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# Gonad Maturation Stages of *Auchenoglanis occidentalis* (Valenciennes 1840) in River Rima, North-Western Nigeria

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## ABSTRACT

The aim of this study was to gain knowledge on the gonad morphology and to determine the stages of gonad maturation in *Auchenoglanis occidentalis* (Cuvier and Valenciennes), based on macroscopic and histological procedures. A total of 800 samples, of which 398 were females, 399 males and 3 with unidentified sex were examined between November 2005 and December 2008. The samples ranged in total length from 6.60 to 33.00 cm with a mean of 19.51±4.50SD and from 2.26 to 462.90 g total weight with a mean of 100.05±70.09 SD. In females, mean ovary weight was 2.74±0.23 SE, mean left ovary length 3.70±0.08 SE, mean left ovary width 0.59±0.03 SE, mean right ovary length 3.65±0.08 SE and mean right ovary width 0.57±0.02 SE. In males, mean testis weight was 0.17±0.03 SE, mean left testis length 2.75±0.06 SE, mean left testis width 0.20±0.01 SE, mean right testis length 2.74±0.06 SE and mean testis width 0.19±0.01SE. This shows that in both sexes, the left gonads were slightly larger than the right ones. Six stages of gonad maturation were established for the females and males namely, immature (I), maturing (II), mature (III), ripe and running (IV), spent (V) and resting (VI). Based on macroscopy, eggs of various sizes and colours were found in each ovary, while histologically, various stages of oocytes and spermatocytes developed in each ovary and testis, respectively, indicating multiple spawning in both sexes. Spawning of the species occurs during the peak of rainy season in River Rima, which is between July and September.

**Key words:** *A. occidentalis*, gonad morphology, gonad maturation stages, macroscopy, histology, River Rima

## INTRODUCTION

The study of gonad stages of maturation has become increasingly important in fish production, notably in induced spawning and hybridization studies (Omotosho, 1993). Knowledge of the gonad maturation stages of fishes is also required for many purposes and these 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).

The use of macroscopic characteristics to classify gonad maturity stages based on external examination may result in individual interpretations of staging characters, even by experienced sampling personnel (Williams, 2007). This is due to unstandardized method of interpretation of the

features in the various stages, leading to confusion in characterization of the maturity stages. Additionally, certain reproductive states such as those not exhibiting active spawning characteristics cannot be reliably identified by macroscopic methods (Hunter and Macewicz, 2001). Moreover, an incorrect assignment of maturity stage can lead to problems in fisheries management, since the managers need to know the real number of spawning fish in a population to be able to manage fishing effort effectively (Costa, 2009). Hence, the validation of gonad maturation stages with histology permits better determination and understanding of the process of gonad maturation by revealing the details of oocyte and sperm development, which present less ambiguity in assigning maturity status (Mendonca *et al*., 2006). Histological analysis of gonads is also used to determine the pattern of oogenesis and thus, the spawning pattern of a fish as well as the types of methods necessary for the estimation of annual fecundity (Morrison, 1990)

Based on macroscopic and histological methods in various fish species, different numbers of gonadal maturation stages, ranging from five to nine were reported. These include the reports of Otobo (1978), Morrison (1990), White *et al*. (1998), Dos-Santos *et al*. (2004), Seoka *et al*. (2007), Williams (2007) and Mahmud (2009).

This study provides information on the gonad morphology and maturation stages of *A. occidentalis* from river Rima in Northwestern Nigeria. It belongs to the Family Claroteidae (Nelson, 2006) and is commonly known as the giraffe nosed catfish. This species grows to about 500 mm length and a weight of 4.5 kg and has flesh of fair quality (Reed *et al*., 1967). Holden and Reed (1972) noted that this species is fairly common, especially in swamps and is of considerable commercial importance. In Nigeria, very little published research works are available on the biology of this species.

