DETERMINATION OF HEAVY METAL LEVELS IN STRAIGHTFIN BARB (*Enteromius paludinosus,* Peters 1852) AND ITS ENDO-PARASITES AS BIOINDICATORS IN LAKE NAIVASHA, KENYA

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been submitted or presented for examination in any institution.

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Recommendation

This thesis has been presented for examination with our approval as official University supervisors

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DEDICATION

In loving memory of my parents, the late John B. Ngesa and Selina A. Ngesa who always had faith in me and moulded me into the person I am today.

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ABSTRACT

Fish parasites such as cestodes are regarded as good bioindicators of environmental contamination with trace elements including heavy metals. Research on their biondicative potentials in Lake Naivasha, Kenya is lacking. This study aimed to determine the levels of selected heavy metals Arsenic (As), Chromium (Cr), Lead (Pb) and Mercury (Hg) in the straightfin barb (*Enteromius paludinosus*) and its endo-parasites as bioindicators in Lake Naivasha, Kenya. Water, sediment and 1307 fish were collected from November 2017 to February 2018. The fish were transported to the laboratory in the Department of Biological Sciences, Egerton University. In the laboratory, their lengths and weights were then measured followed by dissection to examine their gonads, obtain the muscle tissues and the parasites. The samples were analyzed for heavy metals using a Thermal-electron atomic absorption spectrophotometer at the Lake Nakuru Water Quality and Testing Laboratory. The equations for the length-weight relationship of the combined sexes, males and females of *E. paludinosus* were, $\log W = 3.23 \log L + \log 0.11$, $\log W = 2.83 \log L + \log 0.15$ and $\log W = 2.87 \log L + \log 0.15$ respectively. The mean condition factor of the combined sex, males and females were; 0.59, 0.57 and 0.60 respectively. The sex ratio of the fish samples was 1: 1.98 (males: females). The concentrations of heavy metals in the water sample was 0.076 and 0.001 mg/l for Pb and Hg respectively while that of As and Cr were below detection limit. The concentrations of heavy metals in the sediment were 0.17, 1.49, 15.82 and 0.05 mg/kg wet weight for As, Cr, Pb and Hg respectively. In the muscle tissues of the fish, the mean concentrations of As, Cr, Pb and Hg were 5.07, 22.09, 45.21 and 1.55 mg/kg ww respectively. The bioaccumulation factors for *L. intestinalis* were 2.41, 2.19, 5.86 and 5.14 for As, Cr, Pb and Hg, respectively. It was concluded that the population of *E. paludinosus* in Lake Naivasha had a good growth ratio but not in a good condition. Therefore, it is recommended that further analysis of length-weight relationship and condition factor should be carried out for both dry and wet seasons. This study also concludes that the fish are not safe for consumption. This is because of the health risks that could be brought about by the high levels of Pb and Hg in the fish muscle tissue that both exceeded the FAO/WHO and EU maximum permissible limits. Additionally, the cestode, *L. intestinalis* showed a potential of accumulating heavy metal levels from the fish host (*E. paludinosus*). Because of that, it is recommended that more studies should be done on other cestodes and acanthocephalans that infect different fish species in Lake Naivasha. This will aid in establishing their bioindicative potentials in addition to *L. intestinalis*.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectrophotometer
APHA	American Public Health Association
BAF	Bioaccumulation Factors
BCF	Bioconcentration Factors
EU	European Union
FAO	Food and Agriculture Organization
KEBS	Kenya Bureau of Standards
LEL	Lowest effect level in sediment
LWR	Length-Weight Relationship
NEMA	National Environment Management Authority
SDGs	Sustainable Development Goals
SEL	Severe effect concentration in sediment
TEC	Threshold effect concentration in sediment
THQ	Target Hazard Quotient
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
WQTL	Water Quality Testing Laboratory
ww	Wet Weight

CHAPTER ONE
INTRODUCTION

1.1 Background information

Heavy metals are a group of metals and metalloids with atomic density 5 g/cm^3 times greater than water (Duruibe *et al*., 2007). They are also known as trace elements when they occur in minute concentrations in biological systems. Some of these metals are toxic to living organisms even at low concentrations, whereas others are biologically essential and become toxic at relatively high concentrations. The elevated levels of these heavy metals in the aquatic environment are as a result of increased anthropogenic activities such as domestic, industrial, mining, and agricultural practices. These activities consequently lead to contamination thus, exceeding the thresholds set by environment management authorities (NEMA, 2009). Heavy metals such as Lead (Pb), Mercury (Hg), Chromium (Cr) and Arsenic (As) have been identified according to a report on World's Worst Pollution Problems by McCartor and Becker (2013) as having the greatest impact on the health of a large human population hence a top threat to humankind.

For more than thirty years, the need for serious studies on pollutants in aquatic systems and their biota was recognized in Kenya due to the rapid population expansion, growing industries, and increasing urbanization (Onyari, 1981). For instance, there have been a number of investigations on heavy metals and other trace elements on Ramsar sites such as Lakes Baringo, Bogoria, Elementaita and Naivasha (Campbell *et al*., 2003; Kamau *et al*., 2008; Ochieng *et al*., 2007; Yang *et al*., 2017). In general, the studies found some elevated levels of trace elements such as Nickel (Ni), Copper (Cu), Cadmium (Cd), Mercury (Hg) and Arsenic (As), indicating that anthropogenic factor played an increasingly important role in the heavy metal pollution of the four lakes. In Lake Naivasha alone, reports by Ochieng *et al*. (2007), Mutia *et al.* (2012) and Otachi *et al*. (2014) recorded elevated levels of trace elements such as Aluminium (Al), Iron (Fe), Manganese (Mn), Zinc (Zn), Rubidium (Rb), Copper (Cu), Cadmium (Cd) and Lead (Pb) in the sediments compared to the threshold levels provided by Turekian and Wedepohl (1961). Additionally, they reported higher levels of heavy metals in blue spotted tilapia (*Oreochromis leucostictus*) and common carp (*Cyprinus carpio*) than the FAO/ WHO (2011) acceptable limits. In most of these earlier studies, the aims were to determine the levels of heavy metals (Al, Fe, Mn, Zn, Rb, Cu, Cd and Pb) in the fish and assess if they were safe for human consumption (Campbell *et al*., 2003; Mutia *et al*., 2012; Otachi *et al*., 2014). Elsewhere, fish has been extensively studied as bioindicators of environmental contamination arising from heavy metals (Sures, 2001; 2003; Tekin-Özan and Barlas, 2008; Plessl *et al.*, 2017). In most of the studies, different fish organs have been found to have varying abilities to accumulate heavy metals (Mahboob *et al*., 2014; Otachi *et al*., 2014; Otachi *et al*., 2015).

However, as from the 1980, ecologists have been attempting to study the link between parasitism and environmental pollution (Poulin, 1992; Sures, 2001; 2006; Tekin-Özan and Barlas, 2008). Fish parasites have been used as biological tags to assess fish populations and are considered as sensitive probes to monitor a range of environmental factors, among them being stress due to pollution. As a result, the relationship between environmental pollution and parasitism in aquatic organisms and the potential role of endo-parasites as water quality indicators have received increasing attention (Thielen *et al*., 2004; Tekin-Özan and Barlas, 2008). Different parasites both at the population and component level have been found to have variable responses when exposed to pollution of which the responses differ depending on where in the host the parasite lives and the kind of pollutant (Gilbert and Avenant-Oldewage, 2017). Parasites are now regarded as useful indicators of aquatic health because of their sensitivity to environmental change; others are more resistant than their hosts and tend to increase in numbers in polluted conditions (Sures, 2004).

In Africa, information is available on fish cestodes bioaccumulation from South Africa and Kenya (Retief *et al*., 2006; Madanire-Moyo and Barson, 2010; Oyoo-Okoth *et al*., 2010; 2012), fish acanthocephalan in Egypt (Abdou and Mahfouz, 2006), and a fish infesting nematode in Lakes Naivasha (Otachi *et al*., 2014) and Turkana (Otachi *et al*., 2015). This study intends to build on these earlier studies. Heavy metals were determined in water, sediments and tissues of the straightfin barb (*E. paludinosus*) of which there is hardly any information or studies conducted on this fish in Lake Naivasha. Secondly, heavy metals were determined in the endo-parasites (cestodes), *Ligula intestinalis* infecting the fish, to establish their bioindicative potentials for biomonitoring in Lake Naivasha. In order to find out the sub lethal effects of the reported pollutants in the lake, the biological aspects such as the length-weight relationship (LWR), condition factor (K) and sex ratio of *E. paludinosus* were investigated. This study contributes to the achievement of the Sustainable Development Goals number number 2 (on food security), 3 (on good health and well being for people) and 6 (on sustainable management of water and sanitation). This study further establishes the health risks associated with drinking untreated water from Lake Naivasha coupled with consuming *E. paludinosus* as well as the impact to the people who depend on this lake for their livelihood.

1.2 Statement of the problem

Increased human activities such as domestic related, industrial, mining and agriculture has led to waste discharges into the environment and particularly water systems. Consequently resulting to rise in heavy metal levels in the aquatic ecosystems therefore causing contamination. This not only leads to environmental degradation but also can affect aquatic organisms such as fish through their toxicity. Several studies have been conducted on heavy metal levels in fish and fish parasites from Lake Naivasha. However, none of them has reported on the levels of heavy metals in *E. paludinosus* and its endo-parasite the cestode, *L. intestinalis*. Although fish parasites such as cestodes have been reported as good environmental contamination bioindicators of heavy metals, there is limited information on their biondicative potentials in Lake Naivasha. Therefore, this study targeted to establish knowledge on the bioaccumulation potential of the *L. intestinalis* as an indicator of environmental contamination in Lake Naivasha and other related systems.

1.3 Objectives

1.3.1 General objective

This study aimed at determining the levels of heavy metals in the straightfin barb (*E. paludinosus*) and its endo-parasites as bioindicators in Lake Naivasha, Kenya.

1.3.2 Specific objectives

1. To determine some biological aspects of male and female *E. paludinosus* during the rainy season in Lake Naivasha.

2. To determine the concentration and bioaccumulation factors of the selected heavy metals (As, Cr, Pb and Hg) in *E. paludinosus* and its endo-parasite, *L. intestinalis* from the mouth of River Malewa in Lake Naivasha.

1.4 Hypotheses

1. The biological aspects of male and female *E. paludinosus* do not vary significantly during the rainy season in Lake Naivasha.

2. There is no significant difference in concentration levels of the selected heavy metals in *E. paludinosus* and its endo-parasite, *L. intestinalis* from the mouth of River Malewa in Lake Naivasha.

1.5 Justification

Heavy metals are among the top threats recognized as pollutants that jeopardize the health of tens of millions of people. It is estimated that over a hundred million people are at risk from toxic pollution at levels above the international health standards worldwide. The toxic effects of heavy metals in aquatic organisms which have been reported include bioaccumulation in various organs, tissue damage, disruption in growth and reproduction and induction and synthesis of metallothionein. This consequently leads to serious health risks to the fish consumers. Consumption of fish with high levels of heavy metals, for instance Lead (Pb) and Mercury (Hg) could result to health effects such as neurological damage and severe damage to the brain and kidneys and sometimes cause death. Failure to address this issue of heavy metal contamination could have a negative impact on the livelihoods and health of the people depending on fishing activities for income and food. Specifically, this study determines the levels of heavy metals in the cestode, *Ligula intestinalis* to establish its bioindicative potentials hence recommend its innovative inclusion in future biomonitoring activities of Lake Naivasha and other related systems. This study also provides a monitoring tool to identify the health risk towards consumption of fish and more specifically *E. paludinosus* from contaminated systems such as Lake Naivasha. Additionally, it will aid in the achievement of vision 2030, Sustainable Development Goals (SDGs) and Nakuru County Integrated Development Plan (NCIDP) in regards to food security, good health and well being for people and sustainable management of water and sanitation.

1.6 Scope of thesis and limitations

1.6.1 Scope

The main research themes investigated are presented as objectives in separate chapters, however cross-referencing of different chapters will facilitate understanding of the overall objective of the study.

Chapter One: Introduction

This chapter provides the background information of heavy metal pollution in aquatic systems as well as the relationship between environmental pollution, fish and fish parasites in aquatic systems. The statement of the problem, objectives, hypotheses and justification of the study are also stated therein.

Chapter Two: Literature Review

This chapter provides literature review on the sources of trace elements in the water bodies. The effects of heavy metals in humans are discussed. The general categories of fish parasites and their effects on their fish host are explained. The length-weight relationship and condition factor of fish as well as the conceptual issues of biomonitoring in aquatic systems are discussed. Fish and fish parasites as biondicators of pollution is elaborated. The fisheries and fish parasites of Lake Naivasha as well as the biology and ecology of *E. paludinosus* is well stipulated.

Chapter Three: Some biological aspects of straightfin barb, *Enteromius paludinosus* (Peters 1852) during the rainy season in Lake Naivasha, Kenya

In this chapter, the determination of length-weight relationship, condition factor and sex ratio of male and female *E. paludinosus* using the formula $W = aTL^b$ and Fulton's condition Factor are discussed.

Chapter Four: Levels of heavy metals in the straightfin barb, *Enteromius paludinosus* (Peters 1852) from River Malewa, Naivasha, Kenya

In this chapter the Thermal-electron Atomic Absorption Spectrophotometer (AAS) is used to determine heavy metals in the water sample, sediment sample, fish muscle tissues of *E. paludinosus* and its endo-parasite. The methods used are also discussed.

Chapter Five: Summary, Conclusions and Recommendations

This chapter gives the summary of the major findings, conclusions and recommendations for *E. paludinosus* and its endo-parasite, *L. intestinalis* in Lake Naivasha, Kenya.

1.6.2 Limitations

The lack of proper equipment (AAS) in the Biological Sciences department laboratory posed a big challenge. Because of this, the samples were taken for heavy metals analysis in another laboratory outside Egerton University, at the Lake Nakuru Water Quality and Testing Laboratory (WQTL) which took a lot of time to analyze the samples as they are analyzed on a first-come, first-serve basis.

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CHAPTER TWO

LITERATURE REVIEW

2.1 Heavy metals in water bodies

2.1.1 Sources of trace elements in water bodies

Heavy metals are a group of metals and metalloids with atomic density 5 g/cm^3 times greater than water and are one of the most persistent pollutants in aquatic systems. They are also referred to as trace elements when they occur in small concentrations in biological systems (Salem *et al*., 2000). Heavy metals occur in small amounts naturally and may enter into aquatic system through leaching of rocks, airborne dust, forest fires and vegetation (Ogoyi *et al*., 2011). However, the most common toxic heavy metals find their way in water bodies through anthropogenic activities. These include Arsenic (As), Lead (Pb), Mercury (Hg), Cadmium (Cd), Chromium (Cr), Copper (Cu), Nickel (Ni), Silver (Ag), and Zinc (Zn). Their occurrence and accumulation in the environment is a result of direct or indirect human activities, such as rapid industrialization, urbanization and anthropogenic sources such as agricultural runoff, garbage dumps and mining (Ochieng *et al*., 2007).

Several studies have been conducted on heavy metals in different lakes in Kenya, among them Lake Naivasha (Ochieng *et al*., 2007; Njogu *et.al*., 2011; Otachi *et al*., 2014a), and others have already investigated the levels and sources of heavy metals such as Copper (Cu), Cadmium (Cd), Zinc (Zn), Iron (Fe), Lead (Pb), and Nickel (Ni) in Lake Naivasha. In addition, Njogu *et al*. (2011) and Mutia *et al*. (2012) showed that the most important sources of heavy metal pollution in the Lake Naivasha basin are its major tributary, the Malewa River, and some flower farms surrounding the lake. Therefore, the studies of the latter authors on trace elements and heavy metals in the lake have focused on the anthropogenic sources of these metals and concentrations in the muscle of fish tissues of the common carp (*Cyprinus carpio*), largemouth blackbass (*Micropterus salmoides*), tilapia (*Oreochromis leucostictus*), and mirror carp (*Cyprinus spectacularlus*). Studies on heavy metal pollution in aquatic ecosystems have been a major environmental focus (Omwoma *et al*., 2010) particularly due to their potential toxic effects and ability to bioaccumulate in both aquatic and terrestrial ecosystems thus causing adverse effects on organisms.

2.1.2 Bioacumulation of trace elements in aquatic organisms

Bioaccumulation is essentially the buildup of contaminants such as heavy metals or pesticides in living organisms (Abel, 1989). Aquatic organisms are often subject to bioaccumulation because they absorb contaminants from the water around them faster than their bodies are able to excrete them (Arnot and Gobas, 2006). Heavy metals are able to bind to the surface of microorganisms (like phytoplankton in oceans) and sometimes enter the cells themselves. Once they enter the cell, heavy metals can react with chemicals released by the microorganism to digest food, and undergo chemical transformations (Abel, 1989). A good example is mercury becoming methylmercury, which is especially dangerous since it is more easily absorbed by living organisms.

