ASSOCIATION BETWEEN HEAVY METALS AND SELECTED REPRODUCTIVE PARAMETERS IN THE NILE TILAPIA, *Oreochromis niloticus*, ALONG RIVER RUIRU, KIAMBU COUNTY, KENYA

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DECLARATION

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

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DEDICATION

I dedicate my work to my husband Joseph Mokaya and my children Rael Moraa, Samson Moreka, Zablon Nyamora and Calvin Oyaro who gave me all the support I needed during the course of this study.

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ABBREVIATIONS AND ACRONYMS

APHA	American Public Health Association
ANOVA	One way analysis of variance
DNA	Deoxyribonucleic acid
DWAF	Department of Water Afairs and Forestry
DPHCGK	Department of Public Health of the County Government of Kiambu
ELISA	Enzyme Linked Immunosorbent Assay
EIAR	Environmental Impact Assessment Report
FAO	Food and Agricultural Organization
GSI	Gonadosomatic Index
HELCOM	Helsinki Commision
KNBS	Kenya National Bureau of Statistics
MENR	Ministry of Environment and Natural Resources
NEMA	National Environment Management Authority
RNA	Ribonucleic acid
TDSTP	Thika Distric Strategic Plan
UNEPR	United Nations Environmental Programme Report
USEPA	United States of Environmental Protection Agency
WHO	World Health Organization

ABSTRACT

The Nile tilapia, *Oreochromis niloticus*, is a tropical fish species of commercial importance in both aquaculture and in the wild. It plays a great role in human nutrition and food security. Industrial effluents, contaminated with pollutants such as heavy metals are released into water bodies such as streams and rivers, in most countries. River Ruiru is one of the rivers faced with pollution from nearby industries. It is inhabited by various species of fish such as catfish, tilapia and common carp, which, may be harvested by surrounding communities for food. High levels of heavy metals disrupt normal reproductive process in fish. Besides, it has been reported that edible fish contaminated with heavy metals has deleterious effects on the health of humans and other animals that consume them. *Oreochromis niloticus* forms an important source of proteins in many communities. The inhabitants neighbouring River Ruiru, especially those of low socio-economic status obtain fish from the River. This study was aimed at assessing the current status of heavy metal pollution in River Ruiru and their association with selected reproductive parameters in the reproductive cycle of *Oreochromis niloticus*. Fish and water samples were collected monthly, for 8 months, from the downstream and upstream sections. Morphometric measurements, gonadosomatic index (GSI), serum 17β-estradiol (E_2) levels, physicochemical parameters of the river and the levels of five heavy metals (lead, cadmium, copper, iron and zinc) in water and ovaries were determined. The levels of the heavy metals were measured using Atomic Absorption Spectrophotometer (AAS). The level of E_2 was analyzed using Enzyme-Linked Immunosorbent Assay (ELISA). The difference in means of GSI, level of E_2, fecundity, morphometric measurements of the fish from the downstream and the upstream sections of the river was calculated using a two sample t-test. The relationship between the level of heavy metals versus morphometric measurements; GSI; and the level of serum E_2 was determined using Pearson moment correlation. One way analysis of variance (ANOVA) was used to test if there were significant differences in GSI and levels of E_2 between different months. Water in both sampling sites was found to have equal levels of heavy metals implying that both sites were equally polluted. There was no significant difference in gonadosomatic index between the upstream and the downstream sites (t=0.82, p=0.416). Similarly, there was no significant difference in the levels of E_2 between the downstream and the upstream sampling sections. The mean standard length of the mature tilapia, *Oreochromis niloticus,* in the upstream was significantly lower compared to the mean standard length in the downstream (t =2.87, p = 0.008). In the downstream, the levels of lead and iron in fish ovaries were significantly higher compared to the upstream (lead: t = 3.36, p = 0.002; iron: t = 4.920, p=0.001) due to their higher levels of accumulation. The results showed that levels of heavy metals did not associate with the selected reproductive parameters in the Nile tilapia, along River Ruiru. Levels of lead and cadmium both in water and fish ovaries were above allowable concentrations for fish consumption when compared to WHO and United States of Environmental Protection Agency (USEPA) recommended levels. The study recommends that the Ministry of Environment and Natural Resources (MENR) should put measures in place to stop discharging raw effluents into River Ruiru. Also, National Environment Management Authority (NEMA) and Department of Public Health of the County Government of Kiambu (DPHCGK) should hold campaigns for residents of Ruiru in order to safeguard their health by avoiding consumption of fish from the River.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Fish is of great importance for nutrition worldwide, employment and trade in developing countries. According to Food and Agricultural Organization (FAO), fish accounts for more than 40 percent of protein in the diet of two thirds of global population (FAO, 2012; 2014). Tilapia is the second most popular farmed and traded globally (FAO, 2018). It is one of the fish species included in Fish Farming Enterprises Productivity Programme (Charo-Karisa and Gichuri, 2010), in which several fish ponds have been initiated in order to solve food insecurity issues, as envisaged in Vision 2030.

The reproductive cycle of tilapia is complex, originating from oogenesis to fecundity through six stages (Patino and Sullivan, 2002; Ehsan *et al.,* 2013). The cycle is controlled by hormones such as follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol-17β (E_2) (Okuzawa, 2002).

River Ruiru is affected by rural and urban effluents, agricultural and industrial efluents (Muiruri *et al.,* 2013), which make the river polluted with heavy metals. Some of the heavy metals are required for the normal metabolism of organisms such as copper, iron and zinc, when in trace amounts, while others play no significant biological role as lead and cadmium (Bahar *et al.,* 2010). Fish can be contaminated by heavy metals. Excess levels of heavy metals impair gonadal development through suppression of synthesis of 17β-estradiol (Annabi *et al.,* 2013). They can also suppress gamete development and growth (Authman *et al*., 2015). Studies on the effect of heavy metals on reproductive cycle of fish may provide information on population changes due to suppressed reproduction. Fish contaminated with heavy metals is unsafe for human consumption as this is associated with potential health disorders such as immunodeficiency, osteoporosis, neurodegeneration and organ failures (Rzymski *et al.*, 2015). There is no information on the association between heavy metals and reproductive biology of fish inhabiting river Ruiru. The current study was therefore undertaken to study the relationship between heavy metals and selected reproductive parameters in the reproductive cycle of *O. niloticus* to thereby establish correlations between metals in the ovaries and reproductive status of fish in river Ruiru.

1.2 Statement of the problem

River Ruiru harbors various species of fish, which are potential sources of proteins for the surrounding communities. However, the river is influenced by human activities that discharge industrial and domestic wastes which are sources of heavy metals (Kimani *et al.*, 2016). Fish accumulate heavy metals in their tissues through absorption; these are then transported and accumulated in vital organs like liver, kidneys, brain and gonads and if eaten by humans, affect human health (Galanis *et al.*, 2009).

1.3 Justification

Fish form an important source of food. It is therefore important, that fish reproduce at an optimal rate for continuity of their species and to contribute to food security. However, in many instances, the aquatic environment is faced with pollutants such as heavy metals from human activities like industrial and agricultural activities.

Oreochromis niloticus forms an important source of proteins for many communities, and inhabitats neighbouring River Ruiru, especially those of low socioeconomic status. It was therefore important to determine the level of heavy metals in the water and fish ovaries as well as their effect on reproductive performance of *Oreochromis niloticus.* There is no documented information on the association between heavy metals and the reproductive biology of *Oreochromis niloticus* in River Ruiru. The current study therefore was conducted to investigate the association between heavy metals and the selected reproductive parameters in the Nile tilapia, *Oreochromis niloticus,* in the river.

1.4 Research questions

i. What is the relationship between levels of heavy metals and the gonadosomatic index (GSI) of Nile tilapia, *Oreochromis niloticus,* in River Ruiru?
ii. What is the relationship between levels of heavy metals and the serum level of 17β-estradiol in Nile tilapia, *Oreochromis niloticus,* in River Ruiru?
iii. What is the association between heavy metals and the size at sexual maturity of female Nile tilapia, *Oreochromis niloticus*, in River Ruiru?
iv. What is the association between heavy metals and the fecundity of Nile tilapia, *Oreochromis niloticus,* in River Ruiru?

1.5 Hypotheses

i. There is no relationship between levels of heavy metals and gonadosomatic index (GSI) of Nile tilapia, *O. niloticus,* in River Ruiru.
ii. There is no relationship between levels of heavy metals and serum level of 17β-estradiol in Nile tilapia, *Oreochromis niloticus,* in River Ruiru.

iii. Levels of heavy metals have no association with the size at sexual maturity of Nile tilapia, *Oreochromis niloticus,* in River Ruiru.

iv. Levels of heavy metals have no association with fecundity of Nile tilapia, *Oreochromis niloticus,* in River Ruiru.

1 .6 Objectives

1.6.1 General objective

To determine the association between heavy metals and selected reproductive parameters in the Nile tilapia, *O. niloticus,* in River Ruiru.

1.6.2 Specific objectives

i. To determine the relationship between levels heavy metals and the gonadosomatic index (GSI) of the Nile tilapia, *O. niloticus,* inhabiting River Ruiru.

ii. To determine the relationship between levels of heavy metals and the serum level of 17β- estradiol in the Nile tilapia, *O. niloticus,* in River Ruiru.

iii. To determine the association between levels of heavy metals and the size of the female Nile tilapia, *Oreochromis niloticus,* at sexual maturity in River Ruiru.

iv. To determine the association between levels of heavy metals and the fecundity of Nile tilapia, *Oreochromis niloticus,* in River Ruiru.

1 .7 Significance of the study

This study was aimed at providing information on the association between heavy metals and selected reproductive parameters in the Nile tilapia, *Oreochromis niloticus,* in River Ruiru. The findings of the study are expected to be useful in providing biological information that is required for future monitoring and guidance on the conservation and management of fishery resources in River Ruiru and other similar systems**.** Understanding the association between heavy metals and the selected reproductive parameters in Nile tilapia, *Oreochromis niloticus,* will help the Ministry of Environment and Natural Resources to design strategies in establishing measures to protect the fish from heavy metal pollution arising from neighbouring Ruiru communities. The information generated will also provide basis for future research work.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Nile tilapia, *Oreochromis niloticus*

Fish is an important commodity in the developing countries, that have food insecurity and are poverty stricken (Arthur *et al.*, 2015). Fish are widely consumed in many parts of the world due to their high protein content, minerals (iron, zinc, magnesium, phosphorous, calcium and iodine), vitamins (such as A and vitamin C), low saturated fat, and sufficient omega fatty acids with an omega-6 to omega-3 ratio favorable for human health (USEPA, 2004**).** In Kenya, *Oreochromis niloticus* is a commercially important species in the export trade, contributing in both volume and monetary value of Kenya’s total fish exports (FAO, 2015 and 2016).

Oreochromis niloticus is a deep-bodied fish with cycloid scales. It is silver in color with dark vertical body bands. It feeds on phytoplankton, aquatic plants, invertebrates, bottom fauna, detritus and bacterial films (Picker and Griffiths, 2011; FAO, 2012). The method of feeding depends on the source of food. Adult *O. niloticus* are omnivorous, but mostly feed on phytoplankton and algae (blue-green algae). Juveniles feed either via suspension filtering or surface grazing (GISD, 2012). The members of *Oreochromis niloticus* prefer shallow, still waters on the edge of a river accompanied with vegetation. Other workers also observed that this species prefer edges of lakes and wide rivers with sufficient vegetation (Picker and Griffiths, 2011). Their optimum growth temperature is reported to range between 28-36°C and declines with decreasing temperature (FAO, 2012).

2.2 Reproduction in tilapia

Oreochromis niloticus is reported to be a seasonal breeder; breeding being precisely timed (Gomez-Marquez *et al.,* 2003). Favourable breeding site of *O. niloticus* is reported to be shallow vegetated habitats near the shores of the water body (Balirwa, 1998). Just like in other vertebrates, oogenesis marks the base of reproduction in the Nile tilapia. It is divided into six phases: formation of primordial germ cells, transformation of primordial germ cells into oogonia, transformation of oogonia into oocytes, growth of oocytes while under meiotic arrest (vitellogenesis), resumption of meiosis (maturation), and expulsion of ovum from its follicle (Patino and Sullivan, 2002).

Sexual maturity in Nile tilapia species, *Oreochromis niloticus* advances in stages**,** gonad maturation being categorized into six stages. Stage I comprises of fish with transparent gonads that look small, thin and thread like in shape (Duponchelle *et al.,* 2000). Stage II is fish whose ovaries are white in color containing minute white eggs. Stage III is the developing phase with light yellow ovaries in females, and in males testes are enlarged, flattened and white in color. Stage IV is a pre-spawning stage. In *O. niloticus* ovaries are swollen with ripe eggs that are yellow in color (in females), and in males testes are white and swollen. Stage V is a spawning stage where ripened eggs are loosened from the ovary wall, while stage VI is a post-spawning stage where ovaries are flaccid (Duponchelle *et al.,* 2000).

2.3 Heavy metals in the aquatic environment

Rapid population growth, increased urbanization and expansion of irrigation that uses fertilizers, pesticides, and herbicides, have led to the release of pollutants like heavy metals into the aquatic environment from industrial effluents, municipal sewage and polluted runoff from urban and agricultural areas (Budambula and Mwachiro, 2006). Among environmental pollutants, metals are of particular concern, due to their potential toxic effect and ability to bioaccumulate in the aquatic ecosystems (Kithiia, 2012). Examples of heavy metals are lead, cadmium, copper, zinc and iron. The main sources of lead in the water courses include, manufacturing of lead chemicals, consumption of lead products (gasoline), insecticides and sewage containing trace amounts of lead (ATSDR, 2017). Car-wash is one of the activities that take place along River Ruiru, especially at the upstream section, and this may contribute to lead pollution in the river**.** Anthropogenic activities that release cadmium to aquatic environment include, domestic wastewater, non-ferrous metal smelting and refining, and manufacturing of chemicals and metals in the industries (HELCOM, 2002). Domestic waste water from Ruiru town gain access to River Ruiru, hence the need to analyze heavy metals in water and fish ovaries.

Concentrations of copper in the environment are usually related to anthropogenic sources such as electroplating, metal works, textile industries and antifouling paints (Schuler *et al.*, 2008). The main sources of zinc in the aquatic environment include domestic and industrial sewage, road surface runoff, corrosion of zinc alloys and galvanizwd surfaces, and erosion of agricultural soils (Mugera *et al.*, 2014).

Domestic and industrial sewage, corrosion of galvanized roofing iron sheets from Ruiru town and the surrounding communities may get their way into River Ruiru. Thus, there was need to analyze Zinc in the water and fish ovaries. Iron is commonly used as a raw material in the manufacture of articles that include nails, fencing wires**,** dustbins and roofing sheets.

2.4 Association between heavy metals and gonadosomatic index (GSI) in fish

Gonadosomatic Index is the calculation of the gonad mass as a proportion of the total body mass. It is represented by the formula:

GSI = Gonad weight ×100/total body weight (Barber and Blake, 2006).

It is an indicator of the state of gonadal development and maturity (Hama *et al.,* 2015). It reflects the physiological activity of the gonads, where the increase is an indication of the beginning of the breeding season of the fish (Laban, 2007). It is also an important parameter that reflects both the state of population for the continuity of generation and changes in organisms under the effect of heavy metals (Çiftçi1 *et al.,* 2015). The study of gonadosomatic index in different months gives an indication of the spawning season of the species (Mahmoud *et al.,* 2013).

Exposure of fish to various environmental toxicants causes gonadal changes such as decreased GSI due to stressed liver tissues resulting in reduced production of phosphoglycoproteins that form part of the egg yolk (Hama *et al.,* 2015). Heavy metals were reported to cause structural deformation of DNA and an increase in liver ethoxyresorufin ode-ethylase activity leading to a decrease in gonadosomatic index in fish (Martínez-Gómez *et al.,* 2012). Other workers reported that exposure of *Oreochromis niloticus* to cadmium decreases GSI at the beginning probably due to in- hibition of enzymes functioning in synthesis and release of reproductive hormones whereas prolonged exposure to this metal causes increase in GSI due to activation of synthesis of metal binding proteins in gonads (Çiftçi1 *et al.,* 2015). Higher gonadosomatic index was reported in Nile tilapia exposed to agricultural waste water contaminated with copper, nickel, iron and chromium (Mahmoud, 2013). Iron is an essential component of the respiratory pigments (haemoglobin and myoglobin) and various enzymes concerned in tissue oxidation including the cytochromes, catalases and peroxidases. It is also essential for oxygen and electron transport within the body (Gupta, 2014).

