Comparative Studies of Spawning Potentials in Female African Bonytongue *Heterotis niloticus* (Curvier 1829) between Cross River and Great Kwa River, Nigeria

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**Author’s contribution**

The sole author designed, analyzed and interpreted and prepared the manuscript.

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**Original Research Article**

**ABSTRACT**

The spawning potentials of *Heterotis niloticus* from Great Kwa River and Cross River was compared in a twelve-month study. Forty-nine female *H. niloticus* was sampled from Cross River and thirty four from Great Kwa River from January to December 2014. Gonadosomatic index (GSI), fecundity and histological examinations were investigated in the Institute of Oceanography laboratory. Each month of this study recorded at least one female specimen with eggs at different stages of development. In Cross River, the months of August and September recorded one hundred per cent female with eggs, while Great Kwa River exhibited such performance in July, an indication of spawning activities by the fish. Cross River had a major GSI peak in July with a minor one in August, but Great Kwa River did not exhibit any significant GSI peak throughout the study, and no significant difference in GSI between the rivers when compared with T-test (0.22; P>0.05). There was a significant difference (0.02, P<0.05) in fecundity of *H. niloticus* between the population in Cross River and Great Kwa River. Regression analysis of fecundity and body weight showed a positive correlation...
Histological changes in the gonad of the fish showed no disparity between the rivers. *H. niloticus* has the ability to spawn all year round, but in the Cross River, the fish has high reproductive potentials than those in the Great Kwa River.

**Keywords:** *Heterotis niloticus; Great Kwa River; cross river; fecundity.*

1. **INTRODUCTION**

The African bony-tongue is one of the fish species that inhabit the Calabar River and the Great Kwa River. It is a cherished food fish species in Nigeria waters. In Cross River Basin, the highest body size recorded is 8 kg weight and 1.2 m length [1]. The growth index of this species in the Cross River basin is attributed to the high primary productivity of the aquatic environment, since it is a planktvorous species. In their indigenous habitat of Northern Nigeria, highest abundance occurs in July and August each year (rainy season), with rising river level [2]. *H. niloticus* lives mostly in lake, swamps and creeks where the current is not strong. They spawn in swamps and floodplains during the wet season [3]. *Heterotis niloticus* is an example of a fish that builds a large and impressive nest for spawning [4]. The female leaves the nest after spawning, while the fry are within the nest for about 4 to 5 days during which time they are guarded by the male [5,4]. With increasing human exploration which has resulted in environmental degradation, this species has lost about 60% of its breeding and nursery habitat in Nigeria with subsequent reduction in populations [6,7]. Therefore, to effectively regulate stock sizes, biological information such as annual reproductive cycle is essential [8,9]. The factors that affect growth, condition, maturation and fecundity are all crucial in a population of any fishery. In this study fecundity, gonadosomatic index and histological examinations of the ovaries were carried out in Cross River and Great Kwa River to determine and compare the spawning ability and period of the fish species.

2. **MATERIALS AND METHODS**

2.1 **Study Area**

Cross River and Great Kwa River are fresh water bodies found in the South-Eastern Nigeria. Fish specimens used for this study were obtained from Cross River and Great Kwa River. Fish specimens from Cross River were collected at Ayadehe in Akwa Ibom State and those from Great Kwa River were collected at Obufa Esuk. The two rivers play major roles in the economic lives of the people within the state and its environs. Cross River extends between latitude 4.85̊ N and 5.12̊ N and longitude 8.15 and 8.30̊ E, while the Great Kwa River is located at the thick forested belt made up of rain forest, freshwater swamp, forest and mangrove swamp. It lies between latitude 4.45̊ and 5.12̊, longitudes 8.20̊ and 8.31̊ E and has a drainage basin of 1670 km² [10].

2.2 **Sample Collection**

All the fish samples used for this study were obtained from artisanal fishermen fishing in Cross River at Ayadehe in Itu L.G.A of Akwa Ibom State and the Great kwa River at Obufa Esuk in Cross River State for twelve months, twice monthly from January to December 2014. The fishes were transported to Institute of Oceanography laboratory, University of Calabar, Calabar.

2.3 **Laboratory**

In the laboratory, total length (TL) to the nearest 0.1 cm was taken on the measuring board and weight of each of the fish to the nearest 0.1 g was measured using a METLAR MT-5000 D electronic balance. After measurements were taken, each fish was cut open at the visceral region with a pair of scissors and the single gonad which is situated at the left side of the fish was removed. Each gonad was weighed to the nearest 0.01 g, and gonad maturity stages were classified according to [11].

