitrate is nontoxic and it is an important nutrient for algae and macrophytes in fishponds. Excess nitrate accumulation can cause eutrophication of the ponds and associated algal blooms as a result depletion of dissolved oxygen formed (Seema, 2015). According to Stone and Thromford (2004), nitrate is relatively nontoxic to fish, and it does not cause any health hazard except at exceedingly high levels (above 90 mg/l).

In ponds, nitrite is created when total ammonia nitrogen (TAN) combines with oxygen in an aerobic environment. Nitrite is very toxic and known as the fish killer. Without a visible sign, it oxidizes hemoglobin to methamoglobin in the blood, coloring the blood and gills red and impairing breathing. It also damages the fish's neurological system, liver, spleen, and kidney (Jensen, 2003). High temperatures and soft water make it more harmful. Chemical parameters that affect the toxicity of nitrite include the reduction of calcium, chloride, bromide, and bicarbonate ions, as well as the levels of ammonia, dissolved oxygen, and pH (Boyd, 2017). High ammonia levels, low dissolved oxygen, and higher pH can all enhance the harmful effects of nitrites. According to Ekubo and Abowei (2011), freshwater fishponds shouldn't have nitrite concentrations greater than 0.5 mg/l.

2.3 The influence of water quality on infestation of fish parasites

Physio-chemical parameters especially temperature, pH, dissolved oxygen, turbidity and nitrogen compounds play a significant role in the development of diseases in fish (Hossain *et al*., 2007). These factors, when found in excess or if it is below the normal level, the fish under culture are stressed and become more vulnerable to parasitic infestations (Conte, 2004). The chemical imbalance often disturbs the physiological functions of fish organs such as the kidney, skin, and gills (Sabae and Mohamed, 2015). In aquaculture systems, the use of poor water source, improper pond management practices, application of excess fertilizers and feeds cause water quality deterioration which produce stress to fish thereby infestation by different kinds of parasites (Boyd, 2017).

According to Jacobsen (2011), parasites can be naturally present or introduced and establish themselves in culture systems. Parasites can coexist with fish without causing significant harm when the environment is not conducive to their colonization. However, when the environment is favorable for their colonization, their population increases which produce tissue damage to fish (Modu *et al*., 2014). Fish parasite infestations are influenced by several parameters, especially water temperature, dissolved oxygen, and pH levels, which can also affect the stages of free-living parasites. According to Yirenya-Tawiah *et al*. (2011), eutrophication encourages the growth of algae, which raises the density of herbivorous snails, an intermediate host for parasites with intricate life cycles.

For certain parasites to develop and become infested, high-water temperatures are essential. For example, Xu *et al*. (2009), found that, the ideal temperature range for *Ichthyophthirius multifiliis* development is between 20^0C and 25^0C. Furthermore, high water temperatures of 25–30 °C have been linked to significant infections of *Trichodina* and *Chilodonella* sp. (Conte, 2004). On the

other hand, it is known that certain parasites, such as *Trichodina* sp and *Chilodonella piscicola*, can only reproduce in water temperature that is below 20°C. Almeida *et al*. (2008), stated that elevated water temperatures exceeding 30°C, in conjunction with other environmental stressors like pH levels above the ideal range of 6.5 to 8.5, low dissolved oxygen concentrations below 3 mg/l, and high-water temperatures, result in decreased fish developmental stability. This lowers the fish's ability to grow and weakens its immune system, making it more vulnerable to severe infestations.

2.4 Parasites of fish

Fish are susceptible to a wide variety of diseases, like other animals, many of which are brought on by outside factors, such as parasites. Parasitic infestation frequently occurs in fish that causes retarded growth rate, reduction, reduced production, consumer rejection, low reproduction and mass mortality in fish (Outa *et al*., 2021). The most common symptoms of parasitic infestations in fish are weight loss, disruption of reproduction or impotency, blindness, abnormal behavior, epithelial lesions, deformities of gills and others. These all cause eventually economic cause in the fish farming sector and hence parasites are among the important factors responsible for production losses (Nyambi *et al*., 2022). Fish parasites include parasitic protozoans, monogeneans, digeneans, acanthocephalans, nematodes, cestodes and branchiurans, which are the most important fish parasites (Table 2.1).

Table 2.1: Parasites of *O. niloticus*

Parasite group		Species	Location	Effects	Reference
Protozoa	Ciliates	*Chilodonella* spp	Skin/gills	Skin irritation, mucus production, gill damage and flashing.	Abdel-Baki and Al-Quraishy (2014)
		Trichodina spp (Velvet disease)	Skin/gills	Slimy coat, flashing and lethargy.	Marshet Adugna (2017)
		Ichthyophthirius spp	Skin/gills	White cysts on skin and gills, flashing and respiratory distress.	Xu and Klesius (2006)
	Flagellates	*Ichthyobodo* spp	Skin/gills	Skin ulcers, excessive mucus production, gill damage and flashing.	Arguedas *et al*. (2017)
Monogenea (Trematodes)		*Gyrodactylus* spp	Skin	Skin damage, respiratory distress, and anemia.	Marshet Adugna (2020)
		Cichlidogyrus spp *Dactylogyrus* spp	Gills/ skin	Skin erosion, ulcers, and secondary infections.	Meneses *et al*. (2018)
Digenea (Trematodes)		*Clinostomum* spp *Diplostomum* spp *Tylodelphys* spp	Gills/branchial cavity Eye	Gill fusion, respiratory distress, stunted growth White cysts and reduced marketability.	Marshet Adugna (2017)
Cestoda (Tapeworms)		*Proteocephalus* spp *Diphyllobothrium* spp	Intestine	Reduced nutrient absorption and weight loss.	Haji *et al*. (2017)
Nematoda (Roundworms)		*Camallanus* spp *Contracaecum* spp *Anisakis* spp *Capillaria* spp	Intestine, stomach, muscles	Intestinal inflammation, perforation, tissue damage and reduced appetite.	Marshet Adugna *et al*. (2018); Mekonen Hailu *et al*. (2023)
Acanthocephala (Spiny-headed worms)		*Acanthocephalus* spp *gyrinorhynchus* spp	Intestine	Intestinal blockage, bleeding and secondary infections.	Addo *et al.* (2021)
Crustacea (Copepods, Branchiurans and Isopods)		*Ergasilus* spp *Argulus* spp, *Dolops* spp	Gills/ skin	Tissue damage, irritation, secondary infections and reduced growth.	Sriwongpuk (2020); Marshet Adugna (2017)

2.5 Host-parasite relationship

Host-parasite relationship is dependent on three main factors, and they are host, parasite and environment (Khan, 2012). There is a complex relationship exists between host and parasite. As the host uses its defense mechanisms to defend the infestation, the parasite tries to establish itself there. Generally, infestation is influenced by the age, behavior, physiological and immunological state, location and eating pattern of host fishes (Paladini *et al*., 2017).

2.5.1 Prevalence and intensity of infestation in relation to host size.

The prevalence and intensity of infestation depends on the size and condition of fish (Aloo *et al*., 2004). In a marine environment, larger fish tend to harbor a higher number of parasites compared to fry and fingerlings. Blahoua *et al*. (2016) stated that the length group, measuring between 10 and 25 cm showed low levels of monogenean infection (36.7%) while fish with above 25 cm showed 100% prevalence. Shafi (2015) narrated a positive relationship between fish size and parasite infestation. On the other hand, Otachi (2009) reported negative association between fish size and parasite levels in freshwater fishponds and cages.

The relationship between infestation and weight reveals that adult fish have higher infestation rates than juvenile fish. The juvenile fish have a completely developed immunity against parasitic infestation, while adult fish have less immunity. So, fish with larger weight have a greater percentage and quantity of parasites. The percentage of infection rises with increasing size in relation to weight and length (Biu *et al*., 2014). Fish are more susceptible to parasite infection in longer and heavier fish. The reason for this is that larger fish have a greater surface area than smaller fish, which allows the infestation to spread more quickly.

2.5.2 Prevalence and intensity of infestation in relation to host sex

Male fish had higher mean parasite abundance and intensity than female fish. Most studies have found that male fish typically have greater infection rates than female fish (Aloo, 2002) and this difference is attributed to the feeding behavior of fishes. While females lay eggs and hold the eggs in their mouths, during this time, males are constantly moving and they eat more so, its bodies become more parasitized. Ecology may also play a role in the variations in parasite infection between sexes (Leung and Koprivnikar, 2016). According to Guidelli *et al*. (2003), there is a strong relationship between host sex and prevalence, with the females having higher infestation rates than male fish. Female fish were shown to have a higher prevalence of *Contracaecum* sp infestation than male fish.

2.5.3 Growth and condition of fish

Stress levels, feed availability, and water quality parameters like pH, temperature, dissolved oxygen and ammonia concentrations all affect fish condition factor (Nehemia *et al*., 2012). The above criteria need to be routinely checked to produce maximum sized *O. niloticus* in culture systems. As length increases, the Fulton's condition factor frequently falls. Fish are classified as being in excellent condition when their k value is 1.40 or higher and as being in bad condition when it is less than 1.40 (Barnham and Baxter, 1998). A brain tumor, cellular necrosis, mortality, slow growth, emaciation, deformity of the vertebral column, and loss of vision can all occur in heavily infected fish with digenetic trematodes (Iwanowicz, 2011).

2.5.4 Parasitic infestation and hematology of fish

Hematology can also provide information regarding the immunological status and general health of the host. Blood parameter studies are crucial because they provide important information about the physiological capabilities of fish and serve as a tool for evaluating their immune systems. In

addition to providing information about the host health and immune status, hematological parameters may also do so (Tavares-Dias and Moraes,2007). The hematological and physiological status of parasitized fish may show notable alterations. As stated by Tavares-Dias and Moraes (2007), the host's capacity to withstand damage depends on the kind of damage done to the host tissue, the quantity of parasites present, and the host's overall health. Parasites are the source of hematologic illnesses. When they invade fish, the host fish reacts physiologically and behaviorally to stress. Anemia in infested fish was described by Ali *et al*. (2010) who stated that when infestation increases, it affects the host's ability to regulate osmotic balance and decrease the amount of RBCs and hematocrit.

3 MATERIALS AND METHODS

3.1 Description of the study area

The study was conducted in two fish farms viz: Kokeb fish farm of Dara District and the Center for Aquaculture Research and Education (CARE) fish farm, Hawassa University, Sidama Region.

Kokeb Fish Farm: It is located in Bedesa Kebele Dara District, Sidama Region 355 km South of Addis Ababa. It is bordered by Gedeo Zone on the South, Chuko on the Northwest, Aleta Wendo on the North, and Hulla on the Northeast. It is geographically found between 6°36´– 6°54´N latitude and 38°30´– 38°51´E longitude. It has an elevation of 1890 msl and the mean annual rainfall between 1201 and 1600 mm (SAZ, 2005). Kokeb fish farm has 2 ponds; the size of each pond is 10 m X 10 m in length and width. The depth of the pond is 1m at inflow and 1.5 m deep at outflow. It is earthen pond and the source water for the pond is from the adjacent canals.

Figure 3.1: Photograph showing the earthen pond in Kokeb fish farm (Source: Author)

CARE Fish Farm: CARE fish farm is found in Hawassa University Main campus, Sidama Region. It is found in the southern part of Ethiopia at 275 km south of Addis Ababa, the capital city of Ethiopia. It is located at $6^0$54'-$7^0$55'N latitude and $38^0$27'-$38^0$33'E longitude and situated at 1686 m above sea level. The farm has 4 ponds; the size of each pond is 15 m X 10 m; depth at inflow is 1 m and 1.5 m at outflow region. It is concrete pond, the source water for the pond is ground water.

Figure 3.2: Photograph showing the concrete pond in CARE fish farm (Source: Author)

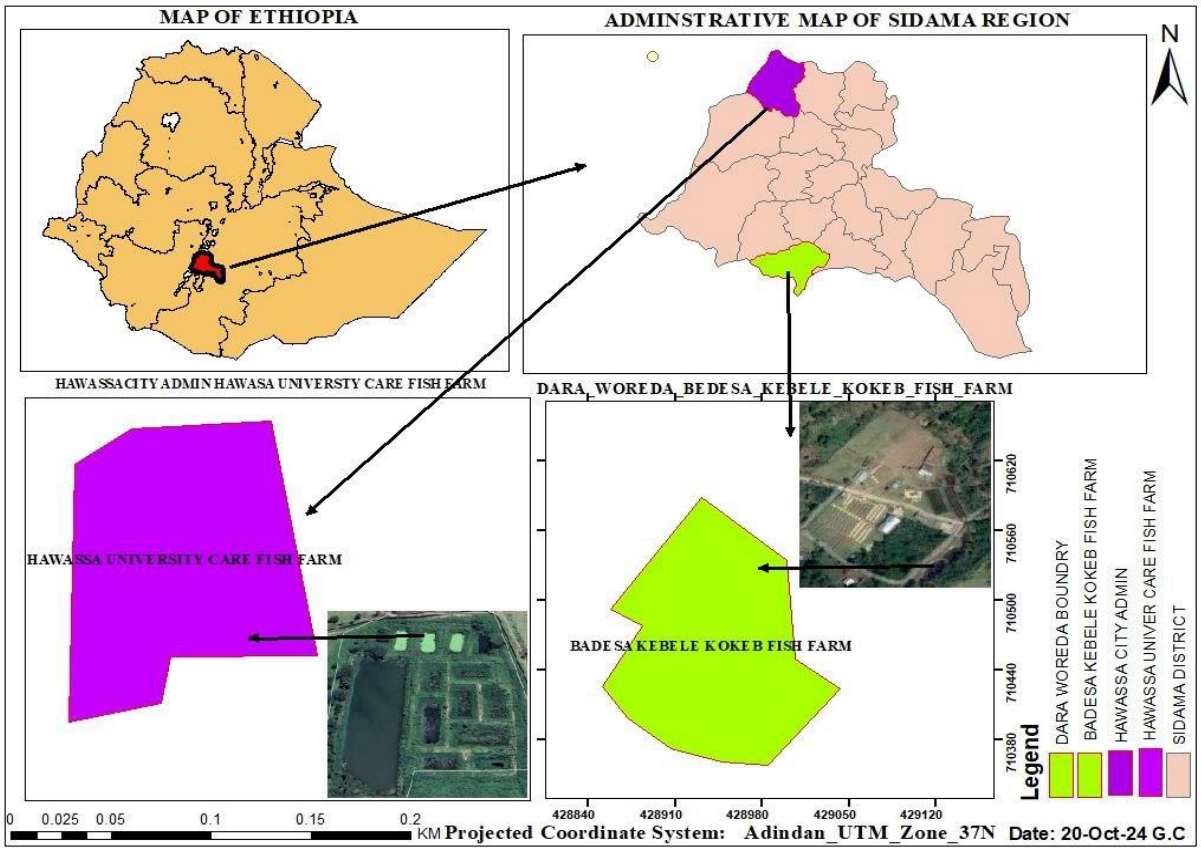

Figure 3.3: Map of the study area (Source: ArcMap, 2024)

3.2 Sampling design and sample size

A cross-sectional study was conducted to collect fish samples for parasitological examination and pond water quality assessment for 6 months from December 2023 to May 2024. A simple random sampling method was used to collect fish samples by using a seine net. The fish selected for the study was *O. niloticus*. A total of 240 *O. niloticus*, each farm with 120 *O. niloticus* were collected and transported to Hawassa University Aquaculture Laboratory in ice box. Morphometric data such as total and standard length were measured by using a measuring board and total weight by digital weighing balance (Elias Dadebo *et al*., 2005). Data was entered in the data register for further study.