2.5 Association between heavy metals and the hormone 17β-etradiol (E_2) in fish

Estrogen is a sex steroid hormone that is essential in maintaining reproductive functions in fish (Pradhan and Olsson, 2015). The hormone is normally produced by both the female gonads (developing ovaries) and inter-renal tissues in response to stimulation by the pituitary gonadotrophins (luitenising and follicle stimulating hormones. It is responsible for stimulating vitellogenesis (formation of egg-york) during the pre-spawning period (Saeed *et al.,* 2009).

Heavy metals are reported to stimulate or inhibit the endocrine system and cause overproduction or underproduction of hormones such as 17β- estradiol in fish (Islam *et al.,* 2015). Heavy metal pollution in the aquatic environment may interfere with reproduction by disrupting the endocrine system through direct cytotoxic effects of metals on reproductive tissues (Tabb and Blumberg, 2006). Increase in heavy metal exposure decreases secretion of estrogen in fishes (Ebrahimi and Taherianfard, 2011**).** Accumulation of heavy metals in the ovaries and other environmental pollutants is reported to disrupt the production of reproductive hormones such as 17β- estradiol, luteinizing hormone and follicle stimulating hormone. This occurs through changes in the physiological processes of the hypothalamic – pituitary – ovarian axis (Bolawa *et al.,* 2014). Studies report that Cadmium inhibits steroidogenesis hence suppressing estrogen production. Lead is also reported to suppress steroidogenesis and hence circulating luteinising hormone, follicle stimulating hormone, and estradiol, leading to a reduction in ovarian primordial follicles (Jackson *et al.*, 2011). However, other researchers reported that estradiol levels significantly increased in dissolved cadmium exposed female *Oreochromis niloticus* (Luo *et al.*, 2015).

2.6 Association between heavy metals and size at sexual maturity of fish

Size at sexual maturity is the length at which gonad development has advanced to at least stage IV in 50% of the individuals (Njiru *et al*., 2006). Sexual maturity in tilapia depends on age, size and environmental factors. In fresh water, growth is reported to be influenced by availability of food and environmental factors such as temperature, dissolved oxygen and pH (Yovita, 2007).

Copper, zinc and iron are essential and necessary in trace concentrations for normal growth and development (Rathore *et al.*, 2011). They are essential trace metals which play a role in several enzymatic processes, such as enzymes involved in cellular respiration, free radical defense, neurotransmitter function and connective tissue biosynthesis (Chojnacka *et al.*, 2017). They become toxic when their levels exceed required levels (Yilmaz, 2005). Pollution due to high level of heavy metals result in a depletion of energy resources such as stored glycogen or body fat and leading in a reduction in fish weight and length (Hama *et al.*, 2015).

At high concentrations, zinc is reported be toxic to fish and may delay or inhibit the growth, sexual maturity and reproduction of fish (Yirgu, 2012). Excess iron limits motion of the fish to resources such as food and oxygen, through its deposition on gills (Teien *et al*., 2008). Exposure of fish larvae to high levels of cadmium reduces the inrease in length and weight (Puvaneswari and Karuppasamy, 2007). Cadmium ions cause a decrease in appetite, food assimilation, locomotion activity and growth rate leading to small sized fish, after exposure of the fish to 10 mg Cd g per liter of food (Szczerbik *et al*., 2006). Adult fish exposed to waste water contaminated with heavy metals to high levels of heavy metals exhibited increased size and weight (Manal *et al.,* 2014).

2.7 Association between heavy metals and fecundity in fish

Fecundity is the number of eggs ripened by the female during a spawning season. Fecundity in tilapia varies depending on environmental factors, food availability and species. It is reported that exposure of fish to high levels of the heavy metals zinc and copper (0.5mg/l repectively, causes atrophy and cytoplasmic leakage in the ova leading to severe degeneration (Tang *et al*., 2013). Heavy metal toxicity hinders the synthesis of vitellogenin leading to reduction in the size and development of oocytes in the fish (Mousa and Mousa, 1999). Other researchers observed that heavy metals cause atresia and necrosis in oocytes leading to a decrease in egg production (El-Morshedi *et al.,* 2014). Different types of pollutants and heavy metals cause deformity and infections on fish gonads (Mazrouh and Mahmoud, 2009). Industrial and agricultural wastes and pesticides have been reported to cause different histopathological effects on the reproductive tissues of fish gonads (Shobikhuliatul *et al*., 2013; Onkar and Sulochana, 2015). These effects may disturb the development of germ cells and may reduce the ability of the fish to reproduc**e** (Hanna *et al.,* 2005).

Lead, Copper and iron are reported to cause oxidative stress in fish (Sevcikova *et al.*, 2011). Stress during reproductive development can have negative effects on fecundity and gamete development and quality in some fish species (Campbell *et al*., 1992). Exposure of an Indian teleost to copper, zinc, or lead, caused disappearance of oocytes in the ovaries (Mazrouh and Mahmoud, 2009). Also, post hatch larvae of *Oreochromis niloticus* subjected to 2 and 5 ppm sublethal levels of zinc for 30 days retained undifferentiated gonads with oogonial proliferation and ovaries of mature tilapia exhibited hyperemia and reduced oocyte number (Caring, 1992). Cadmium prevents egg maturation and hence lowers the number of spawned ova (Karels *et al*., 2003). Lower vitellogenin expression has been reported in the fish ovaries exposed to cadmium, preventing the formation of ova (Luo *et al.,* 2015). Female *Oreochromis niloticus* produces a few hundreds of offspring in a single spawn but under favorable conditions they spawn frequently every 4 to 6 weeks (Darko, 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study location

The study was conducted in River Ruiru, Kiambu County, Kenya. The river passes through Ruiru Town in Ruiru Sub County, which is 3 kilometers away from Nairobi City County border as it joins Athi River. According to KNBS (2010) report, the population was found to be approximately 238,855. The Sub County covers an area of 526 square kilometers (TDSTP, 2010). Two sampling sites along the river were considered during the study period. They are within longitudes 36^0 ‘54’E and 37^0 ‘3’E and latitudes 1^0 ‘12’S and 1^0 ‘8’S (Figure 3.1) within Ruiru Sub County. The mean annual maximum temperature of Ruiru is about 26 degrees Celsius while the mean minimum temperature is around 14 degrees Celsius. The mean annual rainfall averages between 850mm – 1000mm. The average altitude is about 1570 meters above mean sea level (Shadrack, 2011).

The river originates from the slopes of the Aberdare Ranges and flows through several coffee plantations and then through Ruiru Town that is located along the Thika superhighway between Kenyatta University and Juja. To the North-West region, the town’s landscape is steep and divided by Mukuyu and Ruiru Rivers (EIA, 2011). The town hosts several industries including Devki Steel Mills, Brookside Dairy and Spinners Garment Factory that have provided employment and contributed to its economic growth.

There are several settlements along the river without proper sewage disposal system, despite the large population. The river also passes through areas where some industries discharge their wastes into it. Human activities along River Ruiru therefore, affect aquatic animals living along the River (UNEP, 2001). Ruiru is endowed with agricultural land and hence agricultural activities take place in the area. There is extensive use of fertilizers, insecticides and fungicides to improve agricultural production (Kiambu County Government, 2014). In addition, there is intensive housing and industrial development (EIA, 2011). The tropical climatic conditions of the area favor cash crops such as coffee and tea produce that are used for both subsistence and commercial purposes (Makokha *et al.*, 2001).

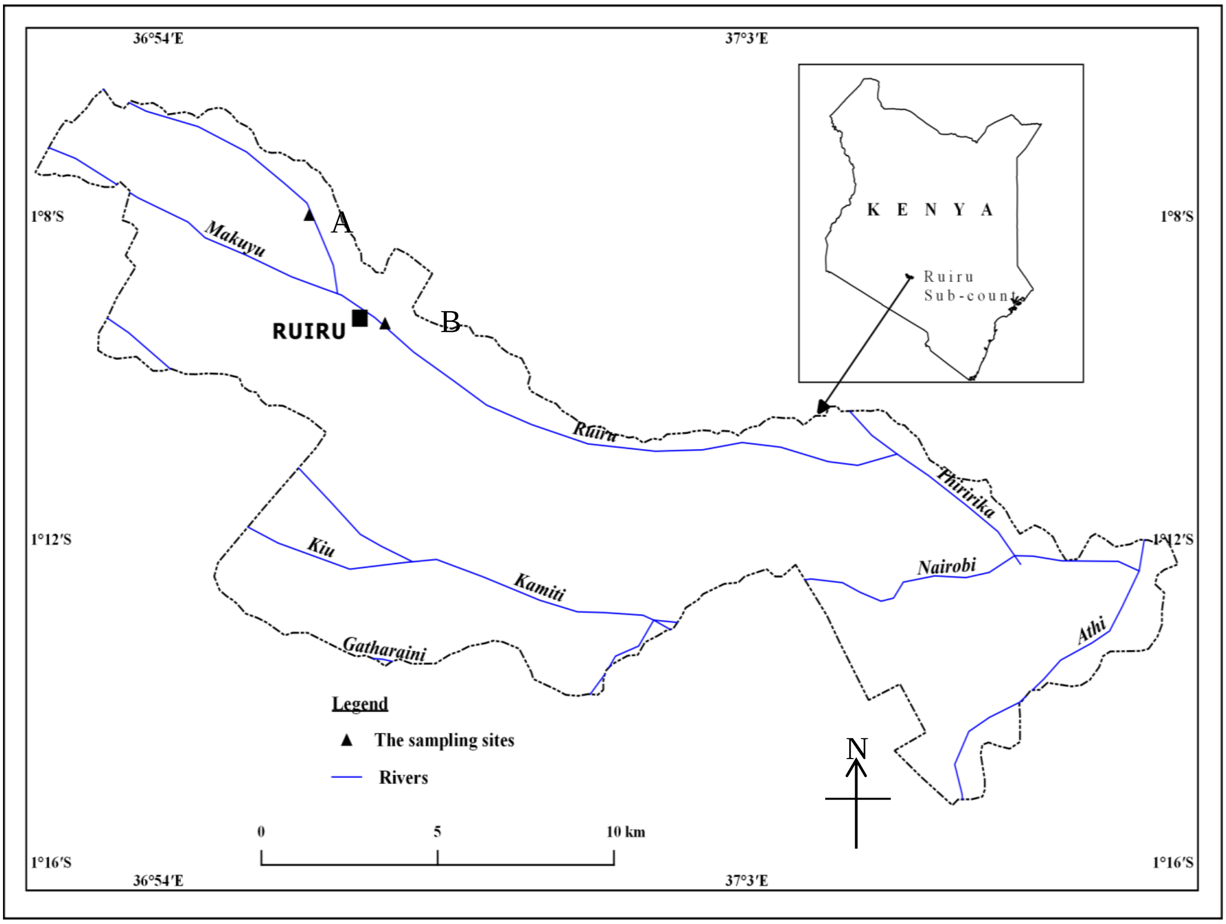

Figure 3.1: Map of Ruiru Sub-county Showing the Location of the Sampling Sites. (Source: Kiambu Topo Map and Kiambu County Government, 2010)

The major cash crop grown in this area is coffee that is locally processed while excess is exported abroad. Some farmers also practice horticulture and floriculture.

Cereals such as maize and sorghum are mainly for domestic purposes, and the surplus is transported to neighboring towns such as Nairobi and Thika. Other farmers keep dairy cattle for the provision of milk, beef and other dairy products. There is also fish farming for subsistence and commercial purposes in Ruiru, following the economic stimulus program (ESP) government project (The Star, 2011).

3.2 Experimental design

Fish were captured using castnet and water samples collected with plastic bottles. Levels of lead, cadmium, copper, zinc and iron in water samples from downstream sampling sites were analysed and compared with levels of metals in water samples collected from the upstream sampling site located along River Ruiru. Ovary samples of *Oreochromis niloticus* from the downstream and upstream sampling sites were also analyzed for the levels of lead, cadmium, copper, zinc and iron. A comparison in the levels of the heavy metals in the ovaries of the fish from the downstream and upstream sites was made. Levels of 17β-estradiol in serum of fish sampled from the downstream parts of the river was determined and comparison made with serum level 17β-estradiol in the fish sampled from the upstream site. Morphometric measumement of fish sampled from the downstream site of the river were determined and a comparison was made with the fish sampled from the upstream site. Also fecundity of fish from the downstream part of the river was determined and comparison made with the fish sampled from the upstream sampling site.

3.3 Sampling and sample size

Sampling sites were chosen based on the surrounding economic activities, proximity of sampling section to settlement areas, point of confluence between River Ruiru and River Mukuyu, point of effluent discharge into the river, and physical appearance of the river water. Sections with *Oreochromis niloticus* samples were therefore purposively identified during the preliminary studies. The course of River Ruiru was divided into downstream and upstream sections with respect to Ruiru Town and one sampling site was chosen on each section. The upstream site was identified as ‘A’ , located upstream along the course of River Ruiru, 3 kilometers past Ruiru Town while the downstream site, ‘B’, was at the downstream section of the river, 750 meters away from Ruiru Town. The sampling sites were 4 kilometers away from one another (Figure 3.1). Each sampling section was 100 meters long. Depth and width of sampling sections were not uniform hence the width and depth were not taken into consideration.

Samples of *Oreochromis niloticus* were taken using a castnet for eight months (November 2014 to June 2015). It was not possible to collect six samples of female Nile tilapia from each sampling site once a month as was planned earlier because they were scarce in both sampling sites. The downstream sampling section was at the entrance of wastes from Ruiru town and the upstream section was adjacent to agricultural land for vegetables and maize with intensive use of insecticides, fungicides and both organic and inorganic fertilisers. Three water samples were collected at 3 different spots within the upstream sampling point and three from the downstream sampling point once a month. The number of *O.niloticus* samples collected from the upstream and the downstream sampling sites depended on their availability during the sampling time. The same procedure was repeated for eight conserecutive months so as to account for the relationship between levels of heavy metals and gonadosomatic index, levels of steroid 17β-estradiol, size at sexual maturity and fecundity of the fish.

3.4 Physicochemical characteristics

Physicochemical characteristics of the water were determined at each sampling site during each session of sampling water. These were turbidity, temperature, pH, electrical conductivity and dissolved oxygen. Measurements were taken during morning hours between 7.00 am and 12.00 pm throughout the study period to avoid temporal variations during the day. Measurements were done at three different points within the upstream and downstream sampling sites once a month.

Turbidity was measured by lowering a Secchi disc of 20 cm diameter deep into the water and the length at which it disappeared was taken. It was raised again slowly and carefully and the length at which it reappeared was noted. The turbidity was calculated as the mean distance where the disc disappeared when viewed from the surface of water and the point when it reappeared upon rising after it had been lowered beyond visibility. Water temperature was determined using a pocket thermometer by dipping its bulb into the water. Measurements were taken at 20 cm deep in the water in the sampling sites in the morning hours between 7.00 am and 12.00 pm when fish were caught. Dissolved oxygen was measured by using oxygen meter by dipping its probe into the water 20 cm deep from the water surface in each sampling site. The pH was recorded using a portable pH meter model 43800-00 by Hach Co and the pH determined by dipping its probe into the water in each study site. Conductivity was determined using the conductivity meter (Model 44600 by HANNA) by diping its probe 20cm deep into the water.

3.5 Collection of water samples

Three water samples were collected by using 200 ml polyethylene sampling bottles from each of the sampling points once amonth. Sampling was done by dipping each bottle at approximately 20 centimeters below the water surface and projecting the open end of the bottle against the flow direction. The collected water samples were then transported to the laboratory and immediately acidified with concentrated nitric acid to dissolve any metals in the water. They were then kept at -20 ^{o} C (freezer) for preservation prior to digestion and analysis of the heavy metals lead, cadmium, copper, zinc, and iron.

3.6 Sampling of fish

Fish samples were collected using castnets that were made up of three parts: the upper section (net band), the middle section (a conical-shaped net mesh), and the lower section, which was lead weighted (0.45 grams of lead masses per foot of radius). Each had a diameter of 4 meters and mesh of 7 millimeters to avoid gilling the fish which hastens their death. Inside the net, lumps of ugali and pieces of flesh were included to attract the fish into an area within the cast nets' range. The nets were casted onto the surface of water in the sampling areas. When the weighted portions of the nets reached the bottom of the sampling section, the top band of the nets were pulled, making the net closed into a sack-like shape within the water. With the weights dragging along the sampling bottom, the nets were slowly drawn back to the shore of the sampling point, ten minutes after the throw to collect the captured fish within the net, if available. Ten throws were made at ten different spots within the upstream sampling point and ten within the downstream sampling point once a month between 7.00 am and 12 noon.

