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Study on gonadal maturation of *Schizothorax labiatus* McClelland inhabiting river Jhelum, Kashmir

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ABSTRACT

The present study was conducted on *Schizothorax labiatus* McClelland fish collected randomly from river Jhelum for a period of 12 months for determination of different phases of gonadal maturation and spawning. The mean monthly GSI in the male fish fluctuated from 1.38±0.11 (August) to 6.19±0.84 (April) while as in females GSI fluctuated from 1.80±0.35 (August) to 11.12±2.09 (April). From September to April mean GSI values showed an increasing trend reaching peak in April then decreasing gradually during the following months, therefore the fish was found an annual breeder and matures in the month of April and spawns in May. Annual gonadal cycle in both the sexes consisted of six stages *viz.,* (i) Immature or resting phase (ii) Preparatory or Developing phase (iii) Maturing phase (iv) Mature phase (v) Spawning phase (vi) Spent phase. Histological studies showed follicular atresia both during pre-spawning and post-spawning period. The study provides an understanding about maturity, gonadal development, environmental influence on gonad activity and thus forms a tool for the development and proper management of *S. labiatus* McClelland fishery in Kashmir.

Keywords: Annual, Jhelum, Gonadal cycle, *Schizothorax labiatus.*

Introduction

High altitude water bodies of Kashmir valley harbors the indigenous Cyprinids like Schizothoracids (snow trouts), among other snow trouts *Schizothorax labiatus* McClelland locally known as 'Chush' is an important food fish inhabiting Jhelum River System. Most fish species inhabiting the Himalayan region are small in size and their size, growth and distribution depends on environmental conditions such as water temperature, velocity of water current, nature of substratum, availability of food and their feeding habits (Yousuf *et al.,* 2003; Bhat *et al*., 2010). The ability to reproduce successfully in a fluctuating environment, determines the success of the fish, as reproduction is a vital component in sustenance, replenishment and progeny maintenance of each and every living organism (Moyle & Czech Jr., 2000). For proper formulation of capture fisheries management policies, reproductive characteristics such as gonadal development, duration of spawning season and associated endocrinal changes which are unique to each fish species are used (Macer, 1974; Johannes, 1978 and Kathirvelu *et al*., 2003). Studies related to reproduction of many species indicates that the reproductive cycle of fishes are closely associated to the environmental changes particularly temperature, day length and food supply influencing gonadal development initiation and fecundity. Reproductive parameters like size at first maturity, fecundity, sex ratio etc. are important in formulation of management measures (Bal and Rao, 1984). Thus this study on GSI, histology of gonads in *S. labiatus* was conducted for understanding reproductive biology required for proper fishery management, since information on these aspects of gonadal maturation and spawning are scarce in this coldwater fish i.e. *S. labiatus*.

Material and Methods

A total of 310 specimens of *Schizothorax*, McClelland of all available sizes, were collected randomly from River Jhelum over a period of twelve (12) months and timely brought to Fishery Resource Management (FRM) Laboratory of Faculty of Fisheries, Rangil, Ganderbal, and fishes were categorized into different size groups.

The fish specimens (both male and female) were dissected open and their gonads were collected to record their length and weight. Various maturity stages of gonads

of both the sexes were determined based on microscopic observation in preserved and macroscopic observations (morphological appearance) in fresh samples following the standards laid down by Lovern and Wood (1937).

The Gonadosomatic index (GSI) was calculated for both the sexes using the formula given by Qasim (1973).

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

After GSI recordings, gonads were immediately fixed in Bouin's fluid for a period of 24 hours and later washed in 70 per cent alcohol in various grades till the yellow colour of picric acid fades away. Further, the gonads were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax. Sections were cut and stretched on albumenized slides and fixed overnight and later deparaffinised in xylene and rehydrated in descending grades of alcohol to distilled water. Sections were stained in haematoxylin, differentiated in alcohol. After washing, sections were stained with eosin and then dehydrated and cleaned. They were then mounted in DPX (Luna, 1968) and stages and changes in gonadal development were recorded through microphotography.

