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Annual Reproductive Cycle of Male Indian Minor Carp Labeobata (Hamilton, 1822)

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Abstract: ***The histological observations of the testis of a minor carp, Labeobata exhibited the annual reproductive events of its testicular function. The gonadosomatic index (GSI) value reached peak in the month of June at the onset of breeding period in their annual reproductive cycle. GSI increased with the proliferation of spermatids and spermatozoa in male fish and decreased sharply during post spawning phase with the release of spermatozoa. The histology of testes revealed five different types of germ cells which are spermatogonia, primary spermatocyte, secondary spermatocyte, spermatids and spermatozoa. Annual reproductive cycle of this fish is divided into five distinct phases such as resting, preparatory, pre spawning, spawning and post spawning phase on the basis of GSI and histological condition of the testes. The spermatogonia are dominated cell types in the resting and preparatory phase but spermatozoa are major cell types during pre - spawning and spawning phase. This study represented that Labeobata is an annual breeder and the testis reached peak maturity at the time of monsoon months, June - July. Number and size of the interstitial cells increased during pre - spawning and early spawning phases indicating their highest activities of gonad. The spermatogenic activities decreased in the post - spawning phase due to release of spermatozoa.***

Keywords: Reproductive cycle, Testis, GSI, Histology, Labeobata

1. Introduction

Studies on reproductive biology of fish are basically required to plan better management strategies of fishery resources [1], and for measuring the influences of environmental variability on the dynamics of fish populations [2]. The reproductive biology is an important topic at the present time. Also for conservation purposes, information on reproduction biology is also useful to select the candidate of fish target from the wild for diversification of fish species in aquaculture industry [3]. The reproductive procedures of a fish species is common to individuals of within species, while the reproductive strategies are varies in response to fluctuations in the environmental parameters [4].

Reproduction in most of the tropical and subtropical fish species is periodic and the peak reproductive event, spawning occurs in the most suitable time of the year to ensure maximum survival and growth of the young. Annual fluctuation in photoperiod and its dependant variable temperature are considered as the primary environmental factors regulating reproductive cycle of fish [5]. Fish reproduction, especially teleost has achieved more attention among fisheries scientists due to economic interest and nutritional requirements for increasing population. The histological description of gametogenesis is the most important for macroscopic staging in the estimation of maturity and reproductive seasonality of a fish [6]. Gonadal development and spawning season are vital to know the spawning frequency of a species for its management [7]. Gonadosomatic index (GSI) is the essential parameter to determine the reproductive status and breeding period of fishes [8].

Information on the maturation cycle of testes is lacking for the important Indian minor carp, *Labeobata*. Accordingly, the purpose of this study is to performed month wise status of the gonadosomatic indices and histomorphological features of the testicular activities to demonstrate the pattern of its annual reproductive cycle.

2. Materials and methods

Procurement of Fish:
The study is conducted on the adult male Indian minor carp, *Labeobata* (Class Teleostomi; Order Cypriniformes; Family Cyprinidae). Adult *Labeobata* (75 - 100g in weight and length 20 - 25 cm in length) is procured from local freshwater ponds located adjacent to the University campus of Visva - Bharati, Santiniketan, West Bengal (Latitude N 23°67″ Longitude E 87° 72″) in second week of each month. Immediately after collection fishes are transported to the laboratory and body weight of each individual of at least 10 fishes are recorded. The fishes are deeply anaesthetized with tricainemethanesulfonate (MS 222, 100 mg L^{-1}) and sacrificed following the guideline of the departmental animal ethics committee for processing of further studies.

Estimation of Gonadosomatic index (GSI):
After scarifying thetestis of each fish dissected out, soaked in blotting paper and weighed with the help of an electronics balance. GSI is estimated as the percentages of gonads weight in body weight using the formula [9]: GSI = Gonad weight (g) × 100 / total body weight (g). Mean GSI of 10 fishes and their standard deviation are calculated in each month throughout the year. The macroscopic characters such as colour, texture, shape, size of the testis and its position in

the body cavity arenoted down throughout the reproductive season.

