Aspects of the Reproductive Biology of *Clarias gariepinus* in Ogbese River

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Background: *Clarias gariepinus* is a freshwater fish that has a wide distribution from South and Central Africa to the Middle East. Gonadosomatic index value and gonadal histology indicates the maturity of fresh water fishes. One of the most important factors necessary in the successful culturing of a fish species is obtaining a basic understanding of its key biological processes which include reproduction. Objective: to study the reproductive aspects such as Sex ratio, Stages of gonad maturation, Gonadosomatic index, Fecundity and a detailed Histological analysis of both ovaries and testes of *C. gariepinus* in Ogbese River, which will serve as a biological basis necessary for proper management and sustainability of *C. gariepinus* in the river. Method: Fish samples were collected from fishermen in River Ogbese using gillnets which were set over-night and were transported to the laboratory with some quantity of water from the river. The samples were immediately examined; the total length, standard length, and total weight of each sample were taken; gonad were detached and weighed; sex and maturity stages were assigned to each sample. Sex ratio, GSI, stages of gonad maturation, fecundity, size at maturity, gonads histology was determined. Statistical analysis of the data obtained was done using correlation and regression. Result: 107 samples of *Clarias gariepinus* were caught between July and September 2014 with 53 females, 54 males. The sex ratio of the species was 1:1 (male: female) indicating no significant difference. The samples ranged in total length from 24.70 - 54.40cm and in total weight from 139.40 - 942.80g. The GSI was higher in females ranging from 0.3 – 12.9%, while male was from 0.1 – 6.2%. Fecundity ranges from 32,619 – 59,081 with mean of 49,294±10,665 for a fish mean weight of 59.62± 8.8g and mean standard length of 44.30±4.00cm. Four stages of gonad maturation were established for the females and males namely; immature (I), maturing (II), matured (III), ripe and running (IV). Based on macroscopy, eggs of various sizes and colors were found in each ovary, while histologically, various stages of oocytes and spermatocytes developed in each ovary and testis respectively. Spawning of the *C. gariepinus* occur during rainy season which is between July and August. Conclusion: The fecundity of both male and female *Clarias gariepinus* observed in this study is relatively high which means that the species can produce large numbers of seeds for pond stocking and thus be useful in artificial breeding.

**Key words:** *Clarias gariepinus*, fecundity, gonadosomatic index (GSI), gonads, histology, sex ratio.

INTRODUCTION

A cheap source of protein is needed to augment for the increasing demand for a balanced diet for the ever increasing population in West Africa Nigeria. *Clarias gariepinus* commonly known as African catfish is a common fish which has a high demand for human consumption; thus knowledge on the fecundity and breeding of this fish species can promote the commercial production of the resource. *Clarias gariepinus* is a predominant fish species in...
water bodies and also of great economic value in terms of food consumption [12]. Previous studies on the reproductive characteristic of fish species include Elaps lacerta [16], Heterobranchus longifilis, Malapterurus electricus, Malapterurus microstoma, Clarias gariepinus, Clarias nigrodigitus, Hepsetus odoe [10]. Emam and Abughrien, [8] studied the effect of different seasons on the histological and histochemical structures of the gonads of mature catfishes (Clarias lazera) of both sexes. The histological results showed that both testes and ovaries of the catfish were degenerated during winter that was considered as resting season of the catfish gonadal activities. Both testis and ovaries began to restore their intact and fully matured structure during spring and continue the same during summer where the testis showed distended seminiferous lobules with all the spermatogenic cells and spermatozoa. Also, the ovaries showed the different developmental stages including matured follicles, therefore, both spring and summer were considered as spawning season of the catfish.

During autumn, both testis and ovaries appeared as spent gonads where the testes showed many empty seminiferous lobules and the ovaries showed many atretic follicles, therefore autumn was considered as post spawning or spent season. The results of the gonadosomatic index (GSI) were coincided with the histological structure of the gonads where they show peak value during spring and summer (spawning season) and showed the lowest value during winter (resting season) Emam and Abughrien [8].

