

MIDLANDS STATE UNIVERSITY



NEMATODE PARASITES OF THE NILE TILAPIA (*Oreochromis niloticus*, Linnaeus, 1758) AND THE SHARPTOOTH CATFISH (*Clarias gariepinus*, Burchell, 1822) IN LOWER UMGUZA DAM

By

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A dissertation submitted in partial fulfilment of the requirements for the degree:

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Submitted by, R165440N in partial fulfilment of the requirements for the degree of Master of Science in Ecological Resources Management (MERM).

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DEDICATION

To my family and friends

ACKNOWLEDGEMENTS

Firstly, and most importantly, I would love to thank God for the invaluable gift of life.

I would like to express my gratitude to my supervisor, Prof. D. Z Moyo whose profound knowledge, expertise, understanding and patience helped me very much. I would also like to thank my family, especially my siblings, for their long-term support through my entire studies. This work would not have been possible without the priceless help rendered by these people.

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ABSTRACT

The study sought to determine abundance and species of nematode parasites of *Oreochromis tilapicus* and *Clarias gariepinus* in Lower Umguza Dam, in the Gwayi catchment area. Fish samples were collected from fishermen and sent to the laboratory for observation and identification using standard parasitological methods and identification guides. 479 nematode parasites were recovered from 160 fish belonging to 2 species; *Oreochromis tilapicus* and *Clarias gariepinus*. Four nematode genera; *Paracamallunus sp.*, *Eustrongylides sp.*, *Contracaecum sp.*, and some unidentified nematode parasites were recovered. Of the 160 host fish samples collected, 78.1% had nematode infections. 43.8% of the nematode parasites recovered were from Nile tilapia and 56.2% from Sharp-tooth catfish. Parasite abundances differed significantly ($P < 0.05$) between the different nematode genera recovered from both *Oreochromis tilapicus* and *Clarias gariepinus*. The most abundant nematodes were *Paracamallunus sp.* (48.6%). *Eustrongylides sp.* and *Paracamallunus sp.* contributed 18% and 33% respectively. Unidentified parasites constituted 0.4% of the total number of nematode parasites collected. The results revealed a positive correlation ($r = 0.681$) between fish size and nematode parasite loads. Nematode parasites between the two host species were significant ($P < 0.05$). Parasite abundances between the different sampling sites were not statistically significant ($P > 0.05$). The results of the study indicated that the general abundances and levels of infestation were low in Lower Umguza Dam and this could be attributed to the above normal rainfall that was received between December 2016 and February 2017. Further studies on comprehensive fish parasite groups are recommended as the study only looked at nematode parasites.

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CHAPTER 1

INTRODUCTION

Background of the study

The ever increasing human population, coupled with the ever increasing frequencies and elongation of droughts has dramatically amplified the need for food, especially in the tropics. As a result, the need for food production has become a one of the most important worldwide issues of concern (Okechi, 2004). Aquaculture and fisheries have a huge role to play in complementing agriculture as the chief sources of food. This realization, together with the advances in science, this has stimulated research into fish ecology. Studies in fish ecology often include assessments of the species present and causes of fish mortality.

The Lower Umguza dam is one of several dams constructed to provide water to the local communities for agriculture; industry and general house hold use. The dam, situated along Umguza River in the outskirts of Bulawayo, serves a very important role in the livelihoods of many families as a source of food as well as income. Several fishermen capture fish and feed families and sell significant volumes to customers in the city centre. This underlines the importance of fisheries and aquaculture to communities in sub-Saharan Africa. The dam is home to a variety of fish species; however, among the most common fish species are the Nile tilapia (*Oreochromis tilapicus*) and Sharptooth Catfish (*Clarias gariepinus*). As any other fish species, Nile tilapia and Sharptooth Catfish serve as host to various nematode parasites. Several scholars, Sinaré *et al.*, 2016; Abdel-Gaber *et al.*, 2015; Onyedineke, *et al*, 2010; Moyo and Yalala (2009) and Yanong, 2006, have it on record that fish serve as definitive, and even, intermediate hosts in the life cycles of many nematode parasites. Parasites have a significant effect on fish health, growth, behavior, fecundity and mortality and they also regulate host population dynamics and their community structure (Marcogliese, 2004). Parasitic infections significantly increase the risk of the occurrence of secondary infections like fungal, bacterial and viral diseases in fish (Sun, 2012).

No meaningful research work has been done on such dams of substantial importance to local communities. In Zimbabwe, data on the helminth parasites of fish are limited to studies that were conducted in Lake Kariba, Lake Chivero and Harare (Barson, *et al.*, 2010; Barson and Marshall, 2003). Even in Southern Africa, little work has been done in as far as the study of fish parasites is concerned, with some regions having no records at all. Information on the roles, functions, and general biology of parasites help to characterize and adequately describe an ecosystem. In-depth knowledge of parasites and parasitic communities also allows researchers to recognize the role of the fish host in the food web or ecosystem (Iwanowicz, 2011).

Apart from the apparent implications to fish health and fisheries, studies into nematode parasites infecting fish could have a huge role to play in our understanding of the ecosystem, especially water bodies that have been subject to documented and worrying levels of water pollution such as Umguza River (Chinyama *et al.*, 2016). Fish parasites are very important to bio-monitoring as the knowledge of fish parasites can also be used in determining the quality of water and hence the health of the aquatic ecosystem (Ramollo, 2008). The biotic integrity of an ecological system is consequently reflected by the characteristics of its entire fauna (Robinson, 1996).

Problem statement and justification

Fish parasites are very common throughout the world and are of particular importance in the tropics (Moyo *et al.*, 2009). However, there is an apparent lack of meaningful research on the helminth parasites of fish in the dams that play a vital role in the livelihoods of local communities. Helminth parasites, particularly nematodes, have significant impacts on the health of the host individuals. They can cause mechanical damage, such as the fusion of gill lamellae and tissue replacement; physiological damage such as cell proliferation, immunomodulation, detrimental behavioral responses and altered growth as well as reproductive damage (Iwanowicz, 2011). In addition to the problems caused by nematode parasites to the fish populations; the exploitation of fish resources for food by human beings opens up many avenues for possible infections by zoonotic diseases.

Nematode parasites have been reported to cause negative health outcomes, including stunting, wasting, diarrhea, organ failure, nutritional deficiencies, mental and developmental retardation, and even death (Cepon-Robins *et al.*, 2014). The in-depth understanding and documentation of the nematode parasites present in water bodies could be a very useful asset in the development of effective strategies to deal with nematode parasites. Nematode parasite management strategies must therefore be based on sound scientific knowledge on the prevalence of parasites, the relationship between host size and parasite infection, the preferred host species for nematode parasites, the preferred location of nematode parasites in the abdominal cavity of the host fish species, as well as knowledge on other aspects as well. Such findings and subsequent discussions could possibly provide a credible platform for future research study.

Furthermore, the study of fish parasites could possibly have a huge role to play in water quality management and pollution remediation, mainly because fish parasites can serve as indicators of water pollution as fish are intolerant to particular environmental conditions. This demonstrates the need for the in-depth understanding, and documentation of fish ecology and parasitology. This study sought to plug an apparent gap in the existing knowledge of fish helminth parasites at Lower Umguza and hence give an idea on the presence and characteristics of helminth parasites in other surrounding water bodies as well as provide a platform for future, related studies. This will ultimately result in better fisheries and aquaculture management principles and strategies as well as better resource utilization, for the benefit of the local communities as well as the economy at large.

Research questions

1. What are the most abundant nematode parasites at Lower Umguza Dam?
2. What is the relationship between host size and parasite loads of fish at Lower Umguza Dam?
3. What are the preferred fish host species for nematode parasites between Nile tilapia (*Oreochromis tilapicus*) and Sharptooth Catfish (*Clarias gariepinus*) at Lower Umguza Dam?

4. What are the differences in the parasite loads between host fish caught at different sites in the Lower Umguza Dam?

Research hypothesis

1. There are no differences in the abundances of nematode parasites between different species at Lower Umguza Dam.
2. There is no relationship between host size and parasite loads of fish at Lower Umguza Dam.
3. Nematode parasites do not show any specific preferred host species between *O. tilapicus* and *C. gariepinus* at Lower Umguza Dam.
4. There are no differences in the parasite loads between host fish caught at different sites in the Lower Umguza Dam.

Objectives of the study

The main objective of this study was to ascertain the presence of nematode parasites in *O. tilapicus* and *C. gariepinus* in the wet season at the Lower Umguza Dam.

The specific objectives of this research study were to:

1. To determine the most abundant nematode parasites at Lower Umguza Dam.
2. To determine the relationship between host size and parasite loads of host fish at Umguza Dam.
3. To determine the specific preferred host species between *O. tilapicus* and *C. gariepinus* for nematode parasites at Lower Umguza Dam.
4. To determine differences in the parasite loads between host fish caught at different sites in the Lower Umguza Dam.

CHAPTER 2

LITERATURE REVIEW

Nematodes, commonly known as roundworms, belong to the phylum Nematoda (Hodda, 2011). The total number of existing nematode species has been estimated to be about 1 million and only about 25,000 species have been described, furthermore; more than half of those are known to be parasitic (Zhang, 2013; Hodda, 2011). Nematodes are thought to account for about 80% of all living individual animals on earth (Platt, 1994); consequently, their manifestation at numerous trophic levels underlines the very important roles they serve in many ecosystems, often in parasitic forms infesting most plants and animals, human beings included (Hsueh et al, 2014). Nematodes are smooth, tubular and comparatively long worms unlike the flatter, segmented tapeworms; and the stouter and shorter monogenes (flukes) as well as the considerably stouter and segmented pentastomids that could be confused with nematodes (Yanong, 2002).



Figure 2.4 Photomicrograph of a *Capillaria* nematode showing typical nematode shape (Source: Yanong, 2002).

Nematode parasites of fish

There over 40 species of adult nematodes, representatives of 9 families from fish in Africa (Khalil, 1971). They range from larval stage nematodes to adult stage nematodes. Adult stage nematodes affect potentially all freshwater and brackish water fish. Predatory fish usually show heavier infections as compared to other fish species; most probably as a result of nematode species that use these fish as intermediate or transient hosts (Yanong, 2006; Paperna, 1997).

Nematode species *Anguillicola* (*A. papernai* Moravec & Taraschewski, 1988) are endemic to South Africa, they have been observed in eels (*Anguilla mossambica*) of the Cape region (Paperna, 1997). The Indo-Pacific eel parasite, *Heliconema anguillae* (syn. *Ortleppina longissima*) (Jubb, 1961), and elver parasite *Paraquimperia* sp. (Jackson, 1978) have all been cited in the same region (Paperna, 1997).

