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Reproductive Biology and Histological characters of male fish *Schizothorax plagiostomus* in River Lidder from Kashmir Himalaya

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ABSTRACT

The present study investigated the reproductive biology of male fish Schizothorax plagiostomus. The fish samples used in the present study was within the range of 25.5cm to 40.33cm in total length and weight ranging from 250g to 580g and the sampling duration was from July, 2013 to June, 2014. The mean gonadosomatic index (GSI) of male fish varied from minimum 4.197±1.335 during September and October to maximum 12.35±2.315 during March-May. Based on the monthly examination of macroscopic and histological gonadal maturity stages and month wise distribution of gonadosomatic index (GSI) it was concluded that the male has a definite spawning season from May to June. The breeding status confirmed by gonadosomatic index (GSI) and histological study of testis showed that there were six stages of spermatogenesis. Results show that S. plagiostomus is a seasonal breeder and has unimodal spawning activity.

Key Words: Schizothorax Plagiostomus, Histology, Testis, Gonadosomatic Index

I INTRODUCTION

The Kashmir valley is famous throughout the world for its waters bodies both lotic and lentic. The lotic habitats include numerous streams like Lidder, Veshow, Dudhganga, Sindh etc spreading throughout the valley The Lidder orginates from the high altitude glacier fed lake Sheshnag, Tarsar and Kolhai glacier. *Schizothorx plagiostomus* is the dominant fish in river Lidder. Fish is locally known as khont and is highly preferred food fish in Kashmir and has an edge over the exotic trouts, because of its taste and good nutritional value. The fish *S. plagiostomus* is delicate and preferred to live in oxygenated, pollution free water.

The study of reproductive biology of any fish species is also important to get information for successfully continuing its culture (Sunder, 1984; Gandotra *et al*., 2009; Bhat, 2012 and Shafi *et al*., 2013). Studies on the reproductive biology of Indian fishes have been made by several workers in past (Allen, 1951; Ali kunhi, 1956; Qasim and Qayyum, 1963; Bhatnagar, 1972; Meijide *et al*., 2005; Zhou, *et al.,* 2014). Reproductive biology of fish is considered as one of the key area to study the breeding season, fecundity and spawning season of different fish species and has long been recognized as widely investigated field (Orlando *et al*., 2003; Sisnero *et*

al., 2004). Most fishes living in the temperate zone exhibit an annual reproductive cycle (Nash, 1999). Reproduction is closely related to the environment which directly affects on gametogenesis and spawning. It occurs when food is available for the offspring in the wild (Wen and Lin, 2001; Tollefsen *et al*., 2002).

II MATERIAL AND METHODS

2.1 Study sites

During the present study fishes were collected monthly from four sites selected along the course of river Lidder at S-I (Pahalgam), S-II (Batkoot), S-III (Ashmuqam) and S-IV (Akura Mattan)as shown in Figure 1.

