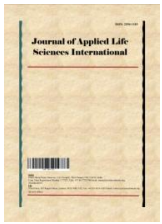

Journal of Applied Life Sciences International

23(9): 31-44, 2020; Article no.JALSI.61180
ISSN: 2394-1103

Association between Heavy Metals and Selected Reproductive Parameters in the Nile Tilapia, *Oreochromis niloticus*, along River Ruiru, Kiambu County, Kenya

Ong'eta M. Kwamboka[1], Syprine A. Otieno[1] and Jemimah A. Simbauni[1*]

[1]Department of Zoological Sciences, School of Pure & Applied Sciences, Kenyatta University, 43844-00100 Nairobi, Kenya.

Authors' contributions

This work was carried out in collaboration among all authors. Author OMK designed the study, performed the practical part including sample collection and supervising laboratory analysis and wrote the first draft of the manuscript. Authors SAO and JAS did over all supervision of the study and writing of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2020/v23i930185
Editor(s):
(1) Dr. Vasil Simeonov, University of Sofia "St. Kliment Okhridski", Bulgaria.
Reviewers:
(1) Raúl Cortés-Martínez, Universidad Michoacana de San Nicolás de Hidalgo, Mexico.
(2) Danijela Kostic, University of Nish, Serbia.
Complete Peer review History: http://www.sdiarticle4.com/review-history/61180

Original Research Article

Received 10 July 2020
Accepted 15 September 2020
Published 03 October 2020

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. River Ruiru is one of the rivers faced with pollution from nearby industries. It is inhabited by various species of fish such as 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. There is no documented information on the association between heavy metals and the reproductive biology of *Oreochromis niloticus* in River Ruiru. This study was aimed at assessing the level of heavy metals in fish ovaries and their association with selected reproductive parameters in the reproductive cycle of *O. niloticus*. Fish

**Corresponding author: E-mail: jasimbauni@gmail.com, jsimbauni@yahoo.com;*

samples were collected monthly, for 8 months, from the downstream and upstream sections. Gonadosomatic index (GSI), serum 17β-estradiol (E_2) levels and levels of heavy metals lead, cadmium, copper, iron and zinc in ovaries were determined. The levels of the heavy metals were measured using Atomic Absorption Spectrophotometer. The level of E_2 was analyzed using Enzyme-Linked Immunosorbent Assay. There was no significant difference in the GSI 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. 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). 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 were above allowable concentrations for fish consumption when compared to WHO levels. The study recommends that the Ministry of Environment and Natural Resources should put measures in place to stop discharging raw effluents into River Ruiru.

Keywords: Oreochromis niloticus; heavy metals; 17β-estradiol; gonadosomatic index; River Ruiru.

1. INTRODUCTION

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 [1,2].

River Ruiru harbors various species of fish, which are potential sources of proteins for the surrounding communities. River Ruiru is affected by rural and urban effluents, agricultural and industrial effluents [3], which make the river polluted with heavy metals. Exposure of fish to various environmental toxicants cause gonadal changes such as decreased gonadosomatic index (GSI) due to stressed liver tissues resulting in reduced production of phosphoglycoproteins that form part of the egg yolk [4]. Other workers reported that exposure of *O. niloticus* to cadmium decreases GSI at the beginning probably due to inhibition 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 [5].

Heavy metals are reported to stimulate or inhibit the endocrine system and cause overproduction or underproduction of hormones such as 17β-estradiol in fish [6]. 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, through changes in the physiological processes of the hypothalamic – pituitary – ovarian axis [7]. However, other researchers reported that estradiol levels significantly increased in dissolved cadmium exposed female *O. niloticus* [8].

It is reported that exposure of fish to high levels of the heavy metals zinc and copper (0.5 mg/l respectively, causes atrophy and cytoplasmic leakage in the ova leading to severe degeneration [9]. Other researchers observed that heavy metals cause atresia and necrosis in oocytes leading to a decrease in egg production [10]. [11], reported that exposure of an Indian teleost to copper, zinc, or lead, caused disappearance of oocytes in the ovaries Also, post hatch larvae of *O. niloticus* subjected to 2 and 5 ppm sub lethal levels of zinc for 30 days retained undifferentiated gonads with oogonial proliferation and ovaries of mature tilapia exhibited hyperemia and reduced oocyte number [12]. Cadmium prevents egg maturation and hence lowers the number of spawned ova [13].

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 [14]. 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.