Bioaccumulation of heavy metals is used for environmental monitoring largely because aquatic organisms are in direct contact with the contaminated water. Tissue metal concentrations in fish are good indicators of aquatic system exposure to the metal contamination (Al-Kahtani, 2009). Heavy metals accumulate in fishes via water, sediments, food such as algae upon which both herbivorous and omnivorous fishes feed on (Khan *et al*., 2011). Gills, skin, and alimentary canal are the entry points of heavy metals which usually build up in metabolically active tissues (Dural *et al*., 2006). Active interaction of the tissue type, with the contaminant, such as, gills with contaminated water and liver with contaminated food, leads to heavy metals finding their way into the respective tissues (Javed and Usmani, 2011).

The liver is extremely active in the storage and uptake of heavy metals and also metallothionein induction has been reported to take place in fish liver (Heath, 1990). Also it has been reported that the accumulation of heavy metals in gills is because of its thin epithelium among all the organs of the body through which metals can easily pass (Bebianno *et al*., 2004). Feeding habits, foraging behavior, and heavy metals concentration in body tissues has been established to be highly correlated in omnivorous and herbivorous compared to carnivorous fishes. This is because omnivorous fishes are known to feed from different levels of the food chain thus, exposed to greater chances of heavy metals biomagnification (Obasohan, 2008).

The degree at which bioaccumulation occurs can be expressed as bioaccumulation factor (BAF). BAF is the ratio of the metal concentration in an organism to that in the surrounding medium, at steady state (Drexler *et al*., 2003) and are generally derived from measurements in natural environments. BAF is determined by using the formula by Drexler *et al*. (2003) given below;

$$\text{BAF}\left(\frac{x}{y}\right) = \frac{\text{Concentration of heavy metal in x}}{\text{Concentration of heavy metal in in y}} \quad \text{(2-1)}$$

Whereby, the variables x and y represent matrices that are compared. For this study, parasites (x) and fish muscle (y).

Bioconcentration factor (BCF) is another aspect that is related to bioaccumulation of heavy metals in aquatic organisms. It can be defined as the ratio of metal concentration in an organism to metal concentration in water, at a steady state (Abel, 1989). BCFs are usually used to determine the ratio of heavy metal concentration between fish and environment (water and sediment), as well as to determine the partitioning of elements between different samples (Davies and Dobbs, 1984). BCF is determined using the formula by Abel (1989) as follows;

$$BCF = \frac{C\ (\text{fish muscle})}{C\ (\text{water})} \quad \ldots\ldots \quad (2\text{-}2)$$

$$BCF = \frac{C\ (\text{fish muscle})}{C\ (\text{sediment})} \quad \ldots\ldots \quad (2\text{-}3)$$

Where: C stands for mean concentration.

2.1.3 Effects of heavy metals in humans

The global health impacts from toxic pollutants such as heavy metals are greater than previously thought. Toxic pollution causes immense harm to humans, especially children (Campbell *et al*., 2003). Human beings usually get exposed to heavy metals in a few different ways, primarily through drinking water or food (crops can uptake metals from contaminated soil or meat and fish products may contain bioaccumulated metals). Bioaccumulation in the food chain starts from the smallest microorganisms and ends in the human body (Drexler *et al*., 2003). Therefore, at every point in this process, heavy metals bioaccumulate in the bodies of each living organism and it is highest at the top consumer level. By the time the contaminated fish are ingested by human beings, the heavy metals are already present in high concentrations. The buildup of heavy metals up the food chain is known as biomagnification (Drexler *et al*., 2003).

Unfortunately, heavy metals can have serious health effects for humans. Many play a role in cancer development or cause internal organ damage, even at low concentrations. For example, the health effects of Lead (Pb) exposure includes neurological damage, anemia, nerve disorders, and a number of other health problems (Mutia *et al*., 2012). On the other hand, Mercury (Hg) is a powerful neurotoxin and can cause severe damage to the brain and kidneys (Campbell *et al*., 2003). Inhalation of mercury can also cause lung, stomach, and intestinal damage, and even death due to respiratory failure. The primary health effects from chromium (Cr) are damage to the gastrointestinal, respiratory, and immunological systems, as well as reproductive and developmental problems (McCartor and Becker, 2013). Arsenic (As) has long been recognized as a poison, and large oral doses can cause death. Lower doses of arsenic can cause decreased production of red and white blood cells, and arsenic poisoning is often characterized by visible changes in the human skin (Senesi *et al*., 1979).

2.2 Parasitism in fish

2.2.1 General categories of fish parasites

Parasites are organisms that live in or on another organism (host) and gain an advantage at the expense of the host (Northrop-Clewes and Shaw, 2000). Parasites are grouped according to their location in/or on the host; ecto-parasites include those organisms living outside the body of the host while endo-parasites are those living within the body of the host. Several studies have revealed rich parasitic fauna in freshwater fishes (FAO, 1996) ranging from ectoparasites to endo-parasites (Auta *et al*., 1999) which affect fish health, growth and survival. The parasitic groups include the protozoans which include microsporideans and myxozoans and helminths such as monogeneans and the digeneans, trematodes (flukes), cestodes (tapeworms), nematodes (roundworms) and acanthocephala (thorny headed worms). The arthropod parasites are represented mainly by the copepods, while the annelid parasites are represented by the leeches. These parasites occupy different locations in/on their host. For instance, tapeworms are located inside the body cavity of the fish while the leeches are found on the fish skin (FAO, 1996).

Cestodes include tapeworms which are widespread in all major water systems of Africa and demonstrate a high degree of host specificity (FAO, 1996). There are two main forms of cestodes; the monozoic forms notably Caryophyllaeidae and the amphilinid represented by the segmented Pseudophyllideans and Proteocephalideans (Van As, 1984). Cestodes occur in the digestive tracts, except the amphilinid, *Nesolecithus africanus*, which occurs in the coelomatic cavity of its host, the mormyrid, *Gymnachus niloticus* and *Polyonchobothrium clarias* in the gall bladder of *Clarias mossambicus*. Cestodes are known to have the ability to accumulate heavy metals far above the levels commonly detected in the tissues of their hosts and the environment (Jirsa *et al*., 2008).

This study focused on the cestode (*Ligula intestinalis*) because of its ability to accumulate the heavy metals from the host fish (Oyoo-Okoth *et al*., 2010) and most importantly because of its specificity to the cyprinid fishes (Hoole, 1994), among them *E. paludinosus* (Britton *et al*., 2009). *Ligula intestinalis* is a tapeworm that infects cyprinid fishes and fish-eating birds which is indigenous to southern Africa (Prudhoe and Hussey, 1977). Its eggs are shed in the birds'faeces and hatch in water to form infective coracidia, which are ingested by copepods (Hoole, 1994). Cyprinid fish become the second intermediate hosts when they eat parasitized copepods. By ingesting the parasitized copepods, the fish get infected by the second-stage larva or plerocercoid. Its final host is the piscivorous bird after ingesting the fish that is infected with the plerocercoid (Hoole, 1994).

2.2.2 Effects of parasitism in fish

There are some effects that come as a result of parasitism in fish. Altered foraging behavior, is one of the effects of parasitism in fish. This consequently force the fish to increase the proportion of time spent foraging than their usual time in order to meet the extra demands of coping with parasite infection (Giles, 1987). Secondly, parasitic infections are known to affect many aspects of host physiology potentially altering its locomotion. Effects such as atrophy of the musculature and pathology of the nervous system are likely to interfere with normal swimming movements of fish (Bone *et al*., 1995). Another effect of parasitism in fish is the morphological changes, such as black or white spots, swellings or color changes resulting from demelanisation. Fishes are often cryptically colored, patterned or counter-shaded in a way that makes it difficult for predators to detect them visually (Ness and Foster, 1999). Thus, infection by certain parasites may disrupt this camouflage and make host fish more visible to their predator.

In a study of the biological and ecological effects of *Ligula intestinalis* infection on cyprinid hosts in Lough Neagh, Northern Ireland, Bean and Winfield (1989) found that *Ligula* infection was associated with changes in the horizontal distribution of larger gudgeon (*Gobio gobio,* Cyprinidae) hosts at certain times of year. During the summer both uninfected and infected fish occupied the shallower inshore waters. However, during the autumn, when uninfected fish moved offshore to in deeper waters, infected fish were found to be randomly distributed in both the shallow and deep waters. Thus, this shows that some infected fish, which presumably have a higher nutritional requirement, delay their autumnal offshore migration to take advantage of the high-quality foraging environment at inshore waters.

2.3 Length-weight relationship, condition factor (K) and sex ratio of fish

Length-weight relationship (LWR) a useful tool in fishery assessment, which helps in predicting weight from length required in yield assessment and in the calculation of biomass (Martin-Smith, 1996). The relationship between the length (L) and weight (W) of a fish is usually expressed by Keys (1928) equation below;

$$W = aTL^b \quad \text{(2-4)}$$

Where W is the weight of fish in grams, a, is the intercept, TL is the total length in centimeters and b is the allometry coefficient (slope of the regression line). Values of the exponent b provide information on fish growth. When b=3, increase in weight is isometric. This means that the weight of the fish depends on the volume of a three-dimensional object which is roughly proportional to the cube of length for a regularly shaped solid. Thus, exists a cubic relationship between weight and length of a fish (Kuriakose, 2017). When the value of b is less than 3 (b<3), this indicates negative allometric growth where the body shape is more elongated. On the other hand, when the value of b is more than 3 (b>3), it indicates a positive allometric growth meaning that specimens have an increased height or width more than their length (Froese, 2006). Therefore, length and weight relationship is a useful tool that provides important information concerning the structure and function of fish populations (Anderson and Neumann, 1996).

The condition factor (K) is an index reflecting interactions between biotic and abiotic factors in the physiological condition of fish. It shows the population's welfare during the various stages of the life cycle (Blackwell *et al*., 2000). Condition factor has been generally described as the well-being or robustness of an individual fish (Blackwell *et al*., 2000) and has typically been estimated by comparing individual fish weight of a given length to a standard weight. Fish condition can be used to characterize components of the environment in which the fish exists for instance, habitat, prey availability, competition. Condition factor is determined using the formula by Le Cren (1951) expressed as;

$$K = \frac{100\ W}{L^b} \qquad (2\text{-}5)$$

Where, W is the weight of fish in grams, L is the total length in centimeters and b is the slope of the regression line obtained from the LWR equation. According to Barnham and Baxter (1998), a K value that is approximately 1.40 indicates a good and well-proportioned fish meaning that the fish is long and heavy (with a lot of flesh) while that of 1.20 indicates a fish of moderate condition. A K value of 1.00 on the other hand shows that the condition of the fish is poor which is long and thin with very little flesh.

A number of studies have been conducted on length-weight relationship and condition factor of different fish species in Lake Naivasha (Britton and Harper, 2006; Aera *et al*., 2014; Outa *et al*., 2014; Keyombe *et al*., 2015; among many others). For instance, Aera *et al*. (2014) reported an isometric growth for male *C. carpio* and allometric growth for females with a condition factor of >1. Outa *et al.* (2014) on the other hand obtained a negative allometric growth for both male and female *O. niloticus* and a condition factor of >1. Additionally, Keyombe *et al*. (2015) reported a positive allometric growth for males, females and combined sexes of *Clarias gariepinus* and a condition factor of <1. A study by Britton and Harper (2006) on *E. paludinosus* reported an isometric growth for combined sexes. Regarding sex ratio of fish from Lake Naivasha, a study by Mutia *et al*. (2010) reported a sex ratio of 1:1.6 on *E. paludinosus* obtaining more females (877) than males (550). Outa *et al.* (2014) on the other hand reported more *O. niloticus* males than females in their different sampling sites and thus recording a sex ratio of 2:1. To the best of my knowledge, none of the studies have reported on the condition factor and sex ratio of *E. paludinosus* from Lake Naivasha. Therefore, this study provides baseline knowledge on population structure of *E. paludinosus* in Lake Naivasha.

2.4 Conceptual issues of biomonitoring in aquatic systems

Biomonitoring is defined as a method of observing the impact of external factors on ecosystems and the development of these impacts over a period, or of ascertaining differences between one impacted location and another (Markert *et al*., 2003). Their study further describe that, measurements or endpoints used for aquatic ecosystems are selected from any level of biological organization such as sub-organismal, organismal, population, community, and ecosystem. Therefore, an ideal bioindicator as described by Hilty and Merenlender (2000) should be characterized by: (1) taxonomic soundness, that is, easy to be recognized by non-specialist; (2) wide or cosmopolitan distribution; (3) low mobility; (4) well-known ecological characteristics; (5) numerical abundance; (6) suitability for laboratory experiments; (7) high sensitivity to environmental stressors and (8) high ability for quantification and standardization. Bioindicators show both the long-term interaction of several environmental conditions as well as reaction to sudden changes of different environmental factors. Macroinvertebrates, periphytons and fishes are the most frequently utilized bioindicators in aquatic ecosystems (Coste *et al*., 2008).

2.4.1 Fish as bioindicators of pollution

The use of an index that evaluates water quality through biological parameters, such as freshwater ecosystem structure and performance, has considerably increased in recent years and has gained recognition as an important measure for calculating the global integrity of freshwater ecosystems (Ollis *et al*., 2006). Indices based on aquatic biota have been widely successful in determining the integrity of aquatic ecosystems (Karr, 1987). Fish and macroinvertebrate assemblages have been highlighted as good bioindicators and have been used for decades in monitoring ecosystem degradation related to farming and forestry, as well as to urban and industrial effluents (Fierro *et al*., 2015; Plessl *et al*., 2017). A notable advantage of using these aquatic biota is the relative simplicity of their capture and sampling (Merrit and Cummins, 2007). Although fish communities may have a high degree of natural variability, they can be useful indicators of ecosystem health. They can be used as both effect and accumulation indicators, whereby the effect indication is the technique which is mostly used (where their presence or absence tells the condition of that particular aquatic ecosystem). On the other hand, the accumulation indication is used by determining the levels of contaminants accumulated in the body organs of the fish to explain the condition of that particular aquatic ecosystem.

Different fish tissues have been found to have different accumulation capacities which are related to the metabolic roles of the heavy metals and the functions of organs, among other factors. Thus, active metabolic organs such as the liver often sensitively accumulate larger amounts of metals than the muscle (Otachi *et al*., 2015a; Plessl *et al*., 2017). For example, Uysal *et al*. (2009) reported higher concentrations of heavy metals in the liver than in the muscle of several fish species belonging to the family Cyprinidae in Enne Dame Lake in Turkey. On the contrary several studies have found concentrations of heavy metals to be higher in the fish muscle than in the liver (Wang *et al*., 2010; Otachi *et al*., 2014a). Intestines have also shown the potential to accumulate heavy metals (Sures *et al*., 1999; Nachev *et al*., 2010). A good example is Manganese (Mn) accumulation in the intestines of Perch (*Perca fluviatilis*), from the subalpine Lake Mondsee, Austria as documented by Sures *et al*. (1999). Heavy metals also accumulate in the skeleton and gills of fish (Moiseenko and Kudryavtseva, 2001; Ondračková, *et al*., 2011). For example, Moiseenko and Kudryavtseva (2001) reported a high Stromium (Sr) concentration in the skeleton and gills of white fish *Coregonus lavaretus* and brown trout *Salmo trutta* in the Kola Region, Russia. However, no single study has reported the levels of heavy metals in *E. paludinosus* and its endo-parasite, the cestode, *L. intestinalis.*

2.4.2 Fish parasites as bioindicators of pollution

Fish parasites have been recognized for their bioindicative potential regarding pollution in the last decades. The main pathways used by assemblages of aquatic parasites in response to pollutants were reviewed by Poulin (1992) who showed that parasite communities are influenced indirectly by pollutants which are toxic both to fish and invertebrate hosts and directly to the parasites and in their free-living stages. Variable responses towards exposure to pollution have been identified at the population and community level of a number of parasites.

Since parasites are sensitive to environmental change, others are more resistant than their hosts and tend to increase in numbers in polluted conditions (Sures, 2004). Parasites can be useful in bioindication in two ways: Effect indicators; for example, where the presence or absence of a certain parasite can indicate environmental conditions and secondly, accumulation indicators where some parasites have been found to have a remarkable ability to accumulate toxins in their tissues (Sures, 2001). For example, certain parasites, particularly intestinal acanthocephalans (Thielen *et al*., 2004) and cestodes can accumulate heavy metals at concentrations that are orders of magnitude higher than those in the host tissues or the environment.

