



# International Journal of Fisheries and Aquatic Studies

ISSN: 2347-5129

IJFAS 2015; 2(3): 74-80

© 2015 IJFAS

www.fisheriesjournal.com

Received: 12-11-2014

Accepted: 18-12-2014

**P. Chakrabarti**

Fisheries Laboratory, Department  
of Zoology, University of  
Burdwan, Burdwan 713 104, West  
Bengal, India

**A. S. Banerjee**

Fisheries Laboratory, Department  
of Zoology, University of  
Burdwan, Burdwan 713 104, West  
Bengal, India

## Histological findings and seasonal distribution of different germ cells in the testicles of freshwater needle fish, *Xenentodon cancila* (Hamilton)

**P. Chakrabarti, A. S. Banerjee**

### Abstract

The cytomorphological changes occurred in the testicles of *Xenentodon cancila* (Hamilton) were elaborated during different reproductive phases. The testicular lobules were synchronously arranged with various stages of germ cells such as spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa. The seminiferous tubules were made up of spermatogenic cells located within cysts during growth phase. However, the seminiferous tubules were dominated by cysts of spermatocytes, spermatids and spermatozoa during maturation phase. Pubertal seminiferous tubules were packed with spermatids and spermatozoa. In the post spawning phase the tubules contained remnants of spermatozoa and spermatogonial cysts. The Leydig cells were close to the blood vessels in the interstitium. The sequential proliferative pattern of various germ cells in the tubular spaces was correlated with gonadosomatic index during different reproductive phases.

**Keywords:** Histology, Seasonal distribution, Germ cells, Testicles, *Xenentodon cancila*

### 1. Introduction

The biological process, especially the reproductive biology is the most important factor concerning the successful management of fisheries and mobilization of seed resources. Teleosts exhibit variations in testicular structure and spermatogenic patterns<sup>[1, 2, 3]</sup>. The fish testis parenchyma is formed from two main compartments, seminiferous lobules and interstitial tissue<sup>[4]</sup>. The anastomosing tubular testis formed compartments which undergo cyclic changes. Histological studies offer a scope to understand the cellular kinetics of gonad, recruitment, development and reabsorption of gonadal cells and finally in staging the maturity state of the gonads. For a proper sustainability of a fish species, a thorough study of maturation cycles and alterations of gonads are important since such a study is aimed in understanding and predicting the annual changes of the population<sup>[5, 6, 7, 8]</sup>. Therefore, the objective of the present study is to investigate the event of spermatogenesis and the annual cyclical changes in the testis of *Xenentodon cancila* (Hamilton). Hopefully, such study will be useful in the development of spawning and population dynamics as well as management of this endemic fish species.

### 2. Materials and Methods

A total number of ten (10) adult male specimens of *X. cancila* (approximately 15 to 20 cm in length and 18 to 42 g weight) were collected from river Damodar, Burdwan, West Bengal, India during December 2012 to November 2013. After sacrificing the fish, the testicles were dissected out and the data on total body weight and testes weight were taken to the nearest milligram to calculate the mean gonadosomatic index (GSI) from the following formula:

$$GSI = \frac{\text{Weight of the testes}}{\text{Weight of the testes}} \times 100$$

For histological studies immediately after collection the testes were quickly removed, cut into small pieces and were fixed in aqueous Bouin's fluid for 18 hrs. After fixation the tissues were washed repeatedly in 70% ethanol, dehydrated properly through ascending series of ethanol, cleared in xylene and embedded in paraffin wax of 56-58 °C under a thermostat

**Correspondence**

**P. Chakrabarti**

Fisheries Laboratory,  
Department of Zoology,  
University of Burdwan,  
Burdwan 713 104, West Bengal,  
India.

vacuum paraffin embedding bath for a period of 1 hr. After routine histological procedure the deparaffinised sections were brought to water through descending series of ethanol and stained with Delafield's haematoxylin-eosin and iron alum haematoxylin stain sections were dehydrated through upgraded ethanol series, cleared in xylene, mounted permanently with DPX and then examined and photographed under Olympus-Tokyo PM 6 compound microscope. The diameter of various spermatogenic cells were measured with the help of reticulo-micrometer and ocular micrometer.

### 3. Results

The testes of *X. cancila* remain attached to the body wall by means of mesenteries. Each testis is enclosed by an outer thin peritoneum beneath which there is an inner thick tunica albuginea made up of dense connective tissue.

#### 3.1. Gonadosomatic index (GSI)

In the present study it has been observed that the values of GSI in *X. cancila* follow a regular cyclical change during growth, maturation, spawning and post-spawning phases. The lowest GSI value was noticed during the end of post spawning phase in November. During December i.e. early growth phase the mean GSI value increased subsequently. During the growth phase i.e. in January and February the GSI value recorded is gradually higher. However, from March onwards when the testes enter into the maturation phase GSI is gradually increased and in June GSI rises sharply. In July the testes are constituted with full of spermatids and spermatozoa and the GSI rises up to peak value and in August the GSI value shows a declining trend. In the post spawning period i.e. in September and October the GSI value is significantly declined as the testes suffered from a regression state (Table 1) (Fig. 11).