2. MATERIALS AND METHODS

2.1 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. Two sampling sites along the river were considered during the study period. They are within longitudes 36° '54'E and 37° '3'E and latitudes 1° '12'S and 1° '8'S within Ruiru Sub County (Appendix 1, Fig. 1). 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 [15].

2.2 Sampling Sites and Collection of Fish Samples

Sampling sites were chosen based on the surrounding economic activities, proximity of sampling section to settlement areas, point of effluent discharge into the river and habitat characteristics such as physical appearance of the river water, type of vegetation and substrate. 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 (Appendix I, Fig. 1). Samples of *O. niloticus* were collected using a cast net. 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 [16]. All the males were returned to the sampling sites and the females were retained for subsequent studies. Same procedure was repeated for eight consecutive 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.

2.3 Blood Samples from the Females

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

2.4 Determination of Sexual Maturity Stages

The fish samples collected from both sampling sites were dissected. Ovary samples were carefully excised and trimmed to remove connective tissues. The maturity stages of the fish gonads were determined separately through visual inspection of the appearance, size and texture, following the procedures by [17] and [18].

2.5 Gonadosomatic Index (GSI)

Gonadosomatic index is the calculation of gonad weight as a percentage of total body weight [19]. 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 separately using the formula by [20]:

$$\mathrm{GSI} = \frac{\text{weight of gonad}}{\text{Weight of fish}} X100$$

2.6 Fecundity (F)

Fecundity is the number of eggs ripened by a female during spawning season [21]. 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 [22].

2.7 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 [23] and [24]. The concentration of 17β-estradiol in serum

samples were interpolated from the standard curve (Appendix II, Fig. 2).

2.8 Digestion of Ovary Samples

Ovary samples were wet digested separately where 1 g of the wet digest was accurately weighed using an electronic balance (Model AAA Adam Co limited). Ten milliliters (10 ml) 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 drop wise 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 [25]. The ovary samples from upstream and downstream were separately 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 [26]. 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.

2.9 Analysis of Heavy Metals

Analysis of the heavy metals lead, cadmium, copper, zinc and iron, in the digested ovary samples were determined separately using Atomic Absorption Spectrophotometer, at wavelengths 283.3 nanometers (nm), 228.8 nm, 324.8 nm, 213.9 nm and 248.3 nm respectively. 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 $mgkg^{-1}$.

2.10 Statistical Analysis

Statistical analysis of data was carried out using Minitab software version 13. 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 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 $p<0.05$.

3. RESULTS

3.1 Maturity Stages

A total of thirty *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 (Table 1).

Table 1. Maturity stages of *O. niloticus* from upstream and downstream sites

Maturity stages	Appearance of ovaries
I.	Tiny and transparent
II.	White
III.	Slightly yellow
IV.	Highly vascularized, dark yellow ovaries
V.	Ripe and loosened eggs from ovary walls
VI.	Shrunkened,flaccid and sac-like ovaries

3.2 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 (Appendix III, Fig. 3). 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 from the downstream sampling section (2.88 ± 0.051). However, there was no statistically significant difference ($t = 0.82$; $p > 0.05$) in these means.

3.3 Levels of Heavy Metals (mg/kg) in the Ovaries of Mature Tilapia from the Upstream and Downstream Sites (Appendix V, Tables 2 and 3 respectively)

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 (Appendix VI, Table 4). There was no significant difference in the mean levels of cadmium, copper and zinc between the upstream and downstream ovary samples ($p>0.05$).

3.4 Relationship between the Level of Heavy Metals in the Ovaries and the Gonadosomatic Index (GSI) of Mature Tilapia Upstream and Downstream

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 both the upstream (Appendix VI, Table 5) and downstream sites (Appendix VI, Table 6).

3.5 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 (Appendix III, Fig. 4). There was a drop in the preceding 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 (Appendix III, Fig. 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 ($t = 1.14$; $P > 0.05$).

3.6 Relationship between Levels of Heavy Metals in Ovary Samples and the Level of 17β-estradiol in Mature Tilapia in the Upstream and Downstream

Pearson moment correlation analysis 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 *O. niloticus* from both the upstream and downstream sampling sites ($P > 0.05$) (Appendix VII, Table 7) and (Appendix VII, Table 8).

3.7 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 Appendix IV, Fig. 5. Fecundity of *O. niloticus* in the downstream section was 921 eggs per female. Fish from the upstream showed lower fecundity at 603 eggs per female. However, 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).

