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Research Article

Reproductive Biology of the Threatened Menoda Catfish, *Hemibagrus menoda* (Hamilton, 1822) in the Kangsha River, Bangladesh

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Abstract

Background and Objective: Knowledge of reproductive biology of a fish species is particularly important for conservation and plays significant role in determining its sustainable management and suitability for culture. Aspects of reproductive biology of *Hemibagrus menoda* from Kangsha River Netrakona district, Bangladesh were studied to evaluate the on-set and period of spawning season and its reproductive potential, which were hitherto not reported. **Materials and Methods:** A total of 479 females and 400 males were collected using Gill nets and Seine nets from March, 2015 to February, 2016. Gonadosomatic index (GSI), fecundity and histology of the gonads were investigated. Regression analysis was used to estimate the relationships between fecundity and standard length (SL), fecundity and body weight (BW) and fecundity and ovary weight (OW). **Results:** The monthly mean GSI of female *H. menoda* started to increase from May to June and reached the peak (12.50 ± 4.97) in July, indicating the peak spawning season of the fish. Mean fecundity estimates based on mature females was 77273.77 ± 276.82 for fishes with mean length of 31.85 ± 2.39 cm and ranged from 22954.99; 25.00 (cm) in May to 222171.8; 40.20 (cm) in July. Fecundity correlated positively with SL ($\log F = 0.0135 + 4.399 \log SL$; $r^2 = 0.774$), BW ($F = 198.7$ BW-47602; $r^2 = 0.805$) and OW ($F = 1066$ OW+6124; $r^2 = 0.832$). Based on histological data, yolk vesicle, premature and mature stages of oocytes were abundant in May, June and July, which further confirms the distinct spawning season of *H. menoda* from May to July. **Conclusion:** *Hemibagrus menoda* spawns once a year and the spawning season extends from May to July with a peak in July. This reproductive pattern helps in development of culture program and conservation of the species.

Key words: Reproductive biology, GSI, fecundity, gonadal histology, spawning season

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fish reproductive biology is important in stock assessment, fishery resource management, fisheries research and profitable aquaculture practices. Knowledge of reproductive parameters such as sex ratio, onset and duration of spawning season, maturity stages, ova diameter, fecundity and histology of the gonads are particularly important for conservation of fish species^{1,2}. *Hemibagrus menoda*, one of the ray-finned, iteroparous and gonochoristic fishes, is among the 8 species in the genus *Hemibagrus* belonging to the family Bagridae. The species is characterized by having dark, vertical spots on its abdomen and is found in the river drainages in Bangladesh and northern India³. However, Hossain⁴ reported that due to natural and anthropogenic induced changes, *H. menoda* have become critically endangered. Moreover, the IUCN⁵ categorized the fish as near threatened.

Spawning season refers to that part of the year in which various fish species get sexually active for spawning⁶. The expulsion of gametes from the body into the surrounding water is called 'spawning', resulting in fertilization. Knowledge of spawning activity is gaining more importance as restoration efforts on large rivers to benefit endangered species and fish diversity requires specific knowledge of when fishes reproduce and the coincidence of environmental conditions⁷. Spawning season can be determined by studying gonadosomatic index (GSI) of the species, external features of the ovaries, such as size and by histology of the gonads. The GSI is often used to follow the reproductive cycle of a species over the year at monthly or less intervals and is a good indicator of gonadal development of fish⁸. The GSI assumes that an ovary increases in size with increasing development by comparing the mass of the gonad with the total mass of the fish. Information on fecundity is also important in order to evaluate a fish for its commercial potentials and to develop numerical relationship between egg production and recruitment^{9,10}. Moreover, to bring a new species in aquaculture, information of reproductive biology is essential in case of mass seed production for supporting commercial aquaculture in Bangladesh, a 5th leading aquaculture producing country in the world. Study of these parameters is necessary in order to arrive at some conclusions on the maturity and breeding season of this species which will aid in its propagation. Histological studies are the most reliable technique to assess the reproductive strategy and tactics of fish species and also to determine peak period of spawning and to understand effective methods for increasing efficiency