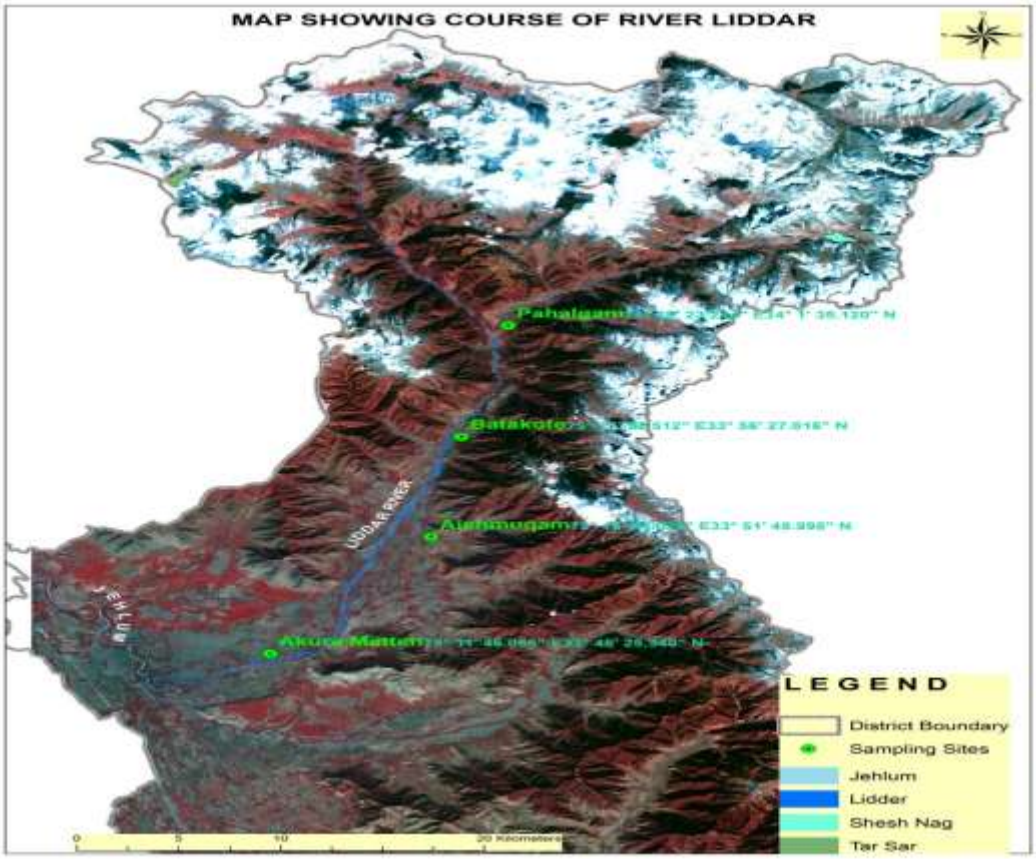


Figure 1; Satellite image of river Lidder showing different study sites along the course of river Lidder

2.2. Collection of specimen

The collection of fish sample was made once in two weeks from the river Lidder, and the specimens were bought to the laboratory. The fish were wiped with tissue paper and the body weight measured using a digital electronic balance (Shimadzu UX320G) with an accuracy of 0.1 g then dissected for macroscopic and microscopic observation of gonads and calculation of GSI.

2.3. Morphological characters of gonads

The examination of the morphological characters of the gonads was done by naked eye as this method is easier and applicable in the field conditions. Macroscopic classification was established by gonadal inspection following the method as described by Qasim (1957).

2.4. Histological examination of gonads

The developmental stages of spermatocyte were determined upon the histological characteristics according to the stages classified by Maruska *et al*., 1996. The technique employed for slide preparation was paraffin embedding technique. In this technique following steps were applied.

- Removal of fixative/washing
- Dehydration
- Clearing
- Paraffin impregnation/embedding
- Casting of blocks

For histological studies portions of the anterior, middle and posterior regions of the gonads were dissected out from the freshly killed specimens, and were fixed in Bouin's fixative for 48 hours, the Bouin's fixative was prepared by mixing 75ml of picric acid, 25ml of formalin and 5ml of glacial acetic acid and the samples were then dehydrated in ethanol with rising concentrations from 70 to 95% [v/v], before final dehydration in absolute ethanol. The time for dehydration of samples in graded alcohols was 1-2 hours in 70%, overnight in 80%, 60 minutes in 90% and again 60 minutes in absolute alcohol. The alcohol contained in the tissues was next eliminated by immersing them in xylene. The tissues were then impregnated with paraffin, which is soluble in xylene at 60°C and embedded in paraffin. The impregnation was achieved by giving 3-4 changes, one hour each, in the molten paraffin bath till the clearing is completely achieved along with the stiffness of the tissues. After processing, 5µm sections using rotary microtome was obtained by using plane-edge type of knives and were stained using haematoxylin-eosin staining procedure. The stained slides were then dehydrated in descending grades of alcohol and cleared in xylene and then mounted in DPX. The stained sections of gonads were examined under binocular microscope (Model TCM-400) under different magnifications and were photographed.

2.5. Gonadosomatic Index (GSI)

For studying the GSI a live male specimen was collected from the source. First the weight and total body length was recorded. The fish was dissected out and the testes were exposed and were taken out carefully in intact form. Weight of gonads was taken on the electronic digital balance (Shimadzu UX320G) with 0.01g accuracy and finally GSI value of male specimen was calculated. GSI was calculated to know the maturity and

to determine the breeding cycle of the fish. This was done as percentage of the gonad weight (GW) in terms of body weight (BW) of the fish (Afonso-Dias *et al*., 2005).

$$\text{GSI} = \frac{\text{GW}}{\text{BW}} \times 100$$

Where, GW and BW are gonad weight and body weight of the fish, respectively.

