

MATURATION AND HISTOLOGY OF THE GONADS OF THREE SELECTED FISH SPECIES OF LOWER RIVER BENUE, BENUE STATE, NIGERIA

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ABSTRACT

The macroscopic and microscopic maturation and histology of the gonads of the three fish species; comprising 189 *Hydrocynus forskahlii* from Abinsi and 214 from Wadata station, 372 *Citharinus citharus* from Abinsi and 366 from Wadata, 384 and 363 *Mormyrus rume* from Abinsi and Wadata, respectively, were caught using gill and cast nets. The specimens were collected fortnightly from fishermen of Lower River Benue, between April 2019 and March 2020. The process of oogenesis and spermatogenesis were studied by macroscopic and microscopic observations, and also utilising histological preparations of ovaries and testes belonging to different gonad maturation stages. The data obtained from this study were subjected to descriptive statistics. The macroscopic and microscopic observations of the fishes gonads corresponded with the method described by Brown-Peterson et al. (2011). There were presences of oocytes at different phases of development indicating the fishes to have prolonged and fractional spawning season, spawning more than once during the spawning season. The histological features corresponding with reproductive cycles and the spawning periods, and the spawning capable phase occurred mostly between May-August. The brooders may be captured during their spawning periods and stocked for aquaculture, or the eggs collected and incubated in the laboratory.

Keywords: Macroscopic, Microscopic, Oogenesis, Spermatogenesis, Histology.

1. INTRODUCTION

To understand the physiology of reproduction, the study of the seasonal developmental changes of gonads through both macroscopic and microscopic observations is necessary. To proceed with the artificial means of reproduction and to produce good quality eggs, it is necessary to have basic information on reproductive biology of fish species (Priyadharsini *et al.*, 2013).

Reproduction involves changes in growth and development of oocytes during the process of gonad maturation and with the advancement of maturation; oocytes accumulate energy reserves and enlarge further for the onset of embryogenesis (Priyadhasini *et al.*, 2013). Classification of ovarian development has been based on both macroscopic (e.g., external appearance of the ovary or gonadosomatic index) and microscopic (e.g., whole-oocyte size and appearance or histology) criteria, and each of these methods has its own type of classification scheme (Murua *et al.*, 2003). Classification terminology for testicular development is equally diverse and inconsistently used (Brown-Peterson *et al.*, 2002).

Among all fishes, reproductive cycles exhibit similar phases of gonadal development including immature, developing, spawning capable, regressing, and regenerating (Brown-Peterson *et al.*, 2011). In addition, the ability of individuals to skip spawning (i.e., to opt out of a reproductive cycle) is increasingly recognized as part of many species' reproductive strategies (Rideout and

Tomkiewicz, 2011). In all fishes, eggs begin as oogonia and go through similar stages of oocyte development (McMillan, 2007; Mommsen and Korsgaard, 2008), which is commonly divided into primary growth and secondary growth (Lowerre-Barbieri *et al.*, 2011b). Secondary growth is typically categorized into the following developmental stages: cortical alveolar stage, vitellogenesis, and oocyte maturation (OM) (Luckenbach *et al.* 2008). Although not all fishes develop cortical alveolar vesicles at this stage (Grier *et al.*, 2009). The function of cortical alveolar in secondary oocyte growth, however, is to make the later stage of vitellogenesis possible (Lowerre-Barbieri *et al.*, 2011b) and cortical alveolar (CA) oocytes is secondary growth oocytes since their formation is gonadotropin dependent (Luckenbach *et al.* 2008; Lubzens *et al.*, 2010). Though, CA oocytes are not vitellogenic and have been considered primary growth oocytes by some (Grier *et al.*, 2009). Fish are not spawning capable until at least some oocytes have completed vitellogenesis (Brown-Peterson *et al.*, 2011), which is the process by which yolk proteins are produced in the liver, transported to the ovary, and stored in the egg to later provide nutrition for the offspring (Senthilkumaran *et al.*, 2004). The presence of vitellogenic oocytes is also commonly used to identify mature females, and this is the criterion for many studies based on macroscopic staging (Kjesbu *et al.*, 2003). The thickness of the ovarian wall is expected to be greater in mature females that have expanded and contracted their ovaries in past reproductive cycles. However, this trait can be difficult to quantify because histological slides are often made from partial cross sections of the ovary, meaning that the ovarian wall is no longer representative of its state in situ. In addition, the thickness of the ovarian wall is highly variable even in a full cross section, making it difficult to obtain a representative measurement (Lowerre-Barbieri *et al.*, 2011b).

