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Histological assessment of gonadal cyclicality of *Chagunius chagunio*

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Abstract

An attempt was made to assess the histological profile of indigenous carp *Chagunius chagunio*. Live specimens of *C. chagunio* were collected from the river Kosi Ramnagar, Uttarakhand, and reared in cement tanks at ICAR-DCFR, Bhimtal. Histological profiling study of gonads was carried out for a complete year. Different histological sections were quite similar and gave an indication towards the prolonged breeding period with batch spawning habit of fish. Findings of the study suggest that the fish might have two possible breeding seasons and fish can successfully achieve its ovarian maturity under captive condition. Testicular development indicates that the males of *C. chagunio* perhaps can produce viable sperms round the year.

Keywords: *Chagunius chagunio*, histological profile, ovarian maturity, testicular development. captive condition

1. Introduction

Chagunius chagunio, commonly known as “Chaguni” belongs to family cyprinidae is one of the economically important indigenous fish of Himalayan region. *C. chagunio* is an endangered species^[1] although IUCN^[2] has declared this species as ‘least concern’ in red list status. As per the CAMP^[3] report the species is not evaluated. Prior to conduct the breeding and seed production studies of any fish species, it becomes very much obligatory to get the precise information concerning gonadal development. Histology is an authoritative tool to study microanatomy of gonads^[4]. Despite the fact that histology is time-consuming it is the most reliable technique for assessing the gonadal status^[5-6]. Histological examination of gonads provides reasonably more precise estimation of fish spawning season^[7]. No records on histological account of gonads of *C. chagunio* is available till date, however, few records of the histological profiling of gonads of some other coldwater cyprinids like *Tor* and *Schizothorax* species are available^[8-10]. Therefore, the present study was accomplished to explore the micro anatomical changes in ovary and testis of *C. chagunio* during its annual maturation cycle, under captive condition.

2. Materials and methods

Live fishes of *C. chagunio* weighing from 57-143 g and length ranging from 17.80 to 23.50 cm were collected from the river Kosi Ramnagar, Uttarakhand (29° 29.03’ N latitude, 79.08’ E longitude and altitude 410 m MSL). Fish were collected using cast net during morning hours and transported in live condition to ICAR-Directorate of Coldwater Fisheries Research, Bhimtal, and reared in cement tanks. They were fed *ad libitum* with conventional carp feed. Fishes were sacrificed to take gonads individually from male and female every month. Gonads were fixed for 24 hours in Bouin’s fixative at room temperature and routine processing up to wax embedding for histological observations was carried out. The paraffin sections were cut at 5 µm thickness and stained with haematoxylin-eosin (H&E) for histological observations. These sections were observed microscopically and microphotographs were taken.

3. Results and discussion

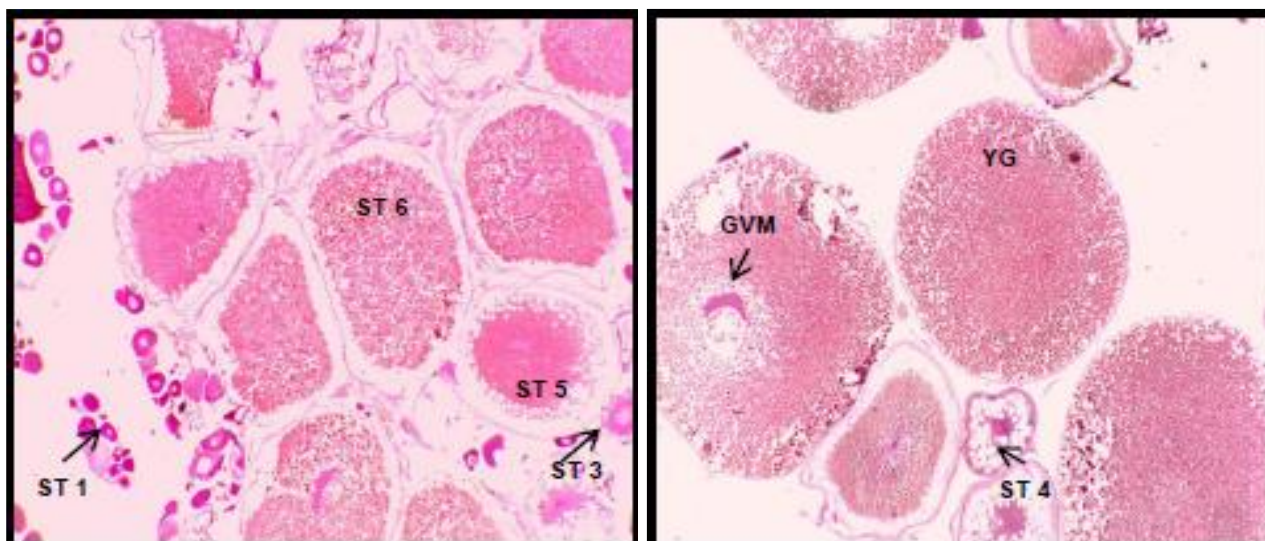
In the present study, histological observation of the gonads of *C. chagunio* revealed that all distinct stages of maturation were present in almost all the samples.

