EFFECT OF WATER QUALITY ON THE PARASITE ASSEMBLAGES INFECTING NILE TILAPIA IN SELECTED FISH FARMS IN NAKURU COUNTY, KENYA

Master of Science Thesis

by

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DECLARATION AND RECOMMENDATION

DECLARATION

I declare that this thesis is my original work and has not been submitted or presented for examination in any institution

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RECOMMENDATION

This thesis has been submitted for examination with our approval as university supervisors according to Egerton University regulations

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DEDICATION

This work is dedicated to the Almighty God, my guardian Maurice, sisters and brothers for their inspiration and moral support, as well as my late parents (Peter and Esther).

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ABSTRACT

Aquaculture has been documented as the most developing food industry in Kenya with increased production since the Government initiated Economic Stimulus Programme (ESP) in 2009. However, the production has not been to a maximum level anticipated in the country, particularly in Nakuru County. This is due to the uncontrolled addition of inputs (Inorganic fertilizers, manure and fish feeds) that deteriorate pond water quality. Poor water quality is one of the most common challenges faced by fish farmers since it impedes fish production and increases the incidences of parasite infections in cultured fish, leading to extensive losses. Therefore, this study investigated the effect of water quality on parasite assemblages infecting *Oreochromis niloticus* Linnaeus 1758 in selected fish farms within Nakuru County, Kenya from November 2016 to February 2017. A semi-structured questionnaire was administered in each fish farm to gather information on the farm management strategies. Selected physico-chemical parameters namely: dissolved Oxygen, temperature, pH, conductivity and turbidity were measured *in situ* using appropriate meters. Water samples from each fish farm were analyzed for nutrients (ammonium, nitrite, nitrate, total nitrogen, soluble reactive phosphorus and total phosphorus) using American Public Health Association (APHA) standard methods. Fish samples were killed by decapitation and examined in the laboratory for ecto- and endoparasites using a compound microscope and the parasitological parameters (prevalence, mean intensity, mean abundance and diversity) calculated. The results indicated that the water quality parameters were significantly different for all the six fish farms (P<0.05). The species of parasites also varied from one fish farm to another. The total number of parasite species recorded during this study were 15, with the highest number of species recorded in Subukia (10 species) and the lowest in Dundori (3 species). The parasite assemblages varied with the water quality parameters between fish farms. Out of 300 fish sampled, 252 were infected by parasites representing an overall prevalence of 84 % with the highest mean intensity of ectoparasites, particularly monogenean parasites compared to the endoparasites encountered during the study. *Trichodina sp* and *Cichlidogyrus halli* were found in all the studied fish farms. There was a positive correlation between water quality parameters and parasite infection levels. This study also recorded a positive correlation between fish size classes and the parasite infection levels (r² =0.3). The Fulton's condition factors of parasitized and non-parasitized *Oreochromis niloticus* showed no significant differences statistically (P>0.05). The fish farms with poor water quality recorded many species of parasites infesting *O. niloticus* compared to others which recorded good water quality parameters. Therefore, regular monitoring and control of water quality in fish ponds is recommended to reduce levels of parasite infestations.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA   Analysis of Variance

APHA   American Public Health Association

ADC   Austrian Development Cooperation

BOD   Biological Oxygen Demand

EC   Electrical Conductivity

DO   Dissolved Oxygen

ESP   Economic Stimulus Programme

FAO   Food and Agriculture Organization

IHE   International Institute for Hydraulic and Environmental Engineering

IPGL   International Training Programmes in Limnology

GOK   Government of Kenya

KES   Kenya Shillings

LWM   Limnology and Wetland Management

SRP   Soluble Reactive Phosphorus

UNESCO   United Nations Educational, Scientific and Cultural Organization

USAID   United States Agency for International Development

TN   Total Nitrogen

TP   Total Phosphorus

NARDTC   National Aquaculture Research Development and Training Centre

NCIDP   Nakuru County Integrated Development Plan

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DEFINITIONS OF TERMS

Abundance   Refers to the number of parasites in/on a single host regardless of whether or not the host is infected (number of parasites divided by number of host-positive and negative-examined).

Decapitation   The complete separation of the head from the body of fish (a humane method of killing fish).

Definitive host   A host in which the parasite reaches maturity and reproduces sexually if applicable (also referred to as final host).

Infection   The process of parasite invading the body of animal or making an animal ill or diseased.

Infestation   The presence of an unusually large number of parasites in an animal typically so as to cause damage or disease.

Intensity   The number of parasites in a single infected host (number of parasites divided by number of positive hosts).

Intermediate host   A host that harbours the parasite only for a short transition period, during which some developmental stage is completed (also called secondary host).

Mean Intensity   The average intensity of parasites among the infected hosts (total number of parasites found in a sample divided by the number of infected hosts).

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).

Morbidity   Prevalence of disease, extent or degree of prevalence of disease, sickly, immobile nearly dying.

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 a percentage when used descriptively).

Zoonosis   Any parasitic disease able to be transmitted from animals, both wild and domestic to human or vice versa.

CHAPTER ONE
INTRODUCTION

1.1   Background information

Aquaculture is the farming of aquatic organisms including fish and other aquatic organisms under controlled or semi-controlled environmental conditions (Erondu and Anyanwu, 2011). It entails the use of external inputs such as clean water and nutrients such as organic fertilizers (manure), inorganic fertilizers and fish feeds for its productivity (USAID, 2013). Its main purpose is to meet the food demands for the growing population with diminishing capture fisheries in most natural water bodies worldwide. Moreover, aquaculture is known to be a set of flexible technologies, species and systems (Allison, 2011). They range from simple ponds receiving little or no inputs and infrequent stocking to massive, high technology systems that can produce up to 100 kilograms (kg) of fish per cubic meter (Dugan *et al*., 2010).

Aquaculture contributes to the global, national and local economies due to its increased fish production used as a commodity for trade internationally (FAO, 2016). For example, the global fish consumption has been estimated to have increased from an average of 9.9 kg in 1960s to 19.2 kg per capita in 2012 (FAO, 2014), due to population growth, increased income and rapid urbanization. Nile tilapia (*Oreochromis niloticus*) is largely produced in many countries and has recorded faster growth globally since 1990. It was introduced and cultured in developing countries on subsistence level to meet local protein demands. Most fish farmers prefer this species because it thrives well under adverse environmental conditions and under intensive cultivation (Bhujel, 2013).

Nile tilapia can be grown under extensive, semi-intensive, intensive and integrated aquaculture systems. These systems differ in the level of management (effort), inputs (inorganic fertilizers, organic fertilizers (manure) and feeds), aeration, stocking densities and production. For instance, in extensive systems there is minimal management and inputs, low fish stocking densities and production (Conte, 2004; Ngugi *et al*., 2007). On the other hand, intensive systems require more inputs, high stocking densities, advanced management, high production even though it is costly (Munguti *et al*., 2014). Semi-intensive systems require all inputs and management practises in moderate quantities (Isyagi *et al*., 2009). Moreover, integrated aquaculture system is connected to other agricultural practices. For example, use of inputs from livestock wastes in fish pond for manuring whereas the water from fish pond is used to irrigate the vegetables in the farm because of its richness in nutrients (Bocek *et al*., 2004). Integration process can be either on-farm system where livestock wastes are derived from the farm and are

channelized into the fish ponds or off-farm systems where the livestock wastes are derived out of the farm (Munguti *et al*., 2014).

Kenya has shown rapid growth in aquaculture nationwide since 2009 (Nyandat and Owiti, 2013). This is because of the government's intervention in promoting aquaculture through the implementation of the Economic Stimulus Programme (ESP). In the programme implemented between 2009 and 2014, the government invested KES. 6 billion in 146 constituencies with aquaculture potential in terms of the availability of resources (water and good soil) to establish and increase the number of fish ponds countrywide (Orina *et al*., 2014). The impact of this ESP was huge and continues to be felt. For instance, there are about 792 operational fish ponds in Nakuru County (Nakuru County Integrated Development Plan (NCIDP), 2013). In fact, the national aquaculture production has grown from 1,000 to 12,000 metric tons between 2000 and 2010 representing an annual increase from 1 % to 7 % of total national fish production respectively (Munguti *et al.*, 2014).

Although there is rapid growth of aquaculture in Kenya, most fish farmers are still facing some challenges that hamper fish production. One of these challenges is an increased incidence of parasite infections caused by poor water quality management leading to economic losses. As a result, contribution of the aquaculture sector to the national economies is still under dismal performance (Allison, 2011). Therefore, to help aquaculture boost fishery sector in a sustainable manner by reducing overexploitation of wild fish populations, fish must be cultured under favourable and managed environmental conditions (Nyandat and Owiti, 2013; FAO, 2014).

The losses in fish farms due to poor management of ponds is mainly prompted by water pollution (Cochrane and Garcia, 2009). The pollution is triggered by fish farmers who have inadequate knowledge and skills on proper pond management. For example, the excessive addition of pond inputs (fertilizers, manure and fish feeds) influence both chemical and physical balances in water (Hossain *et al*., 2011). Surplus manure increases the organic pollution in aquaculture ponds resulting to increased biological oxygen demand (BOD) (Erondu and Anyanwu, 2011). The imbalances create a stressful environment for fish leading to deterioration in its health, making them vulnerable to diseases and parasite infections. Water quality problems cause up to 80 % occurrences of fish diseases and parasites (Keremah, 2013). Consequently, rejection of the fish products by consumers, resulting in losses and low

production (Baidoo and Agbeko, 2015). Therefore, optimum water quality parameters are essential for successful production of fish in aquaculture.

Like other counties, food insecurity is one of the major challenges in Nakuru County (NCIDP, 2013). Hence, aquaculture was introduced as an alternative to bridge the gap between increased demand for food and the rapidly growing human population. However, proper fish farming practices like water quality maintenance and parasite control in fish farms are still a challenge to many fish farmers, leading to increased incidences of parasites in fish farms coupled with low production. Therefore, this study investigated the effect of water quality on the diversity of fish parasites in cultured Nile tilapia (*O. niloticus*) in fish farms.

1.2   Statement of the problem

The Economic Stimulus Programme initiated by the Government of Kenya in 2009 has led to increase in the number of fish ponds in most counties. The aim of ESP was to increase fish protein supply and reduce poverty by creating employment opportunities. However, poor pond management practises resulting to pond water quality problems is still a challenge to most small scale fish farmers countrywide. Poor water quality in fish ponds increases pathogens and parasites, coupled with excessive growth of algae. This is followed by depletion in dissolved oxygen as a consequence of high organic input from algae die-offs. In such circumstances, fish become debilitated and vulnerable to parasite infections leading to reduced fish production in general. This study focused on acquiring information on effect of water quality parameters (physico-chemical such as water temperature, pH, DO, turbidity, conductivity and nutrient concentrations) on parasite assemblages in *O. niloticus* in fish ponds. The data collected will be useful in guiding fish farmers on the status and proper pond management practices to improve small scale fish production in Nakuru County.

1.3   Objectives

1.3.1   General objective

To investigate the effect of water quality on parasite assemblages in Nile tilapia (*Oreochromis niloticus*, L.) fish farms in Nakuru County, Kenya

1.3.2   Specific objectives

1. To determine the relationships between selected water quality parameters (physico-chemical and nutrient concentration) and fish parasites infection levels (intensity, prevalence and diversity) in fish farms in Nakuru County

2. To determine the influence of fish size classes on parasite infestation levels of *O. niloticus* in fish farms in Nakuru County

3. To compare the condition factors of parasitized and non-parasitized *O. niloticus* in fish farms in Nakuru County

1.4 Hypotheses

1. There are no significant relationships between selected water quality parameters (physico-chemical and nutrient concentration) and fish parasite infection levels (intensity, prevalence and diversity) in fish farms in Nakuru County

2. Fish size classes does not significantly influence parasite infestation levels in *O. niloticus* in fish farms in Nakuru County

3. There is no significant difference in condition factors of parasitized and non-parasitized *O. niloticus* in fish farms in Nakuru County

1.5 Justification

The aquaculture sector in Kenya aims to increase fish protein supply, create employment and enhance sustainable management of capture fisheries by restocking and reducing over-reliance on natural water bodies. Increase in aquaculture production depends on effective pond management practises, such as maintenance of good water quality, proper fertilization and parasite control. Improper water quality management stands out to be the main challenge affecting fish growth. For instance, poorly managed water quality in ponds often exposes fish to opportunistic ectoparasites when under stress. Poor water quality may be as a result of unintentional excessive application of pond inputs such as inorganic fertilizers, organic fertilizers (manure) and fish feeds, among other factors. Good water quality is therefore crucial in enhancing aquaculture production and reducing parasite infections. Aquaculture in Nakuru County is relatively a new initiative created by the ESP in 2009. The programme led to an increase in number of fish farms to about 1,500 in Nakuru County compared to 1,531 fish ponds which were recorded in Rift valley province (14 counties) as a whole in 2009. However, the number of the operational fish farms have recently declined to about 792 recorded as active according to the Fisheries Department. Most of the fish farmers established their ponds not more than five years ago, and have not been exposed to extension services regarding pond management practices. Therefore, they have minimal knowledge on maintenance of optimal water quality in the ponds. Moreover, there is no research that has been done on aquaculture practices, water quality and parasite infection levels in fish farms in the county. Therefore, it is necessary to study pond management practises in Nakuru County and their effects on water

quality and consequently how they affect fish condition and health that is, diseases and parasitic infections. Information that has been generated in this study will guide fish farmers to identify sources of pond water pollution that could result in production losses in their farms. In addition, it will form baseline knowledge for consideration in identifying management strategies for water quality maintenance to control parasite infections geared to improving aquaculture production in the county. These will ensure increased fish protein supply, employment opportunities and profits to small scale fish farmers in Nakuru County and in Kenya at large.

CHAPTER TWO
LITERATURE REVIEW

2.1 Global trends in aquaculture growth

Aquaculture production has shown a general increasing trend worldwide faster than other food production sectors (FAO, 2016). As world capture fisheries production is stagnating, aquaculture remains a vibrant and rapidly developing food producing sector (FAO, 2010). Global aquaculture production has grown radically over the past 50 years accounting for about 50 % of the world's fish food supply (Bostock *et al.*, 2010). The annual rate of aquaculture production between 1970 and 2003 was 6.9 % with a marginal decline from 2004 to 2008 at an average of 5.8 % per annum (Bostock *et al.*, 2010). In 2009, the world aquaculture production was 38.2 % of the total production from capture fisheries and aquaculture, this increased to 42.1 % in 2012 and the latest available data in 2014, shows 44.1 % increase (FAO, 2016), with the total production of aquaculture recording 73.8 million tonnes whereby fish contributed 49.8 million tonnes of this. The proportion of workers in aquaculture sector has also increased from 17 to 33 percent from 1990 to 2014 (FAO, 2016).

In Africa, capture fisheries have been heavily exploited and on the extreme, above sustainable levels (Musa, 2013). Rapid development of freshwater fish farming has led to the expansion of the aquaculture sector since 1970s (FAO, 2012). In order to improve fisheries productivity and ecosystem health, aquaculture has been introduced as an alternative source of fish production (Allison, 2011; USAID, 2013). Most aquaculturalists use earthen ponds with a few farmers gradually transitioning to rearing fish in liner ponds, especially in places with porous soil texture that cannot contain water for a prolonged period of time (Dugan *et al.*, 2010). In the East African region, aquaculture is under-developed and contributes less than 1% to global aquaculture production (Mwangi and Mbugua, 2008). A major challenge to the sustainable development of aquaculture is the management of conflicts and competition from other sectors, particularly agriculture, for the limited water resources (Bostock *et al.*, 2010). Moreover, the absence of linkages between farmers and extension services has also been recorded as a main challenge (Jamu and Brummett, 2008). In Kenya, aquaculture is regarded as an alternative option for reducing the broadening gap between fish demand and supply. Aquaculture is further recognized as one of the viable options for restoring the country's economy (Munguti *et al.*, 2014). Cognisant of this potential, the Kenyan government initiated an Economic Stimulus Programme (ESP) in 2009 targeting fish farming to reduce food insecurity and high

unemployment rate (Orina *et al*., 2014). Currently, the aquaculture sector in Kenya is swiftly developing with a 40.5% increase in production between 2009 and 2012 (Musa, 2013). Of these, Nile tilapia (*Oreochromis niloticus*) contributed 75% (16,115 tons) of the total farmed fish production while the African catfish (*Clarias gariepinus*) only contributed to 18 % (3,868 tons) in Kenya (Musa, 2013).

2.2 Biology of the Nile tilapia

Nile tilapia is a tropical species that belongs to the kingdom Animalia, phylum Chordata, class Actinopterygii, order Perciformes, family Cichlidae, sub-family Pseudocrenilabrinae, genus *Oreochromis* and species *Oreochromis niloticus* Linnaeus 1758 (Boyd, 2004). *Oreochromis niloticus* is a freshwater fish species native to Central and North Africa and the Middle East. It is also found in the Nile and Niger River basins in Malawi, lakes including Victoria, Tanganyika, Albert, Edward and George, among others and it prefers to live in shallow water bodies with adequate vegetation. Its body is silver in colour with cycloid scales, and has a deep-body of either olive or grey or black body bars (Picker and Griffiths, 2011). Nile tilapia is distinguished by its dark vertical stripes present in the caudal fin. It has 15-18 dorsal spines for defensive roles (Plate 1). The male usually flushes red colour during breeding season to attract female ones, whereas, the female is a mouth brooder, where it incubates the eggs in her mouth. This species can grow to a maximum length of 62 cm with the average length of 20 cm (Bwanika *et al*., 2004).
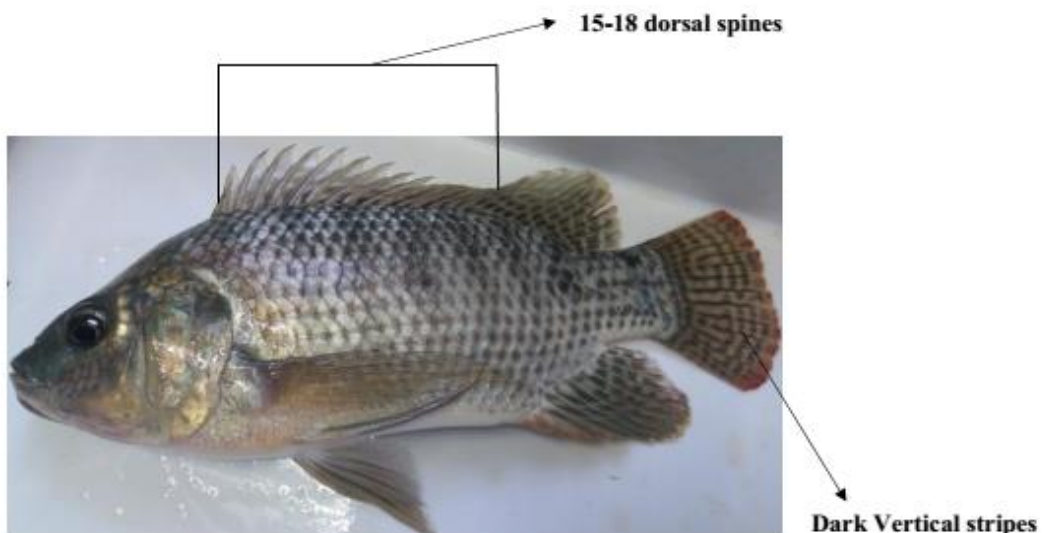
Plate 1: An illustration of Nile Tilapia (*Oreochromis niloticus*) showing important external characteristics (Source: Author)

The feeding habit of Nile tilapia is omnivorous and feeds primarily on phytoplankton, periphyton, aquatic plants (macrophytes), invertebrates (juvenile larval stages of insects in the water), biofilms and detritus (Ng'wala, 2014). The juveniles feed mainly on phytoplankton, zooplankton and detritus, while the adults feed almost exclusively on phytoplankton due to their capacity to filter phytoplankton from water column, hence are classified as filter feeders (Ngugi *et al*., 2007). Therefore, Nile tilapia exhibits change in diet as the fish grows, a phenomena known as ontogenetic shift, which has been reported by Kundu *et al*. (2013). Also Nile tilapia has been reported to feed continuously during the day because of its small stomach and long intestine (Abarike *et al*., 2012). Thus, Nile tilapia are excellent for aquaculture and form the most cultured fish species in Kenya since they readily accept artificial/supplementary diets.