2.4 **Gonadosomatic Index**

Gonadosomatic index (GSI) was calculated according to [12].

\[
GSI = \left( \frac{\text{Gonad weight}}{\text{Whole fish weight}} \right) \times 100
\]

2.5 **Fecundity**

Gonads with ripe ovaries were used to determine the fecundity of *H. niloticus*. Fecundity was estimated from total counts of eggs in the ovaries.
of fish in the advanced state of development [13]. Each weighed ovary was cut into sub-samples of 1 g from the anterior, middle and posterior portions. They were preserved in the modified Gilson fluid inside small glass bottles and shaken periodically to loosen the oocytes, this fluid helps to harden the eggs for counting and diameter measurement. Ovaries of each fish were preserved for a maximum of 5 days before counting the eggs to determine the fecundity [14]. The eggs were then dispersed into Petri dishes and any large clumps were gently separated. Agarose gel were poured into the Petri dishes and carefully mixed to disperse the eggs for even distribution. Counting was done using a stereomicroscope. Fecundity was calculated by multiplying the total weight of ovary by the number of eggs per gram weight of ovary [14]. The relationship between fecundity and body weight (Wt), fecundity and total length (TL), fecundity and ovary weight (OW), fecundity and Gonadosomatic index (GSI) and, fecundity and egg diameter were estimated using the regression analysis.

2.6 Histological Examination of Ovaries

A total of 83 ovaries of *Heterotis niloticus* were sectioned for histological examination during the study period. Some portions of the ovaries were fixed in equal volume of 10% formalin and 0.9% sodium chloride for 24 hours before histological examination according to [12,15]. The fixed ovaries were dehydrated in ascending series of concentration of alcohol (75%, 85%, and 95%). The ovaries were embedded in paraffin wax (melted at a temperature of 55-60°C), in a small square moulds to produce blocks. The moulds were place under running tap water to harden. The resulted blocks were sectioned with microtome machine at 8 µm [12] and floated in water bath. Sections were picked on clean slides and dried at 37°C in the incubator. Each slide was passed through xylene (10 minutes) and through descending concentration of alcohol (absolute alcohol, 95% alcohol, 90% alcohol, 70% alcohol). They were stained in haematoxylin for about 15 minutes, differentiated in 1% acid, rinsed in water and allowed under running water for 30 minutes until sections turned dark blue. One percent eosin
was used to counter-stain the slides before it was dehydrated in alcohol, cleared in xylene and mounted using Distrene Plasticizer Xylene (D.P.X). Slides were examined with light microscope.

3. RESULTS AND DISCUSSION

Forty nine female *H. niloticus* was sampled from Cross River and thirty four from Great Kwa River. *H. niloticus* occurs throughout the year but with the peak from June and August. Monthly variation in mean Gonadosomatic Index of female *H. niloticus* from the Cross River and Great Kwa River between January and December 2014 are shown in Table 1. In Cross Rivers gonadosomatic index was high in the months of July and August, with a minor peak in March. In Great Kwa River no major GSI peak was found during the study, T-test revealed that GSI of fish from both river was not significantly different (0.22) (P>0.05), but fish with different gonad developmental stages were obtained every month during the study. A positive relationship was obtained when regression analysis was used to assess fecundity dependence on body weight (r =0.3686) (P>0.05) with equation F=1.326TW^{0.7544}.

Fecundity range of 508 oocytes (48.1 cm TL; 1.69 kg wt, 5.7 g gonad weight) – 7722 oocytes (69.1 cm TL, 3.01 kg wt, 15.2 g gonad weight) was obtained from Great Kwa River with highest mean of 7468 ± 178 in the month of August (Table 2), while Cross River exhibited higher values ranges of 753 oocytes (58 cm TL, 1.810 kg wt, 6.2 g gonad weight) – 32,240 oocytes (68 cm TL, 2.39 kg wt, 70.2g gonad weight) with high mean of 13387±6861 in the month of march. Fecundity between the rivers was significantly different (0.02; p=0.5)

3.1 Histological Studies

The ovary development stages in the fish were classified according to [11] as presented below:

Stage 1: (Maturing stage) gametes have already been discharged, the swelling process in the cavity of the gonad is complete, and gonads are very small in size, eggs are not visible to naked eyes.

Stage 2: Ripening eggs are visible to naked eyes the gonads increases in size and weight rapidly.

Stage 3: (Ripe stage) gametes get ripe, gonads have reached their maximum weight but the gametes do not run out when light pressure is applied.

Stage 4: (Reproduction) gametes run out when a slight pressure is applied to the abdomen, the weight of the gonads rapidly decreases indicating process of ending the spawning process.

Stage 5: (spent) gametes extruded and cavity of gonads swollen gonads have the appearance of an empty one, usually with a few eggs remaining in the gonad.

Fig. 2. Monthly percentage proportion of *Heterotis niloticus* at different stages of gonadal development
Table 1. Mean monthly gonadosomatic index of female *H. niloticus* from Cross River and Great Kwa River

| River/Year   | January | February | March | April | May   | June | July | August | September | October | November | December |
|--------------|---------|----------|-------|-------|-------|------|------|--------|-----------|---------|----------|----------|
| Cross River  | 0.88 ± 0.00 | 0.31 ± 0.23 | 0.73 ± 0.23 | 0.50 ± 0.37 | 0.15 ± 0.01 | 1.77 ± 0.62 | 0.95 ± 0.49 | 0.60 ± 0.10 | 0.25 ± 0.04 | 0.24 ± 0.04 | 0.50 ± 0.19 |
| Great Kwa River | 0.63 ± 0.09 | 0.39 ± 0.00 | 0.48 ± 0.02 | 0.53 ± 0.06 | 0.62 ± 0.04 | 0.28 ± 0.04 | 0.5 ± 0.08 | 0.32 ± 0.03 | 0.24 ± 0.03 | 0.15 ± 0.04 | 0.38 ± 0.02 |

