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Cyclical changes in the histology of the gonads (ovary and testes) of African pike, *Hepsetus odoe*

Idowu, E. O.

Department of Zoology and Environmental Biology, Faculty of Science, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria.

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Morphology, stages of maturation and histology of gonads of African pike, *Hepsetus odoe* from Ado Ekiti Reservoir were studied from August 2014 to July 2015 in order to establish its reproductive biology. Out of 685 specimens examined 354 were males and 331 were females giving a sex ratio of 1:1.1 (female:male). There was no significant difference (P>0.05) in the sex ratio. The gonads were in pairs. All the gonad maturity stages were encountered in both male and female gonads. These stages were; immature, developing, mature (ripening), ripe and running, and spent. Histology of female ovary showed that six stages were encountered in the oogenesis, namely, oogonium, primary oocyte, primary vitellogenic oocyte, secondary vitellogenic oocyte, tertiary vitellogenic oocyte and hyaline oocyte while in male spermatogenesis five stages were present which are; spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa The oocyte diameter of *H. odoe* is higher when compared to freshwater fishes (the range was from 0.25 to 2.07 mean 1.74 ± 0.16 mm). All the five maturation stages occurred every month throughout the study period except the spent stage (stage V) which occurred only in October and ripe and running stage (Stage IV) which was absent only in December. This indicates that *H. odoe* is a multiple spawner in the reservoir.

Key words: African pike, gonads, maturity stages, histology, oogenesis, spermatogenesis.

INTRODUCTION

Hepsetus odoe was thought and believed to be the only species of the family Hepsetidae until recently when other species were discovered. The work of Decru et al. 2011, 2013 on the revision of the Lower Guinea *Hepsetus* species revealed that three different species occur in Lower Guinea instead of one. *Hepsetus akawo*, recently described from West Africa, is present in the northern part of Lower Guinea; *Hepsetus lineata*, the most widespread species within Lower Guinea, is known from the Sanaga (Cameroon) in the north to the Shiloango (Democratic Republic of Congo) in the south and *Hepsetus kingsleyae* is endemic to the Ogowe Basin.

The reproductive cycle of many fishes is reflected by perceptible changes in the size of the gonads throughout the year (Delahunty and Vlaming, 1980). Studies on the gonadal development of tropical fishes include

E-mail: eunice.idowu@eksu.edu.ng, idowunice@yahoo.com. Tel: +2348039422005.

Chrysichthys nigrodigitatus (Fagade and Adebisi, 1979) and *Clarias gariepinus* (Hogendoorn, 1979; Teugel, 1984; Oladosu et al., 1993), Mormyrid, *Hyperopsus bebe* (Oben et al., 2000), *H. odoe* (Idowu, 2007) and *Auchenoglanis occidentalis* (Shinkafi et al., 2011). The principal external factors which are associated with synchronous reproductive activity in temperate fish have been shown to be day length and temperature (Hyder, 1970). The author also revealed that sunlight and temperature are the most important external factors affecting reproductive pattern in *Tilapia leucostica*, and this subsequently influenced the gonadal development and breeding of the fish. However, Lowe (1959) reported that rainfall is the major external factor stimulating breeding activity in tilapias.

Egg diameter of fish varies from one species to another. It may depend on factors like fecundity which in turn is influenced by environmental factors such as temperature, food availability and mode of spawning. Ekpo (1982) reported that egg diameter of *H. odoe* was 1.36 mm in Lekki Lagoon, Idowu (2007) recorded egg diameter value of 1.74±0.2 mm in Ado Ekiti Reservoir. The egg diameter values of species that are closely related to *H. odoe* have also been reported; *Alestes longipinnis* was 0.99 mm and *A. chaperi* was 0.98 mm (Ekpo, 1982)

Studies on gonad histology according to Oben et al. (2000) and Ayoade (2004) reveal not just more developmental stages of the gonads but throws more light on spawning activities and spawning periods in individual fish species. Classification of gonadal stages of fish is based on morphological and histological examination of the gonads. Through this means many workers have been able to elucidate the maturity stages of various species of fish. Ogueri (2004) recognised seven stages; Ayoade (2004) recognized six stages of gonadal development in fishes. Ugwumba (1984) recognized six stages in the ten pounder *Elops lacerta*. These were immature, immature and developing, ripening, ripe, ripe running and spent. Similar observations on stages of gonadal development were reported for *Hyperopisus bebe* by Oben et al. (2000).

