STUDIES ON OCCURRENCE OF PROTOZOAN AND HELMINTH PARASITES IN NILE TILAPIA (*Oreochromis niloticus* L.) FROM CENTRAL AND EASTERN PROVINCES, KENYA

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DECLARATION AND RECOMMENDATION

Declaration

I certify that this thesis is my original work and has not been presented elsewhere for an award of a degree, diploma or certificate

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Recommendation

This thesis has been submitted for examination with our recommendation as University supervisors.

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DEDICATION

To my wife Lillian, daughter Laura, my parents Peter and Esther.

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ABSTRACT

Aquaculture is an important food contributor to the global and local economy. Diseases and parasitic infections have been recognized as one of the detrimental and limiting factors in the development of aquaculture. No research has been carried out to study the occurrence of parasites in *Oreochromis niloticus* cultured in pioneer integrated cage fish culture in shallow and temporary water bodies (BOMOSA cages) in Kenya. A study was conducted with the aim of finding out the occurrence of protozoan and helminth parasites in *O. niloticus* fish from BOMOSA cages and open ponds. The objectives were: to detect and identify parasites, to determine their prevalence, mean intensity, abundance and distribution, and to relate the occurrence of the parasites to seasonality and biotic factors such as presence of intermediate and definitive hosts. Using routine necropsy and parasitological examination procedures a total of 370 *O. niloticus* fish (57 caged and 313 from open ponds) were examined for the presence of parasites. A total of 3 protozoan and 14 helminths and 1 copepod species were identified. There were no significant differences in parasitic infections between caged and open pond fish (sign test $x=8$, $n=19$, $p>0.05$). In terms of distribution, some parasites seemed more abundant in caged than in open pond fish and vice versa. A few parasites such as the eye fluke *Tylodelphys* spp. seemed to occur at high intensities during the dry season when the water level in the reservoir was low, while the majority of the parasites did not show any relationship with seasons. The prevalence rate and mean intensities of some helminth parasites identified such as eye fluke, *Tylodelphys* spp. and *Clinostomum* spp. warrant for design of control strategies. Even though there were no losses and mortalities recorded during the study period, it should be emphasised that some parasitic infestations can worsen the productive indexes of fish and that some helminth parasites, such as Clinostomatid digeneans, could be pathogenic for man.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF PLATES

CHAPTER ONE

INTRODUCTION

1.1 Background information

Aquaculture, which is broadly defined as the farming of finfish and shellfish in water systems is rapidly expanding in both size and culture systems and is therefore among the fastest growing industries, second to biotechnology (Drucker, 2002). In Kenya, cage culture system is probably the latest development in aquaculture. Fish are reared in cages which are either anchored to piers or in cages which are free floating in water. Information on occurrence, prevalence and pathogenicity of fish parasites and diseases is essential in aquaculture. Such information enables aquaculturists to apply correct control measures for fish diseases which reduces cost of production, and period of growth thereby increasing the profit margins (Akoll, 2005).

Hecht and Endemann (1998) reported that the major constraints observed to hold back productivity in fish farming include parasites and diseases. Therefore to achieve a highly productive and profitable venture, all the constraints faced in aquaculture must be addressed.

1.1.1 History of cage culture

The origins of fish cage culture are a little vague (Michael, 1988). It is likely that the first cages were used by fishermen as holding structures until fish could be accumulated for market. The first true cages for producing fish were developed in Southeast Asia around the end of the 19th century (Beveridge, 1987). Early cages were constructed of wood or bamboo, and the fish were fed with food scraps. The advent of synthetic materials for cage construction in the 1950's marked the beginning of modern cage culture. Cage research had been limited mostly because large scale open pond culture was considered more economically viable, and therefore, receiving more attention in research. However, factors such as increasing consumption of fish, some declining wild fish stocks, and a poor farm economy have produced a strong interest in fish production in cages by both researchers and commercial producers (Helfrich *et al.,* 1984).

1.1.2 Advantages and disadvantages of cage culture

Advantages of cage culture of fish include several aspects. Many types of water resources can be used, including lakes, reservoirs, ponds, strip pits, streams and rivers which could otherwise not be harvested (Michael, 1988). A relatively low initial investment is all that is required in an existing body of water (Strange and Van Gorder, 1980). Harvesting, observation and sampling of fish are simplified. Cage culture allows the use of the ponds for sport fishing or the culture of other species.

A potential fish farmer can produce fish in an existing pond without destroying its sport fishing; does not have to invest large amounts of capital for construction or equipment; and can, therefore, practise fish culture without unreasonable risks.

Disadvantages of fish cage culture include the need that the feed be nutritionally complete; low dissolved oxygen syndrome (LODOS) requiring mechanical aeration (Michael, 1988). The incidence of disease can be high as diseases may spread rapidly; stress due to overcrowding, vandalism and poaching are potential problems which have to be anticipated and precautions taken (Basset and Dillard, 1985).

1.1.3 Disease theory in relation to production, morbidity and mortality

According to Jadwiga (1991) the presence of fish parasites is to a large extent detrimental to fisheries and the fishing industry. Even in well-adjusted host-parasite systems, the host does not remain indifferent to the presence of parasites. There are various modes in which pathogenic effects of parasites on hosts manifest themselves. Epizootics and mass mortality brought about by parasitic infections are very frequent in fishes. More common is a prolonged gradual die-off which may, for some time, go unnoticed, particularly when the few dead fishes are immediately consumed by piscivorous birds. Economic effects of parasites on fishes are mass mortality, rejection of infected fish by the market when parasites and/or lesions are visible and more importantly, retarded growth and weight losses of the infected fish. Furthermore, some fish parasites are potentially pathogenic for man when the parasitized fish are consumed not well cooked, cold smoked, marinated or just raw.

1.2 Statement of the problem

Along with the growing interest in the development of fish culture, particularly in warm water, there is an increasing awareness of the importance of disease as one of the major limiting factors in culturing fish (Paperna, 1980). Whereas the latest attempt to provide sufficient protein food to the communities of Central and Eastern provinces of Kenya through fish culture in cages in reservoirs is a positive step toward ensuring food security in these regions, a major challenge exists: that of diseases and epizootics (Fioravanti *et al.*, 2007). Cages are small thereby presenting a significant stress factor to the fish. Furthermore, some reservoirs being used are uncensored for parasites and pathogens as they are either natural or those that were constructed without the intention of rearing fish. There is therefore a need to study the occurrence of parasites and infections under these culture systems in these water bodies with an aim of controlling them.

1.3 Objectives of the study

1.3.1 Broad objective

The main objective of the study was to determine abundance and distribution of parasites occurring in and on *Oreochromis niloticus* from cages and open ponds.

1.3.2 Specific objectives

The specific objectives of the study were to:

(i) identify and compare the parasites occurring in and on *O. niloticus* from caged and open pond;

(ii) determine the abundance and distribution of parasites in and on *O. niloticus* from the sites under study;

(iii) determine the prevalence and mean intensity of parasites in and on *O. niloticus* from the sites under study;

(iv) determine the association between parasitic infections and abiotic / biotic factors.

1.4 Research hypothesis

HO: There is no significant variation in the type of parasites infesting caged and open pond *O. niloticus.*

HO: There is no significant difference in the abundance and distribution of parasites in caged and open pond *O. niloticus.*

HO: The prevalence and mean intensity of parasites are not significantly different between caged and open pond *O. niloticus.*

HO: There is no association between parasitic infections and abiotic / biotic factors.

1.5 Significance of the study

This study was justified by the fact that aquaculture plays a very important role in the economy of Kenya and also in providing fish food to many rural communities in Kenya. As the development of aquaculture has advanced, it has become increasingly apparent that one essentially unsolved problem is the prevention of economic losses due to mass mortalities and control of mass morbidities. It is a well known fact that animals maintained under crowded conditions are more susceptible to parasitic infections and diseases. This is true of mammals as well as finfish and shellfish populations (Perkins and Cheng, 1990). Parasitic infections of fish can be a major setback in achieving maximum production per unit area of culture. Fish production is in high demand to supplement other food sources which are dwindling with the vagaries of weather for the majority of poor people. In order to achieve some objectives of the Millennium Development Goals on poverty and hunger, we need information on fish infections so that strategies can be devised to fight these enemies of man which compete for our mean resources.

1.6 Definition of terms

In this section some scientific and epidemiological terms are defined as used in this subject of fish parasitology and thesis. These include:

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 hosts – positive and negative - examined).

Epidemiology: is the study of factors affecting the health and illness of populations, useful for identifying risk factors for disease and determining optimal preventive and control approaches.

Definitive host: also called "final or primary host", is a host in which the parasite reaches maturity and (if applicable) reproduces sexually.

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

Paratenic host: is a host that is not needed for the parasite's development cycle to progress, serving as "dumps" for non-mature stages of a parasite which they can accumulate in high numbers (without any biological development).

Intensity: refers to the number of parasites in a single infected host (number of parasites divided by number of positive hosts).

Mean Intensity (MI): is 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 (MA): is 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: is 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).

Runding: Phenomenon where fish exhibit stunted growth, attaining sexual maturity and reproducing when still small.

Zoonosis: any infectious or parasitic disease able to be transmitted from animals, both wild and domestic, to human or *vice versa*.

Morisita's index (I_M): index of similarity which refers to the probability that individuals randomly drawn from each of two communities will belong to the same species, relative to the probability of randomly selecting a pair of specimen of the same species from one of the communities.

Jaccard coefficient (CC_J): Coefficient of community, **(CC_J)** = c/s1+s2-c where c is the number of species common in both communities, s1 and s2 are the number of species in communities 1 and 2, respectively.

Sorensens coefficient (CC_S): Coefficient of community, to quantify community similarity **(CC_S)** = 2c/s1+s2, variable as above.

Shannon-weaner diversity index (H'): Index of species diversity where, **(H') = -∑Pi In Pi,** where Pi = ni/N, proportion of the total number of individuals occurring in species, i. N is the total number of individuals in all the species. In is the natural logarithm, Log_2.

The definitions concerning quantitative descriptors of parasites (Prevalence, Intensity and Abundance) are from Bush *et al.* (1997).

CHAPTER TWO

LITERATURE REVIEW

2.1 The Nile Tilapia

The common names of the host fish under study include: Nile tilapia and Tilapia nilotica. Its synonym is the "Nile mouth brooder" and its scientific name is *Oreochromis niloticus niloticus* (Linnaeus, 1757) (Leo and Schofield, 2008). Trewavas (1983) provided the distinguishing characteristics, synonyms, an illustration, keys and a discussion of hybrids of the Nile tilapia. Axelrod *et al.* (1985) and Axelrod (1993) provided the first photographs of the Nile tilapia fish. This species closely resembles *Oreochromis aureus*. Its maximum size is 63cm and its native ranges are the tropical and subtropical Africa and the Middle East. It is widely distributed in the Nile river basin and Niger river basin and in lakes; Tanganyika, Albert, Edward and George. As well, it is found in many smaller drainages and lakes in western and eastern Africa and in Yarkan river, Israel (Trewavas, 1983). In aquaculture the Nile Tilapia is ranked first among the species of choice for culture because of its characteristics. These include the fact that this fish is a fast grower, acceptable by consumers, accepts and thrills on readily, locally available supplemental feeds, eury-tolerant to environmental conditions and reproduces well in captivity. However, this species has a problem of over-reproduction which leads to "Runding" hence monosex culture is recommended and is vulnerable to diseases and parasites.

2.2 Fish parasites

Parasites can be found in any fish species and within any type of aquatic system. They range from Protozoans (Flagellates, Ciliates, Apicomplexans, etc.) to Metazoans (Myxozoans, Trematodes, Cestodes, Acanthocephalans, Nematodes, and Crustaceans) (Lewis, 1991). Maria *et al.* (2005) related health condition with parasite infestation of *O. niloticus* (Linnaeus, 1757) from Guarapiranga reservoir, Sao Paulo state, Brazil. Here they analysed the hematocrits and the differentiated leukocyte counts. The prevalence of some parasites seemed to be associated with water temperature and the level of dissolved oxygen. The hematocrit and leukocyte cells percentage showed little variation during the

sampling period. Only basophils demonstrated a significant difference between monthly mean values. The percentage of eosinophils was higher in fish parasitised with *Ichthyophthirius multifiliis* and *Cichlidogyrus* spp. than in non parasitised animals.

Even if Paperna (1996) published a scientific manual that compiled both the personal observations and the published information from Africa on aspects of fish health, with particular reference to parasitology, very little work has been done on fish parasites of *O. niloticus* under cage culture systems.

In general, the parasites reported to be problematic in African aquaculture include ectoparasites like protozoans (*Ichthyophthirius multifiliis*, *Chilodonella* spp., *Trichodina* spp.), monogeneans (Dactylogyrids and Gyrodactylids), leeches, crustaceans and larval bivalve mollusks and endoparasites such as protozoans (haemoflagellates, apicomplexans, microsporeans), myxosporeans, larval trematodes (*Bolbophorus* spp., Diplostomatids, Clinostomatids), cestodes, acanthocephalans and nematodes (Hecht and Endemann, 1998).

2.2.1 Protozoa

Protozoa are single celled eukaryotic organisms of the Kingdom Protista, which includes members of the phyla Ciliophora (ciliates), Sarcomastigophora (flagellates), Microsporea and Apicomplexa, with regard to fish parasites. Their life styles range from free-living through various forms of commensalisms to parasitism in most animals, plants and even other protozoans (Lucy and Ernest, 1994; Woo, 1995). More than 65,000 species have been described of which 10,000 species are parasitic with at least 2400 parasitising fish (Lucy and Ernest, 1994).

Structural and morphological characteristics of the protozoan parasites in fish have been described by Lom and Noble (1984), Lom and Dyková (1992), Lucy and Ernest (1994) and Woo (1995). Reproduction and transmission of protozoan parasites is very diverse. Despite of the fact that most of them have a direct life cycle, many have indirect life cycles. During direct life cycle, protozoa are usually released either through faeces, especially for intestinal parasites, or when the host dies and the spores or cysts can also be shed directly into water through lesions. These can gain entrance into a new host through ingestion or active attachment of the trophont. On the other hand, protozoan

parasites with an indirect life cycle like haemoflagellates usually require an intermediate host (usually blood sucking leeches) (Mehlhorn, 1988; Kreier, 1994; Woo, 1995; Overath *et al.*, 1999).

2.2.2 Metazoa

2.2.2.1 Myxozoa

The parasites owing to the phylum Myxozoa are characterized by spores composed of several cells transfigured into 1 to 7 spore shell valves, 1 to 2 ameoboid infective germs (Sporoplasms) and 2 to 7 nematocysts-like polar capsules, the latter containing an extrudible filament with an anchoring function (Lom and Dyková, 1992). They are common multicellular parasites of cold-blooded vertebrates, particularly fish. They undergo complicated development within the hosts, and most of them utilize alternate development within an annelid worm in their life cycle. Many species are highly pathogenic in commercially important fish, particularly in aquaculture. Myxozoans infect a wide variety of tissues (histozoic species) or the lumina of organs such as the gall bladder, urinary bladder or kidney tubules (celozoic species) (Lom and Dyková, 1992).

Concerning the Myxozoan parasites described in African fish, Gbankoto *et al.* (2001a) investigated the gill Myxosporean parasites of 2 euryhaline tilapias from Lake Nokoue, Senegal, *Sarotheredon melanotheron melanotheron* (Ruppel, 1953) and *Tilapia zilli* (Gervais, 1852) from October 1987 and October 1989. They investigated the effects of host sex and size and the seasonal patterns of infection. No significant fish sex effect was found for the different myxosporean parasites. A seasonal pattern was clearly demonstrated for *Myxobolus zillii*, while a host size effect was found for *Myxobolus dossoui*. Gbankoto *et al.* (2001b) investigated *Myxobolus dahomeyensis* infection in ovaries of tilapia species from Benin (West Africa). They determined the prevalence rates in ovaries from 18.3% to 31.6% in *Sarotheredon melanotheron melanotheron* and found that the parasite induced destruction of the oocytes and host castration.

El-Mansy (2005) revised *Myxobolus heterosporus* Baker 1963 (Syn. *Myxosoma heterospora*) (Myxozoa : Myxosporea) in African records using specimens isolated from plasmodia situated in the infected cornea of *Oreochromis aureus*. In addition, he

examined the histological effects of the parasite on the infected tissue. The growth of the plasmodium led to compression and fusion of the epithelial lining of the cornea tissue and occupied a wide area of the cornea tissue.

2.2.2.2 Platyhelminthes

Members of the phylum Platyhelminthes are also called "flatworms" and are relatively simple soft-bodied invertebrate organisms, dorso-ventrally compressed, with a bilaterally symmetrical body that lacks true coelom (aceolomates). Based on classification presented in recent scientific books (Williams and Jones, 1994; Cone, 1995; Cribb *et al.*, 2002; Myers *et al.,* 2005), there are four classes in this phylum namely: Monogenea, Trematoda, Cestoda and Turbellaria. The phylum has over 25,000 species found in marine, freshwater, and even damp terrestrial environments described thus, the largest group without coelom of which some are free living while others are parasitic.

Turbellaria consists of about 3000 species dominating marine environments though some do occur in freshwaters, e.g. Planarians. Although most members of the group are free living, few are reported to be parasitic e.g. order Temnocephalida (Campbell, 2001).

