Pakistan J. Zool., vol. 41(3), pp. 217-234, 2009.

Seasonal and Age-Related Variations in the Ovaries of *Labeo rohita* (Hamilton, 1822): A Detailed Gross and Histological Study of Gametogenesis, Maturation and Fecundity

Khalid P. Lone* and Altaf Hussain**
Department of Zoology, GC University, Katchery Road, Lahore

Abstract.- Seasonal and age-related variations and pattern of oocyte development in the ovaries of female *Labeo rohita* (rohu) were studied during the age of 18-29 months (first maturation cycle). The experiments were conducted from November 2006 to October 2007. Six gross ovarian stages (virgin/immature, developing, early maturing, ripening, mature-gravid and regressing) were defined and photographically discerned. Ovaries were preserved in 10% buffered formalin and histological development followed with age and season. Results showed that *Labeo rohita* is a heterosexual fish. Ovaries are paired structure, joined by a short oviduct. Ovaries were fully developed in June when GSI was the highest (22.73±0.94). Various developmental stages of oogenesis *i.e.*, chromatin nucleolar stage, perinucleolar stage, yolk vesicle (cortical alveoli stage) and vitellogenic stage were observed and characterized. No spawning or postovulatory follicle (POF) was seen. In August, September and October atretic follicles were quite common. Absolute fecundity determined in June, when GSI and oocyte size was maximum, was 469.93 eggs/gram body weight and 2012.55 eggs/gram ovary weight of fish having an average body weight of 1738.76 gram. Environmental parameters including temperature, photoperiod and rainfall appear to affect the growth and development of ovary. Growth of oocytes was triggered by increasing temperatures and photoperiod. This is the first detailed and systematic study on the reproductive tactics of female *Labeo rohita* in Pakistan and the results have been discussed in the light of available literature on the reproductive biology of fish.

Key words: Annual cycle, gonadosomatic index, hepatosomatic index, oogenesis, photoperiod, reproductive biology of rohu.

INTRODUCTION

Morphology, biochemistry and physiology of gonads have been described by a large number of workers in different groups of fishes. Ovaries are not only responsible to produce eggs but also synthesize and secrete hormones of different kinds that have a far reaching effects on the reproductive biology and behavior of the fish. Oogenesis starts from simple proliferation of oogonia up to the formation of mature oocyte and consequently ovulation after final maturation. During this process, size of oocyte increases many folds and this is mainly due to accumulation of yolk granules which are formed in liver under the influence of a specific steroid hormone, 17β-estradiol (Wallace and Selman, 1981) and migrate to oocyte through blood (Droller and Roth, 1966; Wallace, 1978).

Yamamoto *et al*. (1965) divided the oocyte development of rainbow trout into eight stages which includes chromatin nucleolus stage, perinucleolus stage (subdivided into early and late stage), oil droplet stage, primary yolk stage, secondary yolk stage, tertiary yolk stage, and maturation stages. Shrestha (1980) described the ovarian cycle of *Noemacheilus beavani* and divided it into seven distinct phases. Sen *et al*. (2002) divided the development stages of *Labeo rohita* (collected from wild rivers of West Bengal, India) into seven different stages as primary growth phase, perinucleolar stage, pre-vitellogenic or yolk vesicle stage, vitellogenic stage post-vitellogenic stage, germinal vesicle break down stage, and spawning stage. Ganias *et al*. (2004) studied the pattern of oocyte development in the Mediterranean sardine (*Sardina pilchardus sardina*) and divided it into six stages.

Recent improvements in the *in vitro* incubation techniques have allowed an observation of the various morphological events associated with final oocyte maturation (FOM). These may be the: i) migration of germinal vesicle (GVM) to animal pole

* Present address: University of Wah, The Mall, Wah Cantt. Pakistan. E-mail: khalid.lone@gmail.com
** Present address: Department of Zoology, Govt. Islamia College, Civil Lines, Lahore.
0030-9923/2009/0003-0217 $ 8.00/0

where micropyle is situated; ii) the membrane of the germinal vesicle then breaks down, releasing its contents into the cytoplasm (GVBD Stage); iii) in cytoplasm the coalescence of lipid droplets and yolk globules; iv) rapid-size increase of the oocyte caused by hydration; and v) an overall increase in the oocyte translucency.

The oocytes (now eggs) are expelled into the ovarian cavity or peritoneal cavity, a process known as ovulation. The follicular layers that remain behind in the ovary are known as Post-ovulatory follicle (POF). The entire process or at least part of it is hormone dependent (Lone *et al*., 2001, 2008). However, final oocyte maturation and ovulation are not always associated because oocyte of most teleosts do not undergo ovulation following steroid maturation *in vitro* (Narimatsu *et al*., 2007).

Atretic oocytes (also known as corpora atretica) are a very common feature in the teleost ovary (Guraya, 1976a,b; Saidapur, 1978). Follicular atresia involves the hypertrophy of the granulosa and thecal cells and may occur at any stage of oocyte development. Khoo (1975) provided a detailed description of histological changes in follicular atresia in goldfish and classified five consecutive stages, α, β, γ, σ and ε stages.

Factors influencing development and spawning time in female fish are temperature, photoperiod, longitude, latitude, lunar or tidal cycles, rainfall, run-off water, size, age, pollutants, and stress etc. No single factor can be said to be responsible for spawning. The act involves fulfillment of a chain of interrelated conditions as prerequisite to spawning (Lam, 1983; Wang *et al*., 2001; Almatar *et al*., 2004; Berois *et al*., 2004; Yanagimoto and Humphrys Jr, 2005; Juchno *et al*., 2007). For carps, optimum temperature of 22-31°C, long-days, proper spawning ground, monsoon rains and cloudy atmosphere have been reported to be important factors (Kapur, 1981; Jhingran, 1986).

Labeo rohita (rohu) is a major carp and an annual breeder. Based on observations of wild fish, it attains maturity in the end of second year of life (Alikunhi, 1957; Jhingran and Pullin, 1985). However, under pond culture conditions, some fish may mature at the end of first year of life in Indian sub-tropical environment (Orissa, India). In Bangladesh, it matures at an age of 3-4 years (Jhingran and Pullin, 1985). Similarly, in another study spanning over 18 months, Khan (1972) based on wild fish collected from areas around Aligarh (North India) reported that minimum age at first maturity was two years while males matured earlier than females. Fully mature males were of four years and females of 5 years age. Fifty percent maturity point was 2.7 and 2.9 years for males and females, respectively (Khan, 1972).

Although, the major carps have been cultured in Pakistan and India since 2000-3000 years BC, systematic studies on the reproductive biology of these fish are very scanty in general and are particularly missing from Pakistan. In view of this we have started a programme of studying the basic reproductive biology of these fish and the present paper, a part of these studies, describes the development of gonads and histological changes in female rohu during the age of 18-29 months (first maturation cycle ?).

MATERIALS AND METHODS

Fish collection

The fish, Rohu (*Labeo rohita*), were collected from a commercially well managed fish farm, located some 40 kilometers from Lahore on G.T. Road, near Muridkay (latitude 31°58 N, longitude 74°13E). The fish in this farm were raised as a polyculture of Pakistani major carps consisting of rohu, thaila, mori, silver and grass carps in a specified stocking density.

For the present studies a total of about 500 Rohu of the age of about one year were selected at random and were separated from the rest of the stock in a separate pond of size 0.25 hectare to avoid any unnecessary handling stress, except the monthly sampling.

The sampling started in November 2006, when the fish were 18 months of age. The fish were collected through a cast net and a total of 20 fishes taken at random. Without taking any note of the sex. Live fish were packed in polythene bags having water and compressed air. The samples were carefully transported to the laboratory, where they were kept in rectangular cement tanks (4.12 m L × 1.7 m W and 1.0 m D) supplied with running water as a sprinkler. The tanks were covered with a wire

mesh to avoid fish jumping out of water. The fish were kept in these tanks, at least, for one day before the actual sampling was performed in the morning.

Physico-chemical parameters

Atmospheric temperature (°C), water temperature of the pond (°C) and pH of the pond water were measured *in situ* at the time of sampling by portable laboratory meters (WTW, Germany).

Experimental sampling

At the time of sampling, fish were removed from the cemented tank one by one with a scoop net and immediately placed in a water tank containing clove oil (5 ppm) dissolved in absolute alcohol (Merck, Germany) in a ratio of 2:5 (Berka *et al*., 1986) and allowed to stay for 3-5 minutes. The time period was changed according to age and size of fish.

Morphological parameters

Before dissection and removal of gonads total body weight to the nearest gram (g) and total body length, standard body length and body depth (at the start of the dorsal fin level) to the nearest mm were recorded.

Dissection and removal of gonads

After measurements, an incision was made on ventral side of the abdomen from posterior to interior most tip of the fish with a sharp scalpel and a bone cutter. Ovaries were found attached on the lower side of the swim bladder in the abdominal cavity. Features like colour, position of gonadal ducts and any abnormality, if present, were noted. Photographs of the ovaries were taken *in situ*. A complete record of the photographs of the whole fish and their gonads was kept.

Ovaries were separated carefully from the bladder and other tissues, spread on a sheet in original form and photographed and size (length and width) noted to the nearest mm. Immediately after, the weight was taken to the nearest of milligram (mg). After gonads, liver was also removed, weighed, put in a polythene bag and stored at -40°C.

Gonadosomatic index (GSI) and hepatosomatic index (HSI) were calculated according to the formula of Lone and Matty (1980) and Lone *et al*. (2001).