Host specificity of nematodes is variable; several nematodes are known to infect several different fish species while others have been cited in only one particular fish species (Paperna, 1997). The *Camallanidae* have been reported in several diverse fish species; *Procamallanus laevionchus* has been reported from fish hosts of six different families, whereas *Spirocamallanus spiralis* has only been reported from species of *Clarias* and *Synodontis* only. *Paracamallanus cyathopharynx* have only been reported from species of *Clarias*, and *Camallanus kirandensis* from a *Barbus* sp. only (Yanong, 2006; Paperna, 1997; Boomker, 1982).

Rhabdochona congolensis and *Spinitectus allaeri* have been reported from numerous diverse host species, while the other species of the same genera have been reported in one or just a few hosts of closely related species. Species of *Capillaria*, mostly oxyurids and the philometrids

Nilonema gymnarchi and *Thwaitia bagri* have been reported to be very host specific and species of *Anguillicola* will only infect species of *Anguilla* (Moravec & Taraschewski, 1988 in Paperna, 1997).

Larval stage fish parasites that have been reported in Congo and South Africa include *Contracaecum*; it also occurs widely in other African countries (Paperna, 1997). Few larval stage nematode species have been properly studied and documented, possibly because of the difficulties involved with their identification and characterization down to species; mainly as a result of their lack of distinct genital systems and several other features of adult stages which are utilised as taxonomic criteria (Paperna, 1997).

Effects of infestation on fish hosts

Infection of fish by helminth parasites elicits a lot of delirious responses. Parasites can cause mechanical damage (fusion of gill lamellae, tissue replacement), physiological damage (cell proliferation, immunomodulation, detrimental behavioral responses, altered growth) and reproductive damage (Iwanowicz, 2011). Parasites often occur in associations, hence, infection by a type of parasites increases the chances of infection by several other parasite species as well. Infection by nematode parasites greatly increases the infection by many other types of parasites; which result in many other effects. Helminth infection result in a lot of changes in practically the whole body of the fish host individual. Fish parasites, over time, result in the impairment of vision as the fish's eyes are affected. Eye flukes (metacercariae of larval digenean parasites) are known to induce cataracts due to metabolic excretions and mechanical destruction of the lens structures (Shariff *et al.* 1980). Sometimes the eyes may also lose shape and color;

and in the process losing functionality. This results in several undesirable effects to the fish and possibly leads to much more acute effects. The loss of vision impairs the ability of the fish to find suitable forage and nesting sites; it also impairs the ability of the fish to escape predation. (Omeji *et al.*, 2013; Iyaji and Eyo, 2008)

Khalil (1971), reported that 40 species of adult nematodes, representatives of 9 families, have been recovered and documented from fish in Africa and most of them occur in the alimentary system, while only a few enter tissues or inner cavities and these include *Philometridae* and *Anguillicolla*. Parasitic infection by nematodes affects the feeding behavior of fish; the attachment of nematodes on the surface of the gut elicits several responses, irritation included. All these have an effect on the overall health condition of the host (Menezes, 2006). An impaired immune system has profound effects on the ability of the host to survive and acclimatize in a changing environment; hence it makes the fish vulnerable to changing temperature and pollution conditions as it lowers resistance. Thus, the effects of infection are even more profound in fish that harbour water bodies that have been polluted or are at significant risk of pollution such as the Umguza River, which drains into the Lower Umguza Dam, that is fed by tributaries that drain an urban catchment; and as such is prone to pollution due to human activities in the catchment (Chinyama *et al.*, 2016). The presence of nematode parasites puts the persistence of the fish species in the natural water bodies at risk (Iwanowicz, 2011; Paperna, 1996).

Parasites compete with the host for food in the alimentary canal, hence the increased proliferation of parasites in and near the digestive tract results in lesser amounts of food molecules becoming available for absorption into the host's organs, particularly in severe infestation rates. In such situations the feeding behavior of the fish could increase significantly

because of the need to attain a nutrient intake equivalent to the natural condition; when it is free from infection. Low amounts of nutrients available to the host could also result in the loss of weight and reduced immune conditions (Steel, 1974). Parasites often increase significantly in size when in the host; this demonstrates the use of food materials absorbed from the host. Those parasites that remain small certainly do absorb significant amounts of food materials also; as a source of energy for metabolism. Parasites utilise energy reserves of their hosts; this is most obvious in species that undergo some visible growth or development within their fish host (Iwanowicz, 2011; Barber *et al.*, 2000).

Helminth parasites have traditionally been suggested to change the behavior of their hosts by one or several ways. Nematode parasites can migrate to other parts of the body like the neuro-endocrine system of the host. This may alter the behavior of the host by invoking some particular behavioral responses (Barber *et al.*, 2000). Nematodes may also infect other tissues and organs in the body resulting in signs related to the organ system affected and the degree of damage (Yanong, 2002). The migration of helminth parasites to more critical organs could possibly elicit more profound behavior changes and it is not clearly known whether the altered swimming behavior of cyprinid fishes harbouring heavy infections of diplostomatid trematodes is caused by physical damage to the brain (Barber *et al.*, 2000). If the presence of diplostomatid trematodes in the brain can elicit behavior changes then it could also be reasonable to suggest that probably the presence of nematodes in the brain will possibly elicit such behavior changes as well. Metabolic reactions occurring in the bodies of the nematodes could possibly result in the release of some chemical wastes that have significant effects on the host. They have the capability of producing and introducing obnoxious and toxic metabolic by-products that could result in changes in the physiological characteristics of the blood (Bedasso, 2015).

Some species of helminth parasites, such as the trematode (*Euhaplorchis californiensis*), are known to infest California killifish (*Fundulus parvipinnis*) brains, which alters their behavior and causes the fish to swim slowly and in circles at the water surface and when this happens, the susceptibility to predation by birds increase many times. This could be an evolutionary survival strategy by the parasite because when the fish gets preyed upon, then the parasites gets access to the definitive hosts; the birds (Iwanowicz, 2011).

The continued existence of fish in their natural habitats requires them to expend significant amounts of energy and time foraging for food and looking for some possible alternatives in the case of food shortages. Fish also need to move and secure suitable breeding grounds during the mating season. They also need to detect and avoid predators. All these activities need significant amounts of energy and specific behaviors so as to survive successfully. Nematode infections possibly affect the ability of fish to carry out all these activities. Nematode infections are likely to result in some significant changes in the feeding behavior, movement and locomotion, changes in habitat selection as well as changes in sexual behavior and reproduction (Iwanowicz, 2011).

Many parasites have a predilection for sites such as the brain, eye or nervous tissue, and such infections often impair sensory function (Holmes and Zohar, 1990 in Barber *et al.*, 2000). Impairment of sensory and movement capabilities have a huge influence on the successes of the host in foraging activities. For fish species competing for food with other conspecifics, the ability to move quickly to a prey item once detected is important (Barber *et al.*, 2000). Thus, the presence of nematode parasites and their negative effects on the host inflict serious competitive restrictions and reduce survival abilities. Reduced sensory further expose the host to predation;

the survival of the host is also dependent on the ability to detect the presence of predators and escape in time (Barber *et al.*, 2000).

Parasitic diseases of fish are very common all over the world and are of particular importance in the tropics (Omeji *et al.*, 2013). Infection by helminth parasites often result in stunted growth thereby reducing the length and weight of the fish. Nematode parasite loads have a significant effect on the fish size. Data from research provides evidence that parasites may be the cause of the variations and reduced growth in growing fish; and this has a tremendous effect on the population dynamics of fish populations (Masarat, 2016; Sun *et al.*, 2012; Roberts and Janovy, 2000).

Parasite location

Internally, fish parasites are most commonly found in the gut. The digestive tract of many fish has been shown to be a favourite environment for the establishment and growth of pathogenic organisms (Dezfuli *et al.*, 2008). Nematode parasites are known to migrate and establish some populations in the brain, heart, lungs, and even eyes of the host (Iwanowicz, 2011). Nematode parasites can spread to tissues such as the heart, liver, kidney and the brain where they cause several problems. According to Tort and collaborators; the cardiac output of infected hearts was 20-40 % different from that of healthy hearts (Dezfuli *e. al.*, 2008).

These parasites have also been observed in the abdominal cavities of their host (Iwanowicz, 2011). After gaining entry into the body of the host, nematode parasites are known to wander to several body organs. Movement of nematodes to several different parts of the body could

possibly be an evolutionary response to increase their chances of survival in the host and survive several anti-parasite responses by the host's immune system (Iwanowicz, 2011). The occupancy of several such sites probably evolved initially for protection against immune responses and stomach secretions of the host (Iwanowicz, 2011). In bees, nematodes have been found in the ovaries of the female; and here they have been reported to hinder ovary development and even makes the host less active in sexual activities. Helminth parasites that have been shown to result in such negative effects include the ascarid, the hookworms, the pinworms as well as the whipworms. (Lambshhead, 1993).

Biology of nematodes

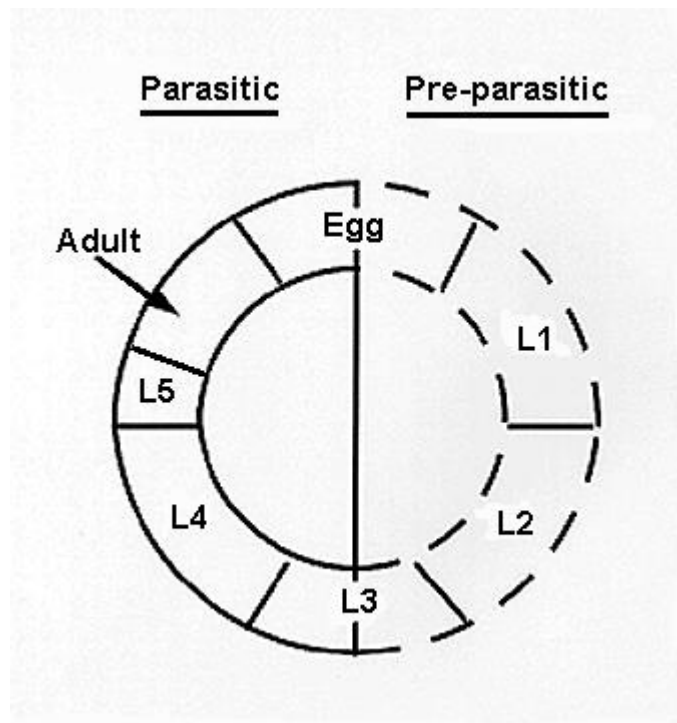


Figure 2.5 Basic nematode lifecycle (Source: Johnstone, 2000)

Nematodes are a diverse animal phylum capable of populating a very extensive range of environments and hence their ways of life and life cycles vary greatly. However, almost all of them can be associated with the same basic pattern shown in Figure 2 (Johnstone, 2000). This basic pattern consists of two distinct phases; the parasitic phase and the pre-parasitic phase. The first phase, the pre-parasitic phase occurs when the nematode lives as a free living individual in the external environment or alternatively inside a second host; the intermediate host. When in the parasitic phase, the individual nematode parasite survives inside the definitive host. As illustrated in Figure 2, this basic life cycle also consists of seven stages, an egg, four larval stages (L1, L2, L3, L4) and two adult stages comprising separate males and females; the sexually immature adult stages can also be termed the L5's (Johnstone, 2000).