The class Monogenea is composed of about 50 families and thousands of described and undescribed species that are parasitic primarily on gills and skin of fish. Monogeneans lack respiratory, skeletal and circulatory systems and have no oral suckers or are weakly developed in most; they attach to host using a posterior attachment hooked organ called the opisthaptor and are capable of stretching and compressing their bodies (Cribb *et al.*, 2002). They have also an anterior attachment structure called prohaptor, a simple digestive system consisting of a mouth opening with a muscular pharynx and an intestine with no terminal opening. Generally, they also are hermaphroditic with functional reproductive organs of both sexes occurring in one individual, and they have a head region that contains concentrated sense organs and nervous tissue (Cribb *et al.*, 2002).

Monogenea are traditionally divided into two subclasses based on the complexity of their haptor: Monopisthocotylea with one main part to the haptor, often with hooks or a large attachment disc, and Polyopisthocotylea with multiple parts to the haptor, typically clamps. Polyopisthocotyleans are almost exclusively gill-dwelling blood

feeders, whereas Monopisthocotyleans may live on the gills, skin and fins (Woo, 1995). The class Monogenea is of great economic importance in regard to fish health and fish farming worldwide. Cribb *et al.* (2002) noted that two monogenean families of Monopisthocotylean, Gyrodactylidae and Dactylogyridae dominate literature and mostly either during description of new species belonging to the genera occurring in fish or as a cause of massive fish mortality. Mauricio *et al.* (2002) diagnosed cases of fish diseases in the state of Sao Paulo, Brazil between January 1999 and December 2000. In 1999, the monogenean was the most important parasite (72.9%), followed by *Piscinoodinium pillulare* (43.2%), *Henneguya piaractus* (34.2%), *Ichthyophthirius multifiliis* (29.8%) and copepodids of *Lernaea cyprinacea* (9.0%). However, in 2000, monogenean showed (78.9%), trichodinids (52.1%), *P. pillulare* (35.7%), *I. multifiliis* (29.8%) and *L. cyprinacea* (11.9%). Laurent *et al.* (2006) studied the systematic of 14 species of monogenean (Ancyrocephalidae) gill parasites from West African tilapiine hosts (Cichlidae) using both morphological and genetic data. Their findings showed that the specificity of monogenean parasites is very high. Monogeneans exhibit a simple direct life cycle, where eggs usually hatch into free swimming larvae called "oncomiracidia" which infect fish (oviparous monogeneans as Dactylogyrids) or well-developed embryos hatch from the adult and the invasion is by adult parasites transferring directly between adjacent hosts (viviparous monogeneans as Gyrodactylids) (Ronald, 1978).

Class Trematoda consists of order Digenea and order Aspidogastrea, both constituting to about 9000 species which are all parasitic usually on both cold-blooded and warm-blooded vertebrates. The order Aspidogastrea is reported to parasitise marine molluscs (Myer, 2001; Cribb *et al*., 2002). The members of class Trematoda have indirect and complex life cycle requiring at least 1 intermediate host (Ronald, 1978) and their adult stage is parasitic of vertebrates. With one exception (*Aporocotyle* genus), digeneans undergo part or all of their larval development in mollusks (Ronald, 1978).

Very few adult-stage digeneans are known to cause significant harm to the fish host, except for extra-intestinal sanguinicoliid and didymozoid parasites, while metacercarial stages can strongly affect growth and survival of fish, and sometimes represent a source for infections in humans (Woo, 1995). Morphologically the Digeneans have an anterior sucker and a ventral sucker, used for attachment and locomotion, a

simple alimentary tract (mouth, pharynx, esophagus and two blind caeca), an hermaphroditic reproductive system. Considerable structural and morphological diversity exist among the digeneans, both at adult and larval stages (Woo, 1995). Their transmission can be through the alimentary tract and also through skin penetration by cercariae (Jadwiga, 1991).

Cestoda are all endoparasites of vertebrates with over 5000 species so far described. Most of them require at least 1 intermediate host and complete their life cycle as adults in the definitive hosts. Two life cycle stages are represented in fish: adults inhabit the intestine, and plerocercoid larvae of the same or different species are found in the viscera and musculature; the first-stage larvae (procercoids) are generally found in aquatic crustaceans (Woo, 1995).

Morphologically the adult cestodes are strongly flattened dorsoventrally, the body consists of the scolex (head), neck, and strobila (body), the latter generally made up of several serial sections (proglottids). Some unsegmented cestodes are also described from fish (*Caryophyllaeus*, *Khawia*, etc.). Scolex is an attachment organ used to fasten the parasite to the host's intestinal mucosa, so it is generally provided with holdfast structures such as suckers and bothria, and additionally hooks and/or proboscids. Cestode have no intestine, the nourishment being absorbed by the tegument covering the whole surface of the body. With a few exceptions, cestodes are hermaphroditic, each proglottid having its own set of male and female gonads (Woo, 1995).

Lenta (2002) reviewed the terminologies associated with the nomenclature of larval or metacestodes as well as the various morphological and developmental characters used to define different types of larval cestodes. Numerous cestodes cause disease in fish (mainly at the plerocercoid larval stage) and in some cases they can be transmitted to humans, as in the case of *Diphyllobothrium* spp., causing a serious fish-borne zoonosis called Diphyllobothriasis.

2.2.2.3 Nematoda

The phylum Nematoda is one of the most common phyla of animals, with over 80,000 different described species (of which over 15,000 are parasitic), diffused in freshwater, marine, and terrestrial environments. The phylum contains both free-living organisms and parasites of plants and animals, including fish (Jadwiga, 1991). They are

also called “roundworms”, as they have an elongated, cylindrical body, circular in section. Nematodes are unsegmented, bilaterally symmetric with a complete digestive system consisting of three sections: anterior (esophagus), middle (intestine), and posterior (rectum) ending with the anus/cloacae (Jadwiga, 1991).

Instead of containing parenchyma as in Platyhelminthes, the body cavity (pseudocoel) is filled with perivisceral fluid imparting turgor to the body. Most nematodes are dioecious, with males usually smaller than females; they are generally oviparous, but some viviparous species occur too. They infect the host fish through the alimentary tract (Jadwiga, 1991).

The life cycle of parasitic nematodes involves a definitive host and one or two intermediate hosts. A larva moults four times (resulting in 4 larval stages) before becoming a sexually mature adult. Fish can be both intermediate and definitive hosts; the nematodes having fish as intermediate hosts reach sexual maturity in the intestine of birds, mammals or predatory fish. The first intermediate host infecting fish are mostly aquatic crustaceans and other invertebrates. Some nematodes parasitising fish at the larval stage, can be transmitted to humans by eating infected raw fish and cause serious diseases (e.g. Anisakiasis) (Jadwiga, 1991).

2.2.2.4 Acanthocephala

Acanthocephalans, also called “spiny or thorny-headed worms”, belong to the separate distinct phylum Acanthocephala with about 1200 species divided into three classes, namely: Archiacanthocephala, Eoacanthocephala and Palaeacanthocephala. They are all intestinal parasites of vertebrates. Among their major hosts are fish, amphibians, birds and mammals (Jadwiga, 1991). Morphologically they are cylindrical worms (from few mm to 70 cm long), with the anterior part provided with an eversible hooked proboscis, without digestive system (they absorb nutritive materials with the whole surface of the body) (Jadwiga, 1991). The acanthocephalans are dioecious, with males usually smaller than females, and oviparous. They exhibit an indirect life cycle with crustaceans as intermediate hosts. They infect host through the alimentary tract by ingestion of the infective larva with food. The fish could be parathenic hosts for some species (the larval stages dwell encysted in the body cavity until eaten by the definitive

host). Mahmoud *et al.* (2006) studied the site adaptation of *Acanthogyrus* (*Acanthosentis*) *tilapiae*; through light and scanning electron microscopy to show the features of parasites obtained from three tilapia species. The pathogenic effects of acanthocephalans are strictly related to the damage caused by the proboscid in the intestinal wall and to the infection intensity.

2.2.2.5 Crustacea

The crustaceans are a large group of Arthropods, comprising almost 52,000 described species, and are usually treated as a subphylum. The majority of them are aquatic, living in either marine or freshwater environments, but a few groups have adapted to life in terrestrial environments. The majority of crustaceans are motile, moving about independently, although a few taxonomic units are parasitic and live attached to their hosts (including sea lice, tongue worms, anchor worms, etc.) (Jadwiga, 1991).

Crustacea parasitic on fish are numerous as species and abundant as individuals, showing strong structural modifications due to the adaptation to parasitism. Three major divisions of Crustacea have to be considered in fish parasitology: Branchiura, Copepoda and Isopoda, the latter found mainly in marine environment (Woo, 1995). The majority of the 2000 species that have since been described as parasites of fish belong to the class Copepoda. This group is very heterogeneous, showing different adaptations to various habitats and to the parasitic life; the range of forms extends from epizoic organisms and commensals to proper parasites (Woo, 1995).

The parasitic copepoda have a direct life cycle, generally shortened if compared to the free-living copepods, with several different larval stages and an adult stage. Copepods are dioecious, with males usually smaller than females. A female carries eggs in egg sacs, either single or paired, attached to the genital segment. The eggs hatch into a nauplius-type larva that will undergo several moults into different larval stages (it depends on the species). The crustacean parasites harm the fish in three ways: they cause pressure atrophy of soft tissues with their hard body, they determine mechanical damage with their attachment structures and they inflict damages of different degrees by feeding at the expenses of the host (some species are hematophagous) (Jadwiga, 1991).

2.3 Environmental factors

2.3.1 Abiotic factors

2.3.1.1 Temperature

Buchmann and Bresciani (1997) while investigating parasitic infections in pond-reared Rainbow trout, *Onchorhynchus mykiss* in Denmark found that prevalence of some parasites such as *Ichthyophthirius multifiliis* increased with temperature (Maximum at 16°C to 20°C) whereas diplomonad had highest prevalence at 1 to 5°C, likewise, the gyrodactyloids occurred more abundantly at lower temperatures.

Muhammad (2003) investigated parasitic infestation in different freshwater fish of Mini Dams of Potohar region, Pakistan i.e. Meinhart and Mangia mini dams. A total of 78 fish belonging to five different species including, *Cyprinus carpio* (common carp), *Hypophthalmicthys molitrix* (silver carp), *Ctenopharyngodon idella* (Grass carp), *Cirrhinus mrigala* (Mori) and *Labeo rohite* (Rohu), were studied. Nine different species of parasites were found in these fishes, viz. *Chilodonella* spp. and *Trichodina* spp. (protozoans), *Lernaea cyprinacea*, *Ergasilus* spp. and *Argulus* spp. (crustaceans), *Contracaecum* larvae and *Rhabdochona charsdensis* (Nematodes), *Gyrodactylus* spp. (monogenean Trematode) and *Piscicola* spp. (fish leech).

Generally, fish were found infected with one species of parasites while mixed infections were far less. This study showed that parasitic infection increased with increase in temperature. Pike and Lewis (1994) too found that there was an optimum temperature for parasitic infections. For *Diplostomum* infection they found that 17.5°C was the optimum temperature above which infection levels decreases.

In another study Stables and Chappell (1986) found that infection of diplostomiasis and transmission from snail to fish does not occur at temperatures below 10°C. Imkovaa *et al.* (2001) studied the influence of water temperature in dactylogyroid species composition in roach, *Rutilus rutilus* . Duane and David (2000) also studied the occurrence of *Ergasilus celestis* (Copepoda) and *Pseudodactylogyrus anguillae* (Monogenea) among wild eels (*Anguilla rostrata*) in relation to stream flow, pH and temperature, and Hafizuddin and Bashirullah (2005) too studied the population and

seasonal distribution of *Procamallanus daccai* in *Eutropiichthys vacha* in Kaptai Lake, Chittagong, Bangladesh. All these studies revealed that intensity and abundance of these parasites fluctuated with seasons with a seasonal preference for the summer period when the temperatures are high.

2.3.1.2 Water quality and quantity

Dzikowski *et al.* (2003) found that the composition of metacercarial communities differ with changing water levels. Changes in habitat conditions which compromise the survival of the intermediate host community are likely to interrupt the transmission of heteroxenous parasites. Pike and Lewis (1994) also found that the flow rates of water have significant effects on occurrence and distribution of *Diplostomum* parasites. Duane and David (2000) while trying to find the relationship between the stream velocity, pH and water temperature to prevalence and abundance of two common gill parasites (monogenean *Pseudodactylogyrus anguillae*) and (Copepod *Ergasilus celestis*) of wild eels (*Anguilla rostrata*) found that the component population, mean abundance and prevalence of both parasites correlated negatively with stream velocity, positively with the pH and temperature.

2.3.1.3 Cages and handling

Christian (1989) found that treating, sorting and trapping of fish causes stress or wounds which act as entry points for pathogens. Perkins and Cheng (1990) indicate that animals maintained under crowded conditions as in cages are more susceptible to diseases.

2.3.2. Biotic factors

2.3.2.1 Body size

Olofintoye (2006) investigated *Tilapia zilli*, *Clarias anguillaris* and *Clarias gariepinus* for helminth parasites. A nematode of the genus *Cucullanus* was accounted for the highest prevalence in the fish species examined. He found that the prevalence of infection in the fish species examined increases with their standard length and body weight. Aloo *et al.* (2004) as well found that larger fish harboured more parasites.

Gbankoto *et al.* (2001a) studied effects of host sex and size and seasonal patterns of infection. No significant fish sex effect was found for the different myxosporean parasites. A seasonal pattern was clearly demonstrated for *Myxobolus zillii* while a host size effect was found for *Myxobolus dossoui*.

2.3.2.2 Biomass density

Christian (1989) reviewed the pathology of farmed tilapias. This study outlined the effects of intensive production systems on the aspects and importance of pathology. The incidences of disease in crowded environments have been found to be high because fish densities provide excellent conditions for the multiplication of parasites, and more particularly those which have a direct reproductive cycle, statistically find the best possibilities to meet their natural host and to ensure their development. Karvonen *et al.* (2003) found that the transmission rate of parasites to fish increases with density.

2.3.2.3 Benthos and Birds

Connelly and McCarthy (1986) studied the ecological factors influencing the composition of the parasite fauna of the European eel, *Anguilla anguilla*. The variations in the occurrence and intensities of the parasites observed were analysed in relation to sampling period, host habitat and characteristics of the eel populations. A variety of factors were shown to be of importance including composition of the fish communities, and distributional patterns of intermediate hosts and piscivorous birds.

Dias *et al.* (2003) found that several mollusc species and fish eating birds have been described as natural first intermediate and definitive hosts for fish parasites respectively. They form an important component of the life cycle of these parasites.

2. 4 Conceptual framework

The conceptual framework (Fig. 1) had three major components which were investigated namely; fish, environment and parasites. Fish interacts with its environment; both abiotic and biotic factors. Fish survival, growth and productivity depend on its ecology. Co-evolutionarily, some parasites have attained equilibrium with their host fishes such as parasites with high host specificity. However, aquaculture which allows controlled culture conditions such as cages introduces significant stress factor owing to

water quality, quantity and its circulation, stocking densities (Biomass), re-stocking, handling among others which disrupt the dynamics of host-parasite balance. A parasitic disease develops as a result of susceptible fish (fish under stress). The specific gaps that were filled in this study included: identifying the parasites occurring in and on Nile tilapia reared in cage and in open pond, establishing their prevalence rates, mean intensities, abundance and distribution in Nile tilapia.

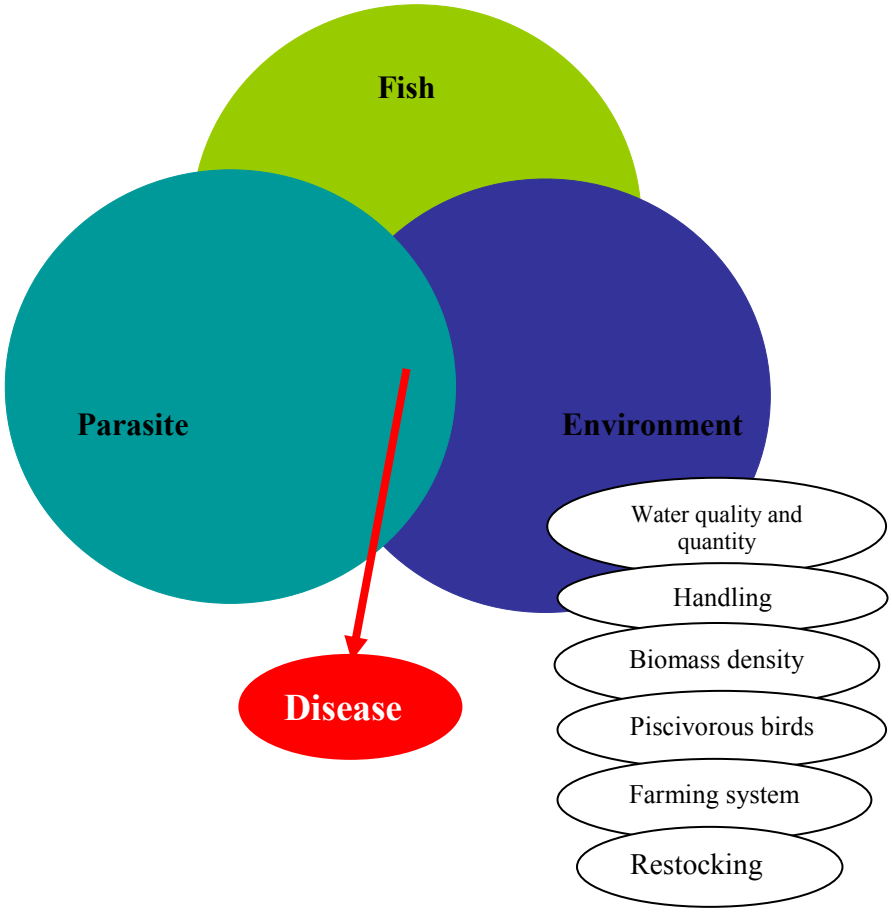


Fig. 1: The conceptual framework of this study showing the interactions between three major factors: Fish, Environment and Disease.