3.3 Water quality parameters

3.3.1 Physico-chemical parameters

The physico-chemical parameters of pond water were measured randomly in-situ in the selected fishponds for 6 months. This included temperature, dissolved oxygen, pH and turbidity. The measurements were conducted weekly between 10 :00 AM and 11 :00 AM. Temperature and dissolved oxygen were measured using a DO meter of DO-810 model. pH was measured by using pen type pH meter of model P301 and turbidity by Turbidimeter (NTU).

3.3.2 Nutrient analysis (NH_3, NO_3 and NO_2)

Water samples for nitrogen compounds were collected monthly over the sampling period from each farm. One large composite bottle and three small sampling bottles were prepared. Before collecting the sample, both the sampling and the composite bottles were rinsed with pond water three times. Sampling bottles were slowly lowered vertically into water until they reached a depth of two meters and at that point, they were vertically pulled up. The water sample was poured into the composite bottle and mixed well. The water sample was put into a polyethylene bottle, and one ampule of sulfuric acid (1 ml of H2SO4 for a 250 ml polyethylene bottle) was added to lower pH and stop reactions in the sample until it can be analyzed. The polyethylene bottle was stored in insulated coolers containing ice and was then transported to Hawassa University Environmental Engineering Laboratory and was kept in a refrigerator at 4^oC for analysis.

Ammonia concentration: Using an acid-base titration technique and a pH electrode, the ammonia content of the sample 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.

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 is reached. The mixture was mixed for one minute, and then two minutes were given. One nitrate tablet was added to the tube, and 10 minutes are then allowed to pass. The sample color was blended with a standard color. The Nitrate Nitrogen Comparator Test Kit was filled with the test tube. 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).

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 dissolved, 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. The Test Kit reported a result in mg/l for nitrogen, and this nitrogen was multiplied by 3.3 to produce Nitrite (mg/l), so N*3.3 = NO2 (mg/l).

3.4 Examination of fish for parasites

Fish samples collected from both fish farms were examined for ecto and endo parasites at Hawassa University Aquaculture Laboratory. Before dissecting the fish, an external examination was carried out to find out if there are any lesions or abnormalities or metazoan parasites which are seen to naked eye on the body surface, fins, eyes, nostrils, gills, buccal and branchial cavities. The body surface was scrapped off with the help of a scalpel, knife or back end of the forceps, and the contents thus collected were placed on Petridish and washed with saturated sodium bicarbonate or saline solution to remove the attached debris and mucus. It was then rinsed for two or three times in freshwater to obtain clean parasites and examined under a compound microscope.

For the collection of endo parasites, an incision was made at the ventral region fish to remove all vital organs which were then separated and kept in separate petri dishes. The musculature and mesenteries were teased and attached larval forms were freed. The body cavity was also looked for the presence of parasites. Scissors, forceps, pointed needles, fine brushes and droppers were used for the collection of parasites.

Figure 3.4: Photograph showing examination of *O. niloticus* for collection of parasites

3.5 Identification of parasites

Identification of parasites collected was based on the distinctive body shapes and the morphological feature of the parasites (Pouder *et al*., 2005; Florio *et al*., 2009). The collected parasites were also cleared in sodium bicarbonate solution and rinsed in tap water to clear the parasites and then preserved in 5% formalin solution (Natarajan, 1987). The specimens were placed in glycerine-ethanol for few days to remove formalin in them and make it more visible under stereo microscope (Woo and Buchmann, 2012).

3.6 Prevalence, intensity and abundance of infestation

The Prevalence (%), mean intensity (MI) and mean abundance (MA) of infestation were calculated by following the method of Bush *et al*. (2001) as follows:

Prevalence (P) = $\frac{\text{Number of infested fish with a particular parasite species}}{\text{Total number of hosts examined}}$ *100

Mean intensity (MI) = $\frac{\text{Total number of parasite species in host species}}{\text{Total number of infested host species}}$

Mean abundance (MA) =$\frac{\text{Number of parasites}}{\text{Number of Examined fish}}$

3.7 Condition factor

The Fulton's condition factor (k) was estimated using weight and total length. The weight of fish was measured using a digital balance to the nearest gram, and the length was measured using a measuring board or meter ruler calibrated in centimeter. The condition factor was calculated using the formula K= $\frac{100W}{L^3}$ (Froese (2006), where K is the condition factor, W is the weight of the fish in gram (g), L is the total length in centimeters (cm). After calculation, the resulting values of condition factor for each fish were compared with the standard values of Barnham and Baxter (1998) as given in Table 3.1.

Table 3.1: The scale of condition factor and fish health status.

K value	**Condition of fish**
1.60	Excellent
1.40	Good
1.20	Fair
1.00	Poor
0.80	Extremely poor

3.8 Hematology

Collection of blood samples: 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 using a 1 ml Terumo medical syringe. The needle was inserted into the musculature along the lateral line behind the anal fin until it reached the spine. The plunger of the syringe was then slowly withdrawn until the syringe was filled with blood. The collected blood was placed in a vacuum tube containing an anticoagulant (EDTA). The tube was then paper labeled. Blood samples were analyzed for RBCs (erythrocyte counts), WBCs (leukocyte counts), hemoglobin concentration, hematocrit, Platelets, and as well as for observation of leukocyte cell differentiation according to the methods of Blaxhall and Daisley (1973).

RBCs (erythrocyte counts): 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).

$$\text{Number of Red Blood Cells} = \frac{\text{No.cells counted X dilution factor}}{\text{Area counted (mm2)}X\text{ depth (mm)}}$$

WBCs (leukocyte counts): 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 hemocytometer and covering it with a glass cover. The number of leukocytes were counted in 5 out of the 16 small compartments in the hemocytometer and converted to obtain the number of cells per mm^3. Leukocyte was counted by using Blaxhall and Daisley (1973) formula:

$$\text{Number of White Blood Cells} = \frac{\text{No. cells counted X dilution factor}}{\text{Area counted (mm2) X depth (mm)}}$$

Hemoglobin concentration: Sahli's method was employed for determining the hemoglobin content. This method is also known as the acid hematin method, where hemoglobin is converted to hematin by dilute hydrochloric acid. For this, 0.1 N HCl was added. The Shali'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 deciliter from the graduated hemoglobin tube.

Hematocrit: Micro hematocrit 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 tub 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 (revolution per minute) 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_2x100 (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.

Platelet: The hemocytometer method was employed to determine the platelet counts. A small drop of blood was placed on a clean glass microscope slide; another slide was used to spread the drop evenly across the slide; this allows the smear to air dry completely before proceeding with staining. Stains such as Wright's stain or Giemsa stain were used for better visualization of platelets. The stained smear was examined under a microscope, and then the number of platelets was counted in a defined area. For a more quantitative approach, a hemocytometer was used. The average platelet count was calculated per microliter based on the observations. The blood sample was diluted appropriately with 1:10 saline solution. The diluted sample was loaded into the hemocytometer, and then the number of platelets was counted in the designated squares.

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 methanol 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:

$$\text{Proportion of a leukocyte cell type} = \frac{\text{Number of the leukocyte cell type X } 100\%}{\text{Total number of leukocytes}}$$

3.9 Data analysis

The collected raw data were entered into Microsoft excel-13 data sheets and analyzed using SPSS Version-26, Statistical Software. Inferential analysis was done to explain statistically significant relationships between variables in the testing of the specific objectives. Physico-chemical parameters of water samples were summarized for the study period as mean ±SE values for each sampling station. Prevalence, mean intensity, and mean abundance were carried out according to Bush *et al*. (2001). One-way ANOVA was used to test the significant differences between the water physicochemical parameters of the two fish farms. Pearson's correlation was used to determine if there is variation among fish size classes and susceptibility to parasite infestations and to determine the possible relationships between the prevalence, mean intensity and mean abundance. Infestation on sex difference of *O. niloticus* was analyzed by chi-square (x^2) test. The effect of parasites on the health status of the host fish was investigated using Fulton's condition factor (k-factor). T-test at

95% confidence level ($P<0.05$) was used to evaluate the significance of differences in hematological parameters between infested and non-infested *O. niloticus*.

4 RESULTS

4.1 Water quality parameters of Kokeb and CARE fish farms

4.1.1 Physico-chemical parameters

The mean monthly physico-chemical water quality parameters of Kokeb and CARE fish farms from December 2023 to May 2024 are presented in Table 4.1. In Kokeb fish farm, the water temperature differed significantly between the sampled months [(F = 55.68; p = 0.0006; df =1, 5)]. The highest mean (30.05 ±0.25^{0}C) temperature was recorded in February and the lowest mean (23.65 ±0.35^{0}C) temperature was registered in May. The mean temperature for the remaining months fell within the acceptable ranges. Similarly, the dissolved oxygen also differed significantly between the sampled months [F = 48.82; p = 0.0008; df =1, 5]. The highest dissolved oxygen concentration (5.90 ±0.10mg/l) was recorded in May and the lowest in February. The highest mean pH (8.74 ±0.16) was registered in February and the lowest (7.05 ±0.45) in May. However, it did not differ significantly across the months studied [F = 4.14; p = 0.056; df =1, 5)]. Turbidity was highest in March (51.85 ±16.45 NTU) and lowest in May (26.30 ±2.10 NTU). There was variation in mean turbidity between sampled months [F = 1.19; p = 0.41; df =1, 5)]. However, the difference was not statistically significant.

In CARE fish farm, the water temperature did not show significant difference between the sampled months [(F = 3.24; p = 0.092; df =1, 5)]. The highest mean temperature was registered in January (26.70 ±0.50^{0}C) and lowest in May (20.45 ±2.25^{0}C). Dissolved oxygen was highest in the month of May (9.05 ±0.75) and lowest in the month of January (4.50 ±0.20 mg/l). There were significant differences in dissolved oxygen between the sampled months [F = 31.41; p = 0.0003; df =1, 5)]. While maximum pH was found in the month of March with a mean value of 7.72 ±0.42, the lowest was in the month of April (6.60 ±0.50). However, the mean pH did not significantly differ across

the months [F = 1.18; p = 0.41; df =1, 5)]. The highest Turbidity was recorded during the month of February with a mean value of 14.70 ±0.60 NTU and the lowest in May with a mean value of 9.75 ±0.55 NTU. There were significant differences in turbidity between sampled months [F = 5.73; p = 0.028; df =1, 5)].

Physico- chemical water quality parameters obtained in the sampled fish farms in terms of mean difference between farms, water temperature showed significant difference between the fish farms [(F = 5.36; p = 0.043; df (1, 11)]. The higher mean temperature (27.20 ±0.92^{0}C) was recorded at Kokeb farm and CARE recorded the lower mean temperature (23.94 ±1.06^{0}C). Similarly dissolved oxygen also showed significant differences between the fish farms [F = 5.54; p = 0.040; df (1,11)], the higher mean dissolved oxygen (6.72 ±0.80 mg/l) was registered at CARE fish farm than in Kokeb fish farm. pH also showed a significant difference between the fish farms [F = 8.70; p = 0.015; df (1, 11)], where the higher mean pH (7.98 ±0.23) was registered in Kokeb farm. In addition, turbidity also showed a significant difference between the fish farms [F =33.01; p = 0.00; df (1, 11)], the higher turbidity (36.84 ±4.28 NTU) was recorded in Kokeb fish farm.

Table 4.1: Mean monthly (±SEM) Physico-chemical water quality parameters in Kokeb and CARE fish farms from December 2023 to May 2024.

