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Seasonal and Age-Related Changes during Pubertal Maturation of Captive Male *Labeo rohita* (Hamilton, 1822): A detailed Gross and Histological Study

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ABSTRACT

Detailed gross and histological seasonal changes during the first reproductive maturation of *Labeo rohita* was undertaken. The study was done throughout the age of 18-29 months. For this purpose, nearly 500 fish were kept in ponds and sampled every month for the histology of the testis. After taking data regarding other somatic traits, the testes were fixed in buffered formalin and processed for routine histology and light microscopy. The annual maturity stages can easily be divided into five gross stages namely, immature and developing, maturing and developed, ripe and running, post spawning and spent-regressed. The testis in *Labeo rohita* were unrestricted and lobular. The maximum GSI (2.89±0.25) was seen in June when the photoperiod and temperatures were highest, while the lowest GSI was seen in October (0.09±0.01). The start of the monsoon rains was a cue for final maturation and spawning and by the middle of July; the testes were already declining and reached the minimum point in October. Both photoperiod and temperature have a strong influence on completion and regression of the spermatogenesis. This detailed study is the first from the Northern latitudes of the Indo-Pak subcontinent on the fish kept in commercial ponds.

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KPL conceived and planned the experiment. IL conducted the practical and lab work under the supervision of KPL. KPL interpreted the data and wrote the manuscript.

Key words
***Labeo rohita*, Testis, Gross stages**

INTRODUCTION

Commercial aquaculture is a relatively new activity in Pakistan as it was not practiced traditionally, which explains its small contribution to overall national fish production. Because of absence of a true fisheries statistics collection system, separate authentic statistics for production from aquaculture is not available. However, it is variously estimated that around 30-50 % of overall inland fish production comes from aquaculture.

Study of reproduction of any species of fish requires the knowledge of its gross gonadal structures, histology and annual dynamics of these parameters. Physiological control of these changes in the form of protein and steroid hormones are also an integral part of such studies. In this connection more work is available for females (Mylonas *et al*., 2010). In contrast to ovarian physiology, surprisingly very little is known about testicular function in fishes, and work is available about very few fish species. Testicular histology and morphology is different among fishes and is studied by Schulz *et al*. (2010) and Uribe *et al*. (2014). In general, Sertoli cells provide physical and chemical sustenance during spermatogenesis. Leydig cells function primarily to synthesize sex steroid hormones (Scott *et al*., 2010) responsible for spermatogenesis, and feedback regulation of pituitary and hypothalamic secretions.

Major carps are an important fishing resource in Pakistan. The history of their culture in Indo-Pak sub-continent is some 4000 years old (Lone, 1983). The main aquaculture system which is in vogue in Pakistan is carp pond culture. It is essentially an extensive culture based on three indigenous carp species (*Labeo rohita, Catla catla* and *Cirrhinus mrigala)* and three exotic species, i.e., *Cyprinus carpio*, *Hypophthalmichthys molitrix* and *Ctenopharyngodon idella*. The farming of *Labeo rohita* along with other major carps is an expanding local industry. The compatibility of rohu with other carps like catla (*Catla catla*) and mrigal (*Cirrhinus mrigala*) made it an ideal candidate for carp polyculture systems. However, not much is known about their reproduction, biology and spawning. *Labeo rohita* (locally known as rohu) is the most important fish among the three Indo-Pakistan major carp species used in polyculture systems. This graceful riverine species is the natural inhabitant of the river system of northern and central India and the rivers of Pakistan. To date, seasonal changes in reproductive endocrinology and concomitant testicular histology in male *Labeo rohita* are unknown particularly from the areas now in Punjab,

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Pakistan. Keeping in view the importance of reproductive biology in control of reproduction and spawning the present study project was undertaken.

The aim of the current project was to describe gonadal development and histology of testis of *Labeo rohita* during the first (pubertal) maturational cycle during the age of 18-29 months in pond culture. The present study is a part of attempt to describe scientifically and systematically the reproductive biology of Pakistani major carps i.e. *Labeo rohita, Cirrhinus mrigala* and *Catla catla* (Lone and Hussain, 2009).

