

Some Aspects of Reproduction in *Amblypharyngodon mola* from Sylhet, Northeast Bangladesh

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ABSTRACT

The mola carplet, *Amblypharyngodon mola* (Hamilton, 1822) is the most common small indigenous freshwater fish species in Bangladesh. The precise information addressing the reproduction in this species including breeding peak, ovarian histology, morphometry, and sex ration in nature is still insufficient. A one yearlong field survey and different laboratory assays have been directed to disclose some key features of reproduction in female *A. mola* from wetland reservoirs in the greater Sylhet region in Bangladesh. The total length and the body weight have shown a strong correlation with gonad weight, occupying statistical r^2 values of 0.89 and 0.91, respectively. However, a Chi-square test at $P < 0.05$ reported no significant deviation in sex ration of the wetland's stock. The maximum fecundity has been reported during the month of May as 12569+620 while the lowest is during the month of July as 9377+455. The highest values for gonadosomatic index have been reported in May while the lowest is in January with another peak in November. The histological study of ovarian cells resulted in the characterization of oocytes, previtellogenic oocytes, vitellogenic oocytes, perinuclear oocytes, and mature cells with yolk mass.

Keywords: *Amblypharyngodon mola*, sex ration, GSI, fecundity, morphometric regression analysis, gonadal histology

INTRODUCTION

The mola, *Amblypharyngodon mola* is one of the most popular small indigenous fish (SIF) species in Bangladesh, which occupy almost all of the shallow freshwater habitats (Mondal & Kaviraj, 2013; Mondal et al., 2019). This fish has drawn significant commercial and cultural value due to its premium nutritional value (Abdulla et al., 2004; Alam et al., 2004; Shikha et al., 2019) and current adoption as ornamental fish in aquarium (Gupta & Banerjee, 2015). Currently this species is also subjected to coculturation with other cyprinid fish or in traditional pond culture technique to supplement domestic food supply in South Asian countries (Neetu & Seema, 2018). This fish is considered as one of the major species to meet the rural nutrition supplement and also constitutes the principle

target of small scale artisanal fishing practices in Bangladesh (Bengal et al., 2017). Rich protein contents and the abundance of micronutrients make this species one of the thriving mediators for securing poor people's nutrition and the economy as well (Ahmed et al., 2012; Hossain et al., 2017; Kohinoor et al., 1998).

Information regarding the body parameters (Khalid et al., 2020), growth factors, and different reproductive indices, i.e., hepatosomatic index, gonadosomatic index and fecundity etc. (Jabed et al., 2020; Jannatul et al., 2015; Rahman et al., 2020), serves as key indicators of reproductive progression and breeding phase of fish (Amzad et al., 2015; Mian et al., 2020; Uddin et al., 2017), which are essential for implementing fishery strategies and conservation approaches (Iqbal & Naeem, 2018; Jannatul et al.,

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2015; Mian et al., 2017). Biometric features of fish are a widely accepted form of data which bears necessary information about the growth, development and stock assessment of the fish (Muchlisin et al., 2010). The major quantitative features in fish (i.e., total length, body weight, gonad weight, and fecundity) provide actual biological modelling of species in a particular geographical area (Emre, 2020; Tharwat et al., 2018). The understanding on major aspects of wild breeding biology, annual reproductive peak, breeding season, ovarian maturation phases, and fecundity will certainly help in the future management of this species (Ahamed et al., 2017; Uddin et al., 2017).

MATERIALS AND METHODS

Study area and duration

Fresh and live random samples of *A. mola* have been collected from different fisher landing stations and local markets of the Greater Sylhet region of Bangladesh. They were transported in insulated ice boxes or in oval fiberglass tanks to bring into the Fish Biology and Genetics laboratory of Sylhet Agricultural University, Bangladesh. This research continued for a full-length year from July 2019 to June 2020.

Recording morphometric data

Shortly after bringing them back to the laboratory, all the fish were washed well with rinsing freshwater and placed on thick tissue towels to reduce the access of water and mucous content within them. Then, the total length and weight of each fish was calculated by using scale (a mm stainless-steel scale attached on a specially designed wooden structure) and electric balance (Ohaus corp. Pine Brook, NJ USA), respectively. Sexual recognition of each fish was confirmed by following the dissection of their gonad, which was weighted and recorded immediately.