Fish farms	Parameters	Months						
		Dec	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Mean ±SE**
Kokeb	**Temp (^{0}C)**	28.00 ±0.40	28.35 ±0.35	30.05 ±0.25	27.60 ±0.20	25.60 ±0.20	23.65 ±0.35	27.20 ±0.92
	DO (mg/l)	4.10 ±0.20	4.20 ±0.10	3.05 ±0.05	4.95 ±0.05	5.35 ±0.25	5.90 ±0.10	4.59 ±0.41
	pH range	7.71 ±0.41	8.30 ±0.10	8.74 ±0.16	8.24 ±0.25	7.85 ±0.15	7.05 ±0.45	7.98 ±0.23
	Turb (NTU)	29.30 ±8.50	29.90 ±3.90	47.25 ±12.45	51.85 ±16.45	36.45 ±5.85	26.30 ±2.10	36.84 ±4.28
CARE	**Temp(^{0}C)**	25.80 ±0.10	26.70 ±0.50	26.10 ±0.30	23.00 ±2.30	21.60 ±1.40	20.45 ±2.25	23.94 ±1.06
	DO (mg/l)	5.65 ±0.25	4.50 ±0.20	5.05 ±0.15	7.15 ±0.55	8.95 ±0.25	9.05 ±0.75	6.72 ±0.80
	pH range	7.30 ±0.10	6.90 ±0.60	7.40 ±0.40	7.72 ±0.42	6.60 ±0.50	6.65 ±0.25	7.09 ±0.18
	Turb (NTU)	9.95 ±0.25	10.26 ±0.93	9.75 ±0.55	12.40 ±1.70	13.50 ±0.20	14.70 ±0.60	11.76 ±0.84

4.1.2 Nutrient concentration

The nutrient content of Kokeb and CARE fish farms is presented in Appendix 1 and mean values are presented in Table 4.2. The nitrogen compound values showed a wide variation in both fish farms. Ammonia concentration was significantly higher in Kokeb fish farm [F = 8.24; P = 0.016; df (1, 11)]. The highest mean concentration of ammonia was 0.86 ±0.23mg/l in Kokeb fish farm. Similarly, nitrate and nitrite concentrations also showed significantly higher values in Kokeb fish farm [F = 5.16; P = 0.046; df (1, 11)], [F =350.15; P = 0.00; df (1, 11)] respectively, with mean values of 2.53 ±0.17 mg/l and 0.31 ±0.11mg/l respectively.

Table 4.2 Mean value of nutrient content of Kokeb and CARE fish farms from December 2023 to May 2024.

Fish farms	Parameters		
	Ammonia	Nitrate	Nitrite
Kokeb	0.86 ±0.23	2.53 ±0.17	0.31 ±0.01
CARE	0.17 ±0.15	2.06 ±0.1	0.05 ±0.04

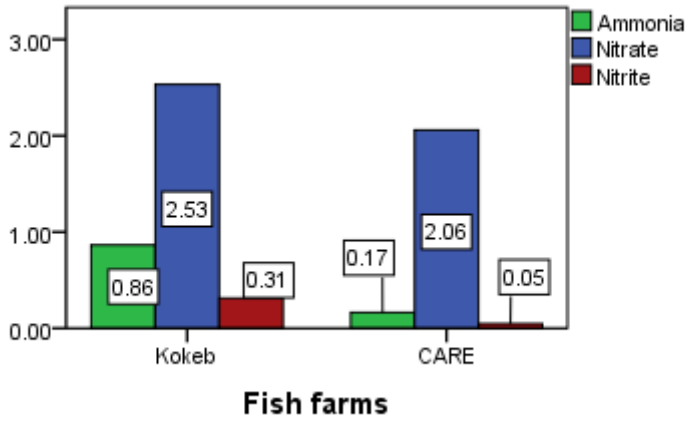

Figure 4.1: Bar graph showing mean values of nutrient content in Kokeb and CARE fish farms.

4.2 Relationship between physico-chemical parameters in Kokeb and CARE fish farms

Water temperatures were negatively correlated with dissolved oxygen in both fish farms, and it was also negatively correlated with pH in Kokeb fish farm and with turbidity in CARE fish farms. Dissolved oxygen was positively correlated with turbidity in CARE fish farm, but in Kokeb fish farm, it was negatively correlated. pH also was negatively correlated with dissolved oxygen in both fish farms (Table 4.3).

Table 4.3: Pearson correlation between physico-chemical parameters in Kokeb and CARE farms

Fish farms	Parameters	Temp	DO	pH	Turbidity
Kokeb	Temp	1	-0.95**	-0.90*	0.50
	DO	-0.95**	1	-0.80	-0.37
	pH	0.90*	-0.80	1	0.70
	Turbidity	0.50	-0.37	0.70	1
CARE	Temp	1	-0.98**	0.44	-0.98**
	DO	-0.98**	1	-0.49	0.95**
	pH	0.44	-0.49	1	-0.52
	Turbidity	-0.98**	0.95**	-0.52	1

**. Significant at 0.01 level and *. Significant at 0.05.

4.3 Parasite infestation in *O. niloticus* in Kokeb and CARE fish farms

Infestation of parasites in Kokeb and CARE fish farms are presented in Table 4.4. A total of 240 *O. niloticus* selected randomly from Kokeb and CARE fish farms were examined for the presence of both ecto and endo parasites from December 2023 to May 2024. Out of 240 *O. niloticus* examined, 109 (45.41%) fish were infested by both ecto and endo parasites. The prevalence of parasites was highest in February in Kokeb farm (90.0%) and in CARE, it was in January (55%). The lowest prevalence of parasites was recorded in May (15.0%) in CARE farm and in Kokeb farm, it was 30 each in April and May. The highest mean intensity of parasites was recorded in February (9.50) in Kokeb farm and in CARE farm it was 5.63 in January. The lowest mean intensity was recorded in April (2.50) in CARE farm and in April in Kokeb farm (4.83).

The highest mean abundance was recorded in February (8.55) in Kokeb farm, and in CARE farm it was in January (3.10). The lowest mean abundance was recorded in April (1.45) in Kokeb farm and May (0.4) in CARE farm. Generally, the highest parasitic prevalence rate, mean intensity and mean abundance were 59.16%, 7.46 and 4.41 respectively in Kokeb fish farm. While in CARE fish farm they were 31.66%, 4.48 and 1.30 respectively (Table 4.4). There was a statistical difference in parasitic prevalence, mean intensity and mean abundance between the two fish farms ($p=0.041$, 0.006 and 0.023) respectively.

Table 4.4: Prevalence, mean intensity, and mean abundance of infestation in *O. niloticus* in Kokeb and CARE farms (n = 240) in relation to different months.

Month/yr.	No of fish Examined		No of fish Infested		Prevalence (%)		No of parasites		MI (No)		MA (No)	
	Kokeb	CARE	Kokeb	CARE	Kokeb	CARE	Kokeb	CARE	Kokeb	CARE	Kokeb	CARE
Dec 2023	20	20	13	7	65	35	87	38	6.69	5.42	4.35	1.90
Jan 2024	20	20	16	11	80	55	116	62	7.25	5.63	5.80	3.10
Feb 2024	20	20	18	7	90	35	171	20	9.50	2.85	8.55	1.00
Mar 2024	20	20	12	6	60	30	91	19	7.58	3.16	4.55	0.95
Apr 2024	20	20	6	4	30	20	29	10	4.83	2.50	1.45	0.50
May 2024	20	20	6	3	30	15	36	8	5.14	2.66	1.80	0.40
Total	120	120	71	38	59.16	31.66	530	157	7.46	4.48	4.41	1.30
	240		**109**		**45.41**		**687**		**6.30**		**2.86**	

4.3.1 Ecto and endo parasites of *O. niloticus*

In this study, 11 species of parasites representing 7 groups were recorded and they are: Protozoa such as *Ichthyobodo* sp, *Trichodina* sp*;* Monogenea such as *Gyrodactylus* sp, *Dactylogyrus* sp, *Cichlidogyrus* sp; Digenea such as *Tylodelphys* sp, *Clinostomum* sp*;* Cestoda such as *Diphylobothrium* sp; Acanthocephala such as *Acanthocephalus* sp; Nematoda such as

Contracaecum sp and Branchiura such as *Dolops* sp. All the 11 species of parasites were represented in the Kokeb fish farm. Out of the 11 species recorded, 5 species of parasites viz: *Ichthyobodo* sp, *Gyrodactylus* sp. *Tylodelphys* sp, *Contracaecum* sp and *Dolops* sp were absent in the CARE fish farm. Most of the external parasites were detected from the gills and skin, whereas the internal parasites were detected from the muscle, stomach and intestine of the fish

4.3.2 Morphological description of parasite species

***Trichodina* sp:** Body shapes vary from cylindrical to discoidal. The peristomial ciliary wreaths always make a complete circle. The aboral disc is typically suctorial with hook like elements possessing both centrifugal process and centripetal rays.

***Ichthyobodo* sp:** Have a small body, ovoid to pyriform (pear-shaped) body with two flagella of unequal size which is used for locomotion and attachment to the host's skin or gills. It has a single, prominent with round or oval shaped nucleus located in the central part of the body.

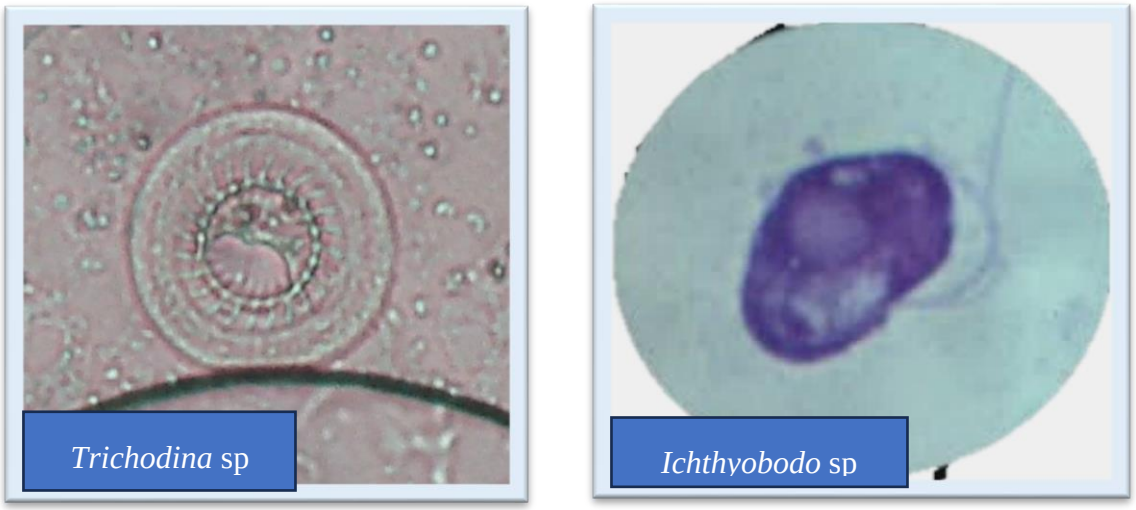

Figure 4.2: Photograph showing protozoan parasites

***Gyrodactylus* sp**: The body is elongated and dorsoventrally flattened, with a streamlined shape adapted for attachment to the host. It has a specialized anterior and posterior attachment organ called a haptor, which is used to attach firmly to the host's skin or gills.

***Dactylogyrus* sp:** It consists of head gland, mouth and two pair eyespots. The eyespots are present on the dorsal surfaces and are visible. Pharynx consists of muscular structure for feeding on mucus, blood, and epithelial tissues of the fish. Posterior attachment organ called haptor which has hooks, clamps, and suckers that firmly grip the fish gills.

***Cichlidogyrus* sp:** The body is flattened and elongated. It possesses a specialized attachment organ called a haptor, at the posterior end of the body which is equipped with numerous hooks, anchors, and accessory pieces that allow the parasite to firmly attach to the host's gills. Mouth is located on the ventral side of the body, near the anterior end and it has one pair of eye spot.

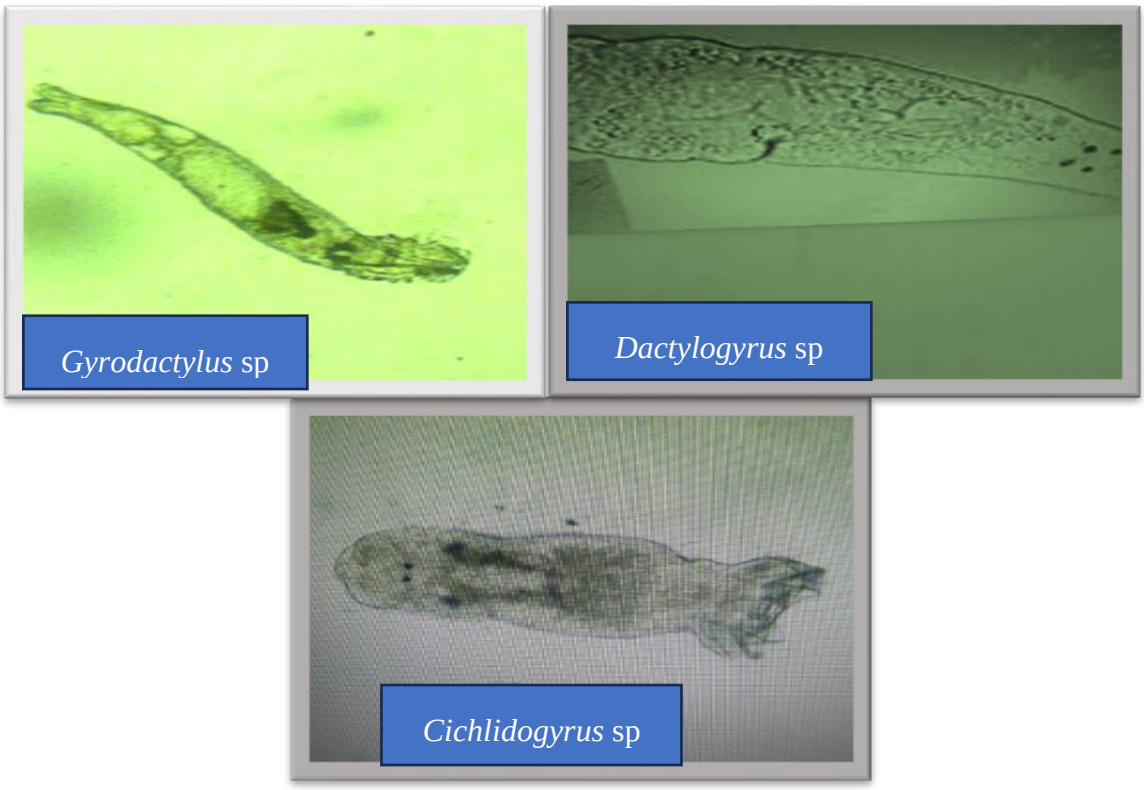

Figure 4.3: Photograph showing monogenean parasites

***Clinostomum* sp:** Body roughly oval with oral and ventral sucker. Oral sucker, located at the anterior end, a muscular structure used for attachment and feeding. Ventral sucker located further back on the body. The digestive system consists of mouth, pharynx, esophagus, and intestinal caeca. The excretory system consists of flame cells and excretory ducts that eliminate waste products.