**Table 1:** Monthly variation of gonadosomatic index (GSI) of testis of *Xenentodon cancila* with average value of fish weight and testis weight.

Phase	Month	Fish weight (mean value in g)	Testis weight (mean value in g)	GSI (mean value)
Post-Spawning	September	17.250	0.036	0.210 ± 0.0105
	October	17.125	0.033	0.189 ± 0.0095
	November	17.777	0.033	0.187 ± 0.0094
	December	18.333	0.047	0.256 ± 0.0128
Growth	January	18.500	0.053	0.290 ± 0.0145
	February	36.667	0.147	0.399 ± 0.0199
	March	40.166	0.175	0.436 ± 0.0218
Maturation	April	37.400	0.182	0.486 ± 0.0243
	May	32.500	0.240	0.738 ± 0.0369
Spawning	June	23.750	0.247	1.042 ± 0.0521
	July	33.000	0.443	1.342 ± 0.0530
	August	18.600	0.187	1.007 ± 0.0504

#### 3.2. Histological architecture of the testicle

Testis of *X. cancila* is composed of anastomosing network of different shaped seminiferous tubules, characterized with germinal epithelium and central lumen. Five types of germ cells viz, spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa are identified in the seminiferous tubules during different reproductive phases (Figs. 1, 3, 5 and 7).

##### 3.2.1 Spermatogonia

These are the largest of all the spermatogenic cells, forming nest and attached to the inner margin of the lobule boundary wall. Each spermatogonium contains prominent cytoplasm and centrally placed nucleus. The diameter range of these cells varies from  $15.3 \times 11.60 \mu\text{m}$  to  $20.5 \times 17.03 \mu\text{m}$  (Figs. 1 and 2).

##### 3.2.2 Primary spermatocyte

The primary spermatocytes contain relatively lesser amount of cytoplasm and the nucleus is deeply stained with haematoxylin. These cells are almost oval or rounded and diameter varies from  $9.32 \times 8.2 \mu\text{m}$  (Figs. 1, 3 and 4).

##### 3.2.3 Secondary spermatocyte

The secondary spermatocytes arising from the division of the primary spermatocytes are smaller and nearly spherical in shape. The cytoplasm of these cells are difficult to distinguish but the nucleus is condensed. The size of these cells approximately measure  $3.61 \times 4.23 \mu\text{m}$  (Figs. 1, 3 and 4).

##### 3.2.4 Spermatids

The spermatids are further reduced in magnitude ( $2.91 \times 2 \mu\text{m}$ ). These cells are deeply stained with iron alum haematoxylin (Figs. 3, 4 and 5). They are characterized with extremely compact and intense basophilic elliptical nuclei.

##### 3.2.5 Spermatozoa

These are the eventual consequence of spermatogenesis and smallest one of all the spermatogenic cells with an average diameter of  $1.90 \times 1.72 \mu\text{m}$ . It has strong affinity to haematoxylin (Figs. 4, 7 and 8).

##### 3.2.6 Interstitial cells

The interstitial cells are round or oval in shape and reside singly or in small groups between the lobular spaces and associated with the blood vessels (Figs. 3, 4, 5 and 8). The interstitial cells undergo changes in morphology in different seasons.

#### 3.3. Cyclical changes during spermatogenesis

On the basis of gonadosomatic index (GSI) and the occurrence of various spermatogenic cells the reproductive phases of *X. cancila* can be disassembled in four phases: growth (December to February), maturation (March to May), spawning (June to August), post spawning (September to November).

##### 3.3.1. Growth phase (December to February)

During early growth phase the predominant spermatogonia are arranged in a definite pattern and few spermatocytes and

spermatids are also present in between them (Figs. 1 and 2). In the late growth phase, the testis is characterized by the presence of all stages of the spermatogenic cells. The primary and secondary spermatocytes are gradually increased in number and cluster of spermatozoa can be seen inside the lumen (Fig. 3).

