

Histological Changes in Testes of Stinging Catfish, *Heteropneustes fossilis* (Bloch) During the Annual Reproductive Cycle

Sandeep Bajpai and Madhu Tripathi

Department of Zoology, Aquatic Toxicology Research Laboratory,
University of Lucknow, Lucknow- 226 007, Uttar Pradesh, India

Abstract: The present study was designed to observe the gonadal activity during the annual reproductive cycle of male *Heteropneustes fossilis*. Developmental activities in testes of catfish exhibited seasonal variations that can be divided into five distinguishable phases on the basis of gonado-somatic index (GSI), morphological aspects and histological changes of testes during different periods of the annual reproductive cycle. These phases are, resting phase (December-January), prepatory phase (February-April), pre-spawning phase (May-June), spawning phase (July-September) and post-spawning phase (October-November). The gonado-somatic index showed regular fluctuation during different months which was found ranging between 0.061 to 0.373. It was found highest during spawning phase and lowest during resting phase.

Key words: Catfish • Testes • GSI • Histology • Reproductive cycle

INTRODUCTION

Reproduction in teleosts is a cyclic phenomenon. It is an essential factor ensuring continuation of specie by recruitment of the next generation. In order to understand the reproductive biology, particularly the reproductive cycle of fish, studies on the duration of breeding, development and gonad maturation are of great significance [1]. The presence of any fish specie in a significant ratio basically depend on the reproductive potential and the environmental factors along with some internal factors which influences the gonadal development resulting in frequent spawning [2]. Photoperiod and temperature are important environmental factors regulating gonadal development and maturation of spermatocytes in most of the seasonally breeding teleosts [3].

Testes of teleosts fishes have a certain reproductive cycle which changes throughout the year. Pattern of these changes in gonads are characteristic for each species. During cyclic changes, many variations occur in testes of fish such as colour, mass, weight, appearance and various developmental stages of spermatogenic cells which have been reported by earlier workers in different fishes [4-12]. The understanding of spawning periodicities is not only a

fundamental value but also applicable in more than one way in drafting fishery policies and conducting experiments. Several reports on reproductive cycle are available for teleosts but freshwater catfish, *Heteropneustes fossilis* has not received much attention regarding this despite its great nutritive as well as experimental value. Keeping all these facts into consideration this study has been designed to observe the histological changes in the testes of catfish during its annual reproductive cycle.

MATERIALS AND METHODS

Collection of Experimental Fish: Adult and healthy male specimens of *H. fossilis* (average length 19 ± 1.5 cm and weight 31 ± 2.5 g) were procured from local fish market of Lucknow city. They were kept in glass aquaria in laboratory for 5-10 days and were sacrificed in midst of each month. Data on total body weight and testes weight of ten fish were recorded for the study of gonado-somatic index (GSI).

Calculation of GSI: Gonado-Somatic Index (GSI) Was Calculated by the Following Formula:

$$\text{GSI} = \text{Gonad weight} / \text{Bodyweight} \times 100$$

Histological Preparation: For histological study, testes were carefully dissected out and immediately rinsed in vertebrate saline. Small pieces of testis were fixed in alcoholic Bouin's fluid for 24 hours. Fixed tissues were washed with 70% alcohol to remove fixative, dehydrated upto absolute alcohol, cleared in cedar wood oil and xylene. Tissues were then embedded in paraffin wax (Merk) of 58-60°C melting point and serial sectioning was done at 5-7µm thickness with a rotatory microtome and stained with hematoxylin-eosin (HE). The prepared slides were examined and selected sections were microphotographed with Olympus research microscope.

RESULTS

Morphology of the Testes: The testes are paired elongated structures, enclosed by an outer thin peritoneum and an inner thick tunica albuginea. Both the membranes are made up of dense connective tissue. The seminiferous tubules are generally of varying shapes and sizes. Each tubule has a definite, thin fibrous wall which is not distinguishable after spawning period. Fibroblasts and tubule boundary cells are the chief components of the wall. The thickness of the lobule boundary wall as well as the shape and size of the lobule boundary cells vary greatly during different phases. The tubule boundary cells are internally lined by means of germinal epithelium. These are provided with a nucleus, but a prominent nucleolus is absent. The inter-tubular spaces are occupied by blood capillaries and interstitial cells. During the breeding season the lobules becomes greatly distended with spermatids and spermatozoa.