2.3 Nile tilapia culture systems in Kenya

The culture of Nile tilapia was introduced first in Egypt in ancient times dating back over 4000 years held in ornamental ponds. In Kenya, warm water fish farming in ponds began in the early 1920s, originally with tilapia and later included the common carp and African catfish though the growth was tremendously low (Orina *et al*., 2014). Later, it was introduced in Japan and Thailand in 1965, then to Brazil in 1971 and lastly to China in 1978 (FAO, 2012). The Government of Kenya popularized rural fish farming in 1960s through "Eat More Fish" campaign which resulted to rapid expansion of aquaculture. However, the growth and the number of productive ponds declined in 1970s mainly due to inadequate extension services, insufficient training for extension services and lack of quality fingerlings (Ngugi *et al*., 2007) and later on in 2009, the ESP program was initiated by GOK to jump-start it. Nile tilapia was cultured and is still being cultured over a wide range of systems from extensive to highly intensive production facilities. Extensive systems involve low fish stocking densities with no or little inputs from external resources to boost fish production. In most cases, this system reconditions itself subject to its natural ability to maintain favourable water quality environment (Conte, 2004). Most fish farmers in Kenya prefer extensive aquaculture especially due to low operational costs. It also requires little knowledge to operate as compared to intensive system (Ngugi *et al*., 2007). Semi-intensive system requires moderate stocking densities of fish and some inputs such as feeds. On the other hand, intensive system involves high stocking densities of fish and more inputs from external sources such as feeds, frequent water exchange and water quality monitoring (Conte, 2004). It requires high cost and advanced knowledge to operate. However, the intensive fish farming is unpopular in Kenya due to the

high cost of infrastructure advancements and adequate knowledge is required (Munguti *et al*., 2014).

Most of the fish farms in Kenya are unintegrated pond systems, which operate as stand-alone ponds without any connection to other agricultural activities such as livestock (Figure 1a). However, integrated aquaculture which involves connection of other agricultural systems to fish farming is growing rapidly. It is a highly sustainable form of agriculture which uses locally available waste resources from one system as input resources to another system (Munguti *et al*., 2014). For instance, use of livestock wastes in fish pond for manuring whereas the water from fish pond is used to irrigate other crops (vegetables and banana plantation) in the farm because of its richness in nutrients (Figure 1b). This type of integrated agriculture helps in optimization of resources by providing the opportunities for intensified production (Munguti *et al*., 2014), coupled with efficient allocation of land, water, labour, equipment and other limited capital. In 2010, about 95% of the fish farms in Kenya were recorded as small scale systems practising both unintegrated and integrated fish farming systems (Otieno, 2011).
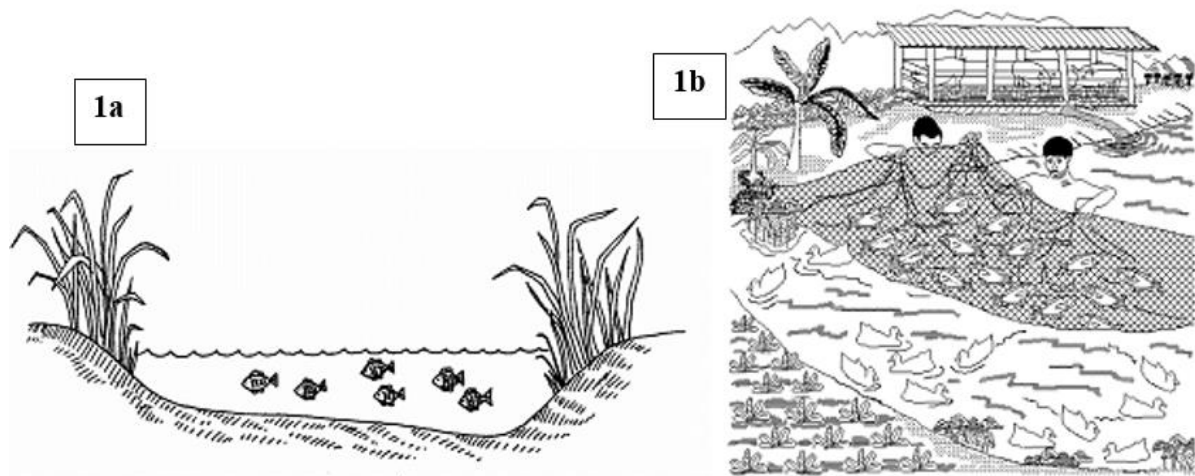
Figure 1: An illustration showing the types of aquaculture systems in Kenya (1a) unintegrated aquaculture pond and (1b) integrated aquaculture pond (Bocek *et al*., 2004)

2.4 Overview of effects of water quality to fish health

Water quality is the physical, chemical and biological parameters in water bodies that affect the production of fish (Boyd, 1998). Pond water quality is the most fundamental and important factor limiting fish production in farmed fish, hence, must be monitored regularly and well-maintained (Ngugi *et al*., 2007). It is also known to be the most difficult aspect to understand, predict and manage by most small scale fish farmers. Condition of optimum temperature,

dissolved oxygen (DO) and pH leads to increased production of healthy fish compared to poor water quality deprived of dissolved oxygen (Boyd, 1998).

Most fish require specific physical and chemical characteristics which when altered, cause fish to become stressed and threatened by increased parasite infestations (Conte, 2004). For example, cultured Nile tilapia survive well under the following conditions: DO levels of 3 mg/l and above, temperature ranges between 20-35 ^{o}C, pH ranges between 6.5-9.0 to maintain alkalinity at or above 40 mg $CaCO_3$/L, ammonia concentration should be kept below 0.5 mg/l to maintain pH level, DO concentration and alkalinity at required levels in order to avoid intoxicating fish (Ngugi *et al*., 2007). The chemical imbalances lead to disruption of physiological functions of the fish organs such as the gills, kidney and the skin. For instance, the ability of fish's skin to protect the body gets hampered, therefore, encouraging invasion by parasites (Boyd, 2009). The deterioration of water quality in ponds is majorly caused by poor pond management that results from excessive application either intentionally or unintentionally of inorganic fertilizers, organic fertilizers (manure) and fish feeds (Boyd, 1998; Liti *et al*., 2005).

2.5 Common parasites infesting Nile tilapia

Parasites are organisms that metabolically depend on their host for survival. Although, most of them do not harm their hosts, but the host suffers as the parasite benefits, a phenomenon called parasitism (Abowei *et al*., 2011). However, the host sometimes dies in cases of intense infection. Parasitic infection in fish refers to a diseased condition in fish resulting from organisms such as protozoan and metazoan species living in or on the fish, commonly found in cultured fish particularly *Oreochromis niloticus* (Martins *et al*., 2011).

The two main categories of parasites are ectoparasites and endoparasites. Ectoparasites are parasites that infect the external surfaces of the fish such as gills, skin, mouth and fins. They exist in three levels: opportunistic, ubiquitous and obligate parasites (Paperna, 1991). Opportunistic parasites have a low degree of adaptability to persist as ectoparasites and they occur in fish that have undergone stress due to adverse environmental conditions. Most of them act as secondary invaders of wounds and lesions (Paperna, 1996). Ubiquitous parasites are known to be well-adapted parasites on fish, however, they are not specific to host or site, for example, members of the genus *Chilodonella, Ichthyobodo* and *Trichodina*. In most cases they damage cells leading to erosion of tissues of fish (Paperna, 1980). The ubiquitous ectoprotozoans are cosmopolitan or trans-continentally dispersed via translocation of their

cultured hosts especially Nile tilapia (Abowei *et al*., 2011). Obligate parasites are heavily specialized parasites with restricted host and site-specific, for example, some nematodes (Biu *et al*., 2014). These obligate parasites are capable of causing massive mortality within a short time and has a morbidity rate of up to 100 % (Emere and Dibal, 2014). Distribution of the more specialized host-specific species follows that of their hosts, but may also be more restricted, sometimes to only one or a few specific environmental characteristics. Most ectoparasites are easily detectable simply by use of microscopic examination of skin and gill scrapings from live or freshly killed fish. Usually, ectoparasites cause huge economic losses in fish farms especially in poorly managed ponds with deteriorated physical, chemical and biological conditions. For instance, the reproduction of *Trichodina spp* is always favoured by excess organic matter and high water temperatures in fish ponds.

Endoparasites occur in internal organs of the fish and most of them are host specific (Florio *et al*., 2009). They include protozoans like haemoflagellates, apicomplexans, microsporeans, myxosporeans, larval trematodes like diplostomatids and clinostomatids, cestodes, acanthocephalans and some nematodes (Otachi, 2009). Most of them require two hosts with at least one intermediate and definitive hosts. The intermediate hosts are mostly aquatic crustaceans and molluscs such as copepods and snails respectively, where the parasite larvae inhabit. The adult endoparasites inhabit the internal organs of fish such as the eyes, gut, kidney, liver, swim bladder, muscle tissue and intestines (Ekanem *et al*., 2011).

2.6 General description of most common parasites in *Oreochromis niloticus*

2.6.1 Protozoans

Protozoans are microscopic, unicellular and complex (eukaryotic) organisms of the Kingdom Protista and includes members of Phylum Ciliophora, Sarcomastigophora, Microsporea and Apicomplexa; with one to several nuclei which can be either identical or divergent (Barger *et al*., 1998). Characteristically, no intermediate host is required for the parasite to reproduce. Consequently, they can be present in very high numbers when fish are crowded causing weight loss, debilitation and mortality (Klinger and Floyd, 2013). The life histories of protozoans range from free-living through various forms of commensalisms to parasitism in most animals, plants and even other protozoans (Otachi, 2009). Protozoan parasites of fishes, particularly *Trichodina* spp. can kill, mutilate and debilitate farmed fish. Besides, they cause damages or injuries in fish tissues that allow the entry of secondary infections (Mumba, 2014).

2.6.2 Metazoans

Metazoan parasites are multicellular organisms of the sub-kingdom metazoa. Some of them are important causes of parasitic diseases in wild and captive fishes (Bassey, 2011). They range from fungal to flatworms that belong to the phylum Platyhelminthes, roundworms (Nematodes), Acanthocephala, crustaceans and also leeches. Flatworms include a large number of parasitic forms, some of which are extremely harmful to humans. For instance, most digenean parasites like *Heterophyes sp* are zoonotic. Examples of Platyhelminthes include: cestodes and trematodes (monogenea and digenea).

Monogenea

The monogenea typically have a simple (direct) life cycle with no intermediate host and they spend their entire life cycle as an ectoparasite on an individual fish. They are small flattened ectoparasites referred to as Platyhelminthes (Florio *et al*., 2009), and are commonly referred to as gill worms (Barger *et al*., 1998). Gill worms have a distinct attachment organ on their posterior end (haptor or opisthaptor) with hardened anchors or specialized clamps with which to pierce the epithelium and hold on to the host. Sclerotized marginal hooks often surround the haptor, bars, disks, scales or hooks may occur on or near the haptor (Otachi *et al*., 2011). The head sometimes has eye spots and specialized holdfast organs. Most reproduce by laying eggs that hatch ciliated larvae called oncomiracidia which quickly mature and attach to the host skin, fins and gills (Otachi, 2009). Monogenean parasites are usually abundant when the water level is reduced and during overcrowding of the fishes (intensive culture) which allows more gill worm offspring to survive and can cause fish kills. Overcrowding of fish into culture ponds coupled with different environmental as well as management factors, promote heavy infestations which can lead to production losses, tissue damages and occasionally mortalities (Scholz, 1999). Gill worms are permanent parasites in the gills, mouth or on the bodies of fishes. They generally feed on mucus or epithelial cells sloughed from the gills or skin. Simple gill worms are common on fishes in all aquatic environments. Examples of Gill worms (Monogenea) include: *Cichlidogyrus spp*, *Dactylogyrus spp* and *Gyrodactylus spp*.

Digenea

Digeneans are parasites that require at least one intermediate host in their life cycles (Otachi *et al*., 2011). They include: *Clinostomum spp* which forms a yellow cysts on the skin below the scales or large cysts behind the gills, while *Euclinostomum spp* form large cysts in anterior and posterior regions of the kidney*, Diplostomum spp* commonly known as eye flukes and *Heterophyes sp* is often found in gills of the fish host (Florio *et al*., 2009). Their infestations

cause damages in the fish eyes (exophthalmia), cataracts, complete collapse of the eye causing blindness and blackening of fish (Otachi *et al*., 2011). On the other hand, *Tylodelphys spp* have been reported from lens and the vitreous humour of fish eyes. Its occurrences are mostly influenced by the differences in temperature (Scholz, 1999). The blind fish rarely see food, thus, feed poorly leading to stunted growth which is of no economic importance. In addition, the greatest damage is caused when the lenses are infected by non-encysted metacercariae of *Diplostomum spp* (Florio *et al*., 2009).

Nematodes

Nematodes are commonly referred to roundworms, threadworms or nematodes. The group of nematodes along with flatworms and thorny-headed worms are sometimes called helminths (Barger *et al*., 1998). Roundworms often infect many different species of farmed and wild fish. Lesser numbers of nematodes usually occur in healthy fish, however, high numbers are found in stressed fish which often cause illness or death (Florio *et al*., 2009), and cause serious diseases and even death in humans. For example *Contracaecum sp, Anisakis spp* and *Eustrongylides sp*, thus they are zoonotic (Barger *et al*., 1998).

Cestodes

Cestodes are endoparasites of vertebrates and most of them require at least one intermediate host and a definitive host (Otachi *et al*., 2011). They are commonly referred to as tapeworms and include: *Diphyllobothrium spp, Proteocephalus sp* and *Amirthalingamia sp*. The commonly reported tapeworm is *Amirthalingamia macracantha* found in fish intestinal wall. Its cyst has fibrous structure and contain red blood cells (Otachi, 2009). The wall around the parasite is usually hypertrophic for chronic inflammation. The cyst often protrude on the outer surface and on the lumen. Its main distinctive feature is the presence of 20 hooks of three sizes which are arranged in two rows in a bilaterally symmetrical pattern (Florio *et al*., 2009).

Acanthocephala

Acanthocephala is also known as thorny-headed worm, morphologically cylindrical worm and its species are all intestinal parasites of vertebrates including fish and humans (Florio *et al*., 2009). The adult females vary from 1 mm to 1 m, but usually 2 cm whereas the males are typically smaller than females (Mumba, 2014). They may appear to be white, red, orange or yellow in colour. The body of Acanthocephala is bilaterally symmetrical, unsegmented or only partially segmented externally and unsegmented internally. They attach in the gut of the fish host with a globular or cylindrical, protrusible, thorny proboscis (Barger *et al*., 1998). The

proboscis pops out like an everting plastic glove, and the thorns fold out and lock like a compact umbrella. Muscles invert the proboscis, and a hydraulic system pop it back out (Klinger and Floyd, 2013). Acanthocephalans lack digestive system and some species have thorns on the body as well. Sexes are separate, fertilization is internal and embryos develop in the body of the female. Shelled larvae (acanthors) are shed through the intestine of the host, eaten by a crustacean, insect or mollusc intermediate host, and develop into acanthella then to an encysted cystacanth larval stage (Otachi, 2009). When the final fish host consumes an infected intermediate host, the cystacanth develops into an adult in the intestine. Adults absorbs nutrients from the gut contents of their hosts. Proboscis hooks can cause some mechanical damage, but this is only serious in a heavy infection (Barber *et al*., 2000). They infect the fish host through the alimentary tract by ingestion of the infective larva via food (Florio *et al*., 2009).

Crustacea

Crustaceans are one of the class of animals with hard segmented shells, particularly referred to as exoskeletons (Barger *et al*., 1998). They are largely aquatic organisms commonly known for causing increasingly serious problems in cultured Nile tilapia (Klinger and Floyd, 2013). They generally have two pairs of antennae, respire through gill or the body surface and anteriorly have paired, segmented, usually biramous appendages. Examples include: Copepods, Brachiura (fish lice) and Malacostraca (Scholz, 1999). Most parasitic crustacea of freshwater fish can be seen with naked eyes, and are usually free-living as well as very important food items for a variety of aquatic life (Barger *et al*., 1998; Klinger and Floyd, 2013). Moreover, the parasitic forms have a direct life cycle (Florio *et al*., 2009). Copepods ordinarily occur on the gills or skin of fishes, but may burrow into the flesh or head sinuses or crawl into the nose or eyes. Parasitic copepods of fishes are permanent parasites, feeding on mucus, sloughed epithelial cells and tissue fluids (Scholz *et al*., 2004). The infected fish may suffer from pressure atrophy of soft tissues and damage of the host tissues through feeding (Florio *et al*., 2009).

2.7 The influence of water quality on fish parasite infection

Parasites are natural components of the environment and may be viewed as an indicator of the relative health of an ecosystem (Adams *et al*., 1997). Majority of the species of parasites present either on body surface or within fish are not hazardous to human health. Those which are hazardous tend to have complex life cycles, involving more than one host for their development such as nematodes which are zoonotic parasites (Khalil *et al*., 2014). Parasites can be occurring naturally or introduced and establish in culture systems of *O. niloticus* depending on both biotic

and abiotic factors (Jakobsen, 2011). When the conditions do not favour their colonization, the parasites will co-exist with fish without massive damage. However, when the conditions favours their colonization, their number increases and damages on fish tissues become pronounced (Modu *et al*., 2014).

Abiotic and biotic factors may have indirect and direct effects on fish parasites either through the occurrence of the intermediate host (Conte, 2004) or infection prevalence. Water characteristics such as salinity, temperature, dissolved oxygen and pH-levels can influence the stages of free-living ectoparasites, consequently, influence parasite infections in fish. Nutrient enrichment in fish farms often increases trematodes in fish farms with frogs as the intermediate host of some trematodes. Eutrophication also promotes algal growth which increases the density of herbivorous snails (Slootweg *et al*., 1993), which are intermediate host of parasites with complex life cycles (Johnson *et al*., 2007). High water temperature is crucial for development and infections of some parasites. For example, temperature ranging from 20 to 25 °C is optimum for development *Ichthyophthirius multifiliis* (Xu *et al*., 2009). Also, massive infections of *Chilodonella* spp. and trichodinids have been reported to occur at high water temperatures ranging from 25-30°C (Conte, 2004). On the other hand, low water temperatures below 20 °C are known to be the optimum requirement for reproduction of some parasites like *Chilodonella piscicola* and trichodinids. High water temperatures above 30 °C coupled with other environmental stressful conditions such as high water pH-levels above optimum (6-9), and low concentration of dissolve oxygen (below 3 mg/l) give rise to decreased developmental stability of fish which results to reduced performance in growth and weaken its immune system to resist parasite infections thus rendering it susceptible to massive infections (Almeida *et al*., 2008).

Biotic factors influencing parasites occurrence include availability of suitable fish hosts, such as intermediate and /or final host suited for digenean parasite to complete its life cycle. These determine the parasite population density, subsequently increase or decrease in transmission rate of the parasites to fish (Ntengwe and Edema, 2008). Biotic factors such as the presence of intermediate and definitive host (mollusc host and fish) provides favourable conditions and host for the parasites (trematodes) especially those with complex life cycles to complete their life cycles and enhance their survival rates, consequently increasing chances of infecting fish in many fish farms with such conditions (Shea *et al*., 2012).

2.8 Host-Parasite relationships and degree of parasite infection

The interaction between hosts and parasites is a complex relationship usually favouring one or the other depending on a number of factors (Khan, 2012). The parasite attempts to establish itself in the host while the latter resists the infection via its defence mechanisms. Consequently, susceptibility and resistance of *O. niloticus* can determine whether or not the infection becomes established. The age, behaviour, physiological and immunological condition, location in the water column, and feeding habits of *O. niloticus* can affect its interaction with parasites (Paperna, 1991). The parasite will mimic its host's (*O. niloticus*) protein composition hence, influencing susceptibility and infectivity. In general, the number of parasites necessary to cause harm to any host varies considerably with size of the host and its health status (Khan, 2012). Information about the mode of transmission and potential intermediate hosts is often crucial to select the most appropriate management action to reduce or eliminate the problem from the system.

Several authors have investigated the relationship between the level of parasite infection and the size of the host fish (Blahoua *et al*., 2016). According to Blahoua *et al*. (2016), monogenean parasite infection on *O. niloticus* is one of the most studied. In his study, monogenean infected all fish size classes even though its lowest prevalence was recorded in the smallest length size ranging between 50 - 100 mm. While a prevalence of 100 % was recorded in fish with standard length greater than 150 mm. This results are similar with findings from the study conducted by Aloo *et al*. (2004), showing that large sized fish harbour more parasites in wild marine environment. However, fry and fingerling stages have been recorded to be more susceptible to parasitic infections than broodstocks (Asely *et al*., 2015). Shafi (2015) found out that the size of fish was positively correlated with parasite infection. However, the study done by Otachi (2009) in freshwater fish ponds and cages showed weak negative correlation between size of fish and parasite infection levels.