**3.3.2. Maturation phase (March to May)**

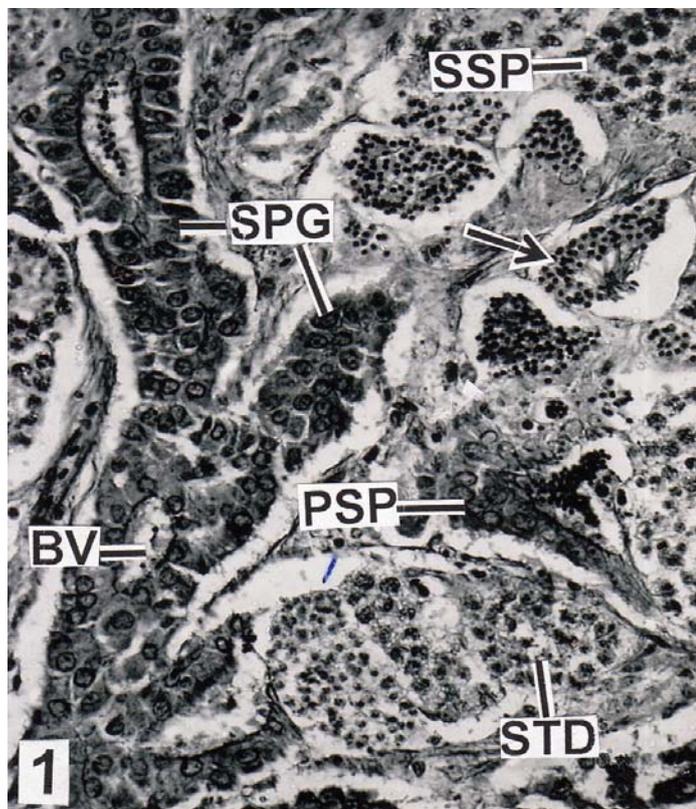
The lobule boundary wall of the testis has become considerably thin and spermatogonia cells are reduced in number and restricted along the boundary wall of the lobules. The spermatocytes are reduced considerably and gradually transformed into the spermatids and spermatozoa (Figs. 4 and 5). The active interstitial cells are noticed in between the lobules (Fig. 5).

**3.3.3. Spawning phase (June to August)**

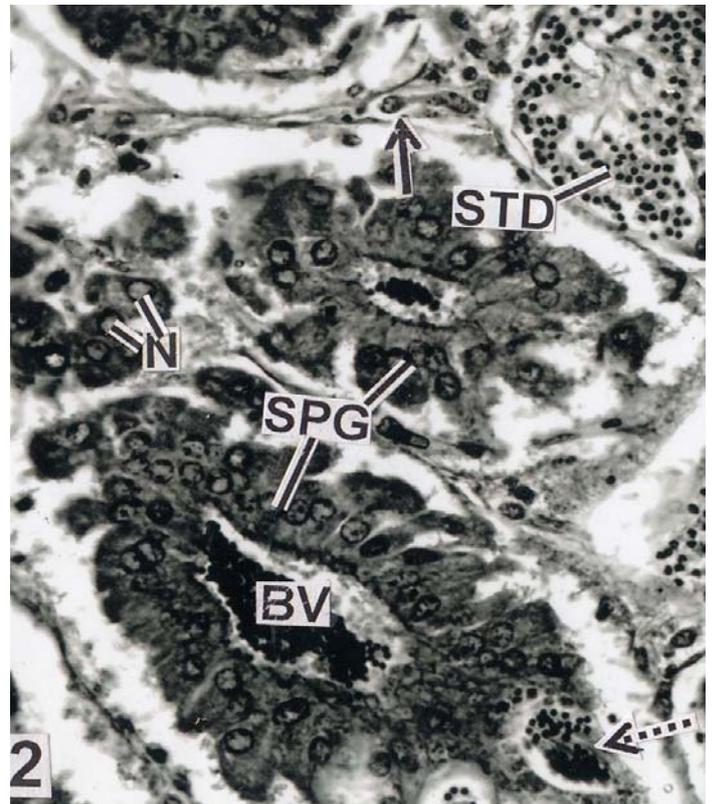
The lobule boundary wall is extremely thin and the spermatogenic activity within the lobules is at their peak. The spermatogonia are reduced in number (Fig. 6). The testicular lobules are full of spermatozoa (Fig. 7) and the maximum activity of interstitial cells can be seen in this stage adjacent to blood vessels as they increase in size (Fig. 8).

**3.3.4. Post spawning phase (September to November)**

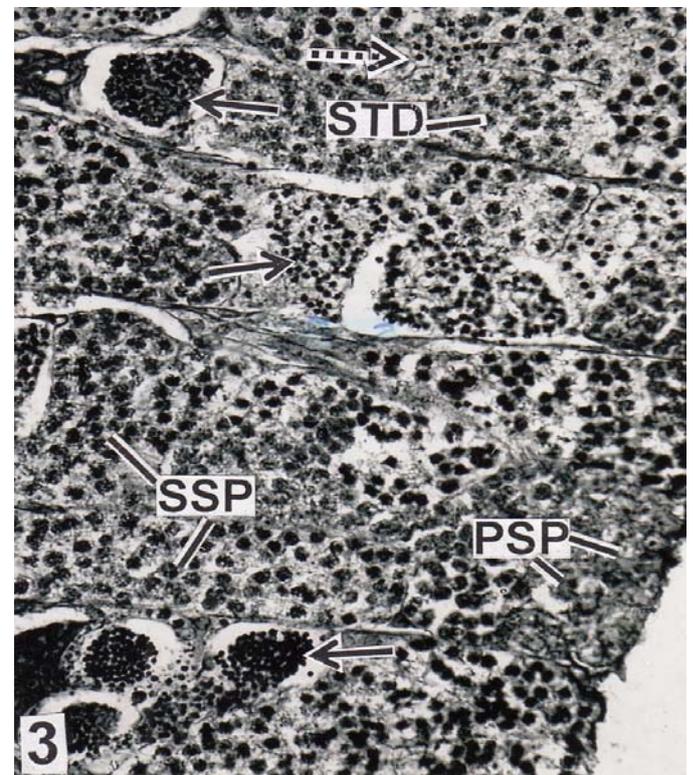
The diameter of lobules decreases due to release of sperms and boundary wall gradually becomes thicker. The spermatogonial cells are present in clusters. The testicular lobules contain residual spermatozoa and few cysts of spermatids (Figs. 9 and 10). The interstitial cells are considerably smaller in size (Figs. 9 and 10).