Histological studies

Whole ovaries were fixed in plastic jars with 400-1000 ml of 10% buffered formalin (4% formaldehyde in phosphate buffer) until processed for histological examinations. Samples were trimmed to approximately 1 cm^3 and loaded to plastic tissue cassettes. The sample loaded cassettes were then processed for routine histology. The samples were first passed through different grades of ethyl alcohol ranging from 30-100% for dehydration and then cleared in two changes of xylene. The tissues were finally taken to embedding centre, transferred to molten wax and xylene in the ratio of 70:30 and in the last changed to molten wax for 4 hours for impregnation of wax. The wax embedded tissues, attached to cassettes, were finally trimmed for microtomy.

The tissues were sectioned at 3–6 μm using a motorized rotary microtome (LEICA) with a well sharpened knife. The sections were floated in warm water 37-40°C for stretching. The stretched sections were placed on glass slides, and put on a hot plate overnight for drying.

The sections were stained with Harris hematoxylin and eosin and mounted with DPX. The photomicrography was done on LEICA PM-5000 microscope with digital camera.

Fecundity

Absolute fecundity was estimated by simple gravimetric method. A small piece of ovary was taken. After cleaning and drying, the sub-sample was weighed. Total number of eggs were then counted. Absolute fecundity was then calculated by using the formula:

$$F = \frac{nG}{g}$$

Where, F =Fecundity
n = Number of eggs in sub-sample
G = Weight of ovaries
g = Weight of the sub-samples

Statistical Analyses of the data

All the data were analyzed using Excel version 2007, while the statistics was applied using SPSS version 16. One-way ANOVA was followed by Tukey test.

RESULTS

Physical and environmental parameters

Table I shows monthly changes in the atmospheric temperature (maximum and minimum), water temperature, photoperiod, and rainfall during the period of study *i.e.* from November, 2006 to October, 2007. The minimum atmospheric temperature of the year was recorded in January (5.49±0.99°C), whereas the maximum temperature was recorded (39.45±1.76°C) in the month of May. The minimum water temperature at 1700 hours was also recorded in January (13.50±1.25°C), while the maximum at 1700 hours was in August (34.0±1.00°C). The maximum day-length of 15.15±0.05h was during June, whereas the minimum day-length of 11.04±0.06 h, was recorded in December. The maximum rainfall was in July (199.86±102.16 mm), and the lowest rainfall was recorded in December as 4.53±6.84 mm (Fig. 1).

Body weight

At the start of the experiment in November, 2006 (age 18 months), the mean body weight was 861.00±14.35 g. In December the weight decreased to 732.37±11.92g probably because of lower temperature and photoperiod. After February, the body weight started increasing gradually and reached 1732.7±127.81g in June when the ovaries were fully developed. After June, it decreased a little in July (1663.13±42.75 g) due to the regression of the ovaries (Table II). After July it started increasing again and was maximum ($P<0.05$) in October (1782.6±137.52 g).

Total body length and standard length

Minimum length was recorded in December, 2006 (age 19 months) as 46.42±0.98 cm (38.03±1.02 standard length). After December the length started increasing and in October, 2007 (age 29 months) it was 53.50±0.41 cm (standard length= 44.38±0.22) in the last month of present studies (Table II).

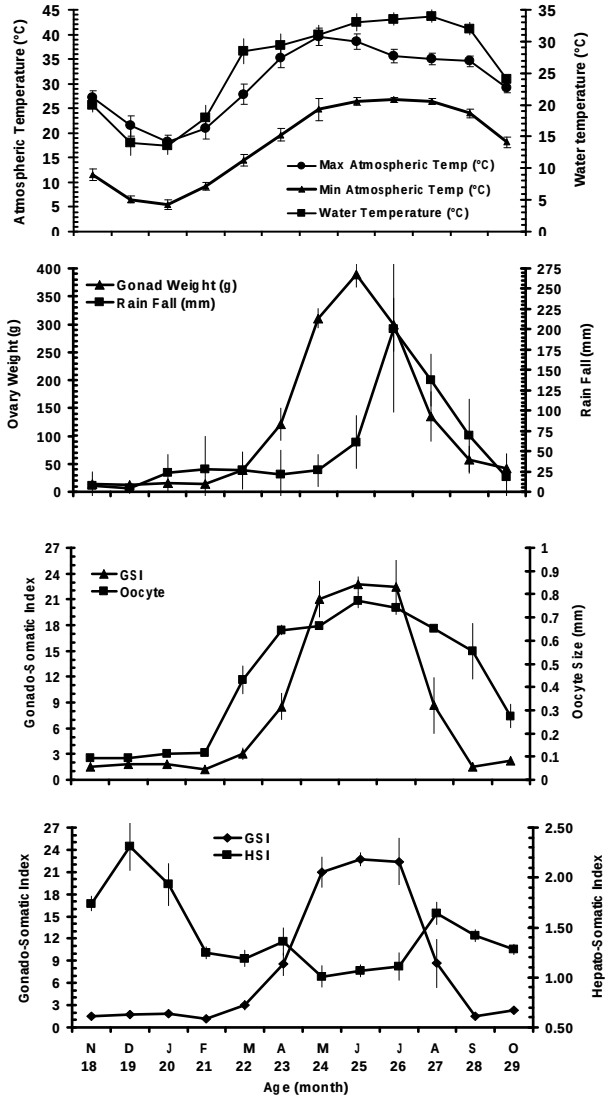

Fig. 1. Monthly and age-related variations in atmospheric and water temperature (°C), rain fall (mm), ovary weight (g), gonado-somatic index (GSI), hepato-somatic index (HSI) and oocyte size (mm) of rohu, *Labeo rohita*. Values given are mean±SEM. For other details see Materials and Methods.

Condition factor (k) and body width

The value of "*k*" was the minimum in December 2006 (0.734±0.04), while the highest value for this parameter was recorded in June 2007 (1.422±0.05). The body width was similar to the trend seen with "*k*" (Table II).

Table I.- Monthly variations (Mean± SEM) of various environmental parameters during the course of the study from November, 2006 to October, 2007.

Month	Age (months)	Maximum atmospheric temperature (°C)	Minimum atmospheric temperature (°C)	Water temperature* (°C)	Photoperiod (Hours)	Rain fall (mm)	Humidity (%) at 8am
Nov.06	18	27.26±1.36	11.52±1.21	20.00±1.20	11.30±0.01	7.96±16.88	78.70±3.36
Dec.06	19	21.48±2.04	6.49±0.70	14.00±1.99	11.04±0.06	4.53±6.84	86.00±4.18
Jan.07	20	18.21±1.45	5.49±0.99	13.50±1.25	11.17±0.06	23.37±22.68	86.90±4.43
Feb.07	21	21.00±2.31	9.14±0.81	18.00±2.00	12.16±0.06	28.12±40.15	77.50±3.71
Mar.07	22	27.79±2.06	14.47±1.25	28.40±1.99	13.03±0.03	26.14±22.78	66.30±6.29
Apr.07	23	35.27±2.09	19.65±1.28	29.00±1.76	14.03±0.00	21.49±29.59	47.00±6.80
May,07	24	39.45±1.76	24.80±2.22	29.00±1.55	14.46±0.05	26.12±19.84	42.70±7.30
Jun.07	25	38.57±1.63	26.49±0.78	33.00±1.40	15.15±0.05	61.28±32.08	55.70±10.07
July, 07	26	35.62±1.43	26.71±0.57	33.50±1.00	15.10±0.05	199.86±102.16	76.30±5.02
Aug.07	27	34.99±1.12	26.40±0.53	34.00±1.00	15.05±0.05	137.16±32.00	77.50±4.03
Sep.07	28	34.57±1.09	24.04±0.86	32.00±1.01	13.05±0.02	69.51±44.81	76.20±6.26
Oct.07	29	29.10±1.02	18.17±1.06	24.00±0.66	12.09±0.00	18.60±28.52	74.10±3.35

*= measured in the afternoon at 1700 hours.

Gonad weight, length and width

The weight of gonad (13.11±2.06 g) was the lowest in December (age 19 months), while the highest values (388.8±23.02 g) were encountered in June (age 25 months). Although the highest value for gonad length was also seen in June, the minimum values for gonad length were observed in November (9.16±0.40 cm). The ovarian width followed ovarian length and weight and was minimum in November (1.702±0.09 cm) while maximum values (5.45±0.15 cm) were in May (Table II).

Gonadosomatic index (GSI)

The monthly distribution of gonadosomatic index (GSI) of female *Labeo rohita* is given in Table II and Figure 1. The GSI values were lowest during the month of February (1.19±0.07), while the maximum were seen in June (22.73±0.94).

Liver weight and hepatosomatic index (HSI)

The lowest values for the liver weight (14.45±1.35 g) were seen in March, while the highest values (27.66±2.16 g) were in August. Similarly the minimum (1.01±0.11) and maximum (2.31±0.24) values of HSI were encountered in May and December, respectively (Table II)

Gross and histological (oogenesis and seasonal) studies of the gonads

Labeo rohita is heterosexual. Its reproductive system consists of an ovary with two lobes, present on each side of the air bladder in body cavity. They were attached with the swim bladder and the length of two lobes was not equal (Fig. 2). In the immature fish the two lobes are separate however, as they grow, they unite posteriorly and form a short oviduct that opens to the exterior through gonopore lying above the anus. Externally each ovary was covered with a thin peritoneal membrane and beneath the membrane lies the tunica albugenia which becomes thinner and thinner as the ovary reaches to full maturity.

Histologically, gonadal development in *Labeo rohita* is group synchronous type. This type, which is found in fish that spawn annually or once in a spawning season, breeders will develop (recruit) a cluster of vitellogenic oocytes and advance synchronously through further stages of development (oogenesis), whereas the rest of the oocyte population remains arrested and is used for next year's cycle. In *Labeo rohita* the ova starts development in earnest in March and increase to full and mature size in June. Its spawning, in wild, probably takes place in late June and July, during the local monsoon season. Sex can be recognized in

(Table II)

breeding season when abdomen is rounded (called "chhalli" in local language) and vent bulged out and becomes reddish in colour.

