



Reproductive biology of *Amblypharyngodon mola* (Hamilton Buchanan, 1822) in pond ecosystem in Assam, North-east India

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ABSTRACT

Reproductive biology of *Amblypharyngodon mola* was studied in Assam during the year 2009-2011. No significant difference was observed in the external morphology between sexes, except during breeding season when the distended and bulging abdomen was vivid in mature female. Gonadosomatic index (GSI) of female *A. mola* showed three peaks in a year, once during March (15.57), second in June (18.74) and the third during September (17.84). In males, GSI was much lower compared to females; with three peaks in a year, during March (1.422), June (1.49) and September (1.398). Higher values of ova diameter was recorded thrice in a year in March (0.68 mm), June (0.748 mm) and September (0.762 mm), indicating that the spawning season of *A. mola* commences during these months. Males attained maturity for the first time in the size group 41-45 mm, while females attained first maturity in the size group 46-50 mm. Length at which 50% of the population mature (M_{50}), was estimated as 53 mm in male and 61 mm in female. Females showed dominance over males all throughout the year with average sex ratio of 1: 1.953 ($\chi^2 = 173.069$, $p < 0.05$, $N = 1660$). Absolute fecundity of *A. mola* varied from 485 (total length 54.4 mm, body weight 1.51 g and ovary weight 0.061 g) to 15786 (total length 91.3 mm, body weight 9.78 g and ovary weight 1.99 g).

Keywords: Absolute fecundity, *Amblypharyngodon mola*, Gonadosomatic index, M_{50} , Ova diameter, Sex ratio

Introduction

Amblypharyngodon mola (Hamilton-Buchanan, 1822) (Family: Cyprinidae), locally known as ‘moah/mowa’ in Assam is a small indigenous fish species (SIS) of immense commercial importance. Among the entire SIS found in Assam, *A. mola* is well known for high content of vitamin A, minerals and quality fatty acids (Goswami and Barua, 1981a, b; Goswami, 1984; 2007). According to Villif and Jorgensen (1993), *A. mola* contains 3 times more calcium and 50 times more vitamin A than that of silver carp and rohu. Recent market study in Assam revealed that the price of *A. mola* has increased tremendously from ₹100-200 kg⁻¹ in 2010 to ₹250-400 kg⁻¹ presently, depending on the size, quality and season. It is also in demand as smoked and dried fish for its superior flavour. Though the species is categorised under the conservation status ‘lower risk least concern’ (LR-lc) (CAMP, 1998), the recent catch statistics shows that the species is gradually becoming scarce in the local markets, while its demand is increasing. Apart from this, *A. mola* also attracts good demand in the international ornamental fish market. It is a self recruiting species and there is lot of potential for its monoculture in small homestead ponds (Borah *et al.*, 2013), polyculture with carps (Roos *et al.*, 2007; Roy *et al.*, 2002; Kohinoor and Wahab, 2003), as well as for introduction in rice fields (Alam *et al.*, 2004) in the north-eastern states of India.

Reproductive biology of *A. mola* has been studied earlier by many workers (Mitra and Jain, 1985; Afroze and Hossain, 1990; Azadi and Mamun, 2004; Saha *et al.*, 2009; Gupta and Banerjee, 2013). However, all these studies are from Bangladesh. Climatic condition of a region has much impact on the reproductive biology of a species and on the development of gonads. The breeding season as well as breeding frequency of a species varies with season, temperature and rainfall. Borah *et al.* (2010) studied certain aspects of breeding of *A. mola* in Assam. However, it was felt that a thorough understanding on the reproductive biology of the species is needed for developing culture strategies under the climatic conditions of Assam and hence the present study on reproductive biology of *A. mola* from pond culture system in Assam was undertaken.