Vitellogenesis is normally a long process during which important and visible changes occur within the oocyte: oocyte size increases noticeably, yolk progressively accumulates in the cytoplasm, and several cytoplasmic inclusions appear (vacuoles, oil droplets, etc.). Vitellogenic oocytes are separated into three stages (primary [Vtg1], secondary [Vtg2], and tertiary [Vtg3] vitellogenesis) based on the diameter of the oocyte, the amount of cytoplasm filled with yolk, and the presence and appearance of oil droplets (in species that have oil droplets) (Brown-Peterson *et al.*, 2011). Oocyte maturation indicates that spawning is imminent and includes two nuclear events: germinal vesicle migration (GVM) and germinal vesicle breakdown (Lowerre-Barbieri *et al.*, 2011b).

The spawning capable phase is defined as the fish being capable of spawning within the current reproductive cycle due to advanced gamete development such that oocytes can receive hormonal signals for OM in females or Sz release occurs in males (Brown-Peterson *et al.*, 2011). Spawning capable females are those that will spawn during the current reproductive cycle and thus contain either vitellogenic oocytes or histological indicators of imminent spawning (late GVM, germinal vesicle breakdown, and hydration) or recent spawning (newly collapsed POFs) (Lowerre-Barbieri *et al.*, 2011b).

Spermatogenic stages follow those outlined by Grier and Uribe-Aranz'abal (2009) and include spermatogonia (Sg), spermatocytes (Sc), spermatids (St), and spermatozoa (Sz), which can be differentiated by a decrease in size and an increase in basophilic staining as development progresses from Sg to Sz (Brown-Peterson *et al.*, 2011).

Histological observation can provide information on the internal structural changes in the germ cells (Priyadharsini *et al.*, 2013). Histological techniques are considered the most accurate means to assess gonadal development (Vitale *et al.*, 2006; Kjesbu, 2009), there is no standard level of

gonadal development considered representative of “mature,” and often the criteria used are not reported (Lowerre-Barbieri *et al.*, 2011b). Microscopic observation is considered as an important method to get detailed information of the germ cell development during gonadal maturation of fishes. Histology offers a powerful tool for reproductive studies and is routinely used for sex verification, assessment of reproductive phase, or quantification of atresia (Blazer, 2002).

The rapidly expanding human population consumes increasing amounts of food from natural aquatic ecosystems. However, environmental degradation, habitat destruction and fishing pressure have caused the decline in the production of these fishery resources from the wild which have diminished greatly. Therefore, the domestication of new fish species with economic importance of known reproductive biology is necessary for intensive cultivation in captivity. There is currently paucity of information on the macroscopic and microscopic maturation and histology of the gonads of these fish species from the Lower River Benue. The study investigated the macroscopic and microscopic maturation and histology of the gonads of *Hydrocynus forskahlii*, *Citharinus citharus* and *Mormyrus rume* in the Lower River Benue.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in the Lower River Benue (Fig. 1) at Wadata and Abinsi. Wadata is located at latitude 7° 43’ 0” North and longitude 8° 32’ 0” East, while Abinsi is located at latitude 7° 45’ 0” North and longitude 8° 45’ 0” East. River Benue originates from the Adamawa Mountains of Cameroun, some 500 km beyond the Nigerian border and flows west across East Central Nigeria (Nedeco, 1959). It is the largest tributary of the Niger River which joins at Lokoja. The highest water levels occur from August to September and the lowest from March to April (Akombo *et al.*, 2011).

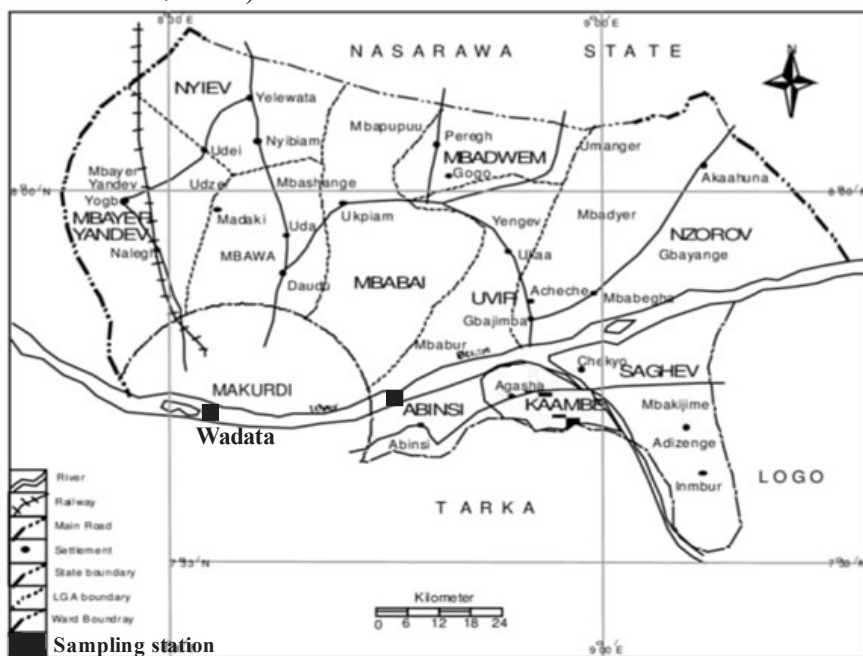


Figure 1: Map of Guma Local Government Area showing the sampling stations in Lower River Benue (Source: Ministry of Lands and Survey, Makurdi, 2013).