The use of intestinal parasites as indicators of environmental quality have attained growing interest over the years (Sures *et al*., 1994; Nachev and Sures, 2009; Plessl *et al*., 2017). Fish, parasites and metal interactions have generated large pool of literature in environmental pollution monitoring perspective (Sures, 2001; Sures, 2003; Thielen, *et al*., 2004; Tekin-Özan and Kir, 2005; Tekin-Özan and Barlas, 2008). Studies have mostly involved endo-helminths of which acanthocephalans and cestodes appear to be the best investigated taxa (Sures, 2001; Sures, 2003; Jirsa *et al*., 2008 and Oyoo-Okoth *et al*., 2010). A major conclusion from these literatures revealed that for different host– parasite associations, intestinal parasites accumulate a number of metals to levels several hundred times higher than the levels in host tissues thus rendering parasites sensitive metal accumulation biomonitors than their fish host but varying considerably from species to species (Sures, 2004). A decade ago, Sures *et al*. (2007) indicated that parasites can also act as metal sinks for their fish host.

2.5 The fisheries and fish parasites of Lake Naivasha

2.5.1 Fisheries

The Lake Naivasha fishery is based on introduced fish species namely; Blue-spotted tilapia (*Oreochromis leucostictus*), Nile tilapia (*Oreochromis niloticus*), Redbelly tilapia (*Coptodon zillii*), Largemouth bass (*Micropterus salmoides*), Common carp (*Cyprinus carpio*) and the riverine Straightfin barb (*Enteromius paludinosus*) (Hickley *et al*., 2002) with the African catfish (*Clarias gariapinus*) being the most recent invader (KMFRI, 2017). In 1959, a commercial gill net fishery was opened in the lake for exploiting tilapias. A fishing ban on *M. salmoides* and *C. zillii*, was enforced in 2001 in order to reduce fishing pressure on these two fish species (Kundu *et al*., 2010). This was followed by an imposed closure for five months from 1st June – 1st October since 2003 (Njiru *et al*., 2017). However, currently fishing is carried out throughout the year.

Between the year 1987 and 2008 the fishery was dominated by *O leucostictus*, *C. zillii*, *M. salmoides* and *C. carpio* (Hickley *et al*., 2004; Ojuok *et al*., 2008). However, as from 2015, the fishery was dominated by *C. carpio*, *O. niloticus*, *O. leucostictus* and *C. gariepinus* (KMFRI, 2017; Njiru *et al*., 2017) clearly showing the decline in abundance of *C. zillii* and *M. salmoides* and the introduction of *O. niloticus* and *C. gariepinus*. Notably, there is hardly any straightfin barb (*E. paludinosus*) fishery documented previously, however it still an important species in Lake Naivasha riverine fisheries. In the fish catches, there have been great variations in the total amount of fish caught over the years with the highest and the lowest documented annual catch of 1181 tonnes per year and 21 tonnes per year in 1970 and in 2015, respectively (Hickley *et al*., 2004; Njiru *et al*., 2017).

2.5.2 Biology and ecology of *E. paludinosus*

Straightfin barb (*E. paludinosus*) is a species of ray-finned fish in the Cyprinidae family. It is a benthopelagic species and occupies a wide range of habitats including large rivers both vegetated and rocky, lagoons both connected to and isolated from main river channels, and small and large streams (Tweddle and Skelton, 2008), and impoundments both large and small. It feeds on a wide range of small organisms including insects, small snails and crustaceans, algae, and detritus. It is preyed upon by the catfish, tiger fish and birds. It spawns among vegetation (Skelton, 2001). Spawning takes place up in the influent rivers during the rainy season. Migration upstream appears to correspond to periods of heavy rainfall or flushing. They are multiple spawners laying from 250-2,500 eggs. Its maximum size is 150 mm SL (Standard Length) (IUCN, n.d) (Plate 1a) with a total of 8 dorsal soft rays and 6-7 anal soft rays (Plate 1b).

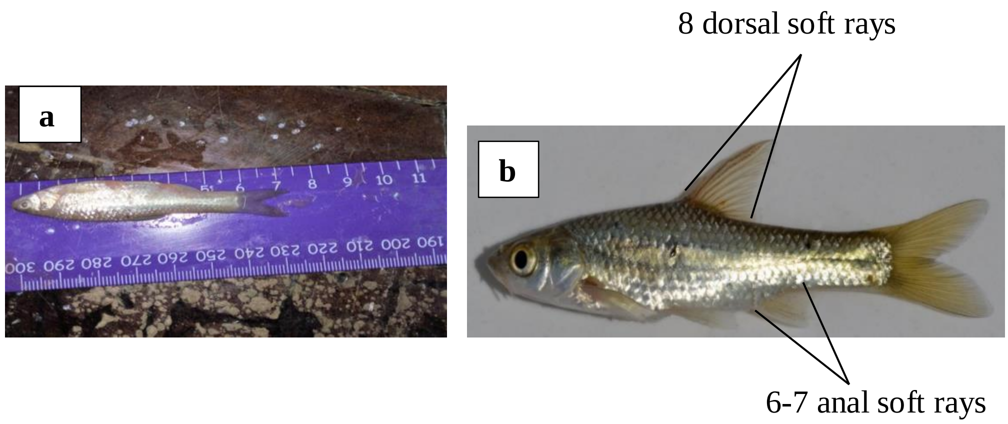

Plate 1: An illustration of *E. paludinosus* showing the general characteristics: (a) Length and size (approximately 7.4 cm) and (b) External characteristics (Source: author)

The fish is abundant in freshwater aquatic systems and its benefits are commercial fisheries and used as food. The distribution of straightfin barb ranges from Ethiopia in the North, throughout east and central Africa, extending westwards into Angola and reaching the southern-most limits of its distribution in Natal, South Africa. In Kenya, it is found in the Lake Victoria basin, Athi and Tana River systems, northern and southern Ewaso Nyiro basins. The fish also occurs in the upper Pangani system, Amboseli swamps, Lake Naivasha and affluents (Seegers *et al*., 2003). In Lake Naivasha, the straightfin barb is usually confined to the north swamp and at the mouth of River Malewa but also exhibits a lake-wide distribution (Aloo *et al*., 2013).

2.5.3 Fish parasites of Lake Naivasha

A number of studies have been conducted on fish parasites in Lake Naivasha (Aloo, 2002; Otachi *et al*., 2014a; Otachi *et al*., 2014b; Otachi *et al*., 2015b; Rindoria *et al*., 2016). Some of the common parasites that affect the fish in Lake Naivasha such as *O. leucostictus*, *M. salmoides*, *C. zillii* and *E. paludinosus* are the helminths, nematodes, acanthocephalans, trematodes and cestodes (Aloo, 2012). Additionally, a study by Rindoria *et al*. (2016) reported gill monogeneans in *O. niloticus* and *O. leucostictus* from Lake Naivasha. Certain parasites such as the cestode, *Ligula intestinalis* are highly host specific (Britton *et al*., 2009). The specific hosts that are infected are cyprinids such as the *E. paludinosus* and *Barbus lineomaculatus* (Britton *et al*., 2009).

Several studies has been conducted on different fish parasites to determine their potential to accumulate heavy metals in Lake Naivasha. For instance, Otachi *et al*. (2014a) reported heavy metal levels in the nematode, *Contracaecum multipapillatum* hosted by *O. leucostictus*. This parasite showed potential to accumulate trace elements but not an efficient accumulation bioindicator since the heavy metal levels accumulated were much lower than those of its host. Britton *et al*. (2009) on the other hand focused on the host specificity and behavioural alterations caused by the cestode, *L. intestinalis*. The study reported that *L. intestinalis* is host specific and especially found in *E. paludinosus* in Lake Naivasha.

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CHAPTER THREE

3.0 SOME BIOLOGICAL ASPECTS OF STRAIGHTFIN BARB, *Enteromius paludinosus* (PETERS 1852) DURING THE RAINY SEASON IN LAKE NAIVASHA, KENYA

3.1 Abstract

This chapter focuses on the first specific objective which was to determine the length-weight relationship (LWR), condition factor (K) and the sex ratio, of the migratory *E. paludinosus*. A total of 1279 straightfin barb were collected from the mouth of River Malewa, a major source of water for Lake Naivasha using seine nets of mesh size 1.2 mm from November, 2017 to February 2018. The fish were caught and transported to the laboratory, then killed humanely by cervical dislocation and thereafter their lengths (cm) and weights (g) determined followed by dissection to examine their gonads. The length-weight relationship was determined using the formula $W= aTL^b$. The condition factor (K) was determined using the formula $K = 100 W/ L^b$. A student's t-test was used to test for any significant differences in the weight and length of both sexes. The sex ratio, expressed as male: female was calculated by dividing the number of females by that of the males. The equations obtained for the LWR of the combined sexes, males and females of *E. paludinosus* were, log W = 3.23 log L + log 0.11, log W = 2.83 log L + log 0.15 and log W = 2.87 log L + log 0.15 respectively. The mean condition factor of the combined sex, males and females of *E. paludinosus* were; 0.59, 0.57 and 0.60 respectively. The sex ratio of the fish was 1: 1.98 (males: females). The results showed that *E. paludinosus* in Lake Naivasha are not in a good condition, K <1. The findings of this study may be attributed to the state of the lake due to the reported pollution increase and ecological changes by recent studies.

3.2 Introduction

Lake Naivasha is one of the most valued resources in the Rift Valley. It is a Ramsar site since 1995, a wetland of international importance (LNRA, 1999). There have been numerous studies in the Lake focusing on different aspects: fish species composition (Siddiqui, 1979; Aloo *et al*., 2013), ecology (Harper *et al*., 1990; Hickley *et al*., 2015), pollution (Onyari, 1981; Kitaka *et al*., 2002; Jimoh *et al*., 2007), fish biology (Oluoch, 1990; Hickley and Harper, 2002), fish parasitology (Aloo, 2002; Otachi *et al*., 2014; Rindoria *et al*., 2016). For instance, a study by Hickley *et al*. (2002) in Lake Naivasha found the following fish species at that time: common carp (*Cyprinus carpio*), the blue-spotted tilapia (*Oreochromis leucostictus*), largemouth bass

(*Micropterus salmoides*), redbelly tilapia (*Coptodon zillii*) and straightfin barb (*E. paludinosus*).

A number of investigations on heavy metals and other trace elements have also been carried out in the lake (Ochieng *et al*., 2007; Mutia *et al*., 2012). Jimoh *et al*. (2007) identified the source of pollutants into the lake from agricultural activities in the catchment which find their way into the lake through River Malewa including runoff from Naivasha town. Kitaka *et al*. (2002) reported an increase in nutrient levels such as phosphorous while Mutia *et al*. (2012) reported an increase in levels of heavy metals such as Lead (Pb), Copper (Cu) and Cadmium (Cd), thus, affecting the quality of the lake. This has consequently stimulated investigations on the health status of the biota including fish and whether or not they get affected by the changes in the quality of the environment that they live in. This is typically done by determining the LWR and condition factor (Britton and Harper, 2006; Outa *et al*., 2014; Keyombe *et al*., 2017).

LWR is a vital aspect in fishery assessment that aids in the estimation of weight from length needed in the computation of biomass and in fish harvest assessment (Garcia *et al*., 1998). For example, a study by Britton and Harper (2006) on LWR of five species which were then found in Lake Naivasha, indicated that fish had a good growth ratio indicated by isometric growth. Several fish species from Lake Naivasha have depicted differences in sex ratios (Siddiqui, 1977; Mutia, 2006; Mutia *et al*., 2010; Outa *et al*., 2014). Different fish species have different sexes dominating in the population. For example, studies on *E. paludinosus* by Mutia (2006) and Mutia *et al*. (2010) revealed a dominating female population as opposed to male fish species like for instance *O. leucostictus* and *O. niloticus* whose males dominate their population (Siddiqui, 1977 and Outa *et al*., 2014) respectively.

Enteromius paludinosus was first recorded in Lake Naivasha in the 1920s and disappeared to reappear later in 1982 (Hickley *et al*., 2002) as it inhabits both River Malewa and the lake. Even though it is not common in the fish markets, it is used as a source of protein by the people who live around the lake (Aloo *et al*., 2013). Several studies have been conducted on different aspects of this fish species in Lake Naivasha. For example, on fish parasites (Britton *et al*., 2009), spawning seasons in the lake (Mutia *et al*., 2010), the LWR (Britton and Harper, 2006) and sex ratio (Mutia, 2006; Mutia *et al*., 2010). However, all these earlier studies were carried out more than a decade ago. A lot of changes have occurred in the lake (increased pollution, rise of heavy metal levels, fish species composition and changes in water levels as well as the ecology of the lake) which may have impacted on this fish species. The main focus of this study was to establish sub lethal effects through assessing the length-weight relationship, condition factor and sex ratio of *E. paludinosus* in Lake Naivasha.

3.3 Materials and methods

3.3.1 Study area

Sampling was done in the month of November, 2017 to February, 2018 in Lake Naivasha. The description of the study site has been vastly done by Gaudet and Melack (1981), Kitaka *et al*. (2002), Odongo *et al*. (2015). Lake Naivasha was declared a Ramsar site in 1995 in recognition of its global importance to wildlife (LNRA, 1999) particularly with more than 400 species of water birds. The surface inflows to the lake come via three main river systems; Gilgil, Malewa and Karati. The Malewa system is the largest with an estimated annual flow of 153 million m^3, Gilgil has an estimated average annual flow of 24 million m^3, whereas Karati only flows intermittently during the rainy season (Ase, 1987). This study was conducted at the mouth of River Malewa (0.714622°S 36.362709°E) as indicated in Figure 1.

3.3.2 Sample collection and analysis

Fish samples were collected twice a month for three months during the rainy season using seine nets of mesh size 1.2 mm from the mouth of River Malewa. The fish were caught then placed into the aerated water tanks. Thereafter, they were transported alive in the tanks to a research laboratory at the Biological Sciences Department, Egerton University. In the laboratory, the fish were killed humanely by cervical dislocation. This was then followed by measurements of the Total Lengths (TL) in cm using a measuring board. The weights of the fish were measured in g using an electronic weighing scale, (ED 4202S, Sartorius AG, Germany). The fish were then dissected to examine the gonads following the standard procedures according to Florio *et al*. (2009).

