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Research article

Ovarian histology of the freshwater catfish *Silonia silondia* (Hamilton, 1822)

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ABSTRACT

Histological characterization of gonadal tissues and their development can divulge vital clues to interpret the onset and progression of the spawning period in fish species and help in wild stock augmentation to conserve the natural fish stocks. In the present study, ovarian histology of female *Silonia silonda* was investigated from the Kangsha River, Bangladesh from March to November 2018. During this investigation, 54 females of *Silonia silondia* was analyzed to study the ovarian development. The histological evaluation revealed five distinct stages of gonadal development (Immature, Developing, Spawning capable, Regression or spent and Regeneration or Re-developing). Six phases of oocyte development were further characterized during the gonadal development stages as under-developed, primary growth, cortical alveolar, vitellogenic, pre-mature, and mature stage. In conclusion, the gonado-somatic index and histological analysis of ovaries indicated that the peak spawning of *S. silondia* occurs in July, and suggesting that the studied fish spawns once per breeding season. The present study recommends year round investigation of gonadal cycle and gonad developmental study of male individuals of *S. silonida*.

1. Introduction

Histological studies on fish reproduction are a prerequisite to determining the peak spawning period of a fish [1]. Understanding links between gonadal development and fish spawning activity carry crucial information on essential ques for restoring and managing endangered species [2,3]. The conservation of threatened fish species to prevent their extinction can be assessed by investigating the reproductive physiology of fish in terms of gonadal maturity, breeding potential, and breeding season.

Previous research shows that fish's spawning season can be determined by studying gonado-somatic index (GSI), by observing macroscopic features of the ovaries such as size, the degree of ovary's opacity, consistency and vascularization, oocyte visibility, overall coloration and microscopically by histology of the gonads [4,5]. Although GSI often reflects a species' reproductive cycle and is considered a good indicator of gonadal development in fish, histological studies can provide the most reliable and objective data in gonad staging. Gonadal histological analysis also has significant importance in determining the maturation cycle of fish [6,7].

Silonia silondia (Hamilton, 1822), commonly known as shilond catfish is a popular food fish with excellent taste and high market

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value in Bangladesh and India [8,9]. It has been abundant in estuaries and rivers throughout Bangladesh in recent decades. However, the natural population rapidly declined from the natural waterbody of Bangladesh due to loss of habitat and overexploitation [9]. Although it has been categorized as the least concern by IUCN Bangladesh in recent years, along with other fish populations, these species are gradually reducing from their natural habitat due to various ecological and anthropogenic factors [10]. The demand for this species is entirely satisfied by capture from natural populations, as captive breeding technologies have not yet been developed [11]. There is a lack of reliable production statistics for this species from the open water bodies of Bangladesh. Given the low resistance of this species to fishing pressures, wild stock conservation becomes imperative [12]. Further, *S. silondia* is listed by FAO [13] as one of the species protected by conservation initiatives. Therefore, it is imperative to have proper documentation of this species in order to effectively conserve, maintain, and ensure the long-term viability of the fishery.

To reduce the pressure on the wild stock, captive breeding and culture of this species could provide appropriate answers to its conservation. Its culture success can offer higher economic benefits for the fish farmer if it can become an aquaculture candidate. To the best of our knowledge and literature review, there are no documented studies on the reproductive biology of this species in their natural habitat. Considering the grander importance and inherent links between gametogenesis and subsequent captive breeding in conserving important fish species like Shilond catfish, this study was designed to examine the gonadal histology and gonado-somatic index (GSI) of Shilond catfish to distinguish between its gonadal maturation and spawning seasons in the natural environment.

2. Materials and methods

2.1. Sampling site

The specimens were collected from three selected sites (Site-1, $25^0$01′36.65 N, $90^0$32′38.53 E; Site-2, $25^0$00′53.06 N, $90^0$32′40.39 E and Site-3, $25^0$01′41.26 N, $90^0$31′36.71 E) of the Kangsha River, Netrakona district, Bangladesh (Fig. 1). The sampling sites were situtated within 3–5 km of each other. Kangsha River (also known as the Kangsai or the Kangsabati) is a river in the northern parts of the Mymensingh and Netrakona districts of Bangladesh. The river flows through Mymensingh, Netrakona, and Sunamganj districts and plays a vital role in the inland fisheries catch of Bangladesh as large quantities of fish are harvested yearly. The river comprises an average length of 26–28 km long course with an average depth 6.87 ± 0.33 m [14].