Spawning usually takes place at night in the shallow in-undated areas of the rivers, lakes and streams. Courtship is preceded by highly aggressive encounters between males. Courtship and mating takes place in shallow water between isolated pairs of males and females. The mating postures, a form of ampexus (the male lies in a U shape curved around the head of the female) is held for several seconds. A batch of milt and egg is released followed by a vigorous swish of the female’s tail to distribute the eggs over a wide area. The pair usually rest after mating (from seconds up to several minutes) and then resume mating.

The oogenesis is a very dynamic process in the ovaries, in which the oocyte passes through various phases of the development that are very similar in different fish species. The ovaries of the fishes have been classified into three types according to the pattern of the oocyte development. In the case of synchronous oogenesis, all the oocytes develop at the same time, ovulation also being simultaneous. The synchronous ovary consists of at least two populations of the oocytes at different developmental stages; Teleosts with this type of ovaries generally spawn once a year and have a relatively short breeding season. In the case of synchronous ovulation, different developmental stages of the oocytes maturation and ovulation in groups may be found within the ovaries.

The testes of African catfish are situated in the dorsal part of the abdominal cavity. They are lobular and appear whitish in color; they are covered by the intestine in such a way that application of pressure cannot easily release milt (semen). Several techniques have been developed by several authors, Nguenga et al., Viveiros et al., Hiemstra et. al., and Pavlov, [13] to preserve fish semen so as to preserve the genetic quality of valid fish.

In the African catfish, male reproductive system can be subdivided into four histological stages that are characterized by the presence of the following germ cell type:

Stage I:Spermatagonia only,
Stage II: Spermatogonia and Spermatocytes,
Stage III: Spermatogonia, Spermatocytes, and Spermatids,
Stage IV: All germ cells including spermatozoa (Melo and Godinho, 2006).

Macroscopic descriptions of gonads maturation stages in C. gariepinus revealed different size and color of eggs in each ovaries, whether in mature or ripe and running stages. Histological description further substantiated multiple stages of the oocytes development in each of the six stages of maturation of the ovaries. Thus, mature stage; secondary vitellogenic oocytes are more abundant, while fewer oogonia and primary oocytes were present. In ripe and running stages, post vitellogenic oocytes which are the characteristics of that stages suggesting imminent spawning were more abundant (even though the earlier mentioned stages were also present). The reproductive biology of a fish might be defined by it reproductive trait and it also expresses the combination of the species-specific reproductive mode.

Vitellogenic stage of oocyte as well as increased gonad weight, GSI value and gonadal histology indicates the maturity of fresh water fishes. The study of gonad stages of maturation as become increasingly important in fish production, notably in induced spawning and hybridization studies. Knowledge of the gonad maturation of the fishes is also required for many purposes and this include determination of stocks that are mature and the size or age at first maturity [6], determination of reproductive potential of fish populations and monitoring of changes in biological characteristics of exploited fish stocks, establishing the reproduction period and length of gonadal maturation to allow for accurate implementation of fishery legislation.

To date, many studies have been carried out to develop reproduction and culture techniques for C. gariepinus [9,7]. Cyclic changes in the gonad (ovaries and testis) have also been examined in a few closely related species, including the African catfish, Clarias lazera, natal mountain catfish, Amphilius natalensis, Japanese catfish, Silurus asotus and fresh water catfish, Mystus montanus [5].

One of the most important factors necessary in the successful culturing of a fish species is obtaining a basic understanding of it key biological processes. The most important of these biological processes is the
reproductive cycle and formation of gametes. However, this study is on the reproductive characteristics of *C. gariepinus* in Ogbese River, Ado Ekiti, Ekiti State, Nigeria.

The objective of this study is to study some of the reproductive aspects such as Sex ratio, Stages of gonad maturation, Gonadosomatic index, Fecundity and a detailed Histological analysis of both ovaries and testes of *C. gariepinus* in Ogbese River, which will serve as a biological basis necessary for proper management of the resource in the river for maximum sustainability.