Nematode reproduction is often sexual, with separate adult male and female individuals coming together and copulating to produce offspring. However, hermaphrodites that are capable of self-fertilization have also been observed and described. In the course of copulation, spicules are inserted into the genital pore of the female to fertilise the eggs. All these processes occur inside the body of an infested definitive host (Johnstone, 2000; *Barnes, 1980*). After successful copulation, the eggs are laid by the female into the external environment and these eggs consequently pass through the three developmental stages (L1, L2, and L3) before the nematode is again infective for another host (Johnstone, 2000). In the nematode life cycle, one stage that is parasitic to a specific host species might not necessarily be parasitic to all possible host species; thus, one stage that passes from the definitive host is not the same stage that is infective for another definitive host (Johnstone, 2000). The larval stage (usually an egg or L1) that passes

from a definitive host must develop through to a stage (usually the L3) that can then infect another host (Johnstone, 2000).

After the fertilization, eggs develop and a first stage larva develops inside an egg and then it hatches. The hatching process is controlled by several factors and these are inclusive of; temperature and moisture levels in the external environment; thus hatching occurs only when environmental conditions are favorable for the survival of the hatched larvae. The combination of the required conditions stimulates the secretion of enzymes to digest the enclosing egg membranes, and hence the exertion of pressure against the weakened membranes to rupture them and release the larvae (Johnstone, 2000). After hatching, the free living larva feeds on bacteria and grows then it molts four times with each successive larval stage. For that reason, molts separate the first and second larval stages (L1 and L2), the second and third larval stages (L2 and L3), the third and fourth larval stages (L3 and L4) and also the fourth larval stages and immature adults (L4 and L5). The L5 consequently grows into a sexually mature adult male or female (Johnstone, 2000). The comparative time taken for the growing nematode parasites to develop from one stage to another is shown in Figure 2.6.

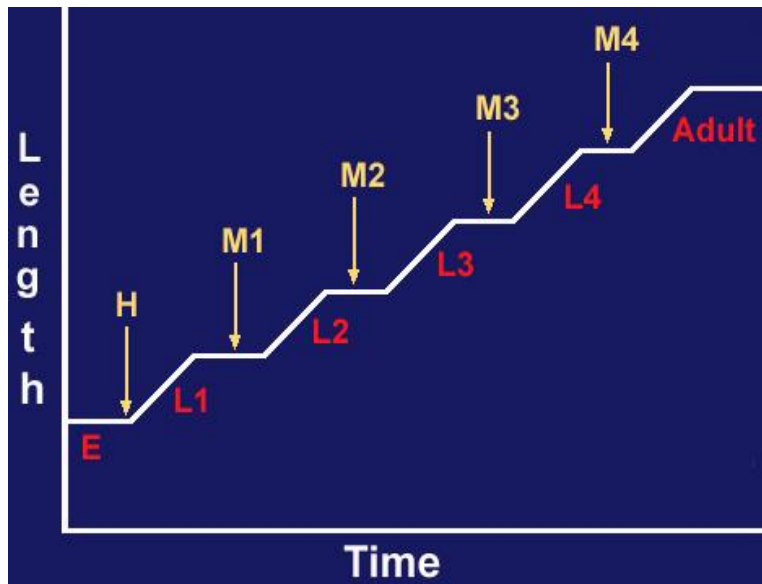


Figure 2.6 Typical growth curve of nematode parasites. (Source: Johnstone, 2000)

Some nematodes species have been observed to follow some slightly different and relatively complicated life cycles as compared to the ones described. Often, these deviations are concerned with the infective stage and whether other hosts, in addition to the definitive host, may possibly play some significant roles in the life cycle. Parasitic nematodes follow either a direct or an indirect life cycle. In the direct life cycles, all pre-parasitic stages are found free-living in the environment and their development may take place either inside the egg or after hatching (Johnstone, 2000). In the nematodes parasites where the first stage larvae hatch from their eggs, subsequent development takes place in the environment and the third stage larva is the infective stage and nematodes that follow this cycle are members of the family Trichostrongylidae. In some nematodes, eggs do not hatch and the pre-parasitic larvae develop inside their eggs such that the infective stage is an egg containing an infective larva and the hatching process is triggered when these eggs are eaten by another host and the infective larva escapes. Nematode species that follow such a cycle include *Ascaris suum* which normally affects pigs (Johnstone,

2000). Such parasitic nematodes can also affect fish hosts; however, they have not been sufficiently documented.

In the indirect life cycles, larvae develop to the infective stage inside an appropriate intermediate host and in these life cycles there are two possible methods of transmission of infective larvae to the definitive host. The intermediate host is ingested by the definitive host and infective larvae are released by digestion in the alimentary tract (Johnstone, 2000). Examples of such parasitic nematodes include *Parelaphostrongylus tenuis*

Importance of fish parasites to bio-monitoring

The knowledge of fish parasites can also be used in determining the quality of water and hence the health of the aquatic ecosystem (Ramollo, 2008). Anthropogenic activities have significantly affected aquatic ecosystems; almost always leaving behind an apparent fingerprint on aquatic ecosystem health (Nachev, (2010). Ecosystem health is a concept that attempts to explain the wellbeing of an ecosystem, that is, the resilience of the system and its ability to resist the disruptive effects of pollution. Nachev, (2010) described a healthy ecosystem simply as one which comprises a balance between system components, stability, diversity and complexity, the absence of disease and the ability undergo homeostasis. The biotic integrity of an ecological system is therefore reflected in the health of its fauna (Robinson, 1996).

Scientists have always sought to understand, characterize and document the activities that have undesirable effects on aquatic ecosystems. They thrive to extend their understanding of the problems emerging as a result of pollution and thereafter try to study in detail every component

of the ecosystem in order to understand the consequences of pollution. Historically, this was exclusively achieved through the study of some biological and physical parameters (Nachev, 2010; Ramollo, 2008). However, the use of biological methods such as the study of fish parasites, promises to provide more effective and reliable ways. Nachev, (2010), reports that there is a close relationship between water organisms and pollution after studying the biological and chemical processes of self-purification running in lotic ecosystems (mostly in River Rhine).

When exposed to significant levels of toxic compounds in water, parasites either die resulting in population size reduction, or they may develop some toleration resulting in competitive advantage and hence accelerated population growth (Nachev, 2010). The resulting numerical changes (increase or decrease of abundance and intensity) of aquatic parasites leading to changes in structure and diversity of parasite communities as a response to different forms of pollution may be used for bio-indication purposes (Nachev, 2010). These studies have been and substantiated and complemented with broader studies and in the process cementing biological methods as important methods of understanding aquatic ecosystems. Fish parasites have provided scientists with useful methods of getting an understanding of these aquatic problems. External parasites such as the copepods and the leeches have been extensively to reflect the quality of water and hence applicability in the field of bio-monitoring, nematode parasites could also be studied for the same purposes (Ramollo, 2008). Pollution leads to changes in the diversity and richness of parasite communities and thus parasites can be used as effect indicators (Nachev, 2010). Consequently, it is very important that the helminth parasites of freshwater fish in specific aquatic ecosystems be known, so as to improve the detection of toxic compounds and monitor ecosystem health.

The use of fish macro-invertebrate assessments provide a useful, integrated and sensitive measurement of environmental problems and assists in the study of assessment of ecological impacts, and consequently, in the management of water resources (Roux, 2001). Water pollution has significant effects on the health of the fish; compromised health, as a result of pollutants, can increase the likelihood of infection. Sures, (2006) articulates that pollution can considerably increase parasitism as a result of its effects on the host defense mechanisms, thus increasing host susceptibility. Pollution can also decrease the levels of parasitism if the parasites are more susceptible to a particular pollutant than the host organism or if the pollution levels compromise the life cycle by eliminating the suitable intermediate host. Either way, the parasite loads can be used to determine and characterize the levels of water pollution (Ramollo, 2008). Knowledge of concepts such as parasite loads in relation to water quality parameters provides a useful way of studying and monitoring ecosystems. In the field of ecological monitoring, researchers are trying to study as many parts of a given ecosystem as possible in order to detect external stress factors, which mostly occurring in the form of contamination (Nachev, 2010).

Bio-indication also makes use some unique capabilities of parasites to absorb and concentrate some dangerous waste material; these include heavy metals (Nachev, 2010). When exposed to high levels of metals, some organisms have got the ability to develop a certain level of tolerance. Where possible, intra-cellular sequestration could happen and the toxins could be stored in specific proteins (Anon.). Research has also shown that some parasites have accumulation capacities and can concentrate toxins. Though the parasites have an ability to absorb toxic substances, the concentrations remain very low. These low concentrations can, however, be detected and hence be used as bio-indicators (Ramollo, 2008).

Apart from giving information on the ecosystem; in-depth studies into fish parasites also reveal useful information about the biology of the host. Barson *et al.*, (2010) asserts that; Parasites can provide much information about the hosts in which they live, such as the type of food they eat, their migration, and also about the ecosystem in which they live. Therefore, the study of fish parasites increases the knowledge we have about the fish themselves, a crucial aspect if we are to effectively utilize fish resources.

CHAPTER 3

MATERIALS AND METHODS

Study Area

The research was done at the Lower Umguza Dam. It is located in the borders of the City of Bulawayo in Matabeleland, Zimbabwe. The climatic conditions in the study area are mainly influenced by the relatively high altitude. Bulawayo lies in the subtropical climatic region. The mean annual temperature is 19.16 °C (66.44 °F). As with much of southern and eastern Zimbabwe, Bulawayo is cooled by a prevailing southeasterly airflow most of the year, and is characterized by three broad seasons: a dry, cool winter season from May to August; a hot dry period in early summer from late August to early November; and a warm wet period in the rest of the summer, early November to April. The hottest month is October, which is usually the height of the dry season. The average maximum temperature ranges from 21 °C (70 °F) in July to 30 °C (86 °F) in October. During the rainy season, daytime maxima are around 26 °C (79 °F). Nights are always cool, ranging from 8 °C (46 °F) in July to 16 °C (61 °F) in January.

The average annual rainfall is 594 mm, which supports a natural vegetation of open woodland, dominated by *Combretum* and *Terminalia* trees. Most rain falls in the December to February period, while June to August is usually rainless. Being close to the Kalahari Desert, Bulawayo is vulnerable to droughts and rainfall tends to vary sharply from one year to another. Abundant rains were recorded in the 2017 rainy season, with some areas in the dam's catchment area flooding.