2.9 Condition factor of cultured Nile Tilapia

The condition factor (k) is one of the main parameters used in fishery research to show the degree of robustness and well-being of the fish in their habitat determined using Fulton's condition factor, expressed by length-weight factor (Githukia *et al*., 2015). The value of k calculated can be used to estimate change in nutritional condition of the examined fish. This factor can also be a measure of various ecological and biological factors such as degree of fitness, development of gonads and the suitability of the environment. Fish condition factor is influenced by level of stress, availability of feeds and water quality parameters such as pH,

temperature, concentrations of DO and ammonia (Nehemia *et al*., 2012). In order to obtain a maximum size of cultured Nile tilapia, the above parameters must be monitored regularly to ensure their optimum levels prevail. The Fulton's condition factor often decreases with increase in length. When k value is 1.40 and above, the fish is considered to be in an excellent condition, whereas, when the value is below 1.40, the fish is considered to be in a poor condition (Barnham and Baxter, 1998). Fish heavily infected with digenetic trematodes may experience loss of vision, reduced growth, emaciation or deformation of the vertebral column, brain tumour, cellular necrosis and death (Iwanowicz, 2011). It has been further shown that these parasites can reduce fish crypsis and escape response and thus increase fish susceptibility to bird predation. The fish under these conditions usually have a very poor condition factor as their feeding ability is despaired (Ndeda *et al*., 2013).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Description of the study area

The study was conducted in selected fish farms from four sub-counties situated in Nakuru County from November 2016 to February 2017. Nakuru County is found within the Great Rift Valley region in Kenya and is located 160 km northwest of Nairobi along the Kenya-Uganda highway (Basweti, 2009). It covers an area of 7,495.1 Km² and lies between Longitude 35º28'and 35º36'E and Latitude 0º13'and 1º10'S (Figure 2). The climatic conditions are strongly influenced by the altitude and physical features. The County has a bimodal rainfall pattern with short rains occurring between October and December while long rains fall between March and May. Temperatures range from a mean annual minimum of 12 ºC and a mean annual maximum of 29.3 ºC (Nakuru County Integrated Development Plan (NCIDP), 2013). It has three climatic (ecological) zones namely II, III and IV. Zone II covers areas with an altitude between 1980 and 2700 m above sea level and receives a minimum rainfall of 1000 mm per annum. Zone III and Zone IV cover areas with the same altitude between 900-1800 m above sea level but receive different rainfall ranges of 950-1500 mm and 500-1000 mm per annum respectively (NCIDP, 2013). The six fish farms were selected from sub-counties in Zone II with elevation ranging from 1942 to 2513 m. The uniqueness of the selected farms lie in the climatic areas which are not suitable for the culture of Nile tilapia, as the temperatures are below the optimum range (20-35 °C) required. Fish farming is practised in Nakuru County, though it is still relatively under-developed. Most fish farmers in Nakuru County practice aquaculture in earthen fish ponds, liner ponds and water reservoirs owned by individuals and/or registered groups. There are approximately 792 operational fish ponds within the County mostly stocked with Nile tilapia (*Oreochromis niloticus*), however, a few fish farmers culture African catfish (*Clarias gariepinus*) (NCIDP, 2013).

3.1.1 Study sites

Six operational aquaculture farms were selected based on the type of pond (earthen and liner ponds), pond inputs i.e. fertilizer, manure or fish feeds, and number of year(s) of operation (minimum 1 year). A total of six fish farms composed of three randomly selected earthen and liner fish ponds were identified. Each fish farm was sampled thrice during the study period for determination of water quality and parasite assemblages. The farms selected were: Arahuka (S 00º15'32.6", E 036º13'49.4") in Bahati Sub-county, Dundori (S 00º09'48.5", E 036º04'04.2") in Bahati Sub-county, Egerton (S 00º 22'11.0" and E 035º 55'58.0") in Njoro sub-county,

Kuresoi (S 00º05'01.7", E 035º40'47.4") in Kuresoi North Sub-county, Njoro (S 00º20'17.3", E 035º56'53.3") in Njoro Sub-county and Subukia (S 00º00'02.3", E 036º14'48.3") in Subukia Sub-county (Figure 2). Dundori, Kuresoi and Subukia fish farms were earthen ponds, while, Arahuka, Egerton and Njoro fish farms were liner ponds.
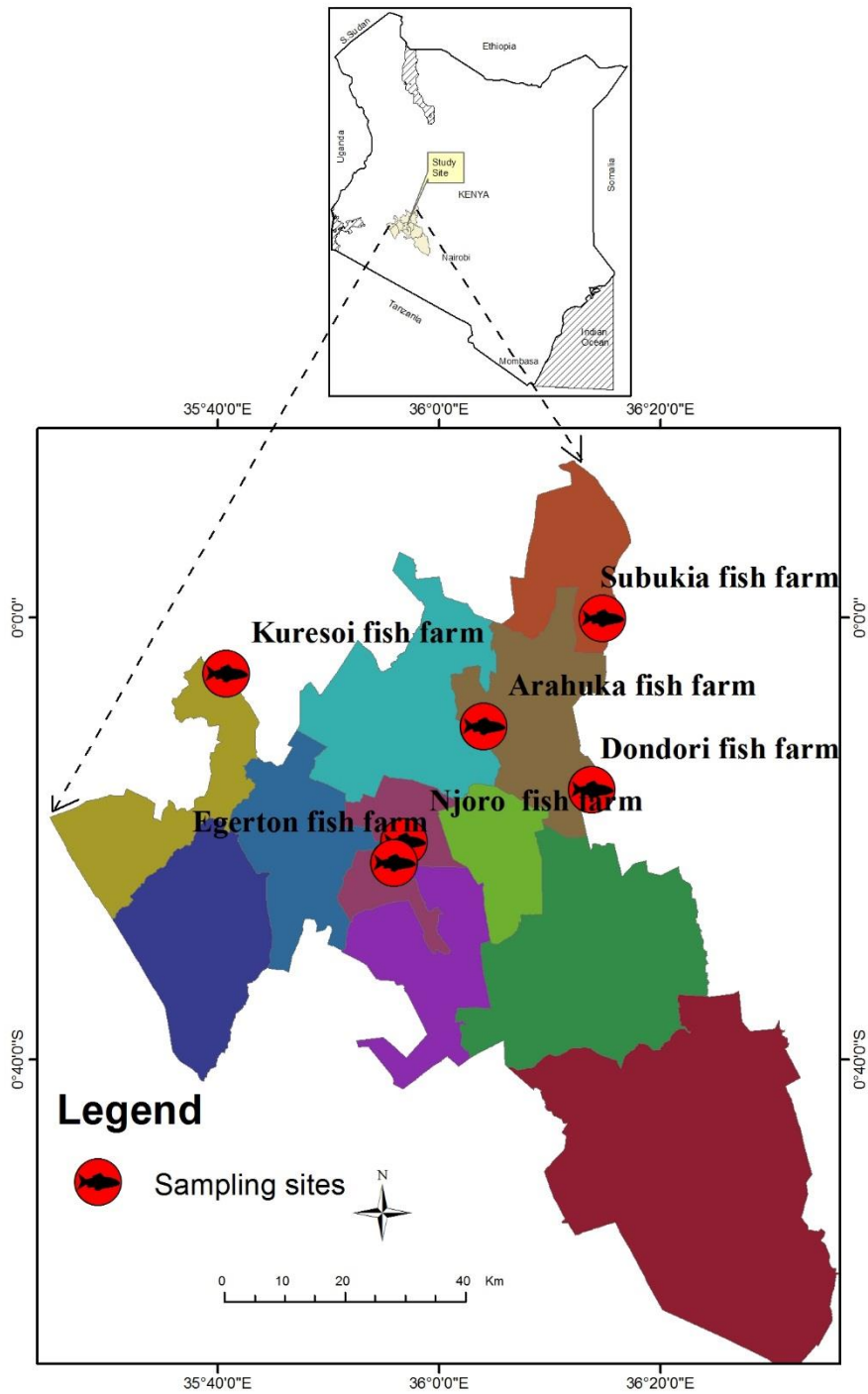
Figure 2: The Geographic position of Nakuru County in Kenya and the six fish farms sampled during this study (Source: Topographical map of Kenya, scale 1:50,000)

3.2 Study design

The study design entailed first a reconnaissance study of the sites. This was done by travelling to eleven sub-counties within Nakuru County with the help of the officers of Fisheries Department to establish the operational fish farms. The fish farms were surveyed and the fish farmers were involved in interviews to acquire details of the farm. The GPS coordinates of each farm visited was recorded. The responses and observations made from the survey, were used to select the suitable fish farms for this study using a purposive sampling method.

3.2.1 Questionnaire survey on pond management

A semi-structured questionnaire was administered face-to-face to the six selected fish farmers to collect data on the type of pond inputs (manure, fertilizers and fish feeds), frequency of water quality monitoring, water replacement, pond liming, fertilization, source of water and seeds (see appendix 1).

3.2.2 Background information of the fish farms under study

The six selected fish farms have different management practises of culturing Nile tilapia as explained below:

Njoro fish farm

Njoro fish farm is located in Njoro sub-county within Nakuru County near River Njoro with the latter being the main source of water for the farm. The fish farm is situated at S 00º 20'17.3'' and E 035 º56'53.3'', 2155 m above sea level (Figure 2) and has an area of 200 m². It is a liner pond (Plate 2) with impermeable durable liner installed into the pond to secure water containment to ensure water conservation, control parasites and prevent groundwater pollution. Njoro fish farm was established in 2014 and has been in operation for 3 years. This fish farm was stocked with 1,000 of *Oreochromis niloticus*. The fingerlings were obtained from a hatchery located in Kendu-bay situated in Homa-bay County which is 212 km away. The management level of the farm is considered to be moderate with weekly water exchange, no fertilizer application to supplement the fish diet and fish are fed once a day. The fish farmer used both commercial and home-made feeds. Birds were the major predators in Njoro fish farm. Njoro fish farm is a community managed pond.

Plate 2: A photograph showing part of the liner pond in Njoro fish farm (Source: Author)

Subukia fish farm

Subukia fish farm is located in Subukia sub-county 43 km from Nakuru town. It is located at S 00º 00'02.3" and E 036º 14'48.3", 2513 m above sea level (Figure 2) and has an area of 800 m². It is an earthen pond created through excavation of natural soil to form a basin which was then filled with water (Plate 3). Subukia farm was established in 2009 and has been in operation for the last 8 years, with a total population of 4,000 *Oreochromis niloticus* and *Clarias gariepinus* in the same pond. The fingerlings were obtained from a hatchery located in Kendu-bay found in Homa-bay County. Main sources of water for the farm operations are borehole and run off stored in a reservoir during the rainy season. The management level of the farm is moderate with water exchange done only when fish deaths occur and fish are fed twice a day to satiation using home-made fish feeds. Poultry manure is often applied in the pond to enhance natural food as supplement fish diet. The major fish predators are birds and otters.

Plate 3: A photograph showing the earthen pond in Subukia fish farm (Source: Author)

Arahuka community fish farm

Arahuka fish farm is located in Bahati sub-county within Nakuru County. It is located at S 00º 09'48.5" and E 036º 04'04.2", 1942 m above sea level (Figure 2) and has an area of 345 m² and is a liner pond (Plate 4). Arahuka farm was established in November 2015 and has been in operation for 1 year, with a total population of 1,000 *Oreochromis niloticus*. The fingerlings were obtained from a hatchery located in Kendu-bay located in Homa-bay County which is 212 km away. Main source of water for the farm operations is supplied and piped from a community owned dam by Menengai water users. The pond management includes only topping up of water and fish are fed once a day with home-made fish feeds which can be categorized as a moderate management. Livestock manure is often applied in the pond to boost natural food production to supplement the fish diet. This farm is mostly predated by birds.

Plate 4: A photograph showing the liner pond in Arahuka fish farm (Source: Author)

Dundori fish farm

Dundori fish farm is located in Bahati sub-county within Nakuru County. It is located at S 00º 15'32.6" and E 036º 13'49.4", 2296 m above sea level (Figure 2) and has an area of 300 m² and is an earthen pond (Plate 5). Dundori farm is situated in a wetland and was established in 2006, and has been in operation for the last 10 years, with a total population of 1,000 *Oreochromis niloticus* and *Clarias gariepinus*. The fingerlings were obtained from a hatchery located in Kendu-bay situated in Homa-bay County. Main sources of water for the farm operations from a nearby stream and underground seepage that recharges the pond. The management level of the farm is moderate with daily water exchange through underground seepage and fish fed once a day with both commercial and home-made fish feeds. Livestock manure is often applied in the pond to enhance natural food production to supplement the fish diet. Birds and otters are the major predators in this farm.

Plate 5: A photograph showing the earthen pond in Dundori fish farm (Source: Author)

Egerton fish farm

Egerton fish farm is located in Njoro sub-county within Nakuru County. It is located at S 00º 22'11.0" and E 035º 55'58.0" (Figure 2). There are a total of ten operational ponds. The pond chosen for this study has an area of 96 m² and is a liner pond (Plate 6). Egerton farm was established in 2009. However, there were management problems and its operations declined. Fortunately, it was rehabilitated in 2014 under a new management and has been in operation for the last 2 years, with a total population of 500 *Oreochromis niloticus*. The fingerlings were obtained from hatchery located in National Aquaculture Research Development and Training Centre (NARDTC) situated about 2 km within Sagana Township in Kirinyaga County. Main source of water for the farm operations is from River Njoro. The pond management includes monitoring weekly, weekly water exchange and fish are fed twice a day with home-made fish feeds which is characterized as moderate management. Poultry manure is often applied in the pond to boost natural food production to supplement the fish diet. Birds are the major predators experienced in this farm.

Plate 6: A photograph showing the liner pond at Egerton fish farm (Source: Author)

Kuresoi fish farm

Kuresoi fish farm is located at Kamara in Kuresoi North sub-county within Nakuru County. It is located at S 00º 05'01.7'' and E 035º 40'47.4'', 2485 m above sea level (Figure 2) and has an area of 750 m² and is an earthen pond (Plate 7). Kuresoi farm is situated in a wetland and was established in 2015 and has been in operation for 1 year, with a total population of 1,000 *Oreochromis niloticus*. The fingerlings were obtained from a hatchery located in Kendu-bay situated in Homa-bay County. Main sources of water in farm is from Rivers Bangala and Chemororoch. The management level of the farm is moderate with daily water exchange through underground seepage and no feeding of fish. However, livestock manure was often applied in the pond to enhance natural food production. This fish farm was majorly an extensive system with little inputs of manure. Fish predators includes birds and otters.

Plate 7: A photograph showing the earthen pond in Kuresoi fish farm (Source: Author)

3.2.3 The sampling design

Fish farms sampled were selected through purposive sampling methods according to their unique characteristics from the others (six farms were chosen out of the sixteen farms visited during the pre-survey in Nakuru County). Information on the management practises of individual fish farmers, for instance, earthen and liner ponds were gathered using a questionnaire. Fish samples were collected using simple random sampling method from the fish farms by the use of seine nets thrice during the sampling period. Figure 3 shows an illustration of sampling design for fish and water samples in fish farms within Nakuru County during this study.
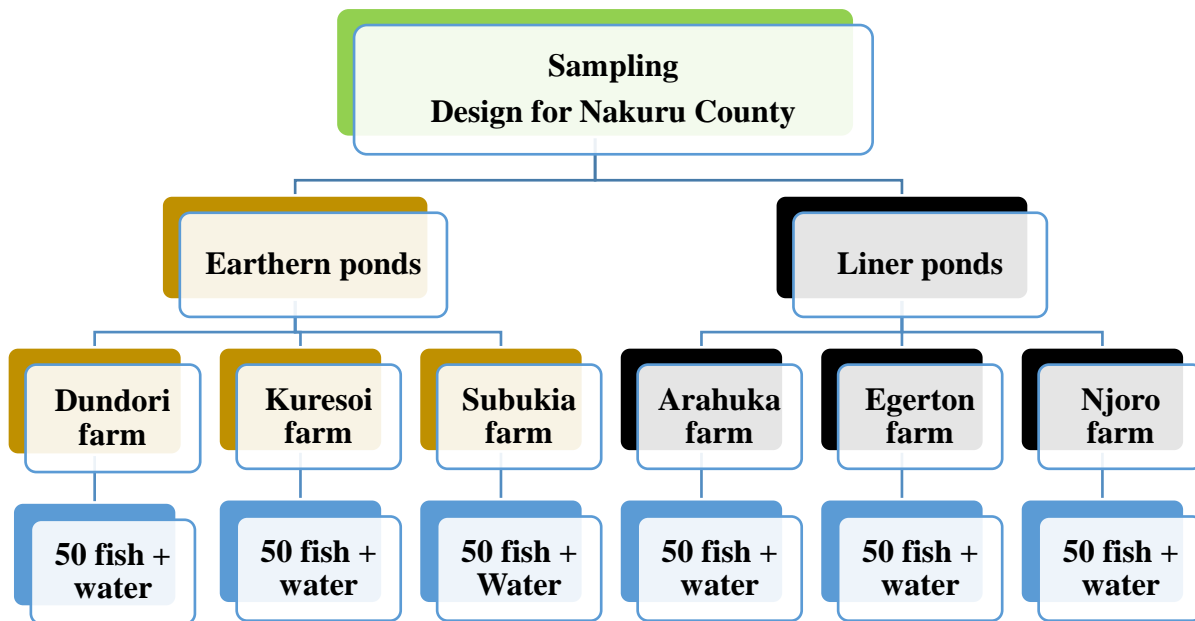
Figure 3: An illustration of sampling design for fish and water samples in selected fish farms within Nakuru County

3.2.4 Measurement of physico-chemical parameters and water sampling

The physico-chemical parameters were measured before collection of the water and fish samples. This included dissolved oxygen (mg/l), temperature (ºC), pH, conductivity (µs/cm) and turbidity (NTU) which were measured randomly *in-situ* in the selected fish ponds in each of the fish farms for 3 months (November 2016-February 2017) using Multi-probe HQ40D meter (HACH LDO; PHC301 & CDC41). The measurements were taken at mid-day (between 10 am - 1 pm) weekly.

Water samples for nutrient analysis (NH_4-N, NO_3-N, NO_2-N, TN, SRP and TP) were collected monthly over the sampling period in triplicates in the six randomly selected fish ponds in each farm using 500 ml acid-washed plastic bottles. The samples were transported in a cool box to Egerton University for analysis immediately on arrival. Nutrients (Nitrogen and phosphorus) concentration were determined calorimetrically following conversion of the concentrations from sample absorbance values in relation to the known standards.

3.2.5 Determination of nitrogen in water samples

Nitrogen components that were determined include: Ammonium-nitrogen (NH_4-N), Nitrite-nitrogen (NO_2-N), Nitrate-nitrogen (NO_3-N) and total nitrogen (TN). Ammonium-nitrogen (NH_4-N) was determined by phenol-hypochlorite method (APHA, 2004). In this method, 2.5

ml sodium salicylate solution and 2.5 ml hypochloride solution were added to 25 ml of filtered water samples from the fish ponds. The samples were then incubated in the dark for 90 minutes and absorbance read at a wavelength of 665 nm from a GENESYS 10 UV scanning spectrophotometer. The absorbance was used to calculate NH_4-N concentration using the equation obtained from the standard calibration curve.

Nitrite-nitrogen (NO_2-N) was determined using the sulphanilamide method, where 1 ml of sulphanilamide solution was added to 20 ml of filtered water sample (APHA, 2004). The samples were left for 8 minutes after which 1 ml of N-Naphthyl-(1)-ethylendiamine-dihydrochloride solution was added and left to settle for 10 minutes. The absorbance was read at a wavelength of 540 nm. The concentration was calculated using the equation obtained from a nitrite standard calibration curve.

Nitrate-nitrogen (NO_3-N) was determined by sodium-salicylate method (APHA, 2004). In this method, 1 ml of sodium salicylate solution was added to 20 ml sample and left to evaporate overnight at 80 ºC to complete dryness. The resulting residue was then dissolved using 1 ml H_2SO_4, followed by 40 ml of distilled water and thereafter 7 ml potassium-sodium hydroxide-tartarate solution. The absorbance was read at a wavelength of 420 nm and concentration calculated using the obtained nitrate standard curve.

Total nitrogen (TN) was determined using persulphate method (Koroleff, 1983). This method oxidized all nitrogenous compounds in unfiltered water sample to nitrate. Approximately 0.5 ml of solution B (mixture of Potassium peroxodisulphate, Sodium hydroxide and Boric acid) was added to 25 ml of unfiltered water samples which topped it to 30 ml. The samples were covered with a cotton plug and aluminium foil and mixed carefully. The flasks containing the samples were autoclaved for one hour at 110 ºC and after cooling water samples were then topped to 50 ml with distilled water in Erlenmeyer flask after which 1ml of Conc. Hydrochloric acid was added and mixed. The absorbance was measured at wavelength of 220 and 275 nm against distilled water and concentration determined from known concentrations of TN standard solutions.

3.2.6 Determination of phosphorus in water samples

Soluble reactive phosphorus (SRP) was analysed using the ascorbic acid method (APHA, 2004). The prepared reagents of ammonium molybdate solution (A), sulphuric acid (B), ascorbic acid (C) and potassium antimonyltartrate solution (D) were mixed in a ratio of A: B: C: D= 2:5:2:1. The resulting mixed solution was added to the filtered water sample at a ratio

of 1:10 and the absorbance read at 885nm wavelength using a GENESYS 10uv scanning spectrophotometer after 15 minutes of reaction. The concentration was determined from known concentrations of SRP standard solutions (APHA, 2004).

Total phosphorus (TP) was determined using ascorbic acid method, after digestion of unfiltered water sample using persulphate to reduce phosphorus present into SRP. After the digestion, evaporated water was replaced and TP analyzed as SRP using ascorbic acid method. The concentration of TP was determined from known concentrations of TP standard solutions (APHA, 2004).

3.2.7 Nile tilapia sampling method and sample size

The sample size of *Oreochromis niloticus* was determined according to Thrusfield (2005) with 95% confidence interval and 5% absolute precision as shown in equation 1.