MATERIALS AND METHODS

Samples collection

The fish was obtained from a fish farm located at 41 kilometers from Lahore (latitude 31˚58 N, longitude 74˚13'E), on the North bound GT road. The fish were kept in a polyculture of carps and the conditions were kept standard as they are in use generally in commercial practice. The sampling was started at 18 months of fish age. For this purpose, a group of 500 fish were collected by cast nets from various production ponds on the farm and were stocked in a new pond so that they may not be disturbed for any reason except the monthly samplings. Every month, on or near the 15th day, the fish were sampled by cast net and a total of 20 fish were randomly collected. Sampling was conducted in afternoon when the water temperatures were comparatively cooler in order to minimize the capture stress. The fish were transported to the wet lab and the whole operation of transportation took around one hour. In the lab, fish were retained in rectangular concrete tanks [1.7 W ×4.12 L × 1.0D (meters)] supplied with water through a sprinkler over it.

Meteorological data

Data about following parameters for Lahore city was collected from Pakistan Meteorological Department Lahore office: monthly total rainfall (mm); monthly mean minimum and maximum temperature (°C); photoperiod (h) and humidity (%).

Anesthetization

At the time of the actual sampling for the blood and gonads, the water of the holding tank was reduced and fish were scooped out one by one by a hand-held scoop net. The fish were given anesthesia with Clove oil (Berka, 1986; Kaiser *et al.,* 2006). The stock solution (clove oil in alcohol 1:2) was mixed in water at a rate of 1 ml for every 100 ml of water. The fish were allowed in the solution for 3 to 5 minutes and time period of anesthesia was changed with age and size of fish.

Measurements of morphological parameters

The morphological parameters total body weight (g), standard body length (mm), total body length (mm), and body depth (mm) were measured. The length and body weights were used for the calculation of condition factor according to the formula, $k = W/L^3 \times 100$ (Lone and Al-Marzouk, 2000).

Dissection and removal of gonads and liver

Lower abdomen of fish was cut from posterior to the anterior end. Testes were found attached to the lower side of air bladder. Gonads were removed with small scissors and freed from any extraneous tissue. Photographs of all gonads were taken after their removal by putting along the side of a scale to compare the developmental changes within a month. Gonads were weighed to the nearest 0.1 mg. Length of gonads was measured to the nearest 1 mm. Liver of fish was removed and weighed and stored at -40 ˚C. Total body weight and gonad weight were used to calculate the gonado-somatic index (GSI) and hepato-somatic index (HSI).

Microscopic studies of testis

Tissues (gonads and livers) were preserved in 10% neutral buffered formalin, and treated for routine hematoxylin and eosin histological examination.

Statistical analyses

All the data were computed and graphed using "MS Excel" version 2013. Statistical analyses were performed by using SPSS (Ver.20).

RESULTS

Seasonal changes in environmental parameters

Various environmental factors: photoperiod, atmospheric temperature, water temperature, rain fall and humidity are presented in Figure 1. These factors correlated with testicular development. Details of body weight, body length, gonad weight, GSI, Liver weight and its index are prsented in Table I.

Gross gonad structure

The month wise gross structure of testis with the minimum and the maximum size is given in Figure 2.

The data regarding the testes weight, GSI and its relation to other parameters are given in Figure 1. At the start of experimental sampling (age= 18 months), the testes were thread like with a mass of 1.03±0.20 g. The testis weight declined in December (0.77 ± 0.21 g)

Table I.- Annual and Age-Related variations of various parameters (Mean±SD) of male *Labeo rohita* during the study period.

