Reproductive aspects of Spotted scat, *Scatophagus argus*, in the South-West coast of Bangladesh

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Abstract. Spotted scat (*Scatophagus argus*) is a delicious and commercially important brackishwater species, but the prevalence of this species is declining in Bangladesh coast due to different natural and manmade interventions. The present study aimed to investigate the reproductive biology of *S. argus* population existing in the South-West coast of Bangladesh. The samples (47) were collected once every month from August 2020 to July 2021. Gonads were collected, the Gonado-Somatic Index (GSI) and fecundity were assessed and histological slides of gonad were prepared for microscopic observation. The mean GSI was found highest in June (20.16±4.87%) and lowest in November (0.882±0.65%). The fecundity range of individual fish was 221,080.75-495,235.29 and the monthly average absolute fecundity was found maximum in June (487,474.69±10,975.13). The minimum length of the youngest mature female was 14.3 cm. The fecundity increased with the increase of total length, body weight and gonad weight, among which a significant linear relationship exists. In contrast, the length-weight relationship (R^2=0.7365) was non-linear, positively correlated and showed a negative allometric growth (b=2.601). Moreover, the gonadal histology demonstrated seven stages of oocyte development. Early perinuclear stage and late yolk granule stage were found in March and June respectively. Based on the GSI, fecundity, egg diameter and gonadal histology, it was revealed that the spawning season of *S. argus* was from April to October with a major peak in June and a minor peak in September. These findings will assist the development of artificial breeding techniques and sustainable management of this species in an open ecosystem.

Key Words: gonadosomatic index, fecundity, gonadal histology, *Scatophagus argus*.

Introduction. The Spotted scat, *Scatophagus argus* (Perciformes: Scatophagidae), also known as Chitra, is among the few economically significant teleost species that could potentially thrive in tropical brackish water fish ponds (Bardach et al 1974). They are dispersed in freshwater, brackish water, and marine environments due to its euryhaline nature. It is widely distributed in the coastal waters of the Indo-Pacific Ocean (Ni & Kwok 1999), which includes the coastal areas from southern India, Bangladesh, and Sri Lanka to the southern part of Japan and Tahiti (Pinto & Punchihewa 1996). This species has a high demand as a food source in Southeast Asia (Barry & Fast 1988; Musikasung et al 2006; Wongchinawit 2007), in addition to its popularity as an ornamental fish (Amarasinghe et al 2002; Bambaradeniya et al 2002). This species contributes significantly to the fishery resources of Bangladesh, both from a commercial and a personal consumption viewpoint. It is also highly demanded on the international market because of its unique taste. It can tolerate extreme fluctuation of environmental variables such as temperature, salinity, dissolved oxygen, tidal movement, river runoff, turbulence and turbidity (Barry & Fast 1992). In Taiwan, this fish species has conventionally been used to limit the growth of algae in ponds (Shao et al 2004). However, the presence of this species in nature is diminishing gradually due to a combination of over exploitation, habitat destruction, climate changes etc. This species is listed in the "least concern" category of the IUCN red list of threatened species (IUCN 2021). To conserve this species, it is essential to develop its induced breeding technique in a controlled culture environment. Similarly, knowledge on breeding biology is a prerequisite to develop artificial breeding techniques and sustainable management of a fish stock. The most essential parameters to determine the spawning cycle of any fish are the GSI, fecundity and gonadal histology. It also evaluates the maturity level of the ovary (Nandikeswari et al 2014). The GSI helps to find out the exact time of the breeding season and breeding frequency of fish species (Ghaffari et al 2011; Shafi 2012; Jan et al 2014). Reliable information on the fecundity of any fish is essential for the evaluation of commercial potential, stock study, life history, culture and the management of fishery (Zin et al 2011). In addition, histology is used to determine the stages of the biological cycle, gonadal maturity stages and spawning (Hasan et al 2018).

Despite the enormous importance of *S. argus* in brackish water aquaculture, the breeding biology of this valuable species has not been approached so far on Bangladesh's coast. Although, there are few published reports on breeding biology and larval development of *S. argus* from far-east and south-eastern countries (Barry & Fast 1992; Zeping et al 2010), there is no exclusive information available on different aspects of the reproductive biology of this species on the Bangladesh's coast. Therefore, considering the importance of this species, a comprehensive study was conducted as a first attempt to explore the reproductive biology including gonadosomatic index, fecundity and gonadal development stages through gonadal histology for determining the peak breeding season of *S. argus* in Bangladesh coast which will helps to develop their induced breeding techniques as well as their proper management in natural environment.