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

Where *n* is the required sample size, 1.96 is the standard deviation unit at confidence level of 95%, *P* is the expected prevalence, and *d* is the desired absolute precision. A prevalence of 90 % and desired absolute precision of 0.05 were used in the determination of sample size. The prevalence of 90% was estimated according to the study done by Florio *et al*. (2009) on prevalence of parasites in fish ponds, cages and wild fish in Eastern Africa. According to that study, ponds in Kenya recorded a higher parasite prevalence value of 86.5 % compared to 85.9 % in cages and 82.1 % in the wild.

Therefore, based on this calculation according to the above formula in equation 1; the total sample size of 138 fish was obtained for this study. However, to increase the precision and accuracy as well as to get a more representative sample, a total of 300 individual fish were sampled, with 50 fish from each farm. The fish samples were collected using seine nets of a mesh size of 1 mm and length of 15 m and width of 2 m. The selected mesh allowed collection of fish of different size-classes. The fish samples were transported alive in a fish tank with pond water to Egerton University, Department of Biological sciences and kept in aquaria as the laboratory analyses and examination of parasite took place for three day.

3.2.8 Determination of the fish condition factor

The Fulton's condition factor (k) of the collected fish specimens was estimated using weight and total length measured from the tip of the mouth to the caudal fin of the collected fish specimens. The weight of fish was measured using a model balance to the nearest grammes (g)

and the fish length was measured using a measuring board or metre rule calibrated in centimetres. The fish condition factor was calculated using Froese (2006) as shown in equation 2:

$$K = \frac{100W}{L^3}$$ (2)

Where K is the condition factor, W is the weight of the fish in grammes (g), L is the total length of fish in centimetres (cm). After calculation, the resulting values of condition factor for each fish was compared with the standard values of Barnham and Baxter (1998) given in Table 1.

Table 1: The scale of condition factor and fish health status (Barnham and Baxter, 1998)

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

3.2.9 Identification of parasites from fish specimens

Fish specimens collected were examined for both ecto- and endoparasites at Egerton University laboratory. The fish were killed by decapitation or by cervical dislocation as described by Schaperclaus (1990). Ectoparasites were determined through gross examination of the external surfaces (skin, fins and gills) for pathological signs and presence of parasites with the aid of a magnifying lens. Skin and gill scrapings were made using a cover slip, then a wet mount was prepared on microscope slide through addition of saline water on the slide with the scrapings then covered by cover slip, and observed under a light microscope. Thereafter, the fish specimens were dissected and the visceral organs placed in petri dishes containing saline solution (0.85% Sodium chloride), and examined for parasites under a dissecting microscope at a magnification of ×100. The body cavity, pericardial cavity and muscle tissues were also examined for endoparasites. Where found, the parasites were counted and preserved in either 4% formalin or 96% Ethanol for further identification and analysis later. The parasites were identified morphologically using standard identification keys and pictorial guides (Paperna, 1980; Paperna, 1996; Kuchta *et al*., 2012; Scholz *et al*., 2004; Pouder *et al*., 2005). The

parameters of infection such as prevalence, mean intensity of infection and abundance of parasites were calculated according to Bush *et al*. (1997) as follows:

$$Prevalence\ (\%) = \frac{Number\ of\ fish\ infested\ \times 100}{Total\ Number\ of\ fish\ examined} \tag{3}$$

$$Abundance = \frac{Total\ Number\ of\ individual\ of\ a\ parasite\ species}{Total\ Number\ of\ host\ examined} \tag{4}$$

$$Mean\ intensity = \frac{Total\ Number\ of\ individual\ of\ a\ parasite\ species}{Total\ Number\ of\ infested\ host} \tag{5}$$

The estimation of the intensity of protozoan parasites infecting *Oreochromis niloticus* was grouped into three categories due to their small sizes as defined by Jirsa *et al*. (2011) as shown in Table 2.

Table 2: Categories used to classify the intensity of protozoan parasites in *O. niloticus* per field of view (Adopted from Jirsa *et al*., 2011)

Categories	Parasite individuals per field of view at ×100 magnification
Low	<10
Medium	11-100
High	>100

3.2.10 Determination of the diversity of parasites in *Oreochromis niloticus* in fish farms
The parameters such as the Shannon-Wiener diversity index for the species community, Simpson species dominance index and Margalef species richness were calculated using an online biodiversity calculator (Young, 2017). For example, the diversity of the parasites infecting *O. niloticus* was determined using Shannon-Wiener diversity index as shown in equation 6.

$$H' = -\sum_{i=1}^{R} Pi\ In\ Pi \tag{6}$$

Where *H'* = Shannon's diversity index, *R* = Total number of species, *i* = the ith species, In = natural logarithm and *Pi* = the proportion of individuals belonging to the ith species and *N* = Total number of individuals of all species of parasites.

3.3 Data management and analysis

Data collected was tested for normality and homogeneity of variance using Shapiro-Wilk test and Levene's test respectively, to determine if variations within the data set were homogenously spread. The water quality data was normally distributed, therefore parametric test using ANOVA was performed.

For objective 1, standard statistical computations viz., prevalence, mean intensity and mean abundance were carried out according to Bush *et al*. (1997) and the measures of diversity of parasites was determined using Shannon-Wiener Index, Simpson index and Margalef richness index. One-way ANOVA was used to test significant differences between the physico-chemical parameters of the six fish farms. Tukey's HSD test was used for post-hoc comparison of different fish farms at $p < 0.05$. Then correlation analysis was performed between key physico-chemical parameters (those significantly different in part one of the analysis) and the diversity of parasites to determine if there were possible relationships between them. The hierarchical cluster analysis was also used to determine the similarities between the fish farms based on physico-chemical parameters, nutrient concentration and parasite assemblages.

Pearson's correlation was used to achieve second objective which was to determine if there is variation among fish size classes and susceptibility to parasite infections and also to determine the possible relationships between the prevalence, mean intensity, mean abundance and the host's (fish) total length.

For objective 3, the effect of parasites on the health status of the fish host was investigated using Fulton's condition factor (k-factor). A Student's *t*-test was used to compare the mean differences in the condition factors of parasitized and non-parasitized *O. niloticus*. Descriptive and inferential data analyses were conducted using R software (version R x64 3.3.0) for descriptive statistics, SPSS software for inferential statistics and MS Excel (2013) was used for data entry and storage.

CHAPTER FOUR
RESULTS

4.1 Pond management practices in the fish farms in Nakuru County

4.1.1 The type of fish cultured in fish farm

This study sought to know the type of fish cultured in the respondent's fish farm. The results showed that *Oreochromis niloticus* (Nile tilapia) and *Clarias gariepinus* (African catfish) were the most common fish cultured in Nakuru County. Three out of the six fish farms cultured both Nile tilapia and African catfish, this applies for Subukia and Dundori where the culture was done in the same pond except at Egerton, where the two fish species were reared in separate ponds, all of the three fish farms reared mixed sex Nile tilapia. While the rest of the fish farms (Arahuka, Kuresoi and Njoro) reared mainly monosex Nile tilapia. Most fish farms were supplied with the fingerlings by the fisheries extension officers. These fingerlings were obtained from a hatchery located in Kendu-bay in Homa-bay County, and were stocked with a total fish population of 1,000 *Oreochromis niloticus* except Subukia and Egerton fish farms. Egerton fish farm obtained its fingerlings or seeds from NARDTC in Sagana, while Subukia uses its own bred fingerlings.

4.1.2 Management practices of water quality of the studied fish farms

The overall result from the interview conducted using questionnaires from the six fish farmers, showed that most pond management practices can be categorized as moderate in this county. It is important to note that all the eleven sub-counties in Nakuru County had fisheries extension officers. In addition, the responses from all the six fish farmers showed they had attended at least one or several trainings on pond managerial skills. However, the major challenge on poor water quality and parasite occurrences in reared fish still occur in most of the fish farms. It is also crucial to note that none of the interviewed fish farmers knew that parasites occur in fish and the majority had no idea about the conditions that cause parasites to infect fish in ponds. Out of the six fish farms surveyed, only Egerton fish farm had its water quality monitored regularly with appropriate meter probe. Whereas two of the fish farms (Subukia and Dundori) monitored their pond water quality through observation. However, the other three (Arahuka, Njoro and Kuresoi) had never monitored the water quality of their farms since they ventured into aquaculture. Surprisingly, Kuresoi fish farm had no other pond inputs apart from livestock manure. On the other hand, the other fish farms were fertilized frequently, fish were fed daily and the water changed more often to maintain the water quality. Three quarters of the respondents confirmed that the contact hours of the extension officers was infrequent and need

to be improved. Three out of the six respondents pointed out that the extension officers were not frequently visiting their fish farms to advise them on feeding methods (frequency and quantity of feeds) and good water quality control. According to the respondents, the major predators in these fish farms were birds and otters. Some fish farms like Subukia, which applied a lot of poultry manure had a lot of Melanoides snails because of the plentiful organic debris at the bottom of the pond (Plate 8). *Melanoides sp* are well-known to be intermediate host of trematode parasites such as *Heterophyes sp* which are zoonotic. Whereas in Kuresoi fish farm, there were a lot of frogs in the fish pond which also hosts some parasites.

Plate 8: Photographs showing molluscs (*Melanoides sp*) found in Subukia fish farms during fish harvesting using a seine net

4.2 Water quality parameters in fish farms in Nakuru County

4.2.1 Variations in physico-chemical parameters of pond water in the fish farms

This study attempted to determine whether water quality parameters can certainly explain the patterns of similarity/dissimilarity in parasite infections observed in *Oreochromis niloticus* in the six fish farms. An overview of the physico-chemical parameters obtained in the sampled fish farms are presented in Table 3. Dissolved oxygen showed significant differences between the fish farms (One-way ANOVA, F = 7.36, df (5, 48), p< 0.05). The highest mean dissolved oxygen level (15.80 ± 1.75 mg/l) was recorded at Egerton fish farm and the lowest level (8.35 ± 1.25 mg/l) in Kuresoi fish farm. It is also important to note that the liner ponds (Njoro, Arahuka and Egerton) studied had higher dissolved oxygen concentration (12.10 ± 4.54 mg/l, 14.06 ± 4.26 mg/l and 15.80 ± 1.75 mg/l respectively) compared to the earthen ponds (Kuresoi, Dundori and Subukia) which recorded lower concentration of DO (8.35 ± 1.25 mg/l, 9.28 ± 2.65 mg/l and 9.31 ± 3.96 mg/l respectively). It could be explained by the increased rate of heat absorbance by the black membrane installed at the bottom which can speed up photosynthetic

rate during the day leading to high concentration of dissolved oxygen and vice versa for earthen ponds.

Similarly, the water temperature also showed significant differences between the fish farms (One-way ANOVA, $F = 6.54$, df (5, 48), $p< 0.05$). The highest and the lowest mean water temperature were 26.66 ± 2.92 °C and 20.52 ± 2.66 °C in Arahuka and Subukia fish farms respectively. In the study, the places with the cold temperature recorded the lowest temperature (Subukia) while places with the warm temperature (Arahuka) recorded the highest. Therefore, the differences in mean water temperature were not brought about by the time the samples were taken since sampling was done almost at the same time for all the farms.

Water pH also showed differences in values ranging between 7.57 and 11.61. The highest pH was recorded in Egerton (11.31) and Subukia (11.61) fish farms with pH ranges of 9.24 - 11.31 and 8.52 - 11.61 respectively, and the lowest pH values in Dundori (7.57) and Kuresoi (7.58) fish farms with pH ranges of 7.57 - 8.90 and 7.58 - 8.38 respectively. In addition, the turbidity also showed significant differences between the fish farms (One-way ANOVA, $F= 39.55$, df (5, 48), $p< 0.05$), where the highest mean turbidity observed was 550.00 ± 215.14 NTU in Subukia fish farm whereas the lowest mean turbidity was recorded at Egerton fish farm (35.64 ± 19.63 NTU). The highest mean conductivity was 412.85 ± 27.47 µs/cm at Egerton fish farm whereas the lowest was 70.36 ± 11.72 µs/cm in Kuresoi fish farm. This showed significant differences between fish farms (One-way ANOVA, $F = 71.62$, df (5, 48), $p< 0.05$).

Tukey's post-hoc text showed differences between some farms while in others there were similarities between fish farms of different physico-chemical parameters. Dissolved Oxygen concentration showed significant differences within three fish farms while, the other three fish farms depicted no significant differences. There was significant differences between Subukia, Dundori and Kuresoi fish farms ($P<0.05$), whereas there was no significant differences between Arahuka, Njoro and Egerton fish farms ($P> 0.05$). On the other hand, water temperature showed no significant differences between fish farms ($P>0.05$) except in Arahuka fish farm which showed a significant difference from the other fish farms ($P<0.05$). Similarly, conductivity also showed significant differences between fish farms ($P<0.05$) except for Kuresoi fish farm ($P> 0.05$). However, turbidity showed significant differences ($P< 0.05$) between all fish farms (Arahuka, Dundori, Egerton, Kuresoi, Njoro and Subukia).

Table 3: Mean values of physico-chemical parameters in each fish farm including pH range values (mean ± SD, n = 18)

Parameters	Fish farms					
	Arahuka	Subukia	Njoro	Dundori	Egerton	Kuresoi
DO (mg/l)	14.06 ± 4.26	9.31 ± 3.96	12.10 ± 4.54	9.28 ± 2.65	15.80 ± 1.75	8.35 ± 1.25
Temperature (°C)	26.66 ± 2.92	20.52 ± 2.66	21.14 ± 2.29	21.10 ± 2.10	21.04 ± 1.71	21.69 ± 1.30
Conductivity (µs/cm)	128.47 ± 20.63	300.63 ± 91.06	167.13 ± 29.40	230.71 ± 34.52	412.85 ± 27.47	70.36 ± 11.72
Turbidity (NTU)	253.55 ± 98.33	550.00 ± 215.14	72.61 ± 12.82	41.99 ± 17.64	35.64 ± 19.63	46.90 ± 25.27
pH range	8.89 - 10.61	8.52 - 11.61	8.63 - 9.93	7.57 - 8.90	9.24 - 11.31	7.58 - 8.38

4.2.2 Nutrient concentration in the six fish farms in Nakuru County

The nitrogen components showed a wide variation in different fish farms. Ammonia-N concentration was significantly higher in Arahuka fish farm compared to other fish farms (One-way ANOVA, F = 3.72, df (5, 48), p< 0.05). The highest mean concentration of ammonia were 531.96 ± 195.67 µg/l and 440.33 ± 214.27 µg/l in Arahuka and Dundori fish farms respectively, with the lowest level being 7.08 ± 2.07 µg/l in Njoro fish farm. Similarly, the Nitrite-N concentration also exhibited a significantly lower level in Njoro fish farms than in other fish farms (One-way ANOVA, F= 10.79, df (5, 48), p< 0.05), where the highest mean concentration of NO_2-N were recorded as 31.21 ± 7.16 µg/l and 11.43 ± 2.29 µg/l in Subukia and Dundori fish farms respectively, and the lowest mean concentration was recorded as 1.03 ± 0.24 µg/l in Njoro fish farm. Correspondingly, Nitrate-N concentration showed significant differences between fish farms (One-way ANOVA, F = 3.43, df (5, 48), p< 0.05), where the highest mean concentration of NO_3-N was recorded in Arahuka fish farm with 0.25 ± 0.11 mg/l and extremely low concentration (0.01 ± 0.01 mg/l) in both Njoro and Kuresoi fish farms. The highest and lowest mean concentration of total nitrogen (TN) were recorded in Arahuka 0.66 ± 0.09 mg/l and Kuresoi 0.14 ± 0.04 mg/l respectively. Therefore, there were significant differences of TN between fish farms (One-way ANOVA, F = 11.16, df (5, 48), p< 0.05). The post-hoc test showed differences for NH_4-N between three fish farms (Njoro, Egerton and Kuresoi) (P<0.05), whereas there were similarities between the other three fish farms (Arahuka, Subukia and Dundori) (P>0.05). All farms were not significantly different in terms of mean concentration of NO_2-N except Arahuka (P<0.05). In addition, there was no significant difference between all fish farms with respect to NO_3-N (P>0.05), however, TN showed a significant difference in one fish farm which is Arahuka fish farm (P<0.05).

The phosphorus components concentrations recorded were significantly different between the fish farms under study. Soluble reactive phosphorus showed significant differences between fish farms (One-way ANOVA, F = 5.40, df (5, 48), p< 0.05), where the highest levels were recorded in Egerton and Subukia fish farms with a mean concentration of 146.25 ± 49.31 µg/l and 127.41 ± 35.72 µg/l respectively, and the lowest level as 3.13 ± 0.99 µg/l in Dundori fish farm. Similarly, the total phosphorus also showed significant differences between fish farms (One-way ANOVA, F = 35.64, df (5, 48), p< 0.05), where the highest level recorded was 780.79 ± 279.35 µg/l in Subukia and the lowest level as 110.79 ± 35.59 µg/l in Kuresoi fish farm. The variation of nutrient concentration in the studied fish farms in Nakuru County are presented in Figure 4. The post-hoc test showed no significant difference (P>0.05) between

fish farms in terms of SRP, whereas the TP showed significant differences between Subukia, Dundori and Kuresoi fish farms (P<0.05) while there were similarities between Arahuka, Njoro and Egerton fish farms (P>0.05).
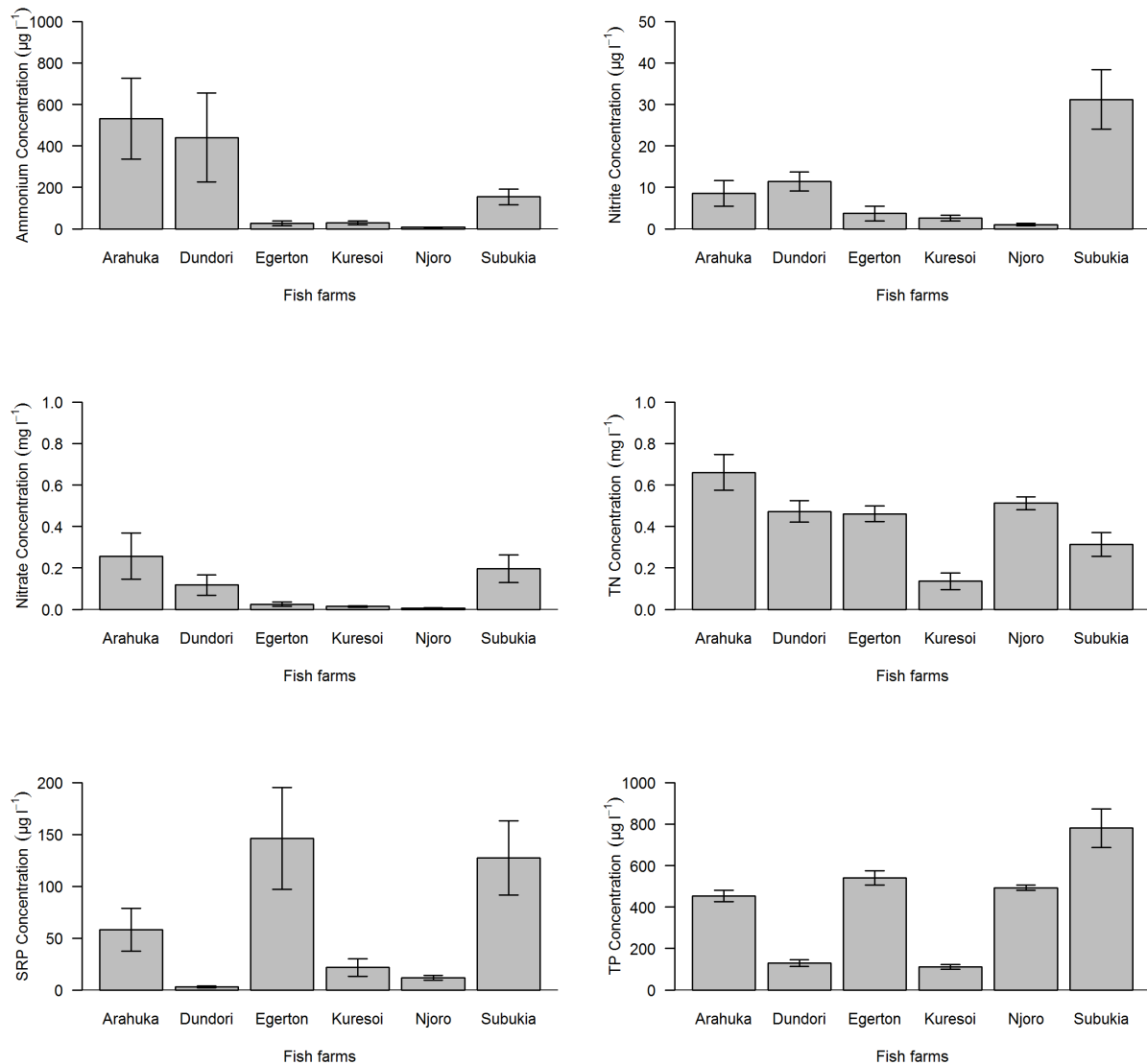
Figure 4: Bar graphs showing mean values of nutrient concentrations (both nitrogen and phosphorus components) in the different fish farms studied (mean± SD, n=18)

4.2.3 Relationship between physico-chemical parameters in the six fish farms in Nakuru County

Water temperature was positively correlated to DO in all the six fish farms surveyed, while turbidity and conductivity were negatively correlated in three fish farms (Arahuka, Njoro and Dundori) as presented in Table 4.