3.8 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) (Appendix VII, Table 9).

Similarly, in downstream sections, there was no significant relationship between the fecundity and any of the heavy metals in the ovary samples of *O. niloticus* sampled from the downstream section of the River (P > 0.05) (Appendix VII, Table 10).

4. DISCUSSION

Metals may enter the body of fish through three possible ways; the body surface, the gill, and the alimentary tract [27]. Ovaries are reported to have a tendency of accumulating heavy metals in them [28]. 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 from 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 0.05 mg/kg for the fish and fish products [29-31] (Appendix VIII, Table 11). This means that consumption of fish from River Ruiru is dangerous to man considering their levels.

Slightly high gonadosomatic index (GSI) in June (upstream) and December (downstream) was due to presence of more eggs in the ovaries in stages III, IV and V. These ovary stages contained ova in stages III, IV and V indicating breeding season [32]. Low GSI recoded during the month of November 2014 in both sampling sites indicated immature (stage II) *O. niloticus* in terms of developing oocytes [33]. 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 [34]. The mean GSI in both sampling sites were similar to the one recoded by [35] but below the one obtained by [11].

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 tolerance of fish to the heavy metals [36]. Mature fish tissues and body fluids are reported to contain certain proteins that react with harsh environmental antigens and provide natural immunity to fish [37]. 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 months 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 mouth brooding phase of female *Oreochromis niloticus* [38], 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- estradiol [39]. It is reported that E_2 stimulates the synthesis of yolk lipid in the oocytes and its increase 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 [40]. The initial estradiol peak observed in January 2015 in female *O. niloticus* in the upstream section may result in the oocytes being maintained through a protective effect. This protection prevents the oocytes from becoming atretic [37]. The second estradiol peak in June 2015 could be due to response to rapid vitellogenic growth phase in the stages IV oocytes [41].

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 [42]. 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 [43,44].

5. CONCLUSION

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 both gonadosomatic index and levels of 17β-estradiol of the *O. niloticus* from both upstream and downstream sampling sites. There was no correlation between the levels of the heavy metals 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.

ACKNOWLEDGEMENT

The authors are grateful to the staff of Department of Zoological Sciences, School of Pure and Applied Sciences, Kenyatta University. The corresponding author sincerely thanks the Editor and the Reviewers in this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. FAO. Cultured aquatic species information programme. Journal of Environmental Internations; 2012.
2. FAO. The State of world fisheries and aquaculture. 2014;223.
3. Muiruri J, Nyambaka H, Nawiri M. Heavy metals in water and tilapia fish from Athi – Galana-Sabaki tributaries, Kenya. International Food Research Journal. 2013;20(2):891-896.
4. Hama S, Abdulrahman N, Wahab N. Correlation between heavy metals in water and some health parameters. International Journal of Plant, Animal and Environmental Sciences. 2015;257.
5. Çiftçil N, Ay Ö, Karayakar F, Cicik B, Erdem C. Effects of zinc and cadmium on condition factor, hepatosomatic and gonadosomatic index of *Oreochromis niloticus.* Environmental Bulletin. 2015;24:11.
6. Islam H, Baruwa B. Kalita J. Estimation of estrogenic heavy metals in water and body of a common fish (*Anabas sp.)* of Borsola Beel, Guwahati City, Assam, India. International Journal of Research in Science and Technology. 2015;5.
7. Bolawa O, Gbenle G, Ebuehi O. Endocrine disruption by the consumption of fish (*Tilapia oreochromis*) from heavy metals polluted river sites and its reversal using zinc. International Journal of Aquaculture. 2014;4.
8. Luo Y, Shan D, Zhong H, Zhou Y, Chen W, Cao J, Guo Z, Xiao J, He F, Huang Y, Li J, Huang H, Xu P. Subchronic effects of cadmium on the gonads, expressions of steroid hormones and sex-related genes in tilapia *Oreochromis niloticus*. Ecotoxicology. 2015;24(10):2213-23.
9. Tang J, Jun-Rong L, Zhong-Liang L, Hua Z, Xiao-Min T, Zhang-Shun C. Effects of Zn^{2+} and Cu on loach ovaries and ova development. Journal of Zoological Research. 2013;34(E4−5):E135−E139.
10. El-Morshedi N, Nadeem A, Kizilbash A, Ahmed A, El-Shebbly A, El Berri A. Effect of heavy metal pollutants on fish population in two Egyptian lakes. International Journal of Advanced Research. 2014;2:408-417.
11. Mazrouh M, Mahmoud H. Some aspects of reproductive biology with emphasis on the effect of pollution on the histopathological structure of gonads in the *Oreochromis niloticus* from Rossetta Branch, Nile River, Egypt. World Journal of Fish and Marine Sciences. 2009;1(3):190-198.
12. Caring VS. Effects of the heavy metal, zinc, on the freshwater fish *Tilapia nilotica* L. Biotropia-The Southeast Asian Journal of Tropical Biology. 1992;6.
13. Karels A, Manning S, Brouwer T, Brouwer M. Reproductive effects of estrogenic and antiestrogenic chemicals on sheepshead minnows (*Cyprinodon variegatus*). Environmental Toxicology and Chemistry. 2003;22(4):855-865.
14. Rzymski P, Tomczyk K, Poniedziałek B, Opala T, Wilczak M. Impact of heavy metals on the female reproductive system. Annals of Agricultural Environmental Medicine. 2015;22(2):259–264.
15. United Nations Environmental Programme Report. Nairobi River Basin Project Annual Report; 2001.