Fish Sample Collection and Identification

Fish samples caught using gill and cast nets were collected fortnightly from fishermen from Wadata and Abinsi, between April 2019 and March 2020. A total of 189 *H. forskahlii* comprising 97 females and 92 males, were sampled from Abinsi station, while 214 comprising 115 females and 99 males were sampled from Wadata station. A total of 372 *C. citharus* comprising 167 females and 205 males were sampled from Abinsi station, while 366 comprising 173 females and 193 males were sampled from Wadata station. A total of 384 *M. rume* comprising 217 females and 167 males were sampled from Abinsi station, while 363 comprising of 204 females and 159 males were sampled from Wadata station. The fish were placed in ice box containing ice block to avoid decomposition and were immediately transported to the General multi-purpose laboratory, at the Department of Fisheries and Aquaculture, Joseph Sarwuan Tarka University, Makurdi for data collection. The fish species were identified using Paugy *et al.* (2003) identification guide.

Macroscopic and microscopic classification of the fishes' gonadal development

Ovary samples were measured microscopically into diameter, circumference, and surface area to the nearest mm, on a random orientation basis using Cosmo-Celestron LCD Digital Microscope, Model 44362. The reproductive cycle of the gonads in different maturity stages throughout the year bi-monthly, was classified based on a modified macroscopic and microscopic classification scheme proposed by Brown-Peterson *et al.* (2011). Five macroscopic and microscopic stages were defined for each sex based on the most advanced gamete stage present and by some distinctive features of the gonads. The gonads were afterward fixed in 10% buffered formalin for the histological analysis.

Histological study of the gonads using haematoxylin and eosin staining technique

The histological analysis was done in the Histopathological Laboratory, Department of Human Anatomy, Ahmadu Bello University, Zaria. The process of oogenesis and spermatogenesis were studied by utilising histological preparations of ovaries and testis belonging to different gonad maturity stages, according to the methods described by Slaoui and Fiette (2011).

The gonads fixed in 10% buffered formalin were removed and passed through ascending grades of methanol from 70% to 90% and 100% for 12 hours to properly dehydrate them. They were cleared in xylene for 2 hours and then infiltrated and embedded in liquid paraffin wax. Sections were dewaxed in xylene for 6 minutes and passed through 100%, 90% and 70% alcohol for 1 minute each, after which they were washed in water and stained in haematoxylin for 10 minutes. The sections were differentiated using 1% acid alcohol for 1 minute and which they were washed with water and further blued in Scott's Tap Water for 2 minutes. Afterwards, the sections were stained in eosin for 2 minutes and washed in water. The sections were dehydrated using ascending grades of alcohol from 70% to 90% and 100% alcohol for 1 minute each and cleared in xylene. The sections were mounted with coverslip using DPX (Dibutylphthalate Polystyrene Xylene). Results from the nuclei stained blue while cytoplasm and other tissues stained pink or red.

The gonads were then cut using Leica Rotary Microtome at 5µm (micron) thickness and the sections were stained using haematoxylin and eosin staining technique using Leica Automatic

tissue processor. Histological examination of specific gonadal features were conducted to confirm the reproductive phase and was classified into stages of development based on the criteria presented by Brown-Peterson *et al.* (2011). The stages for females assigned were primary growth follicles, vitellogenic follicles, oocytes differentiation, and germinal vesicle breakdown. The testicular tissues were also classified into the developmental stages based on Brown-Peterson *et al.* (2011) criteria. The stages assigned for males were primary and secondary spermatocytes and the presence of seminiferous tubules.

Statistical Analysis

Student T-test was used to determine the differences in Seasonal and Sex-wise Gut-length and Gut- weight of the three selected fish species from Lower River Benue. They were analyzed using the procedure of Gayanilo Jr. *et al.* (2005) of the FiSAT II (version 1.2.2) computer software package for fish stock assessment.

3. RESULTS

Macroscopic classification of gonads maturation of the fishes sampled

The results obtained from the macroscopic and microscopic observations of the gonads of the three selected fish species sampled are presented in Table 1.

Five reproductive phases were identified from the results obtained from the macroscopic and microscopic observation of the gonads of the three selected fish species. These phases are immature, developing, spawning capable, regressing and the regenerating.

In the immature phase, the gonads, both the ovaries and the testes were translucent and not visible to the naked eyes.

In the developing phase, the ovaries became visible to the naked eyes. The oocytes diameter of *H. forskahlii* in this phase ranged from 1.82-2.95mm, and *M. rume* ranged from 1.14-1.25mm, while that of *C. citharus* were not visible and could not be measured under the microscope. The testes in this phase were also identified easily, though were small in sizes.