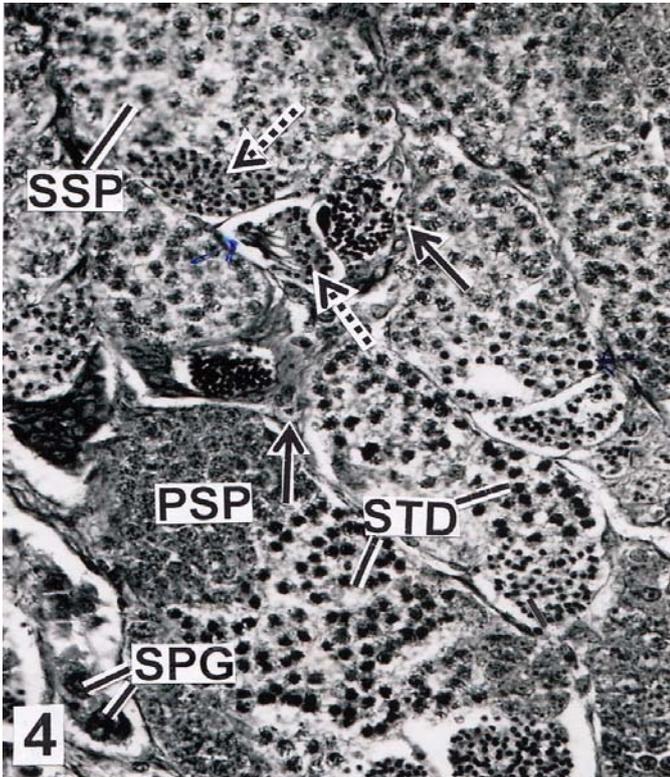
**Fig 1:** Arrangement of spermatogonial cells (SPG) around blood vessels (BV) during growth phase. Note prominent nucleus of SPG, cysts of primary spermatocytes (PSP), secondary spermatocytes (SSP), nest of spermatids (STD) and few spermatozoa (SPZ) (solid arrow); (HE) × 400X.



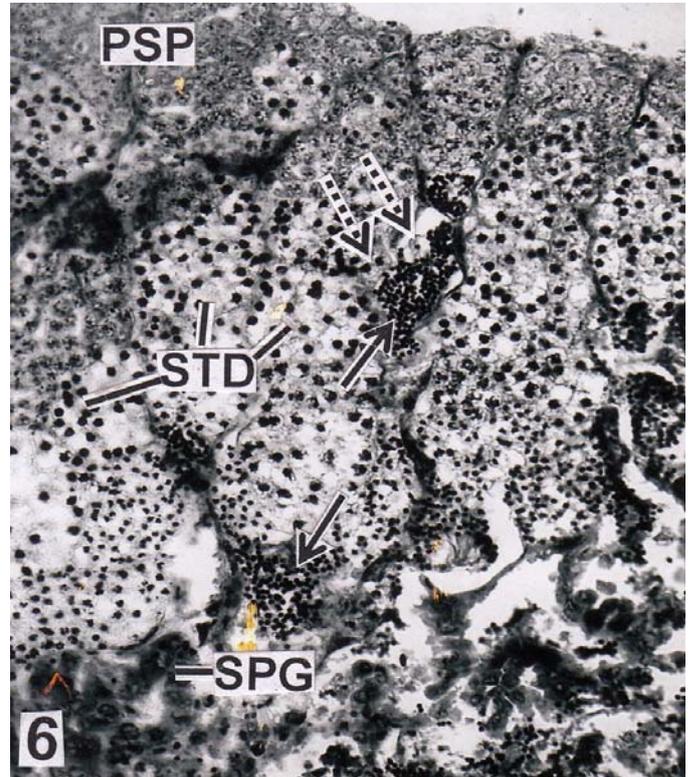
**Fig 2:** Enlarge view of SPG around BV during growth phase with centrally placed nuclei (N) and STD. Note the presence of interstitial cell (IC) (solid arrow) in between the lobular space. Broken arrow indicates SPZ; (HE) × 1000X.



