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Gonadal Maturity of Rainbow Trout (*Oncorhynchus mykiss*) Naturally Inhabited in Balkhila Stream of Uttarakhand, India

Datta Vishal[1*], Bisht H.C.S.[1], Pandey N. N.[2] and Vishvakarma Bipin[1]

[1]Zoology Department, D.S.B Campus, Kumaun University, Nainital 263002, Uttarakhand, India
[2]ICAR-Directorate of Coldwater Fisheries Research, Bhimtal-263136, Nainital, Uttarakhand, India

**Corresponding Author*

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Abstract: In this study an attempt has been made for evaluating the gonadal maturity in the natural stock of rainbow trout inhabited in Balkhila stream of Alakhnanda river at Chamoli district of Uttarakhand, India. In collected stock, about 80% specimen showed full gonadal maturity, which reflects the conducive conditions for the gonadal maturity. The Gonadosomatic index (GSI) values of female rainbow trout ranged from 0.42 to 22.84 with a peak in January and reached its lowest level in March. In case of males, observed minimum value of GSI was calculated as 0.27 during the month of May, whereas the maximum value was 3.44 in the month of December and persisted in month of January and February also with the almost similar values. GSI values reflected the maturity of females during the month of January and February, while males became mature one month advance and remained mature during the December to early March. Hence, non- synchronization situation was observed in the gonadal maturity of both the sexes, which adversely affected the duration of breeding season and is a subject to a further study. In this study, the ovarian and testicular development of rainbow trout showed distinct stages. The oogonia and spermatogonia pass through a number of maturation stages finally leading to the formation of ripe ova and sperms.

Keywords: Rainbow trout, Gonadal maturity, Balkhila stream, Gonadosomatic index, Oogonia

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Introduction

The Balkhila river is an important tributary of Alaknanda river which drains from famous Mandal valley in Garhwal region of Uttarakhand, India. The river rises from Lal Mati glacier and flows in a general direction towards south east to merge with Alaknanda river near Chamoli town. This is a perennial stream having sufficient water in all seasons and continued flow to promote its fishery (Joshi *et al.*, 2016).

The rainbow trout is a native of North America and now, it has become extensively distributed in rivers and streams throughout the world. In India, rainbow trout (*Oncorhynchus mykiss*) constitute the trout fishery in the streams, lakes and reservoirs of Indian Himalayan region (Vass, 2000). Trout is rich in omega-3 poly unsaturated

fatty acid that is needed for the development of brain and retina in infants (Ackman, 1989). In Indian water bodies, the natural seed propagation and production of trout is significantly low. Therefore, ranching of farm raised seed for stocking in streams, lakes etc. is the priority for the development of trout fishery (Vass, 2000). Reliable indicators of reproductive status are essential for good fisheries management and they are also important to evaluate the effects of different biotic and abiotic factors on gonadal maturation of wild fish stock. Gonadosomatic index (GSI) is used to access the reproductive condition of a fish. Changes in the gonadosomatic index (GSI) helps to determine the reproductive season of the fish (Arruda *et al*., 1993). GSI value has been considered by many workers in different fishes for determining spawning season and spawning frequency (Islam *et al*., 2008; Ghaffari *et al*., 2011; Sadekarpawar and Parikh, 2013; Jan *et al*., 2014).

In teleost fish, stress creates inhibitory effect on endocrine function to reduction of gamete and larval quality. Several researchers have investigated fish maturation by histological observations in the natural environment to the evaluation of the efficiency of reproduction.

In this study an attempt has been made for evaluating the gonadal maturity in the natural stock of rainbow trout inhabited in Balkhila stream of Alakhnanda river at Chamoli district of Uttarakhand, India.