Vitellogenic stage of oocyte as well as increased gonad weight; gonadal-somatic index (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 (Omotosho, 1993). 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 (Bagenal, 1978), determination of reproductive potential of fish populations and monitoring of changes in biological characteristics of exploited fish stocks (Williams, 2007), establishing the reproduction period and length of gonadal maturation to allow for accurate implementation of fishery legislation (Goncalves et al., 2006).

Moreover, 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. The most important of these biological processes is the reproductive cycle and formation of gametes. However in the course of this research no reference was crossed on the cytology of *H. ode* gonads; most of the previous works on this species were mainly on food and feeding habits, length weight relationship and other aspects of its biology. The objective of this study therefore was the examination of cyclical changes in the histology of the gonads (ovary and testes) of African pike *H. odoe* in Ado Ekiti reservoir which will serve as a biological basis necessary for proper management and sustainability of the species in the reservoir.

MATERIALS AND METHODS

Sex ratio

Sex of each specimen collected was determined by examination of the gonads after dissection and the ratio of male to female calculated.

Egg diameter

The egg diameter was measured using an ocular micrometer in a binocular microscope. A stage micrometer was used to calibrate the microscope. For each ovary, the egg diameter of about 50 randomly selected eggs were measured and the mean taken as the average egg diameter. Egg diameter was measured in millimetre (mm).

Stages of gonad maturation and gonad histology

Gonad maturity stages were assessed and classified according to a modified classification of Hilge (1977) as follow: Stage I, Immature; II, developing; III, mature; IV, ripe and running; V, spent. The fish were dissected and the gonads at each stage of development were removed. Small pieces from the fore part of the gonads at each stage were cut out and fixed in Bouins fluid before embedding in parafin wax of melting point 56°C. Sections were cut at 10 µm and stained with Erlish Haematoxylin and Mallorys triple stain. Details of the procedures for gonad histology were according to Belelander and Ramaley (1979). Conclusive staging of the gonads were made on microscopic examination of the stained sections and photomicrographs taken.

Statistical analysis

Deviations of sex ratio from the expected 1:1 were determined using the Chi-square test.

RESULTS

Sex ratio

Out of 685 specimens examined, 354 were males and

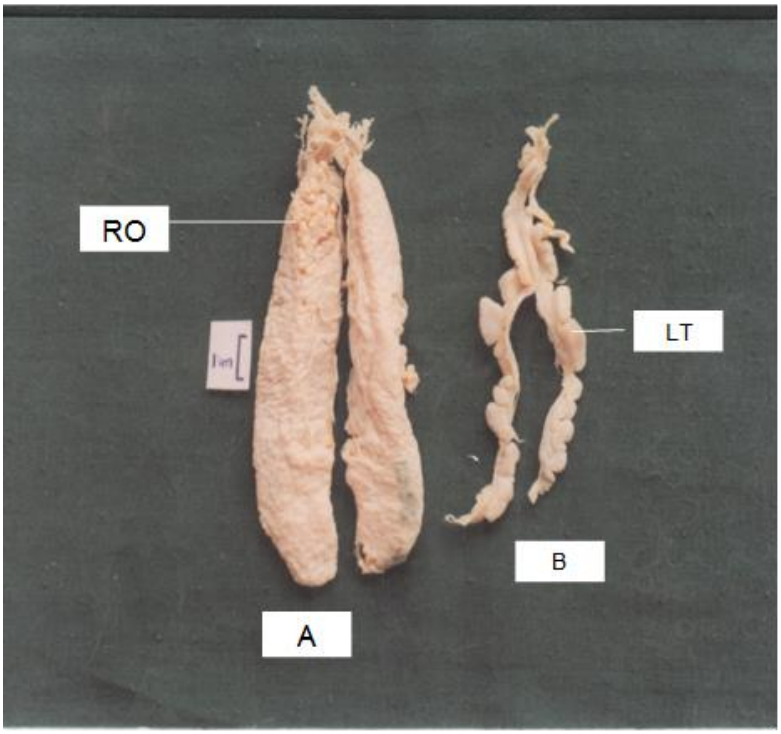

Plate 1. Gonads of *Hepsetus odoe.* A = Ripe ovary of *H. odoe*; B = ripe testes of *H. odoe;* RO = ruptured ovary; LT = lobe of testes.