Results and Discussion

Gonadosomatic Index (GSI)

Besides being useful for determination of fish reproductive period, GSI is good indicator of gonadal development (Rheman *et al.*, 2002). The mean monthly mean GSI during the present study fluctuated between 1.38±0.11 (August) and 6.19±0.84 (April) in males while as in females the values fluctuated between 1.80±0.35 (August) and 11.12±2.09 (April). The minimum and maximum values observed in case of males and females are recorded in Table 1. The mean GSI values were recorded higher in females compared to that of males (Fig. 1). During the period between September to April mean GSI values showed an increasing trend reaching peak in April then decreasing gradually during the following months and reaching a minimum value in August (Fig. 2).

Table 1: Mean (±) GSI values of *Schizothorax labiatus* McClelland

	Minimum	Maximum	Mean± SEM
Male	0.87	14.72	3.80±0.39
Female	0.83	23.12	5.18±0.14

In *S. labiatus* McClelland like most of the other teleosts the reproductive glands (testes in males and ovaries in females) are typically paired structures and their reproductive cycles exhibit various phases varying with seasons.

Ovaries

The ovaries in *S. labiatus* McClelland are similar to those of other teleosts and go through a series of stages which are identified visually by size, colour, transparent or opaque, thus the ovarian cyclicity (Table 2) in *S. labiatus* McClelland during the present study has been observed as:

1) Stage I: Immature or Resting phase (Plate 1 A)
2) Stage II: Developing or Preparatory phase (Plate 1 B & C)
3) Stage III: Maturing phase (Plate 1 D)
4) Stage IV: Mature or Pre spawning phase (Plate 2 A)
5) Stage V: Spawning phase (Plate 2 B & C)
6) Stage VI: Spent phase (Plate 2 D)

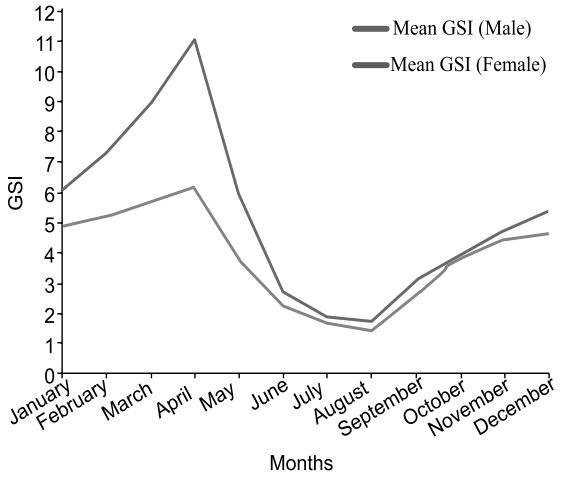

Fig. 1: Comparative analysis of monthly variation in mean GSI of male and female *S.labiatus*

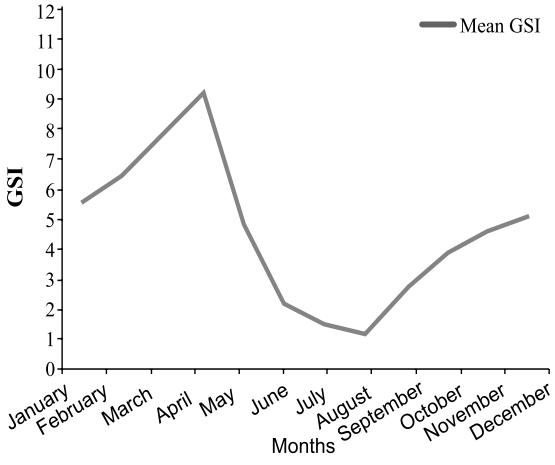

Fig. 2: Monthly variation in GSI values of *S. labiatus*

Table 2: Annual stages of maturation in ovaries of *Schizothorax labiatus*

STAGE		DURATION	GSI value	DESCRIPTION
I	Immature or Resting phase.	Sep - Oct	3.08 & 4.25	Thin, thread like and pale in color ,little vascularization ,less than 1/3 of the body cavity ova in this phase are transparent ,invisible to the naked eye.
II	Developing or Preparatory phase.	Nov - Dec	4.77 & 5.36	Marked increase in size and weight, occupying greater part of the body cavity ,opaque and light yellow in color beginning of maturation, characterized by the presence of yolk vesicle in peripheral ooplasm .
III	Maturing phase.	Jan - Feb	6.10 & 7.32	Increase in size turning yellow in color invaded with blood vessels occupying ¾th of the body cavity. Ova visible to the naked eye.
IV	Mature or Pre spawning phase	Mar - Apr	9.05 & 11.12	Increase in weight and volume, deep yellow in color, attaining maximum weight and were highly vascularized.
V	Spawning phase	May - June	5.90 & 2.61	Large, turgid, deep yellow to orange in color filling the body cavity. The oocytes are large, loose and almost separate from each other in the ovary, a slight pressure on the abdomen evacuates eggs from the belly.
VI	Spent phase.	Jul - Aug	2.01 & 1.80	Reduction in size appearing flaccid and dull in color. Unspawned ova & number of small ova were seen.