Measurement of gonadosomatic index (GSI) and Fecundity

The value of gonadosomatic index for each fish was calculated by using the below formula (Brooks et al., 1997)

$$\text{Gonadosomatic index (GSI)} = (\text{Weight of gonad (g)} / \text{Total body weight (g)}) \times 100$$

Fishes collected during the early to late monsoon season was subjected to the calculation of fecundity. Gravid female fish were selected for fecundity, and Gilson's fluid was used to lessen the oocytes. Finally, absolute fecundity was calculated by using the description of Rahman & Samat, (2020).

The fecundity, $F = n \times G / g$, where "n" denoted the average number of eggs counted in sub-sample, "G" is net weight of the gonads, and "g" is the weight of the sub-sample.

Histology of gonad

The samples from the ovaries were chopped into small pieces and preserved in neutral buffered formalin. The standard protocol for gonad histology described by Van-Dyk & Pieterse, (2008) was followed for the preparation of histology slides. Tissues were embedded and infiltrated in Paraffin, and the resulting blocks were formatted into 3 micrometer slides. Finally, hematoxylin-Eosin staining accompanied the samples to view the cell with a Zeiss microscope (software version 3.0 pro).

Data analysis

The raw data were recorded on excel sheet before further analysis. Data were analyzed by using IBM SPSS Statistics v26 and a one-way ANOVA was tested at $P < 0.05$ to measure the difference between mean values.

RESULTS AND DISCUSSIONS

The ratio between female and male fishes of *A. mola* in the wild natural wetland of the Greater Sylhet region does not vary significantly between months. However, the lowest male to female ratio was accounted in May (1:0.6), followed by January, July, and November (1:0.7), and by March, August, September, and December (1:0.8) (table 1). In contrast, the maximum value for the male to female ratio was observed during the month of October (1:2.03), followed by February, June (1:1.5) and April (1:1.3) (Table 1).

Table 1. Analysis (Chi-square test, $P < 0.05$) of sex ration of *A. mola*

Month	Total	Female		Male		χ^2 -value	Ration (M: F)
	Sample	No.	%	No.	%		
Jan	20	12	60	08	40	1.2	01:00.7
Feb	25	10	40	15	60	1.2	01:01.5
Mar	30	16	53	14	47	0.14	01:00.8
Apr	30	13	43	17	57	0.54	01:01.3
May	30	19	63	11	37	2.13	01:00.6
Jun	25	10	40	15	60	1.2	01:01.5
Jul	20	12	60	08	40	1.2	01:00.7
Aug	30	16	53	14	47	0.13	01:00.8
Sep	30	16	53	14	47	0.13	01:00.8
Oct	30	10	33	20	67	3.33	01:2.03
Nov	30	17	57	13	43	0.53	01:00.7
Dec	30	16	53	14	47	0.13	01:00.8

The body weight (mg) data was plotted against the correspondent of total length (cm) data to disclose the regression analysis of above morphometry. The regression line shows a strong correlation between body weight and total length of *A. mola* in the natural wetlands of Northeast Bangladesh with a r^2 value of 0.89. It shows that about 89 percent of fish showed increasing weight in relation to total length or vice versa (Figure 1). Again, fecundity was also found to have a very strong correlation with increasing body weight of fish, occupying an estimated r^2 value of 0.91, showing that large fish tend to have more fecundity than smaller ones (Figure 2.A). However, the values of the total length show very minor and nonsignificant correlations with ovarian weight (Figure 2.B). The gonadosomatic index of *A. mola* in the natural wetlands of Sylhet, Northeast Bangladesh were reported to be very distinctive on a month-wise distribution. The highest GSI value was recorded in May (16.66) and then fell to 12.58 in June (Figure 3). The two peaks in GSI value were noticed once in May

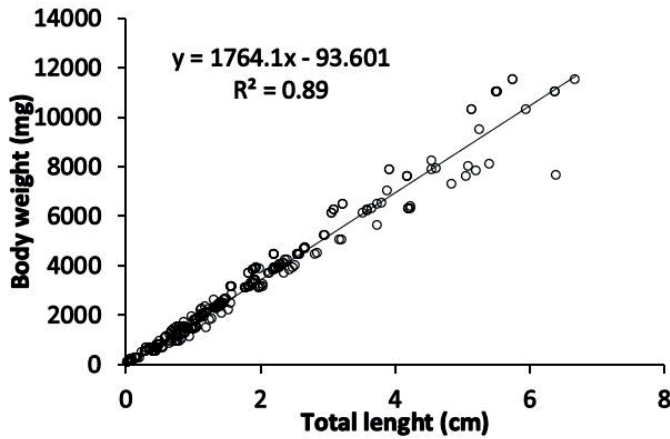


Figure 1. Length weight relationship of *A. mola* in Sylhet, Northeast Bangladesh.