The length-weight relationship (LWR) was calculated using guidelines by Keys (1928) as follows;

$W = aTL^b$ ………………………………………………...………………………... (3-1)

Where

W= the body weight of fish in grams

TL= the total length in centimeters

a= the intercept

b =the slope of the regression line

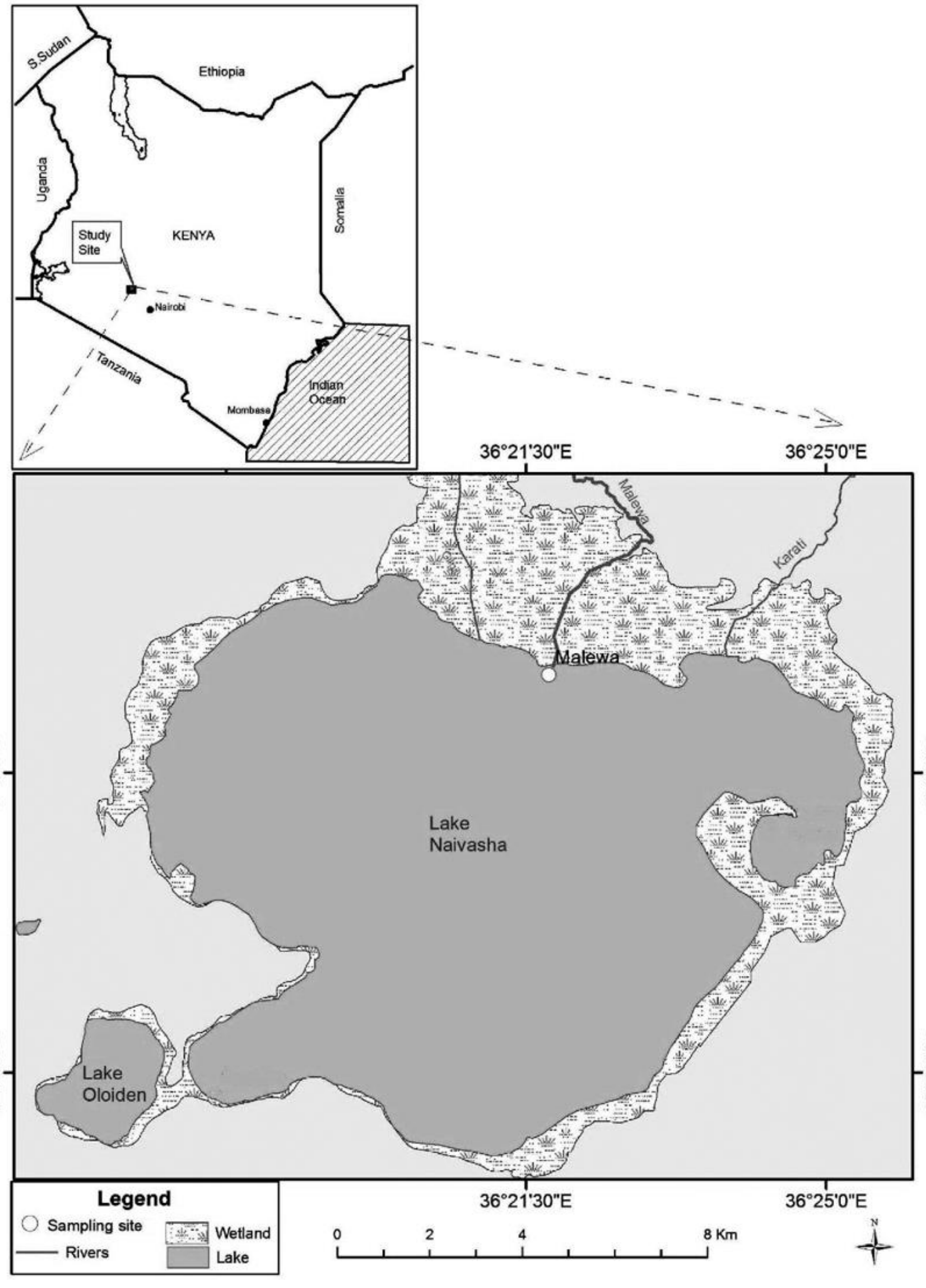

Figure 1: Map of Lake Naivasha showing the sampling point at the mouth of River Malewa (Source: Redrawn from the Survey of Kenya map sheet; No.118/4,119/3 and 132/14)

In this equation, the parameters a, and b, which are termed as length weight parameters were estimated from length-weight data. The parameter a, is a scaling coefficient for the weight per length while parameter b is a shape parameter for the body form of the fish species. Thus, when b = 3, fish is said to be growing isometrically; if the length increases with equal proportion to the body weight for constant specific gravity (Olurin and Aderibigbe, 2006; Taylor *et al.*, 2010) while negative ($b < 3$) or positive ($b > 3$) allometric growth is assumed when the increase in any of the parameters (length or weight) is unproportional to the other.

Since the growth of fish length and weight is not directly proportionate, a linear transformation was made to estimate the a and b parameters using natural logarithm of the observed lengths and weights according to Keys (1928) as follows:

$$\log W = b \log L + \log a \quad \text{(3-2)}$$

The condition factor (K) was determined using the formula by Le Cren (1951) as follows:

$$K = \frac{100\ W}{L^b} \quad \text{(3-3)}$$

3.3.3 Statistical tests

A student’s t-test was used to test the significant differences in the weight and length of both sexes from the data obtained from this study, after log transformation. The sex ratio, expressed as male: female was calculated by dividing the number of females by that of the males and null hypothesis tested with the use of chi-square test.

3.4 Results

3.4.1 Length-weight relationships (LWR)

A total of 1279 fish were measured. Total length ranged from 6 to 11.1 cm and weight from 1.86 to 13.84 g. The mean length and standard deviation (SD) was 8.48 ± 1.02 while that of the weight was 6.20 ± 2.34. The length-weight relationships were separately evaluated for all individuals and grouped by sex (males and females). The results are presented in Figure 2 a, b and c. When both sexes were combined, the regression slope, b (Figure 2 a) obtained a positive allometric growth (b value of 3.23). A negative allometric growth was obtained for the males (Figure 2 b) and females (Figure 2 c) with b values of 2.83 and 2.87 respectively as shown in Table 1 alongside the a values.

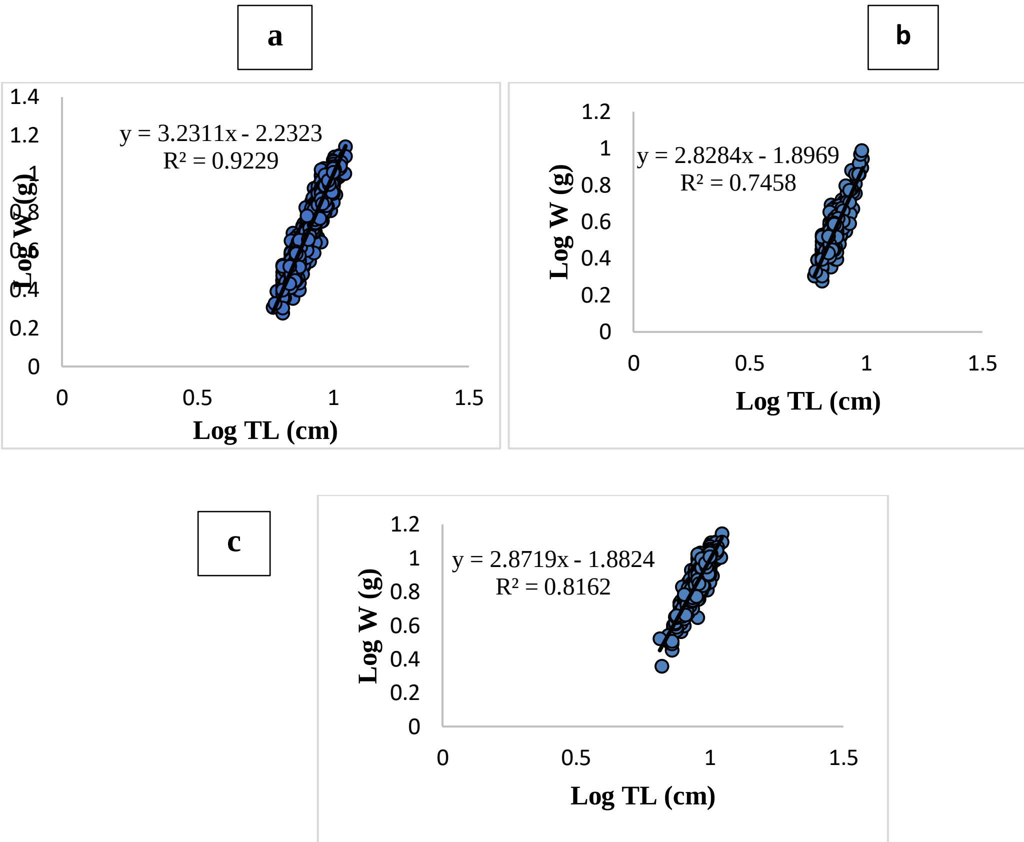

Figure 2: Length-Weight Relationships of *E. paludinosus*; (a) for both sexes, (b) for males and (c) females

Table 1: Length-weight relationship of *E. paludinosus*

Sex	**n**	**Length range (cm)**	**a**	**b**	**R^2**	**p**
Both sexes	1279	6 - 11.1	0.11	3.23 (+)	0.92	<0.05
Male	429	6 - 9.7	0.15	2.83 (-)	0.75	<0.05
Female	850	6.5 - 11.1	0.15	2.87 (-)	0.82	<0.05

n, sample size; (+), positive allometry (b>3); (-), negative allometry (b<3): r^2, coefficient of determination: p, probability

3.4.2 Condition factor

The mean condition factor and the standard errors were 0.59 ± 0.002, 0.60 ± 0.002 and 0.57 ± 0.003 for both sexes combined, the females and males respectively. There was a significant difference between the K factor values of the males and females within the lake (student's t-test, $p < 0.05$).This is illustrated in Figure 3.

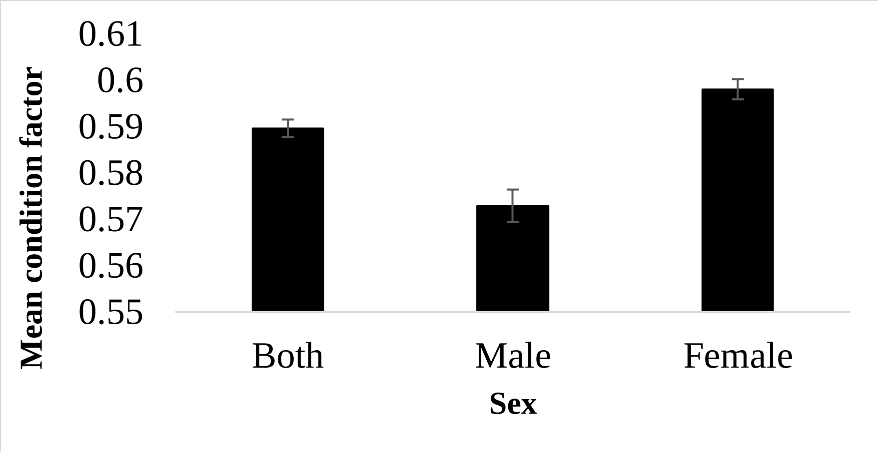

Figure 3: Mean condition factors of combined sexes, male and female *E. paludinosus* in Lake Naivasha

3.4.3 Sex ratio

Out of the total number of the fish samples, female fish dominated (850) while the males were 429. The sex ratio was calculated by dividing the number of female fish by that of the males. The sex ratio was less than 1 (< 1) which was 1: 1.98 (males: females). The Chi-square test showed a significant difference between the sexes ($p < 0.05$).

3.5 Discussion

3.5.1 Length- weight relationship

The length–weight relationships were significant ($p < 0.05$), with values of $R^2 > 0.70$. The expression was log-transformed as suggested by Keys (1928). The equation of both sexes combined was log W = 3.23 log L + log 0.11, indicating a positive allometric growth since b was greater than 3 (b>3), indicating that the fish were in in good growth condition, with the height or width being more than length. The equation for males was log W = 2.83 log L + log 0.15 and females, Log W = 2.87 log L + log 0.15, both indicating a negative allometric growth because b was less than 3 (b<3). The result of combined sexes signified a positive allometric growth. This is different from that of Britton and Harper (2006) who reported a and b values of 0.0173 and 2.904 for *E. paludinosus* indicating an isometric growth (b=3). The variation in LWR of this study could be due to the temporal of the lake variation of some ecological aspects such as water quality, pollution levels coupled with change in ecology of the lake over the last decade since last similar study was carried on. This was evident in the massive fish kills, observed in February 2010, caused by rapid changes in water quality including a decrease in oxygen to lethal levels leading to hypoxic conditions (Njiru *et al*., 2015).

A study by Giannetto *et al*. (2015) on *Barbus pergamonensis*, from the Aegean region in Turkey reported a positive allometric growth of 3.13 similar to observation of this study. Also, Marčeta (2013) on *B. balcanicus* from Slovenia obtained a positive allometric growth with a b value of 3.05. However, Bibak *et al*. (2013) and Dan-kishiya (2013) reported a negative allometric growth for the *B. grypus* (from Dalaki River in Iran) and *B. occidentalis* (from Lower Usuma Reservoir in Nigeria) with b values of 2.93 and 2.11 respectively.

According to Pervin and Mortuza (2008), high b values show a general state of great demand of food as well as the gonad content of the fish. Thus, a remarkable increase in weight is reached upon the consumption of the foods which aid in growth and energy gain (Kamaruddin *et al*., 2012). However, b values could vary due to factors like growth period, sex, gonad development and the contents of the stomach (Pervin and Mortuza, 2008). Moreover, b values depend considerably on conditions such as environmental settings, biological, geographical, seasonality as well as other factors including sampling procedures (Froese, 2006).

3.5.2 Condition factor

The condition factor (K) of both males and females *E. paludinosus* in this study was less than 1 (K= 0.59) indicating that the fish species were in a very poor condition. According to Barnham and Baxter (1998), a K value that is approximately 1.40 indicates a good and well-proportioned fish meaning that the fish is long and heavy (fleshy) while that of 1.20 indicates a fish of moderate condition. A K value of 1.00 on the other hand shows that the condition of the fish is poor (long and thin) with very little flesh. There was a significant difference between the K factor of the males and females ($P < 0.05$). The mean condition factor (K) reported during this study for female fish species was slightly higher (K= 0.60) than that for the males (K= 0.57) indicating that females were in a slightly better condition than the males fish species examined. The environmental conditions could have led to the poor condition of *E. paludinosus* indicated by very low K values such as the sub lethal effects of pollution in the lake as reported in the studies by Ochieng *et al*. (2007), Mutia *et al*. (2012), Otachi *et al*. (2014), among many others. Heavy metals such as Lead (Pb), Copper (Cu) and Cadmium (Cd) were found to be among the pollutants in Lake Naivasha where agricultural activities in the catchment was identified as their main source (Mutia *et al*., 2012). Other heavy metals reported in the lake are Cobalt (Co), Chromium (Cr), Stannum (Sn) and Zinc (Zn) (Ochieng *et al*., 2007). Furthermore, Otachi *et al*. (2014) reported detectable levels of Cd, Pb, Cu, Zn, Stromium (Sr), Rubidium (Rb) and Molybdenum (Mo) from the sediment samples of Lake Naivasha. The presence of such pollutants could have sparked changes in the lake through contamination hence affecting the biota with, *E. paludinosus* included as shown by the low K values obtained in this study. Some of the toxic effects of heavy metals in aquatic organisms reported include bioaccumulation in various organs, tissue damage, disruption in growth and reproduction and induction and synthesis of metallothionein (Bryan, 1971). There is hardly any information on the condition factor of *E. paludinosus* from Lake Naivasha, since the baseline study of Britton and Harper (2006), only provided LWR.

3.5.3 Sex ratio

The sampled fish was dominated by female fish with a sex ratio of 1: 1.98. This finding was slightly different from that of Mutia *et al*. (2010) in Lake Naivasha where the females were 877 and males 550 with a sex ratio of 1:1.6 although similarly the females were more than males. These results shows that female dominance continues despite an eleven-year time difference since the baseline study was conducted. The significant difference in sex ratio could be as a result of a number of factors. There could be behavioral variances between the sexes that might have made females more susceptible during sampling hence easily trapped in the active gear such as seine nets used to catch the fish in this study. Another possibility for the significant difference in sex ratio is the migratory patterns between the males and females that might differ depending on the seasons (Matsuyama *et al*., 1988). Other possible determining factors could be the difference in growth rate between the sexes whereby females attain larger body size than their male counterparts where females dominate when there is plenty of food available (Nikolsky, 1963). It was evident that *E. paludinosus* were migrating upstream for spawning at the time of sampling. This was confirmed by the morphologically ripe gonads observed for both sexes in this study.

3.6 Conclusion

The length-weight relationships of *E. paludinosus* obtained indicated that the population had a good growth ratio as shown by both sexes combined. Combined sexes produced a $b > 3$ which indicated a positive allometric growth while that of the males and females separated produced b values of ($b < 3$) indicating a negative allometric growth. This fish population was not in good condition according to the condition factor values (K) obtained. The results clearly indicate unhealthy population status of this fish species with a probability of depressed procreative potential. In addition, the species sex ratio revealed that there were more females than males confirming an unstable population of *E. paludinosus* in Lake Naivasha.

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CHAPTER FOUR

4.0 LEVELS OF HEAVY METALS IN THE STRAIGHTFIN BARB, *Enteromius paludinosus* (PETERS 1852) FROM RIVER MALEWA, NAIVASHA, KENYA

4.1 Abstract

There have been several studies on heavy metals in Lake Naivasha. However, none of them has reported the levels of Mercury (Hg), Arsenic (As) and Chromium (Cr). Moreover, there are no studies on the heavy metals concentrations in the Straightfin barb (*Enteromius paludinosus,* Peters 1852*)*, a fish species that hosts a parasite (*Ligula intestinalis*), the latter having been reported to have a high ability to absorb heavy metals from its host. This chapter therefore addresses the second specific objective on accumulation of heavy metals namely; Arsenic (As), Chromium (Cr), Lead (Pb) and Mercury (Hg) in water, sediments, tissues of *E. paludinosus* and its endo-parasite, *L. intestinalis* from the mouth of River Malewa in Lake Naivasha, Kenya. A total of 1307 fish were collected from November, 2017 to February, 2018. Water sample, sediment sample, 25 fish muscle tissues (5 infected with *L. intestinalis* and 20 non-infected) were isolated and heavy metal concentrations determined using the Thermal-electron atomic absorption spectrophotometer at the Lake Nakuru Water Quality Testing Laboratory. Additionally, a student's t-test was used to test the significant differences in mean concentrations of heavy metals between the fish and the parasite. The concentrations of heavy metals in the water sample was 0.076 and 0.001 mg/l for Pb and Hg respectively while that of As and Cr were below detection limit. Heavy metals concentrations in the sediment were below the lowest effect level in sediment (LEL), threshold effect concentration (TEC) in sediment, severe effect concentration in sediment (SEL) and the Shale values of sedimentary rocks thus showing no sign of pollution. In the muscle tissues of the fish, As, Cr, Pb and Hg showed high levels with mean concentrations of 5.07, 22.09, 45.21 and 1.55 mg/kg ww respectively. Bioconcentration factors further supported the observation that trace element accumulation was higher in fish compared to sediment and water. The Target Hazard Quotients of As, Cr, Pb and Hg obtained for both the female and male were >1 indicating a possible health risk associated with the consumption of *E. paludinosus*. The Bioaccumulation factors (BAF) for *L. intestinalis* were 2.41, 2.19, 5.86 and 5.14 for As, Cr, Pb and Hg, respectively, indicating the potential of the cestode in accumulation of heavy metals from the host hence qualifying to be used as an accumulation bioindicator.

