PREVALENCE, INTENSITY AND PATHOLOGICAL LESIONS ASSOCIATED WITH HELMINTH INFECTIONS IN FARMED AND WILD FISH IN UPPER TANA RIVER BASIN, KENYA

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A THESIS SUBMITTED TO THE UNIVERSITY OF NAIROBI IN PARTIAL FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE IN FISH SCIENCE

DEPARTMENT OF VETERINARY PATHOLOGY, MICROBIOLOGY AND PARASITOLOGY

FACULTY OF VETERINARY MEDICINE

UNIVERSITY OF NAIROBI

2010

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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This work is dedicated to my mother Rachael Waruguru and my late father, Moses Wanjuki Mathenge.

ACKNOWLEDGEMENTS

I would like to express my sincere and deep gratitude to my supervisors Dr. Mbuthia P.G., Dr. Waruiru R.M. and Professor Ngatia T.A., for their invaluable advice, suggestions, guidance, moral support and encouragement throughout the study period.

I am highly indebted to the Director, Department of Veterinary Services, Ministry of Livestock and Fisheries Development, for allowing me to go on study leave and the award of a scholarship to undertake this MSc programme.

I also wish to acknowledge the Chairman, Department of Veterinary Pathology, Microbiology and Parasitology, Prof. Maingi E. N. for invaluable advice and facilitating the preliminary market study.

I am grateful to the manager and staff, Sagana Aquaculture Centre, Kirinyaga District for their cooperation, friendship and support during the field study.

My very special thanks go to laboratory technologist Mary Mutune of the Department of Veterinary Pathology, Microbiology and Parasitology for her great technical support and encouragement during the research period.

Special thanks to all the staff of the Department of Veterinary Pathology, Microbiology and Parasitology for their encouragement and technical support during my course work and stay at the institution, especially Messrs. Weda E.H., Kibe J. K., Otieno R.O., Muongi J., Gachoka J M. and Mureithi D. Further thanks to Simon Musyoka and other fishermen/mongers at Sagana bridge for their immense cooperation and support during the field study.

My sincere thanks to the Provincial Director of Veterinary Services, Coast Province, my fellow District Veterinary Officers and their staff in the greater old districts of Mombasa and Kwale, respectively, for their immeasurable support and understanding during the study period.

I would also like to express my heartfelt gratitude to my mother Rachael Waruguru Wanjuki, my family (wife Wambui; children Waruguru, Murugi, Gichuki, Mwangi, Mathenge, Ngaruiya and Njenga), brothers and sisters (Mathenge, Wambui, Mihiuko, Muthoni, Gatigi and Kariuki) and their families for their encouragement, moral and spiritual support, without which, this would have been impossible.

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ABSTRACT

Fish have become major source of protein and essential fatty acids for many Kenyans. Thus, many farmers are venturing into fish farming but have challenges due to fish diseases and parasites. This study examined the prevalence, intensity and pathological lesions associated with helminth infections in market, farmed and wild fish in upper Tana River basin, Kenya.

A total of 280 market and field (farmed and wild) fish were examined between July 2007 and March 2008 to determine occurrence and pathology of helminth infections. Forty three fish were obtained from the market while 237 (148 farmed and 89 wild) were from the field. Nile tilapia (*Oreochromis nilotica*), catfish (*Clarias gariepinus*) and other fish species were obtained from Gikomba fish market, Nairobi while farmed and wild tilapia and catfish were from Sagana fish breeding farm, Kirinyaga district and River Tana, respectively. Post-mortem examination was undertaken on the fish, lesions recorded, parasites and tissues collected for histology. Recovered parasites were preserved in 70 % alcohol while tissue sections were fixed in 10 % neutral formalin, processed, stained and examined for microscopic lesions.

Eight genera of helminth were recovered from tilapia and catfish in the field study and had variable prevalences. The prevalence were *Diplostomum* spp. – 35.4 %, *Contracaecum* spp. - 33.8 %, *Paracamallanus* spp. - 24.1 %, *Acanthocephalus* spp. - 13.9 %, *Neascus* spp. – 9.7 %, *Clinostomum* spp. – 8.0 %, *Proteocephalus* spp. – 2.5 % and *Caryophyllaeidea* spp. – 0.4 %. In the market survey,

Contracaecum species were recovered at a prevalence of 30.3 % in tilapia and catfish, respectively. Digenean metacercariae were the most prevalent helminth among the field fish, with *Neascus* and *Clinostomum* spp. being recovered from tilapia. *Contracaecum* spp. prevalence in market tilapia and catfish was markedly higher than in farmed and wild fish at 20 % and 91 %, respectively. There were significant differences in *Contracaecum* spp. infection between tilapia and catfish and between farmed and wild fish of both fish species ($p < 0.05$).

In the market study, *Contracaecum* spp. worm load ranged from 1 – 593, with a mean intensity of 169 worms per fish, while in the field study fish, counts ranged from 1 – 846 worms and a mean intensity of 103.4 worms per fish ($p < 0.05$) for the two fish species. The mean *Paracamallanus* spp. worm count in the field catfish was 6.1 with a range of 1 – 41. There was a significant difference in infestation between the farmed and wild catfish ($p < 0.05$). *Diplostomum* spp. mean count was 5.2 with a range of 1 – 26 parasites per fish ($p < 0.05$) in the two fish species and between farmed and wild fish species. *Clinostomum* spp. mean count in tilapia was 2.4 with a range of 1 – 7 ($p < 0.05$). There was a significant difference in this parasite mean count in tilapia between farmed and wild fish ($p < 0.05$). *Acanthocephalus* spp. mean count was 5.4 with a range of 1 -27, ($p < 0.05$). The mean *Proteocephalus* spp. count was 2.2, with a range of 1 – 4 parasites per fish ($p < 0.05$) for the two fish species. There was a significant difference in infection between farmed and wild catfish. *Caryophyllaeidea* spp. mean count was low.

There were more microscopic than gross lesions observed. Overall, microscopic lesions in both fish species were observed in the:- stomach – 25.7 %, intestines:- 41.8 %, liver – 38.8 %, gills 22.4 %, spleen - 21.9 %, heart- 6.3 %, skin - 23.2 %, muscles – 4.6 %, kidneys - 15.6 %, brain- 19.4 %, testis – 1.7 %, ovaries – 1.3 % and aborescent organ – 0.9 %. *Contracaecum* spp. in the peritoneum caused proliferation of fibroblasts, eosinophils, lymphocytes, plasma cells and hetrocytes. *Contracaecum, Paracamallanus, Acanthocephalus, Proteocephalus* and *Caryophyllaeidea* species caused epithelial desquamation, goblet cell hyperplasia, exudation into the lumen, villi desquamation, necrosis of columnar epithelium and infiltration of eosinophils and other mononuclear inflammatory cells into the lamina propria and muscularis. Other lesions included melanomacrophage infiltration into skin cysts and liver parasitic cysts, bile duct and hepatic blood vessels occlusion and pressure atrophy of epithelial and endothelium accompanied by blood vessel congestion and cholestasis.

In this study the occurrence of *Paracamallanus* spp. in catfish and *Acanthocephalus* spp. in tilapia are reported for the first time in the upper river Tana basin in Kenya. Digenean metacercariae were more prevalent than all other helminths, with farmed males and adult fish having higher helminth prevalence than wild, females and the young fish, respectively. Seven types of ectoparasites namely *Piscinoodinum, trichodina, Ichthyophthirius, ambyphrya, Lamprolegna* and *Ichthyobodo* species and aquarium mites were recovered in varying prevalences. In conclusion, helminths were found to be more prevalent in fish in

the upper river Tana basin and caused pathological lesions in apparently healthy looking tilapia and catfish. More research on the impact of helminthiasis on aquaculture fish production in the River Tana basin is recommended.

CHAPTER ONE

INTRODUCTION

There is a need for more protein sources to feed the ever increasing human population in Kenya estimated at 35 million (Achieng, 1994). Fish farming can assist to bridge this gap as the present fresh fish capture, from Lake Victoria, other major lakes and rivers which account for more than 96 % of the total fish production in the country, is currently overexploited (Greboval *et al.*, 1994).

Annually, the Kenya fish production is estimated to be 200,000 metric tons (mt), accounting for 4.3 % and 0.43 % of total agricultural production and export, respectively (Abila, 2003; FAO, 2003). This earns the fishermen over Kshs. 7 billion and Kshs. 4 billion in foreign exchange which contribute to poverty alleviation. Over 500, 000 people are directly employed by the sector and 1 million benefit from it (FAO, 2003; Gitonga and Ayoki, 2005).

The common riverine fish that are farmed in Upper Tana River basin are tilapia (*Oreochromis* and other *Tilapia* species), common carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*) and catfish (*Clarias* spp.) (Balarin, 1985; Nyandat, 2004; FAO, 2009). There are two major breeding farms in the River Tana basin, Kenya, located at Sagana town (Kirinyaga district) for tilapia and catfish; and Kiganjo (Nyeri district) for rainbow trout fish.

Among the major constraints to fish production are fish diseases especially parasitic infection, few and poorly trained extension staff; and inadequate financial support to the sector (Coche and Balarin, 1982; Fiovaranti *et al.*, 2007). Fish parasites

infections cause clinical or subclinical disease and pathology that may result in economic losses due to mortalities reduced production and increased cost of treatment. Some of the parasites which have been reported to cause mild to severe pathological effects in fish belong to the groups; Platyhelminthes-monogenean and digenean metacercariae, cestodes (larval and adults), aschelminthes and acanthocephalan worms, whose severity depend on infestation intensities (Moravec and Rehulka, 1987; Roberts, 1989; Obiakezie and Taege, 1991). Very little research has been undertaken to determine and document fish parasites, their pathological effects and their effect on production in Kenya (Mbuthia, 1993; Gichohi *et al.*, 2008).

River Tana which originates from Mt. Kenya and the Aberdare ranges transverses through Central Kenya where it supplies water to many aquaculture enterprises on its way to the Indian Ocean. In Central Kenya, aquaculture depends on riverine waters and there is therefore a possibility of the riverine wild fish sharing disease microorganisms and parasites with farmed fish. Wild fish can be reservoirs of parasites for aquaculture fish and vice versa. Thus investigations of parasites of farmed fish need to be carried out together with those of wild fish in these rivers. There is therefore need for research of diseases and parasites of riverine and aquaculture fish in Kenya and their effect on the host fish.

1.1 **Hypothesis**

This study was undertaken to test the following hypothesis:

HO: Farmed and wild fish in the upper River Tana basin are not infested with helminths and when present they do not cause pathological lesions.

H1: That farmed and wild fish in the upper River Tana basin are often infested with helminths that cause pathological lesions in fish.

1.2 **Objectives**

1.2.1 **General objective**

The general aim of the study was to document the occurrence of helminth infections and compare the associated pathological lesions in farmed and wild fish in the Tana River basin.

1.2.2 **Specific objectives**

The specific objectives of this study were to:

1.0 Determine the types, prevalence and intensity of helminth infections in farmed and wild fish in River Tana basin, Kenya.

2.0 Asses and compare the lesions caused by these helminths on farmed and wild fish at their predilection sites.

1.3 **Justification**

Many farmers in Kenya and the River Tana basin in particular are engaged

in fish farming using riverine waters. However, research on fish diseases, parasites and their effect on production have been little and limited to parasite prevalences in farmed fish. There is therefore need for further research on the prevalence of fish diseases and parasites with emphasis on related pathology and their effect on production; with a view to instituting comprehensive control strategies.

CHAPTER TWO

LITERATURE REVIEW

2.1 Kenya fisheries

The Kenya's fisheries sub-sector potential to contribute to the national economy through employment, poverty reduction, enhanced foreign exchange earnings and food security support is high. The sub-sector's growth estimated at 4.1 % in 2005 and its contribution of 0.5 % to the GDP in the year 2006 of this sub - sector could be higher if value additions at various stages of the supply chain are considered and pre and post harvest losses are minimized (Anonymous, 2009).

Kenya development plan sessional paper No.1 of 1986 as well as the millennium goals of 2002, put food security and self-sufficiency as the major goals in enhancing development in the country. Creation of employment (especially in smaller urban centres), will enhance income and foreign exchange generation, thus eradicating poverty (Anonymous, 1997a).

2.1.1 Marine fisheries

Kenya has a 640 km coastline and a 12 nautical mile territorial waters with 200 nautical miles of the Exclusive Economic Zone (EEZ) in the Indian Ocean (Kariuki, 2006). The artisanal fleet comprise some 2, 000 sailing dhows, dugouts, outriggers and other small mostly non-motorized boats. These vessels work out of the larger ports of Lamu, Malindi, Mombasa, Shimoni, and Vanga, as well as from numerous local landing sites. A few freezer trawlers fish the shallow waters of

Ungwana Bay for shrimp, but trawling opportunities are limited because coral outcroppings cover most of the near shore floor and the shelf slopes steeply to depths of a hundred fathoms or more within a few kilometers of the reef (Gitonga and Ayoki, 2005). The contribution of the marine sector to overall national fisheries production is very modest at 3 % to 4 % annually. Food and Agriculture Organization fish statistics estimates for annual marine production in Kenya covering the period 1984 - 1997 indicate a high of 10,103 mt in 1990 and a low of 4,101 mt for 1994. For the most part estimated harvests fluctuated between 6,000 – 10,000 mt during this period. The total marine catch for 2007 is noted as 6,959 mt valued at Kshs 496,224,000.00 (FAO, 2007). There is therefore a need to further explore other fish production areas for more fish production in the country.

2.1.2 **Inland fisheries**

Inland fish production accounts for 96 % of annual national total production for capture fisheries, mainly from Lake Victoria. Other lakes, rivers and aquaculture contribute the balance of the fish. The total inland fishery catch fluctuate between 119,522 and 202,557 mt (Abila, 2003; FAO, 2003). Kenya's portion of Lake Victoria covers about 6 % (4, 080 km^2) of the total lake area. The fish harvested are *Lates niloticus* (Nile perch) 60 %, *Oreochromis niloticus* (Nile tilapia) 10 %, the endemic small pelagic cyprinid *Rastrineobola argentea* ("dagaa or omena") 20 % and other fish 10 %. In 1995, 15, 000 mt of Nile perch fillet valued at US$37.5 million was exported. The next big lake is Turkana with an area of some 7 500

km^2. Its main fish species are *Oreochromis niloticus, Lates niloticus, Labeo horie, Alestes baremose,* and *Hydroryncus forskahli* (FAO, 1990). In the 1990's, fish catches were 1,000 to 2,000 mt/annum. Lake Naivasha covers an area of 115-150 km^2, depending on rainfall cycles. The main fish is tilapia (*O. niloticus*), Common carp (*Cyprinus carpio*) and Black bass *(Micropterus salmoides)* fish. In 2005, it produced 99 mt of fish (Anonymous, 2005). Lake Baringo occupies 130 km^2. Main fish species are *O. niloticus, Barbus gregoris, Clarias mossambicus* and *Labeo cylindricus.* In 2005, total fish catch was 43.3 mt (Anonymous, 2005). Several minor fisheries exist in rivers such as Tana and its reservoirs, Gucha/Migori, Mara, Nzoia, Sondu, Yala, Suam-Turkwell, Kerio, Ewaso Ngiro, Lessos and Turkwell gorge reservoirs; Athi-Galana-Sabaki, and Voi. Of these, River Tana and its dams produces more fish than other rivers (Anonymous, 2005).

2.1.2.1 **Tana River basin**

The Tana River originates from Mt. Kenya and Aberdare ranges and has a drainage area of 42, 217 km^2. It travels for more than 800 km to drain into the Indian Ocean with a catchment area of 100,000 km^2. Peak floods are in the months of November and May. It is divided into upper Tana River and its impoundments that include Masinga, Kamburu, Kiambere, Gitaru and Kindaruma reservoirs. The lower Tana River Basin has floodplains with numerous small lakes, including lakes Balisa and Shakabobo (FAO, 1990).

The upper River Tana basin transverses the sloping ridges of Mt. Kenya and Aberdare ranges in old districts of Nyeri, Kirinyaga, Maragwa, Muranga and Embu to the more flat topography of Kirinyaga, Yatta and Mbeere districts up to Masinga dam (Anonymous 1997 b; Jacobs *et al.*, 2007). This basin lies 100 – 150 kms North and North East of Nairobi, and encompasses the major towns of Nyeri, Embu, Muranga, Sagana, Kerugoya, Karatina, Kagio, and Kutus. The altitude of the basin varies from a high of 4,700 m on Mt. Kenya to a low of 730 m near the Kindaruma dam. Soils vary with the elevation in the basin, with andosols being predominant at higher elevations, nitosols at mid elevation and ferasols and vertisols being predominant at lower elevations (Jacobs *et al.*, 2007). There are numerous aquacultural farms on the upper basin that utilize waters from the river or its tributaries. The main fish breeding farms (Kiganjo and Sagana) are located in this basin. Rainfall follows a similar elevation to that of the soils, with Mt. Kenya and Aberdare areas receiving 1800 mm/yr of rainfall, mid-elevations with 1200-1800 mm/yr and less than 700 mm/yr in elevations below 1000 m. Major land uses within this basin include forests, cropland agriculture (forests, tea, coffee subsistence farming and horticulture), rangelands (livestock farming, irrigation and fishing) and aquaculture (Achieng, 1994).

2.1.3 **Aquaculture**

In 1910, European settlers imported into Kenya, trout (*Onchorynchus mykiss and Salmo trutta*), black bass (*Micropterus salmoides*) and common carp (*Cyprinus*

carpio) through the auspices of Kenya Angling Association. These fish were stocked into rivers for sport fishing but black bass was also stocked into Lake Naivasha (Achieng, 1994). Aquaculture started in 1948, with the establishment of the first two fish culture stations in Sagana and Kiganjo (World Bank, 1980 a and b). Yatta fish hatchery, located on an irrigation channel from the Thika River near Thika town, was built in 1982 and stocked in 1983. The Kenya Department of Fisheries was started in 1954 with a programme of dam and pond construction. Since 1984, eight fish fry production centres (FPC) financed by the United Nations Development Program (UNDP) and the Belgian Survival Fund (BSF) were completed (Achieng, 1994). In the private sector, the Bamburi Cement farm Ltd. in Mombasa (a subsidiary of Bamburi cement Portland cement company) has included a fish culture project in its programmes since 1971, testing ponds, cages, tanks and raceways for tilapia, carp, catfish and *Macrobrachium* spp. culture (Achieng, 1994).

In Kenya, a few thousand farmers practice fish farming amongst other farming activities as the inputs are almost the same. However, information of extent, production, number of small-scale farmers, pond numbers, pond size and small reservoirs fisheries on a national basis is incomplete. Official estimates are some 10,400 small ponds owned by about 7,500 fish farmers (Nyandat, 2004). The total fish production in aquaculture was 1,012 mt in 2003 (FAO, 2003). Due to this, aquaculture is properly seen in the context of agricultural activities. The contribution of this activity at the national level is minor, though at the farmer level

it has important effects on nutrition and income. Most of the production of aquaculture is local for consumption and that which is sold can fetch a high price of upto Ksh 600.00 per kilo. Fish farming can be practiced in most farming areas, thus making it possible for the rural consumer to have fish, which is otherwise mainly a preserve of the urban consumers and the communities mostly around the fisheries.

Aquaculture is one of the fastest growing food-producing sectors in the world, with an annual growth rate of approximately 10 %, as against 2.8 % observed in terrestrial farmed meat production (FAO, 2003). The food and agricultural organization estimates that as the world population increases, the global fish demand w grow by 70 % in the next 35 years. This situation would require a sevenfold increase in aquaculture production by 2025. However, frequent infectious disease outbreaks in the aquaculture industry account for the single largest cause of economic loss ($ 8 million worldwide) thus curtailing the production and economic development of the sector (Subasinghe, 1997). The intensive nature, stress related to environmental conditions like overcrowding, poor hygiene and temperature variations greatly favour disease outbreak and transmission in aquaculture setups (Roberts, 1989; Ghiirardelli *et al.,* 2006).

In Kenya, due to qualitative over fishing, the wild fish capture rates are unlikely to increase much beyond the current production figures of 200,000 mt (FAO, 1990). Thus the gap between the national fish requirement and national production can only be met through fish farming, as the alternative of importing is too expensive.

There is therefore urgent need for further development of fish farming to bridge this gap (Achieng, 1994). Kenya has identified aquaculture development as a core sector for economic stimulus program and allocated 1.12 billion Kenya shillings for its development through the budgetary system (GOK, 2009). *Oreochromis,* S*almonid* and *Clarias* species are the most common farmed species (Balarin, 1985, Nyandat, 2004).

Clarias gariepinus (catfish), synonymous with *C. mossambicus, C. lazeras* and *C. senegalensis,* is endemic to Africa. Its distribution range from Natal and the Orange river in South Africa through Central, West, East and North Africa where it is under culture (Teugels, 1986). The widespread distribution is a reflection of their ability to tolerate a wide range of environmental variations. *Clarias* species have rapid growth, a high reproductive potential and sturdy resistance to environmental variations (Hecht *et al.,* 1988; Macharia *et al.,* 2005). *Clarias gariepinus* spawn naturally in floodplains during the rainy season, and spawning is induced by rise in water levels (Clay, 1977; FAO, 1996a). However, sperms and ova collection from the wild is unreliable and limited to the rainy season. Demand for *C. gariepinus* fingerlings in Kenya, both for aquaculture and as bait has increased substantially in the last few years. The Fisheries Department estimates that the demand for *C. gariepinus* fingerlings for aquaculture activities is 10 million per year (Macharia *et al.,* 2005).

Oreochromis niloticus (Nile tilapia), which is also endemic to Africa, is a member of cichlid fishes commonly referred to as tilapia. The Nile tilapia (*O. niloticus*) was

one of the first fish species cultured. Illustrations from Egyptian tombs suggest that Nile tilapia were cultured more than 4,000 years ago. Positive aquacultural characteristics of tilapia are their tolerance to poor water quality and the fact that they eat a wide range of natural food organisms (Anonymous, 2008). Tilapia is a hardy, prolific, fast-growing tropical fish native to Israel, where it has been farmed for about 2,500 years. It requires water temperatures from 24.4 to 28.9°C. Biological constraints to the development of commercial tilapia farming are due to their inability to withstand sustained water temperatures below 10 to 11.1°C and early sexual maturity that results in spawning before the fish reach market size. They are prolific breeders and were once considered a national pest in Indonesia until the citizens began using them as a food source (Popma and Masser, 1999).

Tilapia inhabit a variety of fresh and less commonly brackish water habitats, from shallow streams and ponds through to rivers, lakes, and estuaries. Most tilapias are omnivorous with a preference for soft aquatic vegetation and detritus. Tilapia can survive on a diversity of food with algae being probably their most common food in the wild. On fish farms, they are fed on a high-protein pelleted feed. When raised in a controlled environment they can achieve growth rates of up to 3 % of body weight per day (average 2 %). All tilapia species are nest builders; fertilized eggs are guarded in the nest by a brood parent. Species of *Oreochromis* are mouth brooders; eggs are fertilized in the nest but parents immediately pick up the eggs in their mouths and hold them through incubation and for several days after hatching (Popma and Masser, 1999). In *Oreochromis* species only females practice mouth

brooding, while in *Sarotherodon* species either the male or both male and female mouth brood. In all *Oreochromis* species, the male excavates a nest in the pond bottom (generally in water shallower than 3 feet) to mate with several females. The female spawns in the nest (about two to four eggs per gram of brood female). After the male fertilizes the eggs the female then picks them and incubates the eggs in her mouth (buccal cavity) until they hatch. Fry remain in the female's mouth through yolk sac absorption and often seek refuge in her mouth for several days after they begin to feed.

Sexual maturity in tilapia is a function of age, size and environmental conditions. Tilapia populations in large lakes mature at a later age and larger size than the same species raised in small farm ponds. For example, the Nile tilapia matures at about 10 to 12 months weighing 350 to 500 grams in several East African lakes. Under good growth conditions, this same species will reach sexual maturity in farm ponds at an age of 5 to 6 months and 150 to 200 grams. When growth is slow, sexual maturity in tilapia is delayed a month or two but stunted fish may spawn at a weight of less than 20 grams. Tilapia has become the third most important fish in aquaculture after carps and salmonids, with world-wide production reaching 1,505,804 mt in 2002 (FAO, 2003).

2.2 **Parasites of fish**

Natural populations of plants and animals always have parasites in a normal complex dynamic equilibrium. Within the fresh water environment, the apex of the

predator – prey pyramid tend to be infected by a considerable parasitic range, sometimes occurring in normally large numbers in the natural environment. However, an unusual event of natural or human origin may disturb this equilibrium leading to an epizootic of one or more of the parasites. Before regulating mechanisms in the environment come into play to establish a new equilibrium, serious loss of fish may occur in the intervening period (Chubb, 1965).

Fish parasites are diverse and number in thousands, many of which are harmless. Many Phyla of the animal kingdom are represented here. These include Protozoa, Platyhelminthes, Aschelminthes, Acanthocephala, Arthropoda, Annelida and Chordates. These will parasitize wild and farmed fish and many are host specific. The number of parasites necessary to cause harm to fish varies considerably with the fish species, size, and health status. However, parasites cause serious outbreaks of disease in farmed fish due to the presence of dense population of fish kept in a particular environment favouring the propagation of certain parasite species (Roberts, 1989; Overstreet and Curran, 2004; Karvonen *et al.*, 2005 and 2006).

2.2.1 **Fish helminths**

2.2.1.1 **Phylum Platyhelminthes**

These are the flatworms where members dorso-ventrally flattened, bilaterally symmetrical and acoelomate. They lack an anus, specialized skeletal, circulatory and respiratory systems. Most of them are hermaphrodites (monoecious) (Myers, 2002).

2.2.1.1.1 **Class Monogenea**

Monogenea are small parasitic flatworms mainly found on skin, fins and gills of fish. They are rarely longer than about 2 cm. Marine forms are generally larger than those found on fresh water hosts. Monogeneans lack respiratory, skeletal and circulatory systems and have none or weakly-developed oral suckers. Monogenea attach to hosts using hooks, clamps and a variety of other specialized structures. They are often capable of dramatically elongating and shortening as they move. Like all ectoparasites monogeneans have well developed attachment structures. The anterior structures are collectively termed the prohaptor, while the posterior ones are collectively termed the opisthaptor. The posterior opishaptor with its hooks, anchors, clamps etc. is typically the major attachment organ. Monogenea are acoelomate. They have a simple digestive system consisting of a mouth opening with a muscular pharynx and an intestine with no anus. They are generally hermaphroditic. Monogeneans are among the lowest invertebrates that possess three embryonic germ layers—endoderm, mesoderm, and ectoderm in addition to a head region that contains concentrated sense organs and nervous tissue (brain).