***Tylodelphys* sp:** Body is flattened, leaf- like with an anterior oral sucker, which is used for attachment and feeding. A ventral sucker used for attachment and locomotion. The digestive system contains branched intestines with no anus. The excretory system consists of flame cells.

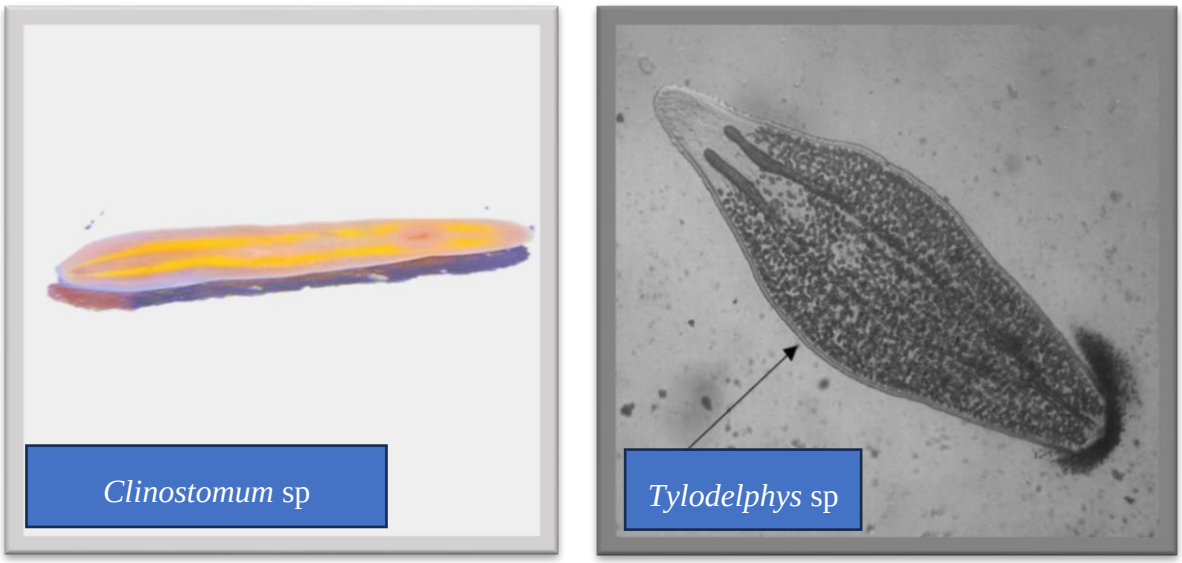

Figure 4.4: Photograph showing digenean parasites

***Diphylobothrium* sp:** Body is long; ribbon-like divided into distinct segments called proglottids. The body contains three regions such as scolex (head), short thin neck, and strobila. Proglottids contain a complete set of male and female reproductive organs.

***Acanthocephalus* sp:** Body is elongated and muscular with an anterior retractable proboscis; it is armed with rows of hooks. The neck is narrow and short.

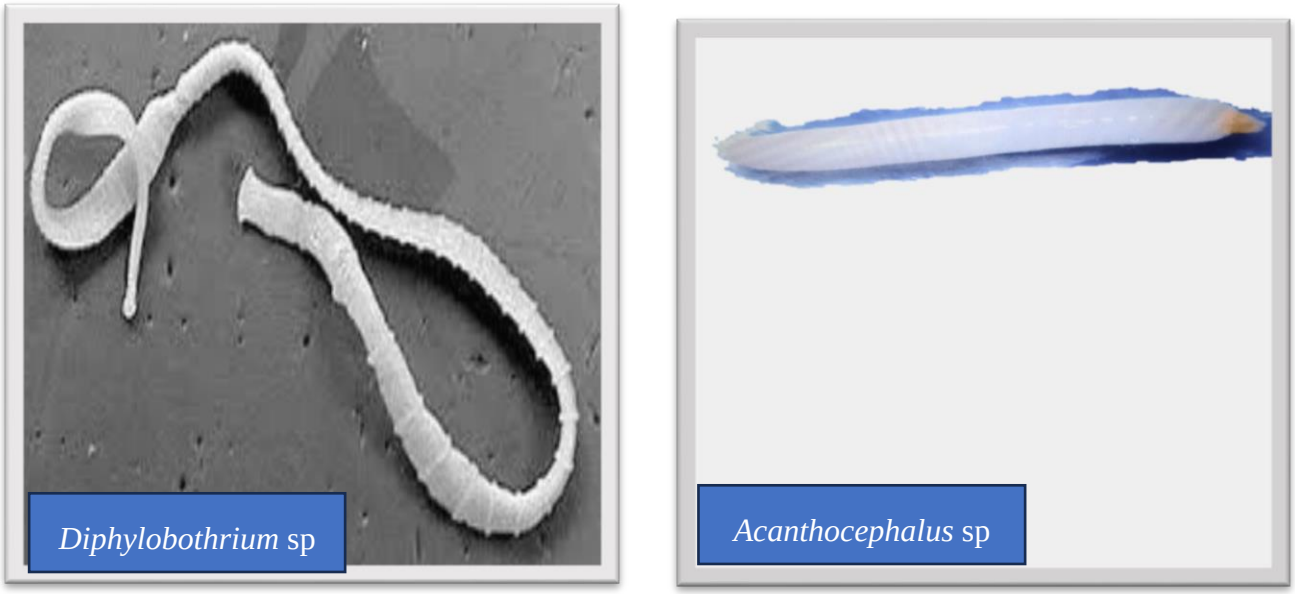

Figure 4.5: Photograph showing cestode and acanthocephalan parasites

***Contracaecum* sp:** Body is long, slender, and cylindrical covered with a tough, protective cuticle. Body is bilaterally symmetrical, caeca at the anterior end.

***Dolops* sp:** A pair of antennae, two compound eyes, two suckers with piercing stylets, a maxilla (suctorial organs), and a proboscis are all found in the cephalothorax. Four pairs of swimmerets are found in the thoracic region; the body is protected by an oval or rounded shaped carapace.

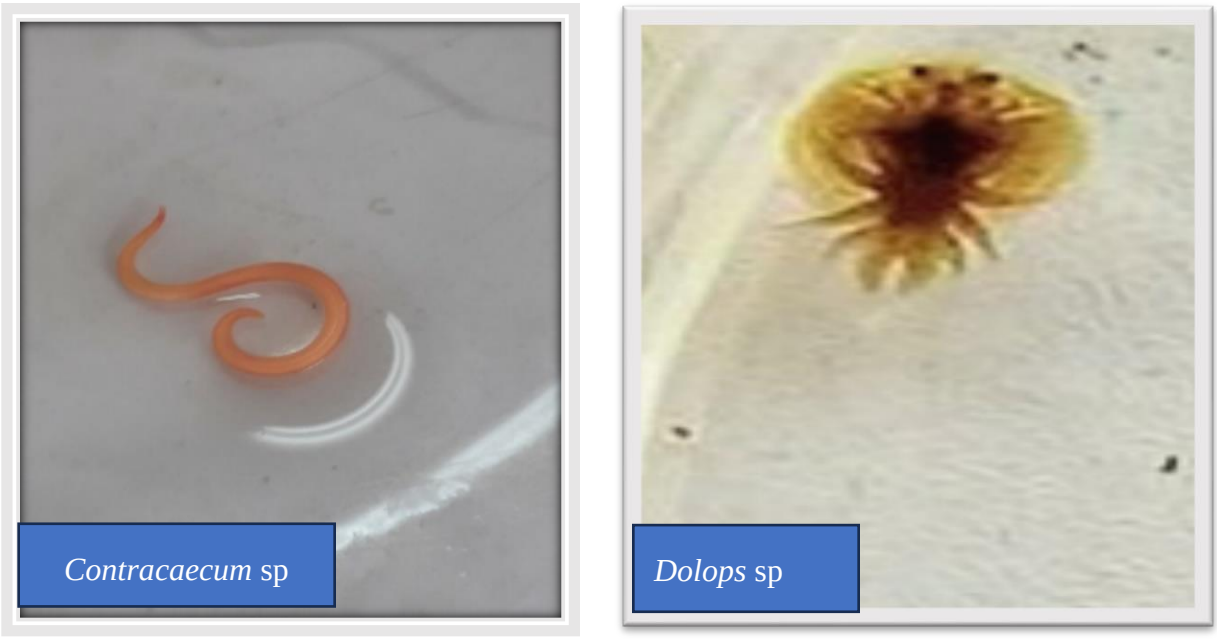

Figure 4.6: Photograph showing nematode and branchiuran parasites

4.3.3 Distribution of parasite species on *O. niloticus*

The distribution of parasites on *O. niloticus* is presented in Table 4.5. Out of 687 parasites collected, the distribution was maximum in *Clinostomum* sp (274) found on the branchial region accounting to 39.88% and the minimum was represented by *Tylodelphys* sp (7) in eye accounting to 1.01%. Although some parasites show distribution in different organs, they show some preferential distribution to organs. In the case of protozoa (*Ichthyobodo* sp and *Trichodina* sp), the distribution was more in gills than skin and in Monogenea while *Cichlidogyrus* sp and *Dactylogyrus* sp showed preference to gills, the skin was less preferred. Digenean parasite, *Clinostomum* sp outnumbered in branchial cavity than gills. While the nematode parasite, *Contracaecum* sp was found in different regions, the preferred site being intestine followed by muscle and stomach. Branchiuran parasite, *Dolops* sp is an ectoparasite and its distribution is in the order of body surface followed by branchial region. Some parasites such as *Dactylogyrus* sp, *Tylodelphys* sp, *Acanthocephalus* sp and *Diplobothrium* sp were specific to one region i.e. gills, eye, and intestine respectively (Table 4.5).

Table 4.5 Distribution of parasites on/in different organs of *O. niloticus*

Parasites		Location	No of parasites	Percentage (%)
Protozoa	*Ichthyobodo* sp	Gills/skin	11/4 (15)	1.60 / 0.58 (2.18)
	Trichodina sp	Gills/ skin	37/13 (50)	5.38 / 1.89 (7.27)
Monogenea	*Cichlidogyrus* sp	Gills/ skin	18/ 9 (27)	2.62 / 1.31 (3.93)
	Gyrodactylus sp	Gills/ skin	2/ 6 (8)	0.29 / 0.87 (1.16)
	Dactylogyrus sp	Gills	37	5.38
. Digenea	*Tylodelphys* sp	Eye	7	1.01
	Clinostomum sp	Branchial cavity, gills	193/81 (274)	28.09 /11.99 (39. 88)
Acanthocephala	*Acanthocephalus* sp	Intestine	132	19.21
Cestoda	*Diphylobothrium* sp	Intestine	24	3.45
Nematoda	*Contracaecum* sp	Intestine/muscle / stomach	26/ 49/ 13 (88)	3.78/ 7.13 / 1.89 (12.8)
Branchiura	*Dolops* sp	Body surface/ b ranchial region	9/ 16 (25)	1.31/ 2.32 (3.63)
		Total	**687**	**100**

4.3.4 Prevalence, mean intensity, and mean abundance of parasite species in fish farms

It is seen that Kokeb fish farm harbored a greater number of parasite species (11 species of parasites), than CARE fish farm which showed only 6 species. In Kokab fish farm, *Clinostomum* sp was the dominant species in prevalence (28.33%), followed by *Acanthocephalus* sp (23.33%),

Contracaecum sp (20.83%), *Dolops* sp (10.0%), *Diphylobothrium* sp (7.50%), *Trichodina* sp (5.80%) *Dactylogyrus* sp (5.0%), *Ichthyobodo* sp (4.16%), *Tylodelphys* sp (4.16%) *Cichlidogyrus* sp (3.3%) and *Gyrodactylus* sp (2.50). In the case of CARE fish farm also, *Clinostomum* sp was the dominant species (12.50%) followed *by Trichodina* sp (10.0%), *Acanthocephalus* sp (8.33%), *Dactylogyrus* sp (6.66%), *Cichlidogyrus* sp (3.30%) and *Diphylobothrium* sp (1.66%). In general, *Clinostomum* sp was the dominant species both in Kokeb and CARE fish farms, the minimum prevalence in Kokeb farm was represented by *Gyrodactylus* sp and *Diphylobothrium* sp in CARE.