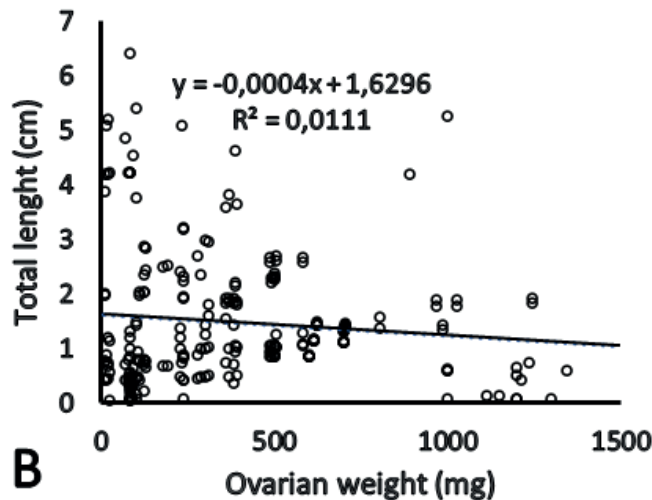
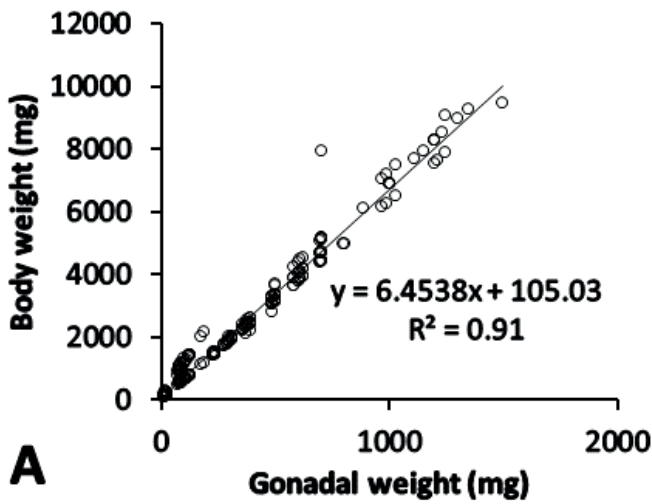


Figure 2. Relation between *A. body weight* and gonadal weight, B. total length and gonadal weight of *A. mola* in Sylhet, Northeast Bangladesh.

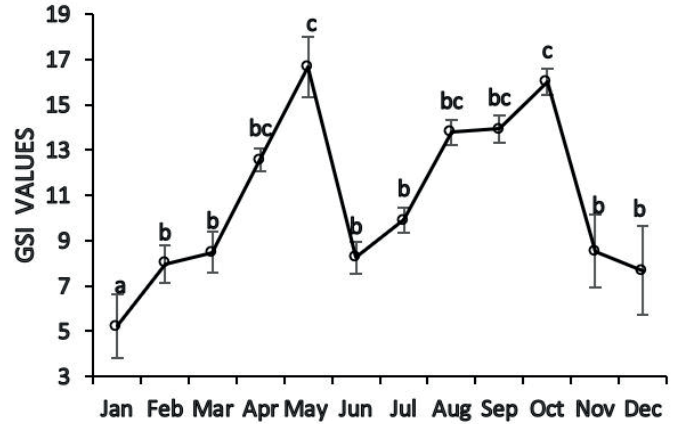


Figure 3. Gonado-somatic index of female *A. mola*.

and another in November 8.54 (Figure 3). The female *A. mola* in the natural wetlands of Sylhet, Northeast Bangladesh have been reported as an intermediate fecundated species. The highest fecundity was observed during the month of May as 125689+620, followed by 11172+160 in August and 10531+305 in June (Figure 4). However, the lowest fecundity was accounted during July 9377+455, followed by 9947+55 in September (Figure 4).

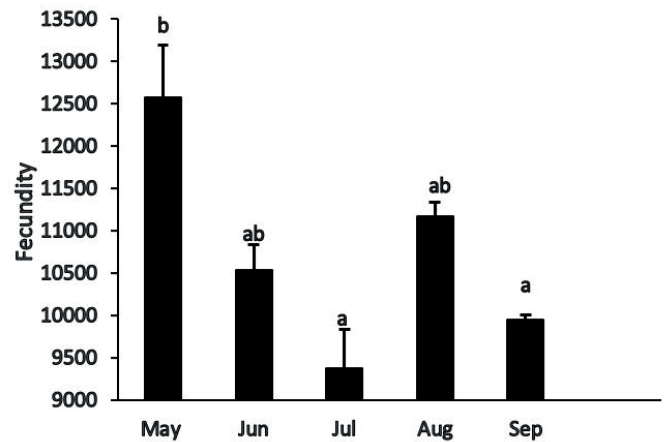


Figure 4. Variation in fecundity of *Amblypharyngodon mola* in Sylhet, Northeast Bangladesh.

