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Testicular Development of the Male Lungfish, *Protopterus annectens* (OWEN) (Pisces : Sarcopterygii) in the Flood Plains of River Niger in Udaba-Ekperi in Nigeria

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Testicular development of the male African lungfish, *Protopterus annectens* (Owen) was investigated histologically. The testis was found to be an elongated structure that possessed two distinct portions : an anterior spermatogenic part that was made up of a system of testicular tubules and a posterior vesicular part that invaded the kidney tissue. Spermatogenesis can be divided into five stages : primary spermatogonia, secondary spermatogonia, spermatocyte, spermatids and spermatozoa. Based on the gonadosomatic index (GSI) and histological changes observed, the reproductive cycle can be divided onto four distinct stages : resting and quiescent (December to February), growing (March to June) ripe and spent (July to August) and postspawning (September to November). The GSI was the maximum on July when reproductive cells were mature, ripe and ready for spawning; and the minimum in August after fish spawned.

Key words: Gonadal development, Testis, *Protopterus annectens* (Owen)

Introduction

The lungfish have for long posed a challenge to the scientific world. Apart from their great evolutionary significance (Smith, 1930, 1935; Johnnel and Svensson, 1954; Rosen et al., 1981; Lander and Lien, 1983; Campbell and Berwick, 1987), their diphasic mode of life has intrigued biochemists and physiologists.

Protopterus annectens (Owen) is an African species that is found in Nigeria. While it is a popular protein delicacy in some Nigerian communities, there are others that have some reservations about the fish. This is partly because of its peculiar appearance and partly because of the taboos about the fish.

Not much scientific information on the biology of this fish is available, especially, in this part of the globe. The more reason why more research work is needed on it, especially, as attempts at fish culture has been geared up to provide more protein for the ever-rising population (Baer et al., 1992). Such information will also remove the various misconceptions about the fish in parts of the country.

The present investigation on the gonadal development of the male fish is an attempt in that direction.

Materials and Methods

Sample collection

Samples were collected on monthly basis between the first and second weeks of each month from January to December 2000 from river Orie and its flood plains around Udaba-Ekperi in Estako East local government area of Edo State of Nigeria. This river being a direct tributary of the bigger river Niger, the whole region can best be described as the flood plains of the

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latter.

A total of 180 adult male fish of 40.5~73.4 ㎝ in total length were used. Fish were collected with dragnets, fish traps, long lines and by manual diggings; and these were immediately conveyed to the laboratory for further analysis.

Histology of the testis

In the laboratory fish were measured (standard and total lengths : ㎝), weighed to the nearest gram and dissected to extract the testis. These were weighed and fixed in bouin's fluid for 24 hours, after which pieces were taken from the anterior spermatogenic and posterior vesicular portions and processed by standard histological procedures, embedded in pure paraffin wax and sectioned at a thickness of 5~7 ㎛. The sections were then stained in Heidenhain's haematoxylin and counter stained in eosin.

The procedures used for getting the dimensions of the germ cells and the estimation of the percentage composition of the different testicular germ cell types were similar to those used by Bhatti and Al-Daham (1978). For each portion of the testis, six randomly selected lobules were examined (i.e. a total of twelve lobules per testis). By means of a graph paper and camera lucida the lobules were demarcated into their boundaries. The areas covered by the different cell types were estimated by counting the squares on the graph paper covered by these cell types. The percentage composition of the cell types was then determined. The dimensions of the germ cells were measured with an ocular micrometer.

Results

Description of the testis

The gross morphology of the testis of *P. annectens* had previously been described by Wake (1979). It is an elongated structure which seldom exceeds 0.3% of the body weight. It is bound to the kidney and the dorsal wall by messenteries. It is often overlain with fats. It is stout medially but tapers laterally. Histological sections show that only the anterior portion of this structure is spermatogenic; the posterior is vesicular and invades the kidney tissue. Sexually mature testis ranged from 6.0~10.0 ㎝ in total length.