Ovarian stages

On the basis of gross size, shape, coloration and other histological features of the ovaries of female *Labeo rohita*, six categories of reproductive and maturity stages were recognized throughout the year. These stages were virgin/immature, developing, early maturing, ripening, mature-gravid and regressing (Table III, Fig. 2). Similarly, female germ cells were classified according to the oogenesis stages encountered during the present studies. These stages were oogonia, pre-vitellogenic stages containing primary oocytes at chromatin-nucleolar stage, peri-nucleolar stage, secondary oocyte at yolk-vesicle stage, vitellogenic stage (various phases), and atretic follicles and oocytes in the regressing ovaries. Since no spawning was observed as *Labeo rohita* do not spawn in captivity, therefore, final oocyte maturation (FOM), and ovulated oocytes (eggs) and post-ovulatory follicles (POF) were not seen during the present studies (Table III, Figs. 3, 4). The details of gross structure and oogenetic cycle are given in Table III.

Fecundity

Absolute fecundity was calculated by simple gravimetric method. It was found that *Labeo rohita* female contained on the average 817,094 eggs in an ovary of 406g weight, whereas the average total body weight of the fish was 1738.76g. It means that *Labeo rohita* female contained 469,929 eggs/kg of body weight and 2,012,546 eggs/kg of ovary weight. Details regarding oocyte size are given in Tables II and III.

DISCUSSION

The annual reproductive cycle of *Labeo rohita* female was studied on the basis of gross appearance, and weight of ovaries. Histologically, this was based on oogenesis, size of oocytes, size and behaviour of nucleus, nuclear membrane, number and location of nucleoli, appearance and distribution of yolk vesicles, yolk granules, appearance of oil droplets (if any), final maturation of oocytes and the GSI values of the ovaries. It is clear from the Tables II and III, Figures 1 and 2 that ovaries during the months of November to February were small in size and contained mainly peri-nucleolar oocytes. The weight of ovaries started increasing in March and peaked in June (388.8±23.02 g). The GSI (22.73± 0.94) was also highest in June with ovaries having vitellogenic oocytes in a group synchronous mode. From July to August, the weight of ovaries and GSI declined and the ovaries were already regressed by October (Fig.1).

Changes in (water) temperature and photoperiod have been shown to correlate well with gonadal weights and therefore with gonado-somatic index (Lam, 1983; Mananos *et al*., 1997; Mylonas and Zohar, 2007). In earlier studies, high temperatures and long photoperiods were positively correlated with growth of gonads (testis and ovary) and related reproductive hormones. Similarly, these conditions of high temperatures and long day-lengths were inhibitory to the gonad growth of fish maturing and spawning in autumn or winter. In summer spawners, water temperature and long days have a key role in both initiating and concluding the spawning season. This has been shown for many fish like major carps (Sen *et al*., 2002; Dey *et al*., 2004, 2005; Bhattacharyya and Maitra, 2006), Japanese sardine (Matsuyama *et al*., 1991). Rose bitterling will initiate its breeding season in rising temperature while gonadal recession will ensue in high temperature but declining photoperiods (Shimizu *et al*., 1994). In blueline tilefish which exhibits a peak in GSI in May to September, a rapidly decreasing photoperiod in October causes gonad regression (Ross and Merriner, 1983). In cyprinid fish, *Gnathopogon caerulescens* and dace, *Tribolodon hakonensis*, photoperiod is important for the spawning season, however, it interacts with temperature and final influence on the gonads is exerted by the temperature (Okuzawa *et al*., 1989; Ma *et al*., 2005). On the other hand, in autumn spawner, *Accheliognathus rhombea*, low temperature and short photoperiod have great influence on gonadal growth and GSI (Shimizu *et al*., 1994). From these studies it can be said that for summer spawners, a day length of more than 13 hours with higher temperatures and for autumn and

Table III.- Description of gonad maturity of female *Labeo rohita* correlated with age (months) and season during the present studies. Gross appearance refers to fresh ovaries.

Maturity stage	Age	Season (Water temperature °C)	Gross appearance	Histological appearance
Virgin (?) / Immature	18-21; 29	October-February (24.0±0.66-18.0±2.0)	It is a long phase and was seen during October to February. However, an odd sample can be seen in other months also. The ovaries at this stage were elongated in shape, brown to pink in colour and opaque in transparency. Vascularization was inconspicuous. The ova were not visible with naked eyes. Mean GSI ranged from 1.19±0.07-1.83±0.22 (Fig. 2A).	The small ovaries at this stage had oogonia and primary oocytes with some oocytes in peri-nucleolus stage. The nucleoli may be one or several, depending upon the size and maturity. Size (µm) of oocytes was the minimum in December (0.092±0.001) while maximum was seen in October (275±50). Tunica was thick. No yolky oocytes were seen. Atresia very rarely seen (Figs. 3, 4).
Developing	22	March (28.4±1.99)	Ovaries grew in size, with colour brown to yellowish. Vascularization increased. Small and thin surface capillaries were seen quite easily. GSI was 3.02 ± 0.69 (Fig. 2A).	Oogenesis has started in earnest. Three types of oocytes can be seen. Oocytes with yolk vesicle, cortical alveoli stage and some with true yolk were present. Size increased up to 430.6±59.0 µm. Atresia scarcely seen (Figs. 3, 4).
Early Maturing	23	April (29.0±1.76)	Ovaries continued growth in size and weight and occupied major portion of the body cavity. They were yellowish in colour; becoming slightly transparent. Tunica was thin. Vascular supply increased further. GSI was 8.52±1.58 (Fig. 2B).	Oocytes increased in size up to 646.9±19.0 µm. The tunica was thinner in bigger ovaries. Three type oocytes were present however, the tertiary oocytes with true yolk granules dominated. The germinal vesicle was centrally located. Atresia present but scarce (Figs. 3, 4).
Ripening	24	May (29.0±1.55)	The stage was predominantly present in May. Ovaries were attaining maximum weight; highly vascularized and having very thin tunica albugenia. Ova could be seen with naked eyes. GSI was 21.01±2.10 (Fig. 2B).	Oocytes further increased in size (664.00±4.0µm) and tertiary oocytes were preponderantly high. Few secondary and primary oocytes could be seen in the crevices of the bigger oocytes. Oocytes of this stage first appeared in April but now were predominant. Atresia scarce (Figs. 3, 4).
Mature-Gravid	25-26	June-July (33.0±1.40-33.5±1.0)	Swelling of belly clearly visible. Ovaries filling the whole body cavity. Ovaries yellow, pale or reddish in colour. Hydrated along with opaque oocytes visible through tunica giving a speckled appearance. No running fish was observed. Eggs did not come out even on pressing the belly. Maximum GSI was seen in these two months. GSI was 22.73±0.94 (June) and 22.41±3.11 (July) (Fig. 2B and 2C)	Size of oocyte reached maximum in June (773.00±31.00 µm) while in July it was 743.00±17.00 µm. However, Germinal vesicle movement (GVM) or breakdown (GVBD) was not seen. No hyaline oocyte or post-ovulatory follicle (POF) seen. Tunica was thin. Atretic follicles or oocytes sparsely seen (Figs. 3, 4).

Continued

Maturity stage	Age	Season (Water temperature °C)	Gross appearance	Histological appearance
Regressing	27-29	August-October (34.0±1.0-24.0±0.66)	During these months ovaries started regressing and showing loose and sometime flaccid appearance. They were shrunken and reduced in volume. Colour became deeper yellow or yellowish red. Both opaque and hydrated oocytes were still visible in August but in few fish in later months also, although the size was clearly small. GSI reduced to 8.66±3.22 in August, 1.48±0.17 in September and 2.27±0.33 in October (Fig. 2C).	Decline in the size of the oocytes started in August (650.00±7.00 µm). Ovaries contained atretic follicles along with tertiary oocytes. Atresia was at full scale in September (553.00±12.00 µm). Some oocytes showed coalescing of the yolk. This decrease in oocyte size caused a decrease in weight and size of the ovary. Primary oocyte started increasing and in October (275.00±5.00 µm) the ovaries again contained primary oocytes only. Some fish however had tertiary oocytes undergoing atresia, which was quite common (Figs. 3, 4).

Note: These Observations are based on the gross morphological and histological studies of *Labeo rohita* ovaries. Oocytes were measured from different stage ovaries. No spawning or POF was observed.

winter spawners and a day length of less than 12 hours and lower temperatures are critical conditions for maturity and spawning. These and related studies have been summarized by Bromage (2001) and Bromage *et al*. (2001).

In the present study also, it seems that both photoperiod and temperature play an important role in gonadal recrudescence and maturation. In our study, the ovaries of rohu started developing in March when the photoperiod increased from the minimum in December and when the water temperatures started increasing from the minimum in January (Fig. 1) and ultimately showed a peak in June. This means that in March when the photoperiod reached spring equinox (12H:12H) and temperature 27.79±2.06°C, the ovary started increasing in weight and showed histological advancement. Later on, when the day-length crossed 14 hours in April and temperature was 29.00±1.76ºC, a rapid development of the ovary was observed. This means that rohu fits very well with the hypothesis that the summer spawners are influenced by the increasing day-lengths (long days) and higher temperatures. These parameters for rohu are a temperature above 25°C and a day length of above 13 hours. The termination of spawning season in rohu seems to have started in August when the photoperiod was already declining from the maximum in June, although the water temperature was still higher (maximum encountered in the present study, *i.e.* 34.00±1.00ºC). Thus, we can conclude that ovarian development and maturation in rohu is initiated by lengthening photoperiods and increasing temperatures in early summer and is terminated by shortened (decreasing) photoperiods with higher temperatures. These observations suggest that for gonadal development and maturation, environmental temperature plays an important role while for the termination of the spawning or end of the maturational cycle is precipitated by photoperiod. However, this hypothesis needs further experimentation and studies for its confirmation.

