Observations of reproductive biology and the gonadal cycle of Gangetic leaffish Nandus nandus (Hamilton, 1822)

Abstract

A study was conducted to determine the breeding season of endangered *Nandus nandus* by observing fecundity, somatic indexes, and gonadal cycle development year-round. Samples were collected from different fish landing centers of Sadar Upazila of Gazipur district. GSI values of *N. nandus* ranged from 4.26 ± 0.45 to 10.28 ± 0.39 while the maximum and the minimum GSI values were observed in July and in October, respectively. However, GaSI values showed an inverse relationship between them. The fecundity of this fish varied from 11176 ±593 to 18030 ± 1704 with the mean fecundity value of 13534.87 ± 87.53. Interestingly the highest fecundity was 18030 ± 1704 in July and the lowest fecundity was 11176 ± 593 in March, implying that the fecundity increased with the increment of time. Histological observation of ovary confirmed the presence of undeveloped oocyte (UO) to mature (M) stages of oocytes in fishes from March to November. The mature stages of oocytes including yolk vesicle's (YV), yolk granule's (YG), and in the case of the male predominance of spermatozoids were found high in proportion during June to July indicating the peak breeding season of *N. nandus*. Finally, these results suggest that *N. nandus* may breed from March with the peak in July and this information may help to provide a guideline for induced breeding and proper management of this fish in a controlled system as well as for conservation from being extinct.

Keywords: Mud perch, gonado-somatic index, fecundity, histology, gonad.

1. Introduction

Evaluation of the stages of gonadal development of fish species is an essential issue in many studies of fish reproductive biology. Fish exhibit different strategies and tactics that maximize reproductively active offspring concerning the available energy (Wootton 1984, Roof 1992, Brown-Peterson et al., 2011). These strategies have been assessed based mainly on germ cell lineage development and fecundity, which are essential to understand the maturation process and oocyte recruitment patterns (Costa et al., 2015). Histology has been used for many decades in health evaluations of wild fishes. Although observation of changes in tissues such as liver, kidney, gill, and spleen as histopathological biomarkers have received much attention for the assessment of the effects of environmental stress (Hinton et al., 1992; Teh et al., 1997), recently gonadal histology has attained the magnetism of scientists for the evaluation of fish reproductive health.

Fecundity appears to bear some broad relationship to the care or nurture accorded to the eggs (Lagler, 1956). It differs in different races of the same species. Fecundity refers to the number of eggs developed for the subsequent spawning by a female of egg-laying animals (Royce, 1972). Fecundity can play a role to get a notion for fish egg production, which is a primary determinant of productivity and hence, contributes to fish culture and population dynamics. Knowledge about the fecundity of a fish is essential for evaluating the commercial potentialities of its stock, life

history, practical culture, and actual management of the fishery (Islam et al., 2012). The gonado-somatic index (GSI) is the ratio of fish gonad weight to body weight. The GSI is particularly helpful in identifying seasons of spawning, as the ovaries of gravid females swiftly increase in size just before spawning. The gastro-somatic index (GaSI) is a relationship between the weight of the alimentary canal and fish weight, which helps determine the feeding condition in different months and seasons.

Gangetic leaffish (*N. nandus*), commonly known as mud perch mostly found in paddy fields where it preys on small organisms. This fish belongs to the order Anabantiformes, family Nandidae, and genus *Nandus* (Nelson et al., 2016). Muddy streams, rivers, pools, marshes are its main habitat. It is an oval-shaped freshwater fish with an arched back and lateral compression. The body coloration may change depending on habit condition, although it is generally greenish-brown in color with brassy reflections and vertically marbled with three broad patchy blotches (Gupta, 2018). Food habit study of *N. nandus* reveals that this species is carnivorous and mainly feed on insect, fish, and shrimp (Paul, 2020). *N. nandus* is a very hardy fish and is of considerable fisheries interest. It is a high-priced fish despite its spinous fins and ugly black bands and blotches all over the body (Talwar and Jhingran, 2001). It is very tenacious and can live in foul water. Therefore, this fish is very suitable for cultivation in muddy water (Bhuiyan, 1964). *N. nandus* is considered delicious, demandable, and high nutritive and medicinal valued fish including protein 17.69 %, fat 4.86%, ash 4.71 %, and high energy 5.43 KJ/g (Hossain et al., 1999). Previously, it was common open-water fish in the market with high demand. Due to different anthropogenic activities with the population explosion, it had significant negative impacts on the abundance of this species. As a result, the International Union for Conservation of Nature (IUCN) in 2010 included *N. nandus* in their red list as an endangered fish.

However, the list of endangered fish species like *N. nandus* will enlarge and they can be added to the extinct list day by day if any initiative could not be taken to save the entire population. In this context, the study of reproductive biology, including fecundity, GSI, GaSI, and gonadal cycle, can play a potential role in conservation. Previously, breeding biology of N. nandus have been studied collecting sample from different region of different countries (Saikia et al., 2021; Mredul et al., 2021; Ray 2013; Goswami and Dasgupta 2004). Since, gonadal development can vary depend on different environmental conditions and food availability (Goswami and Dasgupta, 2004), and the breeding biology and season is essential to know for establishing the induced breeding technique and culture system in controlled environments of a fish, the present study was undertaken to investigate the gonadal development following GSI, GaSI, and fecundity considering the economic importance of *N. nandus*.