Spermatogenesis in *Protopterus annectens*:

Spermatogenesis can be classified into four stages: (1) spermatogonial, (2) spermatocyte, (3) spermatid, and (4) spermatozoon.

(1) Spermatogonial stage : The cell were quite large and spherical with large nuclei. These cells arose from the young undeveloped forms (germ cells) on the basement membrane of the testicular wall. The diameter ranged from 90.3~120.0 ㎛ (mean=103.3±1.70 ㎛). They occurred singly or in cluster of two to many cells. The first spermatogonia to develop were primary spermatogonia which developed to the secondary ones. The only distinguishing feature between the two forms was that the cell membrane of the latter was more obvious compared with the former. Also the primary spermatogonia were slightly larger than the secondary ones. The thickness of the testicular wall ranged from 65.2~81.2 ㎛ (mean=72.8±0.94 ㎛) (Plate I a and Table 2).

(2) Spermatocyte stage : Spermatogonia develop into primary spermatocytes, and the primary spermatocytes were differentiated to secondary spermatocytes by the first meiotic division. These were smaller cells which were mostly oval, but some appeared to be spherical in shape. The nucleus was very distinct but nucleoli were, however, not readily obvious. Their diameter ranged from 70.6~90.7 ㎛ (mean=81.10± 1.15 ㎛). The mean thickness of the testicular wall was 38.6±0.94 ㎛ (range 29.0~45.2 ㎛) (Plate I).

(3) Spermatid stage : These were smaller in size than the spermatotcytes; they were also more numerous. They appeared to taper on both sides. They were more towards the lumen of the testicular lobules. Many of these cells appeared to be irregular in shape. The nucleus was distinguishable. Their diameter ranged from 15.0~28.3 ㎛ (mean=20.8±0.79 ㎛). The testicular wall ranged from 20.1~30.2 ㎛ in thickness (mean=25.8 ±0.56 ㎛) (Plate I d and Table 2).

(4) Spermatozoon stage : These were the smallest of

the male germ cells with diameters which ranged 6.0~9.28 ㎛ (mean=7.6±0.18 ㎛). While some appeared clustered together at the lumen of the lobules, most were free with their tails very obvious (see Plate I e). The thickness of the testicular wall ranged from 15.0~25.9 ㎛ (mean=19.6±0.58 ㎛).

Reproductive cycle with testicular developmental phases

According to Hunter et al (1985), there are four main methods of determining the spawning period in fishes : visual evaluation of the maturity of the gonads, the use of frequency distribution of oocyte diameter, the establishment of the gonadosomatic index (GSI) of the fish and the examination of the histological sections of the gonads. The most commonly used of these are the last two (Gunn et al., 1989, Wang and Chem, 1989, Patzner et al., 1991, Pen and Potter 1991, Hyndes et al., 1992); and infact, De Valming et al (1982) exam-

