



TESTICULAR CYCLICITY OF *TRICHOGASTER LALIUS* DURING BREEDING AND NON BREEDING SEASON

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ABSTRACT

The study was conducted during November 2007 to October 2008 in the laboratory. The testis of *Trichogaster lalius* was paired and unequal in length throughout the year even during Non-breeding (July to February) and breeding (March to June) season also. Both the testis are attached to each other but were not free. It was lying ventral to the kidney and dorsal to the alimentary canal. It was remain attached to the body wall by means of a thin layer of membrane like Mesorchia. The testes were recorded unequal. The right lobe was found bigger than left lobe. The functional part of testis during its seasonal cycle exhibits spermatogenesis *i.e.* a period of activation, growth, maturity, depletion and rest. Histologically, the testicular cycle of the observation period was concerned with the successive stages of slow spermatogenesis, rapid spermatogenesis and spawning. The spermatogenic activity gradually increased from March and reached its peak in the months of May-June when the lobules were full of sperms and active spawning took place.

KEY WORDS: *Trichogaster lalius*, Gonado Somatic Index (GnSI), Maturing Phase, Spawning Phase and Post-spawning Phase.

INTRODUCTION

Kolkata is the major trade center for ornamental fishes, followed by Mumbai and Chennai (Chand, 2003; Mukherjee, 2004). In West Bengal, a local entrepreneur on their own effort develops the production of ornamental fish at present without help of government agency. There is no technological, infrastructural and institutional support from the state for promoting these activities. As a result, the industry is far from being organized. Despite these bottlenecks, it is encouraging to note that a large number of entrepreneurs in West Bengal have started breeding and rearing of ornamental fishes. In West Bengal, 719 units of ornamental fishes are there, where its breeding and culture are the main curricula (W.B. Govt. annual report, 2006-07). There are more than 500 units which have come up in the district of Howrah, Hooghly, South 24-Pargana and North 24-Pargana (Mukherjee, 2004). The demand of ornamental fish is being fulfilled by capturing them from natural resources. Due to over exploitation and indiscriminate destruction of breeding and feeding ground by applying pesticides and insecticides in agricultural field, leads to threatened condition of ornamental fish. Some species are considered as a vulnerable fish species in West Bengal (Menon, 1994; Das and De, 2002). To save this fish population from threatened status, the juvenile of these fish should be recruited to the natural ecosystem as precautionary measures. This is possible through artificial propagation and larval rearing. Agarwal (1996) in his book "Fish Reproduction" has stated "Monthly histological examinations of gonads (testes and ovaries) are taken into consideration to determine the spawning season. Regular histological and histochemical examination of reproduction system could categorically define the size

and age of a fish at first maturity, its reproductive rhythm and the changes in the reproduction cycle in the nature, controlled or in culture system. It is also an effective tool to study the ontogeny. With a view to above, the present piece of work entitled "Testicular cyclicality of *Trichogaster lalius* during breeding and non breeding season" was undertaken in the laboratory condition. It included studies on maturation stages of gonads, histology of gonadal cyclicality, reproduction and breeding period of *Trichogaster*, which would help in further standardization of induced breeding and larval rearing operation.

MATERIAL & METHODS

Rearing of fish in captivity

The study was conducted during November 2007 to October 2008 in the Department of Fisheries Resource Management, West Bengal University of Animal and Fishery Science, Kolkata, West Bengal. Adults of *Trichogaster lalius* ranging from the length 43 mm to 47 mm and weight of 1.1711 g to 1.8474 gm (w/w) were collected from market of Galef Street, ornamental fish market, Kolkata. During the entire rearing period a fresh artificial feed (protein content 40%) was given to the stocked fishes at the rate of 2 to 3 % of their body weight daily *i.e.* during morning and a live feed (Tubifax) at the evening hours.

Sampling: In every fortnight period, sampling was done randomly from the stocked specimen. In each sampling, 30 numbers (15 numbers of male and 15 numbers of female) of healthy and disease free fishes were selected for observation.

Histological study: For histological study, the microscopic slides were prepared by the following procedure as followed by of Agarwal (1996). The

development stages of germ cells in the testes and the change of the oocytes in ovary were studied by following methods.

Collection and fixation of tissue: For histological study, the middle parts of the gonadal tissues (testes and ovary) of *Trichogaster lalius* were collected as stated earlier. The tissues were trimmed into 5 to 6 mm size for better penetration of fixatives into it. The tissues were put into Formaldehyde Saline (Baker, 1944) for 24 to 48 hours as per size of tissues.

