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Breeding biology of snow trout (*Schizothorax plagiostomus*) in Neelum and Jhelum Rivers, Azad Kashmir, Pakistan

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Abstract

Studies on reproduction of fish are important and essential requirements for improvement of fishery resources management and conservation. The fish samples were dissected to ascertain the relationship among fecundity with length and weight of fish and its ovary as well as GSI, ova diameter, for total proteins, glucose, lipids, cholesterol and DNA/RNA ratio during spawning seasons. The fecundity was ranged from 1695 ± 524.44 to 3297 ± 282.99 eggs with total body length 22.52 ± 2.84 to 27.90 ± 1.88 cm and total body weight 172.33 ± 10.9 to 240.9 ± 7.59 g. The total proteins, RNA and total lipids contents were lower in pre-spawning season (37.8 ± 4.1 , 56.4 ± 3.2 and 18.6 ± 4.7 mg/g respectively) and post spawning season 34.7 ± 3.1 , 58.4 ± 4.4 and 14.4 ± 2.2 mg/g) than spawning season (83.9 ± 3.8 , 82.03 ± 3.3 and 36.3 ± 1.72 mg/g). In contrast, the cholesterol and glucose levels were significantly higher in pre-spawning (59.8 ± 2.5 , 58.8 ± 4.51 mg/g) and post-spawning (70.2 ± 1.84 , 61.16 ± 4.73 mg/g) than spawning season (29.8 ± 2.2 , 28.1 ± 1.70 mg/g). The relationship of fecundity with total length, total weight of fish and ovary were found to be linear. It is concluded that by knowing the exact breeding season and relationship of fecundity with various parameters of *Schizothorax plagiostomus*, we could conserve this declining species in natural water bodies by artificial breeding.

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Introduction

Availability of constantly increasing demand of animal proteins is only possible by exploiting all on hand options including natural and artificial breeding and rearing of fish and integrated aquaculture practices as has adopted in many countries who made significant advancement in aquaculture production. The basic knowledge of reproductive life cycle plays an important role for the management and improvement of fisheries resources. The *Schizothorax plagiostomus* is an important species of genus *Schizothorax* of family Schizothoracinae. Geographically, it is distributed in different rivers, lakes, streams and tributaries of all over the Himalayan regions extended to China, Afghanistan, Nepal, Pakistan, Ladakh, Tibet, North East India and Bhutan (Kullander *et al.*, 1999). It is one of the most valuable and suitable fish for the up scaling of the nutritional status.

The population of *Schizothorax* have so far been satisfactory status as per conservation status lists but are declining in the water bodies of Azad Jammu and Kashmir due to habitat destruction, over fishing, competition for food and breeding grounds with exotic carps and lack of captive breeding facilities or has not been bred artificially as yet. Cold water fishes are being influenced by their environment in reaching maturing, ovarian and testicular development, fecundity, spawning and cultural survival. Histological and histo-chemical methods have not been tested on the variety of these fishes to have deeper approaching into the gonadal aspects in relation to environment.

Previous study framed out different parameters such as weight, length, volume, shape of gonads, ova diameter in different reproductive periods (Blaxter and Hempel, 1963; Holliday and Jones (1967), Gonado Somatic Index (Dadzie and Wangila, 1980; Amtyaz *et al.*, 2013), measurements of hemoglobin, serum biochemical values for calcium, glucose, total protein, total urea, total lipids, albumin, cholesterol and creatinine (Sobha *et al.*, 2007) of fish were investigated.

The present study was carried out to investigate the process of gametogenesis, seasonal cyclicity, breeding behavior and other reproductive aspects of *S. plagiostomus*. This study was necessary for the development and practicable management of *Schizothorax* fish. We studied structure and functions of reproductive organs of male and female fish. Thus, it could provide the basic information to the future research scholars and official staff assigned with the duties of protection and conservation of aquatic resources including fisherman about the breeding and non-breeding season of this fish. Hence, the present study would be useful in understanding the relationship of fecundity with various parameters and serve as a tool for better management of indicated resource.

Materials and methods

Ethical Statement

All animal experimental procedures were conducted in accordance with local and international regulations. The international regulation is the Wet op de dierproeven (Article 9) of Dutch Law (International).

Fish Sampling

A total of 330 specimen of *S. plagiostomus* were collected from River Jhelum and Neelum, Muzaffarabad during the 2010-2012 with the help of local anglers (fishermen) using cast net and collected in a hand-held seine and dip net.

The specimens were identified by counting the fin rays and barbels then transferred into large containers and brought alive from the collection site to the Fisheries Laboratory, Department of Zoology, The University of Azad Jammu and Kashmir, Muzaffarabad. After noting down morphometric parameters (body length and body weight) of the fishes in fresh conditions, fishes were dissected.

Observations and Dissection of *S. plagiostomus*

Different measurements i.e., standard length (SL) and body weight were measured upto the nearest using tapeline and 0.01g using electronic balance. Each specimen was dissected and observed the gonads morphology and different stages of maturity.