2. Materials and Method:

The study was conducted to accomplish the present investigation for a period of a year from January to December in the Sadar Upazila of Gazipur district of Bangladesh. During this period, at least twenty fish samples (live or dead) were collected monthly from the landing centers of the Gazipur region of Bangladesh, which are usually collected fishes caught from 'Balai beel' situated in the East region of Gazipur district. Fishes were transferred to the laboratory of the Genetics and Fish Breeding department, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh. First, the total length (cm) and body weight of each fish sample was measured with the help of scale in centimeter and sensitive portable electronic balance (OHAUS Scout pro) in gram respectively. It should be noted that fishes were collected from the landing centers instead of collecting from the beel to reduce the harvesting pressure on nature as this is an endangered species.

2.1 Collection of gonad and gut sample

The ventral side of the samples was cut and opened from the anus towards the lower jaw by using scissors carefully. Muscle, fat tissue, digestive organs, and blood vessels were removed properly. After that, the gonad and gut were taken out by forceps. Then gonad and gut-weight were measured to determine Gonado-somatic index (GSI) and Gastro-somatic index (GaSI) (Kurbah and Bhuyan, 2018). Thereafter, gonad samples were cut into small pieces by using a scissor. Finally, gonad samples were taken into vials filled with 10% buffered formalin and kept at room temperature for histological works.

2.2 GSI, GaSI and Fecundity

The value of GSI is the indicator of the status of gonadal development and maturity of individuals. GSI is a measure of the reproductive cycle based on the assumption that maturation and breeding coincide with the maximum gonad weight. It was calculated using the following formula:

$$GSI \quad _ \frac{\text{Gonad weight (GW)}}{\text{Body Weight (BW)}} \times 100$$

The value of GaSI is the indicator of the status of gut content during the gonad developmental period of individuals. GaSI is frequently applied to determine the feeding content of a fish species over the year at monthly or less intervals. The value of GaSI was calculated using the following formula:

$$\frac{\text{Gut weight}}{\text{Body Weight (BW)}} \quad GaSI= \qquad \times 100$$

For fecundity, a total of fifteen matured fishes were used randomly from March to August. Gravimetric method was applied to determine the fecundity of fish. In this method, the ripe ovaries were carefully taken out and the external connective tissues were removed from the

surface of the ovaries. Then ovaries were weighed and preserved in modified Gilson fluid (Nitric Acid 17ml, Acetic Acid 4ml, Mercuric Chloride 20g, Ethanol 95%, and Distilled water 900ml) according to Ekanem (2000). The mean number of eggs in 0.1 g ovary sample, taken from the anterior, middle, and the posterior portion was determined and then multiplied by the total weight of the ovary, which gave the total number of eggs of respective fish.

The fecundity of the fish was estimated by using the following formula:

$$\text{Fecundity (F)} = N \times \frac{\text{Gonad Weight}}{\text{Sample Weight}}$$

Where N is the number of eggs in the sample.

The diameter of oocytes was measured by an ocular micrometer. Oocytes were taken from anterior, middle and posterior part of the ovary. Randomly ten oocytes were measured from every part of the ovaries of each fish and five fishes were used in the same way. Afterward, mean oocyte diameter was calculated for the respective month.

2.3 Histology of gonads

Histological observations were made in fish samples every month. Small pieces of ovary (5-7 mm) and testis (4-6 mm) from the mid-region were fixed in Bouin's solution for 48 h and then transferred to 10% buffered formalin solution for subsequent use of histological study following Degani (1994). Briefly, the preserved gonads were taken out in a perforated plastic holder, which was covered by perforated steel plates. The cleaning, infiltration, and dehydration process were carried out in an automatic tissue processor "KD-TS6B vacuum tissue processor" using a series of alcohol of increasing concentrations, two changes of xylene, and finally through molten wax (three series). Each piece of tissue, embedded in paraplast wax was sectioned at 10µm and stained with prepared haematoxylene and counterstained with eosin solution. The slides were

observed under a compound microscope (DM 100; Leica, Wetzlar, Germany) to examine monthly developmental variations of gonads.

2.4 Statistical analysis of data

For the statistical analysis of data, Microsoft Excel was used to determine the linear relationship and correlation of determination (R^2) between different variables, and "Statistix 10" software was used for "ANOVA" at 5% level of significance ($P<0.05$). Data were expressed as mean ± standard deviation of the mean.

3. Results

3.1. Gonado-somatic index (GSI) of *N. nandus*

GSI values of *N. nandus* significantly varied throughout the year from January to December ranging from 4.26±0.45 to 10.28±0.39 (Table 1). The highest GSI value 10.28±0.39 was found in July whereas the lowest GSI value was found in October was 4.26±0.45 (Table 1). A continuous increase of GSI was observed from November to July and a sudden decrease in the further months indicates a single breeding period of *N. nandus* (Fig. 1).