Post fixation treatment

Washing: The tissues (testes and ovary) were removed from the fixatives and subjected to overnight washing with flowing clear tap water until the formaldehyde odour was vanished.

Dehydration: The tissues were dehydrated perfectly with graded alcohols, starting from 30%, 50%, 70%, 90% and absolute alcohol (100%) to avoid the brittleness of the tissues.

De-alcoholization: Two changes of xylene (1hr each) were made to clean the tissues from alcohol. For better impregnation of wax into the tissue, the xylene penetrates into the tissue to become transparent and the material comes up to float on the top.

Infiltration: Paraffin wax (melting point 58-60°C of B.D.H) was used for infiltration of tissue. Three changes of wax (45 min each) were made to make tissue xylene free.

Embedding: For the preparation of blocks, pure paraffin wax was melted in water bath in between 58-60°C. Metal 'L' moulds were adjusted according to the size of blocking materials. The melted paraffin was taken from water bath and the blocking disc was filled. After permitting a layer of wax to be solidifying on the bottom disc, the completely infiltrated tissues were carefully taken from the paraffin wax and put inside the different blocking disc according to their size. Care was taken so that the wax on the top of the disc did not solidify during keeping the material in the blocking disc. For this reason, a heated needle or forceps was put only the upper portion or inside the wax of the disc. After the proper positioning of the tissues, the wax inside the disc was allowed to solidify. After few minutes, the 'L' moulds were removed from the wax block. Thus prepared blocks were kept separately inside the labeled polythene packets.

Trimming and sectioning: The paraffin blocks were trimmed carefully to 6 to 7 mm² by sharp blades. The trimmed blocks were fixed to the wooden holder (peg) with the material facing away from it. Molten wax was poured on the holder and the block was kept on it. The block was padded with more wax at the base to make it strong. After being confirm, the blocks were firmly fixed with holder, the sectioning was done by using microtome (SPENCER 820 TYPE). On the microtome, each section was cut into 5µ thickness. The ribbons containing tissues were collected on clear glass slide (already a smear of egg-albumin was kept on that slide) with the help of fine brush.

Spreading and fixing: Glass slides were cleaned properly by Chromic-acid solution, soap and finally with tap water. After cleaning, the slides were air-dried and a thin layer of Glycerin Egg Albumin was rinsed over it. Then the ribbons with materials (about 10 to 15 sections depending on the size) were spread over the clean glass slides. Thin

tissues were made wrinkle free and allowed to fix on slides by keeping them on hot plates (30°C) for 2 to 5 minutes.

De-waxing and staining: Tissues fixed on slides were de-waxed with descending order of alcohols (100%, 90%, 70%, 50% and 30%) and stained by the double staining method with Haematoxylin and Eosin by using standard techniques as described by Agarwal (1996).

Mounting: One or two drops of DPX (mountant) were put on the dried slide which one was ready for mounting. Then, a cover slip or slide was slowly lowered when the mountant would flow ahead of the descending glass without trapping air bubble between the cover slip and slide. The excess of mountant on the slides was removed with xylene soaked cotton. After mounting, the slides were allowed for drying. The excess of mountant on the slides was removed with xylene soaked cotton.

Labeling and storing: Labeling was done on the slide by glass marking pen to avoid future confusion. The slides were stored in slide box to protect them from dust and dirt.

Microscopic observation: The histological sections on the prepared slides were thoroughly observed under Advanced Trinocular Microscope (Olympus, MODEL 8 x 51, Japan) microscope at different magnifications. The developmental stages of germ cells in the testes and changes of the oocytes of ovary were noticed carefully. Colour photomicrographs of selected histological sections were taken as and when required.

Gonado Somatic Index (G_{nsi}): Gonado somatic index values were used as indicator of degree of gonadal development. It was found out by employing the following formula.

$$GnSI = \frac{\text{weight of the gonad}}{\text{total weight of the fish}} \times 100$$

Statistical Analysis: The Mean and Standard error statistical methods were used in the present study

RESULTS & DISCUSSION

Gonado Somatic Index (GnSI)

Determination of maturity and spawning period by observing GnSI is of pivotal significance in the life of fish. The maximum GnSI value was also found to be 0.583±0.043 of male in the month of June (Table 1).

Histology of the testis

Testicular cyclicity, which changes according to the seasonal (12 months = 6 seasons) rhythms are determined according to the presence (in terms of quantity, quality and period) of spermatogenic elements in the testes (Table 1).