Histological preparation:
Testes are cut into small pieces and fixed in Bouin's fixative for overnight. The tissues are rinsed repeatedly in 70% ethanol and dehydrated through graded ethanol. The dehydrated tissues are cleared with benzene and embedded in paraffin wax (56 - 58^0 C, Merck). The tissues are sectioned serially at 5 µm thickness with a rotary microtome (WESWOX, Model MT - 1090A). Sections are stretched on albuminized glass slides and stained with Delafield Haematoxylin and counter stained with 1% Eosin. Slides are mounted with DPX and covered with cover slip.

Microscopic evaluation:
Stained slides are studied under a research microscope (Olympus BX 52). Testes are classified into five different stages of spermatogenic cells on the basis of the characteristic of the cells. The diameters of the testicular cells are measured by the stage and ocular micrometre (Erma, Japan) and photographs are taken by the high resolution digital camera.

Statistical evaluation:
The month wise variation in the GSI of male *Labeobata*is evaluated through one way analysis of variance (ANOVA) at the 1% or less ($p \leq 0.001$) level of significant (using computer programme MINI TAB). The mean and standard deviation (±SD) value of both GSI and cell diameter of testis is represented.

3. Results

Gonadosomatic index (GSI):
Monthly variations of GSI in male *L. bata* are prominent (Figure1). The lowest GSI value is recorded during resting phase in the month of November - December (0.06 ± 0.02). It increases gradually from January to March in the preparatory phase (0.37 ± 0.08) and rapidly during April to May in the pre spawning phase (0.53 ± 0.06). GSI reach the peak in the month of June to July in the spawning phase (1.02 ± 0.2) and again decreased in the post spawning phase during September to November (0.12 ± 0.03). Variations in GSI coincide with the histological conditions of the testes in its different maturation phases.

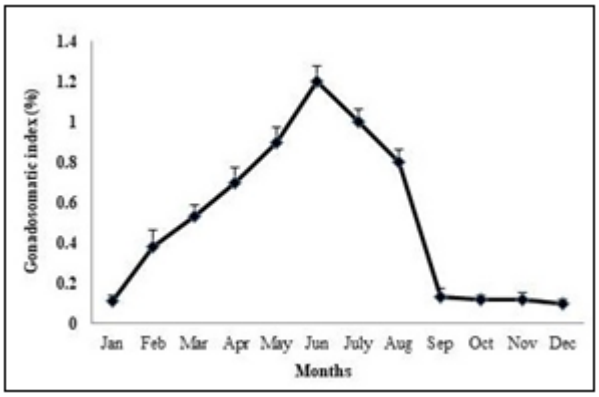

Figure 1: Month wise variation of gonadosomatic index (GSI) in an annual reproductive cycle of male *L. bata.*

Testis morphology:
Testes of *Labeobata*are paired, creamy white, elongated structure lying ventral to the swim bladder (Figure2) and vary in shape and size throughout the year being slender during winter and bulky during rainy season.

Figure 2: Testes of *Labeobata* during spawning phase

Cell types in the testes:
Histology of testes of *L. bata* exhibited five different types of germ cells such asspermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa.

Spermatogonia: These are the largest cell (12.5±1.4µm) with a large nucleus (9.7±1.2µm). Spermatogoniaare distinguishable as primary and secondary spermatogonia, one with compact nucleus, and other with vacuolated nucleus. Cytoplasm iseosinophilic and nucleus took moderate haematoxylin stain (Figure 3: a).

Primary spermatocyte: Primary spermatocytes are large cells (8.6±0.7µm) with prominent, round nucleus (6.4±0.5µm). Cytoplasm is eosinophilic and present as a thin rim around the nucleus (Figure 3: a).

Secondary spermatocyte: These cells are small in size (5.8±0.3µm) with a nucleus (4.5±0.1 µm) and took dark stain with haematoxylin (Figure 3: b).