331 were females giving a sex ratio of 1:1.1 (female: male); there was no significant difference (P>0.05) in the sex ratio.

Egg diameter

Egg diameter ranged between 1.40 to 2.07 mm with mean of 1.74 ± 0.2 mm from ripe/matured stage.

Stages of gonad maturation

Morphology of gonad

The gonads were in pairs. All the gonad maturity stages were encountered. These stages were immature, developing, mature (ripening), ripe and running, and spent.

Female gonad

The description of female gonadal stages was as follows:

Stage 1 (**immature):** Ovaries appeared like pairs of translucent pale white strips and weighed less than 0.5% of fish weight. Eggs were not visible, thus microscopic observations were needed to ascertain sex.

Stage II (immature and developing)**:** Eggs in this stage though minute were visible to naked eye. Ovaries here tended creamy in colour and weighed more than 0.5% fish weight.

S**tage III** (**Ripening):** Ovaries were usually swollen and the lobes were lost. The colour tended yellow and gonad weighed 1-5% of fish weight.

Stage IV (Ripe running stage): ovaries in this maturation stage were fully swollen with anterior bulges and central depression giving a pear like appearance (Plate 1) The ovary wall ruptured and eggs tended reddish due to vascularisation. Ovaries were no longer in strips but occupied more than three quarters (75 to 80%) of the abdominal cavity and rendered alimentary canal and gut almost inconspicuous. Eggs were mostly translucent yellowish and scattered easily on contact. Eggs easily extruded from vent when pressure was applied to the flanks.

Stage V (**Spent**): Ovaries in this stage were brownish towards the vent region. They appeared flaccids.

Males gonads

Stage I (**immature**)**:** The testes in this stage appeared as a pair of white filaments.

Table 1a. Monthly occurrence (by number) of the gonad maturity stages of female *H. odoe* in Ado-Ekiti Reservoir.

Month/year	Gonad maturity stages					
	I	II	III	IV	V	Total number
August, 2014	3	15	-	9	-	27
September	3	12	-	13	-	28
October	12	45	6	9	3	75
November	-	15	3	3	-	21
December	-	3	3	-	-	6
January,2015	-	24	12	15	-	51
February	-	6	9	6	-	21
March	-	6	9	9	-	21
April	6	6	3	9	-	24
May	-	-	3	3	-	6
June	3	6	15	6	-	30
July	6	-	9	9	-	24

Total number (N) = 331.

Table 1b. Monthly occurrence (by number) of the gonad maturity stages of male *H. odoe* in Ado-Ekiti Reservoir.

Month/year	Gonad maturity stages					
	I	II	III	IV	V	Total number
August, 2014	3	12	9	-	-	24
September	15	6	3	-	-	24
October	24	21	15	6	-	66
November	9	3	9	3	-	24
December	6	-	3	-	-	9
January,2015	-	3	3	6	-	12
February	3	3	6	6	-	18
March	15	27	21	9	-	72
April	6	9	6	3	-	24
May	3	-	6	3	-	12
June	3	6	18	12	-	39
July	9	-	6	12	-	27

Total number (N) = 354.

Stage II (immature and developing)**:** The gonads in this stage were still white but increased in size with lobes.

Stage III (ripening): The testes appeared like those in stage II but were larger and softer. The lobes became more prominent.

Stage IV (ripe running): The ripe testes were swollen and multilobed (Plate 1). The colour was creamy white. Blood vessels gave pinkish shades in some areas. When pressure was applied on the flanks, milty drops extruded from the genital pore.

Stage V (spent): Testes in this stage were flabby and pinkish tending brown. Milt still extruded on forced pressure at the flanks.

Table 1a and b show the monthly variations in gonad maturity stages observed in *H. odoe* during the period of investigation. The females had highest ripe eggs (stage IV) in September and January. Stage V (spent stage) was recorded only in October 2014.

Gonad histology

Histology of ovaries

Six stages were encountered in the oogenesis, namely, oogonium, primary oocyte, primary vitellogenic oocyte, secondary vitellogenic oocyte, tertiary vitellogenic oocyte and hyaline oocyte. The development of an oogoniun is shown diagrammatically in Figure 1.