2.2. Sampling and preparation of gonad

Fish samples were collected from the fishermen catch from March to November 2018. Fishermen were mainly catching Shilond catfish by using gill net and hooks in the Kangsha River. After collecting, the live individuals were transported in an ice box to the laboratory at Bangladesh Agricultural University and identified based on the morphological characters reported by Gupta [8]. In the laboratory, the fish were examined for female characters and selected based on their bulging abdomen. Sexually mature female fish were measured for their total length (TL, to the nearest 0.01 cm) and body weight (BW, to the nearest 0.01 g) with a measuring board and digital weighing blance (JC-5202BS, HANDk, Taiwan), respectively. A pictorial view of the studied *S. silondia* is shown in Fig. 2. A

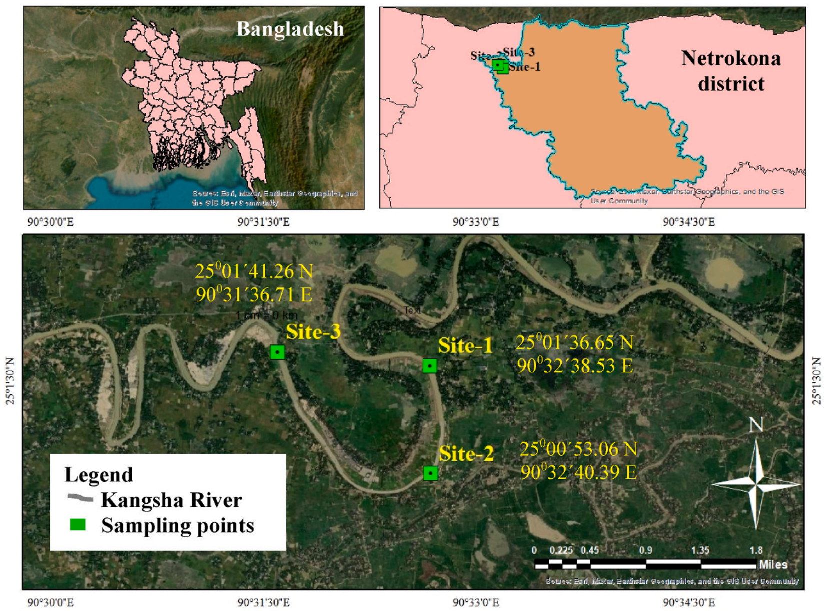

Fig. 1. Fish sampling location in Kangsha River, Netrakona, Bangladesh.

total of 54 sexually mature females were sampled to examine their gonadal histology. Fish were dissected; the gonads were removed and weighed (GW, to the closest of 0.01 g). The collected gonads were then preserved in 10 % buffered formalin for further analysis.

2.3. Determination of gonado-somatic index (GSI)

Quantitative analysis of ovarian development was assessed by GSI, according to the following the formula of Maddock & Burton [15] as follows:

$$GSI = \frac{GW\ (g)}{BW\ (g)} \times 100$$

2.4. Histological analysis

Tissue samples were excised from the rostral, middle, and caudal regions of preserved ovaries and fixed in Bouin's solution for 24–48 h. The samples were then dehydrated in graded alcohol baths and embedded in paraffin wax. The embedded samples were sectioned to 5 μm thick using a microtome machine (HM 325 Manual Micro-tome, MICROM International GmbH, Waldorf, Germany). The sections were stained with haematoxylin and eosin (H-E protocol) and examined with a light microscope (Olympus Magnus, Model no.11F589) connected to a computer with a viewer (Magnus viewer) at 100× magnification. Gonad maturation and histological analysis were based on the characteristics of gonad germ cells [16].