Materials and Method

Study site and sample collection. In the current study, a total of 47 fish specimens were taken from the catches over a 12 months period starting from August, 2020 to July 2021 from the coastal river 'Shibsha' adjacent to the Sundarban mangrove forest (La: 22.330, Lo: 89.292) and connected to the northern coast of Bay of Bengal (Figure 1).

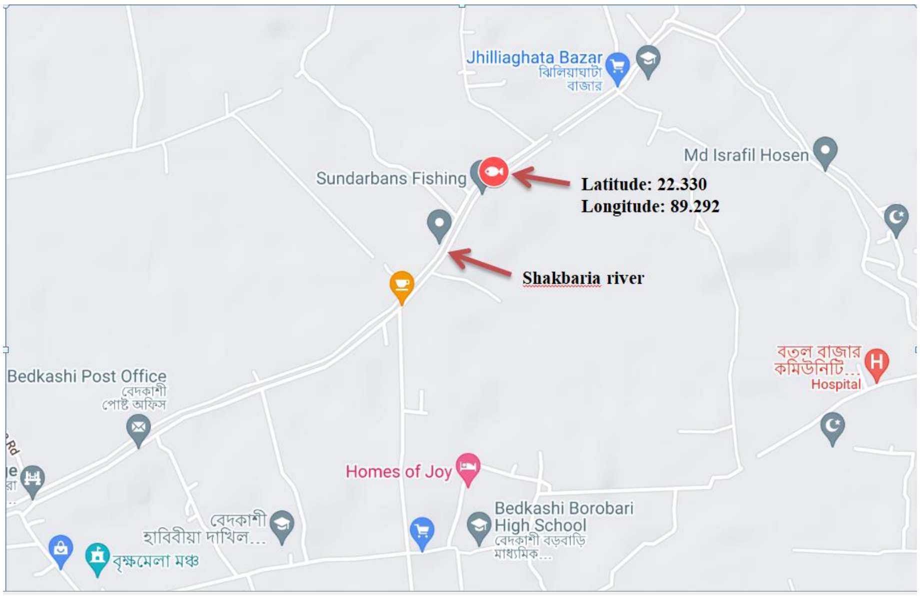

Figure 1. Study area.

Sample processing and measurement. Immediately after collection, length and weight of the specimens were measured using a scale and a digital balance (Model-EK500HA, Japan) with 0.1 g accuracy, in the Biology Laboratory of Brackish water station, Paikgacha of Bangladesh Fisheries Research Institute. After dissection, intact gonads were preserved with 10% formalin in well-labeled vials. The oocyte diameter was measured using an ocular micrometer attached to the microscope's eyepiece (LEICA DM500).

Calculation of the Gonado-somatic index (GSI). The gonad of each female fish was dissected in order to determine the ripeness of the gonad and the breeding period of the fish. The gonadosomatic index (GSI) of the collected samples was calculated as follows (Afonso-Dias et al 2005):

$$GSI = (\text{Gonad weight}/\text{Body weight of fish}) \times 100$$

Estimation of fecundity (F). Absolute fecundity of fish was estimated using the gravimetric method. The weight of ovaries was measured with a digital electric balance (HT224R, Japan) with 0.0001 g accuracy. Then, 0.1 g of eggs was taken from each ovary's anterior, middle, and posterior regions of each lobe. Under a stereomicroscope, the average number of eggs in 0.1 g of sub-sample was determined and then multiplied by the total weight of the ovary to estimate the total number of eggs; that is, the absolute fecundity of the relevant fish (Yeldan & Avsar 2000):

$$\text{Fecundity (F)} = (WOV/WSs) \times NOV$$

Where:
WOV - weight of the ovary;
WSs - weight of the sub-samples;
NOV - number of mature ova in sub-samples.

Histology of gonad. The histological study was carried out in the Fish Disease Laboratory of Bangladesh Agricultural University using the 'animal tissue technique' method (Humason 1972). Initially, the gonads were dehydrated in a series of ethanol concentrations (70 to 100%), followed by xylene clarification and molten wax infiltration using an automated tissue processor (Leica ASP300 S, Germany). With a Microtome (Leica RM2255, Germany), 2 m thick paraffin-embedded blocks were sliced, stained with hematoxylin and eosin, mounted with Canada balsam and covered with a cover slip. A light microscope (OLYMPUS BX 53) was used to examine the slides and take photographs for further observation.