4.2 Introduction

Heavy metals are natural components of aquatic ecosystems (Tayab, 1991; Ochieng *et al*., 2007). However, several human activities such as agricultural, mining, industrial and domestic use have increased their levels in the aquatic environments (Kamau *et al*., 2008). Aquatic organisms can be affected by elevated levels of heavy metals in their environment especially when they occur above threshold concentrations (Ochieng *et al*., 2007). Some of these heavy metals bioaccumulate in various organs of aquatic organisms (Martin, 1979) and may have lethal effects (Eisler, 1973). Bioaccumulation occurs when metal concentrations exceed the fish's capacity to regulate their absorption by body organs (Streit, 1998). When such contaminated fish are consumed by human beings, they pose a risk to their health and have been recognized as one of the top threats to human health (McCartor and Becker, 2013). For this reason, several studies have been conducted in various aquatic ecosystems in Kenya. For example, in Lake Naivasha, despite being a Ramsar site and a world heritage site, it is perceived to be heavily impacted by anthropogenic activities, resulting to a number of investigations on heavy metals (Ochieng *et al*., 2007; Mutia *et al*., 2012; Otachi *et al*., 2014; Yang *et al*., 2017).

Ochieng *et al*. (2007), Mutia *et al*. (2012), Otachi *et al*. (2014) and Yang *et al*. (2017) reported that there were elevated levels of heavy metals such as Aluminium (Al), Iron (Fe), Manganese (Mn), Zinc (Zn), Rubidium (Rb), Copper (Cu), Cadmium (Cd) and Lead (Pb) in the water, sediments and fish. However, none of the studies has investigated Hg, As and Cr. These three heavy metals together with Pb are considered the top four threats to human health (McCartor and Becker, 2013). Furthermore, there are no studies on the heavy metals concentrations in the Straightfin barb (*Enteromius paludinosus,* Peters 1852*)*. This fish species hosts an endo-parasite (*Ligula intestinalis*), that has been reported to have a high ability to absorb heavy metals from its host (Oyoo-Okoth *et al*., 2010). *E. paludinosus* is a benthopelagic fish species which is small in size (maximum size 15cm Standard Length), and occupies habitats such as large rivers, lagoons which are either connected or isolated from their main river channels as well as streams (small or large) (Tweddle and Skelton, 2008). This fish species is a subsistence source of proteins especially for artisanal fishers and their dependents (Aloo *et al*., 2013). The aim of this study was to determine the concentrations of THg, Pb, Cr and As in the fish tissues, associated parasites of *E. paludinosus* and assess the potential health risks for fish consumers around Lake Naivasha.

4.3 Materials and methods

4.3.1 Study area

Sampling was undertaken at the Mouth of River Malewa (0.714622°S 36.362709°E) in Lake Naivasha in the month of November 2017 to February 2018 (sampling location shown on the map provided in chapter three section 3.3.1). The study site has been significantly described by Gaudet and Melack (1981), Hickley *et al*. (2002), Kitaka *et al*. (2002), Otachi *et al*. (2014), Odongo *et al*. (2015). In the year 1995, Lake Naivasha was declared a Ramsar site giving it an international importance due to its freshness and diverse ecology (LNRA, 1999). The catchment soils are mainly sediments that are influenced by the volcanic origins of the basin rocks. Activities in the catchment include; agriculture (mainly the flower farms), production of geothermal power, tourism, recreation, and fishing for commercial purposes (Harper *et al.*, 2002). This has consequently resulted to environmental problems such as water extraction resulting in water level alterations, contamination, eutrophication, and alien species, as well as decline in fish stocks including biodiversity as a whole (Harper *et al.*, 2011).

4.3.2 Collection of water and sediment samples

Measurements of dissolved oxygen, temperature, conductivity and pH were taken *in situ* as described by APHA (2012), at approximately 10 cm beneath the water surface from the sampling site using a Multi probe water quality meter (Model Multi HQ40d, USA). Following the standard methods for water and wastewater examination (APHA, 2012), 500 ml of water sample was collected from similar depth using an acid washed plastic bottle then filtered straight away by a filter pump fitted with Whatman GFC filters into a plastic bottle. 2.5 ml of concentrated nitric acid (Analytical grade) was then added to the filtrate to avoid precipitation of the metals and adsorption at the surface of the bottles.

According to the procedure by IAEA (2003), a stainless Ekman grab sampler was used to collect a sediment sample from the lake bottom. Subsample was carefully taken from the sample that had not touched the metallic parts of the Ekman grab sampler to avoid contamination. The sample was then put in a plastic sample vial, which was then placed inside a cool box and carried to a research laboratory at the department of Biological Sciences, Egerton University. The water and sediment samples were then stored in the refrigerator at a temperature of -20 ^{o}C in the laboratory and later transported to the Lake Nakuru Water Quality Testing Laboratory (WQTL) for heavy metal determination.

4.3.3 Fish sample collection and preparation

1307 *E. paludinosus* were captured twice a month for three months from the mouth of River Malewa by fishermen following the procedure by the Environmental Protection (Water) Policy (2009) using seine nets of mesh size 1.2 mm during the rainy season. The fish specimen were transported alive to a research laboratory at the department of Biological Sciences, Egerton University using a fish water tank whose water is well aerated to provide sufficient air for the fish. In the laboratory, the fish were killed humanely through cervical disconnection. This was followed by taking total lengths (TL) measurements in centimeters (cm) using a measuring board following the description by Scott Weber and Govett (2009). Thereafter, the weights of the fish were measured in grams (g) using an electronic weighing scale (Model ED 4202S, Sartorius AG, Germany). The fish dissection procedure was then carried out following standard measures in parasitological analyses (Florio *et al.*, 2009). The cestode *Ligula intestinalis* was picked using plastic forceps from the body cavity of the fish as described by Scott Weber and Govett (2009). The parasites were placed inside plastic vials after washing them carefully with double distilled water and then stored in the refrigerator at a temperature of -20 $^{\circ}$C for heavy metal determination. The fish tissues (0.2 g) were obtained using a ceramic knife and plastic tweezers, cleaned with double distilled water, placed inside plastic vials and then stored in the refrigerator for heavy metal determination. An image of *L. intestinalis* and *E. paludinosus* is shown in plate 2.

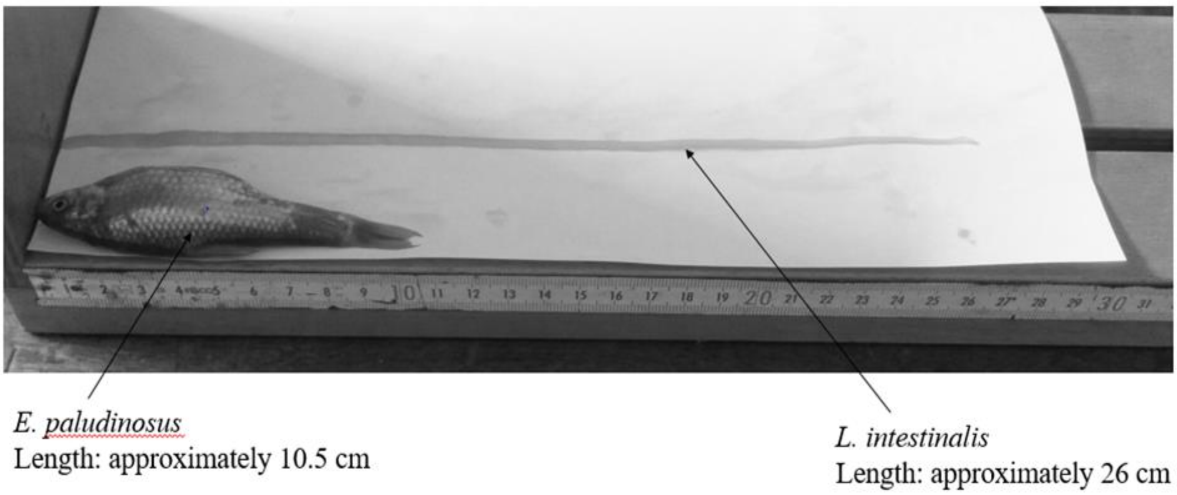

Plate 2: Photograph of *E. paludinosus* alongside its endo-parasite, *L. intestinalis*

4.3.4 Heavy metal determinations in water, sediment, fish muscle tissues and the parasites

Water, sediment, 25 fish muscle tissues (5 infected with *L. intestinalis* and 20 non-infected) and 5 samples of *L. intestinalis* were transported to Lake Nakuru Water Quality Testing Laboratory (WQTL) for heavy metals analysis. 100 ml of the water sample was measured using a clean measuring cylinder and poured into a clean beaker as described by APHA (2012) standard methods. The sample was then digested with 5 ml of 69% concentrated nitric acid (Analytical grade). 50 ml of distilled water was added to the digested sample and immediately heated on a hot plate stirrer at 440 ºC in a fume hood for one hour and evaporated to approximately 25 ml. The solution was cooled and filtered while diluting with distilled water to a final volume of 100 ml using Whatman quantitative filter paper Grade 41 (20-25 µm pore-size and 150 mm diameter) into a volumetric flask ready for heavy metal analysis.

The sediment sample was mixed evenly by pounding with pestle in a mortar. A 2g sub-sample of homogenized sediment was weighed accurately, digested and diluted the same way as the water sample. To avoid cross contamination of the samples, the pestle, mortar, measuring cylinder, beaker and volumetric flasks were acid washed and thoroughly rinsed with double distilled water. Thereafter, a similar procedure was used for the fish tissues (0.2 g) and parasites (0.1 g). Heavy metals concentrations were then determined on the processed samples using direct aspiration by Thermal-electron absorption spectrophotometer (AAS- S series, United Kingdom) at wavelengths of 357.9, 217, 193.7 and 253.7 nm for Chromium (Cr), Lead (Pb), Arsenic (As) and Mercury (Hg) respectively. The heavy metals concentrations were calculated using the equations obtained from the standard calibration curve. The accuracy of the instrument was checked by triplicate analysis of same samples. After every five samples, both a standard and a blank sample were run to check instrumental drift. A serial dilution of a working solution (100 mg/L) made from analytical grade stock solutions (1,000 mg/L) acquired from Merck KGaA, Germany was used to prepare standards for instrument calibration. To get the recovery rates for each heavy metal, one extra sample from the water, sediment, fish muscle tissue and parasite was spiked at a concentration of 0.5 mg/l. The recovery rates shown were 96% for As, 105% for Hg, 98% for Cr and 99% for Pb which were all within the recommended range.

4.3.5 Bioconcentration and bioaccumulation factors

Following the method by Abel (1989), the bioconcentration factors (BCF) were computed to determine both the ratio in concentrations of heavy metals between fish and environment (sediment and water); and partitioning of heavy metals between the dissimilar samples. BCF calculations were done using the mean concentration values of the respective heavy metals obtained in the water, sediment and fish muscle. The BCF was determined using a formula by Abel (1989) as follows:

$$BCF = \frac{C\ (\text{fish muscle})}{C\ (\text{water})} \quad (4\text{-}1)$$

$$BCF = \frac{C\ (\text{fish muscle})}{C\ (\text{sediment})} \quad (4\text{-}2)$$

Where: C stands for mean concentration in fish muscle and sediment both measured in (mg/kg wet weight) and in mg/l in water.

A formula by Drexler *et al*. (2003) was used to determine the bioaccumulation factors (BAF) as follows:

$$BAF\left(\frac{x}{y}\right) = \frac{\text{Heavy metal concentration in x}}{\text{Heavy metal concentration in y}} \quad (4\text{-}3)$$

Whereby x and y variables represent matrices that are compared, in this case, parasites (x) and fish muscle (y).

4.3.6 Risk assessment

To evaluate the health hazard brought about by the heavy metals to individuals who consume *E. paludinosus* in the region, the target hazard quotients (THQ) for heavy metals were established. According to USEPA (2012), THQ is defined as the ratio between the possible exposure to a substance and the reference dose, that is the level at which no antagonistic effects are probable. A THQ of > 1 indicates a potential health risk to the health of fish consumers whereas that of ≤ 1 indicates no important health risk concerning the heavy metals of interest. USEPA (2012) additionally proposed a THQ of 0.1 for noncarcinogens to rationalize additive effects. The THQ was calculated using USEPA (2012) equation:

$$\frac{THQ = EFr \times EDr\ IRFa \times C}{RfDO \times BWa \times AT} \quad (4\text{-}4)$$

Where; C is the concentration of heavy metal in the edible part of fish (milligrams per kilogram wet weight (ww), EDr is the exposure duration (30 years) because some of the adverse effects are experienced after a prolonged exposure to heavy metals , EFr is the exposure frequency (350 days/year), IRFa is the fish consumption per day (0.0123 kg/ day) because per capita is 4.5 kg/year in Kenya (KMFRI report, 2017), BWa is the body weight of an adult male (64.9

kg) and female (61.7), for Kenya (WorldData, n.d), AT is the averaging time for noncarcinogens (365 days/year) while RfDo is the reference dose, oral (milligrams per kilogram per day, according to the updated 2017 Regional Screening Level (RSL) in the fish ingestion table (USEPA, 2017). The mean concentrations of heavy metals were compared with FAO/WHO and EU recommended values.

4.4 Data analysis

The concentrations of heavy metals in the samples were presented as mean with standard deviation (mean ± standard deviation). The differences in mean concentrations of heavy metals between the fish and the parasite was tested using a student's t-test.

4.5 Results

4.5.1 Physico-chemical parameters

The values of physico-chemical parameters obtained at the mouth of River Malewa in Lake Naivasha for temperature, pH, dissolved oxygen, and conductivity are as shown in Table 2. The values were compared with the World Health Organization (WHO, 2011) drinking water desirable limits provided in Table 2 below.

Table 2: Summary of physico-chemical parameters for Lake Naivasha (- means not provided)

Parameter	Mean	WHO drinking water desirable limits
Temperature (˚C)	18.16	-
pH	7.9	6.5 - 8.5
Dissolved Oxygen (mg/l)	7.93	4.50 - 7.50
Conductivity(µS/cm)	129.44	1,500

4.5.2 Heavy metal concentrations in water and sediment

Among the four heavy metals measured, two (As and Cr) were below detection in the water sample as indicated in Table 3. Lead was the highest in concentration in both the water and sediment. Lead was also higher in water than Mercury (Table 3). The concentration of As, Cr, Pb and Hg obtained in the sediment in the order from the highest was Pb>Cr>As>Hg (Table 4). The sediment concentrations were compared with the LEL, TEC, SEL and Shale sediment quality guidelines which are considered to be the normal background level in the Earth's crust as shown in Table 4.

Table 3: Heavy metal concentrations for water samples in comparison with the WHO maximum permissible levels, KEBS natural potable water limits and NEMA water quality standards (mg/l) for recreational waters

Element	**Water (mg/l)**	**LLD**	**WHO**	**KEBS**	**NEMA**
As	B.D	0.001	0.01	0.01	0.05
Cr	B.D	0.01	0.05	0.05	0.1
Pb	0.076	0.004	0.01	0.01	-
Hg	0.001	0.0005	-	0.001	0.001

B.D: Below detection limit, (-): value not provided, WHO: World Health Organization, 2011, KEBS: Kenya Bureau of Standards, 2014, NEMA: National Environment Management Authority, 2006.

Table 4: Concentrations of heavy metals in sediment samples from Lake Naivasha in comparison with different Sediment Quality Guidelines

Element	**Sediment (mg/kg)**	**LEL**	**TEC**	**SEL**	**Shale**
As	0.17	6.0	9.79	33.0	13
Cr	1.49	26.0	43.4	110.0	90
Pb	15.82	31.0	35.8	250.0	20
Hg	0.05	0.2	0.18	2.0	-

LEL: lowest effect level in sediment, TEC: threshold effect concentration in sediment, SEL: severe effect concentration in sediment, (-): value not provided (Buchman, 2008).