In addition to photoperiod and temperature, we also studied the role of rainfall in the final maturation and spawning of rohu. When the data regarding mean monthly rainfall was plotted with the ovarian weight (Fig. 1), it was found that there is a correlation between rainfall and the regression of the ovaries (due to spawning ?). It is known that major carps spawn during the monsoon season when rainfall is at maximum of the year and a casual relation existed between these parameters as was reported by earlier workers working in the wild

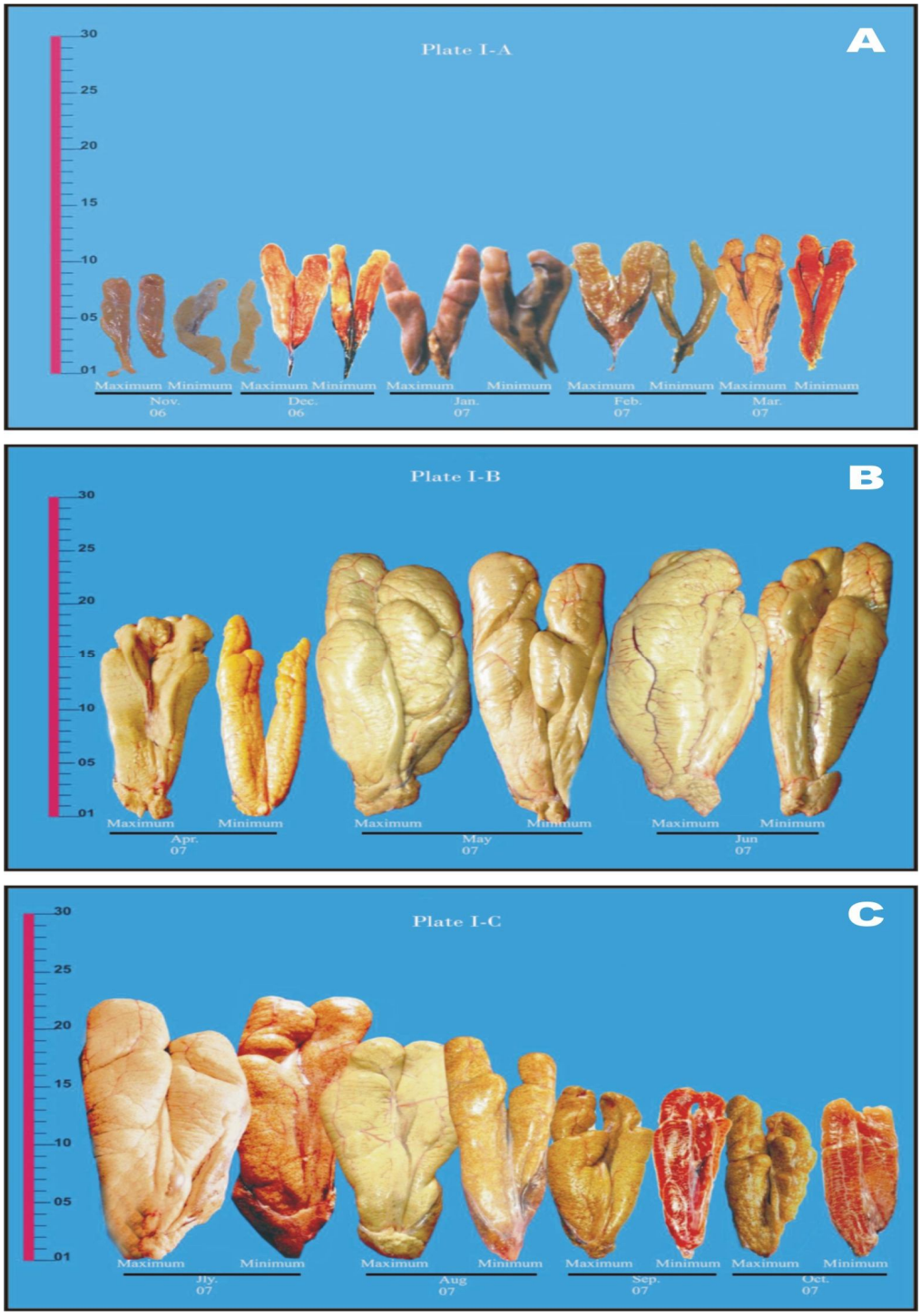

Fig. 2. Size, gross structure and development of *Labeo rohita* ovary. A, November 2006 (age 18 months) to March 2007 (age 22 months). The two lobes of the ovary are separate in the beginning however as they grow, the lobes come together and an oviduct is formed. Also given for each month is the maximum and minimum size (weight) for that month; B, April 2007 (age 23 months) to June 2007 (age 25 months); C, July 2007 (age 26 months) to October 2007 (age 29 months). For other details see Table II and III.

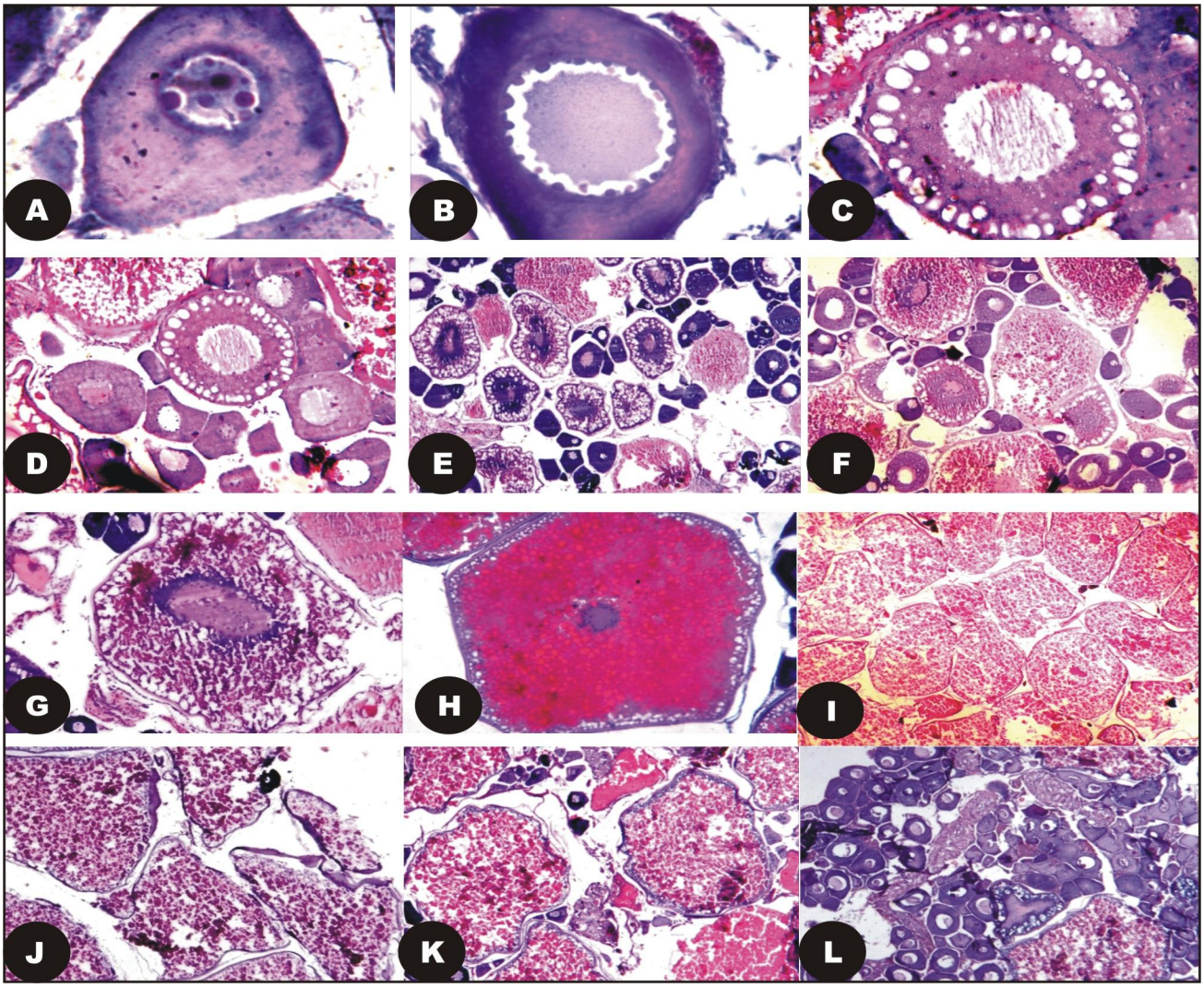

Fig. 3. Formalin fixed and hematoxylin and eosin-stained paraffin sections of *Labeo rohita* ovary showing different stages of oogenesis. (A) primary basophilic oocyte at early nucleolus stage; X400 (B) primary oocyte in peri-nucleolus stage; X400 (C) early secondary oocyte at cortical alveoli (yolk vesicle) stage; X400 (D) view of the ovary showing primary, secondary and tertiary oocytes; X200 (E) section of the ovary showing start of the deposition of yolk (vitellogenesis) in tertiary oocytes; X40 (F) tertiary oocyte with protein and lipid yolk; X100 (G) tertiary oocyte with germinal vesicle containing nucleoli; X100 (H) tertiary oocyte in maturing stage; X100 (I) section of the ovary showing group synchronous type maturation; X40 (J) tertiary oocytes in early stage of atresia; X40 (K) another view of atresia of yolked oocytes; X40 (L) early signs of ovarian regression; X40.