2.5. Statistical analysis

Data were analyzed using the IBM SPSS statistical package (Ver. 20.0) and Microsoft Excel. Data were expressed as mean ± standard deviation (SD). Normal distribution of the data was checked by the Kolmogorov-Smirnov and Shapiro-Wilk tests and the data was found normaly distributed. Monthly variations in TL, BW, GW and GSI were assessed by one-way analysis of variance (ANOVA) followed by the Duncan Multiple Range Test (DMRT) due to the equal sample size. A significance level of $P < 0.05$ was considered in the study.

3. Results

3.1. Morphometric of *S. silondia*

Morphometric parameters of *S. silondia* are shown in Table 1. A total of 54 female fish were assessed for the present study. Total length ranged between 15.05 ± 1.29 (March) to 21.54 ± 1.96 cm (July). Significantly lower body weight was recorded in March (63.31 ± 8.08 g) and the highest in July (149.78 ± 30.72 g). Similarly, gonad weight was significantly higher in July (2.15 ± 0.28 g) and the lowest in March (0.42 ± 0.03 g).

3.2. Gonado-somatic index (GSI) of *S. silondia*

Monthly variations in the mean GSI values of female *S. silondia* are presented in Fig. 3. GSI ranged between 0.62 (May, n = 5) and 1.48 (July, n = 7). A significantly ($P < 0.05$) higher value of GSI was observed during July and a lower during May. The GSI increased gradually from May onwards and decreased from August onward. This alluded to the peak of the reproductive season during June and July.

3.3. Macroscopic observation of gonad

Macroscopic observation showed five gonadal developing stages with distinctive size, colour, and transparency of the gonads (Table 2). Histological results of monthly collected female samples showed that *S. silondia* spawn once a year, and the peak spawning months are June and July.

Fig. 2. Silonia silondia.

Table 1
Morphometric features of *Silonia silondia*.

Months	Sample size (n)	Total length (cm)	Body weight (BW)	Gonad weight (GW)
March	5	15.05 ± 1.29^{g}	63.31 ± 8.08^{e}	0.42 ± 0.03^{e}
April	6	15.65 ± 0.94^{fg}	70.00 ± 4.41^{de}	0.44 ± 0.03^{e}
May	5	16.96 ± 0.14^{ef}	74.04 ± 2.79^{de}	0.46 ± 0.02^{de}
June	9	16.93 ± 0.51^{ef}	84.78 ± 20.57^{cde}	1.17 ± 0.58^{b}
July	7	21.54 ± 1.96^{a}	149.78 ± 30.72^{a}	2.15 ± 0.28^{a}
August	5	18.31 ± 0.42^{de}	101.00 ± 3.44^{bc}	1.05 ± 0.11^{bc}
September	6	20.59 ± 2.14^{ab}	116.65 ± 32.88^{b}	0.84 ± 0.11^{bc}
October	5	19.07 ± 1.09^{cd}	90.39 ± 3.81^{cd}	0.82 ± 0.05^{bc}
November	6	19.81 ± 0.43^{bc}	102.52 ± 8.72^{bc}	0.78 ± 0.05^{cd}
F-value		20.772	13.315	25.575
P-value		<0.001	<0.001	<0.001

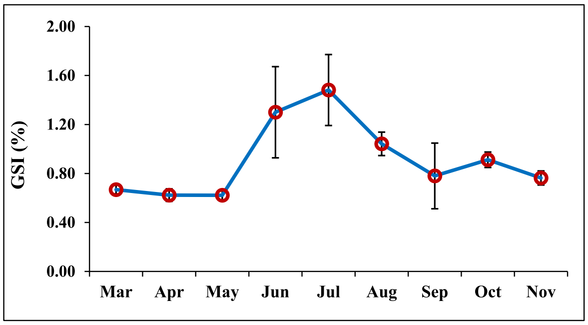

Fig. 3. Monthly variations of gonado-somatic index of female *Silonia silondia*. Points sharing the same letter are not significantly different ($P < 0.05$).