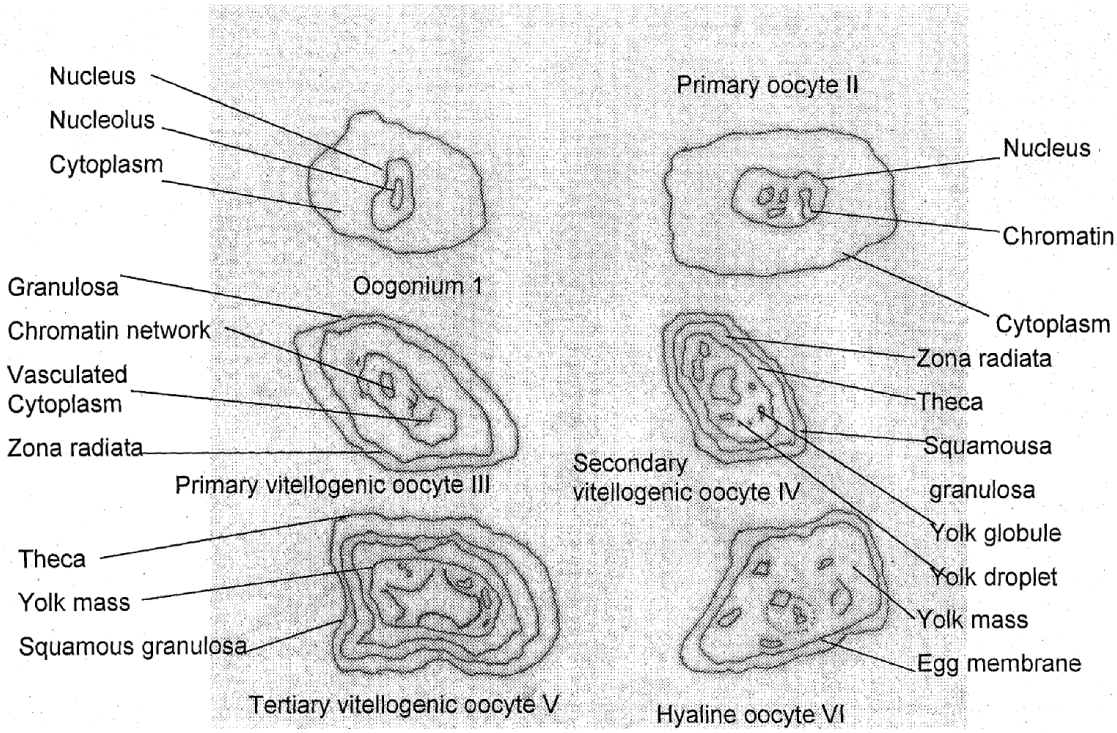

Figure 1. Schematic view of the developmental stages of the egg of *H. odoe* sampled from Ado-Ekiti Reservoir.

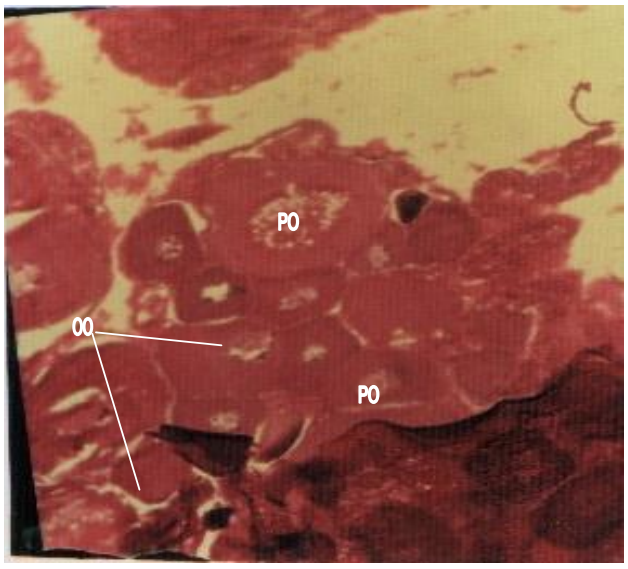

Plate 2. T.S. of stage I (immature) ovary of *H. odoe* showing oogonia (OO) and primary oocytes (PO) H& E (x 100).

Stage 1 (immature): Histological section of stage 1 ovary showed dominance of oogonia and primary oocytes as shown in Plate 2. Oogonia were seen as small spherical cells single or in groups. They were observed in all maturity stages. They were easily identified by the presence of a single large nucleolus in the nucleus.