Materials and methods

The study was conducted at the Fisheries Research Centre, Assam Agricultural University, Jorhat District, Assam (26°48'296"N ; 94°11'961" E) during June 2009 to May 2011. Male and female brooders were collected with utmost care from wild resources in Jorhat District during June 2009 as parent stock and stocked @5 nos. m⁻² in earthen pond of 1000 m² area. Pond preparation and fertilisation was done as per package of practices

recommended for semi-intensive carp culture (Anon., 1997). Length and weight of parent fishes at the time of stocking ranged from 6.5 to 8.5 cm and 2.71 to 6.37 g respectively. The fishes were in maturity stage V (gravid). Fishes were fed with mustard oil cake (50%) and rice bran (50%) at 2-3% of the body weight per day. Gonadal development, maturity stages and sex ratio of 40-45 specimens were studied every month all round the year, for two consecutive years. The physicochemical parameters of pond water during the experimental period were analysed periodically following standard methods (APHA, 1998).

Fishes were sampled randomly from the culture pond every month. Gross and microscopical examination of reproductive organs was done to determine the periodic changes in gonadal morphology as described by Biswas (1993). The maturity stages were studied according to Crossland (1977). Ova diameter was studied under ocular micrometer. For determining the gonadosomatic index (GSI), the total body weight and the weight of gonads were taken in fresh condition using an electronic balance (Dhona) up to the nearest milligram. GSI was then calculated using the formula proposed by Hopkins (1979):

$$GSI = \frac{GW}{BW} \times 100$$

where GW = Weight of gonad in grams and BW = Weight of fish in grams.

Length of the body at first maturity was determined for both the sexes by calculating the percentage of number of males and females in IV and V stages of maturity to the total number of fishes examined in a particular length group.

Length at which 50% of the population mature (M_{50}) was calculated separately for both the sexes as per the method given by Hodgkiss and Man (1978).

Fecundity was estimated by gravimetric method (Biswas, 1993) and the absolute fecundity was calculated using the following formula:

$$F = \frac{NG}{g}$$

where, F = Fecundity, N = No. of eggs in the subsample, G = Weight of ovaries, g = Weight of the subsample.

Relative fecundity (RF) was determined using the formula proposed by Das (1964):

$$RF = \frac{\text{Total number of eggs in the ovary}}{\text{Body weight}}$$

Sex ratio distribution was studied employing Chi-square test (χ^2), following Fisher (1970):

$$\chi^2 = \frac{\Sigma(O - E)^2}{E}$$

where, O = Observed value, E = Expected value

Results

Limnological parameters of water

Water quality parameters recorded during the experimental period are presented in Table 1. The average rainfall during the study period was 0 - 437.8 mm while the humidity was 65-97%.

Table 1. Limnological parameters of culture pond during the study period

Parameters	Range	Mean
Depth of water (m)	1.2-2.2	1.45
pH	7.0-7.8	7.3
Temperature (°C)	17.5-32.0	24.7
Free CO ₂ (mg l ⁻¹)	1.2-2.5	1.6
Dissolved oxygen (mg l ⁻¹)	4.7-7.2	5.6
Total alkalinity (mg l ⁻¹)	68.5-96.3	77.8
Plankton population (mg per 50 l)	3.8-5.3	4.5

Sexual dimorphism

No significant difference in colour was observed between males and females of *A. mola*. However, females were found to be slightly lighter in colour and larger in size than the males. In mature female (Fig. 1), pelvic fins are smooth, caudal fin deeply forked and abdomen soft, distended and bulging. Male is slightly thin and smaller in size than female. Abdomen is not distended in mature males (Fig. 2).