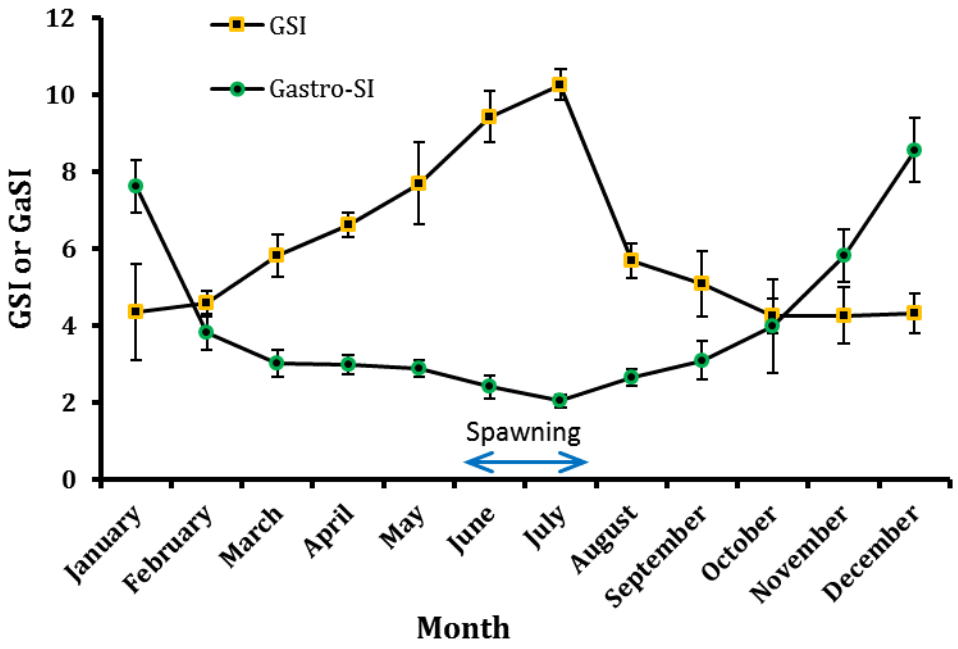


Figure 1. Relationship between GSI and GaSI of *N. nandus*.

3.2. Gastro-somatic index (GaSI) of *N. nandus*

The biometric parameters and their corresponding gut weight are shown in Table 1. GaSI values from the available samples varied from 2.04 ± 0.17 to 8.57 ± 0.84 during the study period. The maximum and the minimum GaSI values, 8.57±0.84 and 2.04±0.17 were observed in December and in July, respectively, indicating that there was an inverse relationship between GSI and GaSI of fish (Figure 1).

Table 1. Mean and standard deviation (±SD) of total length, body weight, ovary weight, and (GSI %) in female N. nandus. Superscripts showing the significance level at P<0.05.

Months	Mean total length (cm)	Mean body weight (g)	Mean gonad weight (g)	Mean GSI (%)	Mean gut weight (g)	Mean GaSI (%)
Jan.	9.95±0.19^{k}	12.41±1.08^{j}	0.55±0.19^{h}	4.37±1.26^{h}	0.95±0.11^{a}	7.63±0.68^{b}
Feb.	11.67±0.13^{g}	22.48±1.41^{f}	1.03±0.10^{f}	4.58±0.34^{gh}	0.86±0.08^{b}	3.83±0.47^{d}
Mar.	12.11±0.15^{f}	24.72±1.21^{e}	1.44±0.12^{e}	5.83±0.54^{e}	0.75±0.08^{cd}	3.03±0.35^{e}
Apr.	12.53±0.12^{e}	25.07±1.04^{e}	1.66±0.03^{d}	6.62±0.31^{d}	0.75±0.06^{cd}	2.98±0.24^{e}
May	13.31±0.16^{c}	30.13±1.66^{c}	2.33±0.42^{c}	7.71±1.07^{c}	0.87±1.07^{b}	2.89±0.20^{ef}
Jun.	13.89±0.18^{b}	35.23±2.05^{b}	3.33±0.34^{b}	9.44±0.67^{b}	0.85±0.08^{b}	2.41±0.29^{fg}
Jul.	15.46±0.84^{a}	40.07±2.59^{a}	4.12±0.30^{a}	10.28±0.39^{a}	0.82±0.04^{bc}	2.04±0.17^{g}
Aug.	12.91±0.10^{d}	26.88±0.95^{d}	1.53±0.13^{de}	5.70±0.46^{ef}	0.71±0.04^{d}	2.65±0.21^{ef}
Sept.	11.36±0.10^{h}	20.47±1.45^{g}	1.04±0.12^{f}	5.09±0.84^{fg}	0.63±0.07^{e}	3.10±0.51^{e}
Oct.	10.91±0.15^{i}	17.69±1.61^{h}	0.75±0.06^{g}	4.26±0.45^{h}	0.69±0.15^{de}	4.00±1.22^{d}
Nov.	10.45±0.13^{j}	14.72±1.24^{i}	0.63±0.12^{gh}	4.26±0.74^{h}	0.85±0.06^{b}	5.82±0.69^{c}
Dec.	9.50±0.10^{l}	10.39±0.99^{k}	0.45±0.06^{h}	4.32±0.51^{h}	0.89±0.08^{ab}	8.57±0.84^{a}

3.3. Fecundity and Oocyte diameter of *N. nandus*

In the current study, fecundity and egg diameter of adult female *N. nandus* were determined month-wise to know the status of gonadal development and maturity of individuals. Fecundity of *N. nandus* ranged from 11176.40±592.51 to 18029.70±1704.24 was determined during the study period (Table 2). The highest and the lowest fecundity were estimated at 18029.70±1704.24 and 11176.40±592.51 found in July and in March, respectively. The oocyte diameter of female *N. nandus* also varied from 0.47±0.07 to 0.68±0.04 mm during the study period (Table 2). Oocyte diameter was significantly increased from March and was peaked in July, indicating a clear gonadal development in the breeding season.

Table 2. Mean and standard deviation (±SD) of total length, body weight, and fecundity in female N. nandus. Superscripts showing the significance level at P<0.05.