4.5.3 Heavy metal concentrations in *E. paludinosus*

In the fish muscle samples, Pb had the highest mean concentration while Hg had the lowest. The order of heavy metal concentration from the highest in the muscle of *E. paludinosus* was Pb>Cr>As>Hg (Table 5).

Table 5: Trace element concentrations in the muscle of *E. paludinosus* fish in Lake Naivasha: values are means (mg/kg wet weight) compared with FAO/WHO and EU standards (n=20)

Element	***E. paludinosus***	**FAO/WHO**	**EU**
As	5.07 ± 2.74	-	-
Cr	22.09 ± 17.90	-	-
Pb	45.21 ± 29.45	0.3	0.3
Hg	1.55 ± 1.80	0.5	0.5

(-): value not provided; Food and Agriculture Organization (FAO) / World Health Organization (WHO), (2011); European Union (EU), (2006)

4.5.4 Target Hazard Quotients

In both females and males, As had the highest THQ value while Cr had the least as shown in Table 6.

Table 6: Target hazard quotients (THQ) for male and female consumers for the four heavy metals (RfDo, reference dose, oral according to USEPA (2017)

		As	Cr	Pb	Hg
RfDo		0.0003	0.003	0.004	0.0001
THQ	Males	92.13	40.14	61.62	84.28
	Females	96.91	42.22	64.82	88.65

4.5.5 Bioconcentration factors

Results of BCF values for heavy metals in water and sediment compared with tissues of *E. paludinosus* indicated that Pb and Hg levels were present in higher concentrations in the fish tissues compared to the water as shown in Table 7. Bioconcentration factors (BCF) could not be determined for As and Cr in water as these elements were below the detection limits of the instrumentation. Arsenic, Chromium, Lead and Mercury levels were present in higher concentrations in the fish tissues compared to the sediments as indicated in Table 7 below.

Table 7: BCF values calculated between mean trace element concentrations in water and sediment and compared to *E. paludinosus* muscles from Lake Naivasha

Element	**Fish/Water**	**Fish/Sediment**
As	N. D	29.65
Cr	N. D	14.82
Pb	1.27	2.86
Hg	2.96	28.63

N. D: BCF value not determined as elements were below detection in water samples

4.5.6 Bioaccumulation factors (BAF) for heavy metals in *L. intestinalis* versus fish muscle tissue (n=5)

Out of the 1307 fish sampled, only 5 were infected by the parasite *L. intestinalis* (prevalence = 0.4%), with only 1 cestode reported per infected fish. *L. intestinalis* contained systematically higher concentration of all the heavy metals than the host fish obtaining mean concentration (mg/kg ww) values of 3.54, 38.05, 7.80 and 1.59 against the mean concentrations in the host fish tissues of 0.69, 6.49, 3.56 and 0.66 for Hg, Pb, Cr and As respectively. All the four heavy metals showed BAF values > 1 (Figure 4). There were significant differences between the mean concentrations of the four heavy metals analyzed between the host and the parasite (t-test, p values < 0.05).

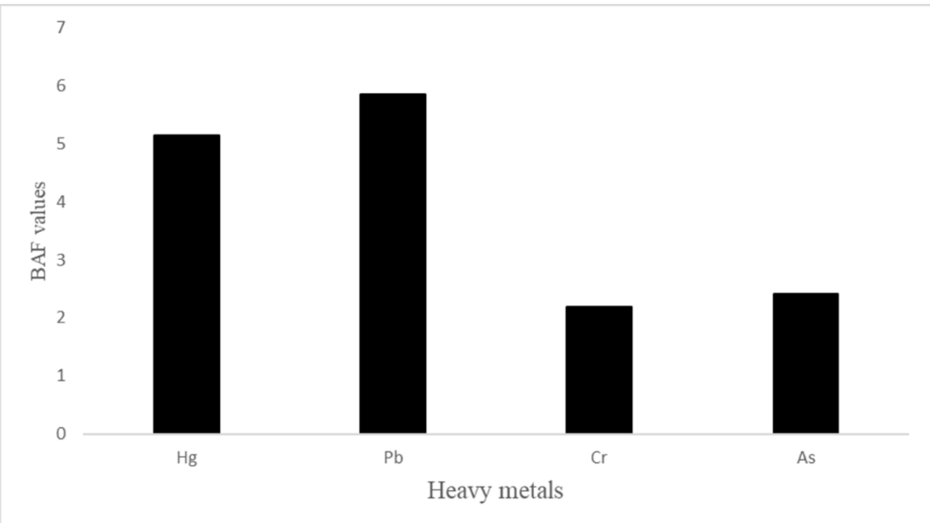

Figure 4: Bioaccumulation factors (BAF) for heavy metals in *L. intestinalis* versus fish muscle tissue (n=5)

4.6 Discussion

4.6.1 Physico-chemical parameters of water

The physico-chemical parameters recorded were within the required WHO drinking water limits in terms of pH range, electrical conductivity and dissolved oxygen. The values of physico-chemical parameters obtained during the study were similar to those obtained by Mutia *et al*. (2012) and Otachi *et al*. (2014) except for the pH and conductivity which were higher. Mutia *et al*. (2012) provided values of 8.66 and 343 μS/cm while Otachi *et al*. (2014) reported 9.0 and 355 μS/cm for pH and conductivity respectively. This could be attributed to the wet season at the time of sampling as well as the location of the study site where River Malewa empties its waters into the lake. On the other hand, Ogendi *et al*. (2014) reported lower values for conductivity and dissolved oxygen of 0.35 μS/cm and 4.5 mg/l during the dry season. Additionally, Njogu *et al*. (2011) and Njiru *et al.* (2015) reported higher temperature values of 19.65˚C and 24˚C during the dry season. The value of pH for this study was similar to that of Njogu *et al.* (2011) and Ogendi *et al*. (2014) in Lake Naivasha.

4.6.2 Heavy metals concentrations in water

Water sample analyzed revealed that most elements were present in lower concentrations compared to those in the sediment and the fish muscle tissue. The Lead (Pb) levels in the present study were however lower than those reported by Mutia *et al.* (2012) from the same lake recording 0.1798 mg/l. Ochieng *et al*. (2007) and Yang *et al*. (2017) on the other hand reported lower Pb concentrations than that of this study with values of 0.0421 and 0.0002 mg/l respectively in the same lake. The comparison of concentrations of heavy metals in this study with the Kenya Bureau of Standards (KEBS, 2014) and World Health Organization (WHO, 2011) revealed that Pb was higher than the KEBS natural potable water limits and WHO maximum permissible level in drinking water. This signifies that the lake may be contaminated with Pb. Unfortunately, National Environment Management Authority (NEMA, 2006) has not provided the maximum limits for Pb. In this study, As and Cr were below the detection limit of instrumentation. This is contrary to the findings of Yang *et al*. (2017) who reported levels of As in other Kenyan Rift Valley lakes obtaining values of 0.0227, 0.00608 and 0.00125mg/l in Lakes Elementaita, Nakuru and Bogoria. Chromium (Cr) was detectable in Lake Elementaita (0.00015 mg/l) and below detection limit in Lakes Nakuru and Bogoria (Yang *et al*., 2017) similar to this study. Mercury levels were within both the KEBS natural potable water limits and NEMA standards. Compared to other Rift Valley lakes, the Pb levels reported in the present study were higher than that of Lakes Elementaita, Nakuru and Bogoria as reported by Yang *et al*. (2017) thus, Naivasha > Bogoria> Nakuru > Elementaita.

4.6.3 Heavy metals concentrations in sediments

The heavy metal levels obtained during this study were compared with the lowest effect level (LEL), threshold effect concentration (TEC), severe effect concentration (SEL) in sediment and Shale values as shown in Table 4. Mutia *et al*. (2012) reported higher Pb levels of 29.48 mg/kg ww than those of the present study while Otachi *et al*. (2014) documented lower Pb levels of 6.94 mg/kg ww. High Pb levels reported in the sediment samples could be because of contamination from the catchment. This is because the mouth of Malewa is one of the key sources of sediment deposition into the lake as it originates from the upper catchment areas and is one the major inlets into the lake. Furthermore, a major highway above the Malewa Bridge could also be a contributing factor with respect to motor vehicle Pb emissions and deposition on roadside soil (Mutia *et al*., 2012). Chromium values obtained in this study were higher than those reported by Ochieng *et al*. (2007) of 1.08 mg/kg ww. This is the first report of As and Hg in the sediment of this lake since there is no study that has reported on the two heavy metals in the sediment of Lake Naivasha. All the four heavy metals had concentrations of below the LEL, TEC, SEL and the Shale values of sedimentary rocks thus showing no sign of pollution in the sediment of Lake Naivasha (Turekian and Wedepohl, 1961). This is because the values obtained in this study were within the normal background level in the Earth's crust.

4.6.4 Heavy metals concentrations in *E. paludinosus* muscle tissues

Compared to the other three heavy metals (As, Cr and Hg) determined, Pb had the highest mean concentration of 45.21 mg/kg ww. The levels of Pb in *E. paludinosus* obtained in this study were lower than those of Mutia *et al*. (2012) in the common carp (*Cyprinus carpio)* from the same lake, with a mean concentration (±SD) of 58.11 ± 0.050 mg/kg ww (wet weight). However, Ogendi *et al*. (2014) and Njogu *et al*. (2011) reported lower mean values of Pb in various fish from Lake Naivasha compared to the present study. For example, Ogendi *et al*. (2014) reported 0.073 ± 0.002 mg/kg ww in *C. carpio* while Njogu *et al*. (2011) reported 1.49, 1.56, 1.51 and 3.22 mg/kg ww in *Oreochromis leucostictus, C. carpio,* and *Micropterus salmoides* respectively. From the findings of this study, the Pb concentrations reported were above both the WHO/FAO and EU maximum permissible level. This infers that *E. paludinosus* from Lake Naivasha is not safe for human consumption as it can cause anemia, neurological damage, nerve disorders, and several other health issues (McCartor and Becker, 2013). The

THQ value of Pb was 61.62 for males and 64.82 for females thus indicating that individuals consuming this fish species are at risk.

In this study, the levels of Cr obtained were higher than those reported by Yi *et al*. (2011) in two fish species (*Coreius guichenoti* and *Leptobotia elongate*) from the middle and lower reaches of the Yangtze River basin in China where, both had 0.805 mg/kg ww. Ahmed *et al*. (2016) reported lower mean concentrations of Cr on five fish species from Buriganga river, Bangladesh (For example, Cr level of 18.84 ± 1.72 mg/kg ww in the muscle tissue of *Labeo rohita*). Zhang *et al*. (2017) also reported low levels of Cr in Crucian carp (3.36 ± 0.0036 mg/kg) from Honghu Lake in China compared to the values obtained in this study. The THQ value of Cr for the males was 40.14 while that of the females was 42.22 indicating the risks involved in consumption of this fish. The health risks of Cr include developing reproductive and developmental problems as well as impairing of the respiratory, gastrointestinal, and immunological systems (McCartor and Becker, 2013).

Total mercury (THg) concentrations in *E. paludinosus* were higher than those reported by Campbell *et al*. (2003), obtaining 0.081mg/kg in the same fish in Lake Naivasha. Yi *et al*. (2011) also reported a mean concentration of 0.054mg/kg ww for *Eriocheir sinensis* from the middle and lower reaches of the Yangtze River basin which is much lower than 1.55 mg/kg ww obtained in this study. Additionally, Gbogbo *et al*. (2018) and Stanek *et al*. (2017) reported lower values of 0.19 ± 0.13 and 0.27 ± 0.03 mg/kg ww in the muscles of *Chrysichthys nigrodigitatu*s and Crayfish (*Orconectes limosus*) from Weija Dam on the Densu River and Lake Gopło, Poland respectively. The findings in this study were similar to those of Andreji *et al*. (2006) who reported a mean concentration of 1.53 ± 0.80 mg/kg ww in the Wels catfish from Lower Nitra River (Slovakia). The Hg THQ values obtained of 84.28 and 88.65 for the males and females respectively, indicates that the health of individuals consuming this fish species at risk thus, prone to severe damage to both the kidneys and brain (McCartor and Becker, 2013). Additionally, Hg levels were above both of the WHO/FAO (2011) and EU (2006) maximum permissible levels making the fish unsafe for consumption.

Higher levels of As were reported in the present study compared to those of Yi *et al*. (2011) in the fish (*Rhinogobio typus*) reporting a mean concentration of 0.039 mg/kg ww, from the middle and lower reaches of the Yangtze River basin in China. In addition, Ahmed *et al*. (2016) reported lower levels of As in *Labeo rohita* from Buriganga river, Bangladesh with values of 0.73 ± 0.03 mg/kg ww. Studies by Gbogbo *et al*. (2018) and Zhang *et al*. (2017) also reported low levels of As in *Chrysichthys nigrodigitatu*s and *Pylodictis olivaris* from Weija Dam and

Honghu Lake in China obtaining 0.37 ± 0.24 and 0.0040 ± 0.0042 mg/kg ww respectively. The As THQ value of 92.13 for males and 96.91 for females shows that the health of the individuals (both males and females) that consume this fish from Lake Naivasha is at risk. Large intake of As (about 50 mg/kg ww) can cause death while lower intake (up to 20 µg/kg ww) can cause a decrease in production of red and white blood cells (FAO/WHO, 2011; McCartor and Becker, 2013).

4.6.5 Bioconcentration

The results showed that heavy metal concentrations were higher in *E. paludinosus* than both in water and sediment. Computation of BCF is important since the values serve as a revelation of the number of times higher a contaminant is in the living organism compared to the environment, as well as, as a means of determining the rationing between fish and environment (McGeer *et al*., 2003). The BCF for As and Cr were not calculated since the concentration in water compared to the fish muscle were below detection limits of the instrumentation. High concentrations of heavy metals in the fish muscles compared to the sediment could be explained by the sedimentation process of the soil particles. Whereby, these soil particles from the catchment could be contaminated with heavy metals which are absorbed by the fish before they settle at the bottom of the lake.

4.6.6 Potential of *Ligula intestinalis* as a bioindicator

A low number of *L. intestinalis* infestations in *E. paludinosus* was reported in this study giving a prevalence of 0.4%. Similarly, *L. intestinalis* prevalence reported by Britton *et al*. (2009) in Lake Naivasha was low, with only 7 of 8665 examined individuals infected between the years 2002 – 2008 (prevalence = 0.1%). The heavy metal with the highest concentration in *L. intestinalis* was Pb (38.05 mg/kg ww). The concentrations of Hg, Pb, Cr and As in *L. intestinalis* obtained in this study were significantly higher compared to the host's muscle. Therefore, *L. intestinalis* showed a potential to accumulate heavy metals. These results are similar to the findings by Tenora *et al*. (2000) on *L. intestinalis* from the body cavity of three cyprinid fish species (*Abramis brama, Rutilus rutilus, Blicca bjoerkna*). These three fish species accumulated greater levels of Pb, Cr and Cd in the fish muscle obtaining bioaccumualation factors of 15.0, 6.0 and 2.6 respectively. Thus, reporting higher bioaccumulation factors than that of this study. The accumulation ability of Pb and Cr by this parasite in the present study was lower than that in the *Rastreneobola argentea/L. intestinalis* host-parasite system reported by Oyoo-Okoth *et al*. (2010) from Lake Victoria, Kenya. They reported BAF values of 11.6 and 10.8 for Pb and Cr with mean concentrations in water (mg/l) of 0.3 and 0.4 for Pb and Cr respectively. The low BAF values in the present study compared to Oyoo-Okoth *et al*. (2010) could be attributed to the relatively high concentrations of Pb and Cr in water from Lake Victoria, compared to the concentrations reported in Lake Naivasha. However, Tenora *et al*. (2000) did not provide the data for the Pb and Cr of their sampling locations and therefore it was impossible to compare and explain why their BAF values were higher than those obtained in this study. Surprisingly, as compared to other cestodes, *L. intestinalis* is ranked lowly in terms of the order of magnitude of accumulation of heavy metals from the host tissues or the environment (Sures *et al*., 1997; Sures *et al*., 1999; Dvoracek *et al.*, 2000; Jirsa *et al.*, 2008). However, when compared to other groups of parasite bioindicators, *L. intestinalis* accumulated much higher levels of heavy metals than the nematode *Contracaecum multipapillatum* infesting *Oreochromis leucostictus* (Otachi *et al*., 2014). However, the limitation of using *L. intestinalis* as a bioindicator, is its low prevalence and abundance experienced during this study and the earlier studies in Lake Naivasha.