A cast net is small enough to be operated by one person. Its round and can be thrown, over a large portion or longer length of the sampling section. Also, weights on the sides of the net help it sink, catching all sizes of fish samples.

Sampling of fish was done once a month from each of the sampling sections during the sampling time. The samples were then transported alive in buckets of water to Nairobi National Museum of Kenya for identification. The fish that were targeted were *Oreochromis niloticus* of all sizes. They were labelled as A1, A2…if from the upstream sampling sitte and B1, B2…if from the downstream site to avoid confusion during the study. They were then transported to the Department of Zoological Sciences, Kenyatta University, for morphometric measurements and preparation before analysis of 17β-estradiol and heavy metals, lead, cadmium, copper, zinc, and iron.

3.7 Sex ratio

All fish samples were macroscopically examined and the sex of each sample was established based on the external morphologies of each sample using a magnifying lens in the laboratory. The sex ratio was determined by using the actual number of fish whose sexes were successfully identified (Mackie and Lewis, 2001). The ratio was determined using the proportions of the number of females to that of males. All the males were discarded and the females were retained for subsequent studies.

3.8 Immobilisation of the fish samples

Fish samples were immobilized by following procedures by Zang *et al.* (2015) to stop them from making movements.They were dipped seperatly in a 10 millimeter glass trough containg a solution of 2-phenoxyethanol (2-PE) whose concentration was 500 parts per million (ppm). The fish took about 2 minutes to stop making movements.The anesthetized fish were lifted from the 2-PE solution by using a skimmer and gently placed on a paper towel soaked with the anesthetics. The fish's head were covered seperately with soft tissue paper also soaked with 2-PE solution to prevent eye dryness. The body surface of the fish were gently dried separately with another dry soft tissue paper.

3.9 Morphometric measurements

Standard length is the measurement taken from the tip of the lower jaw to the posterior end at a point where spiny rays of the caudal fin are attached (Önsoy *et al.,* 2011). Standard length (SL) of each sampled fish in stages II, III, IV, V, and VI from both sampling sections per month, were taken using a tape measure graduated in centimeters. Wet body weight, in grams, of each of the sampled fish in stages II, III, IV, V and VI was taken using an electric weighing balance (Model AAA Adam Co Limited). Wet body weight refers to the weight of the fish including its contained water (Ayo-Olalusi, 2010).

3.10 Blood samples from the females

Five millilters of blood samples were withdrawn from the fish samples of stages II, III, IV, V and VI seperately from both sampling sections, via cardiac puncture using medium sized heparinized needle and 5 milliliter syringes. The blood was then transferred to micro-centrifuge tubes separately and centrifuged to separate the blood serum from plasma. The serum was pipetted into Eppendorf tubes, and then stored in a deep freezer at-20^0 C until analysis for the level of 17β- estradiol.

3.11 Determination of sexual maturity stages

The fish samples collected from both sampling sites were dissected separately. Ovary samples were carefully excised and trimmed seperately to remove connective tissues. The maturity stages of the fish gonads were determined seperately through visual inspection of the appearance, size and texture, following the procedures by Mous *et al.* (1995) and Shoko *et al.* (2015). The stages of the reproductive maturity of each ovary samples were categorized as follows:

Stage I (Immature): ovaries were white and sexes were indistinguishable. Stage II (early developing but immature) white ovary walls with small white dots (eggs) inside. Stage III (developing): ovary walls were yellow with numerous small sized white eggs and large sized scattered yellow eggs. Stage IV (ripening): almost all the eggs were equal in size, attached to the ovary wall and coloured yellow. Stage V (ripe): dark yellow ovaries and loosened large yellow eggs. Stage VI (Spent): Thin, shrunkened and red ovaries containing few eggs of different stages.

3.12 Gonadosomatic index (GSI)

Gonadosomatic index is the calculation of gonad weight as a percentage of total body weight **(**Atiqullah, *et al.,* 2013). Weight, in grams, of the ovary samples of the fish samples obtained from both sampling sites were taken separately using an electric weighing balance (Model AAA Adam Co Limited). The GSI for the fish samples were calculated seperately using the formula by Khallaf and Authman (2010):

$$\mathbf{GSI} = \frac{\textbf{weight of gonad}}{\textbf{Weight of fish}} \boldsymbol{X}\mathbf{100}$$

3.13 Fecundity (F)

Fecundity is the number of eggs ripened by a female during spawning season (Duponchelle *et al.,* 2000). Ovary samples in stages IV and V were carefully dissected separately using a scalpel. The eggs were spread on the dissecting tray and the egg samples physically counted and recorded (Njiru *et al*., 2006).

3.14 Quantitative determination of 17β- estradiol (E_2) concentrations in serum.

The sex steroid 17β estradiol in the blood serum was analyzed using Enzyme-Linked Immunosorbent Assay (ELISA) following the assay kit procedures and methods by Cuisset *et al.* (1994) and Nash *et al.* (2000). Each reagent in the kit is a combination of several other reagents. This assay has high sensitivity and excellent specificity for detection of fish E_2. The standard concentrations (pg/ml) were 0, 40, 100, 400, and 1000. One hundred microlitres (100 µl) of serum sample was dispensed in duplicate into wells of the microlitre plates for ELISA. One hundred microlitres (100µl) of an enzyme antigen conjugate was added to each well (to bind with antibodies on the walls of the wells) mixed and the plates were incubated for 60 minutes at room temperature (20-25^0 C). This was followed by washing three times with wash solution to remove the unbound conjugate. The remaining liquid was removed by tapping the plates upside down on a piece of tissue paper. Then 100µl of a substrate solution was added to each well (to break down the enzyme-antibody complex) and the plates were incubated for 15 minutes at room temperature, followed by addition of 100µl stop solution to stop the reaction of color development which is dependent on the amount of 17β estradiol (E_2) in each well. The intensity of the blue color that developed (absorbance) was measured at 450nm with a microplate reader (BIO-RAD microplate reader Model 550). The concentration of 17β estradiol in serum samples were interpolated from the standard curve (Figure 3.2)

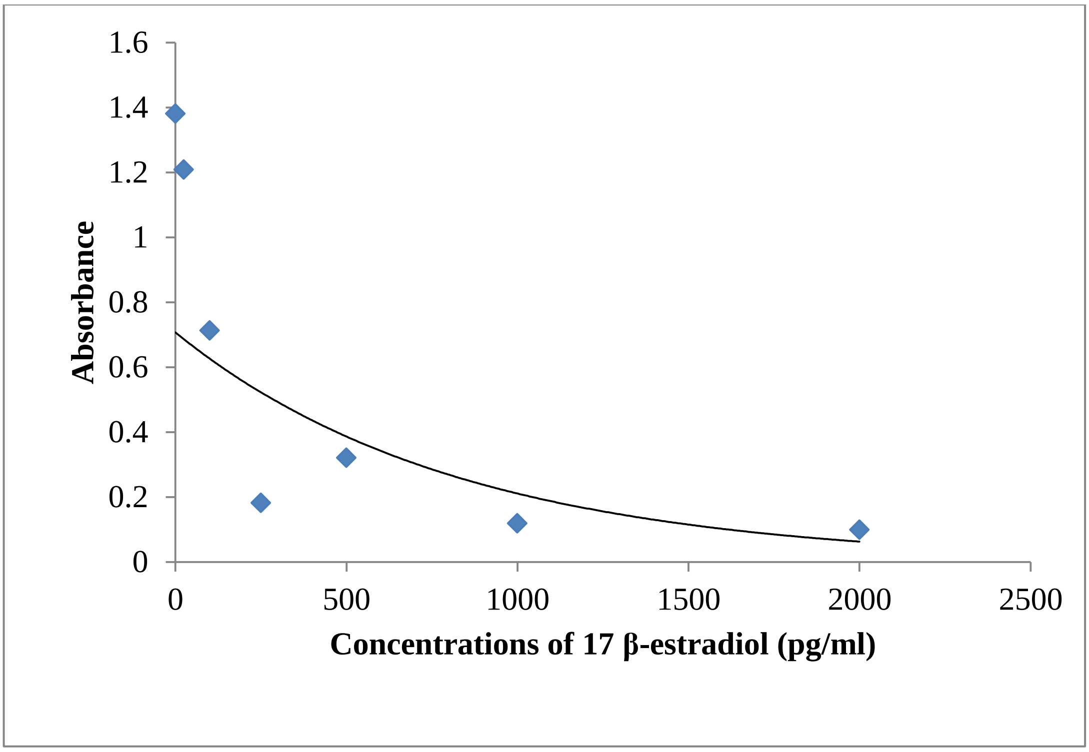

Figure 3.2 Standard curve for 17β- estradiol

3.15 Digestion of ovary samples

Ovary samples were wet digested separately where 1g of the wet digest was accurately weighed using an electronic balance (Model AAA Adam Co limited). Ten milliliters (10ml) of concentrated nitric acid was first added into the ovaries in separate glass beakers and allowed to stand overnight. They were then gently heated on hot plates until dense brown fumes began to appear. Hydrogen peroxide was added dropwise to clear the brown fumes and improve the dissolving power of nitric acid. Digested fish ovaries were allowed to evaporate to about 5 ml to get rid of excess water from the mixture. This was cooled and filtered (using Whatman number 42 filter paper into 100 ml different clean and dry volumetric flasks and then diluted to the mark with distilled water. The ovary samples (thirty from upstream) and (seventeen from downstream) were digested in triplicates then transferred into separate plastic bottles, labeled and stored awaiting analysis of the heavy metals lead, cadmium, copper, zinc and iron. For background correction, six blanks were digested as pre-test samples and each analyzed for lead, cadmium, copper, zinc and iron by atomic absorption spectrophotometer (Türkmen and Ciminli, 2007). Blank solutions were free of the heavy metals lead, cadmium, copper, zinc and iron. Hence they were used to test if the AAS was free from contamination of lead, cadmium, copper, zinc and iron before analysis of the digested water and ovary samples.

3.16 Digestion of water samples

Ten milliliters of the acidified water was measured and put into a clean conical flask and 5 ml of concentrated nitric acid added. The mixture was heated at 100^0 C with the addition of few drops of hydrogen peroxide until there were no brown fumes and the volume reduced to about 5 ml. The mixture was then filtered using Whatman filter paper No. 42 into a 25-ml volumetric flask and topped with distilled water to the labelled mark of 25 milliliters. Sixteen samples of water were digested in triplicates. They were then transferred into separate plastic bottles, labelled and stored awaiting analysis of heavy metals lead, cadmium, copper, zinc and iron (Türkmen and Ciminli, 2007).

3.17 Calibration procedure

Calibration curves for each heavy metal were set to ensure the accuracy of the Atomic Absorption Spectrophotometer (AAS) and to confirm that the results of determination were true and reliable. The calibration of the Atomic Absorption Spectrophotometer (Buck Scientific model 210 VGP) was made with standard solutions. Five working calibration standards were prepared by dilution of concentrated stock solution of 1000 mg/L for lead, cadmium, copper, zinc and iron. These solutions and blanks were aspirated into AAS. Calibration curves of absorbance against concentration for each metal were established and used for determination of metal concentration in the samples of fish ovaries and water. The correlation coefficient of metals was determined from the calibration curves in the appendices.

3.18 Analysis of heavy metals

Analysis of the heavy metals lead, cadmium, copper, zinc and iron, in the digested water and ovary samples were determined separately using Atomic Absorption Spectrophotometer (AAS). The concentrations were read from the standard curves generated, using the standards prepared based on atomic absorption standards made. Each sample was assayed in triplicate (the average values calculated from triplicates were used in statistical analysis). The minimum level of detection for each of the metals (lead, cadmium, copper, zinc, and iron) was 0.001 mg kg^{-1}.

3.19 Statistical analysis

Statistical analysis of data was carried out using Minitab programme version 13. The data on levels of lead, cadmium, copper, zinc and iron in water were analyzed by paired t-test to test whether there was significance difference between upstream and downstream sections. A two sample t-test was used to compare the difference in mean levels of the heavy metals in the ovaries, means of gonadosomatic index, serum level 17β-estradiol, standard length and fecundity of the fish from the downstream and upstream sections of the river. To establish the relationship between the level of heavy metals in the ovaries and gonadosomatic index (GSI), 17β-estradiol, standard length (SL) and fecundity, a Pearson moment correlation was conducted. One way analysis of variance (ANOVA) was used to test if there were significant differences in GSI and levels of 17β-estradiol between different months. The results were expressed as mean ± S.E. Difference in mean values were accepted as being statistically significant at $p<0.05$.

CHAPTER FOUR

RESULTS

4. 1 Characteristics of the sampling sites

4.1.1 Upstream characteristics

The upstream sampling section was relatively vegetated with both emergent and submerged water plants (Plate 4.1). Water was generally dark green and the bottom substrates consisted of decomposing plant materials, intermingled with scorpions and crabs.

Plate 4.1 Upstream sampling section

4.1.2 Downstream characteristics

The downstream sampling site was open with very few emergent plants. Water was cloudy except during the rains when it was brown. The bottom substrate was muddy (Plate 4.2).

Plate 4.2 Downstream sampling section

4.2 Physicochemical parameters of the water in the upstream and the downstream sections of River Ruiru

Overall mean physicochemical parametes of water in the upstream and the downstream sections of River Ruiru are recorded in table 4.3. In the upstream section of the river, the highest electrical conductivity (92.5 ± 3.5 µs/cm) was recorded in the month of April and lowest was recorded in the month of February (44.0 ± 1.9 µs/cm) (Table 4.1). The highest dissolved oxygen was 5.95±0.1 mg/l, recorded in March and the lowest was 4.18 ± 0.22 mg/l in the month of May. The mean pH of the water was highest in the months of April (8.54 ± 0.09) and June (8.5± 0.05) while the lowest was in March (6.95 ± 0.05). Water temperature in the upstream was highest in the month of February (mean 22.0^{o}C). Water transparency was highest in January (mean 82.0±2 cm). Physicochemical parameters of the water in the downstream sites showed that electrical conductivity was highest in May (mean 91.0±3 µs/cm) and lowest in April (44.9±0.95 µs/cm (Table 4.2). Dissolved oxygen was highest in the month of January (6.1 ± 0.3 mg/l) and lowest (3.1±0.25 mg/l) in May. Water was most alkaline (pH, 8.5) in the month of April. Water temperature was highest (22.5^0 C celcius) in March; water turbidity was also higher in the month of March (mean 84.6 ± 0.4 cm) and lowest in April (24.0 ± 0.4 cm).

A paired t-test showed that there was no significant difference ($p > 0.05$) in the monthly means of all the parameters between the upstream and downstream sites (Table 4.3). It was, however, noted that electrical conductivity, dissolved oxygen and pH were slightly higher in the upstream than in the downstream waters. Temperatures and water transparency were slightly higher in the downstream waters than in the upstream water.

