



ASPECTS OF THE REPRODUCTIVE BIOLOGY OF *HETEROBRANCHUS LONGIFILIS* (VALENCIENNES, 1840) IN RIVER OKURA, KOGI STATE CENTRAL, NIGERIA.

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ABSTRACT

The reproductive biology of *Heterobranchus longifilis* was studied from October, 2011 to October 2012. Aspects studied include identification of gonad maturation stages, the sex ratio, breeding season and fecundity of the species. 654 samples of *Heterobranchus longifilis* were obtained from the local fishermen at Ofejiji. They were caught with gill net (4.5 x 4.5cm mesh size), setline with hooks of various sizes (12, 7, and 4). Samples were weighed and measured using the kitchen weighing balance and a meter rule respectively. The gonads were classified into stages I, II, III, IV, V, VI and VII. There was no significant difference in the female to male ratio ($p > 0.05$). There were 28 fecund females used for estimation of fecundity. Fecundity ranged from 17,001 (body weight = 625g, SL = 38.6cm) to 97,122 (body weight = 5158g, SL = 71.5cm) with mean value of 37744 ± 21648 eggs. Gonadosomatic index was observed to be highest in October with value of 2.80 ± 0.2 for female and 1.05 ± 0.1 for male. The frequency of occurrence of gonad stage VI was highest in October with value 85.7% and 74.2% for male and female respectively. In February, frequency of occurrence of gonad stage VI (15.6% for male and 16.7% for female) and mean gonadosomatic index peak were observed for female (1.2 ± 0.1) and male (0.4 ± 0.05). Monitoring the mean monthly gonadosomatic index and frequency of gonad maturation stages, spawning occurred mainly in October. The gonadosomatic index peak and presence of gonad stage VI observed in February could possibly be due to the residual left over eggs in the gonads of the species after its breeding season in October.

Key words: *Heterobranchus, fecundity, gonads, lengths, gonadosomatic*

INTRODUCTION

Sexual maturation and spawning are among the most important aspects of fisheries biology which must be understood in order to interpret population variability. Determination of the timing and duration of spawning may not only explain seasonal variations in the distribution of adults, but is fundamental to the understanding of the distribution and survival of larvae (Winters and Wheeler, 1996). Maturity stages define the degree of ripeness of the ovaries and testes of a fish (FAO, 1974). Knowledge of the gonad maturation stages of fishes is important for determining stocks that are mature and the size or age at first maturity (Bagenal, 1978), establishing the reproduction period and length of gonadal maturation to allow for accurate implementation of fishery legislation (Goncalves *et al.*, 2003). Studies on breeding seasons and factors associated with it are needed to protect new recruits and predict recruitment variability (Morales, 1991). Fecundity is generally defined as the number of ova that are likely to be laid by a fish during its spawning season. It depends on several factors such as age, size of fish, rainfall, and salinity of water and may differ in different races of the same species (Moyle and Cech, 2000). It explains the variation of the level of population and the efforts needed to increase the amount of fish harvest. The information on the fecundity is imperative in the study of fish biology, which is useful in several applied aspects of fishery science, including fishery management of any water body (Lagler *et al.*, 1967). Studies on fecundity gives prerequisite information needed to plan a breeding programme so as to determine how many eggs would be spawned or stripped, the amount of rearing facilities required and the extent to which various culture equipment would be put to use (Eyo and Mgbenka, 1992). Gonado somatic index (GSI) which is one of the parameters used in reproduction studies of fish is used to detect hydrated ovaries and hence reproductive period from increase in weight (Hunter and Macewicz, 2001). The gonadosomatic index and frequencies of matured gonads have been used to monitor the breeding cycles of fish (Islam *et al.*, 2012; Ekokotu and Olele, 2014 and Flores *et al.*, 2015). Because of the diversity of aquatic environment, differences in amount of deposited eggs are observed not only in individual species but also in populations from various water bodies. This study is focused on aspects of the reproductive biology of *H. longifilis* in River Okura with emphasis on its sex ratio; fecundity and breeding cycle. The breeding season of the species was monitored from the gonadosomatic index and frequency of occurrence of matured gonad stage. Hence the reproductive studies of *H. longifilis* in River Okura will form a baseline for comparative studies of the species in many different water bodies. The information will also give an idea on the variation of the level of population, reproductive power of the species, its period of increased weight, its recruitment

pattern etc in the river. All these factors are important in the assessment, management and preservation of this species in River Okura.