of broodstock and ultimately increasing the fish production¹¹. Several studies have been conducted on various aspects of reproductive biology of fishes including sex ratio¹²⁻¹⁴, GSI¹⁵⁻¹⁷, fecundity¹⁸⁻²⁰ and histology of the gonads²¹⁻²³. Not until recently, Jega *et al.*²⁴, studies on the reproductive biology of the menoda catfish were hitherto not reported. In this regard, the purpose of this study was to provide the first hand information on some aspects of reproductive biology such as the GSI, fecundity and histology of the gonads of *H. menoda* from the Kangsha River, Bangladesh, so as to lay foundation for the induced breeding, culture and eventual domestication of this threatened fish species.

MATERIALS AND METHODS

Sampling site: Samples of *H. menoda* were monthly collected from the Kangsha River located at Jaria-Jhanjail, Latitude 25°0'41.10"N and Longitude 9°0'38'27.16"E in the Netrakona district, Bangladesh.

Sample collection: A total of 879 samples, including 479 females and 400 males were collected from the landing sites from March, 2015 to February, 2016. The specimens were identified according to Rahman²⁵ and Hossain²⁶ and sexed according to Norton *et al.*²⁷. Fishing gears used in catching the fish include Gill net, Seine net and by angling. The freshly landed fishes were immediately iced at the sampling site, transported to the Aquaculture Laboratory of Bangladesh Agricultural University, Mymensingh, Bangladesh, where the experiment was conducted. The Total length (TL) of the specimens ranged from 31.55-45.37 and 35.48-43.43 cm for females and males, respectively.

Gonad collection: The ventral side of the fish was cut and opened from the anus towards the lower jaw by using scissors and the whole mass (fat tissues, stomach, liver, ovary, liver, etc.) were removed and the gonads carefully detached from the other visceral organs by the use of needles and forceps. The gonads were then cleaned with tap water and wiped with blotting paper. The ovary weight (OW) was then taken before being kept in 10% buffered formalin (40% formaldehyde; Lab Grade-37% w/v) for study of fecundity and GSI.

Determination of GSI: To determine the sexual maturity, the female GSI was calculated using the formula:

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

Fecundity estimation: Fecundity of *H. menoda* was estimated for 169 matured females following the gravimetric method according to Nandikeshwari *et al.*²⁸, Akter *et al.*²⁹ and Islam *et al.*³⁰. The external connective tissues were removed from the surface of the ovaries and blotting paper used to remove moisture from the surface of the ovaries. A sub sample of about 1-2 mm and 0.20 mg of the gonad was cut from the anterior, middle and posterior parts of the ovary and separately put in a Petri dish. The eggs were separated by using needle and forceps and counted with the aid of magnifying glass. Care was taken to sort the mature and immature eggs. The total number of matured and immature eggs of each ovary sub sample was sorted out and counted with the help of a needle and magnifying glass and estimated using the equation:

$$FI = \frac{\text{Gonad weight}}{\text{Subsample weight}} \times \text{Number of eggs in subsample}$$

By taking the mean number of three sub sample fecundities (F1+F2+F3), the absolute (total) fecundity F_T , for each female fish was then determined using the formula as¹⁹:

$$F_T = \frac{F1 + F2 + F3}{3}$$

Relative fecundity determination: Relative fecundity is the number of eggs per unit length or weight of individual fish and was calculated as³¹:

$$\text{Relative fecundity (Fr)} = \frac{\text{Absolute fecundity}}{\text{Standard length or fish body weight or ovary weight}}$$

Estimation of fecundity-length and weight relationships:

Relationships between absolute fecundity and standard length (SL), body weight (BW) and ovary weight (OW) were determined using the formulae³²: $F = a SL^b$, $F = a + b BW$ and $F = a + b OW$, respectively, where 'a' represents the intercept of the regression with y-axis and 'b' the slope of the regression line.