The areas around the dam have a wide variety of woodland types; these include the Acacia - Mopani, Combretum, Grassland, Gusu - teak, Kopje, Mopane-acacia-marula, Mopani-combretum-acacia, Mopani-terminalia, riverine and vlei. The most common tree species include *Combretum sp.*, *Acacia sp.*, *Ziziphus mucronata*, *Bolusanthos speciosus* and *Colophospermum mopane*. Other tree species that do occur and include *Pseudolachnostylis maprouneifolia* and *Ozoroa reticulate*. Grass species common around the study area include *Heteropogon contortus*, *Melinis repens*, *Aristida rhiniochloa*, *Diospyrus lyciodes*, *Setaria incrassate* and *Aristida congesta* and *Themeda traindra*.



Figure 3.1 Aerial view of the Lower Umguza Dam showing numbered sampling sites. (Source: Google Maps).

Data collection

All fish used in this study were collected from local fishermen at the Lower Umguza Dam from March - April 2017. For each individual fish collected, total length was measured and recorded; there-after the fish were slit open longitudinally in the abdomen, from the genital papillae and extending to the gill region. The open gut region was examined intensively for internal organisms using standard parasitological methods. The abdominal organs were pushed out and collected on standard pill bags labeled with the specific site of collection of the host species from Lower Umguza Dam, host species as well as an assigned sample identification number. The open abdominal regions were washed thoroughly and the washings were also collected. Together with the abdominal organs and fecal matter, these were taken to the Laboratory, prepared for examination using standard parasitology procedures and thereafter observed under light microscopy (Heil, 1999). The observed organisms in the residues were compared with the parasites on a guide and identified. The nematode parasites recovered were recorded using numeric counts.

Physicochemical characteristics

Water samples were collected from the four sampling sites prior to the collection of data. Dissolved oxygen content was measured using a Hach Oxygen 330i meter. Electrical

conductivity was measured using an Oakton CON150 conductivity meter while pH was measured using a sensION+ PH1 pH kit.

Identification of parasites

There are several morphological criteria which allow recognition of helminth parasites of most families and even some genera, and identification in this particular research study referred to the identification guides by; Moravec *et al.*, (2006), Parpena (1980), and Frimeth (1994).

Statistical analyses

All the statistical analyses were done using Statistical Package for Social Sciences (SPSS-21) for Windows (IBM SPSS Inc., Chicago, USA). The comparisons of nematode abundances between the different parasite species were done using one way ANOVA at 5% level of significance. The relationship between host fish length and parasite abundances was tested using linear regression at 5% level of significance. The comparisons of nematode abundances between the 2 different host fish species were done using the Independent samples t-test at 5% level of significance. The comparisons of parasite loads between the 4 different sampling sites were done using the one way ANOVA at 5% level of significance. The Tukey HSD Post-Hoc test was used to show the differences between specific sampling sites.

CHAPTER 4

RESULTS

Physicochemical characteristics

The pH readings at Lower Umguza dam ranged from 7.3 to 8.0. Site B had a slightly acidic reading at 7.3 while Site D was relatively more alkaline; recording 8.0. Dissolved Oxygen at the sampling sites ranged from 5.8mg/L to 6.8mg/L. Site C had the highest reading while Site B had the lowest reading (Table 4.1). Site A had the lowest electrical conductivity (603 μ S/cm) while Site D had the highest readings (680 μ S/cm).

Table 4.1 Physicochemical characteristics of water

Sampling site	pH	Dissolved oxygen	Electrical conductivity
Site A	7.5	6.0mg/L	603 μ S/cm
Site B	7.3	5.8mg/L	580 μ S/cm
Site C	7.8	6.8mg/L	700 μ S/cm
Site D	8.0	6.3mg/L	680 μ S/cm

Nematode parasites abundance

A total of 479 nematode parasites were collected from 160 fish belonging to 2 species; Nile tilapia (*Oreochromis tilapicus*) and Sharp-tooth catfish (*Clarias gariepinus*). Four nematode genera; *Paracamallunus sp.*, *Eustrongylides sp.*, *Contracaecum sp.*, and some unidentified nematodes parasites were recovered. Of the 160 host fish samples collected, 78.1% had

nematode infections. Of those infected, 76.6% had multiple infections and only 23.4% had single infections.

Of the 479 nematode parasites collected, 210 (43.8%) were collected from Nile tilapia and 269 (56.2%) from Sharp-tooth catfish. The abundances of the parasites differed significantly between the different nematode species recovered ($P < 0.05$). The most abundant nematode parasites collected were *Paracamallunus sp.* which contributed 48.6% (Fig 4.2). *Eustrongylides sp.* and *Paracamallunus sp.* contributed 18% and 33% respectively. The unidentified parasites constituted 0.4% of the total number of nematode parasites collected.

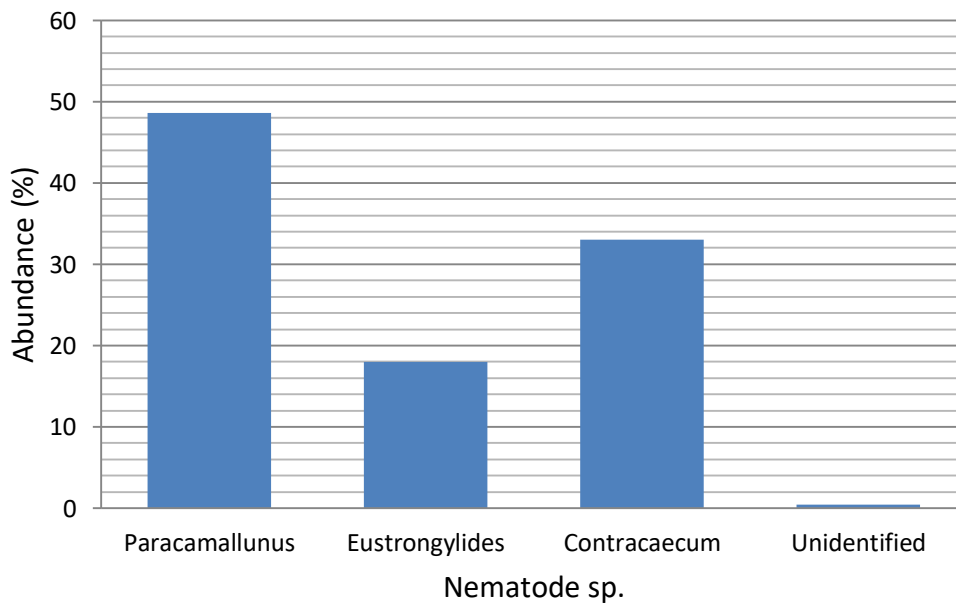


Figure 4.2 Percentage nematode parasite abundance in fish

Relationship between host size and parasite loads of fish

A positive correlation ($r = 0.681$) was observed between fish size and nematode parasite loads (Fig 4.3).

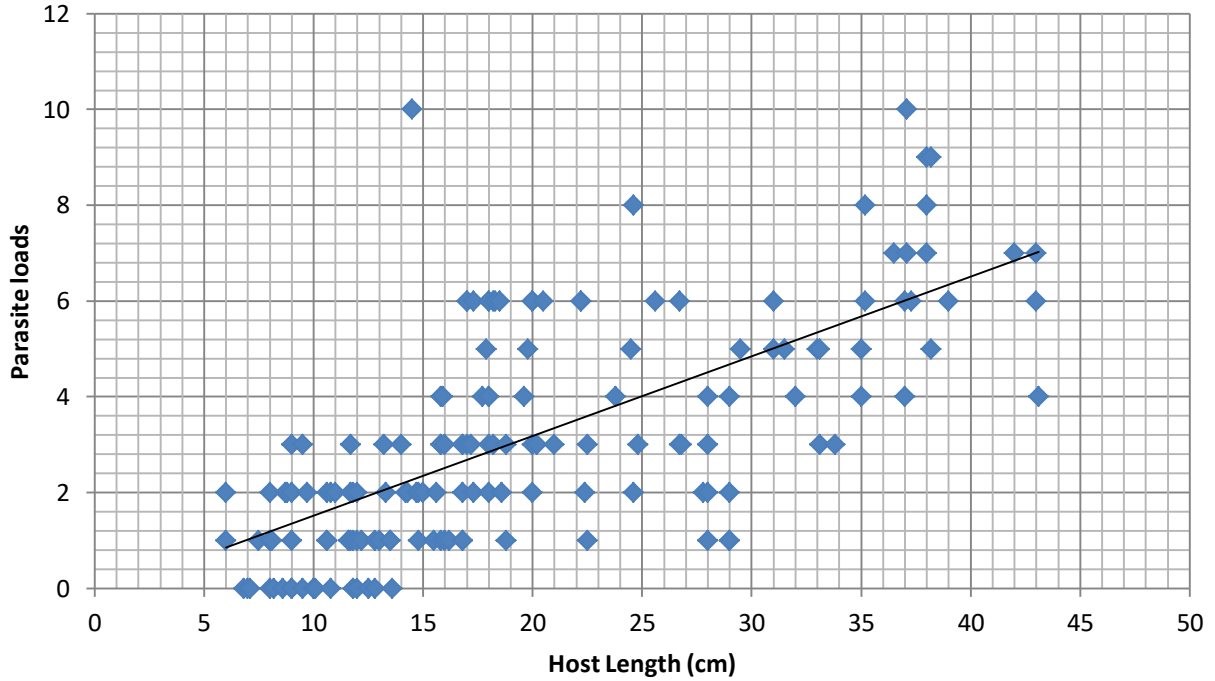


Figure 4.3 Scatter plot showing relationship between host size and parasite loads of fish

Host fish in the 0-9.9cm class had the least average abundances while the 30 - 39.9 cm class and 40 - 49.9 cm class had the highest abundances. Average abundances increased steadily between the aforementioned host length classes (Fig 4.4).