Materials and Methods

The present study on breeding and spawning of Rainbow-trout was conducted at Trout hatchery of Bairangna, Gopeshwer District Chamoli, Uttarakhand state ($30^0$24'45.20988" North, $79^0$19' 10.46064" East). For study of Gonadosomatic index (GSI), natural spawners were collected from Balkhela river by cast net fishing and from state hatchery Bairangna. Gonadosomatic index (GSI) calculated by Gonad weight multiply by hundred and then obtain value divided by body weight. The experimental fish rainbow trout for gonad histology studies were sacrificed and fixed in Bouin's fluid for at least 18-24 h. The fixed tissues were dehydrated in an ethanol gradient, cleared in xylene, infiltrated and embedded in paraffin, sectioned at 6 µm, floated on a heated water bath and mounted on glass slides. After drying overnight, paraffin was removed with a clearing agent; tissue was rehydrated in an ethanol gradient and stained with hematoxylin and eosin (HE) for light microscopic examination. Mature brooders were selected on the basis of sexual dimorphism. During breeding season, female has round body appearance, bloat and soft belly, and swollen and reddened vent, while male has dark and dull in appearance, large pointed snout with hooked lower jaw and oozing of milt.

GSI shows the seasonal changes in the maturity. This factor is affected by environmental conditions and quality of the feed eaten. GSI were calculated by the standard formula:

$$\text{GSI (\%)} = \frac{\text{Weight of gonad}}{\text{Weight of the fish}} \times 100$$

Results and Discussion

Gonadosomatic Index (GSI) has been considered as reliable estimate for gonadal maturity and spawning of any species. The GSI increased with the maturation of fish and reaches to its maximum at the peak period of maturity. Saksena (1987) had suggested the use of GSI and volume of the gonad as indicators of gonadal state while studying Indian fresh water goby, *Glossogobius giuris*. GSI increased with the maturation of fish and reaches to its maximum at the peak period of maturity. The fecundity and GSI have also been studied in *Mystus gulio* (Sarker *et al*., 2002), in *Labeo dyocheilus* (Singh *et al.,* 2008; Pandey *et al.,* 2011,) in *Anabas testudineus* (Marimuthu *et al.,* 2009), in *Labeo rohita* (Alam and Pathak, 2010), in *Labeo calbasu* (Mishra and Saksena, 2012) and in *Neolissocheilus hexagonolepis* (Mahapatra and Vinod, 2011).

In the present study, changes in values of GSI were observed individually for female and male

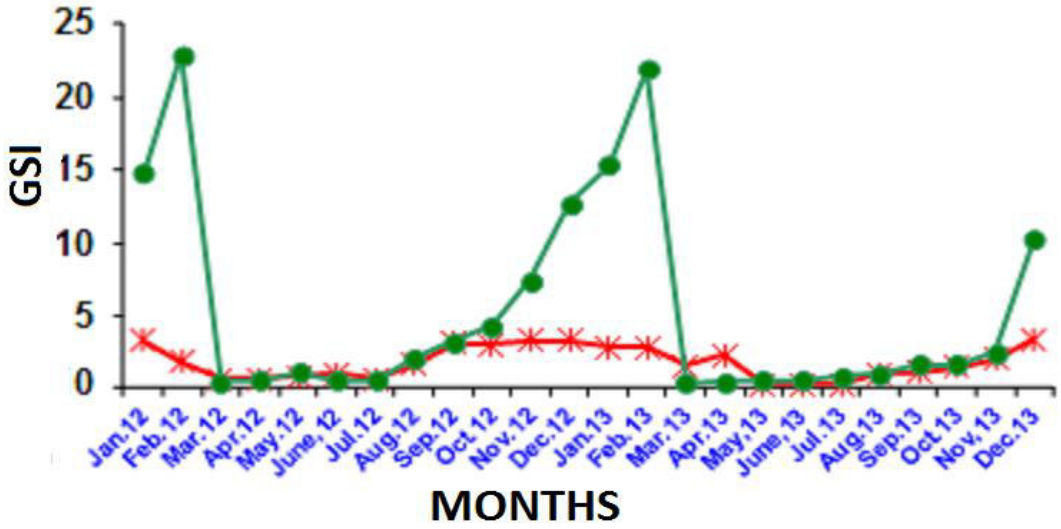

Fig. 1: Gonadosomatic index (GSI) of rainbow trout in Balkhila stream (green line –female; red line –male).

and depicted in Figure 1. The GSI values of female rainbow trout ranged from 0.42 to 22.84. GSI value attained a peak in January and reached its lowest level in March. Minimum value of GSI for male was calculated as 0.27 during the month of May, whereas the maximum value was 3.44 during the period of December to February. Thus, above results showed that males remained mature during the period of December to February, but females showed full maturity one month later. Hence, this non-synchronization in the gonadal maturity affects the duration of breeding season, which has been shrinking for one month in the present study.