2.2.1.1.1.1 **Sub-class Monopisthocotylea**

These are primarily fish ectoparasites which may live on the gills, skin and fins of fish as opposed to order Polyopisthocotylea which are mainly gill dwelling blood suckers (Cone, 1995). They are oviparous or viviparous and with a direct life cycle.

These parasites are small worms of upto 3cm in length, possessing a posterior haptor and an anterior organ attachment. The gyrodactylids- *Gyrodactylus* and *Gyrodactyloides* are 0.3 - 1mm and viviparous in length found on the skin, gills and the fins with species being host specific. *Dactylogyrus* (oviparous), *Cichlidogyrus* and *Gyrodactylus* spp. are main genera endemic in lakes affecting most tropical fish on the skin and gills with the first two causing pale gills and excess mucus secretion (FAO, 1996b).

2.2.1.1.1.2 **Sub-class Polypisthocotylea**

Polyopisthocotylea have multiple parts to the haptor, typically clamps. The Genus *Diclidophora,* which is primarily found in marine fish and primitive freshwater fish like sturgeons and paddlefish while Genus *Protopolystoma,* found in aquatic clawed toads (*Xenopus* species).

2.2.1.1.1.3 **Prevalence and intensity**

High fish densities and introduction of new certain fish species in farms may cause outbreaks. Introduction of *Polypterus senegalus* into aquaria caused an exponential increase in infestations with *Macrogyrodactylus polypteri*, resulting in worm burden of 690–7340 per fish within 20–25 days (FAO, 1996b). High incidences of monogenean infestations (approaching 100%) and intensities of 20–100 or more worms per fish were recorded by Paperna, 1964a, 1979 and Batra, 1984. In the dam of Loumbila, Burkina Faso five monogeneans *Cichlidogyrus*

tilapie (26.7 % - 53.3 %), *Cichlidogyrus rognoni* (6.7 % - 21.4 %), *Cichlidogyrus thurstone* (21.4 % - 73 %), *Cichlidogyrus galli* (20 % - 60 %) and *Scitogyrus longicornis* (14.3 % - 53.8 %) were recorvered in *Oreochromis nilotica*. Otachi, (2009) recovered Monogenean Dactylogyrids and Gyrodactylids at prevalences of 17.85 % ± 6.57 (mean intensity 3.4) and 6.06 % ± (mean intensity – 3.3.) in caged *Oreochromis niloticus* respectively in Machakos, whereas in open ponds the prevalence were 14.95 % + 3.28 (mean intensity – 1.9) and 3.33 % ± 2, respectively. In Sagana open ponds the prevalences were 17.84 % + 8.21 (mean intensity 3.76) and 4.17 % + 2.75 (mean intensity – 11.5), respectively.

2.2.1.1.1.4. **Clinical signs and pathological lesions**

Most monopisthocotyleans cause significant tissue damage through their disruptive attachment and grazing on exposed and vulnerable integument (Cone, 1995). Monogeneans cause corneal opacity, pale gills, excess mucus production, visceral cyst formation and fleshing in fish. Heavily infested fish have thickened cuticle, frayed fins, skin ulcers and damaged gills due to the feeding activities of the parasites and damage of attachment hooks. *Gyrodactylus salaris* has been implicated with the heavy losses of Atlantic salmon juveniles in rivers in Norway (Johnsen and Jensen, 1986).

2.2.1.1.2 **Class Trematoda**

2.2.1.1.2.1 **Order Digenea**

These are endoparasites of vertebrates with a life cycle involving at least one intermediate host (heteroxenous). Both adult and metacercarial stages are found in fish with the exception of genus *Aporocotyle*. All digeneans undergo part or all of their larval development in molluscs. They have two suckers, one anteriorly and the other antero-ventral. The family Heterophyidae, genus *Heterophyes* and *Haplorchis* spp. are found in tilapia in internal organs in cysts while family Clinostomatidae, genus *Clinostomum* spp. are found in tilapia and other cichlids fish in skin and internal organs as cysts called yellow grubs (Malek and Mobedi, 2001). The family Diplostomatidae, genus *Diplostomum* spp. and *Neascus* spp synonymous to *Posthodiplostomum* spp. are found in various fish on the skin, gills and eyes causing blackspot or eye fluke blindness and is one of the most widespread and important disease of fish (Violante-González, *et al.*, 2009). Adult *Gymnarchus niloticus* are found in *Clarias, Bagrus* spp. and *Synodontis victoriae* fish species visceral organs and digestive tract, where worms are visible. *Sanguinicola* spp. were found in *Synodontis and Auchenoglanis* fish species in the blood vessels as adult worms (FAO, 1996b).

2.2.1.1.2.2.1.2 **Prevalence and intensity**

Research and data on blood flukes infecting African fish is scarce. However, Khalil, (1969) reported blood flukes in 6% of Auchenoglanis *occidentalis* examined in the Sudan Nile.

Studies undertaken in Finland have shown that prevalence of *Diplostomum* spp. is directly related to the density of the *Lymnnae* spp. their intermediate hosts (Voutilainen *et al.,* 2008). Otachi (2009) working with farmed open and caged pond tilapia in Central and Eastern Kenya isolated mainly free living diplomastid; *Tylodelphis* spp. in the vitreous humour of fish at prevalences of 47.25 % / 41.58 % (mean intensity – 7/4) in open/caged pond and 25.68 % (mean intensity – 13.5) tilapia in Machakos and Sagana, respectively. Zekarias and Yimer (2008) in Awassa dam in the Ethiopia observed encysted *Clinostomum* spp. in branchial cavity in *O. niloticus* at prevalence of 75.7 %, *Euclinostomum* spp. in branchial cavity, kidney, liver, gall bladder at 30 %, and unencysted metacercariae from brain at 22.5 % in *Clarias gariepinus.* Al-Bassel (1990) reported the occurrence of the monogenean parasite *Gyrodactylus funduli* in a *Tilapia nilotica* fish farm, reaching 10-50 parasites/fish, while Dayhoum (2003), reported the occurrence of the parasite in *Tilapia galilaea* fish farms with an intensity of 9-32 parasites/fish in Egypt. Dayhoum (2003), in Egypt observed *Clinostomum tilapiae* parasitizing fish in very high prevalences in the viscera, while Aloo (2002) found *Clinostomum* spp. on the skin of tilapia fish in Lake Naivasha and Oloiden bay in Kenya. Barson and Avenant – Oldewage, (2004) in Sharptooth catfish, *Clarias gariepinus*, from

the Rietvlei Dam near Pretoria, South Africa found metacercariae of one larval digenean, *Ornithodiplostomum* sp. (prevalence 14 %, mean intensity = 140, $n = 7$), encysted in the muscles.

2.2.1.1.2.2.1.3 **Clinical signs and pathological lesions**

With the exception of extraintestinal parasites like sangunicoliid blood flukes and cyst forming didymozoids, very few adult stage digeneans cause significant harm to fish (Paperna, 1995). Metacercariae encyst in the skin and fins of fresh water fish, with melanin being deposited around the cyst giving them the ugly "blackspots. Metacercariae of the Strigeiod: *Posthodiplostomum cuticola* (*Neascus cuticola*) are commonly found in the skin of cyprinids in Europe and North America causing retarded growth in highly infected young fish and fry. The disease caused by some blood flukes, sanguinicoliasis, primarily results from a large number of fluke eggs sequestered in the afferent brachial arteries and may asphyxiate the host (Bullard *et al.,* 2004). Weight loss, deformities, blindness and death in fish are some of the effects provoked by the presence of metacercariae in the eyes (Shariff *et al.,* 1980; Chappell, 1995). In Kenya, *Diplostomatids* or eye flukes have been recorded in many fresh water fish species in Kirinyaga and Machakos where they caused eyefluke blindness (Mbuthia, 1993, Otachi, 2009). *Clinostomum complanatum* causes yellow grubs in the muscle of fish and make them unsuitable for human consumption (Malek and Mobedi, 2001). There is need

to evaluate the prevalence, intensity and pathological damage of these parasites in riverine and aquaculture set-ups in Kenya.

2.2.1.1.3 **Class Cestoda**

All species of fish are hosts of either larval or adult tapeworms. These are endoparasites with at least one intermediate host in their life cycle. Their body (strobila) is divided into a number of segments called proglottids, each with a single set of reproductive organs, the exception being the order *Caryophyllaeidea* that is not segmented (Schmidt,1986). The scolex (the attachment organ) is present at the anterior end. Adult worms are white in colour and elongated. They are parasitic in the intestines of the host fish, with the larval forms often found encysted amongst the viscera and musculature of host fish. The scolex of the larvae is fully developed but their strobila is short and unsegmented. This class includes tapeworms such as *Bothriocephalus aegypticus, Caryophayllaeus, Ptychobothriidea* and *Protocephalidea* (*Proteocephalus largoprolotis*) found in various fishes in visceral organs and gastrointestinal tract causing granulomatous lesions (Mbuthia *et al.,* 1993). *Amphilinidae* spp. worms are visible in the intestines of infested fishes.

2.2.1.1.3.1 **Prevalence and Intensity**

Gichohi *et al.* (2008) reported the occurrence of *Proteocephalus* spp. in market catfish at 9.1 % while Aloo (2002) reported the occurrence of *Armirthalingamia*

and *Cyclustera* spp. in tilapia in Lake Naivasha and Oliden bay in varying prevalences. Ayanda (2009) reported prevalences of 1.3% for *Amonotaenia* spp. and 3.8% for *Polyonchobothrium clarias in Clarias gariepinus* in Ilorin, North – Central Nigeria. Adikwa and Ibrahim (2004), reported occurrence of *Polyonchobothrium* spp. and *Monobothroids* spp. in *C. gariepinus* in Kano, Nigeria. Dayhoum (2003) reported the occurrence of cestodes at 4 % prevalence in *Clarias* spp. and *Tilapia* spp. in varying prevalences in different inland waters in Egypt. Mean intensity was 0.1 tapeworms per fish, with range of 1 – 4, while Moyo *et al.* (2009) reported prevalences of 60 % *Bothriocephalus acheilognathi* (mean intensity – 5), 80% *Polyonchobothrium clarias* (mean intensity – 5), and 80 % *Proteocephalus glanduliger* (*mean* intensity – 1) in *Claras gariepinus* and no cestode in *Oreochromis macrochir, Oreochromis mossambicus* and *Serranochromis robustus* in Insukamini Dam Zimbabwe. The study of the sharptooth catfish in South Africa, by Barson and Avenanat – Oldewage, (2006) found two cestodes, *Polyonchobothrium clarias* in the stomach at prevalence of 71 %, mean intensity of 5 and *Proteocephalus glanduliger* in the anterior intestines, with a prevalence 14 % and a mean intensity of 2.

2.2.1.1.3.2 **Clinical signs and pathological lesions**

Cestodes plerocercoid infest the body cavity of fish where they may grow to very large sizes causing swollen abdomen, compression and distortion of the viscera, granulomatous lesions when encysted and inhibition of gonadal maturation.

Severity is in older fish, which accumulate the parasites over time. The host cellular response to tapeworm infections may result in total (some *Diphyllobothrium* spp.) or partial (*Ligula* spp.) encapsulation by fibroblasts (Rahkonen and Valtonen, 1998). Initial reaction is infiltration by neutrophils and lymphocytes then macrophages with fibroplasia (Hoole and Arme, 1983).

Diphyllobothrium dentriticum and *D. latum* plerocercoids were reported to cause heavy mortalities in coregonids and brook trout by migrating through the viscera, including the heart (Hoffman and Dunbar, 1961). In fresh water fish they may become encysted among the viscera causing adhesions of the viscera, sterility if the gonads are affected and sometimes death (Williams, 1967).

Digenean metacercariae are zoonotic with members of Opisthorchidae and Heterophyidae being implicated. *Opisthorchis tenuicollis* and *O. sinensis* adults are found in the bile ducts of fish eating mammals including man. *Heterophyes heterophyes* is a common parasite of man in the Middle East and Asia. The broad pseudophylidean, *Diphyllobothrium latum* has been recorded in man in many parts of the world.

Bothriocephalidae aegypticus, Caryocephalidae, Ptychobothriidae and *Protocephalidea* spp. cause granulomatous lesions in visceral organs in cichlids and various, fish in Lake Victoria and Tigoni (Mbuthia, 1993; Mbuthia *et al.,* 1993; Mbuthia, 2000) in Kenya. *Bothriocephalus acheilognathi* is a large cestode of carps and other cyprinids, together with *Caryophyllaeid* spp. cause hemorrhagic enteritis in these fishes (Bauer *et al.,* 1973; Molnar *et al.,* 2003). There are no

studies to show prevalence, intensity and the damage which these parasites may cause in riverine fish in Kenya.

2.2.1.2 **Phylum aschelminthes**

2.2.1.2.1 **Class nematoda**

The class nematode is considered the most economically important helminths of fish. Nematodes are bilaterally symmetrical, coelomate elongate worms with cylindrical body tapering at both ends. They posess a solid resistant cuticle that make them last longer at postmortem conditions. Most are large enough to be seen with a naked eye and are often found in cysts in the liver, muscles, heart, blood vessels, eyes, gonads, viscera surfaces and rarely under the skin (Dick and Choundury, 1995). Their mouth is terminally anterior, with the gut clearly divided into esophagus and intestines. Sexes are separate. Those that parasitize fish require at least one intermediate host (Paperna, 1980).

2.2.1.2.1.1 **Prevalence and intensity**

Zekarias and Yimer, (2008) in a study in Lake Awassa in Ethiopia found *Contracaecum* spp. in the pericardial cavity (39.7 %) and mesentry (80.5 %) of *Oreochromis niloticus* and *Clarias gariepinus,* respectively. In addition, they found the nematodes *Ampliceacum* spp. (41.0 %) and *Eustrongyles* spp. (16.5 %) in the mesentry and musculature respectively, of *C. gariepinus.*

A prevalence of up to 70% and a mean intensity of 9.5 parasites in *H. Malabaricus i*n River Parana in Brazil was observed (Maurício et *al.*, 2003), while similar prevalences and sometimes with even higher infections were recorded in other fish species in Colombian marshes and rivers (Olivero - Verbel *et al.* 2006).

In Kenya, Aloo, (2002) recorded the *Contracaecum* spp. in *Oreochomis leucocystis* and *Tilapia zilli* from Lake Naivasha and Oloiden bay at varying prevalences while Barson (2004) reported the occurrence of the 3rd stage *Contracaecum* species at prevalence of 42.6 % with an intensity of 1–7 worms per fish (mean intensity = 2.2) in the body cavity of catfish (*Clarius gariepinus*) from Lake Chivero, Zimbabwe.

Pericardial *Contracaecum* infection among tilapia in a contaminated pond often approaches 100%, usually with 1–4 worms per fish. In Lake Naivasha, Kenya, 85% of *Tilapia leucosticta* were reported infected with a mean of 9 worms per fish, in L. Baringo 70% of *O. niloticus* with 5 worms per fish, in L. Magadi 30% of *T. grahami* with a mean of 2 worms per fish and in Lake George 30% of (270 mm long) *O. niloticus* with a mean of 1 worm per fish (Paperna, 1974b; Malvestuto & Ogambo Ongoma, 1978).

Barson and Avenant – Oldewage (2006) got a mean intensity of 16.3 (range 3–44) in catfish from Rietvlei dam even though prevalences and intensities of up to 100 % and 700 – 2000 worms per fish have been reported elsewhere (Mashego and Saayman, 1981; Boomker, 1982). However, a study in Insukamini Dam, Zimbabwe, did not observe any *Contracaecum* spp. in catfish but they were there

in *Oreochromi mossambicus* and *Serranochromis robustus* at prevalences of 11.1 % and 40 %, respectively (Moyo and Yalala, 2009). Ninety four per cent of *Oreochromis niloticus*, 82% of *Sarotherodon galilaeus* and 69% of *Tilapia zillii* were reported to have *Amplicaecum* larvae in the sinus venosus, at 2–8 worms per fish in the Nile, Sudan. *Amplicaecum* also occurred in the body cavity of various predatory fishes at prevalence levels of 10–37% with worm burdens of up to 36 per fish (Khalil, 1969).

Adikwa and Ibrahim (2004) recorded two camallanid nematodes (*Paracamallanus* and *Procamallanus* spp.) in catfish with a prevalence of 40.9 % in Kano, while Akinsanya and Otubanyo (2006) recorded low prevalence of *Paracamallanus* spp. in Lekki lagoon in Lagos Nigeria, suggesting that this nematode is a fresh and brackish water parasite in Nigeria. Boomker (1982) reported 53.5 % in South African fresh water fish while Ayanda (2009), in his study of farmed and wild catfish in Nigeria, recovered camallanids (5.625% for *Paracamallanus* spp. and 0.625% for *Procamallanus laevionchus*) in only wild catfish. Akinsanya and Otubanyo, (2006) recorded five helminth parasites in *Clarius gariepinus* including *Paracamallanus* spp. Helminth infection overall prevalence of 4.72% was recorded with the male showing a slightly higher prevalence (5.75%) than the females (3.76%).

Akinsanya *et al.* (2008), found *Raphidascaroides* spp. at a prevalence of 31.2 % in *Synodontis clarias* with male specimens having a higher rate of infection (37.8%) than females at 23.5%.

2.2.1.2.1.2 **Clinical signs and pathological lesions**

Contracaecum spp. in the family *Anisakidae* have been found in the dermis and viscera of tilapia spp, *Bagrus docmac, Clarias mossambicus*, *Protopterus* and *Haplochromis* spp. as granulomatous lesions. Tissue reaction to larval *Contracaecum* spp. involves epithelioid formation, fibrous encapsulation of larvae that may result in multiple mesenteric infections with extensive fibrosis and visceral adhesions in larger fish (Mbahinzireki, 1980; El Din *et al.,* 2009). Heavy camallanids (*Paracamallanus cyathopharynx* and *Procamallanus laevionchus*) infections have been reported in the stomach of *Clarias* spp., and many other catfish. *Spirocamallanus spiralis* have also been reported in the stomachs of other catfish firmly attached to the mucosa without serious harm (Paperna, 1964a,b; Khalil, 1969; Mashego and Saayman, 1981; Boomker, 1982). *Eustrongylides* spp. in the family Dioctophymidae have been found encysted in the dermis and visceral organs. Genus *Philometra* are long (up to 16 cm), thin and red in colour, found in the skin and fins of the fish host. *Philometra lusiana* is found in scale pockets on the anterior part of the body (Bauer *et al.*, 1973) and if larvae lodge in visceral cavity, they disrupt the normal function of the swim bladder causing loss of equilibrium and starvation. Larvae of *Philometra rubra* are found encapsulated in the body cavity of stripped bass in the USA causing severe peritoneal adhesions (Paperna and Zwerner, 1976; Moravec *et al.,* 2003; Genc *et al.,* 2005). Infected fish may develop bad looking ulcers. The larvae of *Anisakis* and *Pseudoterranova* spp. may encyst within muscles of host fish and if ingested raw or undercooked by man

may cause eosinophillic granulomas of the gut (Roberts, 1989). It is not clear wheather some of these parasites occur in rivers in Kenya.

2.2.1.3 **Phylum acanthocephala**

They are also called "spiny or thorny headed worm" and are elongate cylindrical worms armed with an anterior retractile proboscis carrying hooks.. They have no gut and the sexes are separate, with males being smaller than the females. They are mostly gut worms with at least one intermediate host in their life cycle (FAO, 1996b).

2.2.1.3.1. **Prevalence and intensity**

Aloo and Dezfuli (1997) reported cystacanths of *Polyacanthorhyncus kenyensis* infestation prevalences of 30.4 % to 86 %, intensity of 1 – 104 in *Oreochomis leucostiticus;* 33.7 % and intensity of 1 – 12 in *Tilapia zilli* and 6.9 % prevalence and an intensity of 1 – 40 in *Micropterus salmoides* in Lake Naivasha. In Lake Kariba, Doellou, 1992a;b, recorded *Ancathogyrus tilapiae* prevalence of 63 % (range of 1 – 100) in tilapia fish. In Egypt, *Neoechinorrhynchus rutili* infestation in two tilapia fish species was recorded at of 62.9 %, 71.4 % whereas the acanthocephalan parasite *Acanthosentis tilapiae* was found in Bahr Youssef and Fayoum fish farms in the same country with infection intensity ranging from 1-2 parasites/fish to 5-8 parasites/fish, respectively (Dayhoum, 2003). Riverine and aquaculture set-up appear to have low prevalences. In the Sudan Nile, Khalil

(1969) reported abundant *Tenuisentis niloticus* infestation of 93 % (range of 5 – 27), in *Heterotis niloticus,* 26 % *Neoechinorrhynchus* spp. (6 – 43) in *Citharus citharus* and 60 % unidentified acanthocephalan (2 – 5) in *Synodontis batensoda.*

2.2.1.3.2 **Clinical signs and pathological lesions**

The most obvious sign of infection by acanthocephalan worms is fibrotic nodules on the surface of the intestines in some species. The viscera of infected fish may be inflamed, discoloured or enlarged. (FAO, 1996b). *Ancathogyrus tilapiae* was found in various fishes and cichlids in the digestive tract while *Pomphorhynchus* larvae hooks were found embedded in intestinal wall of fresh water fish causing fibrous capsule formation (Hine and Kennedy, 1974). *Acanthocephala jacksoni* was found to cause a number of haemorrhagic ulcers in cultured brook and rainbow trout in Europe and North America (Bullock, 1963). *Ancathogyrus tilapiae* has been found in the digestive tract of cichlids and various fishes in Lake Victoria and other tropical lakes (Paperna, 1964 a) while *Neoechinorhynchus* and *Echinorhyncus* spp. have been found in tilapia in Ghana (Edoh *et al.,* 2008).

Histologically, there is necrosis of cells at site of attachment with accompanying infiltration of fibroblasts, lymphocytes and macrophages into the lamina propria (Dezfuli *et al.,* 1990), causing a chronic fibrinous inflammation with increased connective tissue thickening (Bullock, 1963). Fibroplasia may extend to the muscularis mucosa in some species (de Buron and Nickol, 1994). Their occurrence and effect on riverine and aquaculture fish in Kenya are not well documented,

hence the need for such studies.

2.3 **Diagnosis of fish helminths**

The body size of fish helminths vary from less than one millimetre up to several tens of centrimetres and thus most are observable with the naked eye. The use of binocular microscope is necessary in most cases. A detailed examination of a live and/or freshly caught (dead) fish are preferable. After external examination of the body surface, fins, gills and nasal cavity, the fish body is dissected and individual organs and tissues (digestive tract, liver, urinary bladder, kidneys, gall-bladder, swim-bladder, mesenteries, eyes, brain, pieces of muscles) are thoroughly examined for the presence of endoparasites.

Generally, external parasites are kept in clean freshwater for a few hours without serious damage, but all internal macro-parasites are preserved in saline and fixed within a few minutes to one hour in 70 % ethanol or 2–4 % formalin. Trematodes and cestodes are usually fixed slightly pressed between a glass slide and a coverslip. Trematodes and cestodes are usually stained with carmine, nematodes cleared in glycerine or lactophenol and observed under microscope for morphological differentiation (Lom and Dykova, 1991).

2.3.1 **Phylum platyhelminthes**

2.3.1.1 **Class Monogenea**

Most diagnosis is made by the type, location of the parasite and clinical signs (FAO, 1996b). Monogeneans are diagnosed as hermaphroditic, single testis, ectoparasitic flat worms with sclerotized copulatory organ, attached to skin or gills by special posterior organs. They have ovary and follicular vitelline glands with a few eggs, with the exception of *Gyrodactilidea,* which are viviparous. The anterior end has apical sensory structures, a mouth with or without accessory suckers and special glands or clamps for attachment.

2.3.1.1.1 **Monogenean**

Monopistocotylean have a single posterior haptor with one or two pairs of anchors with 12 – 16 hooks. The functional haptor of the adult develops anterior to larval haptor and has lateral rows of suckers or clamps supported by cuticular sclerites usually acting as clamps (Hoffman, 1999).

2.3.1.2 **Class Trematoda**

2.3.1.2.1 **Adult digenean trematodes**

The diagnosis is based on the recovery and identification of the adult trematode or their eggs from cysts in tissues or body cavities at necropsy. They are smooth, spiny or corrugated, oval, dorsoventrally flattened worms, with anteroventral mouth surrounded by a sucker and an additional sucker or acetabulum. Most are

spiny or corrugated, oval, dorsoventrally flattened worms, with anteroventral mouth surrounded by a sucker and an additional sucker or acetabulum. Most are hermaphrodites. Some *Heterophyes* spp. have a specialized copulatory organ. Sanguinicolidae (blood flukes) have spiny, slender bodies that lack anterior ventral suckers and pharynx. They lay thin shelled unoperculated eggs (FAO, 1996b) while, Didymozoidae are thread-like without ventral suckers and pharynx.

2.3.1.2.2 **Digenean metacercariae**

Diagnosis is based on clinical signs and the recovery of the members of some families or even certain genera through characteristic structural affinities at necropsy, aided by additional features such as the type and location of encystment. To a limited extent, mature trematodes may be obtained from metacercariae through experimental infection of known or suspected definitive hosts such as herons and pelicans in the case of families *Strigeoidea* or *Clinostomatidea* (Ukoli, 1966a,b; Donges, 1974), dogs and cats for family *Heterophyiidea* spp. or in laboratory mice, rats, chicks or ducklings when the trematode is non-fastidious in its choice of definitive host as in case of *heterophyiids* (Sommerville, 1982).

