

Gross and Histological Variations in Testes of a Major Carp, *Catla catla* (Hamilton, 1822), During its First Maturation Cycle in Pond Culture System

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Abstract.- Seasonal variations in testicular development of *Catla catla* were studied during its first maturational cycle from the age of 18 to 29 months. Gross and histological variations were noted from November, 2006 till October, 2007. Based on gross and histological studies, the annual testicular cycle was divided into five stages; immature, maturing, mature and spawning, post-spawning and regressed. In catla, testes were unrestricted and lobular. Peak value of GSI (0.48 ± 0.07 %) was observed in May and first fortnight of June and declined rapidly in later part of June and July (GSI: 0.03 ± 0.07). It shows that catla spawns in high summer when both temperature and photoperiod are at their peak. Moreover, the spawning time is very short and starts in the monsoon and may be over in a fortnight. Testicular development was initiated by an integrated influence of rise in water temperature and photoperiod. Heavy rainfall of monsoon was probably the final cue for spawning. Termination of spermatogenesis and spawning was probably controlled by decrease in photoperiod from summer solstice and decreasing monsoon rainfall. This study is first of its kind from Pakistan (Northern Indo-Pak subcontinent) and describes the detailed gametogenesis in male catla reared in ponds.

Key Words: GSI; photoperiod; rainfall; reproductive biology, seasonal variations, spermatogenesis; temperature.

INTRODUCTION

Pakistan is located in temperate region (Latitude $30^{\circ}0'N$, Longitude $70^{\circ}0'E$). Zoogeographically, the fish fauna is a mixture of Palaearctic, High Asian, West Asian and Oriental elements (Mirza, 2006). Inland aquaculture is meeting around 80% of the total domestic needs, while per capita consumption of fish is 3.7 kg per year. The province of Punjab is contributing around 36.87% (60345 metric tons) of the national aquaculture production (FAO, 2005).

In Pakistan, catla is a valuable and commercially important cyprinid species and is cultured along with indigenous major carps and exotic Chinese carps. This species has been investigated to study its taxonomy, ecology, abundance, feeding and growth parameters, but to date, reproductive biology is quite neglected area of the study (Ayyappan and Jena, 2001, 2003).

Reproductive biology of fish has long been a widely investigated field. Determination and development of sexual maturity is fundamental to fishery science. Due to the importance of these parameters in the dynamics of populations, these are commonly estimated for species of economic importance (Hunter *et al.*, 1992).

In aquaculture, fingerling production systems and breeding programs require optimization of spawning success in young breeding populations. One possible reason for failure of spawning in culture system is lack of information about the reproductive biology of that species.

A fragmented work on reproductive cycle of catla has been done in West Bengal, India (Bhattacharyya *et al.*, 2005; Bhattacharyya and Maitra, 2006). However, the reproductive cycles and their environmental correlates for the same species can be different in tropical and temperate regions (Sivakumaran *et al.*, 2003). The basic elements of the reproductive biology of *Catla catla* in temperate region (Pakistan or Northern India) have not been investigated.

In the present paper we are providing basic information on the reproductive biology of *Catla catla* during second and third year of life, when the fish matures first time in pond culture. These studies are designed to determine the morphological and

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0030-9923/2009/0006-0483 \$ 8.00/0

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histological stages of testicular development and their environmental correlates. These studies first of its kind in Pakistan will provide the base line reference values for reproductive parameters of this fish.

MATERIALS AND METHODS

The fish, *Catla catla* were reared in a commercial fish farm (Latitude 31° 58'N, Longitude 74° 13'E) 40 Km from Lahore. These fish originally came from a stock used for commercial polyculture of major carps and Chinese carps. For the present studies, they were kept for one year to study the complete annual cycle from the age 18 to 29 months in a separate pond with *Labeo rohita* of the same age. Every month (15th ±2 days) fish were collected by cast nets and brought to the lab alive in plastic bags provided with pond water and compressed air. In the lab, the fish were kept in cemented fish tanks (4.12 m (L) × 1.7 m (W) × 1.0 m (D) for 24 hours to be relieved from the stress of capture. Every month 10 male fish were randomly sampled. Because of the lack distinguishing characters between male and female fish, more fish were sacrificed to get the required numbers.

Physical parameters

Data on water temperature, atmospheric temperature, photoperiod and rainfall were obtained from the Meteorological Dept. of Govt. of Pakistan (Lone and Hussain, 2009).

Sampling, anesthetization and bleeding

On the day of sampling the fish were removed from the cemented tanks by a scoop net and anesthetized immediately. Clove oil was used as anesthesia (Berka, 1986; Kaiser *et al.*, 2006). Anesthesia was prepared fresh by dissolving the clove oil into absolute alcohol (Merck, Germany) in a ratio of 1:2. This solution was used as a stock for mixing with water. The fish were removed from anesthetic chamber when completely sedated and blood was drawn from the caudal vein and allowed to clot in centrifuge tubes.