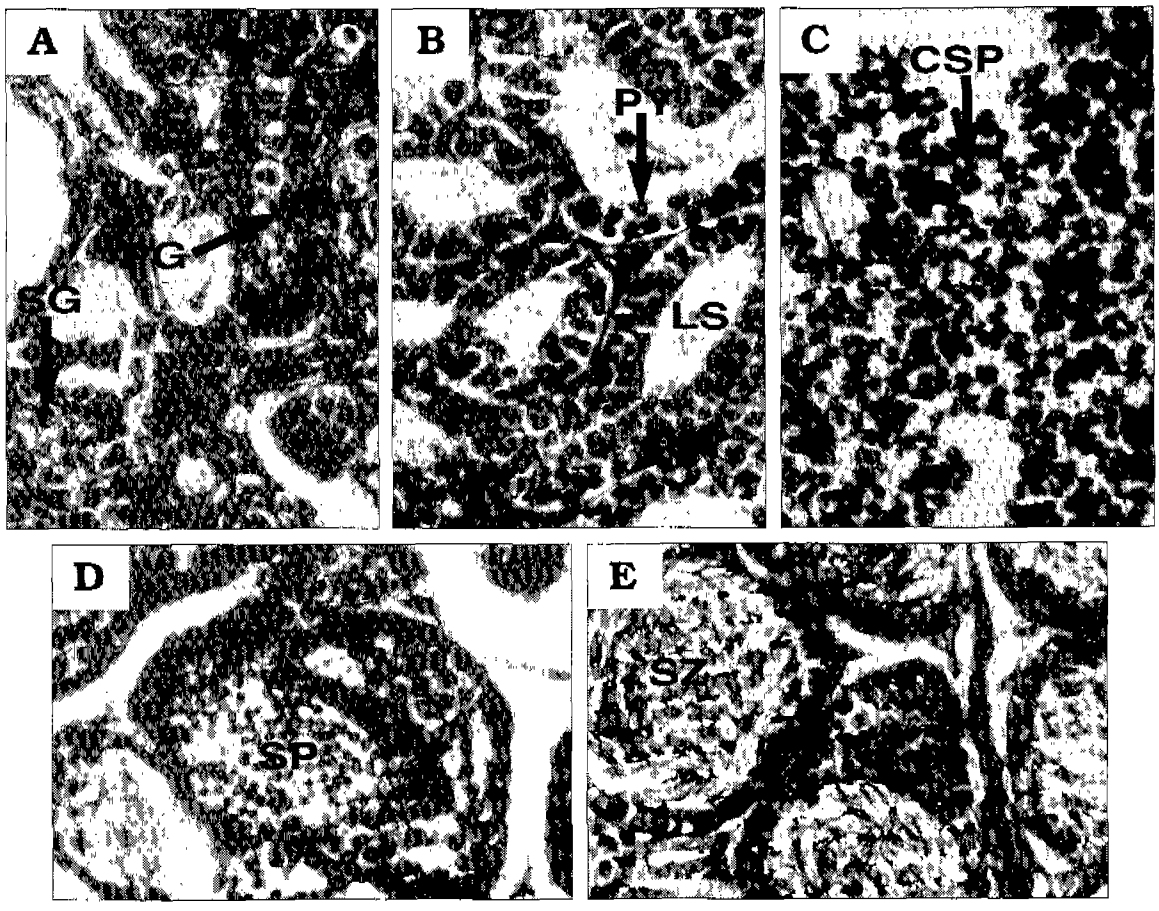

Plate I : Testicular developmental stages of the testis of the African lungfish, *P. annectens*.
A : Section of testicular tubules in the postspawning stage (September to November). Note the primary and secondary spermatogonia in the testicular tubules. B : The tubules in the resting and quiescent stage (December to February). Note a number of spermatogonia and spermatocytes around the lumen of the testicular tubule. C : The tubules in the growing stage (March to June). Note spermatogonia, spermatocytes, spermatids in the tubules. D and E : Section of the tubules in the ripe and spent stage (July to August). Note a number of spermatids and spermatozoa in the lumen of the tubules.
Abbrevations : PG, Primary speramtogonium; SG, Secondary spermatogonium; PY, Primary spermatocyte; SY, Secondary spermatocyte; SP, Spermatid; SZ, Spermatozoon; LS, Lumen of testicular tubules; CSP, Clusters of spermatids. A, B, D, E, ×400; C, ×400.

ined the use of GSI as indicator of the reproductive activity of a stock. In the ocean whitefish, he found that the GSI reflected the main period of reproductive activity adequately, He declared: "..... the gonadosomatic index is a reliable indicator of the spawning period ....." Hence these last two were used here. GSI represents the percentage of gonad weight to body weight during the month. It is assumed that the period when GSI is highest corresponds to the breeding season.