III RESULTS

3.1. Morphological gonadal classification of various maturity stages in males

Male *S. plagiostomus* fish were found to mature earlier and at smaller size than the female. The testis were paired and joined posteriorly by the two vas-deferentia to form a Y-shaped structure which leads to a common sperm duct that opens to the exterior through genital pore. Morphologically the immature testis did not differ much from the ovaries either in shape or size except that they were creamy coloured and multi-lobed. The fully mature male had a pair of white, elongated and turgid testes lying in the body cavity. The mature testes were fringed and filled with milky spermatic fluid and the right and left testis were symmetrical in size.

3.1.1 Stages of maturity

The testes of *S. plagiostomus* were classified into the following six stages of maturity on the basis of macroscopic observations as shown in plate 1.

Stage I: Immature stage

During this stage, testes were small in size, long slender and thread like translucent structures. This stage was found to extend from late September-October in *S. plagiostomus*.

Stage II: Maturing stage

In this stage, testes were slightly bigger ribbon like structures than the first two stages and colour was greyish white and occupied comparatively larger abdominal cavity. The testes became thicker and opaque in this stage. This is sub-divided into early maturing and late maturing stages and there was no conspicuous differential morphological demarcation between early and late maturing stages. It extended from November-December.

Stage III: Mature stage

In this stage, the testes were broad and thick, white in colour and blood vessels were prominent. The testes further increased in size and volume and develop side lobes. It was found that small amount of milt oozed out on applying pressure on the abdomen. They occupied 2/3 of abdominal cavity. It extended from January- February.

Stage IV: Ripe stage

In this stage, the testicular mass increased tremendously, occupying about 90% of the abdominal cavity and occupied almost whole abdominal cavity during the running stage. The testes became turgid and creamy white in colour. It extended from March-April.

Stage V: Running

In this stage the abdominal cavity gets distended and exudes milt on applying slight pressure. The testes were highly vascularised with prominent blood vessels at this stage. It extended from May-June.

Stage VI: Spent stage

In this stage the testes were thin, reduced in size, shrunken and flaccid with harder walls. They were wrinkled in appearance, yellowish white in colour, empty and fleshy in appearance. It extended from July-August.

3.2 Histological characteristics of *S. plagiostomus* testes

Histological characteristics of testes revealed the process of spermatogenesis the process occurred progressively during the annual reproductive cycle.

3.2.1 Histological stages of maturity

The testes of *S. plagiostomus* were classified into the following six stages of maturity on the basis of microscopic observations as shown in plate 2.

Stage I: Immature

The testis consists of a large number of spermatogonia with a few spermatocytes in seminiferous tubules. The spermatogonia were rounded in shape with eccentric ovoid nucleus, surrounded by thin layer of cytoplasm. The homogenous nucleus contains small clumps of chromatin material consisting of chromatin granules and chromatin threads with one or two nucleolus that appeared in the central zone. The spermatogonia divided by mitosis to form primary spermatocytes.

Stage II: Preparatory

In this stage the primary spermatocytes were spherical and smaller than the spermatogonial cells and their daughter cells. They were present in nests. They had no visible nuclear membrane and the chromatin material occupied most of the cell. The lumens of the tubules were noticed in this stage. Primary spermatocytes divided meiotically and produced secondary spermatocytes.

Stage III: Maturing

In the present study, under this stage all stages of spermatogenesis were clearly detectable. Spermatogonia and primary spermatocytes were rarely found, while secondary spermatocytes were found plentiful. The tubules were seen filled with spermatids and spermatozoa. Spermatozoa proliferate moderately within the tubule lumen.

Stage IV: Mature

In this stage the tubules and spermatic ducts were full of spermatozoa and few peripheral spermatogonia and spermatocytes were also reported.

Stage V: Spawning

In this stage the tubules discharged a considerable quantity of sperms and a few sperms were found in the lumen of the tubules. The walls of the tubules become thicker and reduced in size. Spermatogonia were scattered on the walls of the tubule.

Stage VI: Spent

Spent stage shows tubules which were much reduced in size and a few trapped spermatozoa were found in the lumen of the tubules. Spermatogonia were arranged in a row lining the wall of the tubules.

3.3. Gonadosomatic index (GSI)

The mean gonadosomatic index (GSI) of male fish varied from minimum 4.197±1.335 during September and October to maximum 12.35±2.315 during March-May as given in Table 1.