**MATERIALS AND METHOD**

*The study area:*

River Ogbese lies between longitude 5° 26′ and 6° 34′ and latitude 6° 43′E and 7° 17′E. The river runs through a town which is about 5 kilometers from Akure, in Akure North Local Government Area of Ondo State, Nigeria. River Ogbese is one of the major perennial rivers in the south western Nigeria; it took it source from Awo Ekiti in Ekiti State. It flows for approximately 22km from it source to meet River Ose which is 260km long and discharges into the Atlantic Ocean through an intricate series of creeks and lagoons. It vegetation is ever green rain forest type; this type of vegetation favors agricultural practices.

*Collection of fish samples:*

The specimens were examined fresh in the laboratory immediately after collection. On each sample, measurement of total length and standard length (cm) and total weight (g) were taken. Gonads were detached and weighed; sex and maturity stages were assigned to each sample.

*Sex ratio:*

The sex ratio is seen as the proportion of the number of males to that of the female. Sex of each specimen collected was determined by examination of the gonads after dissection and the ratio of male to female calculated, using chi-square ($X^2$) i.e. $X^2 = \frac{E(O - E)^2}{E}$, where $O$= Observed $E$= Expected

*Gonadosomatic index (GSI):*

The relationship between the weight of gonad and body weight of the fish was calculated as follows, After Howand (2002).

$$G.S.I = \frac{Gonad weight}{Fish weight} \times 100$$

The linearity of the gonadosomatic index-weight relationship is determined using the equation;

$$\log Y = a + b \log X$$

Where;

- $Y = $Gonadosomatic index
- $X = $Weight of fish (g)
- $a$ and $b$ are regression constants

*Stages of gonad maturation:*

Fishes were dissected ventrally from the anus to the bare of the operculum to reveal the gonad. Gonad maturity stages were assessed and classified according to a modified classification of Hilge as follows;

- Stage 1 – Immature
- Stage 2 – Developing
- Stage 3 – Mature
- Stage 4 – Ripe and Running
- Stage 5 – Spent

*Fecundity:*

Fecundity was determined by sub sampling using gravimetric method, reviewed by Lagler, Kesteven; Ricker, and Bagenal and Braum, [6]. It was calculated as;

$$N = \frac{n \times w \times W}{Where;}$$

- $N = $Fecundity
- $n = $Average number of eggs in sub samples
- $W = $Weight of ovaries
- $w = $Average weight of ovaries in sub samples
Size at maturity:
The size at maturity is taken as the minimum length at which 50% of the male and female were found with matured gonads.

Macroscopic determination of gonad maturity stages:
Based on macroscopic characteristics, certain features were examined to identify the maturity stages. These are the degree of opacity of the gonads, consistency and vascularization, oocytes or sperm visibility and overall colorations of the gonads.

Histological study of the gonads:
This was conducted following the procedures of Morrison, (1990). Each gonad was fixed in 10% formalin immediately after removal from the fish. The tissue samples were passed through graded concentration of alcohol of 70% in 100ml, after an hour it was passed through another 90% of alcohol, and lastly after another one hour it was passed through 100% alcohol. The reason for soaking the sample in alcohol is for the sample to be fixed and to achieve dehydration. After dehydration, staining of slide was carried out with Safranin O Stain. All the slides were examined under the light microscope. Photomicrographs of the different maturity stages were taken.

Statistical analysis:
All data obtained were expressed as means with standard errors. The data were subjected to the regression and correlation analysis to examine the relationship between and within the data using SAS (Statistical Analysis Software)

Result:
A sample of C. gariepinus is shown in plate 1

Sex ratio:
Out of 107 specimens of Clarias gariepinus collected, 53 were female and 54 were males giving a sex ratio of 1.1:1.0 (males: females).