Table 2
Macroscopic appearance of *Silonia silondia* gonad at different developmental stages.

Stages	Duration	Macroscopic observation of ovary
Immature	March–April	Small, thin, ribbon-like transparent ovary, ovary straight, whitish, poorly developed
Developing	May	Increases in size form lobes, blood vessels visible, yellow orange in color, eggs almost visible, ovary flaccid, and blood vessels were prominent
Spawning capable	June–August	The oocytes are large. Ova visible to the naked eye, loose and almost separate from each other in the ovary, slight pressure on the abdomen evacuate eggs from the belly.
Regression or spent	September	Appearing flaccid, translucent, and reduced in size, dull in color, a small number of un-spawned ova were visible
Regeneration or Re-developing	October–November	Ovaries are firmer, smaller, and lighter in color than in previous stages

3.4. Histological observation of gonad

A monthly count of cell ratios was used to identify the gonadal stages of *S. silondia*. The microscopic observations of the ovarian tissue showed the presence of Nucler chromatin, primary growth oocyte, cortical alveolar, primary vitellogenic oocyte, secondary vitellogenic oocyte, tertiary vitellogenic oocyte, post-ovulatory follicles, migrating nucleus and atresia. Gonad histology of *S. silondia* showed five phases of oocyte development, *viz.* immature, developing, spawning capable, regression or spent and regenerating stages (Fig. 4) with percentage contribution of monthly cell count ratio (Fig. 5). An immature stage is characterized by a conspicuous feature associated with chromatin threads, and oocytes are in the primary growth (PG) stage. The percentage of cells in PG stage was more productive during March and April, alluding to the preparations for the start of the breeding season in the subsequent months. PG stage can be described with a larger nucleus and multiple nucleoli are moving around the periphery of the nucleus. Cytoplasm at this stage stained deeply with haematoxylin, while the nucleus was clear except for the nucleoli. Lampbrush chromosomes were formed in the oocytes (diplotene stage of meiosis), which disappear right before the breakdown of germinal vesicles during oocyte maturation. The cytoplasm tended to lose affinity for haematoxylin. Oil droplets in the perinuclear area characterized the cortical alveolar (CA) stage. The final stage of oocyte development was characterized by the formation of yolk granules in oocytes with fully developed yolk vesicles during the early yolk granule stage. They stained light pink with haematoxylin and eosin. The percentage of PG oocyte gradually decreased in the ovary samples collected in May and was compensated with advanced CA, primary vitellogenic (Vtg1) and secondary vitellogenic (Vtg2) oocytes. During this phase, ovaries were undergoing the developing stage. CA was in a more advanced stage. The