(Jhingran, 1986; Jhingran and Pullin, 1985; Pillay and Kutty, 2005) however no quantitative data were reported. We have for the first time shown that rainfall is correlated quite well with the ovarian weight, ovarian length, GSI and oocyte size. These parameters show peak (in June) when the rainfall is increasing from 26.12±19.84 mm in May to 61.28±32.08 and 199.86±102.16 mm in June and July respectively. These are the months when rohu is reported to be spawning in the wild. After July peak, the rainfall start decreasing and by September had already reached 69.51±44.81 and 18.60±28.52 mm in October when the ovaries are already regressed. Although we did not see the spawning in the present study, however the increase in rainfall coincides well with the decrease in GSI and oocyte

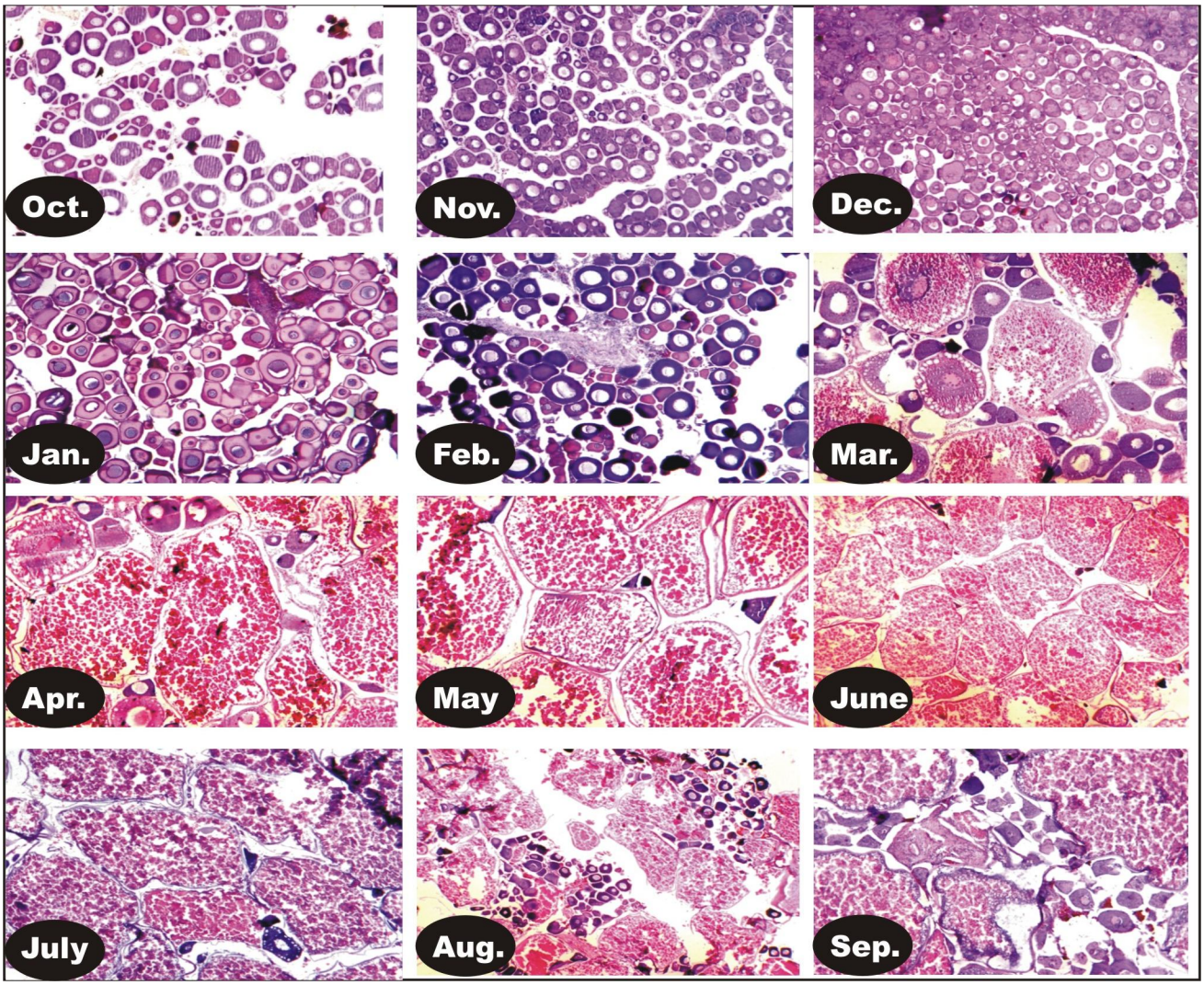

Fig. 4. Seasonal and age-related changes and development of the *Labeo rohita* ovary. The ovaries were fixed in buffered formalin and hematoxylin-eosin-stained paraffin sections were photomicrographed at X40. For more details see Materials and Methods and Table II and III.

size, an indication of spawning as seen in the wild (Fig. 1). Further studies must be carried out to substantiate this conjecture.

As reported earlier, we did not observe any spawning in our fish during the age of 18-29 months, although they had mature ovaries containing yolky oocytes, nevertheless, these oocytes did not enter the final oocyte maturation (FOM) and spawning. Not much is known about this reproductive dysfunction in rohu as it is known that these same fish are routinely spawned in captivity, artificially, after hormonal intervention.

The fish when living in the wild normally develop their gonads according to the environmental conditions and time of the year that best suit them and the newly hatched larvae and young ones. This is the reason for great variability in the development, maturation and spawning tactics of different fish species. However, when the fish are kept in captivity for culture purpose there is always some degree of reproductive dysfunction exhibited. This dysfunction some time is eliminated after few generations when the fish have lived in captivity. However, other fish do not respond to captivity in

this way and even after many generations they still do not breed normally as they in the wild. This necessitates some hormonal therapies for inducing maturation, ovulation and spawning (Zohar and Mylonas, 2001).

There are at least four major types of reproductive dysfunction known. In the first type early gametogenesis do not proceed, as is seen in eels (Miura *et al*., 1991a,b; Ohta *et al*., 1997). The second most common dysfunction is that there is no FOM (although the vitellogenesis takes place normally) and hence no ovulation and spawning. This category includes major carps (including rohu; present study), flatfishes (Berlinsky *et al*., 1996, 1997; Mugnier *et al*., 2000), family serranidae (Tucker, 1994; Watanabe *et al*., 1998), striped bass and white bass (Mylonas and Zohar, 2001), fugu (Yang and Chen, 2004; Chen, 2005), shi drum (Mylonas, 2004), dusky grouper (Marino *et al*., 2003). The third category is found in family salmonidae (Genus: *Oncorhynchus* and *Salmo*) and is the most easy to address. In this case, the fish undergo vitellogenesis, FOM and even ovulation but they do not spawn the eggs in captivity. The reason here is behavioral in nature as gravel bed is not available in captivity (Bromage and Cumarantunga, 1988; Zohar, 1989; Liley and Kroon, 1995). Lastly, there are fish which fail to undergo complete vitellogenesis as is seen in amberjack (Garcia *et al*., 2000) and Mekong river catfish (Donaldson, 1996).

Not much is known for the cause/s of these reproductive dysfunctions but certain points can be elaborated from the available literature. The failure of ovary to initiate gametogenesis, vitellogenesis, FOM or ovulation and spawning in captivity can be due to the lack of complete array of environmental factors that act as cues for favorable changes in the internal *milieu* that force's these activities or processes in the wild fish. The case of major environmental factors like temperature, photoperiod and rainfall has been discussed above. There can be other minor factors (gravel beds for salmonids) that are important at a specific point in the process of complete and successful reproduction. Because of absence of all these or some factors subtle changes in the behavioral response can occur thus blocking the reproductive development. The second major factor in this connection is the stress of captivity. Even if the best possible conditions are provided to the fish captivity itself is a big stress (Schreck *et al*., 2001; Consten *et al*., 2002). Stress induced increase in adrenal cortex steroids particularly cortisol has been shown to be antigonadal in many vertebrates from mammals to fishes (Charpenet *et al*., 1981; Pickering *et al*., 1987; Carragher *et al*., 1989; Mahmoud and Licht, 1991; Moore *et al*., 1991; Norman and Smith, 1992; Foo and Lam, 1993; Coddington and Cree, 1995).

Whatever is the cause of this deficiency for the final completion of the reproductive cycle, it ultimately revolves and ends in the deficiency of hormones from all or one component of the brain-pituitary-gonad axis. Not much is known in this regard for the major carps, however. Studies are available from Europe that points to the fact that failure of the fish either to start gametogenesis, vitellogenesis or ovulation inhibition, the brain-pituitary-gonadal axis is involved. It has been shown that most probably in these conditions the inhibition of the release of LH from the pituitary is the major cause. It is also known that pituitary release of LH is under hypothalamic control therefore, in these fish either the synthesis of LH or release of LH (under control of GnRH from hypothalamus) from the pituitary or both are defective (Zohar *et al*., 1995; Mylonas *et al*., 1998; Steven, 2000; Steven *et al*., 2000; Mylonas and Zohar, 2001). This observation is further substantiated from the observations that all those species that show these inhibitions in the gametogenesis or final maturation do spawn when given injections of GnRH or pituitary extracts, a normal practice in the hatcheries of the world for getting gametes for aquaculture. Studies on this and related aspects are badly needed for major carps so that an insight into this deficiency be achieved for better management purposes.