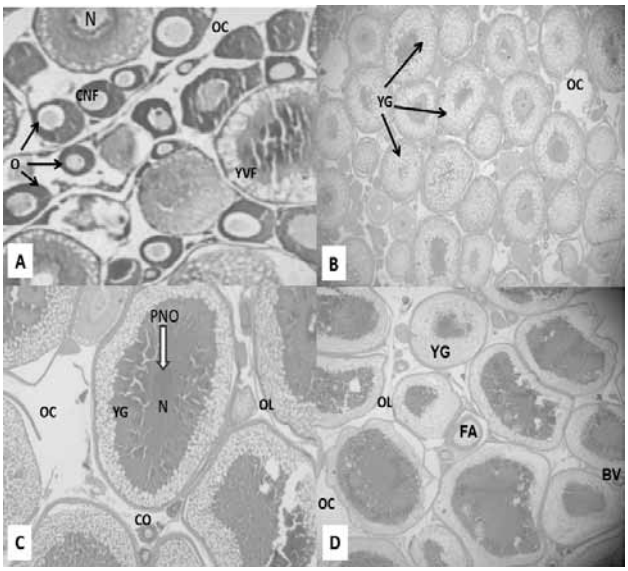

Plate 1. Photomicrographs (50X) of T.S of Ovary of *S. labiatus* showing different stages, A (Stage I), B (Stage II), C (Stage II at 100X) and D (Stage III)

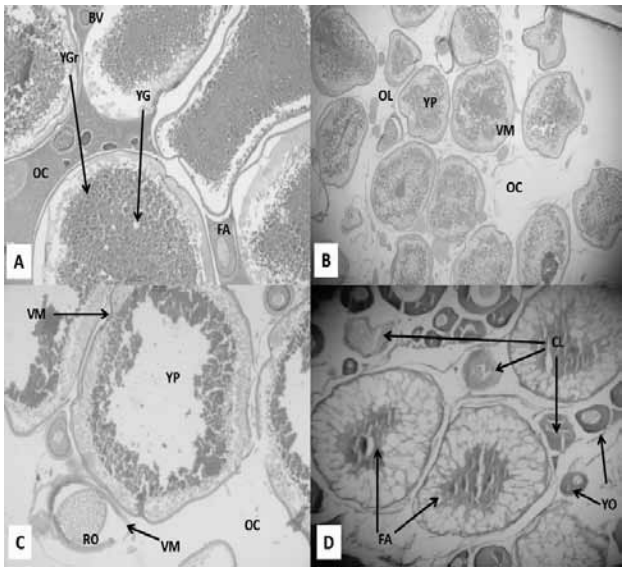

Plate 2. Photomicrographs (100X) of T.S of Ovary of *S. labiatus* showing stages, different A (Stage IV), B (Stage V; 50 X), C (Stage V) and D (Stage VI)

O = Oocyte, YG = Yolk Globules, BV = Blood vessel, FA = Follicle Atresia, CNF = Chromatin nuclear follicle, YVF = Yolk Vesicle Follicle, N = Nucleus, PNO = Perinuclear oocyte, OC = Ovarian cavity, CO = Chromatin nuclear oocyte, OL = Ovarian lamellae, YGr = Yolk Granules, CL = Corpus luteum, VM = Vitelline membrane, OL = Ovarian lamellae, YP = Yolk Plate, YO = Young oocyte, RO = Releasing oocyte.

Testes

S. labiatus McClelland exhibited the following cyclic changes in testes and various phases in annual cycle are described in Table 3.