MATERIALS AND METHODS

Okura River is located in Kogi State, north central Nigeria and lies between latitudes 7°00'N and 7°30'N and longitudes 7°00'E and 7°30'E (Figure 1). It stretches over a distance of about 81.5km. It takes its source from water issuing out from the ground in Igboobe and Olla in Omala local government area of Kogi State and empties into the Anambra River. The sampling location was Ofejiji (N:07°24.55', E:007°19.39') where fishery activity is dominated by occasional fishermen.

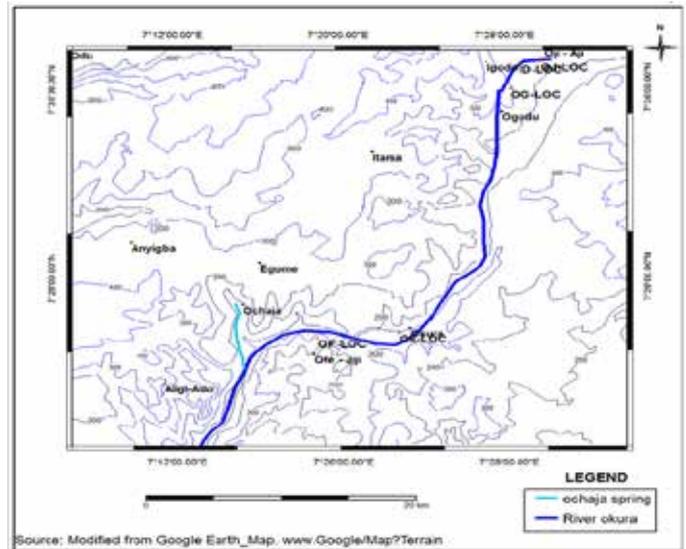


Figure 1: Map of River Okura in Kogi state, Nigeria

Identification and determination of gonad stages

The gonads maturation stages were examined macroscopically and classified into seven (7) stages based on their coloration, appearance, size and length relative to the body standard lengths (Kesteven, 1960 and Mackie and Lewis, 2001).

The sex ratio was determined using quantity of frequency. The sex ratio of male to female in the population is given as the frequency of the male to the frequency of the female. Chi-square test was used to test if the sex ratio is significantly different from 1:1 at 5% level of significance.

The reproductive cycle was monitored from the gonadosomatic index and the frequency of occurrence of matured gonad stage VI. The gonadosomatic index (GSI) was calculated for each specimen as the percentage of gonad weight to that of the fish body weight (Khallaf and Authman, 2010) using the following equation:

$$GSI = W_1/W_2 \times 100,$$

Where W_1 = gonad weight ((ovary or testes in grams) and W_2 = fish weight (grams).

The male and female gonads were examined frequently (monthly) throughout the year for their coloration, size (gonad length and weight relative to their body size). Spawning period is the period when the matured gonads (ovary and testis of stage VI) are being released into the water.

The fully matured ovaries (fecund) were released into the beaker, Gilson's fluid was added. The ovaries were shaken periodically while in the Gilson's fluid to help loosen the ovarian tissue and to ensure rapid penetration of the preservative. After at least 48 hours in preservative the eggs can be completely liberated from the tissues by vigorous shaking (Davenport, 1960).

Fecundity was estimated using the total number of oocytes contained in the ovary of the fish. Absolute fecundity (F) was calculated here as the average number of mature eggs per ovary of fish prior to the spawning season, as indicated by many authors (Duponchelle *et al.*, 2000).

Fecundity was determined by direct count using numerical method. The dried eggs were weighed using the electronic weighing balance. A small quantity of the eggs were sampled out (subsample), weighed and the eggs were counted. This was done about 3 to 4 times. The number of eggs relative to the weight of each of the sub sampling was calculated by direct proportion. This was applied to the whole sample (Khallaf *et al.*, 2003).

RESULTS AND DISCUSSION

Morphological description of gonad stages of *Heterobranchus longifilis*

Seven gonad stages were identified namely immature or juvenile or stage I (Plate 1), early maturation (or stage II) (Plate 2), mid maturation (or stage III) (Plate 3), final maturation (or stage IV) (Plate 4), developed / pre spawners (or stage V) (Plate 5), active spawners (or stage VI) (Plate 6), and Spent /resting stage (or stage VII) (Plate 7). Table 1 gave the description of the various gonad stages of *Heterobranchus longifilis*.