Table 4: Correlation matrix between physico-chemical parameters in the six fish farms

Farm Name	Parameters	DO	pH	Temp	EC	Turbidity
Arahuka	DO	1	0.098	0.963**	0.565	-0.431
	pH	0.098	1	-0.056	-0.563	0.769*
	Temp	0.963**	-0.056	1	0.606	-0.565
	EC	0.565	-0.563	0.606	1	-0.888**
	Turbidity	-0.431	0.769*	-0.565	-0.888**	1
Subukia	DO	1	0.621	0.953**	-0.353	-0.580
	pH	0.621	1	0.651	-0.675*	-0.714*
	Temp	0.953**	0.651	1	-.590	-.779*
	EC	-0.353	-0.675*	-0.590	1	0.961**
	Turbidity	-0.580	-0.714*	-0.779*	0.961**	1
Njoro	DO	1	0.023	0.627	-0.950**	0.898**
	pH	0.023	1	-0.734*	0.214	-0.067
	Temp	0.627	-0.734*	1	-0.799**	0.671*
	EC	-0.950**	0.214	-0.799**	1	-0.908**
	Turbidity	0.898**	-0.067	0.671*	-0.908**	1
Dundori	DO	1	-0.743*	0.095	-0.742*	-0.161
	pH	-0.743*	1	-0.476	0.522	0.570
	Temp	0.095	-0.476	1	0.423	-0.785*
	EC	-0.742*	0.522	0.423	1	-0.071
	Turbidity	-0.161	0.570	-0.785*	-0.071	1
Egerton	DO	1	-0.192	0.418	0.855**	0.233
	pH	-0.192	1	-0.934**	0.065	0.135
	Temp	0.418	-0.934**	1	0.232	0.017
	EC	0.855**	0.065	0.232	1	0.580
	Turbidity	0.233	0.135	0.017	0.580	1
Kuresoi	DO	1	0.419	0.412	0.867**	0.983**
	pH	0.419	1	0.838**	0.030	0.449
	Temp	0.412	0.838**	1	-0.016	0.424
	EC	0.867**	0.030	-0.016	1	0.888**
	Turbidity	0.983**	0.449	0.424	0.888**	1

**. Correlation is significant at the 0.01 level (2-tailed) and *. Correlation is significant at the 0.05 level (2-tailed).

Similarly, water temperature also showed a negative correlation to turbidity in most fish farms except Njoro, Kuresoi and Egerton fish farms, while pH levels had a positive correlation with turbidity in Arahuka, Dundori, Egerton and Kuresoi fish farms except in Subukia and Njoro fish farms. In Subukia and Kuresoi fish farms, conductivity displayed strong positive correlation to turbidity while in Njoro fish farm, it indicated a strong negative correlation. Dissolved Oxygen and conductivity were also positively correlated in Arahuka, Egerton and Kuresoi fish farms, although they were negatively correlated in Subukia, Njoro and Dundori fish farms.

A hierarchical cluster analysis using a rescaled distance cluster combine to identify similarities among different fish farms that were studied based on the selected physico-chemical parameters was performed. These physico-chemical parameters were obtained from a correlation matrix, where the significantly positive correlated parameters (DO, temperature, pH, conductivity and NH_4-N) were used for cluster analysis. These selected parameters are crucial for the cultured fish since they enhance the growth and maintain the health of fish in ponds as noted in most literature. The resulting dendrogram showed two broad clusters of different fish farms categorized as low to high levels of the above mentioned parameters. These dendrogram show two large clusters not closely related but connected with a distance between groups which is equivalent to 25 as presented in Figure 5.

The first cluster consisted of four fish farms namely: Njoro, Kuresoi, Egerton and Subukia fish farms. These four fish farms were found to have similarities based on their low mean values of the physico-chemical parameters used. For instance, the mean ammonia concentration ranged between 7.08 ± 2.07 µg/l and 154.12 ± 38.68 µg/l. It is important to note that the four farms had similar concentrations of the physico-chemical parameters, even though the management practises such as feeding, water exchange, stocking density and fertilization of the ponds were different in each farm.

The second cluster comprised of two fish farms namely: Arahuka and Dundori fish farms had the highest mean ammonia concentration of 531.96 ± 195.67 µg/l and 440.33 ± 214.27 µg/l respectively. These two farms were also having similar concentration of physico-chemical parameters, especially the highest ammonia concentration which may be attributed to by first accumulation of organic matter that decomposed at high rate due to high temperature as seen in Arahuka fish farm, secondly by the presence of hydric soil which has less oxygen (anoxic) as a characteristic of wetland as seen in Dundori fish farm which was constructed in a wetland.
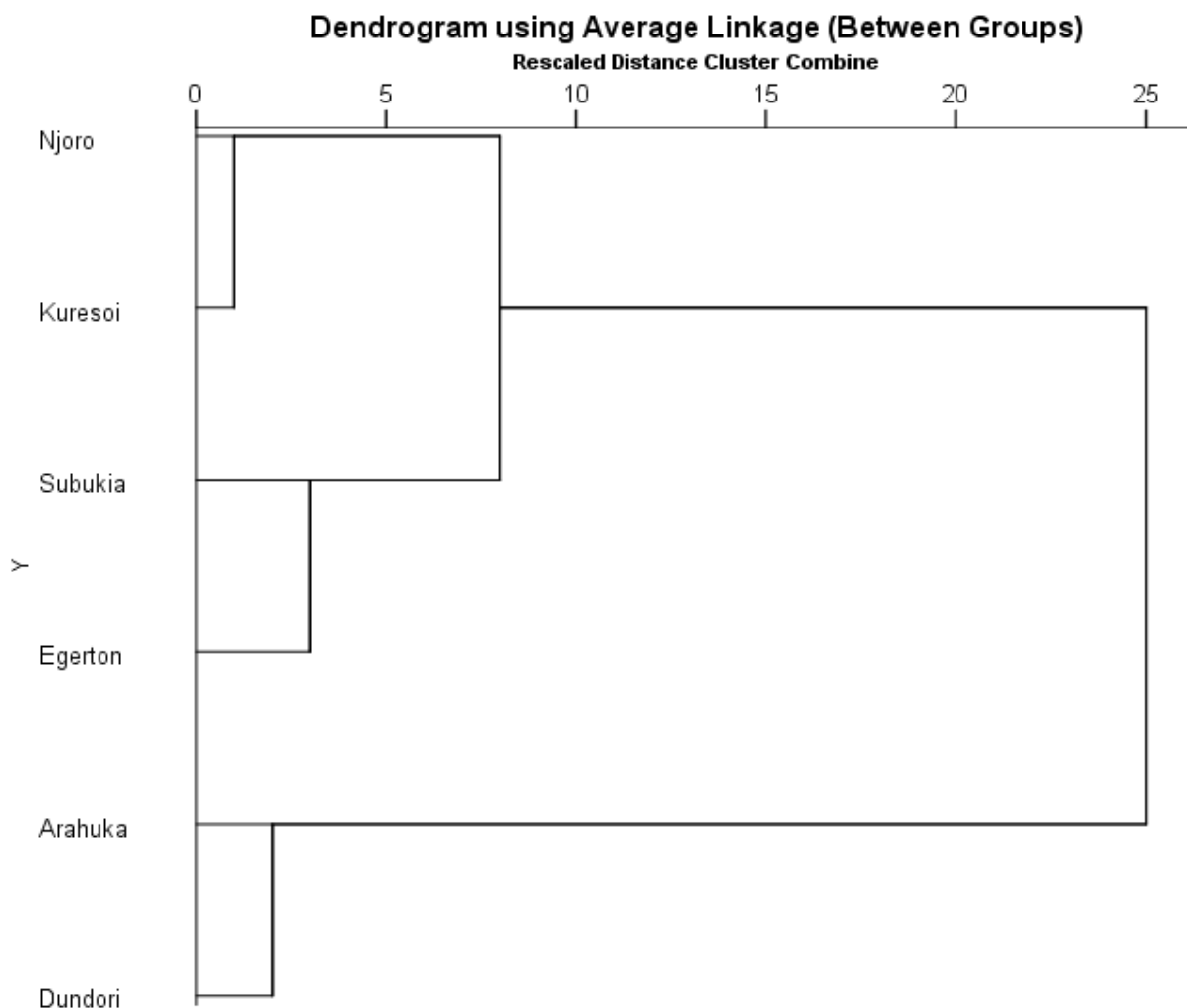
Figure 5: Dendrogram resulting from the similarity matrix based on the mean values of dissolved oxygen, conductivity, pH, temperature and ammonia concentration for the six fish farms

4.3 Results of parasitological analysis of *O. niloticus* in the studied fish farms in Nakuru County

4.3.1 Parasites identified in/on *Oreochromis niloticus*

Out of the 300 fish examined, 252 were found infested with both external and internal parasites. The overall prevalence of parasite infection was 84 %. In this study, 15 species of parasites were recovered. These were: *Lamproglena monodi* (Plate 9a), *Lernaea cyprinacea* (Plate 9b), *Cichlidogyrus halli* (Plate 10a), *Trichodina sp* (Plate 10b), *Gyrodactylus sp* (Plate 11a), *Heterophyes sp* (Plate 11b), *Cichlidogyrus sclerosus* (Plate 12), *Acanthosentis tilapiae* (Plate 13), *Amirthalingamia macracantha* (Plate 14), *Tylodelphys sp.* (Plate 15), *Argulus sp.*, unidentified cestode, *Chilodonella sp.*, *Cryptobia sp.*, *Ichthyobodo sp.* and *Microsporidia sp.*

Most external parasites were detected from the gills, followed by the skin, whereas the internal parasites were detected in the intestinal wall, lumen and eyes of the fish.
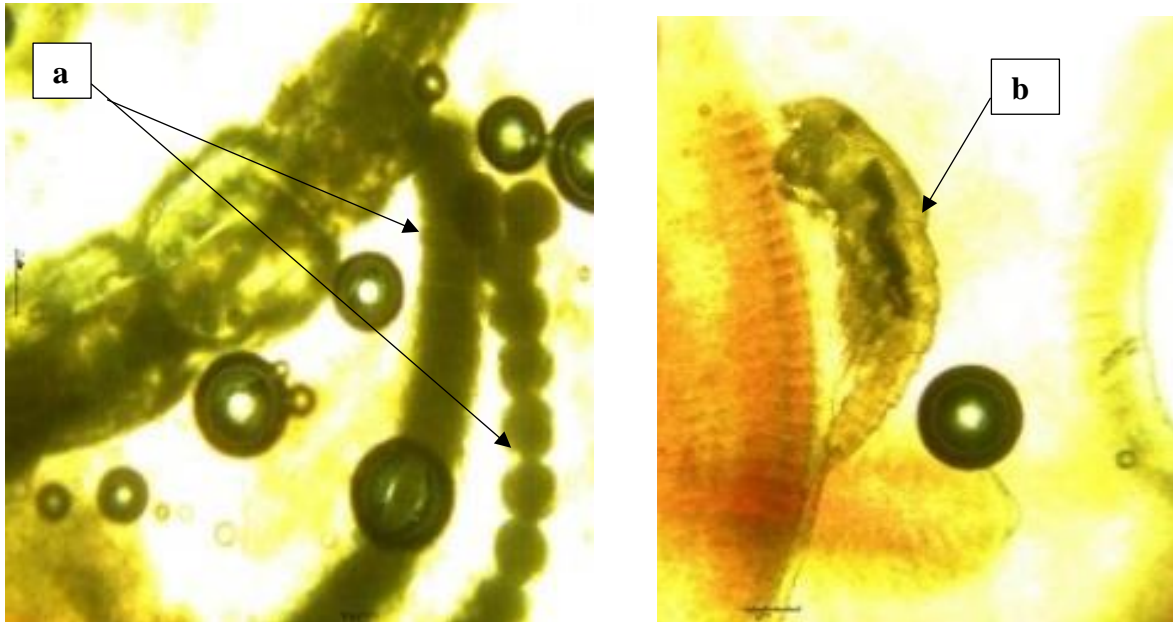
Plate 9: Photographs showing (a) two ovigerous (egg) sacs of *Lamproglena monodi* and (b) *Lernaea cyprinacea* identified during the survey (Magnification ×40)

Plate 10: Photographs showing (a) two *Cichlidogyrus halli* and (b) *Trichodina sp.* identified during the survey (Magnification ×100)
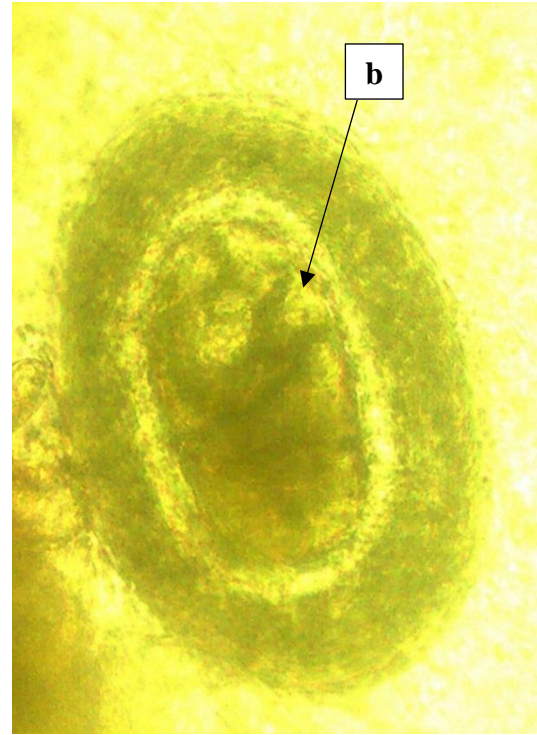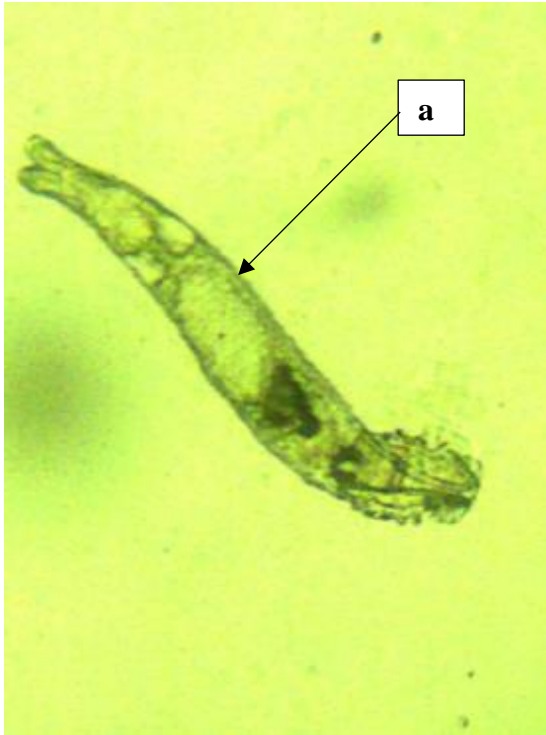
Plate 11: Photographs showing (a) *Gyrodactylus sp*. and (b) *Heterophyes sp*. identified in the gills of fish during the survey (Magnification ×400)

Plate 12: Photographs showing *Cichlidogyrus sclerosus* identified in the gills of fish during the survey (Magnification ×400)

Plate 13: Photographs showing (a) *Acanthosentis tilapiae* and (b) retractile proboscis of the *Acanthosentis tilapiae* (Magnification ×100)
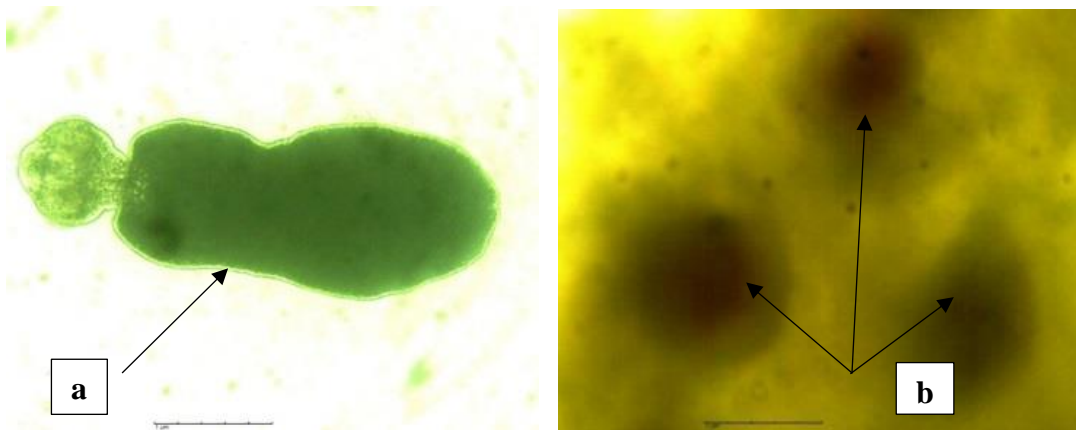
Plate 14: Photographs showing (a) *Amirthalingamia macracantha* and (b) cysts in fish intestinal wall observed during the survey (Magnification ×100)
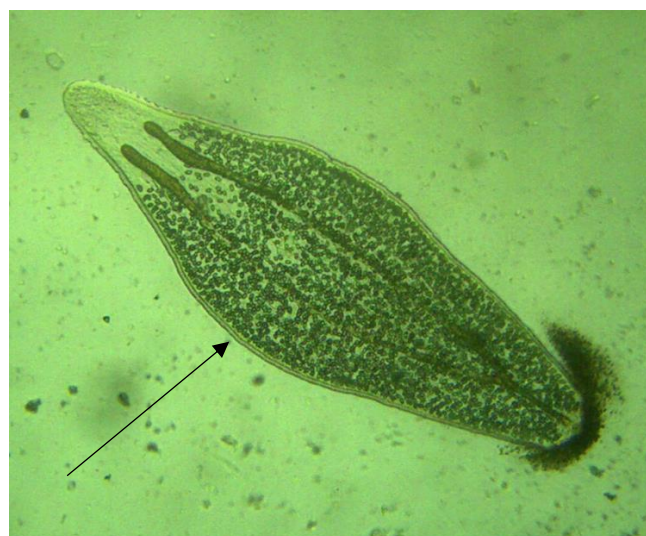
Plate 15: Photographs showing *Tylodelphys sp*. in the eyes of fish observed during the survey (Magnification ×100)

4.3.2 Prevalence, mean intensity and mean abundance of parasites in the fish farms

The prevalence of parasites was calculated for each fish farm and for the species identified from the study. In this study, Subukia fish farm had the highest number of species of parasites (10 species of parasites) followed by Arahuka and Egerton fish farms (9 species of parasites each), Kuresoi fish farm (8 species of parasites), Njoro fish farm (4 species of parasites) and lastly, Dundori fish farm (3 species of parasites).

In Arahuka fish farm, monogenean parasites (*Cichlidogyrus halli*) were dominant in prevalence, mean intensity and mean abundance, followed by protozoan parasites (*Trichodina spp*) which had a higher prevalence with medium intensity and low abundance. According to the findings, the gills were the most infected organs of the fish examined from this farm, followed by the skin with the intestine of one sampled fish recording one *Amirthalingamia macracantha* as presented in Table 5.

Table 5: The prevalence, mean intensity and mean abundance of parasites found in *Oreochromis niloticus* from Arahuka fish farm, n=50

Parasites	Organ infected	Prevalence (%)	Mean intensity	Mean abundance
Protozoa				
Trichodina spp	Gills/skin	62.0	Medium	Low
Ichthyobodo sp	Skin	6.0	Low	Low
Microsporidia sp	Skin	8.0	Low	Low
Cryptobia sp	Skin	14.0	Medium	Low
Monogenea				
Cichlidogyrus halli	Gills	92.0	17.59	16.18
Cichlidogyrus sclerosus	Gills	14.0	2.57	0.36
Cestoda				
Amirthalingamia macracantha	Intestine	2.0	1.00	0.02
Crustacea				
Argulus sp	Gills	2.0	1.00	0.02

In Subukia fish farm, *Amirthalingamia macracantha* were dominant in prevalence, mean intensities and mean abundances compared to all fish farms surveyed, followed by *Cichlidogyrus halli* which had a higher prevalence and low mean intensity and mean abundance. *Trichodina spp*, *Cryptobia sp* and *Acanthosentis tilapiae* also recorded slightly high prevalence with low mean intensity. *Gyrodactylus sp* was only recorded in this fish farm with a high mean intensity of 19.0 than in other fish farms. In this farm, intestinal walls and lumen were the most infected organs of the fish examined, followed by the gills and the skin (Table 6).