Ovarian wall thickness had mean value of 0.08±0.01 mm. The mean oocyte diameter was 0.25 ± 0.21 mm and the range was 0.01 to 0.56 mm.

Stage II (developing): Primary vitellogenic oocyte and primary oocytes were found in the histological sections. The primary oocyte is bigger than the oogonium and is characterized by a large nucleus with many nucleoli around its periphery. They were present in all maturation stages. The follicular cells organize themselves around the developing oocytes which develop a larger cytoplasm. Some are ovoid in shape while some are polygonal. Primary vitellogenic oocytes formed 24 to 30% of cells. Mean oocytes diameter was 0.52 ± 0.15 mm. It ranged between 0.3 and 0.7 mm.

Stage III (matured): There were appearances of secondary vitellogenic oocytes forming 10 to 15% of entire cells. 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 yolk development. There is formation of yolk globules in the cytoplasm of secondary vitellogenic oocytes. Yolk globules were present throughout the cytoplasm at the end of this stage. Cells were enveloped by two layers, sqamous granulosa and cellular theca. The

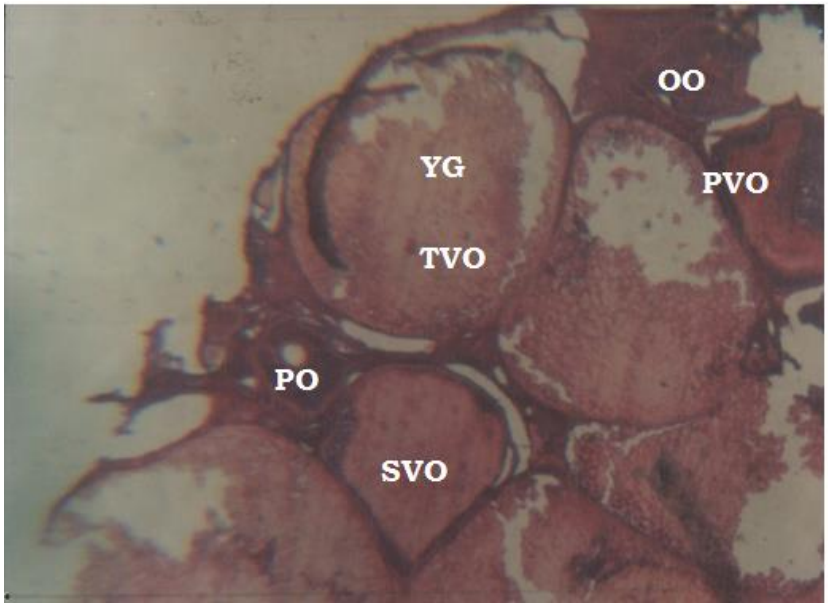

Plate 3. T.S. of stage IV ovary of *H. odoe* showing tertiary vitellogenic oocyte (TVO) with yolk granules (YG), secondary vitellogenic oocyte (SVO), primary oocyte (PO) and oogonium cell (OO) H & E (x 125).

oocyte dimater ranged from 1.26 to 1.76 mm with mean value of 1.53 ± 0.2 mm.

Stage IV (ripe and running): Most of the oocytes were tertiary/mature oocytes and constituted 70 to 75% of entire cells (Plate 3). The interiors were filled with prominent yolk globules and droplets. The two layers enveloping the cells were less defined. The oocyte diameter ranged from 1.40 to 2.07 mm with mean of 1.74 ± 0.16 mm. Mean ovarian wall thickness was 0.01 mm. Yolk globules were prominent in mature oocytes.

Stage V (Spent): There were some ruptured follicles, which appeared empty as if they have released the egg (Plate 4). Hyaline oocytes were also seen and they were filled with yolk and lacked nuclei.

Histology of the testes

Immature (stage I): Sections showed irregular shaped spermatogonia, which aggregated in groups (Plate 5). The spermatogonia were characterized by lightly stained cytoplasm and a large nucleus.

Developing (stage II): Spherical primary spermatocytes predominated. The cell membranes were more defined. The secondary spermatocytes became cusp shaped and were attached to the lobular wall (Plate 6).

Mature (stage III): Sections showed sickle shaped spermatids, which were detached from the lobular wall. Spermatocytes at different stages of development fill the testis at this stage. The testis was filled with spermatids (Plate 7).

Ripe running (stage IV): Empty spaces appeared in the lumen containing loose spermatocytes and spermatozoa (Plate 8).

