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Seasonal Changes in the Ovary of the African Lungfish *Protopierus annectens* (Pices : Sarcopterygii) in the Flood Plains of River Niger in Etsako East Local Government Area of Nigeria

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We investigated the gonadosomatic index (GSI), germ cell development, reproductive cycle of the Afriean lungfish *Protopierus annecteus* (Owen) by histological observations and morphometric data.

Samples were collected from the river Orie and its flood of Nigeria, from January to December 2000. The fish is dioecious and oviparous. Monthly changes in the gonadosomatic index (GSI) showed a similar pattern to change in the mean oocyte diameter and the reproductive cycle. The reproductive period occurred from March to July-August; the spawning period was once a year between July and August, and the main spawning occurred in August when active and voracious feeding occurred during the rainy season. In the resting (dormant) stage after spawning, fish stopped feeding and aestivated during the dry season from December to February.

The reproductive cycle of the species can be divided into five successive stages, quiescent stage (March to April), developing/maturing stage (April to June), ripe/spawning stage (July to August), post-spawning stage (September to November), and resting (dormant) stage (December to February).

Key words: Seasonal changes of ovary, African lungfish, *Protopterus annectens*, River Niger

Introduction

Apart from the great evolutionary importance of the African lungfish, *Protopterus annectens* (Owen) (Johnnel and Svensson, 1954; Grigg, 1965; Roden et al, 1981; Young, 1981), the fish holds a great promise as a rich source of protein (Lander and Lien, 1983; Florey, 1987; Lien, 1987). This is evident in its popularity as delicacy amongst the local populace. Unfortunately, many aspects of the biology of this fish are still very much obscure.

Recent attempts at lungfish culture and breeding as well as its use as biological control agents for disease vectors with aquatic larval stages (Greenwood, 1986; Baer et al, 1992) have made it necessary to focus more attention on its reproductive activity. Little information on the ovarian cycles of this fish is almost non-existent. The purpose of the present study is to understand the reproductive cycle with germ cell development and the spawning season by histological method and morphometric data.

Materials and Methods

Sampling

Samples of the African lungfish, *P. annectens* for the

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histological study were collected monthly between the first and second week of each month by using the dragnets, fish traps and long lines in the river Orie and its flood plains in Etsako East local government area of Edo state of Nigeria from January to December 2000. A total of one hundred and twelve (112) adult female lungfish samples ranging from 35.0 cm to 56.0 cm in total length were used for the present study. After the fish were transported alive to the laboratory, standard and total length (cm) were measured by a Vernier caliper, and their total weights (g) and gonad weights (g) were determined using a chemical balance.

The gonadosomatic index (GSI) was calculated according to the formula of Meien (1927)

$$GSI = \frac{\text{gonad weight (g)}}{\text{total weight (g)}} \times 100$$

and the monthly maxima, minima, means and standard deviations were obtained to determine the general trends of the reproductive activity.

Histological preparations were made for analysis of the gonadal phases by light microscopy. The ovarian tissues were preserved in Bouin's fluid fixative for 24 h, and then washed with running tap water for 12 h. Tissues were then dehydrated in alcohol and embedded in pure paraffin wax and sectioned at 5~7 ㎛ thick using a rotary microtome. The sections were then stained with either Heldenhain's iron haematoxylin-0.5% eosin or Heidenhain's Azan (Pantin, 1959), and examined.

The procedure of measurement of the diameters of the oocytes were similar to those used by Htun-Han (1978). The measurements were done with a calibrated eye-piece micrometer. Cell stages such as oogonium, primary, secondary and tertiary vitellogenesis and so on were not measured by staging as in Nikolsky (1963); instead these stages were identified (and described) by a critical visual examination of the slides month by month. To eliminate bias all the slides were examined at the end of the sampling process.

Results

Position of the ovary

The ovaries in the African lungfish, *P. annectens* are elongated structures extending from close to the heart to the posterior end of the kidneys. They exist in pairs and are attached to the body wall posteriorly and bound tightly to the oviduct by mesentery.