2. 5 Scope and limitations of the study

First, the study was restricted to Sagana Fish Farm in Central province and Ngeki's dam in Machakos, Eastern province, Kenya. Secondly, the study duration of 14 months was a short duration and therefore long-term dynamics of parasite changes in species and abundance with seasons were not fully understood as well as their life cycles. Lastly, that the study was limited to considering abundance, distribution, prevalence and mean intensities of some protozoan and helminth parasites of Nile tilapia and not all the parasites and therefore generalizations were limited to the fish species.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study sites

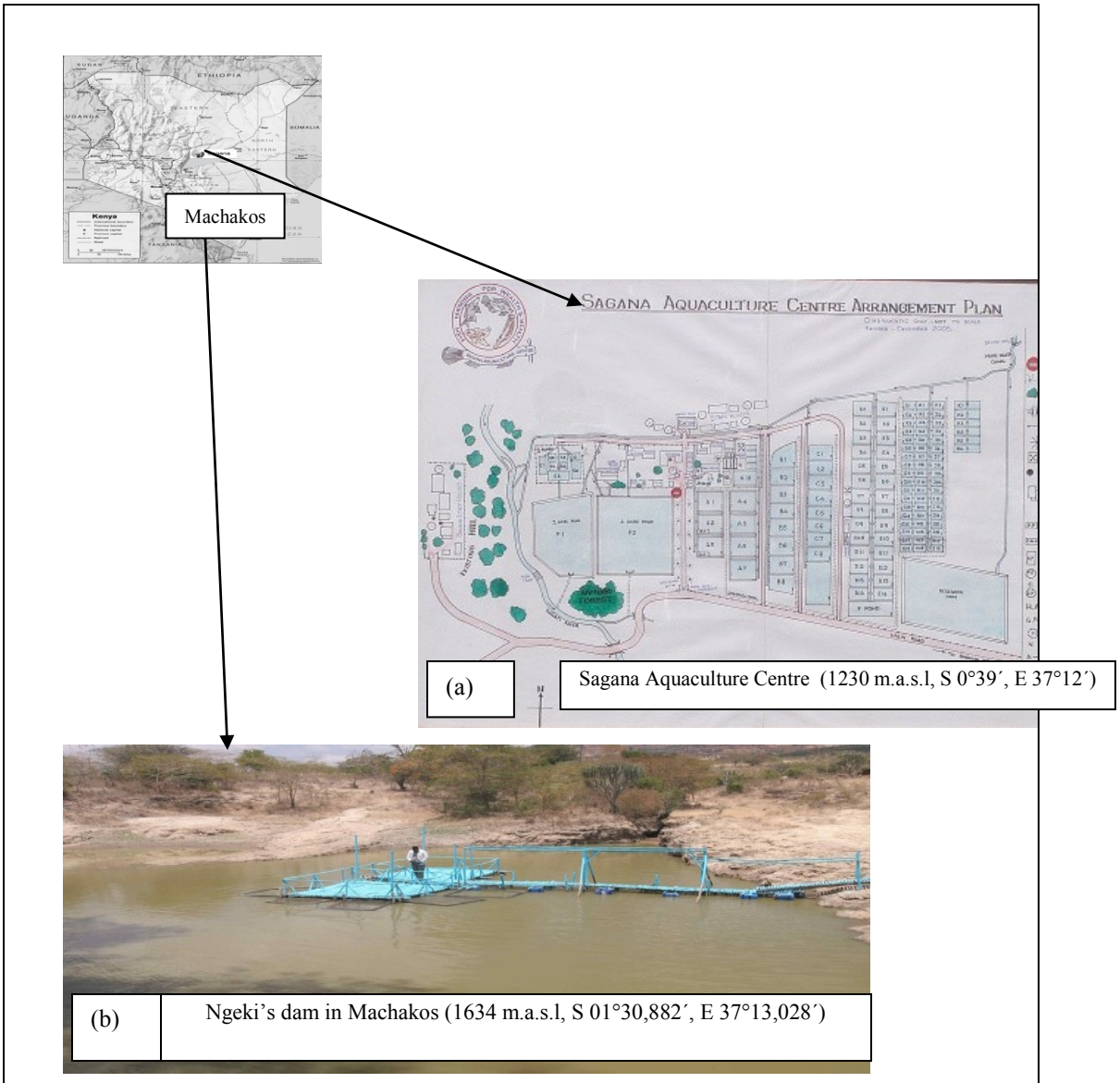


Fig. 2: Location of study sites (a) Sagana Aquaculture Centre in Central province and (b) Ngeki's dam, Machakos in Eastern province (Source: modified from PD/CRSP, 1998)

3.1.1 Description of Sagana Fish Farm

Sagana Fish Farm is sited S 0º39' E 37°12', 1230m.a.sl and comprises 20ha of ponds on a 50ha farm in Kenya's Central Province (Fig. 2). Ponds have been dug in black cotton soils formed from volcanic rocks on a gently sloping plateau approximately 60km south of Mt. Kenya. Sagana Fish Farm is located 2km outside Sagana Township, Kirinyaga District, about 105 km Northeast of Nairobi (PD/CRSP, 1998).

3.1.1.1 Climate

Classified as humid tropical groups; tropical wet and dry type; and, distinct dry and rainy seasons are observed. Temperature daily average ranges from 17-23°C; cool season average; 17-19ºC; warm season average; 19-23 ºC, Daily minimum; 14-19ºC, and a daily maximum of 20-30°C. The 30-year average annual rainfall at Sagana is 1,166mm. Humidity in the highland region surrounding Sagana ranges from around 90% in the early morning to about 40% in the afternoon during the dry season to 50-60% in the rainy season. The warmest period is February through April. There is a distinct cool season between June and August, when rainfall is at a minimum. Even though there is little rain, the skies tend to be overcast much of the day during this period. A rainy period known as long rains fall from March through May with a single month peak of 500mm or more in April, (PD/CRSP, 1998). Short rains period is October to December.

3.1.1.2 Topography

Sagana is situated at the edge of a large plain at the southern foot of Mt. Kenya resulting in a climate that is slightly warmer than areas just 30km further north. The farm is characterised by gently rolling topography with several steep hills in the immediate area. The ponds are located on a relatively flat area that slopes gently from north to south (PD/CRSP, 1998).

3.1.1.3 Geology and soils

Soils are formed on volcanic rocks from Mt. Kenya's latest Pleistocene basalts, phenolites, and pyroclastics. In areas with free drainage conditions on moderate to steep slopes, lateritic and red to reddish brown soils are present. In Sagana, the black cotton

soils indicate that the soils have formed under restricted drainage conditions, which are the result of low rainfall and the presence of level to moderate slopes (PD/CRSP, 1998).

3.1.1.4 Vegetation

The vegetation around Sagana fish farm is mainly riparian trees such as *Acacia* spp. and swamp plants such as *Typha* spp., *Cyperus* spp., *Polygonum* spp., including water grasses; *Graminae* spp. as a result of channelization few water plant species occur in the upper part of Sagana.

3.1.2 Description of Ngeki's dam in Machakos

Ngeki's dam in Machakos is 3km outside Machakos township, situated at 1634 m.a.s.l, S 01°30,882′, E 37°13,028′ located in Machakos district, Southern Kenya between Nairobi and Mombasa in Eastern province, Kenya.

3.1.2.1 Climate

The climatic conditions of this study area are semi-arid, with mean annual temperature varying from 15ºC to 25ºC and a total annual rainfall ranging between 400 mm and 800 mm (NRI, 1990). Depending on altitude and aspect, mean rainfall and temperature vary widely.

3.1.2.2 Topography

Machakos district is hilly and dry; much of the topography is sloping.

3.1.2.3 Geology and soils

The most important geological feature in the study area is the Basement system, although the domal uplift during the Miocene, which formed the Rift Valley, had a strong influence on the geomorphological development (Maree, 2002). The soils in the study area are strongly related to the geology and geomorphology, with the mountains and plains/uplands as the determining landforms. The parent material in combination with a mountainous topography has resulted in the formation of somewhat excessively drained, reddish brown, stony and rocky, sandy, and clay loam soils, that vary in depth (Siderius, 1978).

3.1.2.4 Vegetation

The natural vegetation has been largely cleared for cultivation, but can be found scattered through the area, mainly in the stream valleys (Gicheru and Ita 1987) and on the steepest mountain slopes. The crests of the mountains are covered with government forest. The forest vegetation consists of Eucalyptus trees, shrubs and grasses.

3. 2 Experimental design

The fish were subjected to two treatments namely;

(i) **T1** :Stocked into cages (cage factor) and the conditions here include restricted movement and high densities per unit area of culture

(ii) T2: Stocked in open pond (open pond factor) and the conditions here include free movement and low densities per unit area of culture

Fish in cages were fed with artificial feeds using automatic feeders installed in each cage and natural food while fish in the open pond depended solely on food derived primarily from the biological productivity of the ponds (natural food). Factors which were investigated were the influence of cage and open pond factors in parasitic prevalence and intensities.

3.3 Data collection

3.3.1 Abiotic parameters

(i) Dissolved Oxygen, pH, conductivity, temperature were measured using probes (Universal meter, Model WTW)

(ii) Transparency as a measure of turbidity was measured using a Secchi disc.

3.3.2 Biotic data

(i) Zooplankton

Zooplankton samples were obtained by pulling of plankton net at the surface of the water, preserving the sample in 10% formalin and then examining the sample under both the dissecting and compound microscopes.

(ii) Benthic organisms

Benthic samples were obtained using Perspex corers and the analysis of benthic samples including molluscs, crustacean and annelids was done by sieving of the samples through a graded sieve system and examining the samples to check for potential intermediate hosts of fish parasites under a dissecting microscope (Leica Zoom, model 2000).

(iii) Birds

Presence of fish-eating birds was noted through sighting and photography.

(iv) Fish samples

A seine net was used for open pond fish sampling using line transects (where length of the seine net represented one transect from one edge of pond pulled along the length of the ponds). Cages were lifted and hand nets used to obtain the caged fish at bi-monthly intervals for one year. Ngeki's dam had fish in cages arranged in clusters of 10 cages anchored to piers and other fish in open pond. For each sampling session a single cage was selected by a stratified random sampling technique from which case 10 fish were obtained. At the Sagana fish farm, fish were cultured in an open pond system and at least 10 fish were obtained from a single pond and since there are many ponds in Sagana 3-4 ponds were sampled in each sampling session.

Fish morphometrics (Measuring of standard length) prior to dissection and observation of external fish condition was done. Standard dissection procedures (protocols) in Aloo *et al.* (2004) and identification keys by Paperna (1996) were employed in the laboratory to study the parasitic infections and diseases. Data of the fish parasites observed, identified, counted and preserved were recorded in standard protocols (appendix I).

3.4 Statistical analysis

The numbers of individuals of each parasite species in each host were counted, and their total numbers from all the infected hosts were also determined. The prevalence, intensity, mean intensity, and relative density (abundance) of the parasitic infection of all the detected parasites were calculated seasonally, according to Margolis *et al.* (1982) to correlate parasitic infections with some abiotic factors. The density of the *Trichodina*, *Myxobolus,* Sessiline peritrichs, intestinal cysts and liver cysts infection were reported as the number of individuals per microscopic field under X40 magnification marked by categories (very low, low, medium, high and very high) corresponding to the number of parasites (< 5, $5 - 9$, $10 - 14$, $15 - 19$, ≥ 20, respectively). Shannon-weaner diversity index, Jaccards and Sorensen's coefficients and Morisita's indexes of community similarity were used for comparisons of parasitofauna in the two sites.

A non parametric sign test was used to test variations in parasite type in caged and open pond fish in Machakos. Student's t-test was used in determining whether the differences obtained in mean prevalence and mean intensities in caged and open pond conditions was statistically significant, a simple correlation analysis was carried out to determine relationship between size of fish and parasitic intensities. In all occasions statistical significances' were tested at 5% level of significance. Further ecological quantifications were performed using the Shannon-weaner diversity index, Jaccards and Sorensen's coefficients and Morista's indexes of community similarity (James and Jerrold 1977).

CHAPTER FOUR

RESULTS

4.1 Environmental factors

4.1.1 Abiotic parameters

The mean monthly abiotic parameters monitored during the study (Table 1) were within the normal range required for the successful culture of *O. niloticus*. For all the sites the pH range was 6.47 to 9.44, dissolved Oxygen concentration ranges were between 2.84mg/l to 10.46 mg/l, temperatures ranges of 22.38˚C to 31.8˚C and conductivity ranges of between 93.5µs/cm to 930µs/cm. These parameters were within the tolerable range for *O. niloticus* fish.

Adverse abiotic factors may cause significant stress to the fish hence increasing its susceptibility to disease.

Table 1: Physico-chemical parameters of the study sites as monitored during the study period (Mean ±SE)

	Machakos reservoir	**Sagana pond N18**	**Sagana pond A3**	**Sagana pond A4**	**Sagana pond A7**
pH	7.7-8.9	7.0-9.17	6.87-9.44	6.47	9.21
Dissolved Oxygen (mg/l)	6.2±0.34	4.87±1.8	6.20±3.03	2.84	10.46
Dissolved Oxygen (%sat.)	83.5±8.7	29.15±8.4	124.60±70.4	37.3	166.5
Temperature (°C)	22.38±1.4	24.05±0.68	26.78±1.33	22.75±0.15	31.8
Conductivity (µs/cm)	930±118.3	114.5±19.10	107.25±10.8	93.5±26.561	95

4.1.2 Biotic parameters

Sagana had a higher species richness (8) of birds observed than Machakos (Table 2). Gastropod molluscs were very abundant in Machakos, while they were found in low numbers in Sagana.

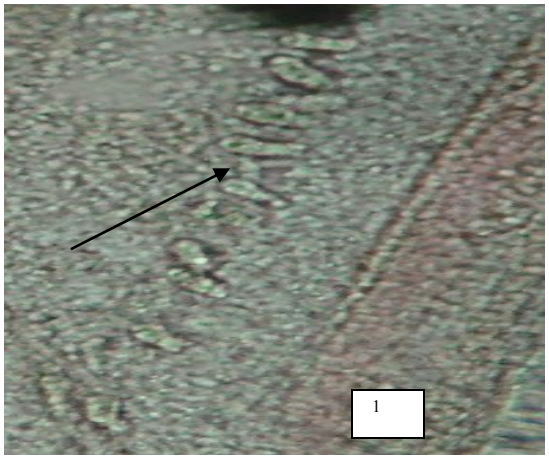
Table 2: Biotic parameters of the study sites

BIOTIC	MACHAKOS	SAGANA (N18,A3)
Zooplankton	Copepods (dominant), Cladoceran, Rotifers	Copepods (dominant), Cladoceran, Rotifers
Birds	Cattle egrets (*Bubulcus ibis),* ducks, Grey herons (*Ardea sp.*) and Hammer kops (*Scopus umbretta*). Few species and low abundance	Cattle egrets (*Bubulcus ibis),* Comorants (*Phalacrocorax auritus*), ducks, Grey herons (*Ardea sp.*), Kingfishers (*Alcedo atthis*) , Sacred Ibis (*Threskiornis bernieri*), and Pelicans.
Benthos	few Chironomids, Oligochaete (Tubificidae) and Snails (dominant in the site)	Chironomids, Oligochaete (dominant) , and Snails

4.2 Parasitological data

4.2.1 Identification and comparison of the parasites occurring in *O. niloticus*

Three Protozoan species were identified, namely: the ciliates sessiline peritrichs (Plate 1) and *Trichodina* spp. (Plate 2), and the flagellate *Cryptobia* spp. Except for *Trichodina* spp., which sometimes occurred also in the skin scrapings, all the other protozoan parasites found in this study were observed exclusively on the gills of *O. niloticus* fish. While *Trichodina* spp. and *Cryptobia* spp. were highly motile, sessiline peritrichs were found attached to the gill epithelium.