Spermatid: These cells are small and oval in shape (2.2±0.1µm) with little cytoplasm (Figure 3: c, d).

Spermatozoa: Spermatozoa are the smallest cells with deeply stained round head (1.72 ± 0.11 µm) (Figure 3: c, d).

Annual maturation cycle of the testes:

Annual testicular activity is synchronous with their female counter parts and can be divided into five distinct phases such as resting, preparatory, pre spawning, spawning and post spawning phase on the basis of GSI and histological condition of the testes. The macroscopic and microscopic features of testis are described below throughout the reproductive phases of this fish (Table 1). The distribution of cell types in the seminiferous tubule are demonstrated by the semi - quantitative method all over the reproductive phases tabulated in the table 2.

Resting phase (November to December): Testes are small thin thread like structure. Small seminiferous lobules contained maximum number of spermatogonial cysts and few primary and secondary spermatocytic cysts (Figure 3: a).

Preparatory phase (January to March): Testes are appeared as thin thread like elongated structure. Seminiferous lobules are small with thick lobule boundary and contained spermatogonia as the major cell type. Primary and secondary spermatotocytes appeared later part of this phase. Some spermatids are observed in this phase and interstitial cells are found in their interlobular space (Figure 3: b).

Pre spawning phase (April to May): Testes are enlarged with creamy white colour. This phase appeared to be the most active phase. Seminiferous lobules enlarged with the proliferation of spermatids and spermatozoa. Initially numerous clusters of primary and secondary spermatocytes appeared and later spermatids and spermatozoa dominated the cell types. Many interstitial cells occupied the interlobular space (Figure 3: c).

Spawning Phase (June to August): Testes are enlarged in size and occupied the whole length of the body cavity. Seminiferous lobule boundary wall distended and confluent with each other with the accumulation of large amount of spermatozoa (Figure 3: d).

Post spawning phase (September to October): Testes are reduced in size, became thin thread like elongated structure. Seminiferous lobules undergo empty due to leaving with large number of spermatozoa and few amount of residual spermatozoa are present in this phase. Spermatogonial cells are appeared in the periphery of the tubules (Figure 3: e).