Biochemistry of S. plagiostomus

After macroscopic examination of reproductive system, gonads were taken out and divided into three parts. First part was used for the estimation of total proteins and glucose through saline extract while the second part was used for the estimation of total lipids, cholesterol and nucleic acid through boiling ethanol solution. The third part was fixed in Bouin's solution for histological studies.

Preparation of Saline Extract

About 25mg of gonads were homogenized with 5ml of 0.9% saline solution using Teflon glass homogenizer to get saline extract. The saline extract was used for the estimation of glucose and total proteins.

Extraction of Lipids, Nucleic Acid and Cholesterol

For the extraction of lipids, cholesterol and nucleic acid, a separate piece (25mg) of gonads was crushed in boiling ethanol with glass rod and centrifuged at 3000 rpm for 20min. The supernatant was collected in separate test tube. The pellet was again mixed with normal ethanol and kept overnight, then centrifuged at 3000rpm. The supernatant was collected. Then the pellet was mixed with the mixture of methanol and ether (3:1 ratio) and kept for 24h, then centrifuged at 3000 rpm for 20min and the supernatant was collected. All the supernatants were mixed and used for the estimation of total lipids and cholesterol contents.

RNA Extraction

The nucleic acids were extracted according to the method by Suzuki *et al.* (2008). The pellet left after the extraction of lipid was dried in vacuum desiccators for 18-24 hrs. For RNA extraction, the pellet was soaked in 10% ice-cold perchloric acid (PCA) for 2h then added with 20% ice cold PCA and kept at 4°C for 18-24h, centrifuged at 3000 rpm for 15 min. Supernatant was isolated and used for the estimation of RNA contents according to Orincol method (Schneider, 1957).

DNA Extraction

Pellet left after the extraction of RNA was soaked and mixed with 10% hot perchloric acid (PCA) and kept at 60°C in the incubator for 2h.

The mixture was centrifuged at 3000 rpm for 15min. The supernatant was separated and used for DNA estimation using diphenylamine method. Both these estimation followed the procedure described by Schneider (1957).

Preparation of Total Proteins Extraction

After the extraction of RNA and DNA contents, the pellet left was digested in 0.5 N NaOH for 24h and used for the estimation of total proteins according to the method described by Suzuki *et al.* (2008).

Estimation of Glucose Contents

Glucose in saline extract and blood plasma was determined Hartel *et al.* (1969) as O-toluidine method. Glucose forms a green compound with ortho-toluidine in acetic acid, which was determined photo metrically at 590nm. Tubes were kept in boiling water bath for 10min then cooled and read at 590nm against blank.

$$\text{Glucose (mg/g)} = \frac{\text{O. D. of test}}{\text{O. D. of Standard}} \times \text{Standard concentration}$$

Estimation of Cholesterol Contents

Cholesterol content was estimated according to the Zak's method by Zak (1957). The adapted procedure was as follow. Test tubes were shaking well and O.D. was determined after 10 min at the wavelength of 610nm against blank.

$$\begin{aligned} \text{Cholesterol (mg /g)} &= \frac{\text{O. D. of test}}{\text{O. D. of Standard}} \\ &\times \text{Standard concentration} \\ &\times \frac{\text{Wt. of tissue}}{\text{Tissue extract}} \end{aligned}$$

Estimation of Protein Contents

Total proteins and soluble proteins were estimated according to the method of Suzuki *et al.* (2008). After mixing tubes were kept for 45min and then read at the wavelength of 750nm against blanks. The O.D. was calculated against standard solution.

$$\text{Protein (mg/g)} = \frac{\text{O.D.of test}}{\text{O.D.of Standard}} \times \text{Standard concentration}$$

*Estimation of Nucleic Acid Contents**Estimation of DNA*

DNA contents were estimated according to the diphenylamine method by Schneider (1957). The procedure was as follow. Heated in boiling water bath for 15min then cooled and read against blank at 600nm.

$$\text{DNA (mg /g)} = \frac{\text{O. D. of test}}{\text{O. D. of Standard}} \times \text{Standard concentration} \times \frac{\text{Wt. of tissue}}{\text{Tissue extract}}$$

Estimation of RNA

RNA was estimated according to Orcinol method (Schneider, 1957). The procedure was as followed.

Mixed and boiled in water bath for 20min. Then cooled and read against blank at 660nm.

$$\text{RNA (mg /g)} = \frac{\text{O. D. of test}}{\text{O. D. of Standard}} \times \frac{\text{Standard conc.}}{\text{Extract used}} \times \frac{\text{Wt. of tissue}}{\text{Total extract}}$$

Estimation of Total Lipids

Total lipids contents were estimated according to Folch *et al* (1957). The procedure was as follow. Mixed well, after 45-50 min, measure the absorbance of sample and read O.D. against blank at 530 nm (Table 1).

$$\text{Total lipids (mg/g)} = \frac{\text{Absorbance of the sample}}{\text{Absorbance of standard}} \times \text{Standard concentration}$$

Table 1. Methodology for estimation of biochemical compounds.