Different cell types in the histological sections were observed at different times of the year. Based on these histological observations as well as the GSI, the reproductive cycle with gonadal development in the testis of *P. annectens* can be divided into the following four stages:

(i) Postspawning stage
(ii) Resting and Quiescent stage
(iii) Growing stage
(iv) Ripe and Spent stage

(i) Postspawning stage: After spawning, the reproductive germ cells at this stage were mainly spermatogonia, hence the testis was small and hardly visible to the naked eyes. Spermatogonia ranged between 82~90% (Table 1). Only very few of the other stages were present. The GSI ranged between 0.92~1.10 (mean=1.02±0.05). The individuals in the postspawning stage appeared from September to November.

(ii) Resting and Quiescent stage: The gonadal development in this fish was investigated during the dry period when fish entered into its annual quiescent phase. The spermatogonia in the testis of the fish that was caught up during the period of drought would not develop further during the period and if any at all it would only be at the early part of the aestivation period. Such development would be minimal and would only involve the production of secondary spermatogonia from the primary forms and possibly few spermatocytes produced. Spermatogonia ranged between 97~98% (mean=97.7±0.20%). The GSI ranged from 0.69~0.70 (mean=0.69±0.01). The drop in GSI at this period must be due to the fact that all energy at this stage is directed at survival rather than development. The individuals in the quiescent stage appeared from December to February.

(iii) Growing stage: The growing stage can be subdivided into the early and late growing stages.

① Early growing stage: As germ cells in the testis began to grow, they were much more larger in size and now visible to the naked eyes. Spermatogonia

Table 1. Annual cyclic changes in the gonadosomatic index (GSI) and the relative abundance of the various spermatogenic stages in the testis of the African lungfish, *Protopterus annectus*

Stage of maturity	Month	Mean body weight (g)	Mean gonad weight (g)	Mean GSI	Mean Spermatogonia (%)	Mean Spermatocytes (%)	Mean Spermatids (%)	Mean Spermatozoa (%)
Resting (postspawning)	September	393.3±58.9	4.3±0.67	1.10±0.06	82.4±0.23	6.1±0.14	2.6±0.15	11.7±0.14
	October	458.9±46.0	4.8±0.58	1.04±0.05	87.6±0.21	7.7±0.16	5.4±0.16	3.9±0.10
	November	510.0±217.3	4.6±1.73	0.92±0.03	96.1±0.19	5.2±0.13	-	-
Dormant/ Quiescent	December	477.7±192.9	3.2±1.17	0.70±0.02	98.1±0.27	2.1±0.14	1.5±0.18	-
	January	563.1±202.0	3.8±1.20	0.69±0.01	97.6±0.15	3.0±0.14	-	-
	February	613.4±292.4	4.2±2.02	0.69±0.01	97.3±017	3.0±0.18	-	-
Young immature (preparatory)	March	392.5±54.3	6.2±0.89	1.00±0.13	96.9±0.77	5.6±0.50	2.4±0.28	-
	April	439.7±88.8	11.9±3.20	1.50±0.26	50.0±0.35	31.0±0.42	10.6±0.36	5.4±0.53
Fast developing maturing (prespawning)	May	510.1±78.8	15.8±4.19	2.00±0.15	20.7±0.32	14.2±0.44	37.4±0.35	50.7±0.44
	June	410.2±56.6	17.2±4.14	4.50±0.12	8.1±0.10	7.1±0.13	30.0±0.13	60.5±0.18
Mature and ripe (spawning)	July	304.4±17.0	16.3±0.87	5.40±0.16	25.0±0.08	7.1±0.12	19.4±0.23	95.7±0.20
	August	328.9±12.10	5.7±0.27	0.5±0.06	60.1±0.16	21.3±0.14	15.1±0.17	27.3±0.24

and spermatocytos were seen to line the periphery of the testicular tubules. There was slight drop in the relative abundance of the spermatogonia (range : 50.0 ~96.9%, mean=73.5±0.56%), while the other more advance stages were becoming more evident and more medially located. The GSI peaked up again and began to increase (range : 1.00~1.30; mean=1.25±0.20). This stage occurred at the end of the dry period and the very early part of the raining season (March~April).