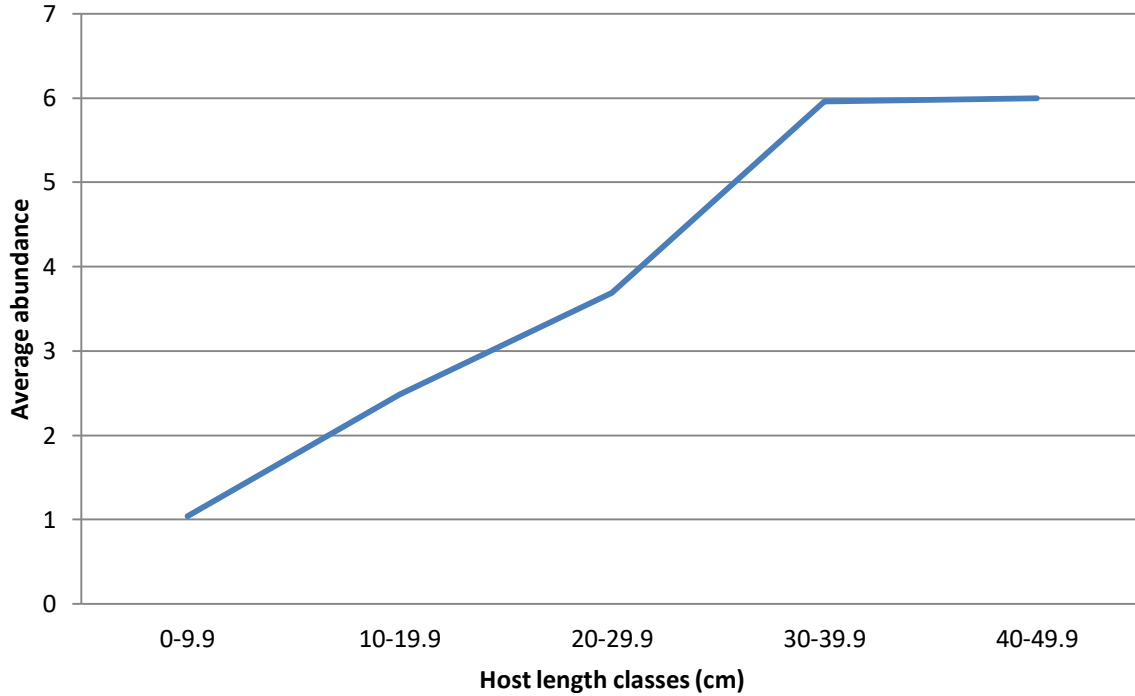


Figure 4.4 Effect of host length on average nematode abundances

Nematode host preferences

Table 4.2 Nematode parasite abundances in relation to the fish species.

Host species	<i>Paracamallunus</i>	<i>Eustrongylides</i>	<i>Contracaecum</i>	Unidentified	Total
Nile tilapia	100	38	70	2	210
Sharptooth catfish	133	48	88	0	269

Relatively high abundances were recorded from the Sharp-tooth catfish (Table 4.2 and Fig 4.5); from whom 56.2% of all nematode parasites were recovered. The remaining nematode parasites,

43.8 %, were recovered from the Nile tilapia. Statistically, the differences in nematode parasites between the two host species were significant ($P < 0.05$).

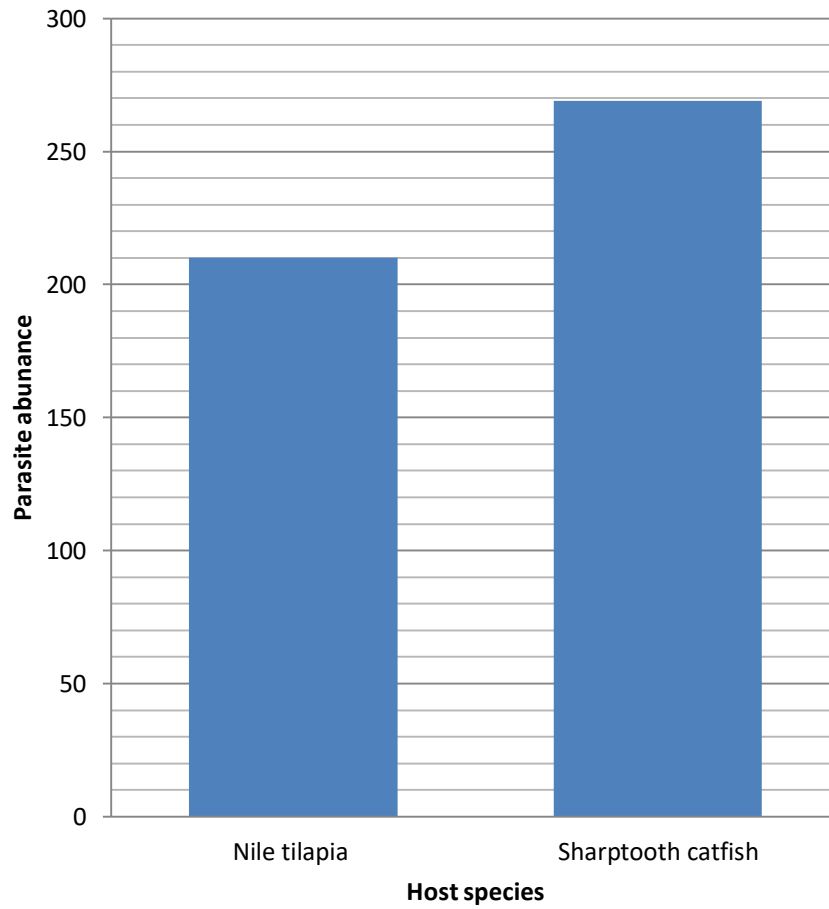


Figure 4.5 Host preferences for nematode parasites

Nematode parasite abundances from the different sample sites

Results showed that parasite abundances were not statistically significant ($P < 0.05$) across all the four sampled sites. Abundances between sites were not statistically significant (Fig 4.6).

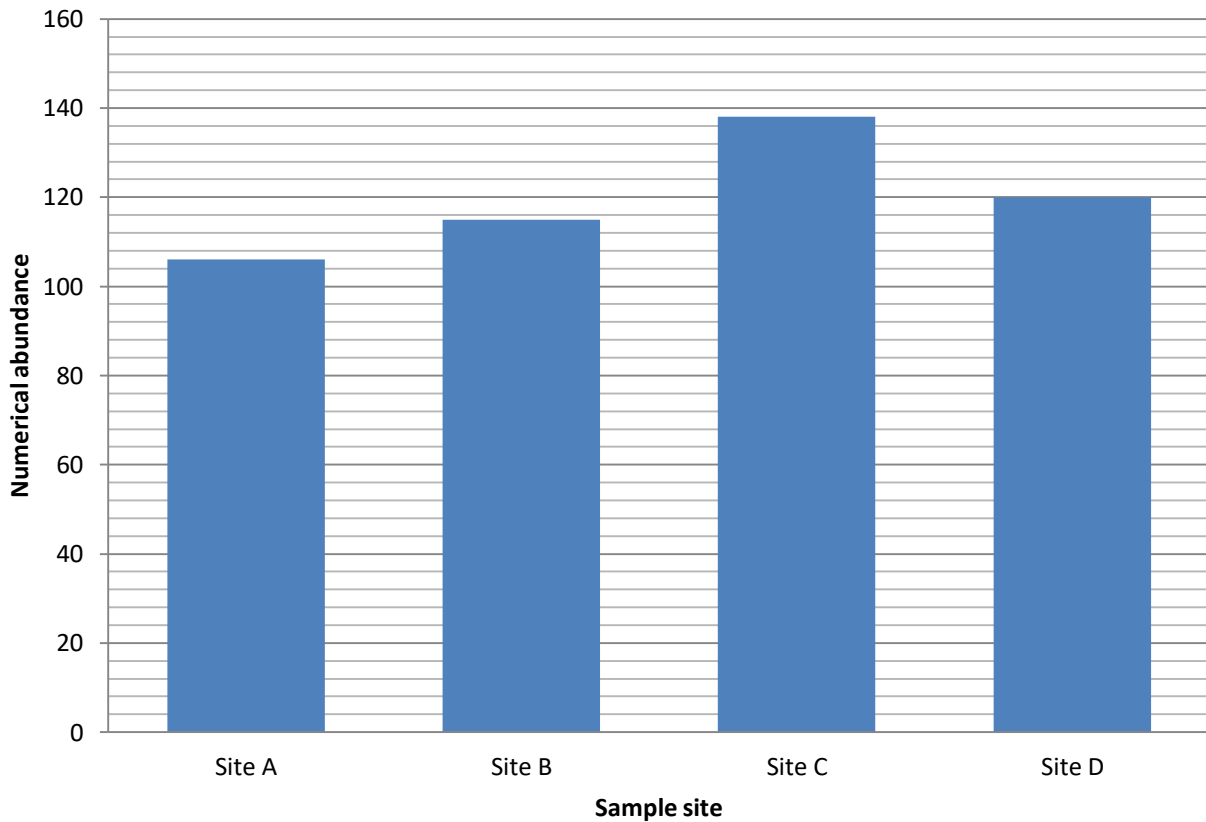


Figure 4.6 Nematode parasite abundances from the different sample sites

As shown in Fig 4.6, Site C contributed the highest number of nematode parasites (138), while Site A contributed the lowest number (106) of nematode parasites. Site B and Site D contributed 115 and 120 nematode parasites respectively.

CHAPTER 5

DISCUSSION

Nematode parasites abundance

The results from this particular study revealed that general abundances and levels of infestation, per individual host fish, by nematode parasites were low as compared to other studies. Mwita (2014) reported abundances as high as 30 per fish. Several other studies have reported such high abundances. This particular study, conversely, recorded relatively low quantities of parasites per fish. This could be a result of several reasons and the most important of these could arguably be the differences in the characteristics of the water bodies involved as well as the climatic seasons in which the studies were carried out. The 2016/2017 rainy season in Zimbabwe was characterised by significantly high rainfall levels which could have resulted in the increased size of habitat for the host fish as water quantities increased and hence new feeding and nesting sites were opened up. This is likely to have reduced the frequency of contact between the fish hosts and the infective stages of the parasites on the old shorelines, feeding and nesting sites, thereby reducing the occurrence and intensity in the fish host.

It is widely documented that there is an apparent relationship between the amount of pollutants in water and the amount of parasites in the water bodies. Collecting basins for all kinds of waste water are unclean and thus capable of harbouring many different kinds of parasites (Edema *et al.*, 2008). As such, water bodies that are polluted like Lower Umguza Dam would be expected to have very high abundances of nematode parasites. Morenikeji and Adepeju (2009) documented that polluted water had the highest percentage of infection as compared to the unpolluted water. Chinyama *et al.* (2016) reported that critical pollutant concentrations were found along the stretch of Umguza River, which drains into the Lower Umguza Dam. Unexpectedly, findings from this study show that nematode parasites were not as abundant as would have been expected. This could be attributed to the very high rainfall received which possibly diluted the water resulting in relatively less polluted water concentrations and hence lower nematode parasite abundances. When water is less dilute and hence relatively more concentrated with organic particles, the oxygen available for aquatic organisms is reduced and this has considerable implications on fish the fish; predisposing them to them to infection. Some of the factors that

enhance parasitic infection in fishes include increase in organic matter in the water and subsequently reduced oxygen content, in the water and the associated poor environmental conditions (Edema *et al.*, 2008). Lower Umguza Dam drains a pollution prone catchment area and increases the quantity of suspended solids, phosphates, nitrates, COD, turbidity and ammonia; this reduces dissolved oxygen and changes such parameters as pH, alkalinity and temperature (Chinyama *et al.*, 2016). Urban effluents promote aquatic pollution, thus making aquatic organisms vulnerable to increased incidence of parasites (Abdel-Gaber, 2015).