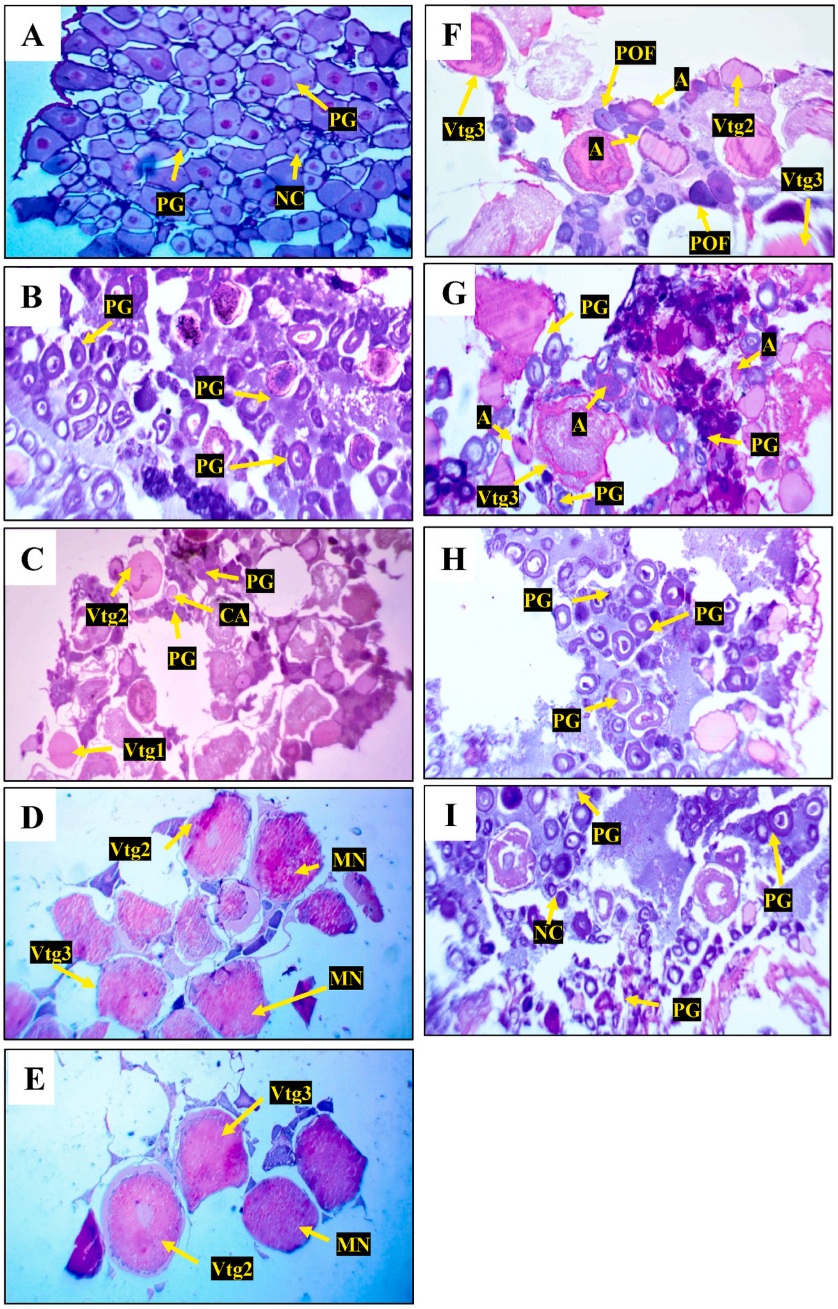

Fig. 4. Histological characterization of ovarian tissue of *Silonia silondia* collected from Kangsha River, Bangladesh. Alphabetic capital letters in each histological section incates the collection month of that ovary, viz., A = March, B = April, C = May, D = June, E = July, F = August, G = September, H = October and I = November. *NC, Nucler chromatin, PG, primary growth oocyte; CA, cortical alveolar; Vtg1, primary vitellogenic oocyte; Vtg2, secondary vitellogenic oocyte; Vtg3, tertiary vitellogenic oocyte; POF, post-ovulatory follicles, MN, migrating nucleus; A, atresia.*

vitellogenic stage was distinguished by the presence of yolk vesicles in the periphery of the oocytes. When the slides were stained with haematoxylin and eosin, they appeared colorless, as they were initially formed as a single row. The size and quantity of these yolk vesicles increased gradually, even though they were initially subtle bodies. The nucleoli were typically located at the periphery of the nucleus; however, they were occasionally observed in other locations within the nucleus. Oil droplets were observed within the cytoplasm, and the diameter of the Vtg2 oocytes increased in tandem with the progression of the yolk granule stage. The yolk granules looked deep pink with haematoxylin and eosin. The ratio of Vtg3 oocytes and late germinal vesicle migration (GVM) was abundant from June to July, and the fish moved into the spawning capable phase. This stage begins with the migration of the nucleus (MN) in GVM. The nuclear membrane broke down, yolk globules coalesced, and no nucleus was observed, although the follicle layer was still