Histological examination of gonads can provide a more accurate estimate of fish spawning periods than GSI (Brown-Peterson *et al.,* 2002). In the majority of teleost fishes, the process of oogenesis may be divided into five, six or eight stages (Fishelson *et al.,* 1996; Nagahama, 1983; Unal *et al.,* 1999; West, 1990; Gokce *et al.,* 2003). In the present study, histological observation of the ovaries and testes of rainbow trout of Balkhila stream revealed five distinct stages of maturation (Figs. 2, 3). Oogonia and spermatogonia passed through these different maturation stages finally leading to the formation of ripe ova and sperms.

In histological sections of ovaries, small, round and transparent oocytes with a central nucleus were observed. All the oocytes were in the pre-vitellogenic stage (Fig. 2). Tunica was found thick. Only primary growth oocytes were present. Usually no atresia was found. This stage was found from May to July, but in the later phase only the size of oocyte was found slightly increased. During the month of September, primary oocytes were dominating, but some early stages of secondary oocytes were also seen. The size of the oocyte was increased because of yolk vesicle deposition. Yolk granules and a very thin follicular layer appeared gradually. Large peripheral nucleoli adjoining the nuclear envelope were distinguished. During November to December, ovaries contained all types of oocytes dominated with tertiary stage oocytes. A few primary oocytes were present, some were in the secondary stage and largest group was of tertiary stage oocytes or yolk vesicle stage oocytes. These oocytes were characterized by a well developed zona radiata, true yolk granules and some lipid droplets. The size and number of yolk granules were also increased.

Histological sections showed that only fully grown yolk vesicle stage oocytes were present during January to mid March. Some oocytes showed germinal vesicle migration. Oocytes were characterized by large mass of yolk and numerous large lipid droplets. The zona radiata was completely thick and had separated from the follicular layers. During April, ovaries become