Relationship between different parameters. To demonstrate the mathematical relationship of fecundity with total length, body weight, and gonad weight, the following statistical formula was used to calculate the coefficient of determination (R^2) (Achakzai et al 2013):

$$Y = a + bX$$

Where:
Y – fecundity;
X - total length (cm) or body weight (g) or gonad weight (g);
'a' & 'b' - regression constants whereas, in the relationship between body weight and gonad weight,
Y - gonad weight (g);
X - body weight (g).
The length-weight relationship was established following the power equation (Froese 2006):

$$W = a \times TL^b$$

Where:
W - the total body weight (g);
TL - the total length (cm);
a and b - constants.

Statistical analysis. Microsoft Excel 2010 and SPSS version 20.0 were employed to determine the linear and non-linear relationships and coefficient of determination (R^2) of fecundity with total length, body weight and gonad weight and of body weight with total length and gonad weight.

Results and Discussion

Total length, body weight and gonad weight. As shown in Table 1, the lowest and highest individual values of both body weight and gonad weight were found in November and June respectively (Table 1). The monthly average value of total length varied between 15.5±1.79 cm in September and 18.11±0.67 cm in April. The monthly average body weight ranged from 128±7.07 g in July to 181.88±24.77 g in April. The highest average value of gonad weight (26.95±13.22 g) was found in June and the lowest average value was found in October (1.2±0.11g). In the current study, the range of length and weight was smaller than those reported (TL of 7.5 cm~25.5 cm and BW of 8.35g~1.2 kg) from the Philippine coast by Barry & Fast (1992). Variations of the total length and body weight might be due to the different sampling area with different feed density, as well as the differences in age, maturity and sex (Yaglioglu et al 2014).

Table1
Mean, maximum and minimum value of different parameters of female *Scatophagus argus*

Parameters	*Minimum*	*Maximum*	*Mean ± SD*
Total length (cm)	12.5	20.2	16.89±1.77
Body weight (g)	85	297	173.51±56.04
Gonad weight (g)	0.47	23.4	11.66±5.898
GSI (%)	0.7692	21.32	7.78±5.47
Fecundity (nos.)	221,080.8	495,235.29	360,777.60±73,505.33

Gonadosomatic index and length at first maturity. The highest monthly average GSI value of *S. argus* females was found in June (20.16±4.87%) and the lowest average value was found in November (0.88±0.65%). GSI values progressively increased from February to reach its major peak in June which indicates the peak spawning season. Thereafter, it showed a sharp fall in July and raised again up to its minor peak in September then descended once again in October to reach a plateau till December (Figure 2a).

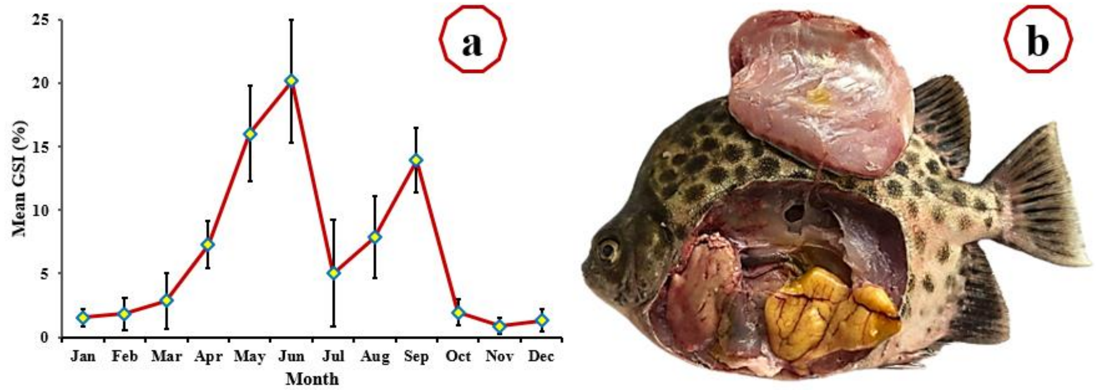

Figure 2. (a) Monthly variation of GSI (Mean±SD) of *Scatophagus argus*; (b) *Scatophagus argus* with fully developed gonad.