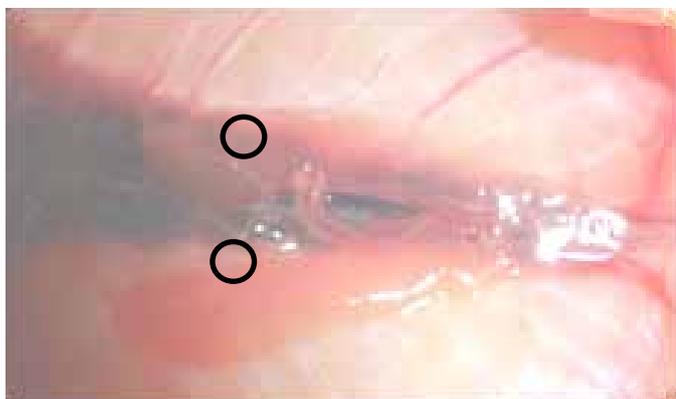


Plate I X1

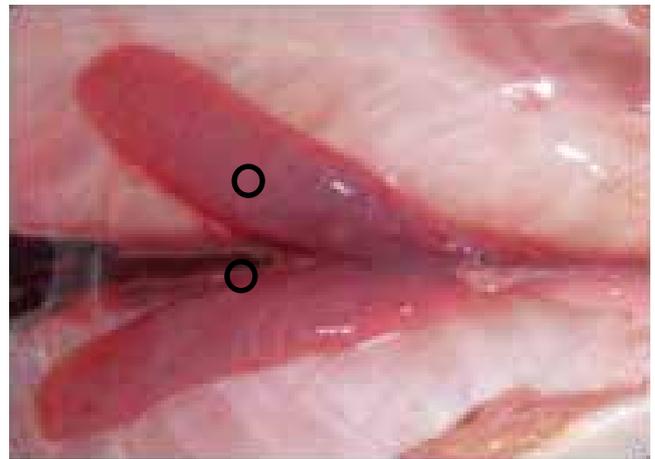


Plate II X 1.25

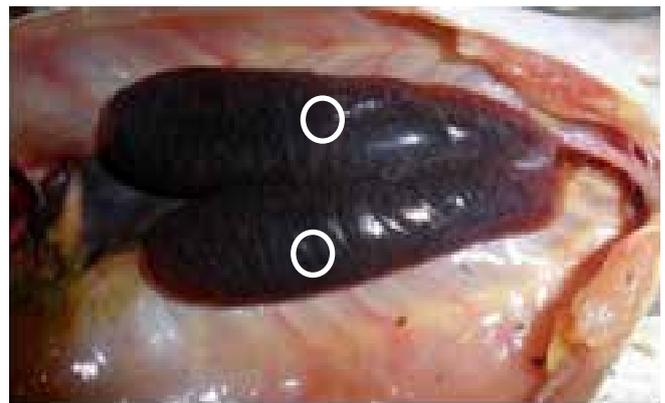


Plate III X 2



Plate IV X1.25

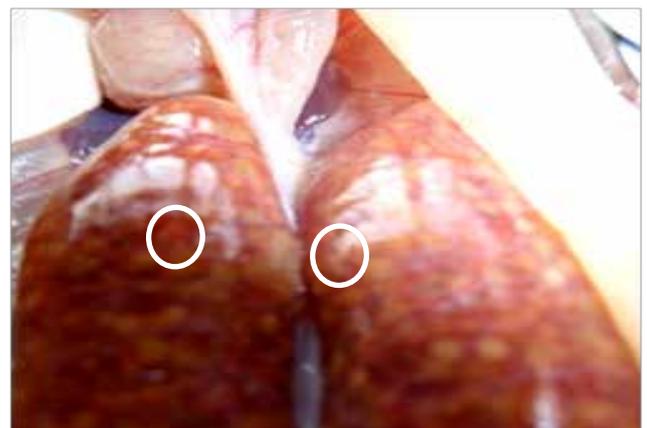


Plate V X 1.5



Plate VI X1.25

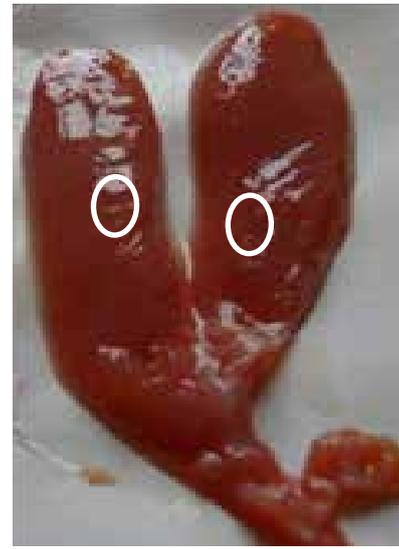


Plate VII X1.