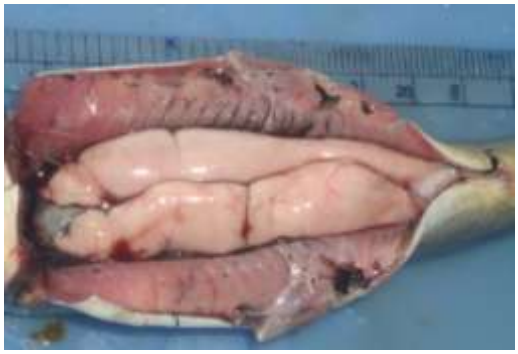
Table 1: The spermatogenic cycle of male *S. plagiostomus* in river Lidder according to gonadosomatic indices (GSI) and Spermatogenic distribution through the period from July 2013 to June 2014.

Stages of Maturity	Morphology and Duration	Average (GSI)	Spermatogenic Distribution
Immature stage	In this stage testes were small in size, slender and thread like. It extends from late September- October.	**4.197±1.335**	Spermatogonia dominated and they divided by mitosis to form primary spermatocytes.

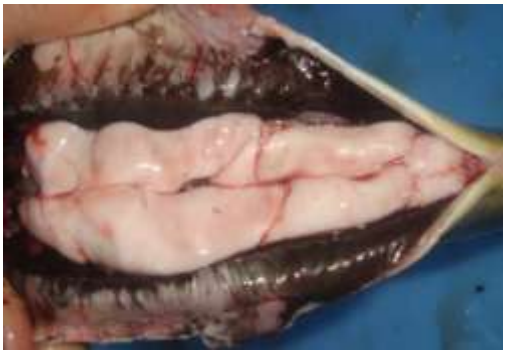
Preparatory stage	In this stage testes showed slightly increased weight and volume .It extends from November- December	**5.382±1.79**	Primary spermatocytes divided meiotically and produced secondary spermatocytes.
Maturing stage	Colour was greyish white, and became thicker and opaque in this stage. It extends from January-February.	**6.246±3.01**	Spermatogonia, spermatocytes and spermatids were formed. The spermatozoa were rarely found.
Mature Stage	In this stage the testes were broad and thick, white in colour and blood vessels were prominent. It extends from March- May	**12.35±2.315**	The tubules and spermatic ducts were full of spermatozoa with few peripheral spermatogonia and spermatocytes.
Ripe and Running Stage	The testes became turgid and creamy white in colour. The abdominal cavity gets distended and exudes milt on slight pressure and was highly vascularised It extends from late June	**7.693±1.776**	In this stage the tubules and spermatic ducts were full of spermatozoa. The tubules discharged a considerable quantity of sperms.
Spent stage	The testes were thin, shrunken and flaccid with harder walls, empty and fleshy in appearance. It extends from July-August.	**4.254 ±0.89**	New generation of spermatogonia and empty tubules were found.

(a) Immature

(b) Preparatory stage



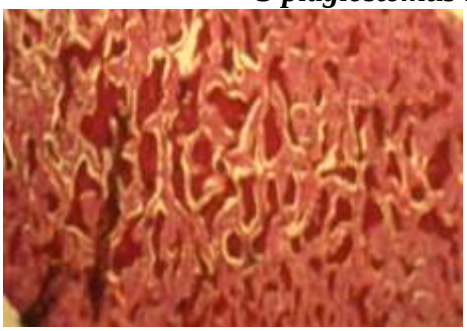
(c) Maturing stage



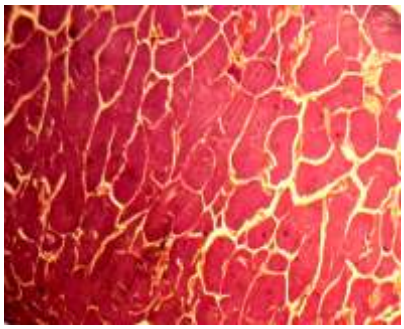
(d) Mature stage

(g) Spent stage

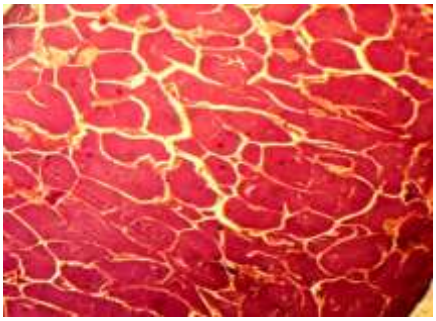
Plate 1. Showing different morphological stages of testicular development in *S plagiostomus* from river Lidder