In the spawning capable phase, the ovaries became large with individual oocytes. The oocyte of *H. forskahlii* in this phase measured between 2.31-2.74mm in diameter, *C. citharus* measured 0.78-1.36mm, and *M. rume* measured 1.93-2.48mm. The testes in this phase also became larger and milt could easily be released with a gentle pressure.

In the regressing phase, ovaries and testes became flaccid and in some samples empty sacs were found.

In the regenerating phase, ovaries were found with thick wall. Testes were smaller and translucent.

Table 1: Macroscopic and Microscopic observation of Gonads Maturation of three selected fish species obtained from the Lower River Benue

Reproductive Phases	Female	Male
Immature	Small translucent ovaries with blood vessels not visible with the naked eyes. Thread-like structures.	Small clear and fine testes (translucent) and thread-like.
Developing	Enlarging ovaries with little blood vessels occupying almost the whole visceral cavity. Ovaries appeared creamy in colour and ova are seen with the naked eyes (<i>H. forskahlii</i> oocytes diameter ranged from 1.82-2.95mm <i>M. rume</i> oocytes diameter ranged from 1.14-1.25 mm).	Small testes and were identified easily, with creamy white colour.
Spawning capable	Large ovaries, vessels blood vessels, and visible individual oocytes (<i>H. forskahlii</i> oocytes diameter ranged from 2.31-2.74mm, <i>C. citharus</i> ranged from 0.78-1.36 mm, <i>M. rume</i> from 1.93-2.48 mm). Ova are creamy yellow in colour, spherical in shape under the microscope and can be separated individually.	Large and firm testes, released of milt with gentle pressure at the abdomen.
Regressing	Ovaries appeared flaccid, sometimes empty sacs, and prominent blood vessels.	Small (reduced in size) and translucent testes, thread-like structures, and flaccid.
Regenerating	Small ovaries with little blood vessels presence. Thick ovarian walls.	Small testes (reduced in size) and thread-like, and translucent.

Histological and microscopic classification of gonads maturation of the fishes sampled

The histological and microscopic classification of the gonads of the three selected fish species are presented in plates 1-4.