Months	Body weight (g)	Total length (mm)	Body depth (mm)	Condition factor (k)	Gonad weight (g)	Gonad length (mm)	Gonad girth (mm)	GSI	Liver weight (g)	LSI
November	834.56 ± 11.12	473.60 ± 4.63	148.84 ± 3.98	0.79 ± 0.03	1.03 ± 0.20	85.90 ± 3.79	3.98 ± 0.66	0.12 ± 0.03	11.95 ± 0.87	1.34 ± 0.11
December	714.35 ± 11.35	434.00 ± 3.72	136.48 ± 6.07	0.88 ± 0.03	0.77 ± 0.21	98.75 ± 2.45	4.93 ± 0.61	0.11 ± 0.03	11.28 ± 0.51	1.58 ± 0.07
January	892.00 ± 40.00	440.00 ± 2.89	153.25 ± 6.77	1.05 ± 0.04	1.04 ± 0.23	87.83 ± 7.22	4.80 ± 0.84	0.12 ± 0.03	11.29 ± 0.57	1.26 ± 0.02
February	1058.33 ± 39.98	450.00 ± 1.15	127.00 ± 0.00	1.16 ± 0.04	1.29 ± 0.12	105.67 ± 3.61	4.80 ± 0.66	0.12 ± 0.02	10.73 ± 0.62	1.02 ± 0.06
March	1161.16 ± 95.98	461.40 ± 11.75	147.32 ± 3.11	1.17 ± 0.04	11.33 ± 3.63	109.90 ± 4.61	10.00 ± 0.79	0.97 ± 0.27	9.74 ± 0.51	0.86 ± 0.06
April	1247.35 ± 20.69	458.50 ± 5.89	158.75 ± 3.67	1.30 ± 0.04	21.79 ± 3.81	128.25 ± 9.27	13.25 ± 1.27	2.09 ± 0.30	13.11 ± 0.71	1.05 ± 0.05
May	1197.46 ± 97.92	473.75 ± 15.46	165.10 ± 6.96	1.12 ± 0.05	24.87 ± 1.35	134.20 ± 4.90	15.54 ±0.76	2.12 ± 0.18	12.40 ± 0.85	1.00 ±0.05
June	1315.40 ± 70.27	470.20 ± 7.36	165.10 ± 4.02	1.26 ± 0.02	38.31 ± 4.41	148.20 ± 6.71	17.42 ± 1.18	2.89 ± 0.25	13.72 ± 0.61	1.05 ± 0.04
July	1251.87 ± 97.91	467.80 ± 10.88	162.56 ± 2.54	1.22 ± 0.07	24.15 ± 4.28	147.60 ± 10.92	15.98 ± 1.91	1.93 ± 0.34	9.48 ± 0.37	0.77 ± 0.05
August	1333.56 ± 89.12	494.20 ± 4.49	149.86 ± 4.75	1.10 ± 0.06	3.76 ± 1.03	108.50 ± 5.16	8.06 ± 0.69	0.28 ± 0.07	16.41 ± 0.66	1.35 ± 0.08
September	1372.12 ± 87.59	496.50 ± 12.51	145.85 ± 6.42	1.16 ± 0.05	2.89 ± 0.33	109.88 ± 5.48	6.65 ± 0.64	0.21 ± 0.01	12.53 ± 1.10	0.92 ± 0.07
October	1365.31 ± 71.86	495.00 ± 7.58	144.78 ± 3.11	1.13 ± 0.05	1.29 ± 0.17	104.40 ± 0.48	5.02 ± 0.47	0.09 ± 0.01	12.60 ± 0.91	0.92 ± 0.05

to provide the lowest values of the year. From November to February no significant change occurred in testes weight and it remained between 1.03 to 1.29 g during the age 18-21 months. After March (testes weight= 11.33±3.63 g), the growth of testes was very fast and the weight reached a peak in June, when it was 38.31 ± 4.41 g, showing a 3.4 times increase in weight. The GSI followed the testicular weight rigorously, and the values ranged from 0.09±0.01 in October to 2.89±0.25 in June when a peak was observed (Fig. 1, Table 1).