Oocytic atresia is a common phenomenon in teleost ovary (Saidapur, 1978; Witthames and Greer-Walker, 1995; McDermott *et al*., 2007). It was also observed in the present study throughout the year and irrespective of age and season. However, the intensity of atresia varied. It was low at the primary and secondary oocyte stages as these stages were encountered during the winter and early summer season when shorter photoperiod prevailed. It increased, as both the temperature and

photoperiod started increasing and the oocytes started developing fast. It was quite prevalent after April (long days and high temperature) when the tertiary oocytes with external vitellogenesis were dominating the ovarian histological picture. Since all signs suggest that our fish did not spawn in pond environment, the atresia was quite prevalent in July, August and September. These are the months when water temperature was quite warm and photoperiod long though decreasing. From our studies it appears that decreasing photoperiod with high temperatures are conditions which cause atresia. However, more studies with fish of other ages are needed to substantiate this hypothesis.

Seasonal changes of HSI in female *Labeo rohita* showed an inverse relationship with GSI (Fig. 1). The HSI reached its minimum level during the month of May when GSI was nearly at its peak. A general reduction in HSI from its maximum values in December can be explained by mobilization of lipid and protein reserves as precursors of vitellogenin (a lipid phosphoprotein) synthesized by the liver. The vitellogenin is released into the blood in response to estrogens produced by the maturing gonads (Rodriguez *et al*., 2001; Maldonado-Garcia *et al*., 2005).

The potential reproductive capacity of an organism or population, measured by the number of gametes (*e.g.* eggs or sperms) is known as fecundity. Fecundity is a measure of fertility, such as sperm count or egg count or the number of live offspring produced by an organism. We measured the absolute fecundity in rohu with ovaries collected from fish at mature-gravid stage in June (Table II). The absolute fecundity values thus obtained were 469.93 eggs/g body weight and 2012.55 eggs/g ovary weight of fish with average weight of 1738.76g. During this time the average size of the oocytes measured from the histological sections was 0.773 mm.

In fish it has been shown many times that the size of the oocyte depends on the size of the fish and the larger fish tend to have bigger and higher number of eggs (Fernandez-Delgado and Herrera, 1995; Olivia-Paterna *et al*., 2002; Juchno *et al*., 2007; Plaza *et al*., 2007). However, it is also known that size of the oocytes/egg can increase to a limit therefore fluctuations in the fecundity may reflect the body and gonad size. It should also be stated here that during evolutionary history the fishes developed two pronged strategy for fecundity. This strategy is a selection that with the same mass of ovary, a small number of large eggs (yielding bigger larvae) can be produced or a large number of small eggs (yielding smaller larvae) be produced. This selection is based on many factors like ambient environment, food availability, season of spawning, predation pressure etc. In rohu, very little is known about the fecundity of fish under cultured conditions as the fish do not spawn naturally in captivity. However, some reports are available from the wild fish. Khan (1972), reported from the area around Aligarh, India, the fecundity of rohu from the age of two to seven year. The fish were 1801 – 9210g in body weight, while the corresponding ovarian weight was 390-2060 g (GSI= 21.6-22.3). There was an increase in the fecundity of fish from second to sixth year (GSI= 21.6-27.1) of life and then declined for the seventh year. The number of eggs per gram of ovary for the two year old fish were 1335 and 535 per gram of the body weight. A number similar to ours on body weight basis but lower than on the ovarian weight basis. The reason for this may be the extra or supplementary food that is given to the fish in captivity however, this conjecture needs to be confirmed. In another study, Khan and Jhingran (1975) also reported fecundity of wild rohu based on the size of the fish and collected from Indian waters. The GSI (ovarian weight) increased from 17.0-29.6% for fish of body weight of 1.75 to 6.75 kg. The number of eggs produced on the body weight and ovarian weight basis were 211 and 1230 and 413 and 1387, respectively. These results clearly show that wild fish has lower fecundity than the cultured fish. Further studies are needed in this connection from Pakistani waters and from various culture systems used in the country.

ACKNOWLEDGEMENT

The authors are thankful to Mr. Mohammad Naveed Khan for his help in preparing graphs.

REFERENCES

ALIKUNHI, K.H., 1957. Fish culture in India. *Farming Bull. Ind. Coun. Agric. Res.*, **20**: 1-144.

ALMATAR, S.M., LONE, K.P., ABU-REZQ, T.S. AND YOUSEF, A.A., 2004. Spawning frequency, fecundity, egg weight and spawning type of silver pomfret, *Pampus argenteus* (Euphrasen) (Stromateidae) in Kuwait waters. *J. appl. Ichthyol.*, **20**: 176-188.

BERKA, R., 1986. *The transport of live fish: a review*. EIFAC Tech. Paper **48**, pp. 52. Food and Agriculture Organization, Rome, UN.

BERLINSKY, D.L., KING, W.V., SMITH, T.I.J., HAMILTON, R.D., II, HOLLOWAY, J., JR., AND SULLIVAN, C.V., 1996. Induced ovulation of Southern flounder *Paralichthys lethostigma* using gonadotropin releasing hormone analogue implants. *J. World Aquacult. Soc.*, **27**: 143–152.

BERLINSKY, D.L., WILLIAM, K., HODSON, R.G. AND SULLIVAN, C.V., 1997. Hormone induced spawning of summer flounder *Paralichthys dentatus*. *J. World Aquacult. Soc.*, **28**:79–86.

BEROIS, N., BOLATTO, C., BRAUER, M.M. AND BARROS, C., 2004. Gametogenesis, histological gonadal cycle and *in vitro* fertilization in the whitemouth croaker (*Micropogonias funrnieri*, Gesmarest, 1823). *J. appl. Ichthyol.*, **20**: 169-175.

BHATTACHARYYA, S. AND MAITRA, S.K., 2006. Environmental correlate of the testicular events in a major carp *Catla catla* in an annual reproductive cycle. *Biol. Rhythm Res*., **37**:87-110.

BROMAGE, N.R., 2001. Recent developments in the control of reproduction of farmed fish. In: *Modern aquaculture in the coastal zone. Lessons and opportunities* (ed. J. Coimbra), pp. 243-260. IOS Press, Amsterdam.

BROMAGE, N.R. AND CUMARANATUNGA, R., 1988. Egg production in the rainbow trout. In: *Recent advances in aquaculture* (eds. J.F. Muir and R.J. Roberts), pp. 63–138. Croom Helm /Timber Press, London.

BROMAGE N.R., PORTER, M., AND RANDALL, C., 2001. The enviromental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. *Aquaculture*, **197**:63-98.

CARRAGHER, J., SUMPTER, J.P., POTTINGER, T.G. AND PICKERRING., A.D., 1989. Effect of cortisol implantation on reproductive function in two species of trout, *Salmo trutta* L. and *Salmo gairdneri* Richardson. *Gen. Comp. Endocrinol.*, **75**:310-321 .

CHARPENET, G., TACHE, Y., FOREST, M.G., HAOUR, F., SAEZ, J.M., BERNIER, M., DUCHARME, J.R. AND COLLU, R., 1981. Effect of chronic intermittent immobilization stress on rat testicular androgen function. *Endocrinology*, **109**: 1254-1258.

CHEN, Y.F., 2005. Induced ovulation and embryonic development of ocellated puffer, *Takifugu ocellatus*. *J. appl. Ichthyol.*, **21**: 136-140.

CODDINGTON, E.J. AND CREE, A., 1995. Effect of acute captivity stress on plasma concentrations of corticosterone and sex steroids in female whistling frogs, *Litoria ewingi*. *Gen. Comp. Endocrinol.*, **100**: 33-38.

CONSTEN, D., LAMBERS, J.G.D., KOMEN, H., AND GOOS, H.J.TH., 2002. Corticoids affect the testicular androgen production in male common carp (*Cyprinus carpio* L.). *Biol. Reprod.*, **66**: 106-111.

DAY, R., BHATTACHARYA, S., AND MAITRA, S.K., 2004. Temporal pattern of ovarian activity in a major carp *Catla catla* and its possible environmental correlate in an annual cycle. *Biol. Rhythm Res.*, **35**: 329-353.

DAY, R., BHATTACHARYA, S., AND MAITRA, S.K., 2005. Importance of photoperiods in the regulation of ovarian activities in Indian major carp *Catla catla* in an annual cycle. *J. biol. Rhythm.*, **20**: 145-158.

DONALDSON, E.M., 1996. Manipulation of reproduction in farmed fish. *Anim. Reprod. Sci.*, **42**: 381–392.

DROLLER, M.J. AND ROTH, T.F., 1966. An electron microscope study of yolk formation during oogenesis in *Lebistes reticulatus* Guppy. *J. Cell Biol*., **28**: 209-232.

FERNANDEZ-DELGADO, C. AND HERRERA, M., 1995. Age structure, growth and reproduction of *Rutilus lemmingii* in a intermittent stream in the Guadalquivir river basin, southern Spain. *J.Fish Biol*., **46**: 371-380.

FOO, J.T.W. AND LAM, T.J., 1993. Serum cortisol response to handling stress and the effect of cortisol implantation on testosterone level in the tilapia, *Oreochromis mossambicus*. *Aquaculture*, **115**: 145-158.

GANIAS, K., SOMARAKIS, S., MACHIAS, A. AND THEODOROU, A., 2004. Pattern of oocyte development and batch fecundity in the Mediterranean sardine. *Fish. Res*., **67**: 13-23.

GARCIA, A., DIAZ, M.V., SCHULZ, R.W. AND AGULLEIRO, B., 2000. Gonadal development and hormonal levels of captive Mediterranean yellowtail (*Seriola dumerilii*, Risso) under photoperiod control. In: *Reproductive physiology of fish* (eds. B. Norberg, O.S. Kjesbu, G.L. Taranger, E. Andersson and S.O. Stefansson), pp. 436–491. University of Bergen, Bergen, Norway.