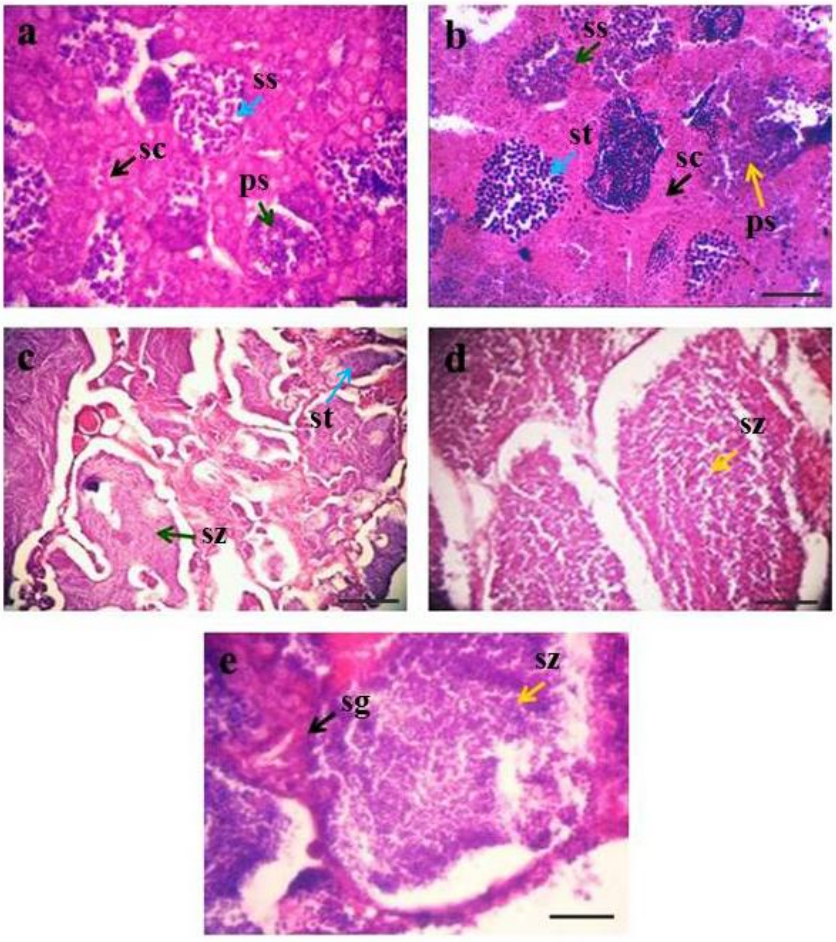

Figure 3: Histological features of the testes in an annual reproductive cycle of *Labeobata*. (**a)** Resting phase - Testes section shows the spermatogonial cysts (sc) as dominant cell types and a few cysts of primary (ps) and secondary spermatocytes (ss) in the seminiferous lobules. **(b)** Preparatory phase - Testes contain spermatogonial cysts (sc), primary spermatocytes (ps) and secondary spermatocytes (ss) and spermatids (st). (**c)** Pre - spawning phase - Testes section shows distended lobules accumulated large amount of spermatids (st) and spermatozoa (sz). (**d)** Spawning phase - Testes section shows lobules are greatly enlarged and confluent and packed only with spermatozoa (sz). (**e)** Post spawning phase - Testes section shows reduced lobules containing residual spermatozoa (sz) and spermatogonia (sg). (Scale bar 100 µm).

Table 1: Macroscopic and microscopic condition of different maturation phases of the testes of *Labeobata*

Phases	Months	GSI	Macroscopic condition	Microscopic condition
Resting phase	November – December	0.06 ± 0.02	Testes are small thin thread like	Spermatogoniaare the main cell type
Preparatory phase	January – March	0.37 ± 0.08	Testes are thin, elongated and creamy white.	Spermatogonia, Primary and secondary spermatocyte are the main cell types.
Pre - spawning phase	April – May	0.53 ± 0.06	Testes are enlarged in size with viscous milt flowing at late stage.	Distended lobules accumulated large amount of spermatids and spermatozoa.
Spawning phase	June – August	1.02 ± 0.2	Testes are very enlarged and free flowing milt.	Lobule greatly enlarged and confluent and packed only with spermatozoa and spermatid.
Post spawning phase	September - November	0.12 ± 0.03	Testes are thin thread like.	Lobules reduced, residual spermatozoa remained and new spermatogonia appeared.

Table 2: Semiquantative scoring of cell types in the testicular tissue of *L. bata* throughout the different reproductive phases

Phases	**Cell types**				
	Spermatogonia	Primary spermatocyte	Secondary spermatocytes	Spermatid	Spermatozoa
Resting	+++	+	+	–	–
Preparatory	+++	++	+	+	–
Pre spawning	–	–	–	+++	+++
Spawning	–	–	–	–	+++
Post spawning	++	–	–	–	++

Note: The number of cell types variation in the different reproductive phases which are as maximum number (+++), moderate number (++) and minimum number (+) in the various field study of histological slides. There are no cell type present in the slides indicates (–) sign.

4. Discussion

Reproduction of all Indian freshwater fish exhibit annual periodicity and maturation of their gonads and spawning occurs in a particular period of the year. Photoperiod, temperature, rainfall and food availability are the important external factors influencing breeding periodicity [10]. This study showed that *Labeobata* is an annual breeder and its gonad reached to the peak maturity stage during monsoon months, June - July.