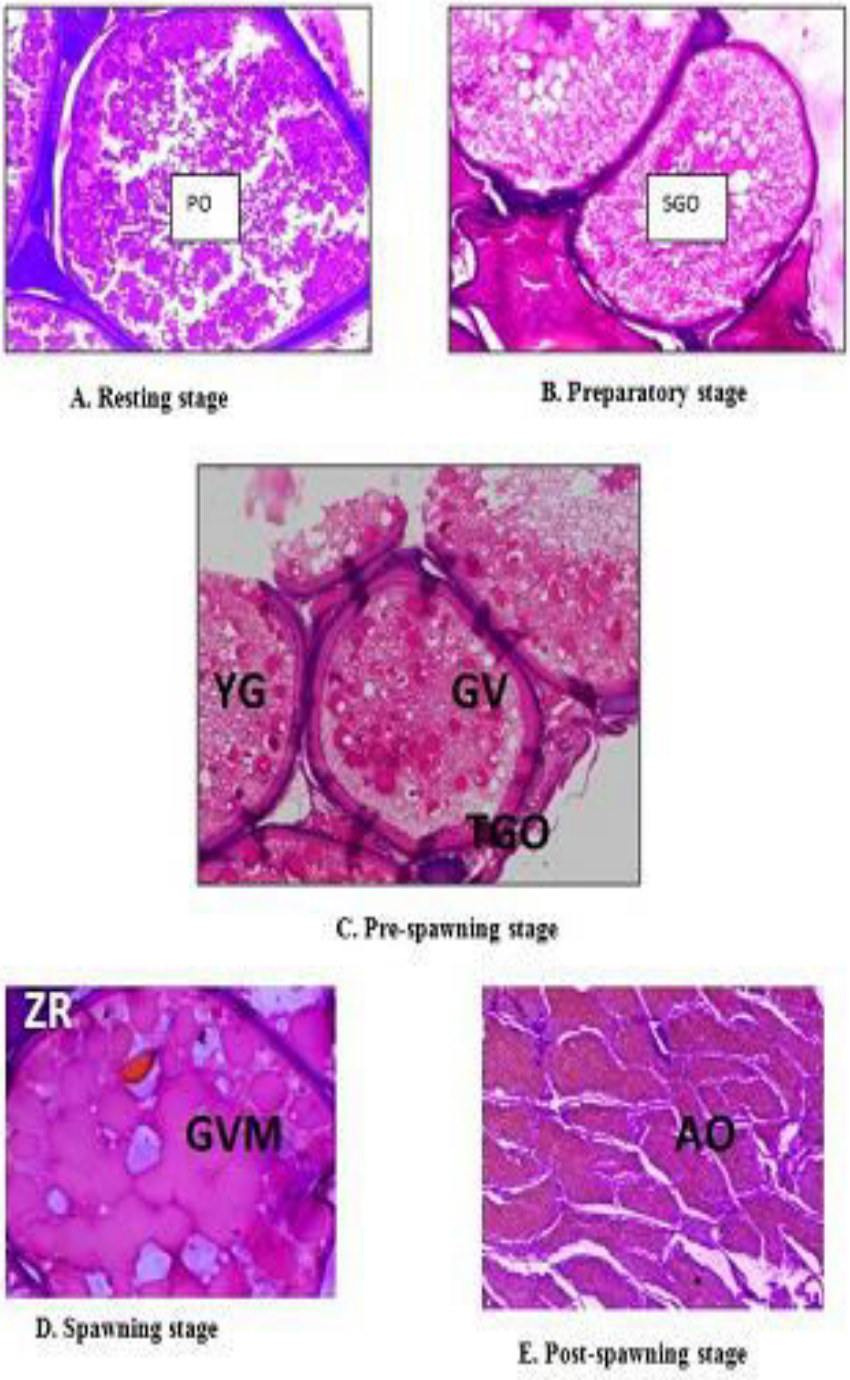

Fig. 2: Histological observation of ovary of rainbow trout.

PO- Primary growth oocyte; SGO-secondary growth oocyte; GV- germinal vesicle; TGO- tertiary growth oocyte; YG-yolk globule; ZR- Zona radiata; GVM- germinal vesicle migration; AO- Atretic oocyte.