On the basis of histological study, in case of *Trichogaster lalius*, 6 stages of testicular cycles were seen during different seasons of a year. These were, -

- 1) Preparatory phase (January to February),
- 2) Pre-spawning phase or Maturing phase (Early maturing phase-March and Late maturing phase -April),
- 3) Peak spawning phase or matured (May-June),
- 4) Post-spawning phase (July-August),
- 5) Resting phase (September-October),
- 6) Late-resting phase (November-December).

In the testes of *Trichogaster lalius*, the sperm mother cells were observed throughout the year but their number were

varying from period to period. In the present study, testes of fish when undergoing reproductive activity (spermatogenesis), about six spermatogenic elements have been identified. The elements of spermatogenesis are produced from sperm mother cell of germinal epithelium and passes through different maturation stages as primary

Spermatogonia (PSG), secondary spermatogonia (SSG), primary spermatocytes (PSC), secondary spermatocytes (SSC), spermatids (SM) and spermatozoa or sperms (SP). The similar investigation was made by Guraya (1976a; 1982).

TABLE 1: Histological changes of testicular cyclicity of *Trichogaster lalius*

Month	GnSI	Histological Changes	Testicular Cycle
November to December	0.331±0.092	Seminiferous tubules were small in size and full of spermatogonia, Interstitial cell and Blood vessel were present in Interlobular junction.	Late Resting Phase
January to February	0.380±0.136	Seminiferous tubules were Slightly large in size and full of spermatogonia. Slow mitotic activity were seen and the spermatogonia start dividing,	Preparatory Phase
March to April	0.480±0.109	Spermatogonia decrease in number and numerous primary and secondary spermatocytes were visible.	Pre-spawning Phase
May to June	0.577±0.052	Seminiferous tubules were large in size and full of sperm, Spermatogonia were few and all stages of spermatogenesis were visible in various lobules.	Spawning Phase
July to August	0.440±0.066	Initially Seminiferous tubules were full of sperm but in terminal stages, tubules were mostly empty. Some sperms along with spermatocytes were present in lumen.	Post Spawning Phase
September to October	0.302±0.121	Empty and collapsing seminiferous tubules were seen, some of which contain residual or unexcellent sperm. After a brief period of rest, the testes start the cycle again.	Resting Phase