Labeobata showed only one GSI peak in the month of June in the female fish during their reproductive cycle [11]. Similarly, in this study the GSI value reached peak in the month of June of male *L. bata* in their annual reproductive cycle. GSI increased with the maturation of gonads and reached to the highest value at the onset of breeding period. GSI values are observed high from June to August indicating the breeding period of the fish. GSI increased with the proliferation of spermatids and spermatozoa in male fish. GSI decreased abruptly during post spawning period with the release of spermatozoa. The month wise variations of GSI are highly significant ($p< 0.001$). Similar pattern of the annual variations in GSI are observed in other teleosts [12], [13].

The study revealed the similar pattern of maturation periodicity in male gonad in *Labeobata* that passed through five maturational phases as like that of female reproductive cycle [11]. The spawning period of the fish occurred during the monsoon months extending from June to August as like that of other fish [14]. The testes are compartmented by seminiferous lobules bounded by connective tissue wall. The connective tissue layers distended with the accumulation of sperm and became confluent each other during breeding period to facilitate the easy passage of sperm. [15] Authors are observed lobule boundary cells instead of interstitial cells in the teleostean testes. However, most of the teleosts possess interstitial Leydig's cells, which are distributed in between seminiferous lobules [16], [17]. Present study revealed interstitial cells instead of lobule boundary cells in between lobules. Annual variations of the interstitial cells are observed in relation to the spermatogenic activities. Number and size of the interstitial cells increased during pre - spawning and early spawning periods indicating their highest activities matching to the requirement for steroidogenesis during these periods.

Spermatogonial stem cells differentiate into spermatogonia [18] and spermatogonia produce millions of spermatozoa through the process of spermatogenesis [19]. Spermatogonial cells in the form of cysts are found throughout the reproductive cycle of *Labeobata* but with a great abundance during the resting phase (November - December) and preparatory phases (January to March). Two categories of spermatogonia such as primary and secondary spermatogoniaare identified in *Labeobata*as in many other teleosts [20], [21]. Spermatogonial division to produce primary spermatocytes began during preparatory phase and primary spermatocyte proliferation reached to the maximum in early pre - spawning phase (April). Primary spermatocytes entered into maturation division and produced millions of secondary spermatocytes and spermatids successively during maturation phase. Spermatids are transformed into spermatozoa by the process of spermeiogenesis during spawning period [22], [23] [24], [21]. With the same process the spawning phase of *L. bata* is characterised by the accumulation of spermatozoa. The spermatogenic activities decreased sharply when the testes entered into post - spawning phase (September). This phase could be distinguished by the presence of empty lobules with a few residual spermatozoa.

Thus, the reproductive cycle of male *Labeobata* can be subdivided in five distinct maturation phase such as resting phase (November December) preparatory phase (January to March), pre - spawning phase (April to May), Spawning phase (June to August) and post - spawning phase

(September to October). The annual reproductive cycle of male *L. bata*varies with the fluctuation of the photo thermal effect of the environment.