## MATERIALS AND METHODS

**Study area:** The fish samples were collected from River Rima, in Sokoto, Northwestern Nigeria on monthly basis for three years, which is from November 2005 to December 2008. Sokoto lies between longitudes 4°8'E and 6°5'E and latitudes 12°N and 13°58'N (Mamman, 2000). The climate of Sokoto is tropical continental, with much of the rains between June and September, while the long dry season is from October and May (Ita *et al*., 1982).

River Rima flows in a South-Western direction over 100 km and joins the major River Sokoto to form the Sokoto-Rima river system. The Sokoto-Rima River flows southwesterly, in a direction up to Zogirma, where it changes direction and run Southwards before emptying into the River Niger. The River is seasonal, usually over flooding its banks during the rainy season in August and September and up to October at times (Mock, 1963).

**Fish samples:** The specimens were examined fresh in the laboratory immediately after collection. On each sample, measurements of total length and standard length (cm) and total weight (g) were taken. Gonads were detached and weighed (g). Length and width (cm) of each gonad lobe were measured. Sex and maturity stage were assigned to each sample.

A total of 800 of the samples, of which 398 were females and 399 males were analyzed, giving a ratio of 1:1. The samples ranged in total length from 6.60 to 33.00 cm with a mean of 19.51±4.50 SD and from 2.26 to 462.90 g total weight with a mean of 100.05±70.09 SD.

**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 colouration of the gonads (White *et al*., 1998).

**Histological study of gonads:** This was conducted following the procedures of Morrison (1990). 1 $cm^3$ of each gonad was fixed in 10% formalin immediately after removal from the fish. The specimens were then washed in water to remove excess fixative from the tissues. The gonads were dehydrated by running them through a series of alcohol in increasing concentrations from 35 to 100% and then cleared. The cleared tissues were pre-embedded with xylene-paraffin and then later fully embedded in pure paraffin wax. This was then poured into a mold and cooled in a freezer. The cooled wax block with the gonad inside was sliced into very thin ribbons at 7 μm thickness using a microtome. Staining of the slides was carried out with haematoxylin and eosin. The slides were finally mounted in DPX mountant and observed under a microscope. Photomicrographs of the different maturity stages were taken using a digital camera.

## RESULTS AND DISCUSSION

**Size of gonads:** Table 1 presents the mean weight of ovaries and testes in *A. occidentalis* samples, based on the six stages of gonad maturation. Ovaries in the mature stage were heavier than those in the ripe and running stage, which were in turn heavier than those in the immature, maturing, spent and resting. Testes were slightly heaviest in the mature stage, followed by the ripe and running stage. The least heavy testes were in the immature and resting stages. In both sexes, gonads were slightly larger in the mature, followed by ripe and running stage and were smallest in the immature, followed by resting stage. The mean values also showed that the left ovary was slightly larger than the right ovary and likewise, the left testis was less noticeably larger than the right testis.

**Macroscopic and histological description of gonad maturation stages of *A. occidentalis*:** The macroscopic and histological descriptions of the six stages of gonad maturation of the female *A. occidentalis* are presented in Table 2, while those of the male are shown in Table 3. The gonads exhibited variations in shape, colour, texture, vascularization and size in each stage of maturity. Histologically, the details of the development of oocytes and spermatozoa (Fig. 1, 2) further validated the macroscopic description of the stages in this species.

**Monthly occurrences of the different stages of gonad maturation:** Monthly occurrences of the different stages of gonad development of the female of *A. occidentalis* are presented in Table 4, while those of the male samples are shown in Table 5. Immature samples of both sexes were found throughout the year due to the presence of samples that have not reached reproductive age. In the early dry season (December to January), only immature gonads were found. Gonads started maturing from March, which is the mid dry season. As the rainy season approaches in May, mature gonads start appearing in the catches up to the end of the rainy season in September. Ripe and running gonads were mostly encountered during the peak of the rainy season in August and, were absent from November, signaling the end of spawning. Spent and resting gonads were more in abundance at the end of the rainy season from October. From the gonad maturation stages of the two sexes recorded, spawning of *A. occidentalis* has its peak from July to September.