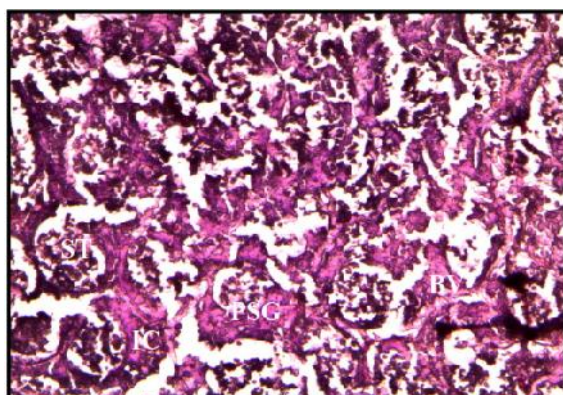


PLATE I: Late resting phase of testis of *Trichogaster lalius*. 40 x 10X

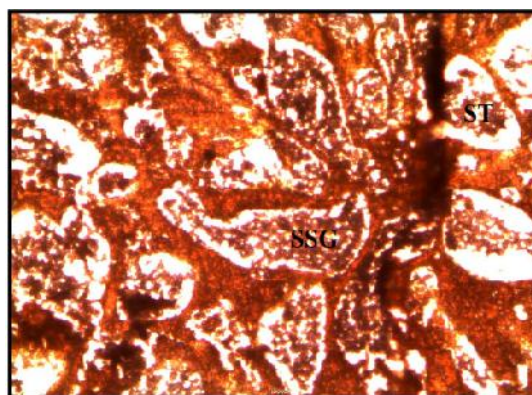


PLATE II: Late resting phase of testis of *Trichogaster lalius*. 40 x 10X

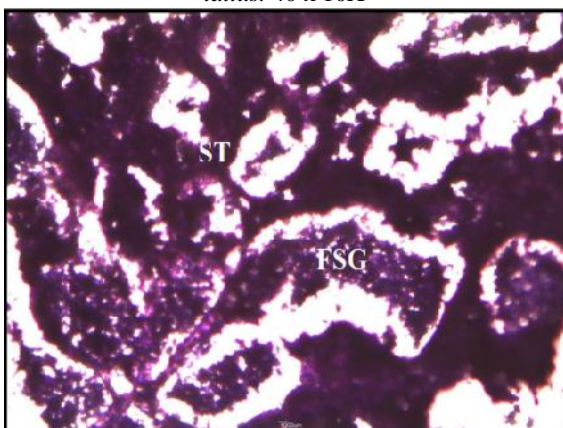


PLATE III: Initial Preparatory Phase of testis of *Trichogaster lalius* 40 x 10X

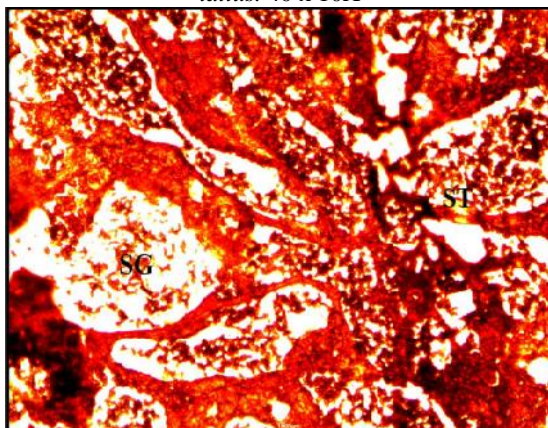


PLATE IV: Terminal Preparatory Phase of testis of *Trichogaster lalius* 40 x 10X

The primary spermatogonia after multiplication by mitotic division are called secondary spermatogonia and later give rise to primary spermatocyte, which undergo mitotic division to form secondary spermatocytes. The life of secondary spermatocytes is relatively shorter and they rapidly divide to form spermatids. The starting from one primary spermatocyte after one complete mitotic division four spermatids is produced. The spermatids transform into mature spermatozoa by recognition of nucleus,

cytoplasmic components and acquire flagellum for mobility (Nagahama, 1983; Agarwal, 1996).

Different species of the teleosts show the different maturity cycle of their gonad. The categorization of the maturation cycle of gonads differs from species to species because of the change of the gonadal product through gametogenesis, which influenced by the maturation of the fish (Rath, 2000).

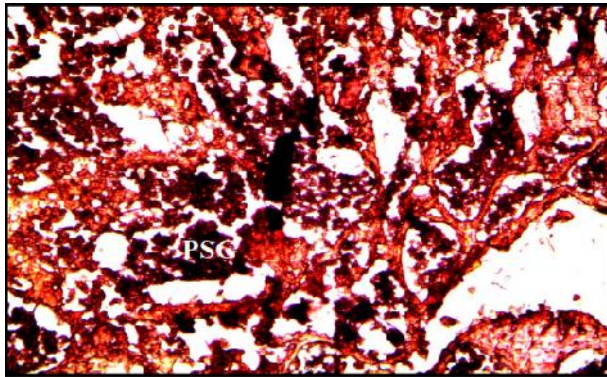


PLATE V: Pre-spawning phases (Early Maturing) of testis of *Trichogaster lalius*. 40 x 10X

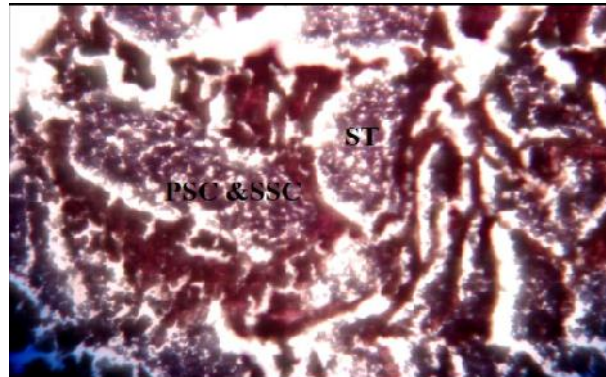


PLATE VI: Pre-spawning phases (Maturing) of testis of *Trichogaster lalius*. 40 x 10X