GSI is a good predictor of the gonadal maturity which increases with the fish maturation until it reaches its maximum and then it declines sharply (Akter et al 2012). However, Gandhi et al (2014) found the highest GSI value (8.6%) in July, during the spawning period (from May to November), on the Mandapam coast of India. Ze-ping et al (2010) observed the spawning duration from April to August in the South China coastal water, with the highest GSI value (14.74%) in June, which strongly supports the present study. Variations of the spawning period and reproductive cycle are influenced by the variety of environmental conditions (Agarwal 2008). Figure 2 displays a scatter plot graph of the total length with the respective GSI. The diagram demonstrated that the smallest mature female measured 14.3 cm in length. The size at sexual maturity of this species in Mandapam coast, India (Gandhi et al 2014), Philippine's coast (Barry & Fast 1992), and Malaysian waters (Morioka et al 2020) was greater than 14 cm TL for females, which strongly supports the result of this study.

Fecundity. As shown in Table 2, the lowest individual value of fecundity (221,080.75) was observed at a total length of 12.5 cm and a body weight of 97 g in September, while the highest individual value (495,235.29) was found at a total length of 17.8 cm and a body weight of 233 g in June. This suggests that large fish have bigger body cavities and contain more energy for egg production, which is consistent with the observation of Rheman et al (2002). Ze-ping et al (2010) found a highest absolute fecundity of 635,755 oocytes, whereas Barry & Fast (1988) recorded a maximum fecundity of 807,000 in this species. On the contrary, Gandhi et al (2014) found a fecundity range from 115,038 to 153,661, which was much lower than in the present study. Discrepancy between the fecundity values is a very usual phenomenon in fish (Reddy & Rao 1991) and is influenced by a variety of factors, including size, age, sex, environmental factors, presence of available food and space (Hunter 1992), fish stock, nutritional condition, and genetical characteristics (Das 1977).

Table 2
The monthly recorded fecundity range and mean fecundity of *Scatophagus argus*

Month	*No. of fish examined*	*Fecundity range*	*Mean fecundity±SD*
April	5	368,798-471,678	405,540.33±57,394.33
May	4	392,612-475,800	434,206±58,822.80
June	4	479,714.10-495,235.29	487,474.69±10,975.13
July	5	281,043-332,047	286,575.37±92,623.39
August	4	271,428.75-329,745.85	306,323.14±30,804.64
September	6	221,080.75-352,070	316,711±30,990.24

Relationship among different parameters

Length-weight relationship (LWR). As shown in Figure 3, the total length and body weight showed a non-linear and positive relationship (R^2=0.7365), according to the following equation: BW=0.107*$TL^{2.601}$ (Figure 3b).

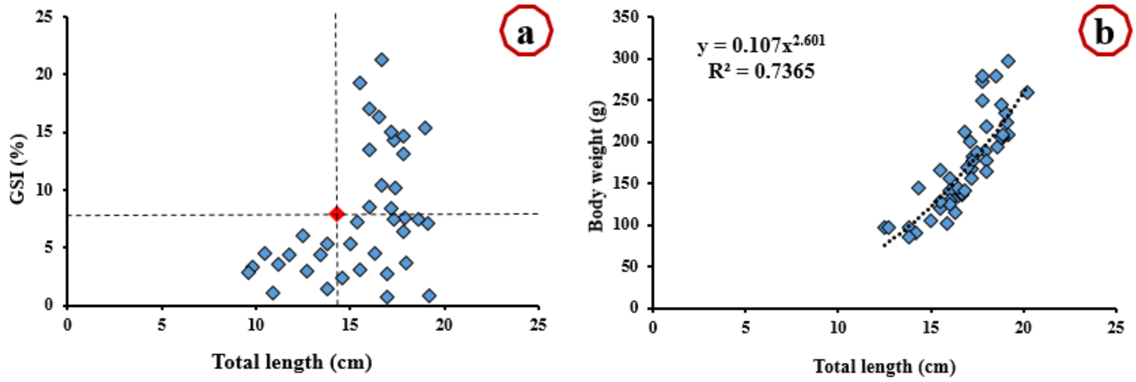

Figure 3. (a) Relationship of total length with corresponding GSI (the point with a red mark indicates the youngest mature female); (b) Relationship between total length and body weight of *Scatophagus argus.*

The exponential expression b, of the length-weight relationship was 2.601 for the combined sexes, which indicates a negative allometric growth (b<3). Similar findings of *S. argus* were observed by Hashim et al (2017) in the Malaysian coast (b=2.76, R^2=0.824), Mandal et al (2020) in the Chennai coast (b=1.14, R^2=0.709) and Musarrat-ul-Ain & Rehana (2018) in the Karachi coast, Pakistan (b=2.88, R^2=0.996). The variations of the LWR depend on the population, season and environmental conditions

(Froese 2007), gonad maturation, sex of individual fish, condition of stomach and wellbeing of fish (Karna et al 2012).