16. Mackie M, Lewis P. Assessment of gonad staging systems and other methods used in the study of the reproductive biology of the Narrow-Barred Spanish Makereri Scomberomorus in Western Australia. Journal of Fisheries Research Report. 2001;99(136):48.
17. Mous P, Goudswaard P, Katunzi E, Budeba Y, Witte F, Ligtvoet W. Sampling and measuring. Fish Stocks and Fisheries of Lake Victoria. 1995;55–82.
18. Shoko A, Limbu S, Mrosso H, Mgaya Y.; 2015. Available:https://doi.org/10.1186/s40064-015-1027-2
19. Atiqullah A, Zaheer M, Usman M. Studies on gonadosomatic index and stages of gonadal development of striped piggy fish, *Pomadasys stridens.* Journal of Entomology and Zoology Studies. 2013;1(5):28-31.
20. Khallaf E, Authman M. Some biological aspects of the Nile mormyrid fish, (*Mormyrus kannume*, Forsskal, 1775), from Bahr Shebeen Nilotic Canal, Egypt. World Journal of Fish and Marine Science. 2010;2:357-375.
21. Duponchelle F, Cecchi D, Corbin J, Legendre M. Variations in fecundity and egg size of female Nile tilapia, *Oreochromis niloticus,* from man-made lakes of Cote D' Voire. Journal of Environmental Biology of Fisheries. 2000;57:155-170.
22. Njiru M, Ojuok JE, Okeyo-Owuor JB, Muchiri M, Ntiba MJ, Cowx IG. Some biological aspects and life history strategies of Nile tilapia, *Oreochromis niloticus*, in Lake Victoria, Kenya. Africa. Journal of Ecology. 2006;44:30–37.
23. Cuisset B, Kuli E, Pradelles P, Kime D, Kulin E, Babin P, Lemenn F. Enzyme immune assay for 11-ketotestosterone using acetylinesterase aslabel: Application to measurement of 11-ketotestosterone in plasma of *Siberian sturgeon.* Composition Biochemistry Physiology. 1994;108:229-241.
24. Nash J, Davail-Cuisset B, Bhattacharyya S, Suter H, Lemenn F, Kime D. An enzyme linked immunosorbent assay (ELISA) for testosterone, 17β-estradiol and 17α, 20β dihydroxy-4-pregnen-3-one using acetylcholinesterase as tracer: Application to measurement of diet patterns in rainbow trout (*Oncorhynchus mykiss*). Journal of Fish Physiology Biochemistry. 2000;22:355-363.
25. Barry C. A method for wet digestion of fish tissue for heavy metal analyses. Transactions of the American Fisheries Society. 1975;104(4):803-804.
26. Türkmen M, Ciminli C. Determination of metals in fish and mussel species by inductively coupled plasma-atomic emission spectrometry. Journal of Food Chemistry. 2007;103:670–675.
27. Afshan S, Ali S, Shaista U, Farid A, Bharwana S, Hannan F, Ahmad R. Effect of different heavy metal pollution on fish. Research Journal of Chemical and Environmental Sciences. 2014;2:74-79.
28. Authman M, Zaki M, Khallaf E, Abbas H. Use of fish as bio-indicator of the effects of heavy metals pollution. Journal Aquatic Research Development. 2015;6:328.
29. FAO/WHO. Evaluation of certain food additives and the contaminants mercury, lead and cadmium. WHO Technical Report, Series No. 505; 1989.
30. Food and Agriculture Organisation (FAO). Fisheries management, 3, managing fishing capacity. Journal of Food and Agricultural Technical Guidelines for Responsible Fisheries, Rome, Italy. 2008;4(3):104.
31. WHO. Drinking water quality guideline. US Government Printing Office; 2008.
32. Laban HA. Biological studies on Egyptian sole, *Solea aegyptiaca,* (Chabanauad). Egyptian Journal of Aquatic Biology and Fish. 2007;11:171-190.
33. Sutthi N, Amornlerdpisan D, Chitmanat C, Kringsak M. Annual growth and reproductive performance in catfish hybrid. Journal of Advanced Agricultural Technologies. 2014;1(2).
34. Authman M. A study on freshwater pollution and its effects on zooplankton and fish *Oreochromis niloticus* in Shanawan drainage canal at Almay, Al-Menoufeya Province, Egypt. 1998;509.
35. Mahmoud M. Reproduction and histomorphology of Nile Tilapia, *Oreochromis niloticus,* collected from two different water sources. Life Science Journal. 2013;10(3).
36. Amiard J, Amiard-Triquet C, Barka S, Pellerin J, Rainbow P. Metallothioneins in aquatic invertebrates: Their role in metal detoxification and their use as biomarkers. Aquatic Toxicology. 2006;76:160–202.

