

Research Article

OVARIAN DEVELOPMENT ACROSS MATURITY STAGES IN *BARILIUS BENDELISIS*: A HISTOLOGICAL STUDY FROM THE MANAS RIVER, ASSAM, INDIA

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ABSTRACT

Histological analysis was conducted on ovarian development in *Barilius bendelisis* collected from Manas River, Assam, India, from November 2019 to November 2021. Five distinct phases of oocyte development were identified during the study. To facilitate the use of both microscopic and macroscopic observations, a gonad maturation scale was devised and adapted to align with the commonly employed unaided visual observation. The four stages correspond with those described macroscopically for various species of Cypriniformes. These ovaries showed few atretic oocytes. The study revealed that *B. bendelisis* is a double-spawner, engaging in two breeding seasons, one from December to April and the other from August to September. Vitellogenic oocytes were specifically observed during the autumn and winter periods.

Keywords: *Barilius bendelisis*, Cypriniformes, Development, Gonad, Oocyte.

INTRODUCTION

Barilius bendelisis (Ham.), commonly known as the Indian hill trout, is a well-known freshwater ornamental fish belonging to the Cyprinidae family. The species is located within the Brahmaputra and Ganga drainage systems along the Himalayan foothills (Talwar and Jhingran, 1991). This fish plays a crucial role in the capture fishery across several areas of the Himalayan region in Arunachal Pradesh, where it is not feasible to successfully cultivate Indian major carps and exotic carps (Sahoo *et al.*, 2009). Despite its significance, basic studies on the histology of *Barilius bendelisis*, particularly regarding its reproductive system, are still limited. However, some research on its reproductive biology has been conducted by Saxena *et al.* (2018) and Jabeen *et al.*, (2016). The most suitable method for determining the reproductive cycle in female fish involves observing seasonal developmental changes in the gonads (Sivakumaran *et al.*, 2003). Histological studies

provide the most reliable and objective information on the spawning season and are essential for detecting details within the maturation cycle (Sivakumaran *et al.*, 2003). Reproductive development and histology in females are best understood through histological techniques, which allow for the determination of reproductive stages. Currently, information on the histomorphological changes in the ovary of *Barilius bendelisis* from natural flowing water is still lacking. Therefore, cytology-based classification systems may offer the most accurate descriptions of the transformations occurring within the gonadal system.

MATERIAL AND METHODS

Study area

The present study was conducted over a period of two years, from November 2019 to November 2021. Monthly

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samples of 20-30 female fish ranging from 6.986-12.035 cm and weight 3.375-18.971g were collected for the study of maturation and ovarian development from three sampling sites of Manas River viz., Mothanguri, B₁ (26°46'90.2"N, 90°57'41.9"E and altitude 87.5 m MSL),

Narayan Guri, B₂ (26°39'63.0"N, 90°59'43.2"E and altitude 56.7 m MSL) and Bekipar, B₃ (26°29'71.1"N, 90°55'16.7"E and altitude 40.8 m MSL). Cast net (90', 1"; 9', 1/2") and gillnet (75x1.3x1.3 m, 2") were used for the collection of the species (Figure 1).