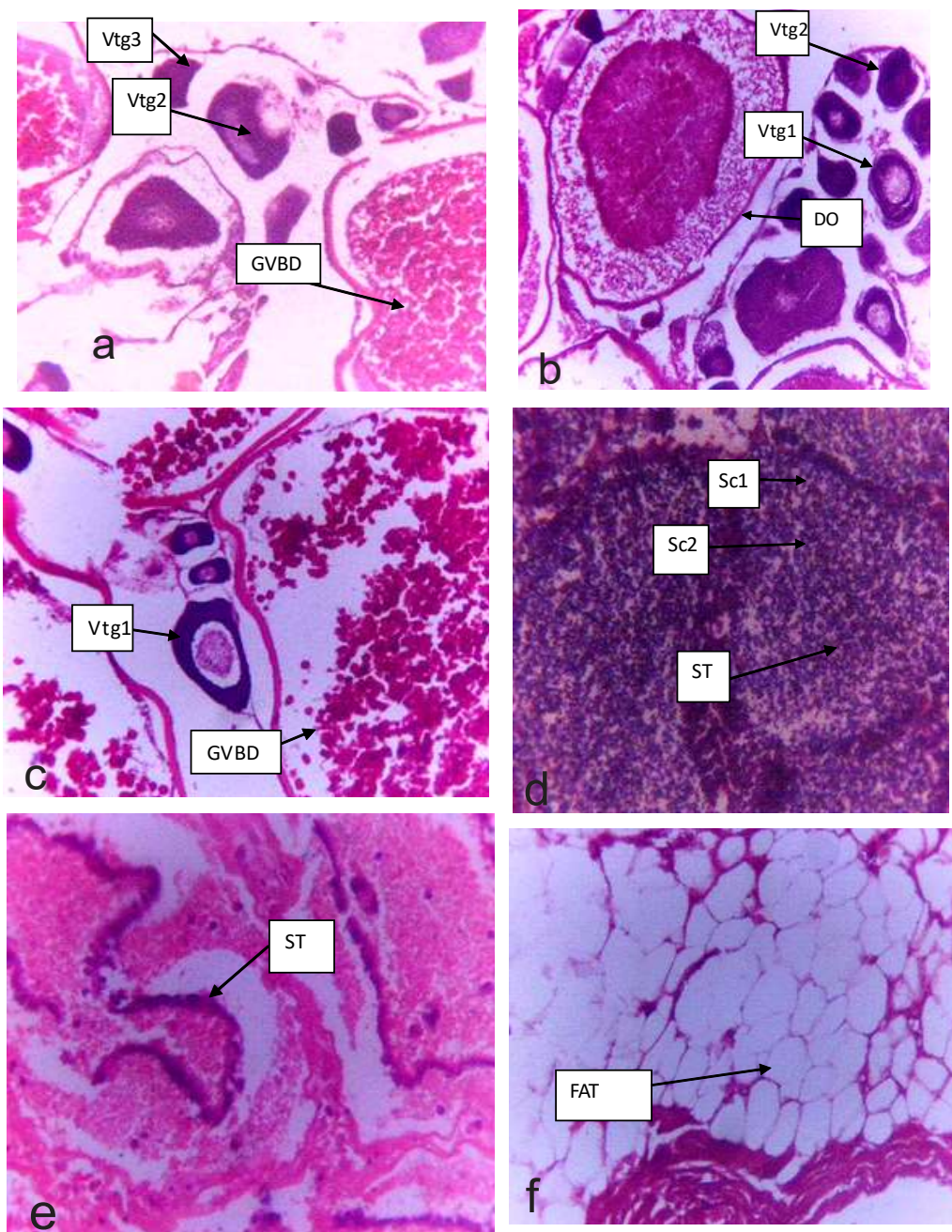


Plate 2: Photomicrographs of Ovarian and Testicular Histology of *H. forskaklii*, demonstrating the Reproductive phases: (a) Spawning capable phase: oocytes undergoing Vtg2=secondary vitellogenesis, Vtg3=tertiary vitellogenesis and GVBD= germinal vesicle breakdown; (b) Developing phase: oocytes undergoing Vtg1= primary vitellogenesis, Vtg2, and OD= oocytes differentiation; (c) spawning capable: oocytes undergoing Vtg1 and GVBD; (d) spawning capable: spermatocysts undergoing Sc1= primary spermatocytes, Sc2= secondary spermatocytes, with presence of seminiferous tubules; (e) Regressing phase: presence of seminiferous tubules; (f) Immature phase: testicles showing fatty tissues

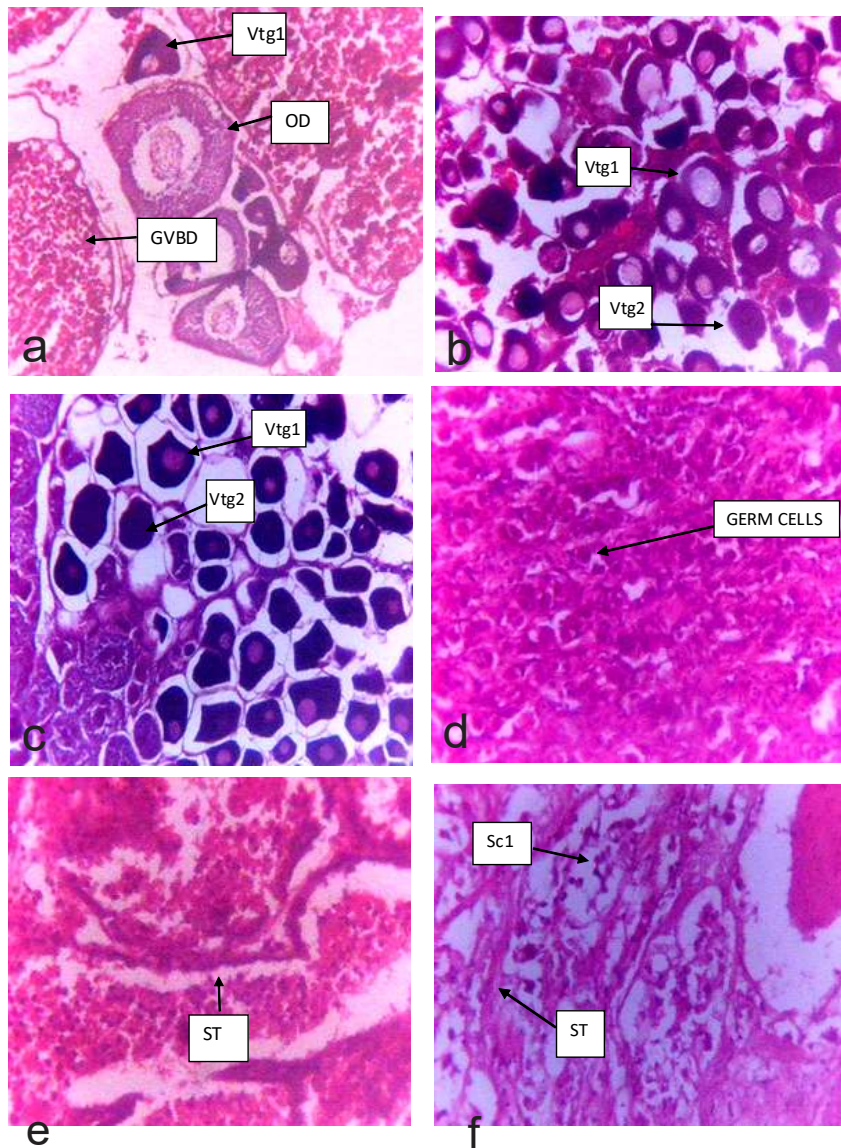


Plate 3: Photomicrographs of Ovarian and Testicular Histology of *C. citharus*, demonstrating the Reproductive phases: (a) Spawning capable: oocytes undergoing Vtg1, OD, and GVBD; (b) Developing phase: oocytes undergoing Vtg1 and Vtg2; (c) Developing phase:

oocytes undergoing Vtg1 and Vtg2; (d) Immature phase: spermatocysts showing a germ cell; (e) regressing phase: presence of seminiferous tubules in the testicles; (f) Regenerating phase: spermatocysts undergoing Sc1 with the presence of seminiferous tubules.