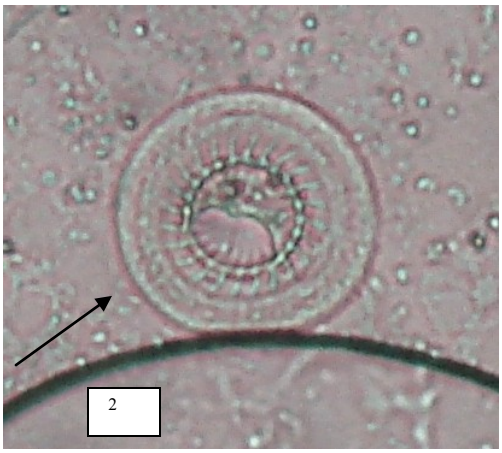
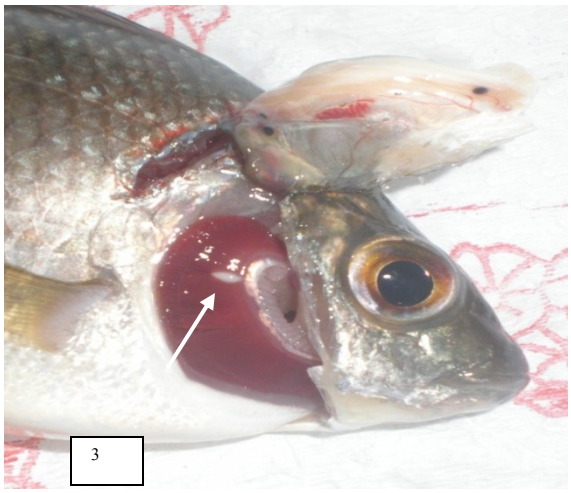


Plate 1: Sessiline peritrichs in gills attached to lamellae (Mg X400) Plate 2: *Trichodina* spp. from gills (Mg X400)

Among Myxozoans, *Myxobolus* spp. spores were often found encysted in white cysts in gills (Plates 3 and 4) and muscle or sometimes they were sparse in intestine and kidney.



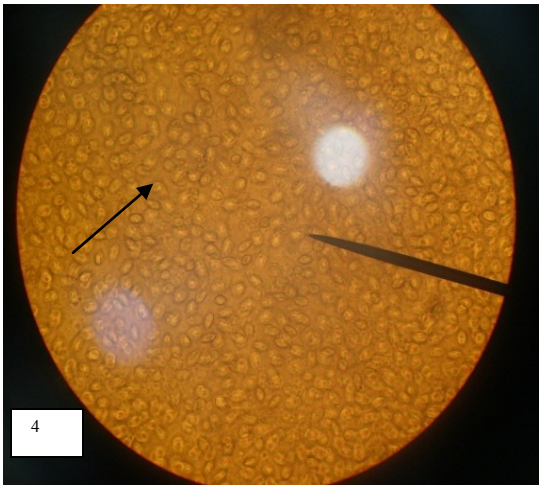


Plate 3 and 4: *In situ* white cyst containing numerous *Myxobolus* spp. in the gills (left, arrow) and *Myxobolus* spores (right) (Mg X400)

A rich helminth community was observed, consisting of 12 different types (family/genus). Among Monogeneans, Dactylogyrids (Plates 5 and 6) and Gyrodactylids *Gyrodactylus* spp. were observed respectively on gills and skin. Among digeneans, *Clinostomum* spp. (Plates 7 and 8) was observed dominantly as yellow cysts on the skin below the scales or as large cysts behind the gills, while *Euclinostomum heterostomum* (Plates 9 and 10) was observed as large round cysts in anterior and posterior regions of

the kidney. Again, encysted Diplostomatid metacercariae (Plate 11) were infrequently found in the eyes, while *Tylodelphys* spp. (Plate 12) was predominantly found swimming actively in the vitreous humour of the eyes. Furthermore digenean metacercariae were found in gill (Plate 13), liver (Plate 14) and skin, in the last localization as "Black spot" (*Neascus* spp. and/or *Posthodiplostomum* spp.) (Plate 15). Larval Cestodes (Plates 16, 17 and 18) *Armithalingamia macracantha* were found free in the gut lumen or encysted in intestinal wall. Nematodes at larval stage (Plate 19) were rarely found in visceral serosae. One species of Acanthocephala; *Acanthosentis* spp. (Plate 20) was observed, generally in the intestinal lumen. In rare cases copepods, *Lamproglena* genera were found in gills (Plates 21 and 22).

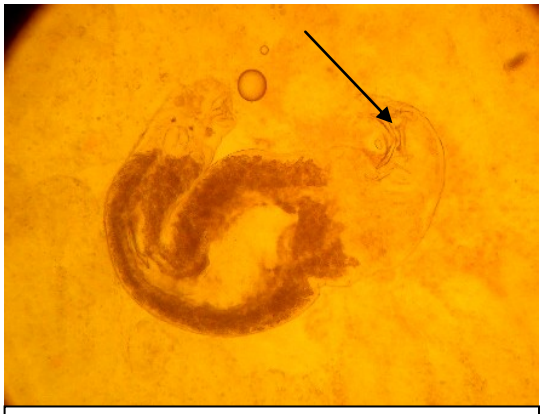


Plate 5: Dactylogyrid monogenean (Mg X200)

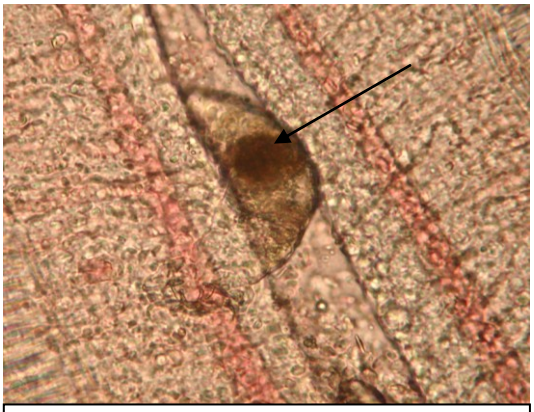


Plate 6: Dactylogyrid monogenean containing a developing egg (Mg X200)

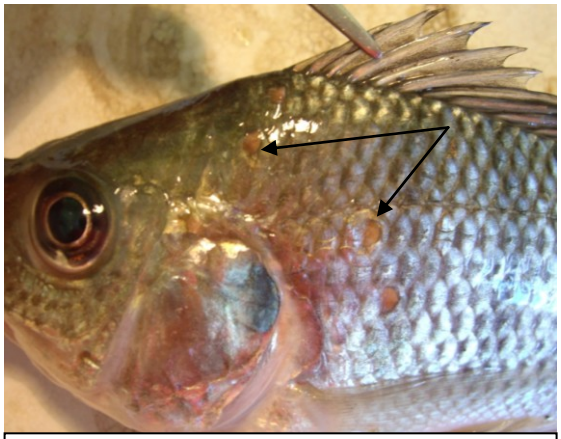


Plate 7: *Clinostomum* spp. cysts in the skin

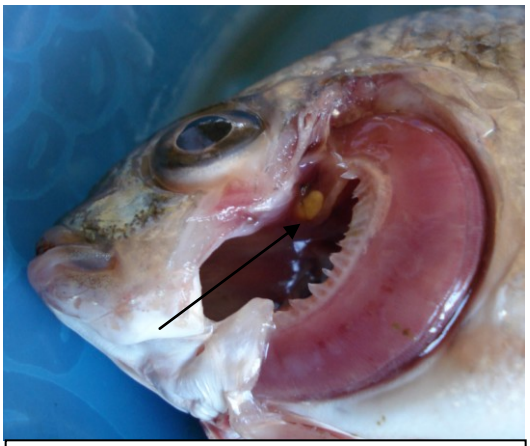


Plate 8: *Clinostomum* spp. close to gill arch

Plate 9: Encysted *Euclinostomum heterostomum* in the kidney

Plate 10: Two excysted *Euclinostomum heterostomum* metacercariae

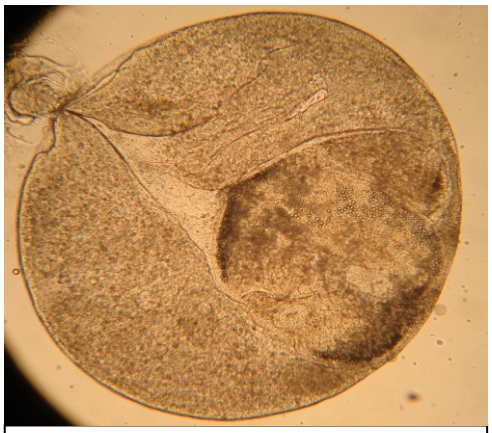


Plate 11: Encysted Diplostomatid metacercaria (Mg X100)

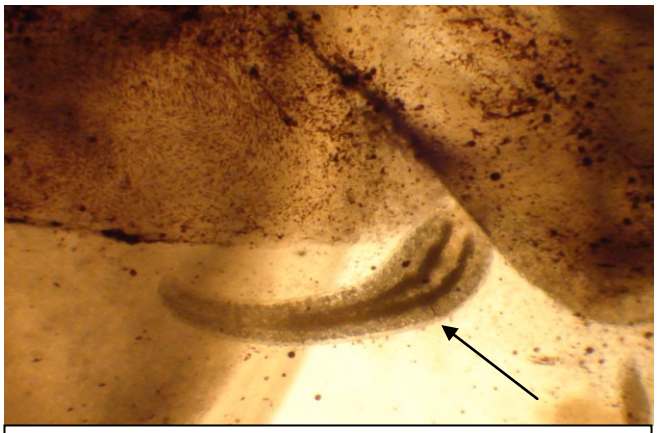
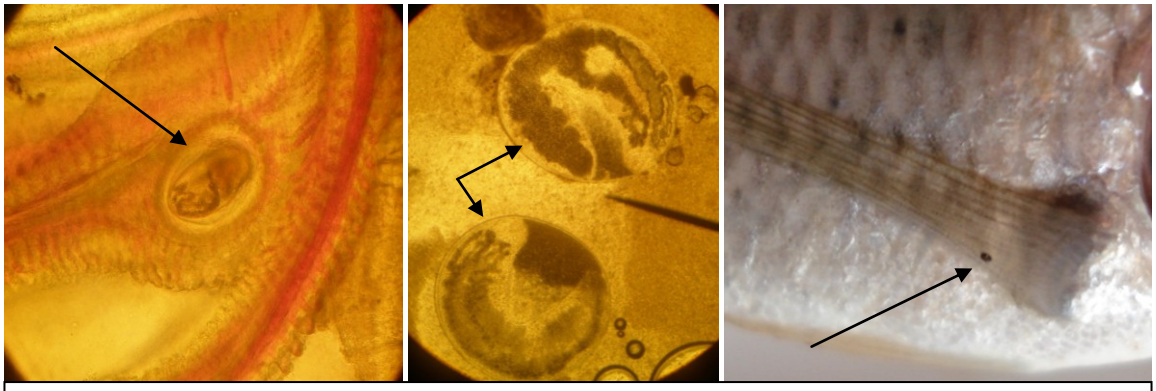
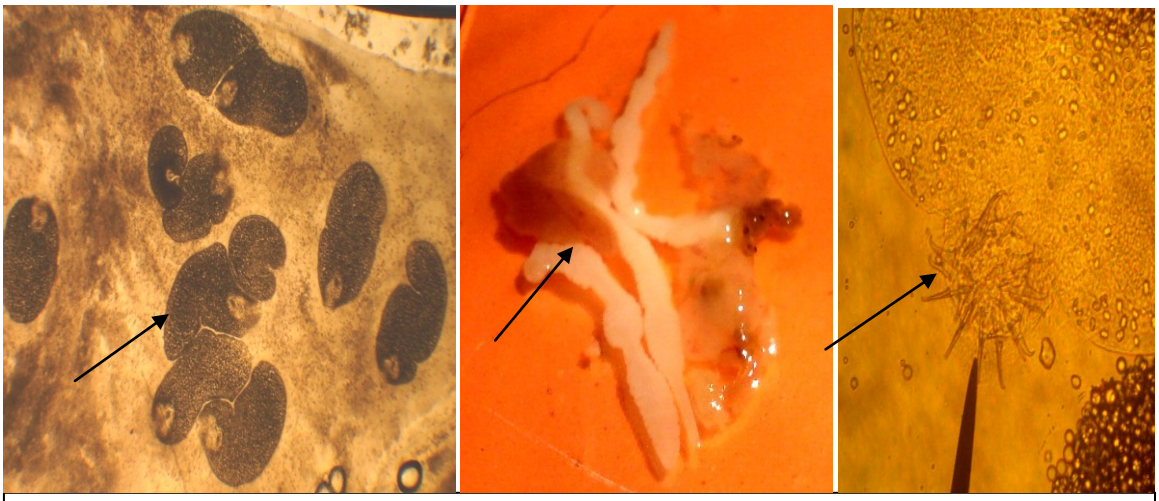


Plate 12: Diplostomatid *Tylodelphys* spp. metacercariae in vitreous (Mg X100)



Plates 13, 14 and 15: Gill metacercaria (left, Mg X200), liver metacercariae (centre, Mg X200) and "Black spot" on the pectoral fin (right).



Plates 16, 17 and 18: Dilepidid cestode *Armithalingamia macracantha*: larval stages free in the gut lumen (left, Mg X100), excysted from gut serosa (centre) and a particular of *rostellum* (right, Mg X400)

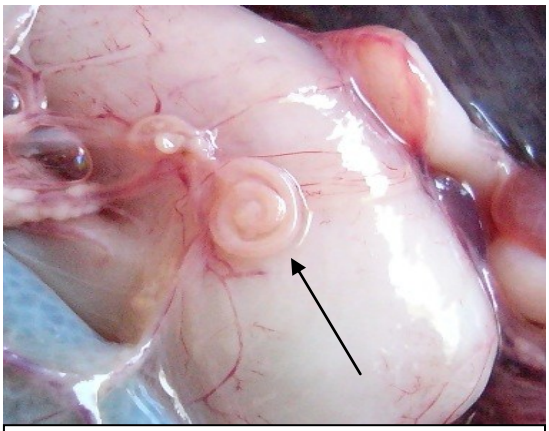


Plate 19: Larval nematode on visceral serosa

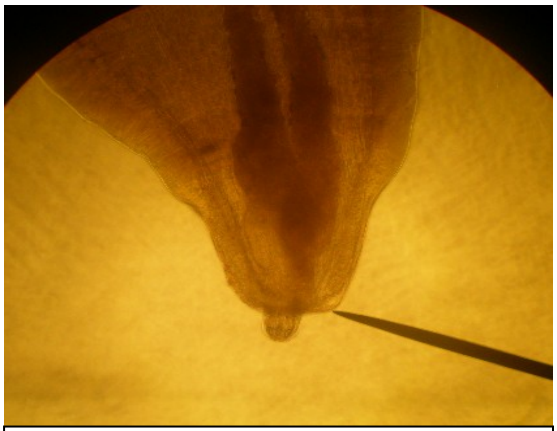


Plate 20: *Acanthosentis* spp. with everted proboscis (Mg X400)

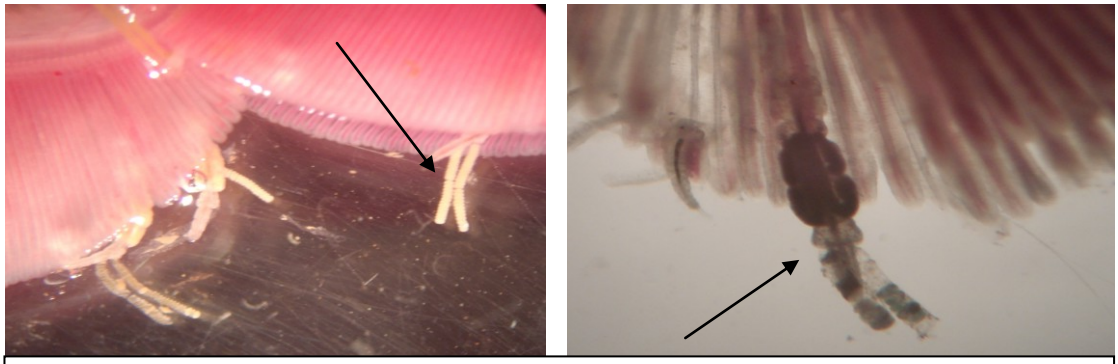


Plate 21 and 22: Copepod crustaceans, Lamproglena genus in tilapia gills

Plates 1-22: showing the parasitofaunal assemblages that were found infecting *O. niloticus* fish. The parasites found in caged *O. niloticus* fish and open pond *O. niloticus* fish were of the same species.

4.2.2 Quantitative parasitological data: prevalence rates, intensities and abundances

A total of 370 *O. niloticus* fish were examined comprising of 57 caged fish in Machakos Ngeki's dam, 86 open pond fish from Machakos Ngeki's dam and 227 open pond fish from Sagana aquacultural centre. The sample size is shown (Table 3).

Table 3: Sample size (n) distribution by date, system and site over the entire study period

	Year 2007					Year 2008	Total
	March	May	July	Sept.	Nov.	Feb*.	
Machakos							
Caged	8	10	12	5	11	11	57
Open pond	13	12	18	11	17	15	86
Sagana							
Open pond	54	17	51	43	30	32	227
							370

Due to the limitation in the number of caged fish few samples of caged fish were examined as compared to open pond fish (Table 3). However, in each case a desired representative sample size (n>30) was achieved.
February represents a deviation from sampling interval done in February 2008 instead of January 2008.

In Sagana open pond *O. niloticus* fish the *Tylodelphys* spp. had a lower prevalence than in Machakos systems but had a high mean intensity (13.5). The monogenean Gyrodactylids too were significant in Sagana with a mean intensity of (11.5). Parasites that were found in Sagana had higher intensities than those of Machakos (fig. 3).

In Machakos open pond *O.niloticus* fish *Tylodelphys* spp. was also dominant in prevalence and mean intensity (fig. 4) as well as the black spot metacercariae (*Neascus* spp. and/or *Posthodiplostomu* spp.). Liver metacercariae spp. too was predominant with a mean intensity (48.8) higher than in the other systems.

