# RESEARCH ARTICLE 

# REPRODUCTIVE BIOLOGY OF BARBUS ALTIANALIS FROM RIVER NYANDO, LAKE VICTORIA, BASIN KENYA 

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#### Abstract

Since 1970s riverine fishes have continuously declined and this has been attributed to anthropogenic factors. Continuous monitoring of riverine fish communities is therefore a requirement. Knowledge on biology of the existing fish species provides a basis for appropriate intervention strategies. This study is aimed at determining the spatial variation in fecundity, gonadal maturity stages of Labeobarbus altianalis along River Nyando. Sampling was done monthly for six months from Dec 2014 to May 2015. Three sites S1 at the upper region; S2 at the mid region and S3 at the lower region closer to the river mouth were sampled. Fish sampling was done using an electro fisher and the sampled fish identified in the field. The length and weight of Barbus altianalis were taken in the field to the nearest 0.1 g . Fresh ovaries were fixed in buffered $10 \%$ formalin for 12 hours and stored in $70 \%$ ethanol for determination of fecundity within the three sampling sites. At all the sampling sites females were significantly higher than males (Chi-square test; $\mathrm{P}<0.05$ ). Highest fecundity occurred in fish sampled from S1 49,440 $\pm 11,432$ eggs $/ L$ ) this could be attributed to the high proportion of females at the ripe/running stage followed by fish sampled from S3 $(37,453 \pm 7895$ eggs $/ L)$ while the least fecundity occurred at site $\mathrm{S} 2(32450 \pm 4235 \mathrm{eggs} / \mathrm{L})$. Gonado somatic indices remained clearly high mostly above 2 for females in all the three stations. Where there were low proportion of ripe/running females At all the sampling sites females were significantly higher than males there could be a possibility of sex reversal where males reverse their sex in response to environment another factor could be male mortality due to greater reproductive investment.


Key words: Fecundity, Nyando, Gonadostomatic index
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## INTRODUCTION

The Nyando River basin covers an area of $3517 \mathrm{~km}^{2}$ of Western Kenya. The river basin drains into the Winam Gulf in Lake Victoria. River Nyando originates near Mount Tinderet and drains the central highlands west of the Rift Valley. The Nyando, which traverses the sugar belt region in Kenya, is apparently polluted by sugar factory effluent (Mugo and Tweddle, 1999). Species abundance and distribution in this river is strongly dependent on habitat type and prevailing conditions (Fayazi et al., 2006). The ripon barbel (Barbus altianalis) is a ray-finned fish species from East Africa in the family Cyprinidae locally known in Kenya as Fuani or Odhadho depending on the place It has been found in Lake and its drainage basin and surrounding areas in the East Africa, including Lakes Kyoga, Edward, Kivu and George (Ntakimazi, 2006). It has also been reported in Rivers Ruzizi, and Kagera. It is an omnivore and often fished for sport and food while in Rwanda (Kagera River population) it is used for commercial purposes (De Graaf et al., 2007).

[^0]Labeobarbus altianalis inhabits inshore waters and rivers and prefers sand and gravel substrate (Eccles, 1992). Juveniles stay in riverine habitats while adults inhabit diverse freshwater habitat which include both riverine and lacustrine habitats (Eccles, 1992). Gastropod molluscs are an important food item in the lake, while insect larvae are of equal importance in hard bottom areas. Plants, fishes, and crustaceans are also eaten. More plant material is consumed by juveniles (Witte and De Winter, 1995). The fish is slow growing and therefore takes long to mature. Widespread and plentiful for such a large fish is attributed to its ecological tolerance and omnivorous habits. As a result of its widespread status, the Ripon barbel is not considered threatened by IUCN. However local stocks face a serious threat from siltation of aquatic habitats resulting into increased turbidity. According to De Graaf et al. (2007) erosion has seriously affected population of this species in Kagera River and must be addressed. Lack of reliable data could have informed IUCN position that does not consider this species threatened. However, recent developments on habitat destruction and fragmentations for example hydroelectric power projects in Sondu Miriu are real threats to this riverine species. The maximum total length ever recorded is 90 cm (Froese, 2011).