Gonadal development stages and maturity cycle

Five maturity stages of ovary and testis were recognised: Stage I (Immature), Stage II (Maturing),



Fig. 1. Gravid female *A. mola*

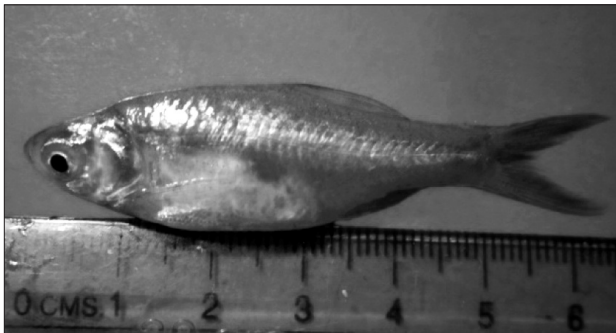


Fig. 2. Mature male *A. mola*



Fig. 3. Fully ripe (Stage IV) ovary of *A. mola*

Stage III (Mature), Stage IV (Ripe) (Fig. 3; 4), and Stage V (Spent). Characteristics of the ovary and testis and the months of their availability are given in Table 2. It was observed that the maturity cycle of *A. mola* extends from February to November. Gravid and spawning fishes (Stage IV) appear in the population during the months of March, June and September. During last part of April, July, October and in the months of November and December, maturing fish (Stage II) with developing gonads were recorded. In the months of April, July and October, spent fishes (Stage V), immature (Stage I) as well as maturing (Stage II) fishes were recorded simultaneously in the population. During September - October spawning was observed for the third time.



Fig. 4. Paired testis (Stage IV) of *A. mola*

Table 2. Characteristics of the gonads in different maturity stages of *A. mola*

Month	Maturity stages		Characteristics of the gonads	
	Stage	Degree of maturity	Male	Female
April, July, October, November	I	Immature	Testis small, whitish, and thread like occupying one sixth of the body cavity close to the vertebral column	Ovaries thin, flat, ribbon-like, whitish yellow in colour, almost transparent, eggs undetectable with naked eye
April, July, October, November, December	II	Maturing	Slight increase in volume and weight, occupy about one third of the body cavity, translucent	Ovaries become thicker, occupy about half of the body cavity, yellowish in colour, eggs visible under magnifying glass.
March - October	III	Mature	Marked increase in volume and weight, whitish to creamy, occupy about half the length of the ventral cavity	Marked increase in volume and weight, occupy three fourth of the body cavity. Eggs visible with naked eye, as granular material, pinkish yellow in colour
March, June, September	IV	Ripe	Testis creamy white, soft and flabby, occupying whole ventral cavity	Ovaries increase in volume and highly distended, occupy almost whole of the body cavity, eggs distinctly visible with naked eye, dark yellowish in colour
April, July, October	V	Spent	Testis shrinking, thin thread like in structure, show considerable reduction in size and weight	Ovaries become flaccid, show reduction in weight, pale whitish in colour. Fully spent ovary with bloodshots in fresh condition, appear empty

Ova diameter

Microscopic study of intraovarian eggs in different months revealed that there were five different developmental stages of ova. Wide fluctuations in the ova diameter and weight of gonads were recorded particularly in the months of April, May, July, August and October, when spawning, spent and maturing fishes appear simultaneously in the population. The ova diameter study showed three peak spawning periods in the breeding cycle of *A. mola* (Fig. 5.)

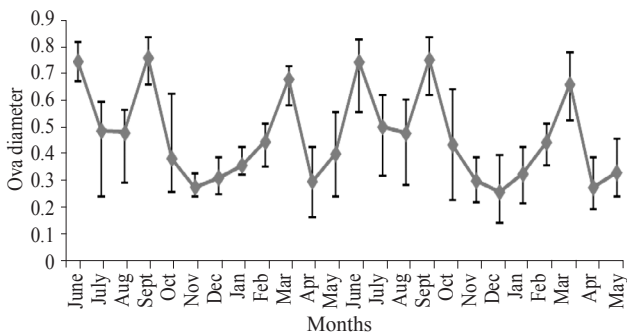


Fig. 5. Seasonal fluctuation of ova diameter in *A. mola*

Gonadosomatic index (GSI)

In female *A. mola*, mean GSI ranged from 2.93 to 18.75 in different months with three peaks in March, June and September (Fig. 6). In the first year, the mean peaks were: 18.51 in June, 17.85 in September and 15.58 in March. Almost similar mean values of GSI were recorded in the second year with 18.75 in June, 17.57 in September and 15.81 in March. Maximum fluctuation in GSI was recorded during the months of July, October and April months. The lowest GSI value was recorded in the month of November (3.71 in first year and 2.93 in the next year).