Gonadosomatic index (GSI)

The GSI values here (Table 1 and Fig. 2)were very low (3.01±0.09~0.91±0.07%) from December to February which corresponded with the heart of the dry season when fish aestivated. The GSI in female increased from March to June (3.02±0.16~5.86±0.13%) when fish had emerged from its stupor to resume active feeding. The GSI reached the maximum in July (9.10±0.12%) which corresponded with the period when the rains were still heavy. A decline was observed in August (2.01±0.12%) followed by gradual increase in September (2.05±0.16%) and these marginal increases continued in the subsequent months (October to December) after which it remained more or less constant. By April the GSI values began to increase again until it reaches the highest peak in July.

Germ cell development in the ovary

The monthly increases in oocyte diameters were taken as the criteria for the development and growth in the ovary. The trends in the GSI and oocyte diameters (Fig. 1,2) show clearly that the reproductive period of *P. annectens* extended mainly from March to July-August when fish spawned. Germ cells (oogonia) appeared immediately after spawning, sometimes in August-September. The growth rate between this time and March of the following year was very minimal coming to complete halt between January and March, which happen to be the heart of the dry season when fish aestivated. Oogonia and oocytes in the post-spawning/resting stages appeared from September to December. At this time, the oocytes have a large

Table 1. Histological changes observed in the ovary of *P. annectens* during the reproductive cycle

State of the fish	Ovarian developmental stage	Months	Sample size	Mean body weight (g)	Mean total length (cm)	Mean GSI	Mean ovum/ oocyte diameter (mm)	Characteristic features of ova/oocytes
Fish prepare to come out of aestivation	Quiescent stage	March	8	293±31.8	36.1±1.35	3.02±0.16	0.16±0.02	Condition remained as in February
Fish emerged from aestivation and commenced active and voracious feeding	Quiescent and developing stage	April	8	350.9±48.7	36.0±1.78	3.13±0.19	0.35±0.03	Ring of vacuoles were clearly visible in the cytoplasm. The younger oocytes at this stage possessed yolk droplets all around the vacuoles in the cytoplasm. The older ones on the otherhand possess larger globules compared with the younger.
	Developing and maturing stage	May	9	374.6±64.5	38.6±2.62	4.70±0.24	0.83±0.03	
	Maturing stage	June	9	507±39.8	45.8±1.27	5.86±0.13	1.23±0.04	
	Ripe and spawning stage	July	11	535.3±55.0	45.5±1.46	9.10±0.12	1.99±0.60	Oval/eggs were large occupying the entire space of the ovary. Yolk at this stage appeared much more homogeneous. Ovary wall thin out. Most cells have irregular shapes.
	Spawning stage	August	11	480.9±30.7	43.7±1.25	2.01±0.12	0.01±0.00	
Active feeding progressively reduced in preparation for aestivation	Post-spawning stage	September	9	420.3±22.2	41.7±1.17	2.05±0.16	0.02±0.00	The oocytes were very small(germ cell stage) and spherical. Nucleus was readily visible with large nucleolus.
	Post-spawning stage	October	9	564.1±51.3	46.5±1.94	2.20±0.12	0.05±0.00	
	Post-spawning stage	November	9	297.2±41.9	34.04±0.92	2.80±0.09	0.11±0.01	Oocyte sizes were larger when compared with the earlier stages. So also were the nuclei. Nucleolus were many and distinct. Easily identified in the nucleus were chromosomes at different stages of meiotic division.
Fish stopped feeding and aestivated. Biochemical activity brought almost to a halt	Resting (dormant) stage	December	9	361.3±75.9	35.7±2.89	3.01±0.09	013±0.02	
	Resting (dormant) stage	January	9	366.7±37.6	40.0±1.36	3.01±0.09	0.16±0.02	The appearances of the oocytes remained as they were in December
	Resting (dormant) stage	February	10	510.5±42.7	42.9±1.61	3.02±0.07	0.16±0.02	