In Machakos caged *O.niloticus* fish *Tylodelphys* spp. was dominant both in prevalence, mean intensity and abundance as shown in (fig. 5). Protozoan parasite; *Trichodina* sp was also important as well as monogenean Dactylogyroid (*Cichlidogyrus tilapiae*) and Gyrodactylus sp. *Clinostomum tilapiae* too was significant in prevalence and mean intensity.

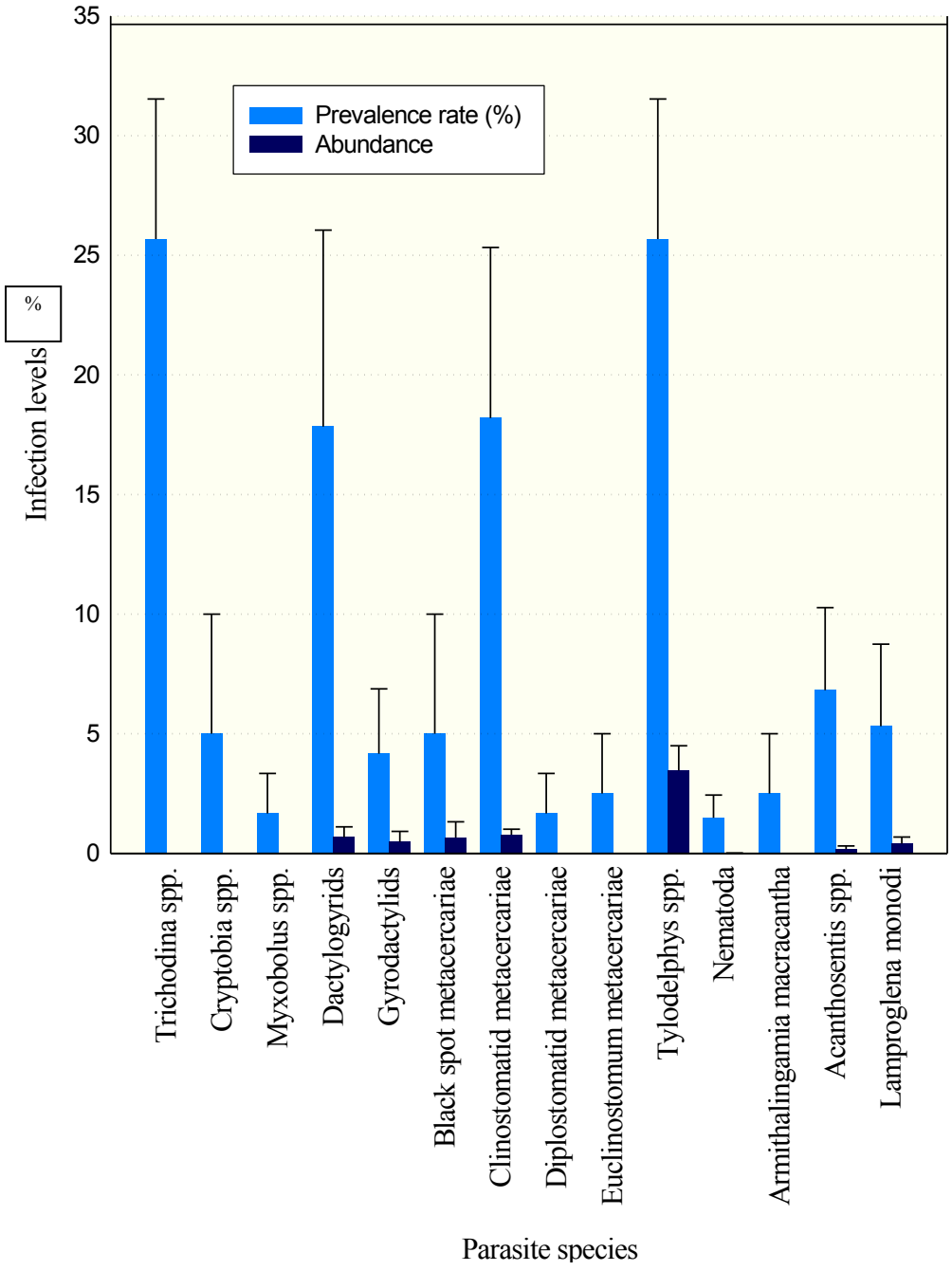


Fig. 3: Shows mean prevalence rates and abundance of parasites in Nile Tilapia fish from Sagana fish ponds at the end of the study.

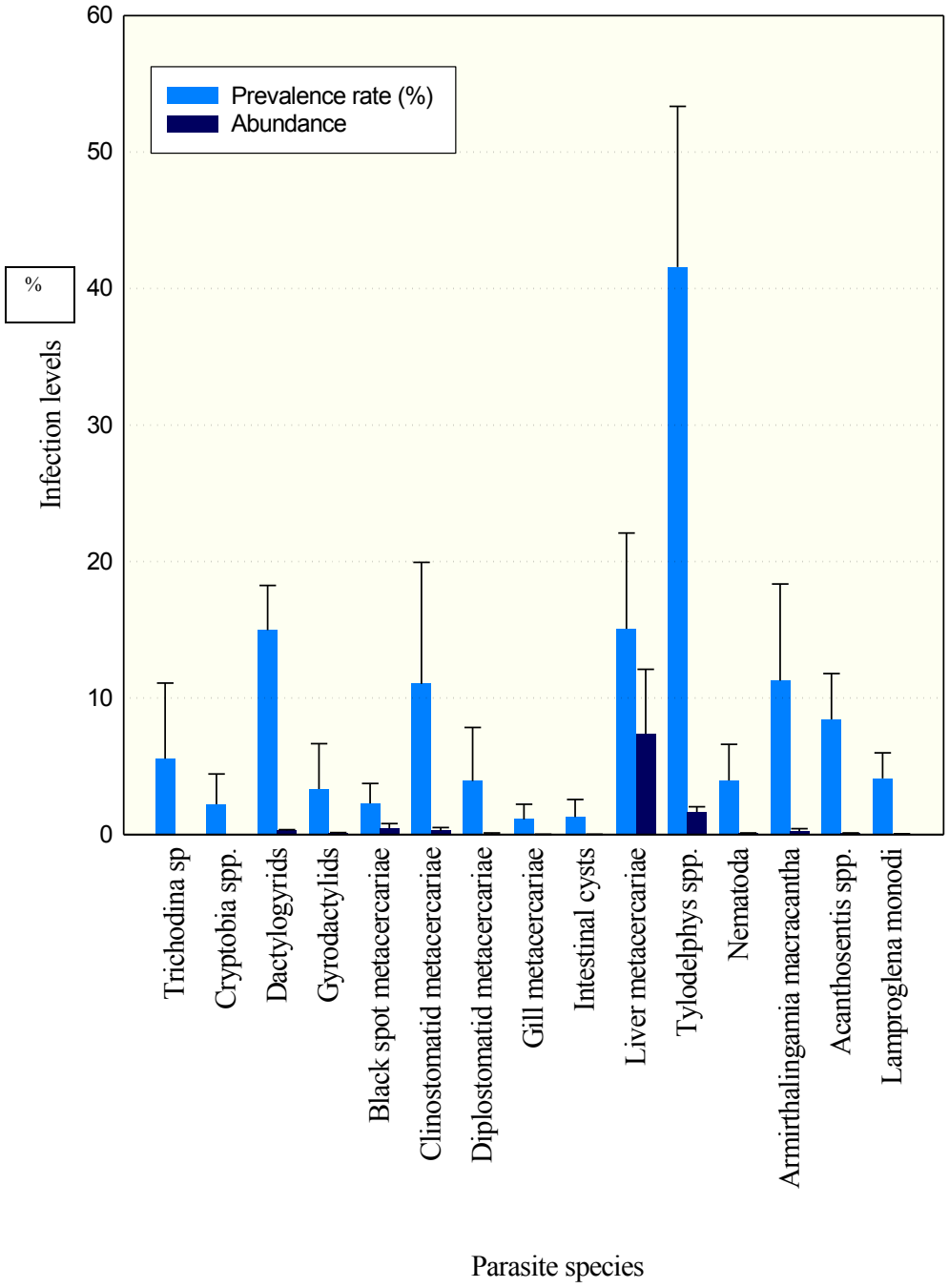


Fig. 4: shows mean prevalence rates and abundance of parasites in Nile Tilapia fish from Machakos open pond system at the end of the study duration.

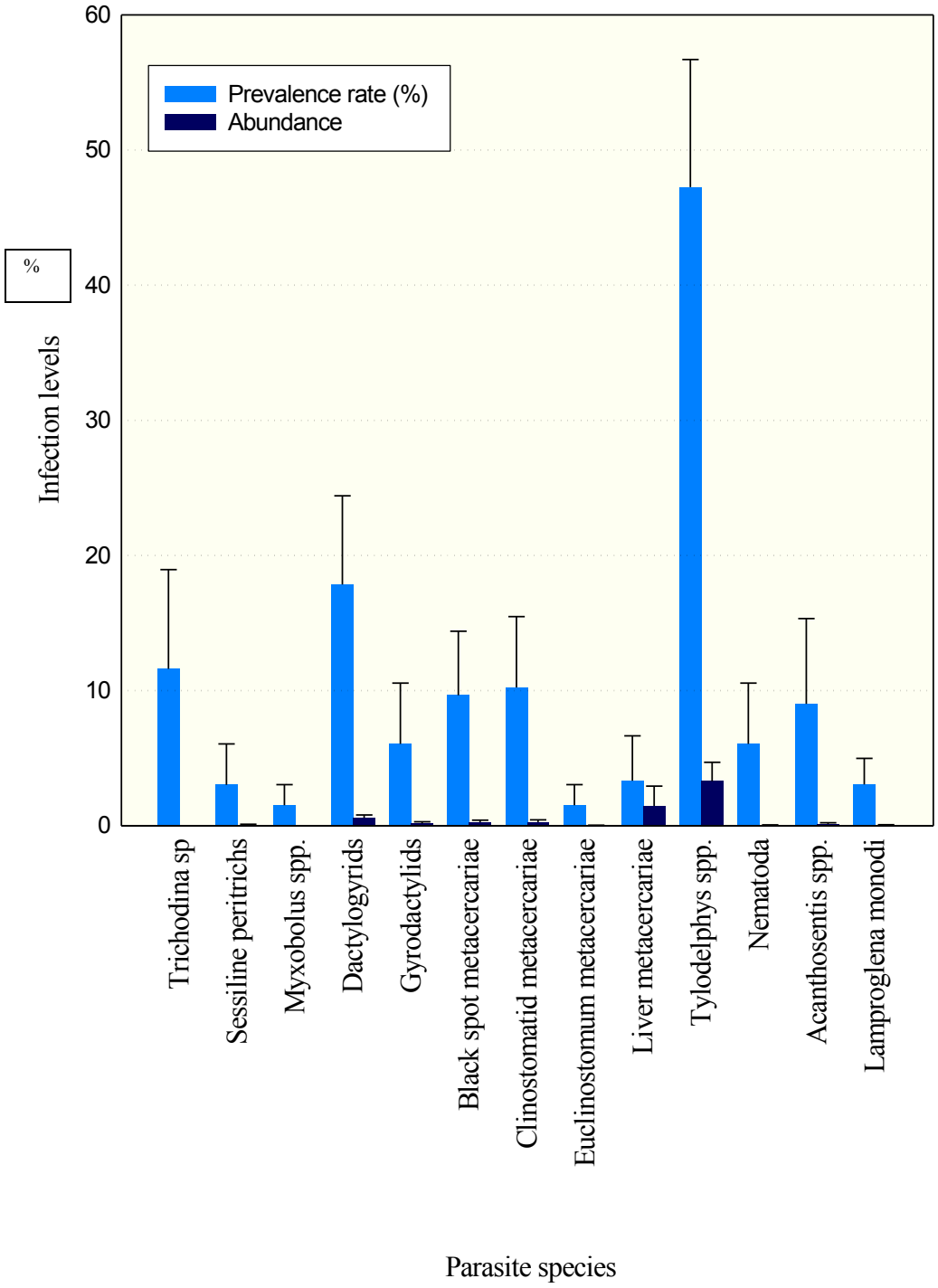


Fig. 5: shows mean prevalence rates and abundance of parasites in Nile Tilapia fish from Machakos caged system at the end of the study duration.

In data analysis a non parametric sign test was used to test variability in the types of parasites infecting caged and open pond fish (where (x) represented the counts of the least sign of difference between caged and open pond *O. niloticus* fish= 8 negatives, α =0.05, n=19. Critical value from statistical tables at n=19, $\alpha/2$ for a two tailed test=4) provided sufficient evidence within accepted degrees of confidence to fail to reject the hypothesis of no significant differences between caged and open pond *O. niloticus* fish in Machakos.

Similarly, there was no statistically significant difference between the type of parasites infecting *O. niloticus* fish in Machakos open pond and *O. niloticus* fish in Sagana open ponds. Further analysis using t-test to test significant differences between prevalence rates and mean intensities between caged fish and open pond in Machakos were insignificant statistically.

The majority of the parasites detected in the *O. niloticus* from the two sites were found in all seasons. These included: *Tylodelphys* spp., *Clinostomum* spp. (Fig. 6, 7, 8, 9, 10, 11, 12 and 13), *Euclinostomum heterostomum*, larval Cestodes, Nematodes, Acanthocephalans, Dactylogyrids and Gyrodactylids. The results indicated that some parasite species exhibited seasonality, such as *Tylodelphys* spp. (fig.12) and *Clinostomum* spp. (Fig. 9).

Some parasites occurred throughout the year at low infection levels and lacked seasonality; larval cestodes, Black spot metacercarie, Acanthocephalans, Nematodes and crustaceans were noted occasionally. A summary of month by month analysis of some parasites found per study site are shown below (Fig. 6-13)

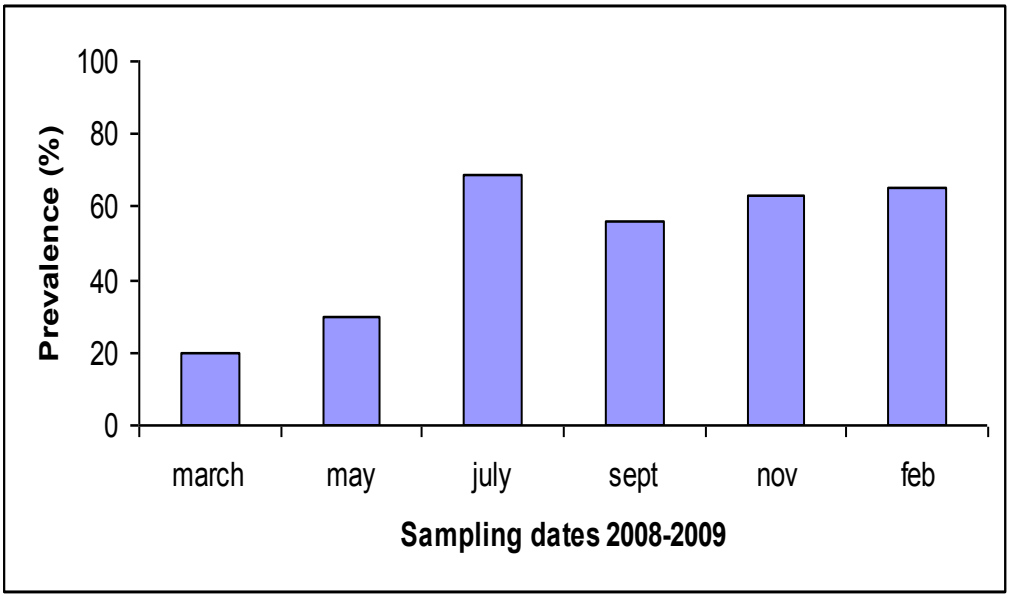


Fig 6: Prevalence of *Tylodelphys* spp. in *O. niloticus* in Sagana open ponds

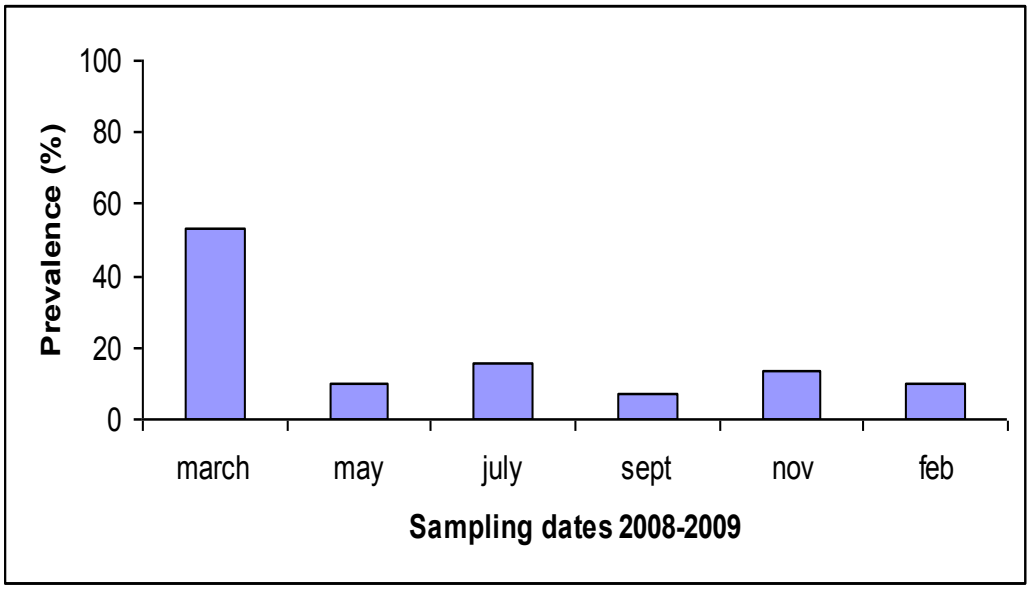


Fig 7: Prevalence of *Clinostomum* spp. in *O. niloticus* in Sagana open ponds

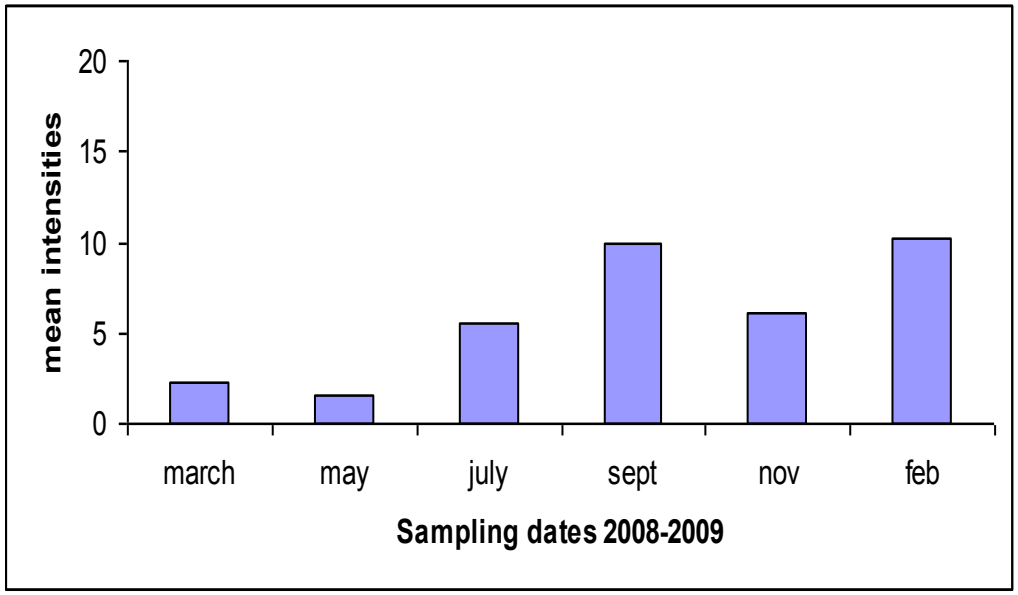


Fig 8: Mean intensities of *Tylodelphys* spp. in *O. niloticus* in Sagana open ponds