37. Łuszczek-Trojnar E, Drąg-Kozak E, Szczerbik P, Socha M, Popek W. Effect of long-term dietary lead exposure on some maturation and reproductive parameters of a female Prussian carp (*Carassius gibelio B.*). Journal of Environmental Science and Pollution Research International. 2014;21(4):2465-78.
38. Cornish D. Seasonal steroid hormone profiles in plasma and gonads of the tilapia, *Oreochromis mossambicus*. Water SA. 1998;24:3.
39. Taghizadeh V, Imanpoor M, Nooshin M. Study the seasonal steroid hormones of common carp in Caspian Sea, Iran. Journal of Springerplus. 2013;2:193.
40. Acharjee A, Chaube R, Joy K. Effects of altered photoperiod and temperature on expression levels of gonadotrophin subunit mRNAs in the female stinging catfish, *Heteropneustes fossili*. Journal of Fish Biology; 2017.
41. Nazan D, Yener A, Rikap Y. Ovary maturatıon stages and histological investigation of ovary of the Zebrafish (*Danio rerio*). An International Journal of Brazilian Archives of Biology and Technology; 2008.
42. Koca Y, Koca S, Yildiz S, Gurcu B, Osanc E, Tuncbas O. Investigation of histopathological and cytogenetic effects on *Lepomis gibbosus* (Pisces: Perciformes) in the Cine stream (Aydin/Turkey) with determination of water pollution. Environmental Toxicology. 2005;20:560–571.
43. Khallaf E, Authman M. The biology of *Oreochromis niloticus* in a polluted canal. Ecotoxicology. 2003;12(5):405–416.
44. Silva J, Marcus R, Iracema D, Francisco, Araúj G. Gonadal development and fecundity of the smooth weak fish *Cynoscion Leiarchus* (Teleostei: Perciformes: Sciaenidae) in a tropical Brazilian bay. Zoologia. 2016;33(6):1984-4689.
45. Kiambu Topo Map and Kiambu County Governemnt; 2010.