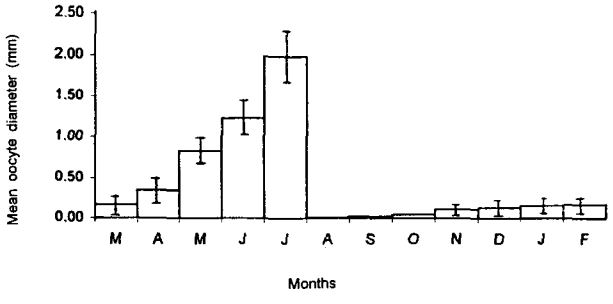

Fig. 1. Monthly changes in the mean oocyte diameter (mm) of lungfish, *P. annectens*. Vertical bars denote standard error of the mean (95% confidence limit).

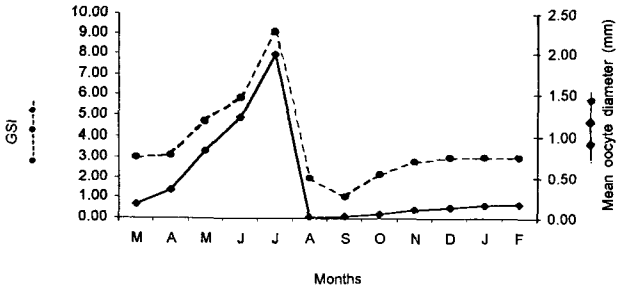

Fig. 2. Monthly changes in gonadosomatic index of lungfish, *P. annectens*.

nucleus with centrally located nucleoli (see Plate 1a). The months of January to March (dry/drought months) were resting stage when fish aestivated and became quiescent (See Table 1 and Plate 1b).

Individuals in the immature and developing stage occurred in April when fish had come out of aestivation and commenced active feeding. Oocytes at this stage were fast developing but immature or obviously representing the previtellogenic phase when oocytes actively developed in preparation for yolk accumulation (vitellogenesis). The individuals in the mature stage appeared from early May when the process of yolk deposition commenced and oocytes rapidly went through primary, secondary and tertiary vitellogenesis. Active vitellogenesis was characterized by yolk droplets and many globules (see Plate 1c). By July the