Gonado-Somatic Index (GSI): The study revealed that the values of gonado-somatic index (GSI) showed a regular fluctuation in different months. The GSI values varied from 0.061 to 0.373. It was recorded 0.106, 0.086, 0.061 and 0.070 during October, November, December and January, respectively i.e. the period of post-spawning and resting phase. During prepatory phase (February to April), the values of GSI gradually increased i.e. it reached from 0.098 to 0.153. During pre-spawning period (May-June), the GSI value increased rapidly. In July it reached upto 0.293. The highest GSI (monthly average) was recorded during spawning phase in the month of September i.e. 0.373 while it was found lowest 0.061 during resting phase, occurred in December (Table 1; Figure 1).

Histology of the Testes: Spermatogenesis involves proliferation of both primary and secondary spermatogonia through repeated mitotic divisions and

Table 1: Seasonal variations in the Gonado-somatic index (GSI) in male *Heteropneustes fossilis*

Months	GSI±Standard Error	Reproductive Phases
December	0.061±0.013	Resting phase
January	0.070±0.050	
February	0.098±0.014	Prepatory phase
March	0.121±0.031	
April	0.153±0.033	
May	0.178±0.052	Pre-spawning phase
June	0.255±0.028	
July	0.293±0.003	Spawning phase
August	0.315±0.023	
September	0.373±0.010	Post-spawning phase
October	0.106±0.016	
November	0.086±0.032	

Values are Mean ± S.E.M., N= 10 (Number of observations per value)