In male *A. mola* also three peaks in GSI values were recorded in the month of March, June and September (Fig. 7). Minimum fluctuation in the GSI values was noticed during the peak spawning period while in the

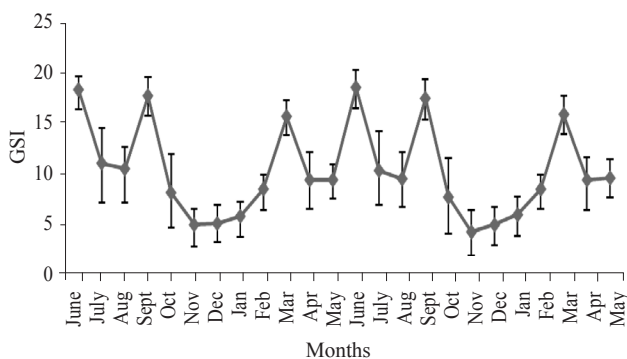


Fig. 6. Seasonal fluctuation of GSI in female *A. mola*

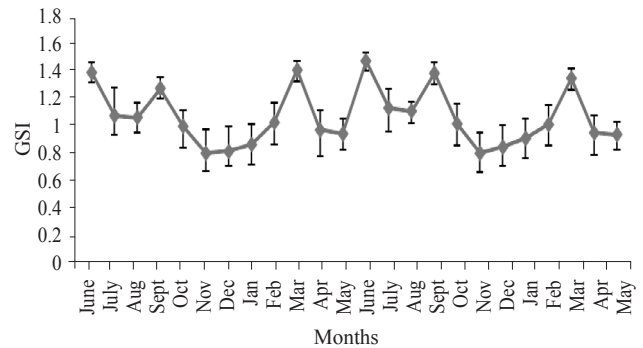


Fig. 7. Seasonal fluctuation of (GSI) in male *A. mola*

other months, a similar trend of fluctuation in GSI was recorded. GSI during June ranged from 1.33 to 1.49 in the first year and from 1.42 to 1.56 in the second year. In the month of September however, GSI values ranged from 1.21 to 1.37 in the first year and from 1.32 to 1.48 in the subsequent year. The third peak, in the month of March showed fluctuation from 1.34 to 1.49 in the first year and 1.27 to 1.44 in the second year.

Length at first sexual maturity and M₅₀

In *A. mola*, it was observed that males attained sexual maturity at an earlier stage than that of females. The gonads in Stage IV and Stage V were regarded as mature gonads. Recognition of the sexes by examination of gonads was possible from the size group of 36-40 mm. It was observed that amongst the fishes of length group 36-40 mm, mature fishes were not encountered in any of the sexes. In the length group of 41-45 mm, 20% maturity was recorded in males, while no mature fish was observed among females. Mature female (7%) was found to appear from 46-50 mm length group. One hundred per cent maturity was observed in the 61-65 mm size group in case of males, whereas all the females belonging to 71-75 mm length group were mature. Total length of the mature female fishes ranged from 71 to 95 mm, while that of mature male fishes ranged from 61-80 mm. Percentage occurrence of different stages of maturity in female *A. mola* in various size groups during the year 2009-11 is shown in Table 3, while that of male is shown in Table 4.

Pond bred generation with maturing gonads first appeared in the population within 130-140 days of their life. Although upon attainment of maturity, the whole batch did not breed at a time. The age at first complete maturity was observed to be 140-160 days when they attain average body length of around 56 mm for both the sexes.

The percentage occurrence of mature male and female individuals is represented in Fig. 8 a and b respectively, from which M₅₀ values for both the sexes of *A. mola* were estimated. M₅₀ value was estimated as 53 mm in male and 61 mm in female.