Histological examination of oocytes: Ovaries belonging to a range of developmental stages were prepared for histological study by fixing in 10% buffered formalin for 3 days. They were then taken out from vials and cross sections from different parts cut and placed on tissue paper so as to absorb the moisture. Each cross section of an ovary was separately put

into one cassette and labeled with a pencil accordingly. Standard histological processes involving dehydration, clearing, infiltration, embedding, trimming and blocking, sectioning, staining, mounting and observation of slides under a light microscope (XSZ-PW107) for identification of gametogenic cell types were performed.

Statistical analyses: Mean GSI values of the female samples were computed monthly. Regression analysis was performed using MS Excel 2007 to estimate the relationships between fecundity and standard length, fecundity and body weight and fecundity and ovary weight.

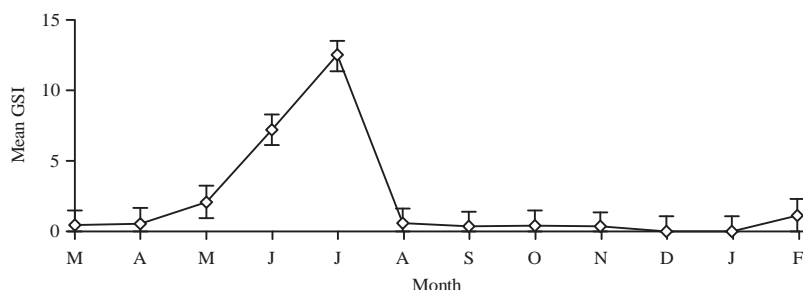
RESULTS

Morphology of the gonads: Macroscopic examination of *H. menoda* during the 12 months study period showed that the gonads in female and male are bilobed, located in the dorsal portion of the body cavity, separated into two equal sizes, joined each other at the caudal region and formed a common duct at the base of uro-genital papillae. Ovaries in the developing and immature *H. menoda* are pinkish, slender and translucent and resemble immature testes in appearance. As they advance in maturity, they become pale green and greenish in colour and expand in length and diameter. Testes are thin, ribbon-like and somewhat segregated in developing (immature) fish but as the fish progress in maturity, they become creamier, whitish and serrated.

GSI: The mean values of GSI ranged from 0.00 ± 0.00 (December) to 12.50 ± 4.97 (July) in female which showed distinct seasonal changes. The GSI started increasing in May, reached the peak in July and then abruptly dropped in August. Consequently, the GSI remained very low throughout the study period. The monthly variations in the mean GSI of female *H. menoda* is shown in Fig. 1.

Fecundity: The mean fecundity was 77273.77 ± 276.82 for fishes with mean SL of 31.85 ± 2.39 cm and mean BW of 628.37 ± 18.60 g (Table 1). The highest fecundity (222171.8) was in July from fishes with mean SL and BW of 40.20 cm and 1074.00 g, respectively. The lowest (22954.99) was in May from fishes with mean SL and BW 25.00 cm and 389.00 g, respectively.

Relative fecundity: The relative fecundity of *H. menoda* in terms of SL, BW and OW is presented in Table 2-4, respectively. Slight fluctuation was observed in the relative fecundity in terms of SL. However, lowest (903.47) relative fecundity was

Fig. 1: Monthly variation in the mean GSI of female *H. menoda*

The vertical bars indicate standard deviation

Table 1: Egg counts of *H. menoda* collected from Kangsha River Netrakona Bangladesh (March, 2015-February, 2017)

Month	Fish No.	SL (cm)	BW (g)	OW (g)	GSI	Absolute fecundity
May	10	31.50	665.00	9.00	1.35	35869.59
May	09	25.00	389.00	5.54	1.42	22954.99
May	10	33.40	478.00	17.05	3.57	39869.59
June	21	28.50	400.00	37.20	9.30	70295.76
June	20	26.70	336.00	17.30	5.15	24025.37
July	34	40.20	1074.00	88.60	8.25	222171.80
July	29	29.50	437.00	78.50	17.96	30821.26
July	36	40.00	1248.00	141.00	11.30	172181.80
Mean \pm SE		31.85 \pm 2.3	628.37 \pm 18.6	49.27 \pm 0.97	7.29 \pm 2.3	77273.77 \pm 276.8
Range		25.00-40.2	336.00-1248.00	5.54-141.00	1.35-17.96	22954.99-222171.8