4.7 Conclusion

The heavy metal concentrations were highest in the parasite followed by the fish muscle tissues, then the sediment and least in water. Arsenic and Chromium were below the detection limit of instrumentation in water. The four heavy metals levels in the sediment were below the LEL, TEC, SEL and the Shale values of sedimentary rocks implying no sign of pollution in the sediment of the lake. Lead concentrations were high in the fish muscle tissue compared to the other three elements. The levels of Pb and Hg in the fish were above the FAO/WHO and EU maximum permissible limits hence not safe for consumption. The Target Hazard Quotient values for As, Cr, Pb and Hg were high with slightly high THQ values for females than male fish. This could put at risk the health of the consumers of this fish as their regular source of protein. The four heavy metals showed bioaccumulation factors (BAF) values of >1 indicating the potential of *L. intestinalis* in accumulation of heavy metals from the host (*E. paludinosus*). This therefore makes the parasite a sensitive biomonitor of metal accumulation than its fish host.

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CHAPTER FIVE

5.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

This study was conducted at the mouth of River Malewa, a major source of water for Lake Naivasha from November 2017 to February 2018 where fish samples of *E. paludinosus* were collected using seine nets of 1.2 mm. The objective was to determine the levels of heavy metals in *E. paludinosus* and its endo-parasite (*L. intestinalis*) as bioindicators in Lake Naivasha. The biological aspects such as the length-weight relationship, condition factor and sex ratio of this fish species were determined. The major findings of the study are as follows: the

Length-Weight Relationship (LWR) of *E. paludinosus* had a good growth ratio with the combined sexes showing a positive allometric growth ($b > 3$). However, the males and females separated showed a negative allometric growth for each with b values of ($b < 3$). The fish species under study population was not in good condition as shown by the condition factor values (K) that is, $K < 1$ for the combined sexes, males and females. In addition there were more female *E. paludinosus* than the males as shown by the sex ratio. The *E. paludinosus* fish parasite (*L. intestinalis)* had the highest levels of heavy metals concentrations with water having the least of the selected heavy metals analyzed in this study {Arsenic (As), Chromium (Cr), Lead (Pb) and Mercury (Hg)}. Both Arsenic (As) and Chromium (Cr) were below the detection limit of instrumentation in the water sample coupled with Mercury (Hg) concentrations being lower than Lead (Pb). All the selected four heavy metals levels in the sediment were below the LEL (lowest effect level), TEC (threshold effect concentration), SEL (severe effect concentration) and the Shale values of sedimentary rocks. Pb concentrations were higher in the fish muscle tissue compared to the other three elements (As, Cr and Hg). Pb and Hg levels in the fish were above the FAO/WHO and EU maximum permissible limits. All the selected four heavy metals showed bioaccumulation factors (BAF) values of >1 rendering the parasite a heavy metal accumulation biomonitor than its fish host (*E. paludinosus*)

5.2 Conclusions

1. The length-weight relationships revealed that the population of *E. paludinosus* had a good growth ratio with both sexes combined producing a $b > 3$ indicating a positive allometric growth. On the other hand, the growth ratio of the males and females separated produced a b value of ($b < 3$) showing a negative allometric growth. The population was not in good condition as the condition factor values (K) obtained for the

fish population revealed. This is a clear indication of the unhealthy population status of *E. paludinosus* with a probability of depressed procreative potential. In addition, the sex ratio revealed that the female *E. paludinosus* were more than the males confirming that this fish species population of Lake Naivasha is unstable.

2. The heavy metals, Arsenic (As) and Chromium (Cr) were below the detection limit in water. The concentration of Lead (Pb) in water was higher than the KEBS natural potable water limits and WHO maximum permissible level of drinking water thus showing that the lake may be contaminated with Lead (Pb). Mercury (Hg) levels on the other hand were within the KEBS natural potable water limits and NEMA standards. In the sediment, As, Cr, Pb and Hg were below the LEL, TEC, SEL and the Shale values of sedimentary rocks implying no sign of contamination. The Target Hazard Quotients (THQ) values of the four heavy metals were more than 1 (>1) thus indicating a potential health risk to the health of fish consumers. All the four selected heavy metals also showed bioaccumulation factors (BAF) values of >1 with regard to *L. intestinalis* thus indicating its high potential in accumulation of the heavy metals from the host (*E. paludinosus*) therefore rendering it a good fish parasite bioindicator in Lake Naivasha and other similar systems.

5.3 Recommendations

1. Based on the health risk assessment results obtained (THQ>1) for all the four selected heavy metals in this study, this study recommends that *E. paludinosus* from the study site should not be consumed as its continued consumption will eventually lead to accumulation in the consumers thus causing health problems. In addition, since *L. intestinalis* showed a high potential in the accumulation of the four selected heavy metals from its host, *E. paludinosus*, it is highly recommended that this fish parasite should be included in the future biomonitoring activities in Lake Naivasha.
2. Further analysis of length-weight relationship and condition factor of E. paludinosus taking water quality into consideration should be carried out for both dry and wet seasons to determine whether different seasons have different effects on the well-being of this fish species in Lake Naivasha.

APPENDICES

Appendix 1: Publication 1 {Levels of heavy metals in the straightfin barb *Enteromius paludinosus* (Peters 1852) from River Malewa, Naivasha, Kenya}

Environ Monit Assess (2019) 191:292
https://doi.org/10.1007/s10661-019-7457-5

Check for updates

Levels of heavy metals in the straightfin barb *Enteromius paludinosus* (Peters 1852) from River Malewa, Naivasha, Kenya

Elizabeth A. Ngesa · Elick O. Otachi · Nzula K. Kitaka

Received: 12 November 2018 / Accepted: 4 April 2019

Abstract There have been several studies on heavy metals in Lake Naivasha. However, none of them has reported the levels of mercury (Hg), arsenic (As), and chromium (Cr). Moreover, there are no studies on the heavy metals' concentrations in the straightfin barb (*Enteromius paludinosus*, Peters 1852), a fish species that hosts a parasite (*Ligula intestinalis*), the latter having been reported to have a high ability to absorb heavy metals from its host. This paper therefore addresses the accumulation of heavy metals, namely arsenic (As), chromium (Cr), lead (Pb), and mercury (Hg) in the tissues of straightfin barb, *Enteromius paludinosus* (Peters 1852) from the mouth of River Malewa in Lake Naivasha, Kenya. A total of 1307 fish were collected during the month of November 2017. Water samples, sediment samples, 25 fish muscle tissues, and its endoparasite, the cestode *Ligula intestinalis*, were isolated, and heavy metal concentrations were determined using the thermal-electron atomic absorption spectrophotometer at the Lake Nakuru Water Quality Testing Laboratory. The concentrations of heavy metals in the sediment were below the lowest effect level in sediment, threshold effect concentration in sediment, severe effect concentration in sediment, and the shale values of sedimentary rocks thus showing no sign of pollution. In the muscle tissues of the fish, As, Cr, Pb, and Hg showed high levels with mean concentrations of 5.0696, 22.0854, 45.2108, and 1.5458 mg/kg ww, respectively. Bioconcentration factors further supported the observation that trace element accumulation was higher in fish compared with sediment and water. The target hazard quotients of As, Cr, Pb, and Hg obtained for both the female and male were > 1 indicating a possible health risk associated with the consumption of *E. paludinosus*. The bioaccumulation factors (BAFs) for *L. intestinalis* were 2.4093, 2.1873, 5.8601, and 5.1395 for As, Cr, Pb, and Hg, respectively, indicating the potential of the cestode in the accumulation of heavy metals from the host; hence, it can be used as an accumulation bioindicator.

Keywords Fish parasites · Bioconcentration factor (BCF) · Lake Naivasha · Target hazard quotient (THQ) · *Ligula intestinalis* · Bioaccumulation factor (BAF)

E. A. Ngesa · E. O. Otachi (✉) · N. K. Kitaka
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P.O. Box 536, Egerton, Kenya
e-mail: elickotachi@gmail.com

Introduction

Heavy metals are natural components of aquatic ecosystems (Tayab 1991; Ochieng et al. 2007). However, several human activities such as agricultural, mining, industrial, and domestic use among others have increased their levels in the aquatic environments (Kamau et al. 2008). Aquatic organisms can be affected by elevated levels of heavy metals in their environment especially when they occur above threshold concentrations (Ochieng et al. 2007). Some of these heavy metals bioaccumulate in various organs of aquatic organisms (Martin 1979) and may have lethal effects (Eisler 1973).

Published online: 22 April 2019

Appendix 2: Publication 2 {Some biological aspects of straightfin barb, *Enteromius paludinosus* (Peters 1852) during the rainy season in Lake Naivasha, Kenya}

Scientific African 4 (2019) e00097

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journal homepage: www.elsevier.com/locate/sciaf

Some biological aspects of straightfin barb, *Enteromius paludinosus* (Peters 1852) during the rainy season in Lake Naivasha, Kenya

Ngesa Elizabeth Adhiambo, Otachi Elick Onyango*, Kitaka Nzula Kivuva

Department of Biological Sciences, Egerton University, P.O. Box 536, Egerton, Kenya

ARTICLE INFO

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Lake Naivasha
Length–weight
Sex ratio

ABSTRACT

This paper focused on determining the sub lethal effects of reported pollutants in Lake Naivasha, Kenya using the straightfin barb (*Enteromius paludinosus*), a decade after the existing baseline study. The specific objectives included determining the length–weight relationship (LWR), condition factor (K) and the sex ratio, of the migratory *E. paludinosus*. A total of 1279 straightfin barb were collected from the mouth of River Malewa, a major source of water for Lake Naivasha using seine nets of mesh size 1.2 mm in the month of November 2017. The fish were caught and transported to the laboratory, then killed humanely by cervical dislocation and thereafter their lengths (cm) and weights (g) determined followed by their dissection to examine the gonads. The length–weight relationship was determined using the formula $W = aTL^b$. The condition factor (K) was determined using the formula $K = 100W/L^b$. A student's *t*-test was used to test the significant differences in the weight and length of both sexes. The sex ratio, expressed as male: female was calculated by dividing the number of females by that of the males. The equations obtained for the LWR of the combined sexes, males and females of *E. paludinosus* were, Log $W = 3.23$ Log L + Log 0.1073, Log $W = 2.83$ Log L + Log 0.1500 and Log $W = 2.87$ Log L + Log 0.1522 respectively. The mean condition factor of the combined sex, males and females of *E. paludinosus* were; 0.5897, 0.5729 and 0.5981 respectively. The sex ratio of the fish was 1: 1.981 (males: females). The results showed that *E. paludinosus* in Lake Naivasha are not in a good condition, $k < 1$. The findings of this study may be attributed to the state of the lake due to the reported pollution increase and ecological changes by recent studies.

Appendix 3: Research permit granted by the National Commission for Science, Technology and Innovation (NACOSTI)

THIS IS TO CERTIFY THAT:
MISS. ELIZABETH ADHIAMBO NGESA
of EGERTON UNIVERSITY, 0-20100
Nakuru,has been permitted to conduct research in *Nakuru County*

on the topic: *DETERMINATION OF HEAVY METAL LEVELS IN STRAIGHTFIN BARB (ENTEROMIUS PALUDINOSUS) AND ITS ENDO-PARASITES AS BIOINDICATORS IN LAKE NAIVASHA, KENYA*

for the period ending:
26th June,2019

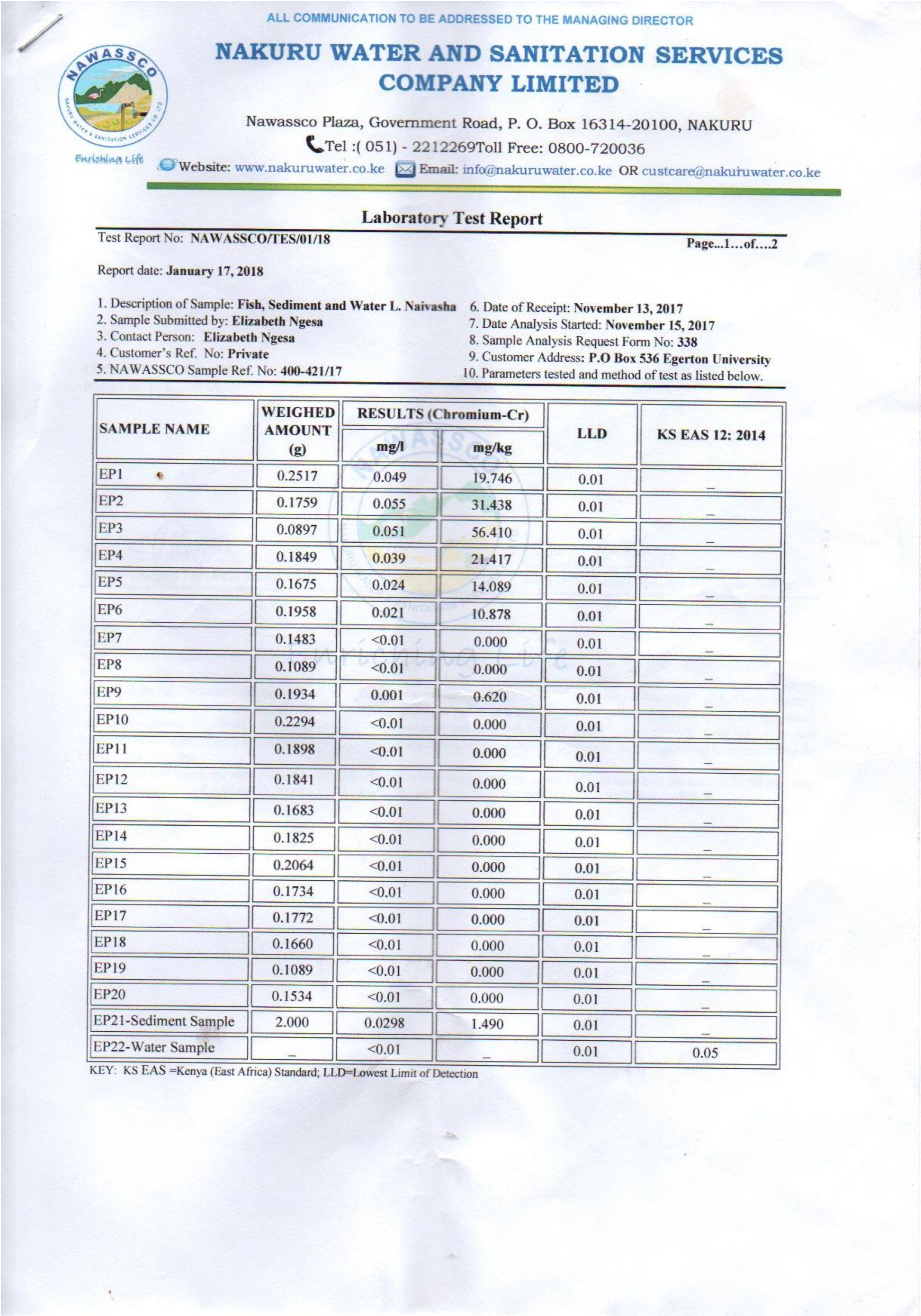
Permit No : NACOSTI/P/18/91067/22732
Date Of Issue : 27th June,2018
Fee Recieved :Ksh 1000

....
Applicant's Signature

....
Director General
National Commission for Science, Technology & Innovation

Appendix 4: Results of heavy metals analysis (*E. paludinosus* muscle tissues, sediment and water) from Lake Nakuru Water Quality and Testing Laboratory (LWQTL)

1) **Chromium (Cr)**



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Enriching Life

Laboratory Test Report

Test Report No: **NAWASSCO/TES/01/18** **Page...1...of....2**

Report date: **January 17, 2018**

1. Description of Sample: **Fish, Sediment and Water L. Naivasha**
2. Sample Submitted by: **Elizabeth Ngesa**
3. Contact Person: **Elizabeth Ngesa**
4. Customer's Ref. No: **Private**
5. NAWASSCO Sample Ref. No: **400-421/17**
6. Date of Receipt: **November 13, 2017**
7. Date Analysis Started: **November 15, 2017**
8. Sample Analysis Request Form No: **338**
9. Customer Address: **P.O Box 536 Egerton University**
10. Parameters tested and method of test as listed below.