In the case of intensity, it was more in *Clinostomum* sp (6.23) and minimum in *Tylodelphys* sp (1.40) in Kokeb farm, while in CARE, the intensity was also more in *Clinostomum* sp (4.13) and unlike Kokeb farm; the minimum intensity was represented by *Diphylobothrium* sp in CARE (1.00). The abundance showed that it was maximum in *Clinostomum* sp (1.76) and minimum in *Tylodelphys* sp (0.05) in Kokeb farm. In CARE the abundance was more in *Clinostomum* sp (0.51) and less in *Diphylobothrium* sp (0.01) (Table 4.4).

Table 4.4: The prevalence, mean intensity and mean abundance of parasite species

Parasites		Kokeb fish farm					CARE fish farm				
		FI	P (%)	PNo	MI	MA	FI	P (%)	PNo	MI	MA
Protozoa	*Ichthyobodo* sp.	5	4.16	15	3.00	0.12	-	-	-	-	-
	Trichodina sp	7	5.83	16	2.28	0.13	12	10.00	34	2.83	0.28
Monogenea	*Cichlidogyrus* sp	4	3.30	15	3.75	0.12	4	3.30	12	3.00	0.10
	Gyrodactylus sp	3	2.50	8	2.66	0.06	-	-	-	-	-
	Dactylogyrus sp	6	5.00	13	2.16	0.10	8	6.66	24	3.00	0.20
Digenea	*Tylodelphys* sp	5	4.16	7	1.40	0.05	-	-	-	-	-
	Clinostomum sp	34	28.33	212	6.23	1.76	15	12.50	62	4.13	0.51
Cestoda	*Diphylobothrium* sp	9	7.50	22	2.44	0.18	2	1.66	2	1.00	0.01
Acanthocephala	*Acanthocephalus* sp	28	23.33	109	3.89	0.90	10	8.33	23	2.30	0.19
Nematoda	*Contracaecum* sp	25	20.83	88	3.52	0.73	-	-	-	-	-
Branchiura	*Dolops* sp	12	10.00	25	2.08	0.20	-	-	-	-	-

P (%) = Prevalence (%), P No = Parasite number, FI= fish infested, MI (No)= Mean intensity, and MA (No)= Mean abundance.

The following Figure 4.7 shows that, the similarity and dissimilarity of parasite species in both Kokeb and CARE fish farms. *Clinostomum* sp, *Acanthocephalus* sp *Cichlidogyrus* sp, *Trichodina* sp, *Dactylogyrus* sp *and Diphylobothrium* sp were found in both fish farms. But *Ichthyobodo* sp, *Gyrodactylus* sp, *Tylodelphys* sp, *Contracaecum* sp and *Dolops* sp were found only in Kokeb fish farm.

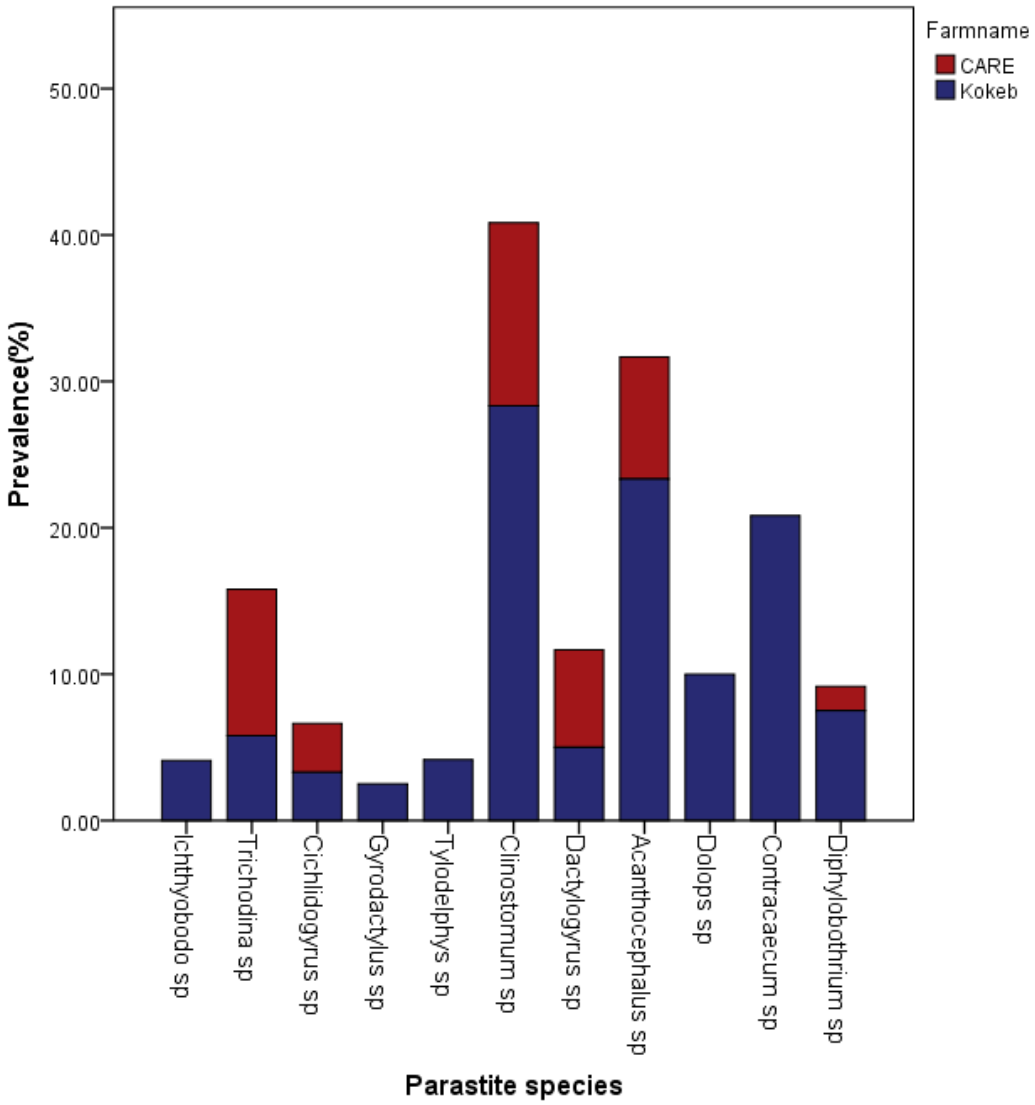

Figure 4.7: Similarity of Parasites distribution in *O. niloticus* in both fish farms, n=109 (fish infested)

4.4 Relationship between water quality and parasite infestation

Water quality parameters showed significant difference between fish farms and parasite prevalence in *O. niloticus*. In both farms, all parasite species showed a significant negative correlation with dissolved oxygen, except *Cichlidogyrus* sp in Kokeb farm which showed insignificant correlation. Similarly, in both farms, all parasite species had a significant positive correlation with temperature and ammonia except *Cichlidogyrus* sp in Kokeb farm, which showed insignificant positive correlation with temperature and *Dactylogyrus* sp in CARE farm, which had insignificant positive correlation with ammonia. All species in both farms had insignificant positive correlation with pH, turbidity and nitrite, but *Clinostomum* sp, *Tylodelphys* sp and *Ichthyobodo* sp in Kokeb farm showed a significant positive correlation with pH. All parasite species in both farms showed a significant positive correlation with nitrate but *Trichodina* sp, *Dactylogyrus* sp and *Cichlidogyrus* sp in Kokeb farm showed insignificant positive correlation (Table 4.6).

Table 4.6: Correlation between water quality parameters and prevalence of parasites in Kokeb and CARE fish farms

Farms	Parameters	Temp	DO	pH	Turbidity	NH_3	NO_3	NO_2
Kokeb	*Clinostomum* sp	0.94**	-0.94**	0.85*	0.48	0.98**	0.90*	0.70
	Acanthocephalus sp	0.86*	-0.90*	0.68	0.49	0.89*	0.84*	0.78
	Contracaecum sp	0.88*	-0.94**	0.67	0.39	0.89*	0.88*	0.74
	Dolops sp	0.93**	-0.90*	0.95**	0.50	0.86*	0.87*	0.52
	Diphylobothrium sp	0.85*	-0.87*	0.73	0.34	0.88*	0.90*	0.80
	Trichodina sp	0.84*	-0.80*	0.87*	0.74	0.85*	0.71	0.69
	Dactylogyrus sp	0.88*	-0.85*	0.72	0.61	0.83*	0.76	0.64
	Cichlidogyrus sp	0.73	-0.78	0.79	0.61	0.81*	0.76	0.80
	Tylodelphys sp	0.87*	-0.92**	0.83*	0.44	0.95**	0.92**	0.76
	Ichthyobodo sp	0.89*	-0.87**	0.88*	0.63	0.92**	0.81*	0.70
	Gyrodactylus sp	0.88*	-0.94**	0.67	0.39	0.89*	0.88*	0.74
CARE	*Clinostomum* sp	0.96**	-0.94**	0.22	0.05	0.95**	0.92*	0.45
	Acanthocephalus sp	0.93**	-0.94**	0.29	0.01	0.96**	0.92**	0.52
	Diphylobothrium sp	0.83*	-0.86*	0.58	0.18	0.86*	0.85*	0.39
	Trichodina sp	0.90**	-0.91**	0.69	0.06	0.82*	0.94**	0.47
	Dactylogyrus sp	0.81*	-0.84*	0.61	0.01	0.77	0.85*	0.51
	Cichlidogyrus sp	0.89*	-0.89*	0.51	0.07	0.83*	0.92**	0.56

**. Significant at 0.01 level and *. Significant at 0.05 level.

4.5 Prevalence and intensity of infestation in relation to length of fish

Prevalence and intensity of infestation in relation to length of fish is presented in Fig. 4.2. It is seen that, the length ≥ 20 cm showed the high prevalence (64.28%), which is higher than other length groups of *O. niloticus*. The length group < 15 cm and between 15 and 20 cm had a prevalence of 18.03% and 49.60% respectively. The prevalence showed a strong positive correlation between fish size and parasite infestation levels (r = 0.978). In general, the number of parasites increases with the increase in fish size. The length group of fish >20 cm had also the highest mean intensity of parasite (7.86), followed by the length group 15-20 cm with 5.80. The lowest mean intensity of parasite was found in the length group <15 cm which was 3.63 as shown in figure 4.8. There was a strong positive correlation between the length of *O. niloticus* and intensity of parasite species (r = 0.999).

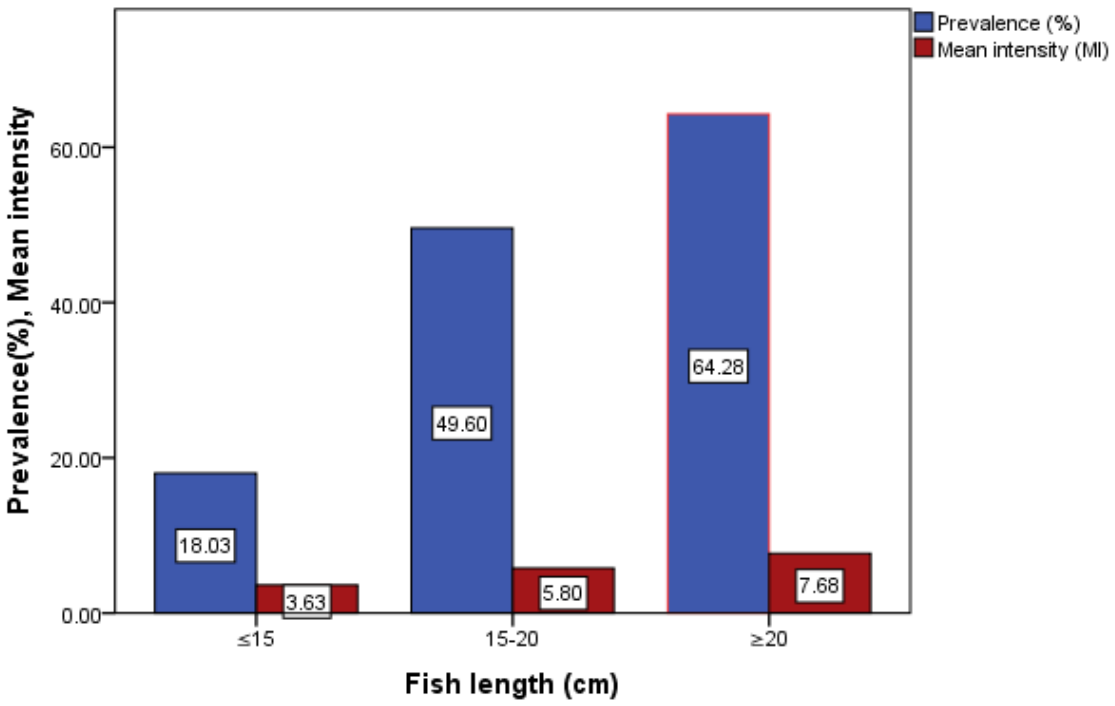

Figure 4.8: Bar graph indicating the prevalence (%) and mean intensity (No) of parasite species based on length of *O. niloticus* in Kokeb and CARE fish farms.

4.6 Prevalence and intensity of infestation in relation to weight of fish

In terms of fish body weight, *O. niloticus* weighing ≥ 150 (g) were highly infested with the prevalence of 75.40%, followed by weight group of 101-150 (g) and 51-100 (g), which had 63.41% and 32.18% infestation rates, respectively. The prevalence rate of 17.64% was in sample weighing ≤ 50 (g). The highest mean intensity of parasites was found in weight group of ≥150 (g) with mean intensity of 7.00, followed by weight group of 101-150 (g) and 51-100 (g) which had mean intensity of 6.30 and 6.00 respectively. The lowest mean intensity was found in the weight group ≤ 50 (g) with mean intensity of 3.66. The p-value ($p < 0.05$) between fish body weight and prevalence of parasites was 0.008, and the mean intensity was 0.04. The Pearson correlation value (r) for weight and prevalence was 0.985, and for intensity it was 0.920.