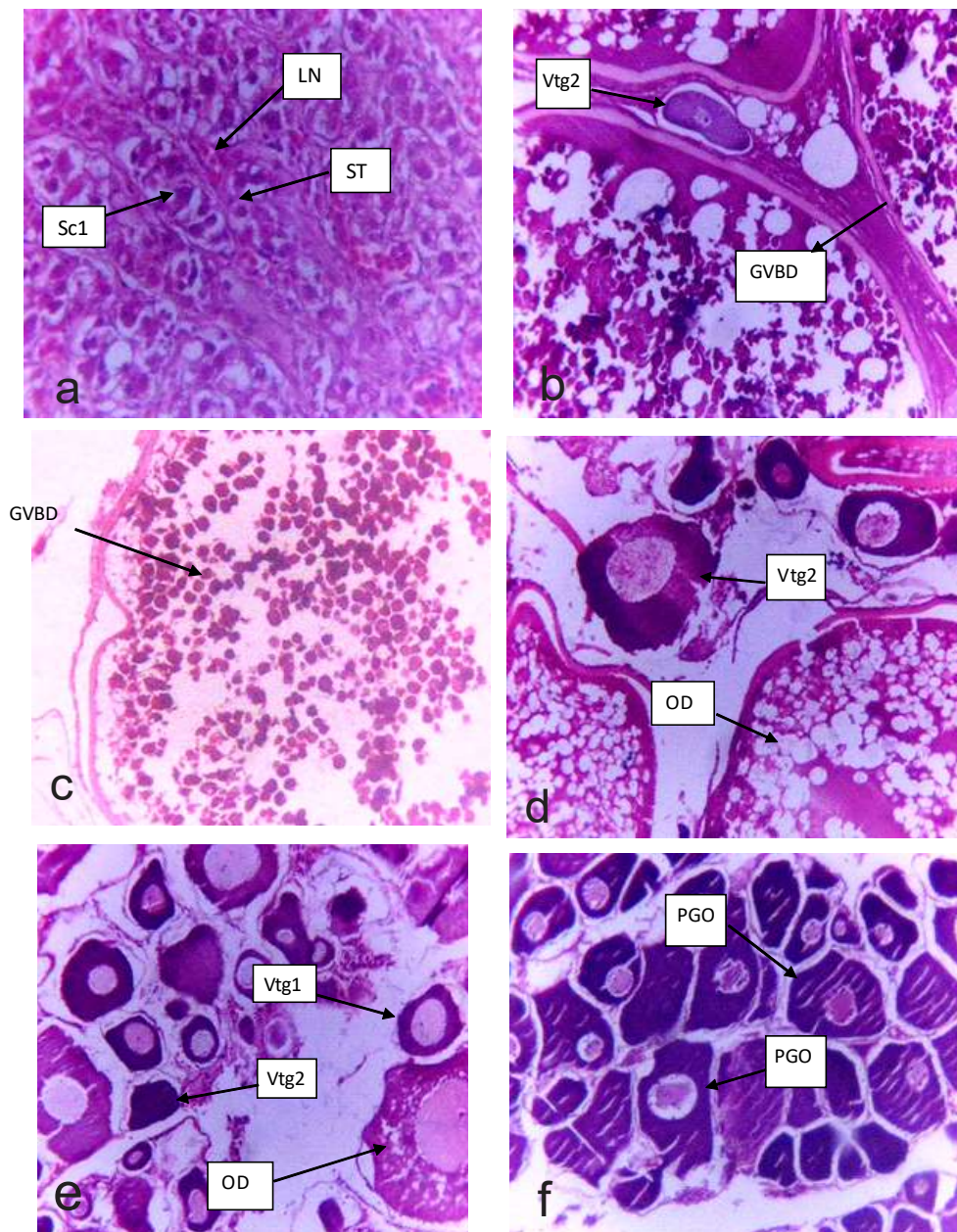


Plate 4: Photomicrographs of Ovarian and Testicular Histology of the fishes, demonstrating the Reproductive phases: (a) Regenerating phase of *C. citharus*: spermatocysts undergoing Sc1, presence of seminiferous tubules, and Wleydig cell necrosis; (b) *M. rume* (Spawning capable): oocytes undergoing Vtg2 and GVBD; (c) *M. rume* (Spawning capable): oocytes undergoing GVBD; (d) *M. rume* (Spawning capable): oocytes undergoing vtg2 and OD; (e) *M. rume*

(Developing): oocytes undergoing Vtg1, Vtg2, and OD; (f) *M. rume* (Immature): oocytes undergoing PGO= primary growth oocytes.