**Fig 3:** Increased number of SSP, STD in between PSP and also cysts of SPZ (solid arrows) in the lumen of seminiferous lobules in the late growth phase. Broken arrow indicates IC in between the lobules; (IAH) × 400X.



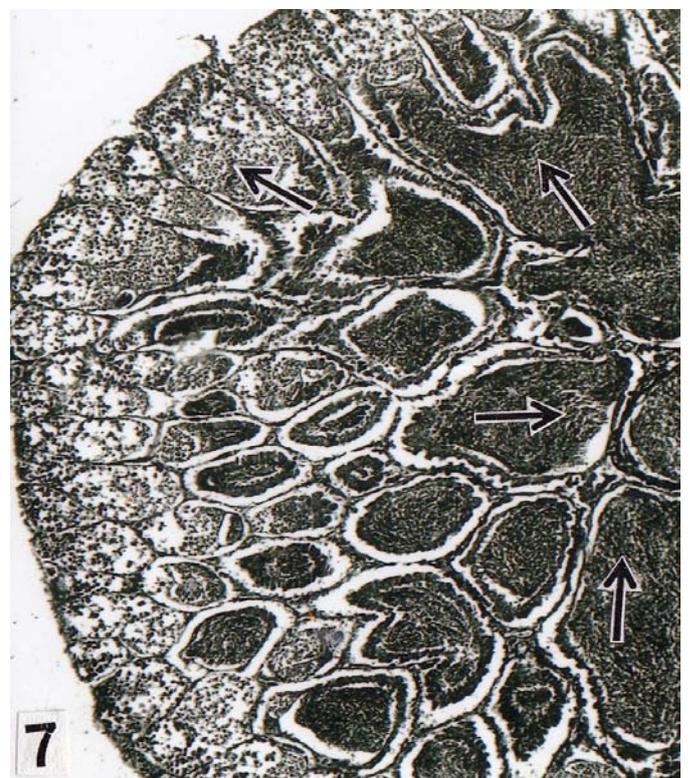
**Fig 4:** Showing reduced number of SPG, PSP, SSP and increased number of STD and SPZ (broken arrows) in the lobules during maturation phase. Note active IC (solid arrows) in interlobular spaces; (IAH) × 400X.



**Fig 6:** Showing cysts of SPZ (solid arrows) in between STD. Note few SPG and PSP in the testicular lobule during spawning phase. Broken arrows mark hyperactive IC; (HE) × 400X.



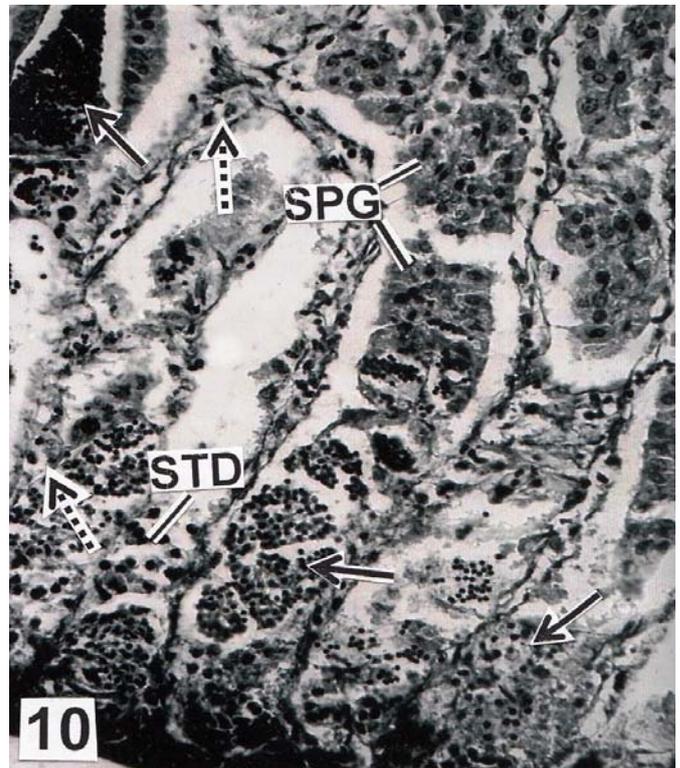
**Fig 5:** Showing packed STD and cysts of SPZ (solid arrows) in the seminiferous tubules during late maturation phase. Broken arrows indicate active IC in between lobules; (IAH) × 400X.