Table 4.1: Physicochemical parameters of the water in the upstream sections of River Ruiru

Month	Electrical conductivity (µs/cm)	Dissolved oxygen (mg/l)	pH	Temperature (^oC)	Water transparency (cm)
Nov.14	69.4±0.8	4.8±0.3	8.3±0	20.4±0.4	23.3±0.02
Dec. 14	76.4±0.4	4.5±0.25	8.3±0.2	20.5±0.5	29.7±4.1
Jan.15	45.4±2.6	5.8±0.2	7.5±0.05	20.4±0.6	82±2
Feb.15	44±1.9	5.4±1	7.55±0.25	22±0.03	81±1
Mar.15	45.5±7.4	5.95±0.1	6.95±0.05	19.7±0.2	80.3±0.1
April.15	92.5±3.5	4.55±0.15	8.54±0.09	21±0.95	23.8±0.05
May.15	88.5±1.5	4.18±0.22	8.2±0.1	21. ±0.5	25±0.15
June.15	83±13	4.4±0.2	8.5±0.05	20±20	34.5±0.5

Table 4.2: Physicochemical parameters of the water in the downstream section of river Ruiru

Month	Electrical conductivity (μs/cm)	Dissolved oxygen (mg/l)	pH	Temperature (^{0} C)	Water transparency (cm)
Nov.14	77.5±0	3.85±0.55	7.9±0.35	19.8±0.25	24.9±1.9
Dec. 14	86.5±2.5	3.2±0.4	8±0.05	20.8±0.25	25.9±2.9
Jan.15	47.7±0.8	6.1±0.3	7.5±0.1	20±0.05	81±3
Feb.15	51±1	5.4±0.1	7.5±0.1	21.9±0.1	83±3
Mar.15	44.9±0.95	4.6±0.15	7.4±0.4	22.5±0.95	84.6±0.4
April.15	87.5±0.5	4.0±0.2	8.5±0.1	20.8±1.2	24±1
May.15	91±3	3.1±0.25	8.2±0.1	21.1±0.85	34.3±0.5
June.15	51.5±5.5	5±0.1	7.7±0.1	21.6±0.8	45.2±0.75

Table 4.3: Comparison of the mean physicochemical parameters between the upstream and downstream

Physicochemical characteristics	Upstream (Mean ± SE)	Downstream (Mean ± SE)	t-value	P-value
Electrical conductivity	68.09 ± 7.21	67.20 ± 7.13	0.189	0.856
Dissolved Oxygen	4.95 ± 0.24	4.406 ± 0.38	2.028	0.082
pH	7.9± 0.20	7.84 ± 0.14	1.103	0.306
Temperature (^{o}C)	20.63 ± 0.25	21.06 ± 0.33	1.060	0.324
Water transparency	47.45 ± 9.94	50.36 ± 9.82	1.654	0.142

4.3 Sex ratio

A total of 88 *Oreochromis niloticus* were caught, where 54 of these fish were from upstream (30 females and 24 males) and 34 from downstream (17 females and 17 males) (Table 4.4). The ratio of male to female was 1.146: 1 in both upstream and downstream. A paired sample t-test showed that the mean number of the female tilapia from the upstream (3.75 ± 0.25) was significantly more than the mean number of females from the the downstream (2.125 ± 0.295), ($t = 4.33$; $p <0.05$). The number of male tilapia in the upstream (mean 3.50 ± 0.54) was significantly higher than the number of male tilapia in the downstream (mean 2.125 ± 0.35), $t = 2.58$; $p < 0.05$. A highly significant number of tilapia (both males and females) was collected from upstream (mean 3.625 ± 0.287) than in the downstream (mean 2.125 ± 0.316) ($t=4.74$; $p= 0.0001$).

Table 4.4: The number of sexually identified (mature and immature) *Oreochromis niloticus* sampled in the two sampling sites of the River

	Upstream			**Downstream**		
Month	**Females**	**Males**	**Sex ratio**	**Females**	**Males**	**Sex ratio**
Nov. 2014	3	2	3:2	2	2	1:1
Dec. 2014	4	2	2:1	2	3	2:3
Jan. 2015	3	2	3:2	2	2	1:1
Feb. 2015	4	4	1:1	1	2	1:2
Mar. 2015	5	3	5:3	2	2	1:1
Apr. 2015	4	2	2:1	2	2	1:1
May 2015	4	6	2:3	4	2	2:1
Jun. 2015	3	2	3:2	2	2	1:1

4.4 Maturity stages

A total of thity *O.niloticus* samples were collected from the upstream and seventeen from the downstream sampling sites. Maturity status of each fish in each month was determined and assigned as stages I, II, III, IV, V and VI. In stage I, gonads were not fully developed and under a magnifying lens, appeared to be in form of two tiny transparent threads. It occupied a small part of the body cavity. In stage II, the difference between males and females was clearly noticed; in females, oocytes were macroscopically distinguishable as quite minute, white and round scattered in the transparent ovaries. Ovaries still maintained their transparency status. Stage III gonads were slightly yellow. A few stage IV ova were noticed scattered among stage III ova. The ovaries appeared expanded and occupied almost two thirds of the abdominal cavity. Stage IV was a prespawning stage. A dense network of blood capillaries ran on the surface of the darkened yellow ovaries that occupied about three quarter of the abdominal cavity. Ripe eggs were slightly stuck on the ovary walls and ovaries occupied almost the entire body cavity. Stage V was a spawning stage. Ripened eggs were loosened from the ovary wall and visible through the ovarian wall. Stages VI were post spawning individuals whose gonads were spent already. Gonads looked shrunken, flaccid, sac like and reduced in volume.

4.5 Standard length and sexual maturity

The results of this study show that the mean standard length at sexual maturity (stages III-VI) of *Oreochromis niloticus* in both sampling sites (upstream and downstream) was within a range of 9-21 centimeters (Tables 4.5 and 4.6), respectively. Spawning occurred at a length of 13-19 cm in the upstream. In the downstream, spawning occurred at a standard length of 15-19 cm. Length of the post-spawning stage ranged 15.1-21 cm both in the upstream and downstream.

Table 4.5: Percentage of *Oreochromis niloticus* in various class standard lengths (cm) and maturity stages at the upstream section of River Ruiru (n=30)

Class size (centimeters)	**Percentage (%) maturity stages**						**% mature**
	N	II	III	IV	V	VI	
5-9	9	66.7	33.3	0	0	0	33.3
9-13	9	44.4	33.3	22.2	0	0	55.5
13-17	8	0	0	25	62.5	12.5	100
17-21	4	0	25	0	25	50	100

Table 4.6: Class standard lengths (cm) and maturity stages of female *Oreochromis niloticus* downstream (n =17)

Class size (centimeters)	**Percentage (%) maturity stages**						**% mature**
	Number	II	III	IV	V	VI	
5-9	3	66.7	33.3	0	0	0	33.3
9-13	1	0	100	0	0	0	100
13-17	3	0	33.3	33.3	33.3	0	100
17-21	10	0	20	40	40	100	100

4.6 Monthly distribution of maturity stages upstream and downstream

A total of thirty samples of fish in stages II, III, IV, V and VI were sampled from the upstream sampling site and seventeen samples from the downstream sampling site for eight months. In the upstream site, stage II fish samples were present throughout the sampling months except in February and May (Figure 4.1) while in the downstream, stage II were found in December 2014 and January 2015 (Figure 4.2). Stage III were caught in February March and April in the upstream site (Figure 4.1), while in the downstream; they were collected in November 2014, December 2014 and January 2015 (Figure 4.2). The prespawning stage (IV) fish in the upstream were caught in December 2014, January 2015, April, and June 2015 (Figure 4.1), while in the downstream, they were caught in the months of February 2015, March 2015 and May 2015 (Figure 4.2). Stage V (spawning stage) samples were observed in five months in the upstream, that is, November 2014, December 2014, March 2015, April 2015 and May 2015 (Figure 4.1) whereas in the downstream, they were collected in November 2014, May, and June 2015 (Figure 4.2). Stage VI (spent stage) samples in the upstream were caught in January 2015 and May 2015 (Figure 4.2) while in the downstream site stage VI samples were sampled in March 2015, April 2015 and May 2015 (Figure 4.2).

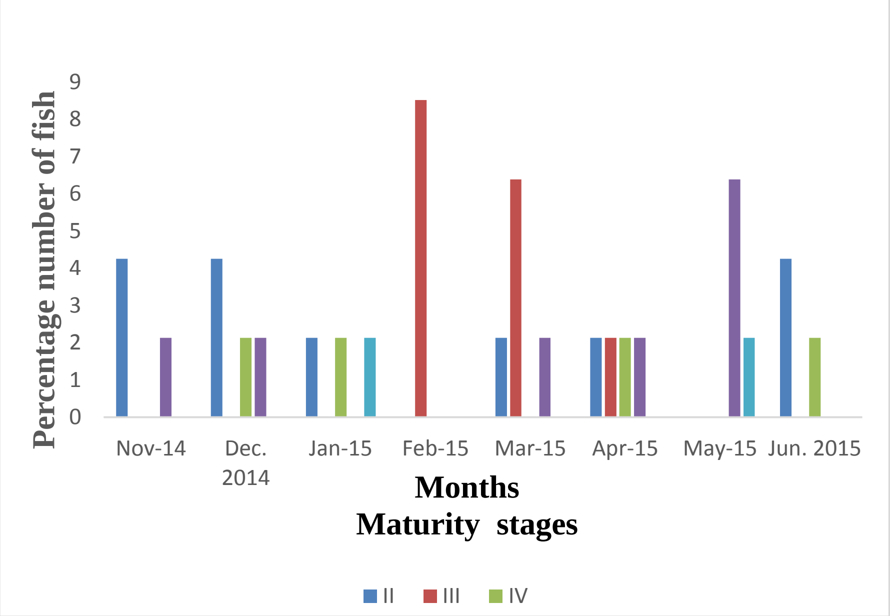

Figure 4.1: Percentage number of fish in different maturity stages from the upstream site

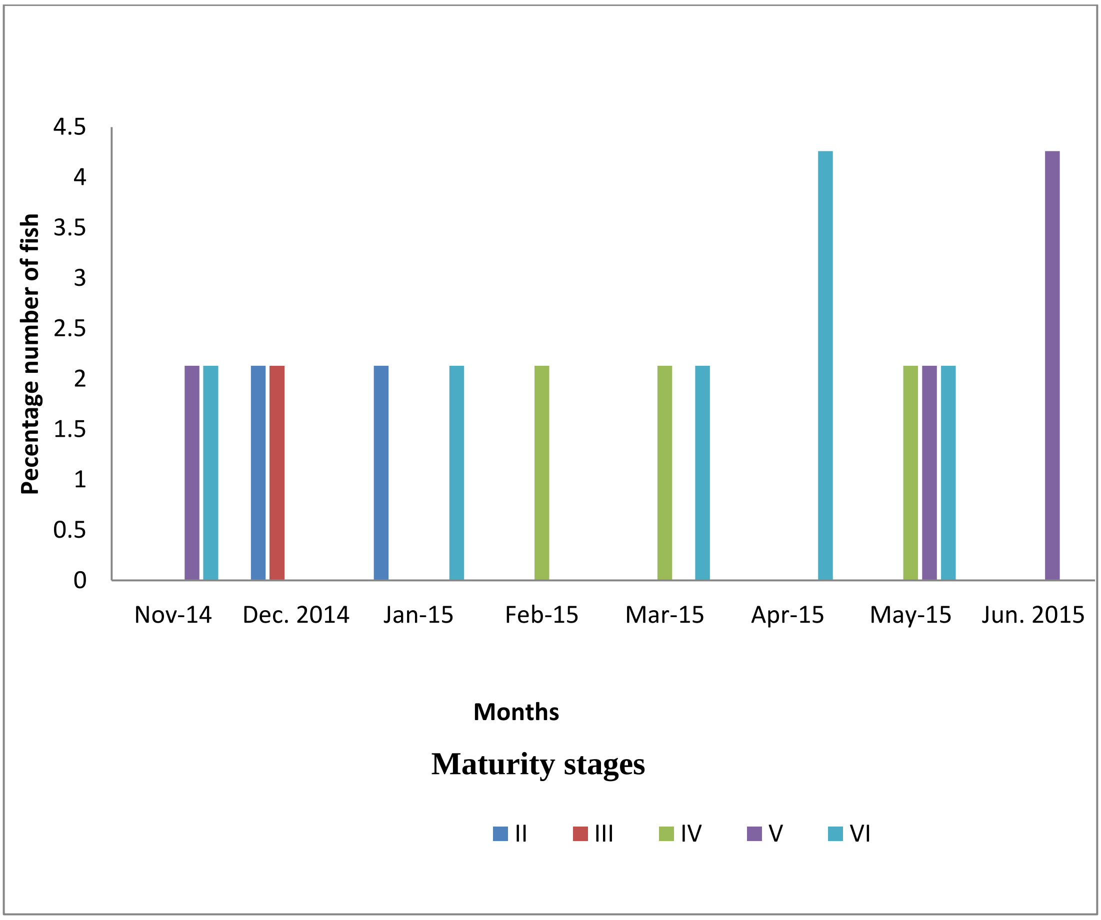

Figure 4.2: Percentage number of fish in different maturity stages from the downstream site

4.7 Gonadosomatic index (GSI) for sexually mature samples

Twenty one ovary samples were mature (stages III, IV, V and VI) from fish sampled from the upstream sampling section while seventeen ovary samples were from fish from the downstream sampling site**.** The mean monthly overall GSI of ovary samples in stages III, IV, V and VI from fish sampled from the upstream sampling section (2.39 ± 0.064) was slightly lower than the mean monthly GSI of the ovary samples in stages III, IV, V and VI of fish sampled fom the downstream sampling section (2.88 ± 0.051). However, there was no statistically significant difference (t =0.82; p >0.05) in these means. The mean GSI of the mature *Oreochromis niloticus* samples from the downstream sampling sections were significantly higher than the

GSI of the ovary samples of the fish samples from the upstream sampling section in the months of December, January, and February ($p < 0.05$). On the other hand mean GSI were significantly higher in upstream sampling sites compared to downstream sampling sites in the months of March, April and June ($p < 0.05$)**.**

The mean gonadosomatic index (GSI) in the fish samples from the upstream sampling section was highest in June 2015 samples (5.72±0.0) while the lowest GSI were recorded in January 2015 (1.04 ±0.86). The findings showed a significant difference between the two means of gonadosomatic index ($p <0.05$). However, mean GSI of the fish samples from the upstream sampling section in the other months were not significantly different ($F = 0.090$; $P > 0.05$). In the downstream sampling section, the mean GSI of the fish sampled in March 2015 (0.89±0.71) was the lowest while December 2014 samples had the highest mean gonadosomatic index (5.89±0.00). However, the GSI of the fish sampled in December 2014 compared to the months of January; February; May and June were not significantly different ($F = 1.93$; $P > 0.05$).

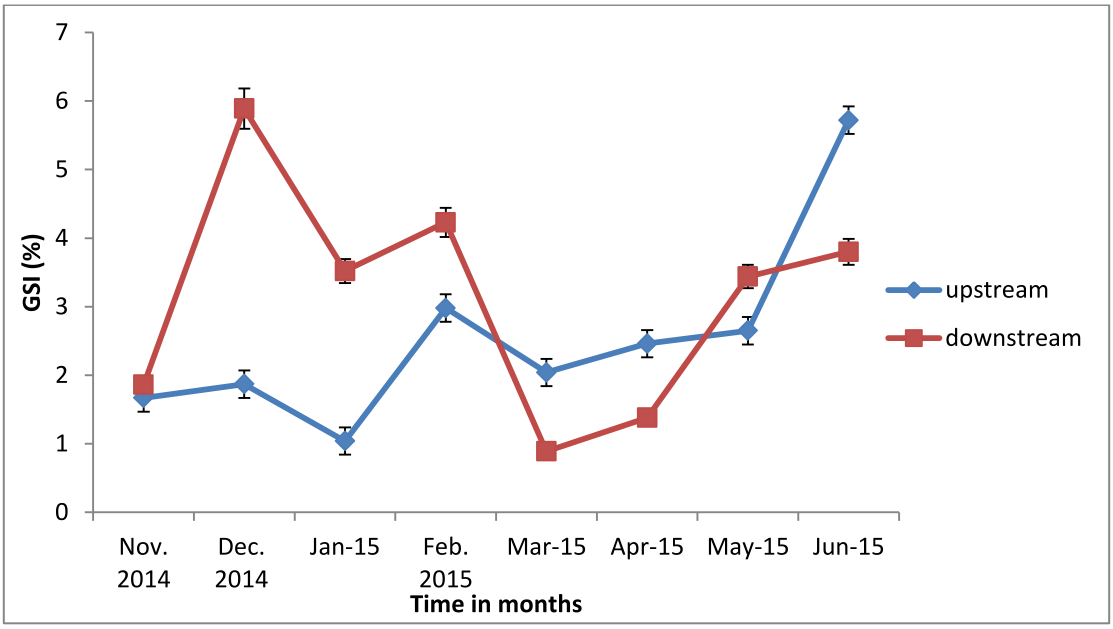

Figure 4.3: Monthly means of GSI for mature *Oreochromis niloticus* from the upstream and downstream

4.8 Monthly means of GSI for immature (stage II) *Oreochromis niloticus* from the upstream and downstream

Nine ovary samples were in stage II from the fish samples collected from the upstream sampling section and two were sampled from fish samples caught from the downstream sampling section for eight months (Table 4.6). That was because the stage II fish samples were rare, probably due to unconducive water characteristics to the fingerlings. Ovary samples from the fish samples collected from the upstream sampling section recorded the highest GSI in the month of November 2014 (0.797±0.I34) and lowest in the month of April 2015 (0.228±0.00). From the downstream samples, gonadosomatic index (GSI) in samples captured in December 2014 (1.316±0.00) was slightly higher than in January 2015 (0.167±0.12). On comparing the mean GSI of stage II ovary samples from *Oreochromis niloticus* samples from the upstream sampling section to those from the downstream sampling section, a two sample t-test showed no significant difference (t = 0.01; P > 0.05).