Six gonad maturation stages were established based on macroscopic or visual examination of the gonads. During development, the gonad stages showed visible morphological changes in colour, shape, vascularization and size, which occurred during the maturation process and follow the same pattern in most oviparous fishes (Barr, 1968). The size of the gonads increased from immature to the maturing and was largest at the mature stages. They declined from ripe and running to spent

Table 1: Gonad dimensions of *A. occidentalis* based on gonad maturation stages

| | Ovary | | | Testis | | |
|---|---|---|---|---|---|---|
| Parameter | No. of samples | Mean | SE | No. of samples | Mean | SE |
| **Gonad weight (g)** | | | | | | |
| Overall | 376 | 2.74 | 0.23 | 382 | 0.17 | 0.03 |
| Immature | 101 | 0.15 | 0.02 | 97 | 0.06 | 0.01 |
| Maturing | 77 | 0.57 | 0.11 | 102 | 0.12 | 0.01 |
| Mature | 83 | 7.82 | 0.56 | 114 | 0.35 | 0.10 |
| Ripe & running | 51 | 5.94 | 0.72 | 17 | 0.23 | 0.08 |
| Spent | 52 | 0.30 | 0.06 | 44 | 0.09 | 0.02 |
| Resting | 12 | 0.17 | 0.06 | 08 | 0.05 | 0.01 |
| **Left gonad length (cm)** | | | | | | |
| Overall | 376 | 3.70 | 0.08 | 382 | 2.75 | 0.06 |
| Immature | 101 | 2.55 | 0.10 | 97 | 1.93 | 0.10 |
| Maturing | 77 | 3.08 | 0.13 | 102 | 2.89 | 0.10 |
| Mature | 83 | 5.20 | 0.14 | 114 | 3.34 | 0.08 |
| Ripe and running | 51 | 4.82 | 0.17 | 17 | 3.39 | 0.16 |
| Spent | 52 | 3.62 | 0.10 | 44 | 2.42 | 0.19 |
| Resting | 12 | 3.53 | 0.26 | 08 | 2.40 | 0.16 |
| **Left gonad width (cm)** | | | | | | |
| Overall | 376 | 0.59 | 0.03 | 382 | 0.20 | 0.01 |
| Immature | 101 | 0.23 | 0.01 | 97 | 0.14 | 0.01 |
| Maturing | 77 | 0.39 | 0.02 | 102 | 0.19 | 0.01 |
| Mature | 83 | 1.18 | 0.04 | 114 | 0.28 | 0.01 |
| Ripe and running | 51 | 1.14 | 0.05 | 17 | 0.24 | 0.02 |
| Spent | 52 | 0.32 | 0.02 | 44 | 0.12 | 0.01 |
| Resting | 12 | 0.19 | 0.04 | 08 | 0.10 | 0.00 |
| **Right gonad length (cm)** | | | | | | |
| Overall | 376 | 3.65 | 0.08 | 382 | 2.74 | 0.06 |
| Immature | 101 | 2.52 | 0.10 | 97 | 1.91 | 0.10 |
| Maturing | 77 | 3.04 | 0.12 | 102 | 2.85 | 0.10 |
| Mature | 83 | 5.09 | 0.14 | 114 | 3.34 | 0.08 |
| Ripe and running | 51 | 4.69 | 0.17 | 17 | 3.39 | 0.16 |
| Spent | 52 | 3.62 | 0.12 | 44 | 2.42 | 0.19 |
| Resting | 12 | 3.53 | 0.26 | 08 | 2.40 | 0.16 |
| **Right gonad width (cm)** | | | | | | |
| Overall | 376 | 0.57 | 0.02 | 382 | 0.19 | 0.01 |
| Immature | 101 | 0.22 | 0.01 | 97 | 0.13 | 0.01 |
| Maturing | 77 | 0.38 | 0.02 | 102 | 0.19 | 0.01 |
| Mature | 83 | 1.12 | 0.04 | 114 | 0.28 | 0.01 |
| Ripe and running | 51 | 1.08 | 0.05 | 17 | 0.24 | 0.02 |
| Spent | 52 | 0.32 | 0.02 | 44 | 0.12 | 0.01 |
| Resting | 12 | 0.19 | 0.04 | 08 | 0.10 | 0.00 |