Liver weight and HSI

The liver weight in November was 11.95 ± 0.87 g. In winter months from November to March the liver weight decreased and in March the weight was 9.74 ± 0.51 g. In spring when the fish became active again, a rise in weight was observed in April (13.11 ± 0.71 g). No significant change occurred in liver weight during peak summer months.

Histological characteristics during seasonal testicular development

November

The testes were thin in circumference, straight and thread like. No sperm duct was present. The blood vessels were less pronounced and testes were slightly reddish in color. The GSI was 0.12±0.03 (Fig. 2).

The sections from caudal, middle and cephalic portion of the testis were examined and found that all were at the same stage of histological development. The testes were immature, primary germ cells and spermatogonia were dominant cells. Stromal tissue was also present. Certain vacuolated areas were also seen. The tunica albuginea was thick and dense containing smooth muscle fibers. No distinction in lobules was seen in immature stages (Table II, Fig. 3).

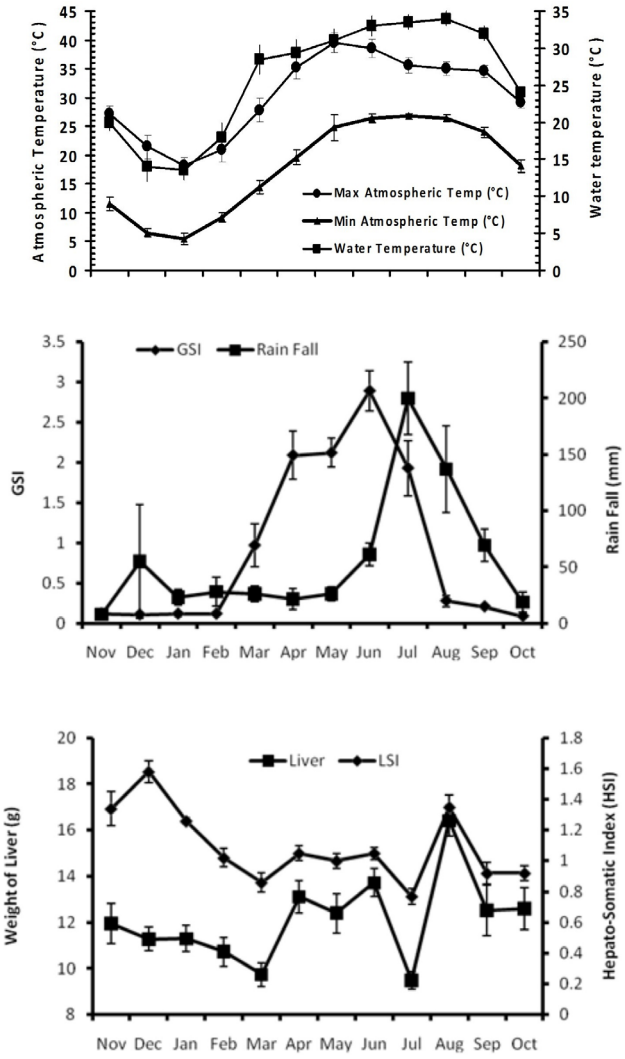

Fig. 1. Environmental parameters (minimum and maximum atmospheric and water temperature) and seasonal variations in Gonadosomatic Index (GSI), rain fall, weight of liver and hepato-somatic index (HSI) of Labeo rohita from age 18 months (November) to 29 months (October). Values given are mean±SD. For more details see Material and Methods.

December

The GSI was lowest (0.11±0.03) in this month corresponding with lower water temperatures. The fatty infiltration had decreased and the island like areas now transformed into the stromal tissue (Table II, Fig. 2-3).

January

No variations were observed in the gross structure of testis which were thin and straight The GSI was 0.12±0.03. Abundant spermatogonia having oval nuclei were seen. Stromal tissue was quite prevalent interspersed between spermatogonia (Figs. 2 and 3).

February

The GSI was still the same (0.12±0.02) since age 19 months. The spermatogenesis was at initial stage with spermatogonia as the predominant cells. The tunica remained thick (Figs. 2 and 3).

