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HISTOLOGICAL OBSERVATION OF GONADS DURING BREEDING AND NON BREEDING SEASON OF *TRICHOGASTER FASCIATUS* IN SHANTI JHEEL, WEST BENGAL.

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ABSTRACT

The study was conducted during March to February months in the laboratory of Department of Fishery Resources Management, West Bengal University of Animal and Fishery Science to understand the gonads histologically during breeding and non breeding seasons of *Trichogaster fasciatus* collected from Shanti Jheel, Nadia, West Bengal. The seasonal development of gonads was also studied histologically to know the breeding season and maturation of fish. Studying the histological characteristics and the gonadal cycle of *Trichogaster fasciatus* was badly categorized into three phases, viz., pre-spawning , spawning and post- spawning phases which included primary spermatogonia, secondary spermatogonia, primaryspermatocytes, secondary spermatoctes and sperm in the case of testes and various oocytic stages from stage-I to stage-VII in the case of ovary. From the present study it can be conclued that *Trichogaster fasciatus* breeds once in a year and its breeding season is from April to July and peak breeding season is in between June-July.

KEYWORDS: *Trichogaster fasciatus*, gonad, histologically and breeding seasons.

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INTRODUCTION

The banded gourami, is a species of gourami found in the rivers and water bodies of Eastern India & Bangladesh. Its distinctive feature is the rainbow colored bands on its body. Size of a fully grown male can be up to 4 inches. *Colisa fasciatus* is an obligatory air-breathing fish found commonly in stagnant waters in ponds and pools with low oxygen content. Aerial respiration is performed by the fish with the aid of a pair of supra branchial chambers, each containing a complicated labyrinthine organ (Munshi 1965, Prasad *et al.*, 1982). The fish respires by surfacing frequently and gulping atmospheric air.

Spawning season of fish can be determined through monthly histological examination of gonads (testis and ovary). Regular histological and histochemical examination of reproduction system could categorically define the size and age of a fish at first maturity, this reproductive cycle in natural and controlled system (Agarwal, 1996). Different authors studied the histomorphological changes in testes and in the ovary, the percentage and duration of different developmental stages of oocytes, the average diameter and the gonosomatic index and distinguished different reproductive phasis during reproductive cycle i.e. resting, preparatory, maturing, pre-spawning, spawning and spent of the teleostan ovary. Growing oocytes have been observed with minor variation and with a few histochemical changes in all the cases (Borah, 2002; Verma *et al.*, 2002 and Sarma *et al.*, 2003).

MATERIAL AND METHODS

A study on histology and pre-spawning fecundity of gonads of *Trichogaster fasciatus* during breeding and non breeding season was conducted in the Department of Fishery Resources Management, Faculty of Fishery Sciences, West Bengal, during March, 2003 to February, 2004.

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 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 fasciatus* were collected by dissecting out the fish. The tissues were trimmed into 5 to 6 mm size for better penetration of fixatives into it. The tissues were put into Formaldehyde Saline 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 (1 hr 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 double stained 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 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.

Table 1: Histological changes and testicular cyclicity of *T. fasciatus* during March to February

Months	Histological changes	Testicular cycle
March	The lobular mass consisted of spermatogenic activity and primary spermatogonia and primary spermatocytes were present.	Pre-spawning Season
April- May	The lobules were completely packed with spermatozoa. The testicular wall and lobular wail became thinner and intra1obular space decreased.	Spawning Season
June -July	The thin lobular wail and full presence of Spermatozoa.	
August	Gradual decrease in spermatozoa in lobules.	
September	Remarkable decrease in spermatozoa and inter-lobular space is more.	Post-spawning Season
October	Still decrease in spermatozoa.	
November-December	Slow miotic activity is seen; spermatozoa start dividing	
January	Increase of spermatogenic activity is seen.	
February	More spermatogec activity is seen. Primary and secondary spermatogonia are seen.	Pre-spawning Season

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Table 2: Histological changes and ovarian cyclicity of *Trichogaster fasciatus* during March to February.

