

Some Aspects of Reproduction in Long Whiskered Catfish, *Sperata aor* (Hamilton 1822), from North-East Bangladesh

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Abstract

Different reproductive aspects such as sexual maturity, spawning, gonadosomatic index, fecundity, and histological changes in gonads of captive reared *Sperata aor* were investigated for a period of one year. Male and female fish were identified by based on morphological characteristics. The spawning season was found to be extended from May-August. Gonadosomatic index reaches maximum in August and lowest in September. Absolute fecundity varied from 59255 to 70586 with an average value of 64920. Absolute fecundity had been reported to be increased with total length, body weight. Histological study of ovary of *S. aor* indicated the presence of four developmental stages viz, early perinucleolar oocytes, late perinucleolar oocytes, yolk vesicle stage and yolk granular stage. Findings reveal that, *S. aor* has group asynchronous manner of ovarian growth and used to spawn numerous times in a year under favorable environmental conditions. The testes histology represents the presence of spermatocyte, spermatid, and spermatozoa. This species builds nests during breeding season and fries are found within the nests.

Introduction

Giant Catfishes belonging to Bagridae family and *Sperata* genus, are very popular and common freshwater habitant of South Asia with a promising nutritive and commercial value (Iqbal *et al.*, 2018). To the date four species i.e., *S. acicularis*, *S. aor*, *S. aorella* and *S. seenghala* belonged to this genus has been identified in Bangladesh. The long whiskered catfish, *Sperata aor* is one of the critically endangered fish species in Bangladesh (IUCN Bangladesh, 2015). Once it was a common dweller of rivers and canals in Afghanistan, Pakistan, India, Nepal, Bangladesh and Myanmar, but now its abundance has been declined due to over harvesting and countless ecological deviations in natural aquatic environment (Khan & Nazir, 2019). Therefore, the *Sperata sp.* is now being considered as a

one of diminishing indigenous fish fauna of India subcontinent (Khan *et al.*, 2016; Nazir & Khan, 2017).

Fishes are diversified vertebrates group and shows very distinctive reproductive strategies based on species, ecology and environment (Bolis *et al.*, 2001). This diversity is tending to be high largely due to enormous numbers of fish species (Vollf, 2005). A detailed information of reproduction is key to proper management of aquaculture and natural fisheries (Hossain *et al.*, 2017; Jannatul *et al.*, 2015; Mian *et al.*, 2020). Different allometric such as length-weight relationship, gonadosomatic index (GSI), fecundity and breeding peaks of fish are vital information regarding conservation and sustainable harvesting of any fishery stock (Mian *et al.*, 2020; Uddin *et al.*, 2017). The values of gonado-somatic index govern the maturation phase and spawning onset in several fish species. Histological

observation of gonad provides onset of maturation phases and spawning term of a species. Reproductive biology is especially concern to hatchery managers who depend on size at first sexual maturity and on the ontogeny spawning development (Neto, 2005). To begin the induced breeding system and culture in indoor controlled environments, it is indispensable to have data on breeding biology, season, and reproductive potentiality of a species. Therefore, current research has been undertaken to find out the key information of reproduction in *S. aor* at captivity.

Materials and Methods

Experimental Design and Nesting Observation

Fishes were stocked in total 6 ponds (each pond is of 40.5 m²) at 20 stocking density. Animals were feed efficiently twice daily around 10:00 am at morning and 4.30 pm at afternoon hours with artificial pelleted feed composed of dietary 38–40% crude protein, 13% moisture, 6% fiber and 3% crude fat. The feeding rate was maintained around 2.5-3% body weight of fish. Live small shrimp were also given weekly as live feed. Three experimental ponds covering each of 80.92 m² area were selected for observation of nesting behavior and morphology of nest formation. The ponds were nearly dry up to observe the nests.

Fish Sampling

Series of monthly field sampling and pond treatment was done for a period of twelve months started from July 2018 to June 2019. Laboratory assays were conducted in the laboratory of Fish Biology and Genetics, Sylhet Agricultural University, Sylhet. Field collected samples were bring back to laboratory to collect morphometrics data, and to identify their sex and maturation stages. Following the dissection, a piece of gonad had been preserved in neutral buffered formalin for histology.