Of the 160 host fish samples collected, only 35 (21.9%) were free from nematode infection. This revealed a very high infection rate consistent with findings from various other studies. 78.1% of host fish were infected with nematode parasites while Umuoeren *et al.* (1988) recorded 60.4%; Sinare *et al.* (2016) recorded an infection prevalence of 65.18% and 100% recorded by Malsawmtluangi and Lalramliana (2016). Some studies have also yielded exceptionally low infection rates, as low as 3.33% (Ekanem *et al.*, 2011), 6.94% (Edema *et al.*, 2008) and 7.7% (Ugwuzor, 1987). Studies by Bekele and Hussien (2015) yielded 20.83% infection rate in Ethiopia while infection rates of 65% were reported by Abdel-Gaber *et al.* (2015). Infection rates from several different areas show some significant discrepancies and Edema *et al.*, (2008) suggests that infection rates vary greatly from one area to another as a result of several factors which include among other things, the nature of the water which is reflected in the human use and the endemicity of infection in the area.

The high infection rates could also be linked with the season when the research study was done. In late summer, the rainy season is past its peak and vegetation growth is at its peak and hence high food availability. Aloo (2002) acknowledged that most fish species, especially tilapia feed primarily on phytoplankton and decomposed organic matter and the quantity of these is highest in the rainy season; and hence greater probabilities of exposure to infection as helminth parasites can be found on phytoplankton and decomposed organic matter. This time period also corresponds to the period of reproduction and proliferation of intermediate hosts; therefore, the increase of intermediate hosts could explain the increased rate of infection observed in the results during this period (Sinare *et al.*, 2016). In separate studies, Usip *et al.* (2010) in Sinare *et al.* (2016) reported that during the rainy season, most species of fish had a high level rate of infection.

Findings from this particular study revealed that at least three nematode parasites were present at Lower Umguza Dam; *Paracamallunus sp.*, *Eustrongylides sp.* and *Contracaecum sp.* For comparative purposes, no records on the presence of nematode parasites could be found from studies at the Lower Umguza Dam and even nearby water bodies. There are only a few reports about the parasites of fish in Zimbabwe (Moyo *et al.* 2009). Nonetheless, these named nematode parasites have been documented in studies from Zimbabwe and Southern Africa. Moyo *et al.* (2009) indicates that all three nematodes were recovered in studies done at Insukamini Dam in the Midlands; Zimbabwe. Several previous studies have documented that prevalent nematode species in Southern Africa are widely distributed as widely as their suitable hosts and these hosts include the intermediate hosts, fish; as well as the definitive hosts and these include piscivorous fish (Boomker, 1982).

The similarities in the nematode parasites present could be attributed to the presence of piscivorous birds such as ducks, herons, darters, egrets, cormorants, pelicans and the king fisher that enhance dispersal from and to the study site, as well as other water bodies in the region. Piscivorous birds can be definitive hosts for helminth parasites (Franson and Custer, 1994; Spalding *et al.*, 1993). Helminth parasites that infect freshwater fish are usually found as adults in fish eating birds, such as cormorants and pelicans (Gebawo, 2015). These birds constantly migrate from water body to both near and far water bodies in search for food and suitable breeding sites. There is scientific evidence that aquatic birds enhance the dispersal of helminth parasites as documented by Salgado-Maldonado and Pineda-Lopez (2003) that the duck *Anas platyrhynchos* that fed on fish infected with *Bothriocephalus acheilognathi* defecated viable eggs of this helminth a few days (1–3 days) after ingesting the fish and these eggs hatched into normal infective helminths four days later.

Often, in older nestlings and adult birds, nematode infection does not result in speedy death hence the birds can survive and traverse huge distances and spread the parasites to other water bodies. Oligochaetes (fresh water aquatic worms), minnows and small fish play important roles in the life cycle of helminth parasites. Eggs hatch within oligochaetes; second-and third-stage larvae are produced within oligochaetes and these are consequently spread to the minnows and small fish which feed on the oligochaetes; minnows and small fish are fed upon by piscivorous

fish which can then possibly transfer these to uninfected water bodies (*Franson and Custer, 1994; Spalding et al., 1993*).

However, Barson, (2004) did not recover any *Paracamallunus sp.*, *Eustrongylides sp.*, and *Contracaecum sp.* from studies at a major water body in Zimbabwe. Nematode *Paracamallunus cyathopharynx* has been reported in Burkina Faso (Sinare, 2016), Nigeria (Ayanda, 2008), and Egypt (Eissa *et al.*, 2011). Besides reports in Southern Africa, *Contracaecum* has also been reported in Ethiopia (Bekele and Hussien, 2015); and even in Mexico (Salgado-Maldonado, 2004).

Gebawo (2015) indicates that nematodes of the genus *Contracaecum* could represent human health risk factors by eating raw or smoked meat of parasitized fish. Encapsulated larval nematodes are known to cause fibrous capsule and the non-encapsulated larvae cause extensive tissue damage by migration (Paperna, 1980). Several traders were observed buying fish from local fishermen for re-sale in the city centre; and this constitutes a significant health risk to the consumers.

The abundances of the parasites differed significantly between the different nematode species recovered. This corroborates the findings of Bekele and Hussien (2015), which reported variations in the abundances of nematode parasites found at a different study site. At Lower Umguza Dam, the most abundant nematode parasites collected were *Paracamallunus sp.* *Eustrongylides sp.* and *Contracaecum sp.* contributed 18% and 33% respectively. These findings are different from those of Bekele and Hussien (2015) who found that *Contracaecum* was the dominant in all species of fish examined. Bekele and Hussien (2015) hypothesizes that the higher prevalence of *Contracaecum* might be due to the fact that the parasite infests wide range of aquatic birds that can serve as final and intermediate hosts; and larval stages are known to occur in most African fresh water fish, including catfish and tilapia. Based on these suggestions, the abundance of *Paracamallunus* at Lower Umguza Dam could be a result of the possible abundance of birds that serve as final and intermediate hosts for *Paracamallunus* as compared to the presence of birds that serve as final and intermediate hosts specifically for *Eustrongylides* and *Contracaecum*. Still, more work still needs to be done so as to fully describe and document these phenomena.

Relationship between host size and parasite loads of fish

Results obtained showed that there was a positive correlation between host size and nematode parasite loads. The findings suggest that an increase in the length of the host fish results in reciprocal increases in the abundance of parasites infesting the host individual. Just like in this study, findings by Abdel-Gaber (2015) also showed that the smallest fish are relatively less infected than the other length groups of the examined samples and the percentage of infection increases with increasing fish lengths. Fish length has some profound influences on the abundance of nematode parasites in the host fish. Host size is suggested as the most significant predictor of parasite abundance on the infested host (Oniye 2004; Amundsen *et al.*, 1997). For most helminth parasite species mean intensity varies significantly among fish of different sizes (Mwita, 2014).

The length of the host fish is a reflection of age, as the fish grows older, it also increases in size; length and weight. Thus, longer fish are likely to be more parasitized as they are older. This agrees with the findings of Olurin and Somorin (2006) who reported that an increase in fish length results in increased susceptibility to parasite infection. This also substantiates the findings of Olurin and Somorin (2006) who reported that the longer the fish, the greater the susceptibility to parasite infection. Parasites generally accumulate over time; thus, parasite abundance will likely correlate with fish length. In addition, older fish individuals are more capable of moving over longer distances and this leads to an increase in exposure to potential infection. Younger and hence shorter fish will also show less parasite abundances because they have been exposed to parasites for a relatively shorter time. Furthermore, older fish consume more food and this increases the probability of feeding on parasitized food sources. Bigger fish tend to cover wider areas in search of food; as a result, they take more food than the smaller ones and this exposes them further to infestation by parasites (Omeji *et al.* 2013). Mwita (2014) suggests that by virtue of bigger size, longer fish have a larger internal environment for parasites to attach and be established.

Amin (1986) in Masarat (2016) observed varying results in the parasitic abundance in different length groups of fish, and these were attributed to changes in the feeding capacity of different ages of the host. Findings by Onyedineke *et al.* (2010) revealed that helminth parasites were most prevalent in fish of 10 to 30cm standard length which is almost similar to the results in this particular study. There were no considerable increases in nematode parasite abundances in host fish that fell in the 40-49.9 cm length class. This suggested that nematode abundances increase with host length until a particular point where increases in host length will not result in significant increases in parasite abundances. This could be a result of the some responses developed by the older host fish in a bid to reduce the effects of infestation and acclimatize to the environment. The development of immunity in older fish that renders them less susceptible to invading parasites has also been cited as possible causes and explains the occasionally inconsistent relationship between host size and nematode parasite abundance, which results in the lower intensity observed in large fish (Mwita, 2014). Morphological, behavioural and dietary changes in large and old fish could also be the reasons for this observation (Mwita, 2014).

Nematode host preferences

Relatively high abundances were recorded from the *C. gariepinus* as compared to the *O. nilotica*. This could be attributed to the different behaviour and activities of the fish species. Peter (2005) suggests that this might be due to the fact that *C. gariepinus* has less discriminatory or omnivorous behaviour when feeding. *C. gariepinus* is generally classified as omnivorous and a predator feeding mainly on aquatic insects, fish and higher plants debris which could be infected (Ayanda, 2008); while *O. tilapicus* is mainly relies on phytoplankton and decomposed vegetation for food. *C. gariepinus* is known to traverse long distances (Mwita, 2014), and this could possibly result in increased exposure to possible infection by nematode parasites. Variations in nematode parasite abundance between different species could also be a result of possible biological factors deriving maybe from feeding differences between the different host species. *C. gariepinus* is likely to move longer distances, eat more and hence accumulate more parasites in the body. More contact with food results in a higher tendency of getting infected with parasites

(Ayanda, 2008). The possibility that the feeding patterns of particular fish species influence the abundance of their larval helminths remains (Luque and Poulin, 2004).

Cohabiting fish species will almost always compete for sites and food, and the species with a competitive advantage will get better access to feeding sites and nesting sites; and this can also mean an increased exposure to infective helminth parasites. Therefore, the higher abundances of nematode parasites in *C. gariepinus* could be a result of these competition dynamics. Nonetheless, there seems to be an apparent lack of in-depth knowledge on what really determines and influences host preferences in nematode parasites that parasitize fish. Ogawa (1996) and Gulelat *et al.* (2013) in Bekele and Hussien (2015) suggest that the problem of parasitic infections is associated with feeding behaviour of fish and these behaviours of fish could expose them to easy transmission of parasites from invertebrate intermediate hosts and fish intermediate hosts. Another reason for the recovery of a large number of helminths in *C. gariepinus* could be related to the large size of Clarias as compared to other fish species (Moyo *et al.*, 2009).

Polanski (1961) in Malsawmtluangi and Lalramliana (2016) summarised that the key elements influential in the determination of the characteristics of parasitic fauna as well as the intensity and incidence of infection include the diet of the host, lifespan of the host, the mobility of the host throughout its life, including the variety of habitats it encounters, its population density and the size attained, large hosts provide more habitats suitable for parasites than do small ones. The interaction of these factors could be responsible for making *C. gariepinus* more susceptible to helminth infections as compared to *O. nilotica*.