Table 6: The prevalence, mean intensity and mean abundance of parasites found in *Oreochromis niloticus* from Subukia fish farm, n=50

Parasites	Organ infected	Prevalence (%)	Mean intensity	Mean abundance
Protozoa				
Trichodina sp	Gill/skin	28.0	Medium	Low
Ichthyobodo sp	Gills	4.0	Low	Low
Microsporidia sp	Gills	2.0	Low	Low
Cryptobia sp	Gills/skin	14.0	High	Low
Monogenea				
Cichlidogyrus halli	Gills	46.0	4.35	2.00
Cichlidogyrus sclerosus	Gills	4.0	1.5	0.06
Gyrodactylus sp	Gills	8.0	19.00	1.52
Cestoda				
Amirthalingamia macracantha	Intestine	64.0	43.25	27.68
Unidentified Cestode	Intestine	2.0	1.00	0.003
Acanthocephala				
Acanthosentis tilapiae	Intestine	76.0	7.11	0.90

In Egerton fish farm, *Cichlidogyrus halli* and *Cichlidogyrus sclerosus* were dominant and had high prevalence, mean intensities and mean abundances. *Tylodelphys sp* was only recorded in one fish at Egerton fish farm with an intensity of 37.0. Gills were the infected organs of the fish examined from this farm, followed by the intestine and the eyes (Table 7).

Table 7: The prevalence, mean intensity and mean abundance of parasites found in
Oreochromis niloticus from Egerton fish farm, n=50

Parasites	Organ infected	Prevalence (%)	Mean intensity	Mean abundance
Protozoa				
Trichodina sp	Gills	18.0	Medium	Low
Cryptobia sp	Gills	4.0	High	Low
Monogenea				
Cichlidogyrus halli	Gills	98.0	49.63	48.64
Cichlidogyrus sclerosus	Gills	76.0	16.34	12.42
Digenea				
Tylodelphys sp	Eyes	2.0	37.00	0.12
Heterophyes sp	Gills	8.0	7.00	0.56
Cestoda				
Unidentified cestode	Intestine	4.0	2.00	0.01
Acanthocephala				
Acanthosentis tilapiae	Intestine	38.0	5.58	0.35
Crustacea				
Lamproglena monodi	Gills	22.0	1.45	0.05

In Dundori fish farm, *Trichodina spp* were dominant in prevalence, mean intensity and mean abundance. In this farm, gills were the only infected organs of the fish examined (Table 8).

Table 8: The prevalence, mean intensity and mean abundance of parasites found in
Oreochromis niloticus from Dundori fish farm, n=50

Parasites	Organ infected	Prevalence (%)	Mean intensity	Mean abundance
Protozoa				
Trichodina sp	Gills	12.0	Medium	Low
Monogenea				
Cichlidogyrus halli	Gills	2.0	1.00	0.02
Crustacea				
Lernaea cyprinacea	Gills	2.0	1.00	0.003

In Kuresoi fish farm, *Cichlidogyrus halli* was dominant in prevalence but had low mean intensity and mean abundance, followed by *Cichlidogyrus sclerosus, Trichodina spp* and *Ichthyobodo sp* which had high prevalence and low mean intensity and mean abundance. In this study, gills were the most infected organs of the fish examined from this farm, followed by intestine which had the lowest infection levels (Table 9).

Table 9: The prevalence, mean intensity and mean abundance of parasites found in *Oreochromis niloticus* from Kuresoi fish farm, n=50

Parasites	Organ infected	Prevalence (%)	Mean intensity	Mean abundance
Protozoa				
Trichodina sp	Gills	24.0	Medium	Low
Chilodonella sp	Gills	6.0	Low	Low
Ichthyobodo sp	Gills	22.0	Low	Low
Microsporidia sp	Gills	4.0	Low	Low
Monogenea				
Cichlidogyrus halli	Gills	80.0	7.05	5.64
Cichlidogyrus sclerosus	Gills	32.0	5.25	1.68
Digenea				
Heterophyes sp	Gills	6.0	1.00	0.06
Acanthocephala				
Acanthosentis tilapiae	Intestine	6.0	2.00	0.02

In Njoro fish farm, *Cichlidogyrus halli* was dominant in prevalence, mean intensity and mean abundance, followed by *Trichodina spp.* and *Cichlidogyrus sclerosus* which had a higher prevalence but low mean intensity and mean abundance. According to the findings, only gills were the most infected organs of the fish examined from this farm (Table 10).

Table 10: The prevalence, mean intensity and mean abundance of parasites found in *Oreochromis niloticus* from Njoro fish farm, n=50

Parasites	Organ infected	Prevalence (%)	Mean intensity	Mean abundance
Protozoa				
Trichodina sp	Gills	16.0	Low	Low
Cryptobia sp	Gills	4.0	Low	Low
Monogenea				
Cichlidogyrus halli	Gills	98.0	21.71	21.28
Cichlidogyrus sclerosus	Gills	12.0	1.17	0.14

There were similarities in terms of the species of parasites recovered between the fish farms. For instance, *Cichlidogyrus halli* and *Trichodina spp* were found in all the six farms. On the other hand, *Acanthosentis tilapiae* was only recorded in three fish farms: Egerton, Kuresoi and Subukia farms (Figure 6).
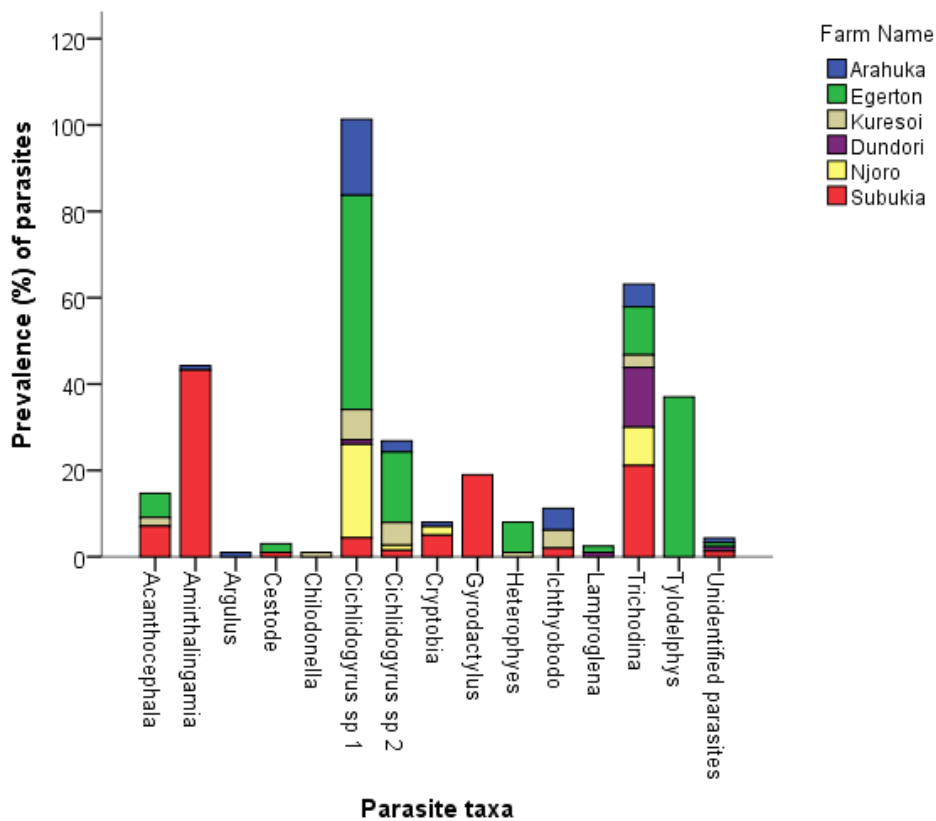
Figure 6: Summary of different parasites found in *Oreochromis niloticus* in different fish farms (six), the patterns represent different fish farms, n=252 (fish infected)

Other species of parasites only existed in one or two but not in all the six fish farms surveyed, for example, *Amirthalingamia macracantha* and *Gyrodactylus* sp which were recorded in Subukia fish farm while, *Lamproglena monodi* was found in Egerton fish farm as summarized in Figure 6. Some of the parasites could not be identified easily as their morphological characteristics were not visible under the microscope (see appendix 2).

4.3.3 Diversity of parasites in *Oreochromis niloticus* in fish farms in Nakuru County

The diversity and abundance of parasites varied widely among the fish farms. There was a high diversity of parasites infecting *Oreochromis niloticus* in Kuresoi with Shannon-Wiener index of 1.20, followed by Subukia with 1.10, Egerton with 0.96 and Arahuka 0.91 (Table 11). Njoro and Dundori fish farms recorded the lowest diversity of parasites as 0.29 and 0.13 respectively. Dundori and Njoro showed the highest Simpson index of dominancy of 0.95 and 0.87 respectively with the lowest of 0.45 and 0.42 recorded in Subukia and Kuresoi fish farms respectively. Species richness was highest in Subukia, Kuresoi, Arahuka and Egerton fish farms (Margalef richness index 1.20, 1.10, 1.00 and 0.98 respectively) compared to Dundori and Njoro with 0.45 and 0.43 respectively.

Table 11: Shannon-Wiener index, Simpson index and Margalef richness index of parasites of *Oreochromis niloticus* in each fish farm

Diversity characteristic	Fish farms					
	Arahuka	Egerton	Dundori	Kuresoi	Njoro	Subukia
Shannon-Wiener Index	0.91	0.96	0.13	1.20	0.29	1.10
Simpson Index	0.55	0.53	0.95	0.42	0.87	0.45
Margalef Richness Index	1.00	0.98	0.45	1.10	0.43	1.20

4.3.4 Relationship between water quality and parasite infection levels

Selected physico-chemical parameters which showed significant differences between fish farms were used to relate the effects of water quality on parasite assemblages infecting *O. niloticus* from the six fish farms. This relationship was done using two species of parasites: *Cichlidogyrus halli* and *Trichodina* spp which were common in all fish farms as summarized in Table 12.

Table 12: Selected physico-chemical parameters and common species of parasites (*Cichlidogyrus halli*) in the six fish farms

Parameters	Fish farms					
	Arahuka	Dundori	Egerton	Kuresoi	Njoro	Subukia
EC (µs/cm)	128.47	230.71	412.85	46.90	167.13	300.63
DO (mg/l)	14.06	9.28	15.80	8.35	12.10	9.31
Temp. (°C)	26.66	21.10	21.04	21.69	21.14	20.52
Abundance of *Cichlidogyrus halli*	809	1	2432	282	1064	100

A scatter plot was used to find out the relationship between water quality and abundance of parasites in the six fish farms (Figure 7). There was a weak positive correlation between abundance of parasites and mean conductivity (R^2=0.2716) although not significant. However, this indicates that the parasite infection rate was increasing with the increase in mean conductivity in the fish farms (Table 13).
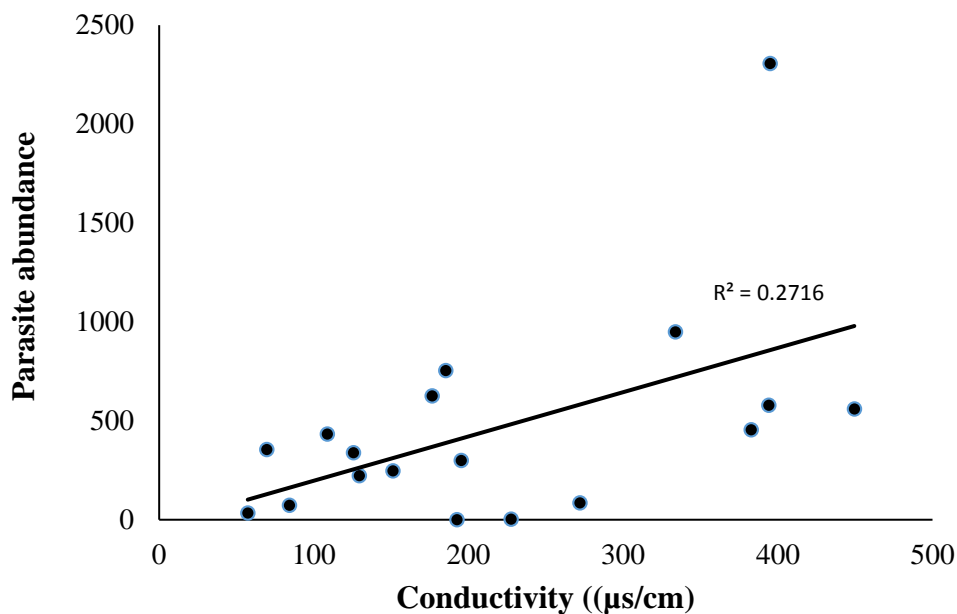
Figure 7: A scatter plot showing the relationship between the selected physico-chemical parameter (Conductivity) and the abundance of parasites in the six fish farms sampled.

Table 13: Pearson correlation matrix between physico-chemical parameters and most common parasite (*Cichlidogyrus halli*) abundance in the six fish farms

Variables	DO	pH	Temp.	Conductivity	*Cichlidogyrus halli*
DO	1	0.474*	0.562*	0.207	0.484*
pH	0.474*	1	0.034	0.412	0.353
Temp	0.562*	0.034	1	-0.356	0.053
Conductivity	0.207	0.412	-0.356	1	0.411
Cichlidogyrus halli	0.484*	0.353	0.053	0.411	1

*. Correlation is significant at the 0.05 level (2-tailed).

4.4 Relationship between fish size and parasite infection levels in fish farms

4.4.1 The intensity of parasites in relation to the fish length (cm)

Most of the fish in each fish farm were ranging between 1-2 years of age with average length of 20 cm. The highest number of fish sampled and examined were those from the category size class 21-25 cm. The majority of the farms had fish of this size class. Generally, the number of parasites increases with the increase in fish length. The common species in all fish farms, especially *Cichlidogyrus halli* were used to determine this relationship. The result indicated an increase in parasite intensities with the increase in fish size. However, the number of parasites peaked at fish length class 21-25 cm after which there was a decrease in number of parasites at length class 26-40 (Figure 8).
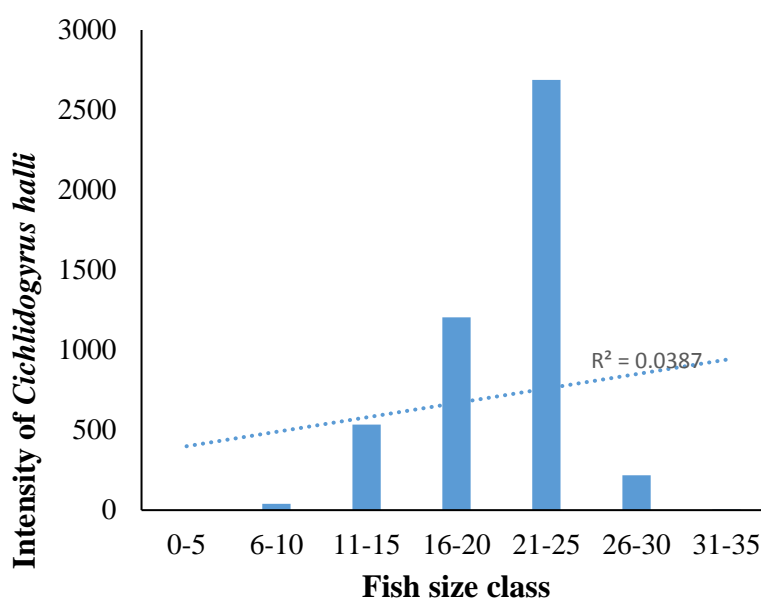
Figure 8: Bar graphs indicating the overall abundance of *Cichlidogyrus halli* in relation to fish size classes of *Oreochromis niloticus* in all the six fish farms, n=300

4.4.2 Prevalence (%) of parasites in relation to fish length

The common species of parasites that occurred in all fish farms were used to compare fish size classes and prevalence of parasites. These were *Cichlidogyrus sp1* and *Trichodina sp*. The most prevalent among them was *Cichlidogyrus halli* followed by the *Trichodina sp* in *Oreochromis niloticus* in class size 21-25 cm. The highest recorded prevalence of *Cichlidogyrus halli* in this size class was 25.3 % and *Trichodina sp* had 9.7 %. However, both species recorded the lowest prevalence of 0.3 % in size class 31-35. The prevalence (%) showed a strong positive correlation between fish size and parasite infection levels (Figure 9) especially for *Cichlidogyrus halli* (r^2=0.94).
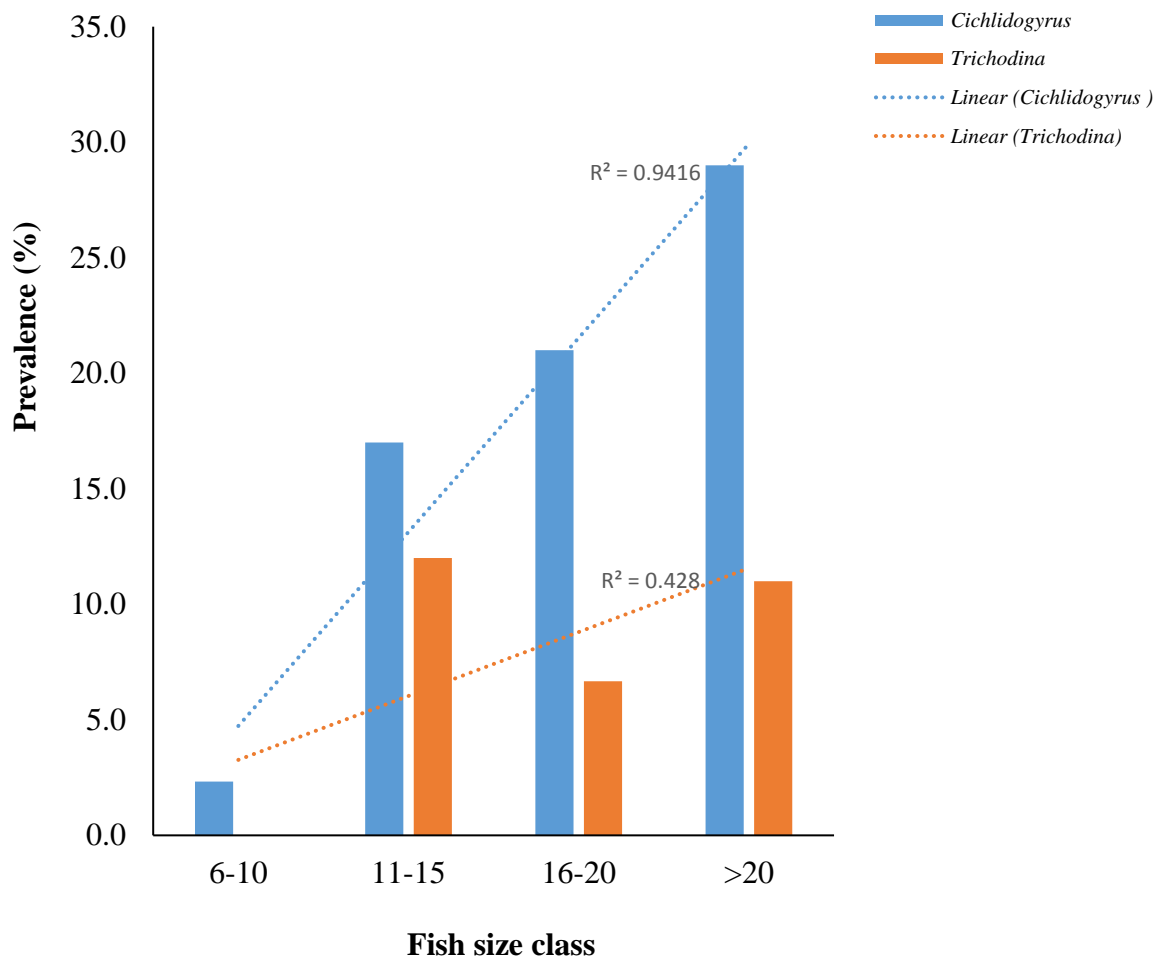
Figure 9: A bar graph showing the prevalence of parasite species in relation to the fish size classes in fish farms in Nakuru County, n=300

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4.5 Condition factors of parasitized and non-parasitized *Oreochromis niloticus*