Relationship of fecundity with other parameters. Positive and linear relationships were found between the gonad weight and body weight with the fecundity and gonad weight. The equation of these relationships, the coefficient of determination (R^2), the 'a', 'b' and '*P*' values are depicted in Figure 4 and Table 3.

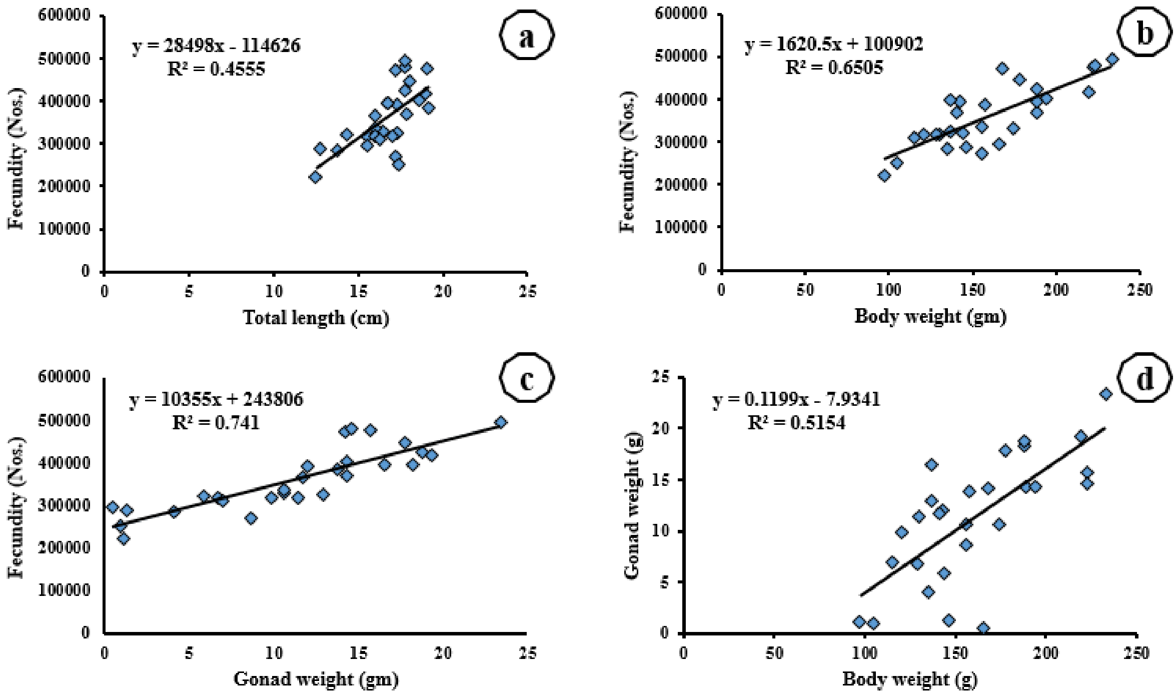

Figure 4. Relationship between a) Fecundity and total length; b) Fecundity and body weight; c) Fecundity and gonad weight; d) Gonad weight and body weight.

A highly positive relationship (R^2=0.741) was observed between the fecundity and gonad weight, whereas a moderate positive relationship was found in case of the fecundity and body weight (R^2=0.6505), and the gonad weight with body weight (R^2=0.5154). The total length and fecundity showed a low positive relationship (R^2=0.4555) (Figure 4). These findings agree with Hashim et al (2017) for *S. argus,* Akter et al (2012) for *Rhinomugil corsula* and Baali et al (2021) for *Sardinella aurita.*

Table 3

Regression equation, coefficient of determination (R^2), 'a', 'b' and '*P*' values of different relationships

Relationship	*Regression equation*	*R^2*	*a*	*b*	*n*	*P-value*
Fecundity (F)-Total length (TL)	F=28498*TL-114626	0.4555	-114626	28498	28	0.006
Fecundity (F)-Body weight (BW)	F=1620.5*BW+100902	0.6505	100902	1620.5	28	0.000
Fecundity (F)-Gonad weight (GW)	F=10355*GW+243806	0.741	243806	10355	28	0.190
Gonad weight (GW)-Body weight (BW)	GW=0.1199*BW-7.9341	0.5154	-7.9341	0.1199	28	0.063
Total length (TL)-Body weight (BW)	BW = $0.107 \cdot TL^{2.601}$	0.7365	0.1173	2.497	47	0.007