Nematode parasite abundances from the different sample sites

The results from this particular study, unlike the widely reported findings in previous studies, showed that parasite abundances were not statistically significant across the different sampled sites. Findings also showed that the physicochemical conditions recorded across the sampling sites were only slightly different. Hence, the resemblances in the parasite abundance across the sampling sites could be attributed to the almost similar physicochemical characteristics of the

water. Mwita (2014) reported that variations in the abundances of nematode parasites among different sampling sites was an indication of the presence of an uneven distribution in terms of species and density of parasites' intermediate hosts, some of which constitute food items for the host fish. Therefore, the different findings in this particular study possibly point to the existence of an even distribution in terms of the density of the parasites' intermediate hosts.

Previous studies have revealed variation in the abundance of nematode parasites of fishes among different sampling stations in large bodies of water (Mwita, 2014). In such cases the variations could be a result of the differences in anthropogenic activities on the different sides of the water body. These activities result in the accumulation of chemical product substances from agriculture; these result in eutrophication which promotes the proliferation of algae and other organic material, thus accelerating the proliferation of intermediate hosts which may increase the chance of infection (Sinare *et al.*, 2016).

Conclusions

Relying on the experimental results of this study, it can thus be concluded that the general abundances and levels of infestation, per individual host fish, by nematode parasites were low at Lower Umguza Dam. This study sought to reveal the most abundant nematode parasites at Lower Umguza Dam and from the evidence gathered, it can be concluded that of the three recovered nematode genera, the *Paracamallunus sp.* nematodes are the most abundant as compared to the *Eustrongylides sp.* and *Contracaecum sp.* The study also sought to ascertain the relationship between host size and parasite loads of fish at Lower Umguza Dam. From the evidence gathered, it can be safely concluded that there was a positive correlation between host size and nematode parasite loads. The findings showed that an increase in the length of the host fish resulted in reciprocal increases in the abundance of parasites infesting the host individual. Furthermore, the study sought to reveal the preferred fish host species for nematode parasites between Nile tilapia (*O. tilapicus*) and Sharptooth Catfish (*C. gariepinus*) at Lower Umguza Dam. From the evidence gathered, it can be concluded that Sharptooth Catfish is the most preferred host species when compared to Nile tilapia. Lastly, the study sought to ascertain the

differences in the parasite loads between host fish caught at different sites in the Lower Umguza Dam and the results showed that the showed that parasite abundances were not statistically significant across the different sampled sites; therefore, it can be concluded that the differences in the similar physicochemical characteristics of the water were not significant enough to influence any changes hence there is bound to exist an even distribution of the density of the nematode parasites at the study site .

Limitations

Due to the financial implication, time implication and complexity associated with studying all the possibly available helminth parasites; this research only delved in-depth, on nematode parasites. The data was collected over a single weather season, late summer (early March to late April), and replication was not done during winter or any other season. For comparative purposes, at least one other different water body should have been studied, however, due to the financial implications; only one study area was used. Data collected were limited to the phenomena discovered from the digestive tract only and did not narrow down to the presence in any other specific body organs of the individual host. Data were only collected from two fish species only; Nile tilapia (*O. tilapicus*) and Sharp-tooth catfish *C. gariepinus*, whereas, for comparative purposes, more fish species should have been included.

Recommendations

Recommendations made from this study are listed below;

1. Given the ubiquitous reports of pollution at Lower Umguza Dam and the widely documented apparent relationship between the amount of pollutants in water and the amount of parasites in the water bodies, it is rather surprising that the research findings yield low nematode abundances. Therefore, it is recommended that further research studies be conducted at the study area so as to fully describe and understand the

unexpected phenomena. These studies should also be done over several weather seasons so as to yield more comprehensive and useful information.

2. There is scanty information concerning fish helminth parasites in the country, especially the western parts of the country. Hence it is recommended that further investigations in other water bodies be carried out to see the possible presence of fish parasites, as well as the effects of ecological and environmental conditions on the population dynamics of these parasites.
3. Unlike the findings in other studies, evidence gathered in this particular research revealed that the parasite loads between host fish caught at different sites in the dam were not statistically significant and thus pointing to similar physicochemical characteristics of the water in all sampling areas. This needs further research so as to ascertain why the physicochemical characteristics of the water could be so similar in such a large water body.
4. Further research could also study the changes of the physicochemical characteristics in relation to seasons and how the abundance of fish parasites fluctuates in response to the weather changes.
5. Given the results collected it is recommended that further research studies be conducted so as to fully understand the phenomena influencing parasite abundance. For comparison purposes, it is recommended that investigations in other water bodies be carried out, and in different weather seasons. There is need for studies to ascertain the possible fluctuation in parasite abundance in response to changing physicochemical characteristics and seasons.

In conclusion; as a result of the extensive reports on pollution at the Lower Umguza Dam and the effects of such pollution on aquatic organisms which are of significant importance to local communities, parasitological information should be recognized as an important resource to ecology, animal production and public health as most fish parasites have the potential to cause zoonotic diseases of concern to other organisms.

References;

- Aloo, P. A. (2002), “A comparative study of helminth parasites from the fish *Tilapia zillii* and *Oreochromis leucostictus* Lake Naivasha and Olodien Bay, Kenya.” *Journal of Helminthology*, **76**:95–102
- Amundsen, P.-A., Kristoffersen, R., Knudsen, R. and Klemetsen, A. (1997), “Infection of *Salmincola edwardsii* (Copepoda: Lernaeopodidae) in an Age-Structured Population of Arctic Charr—A Long-Term Study.” *Journal of Fish Biology*, **51**, 1033-1046.
- Abdel-Gaber, R., El-Garhy, M., and Morsy, K. (2015), “Prevalence and intensity of helminth parasites of African Catfish *Clarias gariepinus* in Lake Manzala, Egypt.” *Advances in Bioscience and Biotechnology*. **6**:464-469 <http://dx.doi.org/10.4236/abb.2015.67048>
- Ayanda, O. I. (2008), “Comparison of parasitic helminth infection between the different age groups of *Clarias gariepinus* from Asadam Ilorin, north-central Nigeria.” *African Journal of Environmental Science and Technology*, **2(11)**:404-406
- Barber, I., Hoare, D., Krause, J. (2000), “Effects of parasites on fish behaviour: a review and evolutionary perspective.” *Reviews in Fish Biology and Fisheries* **10**:131–165.
- Barnes, R. G. (1980) *Invertebrate zoology*. Philadelphia: Sanders College.
- Barson, M., Prikrylova, I., Vanhove., M. M., and Huysei, T. (2010), “Parasite hybridization in African *Macrogyrodactylus* spp. (Monogenea, Platyhelminthes) signals historical host distribution.” *Parasitology*. doi:10.1017/S0031182010000302
- Barson, M. and Marshall, B. E. (2003) The occurrence of the tapeworm, *Ligula intestinalis* (L.), in *Barbus paludinosus* from a small dam in Zimbabwe, *African Journal of Aquatic Science* **28(2)**: 1–4
- Boomker, J. (1982), “Parasites of South African freshwater fish. I. Some nematodes of the catfish [*Clarias gariepinus* (Burchell, 1822)] from the Hartbeespoort dam.” *Onderstepoort J. vet. Res.* **49**:41–51.

- Bekele, J., Hussien, D. (2015), “Prevalence of Internal Parasites of *Oreochromis niloticus* and *Clarias gariepinus* Fish Species in Lake Ziway, Ethiopia.” *J Aquac Res Development* **6**:308
- Chinyama, A., Ncube, R., Ela, W. (2016), “Critical pollution levels in Umguza River, Zimbabwe.” *Physics and Chemistry of the Earth*. Volume 93:76-83
- Dezfuli, B.S., Lui, A., Boldrini, P., Pironi, F., and Giari, L. (2008), “The inflammatory response of fish to helminth parasites.” *Parasite*, **(15)**:426-433
- Edema, C. U., Okaka, C. E., Oboh, I. P., and Okogub, B. O. (2008), “A preliminary study of parasitic infections of some fishes from Okhuo River, Benin City, Nigeria.” *International Journal of Biomedical and Health Sciences*. **4(3)**117-112
- Ekanem, A. P., Eyo, V. O., and Sampson, A., F. (2011), “Parasites of landed fish from Great Kwa River, Calabar, Cross River State, Nigeria.” *International Journal of Fisheries and Aquaculture*, **3(12)**:225-230
- Eissa, I., Gado, M. S., Laila, A. M., Mona, S. Z., Noor, E. (2011), “Field studies on prevailing internal parasitic diseases in male and hybrid tilapia relation to mono sex tilapia at Kafr El-Sheikh Governorate fish farms.” *Journal of Animal Science* **7(3)**:722-728.
- Franson, J. C., and Custer, T. W. (1994), “Prevalence of eustrongylidosis in wading birds from colonies in California, Texas, and Rhode Island, United States of America.” *Colonial Waterbirds*, **17**:168–172
- Gebawo T. B. (2015) “Study on the prevalence and temporal abundance of parasites of fishes in Lake Elan.” *Global Journal of Fisheries and Aquaculture*. **3(7)**:265-269
- Hodda, M. (2011), “Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness.” *Zoo taxa*. **3148**:63–95.
- Heil, N. (Ed.) (1999) National Wildlife Health Survey – Laboratory Procedures Manual. 5.0 Edition. U. S. Fish and Wild-fish Service. Warm Springs, GA.
- Hsueh, Y., P., Leighton, D., H., W., Sternberg, P., W. (2014) Nematode Communication. In: Witzany G (ed). *Biocommunication of Animals*. Springer, 383-407. [ISBN 978-94-007-7413-1](https://doi.org/10.1007/978-94-007-7413-1).