Ingredients	Concentrations (ml)		
	Sample	Blank	Standard
For estimation of glucose contents			
Tissue extract/plasma	0.5
Glucose standard (100 mg in 100 ml water)	0.5
Distilled water	0.5
O-toludine reagent (9 ml O-toludine dissolved in 100 ml of glacial acetic acid)	3	3	3
For estimation of cholesterol contents			
Tissue extract/plasma	0.1
Cholesterol standard (300 mg of cholesterol in 100 ml ethanol)	0.1
Ethanol	0.1
Ferric chloride acetic acid reagent (0.05% solution FeCl ₃ .6H ₂ O in glacial acetic acid)	2	2	2
Tubes were mixed well and kept for 15 min at room temperature and then were added with sulphuric acid (H ₂ SO ₄)	0.5	0.5	0.5
For estimation of protein contents			
Tissue extract/plasma	0.5
Standard solution (100 mg of albumin in 100 ml of distilled H ₂ O)	0.5
Distilled water	0.5
Folin mixture	2	2	2
Mixed and kept at room temperature for 15 min and then added
Folin Cicalteau phenol reagent (1:3) diluted	0.2	0.2	0.2
For estimation of DNA			
Tissue extract	0.4
DNA Solution (100 mg of DNA in 100 ml of 10% PCA)	0.4
PCA 10%	0.4
Diphenylamine (1 g diphenylamine in 100 ml of glacial acetic acid +2.5 ml of H ₂ SO ₄)	3	3	3
For estimation of RNA			
Tissue extract	0.2
RNA solution (100 mg in 100 ml of 10% PCA)	0.2
PCA 10%	0.2
Orcinol reagent (0.5 g Orcinol in 50 ml concentrated HCl + 50 mg of FeCl ₃)	2	2	2
For estimation of total lipids			
Tissue extract/Plasma	0.05
Standard Solution	0.05
Distilled water	0.05
Sulfuric acid, 95-97%	2.0	2.0
Mix well and heat in boiling water for 10 minutes. Cool in cold water for 5 min.
Reaction mixture	0.1	0.1
Sulfuric acid, 95-97%	0.1
Color reagent (Vanillin)	2.0	2.0	2.0

Histology of S. plagiostomus

Tissues were fixed in Bouin's fixative for one week and dehydrated with upgrading of ethanol solution (30-100%). Then these tissues were impregnated in equal amount of Xylene and wax and incubated it for 2h at 65°C and processed for wax embedding by using cavity boxes. 6-8µm thick sections were cut in rotary microtome; sections were stretched in tissue floating bath and then transferred to albumenized slides. The sections were double stained in hematoxylin and eosin and mounted with DPX (Markus *et al.*, 2014).

Estimation of Fecundity

The maturity of fish was measured during the spawning and non-spawning seasons. The weight and length of fresh fish were calculated. The ovaries were taken out and kept on filter paper for 30min to remove the excess water. The volume and weight of ovaries were cautiously measured. The sub samples from three different regions (anterior, middle and posterior) of both ovaries were mixed randomly and subjected to volumetric and gravimetric counts (Muth, 1973).

Volumetric Method

One milliliter of sub samples was taken from homogenized mass of ova and counted. The results were computed to calculate the number of eggs in total volume of ovary. The same counting was repeated three times.

Gravimetric Method

The preserved eggs were kept on filter paper to remove the excess moisture and left for air dry. The fecundity was estimated by weighing the two random samples of 100 eggs and obtained results by putting these values in following formula:

$$\text{Fecundity} = \frac{\text{Total weight of eggs}}{\text{Weight of sub samples}} \times 100$$

The means values of both methods were recorded as the absolute fecundity. Relative fecundity was calculated by the ratio of all ova to total fish weight.

Gonado Somatic Index Measurement

For the estimation of gonadosomatic index, extra wetness/moisture was removed, the total mass of fish was calculated and the gonads were weighted.

The following formula according to de Vlaming (1982) method was used to calculate GSI:

$$GSI = \frac{\text{Weight of Gonads}}{\text{Weight of fish}} \times 100$$

Statistical Analysis

Statistical analyses were performed using Graph Pad Prism for Windows (version 5.03) and also used to plot graphs. To analyze the impact of reproductive seasons on biochemical and physico-chemical parameters, we used one-way analysis of variance and a Dunnett's multiple comparison tests with probability level of 5% as the minimal criterion of significance. Data are presented as mean \pm SEM. Regression coefficient and Correlation were calculated by using Minitab.

Results

Gonado Somatic Index (GSI) was calculated for both male and female fish (Fig. 1A, B). It was observed that in pre-spawning season, the mean value of GSI in both female and male *S. plagiostomus* was $4.758 \pm 0.51g$ and $3.602 \pm 0.70g$ respectively, while at peak (spawning) during May to August both female and male have mean GSI value as $7.25 \pm 0.57g$ and $6.00 \pm 0.43g$ respectively. This raise in GSI weight was the sign of onset of spawning activity. Whereas, the mean value of GSI in both sexes were suddenly decreased from September to December ($3.100 \pm 0.23g$, and $3.109 \pm 0.52g$), were indicating the post-spawning season of *S. plagiostomus*.