Histological observation. The current study revealed that eggs from the ovary develop asynchronously but go through discrete developmental stages, before reaching full maturity. A short overview of the gonad stages based on a microscopic examination of the ovary segments of female *S. argus* using previously standardized guidelines (Wallace & Selman 1981) is given below (Figure 5):

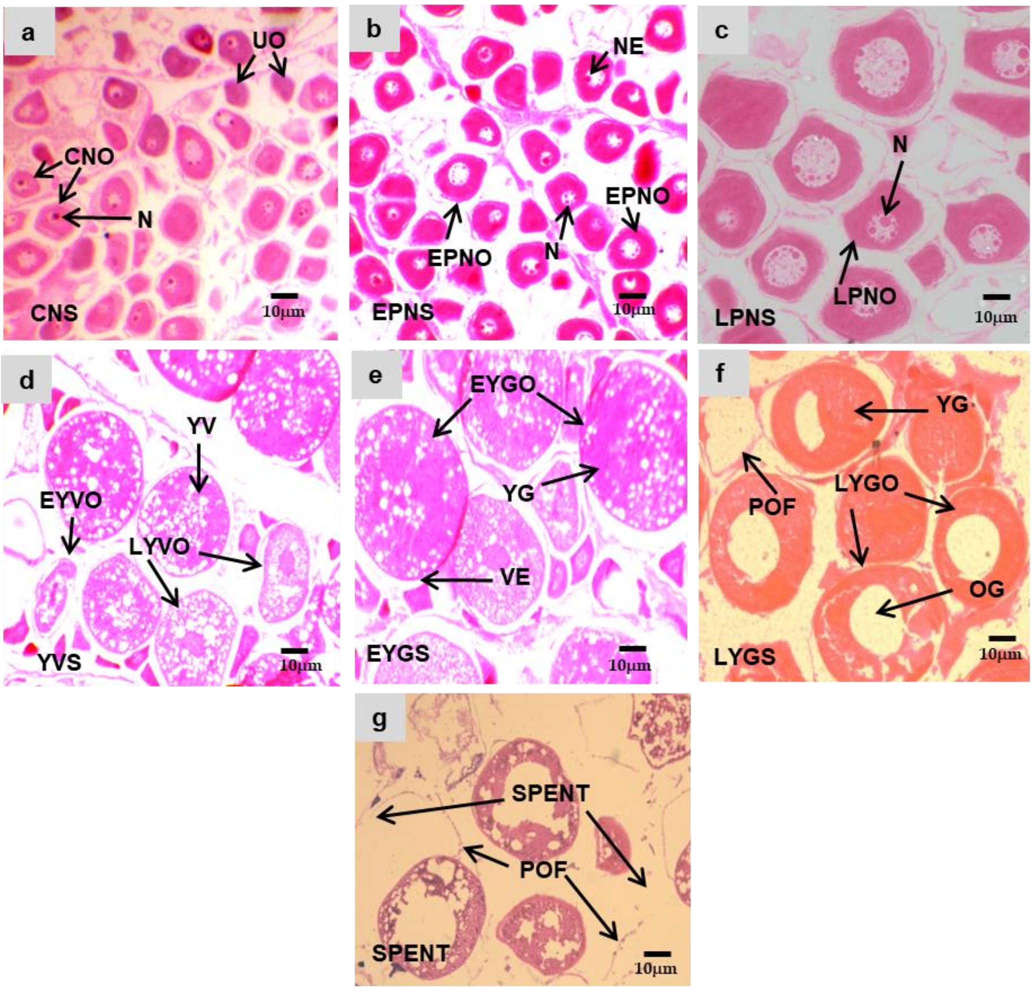

Figure 5. (a) CNS-Chromatin nuclear stage, (b) EPNS-Early Perinuclear stage, (c) LPNS-Late perinuclear stage, (d) YVS-Yolk vesicle stage, (e) EYGS-Early yolk granular stage, (f) LYGS-Late yolk granular stage, (g) SPENT (N-Nucleoli; NE-Nucleolus; YG-Yolk granule; VE-Vitelline envelope; POF-Post ovulatory follicle; UO-Undeveloped oocyte; EPNO-Early perinucleolar oocyte; LPNO-Late perinucleolar oocyte; EYVO-Early yolk vesicle stage oocyte; LYVO-Late yolk vesicle stage oocyte; EYGO-Early yolk granule stage oocyte; LYGO-Late yolk granule stage oocyte; OG-Oil globule).