1) Stage I: Immature or Resting phase (Plate 3 A)
2) Stage II: Developing or Preparatory phase (Plate 3 B)
3) Stage III: Maturing phase (Plate 3 C)
4) Stage IV: Mature or Pre spawning phase (Plate 3 D)
5) Stage V: Spawning phase (Plate 4 A)
6) Stage VI: Spent phase (Plate 4 B)

Table 3: Annual stages of maturation in testes of *Schizothorax labiatus*

	STAGE	DURATION	GSI value	DESCRIPTION
I	Immature or Resting phase	Sep - Oct	2.71 & 3.94	thin, thread like translucent pale in colour
II	Developing or Preparatory phase	Nov - Dec	4.41 & 4.57	The testes appear thin ribbon like transparent and occupy very small portion of the body cavity.
III	Maturing phase	Jan - Feb	4.86 & 5.16	Moderately thick and convoluted , grey-whitish extending up to ½ of the body cavity and visible primary and secondary spermatocytes.
IV	Mature or Pre spawning phase	Mar - Apr	5.65 & 6.19	increase in weight and volume ,deep yellow in color , attaining maximum weight and were highly vascularized.
V	Spawning phase	May - June	3.69 & 2.63	Moderately thick and convoluted, grey-whitish extending up to ½ of the body cavity and visible primary and secondary spermatocytes.
VI	Spent phase	Jul - Aug	1.68 & 1.38	The weight and volume of testes is reduced due to the discharge of sperms making testes look slightly flaccid and flabby.

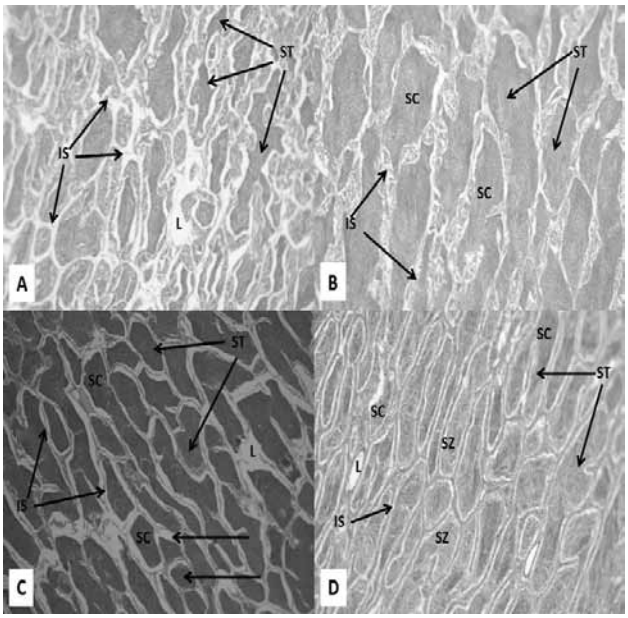

Plate 3: Photomicrographs (50X) of T.S of testes of *S. labiatus* showing different stages, A (Stage I), B (Stage II), C (Stage III) and D (Stage IV)

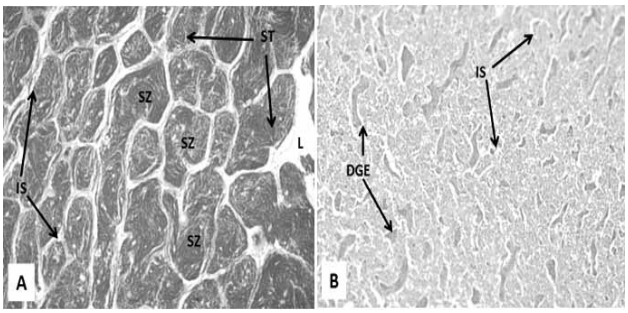

Plate 4: Photomicrographs (50X) of T.S of testes of *S. labiatus* showing, different stages A (Stage V), B (Stage VI)

IS = Interstitial space, **ST** = Seminiferous tubule, **L** = Lumen, **SC** = Spermatocytes, **SZ** = Spermatozoa **DGE** = Discontinuous germinal epithelium