Through observation of different ovarian stages, it was found that the percentage of matured specimens was mostly procured from May onward to August (Fig. 1C, D). A gradual increased in the indices of occurrence of mature individuals in these months indicating the proximity of spawning period. Field observation of spawning ground was carried out throughout the study period. The spawn were occasionally observed in the months of January to March and found abundantly from May to August. The small fry were also catches at the spawning ground from May onward (Fig. 1D). The percentage occurrence of mature fishes, both male and female have been summarized in Fig. 1D.

It gains first maturity at the length of 22.52 ± 2.8 to 27.90 ± 1.8 cm and up to weight of 172.3 ± 10.9 to 240.9 ± 7.5 g. The mean values of ova diameter was calculated in three different spawning seasons.

The mean values of ova diameter was significantly less in pre-spawning season (2.605 ± 1.17 nm) and significantly greater in spawning season (6.348 ± 0.27 nm) (Fig. 1E).

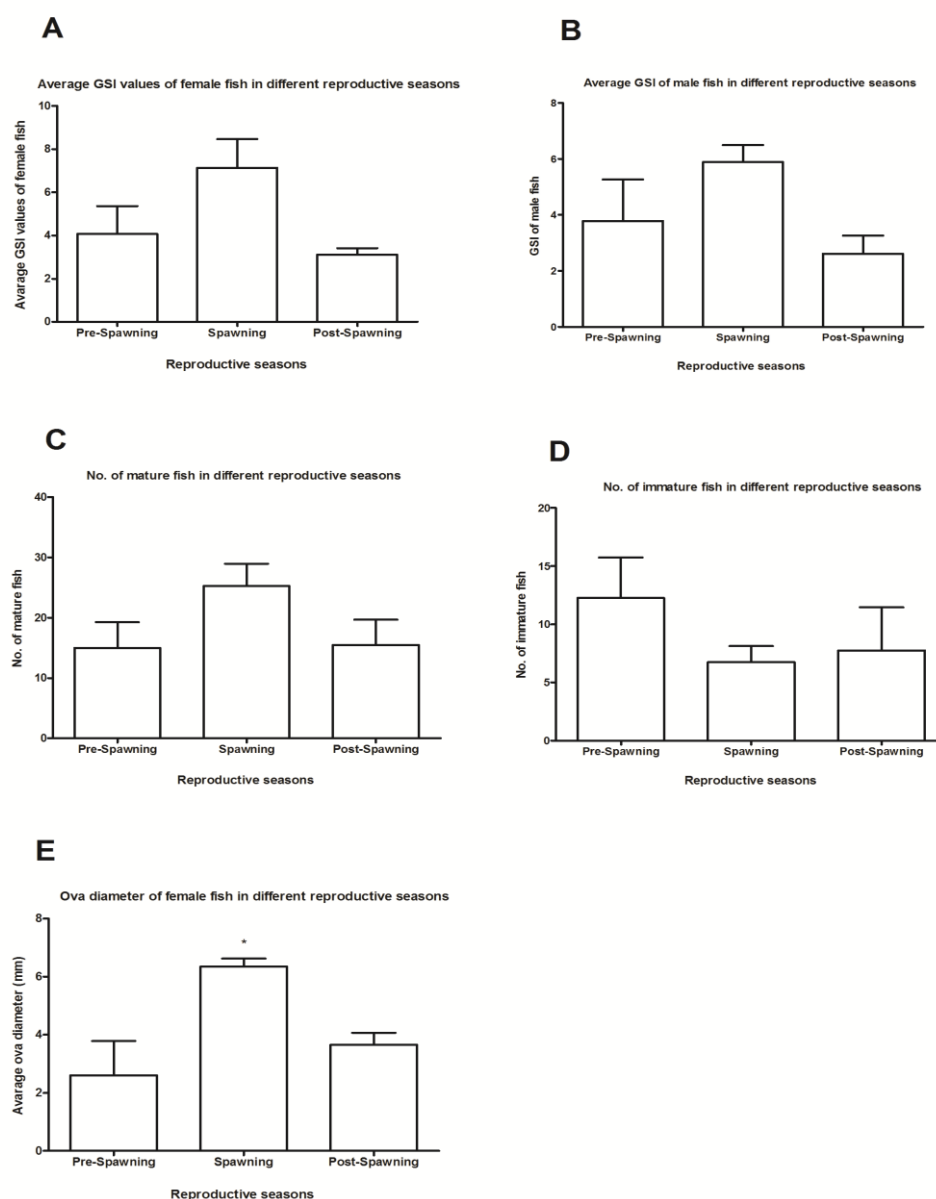


Fig. 1. Reproductive parameters of *Schizothorax plagiostomus* during different reproductive seasons. Key: (*) indicated the significance difference between pre-spawning and spawning seasons, (#) indicated the significance difference between spawning and post-spawning seasons while (\$) denoted the significance difference between pre-spawning and post-spawning seasons.