The histological analysis of ovarian tissue resulted in the occurrence of oocytes and pre-vitellogenic oocytes as the initial phase of gonadal maturation (Figure 5.A). At the end of primary development phase, the nuclear mass of the cell tends to migrate toward the periphery, termed as perinuclear oocytes (Figure 5.B). Mature vitellogenin oocytes are characterized by the presence of yolk globules and yolk vesicles in the cell (Figure 5.C).

The sex ratio for a particular of the species would be different in response to habitat, fishing strategies, and harvesting method also (Oh et al., 2008). A study by Das et al., (2018) recorded sex ratio of 1 : 2.05 for *A. mola* in Dekar Haor of Sunamganj, Bangladesh while a 1:3.04 male to female ratio was reported by Gupta

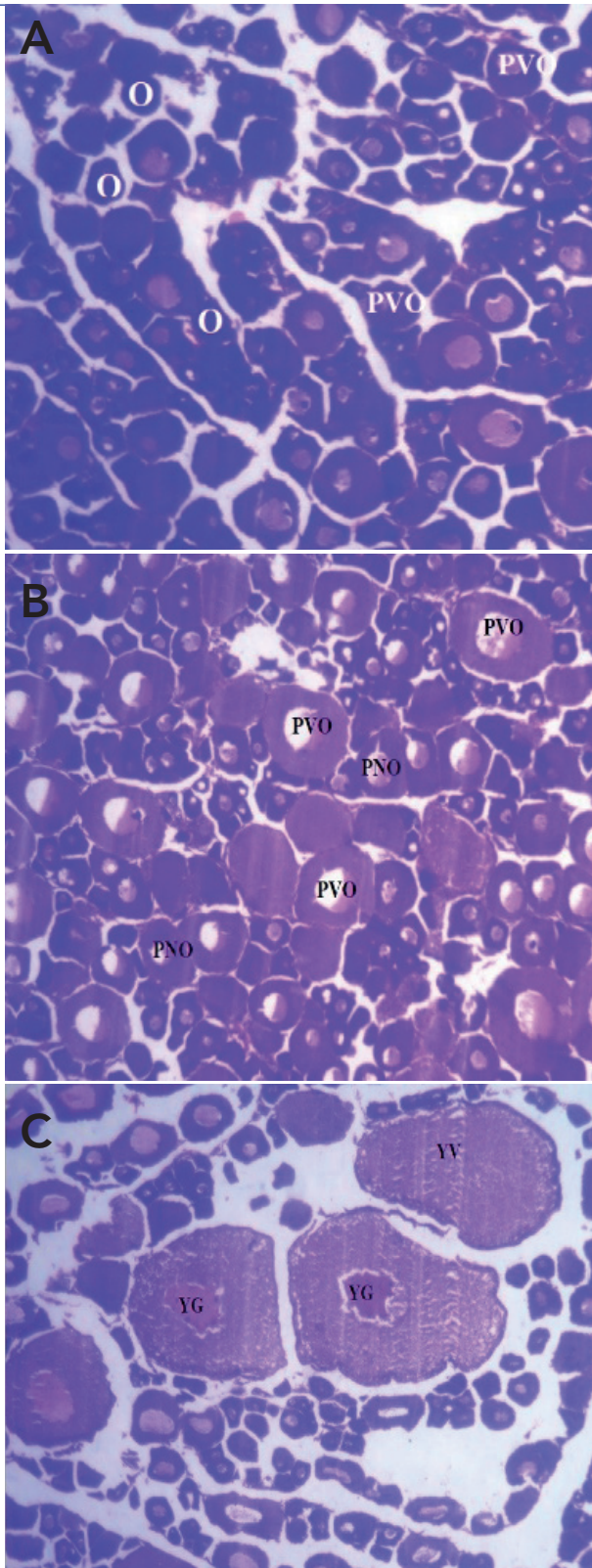


Figure 5. Different stages of development in ovarian cell of *A. mola* (O-oocytes stage, PVO-previtellogenic stage, PNO-perinuclear oocytes, YV-yolk vesicle, YG-yolk globules).