O - Ovary

Table 1: Classification and morphology of gonad stages of *Heterobranchus longifilis* from River Okura

Stage	Degree of maturation	Morphological Description
I	Immature/ Juvenile	The ovary is about 1-1.25mm wide, occupying around 1/2 - 1/3 of the length of the body cavity. It appears as thin, flat paired organs; and translucent, distinguishable from testes by a thicker appearance. It is light soft pink in color.
II	Early maturation.	The ovaries occupy 1/2 to 2/3 of the length of the body cavity; 3-5 mm wide, slightly narrower at posterior end. The color varies from rose in first spawners to dusty deep rose color in repeat spawners and transparent with a cylindrical shape; no single oocytes can be detected with the naked eye. Testes become enlarge, flat, increase in weight and volume, and creamy white in color.
III	Mid Maturation	The ovaries occupy between 2/3 and the entire length of the body cavity; 5-12 mm wide and narrower towards the posterior end; In repeat spawners the color is transparent tropical pink and it varies from early stage translucent to late stage opaque deep regal red in first spawners. The oocytes are easily detectable with naked eyes and appear measly in the ovary bag. What distinguish between first time and repeat spawners are a more reddish color and often a thickened wall. Testes enlarge, increase in weight and volume, light pinkish and thicker in size and look more vascular with blood capillaries.
IV	Final maturation	The ovaries occupy the entire length of the body cavity. The color is deep faded purple in first spawners and blood red in repeat spawners. The sizes of the ovaries are bigger with narrow posterior end. The eggs are visible within the ovary. The texture of the ovary is very crispy. The testes are highly vascularised with indentations at the longitudinal margin.
V	Deve-oped / pre-spaw ners	The ovary occupies the entire length of the body cavity; the lobes are much more enlarged The lobes are much enlarged with the ovaries clearly visible with the naked eyes. The gonad of the first spawner is walnut in color while the repeat spawner is reddish murky green in color. No eggs are released with slight pressure to the abdomen. Testes become pinkish red and increased in weight and volume. Prominent blood capillaries run from the anterior to the posterior end of the testes. The testes are marked with very conspicuous indentations A gentle pressure on the testes runs the milt.
VI	Active spawners	The ovaries occupy the full length of body cavity and greatly enlarged. The ovaries are murky red color in repeat spawners. The eggs are enlarged and clearly visible to the naked eye, a touch with gentle pressure will release eggs. The sacs of ovaries start reducing in volume as eggs are being released. Milt is released from the testes causing the testes to become flabby and thin. It is dull white in color.
VII	Spent /resting stage	The ovaries have released their eggs and they have become flaccid and shrunken with prominent main artery. As the ovary wall contracts the transverse grooves appear again and the ovary becomes more firm. The color of the ovary varies from deep ruby red in first spawners to brownish red in repeat spawners. Gonad shrunken having loose walls. Ovaries are flaccid, shrunken and sac like.. Ovary contains ripped unspawned darkened eggs and a large number of small ova. Testes become flabby, thin and dull white in color.

Sex ratio

654 individuals were examined, 346 were male while 308 were female. The sex ratio of male to female was 1.12: 1. The chi-square test of significance shows that there is no significant difference in the female: male ratio ($p > 0.05$) from the expected 1:1 ratio (Table 2).

Table 2- Sex ratio of fish species of *Heterobranchus longifilis* in River Okura

Sex	Observed (O)	Expected (E)	Residual O-E	(O-E) ²	(O-E) ² /E Component
Male	346	327	19	361	1.104
Female	308	327	-19	361	1.104
Total	654	654			2.208

The chi-square test analysis showed that there is no significant difference in the sex ratio of male and female sexes (df = 1, $\chi^2_{0.05,1} = 3.841$, calculated χ^2 value = 2.28).