Fecundity

Out of 330 samples of *S. plagiostomus* collected from the both rivers Jhelum and Neelum, only 100 samples were found as mature viz., ripe or pre-spawning phase the present.

Study determined that fecundity of *S. plagiostomus* ranged from 1695 ± 524.44 and $3,297 \pm 282.99$ on the average length of fish ranging 22.52 ± 2.8 to 27.90 ± 1.8 cm and average fish weight ranging 172.3 ± 10.9 to 240.9 ± 7.5 g (Table 2).

Table 2. Relationship between fecundity and various body parameters of *S. plagiostomus*.

Seasons	Total no. of female fish	Total fish length* (cm)	Fish weight* (g)	Ovary weight* (g)	Ovary length* (cm)	Fecundity
Pre-spawning season	52	22.52±2.84	172.33±10.9	39.75±12.5	9.37±0.94	1695±524.44
Spawning season	86	27.90±1.88	240.9±7.59	89.27±3.37	10.75±0.43	3297±282.99
Post spawning season	55	31.03±1.00	310.08±9.57	43.06±13.9	10.5±0.2	1827±622.04

*The given values were mean+SEM.

Fecundity Relationship

Fish Length-Weight and Fecundity Relationship

It was observed that fecundity in *S. plagiostomus* increased with the increase in fish length and weight. The maximum numbers of ova 3,297±282.99 were recorded in fish with mean length 27.90±1.88cm and weight of 240.9±7.59g. While, the minimum ova 1695± 524.44 were counting at first maturity (22.52±2.84cm and 172.33±10.9g). However, fishes of same length as well as different length showed variations in fecundity (Table 3). The straight line regression equation of this relationship is

$$\text{Log F} = -2558 + 167 \text{ Log L ;}$$

Where, F=the fecundity and L=total length of the fish. The value of correlation coefficient (r) for this relationship was calculated as 0.560 and fecundity plotted against weights gave the linear relationship and observed correlation coefficient (r) for this relationship was observed as 0.744 which corresponds to a positive correlation.

Ovary Weight-Fecundity Relationship

Ovary weight varied considerably even within the fishes of same length which had direct impact on

ovary weight-fecundity relationship. The fecundity increased as ovary weight is increased. The linear relationship between fecundity and ovary weight has established (Table 3).

The regression correlation coefficient (r) for this relationship was 0.987 which corresponds to a very strong positive correlation.

Ovary Length and Weight and Fish Length Relationship

The mean value of ovary length was 9.37±0.94 to 10.75±0.43cm and ovary weight of fish ranged from 39.75±12.5 to 89.27±3.3g with fish total length ranging from 22.52±2.84 to 27.90±1.8cm (Table 3). The values of correlation coefficient (r) for length relationship was 0.572 and for weight relationship was 0.575.

Ovary Weight and Fish Weight

The mean value of ovary weight of fish ranged from 39.75±12.5 to 89.27±3.37g while the average fish weight ranged from 172.33±10.9 to 240.9±7.59g (Table 3).

The relationship between ovary weight and fish weight was determined by value of correlation coefficient (r) was 0.753.

Table 3. The regression and correlation co-efficient between the dependent variable and independent variable of *S. plagiostomus* with equation.

Dependent variables (Y)	Independent variables (X)	Equation Log Y=log a + b log X	Regression coefficient (b)	Correlation (r)
Fecundity (F)	Body length (BL)	Log F = -2558+167log TL	167	0.560
Fecundity (F)	Body weight (BW)	Log F = - 2128 + 14.6Log TW	14.6	0.744
Fecundity (F)	Ovary weight (OW)	Log F = 282 + 34.7logOW	34.7	0.987
Fecundity (F)	Ovary length (OL)	Log F = - 2730 + 490log OL	490	0.572
Ovary weight (OW)	Total fish length (TL)	Log OW = - 83.8 + 4.88Log TL	4.88	0.575
Weight of ovary (OW)	Total fish weight (TW)	Log OW = - 69.4 + 0.420log TW	0.420	0.753

Biochemical Estimation

The level of cholesterol generally fluctuates at different maturity stages of fish. The tissue cholesterol level in both sexes of *S. plagiostomus* was significantly highest (59.8±2.5 and 70.2±1.84mg/g)

during the pre and post-spawning seasons when the GSI values were lower as compared to spawning season (29.8±2.2mg/g) when the GSI values were maximum (Fig. 2A).

The present study revealed that the lipids concentration is also increased in *S. plagiostomus* in breeding season ($36.3 \pm 1.72 \text{ mg/g}$) and went down ($18.6 \pm 4.7 \text{ mg/g}$; $14.4 \pm 2.24 \text{ mg/g}$) during pre and post-breeding seasons respectively (Fig. 2B). The amount of DNA in a cell is constant (Fig. 2C); other than the amount of RNA. In our study, the RNA level was significantly higher ($82.03 \pm 3.3 \text{ mg/g}$) the spawning season which was significantly dropped during pre and post-spawning seasons ($56.4 \pm 3.22 \text{ mg/g}$) and (58.4 ± 4.4) respectively (Fig. 2D).