Table 3. Percentage occurrence of different stages of maturity in various size groups of female *A. mola*

Length group (mm)	Mean length (mm)	No. of fish observed	I (Immature)		II (Maturing)		III (Mature)		IV (Ripe)		V (Spent)	
			No.	%	No.	%	No.	%	No.	%	No.	%
41-45	43.0	16	16	100	-	-	-	-	-	-	-	-
46-50	48.0	21	19	90.47	2	9.52	-	-	-	-	-	-
51-55	53.0	22	13	59.09	9	40.9	-	-	-	-	-	-
56-60	58.0	19	6	31.57	8	42.1	5	26.32	-	-	-	-
61-65	63.0	23	4	17.39	8	34.78	11	47.82	-	-	-	-
66-70	68.0	17	-	-	5	29.41	8	47.05	4	23.53	-	-
71-75	73.0	24	-	-	2	8.33	7	29.16	11	45.83	4	16.67
76-80	78.0	23	-	-	-	-	5	21.73	13	56.52	5	21.73
81-85	83.0	18	-	-	-	-	3	16.67	10	55.56	5	27.78
86-90	88.0	24	-	-	-	-	2	8.33	13	54.16	9	37.5
91-95	93.0	21	-	-	-	-	-	-	9	42.85	12	57.14
96-100	98.0	15	-	-	-	-	-	-	-	-	15	100

Table 4. Percentage occurrence of different stages of maturity in various size groups of male *A. mola*

Length group (mm)	Mean length (mm)	No. of fish observed	I (Immature)		II (Maturing)		III (Mature)		IV (Ripe)		V (Spent)	
			No.	%	No.	%	No.	%	No.	%	No.	%
36-40	38.0	14	14	100	-	-	-	-	-	-	-	-
41-45	43.0	18	11	61.11	7	38.88	-	-	-	-	-	-
46-50	48.0	22	4	18.18	9	40.9	6	27.27	3	13.64	-	-
51-55	53.0	20	-	-	4	20	10	50	6	30	-	-
56-60	58.0	15	-	-	2	13.34	5	33.34	8	53.33	-	-
61-65	63.0	25	-	-	-	-	8	32	12	48	5	20
66-70	68.0	15	-	-	-	-	3	20	7	46.67	5	33.33
71-75	73.0	20	-	-	-	-	2	10	11	55	7	35
76-80	78.0	24	-	-	-	-	2	8.33	10	41.67	12	50
81-85	83.0	15	-	-	-	-	-	-	-	-	15	100