3.4. Relationship between fecundity and total length

The scatter diagram obtained from the log of fecundity and log of total length relationship showed a nearly perfect correlation. The evident trend from lower left to the upper right showed the regression of fecundity Y on total length to be positive as the correlation determination (R^2). The logarithmic relationship of fecundity on total length (Figure 2) gave a regression coefficient of 2.040; intercept 1.83 and correlation determination of 0.9085. Therefore, the regression equation of fecundity and total length of fish could be expressed as:

$$Log_{10}Y = 1.83 + 2.0401Log_{10}X$$

Months	Mean total length (cm)	Mean body weight (g)	Mean fecundity	Egg diameter (mm)
March	12.11 ± 0.15^{f}	24.72 ± 1.21^{e}	11176.40 ± 592.51^{e}	0.47 ± 0.07^{c}
April	12.53 ± 0.12^{e}	25.07 ± 1.04^{e}	11693.10 ± 553.83^{de}	0.56 ± 0.05^{b}
May	13.31 ± 0.16^{c}	30.13 ± 1.66^{c}	12995.20 ± 487.74^{c}	0.57 ± 0.06^{b}
June	13.89 ± 0.18^{b}	35.23 ± 2.05^{b}	15290.60 ± 894.14^{b}	0.63 ± 0.04^{a}
July	15.46 ± 0.84^{a}	40.07 ± 2.59^{a}	18029.70 ± 1704.24^{a}	0.68 ± 0.04^{a}
August	12.91 ± 0.10^{d}	26.88 ± 0.95^{d}	12024.20 ± 492.71^{d}	0.54 ± 0.07^{b}

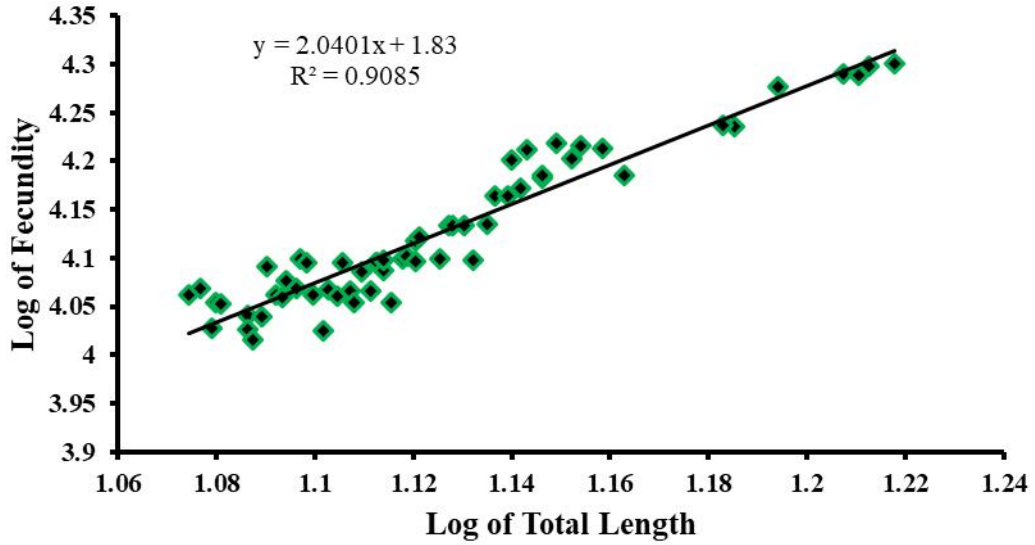


Figure 2. Relationship between log of fecundity and log of total length of *N. nandus*.

3.5. Relationship between fecundity and body weight

The logarithmic relationship of fecundity against body weight (Figure 3) produced a regression coefficient of 0.885, intercept 2.8195, and correlation determination of 0.8608 in *R. rita*.

$$Log_{10}Y = 2.8195 + 0.885\ Log_{10}X$$

A straight line through the origin would fit the points well, showing that the number of eggs was directly proportional to the weight of the fish.