SL: Standard length, BW: Body weight, OW: Ovary weight

Table 2: Relative fecundity of *H. menoda* in relation to standard length (SL)

Number of fish examined	Size group (SL, cm)	Absolute fecundity	Relative fecundity (SL, cm)
40	25.00-27.00	23490.18	903.47
19	27.01-29.00	70295.76	2510.11
19	29.01-31.00	30821.26	1027.20
24	31.01-33.00	35869.59	1120.75
28	33.01-35.00	39869.59	1172.46
39	≥ 35.01	197176.8	5633.62
Mean \pm SE	31.85 \pm 2.39	66253.86 \pm 257.14	2061.27 \pm 42.97
Range	25.00-40.20	23490.18-197176.8	903.47-5633.62

Table 3: Relative fecundity of *H. menoda* in relation to body weight (BW)

Number of fish examined	Size group (BW, g)	Absolute fecundity	Relative fecundity (BW, g)
38	330.00-380.00	24025.37	67.677
37	381.00-430.00	46625.37	114.980
37	431.00-480.00	35345.43	77.590
57	≥ 481.00	143407.73	298.140
Mean \pm SE	468.25 \pm 0.89	62350.97 \pm 58.56	139.60 \pm 2.37
Range	356.00-1248	24025.37-143407.7	67.67-298.16

Table 4: Relative fecundity of *H. menoda* in relation to ovary weight (OW)

Number of fish examined	Size group (OW, g)	Absolute fecundity	Relative fecundity (OW, g)
38	5.00-35.00	28417.44	1420.87
37	35.01-75.00	70295.76	1278.10
37	75.00-105.00	126496.53	1405.51
57	≥ 105.01	172181.80	1638.26
Mean \pm SE	49.27 \pm 1.26	99347.88 \pm 125.52	1435.69 \pm 6.11
Range	5.53-141	28417.44-172181.8	1278.10-1638.27

observed in smallest (25.00-27.00 cm) size group fishes while highest relative fecundity was enumerated in the largest (≥ 35.01 cm) size group (Table 2). Deviations were also

observed in BW related fecundity (Table 3). Lowest (67.677) relative fecundity was recorded in the lowest (330.00-380.00 g) size group fishes while the highest (298.14)

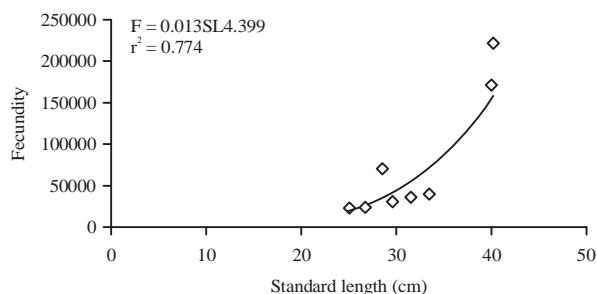


Fig. 2: Correlation between fecundity and standard length of *Hemibagrus menoda*

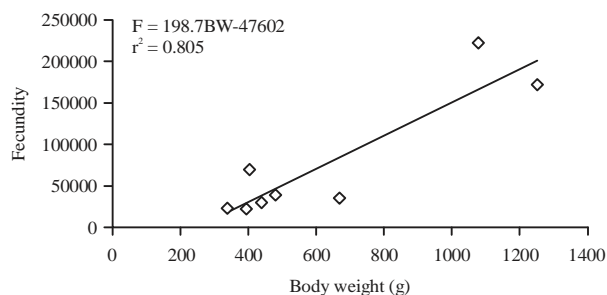


Fig. 3: Correlation between fecundity and body weight of *Hemibagrus menoda*

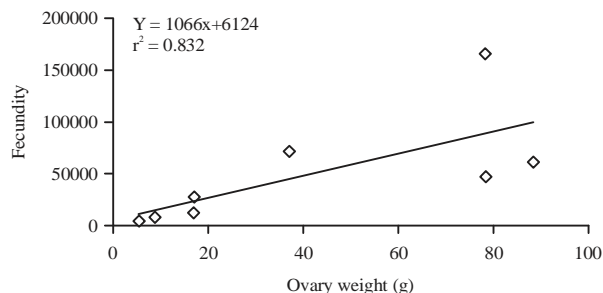


Fig. 4: Correlation between fecundity and ovary weight of *H. menoda*

relative fecundity was in the largest (≥ 481) size group. Results in Table 4 indicated that relative fecundity of *H. menoda* was less dependent on OW. The lowest (1278.10) relative fecundity in terms of OW was in the size group (35.01-75.00 g) while the highest (1638.26) fecundity was in the size group (≥ 105.01 g).