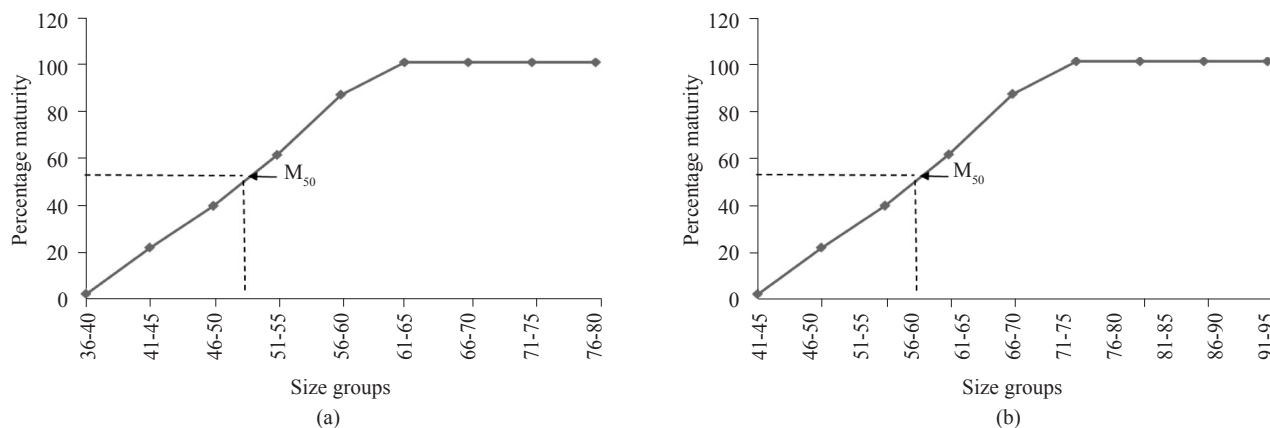


Fig. 8. Percentage of mature fish in different length groups in male (a) and female (b) *A. mola*

Fecundity

Estimation of fecundity based on 46 nos. of specimens of different length and body weight revealed that fecundity of the species varies depending on length, body weight and ovary weight. Absolute fecundity of *A. mola* varied from 485 (for fish of total length 54.4 mm, with body weight 1.51 g and ovary weight 0.06 g)

to 15786 (for fish of total length 91.3 mm, body weight 9.77 g and ovary weight 1.99 g). Relative fecundity was found to range from 240 to 1615 eggs per gram body weight with an average of 865 eggs per gram body weight.

The trends of relationship between fecundity (F) and total length (TL), fecundity and body weight (BW), fecundity and ovary weight (OW) were calculated as :

Log F = -6.29 + 5.231 Log TL ($r=0.691$, $p<0.01$, $SE=0.266$)

Log F = 2.429+1.671 Log BW($r=0.847$, $p<0.01$, $SE=0.223$)

Log F = 3.866+1.035 Log OW($r=0.981$, $p<0.01$, $SE=0.116$)

Sex ratio

Out of 1660 fishes studied, 562 were males and 1098 were females. Monthly ratio between males and females ranged from 1: 1.36 to 1: 3.62, the average being 1: 1.95 ($\chi^2 = 173.0698$, $p<0.05$, $N=1660$) (Table 5). Females significantly ($p<0.05$) dominated over the males all throughout the year.

Discussion

The study indicated five maturity stages of gonads in *A. mola*. Gonads of *A. mola* were found to develop from thin, flat and tiny structure to soft flabby enlarged

of GSI in *A. mola* was reported by Azadi and Mamun (2004). However, the breeding peaks were reported in July, August and October. On the other hand, Mustafa (1990) confirmed that *A. mola* breeds three times a year in April, July and October. Variations observed in the breeding season could be attributed to the prevalent agro-climatic conditions of Assam and the advent of monsoon associated with long photoperiods and rising temperature.

Gonadal maturation in *A. mola* indicated first gonadal maturity with in 140-160 days, when it attained an average body length of 56 mm. Attainment of maturity was found to synchronise in both the sexes. However, the size at first maturity was found to differ considerably. The males appeared to mature for the first time in the size range of 41-45 mm. About 20% of the observed male population was found to attain maturity at this size. However, in

Table 5. Monthly distribution of sex ratio in *A. mola*

Months	No. of fish	Male (Obs. value)		Female (Obs. value)		Ratio of male and female	χ^2 (male + female)	Level of significance
		No	%	No	%			
June	160	41	25.63	119	74.375	1 : 2.9	38.03	$p<0.05$
July	120	26	21.67	94	78.34	1 : 3.62	38.53	$p<0.05$
Aug	100	34	34.00	66	66	1 : 1.94	10.24	$p<0.05$
Sept	140	37	26.43	103	73.57	1 : 2.78	31.11	$p<0.05$
Oct	165	59	35.75	106	64.24	1 : 1.80	13.39	$p<0.05$
Nov	120	47	39.17	73	60.834	1 : 1.55	5.633	$p<0.05$
Dec	135	57	42.23	78	57.78	1 : 1.37	3.266	$p>0.05$
Jan	130	55	42.31	75	57.692	1: 1.36	3.08	$p>0.05$
Feb	150	58	38.67	92	61.34	1 : 1.58	7.71	$p<0.05$
March	125	39	31.20	86	68.8	1 : 2.21	17.67	$p<0.05$
April	145	47	32.41	98	67.58	1 : 2.08	17.94	$p<0.05$
May	170	62	36.47	108	63.529	1: 1.74	12.45	$p<0.05$
Total/ Avg	1660	562	33.86	1098	66.144	1: 1.95	173.07	$p<0.05$