Stages of gonad maturation:
The maturity stages for the ovaries and testes of Clarias gariepinus were classified as immature/virgin, developing, mature, ripe and running.
The description of the female gonadal stages was as follows:
Stage 1 – ovaries were very small, paired with smooth edges and ovaries connected throughout their length by connective tissue; they were greenish in color.
Stage 2 – ovaries were bigger and oval in shape and slightly of unequal size. They were covered with prominent blood vessels and very few tiny eggs discernable with naked eyes.
Stage 3 – paired ovaries free, smooth and transparent orange yellow translucent eggs were clearly visible, the ovaries occupy almost 50% of abdominal cavity. Eggs did not extrude with pressure on the abdomen.
Stage 4 – ovaries were swollen and occupied 70% of the abdominal cavity, egg was translucent. Eggs were extruded with slight pressure on the abdomen.
The description of male gonadal stages were as follows:
Stages 1- the testes appeared as pair of white filaments, testes were very small and not easy to identify, they were fusing appearing like a single structure.
Stage 2 – the gonads were white and increased in size and could be seen with eye and had fingers like outgrowth giving it a labeled appearance.
Stage 3 – finger like outgrowth are more pronounce and swollen and the lobes becoming prominent. Testes translucent are white in color with rough edges.
Stage 4 – the ripe testes were fully swollen multi lobed. The color was creamy white/milky in color. With pressure to the abdomen milt flows out.
Mature and developing gonads of both male and female are found in the sample from July to September.

Histology of ovaries:
Stage 1: immature: histological section of stage 1 ovaries shows dominance of oogonia and primary oocytes (Plate 3).Oogonia were seen as small spherical cells or in group. They were observed in all maturity stages. They were easily identified by the presence of single large nucleolus in the nucleus.
Stage 2: Developing: primary vitellogenic oocytes and primary oocytes were found in the histological sections(Plate 4).The primary oocyte is bigger than the oogonium and it is characterized by a large nucleus with many nucleoli around the periphery. They were present in all maturation stages. The follicular cells organized
themselves around the developing oocytes which develop a large cytoplasm. Some are ovoid in shape while some are polygonal.

Stage 3: Matured: there were appearances of secondary vitellogenic oocytes forming 10 – 15% of entire cells (Plate 5). Primary vitellogenic oocytes and primary oocytes were also present. Primary vitellogenic oocytes were comprised of irregular shaped cells with vasculated double layered cytoplasm. This stage marks the beginning of stage development. There is formation of Yolk globules in the cytoplasm of the secondary vitellogenic oocytes. Yolk globules were present throughout the cytoplasm at the end of this stage. Cells were enveloped by two layers, squamous granulose and cellular theca.

Stage 4: Ripe and running: most of the oocytes were tertiary/mature oocytes and constituted 70 – 75% of entire cells (Plate 6). The interiors were filled with prominent yolk globules and droplets. The two layers enveloping the cells were less defined.

Histology of testes:

Immature (stage I):
Sections show irregular shaped spermatogonia, which aggregated in groups (Plate 7). The spermatogonia were characterized by lightly stained cytoplasm and a large nucleus.

Developing (stage II):
Spherical primary spermatocytes predominated (Plate 8). The cells membranes were more defined. The secondary spermatocytes became cusp-shaped and were attached to the lobular wall.

Mature (stage III):
Sections showed sickle shape spermatids, which were detached from the lobular wall. Spermatocytes at different stages of development filled the testis at this stage. The testis was filled with spermatids (Plate 9).

Ripe running (stage IV):
Empty spaces appeared in the lumen containing loose spermatocytes and spermatozoa (Plate 10).
The monthly occurrence of the gonads maturity stages of *Clarias gariepinus* for female and male is illustrated on table 1 (a) and (b) respectively.

Size at maturity:
The size at maturity for the female *C. gariepinus* was 39.2cm and 35.5cm for males. The minimum length at which 50% of female and males have matured gonads in the river was 41.2±2.0cm and 38.3±2.2cm (Standard length) respectively.