Gonadosomatic Index (GSI) and Fecundity Estimation

Following the dissection of mature male and female fish, the weight of the gonad has been taken carefully to calculate GSI using formula from Brooks *et al.*, (1997).

$$GSI = \frac{\text{weight of gonad}}{\text{weight of body}} \times 100$$

Only matured ovary ready to spawn was selected for determination of fecundity. Total numbers of oocytes in whole ovary were accounted by following the equation from Rahman & Samat, (2020).

$$N=(W_t/W_s) \times N_s$$

Where: W_t = total weight of the gonad, W_s =weight of each subsample, and N_s =numbers of individual oocytes in each subsample.

Histological and Microscopic Observation of Gonads

Standard histological procedure described by Van-Dyk & Pieterse, (2008) has been followed to perform the histological study of gonad samples. Histological slides were examined under compound light microscope and images were taken with the attached digital camera (Olympus Xcam-Alpha, Germany) and maturation stages were identified according to the features described by Feist, (2009).

Data Analysis

All statistical data was treated by inputting in using Microsoft Excel sheet and analyzed in IBM SPSS 26. Variance between group means were justified by one-way analysis of variance (ANOVA) and Duncan's multiple range test and all the significance was established at $P<0.05$.

Results

Sex Determination

Entire body surface of all the male brood fishes was inflamed and bright reddish in color during July-August. A thick milky white fluid secretion or scum formed on their body surface (Figure 1.A-B). Further, dissection and histological investigation also confirmed them as male. All mature male parade a pointed genital papilla which is typical to most of teleost. The abdomen of the female brood fishes was softening, swollen, and rounded, the genital opening tends to be ovate, protruded, and reddish in all mature fish (Figure 1.C-D).

GSI and Fecundity of *S. aor*

The gonado-somatic index showed a well gradual rising from September to August. The GSI values were fluctuated between 0.47 ± 0.04 to 3.00 ± 0.02 and 1.08 ± 0.01 to 0.12 ± 0.01 for female and male *S. aor* respectively during the period of August to July (Figure 2). Higher peaks for GSI were detected during June to August from 2.60 ± 0.03 to 3.00 ± 0.02 and 0.86 ± 0.01 to 1.08 ± 0.01 for female and male respectively (Figure 2).

The fecundity had been accounted by sacrificing 24 randomly allocated mature female brood encompassing total length ranged between 46.83 ± 0.15 cm to 51.33 ± 0.19 cm, weight from 901.41 ± 20.81 gm to 1084.56 ± 12.07 gm and ovary weight from 20.96 ± 0.12 gm to 32.56 ± 0.48 gm. The fecundity was found to be fluctuated from 59255.17 ± 184.03 to 70048.86 ± 131.99 (Table 1). There was no significant difference between the fecundity from May and June as well from between and July August (Table 1).