& Banerjee (2013) for the natural wetland of Baruipur, West Bengal, and 1.00:2.078 was recorded by Azadi & Mamun (2004) at the Kaptai Reservoir in Bangladesh. Current research is supportive of the previous study by Ahmed, et al., (2017), who had reported male to female ratio ranges between 1:1.14 to 1.32 in the Payra River of southern Bangladesh.

Morphometric studies of fish provide beneficial information towards the better understanding of the maturity and life history of particular species (Hossain et al., 2006). Previous research by Gupta & Banerjee (2015) observed a regression correlation value of 0.913 for female *A. mola* in freshwater from West Bengal, India, while Nower et al. (2018) reported a r^2 value of 0.93-0.96 for the same species in the Ganges River, Northwestern Bangladesh. All the above studies closely stand with the present findings for *A. mola* in the Greater Sylhet region, Bangladesh. The fecundity of fish was reported to be boosted with fish size (Jannatul et al., 2015); the relation between fecundity and body weight might have shifted drastically during the onset of breeding season due to dramatic modification of egg size (Rai et al., 2018). A positive correlation between fecundity and body weight was reported for several, i.e., $r^2 = 0.77$ for *Xenotodon cancila* from Assam, India (Borthakur, 2018), $r^2 = 0.75$ for *Mystus cavasius* (Hamilton) from Brahmaputra and Kongsa river, Bangladesh (Islam & Das, 2006), $r^2 = 0.87$ for *Oreochromis Niloticus* from Balochistan (Kausar et al., 2019), and $r^2 = 0.79$ for cyprinid fish *Labeo calbasu* at Faridpur, Bangladesh (Kabir & Quddus, 2015). The present study also revealed a similar trend of morphometric correlation, and slight variations are raised because of differences in study location, season, and sampling strategies.

The values of gonadosomatic indices act as potential marker for addressing the gonadal development stage, maturity, and breeding peaks of individual fish species (Hasan et al., 2018). Gupta & Banerjee (2013) had identified two distinguished GSI peaks for *A. mola* in west Bengal, India, one in June and another one October, and reached the second peak in November while a single peak in July had been observed by Mondal & Kaviraj, (2013) in the floodplain lakes of India. The lowest GSI value had been observed in January while highest value was in June for the same species from Bengal, India (Pal & Mahapatra, 2016). Two peaks in June and November and the lowest value in January had been documented in South Bengal, India (Pal and Mahapatra, 2016). Multiple peaks revealed that this species might spawn several times within year. The deviation of peaks in the current research might vary due to the study's geography and sampling strategies. The fecundity of a species depends upon a variety of intrinsic factors, environmental features, and nutritional properties of the diet (Alam et al., 2004; Kohinoor et al., 1998; Mian et al., 2020), and accurate knowledge in fish fecundity helps in regulating harvesting quotas and also provides key roles in aquaculture and fishery management (Kant et al., 2016; Tracey et al., 2007). The fecundity of mature female of *A. mola* ranged between 3785 to 12590 oocytes in floodplain water of India (Mondal & Kaviraj, 2013), 1,548–4,020 oocytes in the natural waters of South Myanmar (Kulabtong, 2016), varied between 1,280-13,679 in the Kaptai Reservoir in Bangladesh (Azadi & Mamun, 2004), and fluctuated between 1652 to 15,985 in the Ganges River of Bangladesh (Rahman et al., 2018). The findings of present research are also strongly supported by above previous results.

Ovarian histology provides essential data for predicting the reproductive success and peak in several fish species (Alonso-Fernández et al., 2011; Emam & Abughrien, 2014; Javed et al., 2020). Previous studies on different fish species revealed a characterization of oocytes and previtellogenic oocytes during the initiation of reproductive peak (Guraya et al., 1975; Viana et al., 2018). As the development process goes onward, the size of ovum and yolk globules increases (Murata et al., 2014; Quagio-Grassiotto et al., 2014). However, the simultaneous occurrence of different development stages also indicate the nature of several spawning peaks in this fish (Amzad et al., 2015; Javed et al., 2020), which is also aligned with their multiple peaks in GSI values.

CONCLUSIONS AND RECOMMENDATIONS

This research has disclosed a set of necessary information regarding the reproductive aspects of *A. mola* from the natural wetlands of the Greater Sylhet region of Bangladesh. The above information might be used in the aquaculture development, wild stock management, and conservation of this fish in Bangladesh. However, broad studies with the collection of samples from different areas within the country would reflect actual trends of the reproduction in this fish.

Conflict of interests: No conflict of interest has been generated on this research work.

Ethics committee approval: The research has complied and essentially approved with all regional, national, and institutional ethical clearance.

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