Spent (stage V): Testis had unfilled lumen with spermatozoa.

DISCUSSION

The sex ratio observed in this study did not differ significantly from the expected 1:1 ratio. According to Nikolsky (1969) and Idowu (2007) a 1:1 sex ratio represents lack of difference in the longetivity of the sexes. Females were slightly heavier than males. This could be due to additional weight gain in ovaries of females especially during the breeding season.

The eggs of *H. odoe* in this study were relatively large and their sizes were higher than 1.36 mm of the same species in Lekki Lagoon reported by Ekpo (1982). This could be as a result of differences in habitat which may reflect the amount of food available and consumed. The egg size of fish varies from one species to another and are affected by various factors such as the degree of parental care (Fryer and Iles, 1972; Fletcher and Wooton, 1995), mode of spawning (Adebisi, 1987), length of interspawning interval (Townsend and Wooton, 1984), amount of food consumed (Springate et al., 1985;

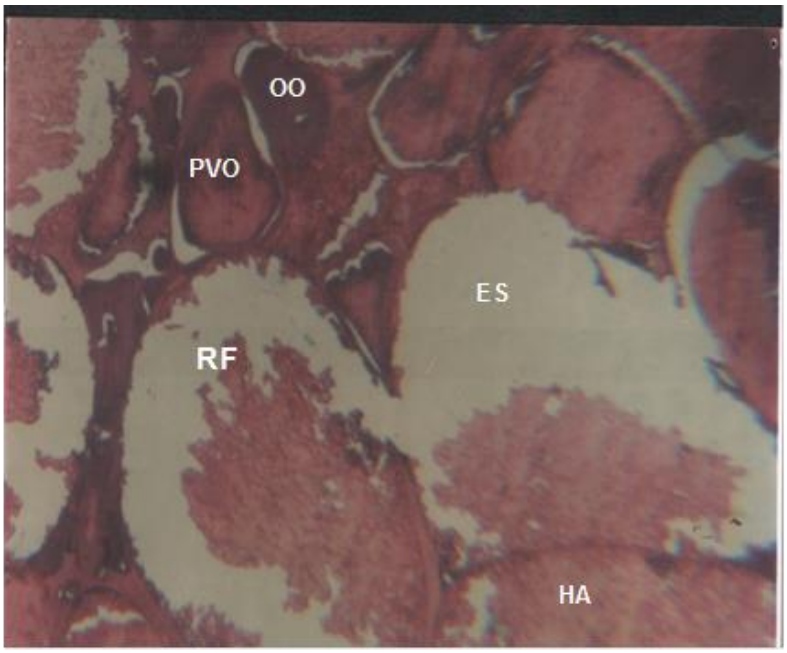

Plate 4. T.S. of stage V (spent) ovary of *H. odoe* showing ruptured follicle (RF), empty space (ES), hyaline oocyte (HA filled with yolk), primary vitellogenic oocyte (PVO) and oogonium cell (OO) H & E (x125), vitellogenic oocyte (PVO) and oogonium cell (OO) H & E (x125).

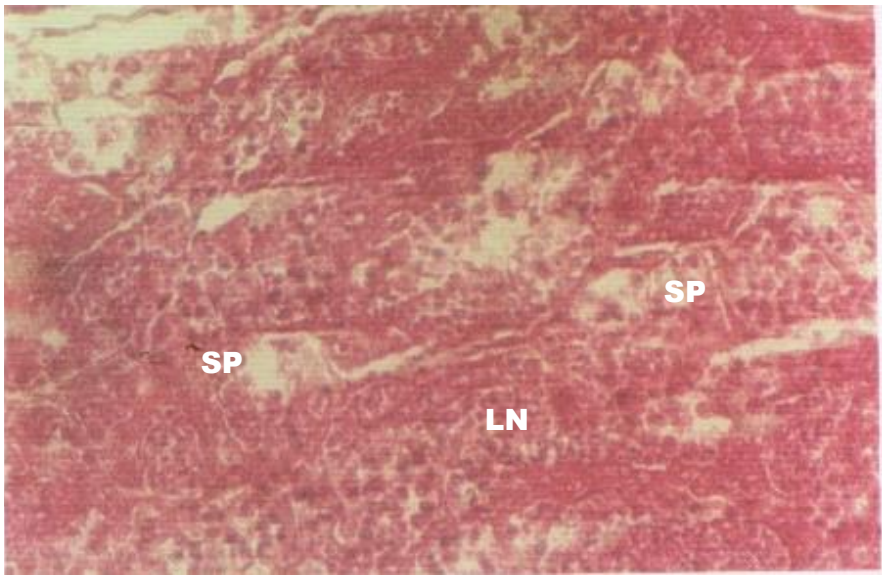

Plate 5. T.S. of stage I (immature) testis of *H. odoe* showing spermatogonia (SP) and large nucleus (LN). H & E (x 400).