② Late growing stage: It was a stage that witnessed a rapid production of the more advance stages of the reproductive cells. There was considerable drop in the relative abundance of the spermatogonial cells (range 8.1~20.7%; mean = 14.4± 21%). On the other hand there was tremendous in crease in the relative abundance of spermatozoa (range 50.7~60.5%, mean=55.6±0.31%). The testis also had increased considerably both in size and weight especially the spermatogenic portion. The tubules were lined with spermatocytes and spermatids in various developmental stages. Spermatids and spermatozoa were very evident in the lumen (Plate I d). GSI had also increased considerably (range 2.00~ 4.50%, mean=3.25±0.14) (Table 1, Fig. 1 and 2). The individuals in the late growing stage occurred between May and June.

(iv) Ripe and Spent stage : These were completely filled with spermatids and spermatozoa, especially, the latter (mean=95.7±0.20%). By early August, the entire spermatogenic part of the testis was filled with spermatozoa, especially, in the lumen while there was sharp decrease in the other stages (Plate I e). These were only evident at the periphery of the tubules. GSI was at its maximum in July (mean=5.40±0.16). Spawning occurred before mid August; hence the drastic drop in the relative abundance of the spermatozoa in August (mean=27.3±0.24%) and the GSI value (mean= 0.50±0.06). By the end of August spermatogonia had shown some considerable increase (mean=60.1±0.16%), showing that as soon as spawning was completed, the fish immediately directed its energy apparatus to the production of new spermatogonia in preparation for a new reproductive cycle. Ripe and spent testis appeared between July and August.

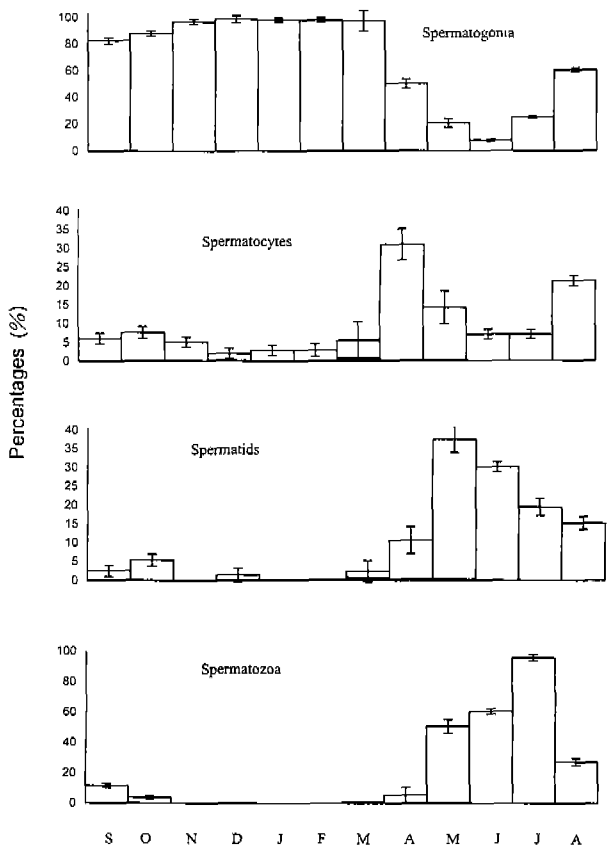

Fig. 1. Monthly changes in percentages of the spermatogenic stages of the testis of *P. annectens*. Vertical bars denote ×10±S.E of the mean (95% confidence limit).