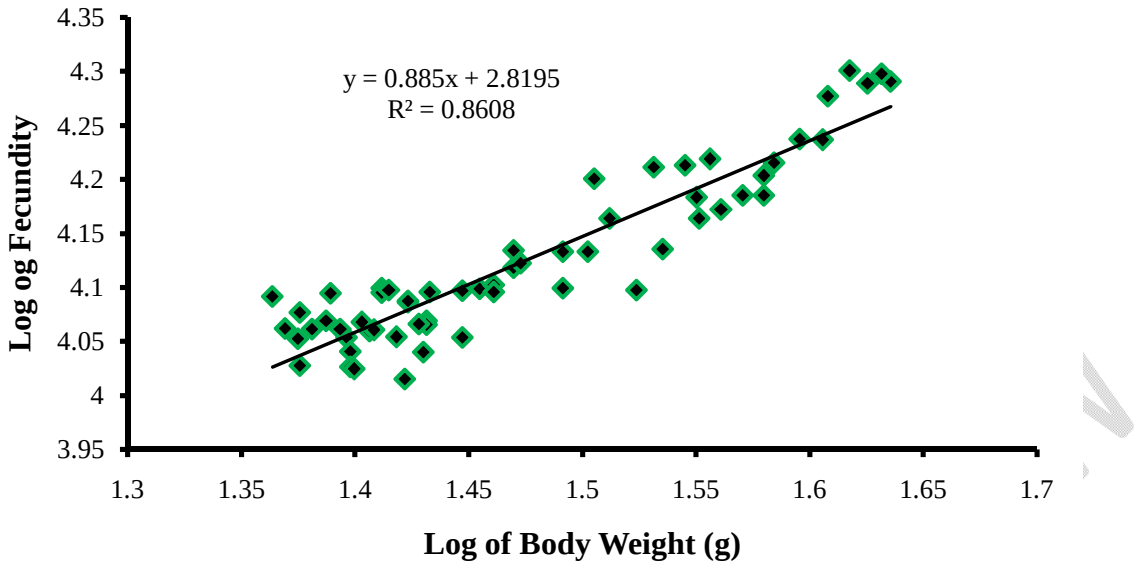


Figure 3. Relationship between log of fecundity and log of body weight of *N. Nandus*.

3.6. Relationship between fecundity and ovary weight

The fecundity increased progressively with the ovary weight of the fishes (Figure 4). In the present study, the regression coefficient (R^2=0.8667) indicates that the linear relationship between log fecundity and log ovary weight was positively correlated. For the regression of log fecundity on log ovary weight the following equation was obtained:

$$Log_{10}Y = 3.9877 + 0.3991Log_{10}X$$

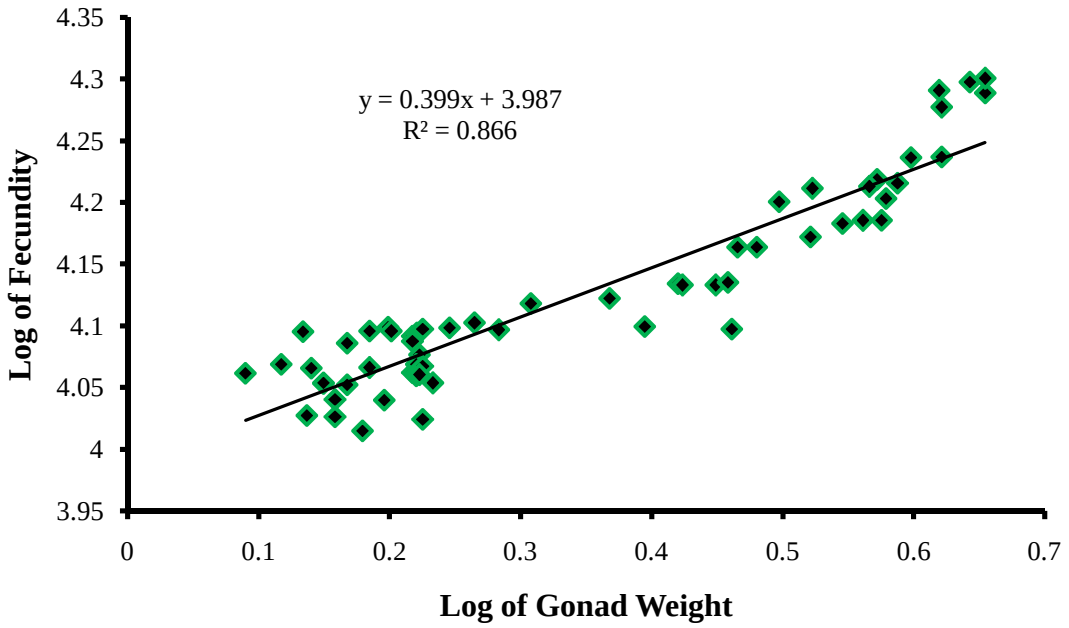
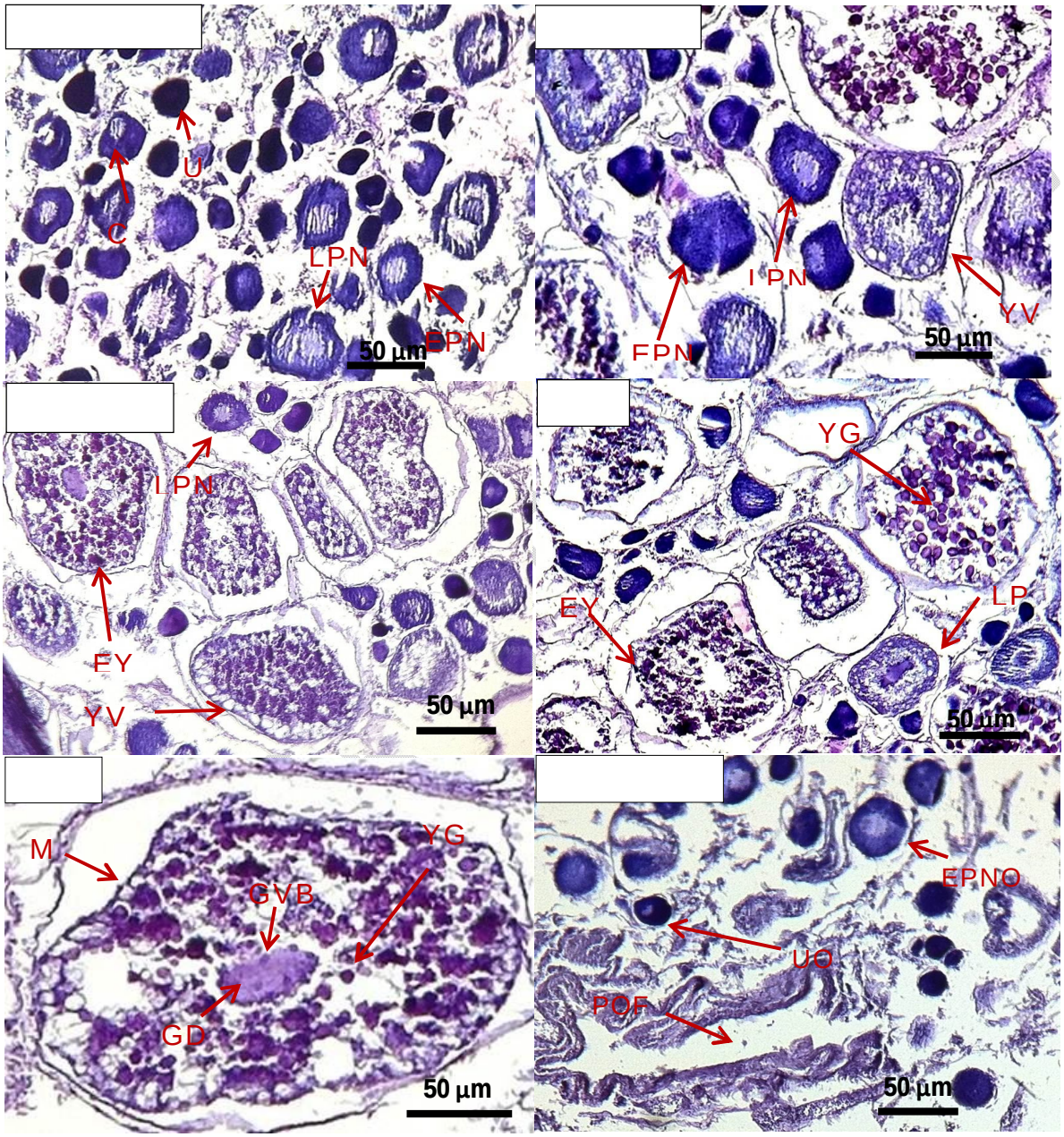