Fecundity-length relationships: The relationship between fecundity and SL for *H. menoda* is presented in Fig. 2. The logarithm transformed fecundity and SL values were plotted in the scatter diagram and yielded a non linear (power curve) equation: $F = 0.0135SL^{4.39}$. The logarithmic transformation gives a linear regression (straight line regression) of fecundity and SL of the fish as:

$$\text{Log } F = 0.0135 + 4.399 \log SL \quad (r^2 = 0.774)$$

Where:

F = Fecundity

SL = Standard length

The estimated coefficient of determination was 0.774 suggesting that 77.4% of the variation in fecundity was due to variation in SL. The slope (4.399) which is greater than 3 in the equation indicated that fecundity increases as the fish gets longer.

Fecundity-BW relationships: The logarithmic relationship between fecundity and BW of *H. menoda* gave a linear equation: $F = 198.7 BW - 47602$; $r^2 = 0.805$ (Fig. 3). The coefficient of determination showed that 80.5% variation in fecundity was due to variation in BW. A straight line through the origin would fit the points well which told that the number of eggs were directly proportional to the weight of the fish.

Fecundity-OW relationships: In order to study this relationship, the fecundity values were plotted against the respective weight of ovaries as a scatter diagram (Fig. 4). Strong coefficient of determination ($r^2 = 0.832$) was found between OW (X) and fecundity (Y). The relationship between fecundity and OW may be expressed using regression equation as:

$$F = 1066 OW + 6124; \quad r^2 = 0.832$$

and in logarithmic form as:

$$\text{Log } F = 1011 + 1.020 \log OW \quad (r^2 = 0.832)$$

Where:

F = Fecundity

OW = Weight of ovary

Variations in the histological features of *H. menoda*: The development stages of the oocytes in female *H. menoda* during oogenesis were identified over the 12 month study period (Fig. 5). Microscopic observations of the different ovary sections examined revealed different stages of development such as undeveloped oocytes (UO), early perinucleolar stage (EPNO), late perinucleolar stage (LPNO), cortical alveolar stage (CA), yolk vesicle stage (YV), early yolk granule stage (EYG), late yolk granule stage (LYG), pre-mature stage (PM) and the mature stage (M) (Fig 5). The UO and EPNO were observed in January and March samples and were found to be minute in size and difficult to recognize.