Morphological parameters

After bleeding, total body weight, total body

length, standard length and body depth were measured before dissection. Afterwards, lower abdomen of fish was cut from posterior to anterior end for removing the testes. Morphological features of testes like its color, thickness, blood vessels, position and color of the opening of sperm duct and any visible abnormality in testes were noted. After removal, gonads were immediately weighed and preserved in 10% buffered formalin following Troyer (1980).

The gonadosomatic index (GSI) and hepatosomatic index (HSI) were calculated as $GSI = 100 (W_G / W^1)$ where W_G is weight of tissue and W^1 is total body weight of fish.

Histological studies

Tissues were processed for routine haematoxyline-eosine staining. Stained slides were microphotographed by high resolution microscope (Leica, Japan) attached with a high resolution digital camera. Thickness of tunica, diameters of germ cells, nuclei, nucleoli and lobular walls were measured by a micrometry software (Motic Images Plus, China).

RESULTS

The details of environmental data are given elsewhere (Lone and Hussain, 2009) and in Figure 1.

Gross structure

Annual testicular cycle of catla reported here was the first reproductive cycle of fish and the study extended from November 2006 (age= 18 months) to October 2007 (age= 29 months). Testes of catla are soft, creamy white, paired glands, suspended antero-posteriorly in the peritoneal cavity on the ventral side of the dorsal air bladder, by thin mesorchium. Testes were posteriorly attached and end into vasa deferens just prior to its opening to exterior through genital pore. The genital pore opens in cloaca along with the openings of excretory and digestive systems. Vasa deferens is very thin, narrow and white in color. Marked increase in length and width of testes was noted only during spawning season. At this time maturing and mature specimens could be identified by release of milt on slightly pressing the abdomen. However, only few drops of very thick

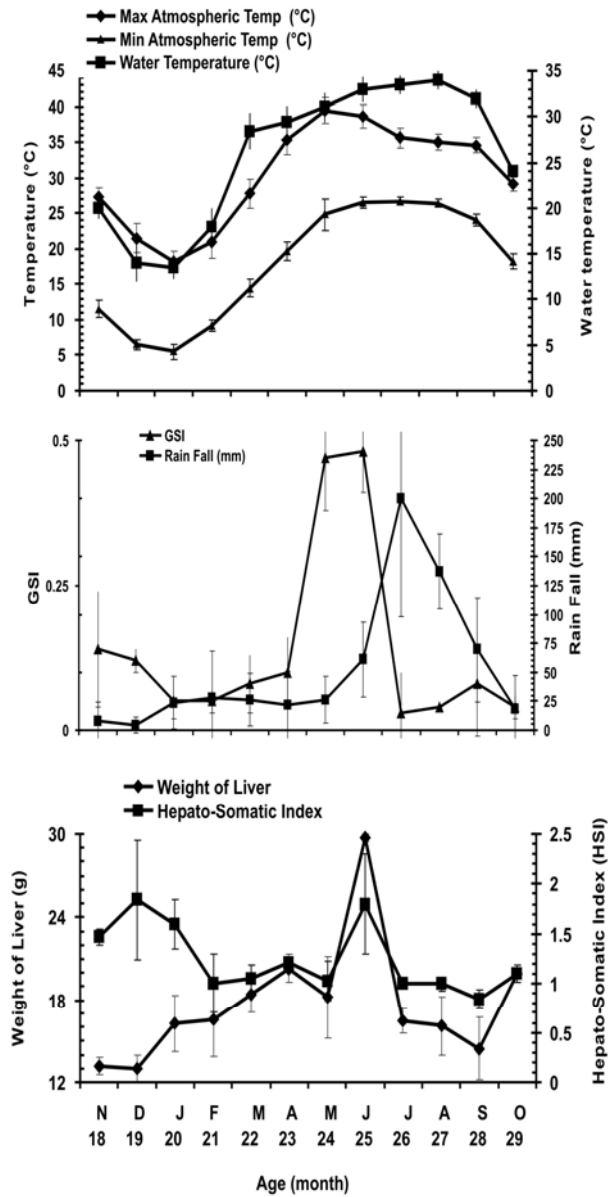


Table I

Fig. 1. Environmental parameters and age-related changes in the gonadosomatic index (GSI), liver weight (g) and hepatosomatic index (HSI) of male *Catla catla*. Values are Mean \pm SEM. For other details see Materials and Methods.

milt were observed even in fully mature specimens (Fig. 2). Details of body weight, liver weight and gonadal parameters are given in Table I, while testicular stages and annual cycle has been summarized in Table II.