GURAYA, S.S., 1976a. Recent advances in the morphology, histochemistry and biochemistry of steroid-synthesizing cellular sites in the nonmammalian vertebrate ovary. *Int. Rev. Cytol.*, **44**: 365-409.

GURAYA, S.S., 1976b. Recent advances in the morphology, histochemistry, and biochemistry of steroid-synthesizing cellular sites in the testes of non-mammalian vertebrates. *Int. Rev. Cytol*., **47:** 99-136.

JHINGRAN, V.G., 1986. Aquaculture of Indian major carps. In: *Aquaculture of cyprinids* (eds. R. Billard and J. Marcel), pp.335-346. Edition Quae, Paris.

JHINGRAN, V.G. AND PULLIN, R.S.V., 1985. *A hatchery manual for the common Chinese and Indian major carps.* ICLARM, Manila.

JUCHNO, D., BORON, A. AND GOLASZEWSKI, J., 2007. Comparative morphology and histology of the ovaries

of the spined loach *Cobitis taenia* L. and allopolyploids of *Cobitis* (Cobitidae). *J. Fish Biol.*, **70**: 1392-1411.

KAPUR, K., 1981. Spawning of major Indian carps-A review. *Acta Hydrochim. Hydrobiol.*, **9**: 163-182.

KHAN, R.A., 1972. *Studies on the biology of some important major carps*. MS Thesis, Department of Zoology, Aligarh Muslim University, India. pp.185.

KHAN, R.A. AND JHINGRAN, V.G., 1975. Synopsis of biological data on rohu, *Labeo rohita* (Hamilton, 1822). *FAO Fish. Synop.* **111**: 1-100.

KHOO, K.H., 1975. The corpus luteum of goldfish (*Carassius auratus L.*) and its functions. *Can. J. Zool*. **53**: 1306-1323.

LAM, T.J., 1983. Environmental influences on gonadal activity in fish. In: *Fish physiology* (eds. W.S. Hoar, D.J. Randall, and E.M. Donaldson), vol. 9B, pp. 65-116. Academic Press, New York.

LILEY, N.R. AND KROON, F.J., 1995. Male dominance, plasma hormone concentrations, and availability of milt in male rainbow trout (*Oncorhynchus mykiss*). *Can. J. Zool.* **73**: 826–836.

LONE, K.P., AL-ABLANI, S. AND ALMATAR, S., 2008. Oogenesis, histological gonadal cycle, seasonal variations and spawning season of female silver pomfret (*Pampus argenteus*, Euphrasen) from the spawning grounds of Kuwait. *Pakistan J. Zool*., **40**: 397-407.

LONE, K.P., AL-ABLANI, S. AND AL-YAKOUT, A., 2001. Steroid hormone profile and correlative gonadal histological changes during nature sex reversal of zobaidy kept in tanks and sea-cages. *J. Fish Biol.*, **58**: 305-324.

LONE, K.P. AND MATTY, A.J., 1980. Effect of feeding methyltestosterone on the growth and body composition of carp, *Cyprinus carpio*. *Gen. Comp. Endocrinol.*, **40**: 409- 425.

MA, Y.X., MATSUDA, K. AND UCHIYAMA, M., 2005. Seasonal variations in plasma concentrations of sex steroid hormones and vitellogenin in wild male Japanese dace (*Tribolodon hakonensis*) collected from different sites of the Jinzu River Basin. *Zool. Sci.*, **22**: 861-868.

MAHMOUD, I.Y. AND LICHT, P., 1997. Seasonal changes in the gonadal activity and the effect of stress on reproductive hormones in the common snapping turtle, *Chelydra serpentina*. *Gen. Comp. Endocrinol.*, **107**-359-372.

MANANOS, E.L., ZANUY, S. AND CARRILLO, M., 1997. Photoperiodic manipulations of the reproductive cycle of sea bass (*Dicentrarchus labrax*) and their effects on gonadal development, and plasma 17β-estradiol and vitellogenin levels. *Fish Physiol. Biochem.*, **16:** 211-222.

MARINO, G., PANINI, E., LONGOBARDI, A., MANDICH, A., FINOIA, M.G., ZOHAR, Y. AND MYLONAS, C.C., 2003. Induction of ovulation in captive-reared dusky grouper, *Epinephelus marginatus* (Lowe, 1834) with a sustained-release GnRHa implant. *Aquaculture*, **219**: 841–858.

MATSUYAMA, M., ADACHI, S., NAGAHAMA, Y., KITAJIMA, C. AND MATSUURA, M., 1991. Annual reproductive cycle of the captive female Japanese sardine *Sardinops melanostictus*: Relationship to ovarian development and serum levels of gonadal steroid hormones. *Mar. Biol.*, **108**: 21-29

MCDERMOTT, S.F., MASLENIKOV, K.P. AND GUNDERSON, D.R., 2007. Annual fecundity, batch fecundity, and oocyte atresia of Atka mackerel (*Pleurogrammus monopterygius*) in Alaskan waters. *Fish. Bull.*, **105**: 19-29.

MIURA, T., YAMAUCHI, K., TAKAHASHI, H. AND NAGAHAMA, Y., 1991a. Human chorionic gonadotropin induces all stages of spermatogenesis *in vitro* in the male Japanese eel (*Anguilla japonica*). *Dev. Biol*., **146**: 258–262.

MIURA, T., YAMAUCHI, K., TAKAHASHI, H. AND NAGAHAMA, Y., 1991b. Hormonal induction of all stages of spermatogenesis *in vitro* in the male Japanese eel (*Anguilla japonica*). *Proc. natl. Acad. Sci.*, **88**: 5774–5778.

MALDONADO-GARCIA, M., GRACIA-LOPEZ, V., CARRILLO, M., HERNANDEZ-HERRERA, A. AND RODRIGUEZ-JARAMILLO, C., 2005. Stages of gonad development during the reproductive cycle of the blakfin snook, *Centropomus medius* Gunther. *Aquacult. Res.*, **36:** 554-563.

MOORE, M.C., THOMPSON, C.W. AND MARLER, C.A., 1991. Reciprocal changes in corticosterone and testosterone levels following acute and chronic handling stress in the tree lizard, *Urosaurus ornatus*. *Gen. Comp. Endocrinol.*, **81**: 217-226.

MUGNIER, C., GAIGNON, J.L., LEBEGUE, E., FOSTIER, A. AND BRETON, B., 2000. Induction and synchronization of spawning in cultivated turbot (*Scophthalmus maximus*, L.) broodstock by implantation of sustained release GnRH-a pellet. *Aquaculture*, **181**: 241-255.

MYLONAS, C.C., PAPANDROULAKIS, N., SMBOUKIS, A. AND DIVANACH, P., 2004.Induction of spawning of cultured greater amberjack (*Seriola dumerili*) using GnRHa implants. *Aquaculture*, **237**: 141–154.

MYLONAS, C.C., WOODS, L.C., III, THOMAS, P. AND ZOHAR, Y., 1998. Endocrine profiles of female striped bass (*Morone saxatilis*) during post-vitellogenesis, and induction of final oocyte maturation via controlled-release GnRHa-delivery systems. *Gen. Comp. Endocrinol.*, **110**: 276–289.

MYLONAS, C.C. AND ZOHAR, Y., 2001a. Endocrine regulation and artificial induction of oocyte maturation and spermiation in basses of the genus *Morone*. *Aquaculture*, **202**: 205–220.

MYLONAS, C.C. AND ZOHAR, Y., 2001b. Use of GnRHa-delivery systems for the control of reproduction in fish. *Rev. Fish Biol. Fish.*, **10**:463–491.

MYLONAS, C.C. AND ZOHAR, Y., 2007. Promoting oocyte maturation, ovulation and spawning in farmed fish. In: *The fish oocyte: from basic studies to biotechnological applications* (eds. P.J. Babin, J. Cerda and E. Lubzens), pp. 437-474. Springer, Dordrecht.

NARIMATSU, Y., YAMANOBE, A. AND TAKAHASHI, M., 2007. Reproductive cycle, age and body size at maturity and fecundity of female willow flounder *Tanakius kitaharai*. *Fish. Sci*., **73**: 55-62.

NORMAN, R.L. AND SMITH, C.J., 1992. Restrain inhibits luteinizing hormone and testosterone secretion in intact males rhesus macaques: Effects on concurrent naloxone administration. *Neuroendocrinology*, **55:** 405-415.

OHTA, H. AND TANAKA, H., 1997. Relationship between serum levels of human chorionic gonadotropin (hCG) and 11-ketotestosterone after a single injection of hCG and induced maturity in the male Japanese eel, *Anguilla japonica*. *Aquaculture*, **153**: 123–134.

OKUZAWA, K., FURUKAWA, K., AIDA, K. AND HANYU, I., 1989. Effect of photoperiod and temperature on gonadal maturation, and plasma steroid and gonadotropin levels in a cyprinid fish, *Ganathopogon caerulescens*. *Gen. Comp. Endocrinol.*, **75:** 139-147.

OLIVIA-PATERNA, F.J., TORRALVA, M.M. AND FERNANDEZ-DELGADO, C., 2002. Age, growth and reproduction of *Cobitis paludica* in a seasonal stream. *J. Fish Biol*., **60**: 389-404.

PICKERING, A.D., POTTINGER, T.G., CARRAGHER, J. AND SUMPTER, J.P., 1987. The effect of acute and chronic stress on the levels of reproductive hormones in the plasma of mature male brown trout, *Salmo trutta* L. *Gen. Comp. Endocrinol*., **68**: 249-259.