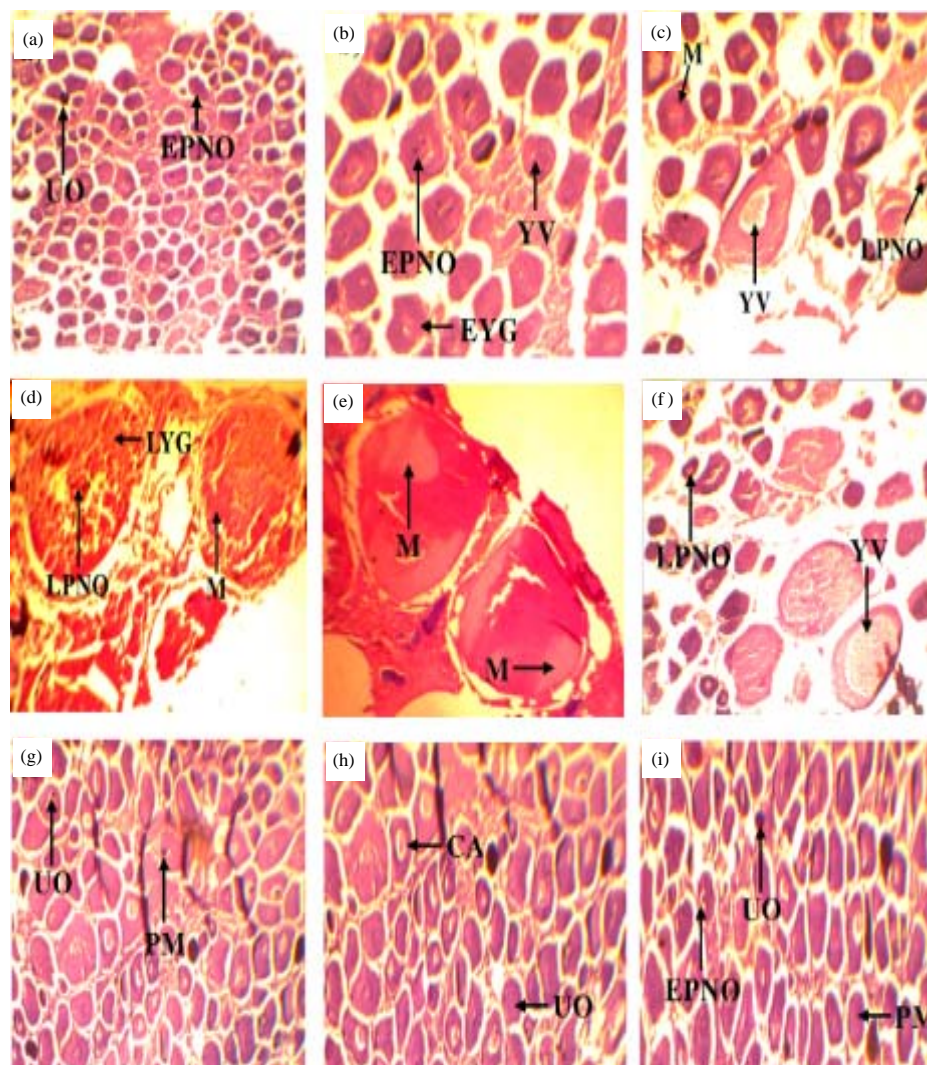


Fig.5(a-i): Haematoxylin-eosin stained sections showing different developmental stages in ovary of *H. menoda* at 10x magnification sampled in (a) March, 2016, (b) April, 2016, (c) May, 2016, (d) June, 2016, (e) July, 2016, (f) August, 2016, (g) October, 2016, (h) December, 2016, (i) January, 2017. UO: Undeveloped oocytes, EPNO: Early perinucleolar oocytes, LPNO: Late perinucleolar oocytes, CA: Cortical alveolar stage, YV: Yolk vesicle stage, EYG: Early yolk granule stage, LYG: Late yolk granule stage, PM: Pre-mature stage and M: Mature stages

The oocytes at this stage are often referred to as oogonia and after staining with haematoxylin-eosin, their sizes ranged from 17-54 μm . Oocytes in the mature stage were found to contain 4 bands of oocytes, LPNO, YV, LYG and M oocytes which predominates the May, June, July and August samples. The YV stage signals the onset of the vitellogenesis and initially appeared colourless on staining with haematoxylin and eosin. The YV stage contains several nucleoli which developed as minute bodies and progressively became enlarged in diameter of the oocytes (60-75 μm). At the LYG stage, oocytes increased concurrently with the advancement of yolk granules and the

yolk granules appeared deep pink with haematoxylin and eosin (June and July). At the end of mature (M) stage, vitellogenesis was completed, oocyte full-grown and yolk globules size increased (about 1060 μm). In the post-vitellogenic oocytes large lipid vacuoles and yolk granules of the remaining oocytes degenerated and underwent atresia (August). Undeveloped oocytes (oogonia) predominate in the later stages (October) of oocytes development characterized by stem cells occurring singly. Subsequently the appearance of CA (December) which stained deeply with haematoxylin and eosin indicated the early stages of secondary growth development.