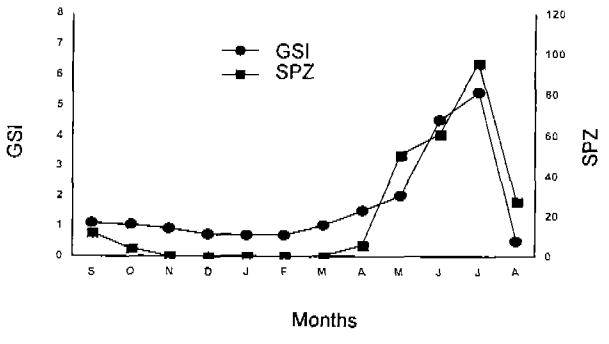

Fig. 2. Monthly changes in mean spermatozoa (SPZ) and mean gonadosomatic index (GSI) of *P. annectens*.

Discussion

The study of gonadal development and the repro-

Table 2. Average sizes and size ranges (in μm) of the spermatogenic cells of the African lungfish, *P. annecteus* (At least 30 cells of each spermatogenic cell type were measured using ten fish)

	Spermatogonia	Spermatocytes	Spermatids	Spermatozoa
Mean cell diameter	103.3±1.70	81.1±1.15	20.8±0.76	7.6±0.18
Range	90.3~116.2	70.6~90.7	15.0~28.3	5.5~9.2
Mean thickness of the testicular wall	72.8±0.94	38.6±0.94	25.8±0.56	19.6±0.58
Range	65.2~81.3	29.0~45.2	20.1~30.2	15.0~25.9

ductive cycle in the testis of the African lungfish, *Protopterus annectens* has revealed four main phases: resting and quiescent stage (December~February), growing stage (March~June), ripe and spent stage (July~August) and postspawning stage (September~November). The different stages of spermatogenesis including spermatogonia were observed in histological sections. Maximum activities were evident during the reproductive period. Grier et al (1980) reevaluated testicular morphology in several orders of teleosts and concluded that fishes could be divided into two different tubular types: (i) salmoniform, perciform and cypriniform fishes and (ii) antheriniform fishes. While the former have spermatogonia distributed along the length of the testicular tubules, the later have theirs only in the distal end of the tubules. The present finding has shown that *P. annectens* falls into the first group. By this it is clear the fish shares the primitive osteichthyan pattern of many teleosts and in this way differs from the amphibian with which it also shares many features (Wake, 1979). Moreover, while spermatogonia have been seen to occur singly in a number of teleosts such as *Tilapia leucotica* and *Oncorhynchus masou* (Hyder, 1969, Hiroi and Tamamoto, 1970), these cells in *P. annectens* occurred both singly and in clusters.

The weights of the testis follow a regular cyclical changes in correlation with the spermatogenic activity being go on within them. This indicates the seasonal changes in the gonadosomatic index (GSI). In September to November and December to February (of the following year), when the testis were in the resting and quiescent/dormant stages respectively, the GSI initially rose slightly before again falling. The spermatogonia were in relative abundance at this stage, an indication that there was no significant cellular activity in the testis at this period. Infact cellular activity is always at a standstill during the dormant/quiescent period of aestivation. Only spermatogonia (usually primary) were produced as at then. No production of the other more advance spermatogenic cells, viz., spermatocytes, spermatids and spermatozoa. In the growing stage (March~July), the GSI increased as a result of a higher level of cellular activity. While there was pronounced the increase in the relative abundance of the more advance reproductive units, there was decrease at the lower level. In July (the prespawning), the GSI reached the peak showing maximum production of spermatozoa at this period. In August (the spawning month), the GSI fell to the minimum due to loss of spermatozoa in the process of spawning. This GSI again began to increase gradually during the postspawning months of September to November.

Moreover, the testicular wall gradually decreased in thickness from 72.8±0.94 μm (at the resting stage) to 19.6±0.58 μm (at the spawning stage) (Table 2). As soon as spawning was over, the testicular wall grew thicker once more. Htun-Han (1978) had suggested that the thinning out of the walls at the spawning stage was probably due to distension caused by the increased weight of the testicular content at spawning and the intense activity within. Also the increased thickness of the wall after spawning was possibly due to the contraction of this after the release of the spermatozoa during spawning.

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