Fecundity:
Fecundity range from 32,619 - 59,081 with mean of 49,294 ±10,665 eggs for a fish of 47.40g-70.30g (mean=59.62g±8.8g) weight at standard length of 40.00-51.00 (mean=44.30±4.00).

Gonadosomatic index:
Gonadosomatic index (GSI) was higher in Females 0.30-12.90 with mean value of 4.02±6.28 than in males 0.10-6.2 with mean value of 0.70±0.17. Logarithmic transformation of gonadosomatic index-weight relationship of male, female and both sexes are illustrated in figures 1, 2, 3 respectively. These can be represented by the following regression equation

Male: $Y = 0.69 + 0.0003x$ ($r = 0.0014$)
Female: $Y = 3.96 + (-0.0002)x$
$Y = 3.96 - 0.0002x(r= 0.0003)$
Both sexes: $Y = 1.05 + 0.0003x(r = 0.03)$
Correlation coefficient values of male ($r = 0.0014$) is significant.
Plate. 1: *Clarias gariepinus*

Plate. 2: gonads of *C. gariepinus*

A = ripe ovary of *Clarias gariepinus*
B = Ripe testes of *Clarias gariepinus*
RO = Ruptured ovary  
LT = Lobe of testes
Plate. 3: L.S. of stage 1 (Immature) ovary of *Clarias gariepinus* showing oogonia (OO) and primary oocytes (PO).

Plate. 4: L.S. of stage 2 (maturing) ovary of *Clarias gariepinus* showing oogonia (OO), primary oocytes (PO) and primary vitellogenic oocytes (PVO)
Plate. 5: L.S. of stage 3 (matured) ovary of *Clarias gariepinus* showing oogonia (OO), primary oocytes (PO), primary vitellogenic oocytes (PVO) and secondary vitellogenic oocytes (SVO)

Plate. 6: L.S. of stage 4 (Ripe and Running) ovary of *Clarias gariepinus* showing oogonia (OO), primary oocytes (PO), primary vitellogenic oocytes (PVO), secondary vitellogenic oocytes (SVO) and tertiary vitellogenic oocytes (TVO) with yolk granules (YG).
Plate 7: L.S. of stage 1 (immature) testes of *Clarias gariepinus* showing spermatogonia (SP) and Large Nucleus (LN).

Plate 8: L.S. of stage 2 (immature and developing) testes of *Clarias gariepinus* showing spermatogonia (SP) and primary spermatocyte (PS), Cell Membrane (CM)
Plate. 9: L.S. of stage 3 (ripening) testes of *Clarias gariepinus* showing secondary spermatocyte (SS) and spermatid (ST)

Plate. 10: L.S. of stage 4 (ripe and running) testes of *Clarias gariepinus* showing spermatid (ST) and spermatozoa (SZ) and Empty space (ES).
Fig. 1: Gonadosomatic index-weight relationship of male *Clarias gariepinus* in Ogbese River.

\[ Y = 0.6975 + 0.0003X, \quad r=0.0014 \]

Fig. 2: Gonadosomatic index-weight relationship of female *Clarias gariepinus* in Ogbese River.

\[ Y = 3.9626 - 0.0002X, \quad r=0.0003 \]
Fig. 3: Gonadosomatic index – weight relationship of combined sexes of *Clarias gariepinus* in Ogbese River.

Table 1a: Monthly occurrence (by number) of the gonad maturity stages of *Clarias gariepinus* Gonad maturation stages of female *Clarias gariepinus*

<table>
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<th>Month/Year</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>Total</th>
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<td>5</td>
<td>11</td>
<td>2</td>
<td>-</td>
<td>19</td>
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<tr>
<td>August 2014</td>
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<td>7</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>16</td>
</tr>
<tr>
<td>September 2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>11</td>
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Total No (N) = 50