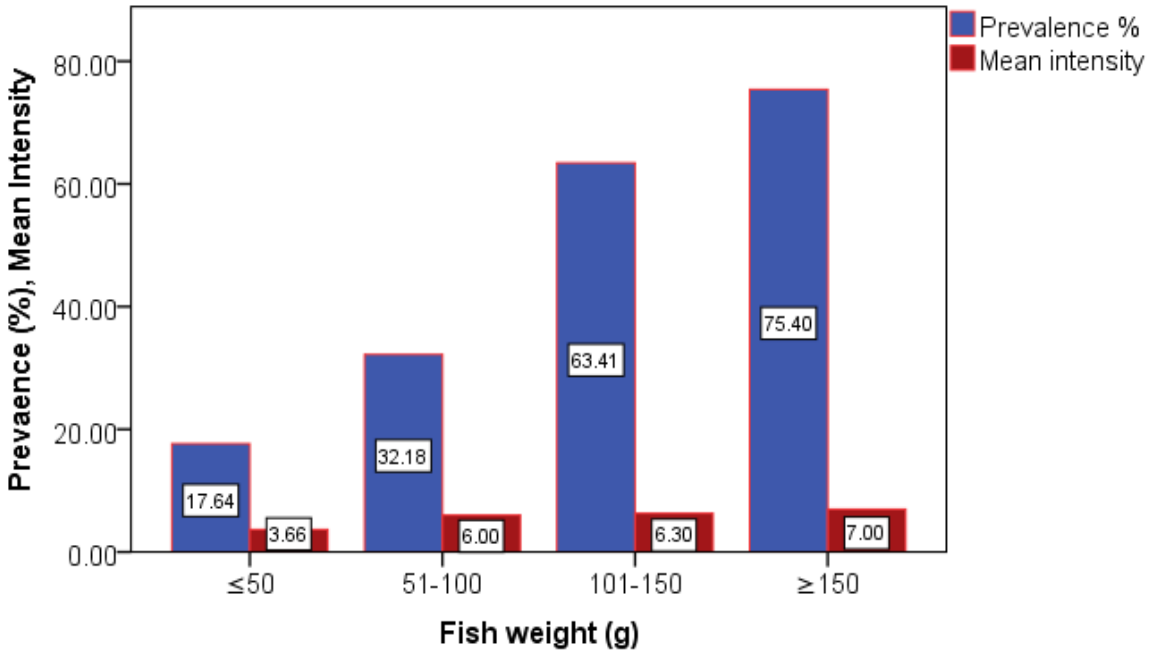

Figure 4.9: Bar graph indicating the prevalence (%) and mean intensity (No) of parasite species based on weight of *O. niloticus* in Kokeb and CARE fish farms.

4.7 Prevalence and intensity of infestation in relation to sex of fish

The total prevalence and mean intensity in male and female *O. niloticus* were assessed to observe their influence on sex of fish in both farms, the results of which are presented in Table 4.8. While analyzing the infestation of 240 *O. niloticus*, 129 *O. niloticus* were males, and 111 *O. niloticus* were females. Out of 129 male *O. niloticus* examined, 68 were infested (52.71%) and out of 111 females, 41 were infested (36.93%). The mean intensity of infestation in relation to sex of fish was 6.58 in male and 5.82 in females. The abundance was 3.47 for males and 2.15 for females. Thus, it is clear that the overall prevalence, mean intensity and mean abundance of infestation were generally more in males than females. The statistical significance of infestation between male and female was $x^2 = 2.776$, $p < 0.05$, which was less than 3.841 ($x^2_{calculated} < x^2_{tabulated}$, $2.776 < 3.841$). There was significant difference of infestation between male and female fish.

Table 4.7: The overall prevalence, mean intensity and mean abundance of parasite species based on sex of *O. niloticus* in Kokeb and CARE fish farms (n = 240).

Sex	Number of fish		Prevalence (%)	No of parasites	MI (No)	MA (No)
	Examined	Infested				
Male	129	68	52.71	448	6.58	3.47
Female	111	41	36.93	239	5.82	2.15
Total	240	109	45.41	687	6.30	2.86

$x^2_{calculated} < x^2_{tabulated}$, $2.776 < 3.841$.

4.8 Condition factor of parasitized and non-parasitized *O. niloticus*

The effect of parasitic infestation on the condition factor of fish was determined by Fulton's condition factor (K) by using the weight and total length of *O. niloticus*. The overall condition factor of *O. niloticus* from the fish farms was above 1.0. The highest K value of individual non parasitized fish was in CARE (2.37) and (1.86) for Kokeb. The lowest K value was in Kokeb fish farm (1.14) and in CARE it was (1.23). This result showed that, the mean condition factor of each fish farm was lower in parasitized *O. niloticus* compared to the mean K value of the non-parasitized *O. niloticus* (Figure 4.4). However, there was no significant difference in condition factor between parasitized and non-parasitized *O. niloticus* in both fish farms ($p > 0.05$).

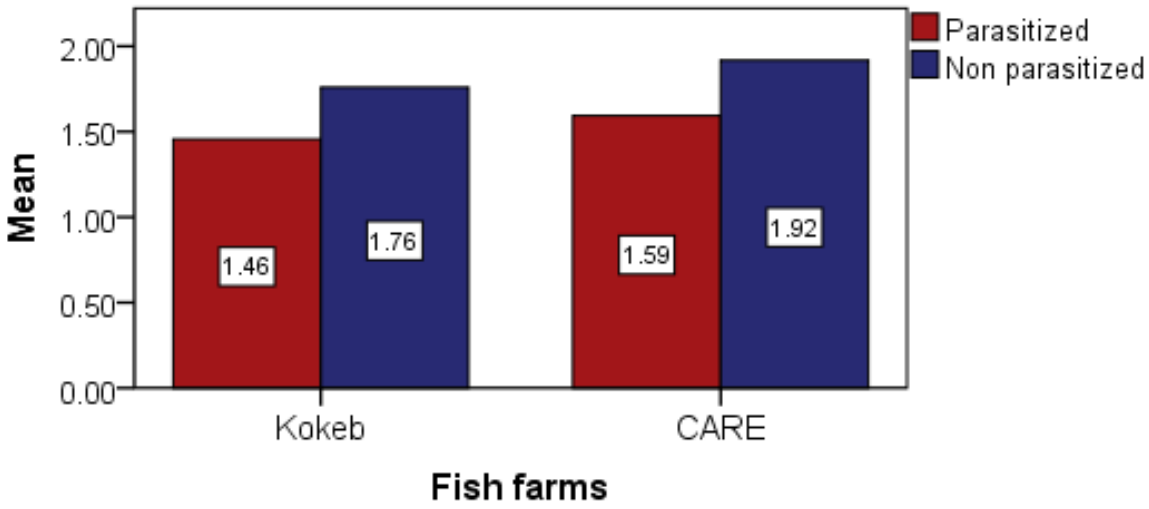

Figure 4.10 : Bar graph showing the comparison between condition factor of parasitized and non-parasitized *O. niloticus* in Kokeb and CARE fish farms.

4.9 Effects of parasites on hematology of *O. niloticus*

For hematological analysis, blood samples from infested and non-infested *O. niloticus* were determined. There was a significant difference in hematological parameters between the two groups of fish. The red blood cells (RBCs), hemoglobin (Hb) and hematocrit (HCT) levels showed significant differences ($p<0.05$) between the two groups. The non-infested group recorded higher blood values compared to infested ones.

Leukocyte count, lymphocyte, monocyte, eosinophil, and basophil showed a significant difference ($p<0.05$) between infested and non-infested *O. niloticus*. Higher values were recorded in the infested group than the non-infested ones. The neutrophil counts however, showed a non-significant difference between infested and non-infested groups. Platelet counts (PL) showed a significant difference ($p<0.05$) between infested and non-infested groups, with higher values in the infested groups (Table 4.8).

Table 4.8: Hematological parameters of infested and non-infested *O. niloticus*

Parameters	Infested	Non infested	p-value
Red blood cells (RBCs) (mm^3)	1.82 ±0.22	2.83 ±0.28	0.021*
Hemoglobin (Hb) (g/dl)	12.54 ±0.97	17.14 ±0.45	0.004**
Hematocrit (HCT) (%)	8.37 ±0.37	9.79 ±0.34	0.02*
Leukocyte count (µl)	10.03 ±0.64	7.78 ±0.32	0.016*
Lymphocyte (%)	89.96 ±2.19	75.18 ±2.94	0.003**
Monocyte (%)	29.61 ±0.96	23.31 ±1.50	0.007**
Eosinophil (%)	2.08 ±0.16	1.22 ±0.08	0.002**
Basophil (%)	6.82 ±0.32	5.45 ±0.18	0.006**
Neutrophil (%)	58.55 ±0.48	57.85 ±1.39	0.65
Platelets (PT) (µl)	84.64 ±1.53	78.06 ±1.55	0.013*

* and ** Indicates significant difference between values of the two groups

5 DISCUSSION

5.1 Water quality parameters in Kokeb and CARE fish farms

Physico-chemical parameters such as temperature, dissolved oxygen, pH, turbidity, ammonia, nitrate, and nitrite are important for health of fish in aquaculture. Temperature plays a significant role as it affects fish metabolic activity (FAO, 2006). It also affects the toxicity and the reaction rate of other chemicals such as ammonia, which are detrimental to fish besides influencing the solubility of gases in water including atmospheric oxygen. Kiran (2010) observed that temperature fluctuation affects the production of phyto and zoo plankton, the natural fish food organisms of cultivable fish species both in natural and in culture systems. High temperature in any aquatic ecosystem led to an increase in metabolic activity of aquatic organisms, reduces the solubility of atmospheric oxygen and contributes to stress in fish, which may lead to fish death. (Reshid *et al*., 2014).

In the present study, the mean water temperature recorded in both fish farms ranged from 20.45 $\pm 2.25^0$C to 30.05 $\pm 0.25^0$C (Table 4.1). The higher temperature level was recorded at Kokeb farm (30.05 $\pm 0.25^0$C) with an overall mean value of 27.20 $\pm 0.92^0$C. There was a significant difference ($p<0.05$) in the level of water temperature between both fish farms. The higher temperature observed on Kokeb fish farm might be due to the trapping of heat by turbid water and suspended sediments. Since the site was located with the presence of relatively high organic inputs, as confirmed by higher NH_3, NO_3 and NO_2, it might generate heat during microbial degradation of organic materials.

In this study, water temperature showed a negative correlation with dissolved oxygen because high temperature reduces the concentration of oxygen in water. The present results of water temperature thus agree with the observations of Otachi *et al*. (2011) who noted that water temperature ranged

between 22.38^{0}C and 31.80^{0}C in Sagana fishponds. Similarly, Ngodhe (2021) also reported that the water temperature range as 24.8^{0}C - 28.8^{0}C in the study of the prevalence of parasitic infection in farmed *O. niloticus*. Worako Adimasu (2015) asserts that metabolic processes of aquatic organisms may speed up, slow down, malfunction, or stop entirely if water temperatures rise, fall, or fluctuate too much.

Dissolved oxygen is one of the environmental parameters that exert a tremendous effect on fish growth and production due to its direct effect on feed consumption and metabolism and its indirect effect on nutrient availability. The dissolved oxygen concentration recorded in this study was in the range of 3.05 ±0.05 - 9.05 ±0.75 mg/l. The higher value of dissolved oxygen content was recorded at CARE fish farm (9.05 ±0.75) with an overall mean value of 6.72 ±0.80 mg/l (Table 4.1). There was a significant difference ($p<0.05$) in dissolved oxygen content between the two fish farms. The higher value of dissolved oxygen content in CARE fish farm may be due to lower turbidity value and comparatively low temperature associated with relatively low biological degradation activity.

The present study observation agreed with the findings of Bagge *et al*. (2004) who reported dissolved oxygen a range of 3.00 - 10.00 mg/l in nine fishponds in Finland. Similarly, Onada *et al*. (2015) further pointed out that, the minimum dissolved oxygen should be 5 mg/l for tropical fish with the optimum levels between 5 - 15 mg/l with a tolerable level of 2 mg/l. In general, the relationship between temperature and dissolved oxygen is inverse; large amounts of dissolved oxygen are released from the water, resulting in low dissolved oxygen levels. Additionally, the breakdown of allochthonous materials (organic matter) would deplete the dissolved oxygen in these areas (Oduor, 2000).

Like temperature and dissolved oxygen, pH also affects the metabolism and the physiological processes of aquatic organisms as well as affecting the toxicity rate of ammonia. The desirable

range for pond pH is 6.5 - 9.5 and acceptable range is 5.5 - 10.0 (Stone and Thomforde, 2004). In the current study, the water pH remained constant in both fish farms with values ranging from 6.60 ±0.50 - 8.74 ±0.16 (Table 4.1). This agrees with the results of Stone and Thomforde (2004). Thus, a good pond productivity and fish health can be maintained if this pH range is maintained. Furthermore, a similar range was obtained by Kamal *et al*. (2007) who reported a range of 7.5 - 8.3. and Boyd (2017) who recorded a range of 6.5 - 8.5. Additionally, Tilahun Adugna and Ayele Wondie (2016) also reported a range of 7.01- 8.01 in Selameko man-made reservoir South Gondor, Ethiopia.