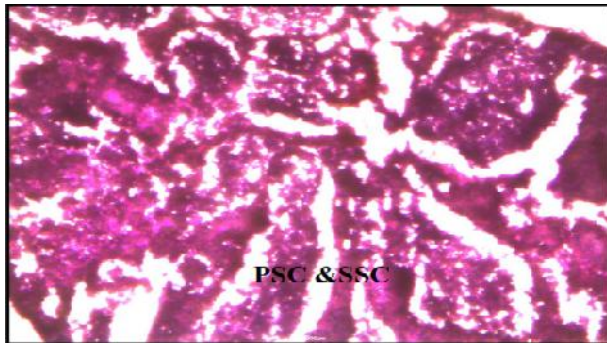


PLATE VII: Pre-spawning phases (Late Maturing) of testis of *Trichogaster lalius*. 40 x 10X

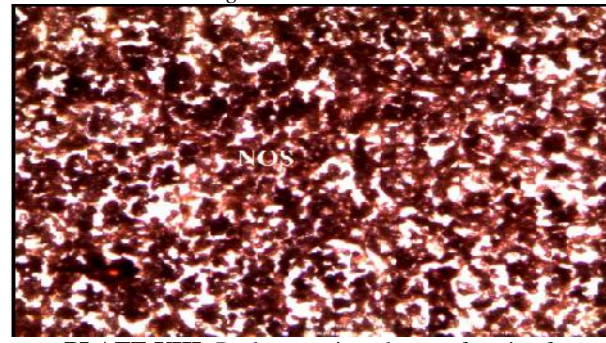


PLATE VIII: Peak spawning phases of testis of *Trichogaster lalius*. 40 x 10X

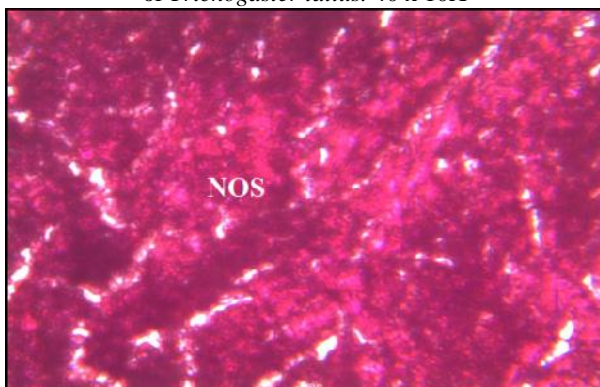


PLATE IX: Peak spawning phases of testis of *Trichogaster lalius*. 100 x 10X

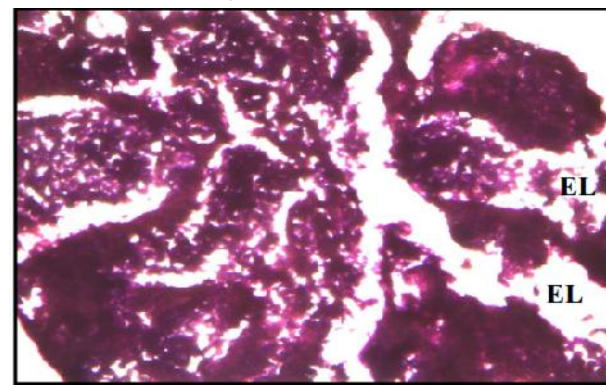


PLATE X: Initial Post-spawning phases of testis of *Trichogaster lalius*. 40 x 10X

Khanna and Shrestha (1976) observed the reproductive cycle of *Schizothorax plagiostomus* was divided into eight phases. But in the present investigation six spermatogenic phases were observed. Similar observation was made by Banu and Bhakta (1985) in *Colisa fasciatus* in Bangladesh and Khan (2004) in West Bengal. Morpho-histological

structure of testis is variable from species to species. Sathyanesan (1959) in *Mystus seenghala*, Rai (1965) in *Barbus tor*, Bisht (1974) in *Schizothorax richardsonii* and Agarwal (1996) in *Schizothorax plagiostomus* had described the anterior region as functional lobule and posterior region as non-functional region (sterile), which

was mean only for the storage and conduction of sperms. In the 'transition' portion between these two regions the germ cells seen towards the periphery in varying quantities which progressively decrease in number further backwards and finally disappear in the posterior region. In the present study all regions of testis of *Trichogaster lalius* are histologically identical like many other teleosts. Similar results have also been observed in *Clarias batrachus* (Lehri, 1967), *Channa gachua* (Sanwal and Khanna, 1972a), *Anabas testudineus* (Das, 2002) and *Colisa fasciatus* Khan (2004). During the preparatory phase (January to February) the sperm mother cells contain the spermatogonia and multiply to give rise the primary spermatocytes, which are smaller in size than spermatogonia and possessed a darkly stained nucleus (Plate III to V). The similar result also made in *Glyptosternum pectinopterus* (Khanna and Pant, 1966). The source of annual supply of the germinal portion of the testes appears to be controversial. Lofts and Marshall (1957) believed in extra-testicular origin of germ cells. While Ahsan (1966) suggested dual origin of germinal cells from certain migrating cells as well as by the division of primary germ cells. Several workers including Sanwal and Khanna (1972a), Bisht (1974), Nautiyal (1983) and