In the present study highest GSI as observed during April indicates that the fish matures in this month and spawning starts in the month of May when the GSI abruptly decreases, however, higher value of GSI were reported from females as compared to males. The gonadosomatic index or maturity index is an indirect method for estimating spawning season of a species (Biswas, 1993). Shafi (2012) observed the Gonadosomatic index (GSI) of *C. carassius* and reported higher values in case of females than in males. Hussain (2014) while studying breeding biology and fecundity of *S. niger* from Dal lake revealed the GSI highest during March i.e peak maturity period of the fish, then it decreased gradually upto June attaining its lowest value in July. Snowtrout, *S. niger* exhibits spawning from mid April to May end (Malhotra, 1966). Shafi (2013 a) while studying breeding biology of *Schizothorax niger* from Dal lake revealed that the GSI recorded was highest during February. The gonadosomatic index increase as maturation progresses and reaches a peak at maturity and decrease abruptly when the fish becomes spent. Generally females exhibit comparatively higher GSI values than males (Khan, 1945; Ganpatti and Chako, 1954; Pathak and Jhingran, 1977; Piska and Devi, 1993). Similar observations were recorded during the current study in *S. labiatus* as well which showed the maximum GSI in the month of April (6.19 in males and 11.12 in females) and minimum GSI in the month of August (1.3 for males and 1.8 for females).

The reproductive glands (testes of males and ovaries of females) of *S. labiatus* McClelland are typically paired structures. Many studies have been performed on histological and morphological changes of ovary in fishes (Biswas, 1993). Cyclic changes in development are seen in mature gonads (Fouche *et al.,* 1985). *S. labiatus* McClelland

clearly exhibited cyclic changes in the gonads and various phases in annual cycle. The testes of *S. labiatus* McClelland were thin, thread/ribbon like translucent in immature and preparatory, opaque in maturing phase, grey whitish in mature phase and creamish in spawning phase when they reach to their maximum development and look slightly flaccid and flabby because of the discharge of sperms during spent phase. In different species, the spermatogenic activity starts at different times of the year (Rai, 1965; Nair, 1966; Shrestha and Khanna, 1976; Nautiyal, 1983). Such variations are probably due to the local physico - chemical and environmental factors (Ahsan, 1966). The ovaries in *S. labiatus* McClelland were thin, thread like pale to light yellow in colour in immature and preparatory phase, yellow in maturing phase, deep yellow in mature phase and deep yellow to orange in spawning phase, flaccid and dull in spent phase. In *S. labiatus* McClelland, atretic oocytes were seen both during the pre spawning and during the post-spawning period. Small atretic previttogenic oocytes are relatively of rare occurrence. Atresia is accompanied by the shrinkage of follicles, resulting in disorganization of nuclear and ooplasmic component. The occurrence of large cortical alveoli is the most characteristic feature of the teleost egg (Donato *et al.*, 1980; Takahashi, 1981), dog fishes and other cartilaginous fishes (Guraya, 1982). Various investigators have termed the cortical alveoli as cortical vacuoles, intra vacuolar yolk, intravesicular yolk, carbohydrate yolk, yolk vesicle etc. In teleosts atretic oocytes may be present throughout the year (Braeckevelt and McMillan, 1967) or only during the post-spawning period (Rajalakshmi, 1966). Several factors have been found responsible for follicular atresia, mostly hormonal of intra-ovarian and extra-ovarian sources (Saidapur, 1978) and unfavourable environmental conditions i.e. photoperiod (Saxena and Anand, 1977), overcrowding, temperature and inadequate feed supply (Lam, 1983). In addition, increasing water pollution by insecticides, pesticides and industrial effluents have also been reported to affect the metabolism of fish ovary resulting in the increased incidence of follicular atresia (Saxena and Bhatia, 1983; Mani and Saxena, 1985). All such factors seem to be responsible for higher follicular atresia in *S. labiatus* McClelland which leads to less spawning/or release of mature ovum from the ovary, which ultimately means less recruitment of fishes. If the aquatic environments of Kashmir valley are not managed properly, it is certain that with the every passing year the new spawn of the fish is going to decrease and the production of fish from natural waters will also decrease.

The fish spawned during the short period of time with peaks during April and May which reveales that the fish is an annual breeder. The gonads of both the sexes of *S. labiatus* McClelland were found to pass through the six phases in the annual cycle. Atretic oocytes were seen during the pre-spawning and post-spawning period of the fish in good numbers. *S. labiatus* McClelland still exists in good conditions and good numbers in river Jehlum. The less number of females as compared to males and the more number of follicular atresia suggest that all is not well with *S. labiatus* in Jehlum River System but if proper fishery management steps are taken at an earliest, the *S. labiatus* fishery can be enhanced for fish production.

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