March

The testes weight increases around 10 times the mean weight during the previous months. The GSI during this month was 0.97± 0.27. The testes were convoluted and pinkish in color. Spermatogenesis was quite active and primary and secondary spermatocytes were seen in the cysts of the lobules (Figs. 2). This picture was a little advanced in the fish that had the maximum weight of the testis (Table II).

Fig. 2. Size, gross structure and developmental stages of the testis of male *Labeo rohita* reared in earthen ponds from the age of 18 month (A) and 29 months (B). For other details, see Tables I and II.

April

The GSI nearly doubled from the March values and was 2.09±0.30. The testes were highly convoluted and creamy in color. Blood supply to the testis was the highest. Sperm duct started to appear, however the testis were still separate. The genital papilla started bulging and two pores

(anal pore and genital papilla) could be seen (Table II, Figs. 2).

Spermatogenesis was well underway and lobules and their cysts were quite discernable. Many cysts had ruptured and released the spermatids into lobular lumen which was full of these cells (Table II). Numerous Sertoli and Leydig cells were present (Fig. 3).

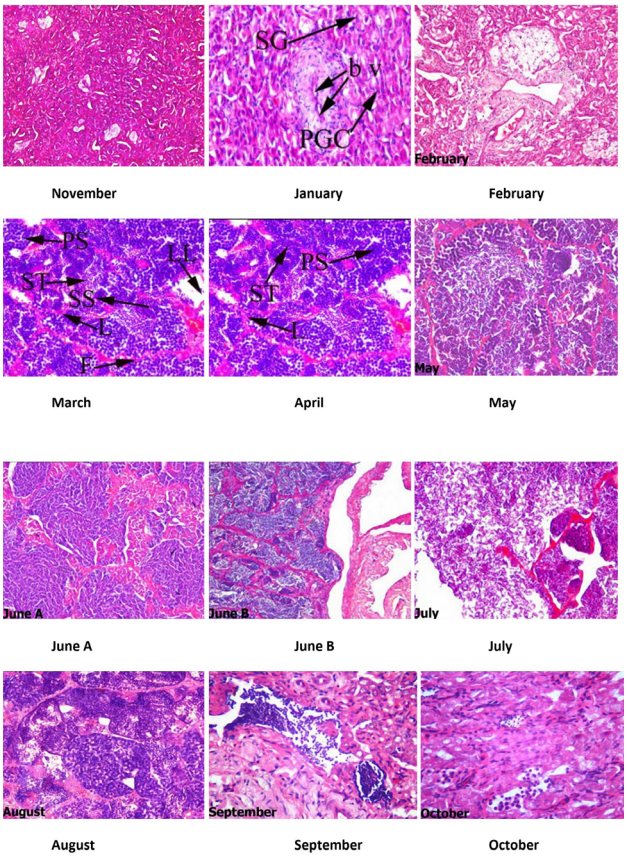

Fig. 3. Seasonal and age-related changes in the testis of *Labeo rohita* during the age of 18 to 29 months. The tissues were fixed in buffered formalin paraffin embedded and 5µ thick sections were stained with hematoxylin-eosin. The stained sections were photomicrographed with high resolution digital camera. For other details, see Table I and II and the section "Materials and Methods". Bv, Blood Vessel; I, Interstitium; LL, Lobular Lumen; PGC, Primary Germ Cell; PS, Primary Spermatocytes; SG, Spermatogonia; SS, Secondary Spermatocytes; ST, Spermatids

May

The GSI for this month was 2.12±0.18. The testicular weight was still increasing. The testes were whitish in color and highly convoluted. The blood vessels were highly developed. A clear demarcation in genital and anal pore was seen (Table II, Fig. 2).

Majority of the cysts and lobules had ruptured however some lobules were still intact and full of these cells (Fig. 3).

June

A peak in GSI (2.89±0.25) was seen in this month. Some of the lobules boundaries were broken and this was more pronounced in the central part of the tissue. A majority of the lobules showed that active spermatogenesis had ceased and the lobules were storing the sperm. The tunica albuginea was thin consisting of connective tissues (Fig. 3).