2.3.1.3 **Class cestoda**

The diagnosis is based on the recovery of eggs or segment residues from faecal material and recovery of tapeworms at necropsy. Tapeworms are identified by the presence and shape of their scolexes, the number and shape of scolex modification (suckers, grooves, hooks, spines and glandular areas), presence and size of the neck;

segmentation of strobila (mono or polyzoic), the type of reproductive organs (monoecious or hermaphroditic), number and shape of ovaries and testis, the type of vitellaria and the opening of the cirrus or atrial pore (Schmidt, 1986; FAO, 1996b).

2.3.1.3.1 ***Bothriocephalus acheilognathi***

These worms have an arrowhead or heart shaped, fleshy scolex with anterolaterally directed narrow slit like openings (bothridia). They lack a distinct neck and their mature strobila are broad, long and acraspedote (Choudhury *et al.*, 2006). The proglottids have rounded edges while gravid segments are longer and broad (Scholz, 1997).

2.3.1.3.2 **Other adults tapeworms**

Diagnosis is based on the recovery of the tapeworms or segments at necropsy. *Caryophyllaeideans* and *Amphillids* are unsegmented (monozoic) whereas *Pseudophyllideans* and *Protoecephalideans* are segmented (polyzoic) (Van As and Basson, 1984).

2.3.1.3.3 **Larval tapeworms**

These are mostly found whole in the body cavities *(Ligula* pleurocercoids) or encysted as cysticercoids *(Cyclophyllideans)* (Mashego, 1982).

2.3.2 **Phylum aschelminthes**

Nematodes are identified using the labial parts, presence of annulations, presence and shape of esophagus, ventriculus and caecum, the presence of cuticle, the structure of excretory system and shape and type of the genital organs (papillae and spicule) (Anderson *et al.*, 1974). The shape and structure of the buccal capsule may also be used to identify the nematodes, like in the family *Camallanoidea* (Chaubad, 1975). Labia are lobes or lips which surround the mouth. Annulations are deep transverse grooves occurring at regular intervals in the cuticle. Ventriculus is glandular modification of the distal portion of the esophagus in some nematodes, which may have a solid appendage of varying length, extending posteriorly into the intestines. Caecum is a blind diverticula or pouch of the intestines. Genital papillae are retractile setae in the anal region of males which may be preanal, postanal or caudal in position. Spicules are sclerotized copulatory structures of male nematodes, usually paired but may be seperate. Most larval nematodes lack a cuticle (Anderson *et al.*, 1974; FAO, 1996b).

2.3.3 **Phylum acanthocephala**

The worms are sac-like, containing lemnisci connected to the proboscis and genital organs opening posteriorly. Their sexes are separate and the male are usually smaller with genital opening within a membranous bursa. An alimentary canal is absent. The number and arrangement of the hooks on the proboscis are the main criteria for differentiation of species (Kabata, 1985). Apart from the anterior lemnisci and the probosci receptacle, the only conspicuous interior organs are the

reproductive system. In males there are two testes followed posteriorly by 1-8 cement glands. The efferent ducts from the testes and the cement glands open posteriorly through a small copulatory organ surrounded by an invaginable bursa. Acanthocephalan ovaries fragment early in development to form ovarian balls, floating freely in the pseudocoelom. The female system terminates in a vagina, regulated by a vaginal sphincter that discharges through the vaginal pore (Amin, 1987).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

Two studies, a market survey and a field study were undertaken in this study. In the market survey four purposive sampling visits were undertaken between January and May 2006 at Gikomba wholesale fish market, Nairobi to purchase fresh fish. A stratified random sampling, based on species was used to select fish that were purchased from those sold at the market. The fish were grouped into four genera *(Oreochromis* (tilapia), *Clarias* (catfish), *Cyprinus* (common carp) and *Barbus* (barbus) species. These are the commonest fish catches landed at various sites on the River Tana basin (Anonymous, 1997b). They were then transported in cool boxes with ice to the laboratory, at the Department of Veterinary Pathology, Microbiology and Parasitology for postmortem examination and other tests.

The field study was carried out at the Sagana aquaculture centre, a department of Fisheries breeding farm in Sagana town, Kirinyaga district (100 km North East of Nairobi, altitude 1230 m, latitude 0°39'S and longitude 37°12'E); and at the upper River Tana basin below the Sagana bridge, where the four districts of Kirinyaga, Mbeere, Machakos and Maragwa intersect **(Fig 1)**. Upper River Tana basin includes the western highland forest catchments of Mount Kenya and the Eastern Aberdare ranges up to the Masinga dam, the greatest regulator of the River Tana system downstream (Jacobs *et al.*, 2007).

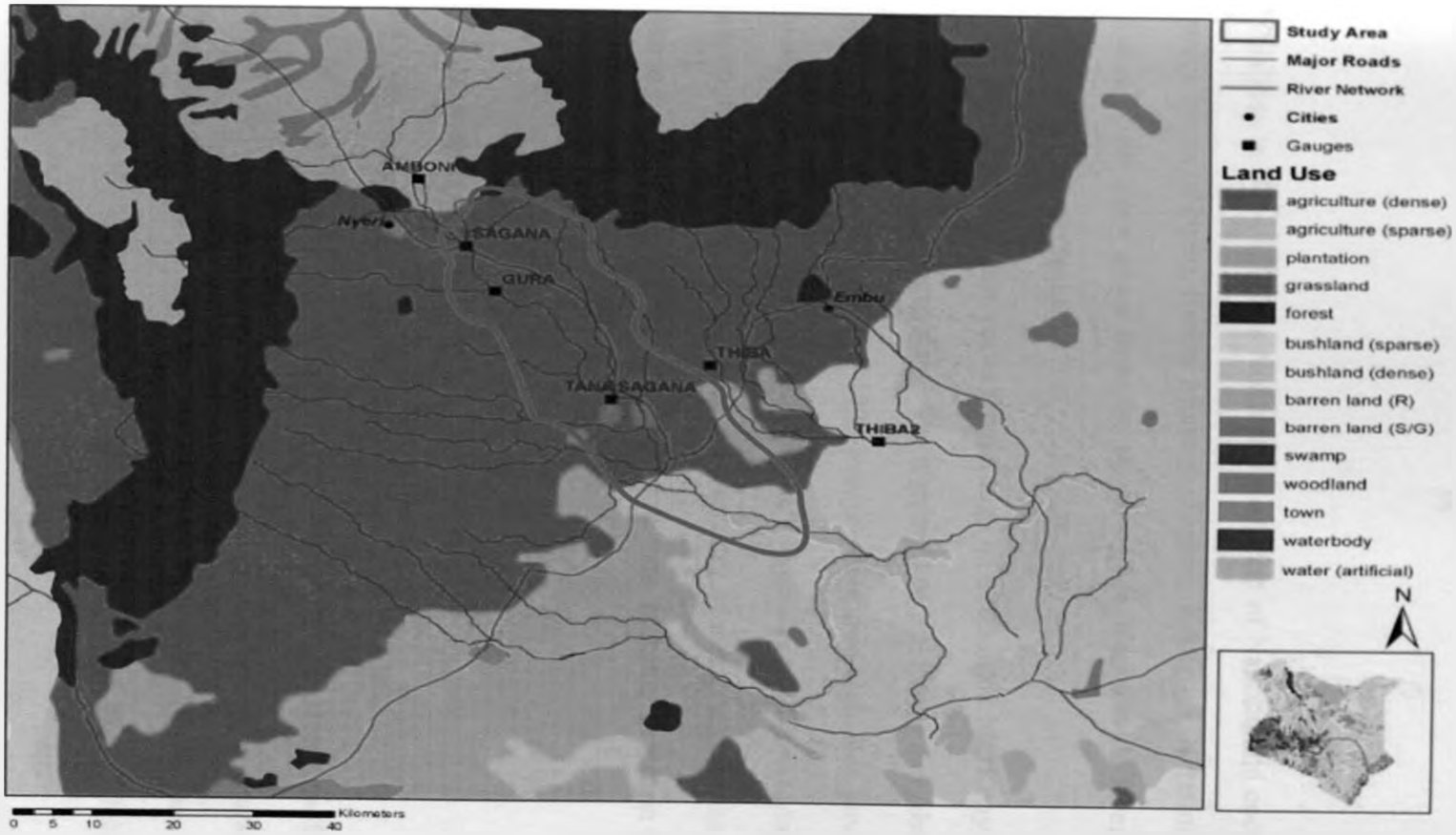


Figure 1: Map of study area (Jacobs et al., 2007)

This area is 100 – 150 kms North and Northeast of Nairobi, and encompasses the major towns of Nyeri, Embu, Muranga, Sagana, Kerugoya, Karatina, Kagio, and Kutus. River Tana arises from the slopes of Mt. Kenya with several tributaries from the Aberdare ranges.

The altitude of the basin varies from a high of 4700 m on Mt. Kenya to a low of 730 m near the Kindaruma dam. The highlands of Mt. Kenya and Aberdare ranges slopes have annual rainfall of 1800 mm/yr while mid-highlands have 1200-1800 mm/yr and lowlands (below 1000 m) have 700 mm/yr and below. Major land uses within the basin include forests, agro-forestry (forests, tea, coffee subsistence farming and horticulture), rangelands (livestock farming, irrigation and fishing) and aquaculture (Jacobs *et al.*, 2007).

3.2 **Study design**

3.2.1 **Market survey**

A total of 43 wild fresh dead fish were randomly selected from five fish mongers dealing with fish from River Tana basin at the Gikomba fish market, Nairobi. These comprised of 15 *Oreochromis* spp., 11 *Clarias* spp., 10 *Cyprinus carpio* and 7 *Barbus* spp. They were transported in cool boxes to the laboratory for postmortem examination

3.2.2 **Field study**

Purposive selection of sampling sites (Sagana fish breeding farm and Masinga Dam) was undertaken due to financial and time limitations. At the sampling sites, systematic random samples of fish were taken where every other fish was selected for examination from the fish presented. This ensured that the sample was representative, evenly distributed, sampling error minimized and that all fish had equal chance of selection. Postmortem examination was performed on all fish at the site of purchase; various fish tissue samples were taken, marked, recorded and transported in preservatives to the laboratory for further processing.

3.2.3 **Field study sample size**

A total of 237 (129 were tilapia and 108 catfish) live fish fish were selected, and examined for lesions and parasites at the site. Eighty-five tilapia fish (*Oreochromis nilotica*) and 63 catfish (*Clarias gariepinus*) were farmed from Sagana Fish breeding farm, while 44 tilapia and 45 catfish were wild fish purchased from the fishermen at the Sagana bridge and fish landing sites in Masinga dam.

Total sample size for the field study was calculated following the formula given by Martin *et. al.* (1987).

$N = 4PQ/L^2$,

Where: N is the estimated sample size, P is the prevalence (estimated at 0.5), Q is 1-P and L = the precision required (+ or – error around the estimate).

A precision (L) of 0.1(10%) was set, while 4 is approximately $(1.96)^2$ for 95% confidence level.

Therefore $N = \frac{4 \times 0.5 \times 0.5}{(0.1)^2} = 100$ fish per species

3.2.4 **Age and sex assessment**

Sex of the fish was determined at postmortem. The age of fish was sourced from farm records for the farmed fish while those from the wild was determined by size and the development of sexual organs at postmortem and recorded as either young or adult.

3.3 **Postmortem examination**

Postmortem examination in the field study was performed on all fish at site of purchase. Live fish were stunned with a single blow to the back of the head and pithed to separate the central nervous system from the spinal cord. All fish were then subjected to postmortem examination and lesions recorded (Roberts, 1989; Theodore, 2000; Untergasser, 1989) as briefly outlined below.

The skin was examined grossly for ectoparasites at site. Thereafter skin scrapings were taken and preserved in 70 % ethanol and transported to the laboratory for further examination of parasites.

Each fish was laid on its side on a paper towel in a dissection tray to prevent slipping, and a midline incision made with a scalpel blade starting at the anterior end of the vent. A lateral incision from the vent side in an arc, on the abdominal

wall of the fish up to the upper corner of the operculum was made to expose the swim bladder and other organs. The body wall was then lifted and the organs observed grossly *in situ*. A third incision connecting the two previous incisions (opercula incision) allowed the skin and muscular flap to be completely removed. The gills, liver, peritoneal cavity, swim bladder, spleen, heart, and musculature were examined grossly and under a dissection microscope for helminths.

Worms collected were preserved in 70 % ethanol prior to identification. Visceral organs, sections of the stomach, intestines, liver, swim bladder, gills, musculature and skin were taken, preserved in buffered 10 % formalin and processed for histopathology (Roberts, 1989; Untergasser, 1989).

3.3.1 **Examination of gastrointestinal content for parasitic infection**

The gut was separated into stomach and intestines, preserved in 70 % ethanol. The contents of each gastrointestinal segment were expressed out and examined in 0.64 % physiological saline and thin transparent intestinal or stomach sections placed on slide and dissected with dissecting needles to expose contents which were examined at various magnifications for worms, worm eggs, flagellates and cysts according to Roberts (1989) and Untergasser (1989).

3.3.2 **Processing of Platyhelminthes and Acanthocephala worms for identification**

On isolation, the worms were fixed in hot formal saline (4 %) solution for 24 hours, washed in distilled water for another 24 hours and then stored in 70 %

ethanol before identification. The Platyhelminthes whole mounts in 70 % ethanol were downgraded to water, stained by Gower's Carmine stain followed by a slow differentiation in 0.5 % hydrochloric acid (HCL) in 70 % ethanol for 1-12 hours depending on the size of worms. Platyhelminthes sections were stained using Heidenhain´s azan stain (Roberts, 1989).

3.3.3 **Helminth identification**

Worms collected from the body cavity were preserved in 70 % ethanol, manually counted and recorded. Trematodes were identified using morphological features as described by Cone (1995) and Paperna (1995). Nematodes were identified using morphological features as described by Anderson *et al.* (1974) and FAO (1996b). Acanthocephalans were identified using morphological features and the species differentiated by the number and arrangement of the hooks on the proboscis (Kabata, 1985) while tapeworms were identified using the size and shape of scolexes, number and modification (armed or unarmed) of suckers, the size of segments, cirrus pouch, number of testis and positioning, the shape and positioning of vitellaria, ovaries and uterus, and the position of uterine diverticula (Schmidt, 1986; FAO, 1996b).

3.3.4 **Helminth infection severity scoring criteria**

Helminths recovered were identified, counted and infection severity scored as:- Mild (1 – 50 worms), Moderate (51 – 100 worms) and Severe (above 100 worms)

for abdominal infections. Branchial *Contracaecum* species infection severity was scored as: - Mild (1-2 worms), Moderate (3-5 worms) and Severe (above 5 worms).

3.3.5 **Identification of ectoparasites**

Ectoparasites were identified using morphological features as described by Untergasser (1989) and Woo (1995).

3.4 **Tissue processing for histopathology**

Standard histological procedures for tissue processing were used as described by Luna (1968). All tissues were trimmed to approximately 1 cm long and labeled using a tag. Formaldehyde was rinsed from tissues using tap water, and thereafter they were passed through ascending grades of alcohol to dehydrate in 80 % for 4 hours and then 96 % in 2 changes for 2 hours. The tissues then underwent through three changes of isopropanol for 1.5 hours each. The alcohol was cleared using amyl acetate for 1 hour, then through xylene in two changes for 2 hours and 2.5 hours, respectively. Two molten wax changes at 60 ^{o}C was used for impregnation each for 3 hours then followed by embedding using molten wax in paper boats. Tissues were then mounted in wax on wooden tissue blocks ready for microtomy. Five-micron thick sections were cut using a microtome (Leitz Wetzlar, Germany) and mounted on labeled glass slides.

The tissues were then dewaxed using xylene for two stages each for 5 minutes, followed by rehydration using descending grades of alcohol. Absolute alcohol in

two stages each for 5 minutes and then 90 %, 80 %, 70 %, and 50 % each for 3 minutes and then placed in water for 5 minutes. They were stained using hematoxylin and then counterstained with 1% eosin and dehydrated using ascending alcohol grades, cleared using two changes of xylene and mounted using Dibutyl phthalate xylene (DPX) mountant. Mounted tissues on slides were then examined under light microscope (X 4, X 10 and X 40 magnification) for any cellular changes. Lesions seen were described and scored as none for no lesion, mild where the inflammatory cells involved were few primary mononuclear inflammatory cells or severe where many mononuclear inflammatory cells were accompanied by fibroblasts and melenomacrophages.

3.5 **Data analysis**

Data for market study was entered in Ms excel, exported to Instat® Statistical package for descriptive statistics (Steel and Torrie, 1980). The one for the field study was entered in Ms Excel, exported to Genstat® (Genstat Discovery Edition 3) for descriptive statistic and for analysis. Z – test, Chi Square test, one and two way ANOVA were used to analyze the data. The prevalence of parasites was defined as the total number of individuals of a host infected with a particular species divided by the number of host examined while the mean parasite intensity was defined as total number of parasites in infected hosts divided by the number of infected hosts (Margolis *et al.*, 1982).

CHAPTER FOUR

RESULTS

4.1 Prevalence of helminth infestations

A total of 43 wild fresh fish of various ages, sexes, sizes and comprising of 34.9% *Oreochromis* spp., 25.6% *Clarias* spp., 23.3% *Cyprinus carpio* and 16.3% *Barbus* spp were examined in the market survey. In the field study, a total of 237 live fish composed of 129 tilapia and 108 catfish were examined. Eighty-five *O.nilotica* and 63 *C. gariepinus* were farmed from the Sagana Fish breeding farm, while 44 tilapia and 45 catfish were wild fish. The examined tilapias were 72 adults and 57 young fish, divided into 75 males and 54 female fish. Catfish were 58 adults and 50 young fish which were 51 males and 57 females fish (**Table 1**).

In the market survey, 43 fish were examined and the main worms observed in the fish were 30.3 % Nematodes, 4.6 % tapeworms (adult and pleurocercoids) and 2.6 % trematodes. Of these, 23.3 % nematodes and 2.3 % cestodes were recovered in catfish while 7 % nematodes, 2.3 % cestodes, 2.3 % trematodes were recovered in tilapia. No helminths were recovered in common carp and barbus fish (**Fig. 2; Appendix 1**). The nematode recovered in the two fish species was 3rd stage *Contracaecum* spp. larvae which was found embedded in fibrin mesh and free in the abdominal cavity and in some cases entangling the visceral organs (**Fig. 3 and 4**).

In the field study, helminths encountered were in the phyla: - Aschelminthes (nematodes), Platyhelminthes (digenean trematodes and cestodes) and

Acanthocephala species. A total of 156 (65.8 %) fish had one or more helminth parasites. The overall prevalence of each helminth was *Diplostomum* spp. 35.4 %, *Contracaecum* spp. 33.8 %, *Paracamallanus* spp. 24.0 %, *Acanthocephalus* spp. 13.9 %, *Neascus* spp. 9.7 %, *Clinostomum* spp. 8 %, *Proteocephalus* spp. 2.5 % and 0.4 % f*Caryophyllaeidea* spp. (**Fig. 5; Appendix 2**). Of these helminthes, *Contracaecum* spp., *Diplostomum* spp. and *Proteocephalus* spp were common to both fish species while *Acanthocephalus* spp., *Clinostomum* spp. and *Neascus* spp. were recorded only in tilapia. *Paracamallanus* spp. and *Caryophyllaeidea* spp. were observed in catfish only (**Fig. 6**). There was no significant difference ($p > 0.05$) in overall helminth infestation between tilapia and catfish sampled in the field study (**Appendix 3**).

Table 1: Total number of wild, farmed tilapia and catfish of different age and sex examined for parasites and lesions

Type of fish examined	Wild fish					Framed fish					
	Adults		Young		Sub-total	Adults		Young		Sub-total	Total
	Males	Females	Males	Females		Males	Females	Males	Females		
Tilapia	12	9	15	8	**44**	29	22	19	15	**85**	**129**
Catfish	18	7	7	13	**45**	12	21	14	16	**63**	**108**
GRAND TOTAL											**237**

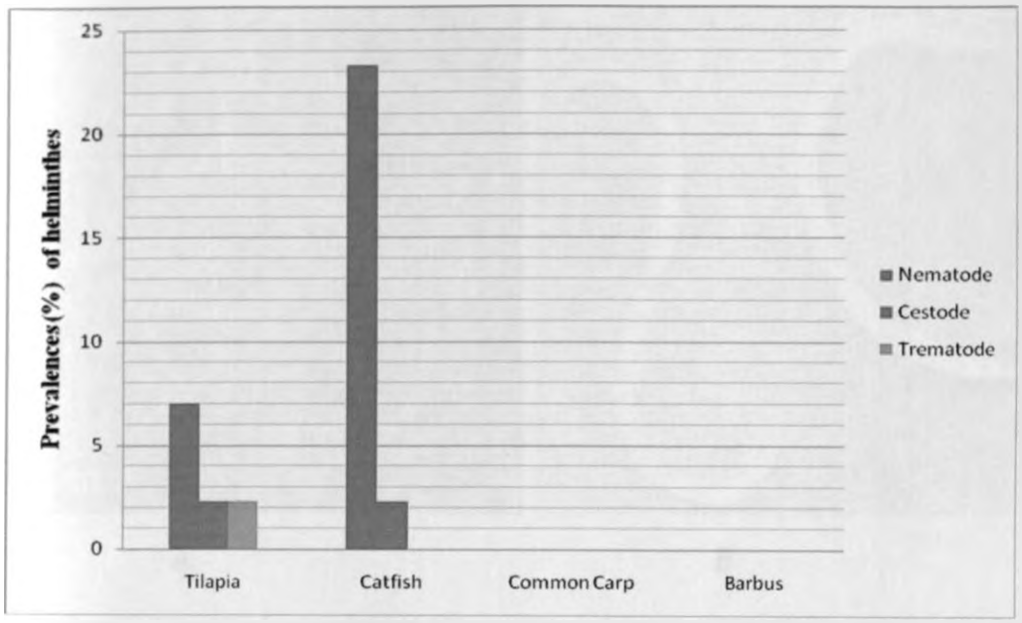


Figure 2: Percentage Prevalence of helminths observed in different fish in the market survey

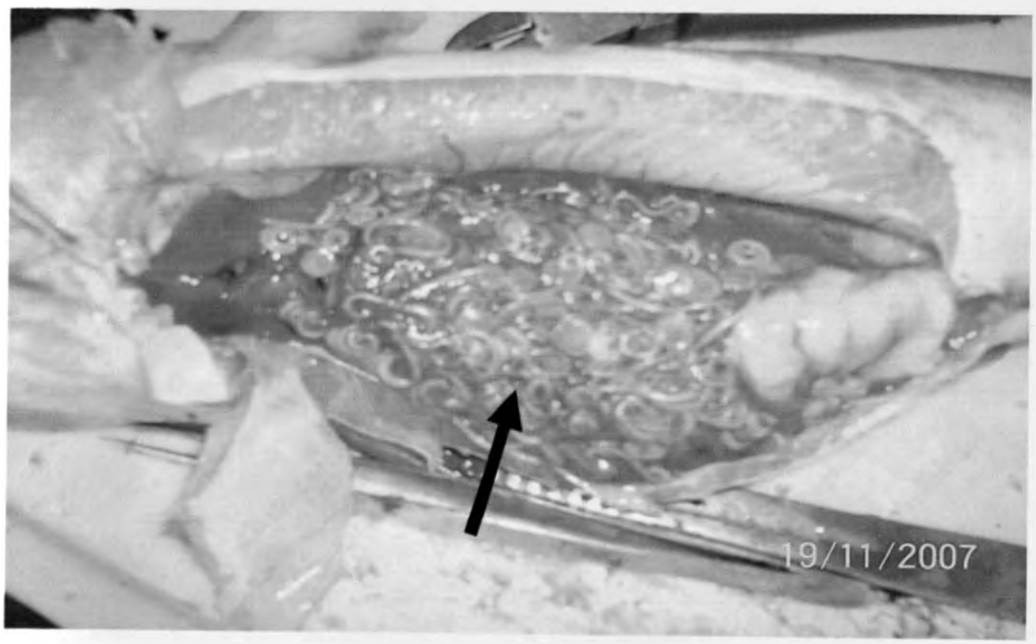
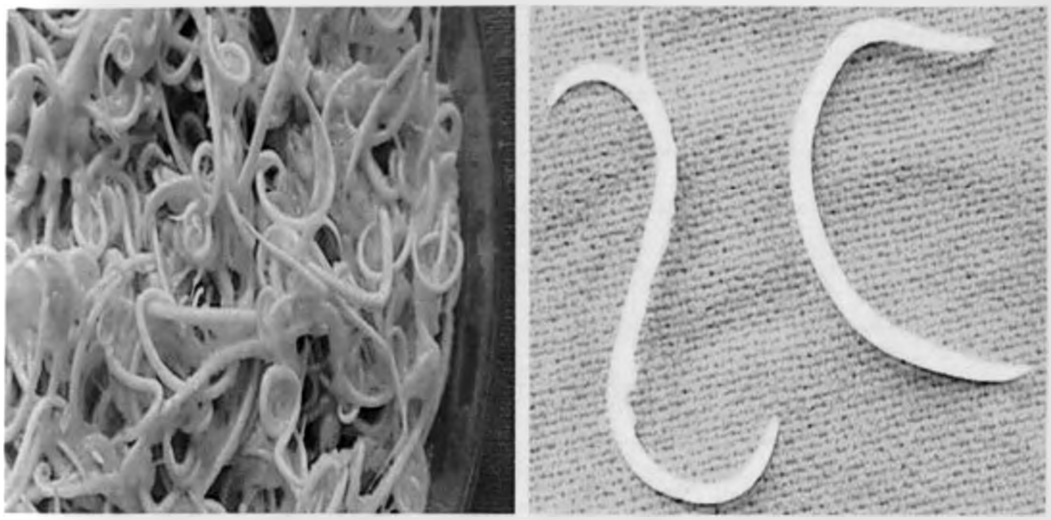


Figure 3: Heavy peritoneal *Contracaecum* species, 3rd stage larvae infestation in a market catfish (arrow)



A B

Figure 4: *Contracaecum* species 3rd stage larvae: enclosed in fibrin strand (A), and two larvae separated from the fibrin mesh (B)