Similarly, the level of total protein was calculated in *S. plagiostomus* throughout the reproductive cycle. Protein contents were found significantly highest ($83.9 \pm 3.8 \text{ mg/g}$) during spawning season as compared to pre and post-spawning season ($37.8 \pm 4.1 \text{ mg/g}$ and $34.7 \pm 3.1 \text{ mg/g}$) respectively (Fig. 2E). The lowest level of glucose ($58.8 \pm 4.51 \text{ mg/g}$) during the spawning season can be correlated with the increased activity and development. It could also be due to its utilization in enhanced fat synthesis which accompanies maturation of gonads (Fig. 2F).

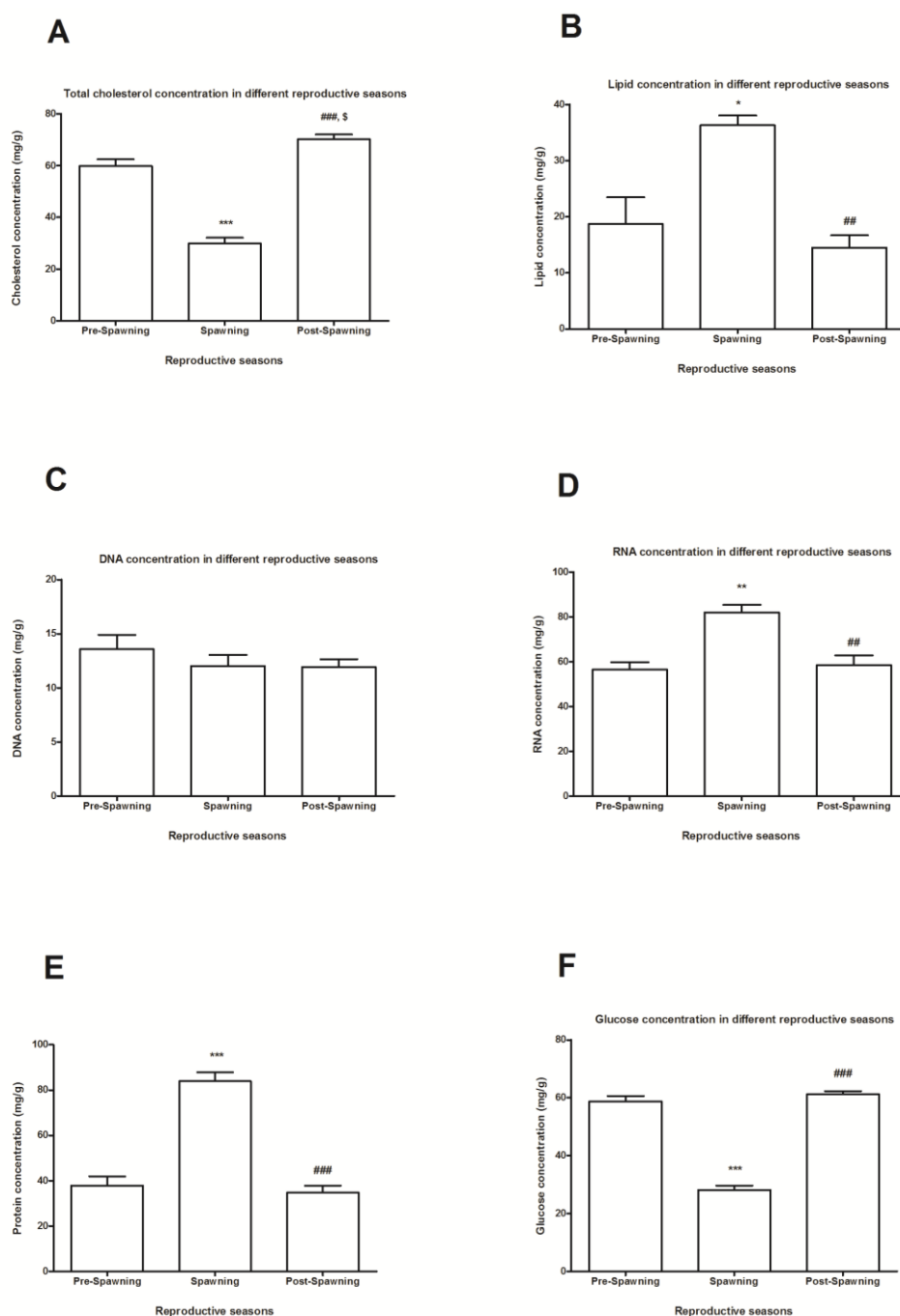


Fig. 2. Biochemical parameters of *Schizothorax plagiostomus* during different reproductive seasons.