Fletcher and Wooton, 1995) and habitat (Elliot, 1986; Ayoade, 2004). Generally, fish species with low egg diameter are usually very fecund while species with large egg diameter are less fecund and may show some degree of parental care (Ekpo, 1982; Adebisi, 1987; Oben et al., 2000).

Fishes with low egg diameters have been reported by some authors. These include; *S. mystus* (Ayoade, 2004), *C. gariepinus* (Abayomi and Arawomo, 1996), *H. bebe* (Adebisi, 1987; Oben et al., 2000), *E. vittata* and *Pellonula afzeliusi* (Ekpo, 1982). Fish species with large egg diameters include, mouth brooding *Tilapia* species

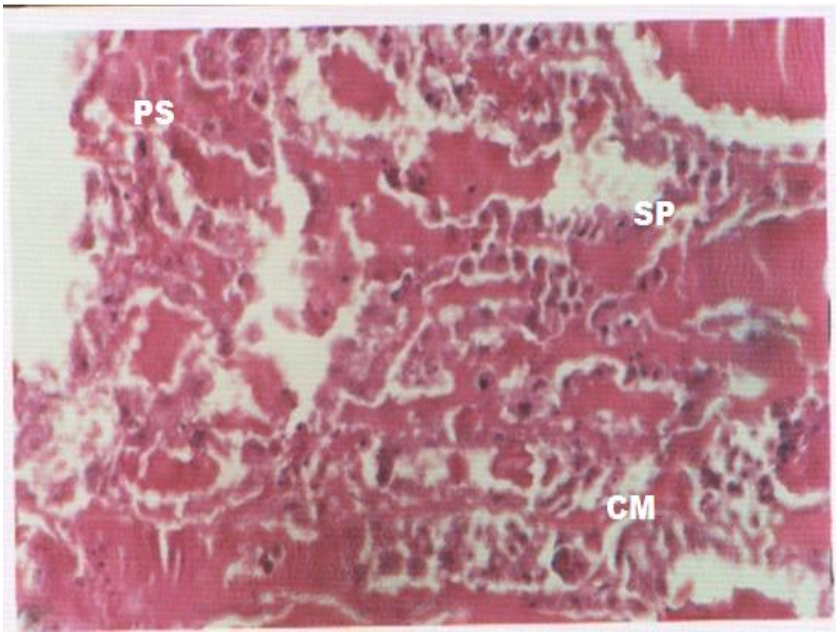

Plate 6. T. S. of stage II (immature and developing) testis of *H. odoe* showing spermatogonia (SP) and primary spermatocyte (PS), cell membrane (CM) H & E (x 400).