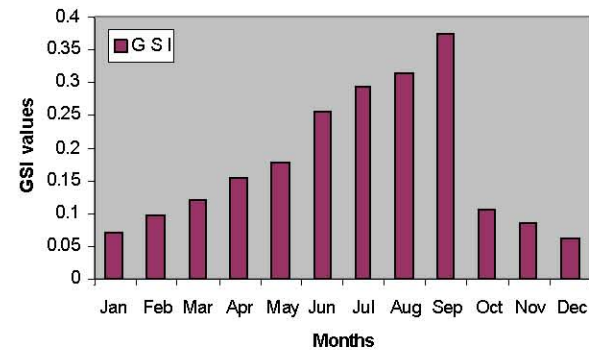


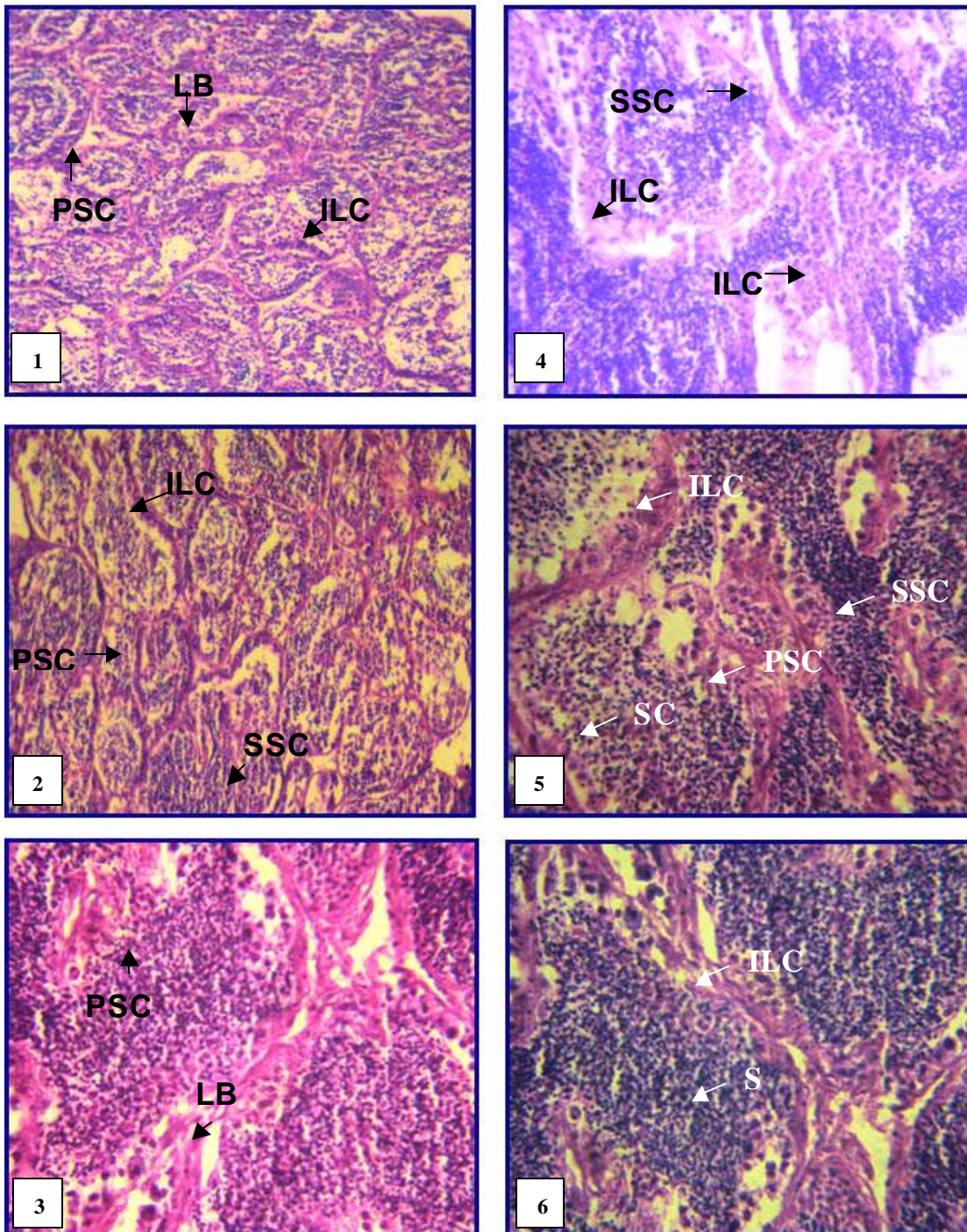
Fig. 1: Gonado-somatic index values (GSI) during annual reproductive cycle in male *Heteropneustes fossilis*

growth to form primary spermatocytes, these then undergo reduction division to form secondary spermatocytes. The division of the secondary spermatocytes produces spermatids which then metamorphose into the active, potential functional, motile gametes (spermatozoa). Spermatogenic cells appear inside the seminiferous tubules at different stages during spermatogenesis i.e. primary and secondary spermatogonia, spermatocytes, spermatids and spermatozoa. All six spermatogenic stages were observed in *H. fossilis*, on the basis of size, shape, nuclear and cytoplasmic characteristics.

Cell Types

Primary Spermatogonia: These are also known as the 'sperm mother cell'. They were the largest among all spermatogenic cell type, spherical in shape and present in cystic form, large nucleus with prominent eccentric nucleolus. They have less cytoplasmic affinity with dyes therefore take dull colouration during staining. (Plate 1; Figs. 1, 2, 3; Plate 2; Figs. 4, 5, 6).

PLATE- 1



- Fig. 1: Late resting phase (January) testis tubules showing disorganized spermatogonia and thin lobular boundary (arrow).
- Fig. 2, 3 & 4: Preparatory phase (February, March & April) testis showing higher frequency of primary and secondary spermatocytes, cells appears highly fused. Spermatogonia formation is seen (arrow).
- Fig. 5 & 6: Pre-spawning phase (May & June) testis showing primary and secondary spermatogonial cells, spermatocytes and spermatids (arrow).