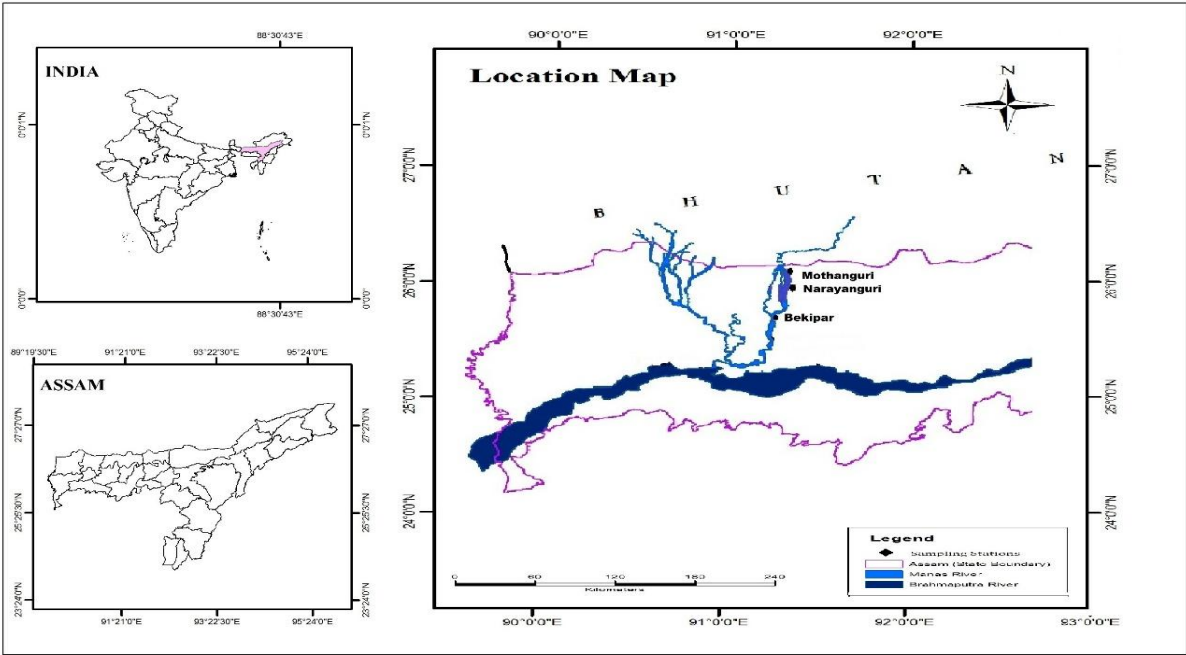


Figure 1. Map of Manas River along with the sampling sites.

After visual morphological observations, ovaries were dissected out and thoroughly wiped to remove moisture using a filter paper and weighed using an electronic balance (Anamed Electronic Balance; sensitivity 0.001g). Measurements of the length of ovaries were taken by digital caliper nearest to 0.1 mm, and then the values were converted into centimeters. For histological studies, the ovaries were fixed in 10% formalin solution for 24-72 h (depending upon the size of the ovary), and after dehydration, they were embedded in paraffin wax and sectioned at 5 µm thickness and stained with haematoxylin/eosin (H and E). The ovarian tissues were observed under a bright-field microscope (Leica make, DM3000).

RESULTS AND DISCUSSION

The current study found that the minor carp *B. bendelisis* spawns biannually in April and September. The length and weight of the ovary ranged from 1.909 to 5.289 cm and 0.006 to 1.621g, respectively. As mentioned by Saxena et al. (2018), *B. bendelisis* has a pair of ovaries and oviducts as its female reproductive system. Ovaries were elongated, extending the length of the abdominal cavity. The ovary was classified into four stages based on the macroscopic and histological stages of the ovary, depending on the maturity stages. As outlined in Table 1, the key histological and macroscopic features of the development stages are described.

Table 1. The Macroscopic characteristics and histological description of the different stages of ovarian maturity in *Barilius bendelisis*.

Stage	Degree of maturation	Macroscopic description	Histological description
I	Maturing stage	Occupying half of the abdominal cavity, pinkish, translucent eggs are visible with the naked eye, pale in colour	Chromatin nuclear oocytes and peri-nuclear oocytes were present. Sometimes, yolk granules, vitellogenic oocytes were also seen.
II	Ripening of the vitellogenic stage	Occupy ¾ th of the body cavity; eggs were large and easily seen with the naked eye; yellowish with conspicuous superficial	Dominated by yolk vesicles oocytes; some yolk globular oocytes present. Minimal atresia.

		blood vessels; having a granular appearance.	
III	Spawning stage	Ovaries distended and containing large translucent eggs which were spherical and filled with yolk; occupy the entire abdominal body cavity; orange coloured.	Ovaries consist of mature eggs. Some atresia present.
IV	Spent	Ovaries flaccid and shrunken, having loose walls, fully empty. A few residual oocytes were occasionally observed.	Widespread atresia of vitellogenic and some cortical alveolar oocytes

Histological observation (Figure 2) showed the presence of oocytes of different maturity stages in the ovary. Change of immature oocytes into developing and mature stage oocytes is associated with the accumulation of lipid globules across the ovary. In the present study, the spent phase of the ovary is observed to be short with a prolonged developing and vitellogenic stage. This may be attributed to the incomplete vitellogenesis and oocyte maturation of certain developing oocytes (Saxena *et al.*, 2018). Several studies on fish ovary development have reported similar findings related to

reproductive dysfunction (Mylonas and Zohar, 2001; Lee and Yang, 2002; Agullerio *et al.*, 2006). Mylonas *et al.*, (2010) stated that the abnormal release of luteinizing hormone, which can be impacted by several environmental and social variables, is the reason why vitellogenesis and oocyte maturation are not completed. In this study, *B. bendelisis* was found to be a double spawner, exhibiting two breeding seasons: December to April and August to September. The presence of vitellogenic oocytes was found during autumn and winter.