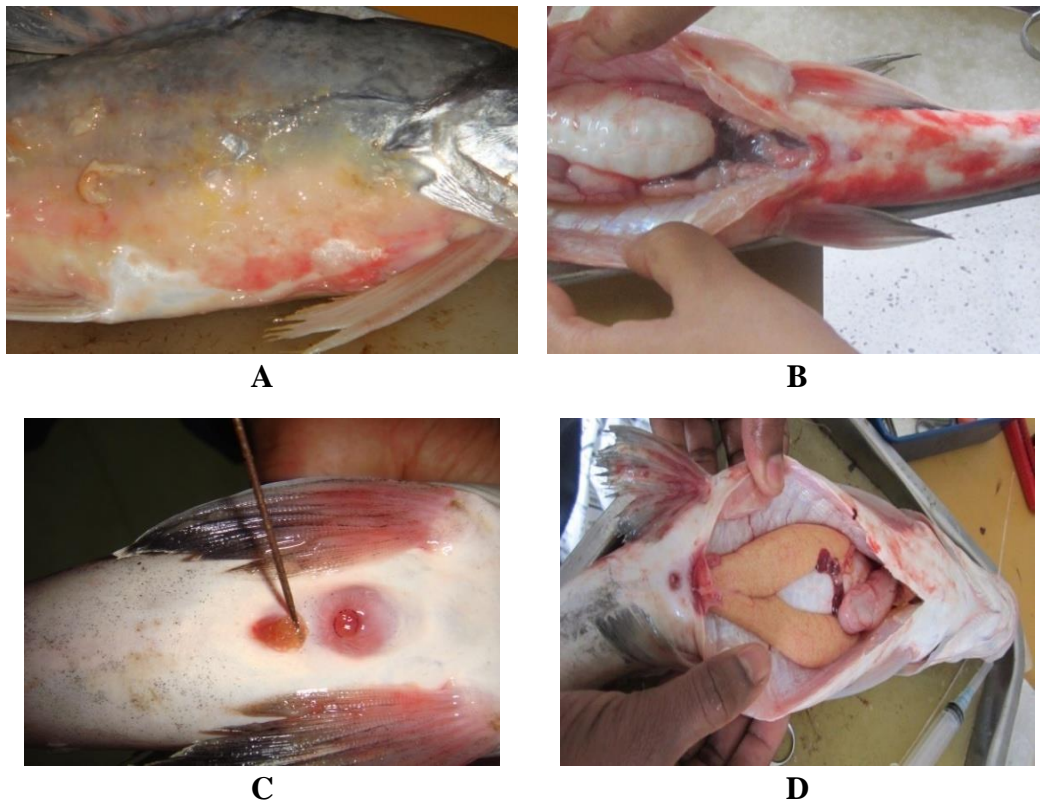


Figure 1. A-B. Thick milk like scum on the mature male *S. aor*; C-D. Female genital opening.

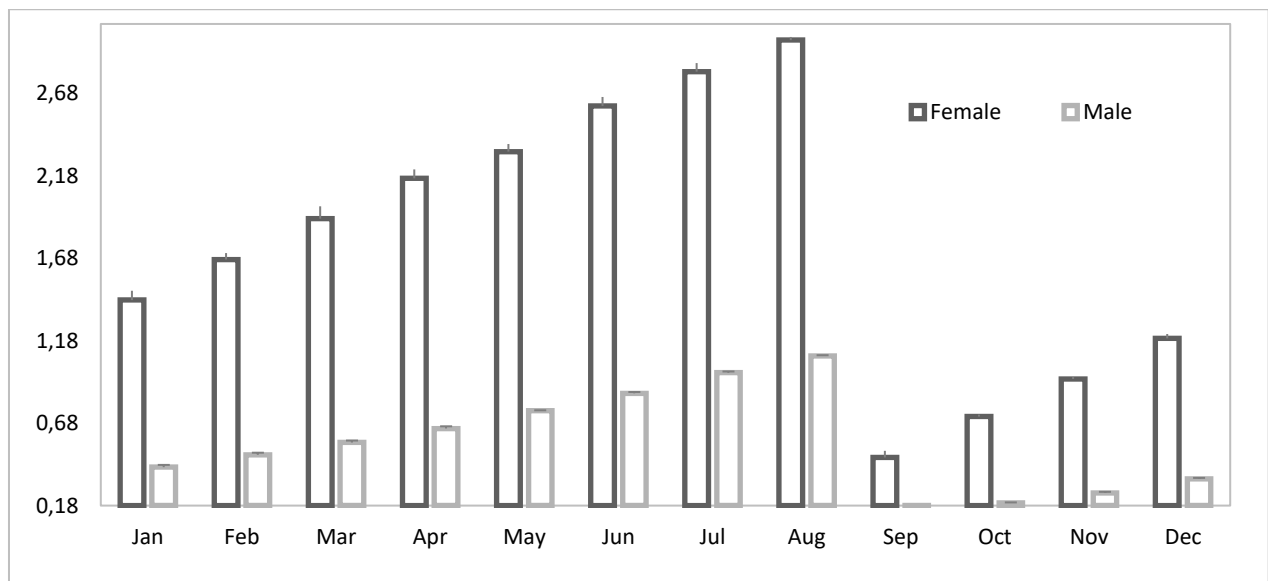


Figure 2. Monthly variation of GSI of female and male *S. aor*.

Table 1: Mean and standard deviation (\pm SD) of body weight, ovary weight and fecundity of *S. aor*

Month	Total length (cm)	Body weight (g)	Ovarian weight (g)	Fecundity
April	43.69 \pm 1.02 ^a	762.59 \pm 26.24 ^a	16.49 \pm 0.18 ^a	*
May	46.83 \pm 0.15 ^b	901.41 \pm 20.81 ^b	20.96 \pm 0.12 ^a	59255.17 \pm 184.03 ^a
June	48.26 \pm 0.28 ^b	966.84 \pm 22.47 ^c	25.17 \pm 0.88 ^b	63314.67 \pm 153.60 ^a
July	51.1 \pm 0.22 ^b	1027.85 \pm 21.36 ^c	28.88 \pm 0.49 ^b	67342.67 \pm 253.71 ^b
August	51.33 \pm 0.19 ^b	1084.56 \pm 12.07 ^c	32.56 \pm 0.48 ^b	70048.86 \pm 131.99 ^b
September	39.85 \pm 1.65 ^a	705.12 \pm 90.55 ^a	3.35 \pm 0.69 ^c	*

*oocytes were too minute in size for counting. In column, means indicated by same letters stands for non-significant different from each other at 5% level of probability by DMRT.