3.1 Ovarian development

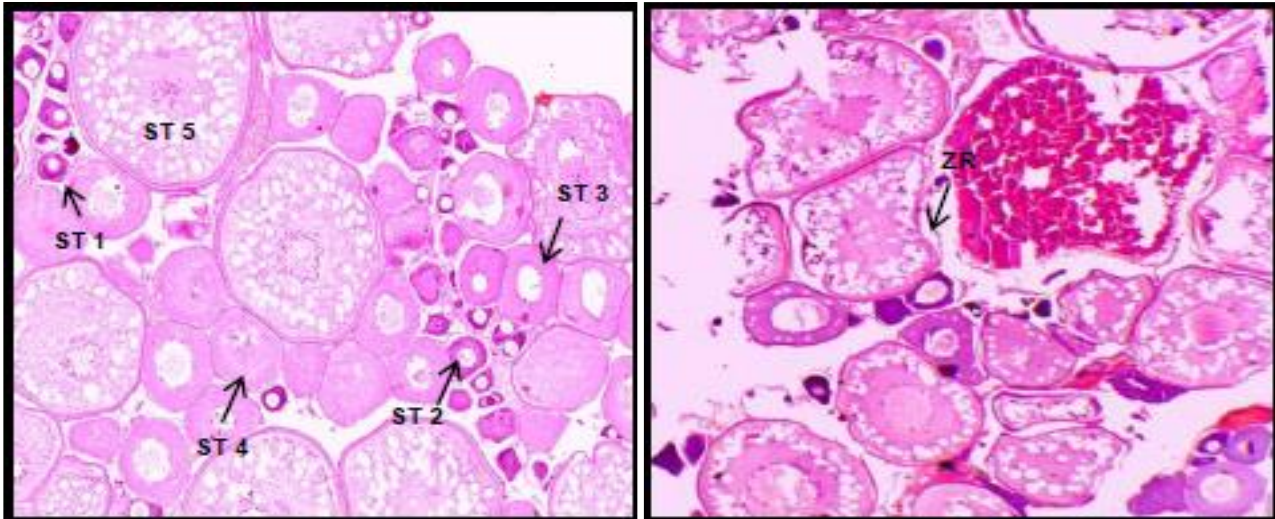
Histological observations of ovarian tissue revealed that a large size range of maturing oocytes were present simultaneously during most of the months and there was a continuous gradation from the smallest to the largest size groups indicating several batches of eggs at different stages of maturity. This reveals that *C. chagunio* is an asynchronous spawner and could possibly breed many times in a year in small batches even within a single breeding season. It could be clearly seen in histological section of the ovaries that there was an increase in oocytes size as the stages of maturity progressed. In the ovarian sections six distinct developmental stages could be seen. Stage I oocyte was characterized with centrally located large nucleus. Stage II oocyte was slightly larger in size in comparison to stage I with heavily stained ooplasm with hematoxylin. In stage III oocyte the volume of nucleus as well as ooplasm was increased whereas the ooplasm was faintly stained than the former one. The nucleus of the stage III oocyte was consisting of many nucleoli. Stage IV oocyte was larger in size and irregular in shape. In this stage the yolk granules started to appear on the periphery of the oocyte and zona radiata could clearly be seen. In oocyte V stage, yolk globules were occupying most of the ooplasm and two distinguishable layers could be seen in the zona radiata. In the oocyte VI stage germinal vesicle started to migrate towards the animal pole and the shape of the germinal vesicle became irregular due to the disintegration of outer membrane. It was observed that the histological sections during April to September were more or less similar having all the maturity stages of oocytes having dominance of advance stages whereas the sections of October to March were close in appearance with dominance of initial stages of oocyte development. Oocytes at different developmental stages are