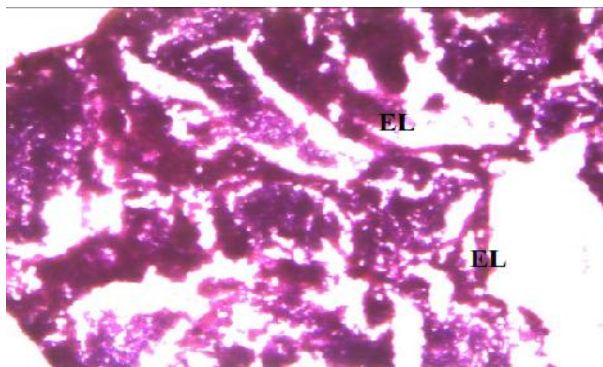


PLATE XI: Terminal Post-spawning phases of testis of *Trichogaster lalius*. 40 x 10X

The testicular cyclicity of *Trichogaster lalius* was concerned with successive stages of slow spermatogenesis, rapid spermatogenesis and spawning. In different species, the spermatogenic activity starts at different times of the year (Rai, 1965; Nair, 1966; Shrestha and Khanna, 1976; Nauriyal, 1983). It was also confirmed in the case of *Trichogaster lalius* that a male could attain its maturity during March to June, which is considered as the breeding season of the fish. But in case of *Colisa fasciatus*, breeding season was observed in June-July Banu and Bhakta (1985) in Bangladesh and Khan (2004) in same species in West Bengal. The speratogenic activities decrease sharply as the testes enter in to post-spawning and resting phase (July-October). These phases are characterized by empty lobules with a few residual spermatozoa or un-expelled sperm, which was collapsing the seminiferous tubules (Plate X and Plate XII). The similar observation was made by Khanna (2002) and Mandal (2007). On the basis of histological study, the testicular cyclicity of *Trichogaster lalius* was divided into 6 testicular cycle and 3 successive stages viz., (1) Pre-spawning phase (slow spermatogenesis and rapid

Agarwal (1996) reported that the new crop of germ cells is produced by the division of the resting or pre-existing sperm mother cells within the lobules of the testes. In the testis of *Trichogaster lalius*, sperm mother cells were observed throughout the observation period but found in lesser number during the spawning period (May to June). So it is believed that spermatogenesis process starts from January and February, the primary spermatocytes are produced during March and April. In spawning period (May-June), the lumens of lobules are emptied indicating the complete release of sperms from testis. As the spermatogenesis is over, these cells were undergone to resting phase. Hence, it may be concluded that the new crop of spermatogonia during reproductive cycle arises by multiplication of the resting mother cells. The primary and secondary spermatocytes were filled in lobular lumen during preparatory phase (Plate III and Plate IV). In pre-spawning phase spermatids were also found in lumen while sperms along with spermatids were observed in spawning phase (Plate VI to Plate IX). Similar observation has been reported on spermatogenic development and differentiation in other teleosts (Agarwal, 1996, Kumar *et al.*, 2003 and Tripathi and Kumar, 2005).

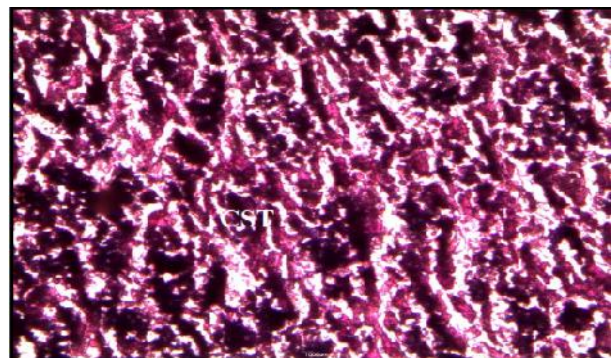


PLATE XII: Resting phase of testis of *Trichogaster lalius*. 40 x 10X

spermatogenesis) (2) Spawning phase (breeding and peak spawning) and (3) Post-spawning phase (resting, late resting and preparatory).

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