Relationship Between Biometric Parameters of Fish

The linear regression relationship of total length and body weight gave correlation coefficient of 0.909 (Figure 3) and represents strong explanation of fish body weight by their length. The relationship of fecundity alongside the body weight produced a correlation coefficient of 0.90 (Figure 4.B), and ovarian weight to body weight was 0.955 (Figure 4.A).

Histological Observation of Gonad

The histological analysis of the ovarian cell during the reproductive season revealed that whole oocytes within an ovary did not matured synchronously and oocyte at different maturation phase were observed as well, which refers that *S. aor* are breed as batch spawner. In initiation of yolk vesicle phase, oocyte disruptions from the epithelium germinal layer and form an enveloped composed of follicular epithelium (Figure 5.I-II). Peripheral nucleoli appear at the bordering area next to the nucleus (thin lines) and cortical alveoli formed (Figure 5.II). The vitellogenesis phase was featured by the entrance of yolk molecules and fat vacuoles in the ooplasmic area of oocytes (Figure 5.III-V). In histological observation of male gonad, the initial maturing stage was characterized by sperm duct (SD), visible but thin spermatocytes (SC) more in number with spermatogonia (S) (Figure 5.VII), while in matured phase, the spermatozoa were noticed (SZ) in the sperm duct (SD) and sperm duct being thicker or fuller due to the fluids in them (Figure 5.VIII)

Nest Building Observations

Most of the nests found near the periphery of the pond at 3-4 feet depth and they were circular in shape. Information recorded over the 12 month of study period. Nest building is one of the important parameters to know the breeding nature of the fish. The average diameter and depth of the nests were 99.67 ± 1.83 cm and 24.63 ± 2.04 cm, respectively (Table 2). While the average total body length and depth of brood fishes were 50.26 ± 1.39 cm and 6.82 ± 0.43 cm, respectively. There was no significance difference between the nest parameters and fish biometrics for all nest in the three-pond system. Fries of *S. aor* were reported in the pond with artificial nests during July-August, while absent in the pond with without artificial nests. So, it can be concluded that the fish breeding and nursing of young took places within the nest.

Discussions

Body constitute of fish is found to be change in response to rearing water quality and season (Dygart, 1990; Hagmayer *et al.*, 2018). Gonadosomatic index used as good an indicator of the spawning peak of teleost (Ali & Kadir, 1996; Vlaming, 1972). The mean GSI values during the one-year study period showed the presence of a single breeding season in *S. aor* from July to August. There was a gradual rise in the GSI values from September to August. The highest gonado-somatic index of female and male *S. aor* was 3.00 ± 0.02 and 1.08 ± 0.01 respectively in August. On the other hand, the

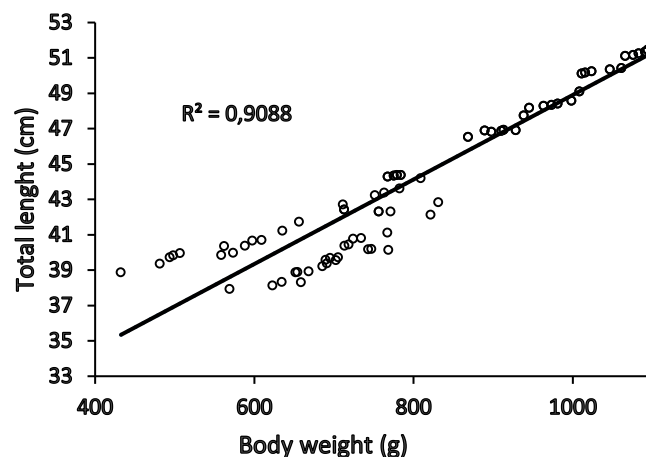


Figure 3. Relationship between total length and body weight of *S. aor*

Table 2. Diameter and depth of nest prepared by *S. aor* on the experimental ponds (No. of total nests=30)