The overall condition factor of *Oreochromis niloticus* from the six studied fish farms was above 1.0. The highest k value of individual fish (*O. niloticus*) recorded was 2.23 and the lowest was 1.03 in the six fish farms. The lowest condition factor was recorded at Egerton and Dundori fish farms of 1.03, while the highest condition factor was recorded in Subukia fish farm of 2.23. This study showed that the mean condition factors of each fish farm was lower in parasitized *O. niloticus* compared to the mean k value of the non-parasitized ones (Figure 10). However, there were no significant differences in condition factor between the parasitized and non-parasitized *Oreochromis niloticus* in all the six studied fish farms (t-test, df =10, P> 0.05).
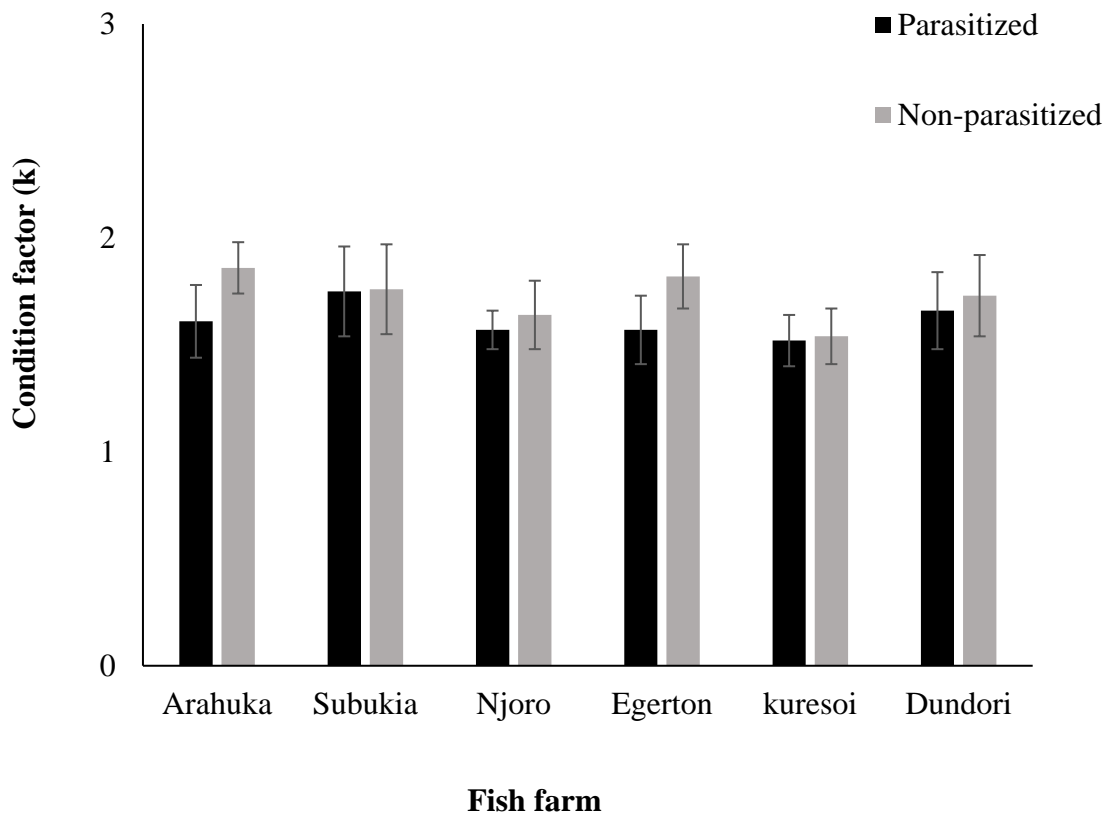
Figure 10: Bar graph showing the comparison between condition factor of parasitized and non-parasitized *Oreochromis niloticus* in fish farms in Nakuru County, n=300

CHAPTER FIVE

DISCUSSION

5.1 Water quality parameters in fish farms in Nakuru County

Generally, some of the physico-chemical parameters analysed such as dissolved oxygen, temperature and conductivity, fell within the desirable and acceptable limits. However, there were some values of ammonia (above 0.5 mg/l), pH (above 6.5-9.0) and turbidity higher than the acceptable limits in the ponds. Njoro and Dundori fish farms were found to be the best fish farms in terms of water quality management with their physico-chemical parameters (Table 3) within the acceptable range. This may be ascribed to the proper management practices in the two farms. The unique technique that was only practised in these farms was the frequent replacement of water in the ponds. For example, Njoro fish farm had a weekly water exchange using water from River Njoro and the released water from the farm was used to water trees in the nursery. Dundori fish farm which was constructed in a wetland had its water mixed daily through underground seepage which help in dilution of nutrients, thus maintaining desirable water quality conditions. Kuresoi fish farm did not add inputs in the pond therefore the water quality was good. However, the remaining three fish farms (Arahuka, Subukia and Egerton) had exceedingly deteriorated water quality with the highest levels recorded in this study. This could be due to the uncontrolled addition of livestock and poultry manure, overfeeding of fish, infrequent replacement of water as well as high stocking densities of the ponds. For example, Egerton fish farm had the highest conductivity which showed that the pond was being over-manured.

The range of the pH obtained from this study was 7.57-11.61, with the highest value slightly above the acceptable limit according to Stone and Thormforde (2003) and also above the standard range. The desirable range for pond pH is 6-9 (Almeida *et al*., 2008) and acceptable range is 5.5-10.0 (Stone and Thormforde, 2003). The optimum range recorded by Otachi *et al*. (2011) on his study conducted in Sagana and Machakos open ponds was 6.47-9.44. The fluctuations in water pH recorded in fish ponds in this study, may be attributed by the result of the interplay of photosynthesis and respiration, thus it is important to lime fish farms (Egerton and Subukia) to reduce fluctuations of pH in the fish farms to increase buffering capacity of the fish ponds, to enhance fertilization and improve fish production, stimulating the growth of phytoplankton and zooplankton which in turn act as food for fish, and also prevent wide swings in pH. Extreme alkaline water (greater than pH 9) is dangerous as ammonia toxicity increases rapidly. High water temperatures often make fish more sensitive to changes in pH levels. It is

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usually an important chemical parameter to consider because it affects the metabolism and other physiological processes of culture fish. The desirable range pH (6-9) should be maintained for growth and production.

The mean water temperature recorded in the six fish farms ranged from 20.52 °C to 26.66 °C (Table 3). In this study, water temperature showed a positive correlation with DO because the sampling was done mostly after mid-day when the rate of photosynthesis by phytoplankton was higher in the ponds releasing higher amounts of oxygen in water as a by-product. This results agreed with the ranges (23.4-24.0 °C) recorded by Liti *et al*. (2005) in the study conducted on the effects of open-pond density and caged biomass of Nile Tilapia (*Oreochromis niloticus* L.) on growth, feed utilization, economic returns and water quality in fertilized ponds. Otachi *et al*. (2011) also noted water temperature range from 22.38 °C to 31.8 °C in Sagana fish ponds. Temperature affects all chemical and biological processes and has a direct effect on important factors such as growth, oxygen demand, food requirements and food conversion efficiency. Increase in water temperature beyond 30 °C increases the activity level, for instance, metabolic rate of fish doubles for every rise of 10 °C, according to the study done by Allan *et al*. (1995) on effects of pond preparation and feeding rate on production of *Penaeus monodon* Fabricius, in model farming ponds. The higher the temperature, the greater the requirements such as food resulting to enhanced growth rate. In addition, temperature also has a crucial role in stimulating fish gonad maturation and spawning activity.

The dissolved oxygen obtained from this study was in the range of 8.35-15.80 mg/l. Dissolved oxygen is one of the environmental parameters that exerts 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. This observation agreed with the findings of Onada (2015), who reported a range of 7.04 - 13.00 mg/l in the study conducted on interrelationship among water quality parameters in earthen pond and concrete tank. Otachi *et al*. (2011) recorded slightly lower values ranging from 2.84 - 10.46 mg/l on the study carried out Sagana and Machakos open ponds, similar to Bagge *et al*. (2004) (3-10 mg/l of DO) study on fish population size as the determining factor of parasite infection in nine ponds in Finland. Onada (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 (Brown *et al*., 2011). However, Liti *et al*. (2005) noted lower levels of DO (ranging from 1.6 - 3.2 mg/l) in the study conducted on the effects of open-pond density and caged biomass of Nile Tilapia (*Oreochromis niloticus* L.) on growth, feed utilization, economic returns and water quality in fertilized ponds

at dawn when respiration rate was dominating. At dawn oxygen depletion is attributed to aerobic organisms such as fish in the pond, whereas high DO levels during the day is a result of photosynthetic activities by phytoplankton when light intensity is high.

Ammonia is the second gas of importance in fish culture; its significance to good fish production is vast. The ammonia level recorded ranges between 0.007 - 0.531 mg/l. Even though, the desirable limit is 0 - 0.05 mg/l and acceptable limit less than 0.5 mg/l. This study obtained ammonia levels that were close to the tolerable level. High ammonia levels can result from overfeeding, protein rich, excess feed decays that liberate toxic ammonia gas, coupled with the fact that fishes, excrete ammonia which may accumulate to dangerously high levels under certain conditions. Fortunately, ammonia concentrations are partially buffered by conversion to non-toxic nitrate ion by nitrifying bacteria. Additionally, ammonia is converted from toxic ammonia (NH_3) to non-toxic ammonium ion (NH_4^+) at pH below 8.0. However, the high levels of ammonia obtained may be due the effect of pH, which was positively correlated with ammonia in this study. Nutrient concentration, particularly ammonia was high in Dundori and Arahuka fish farm. The high ammonia obtained in Dundori could be explained by the fact that the farm was established in a wetland with low oxygen content resulting to low nitrification process hence ammonium accumulation together with frequent fertilization of the ponds. On the other hand, Arahuka experienced low water level and high water temperature, which triggers increase in the decomposition of the organic matter from the bottom of the pond resulting to high ammonia. There was high phosphorus in Egerton and Subukia fish farms due to the frequent fertilization of the pond.

The variable mean water turbidity recorded in this study ranging from 35.64 - 550 NTU could have been as a result of different management approaches practised in the farms. Turbidity in shallow water systems such as ponds normally results from suspended solids (clay) resulting to muddy water which could be harmful for fish by increasing chances of parasite infections. Also high turbidity reduces natural food production in ponds.

5.2 Relationship between water quality and parasites infestation in Nile tilapia

Water quality is one of the most important prerequisite of production of healthy fish (Hossain *et al*., 2007). The occurrence and magnitude of infections are closely related to the sanitary conditions prevalent in the water and also the general health of the fishes themselves. The parasite community of fishes shows considerable variation with the environmental conditions in which they live. Various physico-chemical factors such as water temperature, ammonia, DO,

pH, conductivity and turbidity have strong influence on fish health and their resistance against the disease causing agents (Hossain *et al*., 2007). The physiological and biological features of the host affect the composition of parasites. Poor condition of physico-chemical properties of water is oxygen depletion, excess ammonia in water and temperature change below and above the optimum (20-35 °C). Excess feeding can result in an increase in organic material, a decrease in DO due to oxidation by bacteria and an increase in metabolic wastes (Allan *et al*., 1995).

This study showed significant differences in most of the physico-chemical parameters in the fish farms studied (p< 0.05). Egerton and Subukia fish farms had pH values higher than the tolerable levels by fish (6-9) (Almeida *et al*., 2008). Higher pH values (greater than acceptable level of 10.0) have been reported to have an effect on fish health and parasite infestations as well as infections (Hossain *et al*., 2007). This also applies to this study, where the fish farms with the highest pH such as Egerton and Subukia fish farms recorded the highest mean intensities of parasites (68.9 % and 43.16 % respectively). The lower levels of water pH (lower than the optimum range of 6-9) increases the toxicities of hydrogen sulphide, copper and other metals to fish. Fish are known to become susceptible to parasites infestation and diseases more in acidic waters (Stone and Thormforde, 2003). When pH rises to above 11, the gills, lens and cornea of the fish eyes get destroyed, resulting to fish becoming vulnerable to infections (Hossain *et al*., 2007). However, the results of this study showed that the fish farms with high pH values had more parasites intensities compared to those with low pH values. For example, Egerton fish farm which had the highest pH of 11.31 recorded a total of 2,432 *Cichlidogyrus halli* compared to Dundori fish farm with the lowest pH of 7.57 which recorded 1 *Cichlidogyrus halli*. This implies that toxicity increases the prevalence of the external parasites and vulnerability of the fish (Karvonen, 2012) as the case in this study where more ectoparasites were recorded in Egerton fish farm, for instance, *Cichlidogyrus halli* which recorded a prevalence of 98.0 %.

The water conductivity obtained at Egerton and Subukia fish farms had the highest mean values (412 and 300 µs/cm) compared to other farms. Otachi (2009) recorded conductivity of up to 930 µs/cm in Machakos ponds which was triple the findings of this study. It has been reported that parasites tend to thrive better in ponds with high ionic concentration than those with low conductivity. This is because waters with a high conductivity are more productive hence likely to harbour more intermediate host of parasites. For instance, in this study, Subukia fish farm recorded a huge number of *Amirthalingamia macracantha*. In addition, the higher the fertilization in the pond the higher the productivity, consequently increase in phytoplankton

biomass which serve as food for zooplanktons particularly copepods. Copepods are well known to be the intermediate host of *Amirthalingamia macracantha*. In addition, nutrient concentration in this study was found to influence the snail population in earthen pond (Subukia farm). Similar finding was noted by McKenzie and Townsend (2007) in the study conducted on parasites and infectious disease responses to changes in nutrient availability. This could be explained by the fact that snails consume primary producers such as phytoplanktons and therefore, nutrients can trigger bottom-up effect whereby increased algal growth provides more available food. Figure 11 below elaborates on this relationship and response of parasites and pathogens to changes in water quality.
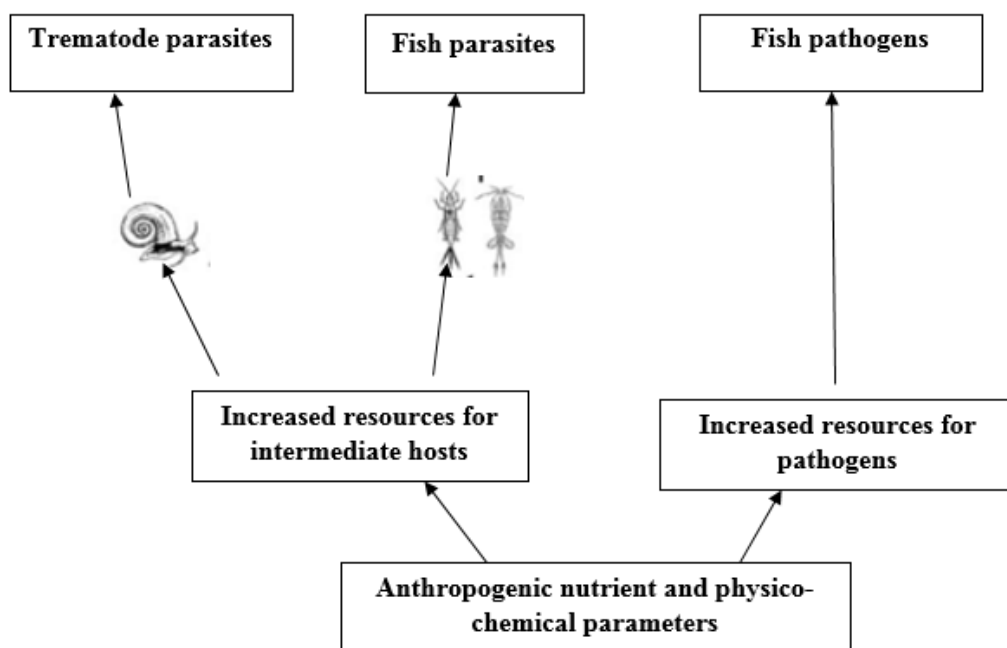
Figure 11: Conceptual model of mechanisms by which parasites and pathogens respond to increased physico-chemical parameters and nutrient concentration (Adopted from McKenzie and Townsend, 2007)

Dissolved oxygen (DO) concentration showed significant differences between fish farms. In this study, when Subukia fish farm recorded low DO level of DO of 5.69 mg/l, *Gyrodactylus sp* were also recorded with a mean intensity of 19 individual parasites per fish host. Similarly, Kuresoi fish farm also recorded *Chilodonella sp* at DO level of 7.51 mg/l. In other findings, low dissolved oxygen concentrations (ranging from 3.0 - 7.5 mg/l) in water coupled with low light conditions has been reported to cause Chilodonellosis outbreak (Hossain and Rahman, 2007). This infers that low oxygen concentrations favours the survival of parasites such as *Chilodonella*. This study recorded *Gyrodactylus sp* in Subukia when low DO was recorded.

Low dissolved oxygen weakens fish's immune system together with increased reproduction of the parasites, resulting to heavy infection of the fish host. Paredes-trujillo *et al*. (2016) noted that the mean abundance of *Gyrodactylus sp* increase in ponds when the DO values are low (5-8 mg/l), according to the study conducted on the geographical distribution of protozoan and metazoan parasites of farmed Nile tilapia *Oreochromis niloticus*. The study further explained that the probability of the increase in transmission and reproduction of *Gyrodactylus sp* could also be ascribed to the absence of the adequate application of management procedures such as appropriate isolation of new fingerlings in a specific quarantine facilities before being released into the production pond.

Water temperature showed variations among fish farms with significant differences. The highest mean water temperature was recorded in Arahuka fish farm of 26.66 °C and the lowest of 20.52 °C in Subukia farm. This is the most important abiotic water characteristic that influences the dynamics of parasitic assemblages, prevalence, and abundance in fish species (Lal and Kumar, 2015). They further noted that temperature sometimes affects the manner in which the parasites develop and establish themselves, how they release their infective stages and their movement to the hosts. Water temperature does not only affect the parasites directly but also indirectly by favouring the occurrence of intermediate hosts. The intermediate hosts thrive in the warm conditions and reduce in numbers during the cold season. This infers that warmer conditions favour the survival of monogenean parasites such as *Cichlidogyrus halli* as recorded in Arahuka fish farm with the highest water temperature, whereas the same species flourished best in Egerton fish farm because of high stocking density and high pH levels recorded.

Monogenean parasites such as *Cichlidogyrus* sp were found to occur in all the fish farms due to the favourable environmental conditions available in the fish farms as well as their simple life cycle which make them easily transmitted from one fish host to another particularly in overcrowded fish farms as shown in Egerton fish farms. Their reproduction rate is favoured by high water temperature. For instance, the parasitic eggs of the monogenean parasite hatch after 13 days from the attachment of the larval when in water temperature of 20 °C, 8 days in water temperature of 25 °C and takes only 6 days in water at 30 °C (Lopesa, 2011), according to the study conducted on environmental quality assessment through analysis of the frequency of the black spot disease in an assemblage of fish. This simply indicates how favourable higher temperature are for fish parasites. It should, however, be noted that different parasites react differently to different water temperature. However, the best temperature for the hatching of

the parasites is 20-25 °C. Although higher temperatures are favourable for the hatching of the parasitic eggs but not for the attachment to the host (Puinyabati *et al*., 2013). Therefore, the larval stage is usually shorter with increase in temperature. The differences of monogenean prevalence in the present study could have been influenced by differences in the water temperatures ranges in the six fish farms. Otachi (2009) also recorded 80 % prevalence of monogeneans such as *Cichlidogyrus sp* in most of Nile tilapia grown in fish farms in Kenya. In addition, Bagge *et al*. (2004) noted that the high stocking densities of cultured fish results in crowding in fish ponds which in turn increases the number of monogenean species occurring on the gills of fish.

Turbidity showed significant differences between fish farms with Subukia recording the highest turbidity of 550.00 NTU and the lowest 35.64 NTU at Egerton farm. The turbidity was found to be high in earthen ponds than liner ponds because the liner ponds are fitted with protective impermeable material to ensure water containment hence no direct contact with the sediment as in the case of earthen ponds. The highest infestation levels in fish in a high turbid farm (Subukia) was by *Amirthalingamia macracantha* which had the highest prevalence of 64 % in Subukia (33 infected fish out of 50 fish examined with a total of 1384 parasites). Whereas *Chilodonella spp* was found to have a high prevalence of 6 % in Kuresoi compared to other fish farms with turbidity of 46.90 ± 25.27 NTU. This result contradicts with the findings by Posthaus (2014) that indicated that water turbidity causes a condition referred to as chilodonellosis. Posthaus (2014) further pointed out that high turbidity conditions allow for the survival of the intermediate hosts of the *Chilodonella*. Similarly, *Amirthalingamia macracantha* were found to survive best in Subukia fish farm which had high turbidity due to high amount of silt available since it was an earthen pond. In addition, the pond had a red pigmentation probably caused by Euglenophytes which are known to grow in rich organic matter ponds. Normally the red pigment is a protective mechanism of the algae to excessive light. The high turbidity in this farm could be ascribed by its polyculture type of farming. It can be attributed by the presence of African catfish in the pond which led to the high turbidity as they disturb the bottom of the pond. High numbers of molluscs were also recorded in this farm and were found to flourish in this turbid fish pond, hence increasing the intermediate host of some parasites.

In this study, Trichodinidae were the second most frequent parasites, with the prevalence ranging from 10 - 65 % depending on the fish farm. This result compares with Pantoja *et al*.

(2012) who obtained a prevalence range between 20-90 %, with a high prevalence and a positive correlation with the physico-chemical parameters of the water.

5.3 Relationship between fish size and parasite infection levels in fish farms

The prevalence of parasites infecting fish was positively correlated with the fish length in Njoro fish farm with $R^2 = 0.9416$ (Figure 9). Results obtained in this study, shows an overall abundance of parasite being highest in the fish in length class 21-25 cm. This is because as the fish (*O. niloticus*) grows its interaction with the environment increases in terms of feeding habits, breeding process and consequently exposure to parasites also increases. This results are in consonance with Akoll *et al*. (2012) who recorded a positive correlation between fish size and the number of parasites infecting *Oreochromis niloticus* in ponds, from the research conducted on the infection patterns of Nile tilapia by two helminth species with contrasting life styles. In addition, Aloo (2002) also observed that larger fish harbour more parasites because of their feeding habits since adult tilapia are known to feed on zooplankters such as copepods which are intermediate host for some parasites, for instance Acanthocephala. However, Biu *et al*. (2014) recorded a contrasting result and noted that small sized fish had more parasites compared to large ones. The reason being that the immune system of the small fish are less developed and therefore, cannot resist parasites whereas the larger ones have fully developed immunity against parasitic infestations.