shown in plate 1. Histological observations of the ovaries of *C. chagunio* clearly show the presence of oocytes of all the stages (From Stage I to stage VI). However, it is also opined that there is no standardized scheme for categorizing the number of stages in staging fish gonads^[11]. Different workers have been described varying number of maturity stages viz five, six, seven, eight, nine and even upto ten in different teleost fishes^[12-23]. The present study is the first attempt regarding the histological study of gonads of this fish. Presence of oocytes in all the maturity stages give an indication that *C. chagunio* is an asynchronous spawner like other cold water cyprinids; mahseer and snow trout and possibly shed eggs over a period of time in small batches. Earlier workers have described the mahseer and snow trout as asynchronous batch spawner^[8, 9, 24-27]. No earlier records are available regarding the gonadal histology of *C. chagunio* which describe the ovarian developmental pattern of this fish. A few records describe the migration pattern and two distinct breeding season of *C. chagunio*. Reported breeding season of *C. chagunio* in natural conditions is May-June^[28], April-June^[29] and August- September^[30]. A study^[31] based on gonadosomatic index of *C. chagunio* also define its two breeding seasons viz April-June and August- September.

As observed in histological sections of the ovary, dominance of initial stages of oocyte development was during October to March indicating a pause in breeding. On the other hand, as per the information collected from local fishermen of Ramnagar area of Uttarakhand, the experimental fish breeds round the year. Although, it is also suggested^[23] that the histological studies of gonads of serially spawning fish may give sometimes misleading information regarding breeding season due to continuous process of shedding of eggs, generation of new oocytes and follicular atresia.



Histological observation during April- September



Histological observation during October- March

Plate 1: Histological observation of ovary of *Chagunius chagunio* (n=3) ST1-ST6: stage 1 to Stage 6 oocytes; GV- germinal vesicle; YG- yolk globule; ZR- Zona radiata; GVM- germinal vesicle migration

3.2 Testicular development

Histological sections of the testis of *C. chagunio* show a similar pattern of development throughout the year. Samples of different months were showing similar conditions and distinguishable maturity stages of spermatogenesis were present simultaneously with free spermatozoa round the year. Based on their nuclear and cytoplasmic characteristics different developmental stages of spermatogenesis were identifiable. It was clearly seen that sperm ducts were containing all the maturity stages (spermatogonia, primary and secondary spermatocytes and spermatids) including free sperms. The complete process of maturity of the testis (spermatogenesis) can be categorized in different stages. Spermatogenesis starts with rapid mitotic division of spherical spermatogonia followed by a meiotic division phase with primary and secondary spermatocytes. Primary spermatocytes were larger in size and the nucleus could be clearly seen in these germ cells. Secondary spermatocytes were smaller in size in comparison to those of primary spermatocytes and were characterized with heavily stained cells with unclear nucleus. The spermatids cells were even smaller than the secondary spermatocytes taking dark stain. Free spermatozoa

were lying into the central lumen appearing like small pinheads were ready to release for spawning. In contrast to the oogenesis, the size of the cells in the spermatogenesis decreases as the advance maturity stages comes. All the samples were showing similar developmental stages which are shown in plate 2. In the present study the histological sections of the testis of *C. chagunio* illustrate a similar pattern of testicular development throughout the year indicating the capacity to produce viable sperms any time during the year. Samples of different months were depicting all the maturity stages of spermatogenesis simultaneously with free spermatozoa in the central lumen. There were no distinguishable maturity stages of testes like resting phase, preparatory phase, pre-spawning phase, spawning phase and post-spawning /regression phase as suggested in many other teleosts [32-37].