testis and yellowish, enlarged ovaries. However, due to the presence of a range of developmental stages in an individual gonad at any one time, the differentiation achieved is inevitably less precise. Ripening eggs at very different stages of development were found at any one time in the same ovary both before and during spawning. These observations indicated that *A. mola* is a partial spawner in which spawning takes place over a prolonged period. Analysis of intra-ovarian egg diameter showed highest values thrice in a year in March, June and September, indicating that the spawning season of *A. mola* commences during these months. These observations on ova diameter study are in accordance with an earlier report on *A. mola* from the Kaptai Reservoir in Bangladesh by Azadi and Mamun (2004). However, they recorded three peaks in the month of July (0.475 mm), August (0.558 mm) and March (0.496 mm).

GSI showed three peaks in a year, once during March, second during June and third in September. Similar pattern

females, only 7% population was observed to attain first maturity in the 46-50 mm size group. Similar findings on early maturity of males was reported in *A. mola* by Gupta and Banerjee (2013) and in *Amblypharyngodon chakaiensis* by Babu and Nair (1983). In the present study, the M_{50} value of *A. mola* was found to be higher in female (61 mm) than in male (53 mm). Gupta and Banerjee (2013) also recorded higher M_{50} value in female (5.5-6 cm) than male (5-5.5 cm) population of *A. mola*. Higher M_{50} value for females (17.6 cm) as compared to males (16.3 cm) was also observed in *Puntius sarana* by Borah (2002).

Fecundity in fish is found to vary from species to species, depending on age, length, weight and environmental condition (Biswas, 1982). In the present study, it was observed that fishes of the same size had different number of eggs, in their ovaries at different stages of maturity. Similar observations were also made by Saha *et al.* (2009) in *A. mola*. Absolute fecundity of

A. mola varied from 485 to 15786 indicating that the fish is highly fecund under pond condition. The average relative fecundity observed in the present study was 865 eggs g body weight⁻¹ which was quite similar to the earlier report of 995 eggs g body weight⁻¹ for *A. mola* by Azadi and Mamun (2004). Fecundity of a species is also dependent on the egg size and high fecundity is often correlated with small egg size (Rath, 2000). As indicated earlier, the egg size of *A. mola* is considerably small, which justifies the present observation of high fecundity. The coefficient of correlation between body weight and fecundity ($r=0.847$) as well as ovary weight and fecundity ($r=0.981$) indicated highly positive correlation. Similar observations were made by Qasim and Qayyam (1961) in *Channa punctatus*, *Ompok pabda* and *Mystus vittatus* and by Azadi and Mamun (2004) in *A. mola*.

Populations inhabiting different regions exhibit different sex ratios (Nikolsky, 1956). In the present study, the average sex ratio (M:F) observed was 1: 1.95 indicating dominance of females. Dominance of females over males has been reported earlier in *A. mola* (Piska and Sarala, 1986; Azadi and Mamun, 2004; Suresh *et al.*, 2007; Hoque and Rahman, 2008; Saha *et al.*, 2009). Higher sex ratio of females has also been recorded in other species of freshwater fishes viz., *Eutropiichthys vacha* (Azadi *et al.*, 1990) and *Amblypharyngodon chakaiensis* (Babu and Nair, 1983).

Results of the present study indicate that maturity cycle of *A. mola* extends from February to November. At any period of observation, the sampled ovaries contained several batches of eggs at different maturing stages which confirm that *A. mola* is a partial spawner with three peaks in a year, in March, June and September. Males attain maturity earlier than females and females show dominance over males all throughout the year.

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