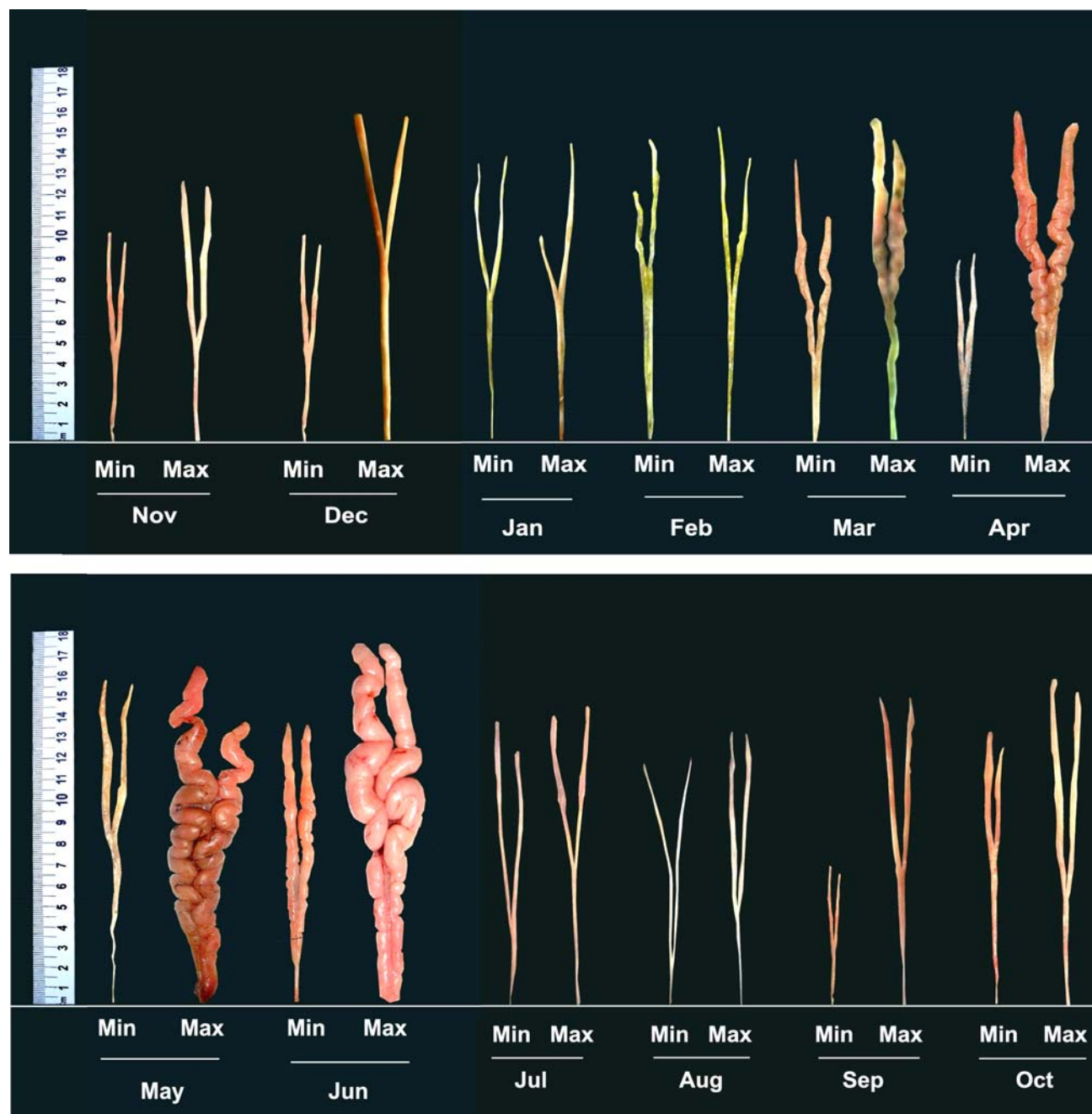


Fig. 2. Size, gross structure and development of the testis of male *Catla catla* reared in ponds from the age of 18 months (November, 2006) to 29 months (October, 2007). For other details, see Tables I and II.

Annual testicular cycle

November (18 months)

Testes were very thin and small in length. Blood vessels were not prominent and testes look like cords. Mean GSI value was 0.14 ± 0.10 (Table

I). The histological picture showed that tunic was rather thick ($4.2 \mu\text{m}$). Myoid cells and connective tissue was present among the germ cells. Spermatogonia were the only germ cells present, sometimes in nests. Nucleolus was not prominent in

all cells. Mean diameter of spermatogonia was $7.6 \pm 1.25 \mu\text{m}$. Few cysts of primary spermatocytes and spermatids were observed also. Overall, testes did not show any sign of testicular activity and were in resting stage (Fig. 3).

December (19 months)

Morphologically testes were like those in November. GSI was 0.12 ± 0.02 . Testes were compact with myoid cells and connective tissue present like November. The testicular tunic was $3.22 \mu\text{m}$ in thickness. Only spermatogonia were present within the testis, having diameter of $10.60 \pm 1.12 \mu\text{m}$. Chromatin was dispersed in nuclei, although few cells were at anaphase stage of mitosis (Fig. 3).

January (20 months)

In January, minimum values of air and water temperature were observed. GSI in this month was very low also (0.05 ± 0.03). Apparently, testes were not different from previous two months. Histologically, testes were more compact than December and its tunic was $1.13 \mu\text{m}$ thick. Spermatogonia were cytologically different from December. A prominent nucleolus had appeared while the nucleus was not darkly stained. The observed diameter of spermatogonia was $8.00 \pm 2.12 \mu\text{m}$, lesser than that in December, indicating the slow multiplying activity of spermatogonia (Fig. 3).