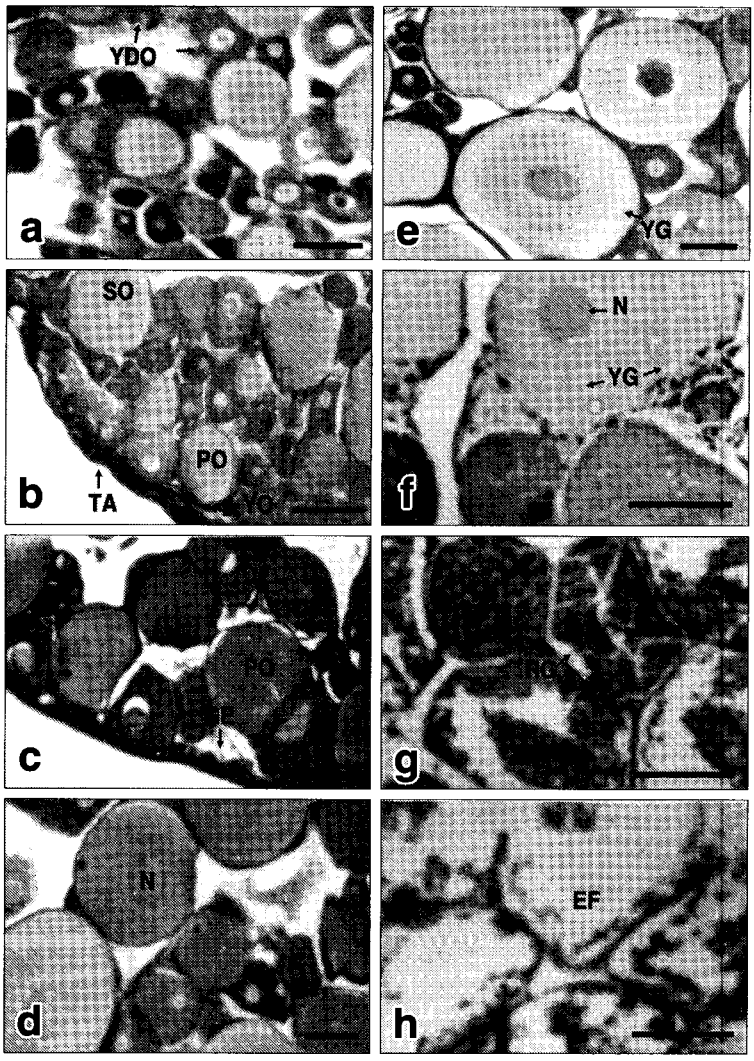

Plate 1. Photomicrographs showing the seasonal changes in the ovary of *Protopterus annectens* (O). (a) Post-spawning (Sept.-Oct.), (b & c) oogonia (YO) develop from germinal epithelium (GE) to produce developing oocytes (DO) between Nov. and March. This stage showed in the dry/drought period. Fish becomes dormant/quiescent. No further development except slight size increase as seen in c. (d & e) Developing stage between April and May when oocytes accumulate yolk granules droplets (YG) and increase rapidly. (f) Oocytes attain the maximum size in June. (g) Oocytes mature and become ripe (RO) ready for spawning (July/August). (h) Post-spawning stage (from September to November) when the ovary appears empty (EF). Scale bar; 25 μm in a, b & c, 60 μm in d & e and 125 μm in f, g & h. Other abbreviations: N = Nucleus; TA = Tunica albuginea; YDO = young developing oocyte.

process of vitellogenesis had been completed and maturation had started and by the end of the month, ova/oocytes were at the hyaline/ripe stage in preparation for ovulation and spawning in late July/early August. Oocyte diameters were at their largest sizes at this time and the period was histologically characterized by many vacuoles in the cytoplasm.

Discussion

A widely used criterion for determination of the spawning periods of a species of fish is the gonadosomatic index (GSI) combined with the histological characteristics of germ cells during the developmental period of the oocytes (Gunn, et al., 1989; Wang and Chen, 1989; Patzner, et al., 1991; Pen and Potter, 1991; Hydes et al., 1992). The period when GSI reached the highest and rapidly dropped has been known to correspond to the breeding season of the fish. Therefore it is assumed that the breeding (spawning) season of this species is between July and August. Monthly changes in the GSI showed a similar pattern to changes in the mean oocyte diameter and the reproductive cycle.

The lungfish, *P. annectens* has two annual phases; the phase of activity (rainy season phase) and that of inactivity/quiescence (dry/drought season phase). From this, it is obvious that fish has to be very active during the short raining period in order to adequately prepare for the dormant life of dry/drought period.

The present investigation shows that the annual ovarian cycle of this fish can be divided into five stages.

(i) *Quiescent stage* : ovary consists of very small spherical oogonia. The individuals in this stage appeared between March and April.

(ii) *Developing/maturing stage* : when oocytes increase rapidly in size due to accumulation of yolk materials in the oocytes cytoplasm during vitellogenesis. The individuals in this stage were found from April to June.

(iii) *Ripe/spawning stage* : when oocytes had accumulated enough yolk, becomes matured and ripe ready for ovulation and spawning. This stage occurred between late July and early August.

(iv) *Post-spawning stage* : fish has spawning and the ovary becomes empty. Soon oogonia appeared in preparation for a new reproductive cycle. The individuals in this stage appeared between September and November.

(v) *Resting stage* : oocytes stop any further development or increases in size as a result of fish becoming quiescent. This stage appeared between January and February.

There is, therefore, every evidence that early part of lungfish aestivation witnesses some level of growth in the oocyte. This is evidenced in the fact that by March (which is the tail end of the quiescent period) mean oocyte diameter had increased slightly from 0.13±0.02 mm in December to 0.16±0.02 mm. Moreover, the pattern of ovarian cycle described as "metachronism" (Nagahama, 1983) whereby fish spawns more than once during a single reproductive cycle (as evident by the presence of oocytes in all gonadal developmental stages) is absent in *P. annectens*. It is assumed that this fish spawns more than once during the spawning period.

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