APPENDIX I

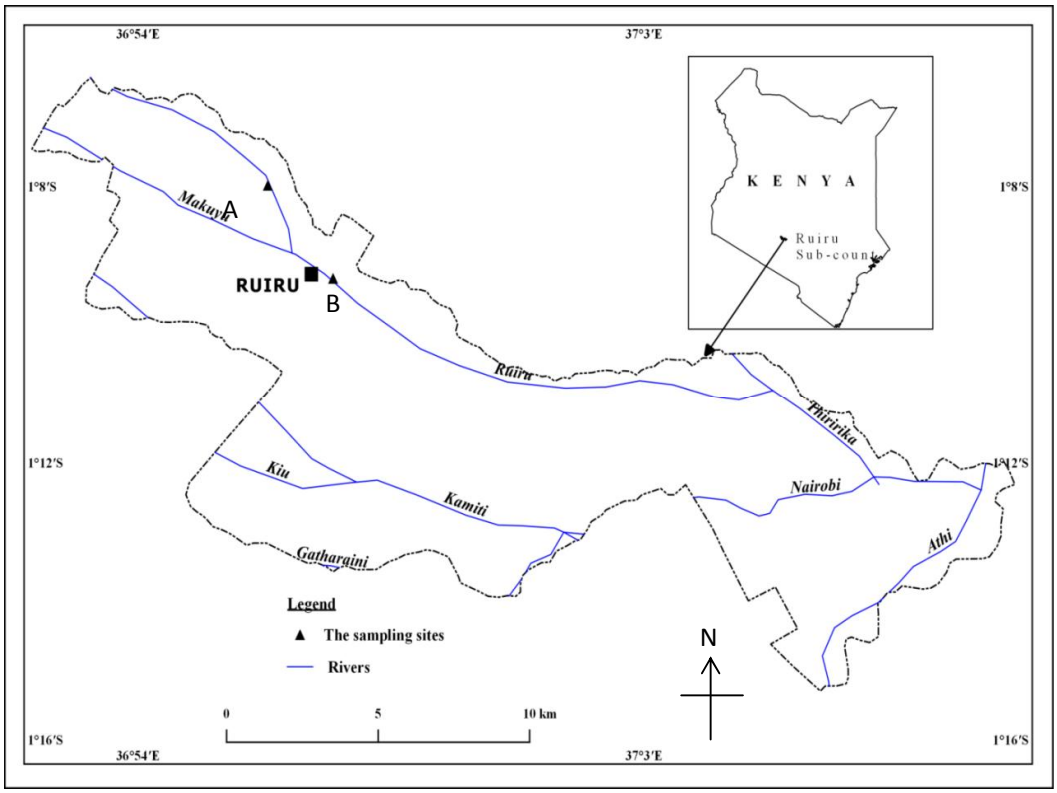

Fig. 1. Map of Ruiru Sub-county showing the location of the sampling sites
(Source: Kiambu Topo Map and Kiambu County Government, 2010) [45]