Figure 1 Map showing River Nyando and the sampling stations

In many temperate and tropical fishes (frequently called multiple, partial, serial, or heterochronal spawners), annual fecundity is seasonally indeterminate and batch fecundity is the only useful measurement. In such fishes the standing stock of yolked eggs, regardless of maturity state, give no indication of annual fecundity because these fishes continuously mature new spawning batches throughout a typically protracted spawning season. In the active ovaries of fishes with indeterminate annual fecundity, the oocytes usually occur in nearly all maturity stages; they range in size continuously from small unyolked oocytes $<0.1 \mathrm{~mm}$ diam. to yolked oocytes $0.4-0.7$ mm diameter, and no large hiatus exists between maturity classes of oocytes except for one between hydrated oocytes and advanced yolked oocytes which is of a temporary nature. Such fishes usually spawn many times during a season. According to (Cadwalladr, 1965, Katunzi, 1985), when ripe female and males congregate on their way to spawn in the riverine environment it leads to recruitment overfishing. Apart from overfishing, most of these species are threatened by modification of riverine regimes by human activities such as farming, settlement and pollution from factories. The decline of the species natural stocks has also been attributed to ecological changes that have taken place in Lake Victoria. As Witte and De Winter, (1995) noted, Barbus spp have not been given the deserved attention despite their socio-economic, socio-cultural and ecological importance therefore it is of importance to re-evaluate existing information on the biology of Labeobarbus altianalis.

## MATERIALS AND METHODS

## Study Area

The study was conducted along River Nyando within Lake Victoria basin, Kenya. River Nyando partly originates from Mau Complex and drains a catchment of high agricultural potential dominated with several agro-based industries such as coffee and sugar factories. The River Nyando is one of the rivers flowing into the Lake Victoria in Kenya and is polluted in some stretches by sugar factory effluent. The various habitats and altitudes along this river is easily accessibility by road.

## Sampling Stations

Three stations were marked along the river to represent upstream, mid-stream and downstream habitats (Figure 1). Station 1 denoted as S 1 was at Fort Tenan Bridge at latitude 0.21208 and longitude 35.30047 . This station was picked to represent the upstream habitat of the river. The second station (S2) which was the mid-stream was at Chemelil bridge (latitude -0.1466 , longitude 35.12586 ) while the downstream habitat was represented by S3 (latitude -0.17222 , longitude 34.9208) located at Ahero Bridge.

Table 1. Female and male gonad maturity stage classifications of Labeobarbus altianalis, as modified from Hopson (1972). Stage VI spent males were not included in Hopson's classification

| Gonad stages | Male | Gonad stages | Female |
| :---: | :---: | :---: | :---: |
| Stage I: <br> Immature | Testes were a pair of thin transparent filaments running longitudinally, along the dorsal wall of the body cavity. Sex was indistinguishable. | Stage I: Immature | Ovary paired, thin, transparent running longitudinally dorsal to body wall. Sex indistinguishable. |
| Stage II: <br> Developing (M1) | Testes semi-transparent greyish-white, flat with longitudinal fat attached by the mesentery to its vertical surface. The testes were small in transverse section. Testes were well vascularised, with no milt. | Stage II: <br> Resting(F1) | Ovaries greyish-white, transparent, smooth cylindrical and lightly vascularised. They did not span over the entire length of the peritoneal cavity. |
| Stage III: <br> Mature/Resting | Testes opaque, white-pinkish, soft and triangular in section, well vascularised and flattened along the edge. The fat was smaller than the testes but still prominent. Milt exuded from the lumen of cut testes. | Stage III: <br> Early <br> maturing | The ovaries were reddish-pink, semi-transparent, pear shaped in transverse section and well vascularised. |
| Stage IV: <br> Mature/Ripe | Testes opaque, ivory-white or pinkish, triangular in cross section, edges rounded with the fat strand reduced, lying in the longitudinal groove on the ventral surface of the testes. Large volumes of milt. | Stage IV: <br> Late maturing | Ovaries were pinkish with clearly visible small, opaque, pear-shaped yolky oocytes. Small blood vessels were present. |
| Stage V: <br> Ripe/running | Testes appearance similar to those in stage VI. A large volume of milt was released through the cloaca on applying gentle pressure on the abdomen. Testes turgid. | Stage V: <br> Ripe | Ovaries were opaque with large, yellow, yolky oocytes discernible through a superficial membrane. Large blood vessels were prominent on the surface of the ovaries. |
| Stage VI: <br> Spent | Testes were flabby, reddish-pink and smaller than in stage V. | Stage VI: <br> Running <br> Stage VII: <br> Spent | Oocytes were yellow-brownish in colour. Slight pressure resulted in eggs exuding from the vent. Ovaries were flabby and loose with a few scattered residual Stage V oocytes. |