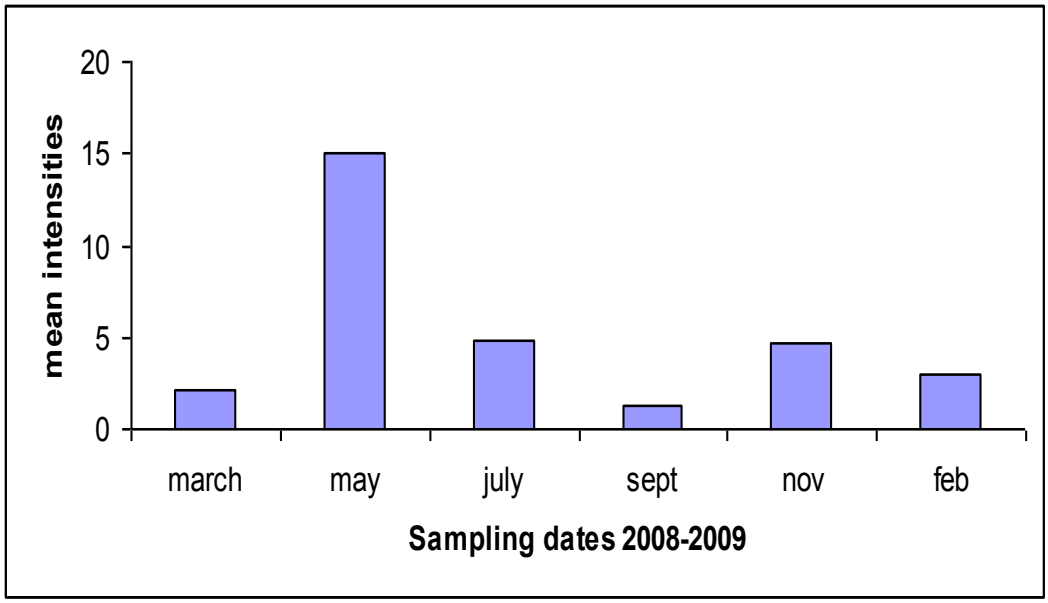


Fig 9: Mean intensities of *Clinostomum* spp. in *O. niloticus* in Sagana open ponds

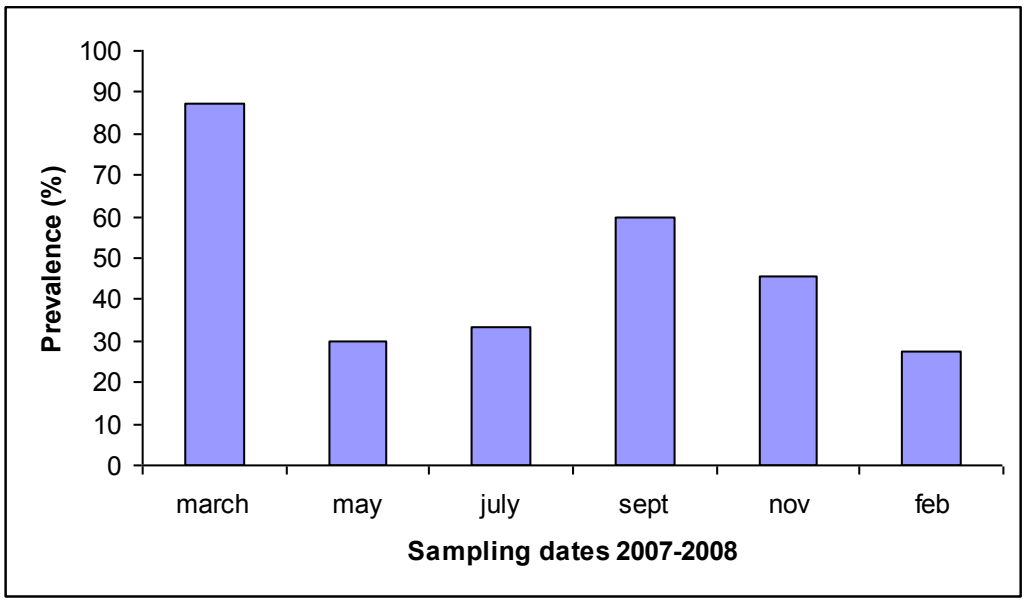


Fig 10: Prevalence of *Tylodelphys* spp. in *O. niloticus* fish in Machakos cages

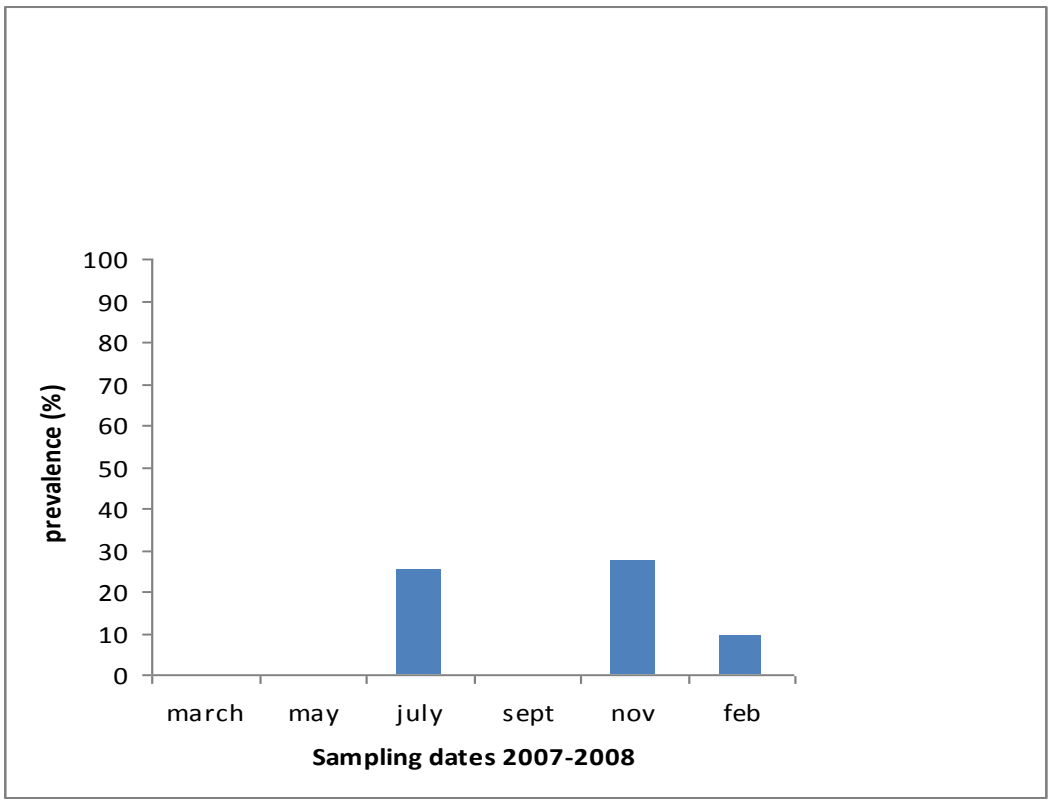


Fig 11: Prevalence of *Clinostomum* spp. in *O. niloticus* in Machakos caged fish

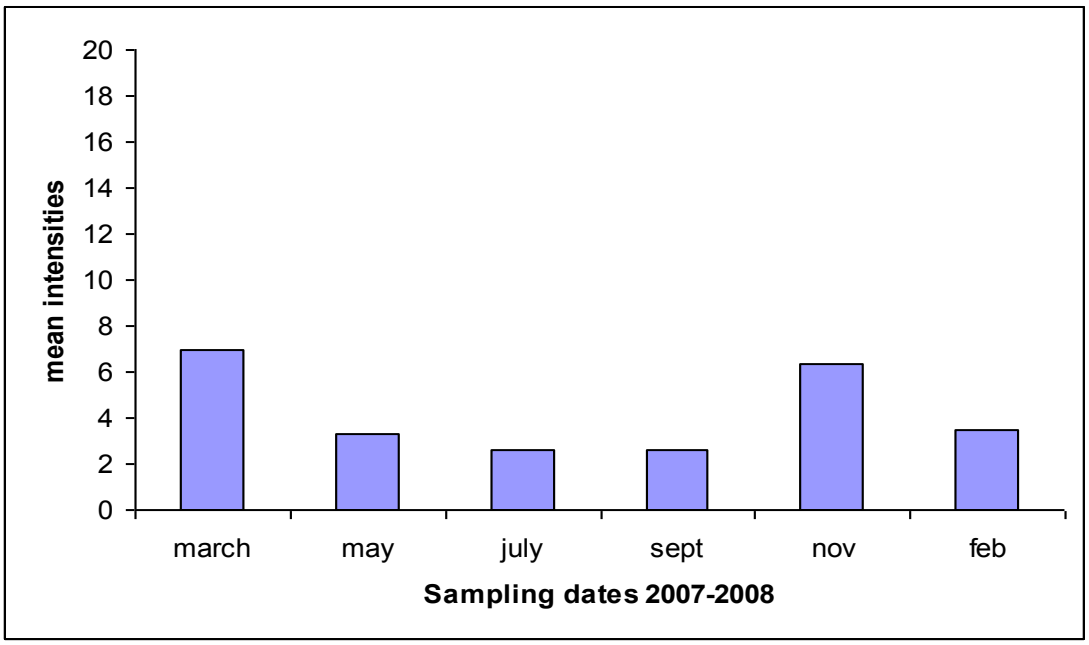


Fig 12: Mean intensities of *Tylodelphys* spp. in *O. niloticus* in Machakos open pond fish

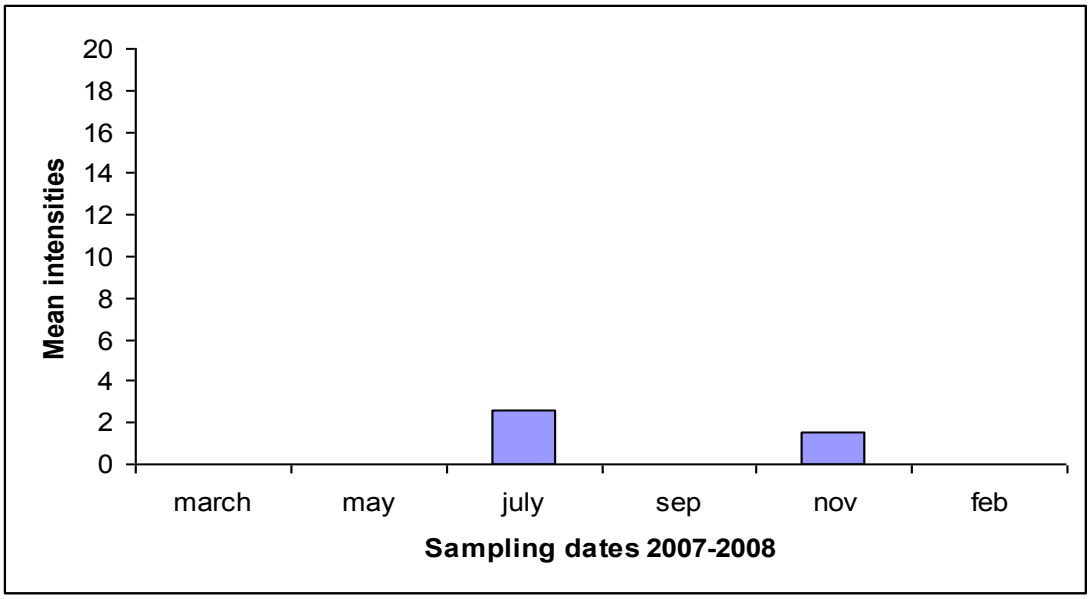


Fig 13: Mean intensities of *Clinostomum* spp. in *O. niloticus* in Machakos open pond fish

Further ecological quantification of parasite community similarities between Machakos open pond fish parasite community and Sagana open pond fish parasite community revealed 0.71 and 0.83 Jaccards (CC_J) and Sorensens (CC_S) coefficients respectively. Since both values were close to 1, then most species found were common in both communities.

Morisita's index (I_M) of community similarity between Machakos caged and Machakos open pond *O. niloticus* parasitofauna, was 0.15 meaning that there was a 15% probability of picking an individual randomly from each of the two communities belonging to the same species, relative to the probability of randomly selecting a pair of specimen of the same species from one of the communities while that (I_M) between the *O. niloticus* parasitofauna of Machakos open pond and Sagana Open ponds was 0.22 implying that there was a 22% probability of picking individuals randomly belonging to the same species from each of the two communities relative to the probability of picking a pair of specimen belonging to the same species from one of the communities and the I_M between the *O. niloticus* parasitofauna of Machakos caged and Sagana open ponds was 0.46. This implied that there was a 46% probability of finding individuals in random sampling belonging to the same species from each of the two communities relative to the probability of randomly selecting a pair of specimen of the same species from one of the communities.

4.2.3 Fish size and parasite intensities correlations

The intensity of infestation was correlated with the size of the host *O. niloticus*.

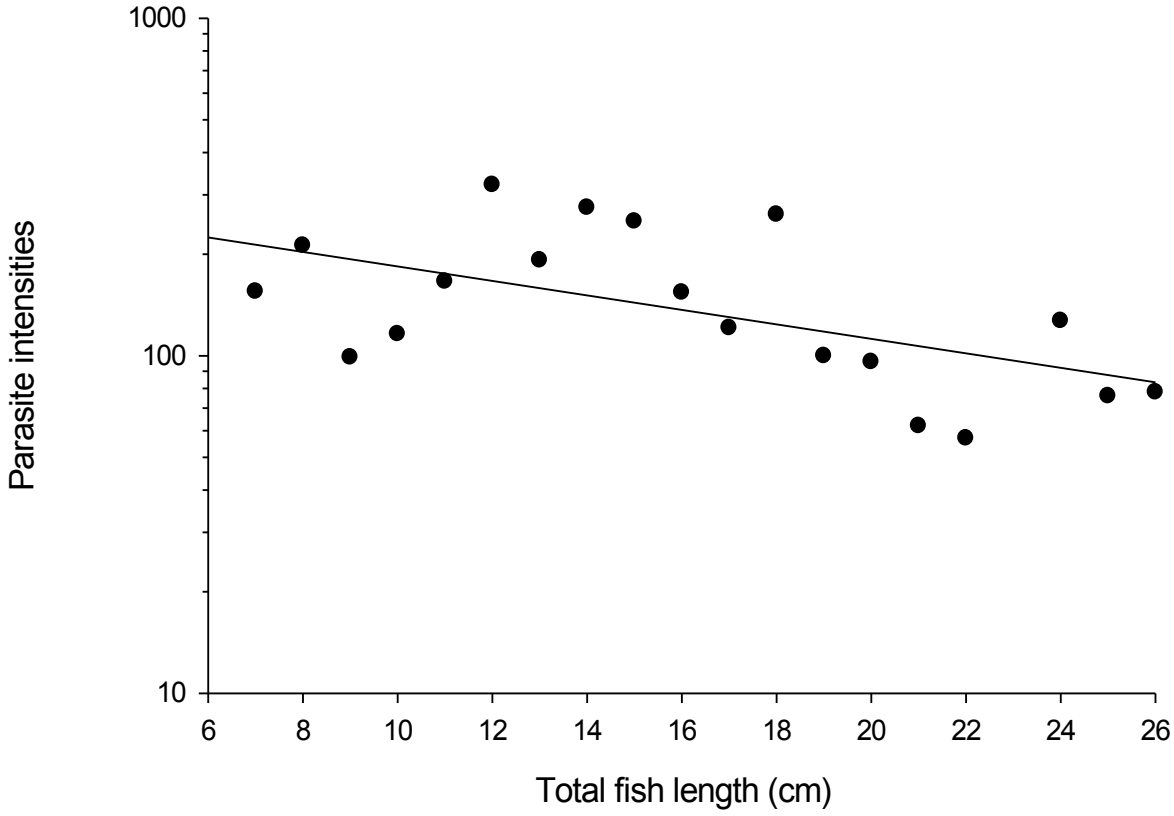


Fig. 14: Semi-log Scatter plot of variation of parasite intensities with size of Nile Tilapia

There was a weak negative correlation which was not statistically significant (R^2=0.278, p>0.05) between the size of the fish and the intensity of parasitic infestation (Fig. 14).