APPENDIX II

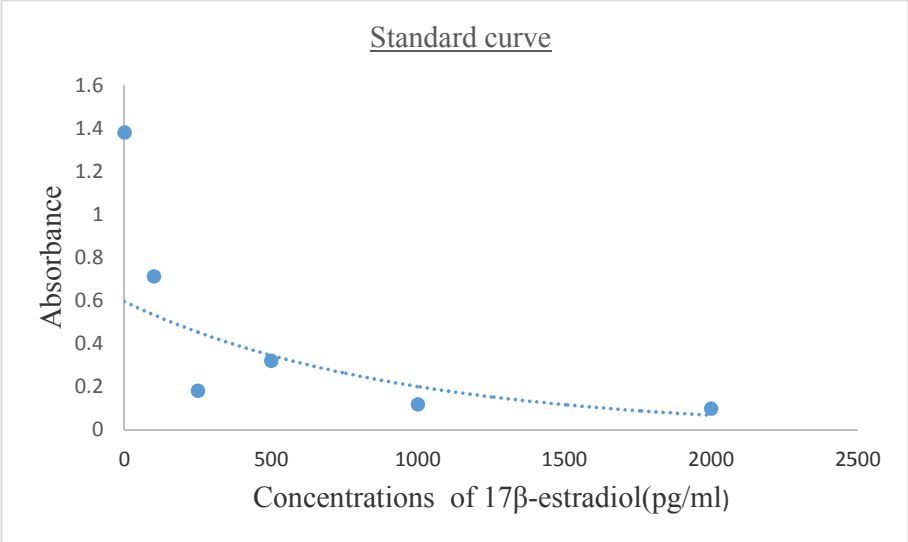

Fig. 2. Standard curve for 17β- estradiol

APPENDIX III

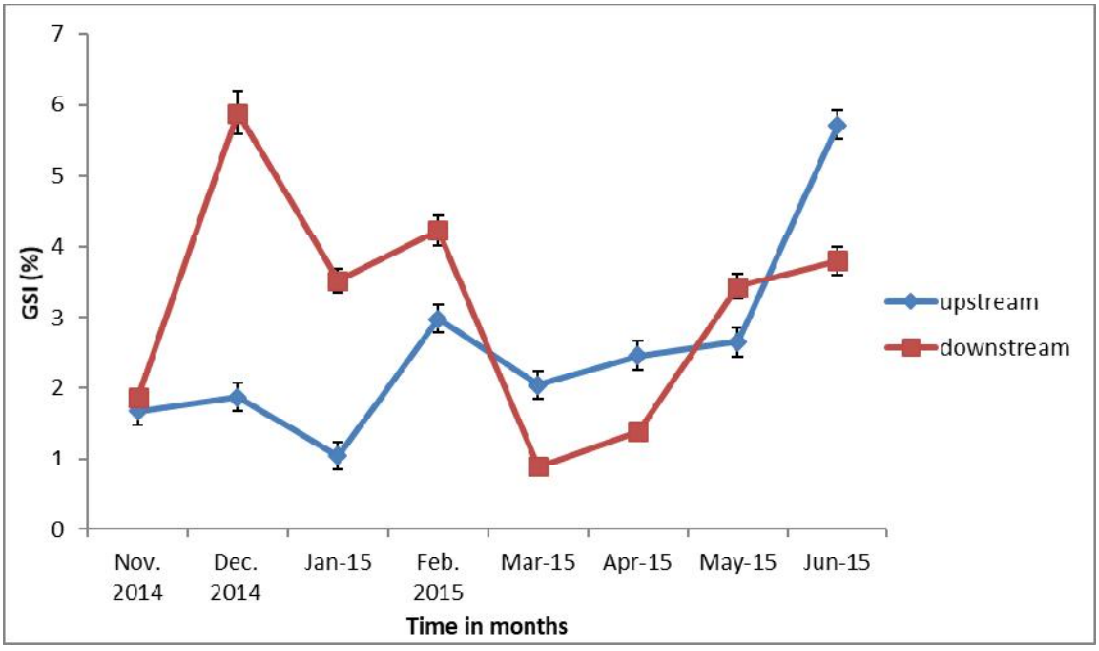

Fig. 3. Monthly means of GSI for mature *Oreochromis niloticus* from the upstream and downstream

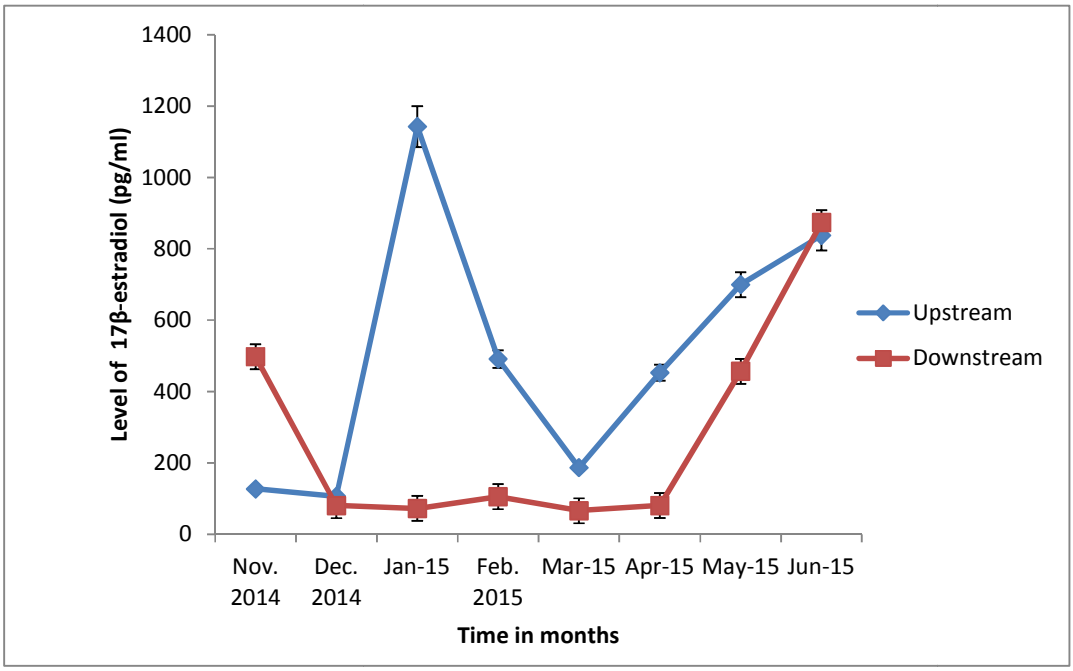

Fig. 4. Mean monthly level of 17β-estradiol (pg/ml) in mature tilapia sampled from upstream and downstream sections of River Ruiru