## Sample collection

Sampling was done monthly for six months from Dec 2014 to May 2015. Fish samples were obtained using an electro fisher which quickly immobilized fishes and caused minimal habitat disturbance. The generator was a Honda GX 2408 HP that produced a current at 400 V and 10 A . The electro-fishing exercise was carried out for a timed period, usually between 15 and 20 min per site, depending on water depth, terrain and catches. This allowed enough time for a 50 M span of operation upstream and downstream of the generator. At each site location data (GPS readings) and physical characteristics were noted. As soon as the fish were landed, they were counted and identified using morphological characteristics. The non target species were returned into the river where as the Labeobarbus altianalis were weighed to the nearest 0.1 g using a potable weighing balance. The Total Length (TL) of the fish was measured from the tip of the anterior part of the mouth to the caudal fin and standard length (SL) was measured from the rostral tip of the upper jaw to the origin of the caudal fin. The TL and SL were measured using a meter rule calibrated in centimeters.

## Reproductive Biology

## Reproductive Seasonality

Individuals were sexed macroscopically; Gonads were removed from each sample of Labeobarbus altianalis and preserved in $10 \%$ buffered formalin. Each gonad sample were then dehydrated in an ascending series of ethanol and cleared in toluene. After dehydration, samples were embedded in paraffin, and histological sections cut at $5 \mu \mathrm{~m}$ using a rotary microtome. Sections were mounted on glass slides and stained with Harris hematoxylin followed by eosin counter stain. The slides were evaluated to determine the stage of the spermatogenic cycle in males and the ovarian cycle in females. Female stages were categorized in accordance with (Goldberg, 1981). Presented in table $1 . S t a g e ~ 1 ~ i s ~ t h e ~ n o n ~ s p a w n i n g ~$ condition consisting mainly of primary oocytes;

Stage 2 consists of slightly enlarged vacuolated oocytes; Stage 3 was characterized by yolk deposition in progress. In Stage 4 (spawning) mature/ripe oocytes predominate and some postovulatory follicles were present. This was done to determine variation of the different stages at the three stations. Cyclic gonadal recrudescence was described by a periodic regression of observed gonosomatic index (GSI) on the sine and cosine of the angular transformation of the independent variable.

## Sexual Maturity

Sex of each individual was determined from gonad inspection following anatomical dissection and/or external characters. Gonad state was assessed using a five-point scale (Bagenal, 1978) this result was useful in determining their variation between the stations.

## Fecundity and Egg Size

For the determination of fecundity and egg size, only fish in the "ripe- and- running" stages were considered since research has shown that only fish at maturity stages have yolked eggs (Tómasson et al., 1984). Appendix (vi) Fresh ovaries were fixed in buffered $10 \%$ formalin for 12 hours and stored in $70 \%$ ethanol. Ovary mass was then recorded and all the eggs from the left ovary emptied into a beaker of water and shaken gently to cause uniform mixing. Volumetric sub samples of 1-2 ml will be pipetted from the mixture and transferred to a Petri dish where all ova were counted using a tally counter. Fecundity was then calculated as
$\mathrm{F}=\mathrm{n} \times(\mathrm{V} / \mathrm{v}) \times(\mathrm{W} / \mathrm{w})$
where n is the number of ova in the sub sample, V is the volume of ova and water, v the volume of sub sample, W the weight of both ovaries and $w$ the weight of the ovary whose eggs were counted. A second sample of ova was obtained from the ovary that was not used for fecundity studies, and their diameter measured along their median axis with a calibrated eyepiece micrometer fitted on a dissecting microscope at $\times 20$
magnifications. Absolute fecundity and egg size was plotted against eviscerated mass and fork lengths and the least-squares regression parameters estimated from the base-10 logarithm transformed data. Elevations and slopes of the regression of the three populations was compared by analysis of interactions in the multiple regression models.