Months	**Histological changes**	**Ovarian cycle**
March	The oocytes of late yolk vehicle stage and early yolk stage are more in number.	Pre-spawning Season
April- May	Increase in size of oocytes. The intra-ova space is decreasing. The ovary is filled up with ripe eggs.	Spawning Season
June -July	The ovary is filled up with ripe eggs. The yolk globules were decreasing in size and occupying whole of the ooplasm. Some unite globules were observed.	
August-September	Number of oocytes gradually decreasing and inter ova space is increasing.	
October	Number of oocytes still decreasing; inter ova space is increasing; ovary shrunken.	Post-spawning Season
November-December	Ovary shrunken and formation of new oogonia in ovary is Seen.	
January - February	More number of immature oocytes stage-I, stage-II and stage-III is seen.	

RESULT AND DISCUSSIONS

Histological observation

Histological changes that were observed in both the sexes (male and female) are presented in tabular form (Tables 1 and 2).

Seasonal testicular cyclicity : On the basis of morphological observation for one year

(March to February), the seasonal testicular cycle of *Trichogaster fasciatus* was categorised as follows:

1. Pre-spawning season: It consisted of
   I. Phase of slow spermatogenesis i.e. early maturing phase (January to February)
   II. Phase of rapid spermatogenesis i.e. maturing phase (February to Mach)
2. Spawning season or matured stage (April to July): It consisted of
   I. Breeding season (April-May)
   II. Peak breeding season (June- July)
3. Post-spawning season: It consisted of
   I. Spent phase (August to November)
   II. Resting phase (November to December)
   III. Preparatory phase (December to January)

Histology of the testis: In the testes of fish, when undergoing reproductive activity (spermatogenesis), about six spermatogenic elements have been identified and described by Guraya *et al.* (1997). The elements of spermatogenesis are produced from sperm mother cell of germinal epithelium and passes through maturation stages as primary spermatogonia, secondary, primary spermatocytes, secondary spermatocytes, spermatids and spematozoa (sperms).

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. In most of the fishes, about 5 to 6 stages of testicular cycles were seen during different seasons of a year. These were early-maturing, maturing, matured, spent, resting and preparatory (Khanna, 1993). On the basis of histological study, the testicular cyclicity of *Trichogaster fasciatus* was distinguished into pre-pawning (slow spermatogenesis and rapid spermatogenesis), spawning (breeding and peak breeding) and post-spawning (spent, resting and preparatory) phases. It was confirmed due to the presence of the above six spermatogenic elements found in testes in different quantity in different periods. During breeding season (April-July) the seminiferous tubules of *T. fasciatus* was larger in size and filled with sperm. Spermatogonia were few and all stages of spermatogenesis were seen in various lobules. In this time, the sperm mother cells were less in number, which is supported by the observation of Das (2002). In the testes of *T. fasciatus*, the sperm mother cells were observed throughout the year but their number were varying from cycle to cycle. During spermatogenesis a lesser number of sperm mother cells had undergone division. So, a new crop of spermatogonia was produced during reproductive cycle. During preparatory phase (January-February) the sperm mother cells contained the spermatogonia and slow mitotic activity was seen. The spermatogonia started dividing to give rise to the primary spermatocytes and then secondary spermatocytes. These are smaller in size than that of spermatogonia and possessed a darkly stained nucleus (Khanna and Pant, 1996) in different fish species.

During August to September (post spawning), the lobule mass consisted of low spermatogenetic activity where less number of sperm was found. The number of primary spermatogonia, primary and secondary

spermatocytes were still less. The lobules were empty and collapsing seminiferous tubules were seen. Some of them contained the residual sperms. During the observation, it was noticed that the testicular wall became thin during breeding season due to presence of large quantity of sperms. During non-breeding season, particularly post-spawning and preparatory phases, the testicular wall was found thick.

On the basis of above histological study, the testicular cyclicity of *Trichogaster fasciatus* was divided into 3 successive stages viz.,

(I) Pre-spawning phase (slow spermatogenic and rapid spermatogenic)

(2) Spawning phase (breeding and peak breeding) and

(3) Post-spawning phase (spent, resting and preparatory).

It was also confirmed in the case of *Trichogaster fasciatus* that a male could attain its maturity during April-July, which is considered as the breeding season of the fish. Similar observations were made by Banu and Bhakta (1985) for *Trichogaster fasciatus* in Bangladesh.