CHAPTER FIVE

DISCUSSION

5.1 Occurrence of Protozoan parasites: Ciliates and Flagellates

The occurrence of *Trichodina* spp., sessiline peritrichs and *Cryptobia* spp. is a common finding for their ubiquitous presence in water environment (open ponds or cages) and their opportunistic action on stressed or traumatised fish (Paperna, 1996). In the examined fish prevalence, mean intensity and abundance values for these protozoan parasites were generally low and only in sporadic cases a high mean intensity was observed for instance for *Cryptobia* spp. in open pond in Machakos.

Trichodina spp. was found in both open pond and cages in Machakos and Sagana but no massive infections were detected. Sessiline peritrichs were detected only in caged fish at very low level of infection, may be due to a net fouling and a less active swimming behaviour of caged fish.

Attached sessilians rarely could produce mechanical damage or peripheral tissue response in the gills and fish mortality can be observed only during a dense cover disrupting gas exchange through the respiratory epithelium (Paperna, 1996).

In our case the low biomass density present in Machakos cages avoided stressful conditions which favour colonization and infection by opportunistic protozoan organisms potentially pathogenic for fish.

5.2 Occurrence of metazoan parasites

5.2.1 Myxozoans

The prevalence of *Myxobolus* spp. observed in this study is lower than that reported by Gbankoto *et al.* (2001b) with regard to *Myxobolus* sp. found in gills of *Tilapia zillii* and by El-Mansy (2005), who found *M. heterosporus* in several organs of *O. niloticus* in River Nile, Egypt. This could have been an indication of seasonality as reported for many myxozoan species and strongly related to the presence of the alternate host (benthic annelids) during the year. In Africa, the first description of myxosporeans was given by Baker (1963). This was followed by a number of studies, such as those of Paperna (1968), Abolarin (1974), Obiekezie and Okaeme (1990), Fomena and Bouix (1994), Kostoingue and Toguebaye (1994) and Fomena and Bouix (1997). Studies in

Egypt include Fahmy *et al.* (1971), Imam *et al.* (1987), Ashmawy *et al.* (1989), Abdel-Ghaffar *et al.* (1995), Koura (2000), Ali *et al.* (2002) and El-Mansy (2005).

The pathogenic effects of several myxosporean parasites have been described, among which there has been myoliquefaction of muscle tissues after the death of the host (Barja and Toranzo, 1993; Pampoulie *et al.*, 1999), reduction of the respiratory capacity (Molnar and Szekely, 1999) or of host fecundity (Swearer and Robertson, 1999) and parasitic castration (Sitja-Bobadilla and Alvarez-Pellitero, 1993). During investigations on myxosporean diseases in Tilapia from Benin (West Africa), *Myxobolus dahomeyensis* (Siau, 1971) was found in the ovaries of some wild species (Sakiti *et al.*, 1991). The occurrence of myxosporean parasites has been investigated extensively in fish farms because their potential pathogenic effect on their hosts (Okaeme *et al*., 1988; Obiekezie and Okaeme, 1990; Tarer *et al*., 1996), mainly in case of histozoic localizations in functionally relevant organs. In Kenya the present study is the first report of these myxozoan parasites in fish.

The presence of *Myxobolus* spp. in caged fish from Machakos and in fish farmed in open ponds of Sagana could suggest the possible introduction of the parasite from Sagana fingerlings to Machakos, but this hypothesis should be verified by a correct identification of the species involved using both morphological and molecular techniques.

5.2.2 Monogeneans

Monogeneans are hermaphroditic ectoparasites having a direct life cycle and requiring only one host on which they attach to the skin and gills (Jadwiga, 1991). In the fish examined Gyrodactylid parasites (viviparous) from the skin and Dactylogyrids (oviparous) from the gills were collected in fish from all the sample sites. In Machakos cages and ponds the prevalence, mean intensity and abundance values were almost similar, even if slightly higher in the caged fish both for Dactylogyrids and Gyrodactylids. This could be referred to the strict confinement and overcrowding of caged fish, favouring the easy contact and fish-to-fish transmission of Gyrodactylids, and invasion by oncomiracidia of Dactylogyrids. Furthermore caged fish may have certain types of physical injuries that are specific to this farming system and, when overstocked, fish may suffer from fin and skin damage caused by net abrasion (Moring, 1982) and are

more susceptible to pathogenic organisms if handled without care. Studies by McGuigan and Sommerville (1985) on the effects of cage culture of fish on the parasite fauna also agree with our findings.

Several faunistic and seasonal studies on dactylogyrid species have been done to date (Valtonen *et al.*, 1990; Lux, 1990; Pojmasńka, 1994; Lacasa-millán and Gutiérrez-galindo, 1995). Some of these studies are mainly focused on the existence of dactylogyrids in connection with host fish size and seasonal changes (Koskivaara *et al.*, 1991; Öztürk, 2002). Such studies have shown that the *Dactylogyrus* species variety and richness changed from locality to locality.

Similarly, this study determined the existence of relevant seasonal differences in prevalence and intensity values of dactylogyrid species in *O. niloticus* fish from both Sagana and Machakos, may be linked to abiotic parameters such as temperature. Temperature is commonly regarded as one of the most important factors determining the existence and abundance of monogenean parasites (Koskivaara *et al.,* 1991). While some of them tend to reproduce more at a higher water temperature, others prefer a cool water temperature (Hanzelová and Žitňan, 1985). However, it should be kept in mind that the seasonal abundance of dactylogyrids is sometimes more influenced by other abiotic and biotic factors than by temperature; e.g. *Dactylogyrus solidus* is very sensitive to oxygen depletion (Bauer, 1962). Similary, Gonzales-Lanza and Alvarez-Pellitero (1985) claimed that the relationship of *Dactylogyrus legionensis* to temperature was not clear, and that other abiotic (light, pH, oxygen and salinity) and biotic (spawning) factors had more influence on the existence of parasite species.

5.2.3 Digeneans

5.2.3.1 Eye flukes

The differences in infection levels of *Tylodelphys* spp. and other diplostomatid metacercariae observed in caged and open pond fish in each system could have resulted from a number of factors. In any sampling season the chance proximity of infected snails could have affected the results at any one time. Concerning to *Tylodelphys* spp., very little variation occurred in the mean intensities from different sampling sites, with slightly higher prevalence values in Machakos caged fish may be for their increased susceptibility to cercarial invasion in consequence of the confinement in cages. These findings are

similar to those reported by Ching (1985) for *Diplostomum beari bucculentum* and Field and Irwin (1994). *Tylodelphys* spp. findings in this study on relationship to temperature are different to findings by Buchmann and Bresciani (1997) that found very low prevalence in Danish rainbow trout. This could have been due to the fact that here in the tropics temperatures are always high as compared to the temperate regions. In Sagana temperatures were always high (>23°C) in all the sampling months, even if the highest values were recorded in November (28.6°C). The eye fluke *Tylodelphys* spp. neither showed seasonality in prevalence nor mean intensities in Sagana because the regulated water levels in the ponds tend to mitigate abrupt fluctuations in temperatures. In Machakos prevalence and intensity of *Tylodelphys* spp. fluctuated with changes in temperature with a high intensity in dry months, may be due to high temperatures which trigger cercarial shedding by snail host. Seasonality of eye fluke infection levels were previously described (Wootten, 1974; Burrough, 1978) and were similar to findings in this study. Eye flukes were found in almost all populations examined, regardless of the site, confirming the studies by Stables and Chappell (1986) and the ubiquitous presence of eye flukes in fresh and marine waters. However these parasites have been reported for the first time in these study sites. Eye infections by *Diplostomum* spp. metacercariae are considered of detrimental impact to fish activity and survival since feeding and therefore growth rates of the infected fish are greatly hampered. Diplostomatid metacercariae may penetrate in the eye socket and cause exophthalmia, or inside the eyeball within the retina, in the vitreous humour or inside the lens. The greatest damage is caused when the lenses are infected by a non-encysted metacercaria of *Diplostomum spathaceum*, while eye fluke that lives in vitreous humour as *Tylodelphys* spp. or other *Diplostomum* species, are not yet correlated to pathological effect (Paperna, 1996). Our findings reveal this kind of non-encysted metacercaria with both a high prevalence and intensities.

In *Diplostomum* spp. infections the damage due to this parasite was evident as exophthalmia, blind eyes and blackening of fish. Blind fish do not see food and therefore feed poorly leading to stunted growth which is of economic importance. The parasite infection level raises some concern as the pathogenicity (Shariff *et al.*, 1980, Brassard *et al.*, 1982) and the growth reducing effect (Sato *et al.*, 1976; Buchmann and Uldal, 1994)

of eye fluke infections are well known for fish hunting by eye as trouts, but concerning tilapias further studies need to be assessed.

5.2.3.2 Clinostomatids

Fish in Sagana showed prevalence and mean intensity values of Clinostomatids higher than Machakos fish. Machakos caged and open pond fish showed an almost equal prevalence with a slightly higher intensity in caged fish. A few hatchery fish examined during the study indicated presence of these parasitic cysts, so the parasites could have been carried from Sagana hatcheries with the fingerlings during stocking to Machakos, where they found a good environment to establish their life cycle.

During this study two different species of clinostomatids were isolated from tilapias, a smaller and whitish one from the skin and a bigger and yellowish one from the basis of gills and pharyngeal area, but the taxonomical status of these parasites actually is not clear and the only review on valid species of genus *Clinostomum* was published by Ukoli in 1966. Further morphological and molecular studies need to be assessed to achieve a correct identification of these parasites.

Despite the large size of both cysts (3-7mm) and excysted metacercariae (1-2cm long), infestation by these so called “yellow grubs” in the visceral organs or under the skin was found generally to have no effect to the fish health, although massive metacercarial infections have sometimes resulted in mortalities of young fish (Woo, 1995).

Clinostomum spp. are of economic importance since heavily infected fish, particularly those with cutaneous infections, when marketed are often rejected by consumers (Kabunda and Sommerville, 1984). Furthermore some human cases caused by Clinostomatids have been reported, pointing out the potential zoonotic role of these parasites.

The presence of *Euclinostomum* sp. has been recorded from the kidney of tilapias in both sites of Machakos and Sagana at low prevalence and mean intensity. The kidney disappeared completely in the area occupied by the parasitic cyst, and massive infestations are reported to cause mortality, mainly in young fish (Paperna, 1996).

5.2.3.3 Black spot metacercariae

Black spot metacercariae showed the higher prevalence in Machakos caged fish in February and November with a high mean intensity in November indicating some seasonality (23°C). In open pond fish, the highest prevalence of the parasite was observed in March (20°C) just after onset of long rains, suitable period for the development of snails, first intermediate host for these parasites. However no distinct seasonality was observed in this case.

The black spots in fishes are produced by metacercariae of at least six species of strigeid flukes. The parasites are not black themselves, but the fish deposit pigment around the encysted metacercariae as a reaction to the presence of the larval parasite (Berra and Au, 1978). Even infestations of these parasites do relatively little damage to the fish, but there is some evidence that heavily infested juvenile fish may suffer stress, weight loss and even death (Baker and Franck, 1985).

5.2.4 Cestodes

Cestodes did not occur in caged fish, and the prevalence values were higher in Machakos open pond fish as compared to Sagana open pond fish. This could have been due to a more suitable environment for these parasites in Machakos than in Sagana, with biotic factors such as the presence of suitable intermediate host (copepods) to be clarified in order to better understand the epidemiology of these parasites in the aquatic environments under study. Concerning the absence of larval cestodes in caged fish, it could be due to their different feeding behavior, as they are fed on supplemental feed by the farmer and don't prey actively zooplankton.

Most of the cestodes were identified as plerocercoids of *Amirthalingamia macracantha* (Cestoda: Gryporhynchidae) on the basis of the appearance of the hooks (rostellum) in the scolex as described by Scholz *et al.* (2004). Aloo *et al*. (2002) also reported these gryporhynchid parasites in fish from Lake Naivasha but not encysted.

One interesting observation was the aggregation of cestode plerocercoids in intestinal wall and the frequent association with Acanthocephala cystacanths, probably due the presence of both larval stages in small crustaceans (copepods and amphipods respectively) in the same level of food chain (Jadwiga, 1991).

5.2.5 Nematodes

Nematode parasites were found with very low prevalence and intensity values in Sagana open pond fish, while in Machakos fish larval stages of nematodes were observed in visceral serosae with higher prevalence, mainly in caged fish.

The identification of the larval nematodes on the basis of morphological features was not possible and further molecular analyses are necessary.

5.2.6 Acanthocephalans

All the acanthocephalans found during this survey were identified as *Acanthogyrus* (*Acanthosentis*) sp. No seasonality in distribution throughout the year was observed. It was slightly more prevalent in Machakos than in Sagana though the mean intensities were higher in Sagana. The development of an acanthocephalan requires an intermediate host, an aquatic crustacean (usually an amphipod), where the acanthella larva shed from the egg develops to a cystacanth larva. When the fish feeds on this intermediate host the cystacanth excysts and infects the intestine of the host. Several fish species can act as paratenic host. Acanthocephalans are commonly considered as parasites with a low specificity to their intermediate, definitive or transport hosts. A broader environmental tolerance of these parasites might account for a higher adaptation to different farming systems, different seasonal periods with various levels of morphological variability within species (Mahmoud *et al.*, 2006).

More than 40 species of *Acanthosentis* are described throughout the world, 6 of which are in Africa where the distribution of these species cannot be accounted for by the dispersal of their fish hosts, except for *A.* (*A.*) *tilapiae*. The other five species have regional distributions in geographically distant and unrelated parts of the continent: *A.* (*A.*) *malawiensis* in Malawi, *A.(A.) maroccanus* in Morocco, *A.* (*A.*) *nigeriensis* in Niger, *A.* (*A.*) *papilio* in Senegal and *A.* (*A.*) *phillipi* in South Africa. In stark contrast, *A.* (*A.*) *tilapiae* has been reported from 30 species of cichlid (28 of the genus *Tilapia*) and three non-cichlid species in Tanzania, Congo, Uganda, Chad, Nigeria, Egypt and Malawi (Amin and Hendrix, 1999). Much of this distribution is attributed to the dispersal of the ubiquitous cichlid and intermediate hosts via the waterways of the River Nile (Amin, 2005). In our case the parasites found seem to be *A. tilapiae* and after morphological confirmation this could be the first report of this species in Kenya.

In juvenile fish a single specimen of *A. tilapiae* obstructed the digestive tube, apparently with no clinical implications. Low to moderate infections with larval stages in visceral organs caused only local changes while heavy infections led to granuloma, fibrosis and atrophy of the affected portion (Paperna, 1996).

Our results showed that this parasite has a tendency for a strong aggregation pattern. Aggregated parasite distributions are common among parasites (Rohde, 1993) and increase the chances of mating (Kennedy, 1976).

5.2.7 Copepods

Parasitic *Lamproglena* spp. are Lernaeid copepods specialized for attachment on the gills and were observed in all the examined sites with similar prevalence values but higher mean intensity in Sagana ponds. Egg sacs were elongated and eggs organised in a string rather than in a clump as in *Lernaea*. This copepod frequently moves from one to another gill septum, leaving behind thickenings and mechanical grasping that prevent the circulation and blood supply, thus decreasing gill capacity, i.e. the exchange of oxygen with the surroundings. This leads to respiratory problems and reduced viability of the fish (Öktener *et al.*, 2008). Paperna (1996) suggested that high infestations in *Lamproglena clariae, L. intercedens* and *L. monodi* may interfere seriously with respiration of their host fishes.

In the fish examined we observed thick layer of mucus in infected gill lamellae, confirming the observations by Paperna (1996) and Öktener *et al.*, (2008). This could greatly hamper oxygen intake by the fish.

5.3. Relationship between fish size and parasitic intensities

The findings on correlation between fish size and parasitic intensities in this study differ from observations by Aloo *et al.* (2004), who suggested that large sized fish harbour more parasites, probably for the different environments taken into consideration: they examined wild marine fish, while we examined fish from controlled (cages and ponds) freshwater environment, where the influence of several abiotic and biotic factors could change the dynamic of parasitic infections and also due to the fact that this study considered protozoan and helminth parasites while Aloo *et al.* (2004) considered only the helminth parasites.