1. Chromatin Nuclear Stage (CNS). This is the most immature stage characterized by the chromatin threads. This stage is identified by the youngest and undeveloped oocyte (UO) and often refers to oogonia. These oocytes are rarely seen at maturity. Beside other stages, this stage was found in the months of March and October (Figure 5a).
2. Early Perinucleolar Stage (EPNS). The real development of the ova begins at this stage, when the oocyte's nucleus begins to expand, and numerous nucleoli of varying sizes are found around the periphery of the nucleus, indicating an immature oocyte. At this stage, the cytoplasm of the oocyte is deeply stained with haematoxylin and becomes darker than the nucleus. This phase was noticed between March and April (Figure 5b).
3. Late Perinucleolar Stage (LPNS). This stage can be distinguished from the previous stage by the amplification of the oocytes. A large number of nucleoli appeared throughout the nucleus. The oocyte is surrounded by a follicular layer. The formation of

the vitelline envelope begins at this stage, which stage was observed primarily in April and partially in May (Figure 5c).

4. Yolk Vesicle Stage (YVS). This stage was characterized by the beginning of the vitellogenic state, as shown by the presence of yolk vesicles at the oocytes' periphery. Initially they were formed as a single row that appeared colorless when the slides were stained with haematoxylin and eosin. These yolk vesicles (YV) began as tiny forms but grew in size and quantity, indicating a late yolk vesicular oocyte. This stage was predominant in May (Figure 5d).

5. Early Yolk Granule Stage (EYGS). The formation of yolk granules in oocytes with fully developed yolk vesicles signaled the end of oocyte maturation. When stained with haematoxylin and eosin, yolk granules turn a light pink color. Some of these oocytes were observed in May, while the others were detected in June (Figure 5e).

6. Late Yolk Granule Stage (LYGS). With the advancement of the yolk granule stage, the diameter of the oocytes and the quantity of yolk granules increased dramatically and oil droplets were evident inside the cytoplasm. The yolk granules were densely filled and occupied almost the entire oocyte. With haematoxylin and eosin, the yolk granules were revealed to be deep pink. LYGS was found predominantly in June and partially in September, when the ovary was fully matured (Figure 5f).

7. Spent phase. At this point, the oocyte starts the ovulatory phase. Although a few mature oocytes were discovered in the ovary, the majority of oocytes were expelled from the body cavity. This refers to the spent and resting phase of the ovary that occurred in July and August (Figure 5g).

The knowledge on the ovarian development and peak breeding period of a species is crucial for the effective management of its population. The histological events regarding the gonadal development found in this study were comparable to those observed in previous studies of scats (Mandal et al 2020; Morioka et al 2020; Shao et al 2004). In accordance with the present study, they discovered that the vitellogenic stage of *S. argus* is characterized by a significant increase in the size of the oocytes, which is induced mostly by the addition of lipid droplets to the cytoplasm of the cell. The final stage of oocyte development was determined by completely hydrated mature oocytes of *S. argus*, similarly to the findings of Mandal et al (2020) in the Chennai coast.

Oocyte diameter and occurrences of monthly maturity stages of female S. argus. Based on the oocyte prevalence percentage, ovarian developmental phases were classified as immature (Stage I), developing (Stage II), maturing (Stage III), mature (Stage IV), and spent (Stage V). The immature stage oocytes (average oocyte diameter 153.26±102 μm) were 85% in January and 48% in March. A quick development of oocytes (average oocyte diameter range: 356.86±113 to 560.27±67 μm) with the shifting from developing (40%) to maturing stage (72%) was evident between March and May. There was a significant mature stage oocyte (91%) observed in June (average oocyte diameter 560.27±67 μm). The occurrence of immature stage oocytes was minimal from May to July (0–24%) while the maximum number of maturing stage (72%) and mature stage (91%) oocytes were present in May and June, respectively. The highest percentage (70%) of spent stage was observed in July (Table 4, Figure 6). Mandal et al. (2020) found highest percentage of mature stage oocytes in August, in the Indian Ocean's waters, slightly later than the present study. The discrepancy of the ecological conditions affects the spawning season and periodicity (Agarwal 2008).