Table 1b: Gonad maturation stages of male *Clarias gariepinus*

<table>
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<th>III</th>
<th>IV</th>
<th>V</th>
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<td>7</td>
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<tr>
<td>August</td>
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<td>8</td>
<td>7</td>
<td>-</td>
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<td>22</td>
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<tr>
<td>September</td>
<td>1</td>
<td>7</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>11</td>
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</table>

Total No (N) = 54

Discussion:

The sex ratio observed in this study did not differ significantly from the expected 1:1 ratio similar to the result of Shinkafi and Ipinloju [14] but at variance with the study of Offem et al. [12] and Taiwo and Aransiola [15]. According to Nikolsky, a 1:1 sex ratio represents lack of difference in the longevity of the sexes, and it also indicates that almost the same number of male and female were caught in Ogbese River, and as such no disparity in the survival of both sexes to the environmental condition of the river. The knowledge of sex ratio can enhance fishery management and allow the movement of sexes in relation to season is known.

The maturity stages observed in both sexes of *C. gariepinus* were mostly at stage 2 and 3 which indicates intensive breeding activity coincident with the rainy seasons (Table 1a and 1b) [4]. The fecundity recorded in *C. gariepinus* in this study is quite lower when compared with the result of Admassu et. al. [4] study of the same species in Lake Babogaya, this could be attributed to differences in body condition and growth between the populations [4]. Abayomi and Arawomo, [2] reported fecundity of 15,667 – 650,626 for *C. gariepinus*. The authors further reported that this could be attributed to the spawning behavior of fish to lay more eggs to account for the losses to predators and adverse external factors.

The histological analyses permitted precise description of the process of development and maturation. According to Elourduy and Ramírez, visual evaluation of maturity of the gonad by microscopic characteristics
and the use of gonadal indices are gross indicators of reproductive activities and also to detect subtle differences between the gonads.

All the five maturation stages occurred every month throughout the study period except the spent stage (stages V), ripe and running stage (stage IV) which was absent only in September. In July and August, the increase in female with gonad maturation stage IV shows the onset of rain, so breeding may occur during raining season. Increase in GSI of females observed in July and August may also indicate the onset of breeding period as it coincided with rainy season [17].

The minimum length at which 50% of female and males have matured gonad in the river was 41.2±2.0cm and 38.3±2.2cm (Standard length) respectively. This therefore shows that maturity occurs in both males and females of Clarias gariepinus in Ogbese River at relatively young age.

**Conclusion:**

The maturity stages of both female and male species of Clarias gariepinus observed in the study showed majority to be at the developing and mature stages indicating that there is intensive breeding activity of Clarias gariepinus in River Ogbese during the rainy season. The fecundity of the species observed in this study is relatively high which means that the species can produce large numbers of seeds for pond stocking and thus be useful in artificial breeding. The result of this study can be applicable in measurement of breeding activities of Clarias gariepinus and other clariid families in River Ogbese and a comparative study on other rivers.

**Recommendation:**

Further research is recommended on the reproductive biology of Clarias gariepinus in Ogbese River particularly in other months of the year that were not covered in the present research. This will enhance all round seasonal knowledge of the reproductive biology of the species in the river for its proper management and sustainability.

**Application of the study to aquaculture:**

Economically productive aquaculture, like Agriculture is heavily dependent upon an adequate supply of seed of fertile eggs and juvenile fish with which to stock the culture enclosures. Artificial propagation constitutes the practicable means of providing enough quality seed for possible introduction of important seed at widely separated geographical areas. Mayer believed that knowledge of the fecundity of fish is a great benefit to fishery scientist in assessing the size of fish stocks. The relatively high fecundity of Clarias gariepinus observed from this study when compared with other lowly fecund species such as Hepsetus odoe could mean that the species can produce large numbers of seeds for pond stocking useful in artificial breeding.

**Contribution to knowledge:**

The knowledge of the fecundity and gonadosomatic index of this species helps in the assessment of the breeding activity of Clarias gariepinus which can be applied in the commercial production of the fish species in captivity.

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**REFERENCES**