- Iwanowicz, D. (2011) Overview on the effects of parasites on fish health. United States Geological Survey. National Fish Health Research Laboratory
- Iyaji, F.O., and Eyo, J. E. (2008), “Parasites and their Freshwater Fish Host.” *Bio-Research*. **6**(1):328 – 338.
- Johnstone, C. (2000) Parasites and Parasitic Diseases of Domestic Animals. University of Pennsylvania. http://cal.vet.upenn.edu/projects/merial/Nematodes/nems_5.htm
- Khalil, L.F., 1971. Check list of the helminth parasites of African freshwater fishes. Commonwealth Institute of Helminthology, St Albans. Technical Communication No. 42.
- Lambshhead, P. J. D. (1993), “Recent developments in marine benthic biodiversity research.” *Oceanis* **19**(6):5-24
- Luque, J., and Poulin, R. (2004), “Use of fish as intermediate hosts by helminth parasites: A comparative analysis.” *Acta Parasitologica*, **49**(4):353–361
- Malsawmtluangi, C., and Lalramliana. (2016), “Prevalence of helminth parasites infecting *Mastacembelus armatus* (Lacepède, 1800) from different rivers of Mizoram, northeast India.” *Science Vision*. **16**(3):2229-6026
- Masarat, S. (2016), “Effect of helminth parasitic load on the length weight-ratio of fresh water fish *Cyprinus* from the river Jhelum of Kashmir Valley.” *Imperial Journal of Interdisciplinary Research*. **2**(7):809-811
- Menezes, R. C., Tortelly, R., Tortelly-Neto, R., Noronha, D., Magalhães, R. (2006), “*Camallanus cotti* Fujita, 1927 (Nematoda, Camallanoidea) in ornamental aquarium fishes: pathology and morphology.” *Mem Inst Oswaldo Cruz*, **101**(6):683-687
- Morenikeji, O. A. and Adepeju, A. I. (2009), “Helminth communities in Cichlids in natural and man-made ponds in south-west Nigeria.” *Researcher*, **1**(3):84-92
- Moyo D.Z., Chimpira C. and Yalala P. (2009), “Observations on the helminth parasites of fish in Insukamini Dam, Zimbabwe.” *Res. J. Agri. Biol. Sci.* **5**:782–785.

- Mwita, C. J. (2014), "The Community of Parasites Infecting *Clarias gariepinus* in the Tanzanian Waters: A Case of Lake Victoria." *Open Journal of Ecology*, **4**:873-882
- Nachev, A. (2010) Bio-indication capacity of fish parasites for the assessment of water quality in the Danube River. Unpublished MSc thesis. Karlsruhe Institute of Technology, Germany.
- Okechi, J. K. (2004) Profitability assessment: A case study of African catfish (*Clarias gariepinus*) farming in the Lake Victoria basin, Kenya. United Nations Programme. Reykjavik, Iceland.
- Olurin, K.B. and Somorin, C.A. (2006), "Intestinal Helminths of the Fishes of Owa Stream, South-west Nigeria." *Research Journal of Fisheries and Hydrobiology*, **1**(1): 6-9
- Omeji, S., Solomon, S. G And Uloko, C. (2013), "Comparative study on the endo-parasitic infestation in *Clarias gariepinus* collected from earthen and concrete ponds in Makurdi, Benue State, Nigeria." *Journal of Agriculture and Veterinary Science*. **2**(1):45-49
- Oniye, S.J., Adebote, D.A. and Ayanda, O.I. (2004), "Helminth Parasite of *Clarias gariepinus* in Zaria, Nigeria." *African Journal of Aquatic Science*, **19**:71-76.
- Onyedineke, N., E., Obi, U., Ofoegbu, P., U., and Ukogo, I. (2010), "Helminth Parasites of some freshwater fish from River Niger at Illushi, Edo State, Nigeria." *Journal of American Science*, **6**(3)
- Paperna, I., and Zwerna, D. E. (1996), "Parasites and diseases of striped bass, *Morone saxatilis* (Walbaum), from the lower Chesapeake Bay." *Journal of fish biology* **12**:267-287
- Paperna, I. (1997) Parasites, infections and diseases of fishes in Africa: An update – Food and Agriculture Organization of the United Nations. Technical Paper. 30:220
- Paperna, I. (1980) Parasites, infections and diseases of fish in Africa. CIFA Technical Paper, 7: 88-89
- Peter, A. (2005) Prevalence and Pathology of Protozoans and Monogenean Parasites from fry and fingerlings of cultured *Clarias gariepinus* (Burchell, 1882) in Uganda. MSc Thesis, UNESCO IHE, Institute for Water Education, 15.

Platt HM (1994). Foreword in Lorenzen S, Lorenzen SA. The phylogenetic systematics of free living nematodes. London: The Ray Society. [ISBN 0-903874-22-9](#).

Ramollo, P. P. (2008) Bioassessing the impact of water quality on the health and parasite composition of *Oreochromis mossambicus* at the Phalaborwa Industrial Complex (PIC) and the Barrage (Olifants River) in the Limpopo Province, South Africa. Unpublished MSc Dissertation, University of Limpopo. South Africa

Roberts, L.S., and Janovy, J. (2000) Foundations of Parasitology, sixth Edition. McGraw–Hill International Edition, Boston. Pp 105-113

Robinson, J. (1996) Evaluation of the Health Assessment Index with reference to bioaccumulation of metals in *Oreochromis mossambicus* (Peters, 1852) and aspects of the morphology of *Lernaea cyprinacea* Linnaeus 1758. Unpublished MSc Dissertation, Rand Afrikaans University, Johannesburg.

Salgado-Maldonado, G., Cabanas-Carranza, Soto-Galera, E., Pineda-Lopez, R., Caspeta-Mandujano, J. M., Aguilar-Castellanos, A. and Mercado-Silva, N. (2004), “Helminth Parasites of Freshwater Fishes of the Panuco River Basin, East Central Mexico.” *Comp. Parasitol.* **71(2)**:190–202

Salgado-Maldonado, G., and Pineda-Lopez, R. F. (2003), “The Asian fish tapeworm *Bothriocephalus acheilognathi*: a potential threat to native freshwater fish species in Mexico.” *Biological Invasions*, **5**:261–268.

Sinaré, Y., Bounou, M., Ouéda, A., Gnémé, A., and Kabré, G. B. (2016), “Diversity and seasonal distribution of parasites of *Oreochromis niloticus* in semi-arid reservoirs (West Africa, Burkina Faso).” *African Journal of Agricultural Research*. Vol. 11(13):1164-1170.

Spalding, M. A., Bancroft, G. T., and Forrester, D. J. (1993), “The epizootiology of eustrongylidosis in wading birds (*Ciconiiformes*) in Florida.” *Journal of Wildlife Diseases*, **29**:237–249

Steel J.W. (1974), “Pathophysiology of gastrointestinal nematodes infections in the ruminant.” *Proc. Aust. Soc. Anim. Prod.* 10:139

Sun, D., Blomberg, S. P., Cribb, T. H., McCormick, A. S., and Grutter, A. S. (2012), "The effects of parasites on the early life stages of a damselfish." *Coral Reefs*, **31**(4):1065-1075

Ugwuzor, G.A. (1987), "A survey of the helminthic parasites of fish in Imo River." *Nigerian Journal of Applied Fisheries and Hydrobiology*, 225-30.

Umuoeren, N.A., Onwuliri, C. O. E., and Anadu, D. I. (1988) Comparative studies in Endohelminth parasites of cultural and incultured fish from Plateau State. *Nigeria Journal of Applied Fisheries and Hydrobiology*. **3**:45-48.

Urban, J. F., Linda, J. R. and Mansfield, S. Shea-Donohue, T., Gause, W. C., and Finkelman, F. D. (1998) Mechanisms of intestinal immunity to nematode parasites and the consequences to invasion by opportunistic bacteria, Old Herborn University Seminar Monograph 11: Immunomodulation of the gastrointestinal mucosa. Germany: 59-69

Yanong, P. E. (2002) Nematode (Roundworm) Infections in Fish. United States Department of Agriculture. UF/IFAS Extension Service, University of Florida. Technical paper.

Yanong, P. E. (2006) Cir 91 document. Tropical Aquaculture Laboratory, Department of Fisheries and Aquatic Sciences. University of Florida. U.S. Department of Agriculture, UF/IFAS Extension Service.

Zhang, Z. (Ed.) (2013) Animal Biodiversity: An Outline of Higher-level Classification and Survey of Taxonomic Richness (Addenda 2013)". *Zootaxa*. **3703** (1): 5–11.

Appendices

Appendix A

ANOVA

Abundances of nematode parasites on host fish

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	186.042	3	62.014	69.262	.000
Within Groups	569.444	636	.895		
Total	755.486	639			

Test of Homogeneity of Variances

Parasites loads on host fish

Levene Statistic	df1	df2	Sig.
104.267	3	636	.000

Multiple Comparisons

Dependent Variable: Parasites loads on host fish

	(I) Parasite species	(J) Parasite species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.0	2.0	.9188 [*]	.1058	.000	.646	1.191
		3.0	.4688 [*]	.1058	.000	.196	.741
		4.0	1.4563 [*]	.1058	.000	1.184	1.729
	2.0	1.0	-.9188 [*]	.1058	.000	-1.191	-.646
		3.0	-.4500 [*]	.1058	.000	-.723	-.177
		4.0	.5375 [*]	.1058	.000	.265	.810
	3.0	1.0	-.4688 [*]	.1058	.000	-.741	-.196
		2.0	.4500 [*]	.1058	.000	.177	.723
		4.0	.9875 [*]	.1058	.000	.715	1.260
	4.0	1.0	-1.4563 [*]	.1058	.000	-1.729	-1.184
		2.0	-.5375 [*]	.1058	.000	-.810	-.265
			3.0	-.9875 [*]	.1058	.000	-1.260

*. The mean difference is significant at the 0.05 level.

Appendix B

Relationship between host size and parasite loads of fish

SPSS Regression Output

Model Summary^b

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.681 ^a	.464	.461	2.1503

a. Predictors: (Constant), Fish length

b. Dependent Variable: Numerical number of parasites

ANOVA^a

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	633.439	1	633.439	137.000	.000 ^b
	Residual	730.536	158	4.624		
	Total	1363.975	159			

a. Dependent Variable: Numerical number of parasites

b. Predictors: (Constant), Fish length

Appendix C

Independent Samples test SPSS Output

Group Statistics

	Fish host species	N	Mean	Std. Deviation	Std. Error Mean
Parasite loads	1.0	260	.808	1.0479	.0650
	2.0	240	1.121	1.2264	.0792

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval	
									Lower	Upper
Parasite loads	Equal variances assumed	3.798	.052	-3.077	498	.002	-.3131	.1018	-.5262	-.1000
	Equal variances not assumed			-3.057	471.892	.002	-.3131	.1024	-.5262	-.1000

Appendix D

The differences in nematode parasite abundances between the sampled sites.

ANOVA SPSS OUTPUT

ANOVA

Parasite loads

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.988	3	.329	.249	.862
Within Groups	655.130	496	1.321		
Total	656.118	499			

Multiple Comparisons

Dependent Variable: Parasite loads

	(I) Sample sites	(J) Sample sites	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.0	2.0	-.0750	.1484	.958	-.457	.307
		3.0	-.1024	.1430	.891	-.471	.266
		4.0	-.1167	.1484	.861	-.499	.266
	2.0	1.0	.0750	.1484	.958	-.307	.457
		3.0	-.0274	.1430	.998	-.396	.341
		4.0	-.0417	.1484	.992	-.424	.341
	3.0	1.0	.1024	.1430	.891	-.266	.471
		2.0	.0274	.1430	.998	-.341	.396
		4.0	-.0143	.1430	1.000	-.383	.354
	4.0	1.0	.1167	.1484	.861	-.266	.499
		2.0	.0417	.1484	.992	-.341	.424
			3.0	.0143	.1430	1.000	-.354