Morphology and Histology of Gonad

In Immature phase, the inactive ovaries are long, thread like, translucent and slightly fleshy in texture. The ova are not visible by the naked eye. Yolk formation has not started. In maturing phase, the ovary become yellowish in color, increase in volume and weight and covers nearly the entire length of body cavity. During preparatory phase, the ovaries increase in volume and become light yellow in color. The blood supply is still inconspicuous. In maturing phase, the ovary become yellowish in color, increase in volume and weight and covers nearly the entire length of body cavity. The color of the ovary becomes yellow with the reddish tinge due to the increased supply of blood.

In spawning phase, the ovaries are greatly enlarged attaining their maximum weight and filling up the entire body cavity and they are now full of ripe and heavily loaded yolked eggs, which may extrude out by slight pressure if applied on the abdomen. The vascular supply increases enormously and numerous blood capillaries are seen distributed in the stroma of the ovary. In recoupment phase, the ovarian wall contract further and becomes more compact and translucent. The degenerating postovulatory follicles, under the process of Artesia are still visible. However, the detailed histological analysis of ovary of these stages of *S. plagiostomus* was conducted (Fig. 3A-F) during this study.

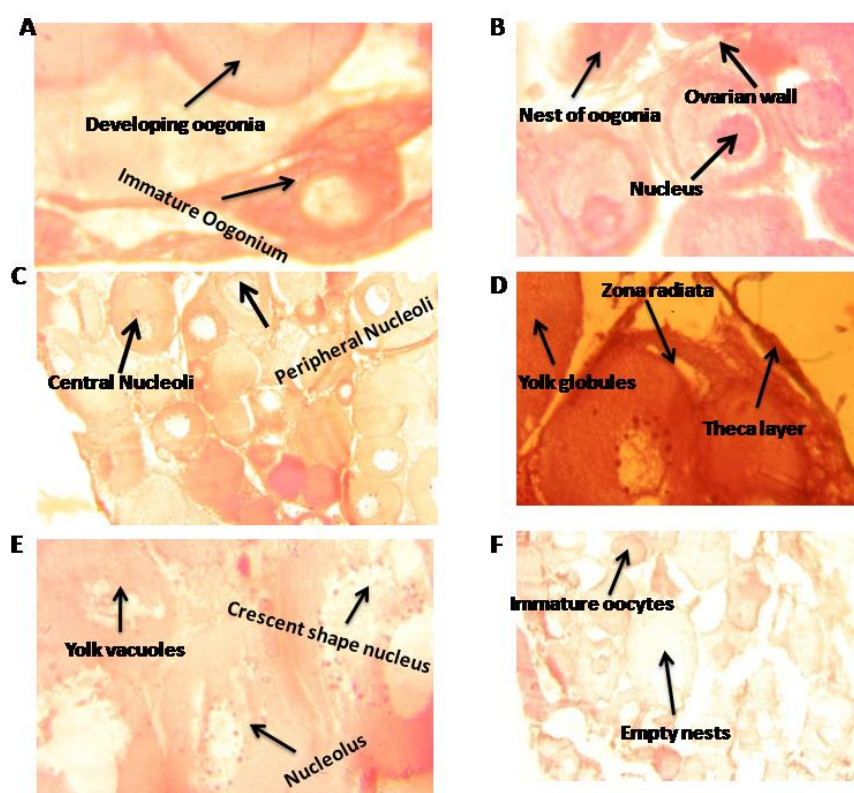


Fig. 3. Cross sections of ovary of fish showing the different stage of maturation.

Discussion

The sustainability of any fish species is ultimately determined by the ability of its abundant brood stock to reproduce successfully in a fluctuating environment (Moyle and Czech, 2000). Therefore, the reproductive strategy based on physiological condition also reflected its anatomical, and behavioral

adaptations are essentially required for the future generations. Fish total length, body weight and gonadal weight indicated that the fish is in good health; which in turn enhance the fecundity in fish. A considerable difference was observed between the GSI values of mature males and female fish. The maturational cycle and monthly variation in gonado

somatic index provided good indication of development of gonads with respect to time of year. Gonad staging on a descriptive scale allows a rapid qualitative assessment of the breeding state and gonads weight gives a quantitative record of changes in the gonads condition (Crossland, 1977). Histological study of gonads, calculation of gonado somatic indices, percentage occurrence of mature fish during the year, the occurrence of large number of spawn and fry in the spawning ground at the particular time of the year were taken into consideration to determine the spawning season of *S. plagiostomus*.

A sudden decrease in the value of gonad somatic index (GSI) was found after August indicated that *S. plagiostomus* spawn during this season, although the gonads were fully mature in the winter season but in a dormant phase because of severe winter and spawned only on return of favorable environmental factors in spring season (Jyoti *et al.*, 1972; Sunder, 1984). Nonetheless, the present study conducted in Rivers Neelum and Jhelum in their natural habitat, we found that *S. plagiostomus* spawn once in a year from May-August, it was also confirmed by Jan *et al.* (2014) for the *S. plagiostomus* collected from the river Jhelum, Kashmir. The size at first maturity depends on the nature of the environment in which the population of fish lives (Moyle and Czech, 2000) and maturity size helps to predict harvestable size of the fish. The length at maturity was suggested as the minimum size that should be allowed to be caught in commercial fishing, as it allows the fish to gain considerable biomass (Snedecor and Cochran, 1980).