Table 4.7: Mean monthly Gonadsomatic Index (GSI) of stage II Tilapia from upstream and downstream

	Upstream			**Downstream**		
Month	**Number of samples**	**GSI**	**S.E**	**Number of fish**	**GSI**	**S.E**
Nov. 2014	2	0.796	0.I34	-	-	-
Dec. 2014	2	0.273	0.106	1	1.316	0.00
Jan. 2015	1	0.271	0.1	1	0.17	0.00
Feb. 2015	-	-	-	-	-	-
Mar. 2015	1	0.255	0.00	-	-	-
Apr. 2015	1	0.228	0.00	-	-	-
May 2015	-	-	-	-	-	-
Jun. 2015	2	2.576	0.607	-	-	-

4 .9 Levels of heavy metals in the water samples

The mean monthly levels of metals in the water samples were compared between upstream and downstream, a paired t-test showed that levels were not significantly different (Table 4.8).

Table: 4.8: Comparison of the levels of heavy (mg/l) metals in the upstream and downstream water samples

	Lead	**Cadmium**	**Copper**	**Zinc**	**Iron**
Upstream	0.33±0.05	0.106±0.01	1.854±0.40	0.473±0.38	2.144±0.03
Downstream	0.40±0.03	0.174±0.03	2.591±0.28	0.413±0.07	3.214±0.31
T-value	1.15	2.03	1.49	1.18	2.33
P-value	0.288	0.082	0.179	0.277	0.052

4.10 Levels of heavy metals (mg/kg) in the ovaries of mature tilapia from the upstream and downstream

The mean level of lead (0.707±0.05) was significantly higher in the fish ovary samples found downstream than those found upstream ($t = 3.36$; $P < 0.05$). The mean level of iron (3.87±0.03) was highly significant in the downstream ($t=4.92$; $p<0.01$) than in the upstream (Table 4.9). There was no significant difference in the mean levels of cadmium, copper and zinc between the upstream and downstream ovary samples ($p>0.05$).

Table 4.9 Comparison of the mean levels (mg/kg) of heavy metals in the ovaries of sexually mature tilapia from upstream and downstream

	Lead	**Cadmium**	**Copper**	**Zinc**	**Iron**
Downstream	0.707±0.051	0.318 ± 0.029	1.627 ± 0.18	0.887 ± 0.11	3.87±0.34
Upstream	0.433± 0.064	0.252±0.049	1.560 ±0.19	0.839 ±0.10	1.83±0.25
t-value	3.36	1.15	0.25	0.32	4.92
P-value	0.002*	0.258	0.801	0.751	0.001*

4.11 The relationship between the level of heavy metals in the ovaries and the gonadosomatic index (GSI) of mature tilapia upstream and downstream

To establish the relationship between levels of heavy metals on GSI of mature tilapia, Pearson moment correlation showed that there was no significant correlation

($P > 0.05$) between the levels of heavy metals (lead, Cadmium, Copper zinc, iron) and the GSI of the Nile tilapia in samples from the upstream site (Figure 4.10).

Table 4.10 Relationship between the level of heavy metals in the ovaries and the gonadosomatic index (GSI) of mature tilapia upstream

Heavy metals	r-values	p-values
Lead	0.317	0.445
Cadmium	0.113	0.789
Copper	-0.243	0.562
Zinc	0.229	0.585
Iron	0.270	0.518

Similarly, levels in GSI in fish samples from the downstream site were not significantly correlated with the levels of the heavy metals in the ovaries ($P > 0.05$) (Table 4.11).

Table 4.11: The relationship between the level of heavy metals in the ovaries and the gonadosomatic index (GSI) of mature tilapia downstream

Heavy metals	p-values	r-values
Cadmium	-0.248	0.145
Lead	0.282	0.096
Copper	0.111	0.519
Zinc	0.178	0.298
Iron	0.222	0.192

4.12 Levels of heavy metals in immature tilapia (stage II) from the upstream and downstream sections

Data on stage II *O. niloticus* samples is scarce due to their unavailability in the sampling sections. In comparing the levels of respective heavy metals in the ovary samples of the immature tilapia from the upstream sampling section and the downstream sampling section, a two sample t-test, showed no significant difference ($P > 0.05$) in the levels of the respective heavy metals (Table 4.12).

Table 4.12: Levels of heavy metals in immature tilapia from upstream and downstream

	Lead	**Cadmium**	**Copper**	**Zinc**	**Iron**
Downstream	0.075±0.07	0.19±0.09	1.05±0.15	1.04±0.16	1.15±0.15
Upstream	0.23±0.1	0.29±0.06	-0.77±0.22	0.5±0.1	0.74±0.15
T-value	1.20	-0.86	1.04	2.62	1.93
P-value	0.28	0.55	0.34	0.23	0.15

4.13 Relationship between the level of heavy metals in immature ovaries and gonadosomatic index (%) upstream

In assessing the relationship between the levels of heavy metals in stage II (immature) tilapia ovaries and gonadosomatic index, there was significant positive correlation between the levels of iron with gonadosomatic index ($r = 0.710$; $p < 0.05$) (Table 4.13). However there was no significant relationship between levels of lead, cadmium, copper and zinc and GSI ($P>0.05$).

Table 4.13: Relationship between level of heavy metals in stages II ovary samples and gonadosomatic index (%) from the upstrem

Heavy metals	r-values	p-values
Lead	0.006	0.987
Cadmuim	-0.135	0.691
Copper	0.194	0.569
Zinc	0.327	0.327
Iron	0.710	0.014

4.14 Serum level of 17β- estradiol (E2) (pg/ml) in mature tilapia upstream and downstream

Twenty one serum samples were taken from mature fish sampled from the upstream sampling section, while seventeen were from the fish sampled from the downstream section. During the month of November 2014, the level of E_2 in serum recorded was 127.08 ± 0 pg/ml upstream while in the downstream; the level was 498 ± 155 pg/ml (Figure 4.4). There was a drop in the preceeding month (December) which recorded 106.35 ± 0 pg/ml in the upstream and 80.36 pg/ml ± 0 pg/ml in the downstream site. This was followed by a drastic rise in serum level E_2 (1143 ± 0 pg/ml) in the upstream whereas there was a drop in the downstream section (72.52 ± 0 pg/ml) in January 2015. The months of February and March recorded a sharp drop of the hormone in the upstream section. However, low serum level E_2 was recorded from fish sampled in the downstream section during the month of February 2015 (105.494 pg/100 ml) and March (66 ±7.79 pg/ml). The months of April, May and June 2015 recorded a rise in the level of serum E_2 in both sampling sites (Figure 4.4).

The mean level of serum 17β-estradiol (E_2) in the upstream was 504.90 ± 187.74 pg/ml while, in the downstream it was 304.08±188.88 pg/ml. However, the two means were not statistically different ($t = 1.14$; $P > 0.05$). In the Nile tilapia sampled upstream, the levels of E_2 were not significantly different ($F = 0.92$; $P > 0.05$). Similarly, the levels of E_2 were not significantly different among the months in the downstream ($F = 1.11$; $P > 0.05$).

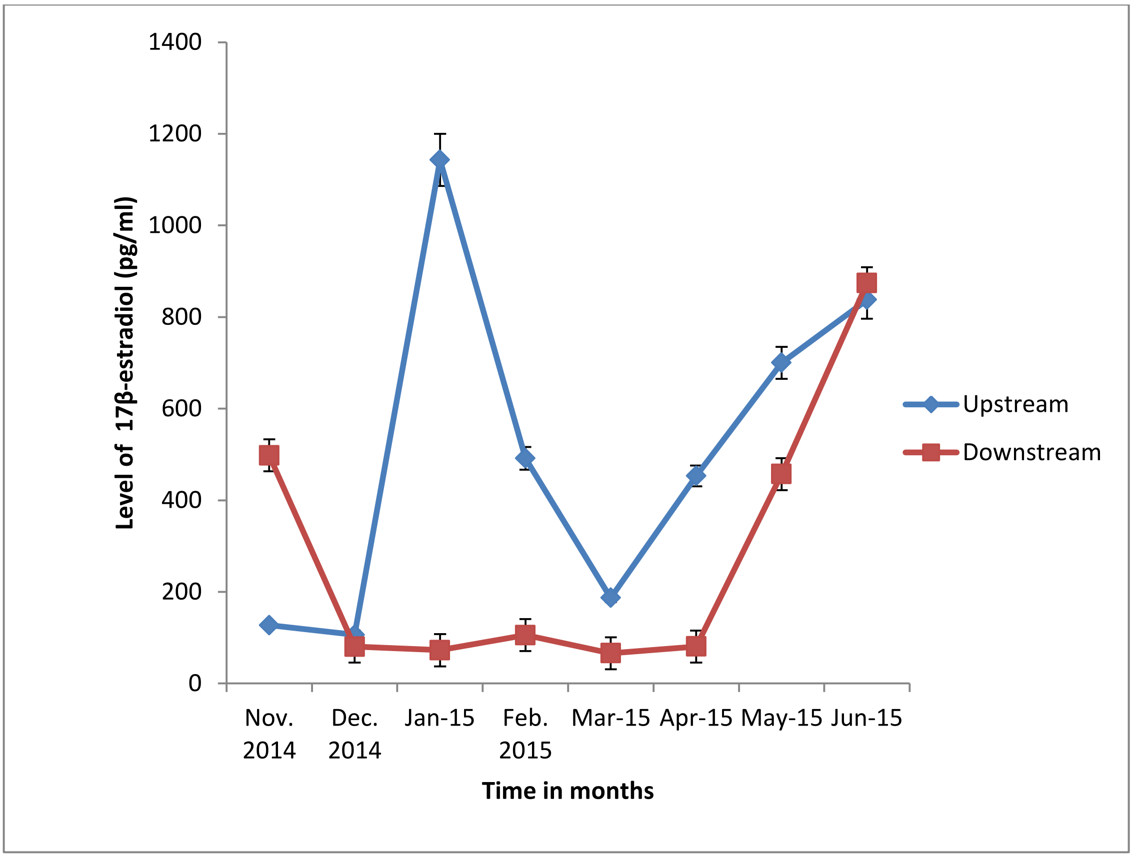

Figure 4.4: Mean monthly level of 17β-estradiol (pg/ml) in mature tilapia sampled from upstream and downstream sections of River Ruiru

4.15 Mean monthly level of 17β- estradiol (E_2) in immature tilapia along River Ruiru

Nine serum samples were taken from *O. niloticus* in stage II (immature) from the upstream section and two were sampled from fish in stage II from the downstream sampling section. Serum E_2 samples were only recorded in the months of November and December (2014), January, March and June, from the upstream sampling site. In the downstream sampling site, E_2 was recorded in Decmber 2014 and January 2015 samples only (Table 4.14).

Table 4.14: Level of 17β-estradiol (E_2) in immature tilapia serum in the upstream and downstream sites

	Upstream			Downstream		
Month	Number of samples	Mean E_2	SE		Mean E_2	SE
November 2014	2	15.11	0.72		-	-
December 2014	2	168.09	0	1	70.740	-
January 2015	1	50.052	0	1	64.733	-
February 2015	-	-	-	-	-	-
March2015	1	342.52	0	-	-	-
April 2015	1	-	0	-	-	-
May 2015	-	-	0	-	-	-
June 2015	2	62.57	14.023	-	-	-

NB *Oreochromis niloticus* in maturity stage II were rare.

4.16 Relationship between level of heavy metals in ovary samples and the level of 17β-estradiol in mature tilapia in the upstream

To establish the association between levels of heavy metals and the level of 17β-estradiol in tilapia, Pearson moment correlation analysis was was used. It showed that there was no significant correlation between levels of heavy metals (lead, cadmium, copper, zinc and iron) and the levels of E_2 of the mature *Oreochromis niloticus* from the upstream sampling site ($P > 0.05$) (Table 4.15).

Table 4.15: Relationship between the level of heavy metals in ovary samples and the level of 17β-estradiol in mature tilapia in the upstream

Metals	r-value	p-value
Lead	-0.577	0.175
Cadmium	0.415	0.355
Copper	0.302	0.510
Zinc	0.421	0.347
Iron	0.441	0.322

4.17 Relationship between the level of heavy metals in ovary samples and the level of 17β-estradiol in mature tilapia from downstream site

Similarly, levels of 17β- estradiol in tilapia samples from the downstream sampling section were not significantly correlated with the levels of the heavy metals in the ovary samples ($P > 0.05$) (Table 4.16).

Table 4.16: Relationship between level of heavy metals in ovary samples and the level of 17β-estradiol in mature tilapia in the downstream

Metals	r-value	p-value
Lead	0.412	0.359
Cadmium	0.420	0.348
Copper	0.437	0.446
Zinc	0.232	0.617
Iron	0.222	0.632

4.18 Relationship between 17β-Estradiol and gonadosomatic index (GSI) in mature tilapia from upstream

There was a slight drop in the level of 17β-estradiol and a slight increase in gonadosomatic index in the months of November and December 2014 (Figure 4.5). In January 2015, gonadosomatic index dropped as the level of 17β-estradiol rose to a peak of 1143 ± 0 pg/ml. Then, there was an increase in gonadosomatic index in the month of February as the level of 17β- estradiol dropped, followed by a decrease in both gonadosomatic index and 17β-estradiol in the month of March. For the remaining three consecutive months (April, May and June), there was an increase in gonadosomatic index after each peak of 17β-estradiol (Figure 4.5).

Pearson moment correlation coefficient showed no significant relationship between gonadosomatic index and the level of 17β-estradiol in Nile tilapia from the upstream (r = 0.238, P >0.05).

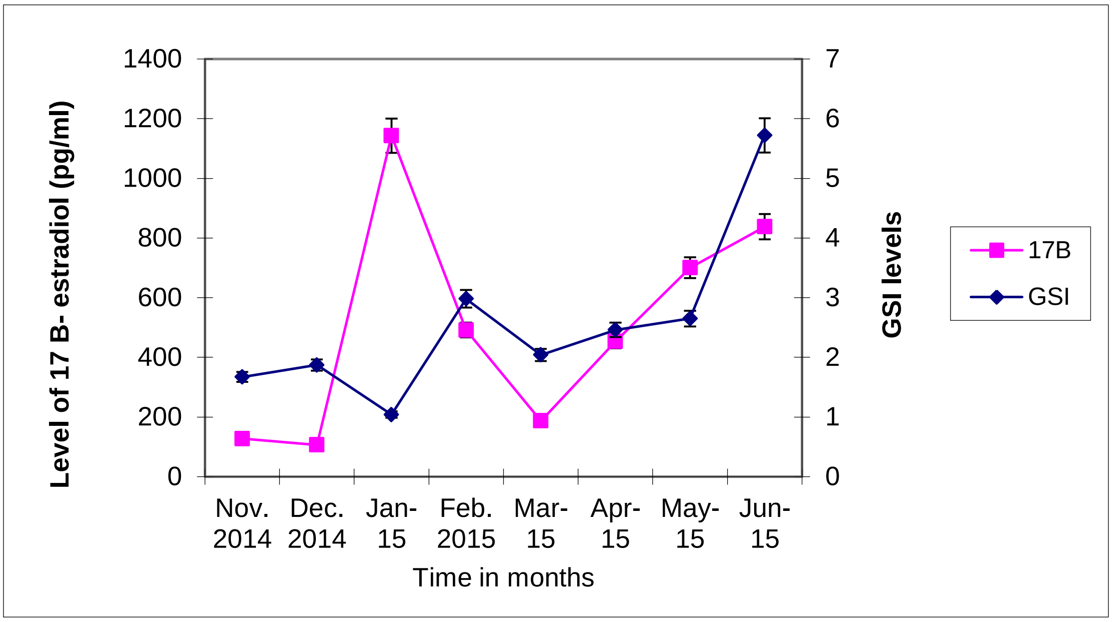

Figure 4.5: Level of 17β-estradiol and gonadosomatic index (GSI) of mature tilapia in upstream 4.23

4.19 Relationship between levels of 17β-Estradiol and gonadosomatic index (GSI) in tilapia from the downstream sampling site

In the downstream sampling sites, gonadosomatic index rose as the level of 17β-estradiol dropped in the month of December 2014 (Figure 4.6). Gonadosomatic index then dropped as the level of 17β- estradiol remained almost constant during the month of January 2015. No serum was collected in the samples collected in the month of February 2015. However, a slight increase in gonadosomatic index was observed. The level of 17β-estradiol still remained constant in the months of March and April. Thereafter, there was a rise in both the level of 17β-etradiol and gonadosomatic index in the three consecutive months of April, May and June 2015. Analysis of the relationship between levels of 17β-estradiol with the gonadosomatic index from the downstream samples showed no significant relationship (r = 0.123; P > 0.05)