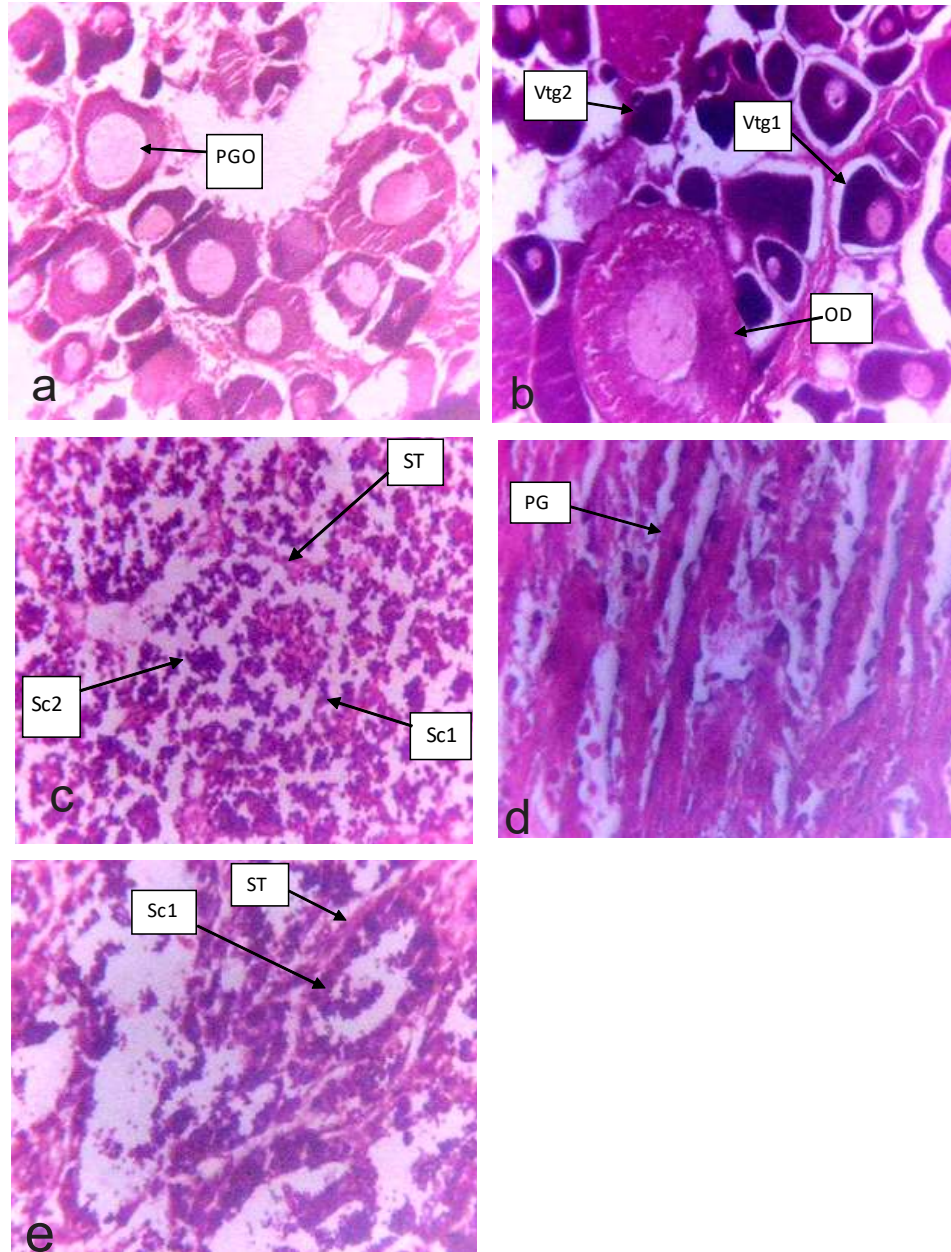


Plate 5: Photomicrographs of Ovarian and Testicular Histology of *M. rume*, demonstrating the Reproductive phases: (a) Immature phase: oocytes undergoing PGO; (b) Developing phase: oocytes undergoing Vtg1, Vtg2 and OD; (c) Spawning capable: spermatocysts undergoing Sc1, Sc2 and presence of seminiferous tubules; (d) Immature phase: spermatocysts undergoing PG= primary spermatogenic cells (e) Regenerating: spermatocysts undergoing Sc1, and presence of seminiferous tubules.