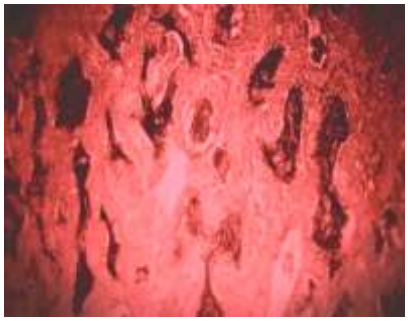
(i)



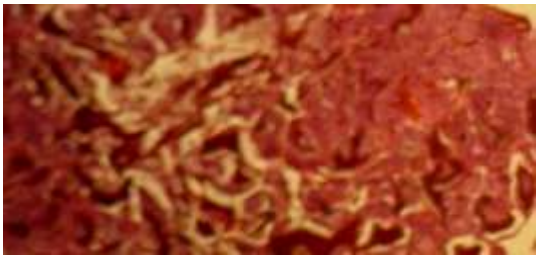
(ii)



(iii)



(iv)



(v)

Plate 2. Photomicrographs of T. S of testes of *S. Plagiostomus* showing different stages of testicular development

(i) Immature stage with primary spermatocytes
(ii) Preparatory stage with secondary spermatocytes
(iii) Maturing stage showing semniferous lobules
(iv) Mature stage showing spermatid spermatogonia and spermatozoa
(v) Spent stage showing empty ducts

IV DISCUSSION

Recent observations have indicated drastic decline of this fish in many areas due to introduction of exotic species, damming, water impoundment and overfishing (Ganai *et al*., 2011).The fish is declining day by day. In order to conserve this fish in natural waters and also to improve its production the study will be carried out to establish data on, reproductive biology which will be useful to improve its culture, management and conservation which is presently very much neglected as compared to exotic trouts and carps. On the basis of the general appearance of gonads, six maturity stages of testis were found. During immature stage, testis were small in size, long slender and thread like translucent structures and gradually it became opaque, enlarged in size and attains greyish white colour in maturing stage. In mature stage, the testes were broad and thick, white in colour, blood vessels were prominent and developed side lobes. Small amount of milt oozed out on applying pressure on the abdomen. Testicular mass increased tremendously, occupying about 90% of the body cavity during the ripe stage. During the running stage the testes were highly vascularised with prominent blood vessels, the abdominal cavity gets distended and exudes milt on applying slight pressure. In spent stage the testes were thin wrinkled and fleshy in appearance. They were much reduced in size, shrunken and flaccid with harder walls.

In reproductively active male *S. plagiostomus* different parts of the testes i.e. anterior, middle and posterior undergo histological as well as morphological changes in different stages. Similar stages of development were seen in all lobes of the testes in different maturity stages. In resting and immature stage ample spermatogonia were observed inside the small seminiferous tubules. The spermatogonia were large, pale spherical cells with a large round, central nucleus, distinct nucleolus and contained dense chromatin material. Slow mitotic activity was also seen in early maturing phase and the spermatogonia started dividing and transformed into sperm mother cells. High grade spermatogenesis was seen during the later part of this phase (developing). Spermatogonia decreased in number and numerous primary and secondary spermatocytes were visible. The

primary spermatocytes were smaller than spermatogonia with dark stained nucleus. They give rise to secondary spermatocytes which are yet smaller than primary spermatocytes with chromatin material in clumps. Whereas in pre spawning phase the seminiferous tubules become highly vascularised, seminiferous lobules gets increased in size and are filled with sperms. All stages of spermatogenesis can be seen in various lobules. The smaller deeply stained spermatids with elliptical nucleus and slightly reduced sperms were seen in this stage. In spawning phase, the seminiferous lobules became empty because of release of sperm. At the end of spermatogenesis the seminiferous lobules were packed with sperm masses. In spent phase, the empty and collapsing seminiferous lobules were seen.

The present study on gonadal histology of *S. plagiostomus* revealed the basic histological architecture and identified the spermatocytes found within the testis. It provides a basic knowledge for the study of reproductive biology and histopathology of the fish.

Observation on maturation of gonad, size frequency, and gonadosomatic index indicate that *S. plagiostomus* from river Lidder breed only once in a year in a synchronized manner. The spawning period of the fish appeared to be short, lasting for three months April, May and June.