In the current study, the macroscopic and histological observations of the gonads, GSI, fecundity, and oocyte diameter showed a good agreement, indicating that the spawning season of *S. argus* extends from April to October, with the major peak in June and the minor peak in September. Ze-ping et al (2010) reported that *S. argus* is a multiple spawner during its breeding cycle and the breeding season on the South China coast runs from April to August, with a major peak between the months of May and July, which is consistent with the current study's findings. Barry & Fast (1992) also identified June-July as the breeding season for this species in Philippine waters and suggested that the monsoon triggers gonadal maturity and spawning. Gandhi et al (2014) discovered that *S. argus* has two spawning seasons in a year, one from June to August and the second from October to December, which is a bit later than in the present study. These modifications may have occurred as a result of regional and environmental differences (Karimi et al 2014).

Table 4
Monthly mean oocyte diameter, gonadal maturity stages and characteristics of *Scatophagus argus* according to Afonso-Dias et al (2005)

Gonad stage	*Macroscopic characteristics*	*Microscopic characteristics*	*Oocyte diameter (µm)*	*Month*
Stage-I: Immature	Ovaries small, cylindrical, and transparent. No visible oocytes.	Chromatin threads with no visible eggs. Oocytes mostly in CN stage but some in EPN stage.	153.26±102	March
Stage-II: Developing	Ovaries developed quickly in this class. Ovaries thicker and whitish yellow color. Some vitellogenic oocytes were observed.	The majority of oocytes were in PNS with one or more nucleoli on the nuclear perimeter. A layer of follicular tissue formed around the oocytes.	356.86±113	April
Stage-III: Maturing	Ovaries large and pinkish yellow in color. Many obscure oocytes were visible.	Yolk vesicles formed in the circumference of the oocytes. Ovaries were occupied by vitellogenic oocytes.	474.13±22	May
Stage-IV: Mature	Ovaries occupy the whole body's cavity with largest and bright yellow oocytes. Egg release with gentle pressure.	Number of yolk granules was sharply increasing. Follicular cells were well developed. Ripe and hydrated oocytes were numerous.	560.27±67	June
Stage-V: Spent	Ovaries are tiny, floppy, pouch-like, and colored red-purple. Numerous voids in the ovaries. Few remaining ova of large size were in a phase of reabsorption.	Oocytes enter the ovulatory phase. Frequent post-ovulatory follicles with a large number of immature oocytes were observed.	364.86±192	July

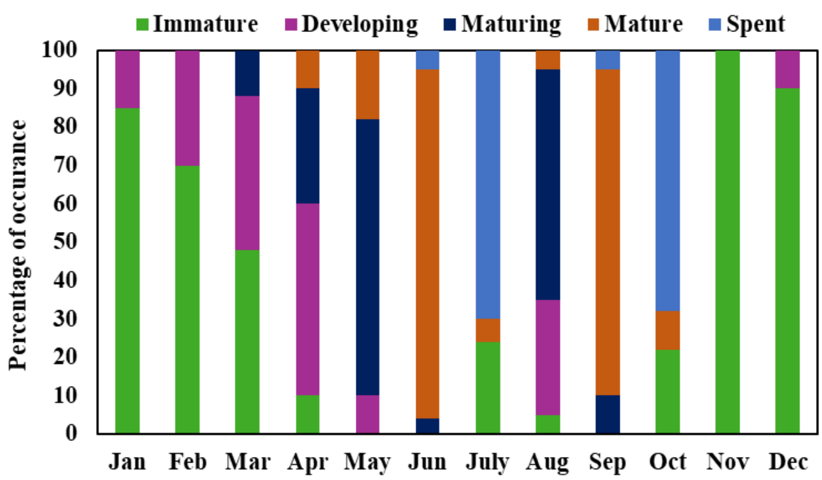

Figure 5. Percentage of monthly maturity stages of oocyte in female *Scatophagus argus.*

Conclusions. This study suggested that *S. argus* has a unique yearly reproductive cycle and an extended spawning period with two annual peaks. The frequency percentage of various stages of oocyte development reveals that *S. argus* is a batch spawner with no synchronized oocyte maturation. The detection and characterization of various gonadal development stages, GSI, fecundity, egg diameter, and minimum length at sexual maturity in this study will serve as a benchmark for future research on the reproductive cycle of other species of scat. In addition, it would be effective for fisheries experts to implement regulations for the control of over-exploitation, which will ensure the sustainable management of this species along the Bangladesh coast.

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Conflict of interest. The authors declare no conflict of interest.

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