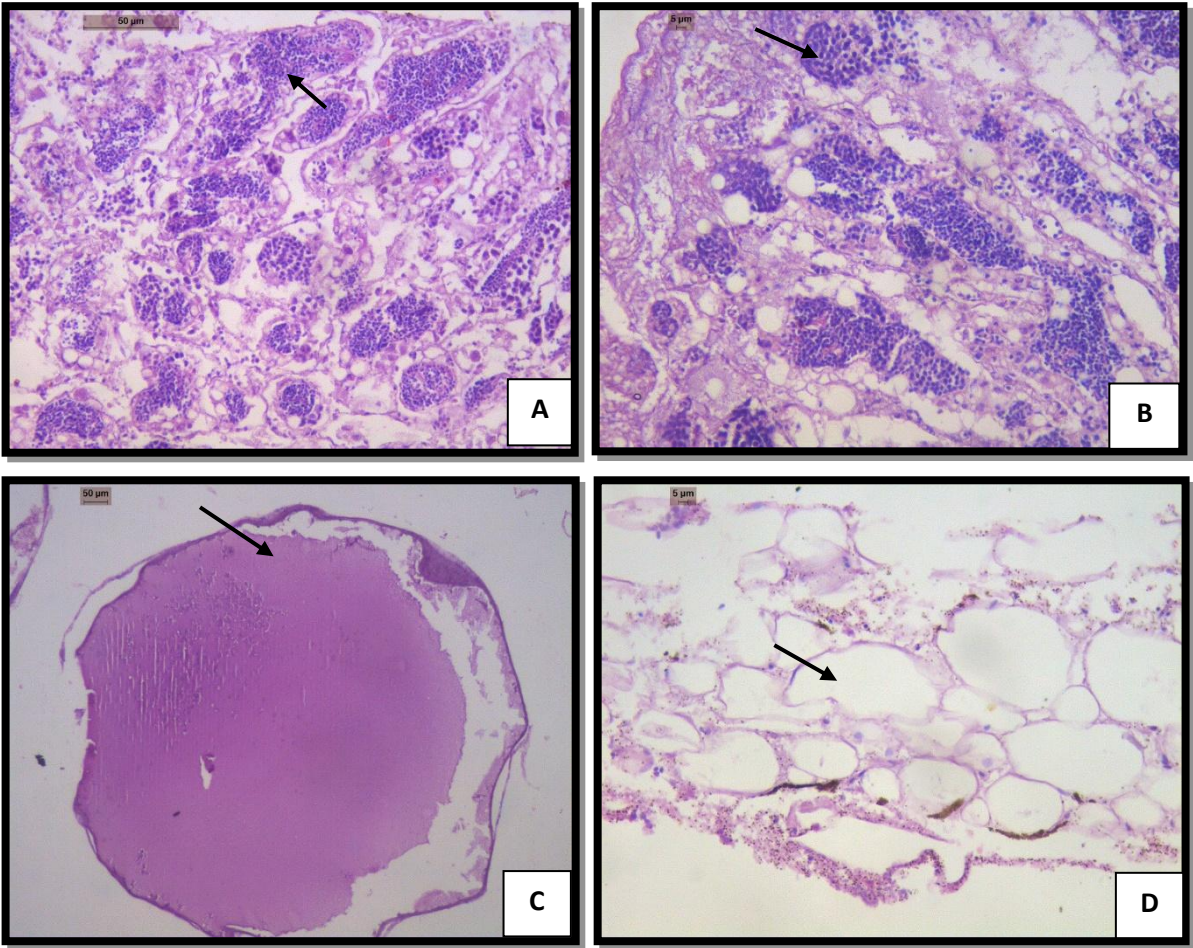


Figure 2. Showing different histological female gonadal development stages of *Barilius bendelisis*, **A:** Perinucleus oocytes stage indicated by arrow in Maturing stage, **B:** Vitellogenic oocytes in vitellogenic stage, **C:** Spawning stage showing mature stage of oocyte, **D:** Spent stage showing empty follicles.

This research also offers foundational data on the biological characteristics of this fish, which is crucial for its commercial production and development. Consequently, gathering all necessary information regarding the growth and reproduction of this species is essential for stock assessment, ensuring the sustainable management of its fisheries.

CONCLUSION

Oocyte development is now considered to encompass four distinct stages in this species' life-to speak more precisely. But it must be said that at all times, there is a fundamental lack of *B. bendelisis* histological information, particularly concerning its reproductive system. It indicates further research is needed in this area. This branch of research says that lays the foundation for further research into the complexities of *B. bendelisis* reproductive physiology, which will be used to help conserve and manage animal populations in their native environment.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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