Turbidity restricts light penetration, limits photosynthesis and production of undesirable macrophytes in ponds (Boyd, 2017). It relates to the amount of materials present in the water, and this could be as a result of input of wastes by the fish farmers into the ponds. The mean water turbidity recorded in this study ranged from 9.75 ±0.55 NTU - 51.85 ±16.45 NTU. A similar finding was obtained by Keremah *at al*. (2014) who recorded mean value of turbidity in earthen ponds was from 20.6 ±3.2 - 45.1 ±15.07 NTU. The turbidity values obtained in this study, from both farms were within the desirable range however, the higher value was found in the earthen pond (Kokeb farm) than concrete pond (CARE) because concrete pond is fitted with the protective material to ensure water contaminant hence no direct contact with sediment. In the case of earthen pond, water has a direct contact with sediments, which could be harmful for fish by increasing the chance of parasite infestations.

Ammonia in water is an indicator of the bacterial decomposition of organic matter. It is also the main indicator of water quality, and the relative concentrations are affected by both pH and temperature. Because it is neutral and can pass through gill membranes more easily than NH_4^+ ions, unionized ammonia (NH_3-N) is toxic. According to studies, the effects of free ammonia alone are

cause total ammonia toxicity (Körner *et al*., 2001). Francis-Floyd *et al.* (2009) assert that fish are killed when ammonia levels exceed 2 mg/l and that a good quality water body must have ammonia levels less than 0.05 mg/l and acceptable limit less than 0.5 mg/l. However, the ammonia concentration in this study ranged from 0.07 mg/l - 1.70 mg/l. High ammonia content was recorded at Kokeb fish farm with an overall mean value of 0.86 ±0.23 mg/l. This study also obtained ammonia levels that were close to tolerable level. Similarly, Keremah *et al*. (2014) reported that mean value of ammonia in earthen fishponds ranged from 0.34 ±0.20 - 0.55 ±0.20 mg/l. Akinwole and Adeola, (2012) obtained the mean values of ammonia in concrete tank and earthen pond are 4.61 ±0.46 mg/l and 5.90 ±0.51 mg/l respectively which are very high. Ammonia is introduced into the ponds through dead phytoplankton, uneaten feeds, dead and decaying organic matter. Fish are very sensitive to unionized ammonia and need an optimum range of 0.02 - 0.05 mg/l.

Nitrate concentration in this study ranged from 1.68 mg/l - 3.20 mg/l. There was a significant difference ($P<0.05$) in the nitrate content between both fish farms. High nitrate content was recorded at Kokeb fish farm with an overall mean value of 2.53 ±0.17 mg/l. The higher nitrate content recorded at this site may be related to the presence and decomposition of inorganic and organic material, those were transported from agriculture land and different wastes around the farm since the farm is an earthen farm. However, this value is low and do not pose any danger to the cultured fish. Similarly, Akinwole and Adeola (2012) reported mean nitrate values in concrete tank and earthen pond were 2.30 ±0.08 mg/l and 2.60 ±0.11 mg/l respectively. Nitrate concentration from 0 - 90mg/l is reported as acceptable and tolerable range (Stone and Thromford, 2004).

Nitrite is a by-product of oxidized NH_3 or NH_4^+ and an intermediatory in the conversion of NH_3 or NH_4^+ into NO_3. The higher nitrite content was also recorded at Kokeb fish farm with a mean value of 0.31 ±0.11mg/l. There was a highly significant difference ($P<0.05$) in the nitrite content between

both fish farms. The mean nitrite value of the farm water was found lower than the permissible limit set by WHO (2017) as 3 mg/l. However, it was found higher than the recommended limit of nitrite for productive fisheries by Bhatnagar and Devi (2013) who reported <0.02 mg/l. Furthermore, the recorded mean value of nitrite was in line with the values of 0.09 ±0.005 mg/l and 0.109 ±0.032 mg/l reported by Goshu and Akoma (2010) who conducted a comparative study of physico-chemical characteristics of some on-farm research ponds.

5.2 Parasite infestation in *O. niloticus* in the fish farms

In the current study, 11 parasite species were recorded. Parasitism, as defined by Marcogliese (2001) reflects a life condition whereby one or more individual organisms derive nutritional benefits at the host's expense, usually without killing the host. All parasites utilize resources that are required for the host's growth, sustenance, development, establishment, and reproduction and as such may harm their host in a number of ways and affect the hosts' production (Barber, 2004). There are many biotic and abiotic factors that affect parasite infestation in fish hosts. Biotic factors include host age, size, and sex while specific abiotic factors are temperature, dissolved oxygen, pH and turbidity among others (Pouder *et al*., 2005). These factors could have also played a role in infestation of the parasite species during the study period.

In this study fish were infested with both ecto and endo parasites viz: *Ichthyobodo* sp, *Trichodina* sp, *Cichlidogyrus* sp, *Gyrodactylus* sp, *Tylodelphys* sp, *Clinostomum* sp, *Dactylogyrus* sp, *Acanthocephalus* sp, *Diphylobothrium* sp, *Contracaecum* sp *and Dolops* sp. The prevalence of these parasites has been observed elsewhere in this study farms and other country in both farmed and capture fish (Mathenge, 2010; Mavuti *et al*., 2017). The overall parasitic prevalence in Kokeb fish farm was 59.16% while CARE fish farm had a prevalence of 31.66% which is lower than 73.1% reported by Waruiru *et al*. (2020) in farmed *O. niloticus* from Kirinyaga County, Keniya.

The prevalence of parasite species from Sebeta fish ponds and private fish farms in Wonchi area Ethiopia, were 71.0% and 82.2%, respectively (Marshet Adugna, 2017). Additionally, Marshet Adugna (2020) also reported that prevalence of parasite species from small-scale fish farms in Amhara region, was 73.2% which is higher prevalence than the present findings.

The parasite prevalence was high in February in Kokeb fish farm and in CARE it was high in January, indicating that parasite prevalence was influenced by the temperature of aquatic environment. Most parasites development, hatching and growth are all temperature dependent. The prevalence of parasitic infestation was also high in Kokeb fish farm; this may be due to relatively compromised water quality parameters and may have been attributed to surrounding vegetation observed around farms which offered good condition for the propagation of the intermediate hosts, like snails. This agrees with the study by Mathenge (2010) who reported that the presence of weeds and other vegetations in the ponds at Sagana fish breeding farm whose presence contributed to the high prevalence of helminthes parasites found in fish.

5.3 Prevalences and mean intensity of parasites in the fish farms

The protozoan parasites recovered during this study from *O. niloticus* in fish farms are *Ichthyobodo* sp *and Trichodina* sp with prevalence and intensity of 4.16% and 3.00 and 5.8% and 2.28 respectively in Kokeb fish farm. Similarly, in CARE fish farm *Trichodina* sp was recovered with prevalence and intensity value of 10.0%, 2.83, which is lower than that of 62.0% prevalence reported from Arahuka fish farm at Keniya (Ojwala *et al*., 2018). Marshet Adugna (2017), reported *Trichodina* sp in Sebata fish ponds and private fish farms with prevalence of 34.6% and 22.2% respectively. Additionally, Taddese (2009) also reported in cultured system in Yemlo and Wonji ponds, *Trichodina* sp with prevalence of 56.67% and 46.7% respectively, which are much higher than the present findings.

Three monogenean parasites such as *Cichlidogyrus* sp, *Gyrodactylus* sp *and Dactylogyrus* sp, were observed. Except *Gyrodactylus* sp, two of them are observed in both fish farms. *Cichlidogyrus* sp was observed in both farms with prevalence and intensity of 3.34%, and 3.75 in Kokeb farm and 3.3% and 3.0 in CARE farm respectively. This is almost similar to the value of 4% and 1.5 recorded in Subukia fish farm at Keniya (Ojwala *et al*., 2018), and much lower than from the value of 76.0%, and 16.34 reported by Ojwala *et al*. (2018) in Egerton fish farm at Keniya. Marshet Adugna (2017) reported that prevalence and mean intensity of *Cichlidogyrus* sp from Sebeta fish ponds were 33.59% and 13.02 respectively, which are also much higher than the present findings.

Gyrodactylus sp was observed only from Kokeb fish farm with prevalence and intensity of 2.5% and 2.66 respectively. Waruiru *et al*. (2020) reported, the prevalence and mean intensity of *Gyrodactylus* sp on *O. niloticus* from Kirinyaga County, Keniya was 0.1% and 2 which is lower than the present findings. The prevalence and intensity of *Dactylogyrus* sp were 5% and 2.16 in Kokeb farm and 6.66% and 3.00 in CARE farm. Similar findings (6.1% and 1.7) were reported by Waruiru *et al*. (2020), in farmed *O. niloticus* from Kirinyaga County, Keniya. Otachi, (2009) stated that, prevalence and mean intensity of *Dactylogyrus* sp (17.85% and 3.4) in Sagana fish ponds Keniya, which are higher than the present results.

Two digenean parasites such as *Tylodelphys* sp and *Clinostomum* sp were observed. *Tylodelphys* sp was observed only from Kokeb fish farm with the prevalence and intensity of 4.16% and 1.4 respectively. Ojwala *et al*. (2018) reported that low prevalence and high mean intensity than present findings (2% and 37) in Egerton fish farm. Additionally, Marshet Adugna (2017) reported, high prevalence and low mean intensity than present findings of *Tylodelphys* sp (12.5% and 0.81) in Sebeta fish ponds. *Clinostomum* sp *was* observed in both fish farms with prevalence and mean intensity of 28.33% and 6.23 in Kokeb farm and 12.50% and 4.13 in CARE farm respectively,

which are higher than that of reported by Waruiru *et al*. (2020) who recorded 3.1%, and 4.6 in farmed *O. niloticus* in Kirinyaga County, Kenya. The prevalence of *Clinostomum* sp from selected fish farms in Amhara region, on the other hand were 32.4% (Marshet Adugna, 2020).

Acanthocephalus sp *was* present in both fish farms with the prevalence and mean intensity of 23.33% and 3.89 in Kokeb farm and 8.3% and 2.3 in CARE farm, which are higher than that of 1.3% and 2 reported by Marshet Adugna (2017) in Wonchi area private fish farms. Similar prevalence (8.8%) finding with CARE farm was also reported by Marshet Adugna (2020) in Amhara region fish farms. *Contracaecum* sp was recorded only in Kokeb fish farm with the prevalence and mean intensity of 20.83% and 3.52. These results are relatively similar with that of Marshet Adugna (2017), who stated prevalence and mean intensity of 17.19% and 5.91 in Sebeta fish farms. Ngodhe (2021) also found *Contracaecum* sp with prevalence of 70% in Winam gulf of L. Victoria area ponds. *Dolops* sp were observed only in the Kokeb fish farm with the prevalence and mean intensity of 10.00% and 2.08. Which are higher than the prevalence and mean intensity (7.81% and 0.2) reported by Marshet Adugna (2017) in Sebeta fish ponds. The present findings are also higher than that of 2.0 % and 1.0, reported by (Ojwala *et al*., 2018) from Arahuka fish farm, Keniya.

5.4 Relationship between water quality and parasite infestation

Water quality parameters such as temperature, dissolved oxygen, pH, turbidity, ammonia, nitrate, and nitrite among others have a strong influence on fish health and their resistance against the disease-causing agents (Hossain *et al*., 2007). The present study found that there was a strong positive relationship between temperature and the prevalence of parasite species on *O. niloticus.* These findings are 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 current findings also

support the results of Waruiru *et al*. (2020) who found a positive relationship between temperature and the prevalence of *Contracaecum* sp in Lake Bogoria Spring. Temperature is an important factor in the egg development, hatching, molting, and other stages of different parasite species. For instance, for *Dolops* sp the higher the temperature, the more the *Dolops* sp infestation. The rising temperature allows *Dolops* eggs to hatch at a high rate, despite the longer hatching time (Sahoo *et al.,* 2013).

The current study reveals that there was a negative relationship between dissolved oxygen concentration and prevalence of parasite species on *O. niloticus*. This recommends that the effect of dissolved oxygen concentration differs among different monogenean parasite species, it means that low dissolved oxygen concentrations favor the survival of parasites and lead to an increase in the prevalence of different monogenean parasites (Paredes-Trujillo *et al.,* 2016). However, the relationship between dissolved oxygen and prevalence of parasite species is inverse as dissolved oxygen level falls the prevalence of parasite species rises and vice versa. The present findings agree with those of Waruiru *et al*. (2020), who reported negative relationship between dissolved oxygen concentration and Parasite species. Similarly, Koyun (2011) documented a negative relationship between dissolved oxygen concentrations and parasite species.

The current study revealed that there was a positive relationship between pH and the prevalence of parasite species. These findings are consistent with those of Sahoo *et al.* (2013); Ojwala *et al*. (2018), who found a positive correlation between water pH and prevalence of parasite species. However, the current findings contradict with the result of Alsarakibi *et al.* (2014), who found a negative correlation between pH and parasite prevalence. The pH variation is highly dependent on the temperature of the water, implying that water temperature and pH have a direct relationship. The overall test results show that there is a relationship between the actual pH and the prevalence

of parasite species on *O. niloticus*, and that pH does affect the prevalence of parasite species on *O. niloticus*. Turbidity showed positive relationship with the prevalence of parasite species. These findings are consistent with those of Ojwala *et al*. (2018) who reported a positive relationship between turbidity and parasite species.

The nutrient parameters (ammonia, nitrate and nitrite) showed a positive relationship with prevalence of parasite species. The current findings agreed with the result of Waruiru *et al*. (2020) who found that there is a positive relationship between nutrient content and prevalence of parasite species. The current results concur with those of Yunikasari and Mahasri (2020), who claimed that there is a positive correlation between ammonia levels and the prevalence of parasites. According to Acosta-Perez (2022), controlling the amount of organic matter entering the water body from various sources is crucial because levels of nitrogenous compounds have been shown to positively correlate with parasite infection. The relationship between these compounds in the nitrification process of the aquatic environment is indicated by the positive correlation between the concentrations of ammonia, nitrites, and nitrates (Setiawan and Rahardja, 2021).