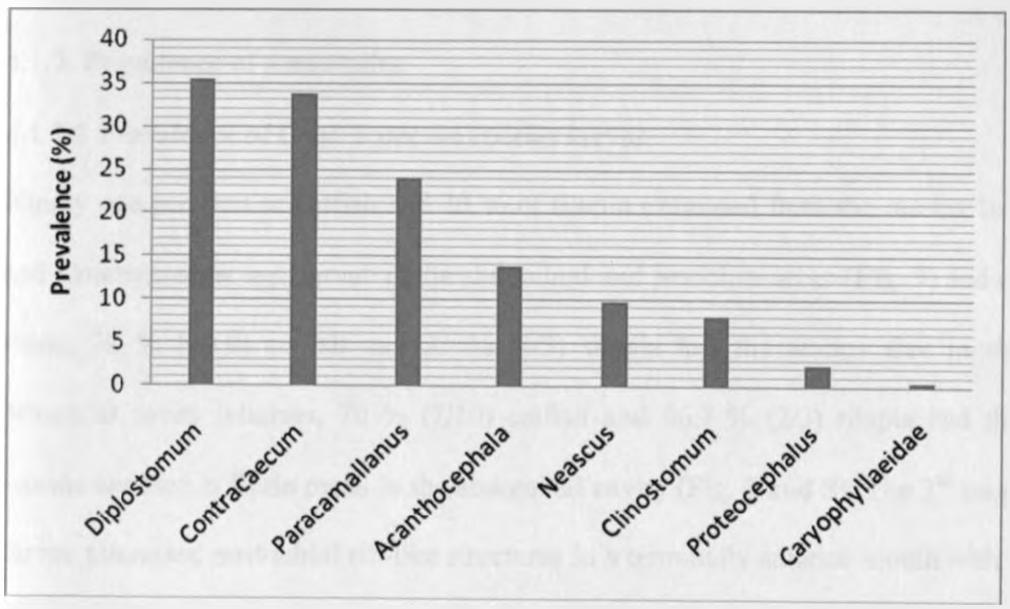


Figure 5: Prevalence of different types of helminthes species recovered in the field fish

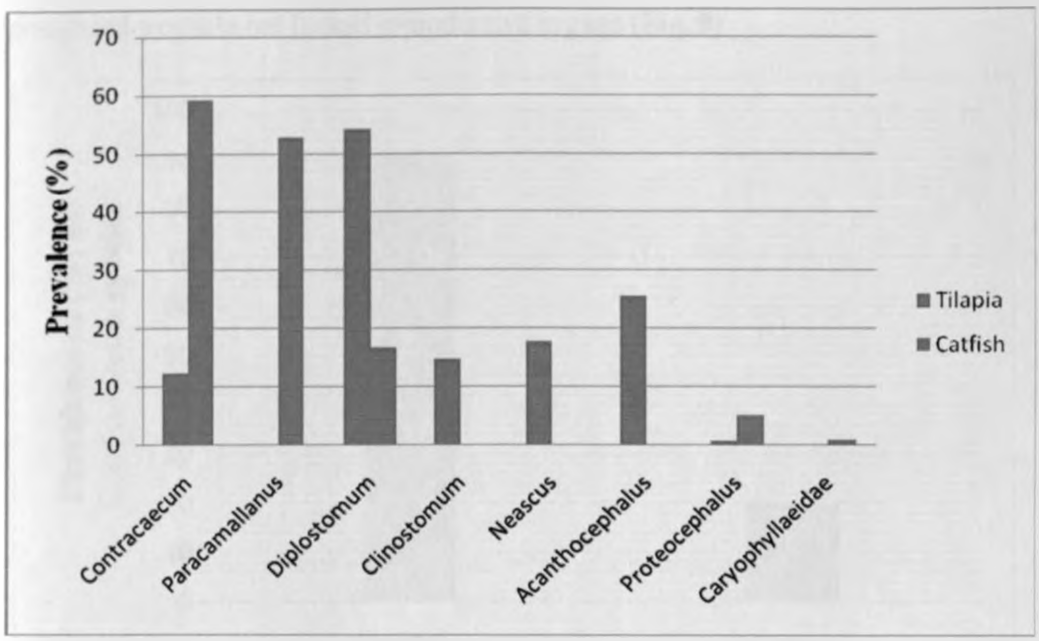


Figure 6: Prevalence of helminthes observed in tilapia and catfish species from the field study

4.1.2. **Prevalence of nematodes**

4.1.2.1 **Prevalence of *Contracaecum* species larvae**

Ninety one per cent of catfish and 20 % of tilapia examined from the market fish had *Contracaecum* spp. larvae in the abdominal and branchial areas (**Fig. 7**) and of these, 30 % (3/10) catfish and 33 % (1/3) tilapia had the worms free in the branchial cavity whereas, 70 % (7/10) catfish and 66.7 % (2/3) tilapia had the worms encased in fibrin mesh in the abdominal cavity (**Fig. 3 and 8**). The 3rd stage larvae possessed post-labial rib-like structures in a terminally anterior mouth with a gut clearly divided into esophagus and intestines with a small globular ventriculus giving off to a posteriorly directed appendix with suture-like depressions. They

possessed a cuticle but lacked reproductive organs (**Fig. 9**).

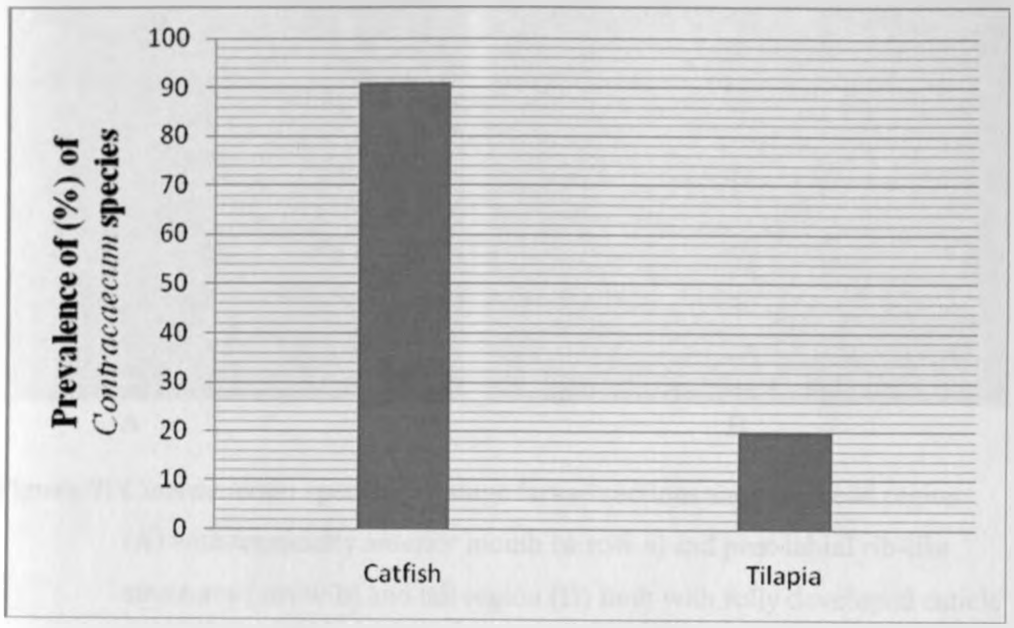


Figure 7: Prevalence of Contracaecum spp. 3rd stage larvae in market catfish and tilapia

Figure 8: A tilapia fish showing a *Contracaecum* spp., 3rd stage larva in the branchial cavity (arrow)

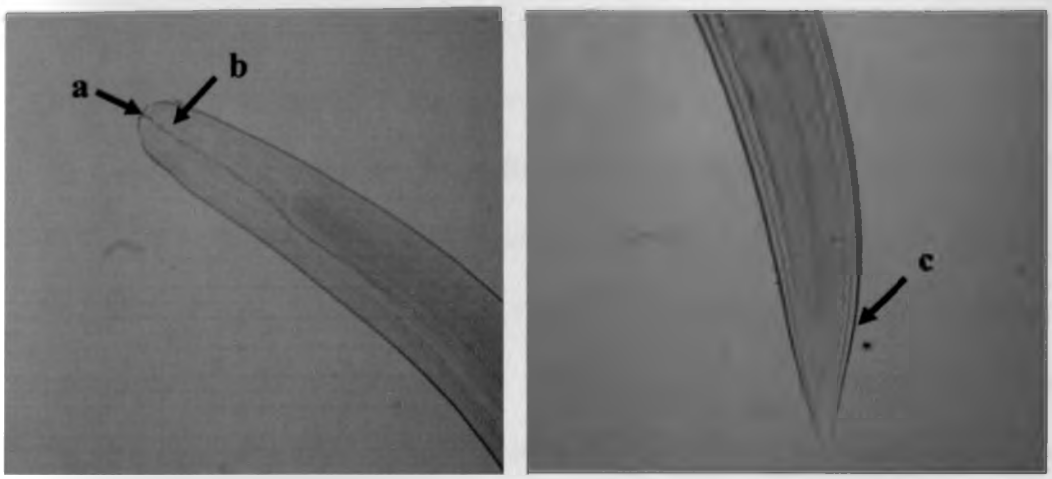


Figure 9: *Contracaecum* species, 3rd stage larvae sections showing head region (A) with terminally anterior mouth (arrow a) and post-labial rib-like structures (arrow b) and tail region (B) both with fully developed cuticle (arrow c) (X10)

In the field study, *Contracaecum* spp. was isolated from both catfish and tilapia species from the abdominal and branchial cavities. Out of the 237 fish sampled, 33.8 % (16 tilapia and 64 catfish) were infected with *Contracaecum* spp. Of these, branchial cavity worm infection accounted for 6.8 % while, abdominal cavity had 27 % (**Appendix 3**). The infection varied from mild (1 worm) to severe (846 worms). There was a significant difference in *Contracaecum* spp. infection between the tilapia and catfish species ($p < 0.05$) (**Appendix 3**) while, no significant difference was noted in the infection between fish sex and the age groups ($P > 0.05$).

The *prevalence of Contracaecum spp. in tilapia* was 12.4 % (n = 129) (**Fig. 6 and Appendix 4**). Branchial worm prevalence in tilapia was 9.3 % compared to 3.1 % for the abdominal worms. Of the 85 farmed tilapia sampled, only one young male fish (1.2 %) had the worm while prevalence in 44 wild tilapia sampled was 34.1 % (15/44) of which 9 were males and 6 females (**Appendix 4**). Adult tilapia had a prevalence of 12.5 % (9/72) while young tilapia had 20.6 % (7/57) worms (**Appendix 4**). Prevalence in male tilapia was 18.5 % (10/54) while female fish had a prevalence of (6/75) 8 %. There was a significant difference in the *Contracaecum* spp. infection in tilapia fish between the farmed and wild fish ($P < 0.05$), while no significant differences in infection was noted between the sex and between age group ($P > 0.05$) (**Appendix 4**). One hundred and eight (108) catfish were examined for *Contracaecum* spp. infection with overall prevalence of 59.3 % recorded. Branchial cavity infection prevalence was 3.7 % while that of abdominal cavity was 55.6 %. An overall prevalence of 18.5 % was recorded in farmed, while wild catfish had 40.7 % (**Appendix 5**). Adult catfish had an overall prevalence of 37.0 % while young ones had 22.2 % (**Appendix 5**). Male catfish had a higher *Contracaecum* spp. prevalence infection than female fish at 32.4 % and 26.9 %, respectively (**Appendix 5**). Sixty - three farmed catfish examined had a prevalence of 31.7 % (20/63) recorded (**Appendix 5**). Of these, 30.2 % (19/63) fish had abdominal and 1.6 % (1/63) had branchial cavity infesctions. Forty five wild catfish were sampled with a prevalence of 97.8 % (44/45) recorded of which 8.9 % (4/45) was branchial infection while abdominal infection accounted for 88.9 %

(40/45). The infection varied from moderate to severe. There was a significant difference in the infection in catfish between the farmed and wild fish ($P < 0.05$), while no significant differences were noted in the infestation between the fish sex and between the age groups ($P > 0.05$) (Apendix 5).

4.1.2.1.2 **Intensity of *Contracaecum* species larvae**

In the preliminary market study, an overall mean intensity of *Contracaecum* spp. was 114. Catfish sampled had a mean worm count of 169 (range 1 – 593) representing a severe infection, whereas the tilapia had a mean of 1 (range 1 -5) which was mild. The overall mean intensity of *Contracaecum spp.* in all the 237 fish sampled in the field study was 103.4 with a range of 1 - 846 worms per fish, with a mean *Contracaecum* count of 3.4 worms for tilapia and 128.3 worms for catfish. The infection varied from Mild (1 – 50), Moderate (51 – 100) to severe (above 100). Worm load per fish ranged from 1-15 in branchial area and 1-8 in the abdominal cavity. The infection in tilapia ranged from mild (1-8 worms per fish) in abdominal cavity to mild (1-2), moderate (3-5) and severe (6-15 worms) in branchial cavity. There were significant differences in the mean worm load between the species and the management (farmed and wild), sexes and the ages (young and adults) ($P < 0.05$).

Farmed catfish mean load was 3.5 worms per fish, wild 185.1; female catfish had a mean load of 77.4 while males had 167.8, whereas adult catfish had a mean count of 161.2 while for the young fish was 69.7. d. In catfish, there was significant differences in the mean worm load between the farmed and wild, and between the

sexe and age ($P < 0.05$). Tilapia mean *Contracaecum* spp. intensity for adults and young was equal at 3.4 worms per fish; farmed fish had a mean intensity of 1 worm, while wild had 3.6 worms per fish. Female tilapia mean *Contracaecum* spp. intensity was 3 worms per fish while males had fish 3.7. There were significant differences in mean worm load within the management (farmed and wild) ($P < 0.05$) but no significant differences were observed between fish sexe and age groups ($P > 0.05$).

4.1.2.2. Prevalence of *Paracamallanus* species

This tiny (<1mm) viviparous worms (**Fig. 10**) was found exclusively in 57 intestines and stomachs of the 108 (63 farmed and 45 wild) catfish in the field study but not in the market fish. The *Paracamallanus* spp. recovered had a bilaterally symmetrical buccal capsule which was divided into two levels; a smaller anterior part with vertical chitinoid plates for attachment to gastrointestinal mucosa and a larger cavity behind the valves (**Fig. 11**). Their esophagi were divided into a shorter anterior muscular part and a longer posterior glandular section. Both adult males and females worms were isolated. The male worms had a ventral caudal papillae and spicule while the females had mid – lateral genital pore (**Fig. 12 and 13**). Overall 24.1 % (57/237) catfish were infested with the *Paracamallanus* spp., out of which 23.2 % (55/237) had the nematode in the intestines; while 0.8 % (2/237) was recorded in the stomach of the catfish (**Appendix 2**).

Overall prevalence of *Paracamallanus* spp. prevalence in the catfish sampled was 52.8 % (n = 108) with the wild catfish having an prevalence of 37 % (n =108), while farmed had a prevalence of 15.7 % (n =108) (**Appendix 6**). Adult catfish had an overall prevalence of 34.3 %, young fish 18.5% whereas male and female catfish had prevalences of 25.9 % and 26.9 %, respectively (**Appendix 6**). The prevalence in farmed catfish was 27.0 % (n = 63), wild 88.9 % (n = 45); males 54.9 % (n = 51), females 50.9 (n = 57) while yong and adults had 41.7 % (n = 48) and 61.7 % (n= 60), respectively. There was a significant difference in the infection between the farmed and wild fish and age group ($P < 0.05$), but no significant difference was noted in the rate of infection between sex in catfish ($P > 0.05$) (Appendix 6).

4.1.2.2.1. **Intensity of *Paracamallanus* species**

Paracamallanus spp. had a mean worm intensity of 6.1 with a range of 1 - 41 worms per fish. Farmed catfish had mean worm load of 7.3, wild – 5.6 worms per fish; adult had 6.8, young – 4.4 worms per fish while female and male catfish had 7.3 worms and 4.9 worms per fish, respectively. There was no significant differences in the mean worm load between farmed and wild, sex and age groups of the catfish examined ($P > 0.05$).

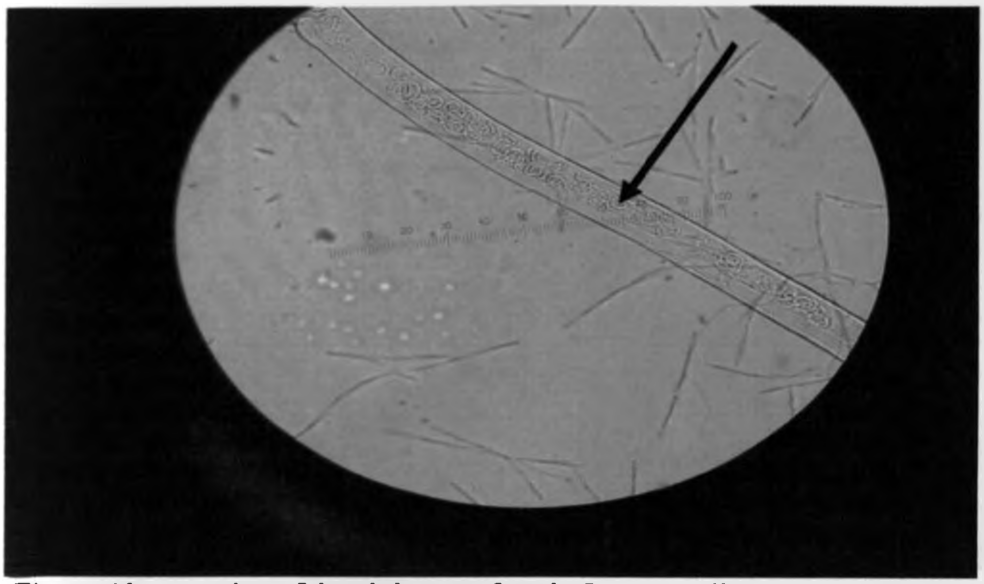


Figure 10: A section of the viviparous female *Paracamallanus* species showing free larval worms in the coelom (arrow) (X 20)

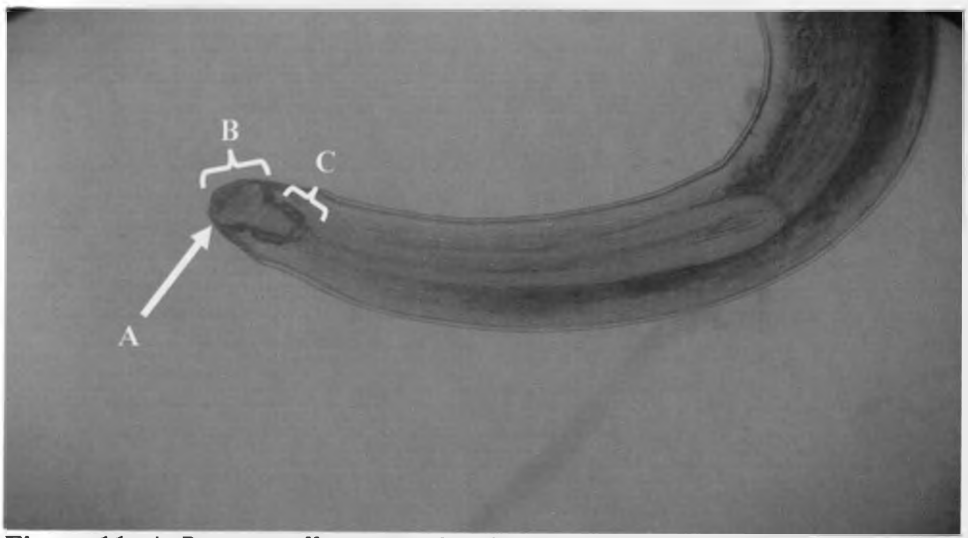


Figure 11: A *Paracamallanus* species showing the vertical chitinoid plates (arrow A) and the buccal cavity divided into two levels:- the upper smaller – (arrow B) and lower larger parts (arrow C) X 10

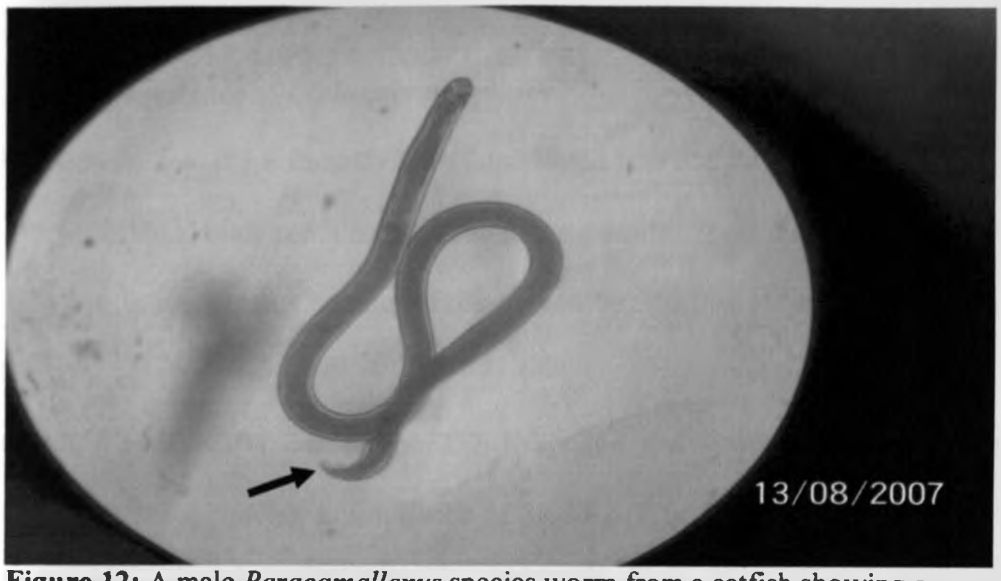


Figure 12: A male *Paracamallanus* species worm from a catfish showing a spicule (arrowed) X 10

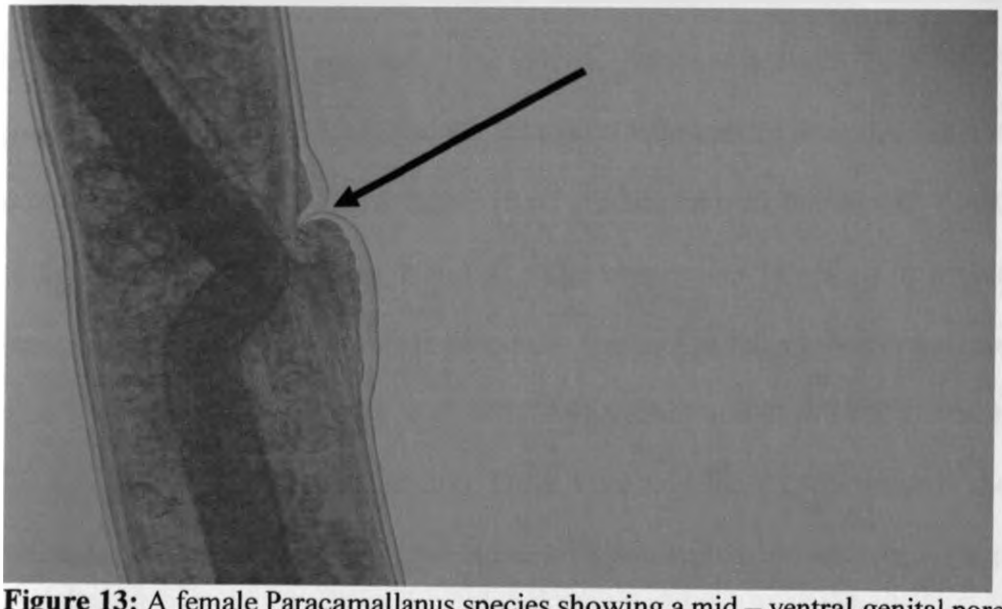


Figure 13: A female Paracamallanus species showing a mid – ventral genital pore (arrow) X 40

4.1.2.3. Prevalence of trematodes (digenean) parasites

4.1.2.3.1. Prevalence of *Diplostomum* species

Diplostomum spp. ("eye flukes") had a cup-shaped fore body with the suckers, and a cylindrical hind body containing the immature gonads. Their metacercariae were found free in the vitreous humour of the eyes in farmed fish of tilapia and catfish (**Fig. 14**).

Overall a total of 84 out of the 237 fish examined were infected with the *Diplostomum* spp., giving a prevalence of 35.4 % (**Fig. 5 and Appendix 2**). In tilapia fish, prevalence was 54.3 % (n = 129) while catfish fish had a prevalence of 16.7 % (n = 108), respectively (**Fig. 6 and Appendix 3**). This parasite was found in farmed tilapia and catfish, only. Of the 85 farmed tilapia examined, 82.4 % had the parasite either free or encysted in the vitreous humor (**Appendix 7**), while 45 farmed catfish examined 28.6 % had the helminth (**Appendix 8**). Prevalence in the adult and young tilapia was 35.7 % and 18.6 %, while for catfish it was 12 % and 4.6 %, respectively (**Appendix 7 and 8**). The prevalences in male and female tilapia was equal at 27.1 %, while in catfish the female fish had a lower prevalence of 15.7 % compared to the male of 17.5 %. (**Appendix 7 and 8**) Wild fish of both fish species did not have the parasite. There were significant differences in the *Diplostomum* spp. infection between the farmed and wild, male and female and between young and adult tilapia fish ($P < 0.05$; **Appendix 7**). In catfish, there was a significant difference between wild and farmed fish ($P < 0.05$) but none between the catfish sex and age group ($P > 0.05$; **Appendix 8**).

Figure 14: A *Diplostomum* spp. metacercariae isolated from the vitreous humour of a catfish (arrow) (X 20)

4.1.2.3.2. **Prevalence of *Clinostomum* species**

The metacercariae of *Clinostomum* spp. ("whitespots") are yellowish or orange in colour and were about 10 cm X 3 cm in size, dorsoventrally flattened, oval, with a smooth body with a sucker around the anteroventral mouth and a additional sucker or acetabulum. They had a digestive system consisting of a pharynx connected to the mouth opening and a short oesophagus and two blind intestinal ceaca (**Fig. 15**). This parasite was isolated from farmed tilapia only and appeared as white spots on the skin between or on scales and in the muscles.