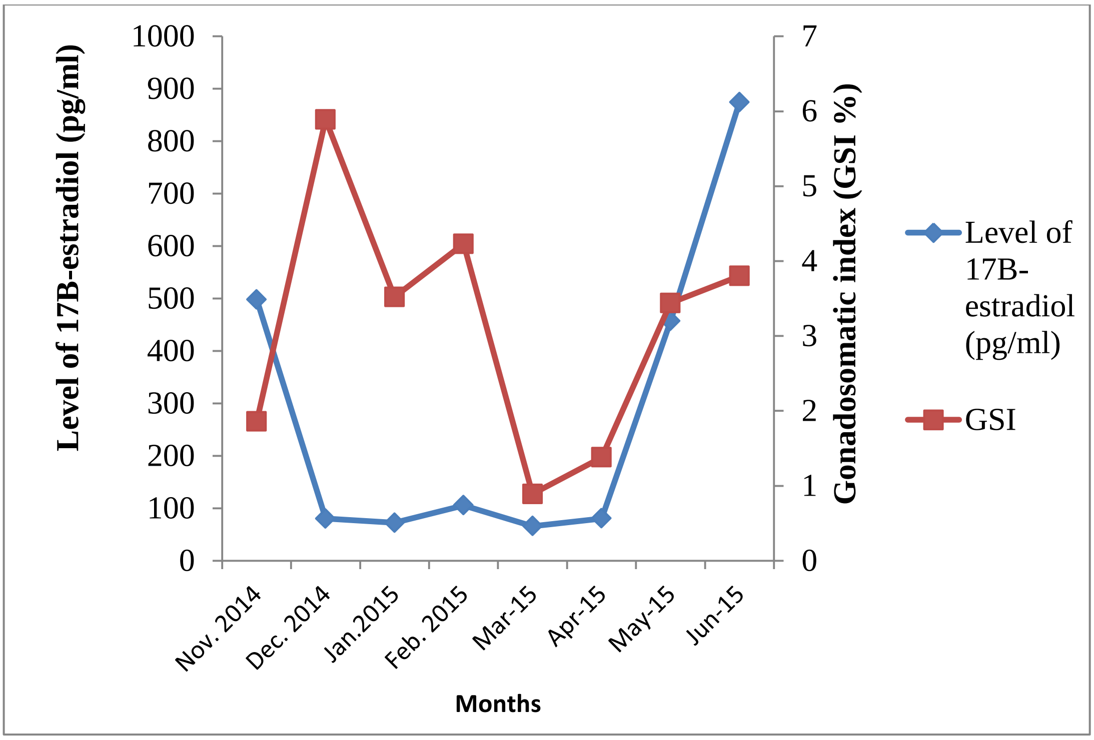

Figure 4.6: Level of 17β-Estradiol and gonadosomatic index (GSI) in the downstream

4.20 Mean monthly standard lengths of mature tilapia in the upstream and downstream

The number of mature *O.niloticus* sampled per month in the upstream and downstream sampling sections is recorded in tables 4.17 and 4.18 below. The mean standard length of mature tilapia in the upstream samples was 13.56 ±1.18 centimeters while in the downstream it was 16.06±0.44 centimeters. A two sample t-test indicated that upstream samples were significantly smaller than downstream samples ($t = 2.87$; $P < 0.05$). Within the upstream section, there was no significant difference among the months ($F = 1.17$; $p > 0.05$), while there was a significant difference among the months within the downstream site ($F = 6.28$; $p < 0.05$).

Table 4.17: Monthly means of standard length (cm) for mature tilapia in the upstream

Month	Number of fish sampled	Mean ± SE
November 2014	1	14.8±0
December 2014	2	13.90 ± 0.40
January 2015	2	17.50 ± 1.50
February 2015	4	11.25 ± 2.45
March 2015	4	11.73 ± 1.85
April 2015	3	13.00 ± 2.00
May 2015	4	14.83 ± 1.24
June 2015	1	11.5±0
Mean standard length (cm)		13.56±1.18

Mean values tested at 95%CI show no significant difference $F = 1.17$; $P = 0.374$

Table 4.18: Monthly means of standard length (cm) for mature tilapia in the downstream

Month	Number of fish sampled	Mean ± SE
November 2014	2	20.3 ± 0.00
December 2014	1	9.50 ± 0.00
January 2015	1	8.20 ± 0.00
February 2015	1	18.00 ± 0.00
March 2015	2	18.75 ± 1.25
April 2015	2	20.50 ± 0.50
May 2015	4	17.63 ± 1.16
June 2015	2	15.60 ± 0.60
Mean standard length (cm)		16.06 ± 0.44

Mean values tested at 95%CI show a significant difference F = 6.28; P = 0.013

4.21 Relationship between standard length (cm) of sexually mature tilapia and level of heavy metals (mg/kg) in the ovaries in the upstream site

In the upstream site, there was a significant positive correlation between the fish standard length and the levels of iron ($r = 0.782$; $P < 0.05$) (Table 4.19). There was no significant relationship between standard lengths of fish with the levels of Lead, cadmium, copper and zinc in the ovaries ($P > 0.05$). However, it was noted that as standard length increased, levels of cadmium also increased ($r = -0.283$; $P < 0.05$).

Table 4.19: Relationship between the standard lengths (cm) of mature tilapia and level of heavy metals (mg/kg) in the ovaries of fish in the upstream site.

Heavy metals	**r-values**	**p-values**
Lead	0.323	0.435
Cadmium	-0.283	0.496
Copper	0.103	0.809
Zinc	0.332	0.422
Iron	0.782	0.022

4.22 Relationship between the standard length (cm) of mature tilapia and level of heavy metals (mg/kg) in the ovaries in fish from the downstream

The correlation between the standard length of sexually mature tilapia and the levels of lead (r = 0.958; P <0.05) and levels of iron (r = 0.946; P < 0.05) was positive and significant. The result showed that there was no significant relationship between fish standard lengths and the level of cadmium, copper and zinc, (P > 0.05) (Table 4.20).

Table 4.20: Relationship between the standard lengths (cm) of sexually mature tilapia and level of heavy metals (mg/kg) in the ovaries in the downstream.

Heavy metals	**r-values**	**p-values**
Lead	0.958	0.0001
Cadmium	0.140	0.741
Copper	0.344	0.404
Zinc	-0.206	0.624
Iron	0.946	0.0001

4.23 Fecundity of *Oreochromis niloticus*

Ripe ova were sampled from eleven ovary samples of fish in stages IV and V fish samples from the upstream sampling section and eight ovary samples in stages IV and V fish samples from the downstream sampling sections. The sampled number per month is represented in the bar graph in figure 4.7. Fecundity of *Oreochromis niloticus* in the downstream section was 921 eggs per female. Fish from the upstream showed lower fecundity at 603 eggs per female. Howeve, a two sample t-test showed that there was no significant difference in fecundity between the downstream and upstream (t= -0.19, p >0.05) (Table 4.20).

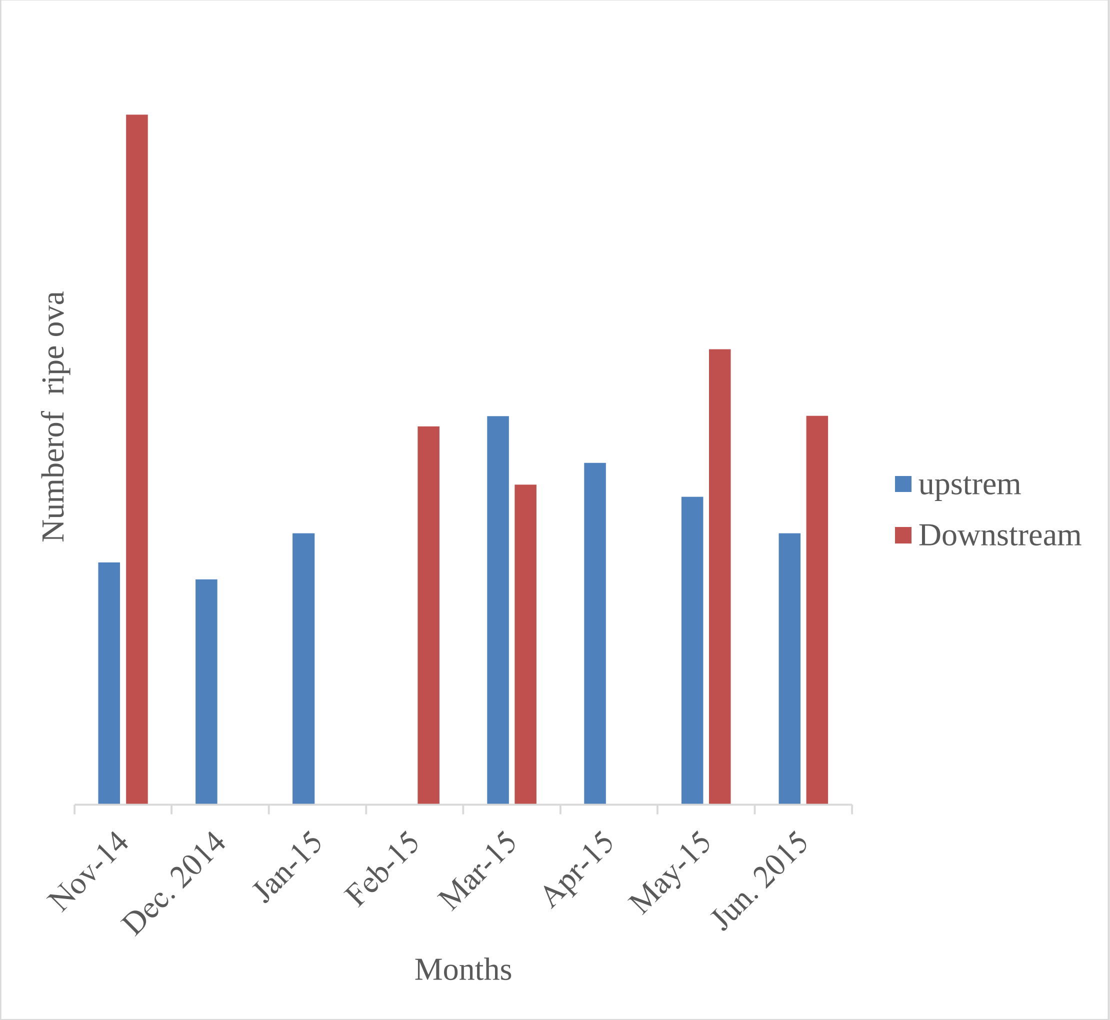

Figure 4.7: Mean monthly ripe ova in the upstream and downstream sections

4.24 Relationship between fecundity and the level of heavy metals (mg/kg) (in the mature ovary samples of Nile tilapia from the upstream and the downstream

In establishing the relationship between fecundity with the levels of heavy metals in the ovaries of fish samples from the upstream, the result showed that there was no significant relationship between the fecundity with any of the heavy metals ($P > 0.05$) (Table 4.21).

Table 4.21: Relationship between fecundity and the level of heavy metals (mg/kg) in the ovaries of mature tilapia from the upstream River Ruiru

Heavy metals	r-values	p-values
Lead	-0.306	0.556
Cadmium	0.329	0.525
Copper	0.654	0.159
Zinc	0.052	0.922
Iron	-0.198	0.707

Similarly, in downstream sections, there was no significant relationship between the fecundity and any of the heavy metals in the ovary samples of *Oreochromis niloticus* sampled from the downstream section of the River ($P > 0.05$) (Table 4.22).

Table 4.22: Relationship between fecundity and level of heavy metals (mg/kg) in the ovaries of mature tilapia from the downstream River Ruiru

Heavy metals	r-values	p-values
Lead	-0.542	0.345
Cadmium	-0.059	0.925
Copper	0.763	0.133
Zinc	-0.173	0.780
Iron	-0.419	0.483

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Physicochemical parameters

The physical, chemical and biological contents of water determine the quality of water. The quality of fresh water for fish should not allow accumulation of pollutants especially heavy metals in fish to such an extent that they are potentially harmful (Water Protection and Sustainability Branch, 2012).

5.1.2 Electrical conductivity

The slightly high electrical conductivity in the upstream sites can be attributed to ions from agricultural, domestic and industial wastes carried and discharged into the river by rain water (Muiruri, 2013, Hadgu *et al.,* 2014). The mean levels of electrical conductivity in the upstream and downstream sites were within the values reported by Hadgu *et al.* (2014) in their study along Ndarugu River in which values ranged between 43 µS/cm and 85µS/cm. However, these mean levels are below the admissible limits of 250 µS/cm and 2500 µS/cm stated by WHO (2008) and EU, (2006) respectively.

5.1.3 Dissolved oxygen

In the present research the mean level of dissolved oxygen was slightly lower in the downstream than in the upstream sections of River Ruiru. This could have been due to slight elevation of water temperature due to industrial wastes and sewage load from Ruiru town **(**Kimani *et al.*, 2016) into the river. This could increase the biological oxygen demand to such a high level that most of the available oxygen may be removed (Maduka, 2004). A few fish can survive in water with dissolved oxygen (DO) of range 2 – 4 mg/l and the range of 4-7 mg/l is good for many aquatic animals (Behar, 1997). The slightly higher mean value of DO recorded in the upstream section may be due to the presence of green water plants, as was observed from the study site. Dissolved oxygen (DO) in the water is important to aquatic organisms, such as fish and therefore its level reflects physical and biological processes taking place in the rivera (Yirgu, 2012).

However, the mean dissolved oxygen values were not significantly different between the two sampling sites, due to the fact that the difference in the level of temperature between the upstream and downstream was not significant. The mean value of dissolved oxygen at downstream and upstream sections was in the range (4.4 – 5.0 mg/L) of moderately polluted water (WHO, 2008). The low dissolved oxygen values, less than 5 mg/L were also reported along River Ogun (Dimowo, 2013) to be most likely as a result of the amount of effluents discharged into the river. Such low oxygen level is likely to cause mass extinction of aquatic organisms (such as fish species) due to anoxic conditions in the River (Mustafa *et al.*, 2012)

5.1.4 pH

The slightly high pH values of water both in the upstream and downstream sampling sites can attributed to fertilisers from the agricultural land adjacent to the river. They were almost similar to the ones recorded along river Nairobi (Budambula and Mwachiro, 2006). Lower pH of 4 is reported to be extremely uncondusive for fish growth and development (Kawamura *et al.,* 2015). However, the values obtained in this study were within the admissible limit (6.5-9.5) (WHO, 2008).

5.1.5 Temperature

The mean level of temperature in the upstream and downstream was lower than the optimal growth temperature for *Oreochromis niloticus* of River Ruiru and this could be due to the canopy of dense vegetation along the shores of the river. It is similar to the value recorded by (Kosgei *et al.,* 2016) and within the water temperatures range (14.8^0 C to 32.1^0 C) obtainened by Budambula and Mwachiro (2006). Hence the value was below the optimal growth temperature for *Oreochromis niloticus* is reported to range between 28^0 C and 36^0 C (FAO, 2012).

5.1.6 Turbidiy

Turbidity is a measure of water clarity. It describes the amount of light scattered or blocked by suspended particles in a water body. High turbidity that was observed in the months of April, May, June, November and December in both sampling sites of River Ruiru is attributed to surface run off into the river, whereas low turbidity recorded in January, February and March is attributed to absence of surface runoff into the water (Kimani *et al.,* 2016). Particles suspended in water affect water clarity (Ifeanyichukwu *et al.,* 2014). High turbidity affects functioning of fish indirectly, in the sense that it lowers dissolved oxygen; prevent light from reaching aquatic plants which reduces their ability to photosynthesize. Also, it lowers visibility and this affects the fish's ability to hunt for food; clog fish' gills and harm eggs (Lene *et al.,* 2014).

5.1.7 Levels of heavy metals in water

The presence of heavy metals in the upstream sampling sites could be attributed to organic and inorganic wastes from runoffs from the agricultural farms closure to the river banks (Kosgei *et al.,* 2016). The downstream section was expected to record more heavy metals than the upstream sampling section, because it receives wastes from Ruiru town where industries, petrol stations and car wash points. However upstream mean level of heavy metals was not significantly different from the downstream mean probably due to recycling of heavy metals in the river and their deposition into the sediments (Kar *et al*., 2008; Kithiia, 2012). Levels of lead, copper, zinc and iron in water in this study were almost similar to the ones recorded by Kimani *et al.* (2016), but higher than the values obtained by Muiruri *et al.* (2013).