According to Yusni and Rambe (2019); Adamba *et al.* (2020), these nitrogenous compounds degrade water quality and make fish more susceptible to parasitic infestations. In general, the prevalence of parasite species in *O. niloticus* is positively correlated with almost all water quality parameters mentioned, except for dissolved oxygen. Temperature, dissolved oxygen, ammonia and nitrate in both fish farms have a significant correlation with the prevalence of parasite species. These results are consistent with those of Ojwala *et al*. (2018), who noted that these parameters were a significant correlation with prevalence of parasite species.

5.5 Prevalence and mean intensity of infestation in relation to length of fish

In the present study, the relationship between the length of O. *niloticus* and prevalence of parasite species (r =0.978) and the length of *O. niloticus* and the mean intensity of parasite were strongly positive (r =0.999). This showed that the prevalence and severity of the parasite species rise with fish length and vice versa. Large fish were more commonly exposed to the parasite infestation. The current research supports the findings of Alom *et al.* (2019), who found that larger fish had higher prevalence and intensity of infestation than smaller fish. This finding is also supported by Ojwala *et al.* (2018) who found the prevalence of parasite species was positively correlated with larger sized fish in Njoro fish farms.

The current results are also in line with the study of Akoll *et al*. (2012), who recorded a positive correlation between fish length and the number of parasites infecting *O. niloticus*. However, Biu *et al*. (2014) recorded a contrasting result and noted that small sized fish had more parasites compared to large ones. The reason attributed for increased infestation in smaller fish was due to less developed immune system in small fish than larger ones, which have fully developed immunity against parasitic infestations.

5.6 Prevalence and mean intensity of infestation in relation to weight of fish

In the present study, the prevalence of parasite species increased with fish body weight. These results are in agree with that of Webb (2008), who found that larger hosts provide a greater surface area for parasite to infest. The highest prevalence of parasite species was observed on fish species dominated by larger individuals. Infested fish were generally heavier than uninfected fish, indicating that large body weighted fish harbor numerous parasites and small body weighted fish harbor a few parasites (Walker *et al.*, 2008). There was a significant positive correlation between

O. niloticus body weight and the prevalence of parasite species. The result supports those of Oniye *et al.* (2004), who found a positive correlation between host weight and parasite prevalence.

According to Webb (2008), larger fish have more surface area for infection than smaller fish. The present study supports the findings of Walker *et al.* (2008), who found that there was significant difference in parasite prevalence and intensity between different host weight groups and larger (heavier) fish appeared to be more susceptible to parasite infestation. According to Omeji *et al*. (2022), variation in parasite prevalence among various sizes of *O. niloticus* existed maximum in bigger fish samples than smaller ones. This could be attributed to their foraging habit, food abundance and availability to the parasite host organisms for consumption.

5.7 Prevalence and mean intensity of infestation in relation to sex of fish

Host sex is one of the biotic factors that is crucial for understanding host-parasite relationships. The physiological, biological, and behavioral differences between male and female fishes, which result in a slight but persistent sexual trend in infection levels, may be the cause of host sex effects on parasitism levels. The present study found that male fish had higher levels of infestation than female fish. The main reason for the differences in parasitic load with sex is believed to be physiological behavior (Aloo *et al*., 2004), and similar findings have been reported in many freshwater fish species (Aloo, 2001; 2002). The present results agree with those of Hayatbakhsh *et al.* (2014), who noted a marginally significant difference in infection between males and females. The increased infestation levels reported in males may be due to higher levels of testosterone, which suppresses the immune system and makes them more vulnerable to parasites.

5.8 Condition factor of parasitized and non-parasitized *O. niloticus*

The analysis of the Fulton's condition factors, an index of the wellbeing of the fish, showed that the parasitized and non-parasitized O. *niloticus* were in good condition and all the values of the

average were above the critical values of 1.0. However, the Fulton's condition factor in parasitized and non-parasitized fish showed no significant difference (t-test, p> 0.05). Omitoyin *et al*. (2013) also reported that higher the condition factor better the condition of *O. niloticus*.

The condition factors (k) usually reflect the variation in fish health, information on the physiological state of the fish in relation to its welfare. Therefore, k values of *O. niloticus* in the fish farms indicated a good condition (k>1) (Barnham and Baxter, 1998). Generally, this study recorded a low intensity of infestation because water quality in both fish farms was within the acceptable limits. Aloo (2002) found that helminth parasites have no effect on condition factor despite of heavy infestation in *Tilapia zillii* and *Oreochromis leucostictus*. It is assumed that they create a dynamic equilibrium co-existence with the fish host especially in well balanced water quality parameters.

5.9 Effects of parasites on hematology of *O. niloticus*

Hematological parameters are indices of the health status of fish. Hematological changes occur in fish when parasitic load increases in host species. Fish parasites can also cause blood disorders such as anemia and leukocytosis (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 the infested fish (Ali *et al*., 2010). Blood samples from fish with and without parasitic infestation revealed notable difference between the two groups.

The current study revealed significant differences (p<0.05) with lower values of RBCs count, hemoglobin, and hematocrit in infested group than non-infested ones. Similarly, Corea *et al*. (2013) recorded low hematological parameters of *Hoplias malabaricus* affected by larvae of *Contracaecum* sp. The current results support the findings of Nashaat and Maghawri (2022), who reported that there was a significant difference with lower value of RBC counts and hemoglobin

cell count in infested group of fish than non-infested fish by *Capillaria* sp. This finding is also corroborated with that of Azevedo *et al.* (2006), who reported that RBC count was slightly decreasing in parasitized *O. niloticus,* and this lowering of blood cells may be due to the feeding nature of parasite species on blood cells.

Hemoglobin is one of the most important proteins found in the RBC, that carries and transport oxygen from lungs or gills to the rest parts of the body. So, the infested fish have low oxygen carrying capacity in case of the parasite affecting RBCs. On the other hand, the present findings conflict with that of Jons and Grutter (2005), who reported that RBC counts can also rise in parasitized fish than non-parasitized as a result of release of blood cells from the spleen that have been stored there, the loss of plasma or the swelling of RBCs as a stress response in a parasitized fish. Furthermore, the current results contradict with that of Ruane *et al.* (2000), who reported that parasitized trout had an increased number of RBCs but no change in their hematocrit. Decreasing RBC number and hematocrit in this study supported the results reported of Tavares-Dias *et al*. (2002) for *O. niloticus* with protozoan parasites; Azevedo *et al.* (2006), for *O. niloticus* ecto and endo parasites, and Nashaat and Maghawri (2022), for *O. niloticus* with *Capillaria* sp. Montero *et al*. (2004), also reported decreased hematocrit in fish parasitized with monogenean parasites.

This study recorded that Leukocyte cells were significantly different ($p<0.05$) between infested and non-infested *O. niloticus* with higher value in infested fish. This result is in line with that of Panjvini *et al.* (2016), who recorded an increase in the number of leukocyte cells in the infested fish. Increasing leukocyte cells may be an indication of the body immune system defenses against parasites. Lymphocyte cells were significantly higher in infested than non-infested *O. niloticus* ($p<0.05$) and this indicates that during infestation lymphocyte cells are transported to infestation site and this enhances their number to overcome the situation. These results confirmed with that of

Haond *et al. (*2003), who suggested that the number of lymphocyte cells are higher in infested Rainbow trout (*Oncorhynchus mykiss*) and this may be due to the migration of lymphocytes out of the blood and epidermis.

In the current study, the percentage of monocyte cells were also significantly higher in infested than non-infested *O. niloticus* ($p<0.05$) and this observation agrees with the findings of Furtado *et al*. (2019), who recorded an increase of monocyte cells in parasitized *O. niloticus.* This finding is also in line with those of Tavares-Dias and Moraes (2007), who reported that infested fish have a higher number of monocyte cells. The percentages of eosinophil and basophil showed significantly higher in infested than non-infested *O. niloticus* ($p<0.05$) and these results are in consistent with those of Ruane *et al.* (2000), who reported that, the parasitized *O. niloticus* had higher percentage of eosinophil and basophil cells.

In the current study, neutrophils showed non-significant difference ($p>0.05$) between infested and non- infested *O. niloticus*, which is confirmed with that of Amriana *et al*. (2021), who reported that there was no significance difference in the percentage of neutrophils between infested and non-infested *O. niloticus*. It is also in line with that of Nashaat and Maghawri (2022), who found insignificant difference in neutrophil counts between infested and non-infested red tilapia with nematode parasite (*Capillaria* sp). There was also a significant difference ($p<0.05$) in platelets in this study with higher value in infested than non-infested *O. niloticus.* Platelet cells could participate in inflammatory responses and their phagocytic activity has been considered as defense function, as in different species of animals (Nagasawa *et al*., 2014).

6 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From the findings of the present study, it is concluded that:

- There was significant variation in water quality parameters between the two fish farms under study. Most of the water quality parameters fell within the acceptable range for the growth and wellbeing of *O. niloticus*. However, the water quality in CARE fish farm was better than that of Kokeb fish farm. This is evidenced by the higher parasite species recovered from the fish in Kokeb fish farm than CARE fish farm.
- There was a positive relationship between water quality parameters (temperature, pH, turbidity, ammonia, nitrate, and nitrite) and prevalence of parasite species recovered from *O. niloticus* in the fish farms except dissolved oxygen, which has a negative relationship in this study. These parameters have a strong influence on fish health and cause stress to the fish, if their values are rise or fall from optimal range. Stressors decrease the capacity of fish immune system to prevent parasitic infestation, and this makes a favorable condition for the spread of parasite species.
- There was a significant difference on parasitic prevalence rate, mean intensity and mean abundance between the culture systems where there was higher prevalence, mean intensity and mean abundance in Kokeb fish farm. There was a positive relationship between the size of *O. niloticus* and prevalence of parasite species. Larger sized *O. niloticus* have higher prevalence of parasite species than smaller weighted fish. Prevalence of parasite species was more in male *O. niloticus* than female *O. niloticus* and this might be due to the physiological difference between the sexes.

- The mean condition factor of parasitized *O. niloticus* was lower (1.5 ±0.11) than that of the non-parasitized (1.83 ±0.12), although not significantly different. Thus, the health status of parasitized and non- parasitized *O. niloticus* was almost similar.
- Parasite species have significant hematological effects on the blood parameters of *O. niloticus.* RBC counts, percentage of hemoglobin and hematocrit are significantly lower in infested *O. niloticus.* Except neutrophil cells, Leukocyte cells, percentage of lymphocytes, monocytes, eosinophils, basophils, and platelets were significantly high in infested *O. niloticus* than non-infested ones.

6.2 Recommendations

The findings of this study, forward the following recommendations:

- Proper pond management practices such as water quality maintenance, fertilization, stocking and feeding strategies of fish have to be followed strictly in accordance with standards in fish culture ponds. This will help maintaining good water quality which in turn would reduce parasite development in water body and infestation in fish farms.
- Zoonotic fish parasite species should be properly identified, and care must be given to prevent their infestation in fish, and human beings properly cooking the fish before consumption.
- Detailed study is recommended on fish diseases in culture systems focusing the relationship between water quality and infestation by different groups of parasites and also different control strategies to protect the health status of human beings and ensuring food security in the region.

- Frequent training and exchange visits should be organized by Fisheries Departments to fish farmers. If these practices are well in place, it will help protect fish farms from any adverse impacts, especially infestation by fish parasites.
- Extension officers have to undertake regular monitoring of fish health by observing the behavior of cultured fish and noting the incidence of infestation by different parasites. They should also educate the farmers how to observe the disease symptoms in fish and to control infestations in culture systems.

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APPENDICES

Appendix Tables

Appendix 1: Nutrient parameters of Kokeb and CARE fish farms from December 2023 to May 2024.

Months/ Yrs	Fish farms					
	Kokeb			CARE		
	NH_3 (mg/l)	NO_3 (mg/l)	NO_2 (mg/l)	NH_3 (mg/l)	NO_3 (mg/l)	NO_2(mg/l)
Dec/ 2023	0.97	3.20	0.31	0.15	2.27	0.06
Jan/ 2024	1.28	2.20	0.30	0.29	2.33	0.08
Fab/ 2024	1.70	2.70	0.36	0.26	2.24	0.02
Mar/ 2024	0.77	2.80	0.30	0.13	2.02	0.04
Apr/ 2024	0.22	2.10	0.28	0.09	1.81	0.05
May/ 2024	0.24	2.20	0.32	0.07	1.68	0.03

Appendix 2: Prevalence and intensity of parasite species in *O. niloticus* in relation to body weight (n= 240).

Weight group (g)	No of fish		P (%)	No of parasites	MI (No)
	Examined	Infested			
≤ 50	51	9	17.64	33	3.66
51-100	87	28	32.18	168	6.00
101-150	41	26	63.41	164	6.30
≥ 150	61	46	75.40	322	7.00

($P < 0.05$) for wieght and prevalence were 0.008, for wieght and intensity were 0.04.

Pearson correlation for weight and prevalence were r= 0.985, for weight and intensity r= 0.920.

Appendix Figures

Appendix 3: Photograph indicating samples of *O. niloticus* for parasite species examination.

Appendix 4: Photograph indicating field sampling

Appendix 5: Photograph indicating laboratory analysis

Appendix 6: Photograph showing blood sample collection for hematology study

Appendix 7: Photograph showing fishing in Kokeb fish farm

Appendix 8: Photograph showing fishing in CARE fish farm