5.4 Condition factors of parasitized and non-parasitized *Oreochromis niloticus*

The analysis of the Fulton's condition factor, an index of the well-being of the fish, showed that the parasitized and non-parasitized *O. niloticus* were in good condition and all the values on the average were above the critical value 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 the higher the condition factor, the better the condition of the fish, according to the study done on the ecological aspects of *Oreochromis niloticus*. The condition factor (k) usually reflects the variations in fish health, information on the physiological state of the fish in relation to its welfare. Therefore, k values of *Oreochromis niloticus* in the fish farms indicated that they were in a very good condition (k>1) according to the standards of Barnham and Baxter (1998). In general, this study recorded low intensities of parasites because the water quality in most of the fish farms was within the acceptable limits. Blahoua *et al*. (2016) found significant and positive correlation of the condition factor of *Oreochromis niloticus* with the abundance of a species of a monogenean in the study carried out on the distribution of gill Monogenean parasites from *Oreochromis niloticus*. He further

noted that parasites such as Acanthocephalans and *Amirthalingamia macracantha,* which were also found in this study, cause visible lesions in the intestinal wall but the heavily infected fish does not appear to have any effect on its condition factor. In addition, Aloo (2002) found out that *Acanthocephala* have no effect on condition factor despite of its heavy infection in fish and insertion of the spiny proboscis in the liver and intestine, according to the comperative study conducted on helminth parasites from the fish *Tilapia zillii* and *Oreochromis leucostictus* in Lake Naivasha and Oloidien Bay, Kenya. It is assumed that they create a dynamic equilibrium co-existence with the fish host especially in well balanced water quality parameters.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From objective 1, this study concluded that there was a positive relationship between water quality parameters and parasite infection levels among fishes in the fish farms in Nakuru County. The fish farms with poor water quality had more species of parasites than the ones with good water quality. For instance, Dundori fish farm which had its water quality parameters within the acceptable limits had a lower overall prevalence of 18 % with fewer parasite species (three species) infecting cultured *O. niloticus* compared to Subukia fish farm which had an overall prevalence of 96 % with more species (10 species). Therefore, the null hypothesis that there is no significant relationships between selected water quality parameters (physico-chemical, nutrient content) and fish parasite infection levels (intensity, prevalence and diversity) in fish farms in Nakuru County is rejected in this study.

From objective 2, this study concluded that there was a positive correlation between fish size and parasite infection levels. Parasite infection levels were found to be higher in larger fish than small sized fish in all the six fish farms as in the case with *Cichlidogyrus halli*. Hence, the second null hypothesis that states that fish size does not significantly influence parasite infestation levels in *O. niloticus* in fish farms in Nakuru County is rejected according to this study.

From objective 3, the mean condition factor of parasitized *Oreochromis niloticus* was lower (1.61) than that of the non-parasitized (1.73), although not significantly different. Thus the health status of non-parasitized and parasitized *O. niloticus* were similar. This is because the infection levels of parasites were generally low in all fish farms to cause serious damages to the fish health. Therefore, this study failed to reject the third null hypothesis.

Some fish farms (Egerton and Kuresoi) had *Heterophyes sp* though in low abundance *Heterophyes sp* are known to be zoonotic to human beings.

6.2 Recommendations

This study recommends proper pond management practises such as water quality monitoring, liming of ponds, fish parasites control, using the required amount of fish feeds to feed fish to avoid under- and overfeeding, and avoidance of over-manuring or over-fertilization of the ponds. This will help in maintenance of good water quality that will in turn reduce the infestation and infection of parasites in fish farms which may result in disease outbreaks.

Extension officers should also be ready to work closely together with fish farmers, and advise them on their daily farm operational activities. Many of the fish farmers have little knowledge on how to monitor and maintain water quality as well as how to control parasites in ponds. Frequent trainings and exchange visits should also be organized by Fisheries department to fish farmers as most of them have not been exposed or have minimal exposure (having only attended trainings once or a few times) to trainings related to aquaculture. If these practices are put in place it will ensure that the fish farms are free from parasites.

The study showed that the health of all fish examined were in good condition even though they contained a number of parasites either in and/or on their internal organs and external surfaces. Therefore, the study recommends that the fish to be cooked thoroughly before eating or preserved in a deep freezer of -20 °C. The exposure to high temperatures when cooking and lower temperature in the freezer are believed to kill parasites in fish. Fish infected with harmful parasites (zoonotic parasites) should be avoided by human beings, as they transmit parasitic diseases to human resulting to diarrhoea.

Further research should be extended to more fish farms within the county and particularly focusing on the relationship between parasite intensities and fish size. From this study, it can be recommended that the larger fish should be handled properly and monitored frequently for parasites. This will enable the fish farmer to place correct measures to reduce or control the infection levels of parasites in pond reared fish and in turn produce fish of excellent condition in terms of health status.

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APPENDICES

Appendix 1: Questionnaire

Table 1: Background information

Name of the enumerator	
Phone number of enumerator	
Interview date	
Name of respondent/farmer	

A) Record keeping of the farms

1. a) Do you keep records of your fish farm? Yes or No_____

   b) If yes, which types of records? _____

2. How long have you been operating your fish farm(s)? _____

   (One year, Two years, Three years, More than three years, any other)

3. Which species of fish do you culture? _____

   (Nile tilapia, Catfish, both Nile tilapia and catfish, any other)

4. What is the size of your pond(s)? _____

   ($100m^2$, $200m^2$, $500m^2$, more than $500m^2$, any other)

5. When did you stock your fish? _____

6. What is the total fish population in each pond? _____

   (Less than 1000, 1000, 1500, 2000, any other)

7. Where do you get the seeds (fingerlings) _____

   (From the wild, from the hatchery, any other)

8. What is the source of water for daily pond operation? _____

B) Pond management practises

9. Do you monitor water quality of the pond? _____

   a) If yes, how often?

   b) How frequent do you change water in the pond? _____

   (Daily, once a week, twice a week, monthly, only when there is a problem detected in the fish farm, any other)

10. Which type of fertilizers do you use? _____

    (Manure, DAP, UREA, others specify)

11. What quantity of fertilizer do you apply _____

12. How often do you apply fertilizer in the pond? _____

(Daily, weekly, twice a week, monthly, only when there is a problem detected in the fish farm, any other)

13. When did you apply fertilizer or lime in your pond last?

(Last week, last month, two months ago, not sure, any other)

14. Which type of fish feed do you use? _____

(Commercial feed, home-made feed, any other)

15. How frequent do you feed fish? _____

(Once a day, twice a day, weekly, others specify)

16. What is the quantity of feeds do you give your fish? _____

(20g, 50g, 100g, any other)

17. How often do you monitor water quality of the pond _____

(Daily, weekly, monthly, others specify)

18. If so what tool do you use? _____

19. Where do you discharge waste water from the pond(s)?

20. Do you experience predation in the pond(s)? _____If so, which predator? _____

(Otter, birds, humans, any other)

21. Have you ever attended any course on pond management practises (extension services)? _____

22. If so how frequent_____ (once, twice, several times, any other)

23. Any other management practise that you do which is not mentioned above? _____

24. What would you like to do or change to ensure your pond(s) perform successfully?

Thank you for Participating

Appendix 2: Additional results on water quality parameters and fish parasites

Table 2: Mean values and standard error (±SE) of nutrient concentration in the six studied fish farms, n = 18

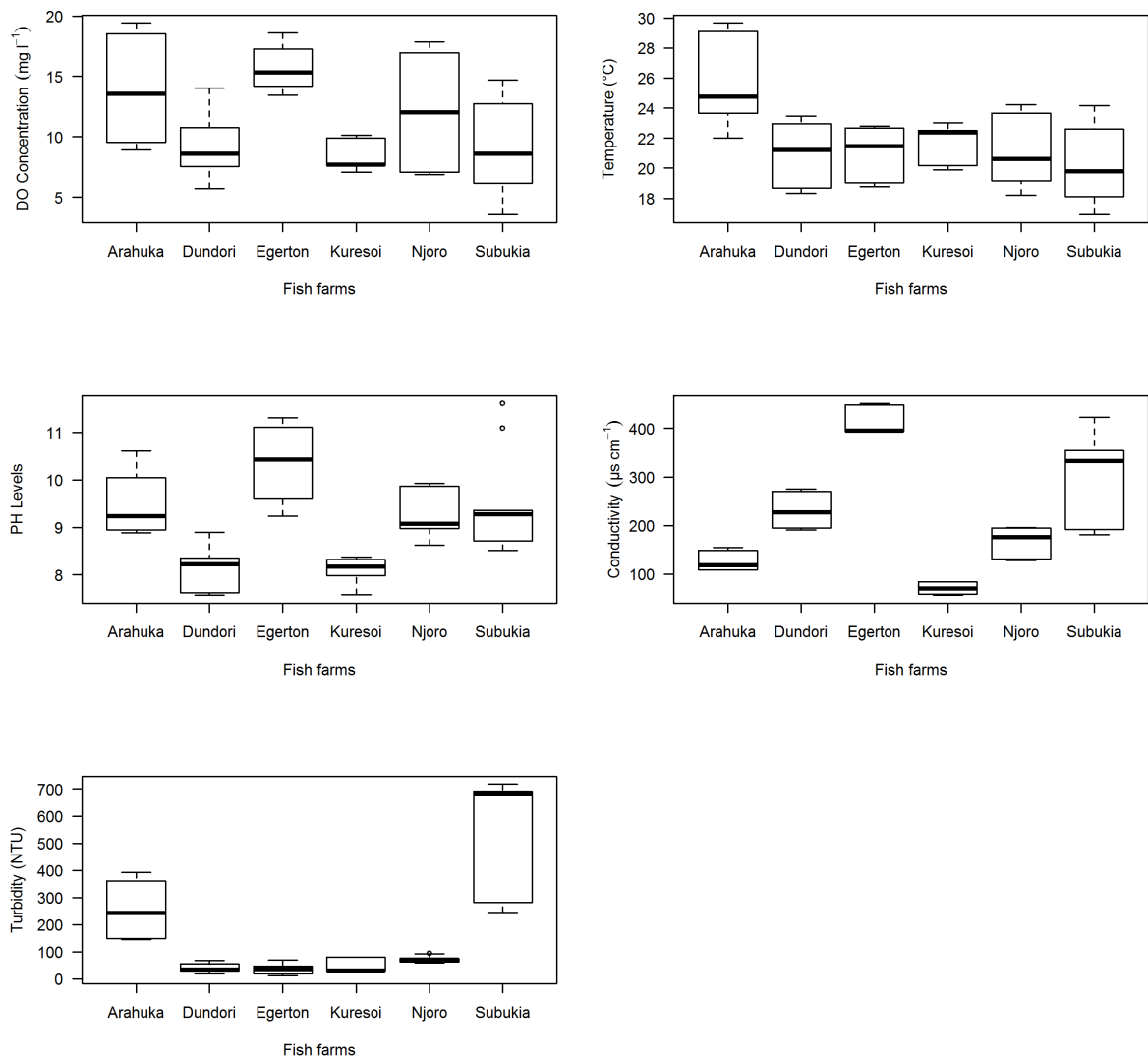| Parameter	Fish farms					
	Arahuka	Subukia	Njoro	Dundori	Egerton	Kuresoi
NH_4-N (µg/l)	531.96 ± 195.67	154.12 ± 38.68	7.08 ± 2.07	440.33 ± 214.27	26.41 ± 12.24	28.90 ± 8.99
NO_2-N (µg/l)	8.56 ± 3.11	31.21 ± 7.16	1.03 ± 0.24	11.43 ± 2.29	3.71 ± 1.79	2.60 ± 0.70
NO_3-N (mg/l)	0.26 ± 0.11	0.20 ± 0.07	0.01 ± 0.002	0.12 ± 0.05	0.02 ± 0.01	0.01 ± 0.004
TN (mg/l)	0.66 ± 0.09	0.31 ± 0.06	0.51 ± 0.03	0.47 ± 0.05	0.46 ± 0.04	0.14 ± 0.04
SRP (µg/l)	58.11 ± 20.87	127.41 ± 35.72	11.77 ± 2.12	3.13 ± 1.00	146.25 ± 49.31	21.81 ± 8.51
TP (µg/l)	453.29 ± 28.11	780.79 ± 93.12	492.45 ± 12.60	129.31 ± 15.44	540.37 ± 33.90	110.79 ± 11.86

Figure 1: Box plots showing variations of physico-chemical parameters of six fish farms in Nakuru County (n=18)

Table 3: The species of parasites found infecting *Oreochromis niloticus* in fish farms with water quality above acceptable limits in different sampling dates, n=300

Date	Fish farms	Parasites		
		Parasite Name	No. of fish	No. of parasites
05/01/2017	Egerton	*Acanthosentis tilapiae*	6	28
		Cichlidogyrus halli	29	1429
		Cichlidogyrus sclerosus	27	577
		Lamproglena sp	6	9
		Heterophyes sp	3	27
		Cryptobia sp	2	Low (<10)
		Trichodina sp	17	199
		Tylodelphys sp	1	37
18/01/2017	Egerton	*Acanthosentis tilapiae*	8	53
		Cichlidogyrus halli	10	480
		Cichlidogyrus sclerosus	7	37
		Lamproglena sp	2	4
		Cestode	2	4
		Unidentified parasites	1	1
27/01/2017	Egerton	*Acanthosentis tilapiae*	5	25
		Cichlidogyrus halli	10	523
		Cichlidogyrus sclerosus	4	7
		Lamproglena sp	3	3
		Heterophyes sp	1	1
		Trichodina sp	1	1
6/12/2016	Subukia	*Acanthosentis tilapiae*	20	134
		Cichlidogyrus halli	12	25
		Amirthalingamia macracantha	13	286
		Cestode	1	1
		Trichodina sp	12	296
		Microsporidia sp	1	5

		Unidentified parasites	5	7
20/01/2017	Subukia	*Acanthosentis tilapiae*	8	83
		Cichlidogyrus halli	5	28
		Cichlidogyrus sclerosus	2	3
		Amirthalingamia macracantha	10	832
		Oligochaete	1	1
		Unidentified parasites	2	2
03/02/2017	Subukia	*Acanthocephala*	10	53
		Cichlidogyrus halli	6	47
		Gyrodactylus sp	4	76
		Amirthalingamia	9	266
		Macracantha	2	Low
		Cryptobia sp	2	4
		Ichthyobodo sp	2	4
		Unidentified parasites		
30/11/2016	Arahuka	*Argulus sp*	1	1
		Cichlidogyrus halli	26	150
		Trichodina sp	25	162
		Ichthyobodo sp	3	15
		Cryptobia sp	7	medium
		Microsporidia sp	4	10
20/01/2017	Arahuka	*Cichlidogyrus halli*	10	415
		Cichlidogyrus sclerosus	7	18
03/02/2017	Arahuka	*Cichlidogyrus halli*	10	244
		Amirthalingamia macracantha	1	1
		Unidentified parasite	1	1

Table 4: The species of parasites found infecting *Oreochromis niloticus* in fish farms with water quality within acceptable limits in different sampling dates, n=300

Date	Fish farms	Parasites		
		Parasite Name	No. of fish	No. of parasites
14/01/2017	Kuresoi	*Acanthosentis sp*	3	6
		Cichlidogyrus halli	24	177
		Cichlidogyrus sclerosus	16	84
		Chilodonella sp	3	3
		Heterophyes sp	3	3
		Ichthyobodo sp	11	46
		Trichodina sp	11	34
		Microsporidia sp	2	2
31/01/2017	Kuresoi	*Cichlidogyrus halli*	10	72
		Trichodina sp	1	1
02/02/2017	Kuresoi	*Cichlidogyrus halli*	6	33
15/12/2016	Njoro	*Cichlidogyrus halli*	10	218
		Cryptobia sp	2	4
24/01/2017	Njoro	*Cichlidogyrus halli*	30	551
		Trichodina sp	6	67
		Cichlidogyrus sclerosus	6	7
31/01/2017	Njoro	*Cichlidogyrus halli*	10	295
		Trichodina sp	2	4
21/12/2016	Dundori	*Cichlidogyrus halli*	1	1
		Trichodina sp	5	82
		Unidentified parasites	2	2
28/01/2017	Dundori	*Trichodina sp*	1	1
		Macroinvertebrate	1	1
		Lamproglena sp	1	1
30/01/2017	Dundori	None	10	0

Appendix 3: Photos of some unidentified organisms/parasites encountered during the study in/on *O. niloticus* (Magnification ×100)
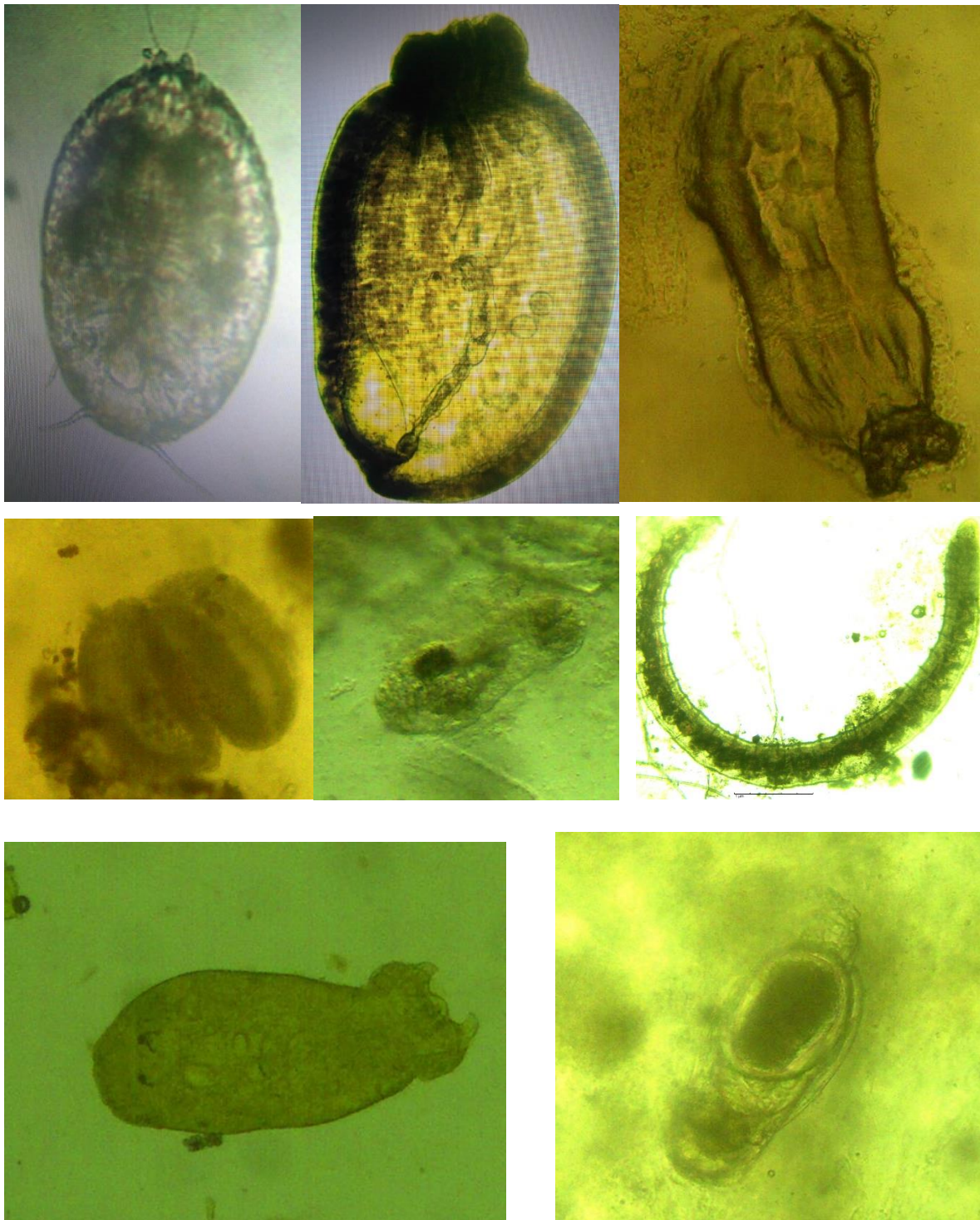
Appendix 4: Photos for field sampling and laboratory analysis of parasites in fish

Fishing
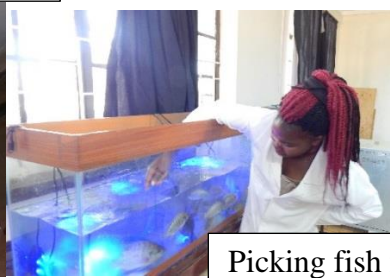

Measuring physico-chemical parameters

Harvested fish

Transporting fish in a fish tank

Picking fish from aquarium

Decapitating fish

Measuring fish length

Weighing fish

Adding saline water on fish organs

Scrapping the gills

Examining the internal organs

Examining gill scrapings under the compound microscope

Nutrient analysis using APHA standard methods