Breeding Season

It was monitored from the period of high gonadosomatic index and the maturation stages of gonads.

a. Mean gonadosomatic index of *Heterobranchus longifilis*

The mean gonadosomatic index (GSI) ranged from 0.13 to 1.10 with mean value of 0.47 ± 0.04 in males. In females it ranged from 0.29 to 2.80 with mean value of 1.21 ± 0.26 . Two peaks of GSI were observed throughout the year for both sexes; in October and in February. In February mean monthly GSI were 0.4 ± 0.05 and 1.2 ± 0.1 for male and female species respectively; and another peak was in October with mean monthly GSI of 1.05 ± 0.1 for male 2.80 ± 0.2 for female (Figure 2).

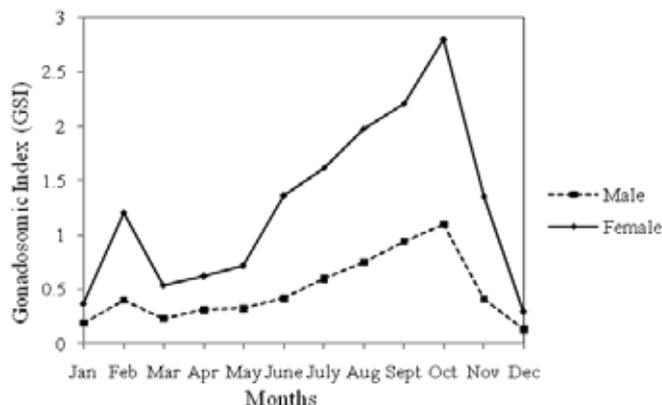


Figure 2: Mean monthly gonadosomatic index of *Heterobranchus longifilis* in River Okura

Table 3: Percentage frequency of gonad stages of *Heterobranchus longifilis* in 2011/2012 in River Okura

Percentage frequency of gonad stages																
	I		II		III		IV		V		VI		VII		Total	
Month	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M
Sept	0	0	0	0	5	4.2	5	33.3	80	54.2	10	8.3	0	0	20	24
Oct	0	0	9.7	0	6.5	7.1	0	0	3.2	3.6	74.2	85.7	6.5	3.6	31	28
Nov	7.7	1.8	46.2	35.3	0	11.8	0	0	23.1	0	23.1	29	7.7	11.8	13	17
Dec	8.3	15.4	41.7	30.8	41.7	23.1	0	0	0	0	0	0	8.3	7.7	12	13
Jan	12.5	17.4	31.3	30.4	6.3	21.7	12.5	0	37.5	30.4	0	0	0	0	16	23
Feb	10	4.4	26.7	17.8	30	44.4	13.3	13.3	3.3	4.4	16.7	15.6	0	0	30	45
Mar	9.5	5.7	14.3	22.9	23.8	42.9	47.6	28.6	4.8	0	0	0	0	0	42	35

Apr	4.2	6.5	8.3	6.5	16.7	22.6	33.3	41.9	37.5	22.6	0	0	0	0	24	31
May	4.8	5	21.4	7.5	35.7	37.5	31	45	7.1	5	0	0	0	0	42	40
June	0	0	9.1	0	22.7	7.1	59.1	39.3	9.1	53.6	0	0	0	0	22	28
July	0	0	3.7	0	7.4	6.1	37	36.4	51.9	48.5	12.5	9.1	0	0	27	33
Aug	0	0	9.4	0	9.4	6.3	12.5	31.3	68.8	59.4	6.3	3.1	0	0	32	32

Spawning period is the period when the matured gonads (ovary and testis of stage VI) are being released into the water i.e. the period there were high frequencies of gonad stage VI. There was prolonged spawning from July to October in the species, it was highest in October. In February 16.7% and 15.6% frequency of gonad stage VI were observed for male and female respectively.

Fecundity of *Heterobranchus longifilis* in River Okura

There were 28 fecund females used for estimation of fecundity. Fecundity ranged from 17,001 (body weight = 625g, SL = 38.6cm) to 97,122 (body weight = 5158g, SL = 71.5cm) with mean value of 37744 ± 21648 eggs (Table 4).