Figure 4. Relationship between log of fecundity and log of gonad weight of *N. nandus*.

3.7. Maturation stages of oocytes

Ovarian development of *N. nandus* was examined to study the pattern and timing of the growth phase and maturation stages of germ cells in the gonad (Figure 5, Plate 1-7) . It was observed that fish exclusively in immature stages (early and late perinucleolar stage oocytes) of maturity was mostly available in the months from December to March (Plate 1-2). Yolk- vesicle stage oocytes appeared from April to May (Plate 3). Both yolk vesicle and yolk granular stages were found in June (Plate 4) but most of the eggs in July (Plate 5) were in the yolk granule stage. In August and September (Plate 6), post-ovulatory follicles were observed and then in the later months oocytes were start to develop again in the ovary of *N. nandus* (Plate 7). Histological data revealed that the spawning season of *N. nandus* starts from March and contained till August. It was configured by the presence of a large number of mature eggs in June and July. This result was consistent with the value of GSI as the values peaked in July. From the histological study of

ovaries at successive months, it was observed that oocytes did not develop synchronously and oocytes at various maturation stages were observed in paired ovaries in the pre-breeding period whereas in the breeding period most of the oocytes were found homogenously developed.



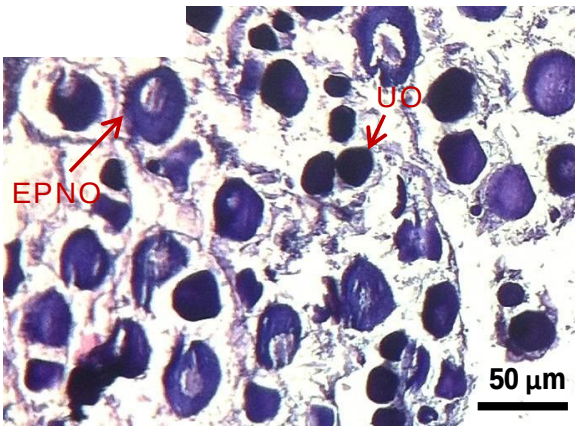


Figure 5. Seasonal changes in ovarian histology in *N. nandus* collected in 2021: Decm - Jan (Plate 1), Feb-Mar (Plate 2), Apr-May (Plate 3), June (Plate 4), July (Plate 5), Aug-Sept (Plate 6), and Oct-Nov (Plate 7). UO, undeveloped oocyte; CN, chromatin nuclear oocyte; EPNO, early perinucleolar oocyte; LPNO, late perinucleolar oocyte; M, mature stage; PM, premature stages; YV, yolk vesicle stage; EYG, early yolk granule; YG, Yolk granule stage; GD, eccentic germinal disk; POF, Post ovulatory follicle. Scale bars: 50 µm.

3.8. Maturation stages of spermatocytes

From the histological study of testis at successive months, it was observed that spermatocytes did not develop synchronously and spermatocytes at various maturation stages were observed in paired testes (Figure 6). In the case of male *N. nandus*, spermatogonia were observed in immature males, whereas males were in maturation, showing the presence of spermatogonia (SG) and spermatocytes (SC). The mature male showed the predominance of spermatozoids (SZ). Both stages were found in testis collected from *N. nandus* in May to August periods. The spent male showed testicular lumen in the testes.