Table 2. Mean monthly fecundity of *H. niloticus* from the Cross River and Great Kwa River

| River/Year | Month | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|------------|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cross River | 5723±0.00 | 2636±1882 | 13387±9861 | 9588±787 | 1217±366 | 5013±421 | 1576±5619 | 11145±6084 | 5902±775 | 5279±1183 | 4046±910 | 5786±1503 |
| Great Kwa River | 4674±1118 | 4572±0 | 4911±738 | 5046±606 | 4979±385 | 3471±1194 | 6167±704 | 7468±178 | 4741±1030 | 4394±629 | 4046±910 | 5786±1503 |

Table 3. Monthly mean and range of total fish length

| Month | Mean length (cm) Cross River | Mean length (cm) Great Kwa River | Fish total length range (CR) | Fish total length range (GKR) |
|-------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| January | 46.9 ± 0 | 49.2 ± 99.0 | 48.9 - 48.9 | 48.6 - 48.7 |
| February | 56.6 ± 2.06 | 61.7 ± 0 | 55.1 - 58 | 61.7 - 61.7 |
| March | 71.6 ± 7.33 | 56.6 ± 8.56 | 63.4 -81.2 | 47.1 - 63.7 |
| April | 73.1 ± 7.10 | 59 ± 5.42 | 65.9 - 80.1 | 53.1 - 63.8 |
| May | 57.7 ± 6.01 | 54.6 ± 5.94 | 53.4 - 61.9 | 50.4 - 58.6 |
| June | 58 ± 9.34 | 63.1 ± 7.31 | 46.7 - 71.5 | 55.6 - 70.2 |
| July | 59.37 ± 6.73 | 61.9 ± 9.65 | 51.7 - 64.3 | 48.4 - 72.4 |
| August | 62.43 ± 41 | 67.43 ± 6.73 | 54 - 68 | 59.8 - 72.5 |
| September | 62.41 ± 4.63 | 65.97 ± 9.46 | 56.5 - 68.4 | 55.8 - 74.5 |
| October | 75.23 ± 5.31 | 69.36 ± 8.51 | 70.3 - 81.2 | 55.7 - 76.2 |
| November | 69.42 ± 6.15 | 57.2 ± 7.74 | 60.3 - 76 | 48.5 - 63.5 |
| December | 61.7 ± 8.05 | 61.75 ± 2.90 | 52.7 - 72.1 | 59.7 - 63.8 |
At least one female *H. niloticus* with eggs at various stages of development was collected each month of the year. This finding confirms the report by [16] that *H. niloticus* is an all year round spawner. One hundred percent female with eggs were found in the month of August and September for Cross River and in July for Great Kwa River, indicating peak spawning activities by the fish. These correlated positively with the high gonadosomatic index and fecundity during this period of the year as a pointer to the main spawning season of the fish. Though gravid fish was found every month of the year, the level of eggs development varied in both rivers. The different eggs size found in the gonad showed that breeding is done in batches as reported by [14] and [17]. Peak in GSI value observed in July and August were confirmed by high frequency of the mature stage (3) and spawning stages (4) in majority of the months (Fig. 2). Cross River showed a major GSI peak in July but Great Kwa River did not exhibit any major GSI peak throughout the study but minor peaks were noticed in March and December. According to [18] gonadosomatic index provide good population level information of reproductive performance. Fecundity range of 508 oocytes–7722 was obtained from Great Kwa River, and 753 oocytes - 32,240 oocytes from Cross River (Table 2). The observed variations in fecundity from the two rivers might have resulted from difference in food abundance and other environmental factors, and is higher than that previously reported [19] 3572 – 15246 oocytes in Oubangui River, Central African Republic, and [16] 2697 - 27508 oocytes in Sô River floodplain respectively. [20] reported high fecundity of 63574, 49067, 39952 in Labeo rohita injected ovaprin-C, this showed that *H. niloticus* is a less fecund fish but might be boosted if such trial is undertaken. A significant difference (P=.05) in fecundity of *H. niloticus* was observed between the population in Cross River and Great Kwa River Histological evidence showed that vitellogenesis in *H. niloticus* is an asynchronous procedure, indicating multiple spawning characters in the fish, and sexual development proceeded throughout the year.

### 4. CONCLUSION

*Heterotis niloticus* from Cross River and Great Kwa River showed a disparity in peak spawning months, which may be due to differences in biological and environmental factors. Females *H. niloticus* were found throughout the year with various stages of gonadal development, indicating that the fish spawns all year round. Five gonadal maturity stages based on microscopic characteristics were found in *H. niloticus* for the two rivers. *H. niloticus* in the Cross River has high reproductive potentials than those in the Great Kwa River. This, therefore, calls for further research on the ecology of the fish in the two rivers.

### ETHICAL DISCLAIMER

As per international standard or university standard written ethical permission has been collected and preserved by the authors.

### COMPETING INTERESTS

Author has declared that no competing interests exist.

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