5 .1.8 Levels of heavy metals in fish ovaries

The heavy metals lead, cadmium, copper, zinc and iron detected in the ovaries of *Oreochromis niloticus* in both sampling sections can be attributed to their presence in the river water. Metals may enter the body of fish through three possible ways; the body surface, the gill, and the alimentary tract (Afshan *et al.,* 2014). Ovaries are reported to have a tendency of accumulating heavy metals in them (Authman *et al.,* 2015**).** Mean higher levels of lead and iron in the fish ovaries sampled from the downstream site than from the upstream section can be associated with wastes fom industrial and urban centers that are closer to the section. Runoff from carwash and petrol stations in Ruiru Town gain access into the river at the downstream section. The levels of lead and cadmium exceeded the recorded permissible level of 0.001-0.01 mg/l lead and 0.01-0.003 mg/l cadmium for drinking water and 0.05 mg/kg for the fish and fish products (FAO, 2008; WHO, 2008). This means that drinking or consumption of fish from River Ruiru is dangerous to man considering their levels. Exceeding levels than the permissive levels of heavy metals lead and cadmium for drinking water and fish have also been reported in other localities (Thakur and Mhatre, 2015).

5.1.9 Association between heavy metals and gonadosomatic index (GSI)

Slightly high GSI in June (upstream) and December (downstream) was due to presence of more ovaries in stages III, IV and V. These ovary stages contained ova in stages III, IV and V indicating breeding season (Laban, 2007). Low GSI recoded during the month of November 2014 in both sampling sites indicated immature (stage II) *Oreochromis niloticus* in terms of developing oocytes (Sutthi *et al.,* 2014).

In this study, results show that there was no correlation between the mean GSI and the mean levels of heavy metals both in upstream and downstream of River Ruiru. This could be due to an adaptive response to drastic conditions concerning various heavy metal pollutants in the river (Authman, 1998). The mean GSI in both sampling sites were similar to the one recoded by Mahmoud (2013) but below the one obtained by Mazhrouh and Mahmoud (2009).

5.1.10 Association between heavy metals and level of 17β estradiol (E_2)

There was no significant relationship between the levels of heavy metals and 17β-estradiol in mature *Oreochromis niloticus* from both sampling sites. This can be attributed to torelance of fish to the heavy metals (Amiard *et al.,* 2006). Mature fish tissues and body fluids are reported to contain certain proteins that react with harsh environmental antigens and provide natural immunity to fish **(**Łuszczek-Trojnar *et al.,* 2014).

In the upstream, serum levels of 17β- estradiol gradually increased from December to January (peak). Thereafter it dropped in February and March 2015, whereas in the downstream, it dropped in December and almost remained constant in the monhs of January and February 2015. This may be due to a decline in steroidogenic postovulatory follicles (stage VI). It also suggests that this period corresponds with the major mouthbrooding phase of female *Oreochromis niloticus* (Cornish, 1998), though fries were never found in the mouths of stages VI *O. niloticus* during sampling. Gonadal estradiol levels gradually increased from March to reach a peak in June 2015 in both sampling sites. This is related to a response of the developing ovaries to gonadotrophin hormones, produced during the prespawning (stage IV) and spawning (stage V) time, to secrete 17β- stradiol (Taghizadeh *et al.,* 2013). It is reported that E_2 stimulates the synthesis of yorklipid (vitellogenin) in the oocytes and its ncrease in levels confirms an increase in the immediate pre- spawning activity. It also reflects a continuous maturing (stages III and IV) of females to prepare for the following spawning cycle (Acharjee *et al.,* 2017). The initial estradiol peak observed in January 2015 in female *Oreochromis niloticus* in the upstream section may result in the oocytes being maintained through a protective effect. This protection prevents the oocytes from becoming atretic (Cornish, 1998). The second estradiol peak in June 2015 could be due to response to rapid vitellogenic growth phase in the stages IV oocytes (Nazan *et al.,* 2008).

5.1.11 Relationship between 17β estradiol and gonadosomatic index (GSI)

In the present study, the level of serum17β estradiol in female *Oreochromis niloticus* did not correlate with GSI. This may be due to the fact that sometimes only a proportion of follicles have oestrogenic capacity at sexual maturity, hence absence of high levels of 17β-estradiol **(**Ali *et al.,* 2016). The later also reported that stimulatory effect on steroidogenesis is associated with preovulatory increases in gonadotropin. A preovulatory increase in estradiol may be excreted via the urine. The oestrogenic excretory products may act as a type of pheromone to prepare and attract males for the female ovulatory phase (Kidd *et al.,* 2010). The later also reported that photoperiod and temperature can modify gonadal development in fish. However, there was a gradual parallel rise in both serum level of 17β-estradiol and gonadosomatic index in both sampling sites from the months of March to June 2015. Estradiol is responsible for stimulating vitellogenesis and hence it is secreted by female gonads during the pre-spawning period until gonad maturity and ovulation (Saeed *et al.,* 2009). Similar results were also obtained by Sutthi *et al.* (2014).

5.1.12 Association between heavy metals and size at sexual maturity

Findings from this study indicate that the standard length of sexually mature *Oreochromis niloticus* positively correlated with levels of both lead and iron in the ovaries from the downstream (downstream) and iron (upstream ovaries), ($p < 0.05$). This could be due to accumulation of high levels of heavy metals with the increase in size of the fish (Ali, 1998). These results are comparable to the ones reported by Rajkowska and Protasowicki (2013) but different from Gomez-Marquez *et al.* (2003).

There was no relationship between the body length with levels of cadmium, copper and zinc. This may be due to elimination mechanisms and metal detoxification (Ahearn *et al.,* 2004). Fish have adaptive capacity under conditions of sub lethal chronic metal given by physiological changes that result in acclimation with increased biosynthetic processes (enhanced metal binding proteins synthesis such as metallothionein) and up-regulation of other pathways to counteract or compete with the deleterious effects of the metal ion regulation (Tabouret *et al.,* 2011).

Higher body length in the downstream fish samples than in the upstream can also be attributed to migrations of fish from the upstream to the downstream to reach very specific spawning or feeding locations in the water (Notch, 2017). Studies by other researchers state that a reduction in body length may also be related to accelerated catabolism in small sized fish in the upstream in response to harsh environmental conditions (Bryan *et al.,* 1995).

5.1.13 Association between heavy metals and fecundity

Based to the results of this study, there was no correlation between mean levels of heavy metals and fecundity of fish from both sampling sites. This could be due to adaptability of tilapia to heavy metals. Fish is reported to have Kupffer cells, responsible for detoxification and elimination of toxic ions (Koca *et al.,* 2005). Fecundity recorded in this study was lower than in other similar water systems, which could be due to other factors such as environmental factors and body size (Khalafu and Authuman, 2003; Silva *et al.,* 2016).

5.2 Conclusions

i. Based on the results obtained in this study, there was no significant correlation between the levels of heavy metals (lead, Cadmium, Copper zinc, and iron) and gonadosomatic index of the *O. niloticus* from both sampling sites.

ii. There was no significant correlation between the levels of heavy metals (lead, Cadmium, Copper zinc, and iron) and Levels of 17β-estadiol sampling sites from both Upstream and Downstream sites.

iii. In the upstream, there was a significant positive relationship between the fish standard length and the levels of iron but there was no significantrelationship between fish standard lengths and the levels of lead, cadmium, copper and zinc. In the downstream sampling site, there was a significant correlation between the standard lengths of sexually matures tilapia and the levels of lead and iron, that is, as the standard lengths of *O.niloticus* increased, also levels of these heavy metals increased.

iv. There was no correlation between the levels of heavy metals (lead, cadmium, copper zinc, and iron) and fecundity in both sampling sites. This implies that there is no relationship between heavy metals and the selected reproductive parameters in Nile tilapia, along River Ruiru.

5.3 Recommedations

5.3.1 Policy recommendations

Based on results from this study, mean levels of lead, cadmium, copper, zinc and iron in both sampling sites were above the maximum allowable concentration for the fish and fish products implying that consumption of fish from River Ruiru is dangerous to man. Therefore,

i. The Ministry of Environment and Natural Resources should put measures in place to regulate the indiscriminate discharge of raw effluents into River Ruiru.

ii. National Environment Management Authority and Department of Public Health of the County Government of Kiambu need to hold campaigns especially for residents that border River Ruiru in order to safeguard their health by avoiding consumption of fish from the River.

5.3.2 Recommendations for further studies

i. A study on the association between pesticides and the reproductive cycle of O. *niloticus* along the River Ruiru should be conducted.

ii. Study on the association between heavy metals and selected reproductive parameters of other species of fish such as common carp and catfish along River Ruiru should be conducted.

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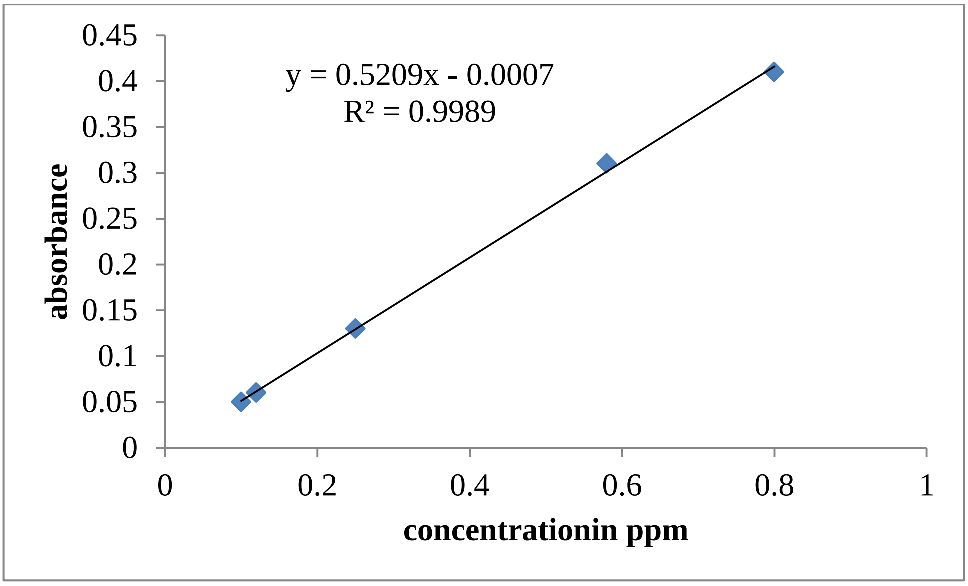
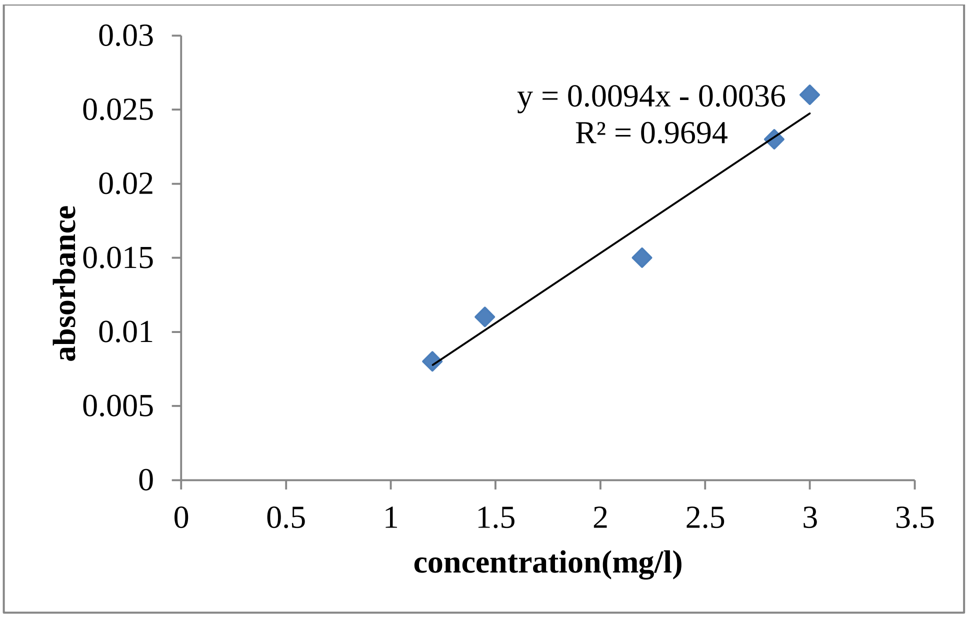
APPENDICES