February (21 months)

GSI value in February (0.05 ± 0.02) was similar with January. However, a slight increase in temperature (around 4°C) was observed (Fig. 1). There was no morphological difference between January and February testes. Histologically, testes were more compact. Tunic of testes was $1.33 \mu\text{m}$. Only spermatogonia were present within the testes along with few primary germ cells. Diameter of spermatogonia noted in February was $7.10 \pm 0.66 \mu\text{m}$ (Fig. 3).

March (22 months)

An improvement in the environmental factors caused a slight increase in GSI in this month 0.08 ± 0.05 (Fig. 1). Morphologically, the testes were broader and pink in color with appearance of surface

blood vessels. Histologically, testicular tissue showed lobular and cystic development and the tunic was $1.5 \pm 0.07 \mu\text{m}$. Lobular walls ($0.73 \mu\text{m}$) surrounded the cysts and a vigorous division of germ cells was seen. Primary spermatocytes having the diameter of $4.50 \pm 0.75 \mu\text{m}$ were found along with the primary germ cells. Interstitial and stromal tissue was decreasing as most of testicular space was being occupied by dividing germ cells. Lobular development was synchronous in peripheral and middle portions of testes (Fig. 3).

April (23 months)

The increasing trend in testicular weight continued (Fig. 1). The mean GSI value in this month was 0.10 ± 0.06 . Morphologically, testes were reddish; more convoluted and having pronounced blood supply. A little amount of milt was released from genital pore on tightly pressing the abdomen of some fish having the heaviest testis. Some fish still had immature testes. Lobular demarcations were very clear; having the thickness of $1.00 \mu\text{m}$. Thickness of testicular tunic was less than previous month ($1.35 \pm 0.01 \mu\text{m}$). Lobules present near the mesorchium on dorsal side were fusing with each other, forming the central duct. All stages of spermatogenesis till spermatids ($2.00 \pm 0.20 \mu\text{m}$), could be observed. Only a few spermatogonial cysts were present. The immature testis had only spermatogonia (Fig. 3).

May (24 months)

May was the hottest month with temperature of $39.45 \pm 0.56^\circ\text{C}$ and photoperiod of 14.65 hours. Gonadal length and width increased further with mean GSI value reaching 0.47 ± 0.09 . Testes were with the maximum blood supply, much convoluted and even the lower most joined portion was now fully developed. Few drops of milt oozed out on pressing the abdomen. Tunic of testes was thinner; having thickness of $1.21 \pm 0.06 \mu\text{m}$. Tubular walls were much thicker ($3.40 \mu\text{m}$) than lobular walls ($0.64 \mu\text{m}$). Central duct was filled with sperms. Although all stages of spermatogenesis could be seen but sperms ($1.40 \pm 0.10 \mu\text{m}$) were the most prevalent germ cells. Cysts of spermatids were still present (Fig. 3).