No earlier literature is available describing the testicular histology of *C. chagunio*, however, some authors have suggested the similar situation in males of other coldwater cyprinid, mahseer [9-10]. Similarly, local fishermen of Ramnagar area of Uttarakhand, also found the experimental fish reproducing round the year (personal communication)

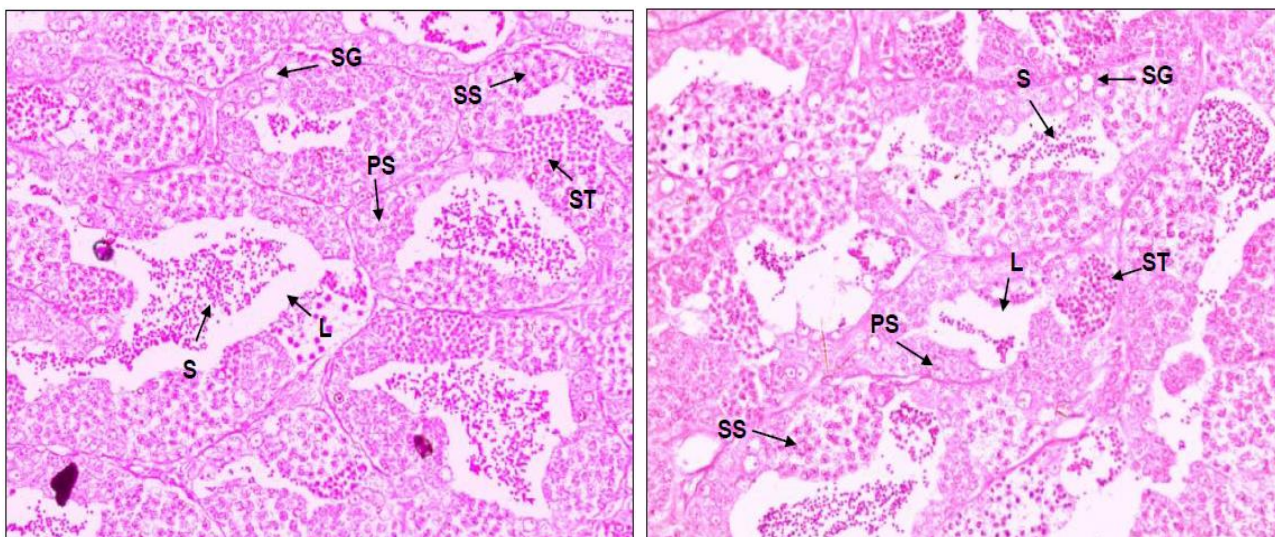


Plate 2: Histological observation of testis of *Chagunius chagunio* (n=3) L- Central Lumen; SG- Spermatogonial cells; PS- Primary spermatocyte; SS-Secondary spermatocyte; ST- Spermatid; S- sperm.

4. Conclusion

The present study is the first one regarding the histological profiling of *C. chagunio* under captive conditions. These findings suggest that *C. chagunio* exhibits asynchronous type of ovarian development with multiple batches of eggs at different maturity stages as well as round the year sperm production by males. These observations not only describe the microanatomy of gonads but also indicate that the fish can successfully achieve its gonadal maturity under captive condition. Present findings will serve as the base line information regarding gonadal development of *C. chagunio* and torch light to the academicians and researchers for the development of breeding and seed production protocol of this species.

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