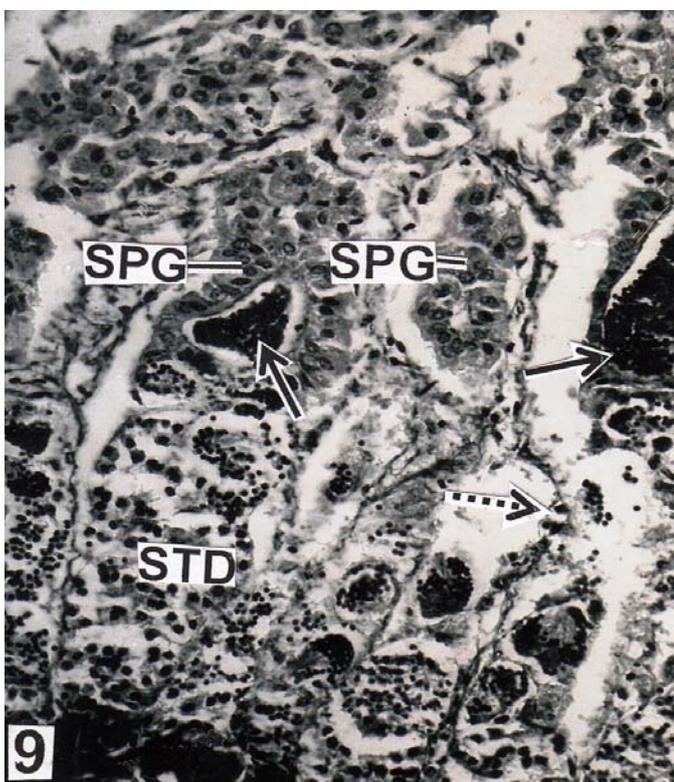
**Fig 7:** Showing dense aggregation of SPZ (solid arrows) within the testicular lobules during spawning phase; (IAH) × 200X.



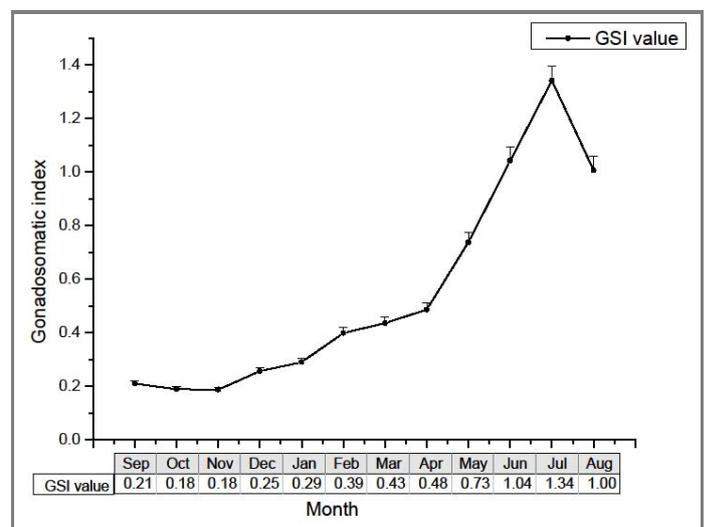
**Fig 8:** Maximum population of SPZ (solid arrows) with deeply stained sperm head during spawning phase. Note the presence of new crops of SPG and hypertrophied IC adjacent to BV; (HE) × 400X.



**Fig 10:** Testicular lobule with residual SPZ (solid arrows) and few STD during post-spawning phase. Note the presence of clusters of SPG adjacent to lobule boundary wall. Broken arrows mark IC; (HE) × 400X.



**Fig 9:** Showing residual SPZ (solid arrows) along with few STD within the lobules during post-spawning phase. Note the cysts of SPG and IC along the lobule boundary wall; (IAH) × 400X.



**Fig 11:** Variations of gonadosomatic index (GSI) of male *Xenentodon cancila* during different months.

#### 4. Discussion

The present histological studies have revealed the fact that *X. cancila* is a seasonal breeder and the testes exhibit remarkable variations in their size and volume. Prior to spawn the testes undergo preparatory stages during the remaining part of the season which includes various degrees of cytological changes in relation to spermatogenetic activity. Accordingly the GSI values vary greatly during the different months of the year. It

remains very low in post spawning phase when the spermatogenic activities are almost ceased. The testes gradually increase in volume and weight from the growth phase and the GSI value gradually increases in the late maturation and becomes highest during the spawning phase. This phenomenal variation in the testis is due to active proliferation of the later stages of spermatogenic cells. Similar changes of the GSI values in relation to spermatogenic activity in the testis of different teleosts have also been observed by Thakur<sup>[9]</sup>, Jayaprakash and Nair<sup>[10]</sup>, Mukhopadhyay and Sinha<sup>[11]</sup>, Chakrabarti and Gupta<sup>[12]</sup>, Rheman *et al.*<sup>[13]</sup>, Chakrabarti and Bose<sup>[14]</sup>. On the basis of GSI value it has been observed that peak level of GSI in the month of June and July is closely related with the maturity and spermiation of the fish under study. Similar observations have been recorded by Llewellyn<sup>[15]</sup>, Pollock<sup>[16]</sup>. According to Hoar<sup>[17]</sup> spermatozoa are formed from spermatogonia through a series of cytological changes known as spermatogenesis. In the present investigation the dormant nests of spermatogonia occur in the post spawning period. Similar type of dormant spermatogonia has been reported in other teleosts by Htun-Han<sup>[18]</sup>, Sinha and Mandal<sup>[19]</sup>, El-Boray<sup>[20]</sup>. In the present investigation, the seminiferous lobules of the testes of *X. cancila* are of cystic type. During the process of differentiation of spermatogonia to spermatozoa within the cyst the cytoplasm and nuclei of spermatogonia progressively decrease in size and volume and finally the spermatids are metamorphosed into spermatozoa. The cystic type testes have also been reported by Loir *et al.*<sup>[21]</sup>, Chaves-Pozo *et al.*<sup>[22]</sup>, Suwanjarat *et al.*<sup>[23]</sup> and Van Dyk and Pieterse<sup>[24]</sup>.