PLATE- 2

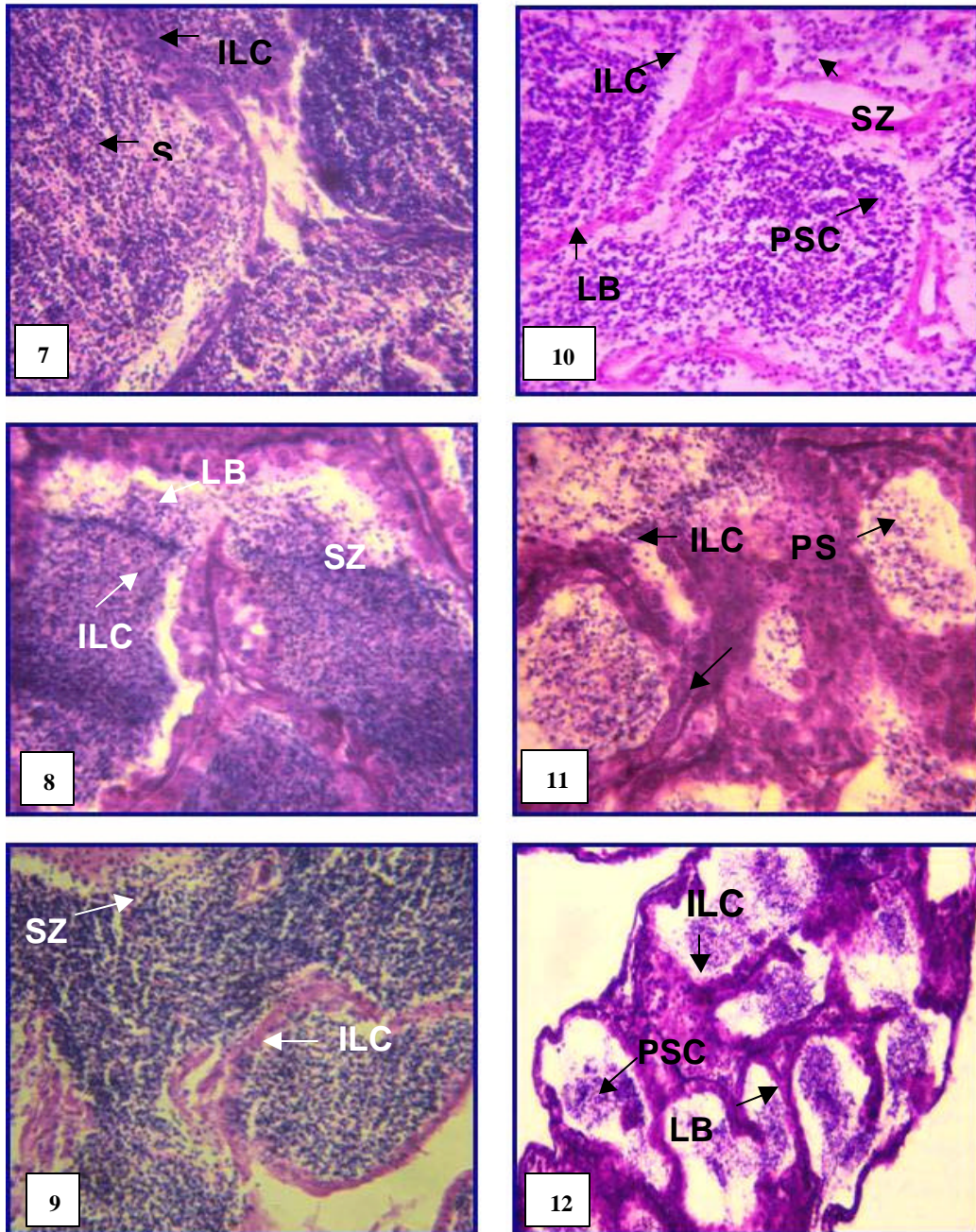


Fig. 7, 8 & 9: Spawning phase (July, August & September) testis showing all spermatogenic cells in seminiferous tubules with maximum concentration of spermatids and spermatozoa (arrow).