## Data Analysis

All the data generated from the sampling was entered into excel spreadsheet for the purpose of management and storage. All statistical analysis were done using Statistical Package for Social Sciences (SPSS version 17.0). Significance was accepted at $\mathrm{P}<0.05$. The proportions of each gonadal maturity stage for Barbus altianalis was determined and represented in bar charts for each station. The stations were then compared for each gonadal maturity stage and spatial variation determined using chi-square statistics ( $\mathrm{P}<0.05$ ).Variation in fecundity with total length was determined at each station using regression analysis.

## RESULTS

Sex Ratios: The sex ratios of fish sampled from the three sites along River Nyando is provided in Table 4.2. At all the sampling sites females were significantly higher than males (Chi-square test; $\mathrm{P}<0.05$ ). At site S 2 two fifth of the population were female and significantly higher than males ( $\chi^{2}$ $=5.432, \mathrm{df}=2, \mathrm{P}=0.0043$ ). At site S 2 upto $58.3 \%$ of the sample was dominated by females that were significantly higher than males ( $\chi^{2}=5.242, \mathrm{df}=2, \mathrm{P}=0.0063$ ). Similarly, site S3 had $66 \%$ being female and $34 \%$ being males were found to be significantly different $\left(\chi^{2}=7.432, \mathrm{df}=2, \mathrm{P}=\right.$ 0.0012 ).

Table 2 Number of male and female fish sampled at three sampling stations of River Nyando [\% are in parenthesis]


## Gonado-somatic Index and Gonadal Maturity Stages

The mean gonadosomatic index of Labeobarbus altianalis sampled at three sites along River Nyando is provided in Figure 2.There was a higher Gonadosomatic index recorded by females more than males in all the stations with the highest in S1 and lowest in S2. For males Gonadosomatic index was high at S1 and lowest at S2

## Fecundity

The average numbers of eggs per litre at the three sampling sites are shown in Figure 3. Fecundity was found to differ significantly among the sampling locations (One-Way ANOVA; $\mathrm{F}=20.5423, \mathrm{df}=2, \mathrm{P}=0.0043)$. Highest fecundity occurred in fish sampled from S1 49,440 $\pm 11,432$ eggs/L) followed by fish sampled from S3 (37,453 $\pm 7895 \mathrm{eggs} / \mathrm{L})$ while the least fecundity occurred at site S2 ( $32450 \pm 4235$ eggs/L). The modeled relationships between fecundity and length found that it was exhibited a binomial response (following quadratic response) increasing with length from about 14 cm to a maximum of about 54,000 eggs $/ \mathrm{L}$ at length 15 to 16 cm before declining (Fig 4).


## Sampling sites

Figure 2 The mean gonadosomatic index of Barbus altianalis sampled at three sites along River Nyando


Figure 3 Average number of eggs/L at the three sampling sites along River Nyando


Figure 4. Variation fecundity with TL of fish at the three sampling sites along River Nyando Maturation Stages and Cyclicity


Figure 5. Variations in maturity stages for Labeobarbus altianalis at the three sampling stations along River Nyando
Size at Various Maturation Stages of Labeobarbus altianalis


Figure 6. Variation in mean TL at each maturity stages of Barbus altianalis along River Nyando