On the basis of spermatogenic activity of testicular lobules and variations in GSI values obtained in the present study the entire reproductive cycle is divided into four distinct phases viz., growth, maturation, spawning and post spawning. Similar division has also been observed by some workers<sup>[18, 25, 26]</sup>. In the present study growth phase is characterized by maximum number of spermatogonial cells. However, gradual cellular activity is found to be associated with the testicular lobules during the late growth phase which in turn is characterized by an increased activity in the conversion of spermatogonia to primary and secondary spermatocytes, few spermatids and spermatozoa. The number of spermatocytes increases gradually reaching the maximum number in the maturation phase. However, during the end of this phase the secondary spermatocytes are rarely seen and enormous number of cysts of spermatids and spermatozoa are almost completely filled up the entire lumen of testicular lobules. Similar feature has also been reported by Dziewulska and Domagala<sup>[27]</sup> in salmonid testes. The early spawning phase characterized by the presence of maximum number of spermatozoa and spermatids in the testicular lobules of *X. cancila*. This is due to the rapid spermiogenesis, during this phase<sup>[27]</sup>. The spermatogenic activity is decreased sharply following the regressive period and the testes finally enter into the post spawning phase. This phase is characterized by almost empty follicles containing residual spermatozoa and few cysts of spermatids along with few dormant spermatogonial cells. Davis<sup>[28]</sup> and Dziewulska and Domagala<sup>[27]</sup> opined that the mature spermatozoa are released from gonads during spawning but some spermatozoa are always left in the testis. They are observed in the gonads for a long period until the beginning of a new spermatogenic cycle. Similar testicular cycles have been reported in different teleosts<sup>[23, 29, 30, 31]</sup>. In the present study the variations of number and size of interstitial cells have been observed

corresponding to the changes in the state of spermatogenesis. The number and size of the cells undergo enlargement during maturation and spawning period thus indicating their role in spermatogenesis. Chung *et al.*<sup>[32]</sup> opined that the interstitial Leydig cells are typical steroidogenic cells exhibiting several cytoplasmic characteristics and are actively involved in spermatogenesis in male.

## 5. Acknowledgements

The authors are thankful to Dr. Anupam Basu, Head of the Department of Zoology, The University of Burdwan for providing necessary laboratory facilities.

## 6. References

- Grier HJ. Cellular organization of the testis and spermatogenesis in fishes. *American Journal of Zoology* 198; 21:345-357.
- Grier HJ, Taylor RG. Testicular maturation and regression in the common snook. *Journal of Fish Biology* 1998; 53:521-542.
- El-Gohary NMA. The effect of water quality on the reproductive biology of the Nile tilapia, *Oreochromis niloticus* in Lake Manzalah. Ph.D. Thesis. Faculty of Science, Ain Shams University, 2001.
- Schulz RW, de Franca LR, Lareyre JJ, Le Gac F, Chiarini-Garcia H, Nobrega RH. Spermatogenesis in fish. *General and Comparative Endocrinology* 2010; 165:390-411.
- Thorpe JE, Talbot C, Miles MS, Keay DS. Control of maturation in cultured Atlantic salmon, *Salmo salar* in pumped seawater tanks by restricting food intake. *Aquaculture* 1990; 86:315-326.
- Jobling S, Coey S, Whitmore JG, Kime DE, Vanlook KJW. Wild intersex roach (*Rutilus rutilus*) have reduced fertility. *Biology of reproduction* 2002; 67:515-524.
- Tomkiewicz J, Tybjerg L, Jespersen A. Micro and macroscopic characteristics to stage gonadal maturation of female Baltic cod. *Journal of Fish Biology* 2003; 62:253-275.
- Shein NL, Chuda H, Arakawa T, Mizuno K, Soyano K. Ovarian development and final oocyte maturation in cultured seven band grouper *Epinephelus septemfasciatus*. *Fisheries Sciences* 2004; 70:360-365.
- Thakur NK. On the maturity and spawning of an airbreathing catfish, *Clarias batrachus* (Linn.). *Matsya* 1978; 4:59-66.
- Jayaprakash V, Nair NB. Maturation and spawning in the pearl spot, *Etroplus suratensis*. *Proceedings of Indian National Academy of Science* 198; 47:828-836.
- Mukhopadhyay S, Sinha GM. Annual cyclical changes in the testicular activity of an Indian fresh water major carp *Labeo rohita* (Ham.). *Jahrbuch Morphologische Jahrbucher* 1986; 132:303-321.
- Chakrabarti P, Gupta AK. A histological study of the testes during growth, maturation and spawning phase in the exotic fish *Puntius javanicus* (Blkr.) *Advances of Biosciences* 1994; 13:11-12.
- Rheman S, Islam ML, Shah MMR, Mondal S, Alam M. Observation on the fecundity and gonadosomatic index (GSI) of the grey mullet *Liza parsia* (Ham.). *Journal of Biosciences* 2002; 2:690-693.
- Chakrabarti P, Bose S. Cyclical rhythms in the cytormorphology of testis of brackish water grey mullet *Liza parsia* (Hamilton, 1822) inhabiting south-eastern coast of India. *Journal of Entomology and Zoology*