July

A decline in testes weight started from this month. Few resting spermatogonia were seen while the whole part of the testes contained sperms (Fig. 3).

August

The mean GSI value for this month was 0.28±0.7. A decrease in testis weight (3.76±1.038 grams) was also observed. Some lobules were still full of spermatids and sperms (residual sperms) and this was particularly true of the central portion and duct of the testis tissue (Table II, Fig. 3).

September

The testis weight had gone down tremendously while the testis was regressing in the body cavity compared to other organs (Fig. 2). The mean GSI value for this month was 0.21±0.01. Some degenerating cells were scattered among the stromal tissue which was proliferating quite rapidly (Fig. 3).

October

The lowest GSI value of the annual seasonal cycle was seen during this month. Very few old spermatozoa were present within the fibrous stroma. No lobule or cysts were seen (Fig. 3).

DISCUSSION

Environmental clues are vital for reproductive growth and entrainment to occur in male and female poikilotherms. The most significant environmental factors are photoperiod and temperature (Vergilio *et al*., 2012). The growth of fish was affected by stock density and specific physiochemical parameters including temperature, pH and alkalinity (Jalbani *et al*., 2018). Studies on the temperate fish has shown that the effects of different lengths days in different fish indicated that the photoperiodic history of the species

Table II.- Gross, macroscopic and histological description of testicular cycle and gonado-somatic-index of male *Labeo rohita* from the age of 18-29 months. Values given are Mean±SD.

Maturity stage	Duration (age)	Testicular weight (g)	GSI	Gross and macroscopic description	Histological description
Immature and developing	Nov-Feb (18-21)	1.03±0.20-1.29±0.12	0.12 ± 0.03-0.12± 0.02	Testis immature and development in testis starts in February. Testes were thin in circumference, straight and thread like. No sperm duct present and no pronounced blood vessels. Testicular weight lowest in December. Both testes were separate. The urogenital pore was not prominent.	Primary germ cells and spermatogonia were the most prevalent cells. Stromal tissue was quite prevalent interspersed between spermatogonia. The tunica albuginea was thick and dense containing smooth muscle fibers. No lobules were seen in immature stages. Few aged spermatozoa were also seen in the ducts.
Maturing and developed	Mar-May (22-24)	11.33± 3.63-24.87± 1.35	0.97± 0.27-2.12± 0.18	In March the testes weight increased 10 times the mean weight during the previous month. Testes were enlarged, convoluted with prominent blood vessels and Pinkish in color. In April Sperm duct started appearing but the testes were still separated. In May, a majority of the fish exuded milt on exerting slight pressure on the belly. The genital papilla clearly showed two bulging pores.	In March, Testes were seen to have rays of connective tissue radiating from the centre towards the periphery throughout the entire mass of the tissue. In April, spermatogenesis was quite active but the lobules and cysts were intact, primary and secondary spermatocytes were seen in the lobular cysts. Some of the small sperm ducts that drain the lobules from different areas can also be seen. With the increase of spermatocytes, the spermatogonia steadily decreased. Even the spermatids could also be seen in the cysts. Interstitial tissue was quite elaborate. In April, Peripheral lobules had no lumen while central lobules were large and had lumen. Cysts with spermatids were numerous. Sertoli and Leydig cells were present. Tunica was thinner than previous months. In May, cysts had ruptured however the lobules were intact and full of spermatids/ spermatozoa. Lobules were loosening up for rupturing and the lobular space and ducts were forming. Tunica was thin. Some fish gave a small of milt on pressing the belly.