Table 4: Fecundity of actively spawning *Heterobranchus longifilis*

Sample size	Wt(g)	SL(cm)	Gonad wt(g)	GSI	Fecundity
	28	28	28	28	28
Minimum	472	35.7	14.4	1.7	17001
Maximum	5158	71.5	145.1	3.8	97122
Mean	1338.5	43.6	34.5	2.6	37743.7
SD	1054.6	9.5	29.4	0.6	21648.1

Gonads of *H. longifilis* were classified into seven stages. The correct classification of gonads of *H. longifilis* into developmental stages is necessary to determine maturity status of the species in River Okura, a prerequisite for setting annual catch quotas using a harvest rate strategy based on spawning biomass estimates. Correct evaluation of macroscopic gonad maturity is a rapid and inexpensive manner of determining the reproductive status of fish, and it allows for many fish to be assessed in the field (Tomkiewicz *et al.*, 2003). Different numbers of gonadal maturation stages ranging from four to nine have been reported in fish (Kesteven, 1960; Stahl and Kruse, 2008; Costa, 2009 Shinkafi *et al.* 2011 and Abobi *et al.* 2015). The description of the various gonad stages in this study is peculiar to *H. longifilis*. Irrespective of the number of stages gonad maturation has been grouped, it is important to know the distinction between females in active and inactive maturity stages.

Most fish in natural populations have a sex ratio of 1:1 but when one of the sexes has some particular advantage, a tendency towards the production of a greater number of this sex can occur. Preponderance of one sex over the other has been observed in populations of other fishes in some Nigerian water bodies (Ekanem, 2000, Taiwo and Aransiola, 2001 and Offem *et al.*, 2008). The dominance of male species over the female *H. longifilis* has been observed in other rivers and this was attributed to the relatively few spawning females susceptible to catch since after fertilization of eggs males emigrate from spawning grounds towards feeding grounds in shallow part (where they are easily captured) and females go towards submerged vegetation and rocky areas to avoid gear and carry out incubation and protection of offspring (Pena-Mendoza *et al.*, 2005; Offem *et al.*, 2008; Shalloof and Salama, 2008; Olele, 2010). The studied species showed no predominance of either sex over another during the sampled periods in the river. The sex ratio of 1.12: 1 male to female observed in River Okura is statistically similar (Table 2). The sex ratio of 1.12:1, male to female observed in River Okura showed that the population of the species in the river is an ideal one.

The spawning or breeding season corresponded to period when GSI was at its peak and when the gonad stage VI was frequent. Two peaks of GSI were observed throughout the year for both sexes; in October and in February. In October mean monthly GSI of female and male were 2.80 ± 0.2 and 1.05 ± 0.1 respectively; and in February mean monthly GSI were 0.4 ± 0.05 and 1.2 ± 0.1 for male and female species respectively. Frequency of occurrence of stage VI was highest in October with values of 74.2% for female and 85.7% for male. Spawning period is the period when the matured gonads (ovary and testis of stage VI) are being released into the water i.e. the period there was high frequencies of gonad stage VI; which was in

October. In February a few gonad stage VI were observed for the male and female species (16.7% and 15.6% frequency of occurrence for male and female respectively).

The life history of fish is determined by the magnitude of food available as well as the season. Spawning of most West African fishes appears to occur during

rainy season (Olaosebikan and Raji, 2004). October forms part of the late rainy season which signifies the major breeding month when GSI was at its peak for both sexes of *H. longifilis* in River Okura (2.80 ± 0.2 in female and 1.05 ± 0.1 in male). This period was also confirmed by high frequency of gonad stage VI (85.7% and 74.2% of occurrence for female and male respectively).

In February, GSI peak were observed for female 1.2 ± 0.1 and male (0.4 ± 0.05). The frequency of occurrence of gonad stage VI at this time was 15.6% for male and 16.7% for female. This signifies brief spawning period. This could possibly be due to the residual left over eggs in the gonads of the species after its breeding season in October.

Fecundity ranged from 17,001 (body weight = 625g, SL = 38.6cm) to 97,122 (body weight = 5158g, SL = 71.5cm) with mean value of 37744 ± 21648 eggs. These values are higher compared to the observation of fecundity of *H. longifilis* from other water bodies. Offem et al (2008) recorded fecundity of *H. longifilis* to be between 5515 and 36,800 eggs in inland wetland of Cross River, Nigeria. Inyang et al., (1997) recorded fecundity range of between 6001 to 51216 eggs per female (mean 24816 ± 14676) in Idodo river basin of southeast Nigeria. Variation in fecundity of individual's fish may be due to differential abundance of food. Nutritional resources are known to play critical roles in regulating variations in fecundity (Wooten, 1973 and Bagenal, 1978).

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