DISCUSSION

The reproductive biology of *H. menoda* in terms of onset and duration of spawning through GSI, fecundity and histology of the gonads were studied as important inputs in the assessment and management of the fish stock. The GSI is often used to identify the gonadal maturity and spawning season of fish species. Examination of the ovary of *H. menoda* revealed a distinct relationship between GSI and gonad maturation in the fish. The high values of GSI in May, June and July obtained in this study indicates the spawning season of *H. menoda*. The highest value obtained only in July indicates one peak spawning season. The onset of spawning season in May observed for *H. menoda* coincides with the onset of rainy season in the region. It could be inferred that maturation of the gonads were influenced by the rainy season. This corroborates with Kiran¹⁵ that the GSI of gonads of cyprinid fish *Salmostoma untrahi* increased at rainy season whereas, lowest GSI was in winter. Recently, similar results were obtained in *Labeo bata* where high GSI values were observed from May to August and only one GSI peak in the month of June³³. High GSI was recorded for *Pomadasys jubelini* in July to September³⁴. The findings of this study agree with Al Mahmoud *et al.*³⁵ who recorded higher GSI for *Channa striata* from March to July with a peak in July. Siddiquee *et al.*¹⁶ reported similar results in *Channa marulius* from Sylhet basin whereby highest GSI was obtained in July. Similarly Gupta and Banerjee³⁶ stated that GSI in female *Mystus tengara* reached peak once a year during the month of July. Khan *et al.*³⁷ mentioned that mature *Plotosus canius* (Grey-eel or Brackish water catfish) became available from April to August, the peak being July. Contrary to our findings, GSI of silver grunt, *Pomadasys argenteus* was highest in March and an additional small peak in October in the females³⁸. The breeding period (GSI) of female *Oreochromis niloticus* exhibited several peaks in March, April, June and September²¹ suggesting that females breed more than once a season (partial spawning), which is contrary to our findings. Findings from this study showed that *H. menoda* is a single spawner and the breeding season expands from May-July with July being the peak.

Fecundity estimation of fishes is vital to having information on the spawning season, reproduction potential and seed production capacity of the species concerned. In the present study, the minimum fecundity was 22,954.99 (SL = 25.00 cm) while the maximum fecundity was 222,171.8 (SL = 40.20 cm). Thus, the fecundity increased with increase in length of *H. menoda*. Bailung and Biswas²⁰ reported minimum fecundity of $6,965 \pm 889.38$ in fish of 6.5-9.0 cm and maximum fecundity of $12,338.57 \pm 1241.04$ in length group of

11.5-14.0 cm for *Mystus dibrugarensis*, which is lower than that of *H. menoda*. Lower range was also reported by Karmakar and Biswas¹⁸ for *Tetraodon cutcutia* (Pisces: Tetraodontidae) from Meleng River in Jorhat district, Assam with minimum fecundity of 447.48 (TL = 5.86 ± 1.19 cm) and maximum fecundity of 1540.08 (TL = 6.55 ± 1.36 cm). Recently, lower minimum and maximum fecundity of 2230 (TL = 4.7-5.6 cm) and 8450 (TL = 7.7-8.6 cm) were recorded in *Perthia ticto* in the study of Gorai River¹⁹. Fecundity within a stock varies annually, undergoes long-term changes and has been shown to be proportional to fish size and condition^{21,39}. Within a given species, fecundity may vary as a result of different adaptations to environmental habitats⁴⁰. The mean fecundity of *H. menoda* was 77273.77 ± 276.82 for fishes with mean length of 31.85 ± 2.39 cm. This mean fecundity of *H. menoda* obtained in this study was higher than those obtained for *Alburnoides* sp., *Perthia ticto* and *Securicula gora* with average fecundity of 1722.92 (TL = 98.72 mm), 2586 ± 700 and $23,860.12 \pm 4980.21$, respectively^{31,41,42}. It could be inferred that *H. menoda* is a highly fecund fish.