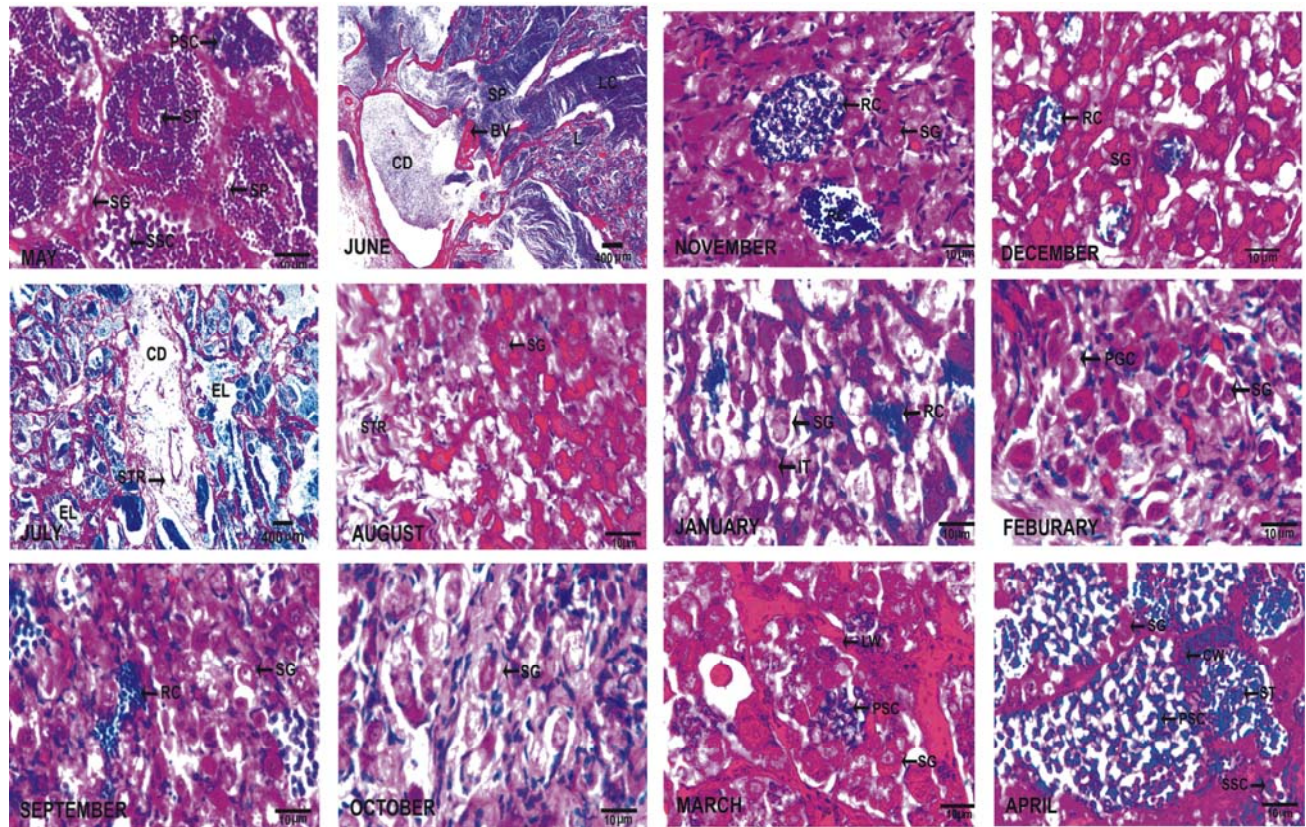


Fig. 3. Seasonal and age-related changes in the testis of *Catla catla* during the age of 18 (November, 2006) to 29 (October, 2007) months. The tissues were fixed in buffered formalin and hematoxylin-eosin stained paraffin sections were photomicrographed with high resolution digital camera. For other details, see Table I and II and Materials and Methods.

June (25 months)

Peak GSI (0.48 ± 0.07), width (0.57 ± 0.10) and length of testes (13.87 ± 0.53) was noted during this month coinciding with near peak values for water temperature and peak photoperiod (Fig.1). Testes were creamy white, highly convoluted, filled with milt, which released from genital pore on handling or pressing the abdomen but in little quantity, not running freely. Genital pore was red and bulged out.

Histologically, testes were at the maximum developmental stage and fish were fully ripe to spawn. Tunic was the thinnest in its thickness i.e. $1.20 \pm 0.17 \mu\text{m}$ because of enlargement of testes. Thickness of lobular wall was only $0.63 \mu\text{m}$, while ductal wall was $6.70 \mu\text{m}$ in its thickness. Lobules were rupturing because of the milt pressure.

Sperm duct had narrow lumen lined by both

connective and epithelial tissues. Blood vessels were also present within the sperm duct tissues. Most of the ducts contained sperms ($0.70 \mu\text{m}$). Unlike previous months, diameter of spermatogonia did not show any significant variation to be categorized into two types (Fig. 3).

July (26 months)

The maximum (33.50°C) water temperature with the heaviest rainfall of monsoons (199.86 ± 32.31) was seen during this month. The rainfall was more than three times the rainfall seen in June. A sudden drop in testicular weight was recorded during this month. Testes became very thin, light pink and no surface blood vessels were observed. They looked like immature gonads. These pronounced morphological changes were also

responsible for sudden drop in GSI (0.03 ± 0.07) and pointed that spawning probably occurs during this month in nature caused by the increased water level and currents because of monsoon rains (Fig. 1).

Histological study, confirmed the above assumption of occurrence of spawning in the last days of June and up to middle of July. July sample was taken in the fourth week of this month. Testicular tunic was $4.37 \mu\text{m}$ in thickness. Collapsed lobules were almost empty of sperms. Central duct had also collapsed and was filling with stromal tissue. Spermatogonia ($5.80 \pm 1.36 \mu\text{m}$) were present as were secondary spermatocytes ($2.40 \pm 0.45 \mu\text{m}$) and sperms ($1.4 \pm 0.15 \mu\text{m}$) (Fig. 3).

August (27 months)

Grossly, testes were in regressed form (GSI = 0.04 ± 0.00). Cystic structure ceased and granulocytic activity was quite common. Spermatogonia were the only gametic cells present. Spermatogonial size was $10.10 \pm 1.54 \mu\text{m}$. Myoid, connective and stromal tissues were pronounced. Thickness of tunic was $6.63 \pm 1.61 \mu\text{m}$ (Fig. 3).

September (28 months)

GSI was low (0.08 ± 0.09), although slightly more than that of August. Testes showed the presence of only spermatogonia ($12.20 \pm 1.53 \mu\text{m}$) of almost same size. Few cysts with primary and secondary spermatocytes could be seen. Interstitial, connective and myoid tissues were noted. Tunic was $2.37 \pm 0.36 \mu\text{m}$ (Fig. 3).