PILLAY, T.V.R. AND KUTTY, M.N., 2005. *Aquaculture: Principles and practices.* 2nd ed. Blackwell Publishers, London.

PLAZA, G., SAKAJI, H., HONDA, H., HIROTA, Y. AND NASHIDA, K., 2007. Spawning pattern and type of fecundity in relation to ovarian allometry in the round herring *Etrumeus teres*. *Mar. Biol*., **152**: 1051-1064.

RODRIGUEZ, L., ZANUY, S. AND CARRILLO, M., 2001. Influence of day-length on the age at first maturity and somatic growth in male sea bass (*Dicentrarchus labrax*). *Aquaculture*, **196**: 159-175.

ROSS, J.L. AND MERRINER, J.V., 1983. Reproductive biology of the blueline tilefish, *Caulolatilus microps*, off North Carolina and South Carolina. *Fish. Bull.*, **81**: 553-568.

SAIDAPUR, S.K., 1978. Follicular atresia in the ovaries of nonmammalian vertebrates. *Int. Rev. Cytol.*, **54**: 225-244.

SCHRECK, C.B., CONTRERAS-SANCHEZ, W. AND FITZPATRICK, M.S., 2001. Effects of stress in fish reproduction, gamete quality, and progeny. A*quaculture*, **197**: 3–24.

SEN, U., MUKHERJEE, D., BHATTACHARYYA, S. P. AND MUKRERJEE, D., 2002. Seasonal changes in plasma steroid levels in Indian major carp *Labeo rohita*: Influence of homologous pituitary extract and steroid production and development of oocyte maturational competence. *Gen. Comp. Endocrinol.*, **128**: 123-134.

SHIMIZU, A., AIDA, K. AND HANYU, I., 1994. Effect of photoperiod and temperature on gonadal activity and plasma steroid levels in an autumn-spawning bitterling, *Acheilognathus rhombea*, during different phases of its annual reproductive cycle. *Gen. Comp. Endocrinol*., **93**: 137-150.

SHRESTHA, T.K., 1980. Rhythm of oocyte maturation and reproductive cycling in a female stone loach, *Noemacheilus beavane* (Gunther). *Intl. J. Acad.*, **1**: 19-20.

STEVEN, C., 2000. *Studies on the GnRH-GtH system of female striped bass (*Morone saxatilis*): effects of GnRH agonist therapy and comparison of reproductive endocrine parameters between wild and captive fish*. M.Sc. dissertation, University of Maryland, College Park.

STEVEN, C., GOTHILF, Y., HOLLAND, M.C.H., STUBBLEFIELD, J., MYLONAS, C.C. AND ZOHAR, Y., 2000. Differential expression of the three GnRH genes in wild and captive striped bass, *Morone saxatilis*, in response to natural and hormonally induced maturation. In: *Reproductive physiology of fish* (eds. B. Norberg, O.S. Kjesbu, G.L. Taranger, E. Andersson, and S.O. Stefansson), pp. 66. University of Bergen, Bergen.

TUCKER, J.W., 1994. Spawning of captive serranid fishes: a review. *J. Wld. Aquacult. Soc.*, **25**: 345–359.

WALLACE, R.A., 1978. Oocyte growth in nonmammalian vertebrates. In: *The vertebrate ovary* (ed. R. E. Jones), pp. 469-502. Plenum, New York.

WALLACE, R.A. AND SELMAN, K., 1981. Cellular and dynamic aspects of oocyte growth in teleosts. *Am. Zool*., **21**: 325-343.

WANG, H.- Y., WENG, C.-F., TU, M.-C. AND LEE, S.-C., 2001. Synchronization of plasma sexual steroid concentrations and gonadal cycles in the sleeper, *Eleotris acanthopoma. Zool. Stud.*, **40**: 14-20.

WATANABE, W.O., ELLIS, E.P., ELLIS, S.C. AND FEELEY, M.W., 1998. Progress in controlled maturation and spawning of summer flounder (*Paralichthys dentatus*) broodstock. *J. World Aquacult. Soc.*, **29:** 393–404.

WHITTHAMES, P.R. AND WALKER, M.G., 1995. Determinacy of fecundity and oocyte atresia in sole (*Solea solea*) (Pisces) from the channel, the North Sea and the Irish Sea. *Aquat Living Resour.*, **8**: 91-109.

YAMAMOTO, K., OOTA, I., TAKANO, K. AND ISHIKAWA, T., 1965. Studies on the maturing process

of the rainbow trout, *Salmo gairdneri irideus*. 1. Maturation of the ovary of a one-year old fish. *Bull. Jpn. Soc. Sci. Fish.*, **31**: 123-132.

YANAGIMOTO, T. AND HUMPHREYS Jr, R.L., 2005. Maturation and reproductive cycle of female armorhead *Pseudopentaceros wheeleri* from the southern emperor-northern Hawaiian Ridge seamounts. *Fish. Sci.*, **71**: 1059-1068.

YANG, Z. AND CHEN, Y.-F., 2004. Induced ovulation in obscure puffer *Takifugu obscures* by injections of LH-RH-a. *Aquacult. Int.*, **12**: 215-223.

ZOHAR, Y., 1989. Fish reproduction: its physiology and artificial manipulation. In: *Fish culture in warm water systems: problems and trends* (eds. M. Shilo and S. Sarig), pp. 65–119. CRC Press, Boca Raton,

ZOHAR, Y., HAREL, M., HASSIN, S. AND TANDLER, A., 1995. Gilthead sea bream (*Sparus aurata*). In: *Broodstock management and egg and larval quality* (eds. N.R. Bromage and R.J. Roberts), pp. 94–117. Blackwell Science, Oxford.

ZOHAR, Y. AND MYLONAS, C.C., 2001. Endocrine manipulations of spawning in cultured fish: from hormones to genes. *Aquaculture*, **197**: 99–136.

(Received 2 February 2009, revised 20 March 2009)

Table II.- Monthly variations (Mean± SEM) of various parameters of female *Labeo rohita* during the study period.

Month (year)	Age (Month)	N	Body weight (g)	Total body length (cm)	Standard length (cm)	Condi-tion factor	Body width (cm)	Gonad weight (g)	Gonad length (cm)	Gonad width (cm)	Oocyte size (mm)	Liver weight (g)	GSI	HS I
Nov.06	18	5	861.80± 14.35	47.70± 0.62	38.96± 0.63	0.794± 0.03	13.70± 0.40	13.68± 1.08	9.16± 0.40	1.702± 0.09	0.094± 0.006	15.08± 0.45	1.48± 0.15	1.74± 0.07
Dec.06	19	7	732.37± 11.92	46.42± 0.98	38.03± 1.02	0.734± 0.04	13.78± 0.33	13.11± 2.06	11.48± 0.63	1.687± 0.04	0.092± 0.001	17.22± 1.98	1.77± 0.25	2.31± 0.24
Jan.07	20	8	876.05± 56.34	46.98± 0.63	38.46± 0.68	0.847± 0.06	13.81± 0.28	15.65± 1.48	12.43± 0.30	2.037± 0.09	0.114± 0.002	16.46± 1.36	1.83± 0.22	1.93± 0.21
Feb.07	21	8	1210.51± 29.36	47.36± 0.36	38.65± 0.28	1.132± 0.01	13.49± 0.23	14.61± 1.04	13.00± 1.05	1.920± 0.10	0.117± 0.004	15.4± 0.81	1.19± 0.07	1.25± 0.06
Mar.07	22	6	1224.70± 111.73	47.96± 0.81	39.15± 0.32	1.098± 0.07	14.18± 0.21	38.86± 11.04	13.60± 1.30	2.630± 0.29	0.430± 0.059	14.45± 1.35	3.02± 0.69	1.19± 0.08
Apr.07	23	7	1338.10± 103.93	47.82± 0.38	39.15± 0.31	1.294± 0.08	17.05± 0.61	121± 28.98	16.61± 1.22	3.294± 0.31	0.646± 0.019	17.73± 1.14	8.52± 1.58	1.36± 0.14
May.07	24	5	1535.84± 171.89	48.96± 0.77	40.05± 0.51	1.306± 0.10	17.27± 0.31	310.72± 18.33	22.50± 0.34	5.446± 0.15	0.664± 0.004	14.98± 0.83	21.01± 2.10	1.01± 0.11
Jun.07	25	5	1732.70± 127.81	49.50± 1.73	40.84± 1.37	1.422± 0.05	18.79± 0.50	388.80± 23.02	22.82± 0.91	5.298± 0.39	0.773± 0.031	18.52± 1.28	22.73± 0.94	1.07± 0.06
July.07	26	5	1663.13± 42.75	48.96± 0.78	40.06± 0.40	1.322± 0.09	16.00± 0.64	299.70± 48.23	22.70± 1.04	5.032± 0.53	0.743± 0.017	15.11± 2.67	22.41± 3.21	1.11± 0.14
Aug.07	27	5	1691.90± 150.68	52.28± 0.76	42.74± 0.73	1.172± 0.07	16.25± 0.25	135.49± 44.98	19.20± 0.98	3.204± 0.33	0.650± 0.007	27.66± 2.16	8.66± 3.32	1.64± 0.11
Sep.07	28	6	1590.60± 85.60	51.86± 0.81	43.41± 0.65	1.130± 0.03	16.08± 0.26	57.67± 24.37	14.59± 1.31	2.580± 0.41	0.553± 0.120	22.63± 1.44	1.48± 0.17	1.42± 0.06
Oct.07	29	5	1782.60± 137.52	53.50± 0.41	44.38± 0.22	1.154± 0.06	16.25± 0.47	41.61± 8.71	13.34± 0.83	2.368± 0.10	0.275± 0.050	22.86± 1.89	2.27± 0.33	1.28± 0.05