Appendix I

Standard solution for metals analyzed

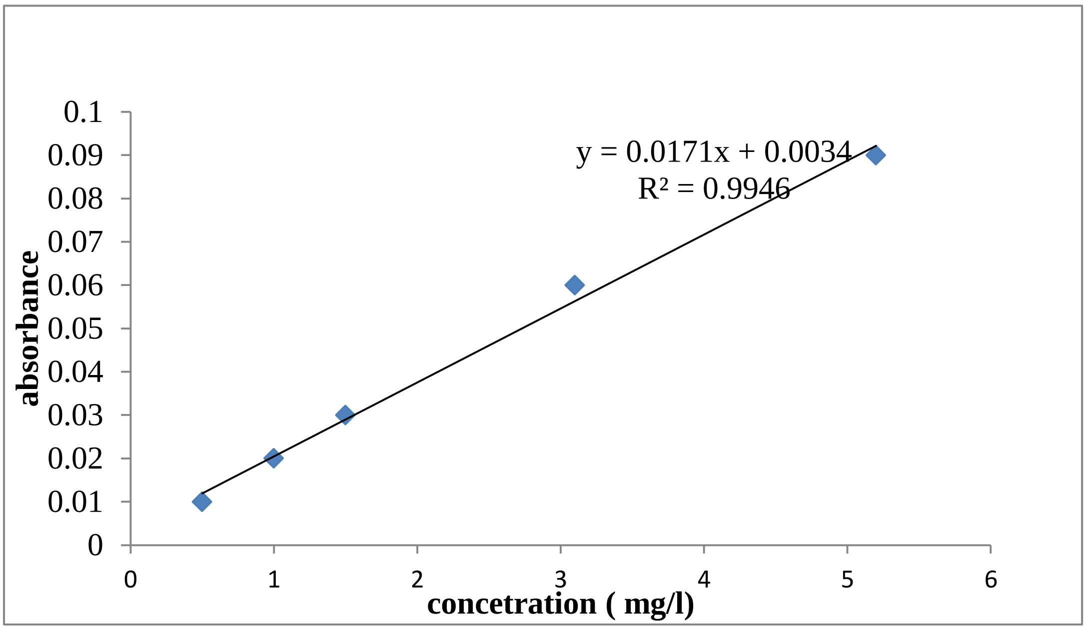


Calibration curve for lead

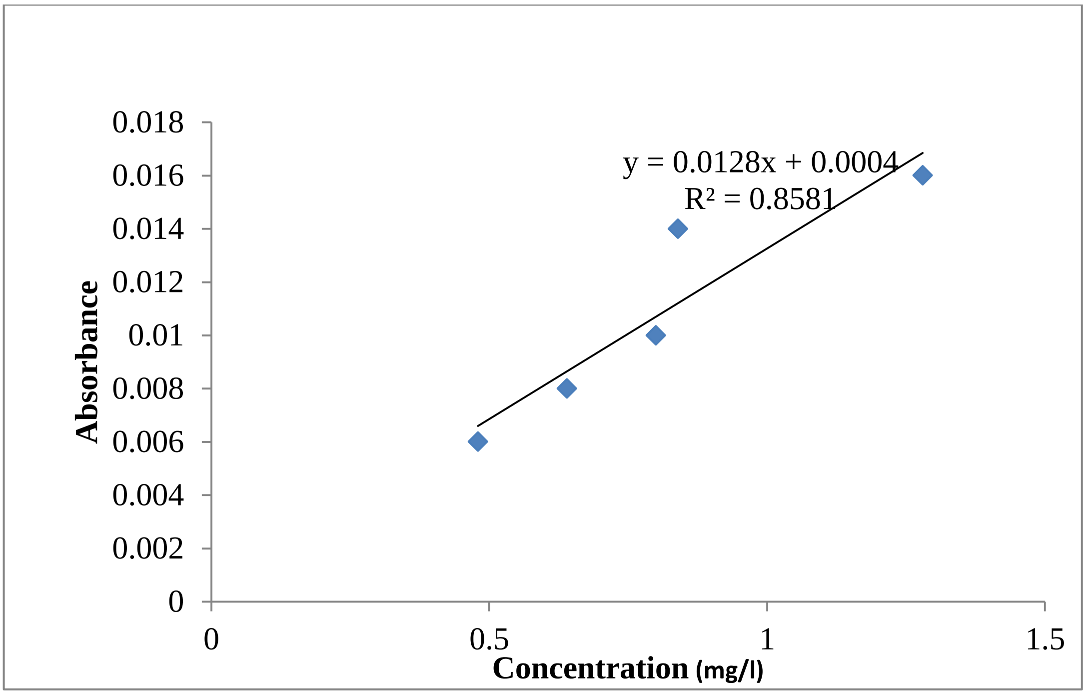


Calibration curve for cadmium

Appendix II

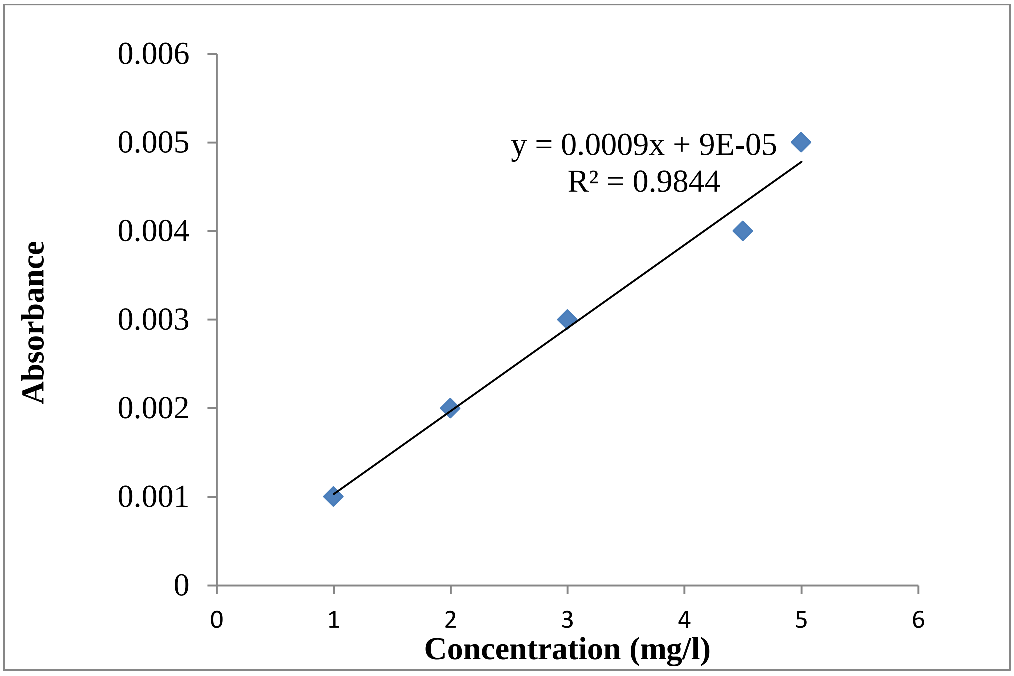


Calibration curve for copper



Calibration curve for zinc

Appendix III



Calibration curve for iron

Sample of *O. niloticus* from River Ruiru

Appendix V

Percentage distribution of different maturity stages of *Oreochromis niloticus* from the upstream section

Month	II	III	IV	V	VI
Nov-14	4.26			2.13	
Dec. 2014	4.26		2.13	2.13	
Jan-15	2.13		2.13		2.13
Feb-15		8.52			
Mar-15	2.13	6.39		2.13	
Apr-15	2.13	2.13	2.13	2.13	
May-15				6.39	2.13
Jun. 2015	4.26		2.13		

Percentage distribution of different maturity stages of *Oreochromis niloticus* from the downstream section

Month	II	III	IV	V	VI
Nov-14				2.13	2.13
Dec. 2014	2.13	2.13			
Jan-15	2.13				2.13
Feb-15			2.13		
Mar-15			2.13		2.13
Apr-15					4.26
May-15			2.13	2.13	2.13
Jun. 2015				4.26	

Appendix VI

Mean monthly gonadosomatic Index (GSI) of the mature tilapia ovaries in the upstream and downstream

	Upstream (= 21)		Downstream (n =15)	
Month	**Mean**	**SE**	**Mean**	**SE**
Nov. 2014	1.67a	0.00	1.86a	1.42
Dec. 2014	1.87a	0.63	5.89a	0.00
Jan 2015	1.04a	0.86	3.52a	0.00
Feb. 2015	2.98a	0.24	4.23a	0.00
March 2015	2.04a	0.89	0.89a	0.71
April 2015	2.46a	1.16	1.38a	0.81
May 2015	2.65a	1.18	3.44a	0.81
June 2015	5.72a	0.00	3.80a	0.46

Mean values in same column denoted by the same letters are not significantly different at $P \leq 0$.

Appendix VII

Levels of heavy metals (mg/kg) in the ovaries in the mature tilapia sampled from the upstream section

Month	Lead	Cadmium	Copper	Zinc	Iron
Novembr 2014	0.67	0.285	0.65	0.65	1.3
December 2014	0.89± 0.06	0.25± 0.04	1.33 ±0.48	1.08± 0.21	2.35± 0.35
January 2015	0.42 ±0.22	0.15±0.06	1.70± 0.20	1.17± 0.45	3.35± 0.65
February 15	0.37± 0.13	0.44 ±0.19	0.70± 0.37	0.52± 0.26	1.52± 0.51
March 2015	0.37± 0.16	0.19± 0.13	1.71± 0.68	0.70± 0.36	1.50± 0.52
April 2015	0.49± 0.07	0.26 ±0.11	1.97± 0.58	1.03 ±0.21	1.53± 0.96
May 2015	0.31± 0.17	0.20 ±0.07	2.15±0.12	0.83±0.19	1.93± 0.68
June 2015	0.1	0.099	1.9	1.2	1.3

Monthly mean levels of heavy metals (mg/kg) in the ovaries fom the mature tilapia sampled from the downstream

Month	Lead	Cadmium	Copper	Zinc	Iron
November 2014	0.74± 0.09	0.19 ± 0.09	1.45± 0.45	0.71± 0.26	4.65± 0.35
December 2014	0.25	0.275	2	0.88	1.3
January 2015	0.25	0.21	0.35	0.88	1
February 2015	0.83	0.1	1.9	0.88	3
March 2015	0.83± 0.00	0.35 ± 0.06	0.50± 0.00	0.59± 0.37	4.85± 0.15
April 2015	0.80 0.03	0.31± 0.03	2.35± 0.25	0.76 ± 0.20	4.85 ± 0.15
May 2015	0.75± 0.04	0.41± 0.02	1.92 ±0.12	1.23± 0.29	4.35± 0.30
June 2015	0.76± 0.07	0.41 ± 0.00	1.95± 0.05	0.83± 0.22	3.33± 0.00

APPENDIX VIII

Levels of heavy metals (mg/l) in water from the upstream section

Month	Lead	Cadmium	Copper	Zinc	Iron
Nov 2014	0.323±0.12	0.1485±0.0	2.275±0.0175	0.53±0.03	2.0±0.0
Dec. 2014	0.485±0.095	0.0995±0.0005	3.75±0.35	0.545±0.065	2.15±0.15
Jan 2015	0.296±0.096	0.05±0.0495	2.05±0.05	0.45±0.05	1.15±0.1
Feb 2015	0.149±0.05	0.1±0.0	1.075±0.075	0.5075±0.008	1.0±0.0
Mar 2015	0.232±0.218	0.0995±0.0005	0.46±0.44	0.56±0.0	1.15±0.15
April 2015	0.2±0.19	0.099±0.0	0.8±0.0	0.33±0.1	3.2±0.5
May 2015	0.513±0.068	0.149±0.009	3.0±0.05	0.28±0.06	4.0±0.3
June 2015	0.485±0.092	0.099±0.09	1.425±0.075	0.585±0.025	2.5±0.2
Mean	0.33±0.05	0.11±0.01	1.86±0.40	2.14±0.38	0.40±0.03

Levels of heavy metals (mg/l) in water from the downstream section

Month	Lead	Cadmium	Copper	Zinc	Iron
Nov. 2014	0.39±0.0	0.099±0.049	3.6±0.0	0.5±0.0	2.975±0.075
Dec. 2014	0.415±0.165	0.28±0.05	2.975±0.225	0.22±0.12	4.35±0.65
Jan.2015	0.417±0.028	0.165±0.165	1.7±0.0	0.42±0.08	3.2±0.5
Feb. 2015	0.225±0.025	0.145±0.051	1.6±0.1	0.595±0.015	3.15±0.15
March.2015	0.4175±0.298	0.1155 ±0.116	3.325±0.125	0.44±0.12	2.85± 0.15
April.2015	0.5125±0.0675	0.0495±0.0495	2.525±0.075	0.15±0.01	4.5±0.5
May.2015	0.5125±0.068	0.239±0.092	1.9±0.2	0.26±0.16	3.3±0.0
June.2015	0.295±0.095	0.295±0.036	3.1±0.4	0.715±0.155	1.385±0.915
Mean	0.40±0.0.03	1.8±0.03	2.59±0.28	0.41±0.07	3.22±0.31

Appendix IX

Mean monthly level of 17β- estradiol (pg/ml) in serum of mature tilapia from the upstream and downstream sections of River Ruiru

	Upstream		Downstream	
Month	Mean	SE	Mean	SE
Nov. 2014	127.08a	0.0	498b	155
Dec. 2014	106.35a	0.0	80.36b	0.0
Jan 2015	1143.0a	0.0	72.52b	7.79
Feb. 2015	491.4a	90.7	105.494b	0.0
March 2015	186.9a	85	66.01b	7.63
April 2015	453a	238	80.67b	0.0
May 2015	700a	336	457b	187
June 2015	837.86a	0.00	874b	587
Mean	**505.900**	**187.74**	**304.08**	**188.88**

Mean values in same column denoted by the similar letters are not significantly different at P ≤ 0.05

Mean monthly levels of heavy metals in mature ovaries (mg/kg) and 17 β estradiol: upstream

		Heavy metals in mature ovaries (mg/kg) upstream				
Month	17β estradiol	Lead	Cadmium	Copper	Zinc	Iron
Nov. 2014	127.08	0.67	0.285	0.65	0.65	1.3
Dec. 2014	106.35	0.89± 0.06	0.25± 0.04	1.33 ±0.48	1.08± 0.21	2.35± 0.35
Jan. 2015	1143	0.42 ±0.22	0.15±0.06	1.70± 0.20	1.17± 0.45	3.35± 0.65
Feb. 15	491.4	0.37± 0.13	0.44 ±0.19	0.70± 0.37	0.52± 0.26	1.52± 0.51
Mar. 2015	186.9	0.37± 0.16	0.19± 0.13	1.71± 0.68	0.70± 0.36	1.50± 0.52
Apr. 2015	453	0.49± 0.07	0.26 ±0.11	1.97± 0.58	1.03 ±0.21	1.53± 0.96
May 2015	700	0.31± 0.17	0.20 ±0.07	2.15±0.12	0.83±0.19	1.93± 0.68
Jun. 2015	837.86	0.1	0.099	1.9	1.2	1.3

Appendix X

Mean monthly levels of heavy metals in mature ovaries and 17β estradiol (pg/ml): downstream

		Heavy metals (mg/kg)				
Month	17β estradiol	Lead	Cadmium	Copper	Zinc	Iron
Nov. 2014	498	0.74± 0.09	0.19 ± 0.09	1.45± 0.45	0.71± 0.26	4.65± 0.35
Dec. 2014	80.36	0.25	0.275	2	0.88	1.3
Jan. 2015	72.52	0.25	0.21	0.35	0.88	1
Feb.15	106.607	0.83	0.1	1.9	0.88	3
Mar. 2015	66.01	0.83± 0.00	0.35 ± 0.06	0.50± 0.00	0.59± 0.37	4.85± 0.15
Apr. 2015	80.67	0.80 0.03	0.31± 0.03	2.35± 0.25	0.76 ± 0.20	4.85 ± 0.15
May 2015	457	0.75± 0.04	0.41± 0.02	1.92 ±0.12	1.23± 0.29	4.35± 0.30
Jun. 2015	874	0.76± 0.07	0.41 ± 0.00	1.95± 0.05	0.83± 0.22	3.33± 0.00

Mean monthly body weight (g) of female tilapia at sexual maturity from upstream

Month	**Mean upstream**	**Mean downstream**
Nov. 2014	150a	236.3a
Dec. 2014	86.10a	75.5a
Jan 2015	167.65a	65.0a
Feb. 2015	88.00a	208.0a
March 2015	73.8a	207.5a
April 2015	90.4a	216.8a
May 2015	116.6a	196.2a
June 2015	49.0a	128.5a

Mean values in same column denoted by the same letters are not significantly different at $P \leq 0.05$

Appendix XI

Standard lengths (cm) and the level of heavy metals in the ovaries of mature tilapia from the upstream site

Month	Standard Length(cm)	Lead	Cadmium	Copper	Zinc	Iron
Nov.14	14.8	0.67	0.285	0.65	0.65	1.3
Dec.14	13.9± 0.40	0.89± 0.06	0.25± 0.04	1.33 ±0.48	1.08± 0.21	2.35± 0.35
Jan.15	17.5±1.50	0.42 ±0.22	0.15±0.06	1.70± 0.20	1.17± 0.45	3.35± 0.65
Feb.15	11.25± 2.45	0.37± 0.13	0.44 ±0.19	0.70± 0.37	0.52± 0.26	1.52± 0.51
Mar.15	11.73± 1.85	0.37± 0.16	0.19± 0.13	1.71± 0.68	0.70± 0.36	1.50± 0.52
Apr. 15	13.0±2.00	0.49± 0.07	0.26 ±0.11	1.97± 0.58	1.03 ±0.21	1.53± 0.96
May.15	14.83± 1.24	0.31± 0.17	0.20 ±0.07	2.15±0.12	0.83±0.19	1.93± 0.68
June.15	11.5	0.1	0.099	1.9	1.2	1.3

Standard length (cm) of sexually mature tilapia and the levels of heavy metals: downstream

Month	Standard Length	Lead	Cadmium	Copper	Zinc	Iron
Nov	18.4± 1.90	0.74± 0.09	0.19 ± 0.09	1.45± 0.45	0.71± 0.26	4.65± 0.35
Dec	9.5±0.00	0.25	0.275	2	0.88	1.3
Jan	8.2±0.00	0.25	0.21	0.35	0.88	1
Feb	18± 0.00	0.83	0.1	1.9	0.88	3
March	18.75± 1.25	0.83± 0.00	0.35 ± 0.06	0.50± 0.00	0.59± 0.37	4.85± 0.15
April	20.5 ± 0.50	0.80 0.03	0.31± 0.03	2.35± 0.25	0.76 ± 0.20	4.85 ± 0.15
May	17.6± 1.16	0.75± 0.04	0.41± 0.02	1.92 ±0.12	1.23± 0.29	4.35± 0.30
June	15.6± 0.60	0.76± 0.07	0.41 ± 0.00	1.95± 0.05	0.83± 0.22	3.33± 0.00

Appendix XII

Monthly catch of stages IV and V *Oreochromis niloticus* and mean number of mature oocytes in River Ruiru

Month	Upstream			Downstream		
	No. females	No. of eggs	Average No.of eggs per female	No. females	No. of eggs	Average No.of eggs per female
N	1	500	500	1	1423	1423
D	2	930	465	0	0	
J	1	560	560	0	0	
F	0	0	0	1	780	780
M	1	801	801	1	660	660
A	2	1410	705	0	0	
M	3	1905	635	3	2816	939
J	1	560	560	2	1603	802
Totals	11	6666	4226	8	7282	4604
Fecundity			603			921

Maximum allowable concentration of selected water quality variables in drinking water and fish, by various organizations

	IN DRINKING WATER			IN FISH
VARIABLE	EU (1998)	USEPA (2006)	WHO (2008)	FAO 2008
Pb	0.01 mg/l	0.015	0.01	-
Cd	0.005	0.005	0.003	-
Cu	2.0	0.03	2.0	0.03
Zn	NG	5	NM	0.04
Fe	0.2	0.3	0.3	0.1
EC (µS/cm)	2500µs/cm	NM	250µs/cm	-
PH	6.5 – 9.5	6.5 – 8.5	6.5 – 8.5	-

NM: Not mentioned
NG: No guidelines