Maturity stage	Duration (age)	Testicular weight (g)	GSI	Gross and macroscopic description	Histological description
Ripe and running	Jun-Jul (25-26)	38.31± 4.41-24.15± 4.28	2.89± 0.25-1.93± 0.34	A peak (2.89±0.25) in GSI was seen in June. Creamy white testes were highly convoluted and the largest organs in abdominal cavity. The milt dripped out automatically through genital pore on handling and the consistency was thick while volume little. All the fish were found to be running. The main sperm duct could be seen on the inner medial side along the whole length of the testis with prominent blood vessels. The genital papilla was bulged out and of red color. Testes were in color. In July majority of the fish were seem to have spawned. Milt pH was 5.5. A decline in testes weight started and GSI was	In lobules, different cysts showed asynchronous development while the growth within the cyst was synchronous. Majority of the lobules had lumen. Central lobules had their boundaries broken. Few cysts containing primary spermatocytes were seen closed to the lobule boundaries in the peripheral portion of the testis. Majority of the lobules were filled with spermatozoa. Active spermatogenesis had ceased in majority of the samples and the lobules were just storing the sperm. The tunica albuginea was thin consisting of connective tissue. In July Few resting spermatogonia were seen while the whole part contained sperms which oozed out on just handling the fish which caused a decline in testicular weight and GSI. Connective tissue has started proliferating at places.
Post-Spawning	Aug-Sep (27-28)	3.76±1.03-2.89±0.33	0.28± 0.07-0.21± 0.01	A significant decrease in testis weight and circumference was observed. Testis became small, thin and straight. In August some milt still oozed out on pressing the belly in some fish. The general outline of the testes was little loose. This was the month when the testis started regressing which started from the cephalic end. In September the testis weight had gone down tremendously while the testis.	`Both organ weights and GSI declined drastically clearly depicting the picture of post-spawning. The testes were loose in outline however; the histological picture was as if spawning is going on. Some lobules were full of spermatids and sperms particularly in the central portion of the testis. Stromal tissue has started creeping in from the peripheral portion of testis towards inner side. In September, the lobular structure was disappearing; although few with spermatozoa were still present. Few remnant spermatozoa were present within the fibrous stroma. No milt even on pressing the belly area.
Spent and regressed	October (29)	1.29±0.17	0.09± 0.01	The lowest GSI value of the annual seasonal cycle was seen during October. Testes were again thin and thread like although their color had changed from cream to dirty white.	No lobule or cysts were seen in October. .The residual sperms were found in the ducts. Only spermatogonia were present. Tunica had become thicker again.

and the change of direction of photoperiod have a far greater importance than a particular critical day length on the gonadal development in fish (Bromage *et al.,* 2001).

The present study reveals that male *Labeo rohita* attains sexual maturity in the month of June when the age of the fish was 25 months (Tables I, II). From the present studies it has been observed that seasonal sexual maturity in *Labeo* correlate well with age, season and body weight. However, few uncertainties were also seen. This are concerned with the size of the males collected during the breeding period. It appears as if the bigger males of the same age mature first, followed by the smaller males (Kumar *et al*., 2003). In present work the fish were virgin and going to mature first time during the summer months (May-July) when they were going to become 2-years old. At best, we can say that the fish were entering the puberty during the first 5 months (November to March) of the study. This information may also be seen in the light of studies on major carps collected from the wild (Kumar *et al*., 2003).

The fast rate of growth was observed in summer when the temperature was high, days long while decrease in temperature and photoperiod retarded the growth (Table I). In *Catla catla,* a fish living in the same range of environment, artificial long photoperiods were shown to be successful in advancing testicular maturation, while shorter daylength-temperature combination can stall maturation in the prespawning phase of the reproductive cycle (Bhattacharyya *et al.,* 2005).

The gonado-somatic-index showed no change from November to February when temperature and photoperiod were quite low. With the advent of spring, both the photoperiod and temperatures started rising gradually with the peak reaching in these parameters around June and therefore a peak was seen in the weight of testis and GSI concomitantly (Fig. 1). In some fishes the gonadal development depends on day length (Lam and Munro 1987), while others respond to the changes in temperature and still in some other fish, gonadal functions are thought to be dependent on a combination of both (Koya and Kamiya 2000). Seasonal fluctuation of photoperiod is the major environmental factor associated with the seasonal reproductive activity of the carps. Ambient temperature appeared as a dependent variable of photoperiod, and thereby, have substantial influences on the development of testis in *Catla catla,* a fish cultured along with *Labeo*. Rainfall, showed significant correlation only with the peak reproductive activity, i.e. the act of spawning (Bhattacharyya and Maitra, 2006).