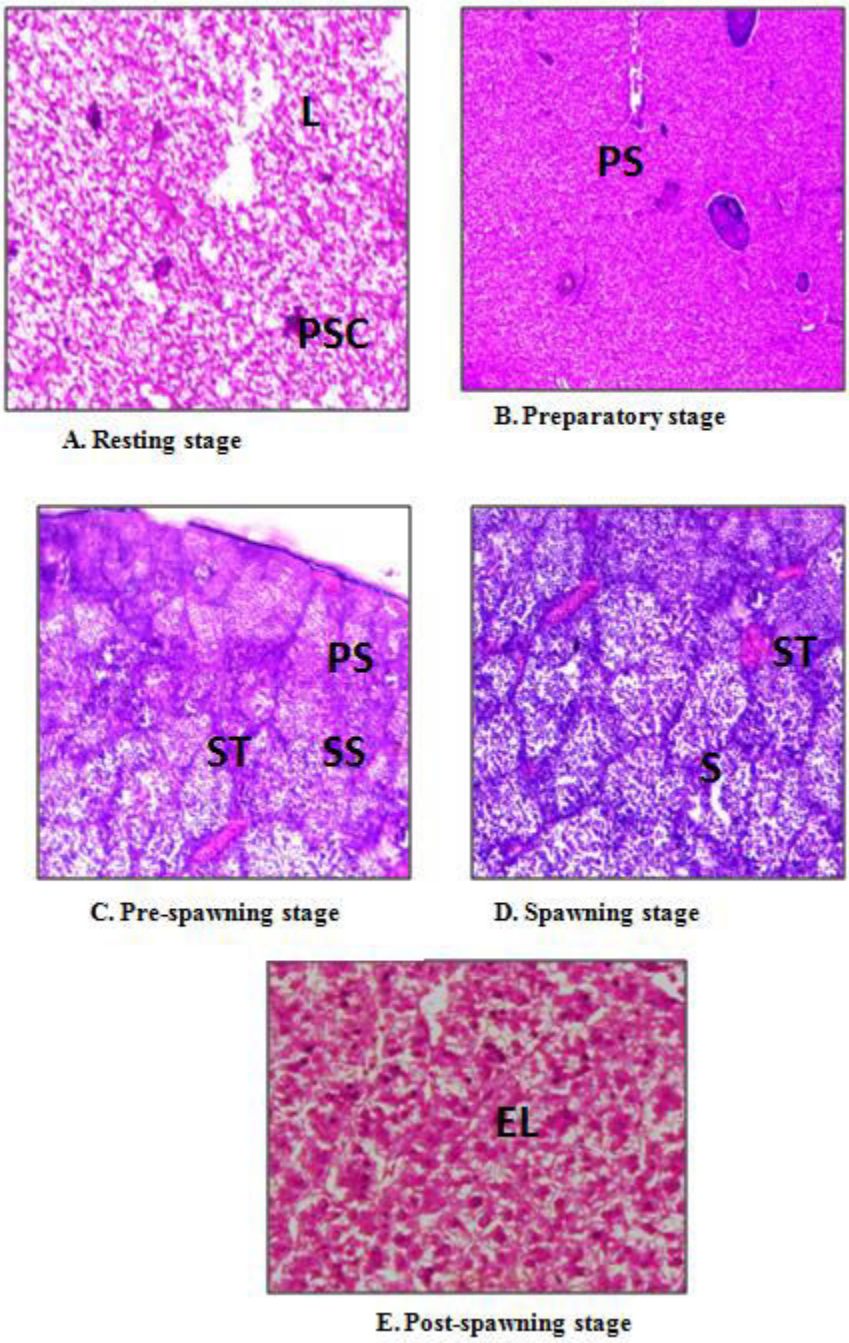

Fig. 3: Histological observation of Testis of rainbow trout.

L- Seminiferous lobule; PSC- Primary spermatogonial cells; PS- Primary spermatocyte; SS-Secondary spermatocyte; ST- Spermatid; S- sperm; EL-empty lobule.

flaccid due to the shedding of mature eggs after spawning. Histological observations of empty/ atretic follicles in the spent ovary indicated the post-spawning stage. Sharma *et al.* (2014) reported that in rainbow trout oocytes was divisible in seven stages, based on morphological basis viz. stage I-chromatin nucleolus stage, stage II– peri nucleolus stage, stage III- cortical alveolus stage, stage IV- primary vitellogenic stage, stage V- secondary vitellogenic stage, stage VI- maturation stage and stage VII- spent stage. These oocytes were characterized by features like cortical alveoli, yolk globules, zona radiata, zona interna, zona externa, granulosa cells, thecal cells.

In this study there was no morphological difference in testes during May to July. In the testis, tunica was thicker. Only spermatogonia were present within the testes along with few primary germ cells (Fig. 3). Spermatogonia had a light cytoplasm and a large nucleus. The proliferation of spermatogonia was at initial stage and the scanty presence of spermatocytes in most lobules gave appearance of empty space. In the month of February, the lobules increased in size and possessed good number of primary and secondary spermatogonial cells but spermatocytes were not present. During the month of September, the testis of fish compactly filled with spermatogenic cells. Primary spermatocytes were also found. In the month of October, some lobules had a big and empty tubule lumen with no spermatogonial clusters. Primary and secondary spermatocytes, spermatid and sperm were observed in the tubules in the month of June. In this stage, the number of large cells got decreased but the number of small cells (spermatids and sperms) was increased progressively. During December to mid March, most of the lobules were characterized with sperm masses with a few secondary spermatocytes and large numbers of spermatids on their inner center. The majority of tubules were found empty in the month of April. Testes contained only primary spermatogonia and primary germ cells. Few sperm were left in the tubules.

Conclusion

Results of the present study clearly demonstrated the GSI values of females showed significant difference between different months for trout fish populations in Bairangana river. The increasing GSI values appeared from January to February with the peak in February, indicating the onset of the breeding season for rainbow trout. The GSI value declined rapidly from March to August after spawning. Therefore, it was confirmed that the fish spawned once in a year with peak spawning during the month of January to February. Present study contributes to better understanding of the cyclic and seasonal ovarian changes in rainbow trout which in turn will help in conservation plan and captive maturation of this valuable species. This information also helps in understanding the histological cell pattern of rainbow trout reproduction in Balkhila river.

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