October (29 months)

An overall decrease in all gonadal and environmental parameters was observed (Fig. 1). GSI at this stage was 0.04 ± 0.02 . Grossly, testes were like immature gonads. Tunic of testes was thick ($2.85 \mu\text{m}$). Gonads were at a complete regressed phase. Testes contained only spermatogonia ($12.64 \pm 1.56 \mu\text{m}$) and primary germ cells. Few residual cysts and connective tissue could also be seen (Fig. 3).

DISCUSSION

The results indicate that *Catla catla* undergoes specific seasonal fluctuations in its

gonadal parameters. GSI data show that catla became fully mature at the age of 24-25 months and this was probably the first maturity. Almost the same range of age (22-24 months) had been reported by Alikunhi (1957) and Dholakia (2004) from India. On the basis of macroscopic and microscopic studies, annual reproductive cycle of catla could be classified into five stages (Table 2) *i.e.* immature, maturing, mature/spawning, post-spawning and regressed as reported in many earlier studies on teleosts (Johnson *et al.*, 1998; Cussac and Ortubay, 2002; Maldonado-Garcia *et al.*, 2005; Tominaga *et al.*, 2005; Bhattacharyya and Maitra, 2006).

The values of GSI indicated that testicular weight remained almost unchanged during the months of low temperatures and photoperiod (less than 12 hours) (October-February). Slightly raised values of GSI in November and December, not fitting in ideal trend, were because of lesser body weights of fish during these months as they were younger in age (Billard *et al.*, 1992) and comparatively milder water temperatures. The low values of GSI during winter months were followed by a gradual increase towards a single seasonal peak in June and a sharp fall afterwards. Same results were reported in other cyprinids like *Cyprinus carpio* (Billard *et al.*, 1992) and *Labeo rohita* (Sen *et al.*, 2002; Lone *et al.*, unpublished data).

The mature phase was marked by spermeiogenesis and sperms were identified by their clustered tails in the lobule's lumen and in collecting ducts. This was opposite to that reported in catla by Bhattacharyya *et al.* (2005) and Bhattacharyya and Maitra (2006). These authors did not observe sperms within the testicular tissues, but only in the milt. However, Billard *et al.* (1992) noted sperms within lobular lumen in a related species, *Cyprinus carpio*.

Following June, GSI suddenly dropped in second half of July, indicating spawning. On contrary, no spermiation was observed in pond conditions as was reported in earlier literature about catla, labeo and bester (Jhingran, 1968; Amiri *et al.*, 1996; Thomas *et al.*, 2003). During sampling very low quantity and thickness of released milt probably indicated (?) no spawning. It can also be associated with the age and reproductive experience as in *Cyprinus carpio*, no spermiation was observed till

the second year of age, although fish had attained maturity in the first year. However, in third and fourth year of age, freely spermiating males were found in pond conditions (Billard *et al.*, 1992). Some authors had ascribed the failure in reproduction in captivity due to the lack of ecological and behavioral factors, such as water currents, temperature and suitable spawning substrates. These factors are critical for the process of sexual maturation under natural conditions and transduce their effect through neuro-endocrine reflexes (Donaldson and Hunter, 1983). It is also attributed with the stress of captivity and high stocking density (Billard *et al.*, 1992). These factors also influence upon the brain-pituitary-gonadal index (Lone and Hussain, 2009).

Regressed phase is marked with the resorption of residual cysts by granulocytes containing large number of lysosomes. This phagocytic activity of granulocytes prepare the gonads for the next wave of spermatogenesis (Lone and Al-Marzouk, 2000; Lone *et al.*, 2001). However, the presence of residual cysts in succeeding months probably indicates the slow performance of immune system in catla or some progression in spermatogenesis by milder temperatures.

Present study very clearly depicts the modulation of spermatogenesis by environmental factors. Influence of environmental factors on catla reproduction has been studied scantily and all of the available studies are from the sub-tropical region of East- and South India (Khan, 1942; Chacko and Kuriyan, 1950; Dubey and Tuli, 1961; Bhattacharyya *et al.*, 2005; Bhattacharyya and Maitra, 2006). Annual reproductive cycle of a fish species shows different entrainment patterns by a single or a set of environmental cues in tropical and temperate regions (Sivakumaran *et al.*, 2003). Therefore, marked differences in environmental control of both tropical and temperate teleost species had been reported (Bye, 1984).

Gametogenesis in cyprinids is finely modulated by temperature and photoperiod (Bye, 1984). Influence of these environmental factors varies among different species at different reproductive phases. Long days and high water temperatures enhance gonadal activity in summer

spawners (Barcellos *et al.*, 2001; Yi-Wang *et al.*, 2001; Dey *et al.*, 2005; Bhattacharyya and Maitra, 2006). On the other hand, many species exhibit gonadal development in response to short days and low temperature (Tranger *et al.*, 2003; Maldonado-Garcia *et al.*, 2005; Goncalves *et al.*, 2006; Narimatsu *et al.*, 2007; Christopher *et al.*, 2008). Influence of environmental factors on gametogenesis in catla seems to be similar to the long day spawners.