and finally up to resting stages and back to immature when the cycle commences again. The increase in weight and size and contents of gonads at stages III and IV was consequent to a rapid accumulation of trophic substances in the reproductive cells. The left lobes of testes and ovaries were found to be slightly larger than the right ones. Similar finding was reported for *O. niloticus* (Omotosho, 1993).

Table 2: Macroscopic and histological description of the gonad maturation stages of female *A. occidentalis*

| Maturity stage | Macroscopic description | Histological description |
|---|---|---|
| Immature (I) | Ovaries very small, elongated, paired with smooth edges and pale pink in colour. Ovaries connected throughout their length by connective tissue. GSI ranged from 0.03±0.94% with a mean of 0.24±0.20SD. | The ovary was composed of oogonia (O) cells and primary oocytes (PO). The wall of the ovary thick (Fig. 1a) |
| Maturing (II) | Ovaries bigger and oval in shape and slightly of unequal size. They were covered with prominent blood vessels and lighter in colour (Pale pinkish). Very few tiny eggs discernible with naked eyes. Ovaries free at about ½ of their length, while the rest of ¾ joined by connective tissue. GSI from 0.04-4.12% with a mean of 0.64±0.66SD. | Primary oocytes (PO) larger and more in number, with fewer oogonia (O). The wall of ovary was thicker and some oocytes beginning to undergo vitellogenesis (PVO), with yolk granules on the cytoplasm (Fig. 1b) |
| Mature (III) | Paired ovaries free, smooth and transparent. Orange yellow translucent eggs were clearly visible. Ovaries covered with prominent blood vessels. In most cases, left ovary slightly larger than the right. Eggs not extruded with pressure on the abdomen. GSI from 0.09±11.03%, with a mean of 5.47±2.57SD. Mean egg size was 1.38±0.33 SD. | Wall of ovary was thinner. Oocytes larger and more mature with migratory nucleus and more yolk accumulation (SVO). Some few oogonia were still present (Fig. 1c) |
| Ripe and running (IV) | Ovaries smooth and less firm, with small whitish eggs found at the posterior and pale yellowish translucent eggs towards the posterior. Eggs were extruded with slight pressure on the abdomen. Blood vessels still prominent. Egg released at intervals, with one ovary releasing eggs at a time. GSI from 0.43-8.94%, with a mean of 4.25±2.56SD. Mean eggs size was 1.23±0.30 SD. | Consisted mainly of post vitellogenic oocytes (PsVO), with the nucleoli at the center of the nucleus and more yolk accumulation. The micropyle also present. Very few oogonia still present. The wall of the ovary thinner than before (Fig. 1d) |
| Spent (V) | Ovaries opaque, blood red and empty deflated sacs. Few bloody eggs still pressed from sample. Blood vessels were not prominent. Ovary is shrunken in size. GSI decreased, with a range of 0.01 to 0.74% and mean of 0.22±0.18 SD. | Post vitellogenic oocytes absent. Oogonia (O) scattered in between few secondary vitellogenic oocytes (SVO). Large empty spaces also seen where there were post vitellogenic oocytes before. Ovary wall very thin or thinner than before (Fig. 1e). |
| Resting (VI) | Fatty tissues more than in spent stage. Ovaries firmer, smaller and lighter in colour than in previous stage, but still pale red in colour. Ovaries with no blood vessels seen on them. GSI range from 0.01±0.25%, with a mean of 0.08±0.08 SD. | More spaces seen in sections with few primary oocytes and some few oogonia (Figure 1f). PVO, SVO and PsVO absent |