5.4 Occurrence and distribution of parasites in the two sites

Results for abundance of intermediate hosts indicated a very high abundance and diversity of birds in Sagana as compared to Machakos due to their historical differences in aquaculture establishments (Table 2). This correlated well with findings of this study on helminth parasites. Sagana fish had a rich and diverse helminth community (Shannon-weaner diversity index, H'= 1.314) as compared to Machakos open pond fish (Shannon-weaner diversity index, H'= 1.174). These findings are similar to findings by Hechinger and Lafferty (2005) and Marcogliese (2005) that a high density of host populations can increase the rate of transmission of parasites and propagation of disease. This is because rich communities and high abundance of intermediate hosts fosters parasitism because many parasites rely on a predator-prey relationship to reach the next hosts in their life cycles. The occurrence of a parasite in a host organism not only indicates the presence of other organisms that participate in the parasite's life cycle, but also trophic pathways in which the hosts participate both up and down the food chain. Low regulated water levels foster parasitism among certain species of parasites of freshwater fish. Slow currents increase parasitism by trapping infectious parasites in the free stage. Hence, for parasites that sequentially use different host species throughout complex life cycles, parasite diversity and abundance in "downstream" host logically increases with the diversity and abundance of "upstream" hosts (which carry the preceding stages of parasites) (Marcogliese, 2005).

Although with some differences, both in Sagana and Machakos environments the study of biotic factors revealed the presence of snails and birds, indicating that the colonisation of parasites with a complex life cycle is strongly favoured.

Findings in this study on abundance of copepods and their probable role as intermediate hosts of cestodes and nematodes are not similar to findings by Buchmann and Bresciani (1997), Wootten (1972) and Wojciech *et al.* (2004), but the very low prevalence of positive copepods usually reported in the life cycle of cestodes and nematodes, requires further studies on the planktonic fauna present in the two sites, Machakos and Sagana Farm.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

There were no significant variations in parasite types that infected caged and open pond *O. niloticus* fish. Three protozoans, fourteen helminths and one copepod genus were identified in *O. niloticus* fish in this study.

No significant differences in mean prevalence, intensities and abundances of these parasites were found between caged and open pond fish. However, enclosure of fish in cages could influence the interaction of fish with intermediate hosts of digenetic trematodes, cestodes and nematodes.

Certain parasites were found to have higher prevalence in caged than in open pond fish, such as parasites with a direct life cycle, i.e. protozoans and monogeneans.

Contrary to the expected high risk of disease and parasites in caged fish, our findings revealed that indeed open pond fish are more susceptible to some parasites, e.g. larval cestodes and liver metacercarial cysts, because of their exposure to infective stages of these parasites in open waters.

The effect of seasonality was not clearly seen in this study and this could be attributed to the bi-monthly sampling designs but there were general increases in intensity of some parasites such as *Tylodelphys* spp. during the dry seasons.

Most of the prevalent parasites were detected in all seasons but the low prevalence of most parasites precluded statistical analysis of changes with season and system of culture.

The fact that no mortalities due to parasitic infection were recorded in caged fish means that the good management of cage systems could minimise the risk of loss of fish due to diseases.

6.2 Recommendations

This study recommends the use of cages in *O. niloticus* fish farming since no significant differences in parasitic infections were obtained between caged and open pond cultured *O. niloticus* fish.

The occurrence of parasites in this study leads to the conclusion that the source of fingerlings is an important determinant of the distribution and occurrence of parasites. Therefore, it is recommended that thorough screening and sanitary control of fingerlings from hatcheries is necessary before restocking activities in any farm.

This study recommends that restocking in reservoirs such as Machakos Ngeki's dam be done a month after the onset of the rainy seasons to effectively avoid the seasonal high abundance and intensity of some parasites such as the Eye fluke *Tylodelphys* spp before the onset of the rainy seasons.

This study recommends and reinforces the "rule of thumb" of "proper cooking of fish" or eating of "properly cooked fish" as preventative mechanism to zoonotic and potentially zoonotic parasitic infections.

This study also recommends further studies on life cycle of major parasites observed such as diplostomatids, clinostomatids, nematodes, acanthocephalans, in order to improve the measures useful to prevent and control these parasites.

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APPENDIX I: PROTOCOL ON PARASITOLOGICAL EXAMINATION OF FISH

Date:……..

Case No:…….

Farm information:

BOMOSA site:	Country:

Sample Information:

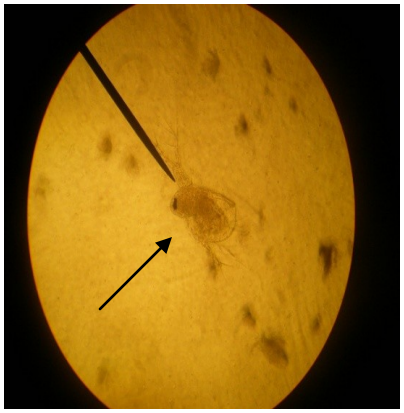
Fish species	Age/Size:	No. subject:
Sample conditions: alive □ fresh □ not fresh □ → Suitable □ yes □ no frozen □ preserved in formalin □ preserved in alcohol □		
Mortality: no □ yes □ → low □ moderate □ high □ for ……..…days	Treatment: no □ yes □ → with…....……	
Symptoms		

L=Low intensity M=medium intensity H=High intensity

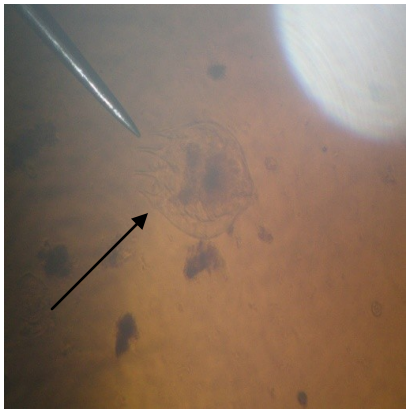
Organs	Parasite	Intensity	Note
Gill			
Skin/Fins			
Stomach/Intestine			
Gall bladder			
Swim bladder			
Kidney			
Muscle			
Other			

Signature ……

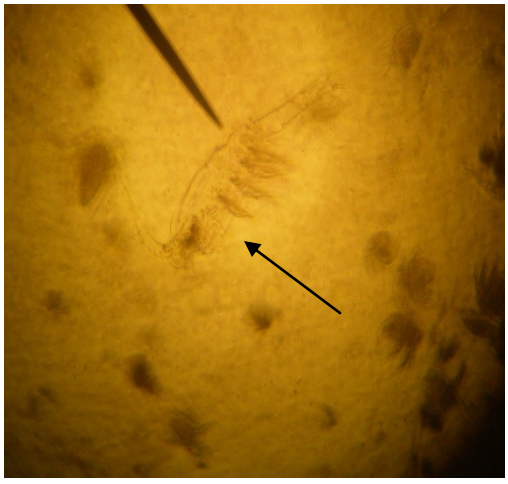
APPENDIX II: PHOTOGRAPHS OF OBSERVED ZOOPLANKTON, BENTHOS AND BIRDS



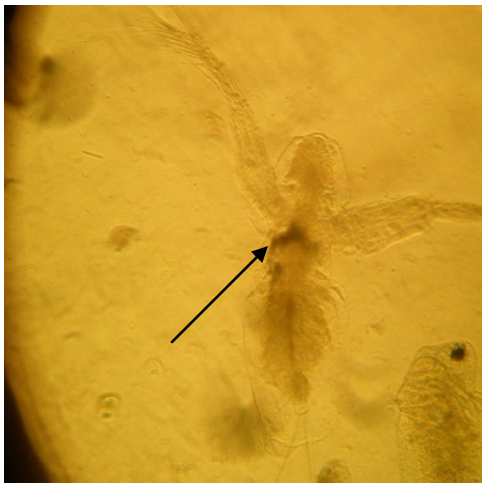
(a)



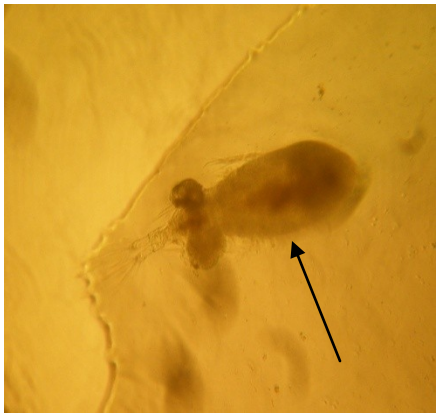
(b)

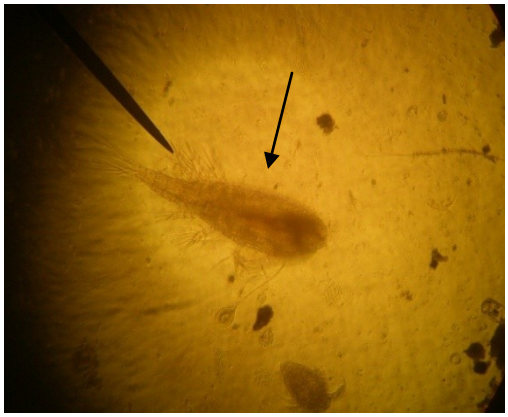


(c)



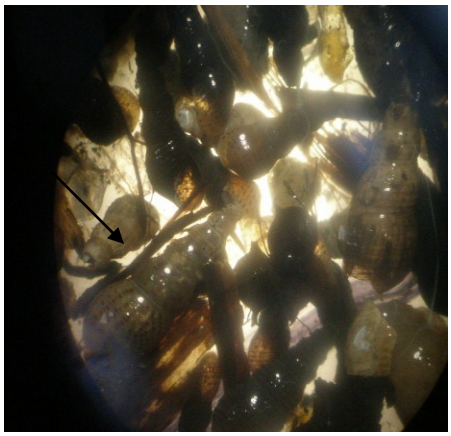
(d)





(e) (f)

Zooplankton observed plates (a) cladocera (b) rotifer (c) copepod (d) unidentified (e) female Cyclopoid copepod (f) Cyclopoid copepod



(a) (b)

Benthos observed plates (a) sorted snails (b) under Leica Zoom microscope

Plate 1 : Cattle Egrets on cages in Machakos' Ngeki's dam.

Plate 2 : Comorants in Sagana ponds

Plate 3: Ducks in water, Egrets and Comorants in a pond in Sagana

Plate 4: Sacred Ibis in Sagana fish ponds

APPENDIX III: QUANTITATIVE PARASITOLOGICAL DATA IN MACHAKOS CAGED SYSTEM *O. niloticus* FISH (Mean ±SE).

Parasite	P	MI	A
Protozoan			
Trichodina sp.	11.61±7.35	high	low
Sessiline peritrichs	3.03±3.03	2	0.06±0.06
Myxozoan			
Myxobolus spp.	1.52±1.52	very high	high
Helminthes			
Monogenean trematodes			
Dactylogyrids	17.85±6.57	3.4	0.59±0.21
Gyrodactylids	6.06±4.49	3.3	0.17±0.13
Digenean trematodes			
Black spot metacercariae	9.66±4.73	2.5	0.26±0.15
Clinostomatid metacercariae	10.23±5.24	2.5	0.27±0.17
Euclinostomum metacercariae	1.52±1.52	1.3	0.02±0.02
Liver metacercariae	3.33±3.33	1	1.47±1.47
Tylodelphys spp.	47.25±9.45	7	3.3±1.40
Nematoda	6.06±4.49	2	0.04±0.03
Acanthocephalan			
Acanthosentis spp.	9.02±6.31	1.4	0.13±0.09
Crustacean			
Lamproglena monodi	3.05±1.94	1.7	0.05±0.03

P Prevalence (%)
MI Mean Intensity
A Abundance

Scales of measurement adapted from (Fioravanti *et al.,* 2007): >50 individuals per field: Very high - 30-50 individuals per field: High - <10individuals per field: Low

APPENDIX IV: QUANTITATIVE PARASITOLOGICAL DATA IN MACHAKOS OPEN POND *O. niloticus* FISH (Mean ± SE)

Parasite	P	MI	A
Protozoan			
Trichodina spp.	5.55±5.55	low	0.12±0.12
Cryptobia spp.	2.22±2.22	high	low
Helminthes			
Monogenean trematodes			
Dactylogyrids	14.97±3.28	1.9	0.28±0.08
Gyrodactylids	3.33±3.33	2	0.067±0.067
Digenean trematodes			
Black spot metacercariae	2.28±1.46	19	0.44±0.36
Clinostomatid metacercariae	11.04±8.9	2.6	0.27±0.24
Diplostomatid metacercariae	3.92±3.92	1.7	0.06±0.06
Gill metacercariae	1.11±1.11	1	0.012±0.012
Intestinal cysts	1.28±1.28	1	0.012±0.012
Liver metacercariae	15.07±7.02	48.8	7.37±4.73
Tylodelphys spp.	41.58±11.77	4	1.65±0.38
Nematoda	3.95±2.66	2	0.07±0.05
Acanthocephalan			
Acanthosentis spp.	8.43±3.36	1	0.08±0.03
Cestoda			
Armirthalingamia macracantha	11.27±7.09	2.1	0.25±0.18
Crustacean			
Lamproglena monodi	4.11±1.87	1	0.04±0.018

Abbreviations as given in appendix III.

APPENDIX V: QUANTITATIVE PARASITOLOGICAL DATA IN SAGANA OPEN PONDS *O. niloticus* FISH (Mean ±SE)

Table 6: Quantitative parasitological data in Sagana open pond *O.niloticus* (Mean ±SE).

Parasite	P	MI	A
Protozoan			
Trichodina spp.	25.68±5.85	low	low
Cryptobia spp.	5.0±5.0	low	low
Myxozoan			
Myxobolus spp.	1.67±1.67	low	low
Helminthes			
Monogenean trematodes			
Dactylogyrids	17.84±8.21	3.76	0.67±0.44
Gyrodactylus spp.	4.17±2.71	11.5	0.48±0.44
Digenean trematodes			
Black spot metacercariae	5.0±5.0	13.2	0.66±0.66
Clinostomatid metacercariae	18.2±7.13	4.3	0.78±0.23
Diplostomatid metacercariae	1.67±1.67	low	low
Euclinostomum metacercariae	2.5±2.5	1	0.025
Tylodelphys spp.	25.68±5.85	13.5	3.47±1.03
Acanthocephalan			
Acanthosentis spp.	6.82±3.45	2.64	0.18±0.13
Cestoda			
Armirthalingamia macracantha	2.5±2.5	low	low
Nematoda	1.49±0.95	2	0.008±0.008
Crustacean			
Lamproglena monodi	5.32±3.43	7.76	0.41±0.27

Abbreviations as given in appendix III.

APPENDIX VI: WORK PLAN

ACTIVITY	**2007**										**2008**			
	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan-Feb	Feb-Mar	Apr-May	Jun
Proposal defence			↔	↔										
Data Collection	←	←	←	←	←	←	←	←	←	←	←	→		
Data Entry			←	←	←	←	←	←	←	←	←	←	→	
Data Analysis									←	←	←	←	→	
Thesis Preparation													↔	↔
Thesis defence														↔

APPENDIX VII: BUDGET

Item	Units	Unit cost (Kshs)	Total cost (Kshs)
Equipments and materials costs			
Universal meter (WTW)	1	80,000	80,000
Laptop computer	1	80,000	80,000
Digital camera	1	30,000	30,000
A pair of waders	1	2000	2000
Hot plate	1	1000	1000
Microscope glass slides	6 packets	500	3000
Cover slips	10 packets	200	2000
Wash bottles	5	150	750
Fine forceps	5	600	3000
Petri-dishes (Glass)	50	200	10,000
Dissecting kits	3	1500	4500
White trays	5	1000	5000
Droppers	10	100	1000
Saviet rolls (Non absorbent)	20	150	3000

Erlenmeyer flask	5	600	3000
Conical flasks	5	2000	10000
pipettes	3	1000	3000
SUB TOTALS			**241,250**
Consumable supplies			
Formalin 40%	3L	1000	3000
Ethanol 70%	3L	700	2100
Wrights or Klein's silver stain	1L	1000	1000
Mountants; permount	1L	500	500
Glycerine	1L	800	800
Distilled water	50L	20	1000
Gloves	10 packets	500	5000
Cotton wool	5 rolls	400	2000
Methylated spirit	2L	300	600
SUB TOTAL			**40,000**
Travelling costs			
Fuel (Egerton to Sagana, Machakos and back)	6 samplings, each 5 days	20,000	120,000
Driver's allowance	5 days for 6	@1500 PDM	45,000

	samplings		
Student's allowance	5 days for 6 samplings	@1500 PDM	45,000
Supervisor's allowance	5 days for 6 samplings	@ 6000 PDM	180,000
SUB TOTAL			**390,000**
Stationery and communication costs			
Printing papers	10 rims	350	3500
Photocopy			1000
Telephone			2000
Postage			5000
E-mail			1000
Internet browsing			10,000
Binding services			3000
USB drive 2GB	1	3000	3000
CD-RW	1 packet	700	700
SUB TOTAL			**29,200**
Book allowance/ stipend	12 months	@20,000 pm	240,000
SUB TOTAL			**240,000**
TOTAL			940,045

Contigency (10%)			94,045
GRAND TOTAL			**1,034,495/=**

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