Testicular development remained recessed during winter months till February (water temperature, $18.00 \pm 1.00^\circ\text{C}$; photoperiod = 11.90 ± 0.00 H), when days were short and water temperature low. An integrated influence of rise in both temperature and photoperiod was observed in spring, resulting in advancement of spermatogenesis. Initiation of gametogenesis seems to be entrained by both longer photoperiod and high temperature (Scott and Pankhurst, 1992; Migud *et al.*, 2004; Bhattacharyya *et al.*, 2005; Bhattacharyya and Maitra, 2006). Bhattacharyya *et al.* (2005) had reported significant role of long photoperiod (16 hours) in testicular development of wild catla in the prespawning phase and it led to precocious maturation of testis. Whereas, either long or short (8 hours) photoperiod did not influence testicular gametogenesis in the remaining part of the gonadal cycle. In present study, peak in GSI is significantly correlated with the near maximum water temperature ($33.00 \pm 1.00^\circ\text{C}$) and the maximum photoperiod (15.11 ± 0.00 H). In July, a sudden fall in GSI points towards occurrence of spawning followed by a slight decline in photoperiod (14.89 ± 0.00 H), although water temperature was the maximum ($33.50 \pm 1.00^\circ\text{C}$). At this point the role of rainfall should also be kept in mind, which showed maximum values in July. From all this it appears that shortening photoperiods along with decrease in rainfall (water levels and current) were cues for regression of gonads despite the fact that water temperature was quite high. This situation we have also seen in catla females, *Labeo rohita* male and females (Lone and Hussain, 2009; Lone *et al.*, unpublished data).

Termination of maturational cycle in catla seems to be modulated by shortening of photoperiod, when the water temperature is high

was also observed in *Dentex tumifrons* (Tominaga *et al.*, 2005). Full maturation (present study) and spawning (in nature) in catla seem to be a short time act and is from mid June till mid July. This period is marked with heavy monsoon rains in Northern and Central Punjab. Termination of testicular development (maturity and spawning) was significantly correlated with the decrease in heaviest monsoon rainfall (199.86 ± 32.31 mm in July to 137.16 ± 38.65 in August and 69.51 ± 14.20 in September) in later part of July, August and September. Rainfall in present study seems to be an exogenous cue for spawning as was also reported by earlier workers from wild (Khan, 1947; Ganapati and Chacko, 1954; Chakraborti, 1998; Bhattacharyya and Maitra, 2006). The temperature and photoperiod range for catla, noted in present study differed from those reported in past studies (Khan, 1942; Chacko and Kuriyan, 1950; Dubey and Tuli, 1961; Chondar, 1999; Bhattacharyya and Maitra, 2006). Moreover, the spawning period (?) in Lahore (mid June-mid July) is around two months earlier than that reported by Menon *et al.* (1959), Hora and Pillay (1962), Talwar and Jhingran (1991) and Bhattacharyya and Maitra (2006) from India. This variation in timing to spawn is because of geographical differences *i.e.*, differences in the longitudes and latitudes (Garg and Jain, 1984).

Overall, gametogenesis in male catla is a fast phenomenon needing a photoperiod of above 13.5 hours and water temperature between 29-33 °C. A single peak of GSI within a short demarcated period points to a quick maturation/spawning time during monsoon.

ACKNOWLEDGEMENTS

The authors are thankful to Prof. Dr. M. Tahir and Mr. Naveed Abbass for providing facilities for histology and microphotography and Mr. Naveed Khan for help in preparing graphs.

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(Received 13 June 2009, revised 29 July 2009)

Table I.- Annual and age-related variations of various parameters (Mean±SEM) of male *Catla catla* during the study period.