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References

[1] Muchlisin ZA, Musman M, Siti - Azizah MN. (2010). Spawning seasons of Rasboratawarensis in Lake LautTawar, Aceh Province, Indonesia. *ReprodBiolEndocrin*8: 49.
[2] Schlosser IJ. (1990). Environmental variation, life - history attributes, and community structure in stream fishes: implications for environmental management and assessment. *Environ Manage*15: 621 - 628.
[3] Muchlisin ZA. (2013). Study on potency of freshwater fishes in Aceh waters as a basis for aquaculture and conservation development programs. *J Iktiologi Indonesia*13 (1): 91 - 96.
[4] Roff DA. (1992). The evolution of live histories: Theory and analysis. Chapman and Hall, New York. *Aceh Int J SciTechnol*3 (1): 43 - 52.
[5] Bhattacharyya S, Maitra SK. (2006). Environmental correlate of the testicular events in a major carp, Catlacatla in an annual reproductive cycle. *Biol Rhythm Res*37: 87 - 110.
[6] Booth AJ, Weyl OLF. (2000). Histological validation of gonadal macroscopic staging criteria for Labeocylindricus (Pisces: Cyprinidae). *AfrZool*35: 223 - 231.
[7] Chakraborty BK, Mirza ZA, Miah MI, Habib MAB, Chakraborty A. (2007). Reproductive cycle of the endangered sarpunti, Puntiussarana (Hamilton, 1822) in Bangladesh. *Asian Fisher Sci*20: 145 - 164.
[8] Rao LM, Reddy KS, Hymavathy V. (1999). Breeding biology and fecundity in Mystus species from Mehadrigedda stream of Vishakapatnam. *EcolEnv Cons*5 (1): 25 - 28.
[9] Roff DA. (1983). An allocation method of growth and reproduction in fish. *Can J AquatSci*40: 1395 - 1404.
[10] Miranda LA, Strussmann CA, Somoza GM. (2009). Effects of light and temperature conditions on the expression of GnRH and GtH genes and levels of plasma steroids in *Odontesthesbonariensis* females. *Fish PhysolBiochem*35: 101 - 108.
[11] Roy K, Mandal DK. (2015). Maturity stages of ovary of a minor carp, *Labeobata* (Hamilton - Buchanon, 1822). *Int j Fish aquatstud* 2 (6): 19 - 24.
[12] Olurin KB, Savage OD. (2011). Reproductive biology, length - weight relationship and condition factor of the African snake head, *Parachannaobscura*, from River Oshun, South - west Nigeria. *Int J Fish Aquacult*3 (8): 146 - 150.
[13] Gupta S, Banerjee S. (2013). Studies on reproductive biology of *Mystustengra* (Ham - Buch., 1822) a freshwater catfish of West Bengal, India. *Int J Aqua Biol*1: 175 - 184.
[14] Guraya SS, Kaur R, Saxena PK. (1975). Morphology of ovarian changes during reproductive cycle of the fish, *Mystustengara* (Ham). *ActaAnat*91: 222 - 260.
[15] Lofts B, Marshall AJ. (1957). Cyclical changes of the testis lipids of a teleost fish, *Esoxlucius*. *Quart J Micros Sci* 3 (41): 79 - 88.
[16] Hyder M. (1970). Histological Studies in the studies of pond specimen of *Tilapia nigra* (Gunther) (pisces: Cichlidae) and their implication of the pituitary testis relationship. *Gen Comp Endocrinol*14: 198 - 211.
[17] deVlaming VL. (1974). Environmental and endocrine control of teleost reproduction. In: Schreck CB, editor. Control of sex in fishes. Blacksburg: Virginia Polytechnic Institute and State University, pp.13 - 83.
[18] De Rooij DG, Russel LD. (2000). All you wanted to know about spermatogonia but were afraid to ask. *J Androl*21: 776 - 798.
[19] Hess RA, Franc ALR. (2005). Structure of the sertoli cell. In: Skinner MK, Griswold MD (eds.), Sertoli Cell Biology. San Diego, CA: Elsevier Academic Press, pp 19 - 40.
[20] Bhatti MN, Al-Daham NK. (1978). Annual cyclical changes in the testicular activity of a freshwater teleost, *Barbusluteus* (Heckel) from Shatt-Al-Arab, Iraq *J Fish Biol* 13 (3): 321 - 326.
[21] Abu-Hakima R. (1987). Aspects of the reproductive biology of the grouper, Epinephelustauvina (Forskål), in Kuwaiti waters. *J fish biol* 30 (2): 213 - 222.
[22] Htun - Han M. (1978). The reproductive biology of dab, *Limandalimanda* in the North Sea: seasonal changes in the ovary. *J Fish Biol*13: 351 - 359.
[23] Chan EH, Chua TE. (1980). Reproduction in the greenback grey mullet Liza subviridis. *J Fish Biol*16: 505 - 519.
[24] Billard R, Fostier A, Weil C, Breton B. (1982). Endocrine control of spermatogenesis in teleost fish. *Can J Fish AquatSci* 39 (1): 65 - 79.