4. DISCUSSION**Macroscopic and Microscopic observation of Gonads maturation of three selected fish species sampled**

The macroscopic and microscopic observation of the fishes' gonads corresponded with the method described by Brown-Peterson et al. (2011). All the fish species exhibited similar phases of gonadal development which is in line with Brown-Peterson et al. (2011), who states that all fishes' reproductive cycles exhibit similar gonadal development including immature, developing, spawning capable, regressing, and regenerating phases. It was observed from this study that the gonads of the fishes first developed on the left side.

Histological and microscopic classification of gonads maturation of the fishes sampled

The histological observations of the gonads made from this study in respect to their reproductive phases are in line with the criteria of reproductive cycle presented by Brown-Peterson et al. (2011). The author proposed that, primary growth oocytes (PGO) and primary spermatogonia (Sg1) could be found in the immature phase; primary vitellogenic oocytes (Vtg1), secondary vitellogenic oocytes (Vtg2), primary spermatocytes (Sc1), and secondary spermatocytes (Sc2) in developing phase; Vtg2, Vtg3, germinal vesicle breakdown (GVBD), Sc1 and seminiferous tubules (sperm ducts) in spawning capable phase; Vtg1, Vtg2, and seminiferous tubules in regressing phase; and PGO and little of sperm ducts (seminiferous tubules) in regenerating phase. This is in line with the finding of this study as these reproductive features were found in the gonads of the fishes at different maturation stages as described by the author.

The histological features at different developmental stages also corresponded with the reproductive cycles and the spawning periods or seasons of these fishes, as stated by Brown-Peterson et al. (2011). In line with the author, the immature phase were characterised by PGO in females, and PG spermatogenic cells and sg1 (primary spermatogonia) in the germinal epithelium of males. The developing phases were characterized with Vtg1, and Vtg2 in females, while Sc1 (primary spermatocytes) and Sc2 in the germinal epithelium of males.

The spawning capable phases were characterised with the presence of Vtg2, Vtg3, and GVBD in females and Sc2, seminiferous tubules in the spermatocysts, sz in the lumen of the lobules and in the sperm ducts of males. The spawning capable phase occurred between May-August. This is also in line with Igejongo et al. (2018), who reported the ripe and spawning stage of *M. rume* to be the most dominant group in the month of May and June. The study also agree with the author on the report that, the presence of oocytes at different stages of development is an indication that the fish has prolonged and fractional spawning season, which resulted to the fishes spawning more than once during the spawning season. Similar reports were given from Shinkafi and Mamman (2011) on *Synodontis eupterus* in River Rima North-western Nigeria, and Mohamed (2010) on *Merluccius merluccius* from Egyptian Mediterranean water. On the contrary, Olele and Obi (2004) reported *C. citharus* to be a total spawner which releases a single batch of eggs per breeding season. According to Lowerre-Barbieri et al. (2011a), species with continuous recruitments have indeterminate fecundity; which is an indication that fecund oocytes are repeatedly recruited into vitellogenesis throughout the spawning season. The finding of this study is also in line with Brown-Peterson et al. (2011) who stated that batch spawners with evidence of previous spawning (POFs in females and sz in the sperm ducts of males) in combination with the presence of vitellogenic oocytes in females is an indication of the spawning capable phase, which is an indication that these fishes were capable of spawning future batches during that current cycle. The author also reported that actively spawning sub-phase within the

spawning capable phase indicates imminent release of gametes and it is defined as the presence of late GVM, GVBD, hydration, ovulation, or newly collapsed POFs in females and spermiation (macroscopic observation of the release of milt) in males.

The finding of this study is also in line with Brown-Peterson et al. (2011), who reported the regressing phase to be characterised by few (if any) healthy vtg2 or vtg3 oocytes in females and depleted stores of sz in sperm ducts and the lumen of the lobules, as there were presence of seminiferous tubules in the spermatocysts of males. The regenerating phase was characterised by small ovaries containing PG oocytes, Vtg1, Vtg2, and OD, with a thicker ovarian wall, and the presence of more space as reported by (Brown-Peterson et al. (2011). The regenerating phase of the males was characterised by the presence of scl and some elements of sperm ducts (seminiferous tubules) in the testicles. The regenerating phase is when the fish are sexually mature but, reproductively inactive. In accordance with the report of Brown-Peterson et al. (2011), regenerating phase could also be characterised by the presence of some elements of sperm ducts (seminiferous tubules) in the testicles.

Histological changes in the gonads of the fishes also correspond with the GSI between the seasons. This is close in line with the reports of Priyadharsini et al., (2013).

5. CONCLUSION

The presence of oocytes at different phases of development was an indication that the fishes had prolonged and fractional spawning season, spawning more than once during the spawning season. The histological features at different maturation stages also corresponded with reproductive cycles and the spawning periods or seasons as stated by Brown-Peterson et al. (2011), as the spawning capable phase occurred mostly between May-August.

Therefore, the sexually matured fishes may be captured during this period and used for aquaculture purpose, or the eggs collected and incubated in the laboratory since the fishes also produces large numbers of eggs.

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