Macroscopic descriptions of gonad maturation stages in *A. occidentalis* revealed different sizes and colours of eggs in each ovary, whether in mature or ripe and running stages. Histological description further substantiated multiple stages of oocyte development in each of the six stages of maturation of the ovaries. Thus, in mature stage, secondary vitellogenic oocytes are more abundant, while fewer oogonia and primary oocytes were present. In the ripe and running stage, post vitellogenic oocytes which are the characteristic of that stage suggesting imminent spawning were more abundant (even though the earlier mentioned stages were also present). This gradation in oocyte maturation suggests that the oocytes do not mature at the same time in all the follicles and is a confirmation of multiple spawning in *A. occidentalis*. Same trend was observed in the males, where the histological study of the testes revealed various stages of sperm development, with spermatogonia and primary spermatocytes seen in all the six stages, but were more abundant in the immature and resting stages.

Table 3: Macroscopic and histological description of the gonad maturation stages of male *A. occidentalis*

| Maturity stage | Macroscopic description | Histological description |
|---|---|---|
| Immature (I) | Testes very tiny, paired, elongated and transparent with rough edges. The 2 testis lobes are fused, appearing like a single structure. GSI ranged from 0.01-0.97% with a mean of 0.10±0.12 SD | Many spermatogonia (S). Sperm ducts not very visible and few primary and secondary spermatocytes (SC) thinly scattered (Fig. 2a) |
| Maturing (II) | Testes darker than before, appearing pale red in colour, with rough edges. Testes not connected to each other, with no visible vascularization and of equal size. GSI ranged from 0.02 to 0.52%, with a mean of 0.14±0.10SD. | Spermatocytes (SC) more in number with the spermatogonia (S). Sperm ducts (SD) still thin, but more visible than in immature stage (Fig. 2b) |
| Mature (III) | Testes translucent and white in colour with rough edges. Each testis was convoluted like a twisted rope, with the two lobes close but not joined together and the left very slightly larger than the right. GSI ranged from 0.06 to 5.80% with a mean of 0.28±0.53SD. | Primary and secondary spermatocytes (SC) more in abundance with few spermatids (ST) and spermatozoa (SZ) in the sperm ducts. The sperm ducts (SD) thicker or fuller due to the spermatozoa and seminal fluid in them (Fig. 2c) |
| Ripe and running (IV) | Testes milky in colour and less translucent with the convolutions less firm. With pressure to the abdomen, milt flows out. GSI ranged from 0.05 to 0.51%, with a mean of 0.17±0.14SD. | More spermatozoa (SZ) in the sperm ducts which are distended. Fewer spermatocytes (SC) present than before (Fig. 2d) |
| Spent (V) | Testes darker, but still milky, opaque, flaccid and shrunken in size. With pressure to the abdomen, bloody milt comes out. GSI ranged from 0.01-1.17% with a mean of 0.09±0.18SD. | Few spermatozoa in sperm ducts and more spermatocytes seen. Conjunctive tissues beginning to form (Fig. 2e) |
| Resting (VI) | Testes lighter, pale white and shrunken in size but firmer than in spent condition. No bloody milt oozes out with pressure. The two testes clearly separated from each other. GSI ranged from 0.10 to 0.06%, with a mean of 0.03±0.02SD. | Sperm ducts (SD) empty with very few residual spermatozoa (SZ). Beginning of spermatogonia activity (S) (Fig. 2f) |