Overall, of all the 237 fish examined 8.0 % had *Clinostomum* spp., white spots (**Fig. 5 and Appendix 2**). A prevalence of 14.7 % (n = 129) in tilapia fish was recorded (**Fig. 6; Appendix 3**). Adult tilapia fish had a prevalence of 10.1 % (n =

129), young 4.7 %, while female and male tilapia had prevalences of 8.5 % (n = 129) and 6.2 % (n = 129), respectively (**Appendix 9**). Prevalence in farmed tilapia was 22.4 % (n = 85) while wild had no parasite. Male tilapia had a prevalence of 20. 4 % (n = 54), female 10.7 % (n = 75) while in young and adult tilapia the prevalences were 10.5 % (n = 57) and 18.1 % (n = 72), respectively. Overall a significant difference in the infection between the two fish species was observed ($P < 0.05$), (**Fig. 6**). In tilapia fish, a significant difference in infection between farmed and wild fish was observed ($P < 0.05$), but there was no significant difference in the infestation rate between sexes and the age groups of the tilapia ($P > 0.05$; **Appendix 9**).

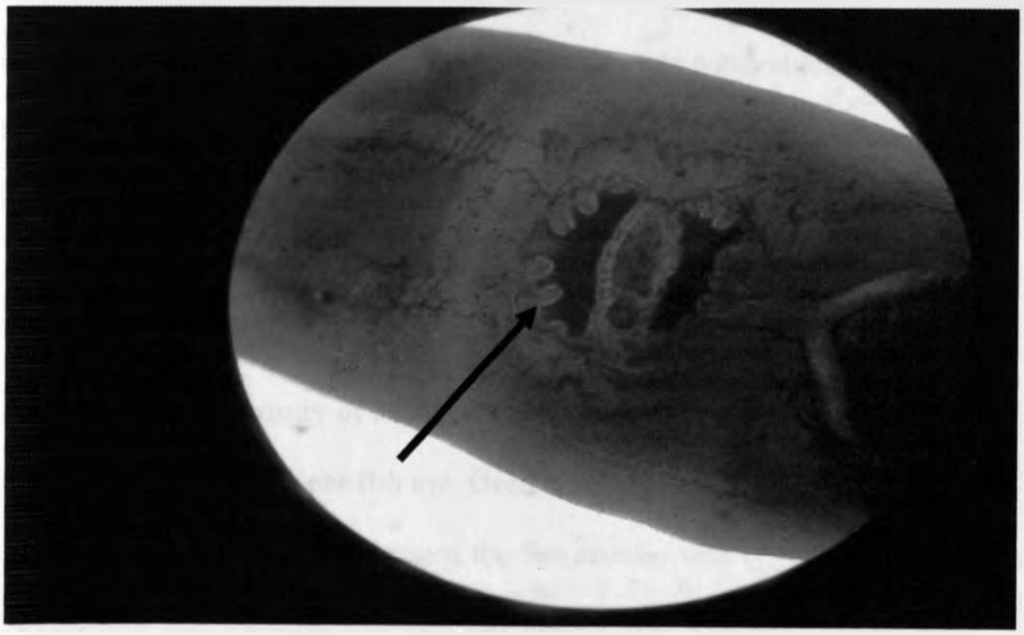


Figure 15: A photomicrograph of *Clinostomum* spp. metacercariae expressed from a white spot from the skin of a tilapia showing the ventral acetabulum (arrow) (X10)

4.1.2.3.3. **Prevalence of *Neascus* species**

The metacercariae of the genus *Posthodiplostomum* or *Neascus* spp. ("blackspots") were morphologically similar to that of *Diplostomum* spp. and were isolated from black spots on the skin and muscles of farmed tilapia fish only. Overall prevalence was 9.7% (n = 237), (**Fig. 5; Appendix 2**). A total of 129 tilapia fish were examined, out of which 17.8 % had the parasite encysted in the skin or in muscles (**Fig. 6; Appendix 10**). Overall prevalences for adult tilapia was 10.9 % (n = 129), young 7 % (n = 129) while female and male fish had 10.1 % and 7.8 %, respectively (**Appendix 10**). Prevalence in farmed tilapia was 27.1 % (n = 85); in male tilpia prevalence was 18.5 % (n = 54), female 17.3 % (n = 75) while in young and adult tilapia the prevalences were 15.8 % (n = 57) and 19.4 % (n = 72), respectively. In tilapia fish, a significant difference was noted in infection between the farmed and wild fish ($P < 0.05$) while there was no significant differences in the infection rate between fish sex and age groups ($P > 0.05$; **Appendix 10**).

4.1.2.3.4 **Mean intensity of digeneans**

The overall mean intensity of *Diplostomum* spp. infection was 5.1 parasites with a range of 1 – 26 parasites per fish eye. Overall, there were significant differences in the mean intensity infection between the fish species wild ($P < 0.05$). The mean *Diplostomum* spp. intensity in tilapia fish was 6.1 with a range of 1 – 26 parasites per fish eye. A mean intensity of 6.1 for farmed and 0 for wild tilapia helminth per fish eye was recorded. Adult tilapia had a mean intensity of 5.7 and young 7, while,

female tilapia had a mean of 6.9 and males 5.8 parasites per fish eye. A significant difference was observed in the mean eyefluke intensity between farmed and wild tilapia ($P < 0.05$). No significant differences were seen in the mean intensity infestation in the sex and age group of fish ($P > 0.05$). In catfish, the mean intensity of the eye flukes infection was 1.75, with a range of 1 – 5 per fish eye. Mean intensity of 1.75 and 0 for farmed and wild catfish, respectively was observed, while 2 and 1.4 was noted for females and male of the catfish, respectively. Adult catfish had a mean intensity of 2, while, young had a mean of 1. There wasa significant difference in maen imtensity of *Diplostomum* spp. infection between farmed and wild fish ($P < 0.05$). No significant differences were observed in the mean intensity of the eye fluke's infection per eye between the sex and age groups ($P > 0.05$).

The overall mean intensity of *Clinostomum* spp. in all fish sampled was 2.4 with a range of 1 – 7 cutaneous cysts per fish and a mean worm load of 0 and 2.4 in catfish and tilapia, respectively. The overall mean intensity for adult tilapia fish was 2.3 and 2.7 for young fish, while in farmed and wild tilapia it was 2.4 and 0 cysts per fish, respectively. Male tilapia fish had a mean intensity of 2.5 while the females had a mean intensity of 2.3 cysts per fish. There were significant differences in the mean intensity of *Clinostomum* spp. cysts between farmed and wild ($P < 0.05$), but no significant differences were observed in the mean intensity between the fish sex and age groups ($P > 0.05$).

Infection with *Neascus* species ranged from mild (1 – 50 cysts), moderate (51 – 100 cysts) to severe (over 100). The overall mean intensity of *Neascus* spp. was 9.5 with a range of 1 - 151 cysts per fish, while mean intensity in tilapia was 17.4. Farmed tilapia had a mean intensity of 26.4, females 85, males 115.5, adults 116.3 while young tilapia had a mean intensity of 68.4 cysts per fish. There was significant difference in infection between farmed and wild tilapia, the sex and age groups ($P < 0.05$).

4.1.2.4. **Prevalence of cestodes**

4.1.2.4.1 **Prevalence of *Proteocephalus* species**

Proteocephalus species observed were polyzoic tapeworms with lateral follicular, vitellaria and a scolex with 4 simple suckers (**Fig. 16**). The tapeworm had a median uterus with lateral branches and lateral vitalleria with irregularly alternating genital pores (**Fig. 17**). *Proteocephalus* spp. were isolated in only farmed tilapia and wild catfish with an overall prevalence of 2.5 % (n =237), (**Fig. 5; Appendix 2**). Overall prevalence in tilapia was 0.8 % (n = 129) and in catfish 4.6 % (n = 108) (**Appendix 3**). Farmed tilapia had a prevalence of 1.2 % (n = 85), adult 1.4 % (n = 72) and male tilapia had 1.9 % (n = 54). Wild tilapia, all young and females did not harbour the cestode (**Appendix 11**). Wild catfish had a prevalence of 11.1 % (n = 45), adult 8.3 5 (n = 60) and male catfish had a prevalence of 4.6 % (n = 51). Farmed catfish, all young and females did not harbor the cestode (**Appendix 12**). Adult and male farmed tilapia had a prevalence of 0.8 % (**Appendix 11**) each while adult and male

catfish had a prevalence of 5.2 % each (**Appendix 12**). There was a significant difference in the tapeworm infection between the fish species ($P < 0.05$) (**Fig. 6; Appendix 3**). In tilapia, no significant differences were observed in the infection between the fish sex, the age groups, the farmed and wild fish ($P > 0.05$) (**Appendix 11**) whereas in catfish there were significant differences in the infection between the fish sex and between the farmed and wild fish ($P < 0.05$) but none ($P > 0.05$) between age group (**Appendix 12**).

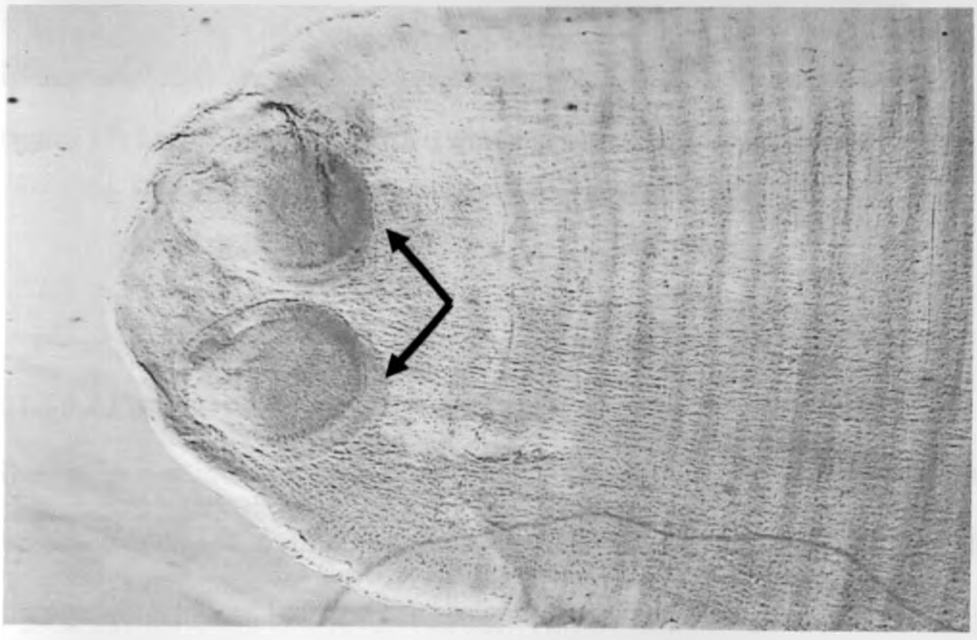


Figure 16: Head region of an adult *Proteocephalus* spp. tapeworm from tilapia fish with obvious suckers (arrows) X 20

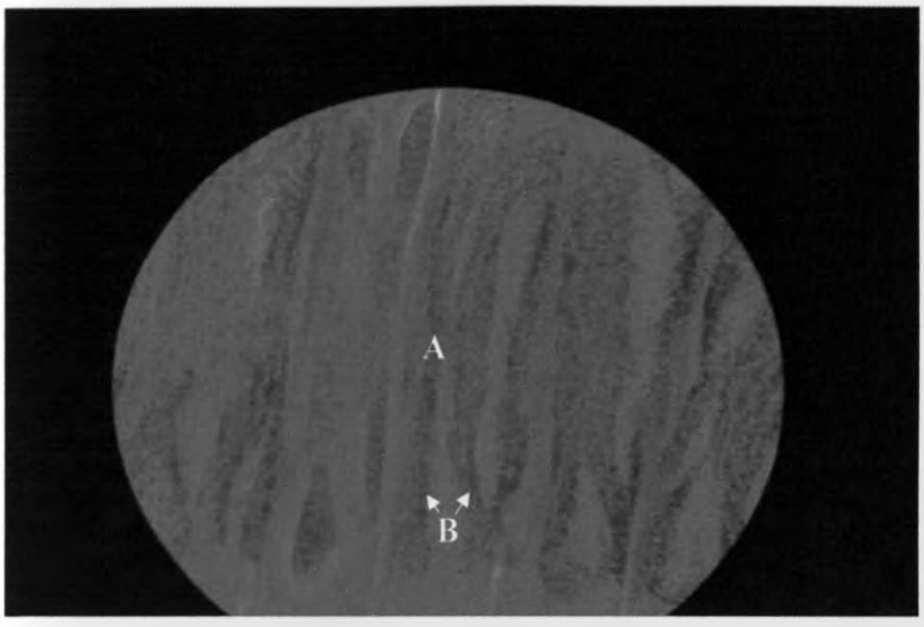


Figure 17: Lateral vitalleria (arrow) with median uterus (A) with lateral branches (B) in *Proteocephalus* spp. proglottid from a catfish (X 40)

4.1.2.4.2 **Prevalence of *Caryophyllaeidea* species**

This is non-segmented (monozoic) tapeworm with single set of male and reproductive organs, a simple scolex with shallow grooves and suckers (**Fig. 18**). The tapeworm had mid-ventral and posteriorly located genital pores.

This tapeworm was isolated in a wild catfish with an overall prevalence of 0.4 % (n =237), (**Fig. 5; Appendix 2**). Prevalence in the catfish species, farmed, young and female catfish was 0.9 % (n = 108). There were no significant differences in the infection in the catfish between age groups, sex and the farmed and wild fish ($P > 0.05$).

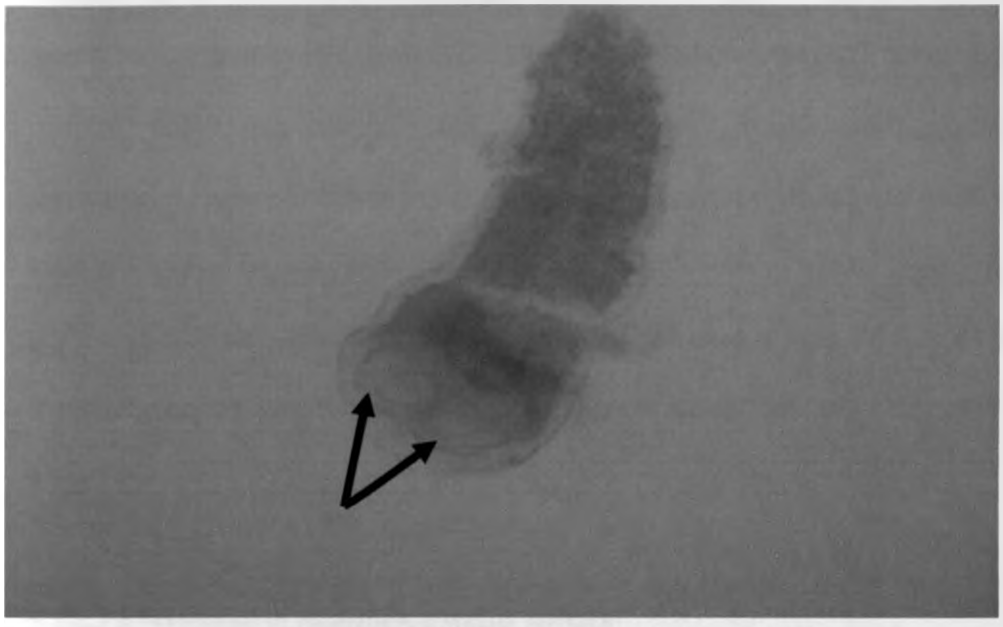


Figure 18: A monozoic *Caryophyllaeidea* spp. tapeworm from catfish showing 2 suckers (arrows) (X10)

4.1.2.4.3 **Mean intensity of cestodes**

The overall mean intensity of *Proteocephalus* spp. in all fish was 2.2, with a load of 1 – 4 parasites while *Caryophyllaeidea* spp. had a mean of 1 per fish. The overall mean intensity of *Proteocephalus* spp. in adult and young fish was 2.4 and 0, respectively. The catfish had a mean of 2.4 while tilapia had 0.01. Farmed tilapia fish had a *Proteocephalus* spp. overall mean intensity of 1, wild had 0. There were significant differences in the overall mean intensities of *Proteocephalus spp.* infection between the species and the farmed and wild tilapia and catfish ($p < 0.05$). No significant difference was observed in the mean

intensities between the sex and age groups of both fish species ($P > 0.05$). The overall mean intensity of *Caryophyllaeidea* spp. infection in the catfish and tilapia was 0.01 and 0, respectively, while in adult and young fish it was 0 and 0.01, respectively. Farmed fish had an overall mean intensity of 0 while wild had 0.01. Male fish had an overall mean intensity of 0.01 while females had 0. There was a significant difference in the overall mean intensity between ages ($P < 0.05$) but none between the species, the sex and between the farmed and wild catfish ($P > 0.05$).

4.1.2.5 **Prevalence of *Acanthocephalus* species**

These worms were tiny, bilaterally symmetrical worms, pseudocoelomate and lacked an alimentary canal. They possed a spined retractable proboscis, (thus "thorny headed worms"), that was sometimes invaginated in a saccular receptacle and used for attachment to the intestinal mucosa (**Fig. 19 and 20**). A big number of immature acanthors with ovarian balls in the coelom were also seen (**Figure 21**). Both male and female worms were isolated, with an obvious membranous bursa of the male being prominent.

Overall prevalence of *Acanthocephalus* spp. infection was 13.9 % (**Fig. 5; Appendix 2**). These worms were isolated in tilapia fish only with a prevalence of 25.6 % (**Fig. 6; Appendix 3**). Farmed tilapia had a prevalence of 15.5 % (n = 129), wild 10.1 % (n =129); male tilapia had a prevalence of 13.2 % (n = 129), female 12.4 % (n = 129), whereas young and adukt tilapia had prevalences of 5.4 % (n =

129) and 20.2. % (n = 129), respectively (**Appendix 14**). Prevalence of in farmed tilapia was 23.5 % (n = 85), wild 29.5 % (n = 44); in males prevalence was 31.5 % (n = 54), in females 21.3 % (n = 75) while in young and adult tilapia the prevalence were 12.3 % (n = 57) and 36.1 % (n = 72), respectively. A significant difference was observed in infection between adult and young tilapia ($P < 0.05$) but none ($P > 0.05$) in between the sex and farmed and wild tilapia **(Appendix 14).**

4.1.2.5.1 **Mean intensity of *Acanthocephalus* species**

The overall mean intensity of *Acanthocephalus* spp. in tilapia fish was 5.4 with a range of 1 – 27. Mean intensity in wild tilapia fish was 8.2 while farmed tilapia had 3.5 worms per fish. Female and male tilapia fish had worm intensities of 5.5 and 5.2 worms per fish, respectively. Mean intensity in farmed and wild tilapia was 3.5 and 8.2 worms per fish while adult and young tilapia mean intensities were 4.7 and 7.7 worms per fish, respectively. There were significant differences in the mean worm intensities between the farmed and wild tilapia and between the age groups ($P < 0.05$). There were no significant differences in the worm intensity between the sex of tilapia fish ($P > 0.05$).

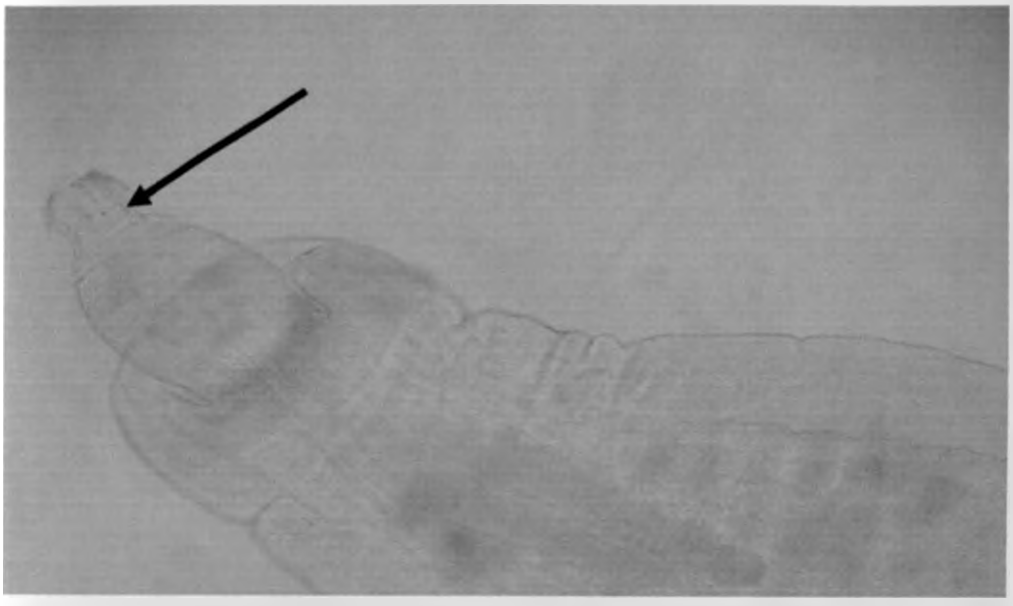


Figure 19: An Acanthocephalan worm with a protruding probosci armed with hooks (arrow) from tilapia fish (X40)

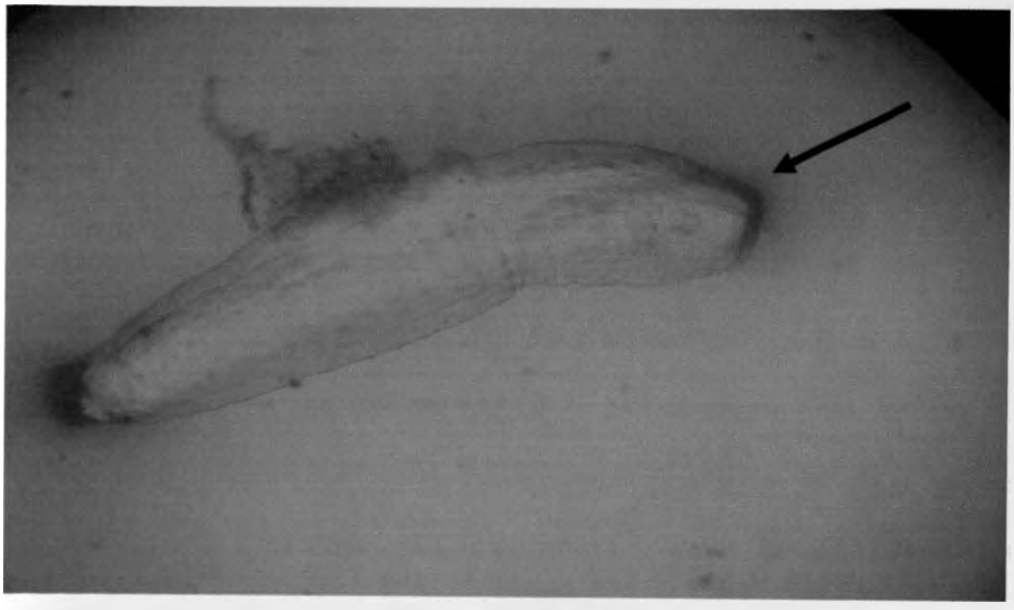


Figure 20: *Acanthocephalus* spp. with an invaginated proboscis (arrow) from a tilapia fish (X10)

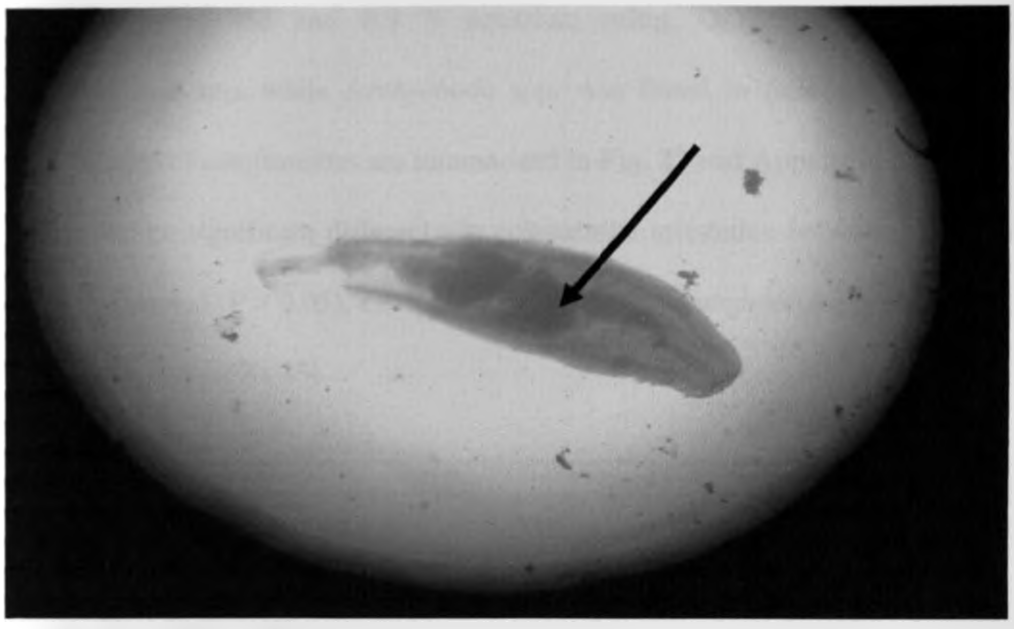


Figure 21: A female Acanthor from an intestinal cyst in tilapia fish showing free ovarian globules in the coelom (arrow) (X 10)

4.2 **Prevalence of ectoparasites**

Out of the 237 tilapia and catfish examined, 85.7 % (203/237) harboured ectoparasites. The major ectoparasites encountered were in the genus *Piscinoodinum* 25.3 %, *Trichodina* 18.6 %, *Ichthyophthirius* 2.1 %, *Ambyphrya* 1.3 %, *Amyloodinum, Ichthyobodo* spp 1.3 %. and aquarium mites. Farmed fish had a higher prevalence of ectoparasites (tilapia – 38.8 %; catfish – 30.6 %) compared to wild fish (tilapia – 6.2 %; catfish – 1.9 %). Prevaence of ectoparasites in tilapia was 27.9 % *Piscinoodinum*, 24.1 % *Trichodina*, 15.5% 7% *Lamproglena* spp. 3.9 % aquarium mites and 2.3 % *Ichthyophthirius*. Prevalence in catfish was 12.0 % *Trichodina*, 1.9 % *Ichthyophthirius*, 2.8 % *Ichthyobodo*, 27.9 %

Piscinoodinum, and and 0.9 % aquarium mites. Only farmed tilapia had *Lamproglena* spp. while *Ichthyobodo* spp. was found in farmed catfish. The prevalences of ectoparasites are summarized in **Fig. 22 and Appendix 15.** Overall, there was no significant difference in ectoparasite infestation between tilapia and catfish sampled ($P > 0.05$), except in *Trichodina and Lamprolegna spp* infestation ($P < 0.05$; **Appendix 15**).