Testicular morphology is as diverse in fishes as their habitats and is studied by Grier *et al*. (2009). In general, Sertoli cells are directly associated with germ cells and provide physical and chemical support during spermatogenesis. Leydig cells are found in adjoining connective tissue where they function primarily to synthesize sex steroid hormones responsible for spermatogenesis, expression of secondary sexual characteristics (Knapp and Carlisle, 2011).

The work on the staging of *Labeo cylindricus* testes was conducted by Booth and Weyl (2000) who placed more emphasis on the spermatogenesis, after comparing with the gonadal staging of testes within the African "labeine" species (Booth and Weyl, 2000). It was noted that the "spent" testes did not show all stages of spermatogenesis and were dominated by Type A spermatogonia and old spermatozoa rather than by spermatocytes. It is generally concluded that gross observations alone are not sufficient to stage the testes since the "spent" and "maturing" testes can almost be identical in appearance, but histologically different (Fig. 2, Table II).

In *Labeo rohita,* with the development of testis, sperm duct appears but the testis remained separate (Fig. 2). After the spawning season, the process of testicular regression was gradual, starting from the rostral to caudal region of the testes. This is a cue that exudation of milt is a gradual process during the spawning season. This picture is similar to the one described by (Rutaisire *et al.,* 2003) for *Labeo victorianus*.

The histological sections of testes showed that from November to February when testes were immature, only primary germ cells were dispersed in the stromal tissues. Few old residual spermatozoa were also seen. The spermatogonial and primary germ cells were scattered and no "nest" of these cells had formed yet. Spermatogenesis involves an initial proliferation of spermatogonia, through repeated mitotic divisions, and ultimately transform into primary spermatocytes. Primary spermatocytes then undergo meiosis to form secondary spermatocytes. Division of secondary spermatocytes, whose life is very short, results in the production of spermatids, which then undergo metamorphosis to the motile spermatozoa (Schulz *et al*., 2010).

Fish spermatozoa in the testis have already completed two meiotic divisions but they may not be fertile. For example, salmonids spermatozoa in the testis are immotile, and acquire their motility during passage through the sperm duct (Morisawa and Morisawa, 1986), where, probably the titer of the male hormones are higher. In the present study, sperms were seen in the milt in June and July. The testes, at that time, were composed of interstitial and lobular compartments. The interstitium between lobules consisted of Leydig cells, fibroblasts, and blood and lymph vessels. It is at this time when sex steroids are highest in concentration in the blood (Suresh *et al*., 2008).

With the decrease in GSI in middle July which coincided quite nicely with the maximum rains of Monsoon, testicular regression had started. Although, the spermatogonia were present throughout the year they became quite notable, when the testes were in regression. Despite this, the residual sperms or spermatocytes remains in testis long after the spawning season and sometime during the winter months also. During the first maturity season, some males were observed with smaller testes and not all males matured (age 18-29 months) and remained at the same stage weather maturing or regressing. Some males remained immature even during the spawning season. This may points to their genetic diversity, as the fish came originally from induced breeding of the brood stock of diverse background and age however, they were spawned collectively on the same day (Maitra and Chattoraj, 2007).

From the picture described here, it appears that the *Labeo rohita males* used in the present study were similar to the *Labeo rohita* whose biology was described earlier from Bengal, India (Bhattacharya *et al*., 2005), but since they (present study fish) came from more Northern latitude, the overall maturity was delayed somewhat pointing to the fact that final maturation of these fish is more dependent upon temperature and rainfall than the overall effect of the photoperiod.

Statement of conflict of interest

The authors declare no conflict of interest.

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