Fig. 10 & 11: Post spawning phase (October & November) testis showing dense concentration of spermatozoa in seminiferous tubules (arrow).

Fig. 12: Early resting phase (December) testes showing complete disorganize spermatogonia, few primary spermatogonial cells and thin lobular boundary (arrow).

LB – Lobular Boundary

ILC- Interstitial Leydig Cells

PS- Primary Spermatocytes

SC - Secondary Spermatocytes

PSC- Primary Spermatogonial Cells

SSC- Secondary Spermatogonial Cells

S- Spermatids

SZ - Spermatozoa

Secondary Spermatogonia: They were also small and rounded in shape and present in groups having centrally placed nucleus with visible chromatin threads and nucleolus having less cytoplasmic content (Plate 1; Figs. 1, 2, 3; Plate 2; Figs. 4, 5, 6).

Primary Spermatocytes: They were smaller than spermatogonias, found just below the spermatogonial cells. Nucleus was strongly stained with hematoxylin and cytoplasm has little affinity for dyes. (Plate 1; Figs. 2, 3, 4, 5).

Secondary Spermatocytes: They were slightly different from the primary spermatocytes in morphology. These are somewhat smaller than primary spermatocytes with nucleus showing lesser affinity to dyes. Nucleolus was not visible and they were having less cytoplasmic content. (Plate 1; Figs. 4, 5, 6).

Spermatids: These were oval and small cells originate from secondary spermatocytes, usually found in densely packed cluster in the interior of the seminiferous tubules. Nucleus has denser and more uniform chromatin. Nucleolus is absent and the cells have scanty cytoplasm (Plate 1; Fig. 6; Plate 2; Fig. 7).

Spermatozoa: They were the smallest, rounded deeply stained structures present in clusters excluding length of the tail. They occur in the interior of the seminiferous tubules and sperm duct with highly basophilic nucleus and large eosinophilic tail. (Plate 2; Fig. 8, 9, 10).

Annual Variations in the Testes: Annual testicular activities of *Heteropneustes fossilis* are synchronous with their female counterpart and can be divided into five distinct phases viz. prepatory, pre-spawning, spawning, post-spawning and resting phases on the basis of GSI and histological observations of the testes.

Prepatory Phase (February to April): Testes appeared thin, elongated and pale yellow in colour. Seminiferous lobules are small with thick lobular boundary wall. Primary and secondary spermatocytes gradually appear in later part of this phase. Spermatogonia formation is also seen. Interstitial cells are found in the inter lobular spaces (Plate 1; Figs. 2, 3, 4).

Pre-Spawning Phase (May to June): It is the most active phase. Testes are enlarged due to proliferation of spermatids and spermatozoa showing white to pink

colour and occupying about one-third of abdominal cavity. Initially testes exhibit numerous primary and secondary spermatocytes while in later phase spermatids and spermatozoa are dominant cells in the testicular lobules (Plate 1; Figs. 5, 6).

Spawning Phase (July to September): Testes white in colour, distended to the highest volume occupying less than half of the abdominal cavity with large and swollen seminal vesicle. Seminiferous lobules are enlarged due to huge accumulation of spermatozoa and the lobular boundary appears thin (Plate 2; Figs. 7, 8, 9).

Post-Spawning Phase (October to November): Testes bright yellow in colour, reduced in size due to release or re-absorption of spermatozoa leaving seminiferous lobules empty or having residual spermatozoa. Lobular boundary appears thick. Spermatogonia appear in the periphery of lobular boundary (Plate 2; Figs. 10, 11).

Resting Phase (December to January): Testes appeared thin, thread like and almost unrecognizable. Seminal vesicle cannot be seen. Tubules showed disorganized spermatogonia. Lobular boundary wall became thicker having interstitial cells (Plate 1, Fig. 1; Plate 2, Fig. 12).