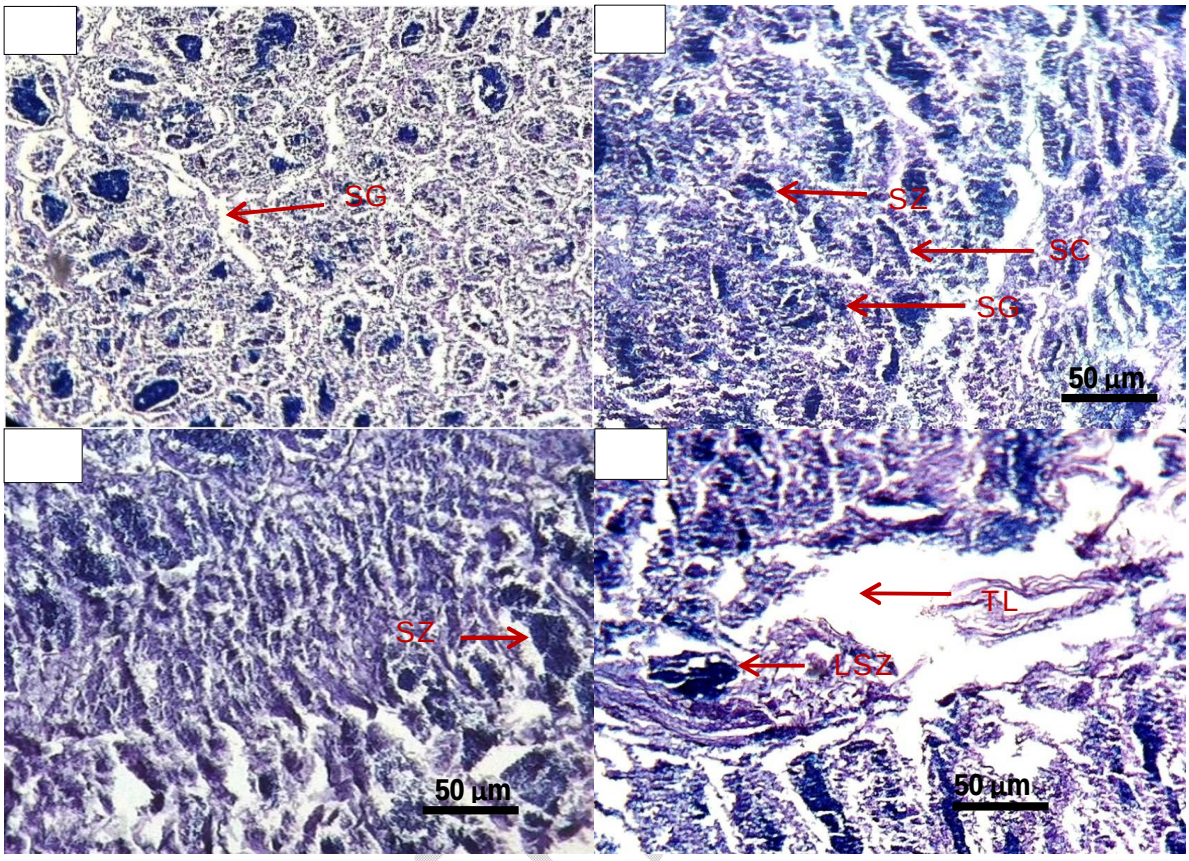


Figure 6. Gonad maturation stages of male N. nandus were found in April to July (A) Immature male, showing the presence of spermatogonia. (B) Male in maturation, showing the presence of spermatogonia (SG) and spermatocytes (SC). (C) Mature males show the predominance of spermatozoids (SZ). Both (B) and (C) stages were found in May to August periods. (D) Spent male showing testicular lumen (TL) and some left spermatozoids (LSZ) in the testes.

3.9. Peak breeding season of *N. nandus*

Histological data revealed that the spawning season of *N.nandus* starts from March and is retained till August. It was configured by the presence of a largenumber of mature eggs in March. This result was consistent with the value of GSI as the values peaked and the value of GaSI as the values lowest in July. A sudden drop in GSI values after August indicated the higher spawning pulse of this species in this habitat. Spawning fully terminated by September as no

other mature eggs were present in the histological observations of ovaries collected during September.

4. Discussion

The mean GSI was found at 6.03 year-round. The GSI was minimum in October (4.26), began to increase in December, and reached the stage of complete sexual maturity in July (10.28), where the ovaries were ripe and mature (Table 1; Fig. 1). Our result showed the highest GSI in the summer months (June and July) which could be the possible breeding season of *N. nandus*. A recent study on the GSI of *N. nandus* suggested peak breeding season also in July gaining average GSI 6.99, though highest was about 16 in the July (Saikia et al., 2021). Moreover, a wide breeding season from March to July reaching the highest GSI (7.448) in May was also reported by Mredul et al., (2021). Similar to our report, Ray (2013) studied with *N. nandus* in India and found a mean gonado-somatic index of 7.06. Goswami and Dasgupta (2004) conducted another comparative study with the biology of *N. nandus* in the beel and pond where the higher mean GSI (6.71) was found in the pond but was significantly lower (2.0) in the *N. nandus* collected from the beel, which though differ our present mean GSI result. We found higher mean GSI values similar to GSI found in the pond in the above experiment which may be due to different environmental conditions as gonadal development can vary due to better environmental conditions and food availability (Goswami and Dasgupta, 2004) that need a further ecological study to support the present findings.