Phukon and Biswas (2002) have studied maturity and spawning of an ornamental fish *Erethistes pussilus* from Brahmaputra river system in upper Assam. They stated that the fish has a short breeding season from May to August based on histological studies of developing gonads. It differed from the present investigation, which may be due to the impacts of environmental factors (Goldberge and Herring, 1981; Xie and Thang, 1990; Soto *et al.*, 1992; Santos, 1995). Incomplete development of sperm in some portion of testes and four stages of gonadal maturation were observed by Sarma *et a!.*, (2003) in the hill state of Meghalaya and it might be due to different geographical area with different temperature and altitude. Kumar *et al.* (2003) studied the annual reproductive cycle of male Rohu in Tarai region of Uttaranchal. They stated four spermatogenic phases of testes. But in the present study six spermatogenic phases were observed. It might be due to rearing of fishes under artificial condition in the laboratory.

Seasonal ovarian cyclicity: On the basis of gross morpho-histological changes occurred in the ovary, occurrence of different stages of developing occytes, nature of ovarian wall and changes in gonadosomatic index, the ovarian cyclicity was divided into following phases:

1. Pre-spawning phase (January to March)
2. Spawning phase (April to July)
3. Post-spawning phase (August to December)

Histology of ovary: Histological observation of ovaries showed the different stages of ova in different quantity, which in turn regulate the ovarian cycle. By the process of oogenesis, ova under 7 stages of oocytes inside the follicle of ovary became a ripe egg (Khanna, 1993). But their distribution in the ovary at different season determines the maturation cycle of the ovaries as resting phase, early maturing phase, advance maturing phase, mature phase, spawning phase and spent phase. In the present study, the maturation cycle of *Trichogaster fasciatus* was broadly divided into three phases, viz., pre-spawning (slow oognesis and rapid oogenesis), spawning (breeding and peak breeding) and post-spawning (spent phase, resting phase and

preparatory) phases. For the better understanding, the entire oocytic cycle and maturity stages of ovary was broadly divided into above three parts.

In the histological section, it was noticed that the oocytes were visible in various stages of development. The germ cells or oogonia were found in the lamellae and probably originate from germinal epithelium. Post spawning season (August-December) includes spent phase, resting phase and preparatory phse. In the spent phase, the ovary became flaccid, less bumber of unspawned ova were noticed in the ovary. In the resting phase, the ovary became thin and the ovarian wall became thick. Almost germinal epithelium was distinguishable. In the preparatory phase, large number of vigorous lamellae hung into the ovarian cavity from the germinal epithelium. Here very less number of oogonia stage were observed but stage-I and stage-II are found more in number in the vigorous lamellae. All these three phases were found in different and broadly categorized into post-spawning phase.

In the pre-spawning season (January-March), the ovary of *Trichogaster fasciatus* is categorized into slow oogenesis and rapid oogenesis. During slow genesis, a large number of stage-III and stage-IV oocytes were found. The time required from stage-III to stage-IV was comparatively more. That is why it is known as slow oogenesis. But in the case of rapid oogenesis the stage-IV, stage-V and stage-VI were seen. The change of stage this phase was so quick that it is very difficult to disguise their differences. That is why it is categorized under rapid oogenesis. Spawning season (Aptil-July) includes breeding phase (April-May) and pre-breeding phase (April-May) when the oocytes of stage-I and stage-II were seen. But stage-1 was found comparatively more than stage-II. But during the peak breeding phase (June-July) a large number of matured oocyte was seen in the ovary. 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 gametogenes which influenced by the maturation of the fish (Rath, 2000). Structure and variation of various oocytes were studied by various scientists (Shashi and Srivastava, 1998; Shashi and Singh, 1998). Verma *et al., (*2002) found seven growing stages of oocytes in the ovary of Rohu. Among them 3 stages had no yolk and rest four stages were vitellogenic, which resembled the present work. But Sarma *et al*., (2003) found five stages in the ovary of carp at mid altitude and low temperature region of Meghalaya. The existence of diffete1ce in gonadal stages of two regions mainly due to environmental impacts on gonadal evelopment (Soto *et al*., 1992; Santos, 1995; Sarma *et al*., 2003).

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