Table 4: Monthly occurrence of gonad maturation stages of female *A. occidentalis*

| | | Gonad maturation stages | | | | | |
|---|---|---|---|---|---|---|---|
| Month | Total No. examined | I | II | III | IV | V | VI |
| January | 05 | 05 | | | | | |
| February | 12 | 12 | | | | | |
| March | 23 | 14 | 08 | 01 | | | |
| April | 28 | 18 | 10 | | | | |
| May | 49 | 18 | 23 | 08 | | | |
| June | 69 | 23 | 29 | 16 | 01 | | |
| July | 72 | 02 | 05 | 42 | 11 | 12 | |
| August | 46 | | 02 | 14 | 24 | 06 | |
| September | 46 | | | 02 | 15 | 29 | |
| October | 18 | 07 | | | | 03 | 08 |
| November | 19 | 13 | | | | 02 | 04 |
| December | 11 | 11 | | | | | |
| Total MS | 398 | 123 | 77 | 83 | 51 | 52 | 12 |
| %MS | 100% | 30.90 | 19.35 | 20.85 | 12.81 | 13.07 | 3.02 |

MS: Gonad maturation stages

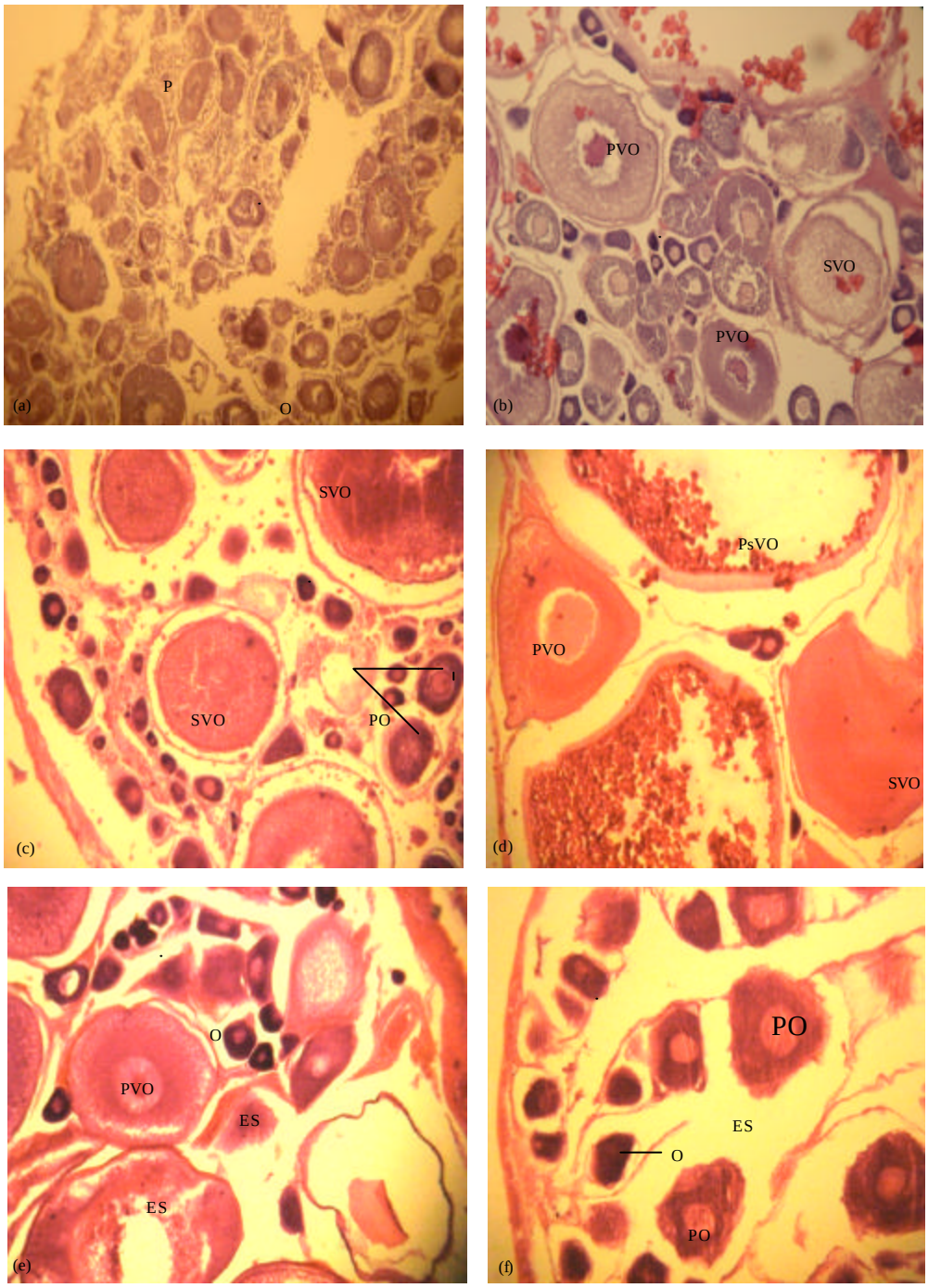


Fig. 1: (a-f) Ovaries of *A. occidentalis* at different stages of maturation-(a) immature; (b) maturing; (c) mature; (d) ripe and running; (e) spent and (f) resting. O: Oogonia; PO: Primary oocyte; PVO: Primary vitellogenic oocyte; SVO: Secondary vitellogenic oocyte; PsVO: Post vitellogenic oocyte; OW: Ovarian wall and ES: Empty spaces