GaSI of female *N. nandus* was also calculated and the highest and the lowest GaSI values were observed 8.57 and 2.04 in December and in July, respectively. Although there was no study available on GaSI of *N. nandus*, Goswami and Dasgupta (2004) studied GaSI in May that is 2.88 which was similar to our findings (2.89) in the same month. In the present study, a relationship

was made between GSI and GaSI (Fig. 1) and found an inverse relation. The Lowest GaSI against the highest GSI in July suggests that *N. nandus* may breed in this month and take the least food during breeding.

The fecundity of female *N. nandus* was estimated from March to August due to the availability of matured eggs on the ovaries. The highest fecundity was estimated in July (18030) and the lowest was in March (11176). The average mean of fecundity was estimated at 13534.87. Moreover, the mean ova diameter gradually increased in size from March onwards and rapidly reached the maximum size (0.68±0.04 mm) in July (Table 2). Parameswaran et al. (1971) reported a wide range of fecundity ranging from 1573 to 23546 and found the highest fecundity and mean egg diameter in July that supported our results. Recently, Saikia et al., (2021) also found similar fecundity (25212.89±5536.47) in the highest size group of fishes collected from Borsola Beel, Jorhat, Assam. However, in contrast to previous and our report, Mustafa et al. (1980) found a significantly varied range of fecundity from 7381 eggs for a fish to 46222 eggs. In addition, fecundity ranges of 1106.25-37922 and 3937-42460 have been documented by Goswami and Dasgupta (2004) and Ray (2013) respectively for this fish species. The variations are probably because the fecundity of fishes varies from species to species, also within the same species due to different factors such as age, size, body and gonad weight, ecological conditions of the water body, (Lagler, 1956).

In addition, relationship of fecundity-total length (a=1.83, b= 2.040), fecundity-body weight (a= 2.8195, b=0.885) and fecundity-ovary weight (a=3.9877, b= 0.3991) was found to be linear. The present study also indicates that *N. nandus* belonging to the same size group had a varying number of eggs in their ovaries. Ray (2013) studied with *N. nandus* and found a relationship between biological parameters, such as fecundity and weight (a=265.41, b=1.06), length, and

fecundity (a=0.1002, b=4.5), ovary weight, and fecundity (a=4256.29, b=0.98 which support current study. Another study in the mathematical relationship between body length-fecundity, body weight-fecundity, and gonad weight-fecundity of *N. nandus* was found to be linear (Mustafa et al. 1980), which is consistent with the present study.

Histological observation shows the evidence of gonadal maturation and spawning season. The reproductive potential of a population is one of the basic needs to designate the individuals of that population regarding their gonadal conditions (Jhingran & Verma, 1972). However, to our knowledge, this is the first ovarian histological observation of *N. nandus* to determine the breeding cycle of *N. nandus.* Here, we also observed testicular developmental stages year-round.

In the present study, the gonadal maturity stages of *N. nandus* were identified in females based on the description mentioned by different authors with slight modifications (Coward and Bromage 1998; Wright 2007). Besides other reproductive parameters, histology revealed major chromatin nucleolar stage, peri-nucleolar stage, yolk-vesicle stage, and yolk-granule stage which were previously observed in different species (Rahman and Mollah, 2013; Hossain 2012; Akhter, 2011). Generally, in a tropical country like India, Bangladesh, the vitellogenesis process inaugurates shortly before the onset of the spawning season. Therefore, vitellogenic oocytes (VO), oocyte maturation (OM), and post-ovulatory follicles (POFs), any of these three indicators are being used for assessing the spawning season in most teleost (Lowerre-Barbieri et al., 2011). Moreover, spawning capable females could be identified either as imminent spawning or recent spawning by ovarian stages at late GVB (germinal vesicle breakdown) and hydration or newly collapsed POF, respectively (Hunter and Goldberg 1980; Hunter and Macewicz 1985). In the present study, the monthly distribution of ovarian stages showed that fishes were available at all

three maturity stages (VO, OM, POFs) throughout the year. In June and July females were available with ovaries occupying mature oocytes (granule stage).

In addition, histomorphology of male *N. nandus* testis was observed parallel to female ovaries throughout the year. The present study was consistently found similar to the previous study performed by Raizada (1975) on morphological and histological changes in the testes during different months of the year where the reproductive cycle of *N. nandus* was divided into post-spawning (October to December), pre-spawning (January to March) and spawning (April to August) periods.

Our results demonstrated the oocyte diameter, GSI, and GaSI values of females showed a significant difference between different months. The increasing GSI values appeared from March to July with the peak in July and the value declined rapidly from 10.28 to 5.70 in August after spawning signifying the onset of the spawning season. In the gonadal histology, the presence of mature oocytes from June to July and POF in August confirmed that the *N. nandus* spawned once a year with peak spawning in July.

Finally, it can be concluded that the current study will contribute to identifying the particular gonadal maturity stage and seasonality of *N. nandus* spawning which in turn will help to conserve and breed this valuable species in captive conditions. This study can also be helpful for sustainable fishery management of *N. nandus* in its original habitat.

Ethical approval

Since the sample fish is endangered, the use of fish for the experiment was kept to an absolute minimum required to achieve statistical significance for validation purposes. All procedures were

conducted in accordance with the United Kingdom Animal (Scientific Procedures) Act 1986, approved by the Ethical Review Committee (ERC) of the Institute of Biotechnology and Genetic Engineering (IBGE), Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur-1706, Bangladesh and conducted under the authority of the project License BSMRAU/IBGE/14. An ethical statement is available as supplementary material.

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