Relative fecundity also contains information useful for comparing the reproductive strategies in fish and could be provided in terms of body and ovary weight^{2,43}. In this study, though highest relative fecundity was found in fishes belonging to the largest size group in terms of body weight and ovary weight, some fluctuations exist as it was observed that relative fecundity was not strictly dependent on body and ovary weight of *H. menoda*. The relative fecundity of 77.59 oocytes was found in 431.00-480.00 body weight group which is lower than the 114.98 oocytes found in the 381.00-430.00 weight group. Similar trend was found in relative fecundity in terms of ovary weight of *H. menoda*. Khan *et al.*³⁷ reported 57.664 ova per ovary weight in *Plotosus canius* with average weight of ovary 21.287 g which was higher than the 14.022 ova per weight of ovary in a fish with ovary weight of 160.460 g. Therefore, this data somewhat conflicts the theory that relative fecundity is ovary weight dependent but corroborates with the theory that fecundity is affected by species, age, feeding, season and environmental conditions⁴⁴.

Fecundity in fishes is often correlated with length, body weight of fish and also with weight of ovary⁴⁵. In this study it was found that fecundity increased with SL, BW and OW. Moreover, it was also found that in *H. menoda*, OW ($r^2 = 0.832$) and BW ($r^2 = 0.805$) are more responsible to increase in fecundity than SL ($r^2 = 0.774$). Similar results have been reported for freshwater fishes *Puntius stigma* and *Monopterus albus* and concluded that the correlation of fecundity with gonad weight is more significant compared to that of fecundity with other body parameters^{46,47}. Results from

this study agree with Agarwal⁴⁵ in his study of fecundity of *Schizothorax plagiostomus* that a close correlation is usually expected between number of eggs and ovarian weight and volume.

From the foregoing, it is evident that *H. menoda* is a highly fecund fish and its reproductive potential is quite high in comparison to other fishes probably due its large size and feeding habit as well as food productivity, temperature, salinity and genetic difference of the stock.

Histological features of oocyte development stages were also studied to ascertain the breeding season of *H. menoda*. Histological observation revealed one spawning season in *H. menoda*, from late April to July, which corresponds to the monsoon season. In this study, oocytes maturation stages were classified into undeveloped oocytes (UO), perinucleolar oocytes (PNO), cortical alveolar stage (CA), yolk vesicle stage (YV), yolk granule stage (YG), premature (PM) and mature (M) stage. Al Mahmoud *et al.*³⁵ and Milton *et al.*²³ used similar classification for *Channa striata* and *Channa gachua*, respectively. In fish ovary, the premature (PM), yolk granule (YG) and mature (M) oocytes are generally observed at the most advanced stage of ovary when the fishes are ready for spawning. In this study, YG, PM and M oocyte stages were abundant from late April to July samples of ovary, indicating the spawning season of *H. menoda*. The occurrence of M stage oocytes was highest in July, indicating the peak breeding season. Current findings are similar to Erkmen and Kirankaya²² who observed that mature oocytes in Mirror Carp and Scaled Carp (*Cyprinus carpio* L., 1758) were first observed from the beginning of April until July that corresponds with monthly changes observed in GSI. The findings from GSI are in concurrence with the histological observations, further confirming the peak breeding season of *H. menoda* in July. Undeveloped oocytes (UO), EPNO and LPNO stage oocytes were abundant in October to January samples, indicating spent stage of ovary of *H. menoda*.

CONCLUSION

The GSI revealed that *H. menoda* spawns once a year and the spawning season extends from May to July with the peak in July. Further histological examination of the ovaries of female *H. menoda* confirmed this finding as the GSI values increased with increasing histological changes. Fecundity studies revealed that *H. menoda* is highly fecund fish and varies in relation to length and body weight of the fish. Knowledge of reproductive biology of *H. menoda* has application in the management and assessment of the stock in open water bodies and in the development of artificial seed production techniques for commercial aquaculture.

SIGNIFICANCE STATEMENT

This study discovered that *H. menoda* is a highly fecund fish and provides the first information on the spawning season (from May to July) of this species. In view of the recurrent flood, excessive rains and water pollution that leads to devastation of habitat and biodiversity in the Kangsha River Netrakona which has inadvertently led to the population decline of many economic species including *H. menoda*, this study can be of immense benefit to conservationists and aquaculturists in planning future management strategies as well as breeding and culture program for this fish species. Based on this discovery, a new cultured fish may soon be in the market.

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