APPENDIX IV

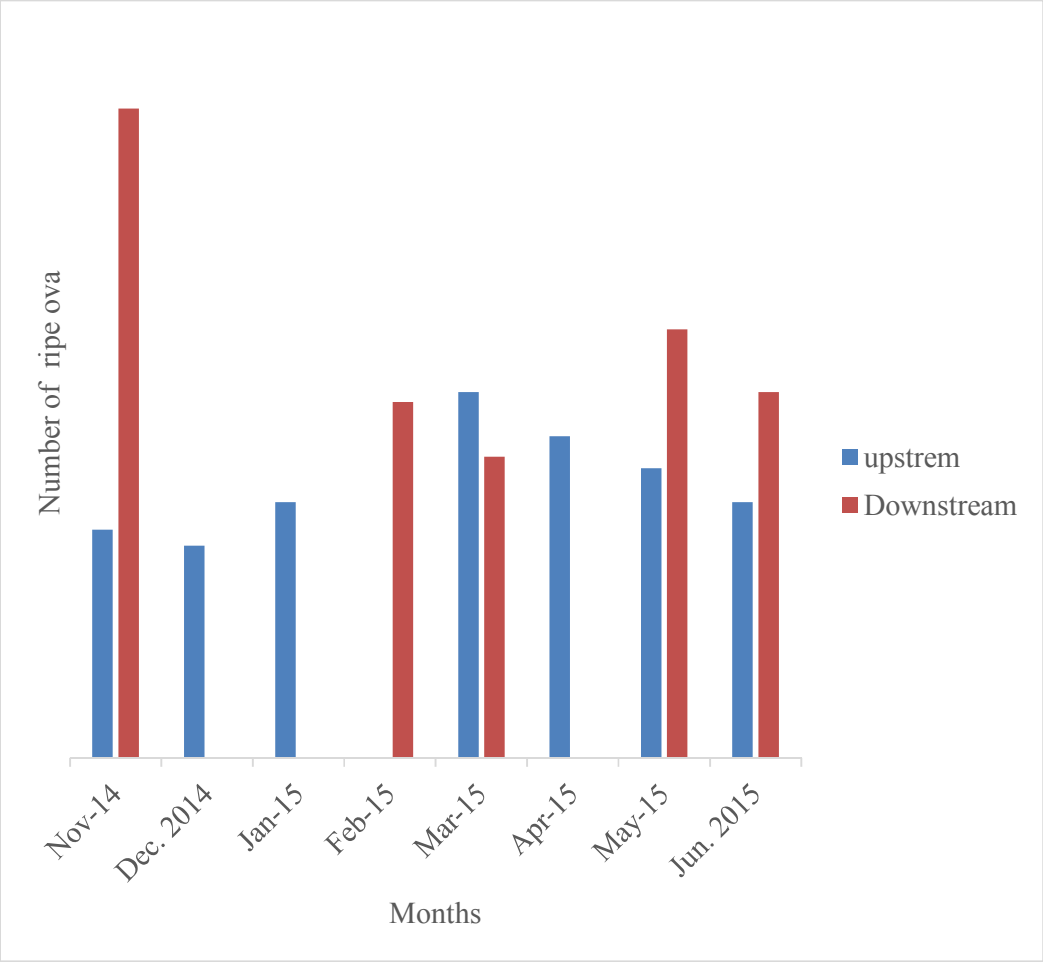

Fig. 5. Mean monthly ripe ova in the upstream and downstream sections

APPENDIX V

Table 2. Levels of heavy metals (mg/kg) in the ovaries in the mature tilapia sampled from the upstream section

Month	Lead	Cadmium	Copper	Zinc	Iron
November 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

Table 3. Monthly mean levels of heavy metals (mg/kg) in the ovaries from 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 VI

Table 4. 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*

Table 5. 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

Table 6. 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

APPENDIX VII

Table 7. Relationship between the level of heavy metals in ovary samples and the level of 17β-estradiol in mature tilapia in the upstream site

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

Table 8. 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

Table 9. 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

Table 10. 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

APPENDIX VIII

Table 11. Maximum allowable concentration of selected water quality variables in drinking water and fish, by various organizations

Variable	In Drinking Water (mg/l)			In Fish (mg/kg)	
	EU (1998)	**USEPA (2006)**	**WHO (2008)**	**FAO/WHO (1989)**	**FAO 2008**
Pb	0.01 mg/l	0.015	0.01	0.0005	-
Cd	0.005	0.005	0.003	0.0005	-
Cu	2.0	0.03	2.0	0.03	0.03
Zn	NG	5	NM	0.04	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

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/61180