Month (Age)	Total body weight (g)	Condition factor (k)	Gonad weight (g)	Gonad width (cm)	Gonad length (cm)	GSI	Liver weight (g)	HSI
November (18)	892.08 ± 15.12	1.30 ± 0.17	1.25 ± 1.25	0.34 ± 0.02	12.40 ± 1.01	0.14 ± 0.10	13.17 ± 0.60	1.47 ± 0.08
December (19)	680.42 ± 41.40	1.40 ± 0.17	0.80 ± 0.18	0.29 ± 0.02	13.38 ± 1.16	0.12 ± 0.02	13.00 ± 1.00	1.84 ± 0.60
January (20)	1031.94 ± 45.16	2.00 ± 0.12	0.57 ± 0.07	0.29 ± 0.01	12.50 ± 0.74	0.05 ± 0.03	16.27 ± 2.07	1.60 ± 0.25
February (21)	1809.27 ± 115.00	2.53 ± 0.10	1.00 ± 0.11	0.29 ± 0.09	13.97 ± 0.50	0.05 ± 0.02	16.56 ± 2.70	1.00 ± 0.30
March (22)	1838.56 ± 165.44	2.61 ± 0.70	1.53 ± 0.37	0.33 ± 0.01	13.82 ± 0.51	0.08 ± 0.05	18.44 ± 1.40	1.05 ± 0.14
April (23)	1669.26 ± 231.02	2.60 ± 0.70	1.90 ± 1.70	0.34 ± 0.03	13.78 ± 0.84	0.10 ± 0.06	20.33 ± 1.00	1.21 ± 0.05
May (24)	1780.39 ± 87.60	3.00 ± 0.75	8.02 ± 2.72	0.62 ± 0.13	13.90 ± 0.80	0.47 ± 0.09	18.21 ± 3.00	1.03 ± 0.20
June (25)	1663.18 ± 130.00	2.76 ± 0.60	8.23 ± 1.90	0.57 ± 0.10	13.87 ± 0.53	0.48 ± 0.07	29.72 ± 0.28	1.80 ± 0.50
July (26)	1723.75 ± 79.00	2.75 ± 0.10	0.60 ± 0.12	0.27 ± 0.01	13.00 ± 0.30	0.03 ± 0.07	16.49 ± 0.90	1.00 ± 0.05
August (27)	1716.85 ± 153.20	2.76 ± 0.12	0.75 ± 0.11	0.23 ± 0.00	12.00 ± 1.73	0.04 ± 0.00	16.13 ± 2.15	1.00 ± 0.07
September (28)	1705.50 ± 22.70	2.66 ± 0.16	1.33 ± 0.15	0.29 ± 0.20	09.80 ± 0.94	0.08 ± 0.09	14.45 ± 2.31	0.84 ± 0.10
October (29)	1871.10 ± 170.10	2.73 ± 0.22	0.68 ± 0.07	0.24 ± 0.07	13.98 ± 1.88	0.04 ± 0.02	19.96 ± 0.01	1.10 ± 0.09

Table II.- Classification of annual testicular cycle of *Catla catla* between the age of 18-29 months.

Maturity stage	Duration (Months)	GSI (%)	Thickness of tunica (µm)	Diameter of germ cells (µm)	Macroscopic appearance	Microscopic appearance
Immature (Stage 1)	Sep. – Feb .	0.05±0.02- 0.08 ± 0.09	1.33±0.30 – 2.37±0.36	SG: 7.10±0.66- 12.20±1.53	Testes were thin thread like. Colour was white to light pink. Blood vessels were not prominent.	Tunica was thick. Development of spermatogonia into nests and start of lobular structure in later months. Primary germ cells were also identified along with some residual cysts, connective and myoid tissue. All stages of spermatogenesis were observed. Interstitial tissue was reduced. Lobular structure of testes clearly seen. Spermatocysts, enclosing cells of same stage were found. Narrow central duct was formed towards mesorchium. Tunica was thin.
Maturing (Stage 2)	Mar. – April	0.08±0.05- 0.10±0.06	1.35±0.010- 1.50±0.70	SG: 6.50±2.50- 7.00±1.82 PSC: 4.50±1.84- 4.50±0.01 SSC: 3.50±0.60 ST: 2.00±0.31	Pinkish red testes with thin blood vessels. Testes were convoluted and released a drop of milt on tightly pressing the abdomen. Sperm duct was clearly differentiated in April samples.	Lobules were disintegrating. Central duct became wider, containing sperms. All stages of spermatogenesis were observed. Spermatogonia were few, present near the lobular walls. Most of the lobules were filled with sperms. Interstitial tissue almost diminished. Tunic was very thin.
Mature & Spawning (Stage 3)	May – June	0.47±0.09- 0.48±0.07	1.21±0.60- 1.20±0.17	SG: 6.50±1.40- 8.00±1.12 PSC: 4.40±0.12 SSC: 3.50±0.42- 4.00±0.57 ST: 2.00±0.20- 2.10±0.34 SP: 0.70±0.03- 1.40±0.10	Testes were greatly convoluted. Blood vessels were now clearly observable on the testicular surface. Color of testes was creamy pink and creamy white as they were filled with milt, which oozed out on slightly pressing the abdomen.	Lobules were largely empty however some still with few sperms. Both central duct and lobules were degenerating. Stromal tissue was very prominent. Granulocyte activity was dominant. Tunica started becoming thicker.
Post-Spawning (Stage 4)	July – Early August	0.03±0.07- 0.04±0.001	1.20±0.17- 4.37±0.80	SG: 5.80 ±1.36- 8.00±1.12 SSC: 2.40±0.45- 3.50±0.42 SP: : 0.70±0.03- 1.40±0.15	Very thin testes, white in color. Testes were like those at immature stage except in length. No milt, even after pressing the abdomen.	Only spermatogonia were seen. Stromal tissue was predominantly present. Few residual cysts were observed. Tunic was thick.
Regressed (Stage 5)	Late Aug. – Sept.	0.04±0.001 0.08±0.09	4.37±0.80- 6.63±1.61	SG: 6.00±1.51- 10.10±1.54	Testes were very thin and white in colour. No blood vessel was observed. .	

SG, Spermatogonia; PSC, Primary spermatocytes; SSC, Secondary spermatocytes; ST, Spermatids; SP, Spermatozoa.