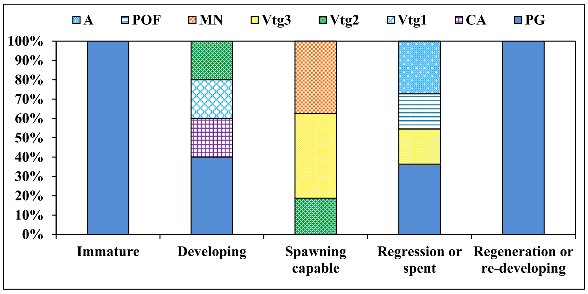

Fig. 5. Gonadal cell ratios persistence during the five distinct stages of ovary development in *Silondia silondia* (*PG, primary growth oocyte; CA, cortical alveolar; Vtg1, primary vitellogenic oocyte; Vtg2, secondary vitellogenic oocyte; Vtg3, tertiary vitellogenic oocyte; POF, post-ovulatory follicles, MN, migrating nucleus; A, atresia*).

visible and considered atretic oocyte or atresia. Finally, the complete vision of yolk globules was evident, and an overall increase in oocyte translucency was observed. The fishes with the regressing or spent ovary were abundant during August and September with atresia and some healthy Vtg2 and Vtg3 oocytes. The ovaries with a higher percentage of nuclear chromatin, and PG during October and November indicated a regeneration stage. In this stage, the ovary wall becomes thick, and the ovaries are more likely in the immature stage.

4. Discussion

The oocyte maturation stages were identified as: immature, developing, spawning capable, regression or spent and regeneration phases consisting of nuclear chromatin, PG oocytes, CA, Vtg1, Vtg2, Vtg3, and atresia. There are no previously published reports on the gonadal histology of *S. silondia*; therefore, it is impossible to provide a clear comparison. However, different gonad developmental stages observed in the present study have nearly corresponded with those described microscopically for two catfish, namely *Mystus vittatus* [17] and *Mystus montanus* [18]. A slight difference in the present study could be due to the rainfall pattern and intensity, temperature, and photoperiodism, as Miranda et al. [19] and Bhattacharyya and Maitra [20] reported in *Odontesthes bonariensis* and *Catla catla*, respectively. Most authors divide the oogenesis process in teleost fishes into four to eight stages of oocyte development based on species and the choice of criteria used [16,21], and our results have well corresponded with those criteria.

In the present study, the higher percentage of PG oocytes was recorded from March to May, while the higher percentage of Vtg2, Vtg3 oocytes and MN in GVM was recorded in June and July, indicating the spawning season of *S. silondia*. A similar trend of oocyte developments in catfish *Ompok pabda* was reported by Chakraborty et al. [2] based on ovarian histology.

GSI and histological analysis have corroborated that *S. silondia* breeds once in a breeding season. The highest percentage of GSI and mature oocytes in the ovary indicated the possible peak spawning season of *S. silondia* in July. However, a nominal peak value of GSI was also observed in June, alluding to the possibility of spawning this month. Therefore, the results indicate that *S. silondia* females may spawn in June and July. A similar outcome was observed in freshwater catfish (*Rita rita),* displaying a peak breeding activity in June and July [22].

These novel outcomes have demonstrated the GSI values of females show a significant difference between different months characterized by the distinct breeding phases. The increasing GSI values appeared in May, peaked in July, and decreased from August onward. Histological observation also showed abundant Vtg2 and Vtg3 oocytes from June to August, indicating the final phases of egg maturation and spawning. Furthermore, it was established that the fish spawns once a year, with the peak of spawning in July.

5. Conclusion

It can be concluded that the current study will help better understand the monthly ovarian stages in *S. silondia,* subsequently assisting in selective breeding under captivity. Further, this study lays the foundation for the large-scale breeding efforts of this species in the hatchery, paving the way for its aquaculture. Furthermore, the findings of the study will provide valuable insights for the future development of fisheries conservation strategies and enhance the effectiveness of management options for endangered fish species. The study recommendes conducting investigations throughout the year, spanning two consecutive years, in order to obtain clear conclusions about the gonadal maturation of *S. silonida*. The study of the gonadal development of male individuals is also recommended.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Farjana Jannat Akhi: Investigation, Data curation. **Shahroz Mahean Haque:** Supervision, Funding acquisition. **Md Idris Miah:** Validation, Supervision. **Md Ayenuddin Haque:** Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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