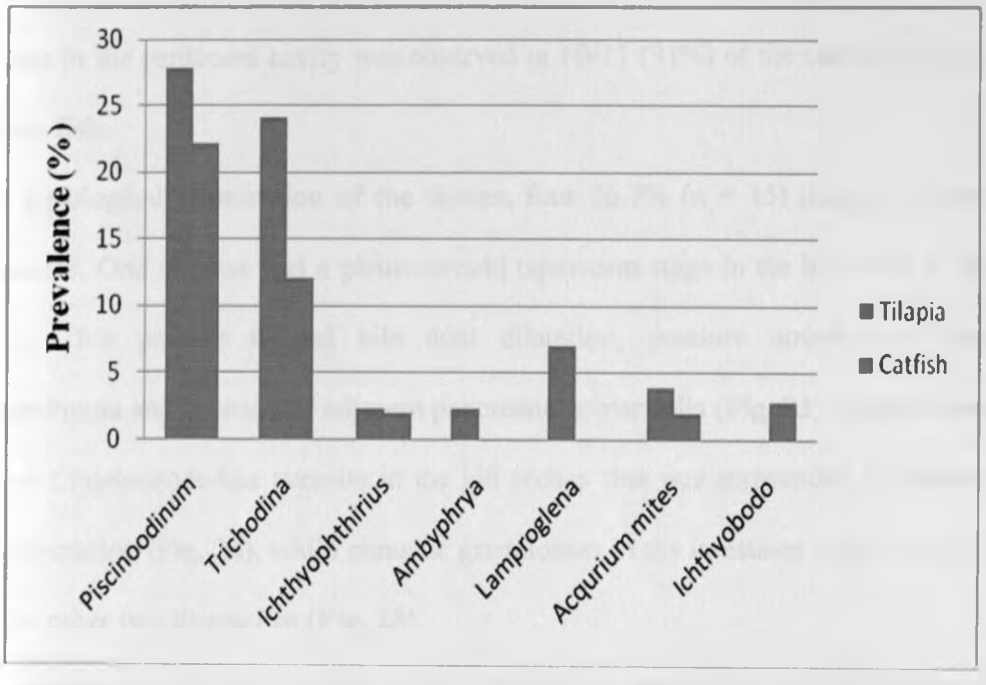


Figure 22: Percent prevalence of ectoparasites recovered from tilapia and catfish species in the field study

4.3 Gross and microscopic lesions in tilapia and catfish

In the market study, grossly, the common carp *(Cyprinus carpio)* and *Barbus* spp. (barbus) did not have worms although they had worm lesions. A total of 5/43 (2/43

tilapia, 2/43 barbus and 1/43 common carp) had swellings around the mouth, while 2 tilapia had grayish lesions on the intestines. Other lesions observed grossly were heamorrhages on the skin, base of fins and eyes in 25/43 (7/43 catfish, 7/15 tilapia, 5/7 common carp and 6/10 Barbus) fishes. Muscular haemorrhages were seen in 15/43 fishes (8/11 catfish, 3/15 tilapia, 3/10 barbus and 1/7 common carp) and exophthalmia was seen in one tilapia. Severe peritonitis characterized by blood stained ascitis, fibrin and adhesions around the *Contracaecum* spp. larvae and organs in the peritoneal cavity was observed in 10/11 (91%) of the catfish and one tilapia fish.

On histological examination of the tissues, four 26.7% (n = 15) tilapia fish had parasites. One of these had a pleurocercoid tapeworm stage in the bile duct in the liver. This parasite caused bile duct dilatation, pressure atrophy of liver parenchyma and necrosis of adjacent pancreatic acinar cells (**Fig. 23**). Another one had a *Cryptocotyle*-like parasite in the gill arches that was surrounded by intense inflammation (**Fig. 24**), while parasitic granulomas in the intestines were observed in the other two tilapia fish (**Fig. 25**).

All *Contracaecum* spp. infected catfish and tilapia showed severe infiltration of mononuclear and polymorphonuclear cells as well as fibroblasts into the mesenteries, intestinal and stomach serosal surfaces. The fish with the swollen mouth areas showed inflammation of the bucco-pharyngeal region with pyogranulomatous lesions, characterized by heterophils, plasma and other

mononuclear cells. One barbus spp. had a purulent peritonitis characterized by heterophils and mononuclear cells (**Fig. 26 and 27**).

In the field study, a total of 237 fish were examined for gross and microscopic lesions in the gastrointestinal tract and other organs. A total of 15.6 % of the fish examined had gross lesions of which tilapia fish accounted for 51.9 % while those of catfish were 48.1 % (**Appendix 16**). Farmed tilapia had a higher mean prevalence of gross lesions than wild tilapia at 6.7 % and 3.1 %, respectively while in catfish, the wild (13 %) had higher than the farmed (6.5 %) (**Appendix 17 and 18**). Peritoneal and mesenteric adhesions were seen in 28.3 % of *Contracaecum* spp. infested fish. Gross lesions in tilapia had a mean prevalence of 9.7 % with visceral organs peritoneal adhesions having a prevalence of 3.1 % (**Appendix 17**). In catfish, the mean prevalence of gross lesions was 19.4 % with peritoneal adhesions having a prevalence of 58.3 %. (**Appendix 18**). The mean prevalence of microscopic lesions in all fish was 17.2 % (**Appendix 19**) with tilapia having a prevalence of 22.7 % (**Appendix 20**), while catfish had 18.0 % (**Appendix 21**). A significant difference was noted in organ adhesions between tilapia and catfish ($P < 0.05$) but none was noted in the overall gross lesions between the tilapia and catfish ($P > 0.05$), (**Appendix 17 and 18**).

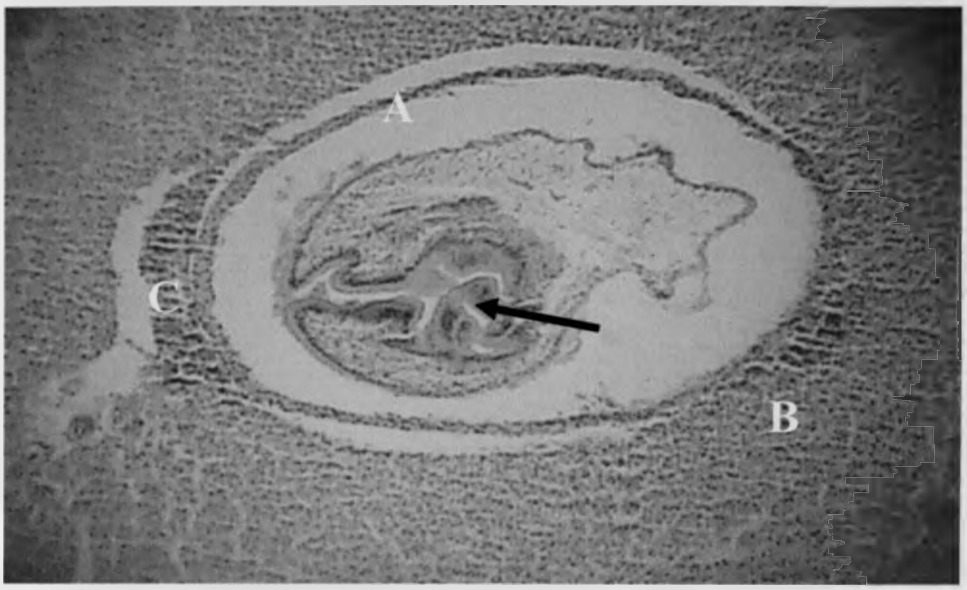


Figure 23: Tapeworm pleurocercoids (arrow) in the bile duct of a tilapia causing pressure atrophy of bile duct epithelium (A), liver parenchyma (B) and necrosis of pancreatic acinar (C) (H & E; X 40)

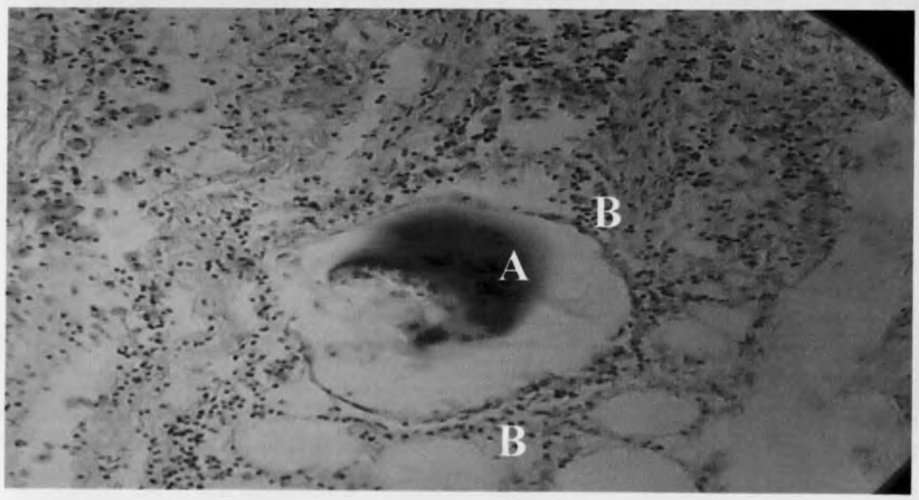


Figure 24: *Cryptocotyle* – like parasite (A) on the gill arch and accompanying cellular inflammation (B), (H & E; X 40)

Figure 25: Numerous parasitic granulomas in the intestinal wall of a tilapia fish (arrows) (H & E; X 40)

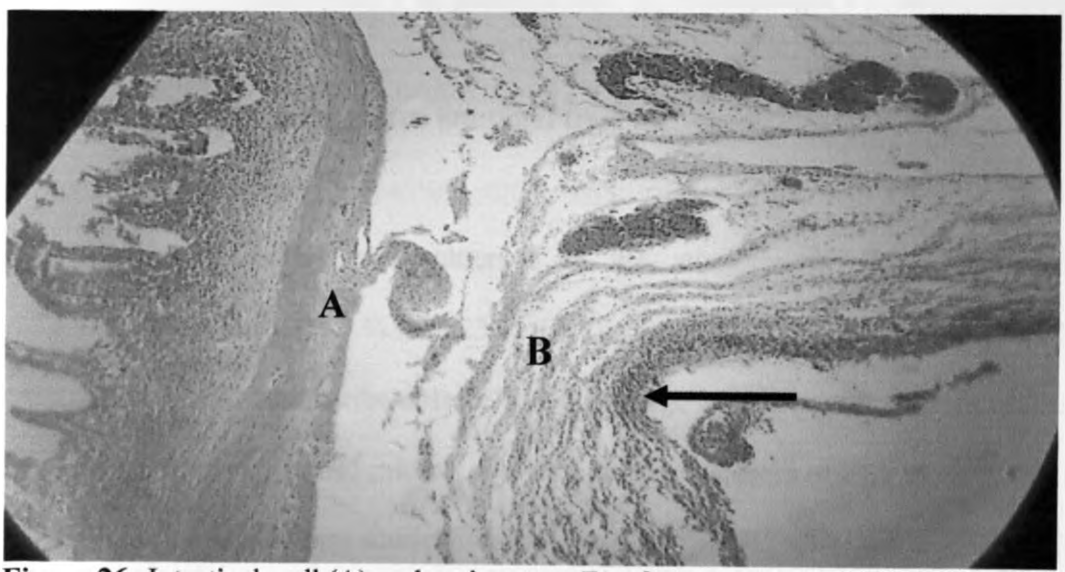


Figure 26: Intestinal wall (A) and peritoneum (B) of *Barbus* spp. fish showing severe inflammation (arrow) (H & E; X 10)

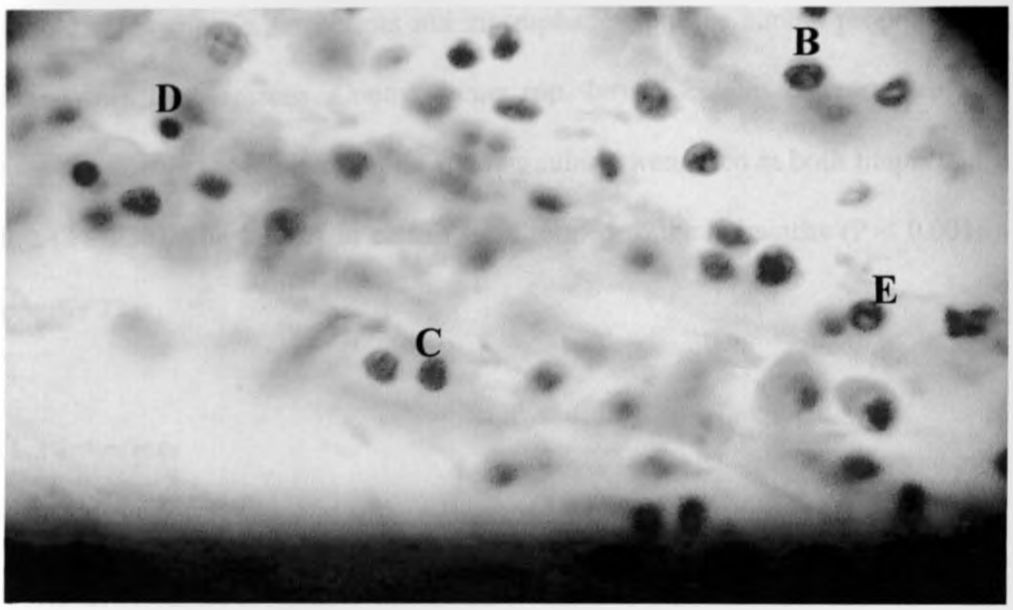


Figure 27: A higher magnification of figure 25, showing inflammatory cells: heterophils – B, plasma cells – C, macrophages – E and lymphocytes – D (H & E; X 40)

4.3.1 **Lesions in the gastro intestinal tract**

4.3.1.1 **Stomach**

No gross lesions were observed in the stomachs of fish examined but microscopically, 25.7 % (n = 237) of all fish examined had changes of which 23.4 % (n = 64) was attributable to parasites (**Fig. 28; Appendix 19**). Stomachs in tilapia and catfish had microscopic lesions at prevalences of 27.1 % (n = 129) and 26.9 % (n =108), respectively (**Appendices 20 and 21**) while tilapia, 8.6 % of the lesions were associated with *Contracaecum* spp. larvae, in catfish 41.4 % of the lesions were attributed to *Contracaecum* spp. and *Paracamallanus* spp. In both tilapia and catfish, the lesions observed were desquamation of the epithelium, hyperplasia of goblet cells and exudates in the lumen (**Fig. 29**). Infiltration of

eosinophils, neutrophils, fibroblasts and macrophages into the lamina propria was observed in these stomachs. *Contracaecum* spp. larvae sections encapsulated in fibrous capsules in the lamina propria and muscularis were seen in both tilapia and catfish (**Fig. 30**). The lesions in catfish were related to the helminths ($P < 0.001$; **Appendix 22**).

4.3.1.2 **Intestines**

Grossly, 0.42 % (1/237) intestines examined had white parasitic cysts visible on the serosal surface of the intestines (**Appendix 16**). On incision, the cysts contained juvenile acanthocephalans, acanthors, which had free ovarian globules free in the coelom. Of the intestines examined, 41.8 % (n = 237) had lesions, of which 67.7 % (n = 99) were attributable to helminth parasites. (**Fig. 28; Appendix 20**). Of the intestines of tilapia examined microscopically, 38 % (n = 129) had lesions with 51 % (25/29) of these, attributed to *Contracaecum* larvae, *Acanthocephalus* spp. and *Proteocephalus* spp. (**Appendix 20**). In catfish, 46.3 % (50/108) intestines had lesions with 84 % (42/58) of these attributed to the helminths in the genus *Paracamallanus* spp, *Contracaecum* spp, *Proteocephalus* and the family Caryophyllaeidae (**Appendix 21**). The lesions observed were desquamation of villi, necrosis and flattening of the columnar epithelium and pinkish detritus exudates into the lumen. Infiltration of macrophages, lymphocytes, plasma cells and eosinophilic granulocytes (**Fig. 31**) into the lamina propria was also noted in

the intestines affected. The helminths were related to the pathological lesions ($P < 0.001$; **Appendix 22**).

4.3.2 **Lesions in other organs**

4.3.2.1 **Eyes**

Forty seven point eight percent (113/237) of the eyes examined, had gross lesions. Of these tilapia had 77.9 % (88/113), while catfish had 22.1 % (25/113), (**Appendix 16**). These included heamorrhages, cysts, exophthalmia, opacity and congestion. There aws a relationship between opacity and the parasites ($P < 0.001$; **Appendix 22**).

4.3.2.2 **Liver**

Grossly, 4.2 % (n =237) of the livers examined had lesions, which included paleness, heamorrhages and white-grayish nodules. Of the liver gross lesions, tilapia fish had a higher prevalence of 90 % (9/10) while catfish accounted for 10 % (1/10), (**Appendix 16**). Microscopically, 38.8 % (n = 237) of the livers had lesions of which, 84.9 % (n = 92) were attributable to parasites (**Fig. 28; Appendix 19**). Forty three point four percent (n = 129) tilapia livers had microscopic lesions, with those attributable to parasites being 48.2 % (n = 56), while in catfish 33.3 % (n = 108) had microscopic lesions, with none being attributable to parasites (**Appendix 19**). These lesions were centri-lobular necrosis of hepatocytes, parasitic tracts in the liver parenchyma and extensive infiltration of

melanomacrophages (**Fig. 32 and 33**). Sections of *Acanthocephalus* spp. worms in bile ducts and portal vessels caused vasculitis, pressure atrophy of the bile duct columnar epithelium and destruction of the endothelium. Cholestasis due to blockage in the bile ducts by the parasites was also observed. Infiltration by melenomacrophages, lymphocytes, plasma cells and eosinophilic granulocytes was also noted. The helminths associated with the lesions in tilapia fish were identified as *Acanthocephalus* spp. (**Fig. 34 and 35**). The lesions in tilapia were related to helminths ($P < 0.001$; **Appendix 22**).

4.3.2.3 **Skin**

Of the fish skins examined grossly, 52.3 % (n = 237) had lesions of haemorrhages and parasitic cysts of which, tilapia fish had 62.1 % (n = 124) while catfish had a prevalence of 37.9 % (**Appendix 16**). Microscopically, 23.2 % (n = 237) of the skin sampled had lesions, of which 78.2 % (n = 55) were associated with parasites (**Fig. 28; Appendix 19**). Of the tilapia skins sampled, 52.7 % (n = 129), had microscopic lesions, with 63.2 % (n = 68) related to parasites while in catfish, 11.1 % (n = 108) of the skins had microscopic lesions which were not related to any helminth (**Appendix 20 and 21**). Microscopic lesions included fibrous encapsulation of metacercariae, proliferation of goblet cells, and infiltration of melanomacrophages into the dermis around the cyst and pressure atrophy of neighbouring tissue (**Fig. 36**). The lesions were related to parasites in tilapia ($P < 0.001$; **Appendix 22**).

4.3.2.4 **Skeletal muscles**

Skeletal muscle hemorrhages were the gross lesions observed in all the fish examined. Overall prevalence of these hemorrhages was 23.2 % (n = 237) of which tilapia and catfish accounted for 3.6 % (n = 55) and 96.4 % (n = 55), respectively (**Appendix 16**). In the tilapia fish examined, 1.6 % (n = 129) of the muscles had gross lesions, while in catfish it was 49.1 % (n = 108), (**Appendix 17 and 18**). Microscopically, 4.6 % of all muscles examined had lesions (**Fig. 28; Appendix 19**). In tilapia, microscopic muscle lesions prevalence was 5.4 % while in catfish it was 3.7 % (**Appendix 20 and 21**). The lesions seen were sections of metacercariae in fibrous capsules infiltrated by melenonomacrophages with atrophy, degeneration and necrosis of muscle fibers.

4.3.2.5 **Heart**

Of the hearts examined grossly, none had gross lesions, but 6.3 % (**Fig. 28; Appendix 16**) had microscopic lesions that involved necrosis of myocytes, infiltration of mononuclear inflammatory cells and eosinophilic granulocytes into the spongy and compact muscle layers of the ventricle. In tilapia, 7.8 % of the hearts and 4.6 % in catfish had microscopic lesions (**Appendix 20 and 21**). The lesions were not related to the helminth parasites ($P > 0.001$).

4.3.2.6 **Spleens**

Twenty-one point nine percent (n = 237) of the fish spleens examined had microscopic lesions but no gross ones (**Fig 28; Appendix 19**). In tilapia 18.6 % (n = 129) of the spleens had microscopic lesions while in catfish the prevalence was 25.9 % (n = 108) (**Appendix 20 and 21**). The lesions were mainly severe proliferation of melanomacrophage centres, congestion and increase of the white pulp and were not related to the helminth parasites ($P > 0.001$).

4.3.2.7. **Gills.**

Grossly 4.6 % (n = 237) of the gills had gross lesions which were heamorrhages and paleness of which; tilapia fish had a prevalence of 45.5 % (n = 129), while catfish had 54.5 % (n = 108) (**Appendix 16**). On microscopic examination, 22.4 % (n = 237) of all the gills examined had lesions (**Fig. 28; Appendix 19**). In tilapia, the prevalence of microscopic lesions was 13.9 % (n = 129), while in catfish 13.9 % (n = 108) (**Appendix 20 and 21**). These microscopic lesions were proliferation of goblet and chloride cells at distal lamellae, telangiectasis, hypertrophy and necrosis of the epithelium leading to clubbing. Some sections of ectoparasites were observed in the epithelium. There was also infiltration of macrophages, lymphocytes and eosinophils in the interstitial spaces. There was no statistical association of the lesions with the helminth parasites.

4.3.2.8 **Kidneys**

One point seven percent (n = 237) and 15.6 % (n = 237) of all kidneys examined had gross and microscopic lesions (**Fig. 28; Appendix 16 and 19**). Kidney gross lesions were white cysts and paleness, while microscopic lesions showed the proliferation of melanomacrophages centres, pinkish exudates in the tubules and infiltration of lymphocytes, heterophils and eosinophillic granulocytes into the interstitium. There was no relationship of the lesions to the helminth parasites (P > 0.001).

4.3.2.9 **Other organs**

Overall, grossly 11.4 % of the operculum, 2.1 % catfish aborescent organs, and 0.4 % buccal cavities had heamorrhages (**Appendix 16**). Minimal lesions were observed in the reproductive organs, with 0.4 % of testis examined showing gross lesions of heamorrhages (**Appendix 16**) and 1.7 % showing microscopic lesions of proliferation of melanomacrophages, infiltration of eosinophillic granulocytes and loss of germinal epithelium (**Fig. 28; Appendix 19**). There was no of the lesions to the helminth parasites (P > 0.001).