Pond	No. of nests	Nest		Fish	
		Diameter (cm)	Depth(cm)	Total length (cm)	Body depth (cm)
1	10	99.88 ± 1.83^a	25.73 ± 1.95^a	50.38 ± 1.38^a	6.75 ± 0.45^a
2	10	94.47 ± 1.92^a	23.53 ± 1.80^a	48.15 ± 1.46^a	6.89 ± 0.42^a
3	10	97.90 ± 0.93^a	21.53 ± 1.90^a	46.38 ± 1.42^a	6.69 ± 0.47^a

*All values are expressed as Mean \pm SE. In column, means indicated by same letters stands for non-significant different from each other at 5% level of probability by DMRT

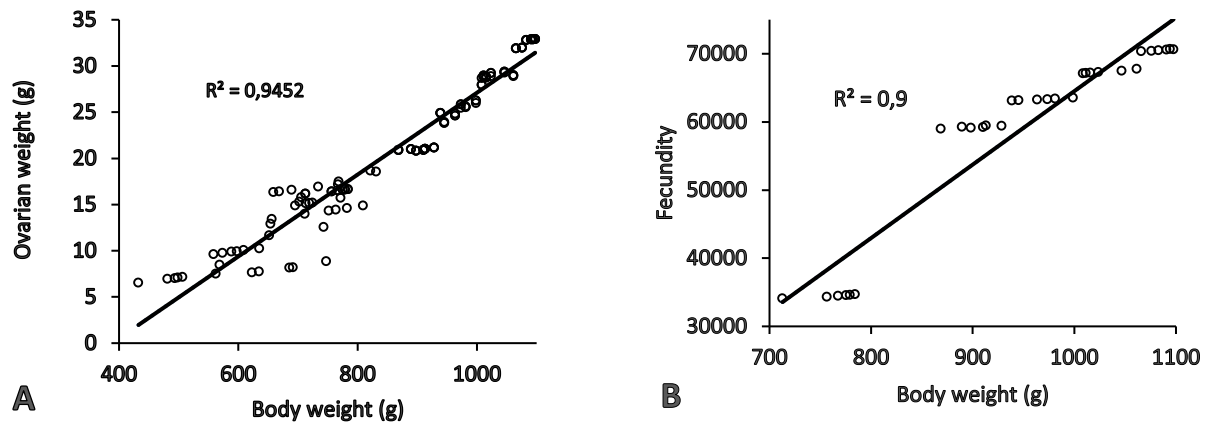


Figure 4. A. Relationship between ovarian weight and body weight, and B. fecundity and body weight of female *S. aor*