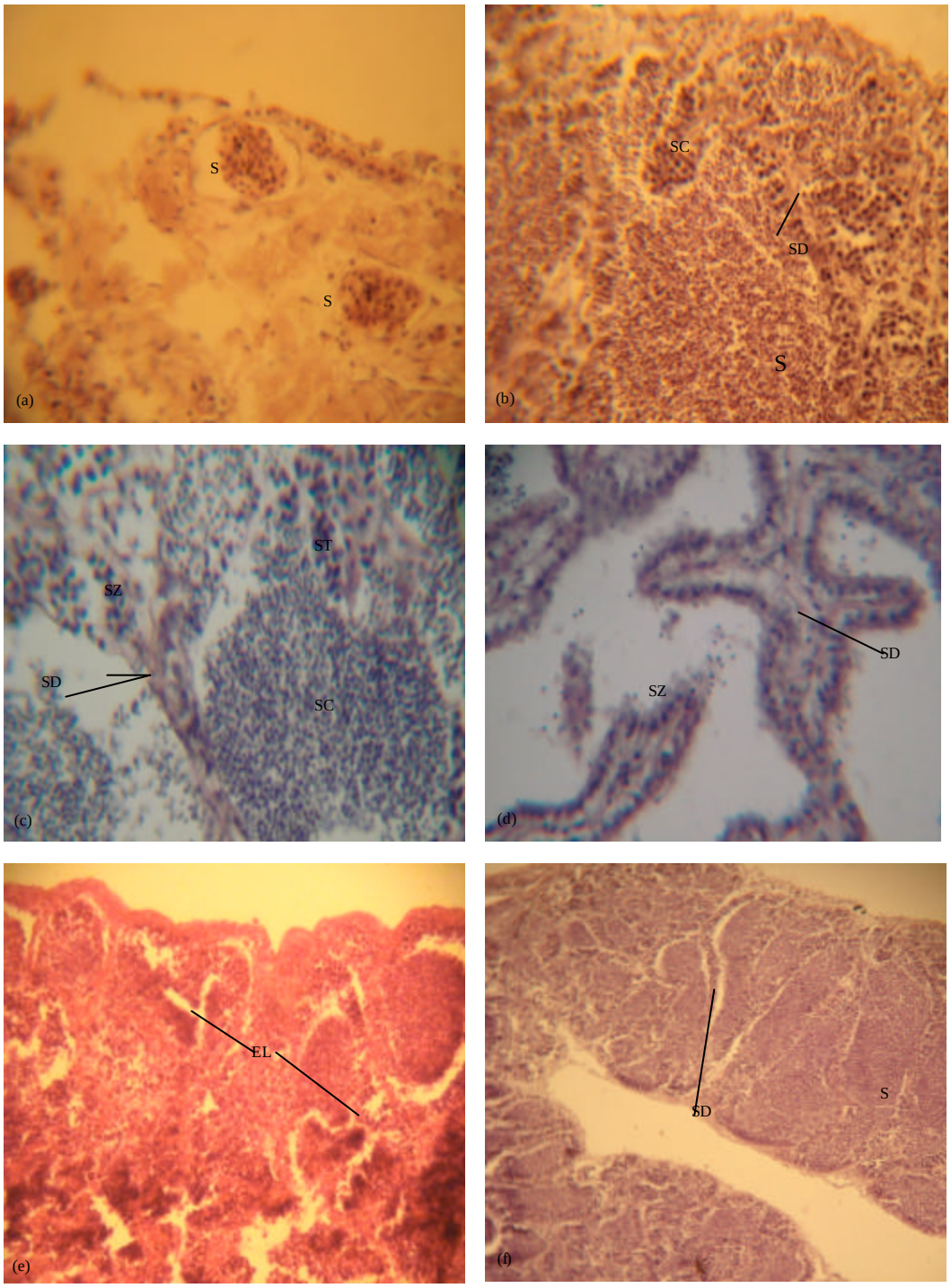


Fig. 2: (a-f) Testes of *A. occidentalis* at different stages of maturation-(a) immature; (b) maturing; (c) mature; (d) ripe and running; (e) spent and (f) resting. S: Spermatogonia; SC: Spermatocytes; SD: Sperm duct; ST: Spermatids; SZ: Spermatozoa and EL: Empty lumen

Table 5: Monthly occurrence of gonad maturation stages of male *A. occidentalis*

| | | Gonad maturation stages | | | | | |
|---|---|---|---|---|---|---|---|
| Month | Total No. examined | I | II | III | IV | V | VI |
| January | 15 | 15 | | | | | |
| February | 13 | 13 | | | | | |
| March | 23 | 22 | 01 | | | | |
| April | 30 | 22 | 08 | | | | |
| May | 36 | 10 | 24 | 02 | | | |
| June | 68 | 05 | 50 | 13 | | | |
| July | 81 | 01 | 18 | 60 | 01 | 01 | |
| August | 50 | 02 | 01 | 35 | 07 | 05 | |
| September | 50 | 01 | | 04 | 09 | 36 | |
| October | 10 | 05 | | | | 03 | 02 |
| November | 19 | 13 | | | | | 06 |
| December | 04 | 04 | | | | | |
| Total MS | 399 | 113 | 102 | 114 | 17 | 45 | 08 |
| % MS | 100% | 28.32 | 25.56 | 28.57 | 4.26 | 11.28 | 2.01 |

MS = Gonad maturation stages

The pattern of gonad maturation in this study revealed that *A. occidentalis* has an annual spawning cycle which has its peak between July and September in River Rima, which is during the rainy season. The reports of Daget (1954), Reed *et al.* (1967), Imevbore (1970), Reynolds (1974) and Olatunde (1989) further confirmed that spawning of most West African fishes appear to occur during rainy season. According to Hickling and Rutenberg (1936), species with a short spawning period have all the maturing eggs at about the same size, but in those with long or continuous spawning, there is a great succession of size between mature oocytes and immature follicles, as the *A. occidentalis* of this study.

## CONCLUSION

Based on macroscopic and histological procedures, six stages of gonad maturation, with an annual spawning season which occurs during the peak of the rainy season, was determined for *A. occidentalis* in River Rima. The species was also found to be a multiple spawner, releasing its reproductive products in batches over a long spawning period of about three months, which is from July to September.

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