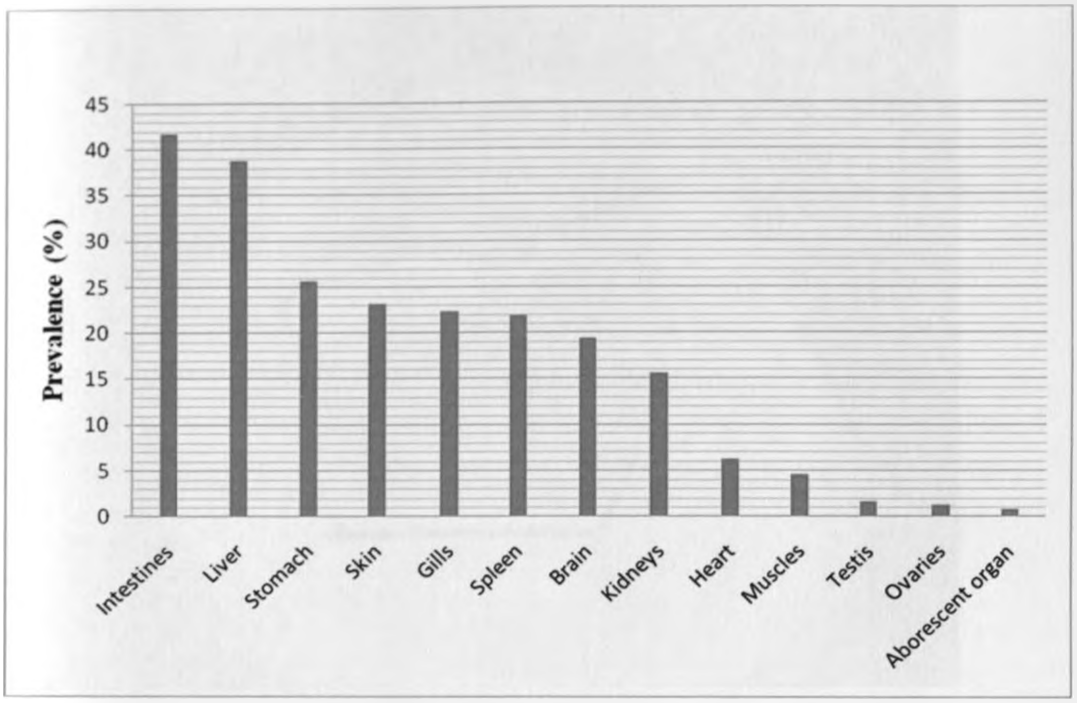


Figure 28: Overall prevalence of microscopic lesions in different organs of all fish examined

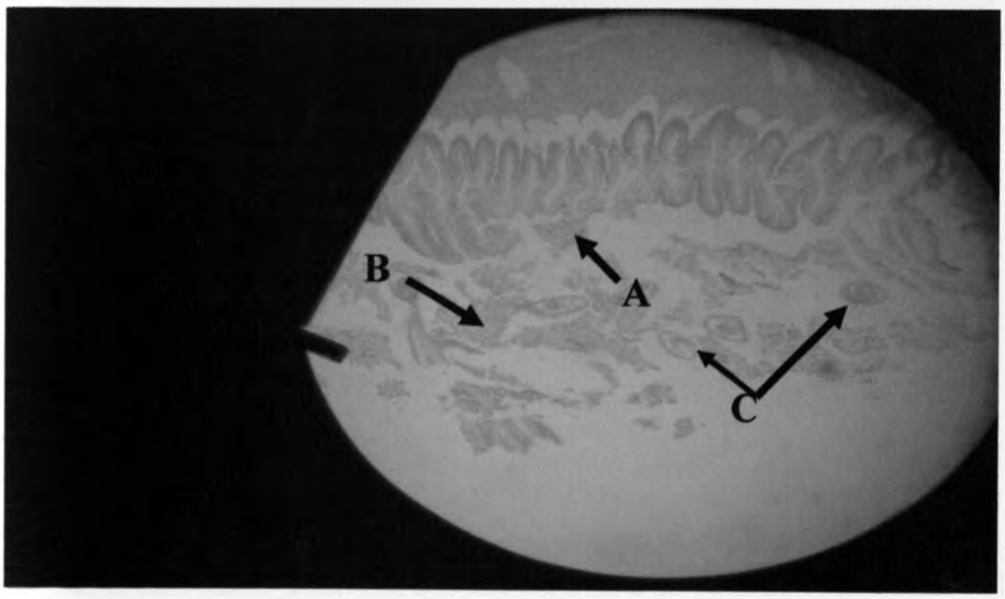


Figure 29: Desquamated villi (A), luminal exudates (B) and helminth sections (C) in a catfish stomach (H & E; X 20)

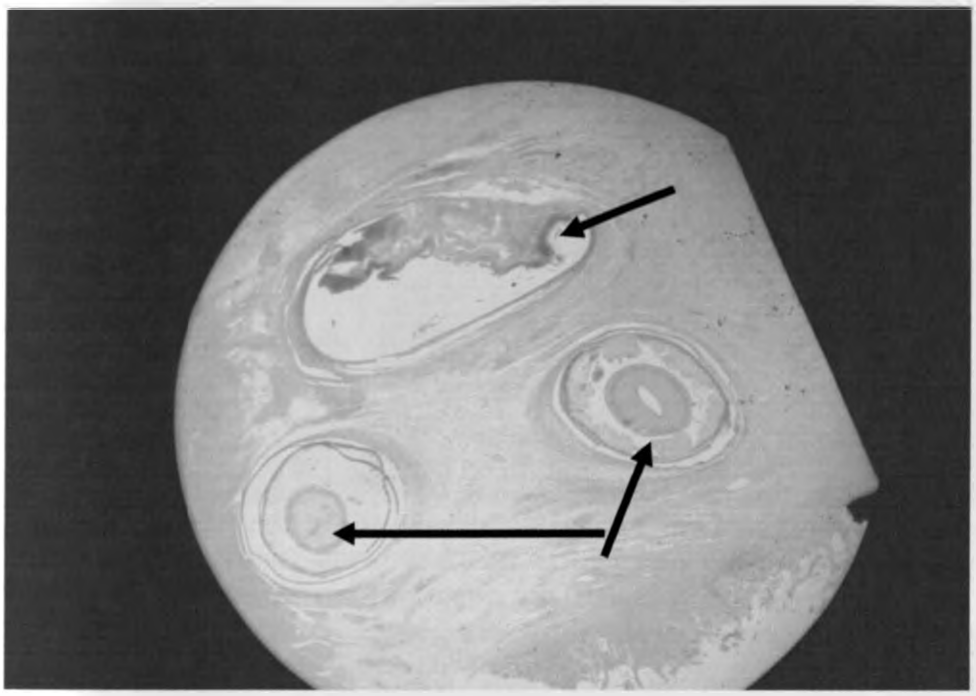


Figure 30: Lamina muscularis of a catfish stomach with cross section of the *Contracaecum* 3rd stage larvae (arrows) in a fibrous capsule (H & E; X 20)

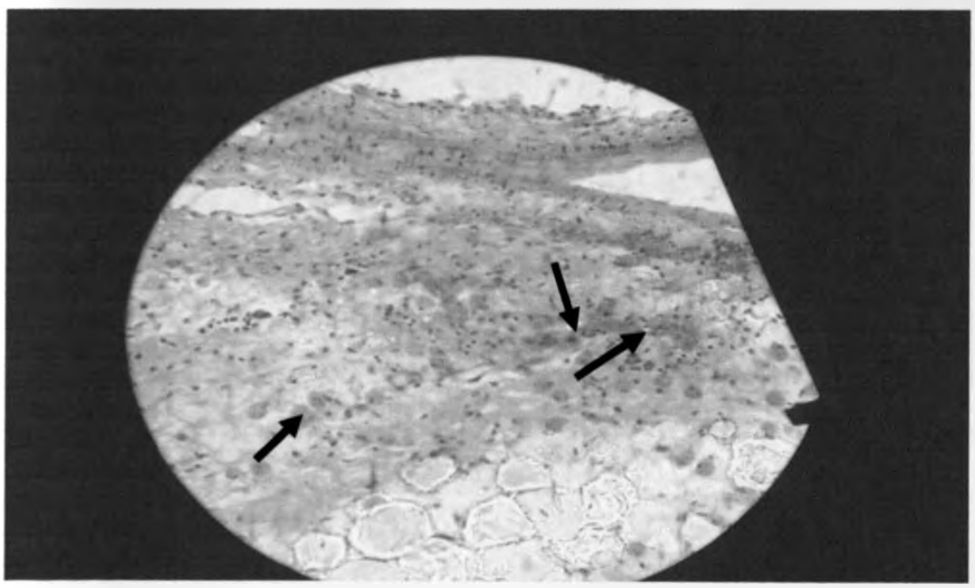


Figure 31: Lamina propria of a tilapia intestine with infiltration of eosinophillic granulocytes (arrows) X 40

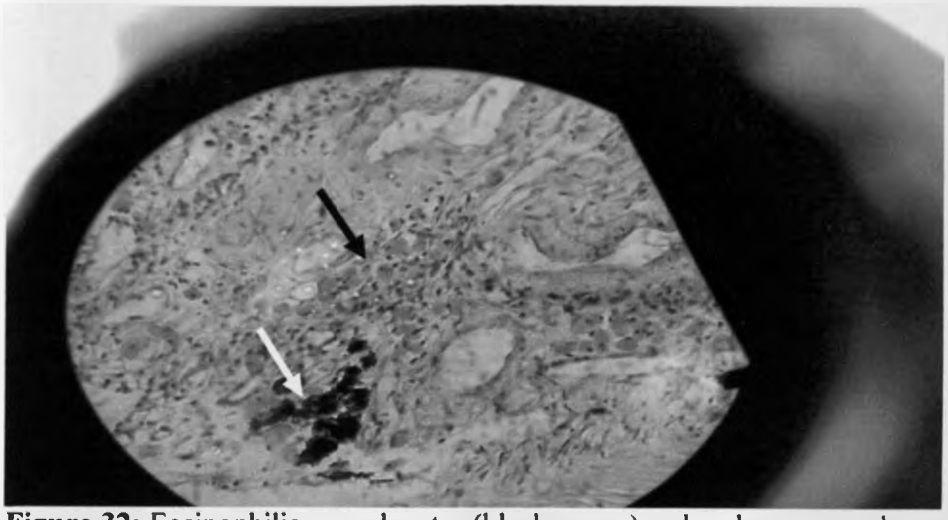


Figure 32: Eosinophilic granulocytes (black arrow) and melanomacrophages (white arrow) and other inflammatory cell infiltration into the liver parenchymaof a tilapia fish (H & E; X 20)

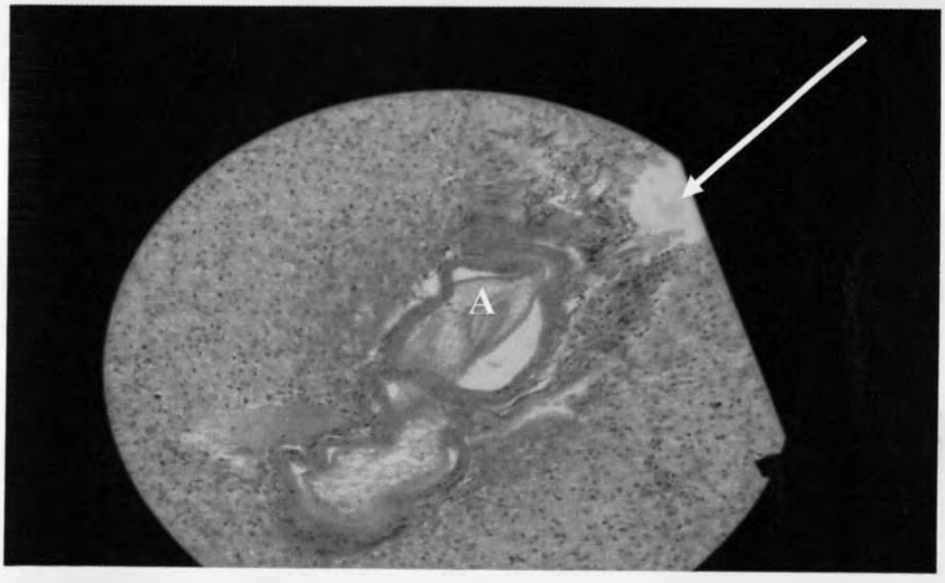


Figure 33: A tilapia liver with a section of a helminth (A) on its migration tracts (arrow) (H & E; X 40)

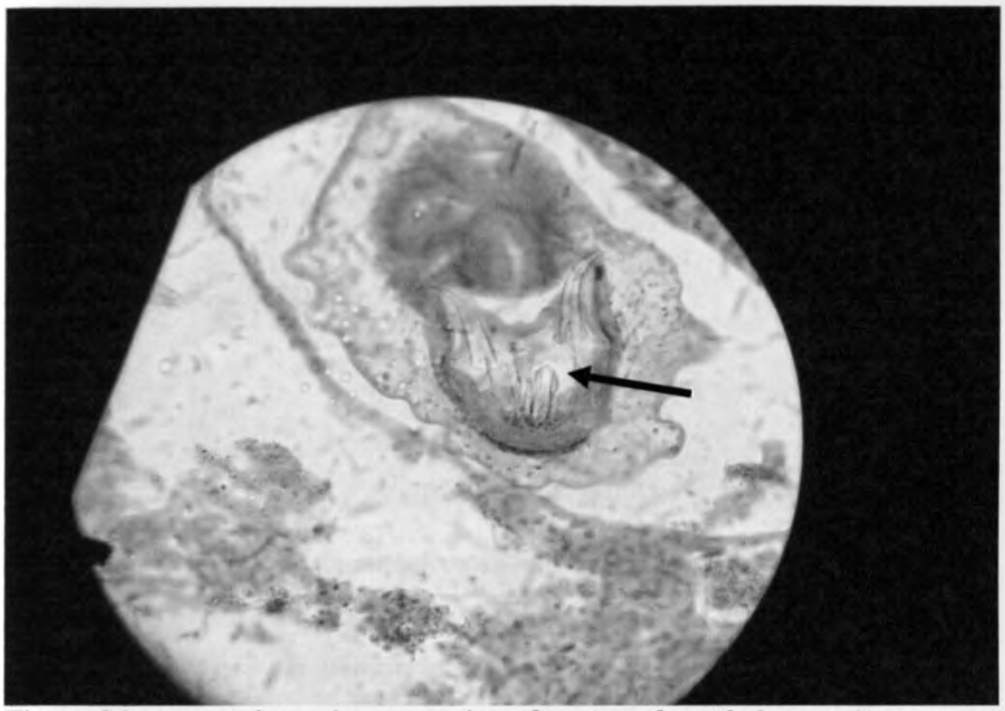


Figure 34: An anterior end cross section of an *Acanthocephalus* spp. in a tilapia bile duct showing the hooks with accompanying epithelial atrophy (arrow) (H & E; X 40)

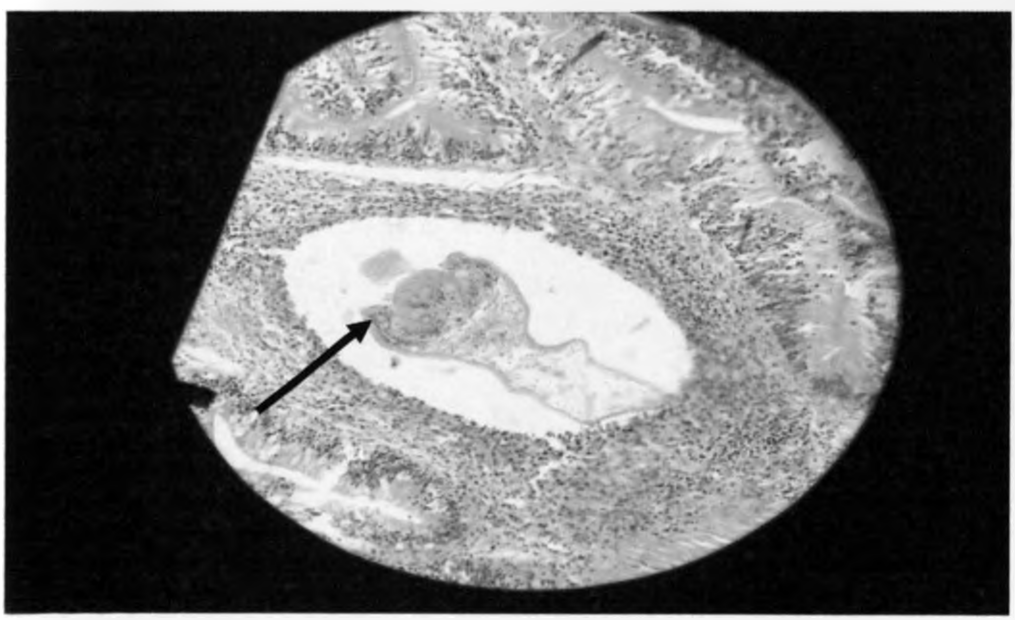


Figure 35: A section of *Acanthocephalus* spp. (arrow) within a hepatic blood vessel in a tilapia fish (H & E; X 40)

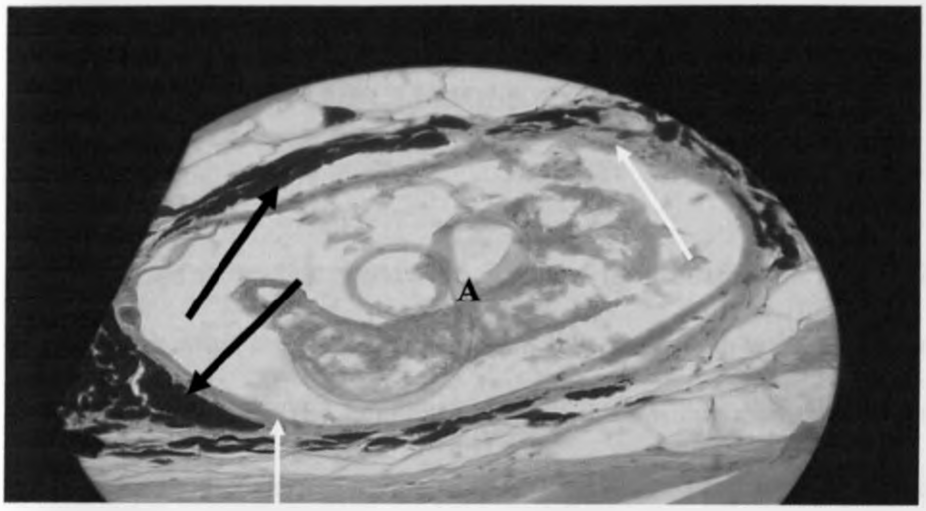


Figure 36: A *Neascus* spp. metacercariae (A) in the dermis of a tilapia encapsulated by double wall of inner fibrous tissue (white arrows) and melano-macrophages (black arrows) (H & E; X 40)

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

This study has shown that market fish, wild and farmed fish from Tana River basin in Kenya are often infected with helminths that cause both gross and microscopic pathological changes. The helminths recovered from this study were nematodes: *Contracaecum* and *Paracamallanus* spp.; trematodes (digeneans): *Diplostomum*, *Clinostomum*, *Neascus* spp.; cestodes: *Proteocephalus* and Caryophyllaeidea spp.; Acanthocephalans: *Acanthocephalus* and Cryptocotyle-like spp.

Overall, helminth prevalence was 65.8 % (n = 237) for the field study fish while the market fish had a prevalence of 50 % (n = 43). The difference could be due to the less fish with trematodes that were possibly rejected before reaching the market on aesthetic reasons, though this was not confirmed. Ectoparasites were also prevalent (7.8 %) in the field fish, with *Piscinoodinum* and *Trichodina* spp. being most prevalent. Most ectoparasites could have died off or lost in handling during transportation to sampling sites. There was no significant difference observed in the mean prevalence of total helminth infestation between tilapia (68. 4%) and catfish (61.2 %) examined from the field study, thus suggesting that helminth are a general problem in the study area.

Nematodes comprised the larger percentage of helminths recovered at a prevalence of 30.3 % and 45.3 % in the market and field study, respectively. Trematodes (digenean) parasites (41.5 %) and tapeworms (4.6 %) in the field and market

studies, respectively, were the next prevalent helminths. This is similar to other previous findings of helminth infections in Africa by other works (Douellou, 1992 a, b; Barson and Avenant-Oldewage, 2006). The nematodes were more prevalent in wild fish compared to farmed ones, while the digeneans were more prevalent in the farmed fish. This high prevalence of the nematodes in the wild may be attributed to the high abundance of the definitive piscivorous birds in the wild, especially around Masinga dam. In the farmed fish, management practices such as proper stocking, proper environmental conditions, reduced pollution and enhanced predator control may have contributed to the lower incidence of nematodes. The high prevalence of digenean helminths in the farmed fish may be attributed to the good mesotrophic ponds with a good continuous water exchange, fringed by trailing weeds in the Sagana Fish Breeding farm which offered good conditions for the propagation of the intermediate hosts, the snails. These conditions may further attract the definitive hosts, the piscivorous birds, (Paperna, 1980).

The two nematodes recovered in this study have been reported elsewhere in African and this country at variable prevalences (Malvestuto and Ogambo-Ongoma, 1978; Boomker, 1982; Aloo, 2002; Adikwa and Ibrahim, 2004; Barson, 2004) in lakes, but have not been reported in aquacultural and riverine systems in Kenya. In addition, Zekarias and Yimer (2008), in their study at Lake Awassa in Ethiopia, found the nematode *Eustrongylides* spp., which was absent in this study. *Contracaecum* spp. was more prevalent in both tilapia and catfish at 33.8 %, while *Paracamallanus* spp. was recorded in catfish only at a prevalence of 52.8 %.

Contracaecum spp. as in most of the Anisakidae family are intermediate host unspecific. Similar results and sometimes with even higher infections were recorded in other fish species in Colombian and Brazilian marshes and rivers (Maurício et *al.,* 2003; Olivero - Verbel *et al.* 2006). In Kenya, Aloo, (2002) recorded the *Contracaecum* spp. in *Oreochomis leucocystis* and *Tilapia zilli* from Lake Naivasha and Oloiden bay while Barson (2004) reported the occurrence of the 3rd stage *Contracaecum* species in catfish (*Clarius gariepinus*) from Lake Chivero, Zimbabwe. However, there are no previous records of its occurrence in River Tana and farmed fish in Kenya. Neither has it been reported in catfish in Kenya.

In the field study, *Contracaecum* spp. infection in catfish (59.2 %) was higher compared to tilapia (12.4 %). Branchial area infection in tilapia was higher (9.3 %) than abdominal cavity (3.1 %), whereas in catfish abdominal cavity infection was higher (55.6 %) than branchial (3.6 %). There could be a predilection difference in the two fish under the study. Comparison of abdominal and branchial *Contracaecum* spp. infestation has not been done before. These findings suggest that *Contracaecum* spp. predilection site in tilapia is the branchial area whereas in catfish, it is in the abdominal cavity. The fish immune systems may be involved in the arrest of their migration forming fibrous capsules around them. These findings are like those by Paperna (1974), Malvestuto and Ogambo - Ongoma (1978) and Barson and Avenant-Oldewage (2006) in overall prevalences in tilapia and catfish; however, these authors did not compare *Contracaecum* spp. infection prevalences

between catfish and tilapia; branchial and abdominal infectivity and intensities as in this study. The overall mean intensity of the *Contracaecum* spp. at 103.4 with a range of 1– 846 in all fish, tilapia 3.4 (1- 15 worms) and catfish 128.3 (1 – 846) worms per fish was much higher than that reported by Barson and Avenant – Oldewage (2006) who got a mean intensity of 16.3 (range 3–44) in catfish from Rietvlei dam even though prevalences and intensities of up to 100 % and 700 – 2000 worms per fish have been reported elsewhere (Mashego and Saayman, 1981; Boomker, 1982).

Prevalences of *Contracaecum* spp. in wild tilapia (11.6 %) and catfish (40.7 %) were higher than those in farmed fish at 0.8 % and 18.5 %, respectively. This may be attributed to the high abundance of the definitive piscivorous birds in the wild compared to the farms where the use of repulsive and deterrence methods of shotguns, slings and scare-crows was observed. Alternatively, it may be a parasite of wild fish more than farmed fish. The prevalence and intensities in catfish of *Contracaecum* spp. infection was higher than in tilapia. This could be a parasite of catfish more than tilapia. However, tilapia may have a low prevalence due to the fact that the fish are more of herbivores and are more likely to get the infection directly from the environment than the catfish which are voracious omnivorous that will feed on all intermediate hosts including other smaller fish including their young ones, who may be infested, thus accumulating the worms (Malvestuto and Ogambo-Ongoma, 1978).

There was a higher prevalence and intensity in adult than young tilapia and catfish may be due to the accumulation of the worms over a longer period and their larger size which tend to be too big for the piscivorous bird, which will feed on small and medium sized fish (Gichohi *et al.,* 2008; Zekarias and Yimer, 2008). However, a study in Insukamini Dam, Zimbabwe, did not observe any *Contracaecum* spp. in catfish but they were there in *Oreochromi mossambicus* and *Serranochromis robustus* at prevalences of 11.1 % and 40 %, respectively (Moyo and Yalala, 2009). They did not however compare the prevalence in the age and sex groups of fish. The male fish in catfish had higher prevalence and intensity compared to the female ones. Maybe the male fish are more aggressive and tend to spend more time feeding than the females, thus collecting more helminths from the water enviroment. There were no significant differences in the prevalence and intensities between the sex in both fish species.

This study documents for the first time *Paracamallanus* spp. in catfish from River Tana basin, Kenya. These helminths were isolated in catfish only and mainly from the intestines with a few from the stomach and are generally parasites of the gastrointestinal tract as reported by FAO (1996 b) and Ayanda (2009). The *Paracamallanus* spp. prevalence in catfish this study was 52.8 % while mean intensity was 6.1 worms per fish (range 1– 41), while Boomker (1982) reported 53.5 % in South African fresh water fish which was much higher than that recorded by Ayanda, (2009) in catfish of 5.625 % in Nigeria. There has been no other such study done in Kenya. Adikwa and Ibrahim (2004) recorded two

camallanid nematodes (*Paracamallanus* and *Procamallanus* spp.) in catfish with a prevalence of 40.9 % in Kano, while Akinsanya and Otubanyo (2006) recorded low prevalence of *Paracamallanus* spp. in Lekki lagoon in Lagos Nigeria, suggesting that this nematode is a fresh and brackish water parasite in Nigeria. In this study *Procamallanus* spp. were not found. Akinsanya *et al.* (2007), also found *Paracamallanus* spp. in African silver catfish (*Chrysichthys nigrodigitatus*), suggesting that the helminth may be genus specific in catfish but species unspecific. Infection in the wild catfish (37 %) was higher than in farmed (17 %) which maybe due to the better availability of the copepod intermediate host. Ayanda (2009), in his study of farmed and wild catfish in Nigeria, recovered camallanids (5.625% for *Paracamallanus* spp. and 0.625% for *Procamallanus laevionchus*) in only wild catfish. Like in studies with silver catfish, elsewhere there were no significant differences in prevalence and intensities of *Paracamallanus* spp. infection in *Clarias gariepinus* between the sex, but there was significant difference in infestation in the silver catfish between the length and the weights (Akinsanya *et al.*, 2007), which were not measured in this study.

Three species of digenetic trematodes were recorded in this study. These were *Diplostomum*, *Clinostomum* and *Neascus* spp. *Diplostomum* spp. overall prevalence was 35.4 % with a mean intensity of 5.2 metacercariae per fish and a range of 1 – 26. Prevalence in tilapia was higher at 54.3 % with a mean intensity of 2.4 worms per fish and a range of 1 – 26, while catfish had a prevalence of 18 % and mean intensity of 1.8 worms and range of 1 – 5 per fish. This was in

agreement with the findings by Violante-González *et al.* (2009) in Mexico in tilapia. Otachi (2009) working with farmed open and caged pond tilapia in Central and Eastern Kenya isolated mainly free living diplomastid; *Tylodelphis* spp. in the vitreous humour of fish at prevalences of 47.25 % / 41.58 % (mean intensity – 7/4) in open/caged pond and 25.68 % (mean intensity – 13.5) tilapia in Machakos and Sagana. He did not compare the Diplostomastid infection in tilapia and catfish, neither did he examine the fish in the wild. Only farmed tilapia and catfish had the parasite indicating that the intermediate host, the common snails (*Bulinus truncates*, *Lymnnae* and *Melanoides tuberculata*), were in abundance in the farms as compared to the riverine system. Studies undertaken elsewhere have shown that prevalence of *Diplostomum* spp. is directly related to the density of the *Lymnnae* spp. intermediate hosts (Voutilainen *et al.*, 2008). In addition, *Diplostomum* spp. metacercariae eye infections have been found to increase the vulnerability of the affected fish to predators by changing their behavior and by coming much often to the surface, where they become easy targets for the predators (Seppälä *et al.*, 2004).

Due to control of predators in fish farms in contrast to the wild, *Diplostomum* spp. infested fish will tend to survive more compared to the wild where they will certainly be predated by the piscivorous birds. Otachi (2009), attributes the differences in prevalences to varying water temperature and caging. In addition, the increased stocking density in fish farms compared to the wild may enhance the infestation rates in those fish (Karvonen *et al.*, 2005; Violante-González *et al.*,

2009). Significant differences in the infestation between the ages which has not been compared elsewhere may be attributed to longer exposure period of the adults.