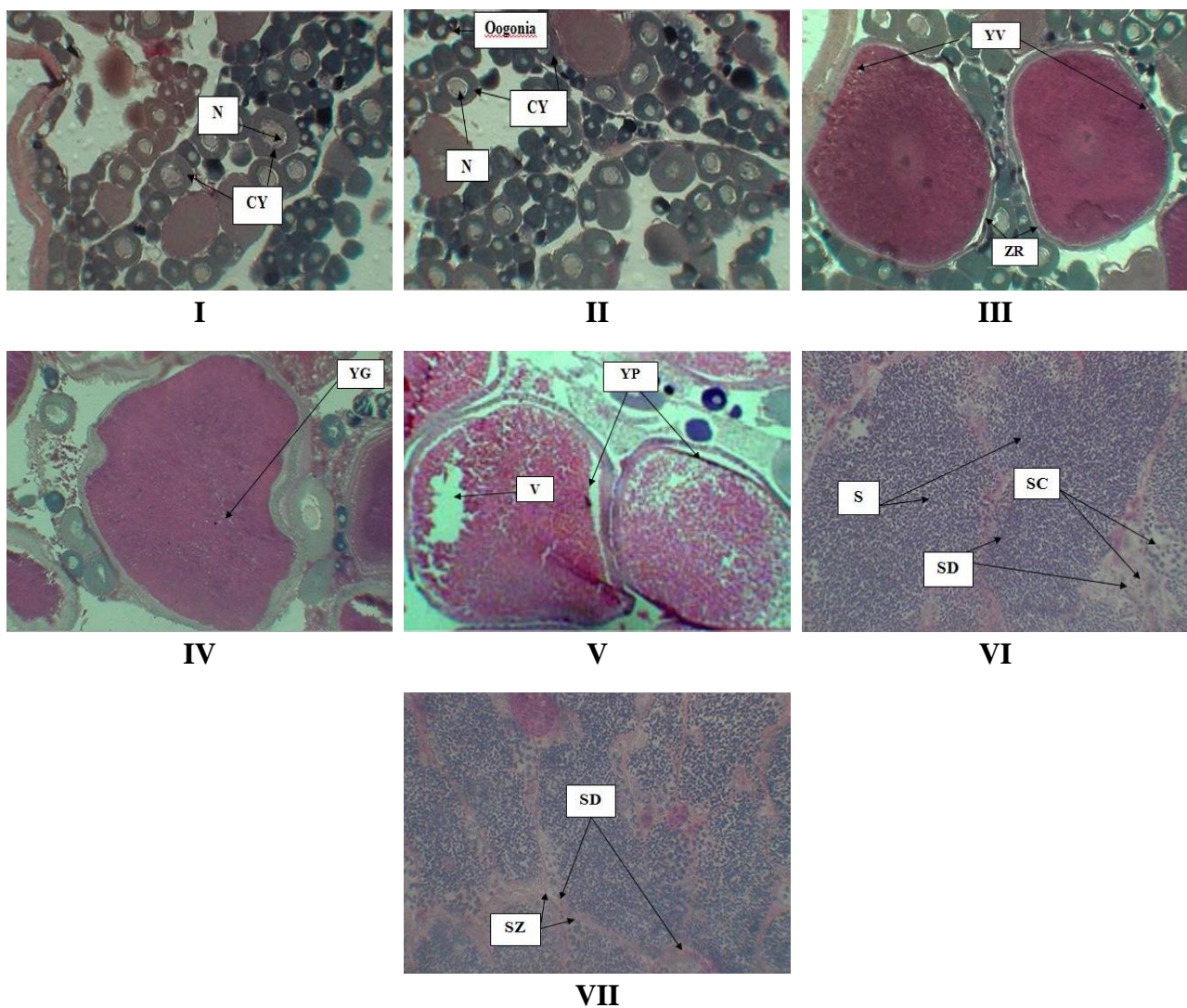


Figure 5. Plate I: Early perinucleolar stages in the ovary (January to February, H&E $\times 10$); Plate II: Late perinucleolar oocyte (LPO) stage in the ovary (March to April, H&E $\times 10$); Plate III: Yolk vesicle stage of oocytes (YVO) in the ovary (May and June, H&E $\times 10$); Plate IV: Early yolk-granule stage of oocytes (YGO) in the ovary (July, H&E $\times 10$); Plate V: Late yolk-granule stage of oocytes (YGO) in the ovary (August, H&E $\times 10$); Plate VI: Maturing stage of testis of male *S. aor* in May and June (H&E $\times 10$); Plate VII: Mature stage of testes of male *S. aor* in July and August (H&E $\times 10$). (N-nucleus, CY-cytoplasm, YV-yolk vesicles, ZR-zona radiata, YG-yolk globules, YP-yolk protein, V-fat vacuoles, S-spermatogonium, SC-spermatocytes, SD-spermatids, SZ-spermatozoa).