DISCUSSION

Observation of the present study revealed increase GSI value from resting to spawning phase. GSI is a good indicator of reproductive activity thereby is used in determining reproductive stages in fishes. Increasing GSI values are mainly associated with the gonadal maturation whereas decreasing values are related to gametic extrusions or reabsorption [13]. The highest GSI values in male teleosts is due to the maturation and active proliferation of advanced stages of spermatogenic cells from spermatogonia resulting in the relative increase in testis size and weight. The reduction of GSI is due to the decreased spermatogenic activities which results in the decreased weight and size of the testis [14-17, 10, 11]. The highest values of GSI in teleosts were due to active somatic energy accumulation and lowest GSI due to somatic energy depletion [18, 19].

As regards the changes at cellular level in testis during immature phase in male fishes, the spermatogonial cells and spermatocytes were mainly observed and no activity was seen in these cells, but gradually the cells became active and spermatogenesis was observed during

pre-spawning phase. Primary spermatocytes were transformed into secondary spermatocytes, spermatids and sperms. On commencement of mature phase all forms of developmental stages of testicular cells were observed in testis and it was found fully turgid due to active proliferation and rapid nuclear and cytoplasmic division. In ripe stage the testis was found to reveal initial spawning activity with maximum number of active spermatozoa. Almost similar cyclic changes in testis were reported by Kumar *et al.* [20, 9] in *Labeo rohita*; Tripathi and Kumar in *Clarias batrachus* [10] and Mandal in *Labeo bata* [11].

The interstitial cells were distributed singly or in small groups in between the testicular lobes of fishes [21, 10]. In the present study they were observed to be smaller in size than spermatogonia and spermatocytes, but larger than spermatids and spermatozoa. Increased activity, number and size of interstitial cells during preparatory to pre-spawning period were an indication of steroidogenesis and spermatogenetic proliferation. The maximum development and activity of interstitial cells have been found just before spawning period and low shortly after spermatogenic activity in present study. These results are in accordance with the findings of Hyder [22] and Guraya [23]. Similar observation of decreased number and size of interstitial cells during spawning phase have also been reported by Pandey and Misra [24] as well as Chakrabarti and Gupta [7].

On the basis of the above findings it can be concluded that activities of male gonads in *Heteropneustes fossilis* show seasonal fluctuations and pass through five maturational phases during its reproductive cycle. The breeding season of the fish are monsoon months extending from July to September.

ACKNOWLEDGEMENT

The authors are grateful to Prof. A.K. Sharma, Head, Department of Zoology, University of Lucknow for providing necessary laboratory facilities needed to carry out this study.

REFERENCES

1. Wang, H.Y., C.F. Weng, M.C. Tu and S.C. Lee, 2001. Synchronization of plasma sexual steroids concentrations and gonadal cycles in the sleeper, *Eleotris acanthopoma*. Zoological Studies, 40(1): 14-20.
2. Shanker, D.S. and R.S. Kulkarni, 2005. Somatic condition of the fish, *Notopterus notopterus* (Pallas) during different phases of there reproductive cycle. J. Environ. Biol., 26(1): 49-53.
3. Lam, T.J., 1983. Environmental influences on gonadal activity in fishes. In: Fish Physiology W.S. Hoar, D.J. Randall and E.M. Donaldson, (Eds) Academic Press, New York, IXB: 65-116.
4. Sanwal, R. and S.S. Khanna, 1972. Seasonal changes in the testes of a freshwater fish, *Channa gachua*. Acta. Anat., 83: 139-148.
5. Chan, E.M. and T.E. Chua, 1980. Reproduction in the green back grey mullet, *Liza subviridis* (Val.). J. Fish Biol., 16: 505-519.
6. Sinha, G.M. and S.K. Mondal, 1981. Detection and localization of alkaline phosphatase, DNA, glycogen, mucopolysaccharides and bound lipids in the testis of a teleosts fish, *Channa punctatus* (Bloch) during the annual cyclical changes by histochemical methods. Mikroskopie., 38: 228-240.
7. Chakrabarti, P. and A.K. Gupta, 1994. A histological study of the testes during growth maturation and spawning phase in the exotic fish *Puntius javanicus* (BLKR). Ad. Bies., 13(1): 11-22.
8. Pandey, K. and A.K. Pandey, 1997. Morpho-histological changes in seasonal cycle of testis in freshwater teleosts, *Anabas testudineus* (Bloch). Him. J. Env. Zool., 11: 55-60.
9. Kumar, A., I.J. Singh and R.N. Ram, 2003. Annual reproductive cycle of male rohu, *Labeo rohita* (Ham) in Tarai region of Uttaranchal. Indian. J. Fish., 50(2): 231-241.
10. Tripathi, M. and A. Kumar, 2005. Seasonal histological changes in testis of freshwater catfish, *Clarias batrachus* (Linn). Environment and Ecol., 23(3): 498-502.
11. Mandal, D.K., 2007. Annual histological changes in the testis of a minor carp, *Labeo bata* (Ham). J. Inland. Fish. Soc. India., 39(1): 1-6.
12. Dalgic, G., S. Karayucel and I. Okumus, 2009. Reproduction cycle of striped venus *Chamelea gallina* from Black sea coast of Turkey. J. Anim. Vet. Advan., 8(10): 2009-2012.
13. Agostinho, A.A., L.C. Gomes and D.R. Fernandez, 2002. Fish ladders as an instrument for the conservation and management of fishing resources in Brazil. Regulated River: Research and Management, 18(3): 299-306.