Studies 2014; 2:110-118.

15. Llewellyn LC. Some observations on the spawning and development of the Mitchellian fresh water hardy head *Craterocephalus fluviatilis*. Australian Zoology 1979; 20:269-288.
16. Pollock BR. Spawning period and growth of yellow fin bream, *Acanthopagrus australis* (Gun.) in Moretoun Bay Australia. Journal of fish Biology 1982; 21:349-355.
17. Hoar WS. The gonads and reproduction. In. The Physiology of Fishes (ed. Brown ME). Vol 1, Academic Press, New York, 1957, 254-285.
18. Htun-Han M. The reproductive biology of the dab, *Limanda limanda* (Ham.) in the North Sea: Seasonal changes in the testes. Journal of Fish Biology 1978; 13:361-368.
19. Sinha GM, Mandal SK. Detection and localization of phosphatases, DNA, glycogen, mucopolysaccharides and bound lipids in testis of a teleost fish, *Anabas testudineus* (Bloch) during the annual cyclical changes by histochemical methods. Mikroskopie 1982; 39:1-13.
20. El-Boray KF. Histological changes in the testes of the fish *Gerres oyena* (Forsskål, 1775) during the reproductive cycle in Suez bay, red sea, Egypt. Egyptian Journal of Aquatic Biology & Fisheries 2001; 5: 83- 93.
21. Loir M, Cauty C, Planguetle P, Le Bail PY. Comparative study of the male reproductive tract in seven families of South-American catfishes. Aquatic Living Resources 1989; 2:45-46.
22. Chaves-Pozo E, Mutero V, Meseguer J, Ayala AG. An overview of cell renewal in the testis throughout the reproductive cycle of a seasonal breeding teleost, the gilthead seabream (*Sparus aurata*). Biology of Reproduction 2005; 72:593-601.
23. Suwanjarat JT, Amornsakum T, Thongboon L, Boonyoung P. Seasonal changes of spermatogenesis in the male sand goby *Oxyeleotris marmoratus* (Bleeker). Songklanakarin Journal of Science and Technology 2005; 27:425-436.
24. Van Dyk JC, Pieterse GM. A histo-morphological study of the testis of the sharp tooth catfish (*Clarias gariepinus*) as reference for future toxicological assessments. Journal of Applied Ichthyology 2008; 24:415-422.
25. Hoffmann R, Wordark P, Groth W. Seasonal anatomical variations in the testis of European pike, *Esox lucius* (Linn.). Journal of Fish Biology 1980; 16:475-482.
26. Pandey K, Misra M. Cyclic changes in the testes and secondary sex characters of fresh water teleost *Colisa fasciata*. Archives de Biology 1981; 92:433-449.
27. Dziejulska K, Domagala J. Histology of salmonid testes during maturation. Reproductive Biology 2003; 3:47-61.
28. Davis TLO. Reproductive Biology of the freshwater catfish, *Tandanus tandanus* (Mitchell) in the Wydir river Australia. Australian Journal of Marine and Freshwater Research 1977; 28:139-158.
29. Cek S, Yilmaz E. Gonad development and sex ratio of sharp tooth catfish *Clarias gariepinus* (Burchell) cultured under laboratory conditions. Turkish Journal of Zoology 2007; 1:35-46.
30. Lawson EO. Testicular maturation and reproductive cycle in mudskipper, *Periophthalmus papilio* (Bloch & Schneider) from Lagos lagoon, Nigeria. Journal of American Science 2011; 7:48-59.
31. Ahmed YA, Abdel Samei NA, Zayed AZ. Morphological and histomorphological structures of testes of the catfish

*Clarias gariepinus* from Egypt. Pakistan Journal of Biological Science 2013; 16:624-629.

32. Chung EY, Yang YC, Kang HW, Choi KH, Jun JC, Lee KY. Ultrastructure of germ cells and the functions of Leydig cells and sertoli cells associated with spermatogenesis in *Pampus argenteus* (Teleostei: Perciformes: Stromateidae). Zoological Studies 2010; 49:39-50.