lowest gonadosomatic index of female and male and the lowest was 0.47 ± 0.04 and 0.86 ± 0.01 respectively in September. This indicates that the breeding season of *S. aor* lasts for long duration from April to August and peak in July to August. Similar trend in GSI value has been observed by several previous researchers who had recorded maximum value of gonadosomatic indices during breeding season for both sexes as 6.363 in *Pomadasys stridens* (Amtyaz *et al.*, 2013), $22.85\% \pm 20.00$ in *Chrysichthys auratus* (Ragheb, 2016) and 12.50 ± 4.97 in Menoda Catfish, *Hemibagrus menoda* (Shehu Jega *et al.*, 2018). As gonads continued to be matured their weight tend to be increased for deposition of maturing molecules and also ovarian mass risen dramatically due to the reception of vitellogenine protein from liver in breeding season. The fecundity variation is common in fish and correspondent to species, size, and area (Gupta, 2014; Tessema *et al.*, 2020). The low oocytes number is related with strong maternal and paternal attention (Cimadomo *et al.*, 2018; Fávoro *et al.*, 2005; Milachich & Shterev, 2016). The absolute fecundity in present research ranged between 59255 to 70048 and seems to be highly fecundated in comparison to other catfish species. The value of absolute fecundity was between 785-14066 oocytes in female feral catfish, *Clarias macrocephalus* (Ali, 1993), 2000- 20600 eggs for female *T. tandanus* in the Gwydir River (Davis, 1977). Current findings were correlated with previous research in Asian striped catfish *Mystus vittatus* (Hossain *et al.*, 2006), Striped Dwarf Catfish *Mystus tengara* (Mitu *et al.*, 2014), and in *Mystus cavasius* (Latif *et al.*, 2018). Total length, body weight and fecundity seem to be correlated in different fish species (Hossain *et al.*, 2017; Kohinoor *et al.*, 2013; Mian *et al.*, 2020). Present study also indicates that fecundity of *S. aor* correlates with body weight. Several researches have noted positive r^2 value for total length and body weight (Schwartz & Jachowski, 1965). A research revealed r^2 of 0.965 for *Pseudomystus siamensis* (Jonathan *et al.*, 2013), $r^2=0.836$ for *H. nemurus* (Jonathan *et al.*, 2013) and $r^2>0.9$ for Long snouted Catfish, *Plicofollis argyropleuron* in Malaysia (Rosli & Isa, 2012). The regression results between fecundity, ovarian weight and body in the present study indicated that the fecundity of *S. aor* had a linear relationship with the body weight where $R^2>0.9$ for both cases. The oocytes output is found to be increased with increasing size and large fishes tend to have more gonadal mass as well as total oocytes numbers (Hagmayer *et al.*, 2018; Kulabtong, 2016). However, seasonal influences are also key in determining the sequential changes in the ovary, size of fish as well as their spawning terms (Helmizuryani *et al.*, 2020; Kapil *et al.*, 2011).

Histological features of gonadal cell provide key information regarding maturation phases, and spawning schedule (Cimadomo *et al.*, 2018; Jannatul *et al.*, 2015; Jega *et al.*, 2018; Uddin *et al.*, 2017). Ovarian growth phases in *S. aor* was investigated in order to disclose the pattern and schedule of designated growth phase and

development phase of gonadal germ cells. It has been noted well that the fish found solely in immature phases during November to April. Ovarian yolk vesicle phase acted in the month of May to June whereas granular phases of yolk growth were located in the month of July to August. The ovarian development of *S. aor* was group asynchronous in nature revealed from histological examination. The present results were agreed with other investigation on *Rita rita* (Rahman & Mollah, 2014), *Clarias lazera* (Emam & Abughrien, 2014) and *Clarias gariepinus* (Elias *et al.*, 2018; Okoye *et al.*, 2018; Tyor & Pahwa, 2017). Following the histological examination of male gonad, the testicular germ cell stages of spermatogonia (S), spermatocyte (SP), spermatozoa (SZ) were observed during May to August. Current findings agreed with former research in other catfish *P. hypophthalmus* (Manosroi *et al.*, 2004) and *Pangasianodon hypophthalmu* (Hassan *et al.*, 2011).

The result of nesting in current study give an assumed of constructing nests by the brood fishes that are nearly two times larger of their total length, although nest's depth is counted as half of their total length. Nesting is one of the important aspects to know the breeding habit, breeding season and parental care of fish and also serve to attract the potential partner for successful breeding and courtships behavior (Navarrete-Fernández *et al.*, 2014; Pärssinen *et al.*, 2019). The purpose of nesting in *S. aor* might have both parental nursing and reproductive functions as nest were found in both pre-spawning and post-spawning periods.

Conclusions

Information on reproductive biology is important for planning, conservation, and management of a threatened fish species. The results of the study would be effective tools towards manipulation of management and protection plans for sustainable conservation and captive growth. The information gathered through the present experiment may be used for better management of current *Sperata aor* fishery in Bangladesh as well as their future conservation strategies and also for induction this species as a potential candidate into commercial aquaculturist.

Ethical Statement

The research has been fully conformed with all sorts of regional, national, and institutional animals' ethics approval.

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Author Contribution

The manuscript comprises postgraduate research project of first author. The order of other authors list

reflects the chronology of contribution by different author in current research work. However, the last author will be treated as team leader as per the university rules of authorship in teamwork.

Conflict of Interest

The authors declare that they have no conflict of interest.

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