Fecundity is the numbers of ripening eggs found in the female just prior to spawning and results of fecundity have been expressed in term of the number of eggs/unit of body weight (Begenal, 1971). In teleosts, fecundity estimation fluctuates from few hundred to several lacs. Fecundity of *S. richardsonii* varies from 2,598 to 27,846 eggs (with average fecundity 12,744 eggs) at maturation (Qadri and Yousuf, 1983). This low fecundity potential is also confirmed by Baloni and Tilak (1985) as fishes inhabiting the cold water streams and lakes have

comparatively low fecundity. In the present study, the number of eggs was found to increase linearly with the increase in total length, body weight, ovary length and ovary weight. These findings are supported by the findings of previous workers, Hussain *et al.* (2003), Mohan (2005), Bahuguna and Khatri (2009). All the relationships between fecundity and total length, body weight, ovary length and ovary weight found to be positively correlated. Significant relationships between fecundity and these variables were also reported by Hussain *et al.* (2003), Bahuguna and Khatri (2009). The value of correlation coefficient 'r' in the present study indicate that among the above parameters studied, closest correlation of fecundity was observed with the ovary weight ($r=0.987$) followed by total fish weight ($r=0.753$), body weight ($r=0.744$) total fish length ($r=0.575$), ovary length ($r=0.572$) and body length ($r=0.560$). Similar results were reported by Bahuguna and Khatri (2009)

The mean values of ova diameter was calculated in three different spawning seasons. The mean values of ova diameter was significantly less in pre-spawning season and significantly greater in spawning season. Ova diameter and distribution frequency indicating that the fish at different time intervals during a year was determinant of the preparation of itself for spawning (Macer, 1974) and intra-ovarian diameter for more than a year gave a spawning periodicity (Biswas, 1993).

The information of the biochemical characteristics is a significant tool that can be applied as a sensitive and effective index to monitor pathological, physiological and seasonal changes in fishes (Xiaoyun *et al.*, 2009). These parameters also confirm its spawning season through increasing and decreasing their level. The level of cholesterol generally fluctuates at different maturity stages of fish. The tissue cholesterol level in both sexes of *S. plagiostomus* was significantly highest during the pre and post-spawning seasons as compared to spawning season. The cyclic alteration in the gonadal cholesterol levels is caused by gonadal activity during pre-spawning and spawning season. Our results are also in conformity with the findings of Nayyar and Sundararaj (1970) that during spawning

period, the tissue cholesterol level was decreased significantly in fish, while cholesterol level increased both in pre and post-spawning season.

The present study revealed that the lipids concentration is also increased in *S. plagiostomus* in breeding season and went down during pre and post-breeding seasons. Growth rate during specific periods of the year manipulates the rate of early fish maturation (Anonymous, 2003). The amount of DNA in a cell is constant other than the amount of RNA. In our study, the RNA level was significantly higher the spawning season which was significantly dropped during pre and post-spawning seasons. Similar results have been reported (Tripathi *et al.*, 2002). The level of total protein was calculated in *S. plagiostomus* throughout the reproductive cycle. The low level of protein in pre-spawning phase would be due to active utilization of this protein by ovaries during the process of vitellogenesis. These findings correlate positively to the results of previous reports (Love, 1970; John, 1977). During spawning phase, the protein level was increased that was also attributed to favorable condition.

The glucose level of fish species differs seasonally and this is associated to gonadal cycle, metabolic activities and feeding intensity (Tandon and Joshi, 1974). The present study found that *S. plagiostomus* has highest blood glucose level during the winter (post-spawning season). This is suggested that this could be due to the continuous feeding activity, showing that the low temperature does not affect the feeding rhythm of fish. The lowest level of glucose during the spawning season can be correlated with the increased activity and development. It could also be due to its utilization in enhanced fat synthesis which accompanies maturation of gonads. Various studies on fish reproduction have been limited to macroscopic descriptions of maturation cycle and for the estimations of parameters used in fish biology, such as length at first maturity and fecundity (Duhamel, 1994). However, the detailed histological analysis of the gonads of female *S. plagiostomus* was conducted (Fig. 3A-F) during this study.

The study concluded that *S. plagiostomus* breed once in a year from May to August in wild/natural habitat. It gains first maturity at the length of 22.52 ± 2.8 to 27.90 ± 1.8 cm and up to weight of 172.3 ± 10.9 to 240.9 ± 7.5 g. The fecundity of fish point toward breeding potential of *S. plagiostomus* on which the future population depends. This study would be a base line towards future ecological study, breeding biology, conservation and management of *S. plagiostomus*; a declining species in natural habitat.

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