## DISCUSSION

## Sex Ratios of Barbus altianalis

According to the sex ratio model (Fisher, 1999), animals should produce offspring of a balanced sex ratio. Therefore, a population with an unbalanced sex ratio will be exposed to frequency-dependent selection for the minor sex. In general, a population with an imbalanced sex ratio due to unusual environmental conditions can be considered to be disturbed or maladapted for the given conditions. In the Atlantic silverside Menidiamenidia (Conover and Van Voorhees, 1990), an unbalanced sex ratio induced by high temperature may become balanced under warm, but stable environmental conditions within a few generations in artificial systems. In thermal springs, the environmental conditions are generally warmer and more stable than in surrounding habitats and effluent rivers. Therefore, Fisher's principle should apply in a similar manner as in the experiments of Conover \& van Voorhees (1990). If the sex ratio of an immigrating fish species were biased at first by the environmental conditions, it would be expected to rebalance in time From this study females dominated in the three stations the significant difference in sex ratio could be attributed to a number of factors one there is a possibility of sex reversal whereby males reverse their sex in response to changes in environmental parameters also there is a possibility that there is high male mortality probably attributed to greater reproductive investment, food supply could be a determining factor where females predominate when food is abundant, while male predominate in oligotrophic environments (Nikolsky, 1963). A more detailed study could unravel the bias of population towards females

## Fecundity, Gonado-somatic Index and Gonadal Maturity Stages of Labeobarbus altianalis

The fecundity of Labeobarbus altianalis in the three sites was also investigated since it is known to vary among populations, and at times, between strains of fish species (Bromage et al., 1990, Jonsson and Jonsson, 1999). The determination of actual numbers of eggs produced would be more useful for analysis of stock dynamics (Mason, 1985, Cadwalladr, 1965). Fecundity differed significantly among the three sampling sites as S1 had the highest fecundity this could be attributed to the high percentage of females at the ripe and running stage (Stage 6 ) within. S3 followed as the proportion of females at ripe and running stage and the site with the lowest fecundity was S2as the proportion of ripe and running females was lowest at this site Such variations could also be attributed to the differential abundance of food (Coward and Bromage, 2000) and the effects of age, egg size and genetic factors (Wootton, 1979). The observed differences of these Labeobarbus altianalis could be a result of the complex interaction of the abovementioned factors. Fecundity increased with length as expected. Gonadosomatic indices remained clearly high mostly above 2 for females in all the three stations being higher in Station, the high fecundity also coincided with high levels of dissolved oxygen and low temperature at the station which is suitable for spawning this implied Labeobarbus altianalis females are is characterized by high (GSI value $>2$ ) gonad activity throughout the three stations .However, even if breeding takes place throughout the three stations, peak spawning activities occur in station 1. It was of interest to note while fish with ripe ovaries (stage IV) occurred in the samples
almost throughout the year fish with running ovaries (stage V ) were found in all the stations sampled. Fish maturity stage largely depends on the age of fish and thus affects the sizes. It is expected that the stage 1 fish of the same species are smaller than the other stages though this may vary due fluctuating environmental condition. Information on sex and gonad maturity stages is thus very crucial in trying to explain the cause of spatial variation in sizes of same fish species within a system. Since this is lacking for Labeobarbus altianalis in Nyando River, this study targets not only to explain the cause of varying sizes but to bridge the information gap. Maturation stages of males varied from the three stations at S1 the males that were mature /ripe attained the highest length of 23 cm which was lower and then S2. Females at S1 immature measured 5 cm which was the lowest and the highest length attained by females at S 1 was 20 cm followed by station 2 and the lowest was station 3 .

## Percentage of Maturity Stages

At S1 males that were mature dominated this could be because of followed by stage 3 maturing resting stage and the ones with the lowest proportion were those at stage 2 developing which was equal to the mature. At S 2 males with spent was at a higher proportion followed by stage 3 maturing/resting and then those at stage 4 mature/ripe the least was those at stage 5ripe/running. At S3 males had a higher proportion of mature/ripe stage 4 followed by those at stage 3 maturing/resting and then followed by the developing stage 2 then followed by those at stage 5 ripe and running the least were those at stage 6 spent. For females at S 1 the highest proportion were the early maturing at stage 3 followed by those at stage 2 resting and then at stage 6 ripe/running the lowest proportion were those at stage.

## Conclusion and recommendations

The upper station had a higher fecundity of Barbus altianalis because of the presence of mature females; it was greatly influenced by water quality status that dictated food availability and accessibility. The varying fish sizes along the river is thus as a result of sex and gonad maturity composition; food items consumed; and influence of dissolved oxygen, pH and Turbidity. Since this work has been done for a relatively short period of time, influence of these parameters on the sizes of Barbus altianalis should be verified over a long duration.

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