SAMPLE NAME	WEIGHED AMOUNT (g)	RESULTS (Chromium-Cr)		LLD	KS EAS 12: 2014
		mg/l	mg/kg		
EP1	0.2517	0.049	19.746	0.01	_
EP2	0.1759	0.055	31.438	0.01	_
EP3	0.0897	0.051	56.410	0.01	_
EP4	0.1849	0.039	21.417	0.01	_
EP5	0.1675	0.024	14.089	0.01	_
EP6	0.1958	0.021	10.878	0.01	_
EP7	0.1483	<0.01	0.000	0.01	_
EP8	0.1089	<0.01	0.000	0.01	_
EP9	0.1934	0.001	0.620	0.01	_
EP10	0.2294	<0.01	0.000	0.01	_
EP11	0.1898	<0.01	0.000	0.01	_
EP12	0.1841	<0.01	0.000	0.01	_
EP13	0.1683	<0.01	0.000	0.01	_
EP14	0.1825	<0.01	0.000	0.01	_
EP15	0.2064	<0.01	0.000	0.01	_
EP16	0.1734	<0.01	0.000	0.01	_
EP17	0.1772	<0.01	0.000	0.01	_
EP18	0.1660	<0.01	0.000	0.01	_
EP19	0.1089	<0.01	0.000	0.01	_
EP20	0.1534	<0.01	0.000	0.01	_
EP21-Sediment Sample	2.000	0.0298	1.490	0.01	_
EP22-Water Sample	_	<0.01	_	0.01	0.05

KEY: KS EAS =Kenya (East Africa) Standard; LLD=Lowest Limit of Detection

COMMENTS/REMARKS
Results are as shown above

For: ……………………………………
Antony Chege – Technical Manager

WATER QUALITY TESTING LABORATORY
29 JAN 2010
TECHNICAL SIGNATORY

For ……………………………………………………
Andrew Kulecho – Quality Assurance Officer

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2) Arsenic (As)

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Laboratory Test Report

Test Report No: **NAWASSCO/TES/04/17** **Page...1...of....2**

Report date: **January 26, 2018**

1. Description of Sample: **Fish, Sediment and Water L. Naivasha**
2. Sample Submitted by: **Elizabeth Ngesa**
3. Contact Person: **Elizabeth Ngesa**
4. Customer's Ref. No: **Private**
5. NAWASSCO Sample Ref. No: **400-421/17**
6. Date of Receipt: **November 13, 2017**
7. Date Analysis Started: **November 15, 2017**
8. Sample Analysis Request Form No: **338**
9. Customer Address: **P.O Box 536 Egerton University**
10. Parameters tested and method of test as listed below.

SAMPLE NAME	WEIGHED AMOUNT (g)	RESULTS (Arsenic)		LLD	KS EAS 12: 2014
		mg/l	mg/kg		
EP1	0.2517	<0.001	0.000	0.001	_
EP2	0.1759	<0.001	0.000	0.001	_
EP3	0.0897	<0.001	0.000	0.001	_
EP4	0.1849	<0.001	0.000	0.001	_
EP5	0.1675	<0.001	0.000	0.001	_
EP6	0.1958	<0.001	0.000	0.001	_
EP7	0.1483	<0.001	0.000	0.001	_
EP8	0.1089	<0.001	0.000	0.001	_
EP9	0.1934	<0.001	0.000	0.001	_
EP10	0.2294	<0.001	0.000	0.001	_
EP11	0.1898	<0.001	0.000	0.001	_
EP12	0.1841	0.013	7.061	0.001	_
EP13	0.1683	<0.001	0.000	0.001	_
EP14	0.1825	<0.001	0.000	0.001	_
EP15	0.2064	0.002	0.969	0.001	_
EP16	0.1734	0.013	7.497	0.001	_
EP17	0.1772	0.011	6.207	0.001	_
EP18	0.1660	0.006	3.614	0.001	_
EP19	0.1089	<0.001	0.000	0.001	_
EP20	0.1534	<0.001	0.000	0.001	_
EP21-Sediment Sample	2.000	0.011	0.171	0.001	_
EP22-Water Sample	_	<0.001	_	0.001	0.01

COMMENTS/REMARKS
Results are as shown above

For: ....
Antony Chege – Technical Manager

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3) Lead (Pb)

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Laboratory Test Report

Test Report No: **NAWASSCO/TES/02/18** **Page...1...of....2**

Report date: **January 17, 2018**

1. Description of Sample: **Fish, Sediment and Water L. Naivasha**
2. Sample Submitted by: **Elizabeth Ngesa**
3. Contact Person: **Elizabeth Ngesa**
4. Customer's Ref. No: **Private**
5. NAWASSCO Sample Ref. No: **400-421/17**
6. Date of Receipt: **November 13, 2017**
7. Date Analysis Started: **November 15, 2017**
8. Sample Analysis Request Form No: **338**
9. Customer Address: **P.O Box 536 Egerton University**
10. Parameters tested and method of test as listed below.

SAMPLE NAME	WEIGHED AMOUNT (g)	RESULTS (Lead-Pb)		LLD	KS EAS 12: 2014
		mg/l	mg/kg		
EP1	0.2517	<0.004	0.000	0.004	_
EP2	0.1759	0.058	33.144	0.004	_
EP3	0.0897	0.084	93.199	0.004	_
EP4	0.1849	<0.004	0.000	0.004	_
EP5	0.1675	0.096	57.313	0.004	_
EP6	0.1958	0.047	24.208	0.004	_
EP7	0.1483	<0.004	0.000	0.004	_
EP8	0.1089	<0.004	0.000	0.004	_
EP9	0.1934	0.007	3.413	0.004	_
EP10	0.2294	0.129	56.103	0.004	_
EP11	0.1898	0.012	6.112	0.004	_
EP12	0.1841	0.168	91.526	0.004	_
EP13	0.1683	0.085	50.386	0.004	_
EP14	0.1825	0.043	23.507	0.004	_
EP15	0.2064	0.014	71.124	0.004	_
EP16	0.1734	0.1054	60.784	0.004	_
EP17	0.1772	<0.004	0.000	0.004	_
EP18	0.1660	<0.004	0.000	0.004	_
EP19	0.1089	0.057	52.158	0.004	_
EP20	0.1534	0.015	9.974	0.004	_
EP21-Sediment Sample	2.000	0.316	15.820	0.004	_
EP22-Water Sample	_	0.076	_	0.004	0.01

KEY: KS EAS =Kenya (East Africa) Standard; LLD=Lowest Limit of Detection

COMMENTS/REMARKS
Results are as shown above

For: ....
Antony Chege – Technical Manager

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4) Mercury (Hg)

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Laboratory Test Report

Test Report No: **NAWASSCO/TES/03/18** **Page...1...of....2**

Report date: **January 17, 2018**

1. Description of Sample: **Fish, Sediment and Water L. Naivasha**
2. Sample Submitted by: **Elizabeth Ngesa**
3. Contact Person: **Elizabeth Ngesa**
4. Customer's Ref. No: **Private**
5. NAWASSCO Sample Ref. No: **400-421/17**
6. Date of Receipt: **November 13, 2017**
7. Date Analysis Started: **November 15, 2017**
8. Sample Analysis Request Form No: **338**
9. Customer Address: **P.O Box 536 Egerton University**
10. Parameters tested and method of test as listed below.

SAMPLE NAME	WEIGHED AMOUNT (g)	RESULTS (Mercury-Hg)		LLD	KS EAS 12: 2014
		mg/l	mg/kg		
EP1	0.2517	0.0175	6.942	0.0005	
EP2	0.1759	0.0018	1.053	0.0005	
EP3	0.0897	0.0012	1.293	0.0005	
EP4	0.1849	0.0011	0.621	0.0005	
EP5	0.1675	0.0011	0.692	0.0005	
EP6	0.1958	0.0011	0.563	0.0005	
EP7	0.1483	0.0012	0.789	0.0005	
EP8	0.1089	0.0008	0.783	0.0005	
EP9	0.1934	0.0006	0.329	0.0005	
EP10	0.2294	0.0005	0.218	0.0005	
EP11	0.1898	0.0021	1.120	0.0005	
EP12	0.1841	0.0087	4.724	0.0005	
EP13	0.1683	0.0007	0.398	0.0005	
EP14	0.1825	0.0058	3.195	0.0005	
EP15	0.2064	0.0039	1.917	0.0005	
EP16	0.1734	<0.0005	0.000	0.0005	
EP17	0.1772	0.0011	0.628	0.0005	
EP18	0.1660	<0.0005	0.000	0.0005	
EP19	0.1089	0.0012	1.085	0.0005	
EP20	0.1534	<0.0005	0.000	0.0005	
EP21-Sediment Sample	2.000	0.0011	0.054	0.0005	
EP22-Water Sample	_	0.0010	_	0.0005	0.001

KEY: KS EAS =Kenya (East Africa) Standard; LLD=Lowest Limit of Detection

COMMENTS/REMARKS

Results are as shown above

For: ……………………………………

Antony Chege – Technical Manager

For ……………………………………………

Andrew Kulecho – Quality Assurance Officer

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Appendix 5: Results of heavy metals analysis (*E. paludinosus* muscle tissues and its endo-parasite - *L. intestinalis*) from Lake Nakuru Water Quality and Testing Laboratory (LWQTL)

1) Chromium

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Laboratory Test Report

Test Report No: **NAWASSCO/TES/32/18** **Page...1...of....2**

Report date: **May 29, 2018**

1. Description of Sample: **Fish and Parasite L. Naivasha**
2. Sample Submitted by: **Elizabeth Ngesa**
3. Contact Person: **Elizabeth Ngesa**
4. Customer's Ref. No: **Private**
5. NAWASSCO Sample Ref. No: **326--335/18**
6. Date of Receipt: **April 04, 2018**
7. Date Analysis Started: **April 23, 2018**
8. Sample Analysis Request Form No: **356**
9. Customer Address: **P.O Box 536 Egerton University**
10. Parameters tested and method of test as listed below.

SAMPLE NAME	WEIGHED AMOUNT (g)	RESULTS (Chromium-Cr)		LLD	KS EAS 12: 2014
		mg/l	mg/kg		
EP 1303	0.2404	0.00084	3.494	0.01	_
EP 1304	0.2911	0.00093	3.195	0.01	_
EP 1305	0.2045	0.00102	4.988	0.01	_
EP 1306	0.2311	0.00076	2.639	0.01	_
EP 1307	0.2680	0.00094	3.507	0.01	_
PR 1303	0.1358	0.00119	8.763	0.01	_
PR 1304	0.1432	0.00111	7.751	0.01	_
PR 1305	0.1141	0.00064	5.609	0.01	_
PR 1306	0.0892	0.0061	8.520	0.01	_
PR 1307	0.0983	0.00082	8.342	0.01	_

KEY: KS EAS =Kenya (East Africa) Standard; LLD=Lowest Limit of Detection

COMMENTS/REMARKS
Results are as shown above

WATER QUALITY TESTING LABORATORY
29 MAY 2018
TECHNICAL SIGNATORY

Technical Manager - Andrew Kulecho

Eliud Rotich – Technical Signatory

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"Unless otherwise stated, the results shown in this test report refer only to the sample(s) tested and such sample(s) are retained for 90 days only (If not perishable) Note that all details in respect of the source and test results of similar samples are not verified or confirmed.

2) Arsenic (As)

ALL COMMUNICATION TO BE ADDRESSED TO THE MANAGING DIRECTOR

NAKURU WATER AND SANITATION SERVICES COMPANY LIMITED

Nawassco Plaza, Government Road, P. O. Box 16314-20100, NAKURU
Tel :(051) - 2212269Toll Free: 0800-720036
Website: www.nakuruwater.co.ke Email: info@nakuruwater.co.ke OR custcare@nakuruwater.co.ke

Laboratory Test Report

Test Report No: **NAWASSCO/TES/34/18** **Page...1...of....2**

Report date: **May 29, 2018**

1. Description of Sample: **Fish and Parasite L. Naivasha**
2. Sample Submitted by: **Elizabeth Ngesa**
3. Contact Person: **Elizabeth Ngesa**
4. Customer's Ref. No: **Private**
5. NAWASSCO Sample Ref. No: **326--335/18**
6. Date of Receipt: **April 04, 2018**
7. Date Analysis Started: **April 23, 2018**
8. Sample Analysis Request Form No: **356**
9. Customer Address: **P.O Box 536 Egerton University**
10. Parameters tested and method of test as listed below.

SAMPLE NAME	WEIGHED AMOUNT (g)	RESULTS (Arsenic)		LLD	KS EAS 12: 2014
		mg/l	mg/kg		
EP 1303	0.2404	0.0011	0.4576	0.001	_
EP 1304	0.2911	0.0019	0.6527	0.001	_
EP 1305	0.2045	0.0012	0.5868	0.001	_
EP 1306	0.2311	0.0019	0.8222	0.001	_
EP 1307	0.2680	0.0021	0.7836	0.001	_
PR 1303	0.1358	0.0021	1.5464	0.001	_
PR 1304	0.1432	0.0025	1.7458	0.001	_
PR 1305	0.1141	0.0015	1.3146	0.001	_
PR 1306	0.0892	0.0019	2.1300	0.001	_
PR 1307	0.0983	0.0012	1.2208	0.001	_

COMMENTS/REMARKS
Results are as shown above

For ....
Technical Manager - Andrew Kulecho

WATER QUALITY TESTING LABORATORY 29 MAY 2018 TECHNICAL SIGNATORY

....
Eliud Rotich – Technical Signatory

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3) Lead (Pb)

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Enriching Life

Laboratory Test Report

Test Report No: **NAWASSCO/TES/31/18** **Page...1...of....2**

Report date: **May 29, 2018**

1. Description of Sample: **Fish and Parasite L. Naivasha**
2. Sample Submitted by: **Elizabeth Ngesa**
3. Contact Person: **Elizabeth Ngesa**
4. Customer's Ref. No: **Private**
5. NAWASSCO Sample Ref. No: **326–335/18**
6. Date of Receipt: **April 04, 2018**
7. Date Analysis Started: **April 23, 2018**
8. Sample Analysis Request Form No: **356**
9. Customer Address: **P.O Box 536 Egerton University**
10. Parameters tested and method of test as listed below.

SAMPLE NAME	WEIGHED AMOUNT (g)	RESULTS (Lead-Pb)		LLD	KS EAS 12: 2014
		mg/l	mg/kg		
EP 1303		0.010	4.201	0.004	–
EP 1304		0.006	4.050	0.004	–
EP 1305		0.029	14.572	0.004	–
EP 1306		0.015	6.361	0.004	–
EP 1307		0.009	3.284	0.004	–
PR 1303		0.033	24.595	0.004	–
PR 1304		0.045	15.355	0.004	–
PR 1305		0.125	109.290	0.004	–
PR 1306		0.024	26.682	0.004	–
PR 1307		0.014	14.344	0.004	–

KEY: KS EAS =Kenya (East Africa) Standard; LLD=Lowest Limit of Detection

COMMENTS/REMARKS
Results are as shown above

WATER QUALITY TESTING LABORATORY
29 MAY 2018
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Technical Manager - Andrew Kulecho

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4) Mercury (Hg)

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Laboratory Test Report

Test Report No: **NAWASSCO/TES/33/18** **Page...1...of....2**

Report date: **May 29, 2018**

1. Description of Sample: **Fish and Parasite L. Naivasha**
2. Sample Submitted by: **Elizabeth Ngesa**
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4. Customer's Ref. No: **Private**
5. NAWASSCO Sample Ref. No: **326--335/18**
6. Date of Receipt: **April 04, 2018**
7. Date Analysis Started: **April 23, 2018**
8. Sample Analysis Request Form No: **356**
9. Customer Address: **P.O Box 536 Egerton University**
10. Parameters tested and method of test as listed below.

SAMPLE NAME	WEIGHED AMOUNT (g)	RESULTS (Mercury-Hg)		LLD	KS EAS 12: 2014
		mg/l	mg/kg		
EP 1303	0.2404	0.0012	0.4992	0.0005	
EP 1304	0.2911	0.0086	1.3268	0.0005	
EP 1305	0.2045	0.0014	0.6846	0.0005	
EP 1306	0.2311	0.0013	0.5625	0.0005	
EP 1307	0.2680	0.0011	0.3731	0.0005	
PR 1303	0.1358	0.0016	1.1782	0.0005	
PR 1304	0.1432	0.0019	1.3268	0.0005	
PR 1305	0.1141	0.00368	3.2296	0.0005	
PR 1306	0.0892	0.00447	5.0157	0.0005	
PR 1307	0.0983	0.00684	6.9613	0.0005	

KEY: KS EAS =Kenya (East Africa) Standard; LLD=Lowest Limit of Detection

WATER QUALITY TESTING LABORATORY 29 MAY 2018 TECHNICAL SIGNATORY

COMMENTS/REMARKS

Results are as shown above

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Technical Manager - Andrew Kulecho

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