In the present study from April onwards the temperature of the water rises rapidly, and the final stage of maturation occurs during this season. Whereas during the month of June, when water temperature was on maximum the fish reached the spawning period. Highest value of GSI was observed during the mature stage when the testis attains their maximum size and weight. The spermatogenic cycle of male *S. plagiostomus* in river Lidder in relation with the gonadosomatic indices (GSI) and Spermatogenic distribution is shown in Table1.

V ETHICAL APPROVAL

Not required as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

VI ACKNOWLEDGEMENTS

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VII CONFLICT OF INTEREST

There is no conflict of interest to disclose

REFRENCES

1. Afonso-Dias, I., Reis, C. and Andrade, J. P. (2005) Reproductive aspects of *Microchirus azevia* (Risso, 1810) (Pisces: Soleidae) from the south coast of Portuga. *Scientia Marina* **69:** 275-283.

2. Alikunhi, K. H. (1956) Observation on the fecundity, larval development and early growth of *Labeo bata* (Ham.). *Indian Journal of Fisheries*, **3:** 216-229.
3. Allen, K. R. (1951) The Horokiwi stream: A study of trout population. Fisheries Bulletin New Zealand Wellington, **10:** 1-238.
4. Bhat, A. A. (2012**)** Determination of fecundity of *Schizothorax esocinus* from river Lidder Kashmir. *Report and opinion*, **4:** 55-57.
5. Bhatnagar, G. K. (1972) Maturity, fecundity, spawning season and certain related aspects of *Labeo flimbriatus* (Bloch) of river Narmada near Hoshangabad. *Journal of Inland Fisheries Society*, **4:** 26-37.
6. Gandotra, R., Shanker, R. and Sing, D. (2009) Studies on the fecundity of snow trout, *Schizothorax richardsonii* (Gray) from the lotic waters of the Rajouri district, Jammu and Kashmir. *Current World Environment*, **4:** 127-132.
7. Meijide, F. J., Nostro. F. L. and Guerrero, G. A. (2005) Gonadal development and sex differentiation in the cichlid fish, *Cichlaso madimerus* (Teleostei, Perciformes): A light and electron microscopic study. *Journal of Morphology*, **264:** 191-210.
8. Nash, J. P. (1999) Seasonal reproduction fish. In: Encyclopaedia of reproduction. Knobil, E. Neill, J. P. eds. San Deigo London. Boston, New York, Sidney, Tokyo, Toronto: Academic Press, **4:** 329-340.
9. Qasim, S. Z. (1957) The biology of *Centronotus gennellus* L. (Teleostei). *Journal of Animal Ecology*, **26:** 389-401.
10. Qasim, S. Z. and Qayyum, A. (1963) Fecundity of some freshwater fishes. *Proceedings of National Institute of Science*, **20:** 373-382.
11. Shafi, S., Yousuf, A. R. and Parveen, M. (2013) Breeding biology and fecundity estimation of *Schizothorax nige*r (Heckel, 1838) from Dal lake, Kashmir. *International Journal of Innovative Research and Studies*, **2:** 112-122.
12. Sisneros, J. A., Forlano, P. M., Knapp, R. and Bass, A. H. (2004) Seasonal variation of steroid hormone levels in an intertinal-nestring fish, the vocal plain fin midshipman. *General and Comparative Endocrinology*, **136:** 101-116.
13. Sunder, S. (1984) Studies on the maturation and spawning of *Schizothorax curvifrons* (Heckel) from river Jhelum Kashmir. *Journal of Indian Institute of Science*, **65:** 41-51.
14. Tollefsen, K. E., Meyes, J. F., Frydenlund, J. and Stenersen J. (2002) Environmental estrogens interact with and modulate the properties of plasma sex steroid- binding proteins in juvenile Atlantic salmon, (*Salmo salar*). *Marine Environmental Research*, **54:** 697-701.
15. Wen, H. and Lin, H. (2001) Effect of environmental factors on gonadal maturation as well as its ovulation and spawning in teleosts. *Ying Yong Sheng Tai Xue Bao*, **12:** 151-155.
16. Zhou, X. J., Xie, C. X., Huo, B., Duan, Y. J., Yang, X. and Ma, B. S. (2015) Reproductive biology of *Schizothorax waltoni* (cyprinidae: schizothoracinae) in the yarlung zangbo river in Tibet, China. *Environmental Biology of Fishes*, **98:** pp. 597.