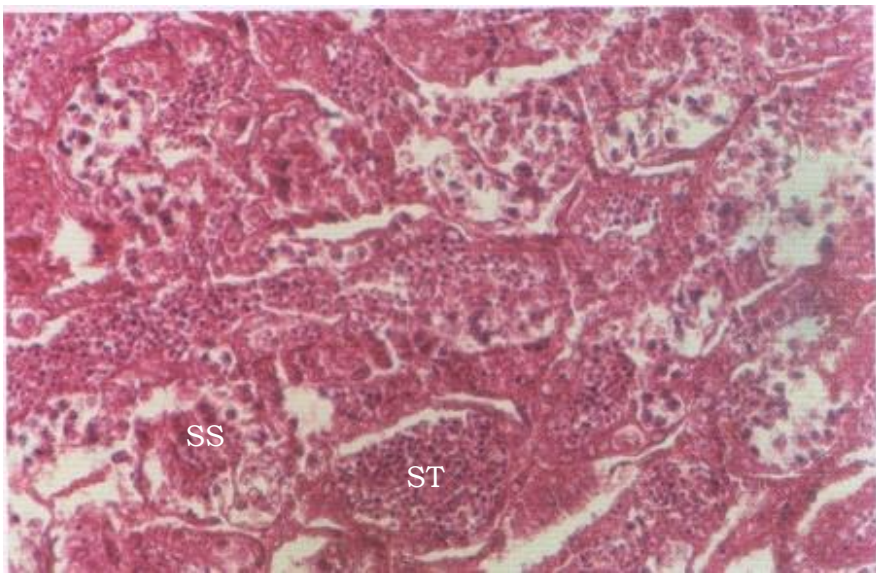

Plate 7. T.S. of stage III (ripening) testis of *H. odoe* showing spermatogonia (SP) and primary spermatocyte (PS), cell membrane (CM) H & E (x 400).

such as *S. galileaus*, *T. aurea,* (Dadzie, 1970). Other fish with large egg diameters include *X. nigri, Pollimyrus adspersus* and *Petrocephalus sanvagii* (Ekpo, 1982). The egg diameters of species that are closely related to *H. odoe* have also been reported to be large. These fish species include *A. longipinnis* and *A. chaperi* (Ekpo, 1982).

The histological analyses permitted precise description of the process of development and maturation. According to Elourduy - Graray and Ramirez - Luna (1994), visual evaluation of maturity of the gonads by microscopic characteristics and the use of gonadal indices are gross indicators of reproductive activity but not accurate enough to establish the stage of gonadal development or

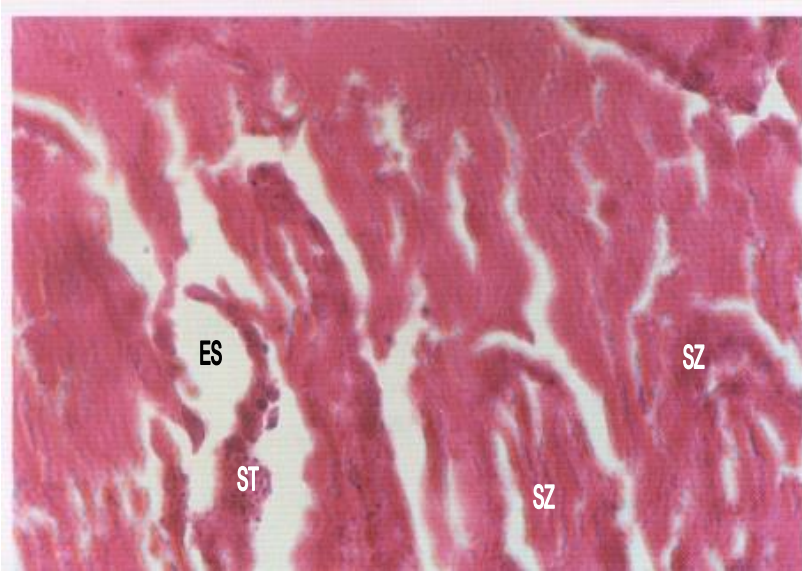

Plate 8. T.S. of stage IV (ripe running) testis of *H. odoe* showing spermatid (ST.) and spermatozoa (SZ) and empty space (ES) H & E (x 400).

to detect subtle differences between gonads. The oocyte diameter of *H. odoe* is higher when compared to freshwater fishes (the range is from 0.25 to 2.07, mean 1.74 ± 0.16 mm). However, Oben (1995) recorded oocyte diameter of 0.17 to 1.23 mm for *Mormyrus rume* while Ayoade (2004) recorded oocyte diameter of 0.06 to 0.66 mm for *Schilbe mystus* in Oyan Lake.

All the five maturation stages occurred every month throughout the study period except the spent stage (Stage V) which occurred only in October and ripe and running stage (Stage IV) which was absent only in December. This indicates that *H. odoe* bred throughout the year (multiple spawner). Abundance of ripe fish was more in October and January, although it was abundant in all other months. This shows that *H. odoe* bred both during the rainy and dry seasons with the peak breeding in these periods of peak abundance of ripe fish.

The peak periods of *H. odoe* abundance were in January, June and September to October. These periods coincided with peak spawning periods. Spawning enhanced new recruits and increased the population during these periods. To avoid the catch of young ones, fishing activities should be reduced or restricted by reduction in number of fishermen and number of fishing periods per day during these peak periods of spawning to allow for steady and adequate recruitment of the species into the population. This will ensure rational exploitation which is the ultimate in sustainable fisheries management.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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