14. Thakur, N.K., 1978. On the maturity and spawning of an air breathing catfish, *Clarias batrachus* (Linn). Matsya, 4: 59-66.
15. Ganguly, S. and P. Chakrabarti, 1995. Annual cyclical rhythm in the cytology of the testis of the freshwater featherback *Notopterus notopterus* (Pallas) J. Freshwater Biol., 7(3): 187-193.
16. Agarwal, N.K., U.S. Rawat, B.L. Thapliyal and S.K. Raghuvanshi, 2004. Breeding biology of snow trout, *Schizothorax richardsonii*, inhabiting the Bhagirathi river of Garhwal Himalayas. J. Inland. Fish. Soc. India, 36(1): 1-8.
17. Pal, S. and P. Chakrabarti, 2004. Correlative changes in the adrenal and testicular activities in relation to reproductive cycle of *Mastacembalus pancalus* (Ham.) J. Inland. Fish Soc. India, 36(2): 7-12.
18. Encina, L. and C. Granado-Lorencio, 1997. Seasonal changes in condition, nutrition, gonad maturation and energy content in barbel *Barbus sclateri* inhabiting a fluctuating river. Environmental Biology of Fishes, 50: 75-84.
19. Arockiaraj, A.R., M.A. Haniffa, S. Seetharaman and S. Singh, 2004. Cyclic changes in gonadal maturation and histological observations of threatened freshwater catfish narikeliru, *Mystus montanus* (Jerdon, 1849). Acta Ichthyologica Piscatorial., 34(2): 253-266.
20. Kumar, A., I.J. Singh and R.N. Ram, 2001. Correlative cyclicity of certain biological indices and testicular development in *Labeo rohita* (Ham) under Tarai conditions of Uttaranchal. The Indian J. Anim. Sci., 71(11): 1087-1089.
21. Yasin, S. and S.N. Ahsan, 1980. Annual cycle of the spermatogenic and endocrine components of testis in the Indian climbing perch. *Anabas testudineus*. Ann. Zool., 16(2): 49-76.
22. Hyder, M., 1970. Histological studies in the testis of pond specimens of *Tilapia nigra* (Gunther) and their implications of the pituitary testis relationship. Gen. Comp. Endocrinol., 14: 198-211.
23. Guraya, S.S., 1976. Recent advances in morphology, histochemistry and biochemistry of steroid synthesizing cellular sites in the testis of the non-mammalian vertebrates. Inter. Rev. Cytol., 47: 99-136.
24. Pandey, K. and M. Mishra, 1981. Cyclic changes in the testes and secondary sex characters of freshwater teleost, *Colisa fasciata*. Arch. Biol. Bruxelles., 92: 433-449.