Neascus and *Clinostomum* spp. were isolated in only farmed tilapia at prevalence of 17.8 % (N = 129) and 14.7 % (N = 129), respectively, but not in catfish. The infection in the farmed tilapia like in *Diplostomum* spp. infection was probably due to the abundance of the intermediate hosts, the common snails and the increased stocking density. Significant differences were observed in infection between the farmed catfish and tilapia as the parasites seem to prefer the tilapia fish where it may encyst under the scales. Fish digeneans are intermediate host specific, their occurrence is dependant on the availability of particular snail species in the particular waters. They have thus been suggested as possible biological monitors of fish migration and water quality (Overstreet and Howse, 1977; Mackenzie, 1983; Lester, 1990).

The predilection site of *Clinostomum* spp. in this study was the skin, while Zekarias and Yimer (2008) in Awassa dam in the Ethiopia observed *Clinostomum* spp. in branchial cavity in tilapia, suggesting that these were different species. In addition they also found *Euclinostomum* spp. in the branchial cavity, liver, kidney and gall bladder and an unidentified large digenean spp. in between the teeth and cranium in tilapia and a digenean metacercaria in brain tissue in *Clarias gariepinus.* These digeneans were not found in this study. Dayhoum (2003), in Egypt observed *Clinostomum tilapiae* parasitizing fish in very high prevalences in

the viscera, while Aloo (2002) found *Clinostomum* spp. on the skin of tilapia fish in Lake Naivasha and Oloiden bay in Kenya. FAO (1996 b) and Ukoli (1969) suggest transcontinental distribution of metacercariae infestation of *Neascus, Clinostomum, Euclinostomum, Centorcestus, Phagicola and Haplorchis* spp. following the migration pattern of the definitive hosts, the piscivorous birds.

In this study two species of tapeworms *(Proteocephalus and Caryophyllaeidea spp.)* were recovered. *Proteocephalus* spp. was more prevalent (2.5 %) than *Caryophyllaeidea* spp. (0.4 %). Gichohi *et al.* (2008) reported the occurrence of *Proteocephalus* spp. in market catfish at 9.1 % while Aloo (2002) reported the occurrence of *Armirthalingamia* and *Cyclustera* spp. in tilapia in Lake Naivasha and Oliden bay. Ayanda (2009) reported prevalences of 1.3% for *Amonotaenia* spp. and 3.8% for *Polyonchobothrium clarias in Clarias gariepinus* in Ilorin, North – Central Nigeria. Adikwa and Ibrahim (2004), reported occurrence of *Polyonchobothrium* spp. and *Monobothroids* spp. in *C. gariepinus* in Kano, Nigeria. Dayhoum (2003) reported the occurrence of cestodes at 4 % prevalence in *Clarias* spp. and *Tilapia* spp. in varying prevalences in different inland waters in Egypt. Mean intensity was 0.1 tapeworms per fish, with range of 1 – 4, while Moyo *et al.* (2009) reported prevalences of 60 % *Bothriocephalus acheilognathi* (mean intensity – 5), 80% *Polyonchobothrium clarias* (mean intensity – 5), and 80 % *Proteocephalus glanduliger* (*mean* intensity – 1) in *Claras gariepinus* and no cestode in *Oreochromis macrochir, Oreochromis mossambicus* and

Serranochromis robustus in Insukamini Dam Zimbabwe, which was much higher than in this study.

Acanthocephalus spp. was observed in tilapia (*Oreochromis nilotica*) fish at a prevalence of 25.6 %, with a mean intensity of 5.4 and a range of 1 – 27 worms per fish. This is unlike previous findings of 63 % (range of 0 – 100) prevalence recorded in *Ancaththogyrus tilapiae* infection in Lake Kariba (Doellou, 1992 a; b), *Neoechinorrhynchus rutili* infestation seen in two tilapia fish species in Egypt of 62.9 %, 71.4 % (Dayhoum, 2003). Aloo and Dezfuli (1997) reported prevalences of 30.4 % to 86 % of cystacanths *Polyacanthorhyncus kenyensis* infestation in *Oreochomis leucostiticus* in Lake Naivasha. Riverine and aquaculture set-up appear to have low prevalences. In the Sudan Nile, Khalil (1969) reported abundant *Tenuisentis niloticus* infestation of 93 % (range of 5 – 27), in *Heterotis niloticus,* 26 % *Neoechinorrhynchus* spp. (6 – 43) in *Citharus citharus* and 60 % unidentified acanthocephalan (2 – 5) in *Synodontis batensoda,* whose intensities are close to those in this study. There were no significant differences in the prevalences between the farmed and wild tilapia (*Oreochromis nilotica*) and between the sex .However a significant difference was observed in the age groups ($P > 0.05$). This is probably because the intermediate hosts, the amphipod; copepods, isopods, and ostracods maybe well distributed in these waters.

Ectoparasites showed a low overall prevalence in all fish examined, with *Piscinoodinum* spp. and *Trichodina* spp. being the most prevalent. Ubiquitous ectoprotozoan parasites are cosmopolitan due to transcontinental dispersion

through cultured tilapia and carp and have been implicated in mass mortalities of tropical marine and fresh water fish (Paperna, 1980). The higher infection in farmed fish may be attributed to the higher stocking density of fish in the farm thus aiding in spread through contact and can take the infection to wild fish.

Gross and microscopic lessons associated with the helminths were recorded in different organs and tissues of apparently healthy looking fish in this study. Grossly, there was haemorrhages of the abdominal wall muscles in the *Contracaecum* spp. infested fish, some of these lesions were similar to those reported by Mbahinzireki (1980), FAO (1996 b) and Elarafi (1991, 1992) in various fish species, however they did not describe severity of the lesions. The microscopic examination of the stomach infested with *Contracaecum* and *Paracamallanus* spp. showed desquamation of the epithelium, proliferation of goblet cells and pinkish exudates in the lumen. Cellular reaction involved infiltration of eosinophils, lymphocytes, fibrocytes and macrophages in the lamina propria. Some worm sections encased in fibrous capsule in the lamina muscularis were also observed. El-Din *et al.* (2009) reported secretion of mucus on mucosal epithelium, hyperplasia of goblet cells, cellular infiltration, degeneration of connective tissue and formation of compact connective tissue around the *Procamallanus laevionichus* in *Clarias gariepinus* in Egypt. The lesions in this study were mild to severe and would most probably affect the growth of fish. Though most authors declare that there is no clear observable effect of the *Contracaecum* spp. infestation in fish, no studies have been undertaken to prove

this, by comparing growth rates of infested and non infested fish of same age and size.

The intestines of the catfish which had the nematodes *Paracamallanus* and *Contracaecum* spp and cestodes *Proteocephalus* and *Caryophyllaeidea* spp showed villi desquamation, necrosis and flattening of the columnar epithelium. Pinkish exudate in the intestinal lumen, with infiltration of heterophils, lymphocytes and eosinophilic granulocytes into the lamina propria was also noted. These lesions will most probably affect food absorption efficiency, and subsequent reduction in growth rates. Some parasite sections were also observed in the lamina muscularis of the intestines, with accompanying proliferation of fibroblasts and other mononuclear inflammatory cells. There is very little information from previous studies on pathology of tissue dwelling nematodes as shown in this study, with those reported so far being of other nematode and fish species by Meguid and Eure (1996), Heupel and Bennet (1998), Khatoon and Bilqees (1996), Ramallo *et al.* (2000), Moravec *et al.* (2003), Menezes *et al.* (2006) and El Din *et al.* (2009). Similar lesions were also observed in tilapia fish infected with *Acanthocephalus spp.* and *Proteocephalus* spp. In one fish case, nodular granulomatous lesions of the intestines contained the parasite lesions similar to those observed by Wabuke-Bunoti (1980) in the intestines and liver of *Clarias* spp infested with *Polyonchobothrium clarias* in Lake Victoria. Though cestode intensities observed was low in this study, the intestines of infected fish may become plugged by the worms and in some instances get perforated as observed elsewhere by Hoffman

(1980). In the tilapia livers, sections of the *Acanthocephalus* spp. in the bile ducts, portal veins and parenchyma caused pressure atrophy of the epithelial lining the vessels, cholestasis, congestion and necrosis of hepatocytes. There was also infiltration of eosinophils, heterophils, lymphocytes and melanomacrophages. The lesions observed showed that though grossly, the fish may look unaffected, microscopic lesions due to parasitism occur and these will most probably lead to sub-clinical losses, which cause more economic losses than clinical parasitism. Though these lesions are similar to those associated with nematode and cestode infections of other fish as described by Dick and Choundry (1995) and FAO (1996 b), this study has in addition described and documented host fish cellular response to these helminths.

Overall, an association between opacity and *Diplostomum* spp. eye infection was demonstrated. The eye opacity was either mono or bilateral in occurrence. *Diplostomiasis* in young fish may cause severe mortalities especially during the migration phase to the eyes, since they cause serious damages (Overstreet and Curran, 2004). In skins examined, tilapia fish had gross lesions of white and black cysts and hemorrhages attributed to injury during capture. *Clinostomum* spp. (white spots) seen as unsightly cysts caused pressure atrophy in contrast to the finding by Coulibay *et al.* (1995), who did not look at the microscopic lesions. The double walled metacercariae cysts are produced by the epithelia of the fish in reaction to infection. This may also cause inrritation on the skin and excess mucus secretions leading to an inflammatory response at the site of attachment (Adeyemo

and Agbede, 2008). Microscopically, infiltration of eosinophilic granulocytes into the dermis seen in this study was similar to that seen by Garcia *et al.* (1993) in *Clinostomum complanatum* infection on the skin of tilapia species.

The *Neascus* spp. cysts, some of which were in the muscles, were fibro-capsules infiltrated by melanomacrophages. Microscopic lesions observed were proliferation of goblet cells, infiltration of eosinophilic granulocytes and melanomacrophages. The lesions observed were similar to those described by Hoffman (1960) and Ukoli (1969). Spleen reaction of the proliferation of melanomacrophage centres seemed to be a typical reaction of the chronic fish infections or parasitim (Ferguson, 1989).

These parasites and the lesions they cause in fish will most probably affect the health of the fish in addition to causing economic loss to the farmer or fishermen due to depressed growth, mortality and rejection in the market of the affected fish on aesthetic reasons. Maria *et al.* (2005), has related prevalence of some parasites to the health of *Oreochromis niloticus* by changes observed in the hematocrit and differentiated leucocyte counts in Brazil, this was not done in this study.

5.2 CONCLUSIONS

1. Helminths and ectoparasites were prevalent in tilapia and catfish in the Upper River Tana Basin.
2. The trematode, *Diplostomum* spp. was the most prevalent helminth occurring in the eyes of fish causing opacity.

3. The nematode *Paracamallanus* spp. was found in farmed and wild catfish, in Kenya for the first time.
4. *Acanthocephalus* spp. was found in farmed and wild tilapia in the River Tana basin for the first time.
5. Prevalence and intensity of helminths in farmed tilapia was higher than in the wild tilapia.
6. Adults of both tilapia and catfish species had more parasites than the young fish.
7. The fish helminths recovered caused gross and microscopic lesions in affected fish, subclinical infections, which may lead to economic loss especially to aquaculture farms.

5.3 RECOMMENDATIONS

1. More research on seasonal prevalence and pathology of endo and ecto parasites of fish should be undertaken.
2. A detailed study on the impact of helminth infestations on production of fish and probable intervention measures is recommended.

CHAPTER SIX

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CHAPTER SEVEN

APPENDICES

Appendix 1: Types of helminths and overall prevalence in fish examined in the market survey

Fish species (n = 43)	Helminths					
	Contracaecum spp.		Cestode		Trematodes	
	No infested	Prevalence (%)	No infested	Prevalence (%)	No infested	Prevalence (%)
Tilapia	3	7 %	1	2.3	1	2.3
Catfish	10	23.3	1	2.3	0	0
Common carp	0	0	0	0	0	0
Barbus	0	0	0	0	0	0
Total	**13**	**30.3**	**2**	**4.6**	**1**	**2.3**

Key

No. – number infested

% - percentage

Appendix 2: Types of helminths, their location in the fish body and overall prevalence in examined fish (N = 237)

Helminth species Observed	Predilection site	Prevalence	Overall percentage Prevalence	P value
Diplostomum	Vitreous humour	35.4	35.4	
Contracaecum	Branchial area	6.8	33.8	
	Peritoneal cavity	27.0		
Paracamallanus	intestines	23.2	24.0	
	Stomach	0.8		
Acanthocephala	Intestines	13.9	13.9	
Neascus	Skin/muscle	9.7	9.7	
Clinostomum	Skin/muscle	8.0	8.0	
Proteocephalus	Intestines	2.5	2.5	
Caryophyllaeidea	Intestines	0.4	0.4	

Appendix 3: Types of helminths recovered, number of fish infested and their prevalence in tilapia and catfish

Helminth Species	Tilapia (N = 129)	Catfish (N = 108)
	Prevalence (%)	Prevalence (%)
Contracaecum	12.4	59.3
Paracamallanus	0	52.8
Diplostomum	54.3	16.7
Clinostomum	14.7	0
Neascus	17.8	0
Proteocephalus	0.8	5.2
Caryophyllaeidea	0	0.9
Acanthocephalus	25.6	0

Appendix 4: *Contracaecum* species infection in tilapia fish from the field study (N = 129)

Factor	Variable	Number of fish examined	Number infected	Overall prevalence (%)	Prevalence (%)	P value
Type	Farmed	85	1	0.8	1.2	<0
	Wild	44	15	11.6	34.1	
Sex	Male	54	10	7.8	18.5	0.129
	Female	75	6	4.7	8.0	
Age	Young	57	7	5.4	12.3	0.8173
	Adults	72	9	7.0	12.5	

Appendix 5: *Contracaecum* species infection in catfish from the field study (N = 108)

Factor	Variable	Number of fish examined	Number infected	Overall prevalence (%)	Prevalence (%)	P value
Type	Farmed	63	20	18.5	31.7	<0
	Wild	45	44	40.7	97.8	
Sex	Male	51	35	32.4	68.6	0.0467
	Female	57	29	26.9	50.9	
Age	Young	48	24	22.2	50.0	0.1311
	Adults	60	40	37.0	66.7	

Appendix 6: *Paracamallanus* species infection in catfish from the field study (N = 108)

Factor	Variable	Number of fish examined	Number Infected	Overall prevalence (%)	Prevalence (%)	P value
Type	Farmed	63	17	15.7	27.0	<0
	Wild	45	40	37.0	88.9	
Sex	Male	51	28	25.9	54.9	0.822
	Female	57	29	26.8	50.9	
Age	Young	48	20	18.5	41.7	0.0608
	Adults	60	37	34.3	61.7	

P < 0.05 – Significantly different

Appendix 7: *Diplostomum* species infection in tilapia from the field study (N = 129)

Factor	Variable	Number of fish examined	Number infected	Overall prevalence (%)	Prevalence (%)	P value
Type	Farmed	85	70	54.2	82.4	- 0
	Wild	44	70	0	0	
Sex	Male	54	35	27.1	64.8	0.0001
	Female	75	35	27.1	46.7	
Age	Young	57	24	18.6	42.1	0.0221
	Adults	72	46	35.7	63.9	

Appendix 8: *Diplostomum* species infection in catfish from the field study (N = 108)

Factor	Variable	Number of fish examined	Number infected	Overall prevalence (%)	Prevalence (%)	P value
Type	Farmed	63	18	16.7	28.6	- 0
	Wild	45	0	0	0	
Sex	Male	51	8.0	7.4	15.7	0.9992
	Female	57	10	9.3	17.5	
Age	Young	48	5.0	4.6	10.4	0.1939
	Adults	60	13	12.1	21.7	

Appendix 9: *Clinostomum* species infection in tilapia from the field study (N = 129)

Factor	Variable	Number of fish examined	Number infected	Overall prevalence (%)	Prevalence (%)	P value
Type	Farmed	85	19	14.7	22.4	0.0009
	Wild	44	0	0	0	
Sex	Male	54	11	8.5	20.4	0.1998
	Female	75	8	6.2	10.7	
Age	Young	57	6	4.7	10.5	0.1716
	Adults	72	13	10.1	18.1	

Key
% - percent
P < 0.05 – Significantly different

Appendix 10: *Neascus* species infection in tilapia from the field study (N = 129)

Factor	Variable	Number of fish examined	Number Infected	Overall prevalence (%)	Prevalence (%)	P value
Type	Farmed	85	23	17.8	27.1	0.0004
	Wild	44	0	0	0	
Sex	Male	54	10	7.8	18.5	0.953
	Female	75	13	10.1	17.3	
Age	Young	57	9	7.0	15.8	0.7596
	Adults	72	14	10.9	19.4	

Appendix 11: *Proteocephalus* species infection in tilapia from the field study (N = 129)

Factor	Variable	Number of fish examined	Number Infected	Overall prevalence (%)	Prevalence (%)	P value
Type	Farmed	85	1	0.8	1.2	0.7384
	Wild	44	0	0	0	
Sex	Male	54	1	0.8	1.9	0.8697
	Female	75	0	0	0	
Age	Young	57	0	0	0	0.9084
	Adults	72	1	0.8	1.4	

Appendix 12: *Proteocephalus* species infection in catfish from the field study (N = 108)

Factor	Variable	Number of fish examined	Number Infected	Overall prevalence (%)	Prevalence (%)	P value
Type	Farmed	63	0	0	0	0.8658
	Wild	45	5	4.6	11.1	
Sex	Male	51	5	4.6	9.8	0.9537
	Female	57	0	0	0	
Age	Young	48	0	0	0	0.1127
	Adults	60	5	4.6	8.3	

Key
% - percent
P < 0.05 – Significantly different

Appendix 13: *Caryophyllaeidea* species infection in catfish from the field study (N = 108)

Factor	Variable	Number of fish examined	Number Infected	Overall prevalence (%)	Prevalence	P value
Type	Farmed	63	0	0	0	0.8658
	Wild	45	1	0.9	2.2	
Sex	Male	51	0	0	0	0.9537
	Female	57	1	0.9	1.8	
Age	Young	48	1	0.9	2.1	0.9116
	Adults	60	0	0	0	

Appendix 14: *Acanthocephalus* species infection in tilapia from the field study (N = 129)

Factor	Variable	Number of fish examined	Number Infected	Overall prevalence (%)	Prevalence (%)	P value
Type	Farmed	85	20	15.5	23.5	0.5961
	Wild	44	13	10.1	29.5	
Sex	Male	54	17	13.2	31.5	0.2718
	Female	75	16	12.4	21.3	
Age	Young	57	7	5.4	12.3	0.004
	Adults	72	26	20.2	36.1	

Appendix 15: Ectoparasites infection in the tilapia and catfish from the field study

Ectoparasite spp.	Tilapia (N = 129)		Catfish (N = 108)		P value
	number infested	Prevalence (%)	number infested	Prevalence (%)	
Piscinoodinum	36	27.9	24	22.2	0.3937
Trichodina	31	24.1	13	12	0.028
Ichthyophthirius	3	2.3	2	1.9	0.3101
Ambyphrya	3	2.3	0	0	0.3101
Lamproglena	9	7.0	0	0	0.0139
Aquarium mites	5	3.9	1	1.9	0.3059
Ichthyobodo	0	0	3	2.8	0.1858

Key

% - percentage

P < 0.05 – Significantly different

Appendix 16: Gross lesions and their distribution in tilapia and catfish from the field study

Organ	Number of fish with gross lesions (N = 237)	Prevalence (%) (overall)	Distribution of lesions by species			
			Tilapia (N = 129)		Catfish (N = 108)	
			number with lesions	revalence (%)	number with lesions	Prevalence (%)
Skin	124	52.3	77	59.7	47	4.6
Gills	11	4.6	5	3.9	6	5.6
Operculum	27	11.4	16	12.4	11	10.2
Fins	56	23.6	13	10.1	43	39.8
Eyes	113	47.8	88	6.8	25	23.1
Liver	10	4.2	9	7.0	1	0.9
Muscles	55	23.2	2	1.6	53	49.1
Kidneys	4	1.7	4	3.1	0	0
Peritoneum	73	30.8	10	7.8	63	58.3
Testis	1	0.4	1	0.8	0	0
Aborescent organ	5	2.1	0	0	5	4.6
Buccal cavity	1	0.4	0	0	1	0.9
Intestines	1	0.4	1	0.8	0	0

Key
% - percentage

Appendix 17: Gross lesions, number of fish affected and their prevalence in farmed and wild tilapia fish (N = 129) from the field study

			Prevalence (%)		
Gross Lesion	**Tissue**	**Number of fish Affected**	**Wild fish**	**Farmed fish**	**Total**
Hemorrhages	Skin	34	16.3	10.1	26.4
	Operculum	16	4.6	7.8	12.4
	Fins	13	6.1	3.9	10.1
	Eyes	17	13.2	0.0	13.2
	Liver	1	0.0	0.8	0.8
	Muscles	2	1.6	0	1.6
	Testis	1	0.0	0.8	0.8
	peritoneum	3	0.0	2.3	2.3
Parasitic Cysts	Skin	43	4.7	28.7	33.3
	Kidney	1	0.0	1.2	0.8
	Eyes	6	0.0	8.6	8.6
	peritoneum	3	0.0	2.3	2.3
	Intestinal	1	0.0	1.2	0.8
Paleness	Liver	8	0.8	5.4	6.2
	Gills	5	0.9	3.1	3.9
	Kidney	3	0.0	2.3	2.3
Exophthalmia	Eyes	36	5.4	22.5	27.9
Congestion	Eyes	35	1.5	25.6	27.1
Adhesions	peritoneum	4	3.1	0.0	3.1

Key
% - percentage

Appendix 18: Gross lesions, number of fish affected and their prevalence in farmed and wild catfish fish (N = 108) from the field study

			Prevalence (%)		
Gross Lesions	**Tissue**	**Number affected**	**Wild fish**	**Farmed Fish**	**Total**
Hemorrhages	Skin	47	30.6	13	43.6
	Muscles	53	34.3	14.8	49.1
	Fins	43	24.1	15.7	39.8
	Operculum	11	4.6	6.5	8.5
	gills	2	0.0	1.9	1.9
	Eyes	15	11.6	0.0	11.6
	Buccal-pharyngeal	1	0.0	0.9	0.9
	Aborescent organ	5	4.6	0.0	4.6
Paleness	Gills	4	2.8	0.9	3.7
	Liver	1	0.0	0.9	0.9
Opacity	Eyes	10	3.1	4.7	9.3
Adhesions	peritoneum	63	39.8	18.5	58.3

Key
% - percentage

Appendix 19: Occurrence of microscopic lesions and their prevalence in various organs of tilapia and catfish from field study

Organ	Lesions alone	Lesions with Parasite	Parasite (s) Alone	Without parasite or lesion	Prevalence (%)
Stomach	49	15	6	167	25.7
Intestines	32	67	34	104	41.8
Liver	14	78	7	138	38.8
Gills	33	20	31	153	22.4
Spleen	52	0	0	185	21.9
Heart	13	2	12	111	6.3
Skin	12	43	16	166	23.2
Muscles	7	4	0	226	4.6
Kidneys	37	0	0	200	15.6
Brain	44	2	0	191	19.4
Testis	4	0	0	233	1.7
Ovaries	0	3	0	234	1.3
Aborescent	2	0	3	230	0.9

Key
% - percentage

Appendix 20: Occurrence of microscopic lesions and their prevalence in various organs of tilapia from field study

Organ	Number with lesions Alone	Number with lesions and with parasite	Number with parasite alone	Number with without parasite or lesion	Prevalence (%)
Stomach	32	3	1	93	27.1
Intestines	24	25	13	67	38.0
Liver	29	27	7	66	43.4
Gills	21	17	26	65	29.5
Spleen	24	0	0	115	18.6
Heart	10	0	11	108	7.8
Skin	25	43	0	61	52.7
Muscles	5	2	0	122	5.4
Kidneys	14	0	0	115	10.9
Testis	4	0	0	125	3.1
Brain	16	1	0	112	13.2

Key
% - percentage

Appendix 21: Occurrence of microscopic lesions and their prevalence in various organs of catfish from field study

Organ	Number with lesions alone	Number with lesions and with parasite	Number with parasite alone	Number without parasite or lesion	Percentage (%) prevalence
Stomach	17	12	5	74	26.9
Intestines	8	42	21	37	46.3
Liver	36	0	0	72	33.3
Gills	12	3	5	88	13.9
Spleen	28	0	0	80	25.9
Heart	3	2	1	102	4.6
Skin	12	0	0	96	11.1
Muscles	2	2	0	104	3.7
Kidneys	23	0	0	85	21.3
Ovaries	0	3	0	105	2.8
Testis	0	0	0	108	0
Brain	28	1	0	79	25.9

Appendix 22: Pearson's Two by two tests of association

a - Stomach – catfish

	Helminth +	Helminth -	Total
Lesion+	12	17	29
Lesion -	5	74	79
Total	17	91	108

$X^2 = 19.65$ with 1 df.

$p < 0.001$

b - Intestines - tilapia

	Helminth +	Helminth -	Total
Lesion+	25	24	49
Lesion -	13	67	80
Total	38	91	129

$X^2 = 17.68$ with 1 df.

$p < 0.001$

c - Intestines -catfish

	Helminth +	Helminth -	Total
Lesion+	42	8	50
Lesion -	21	37	58
Total	63	45	108

$X^2 = 25.23$ with 1 df.
$p < 0.001$

Appendix 22: Pearson's Two by two tests of association (continued)

d - Liver - Tilapia

	Helminth +	Helminth -	Total
Lesion+	27	29	56
Lesion -	7	66	73
Total	34	95	129

$X^2 = 24.36$ with 1 df.

$p < 0.001$

e - Eye opacity (catfish and tilapia combined)

	Helminth +	Helminth -	Total
Lesion+	26	10	36
Lesion -	62	139	201
Total	88	149	237

$X^2 = 22.39$ with 1 df

$p < 0.001$

f – Skin – Tilapia

	Helminth +	Helminth -	Total
Lesion+	43	25	68
Lesion -	5	56	61
Total	43	86	129

$X^2 = 41.69$ with 1 df

$p < 0.001$