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INDUCED BREEDING OF ENDANGERED *AMBLYPHARYNGODON MOLA* (HAMILTON, 1982) UNDER A HATCHERY SYSTEM

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Abstract: The goal of the study was to identify the artificial breeding period for *Amblypharyngodon* mola by monitoring fecundity and the gonadosomatic index. The highest fecundity and GSI values were found in June and October. The study examined how the hormones PG and synthetic SGnRHa affected the reproduction of male and female brood fish during the months of April, May, June, July, and October. The effects of the hormones PG and SGnRHa on the ovulation, fertility, and hatching rate of *A. mola* were assessed under closely observed conditions. Males receiving a single dosage of PG and SGnRHa had better spermiation results. The highest fecundity and GSI value were recorded in fish weighing 5.96±0.07 g, while the lowest fecundity and GSI value were found in fish weighing 0.42±0.04 g. The highest fertilization (97.04±3.12%) and hatching (86.02±2.10%) were found by the PG treatment, while SGnRHa resulted in the highest rates of both (98.04±3.12%) and hatching (87.12±2.40%). The finding was that PG and SGnRHa both work as well to induce ovulation, fertilization, and hatching in *A. mola*. The purpose of the study was to determine the ease of artificial breeding under controlled hatchery conditions utilizing both PG extract and synthetic SGnRHa inducing chemicals.

Keywords: Fecundity, Fertilization, GSI, Hatchling, Induced breeding, Pituitary gland, SGnRHa.

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INTRODUCTION

Local names for *Amblypharyngodon mola* (Hamilton), commonly referred to as the mola carplet or pale carplet, include Moa, Mowka, Moraru, Mouchi, Mowrala, Mola, Moya, or Molongi. The Mola carplet is found in flooded fields, beels, baors, ditches, rivers, streams, and ponds all over Bangladsh (Bhuiyan, 2004). Almost all shallow freshwater habitats in Bangladesh are home to the *A. mola*, one of the most prevalent small indigenous fish (SIF) species (Mondal and Kaviraj, 2013; Mondal *et al.*, 2019). Due to its exceptional nutritional value (Alam *et al.*, 2004; Shikha *et al.*, 2019) and present use as attractive fish in aquariums (Gupta and Banerjee, 2015), this species has gained significant commercial and cultural significance. This species is currently also utilized in South Asian countries to supplement home food supply through acculturation with other cyprinid fish or traditional pond culture methods (Neetu and Seema, 2018).

Fecundity literally denotes the capacity for large-scale reproduction (Chakraborty, 2020). However, the total quantity of eggs produced is referred to as fecundity in the language of animal reproductive biology. Any species' reproductive biology must be studied in order to evaluate the stock's commercial potential, life history, culture methods, and real fishery management (Doha and Hye, 1970). Because of its excellent nutritional value, which includes a considerable quantity of protein, minerals, and vitamin A, as well as its tasty flavor, the Mola Carplet, also known locally as mourala, is one of the little native fish species that is highly popular in Bangladesh and India (Zafri and Ahmed, 1981; Saha *et al.,* 2009). Many fishermen rely on this species not just for food but also for revenue and livelihood prospects.

Although, Bangladesh provides a good habitat for different fishes (Chakraborty, 2021; Chakraborty *et al.,* 2021a) but both natural and human-caused factors are contributing to the decline of *A. mola* population, as is the case with other fish populations (Chakraborty *et al.,* 2021b; Chakraborty and Mome, 2022; Verma and Prakash, 2022; Chakraborty and Tabasum, 2024). Thus, determining the ovarian maturity stage, fecundity, and reproductive periodicity as well as the effects of artificial breeding with PG extract and synthetic SGnRHa inducing substances under a regulated hatchery setting were the goals of the current work.

MATERIALS AND METHODS

At the Faizuddin hatchery in Sibpur, Gouripur, Mymensingh, the experiment was conducted. The formula for calculating GSI is (gonad weight/total weight) ×100, and it is commonly used to ascertain the frequency of spawning for fishes and crustaceans. Male and female fish were gathered each month in order to determine GSI (Lagler, 1956).

Induced breeding tests were carried out in 2023 from April to November. The mature male and female brood fish were removed from the raising pond and placed in a different breeding tank using a seine net. The ripe fish were identified by physical and visual examination of the abdomen, genital entrance, and pectoral fin (Jhingran and Pullin, 1985). The mature brood stocks of *A. mola* were selected based on their maturity level.

Females whose abdomens obviously buckled and were pink when carrying eggs were chosen, as were males that flowed milk when their abdomens were gently squeezed. Mola carplet-induced breeding was the practice of using hormonal injections, often PG extract and synthetic gonadotropin-releasing hormone analogs (SGnRHa), to promote ovulation and spawning in restricted water. Either below the dorsal fin or above the pectoral fin, an intramuscular injection was given. After groups of 45 female fish each received injections of PG extract (Fig. 1a) and Synthetic SGnRHa (Fig. 1b), the fish were then kept in different spawning tanks at different times. Females need 1.0 to 2.0 mg of PG extract per kilogram of body weight for the first injection. At the time of the second injection, male fish received injections of 2.0 mg/kg body weight of PG extract, while female fish received injections of 3.0-4.0 mg/kg body weight, depending on their maturity level. Using synthetic SGnRHa, the fish's reproductive organs were activated once more, causing the females to release eggs (ovulation) and the males to create sperm. Males typically received 0.25 mL per kilogram of body weight from SGnRHa, whereas females typically received 0.3-0.5 mL per kg of body weight.

Fig.1a: PG abstract.

Fig.1b: Synthetic SGnRHa

The injected male and female fish were released in a hapa in the breeding tank. Breeding habits and spawning activities were observed till ovulation. In order to collect eggs, 45 females and 45 males were taken out of the breeding tank and undressed. The egg was fertilized using the dry stripping method. The eggs were placed on a plastic dish or enamel tray. The male fish's milt could be collected by gently pressing on its abdomen. The eggs and milt were well stirred with a clean, soft feather. A few drops of water were added, and the dish was continually whirled for 5 to 6 minutes.

Following a series of freshwater washings, the eggs were moved to additional hatching jars equipped with a continuous water circulation system once they had swollen. Over the course of the incubation period, the water flow in the jar was kept between 600 and 800 ml/min. The temperature that was recorded varied between 26.0 and 29.5 °C around another 2.0 to 4.0 hours, the process was finished, and hatchlings began to come out of the egg shell around 21 to 24 hours. Within an hour of hatching, unfertilized eggs and eggshells were removed from the hatchling jar to prevent fungal infection of the larvae. The fertilization rate (Okomoda *et al.*, 2017) and hatching rate was calculated by the following formula:

$$\text{Fertilization rate} = \frac{\text{Number of fertilized eggs}}{\text{Number of total eggs}} \times 100$$

$$\text{Hatching rate} = \frac{\text{Number of hatchlings}}{\text{Number of fertilized eggs}} \times 100$$

A. mola seemed to be in the early stages of development until 60.0–70.0 hours following the fertilization of the egg. The eggs were collected at random from the hatching tank. The boiled chicken egg's yolk is combined with water and squeezed through glass nylon cloth. Water was mixed with the fine egg yolk emulsion when the hatchlings were ready to be fed. After being removed from hatching jars, larvae from various parent couples were placed in a number of previously prepared nursery ponds. The water's temperature was noted during the experiment.

Statistical analysis

The data were analyzed using one-way ANOVA using MSTAT Software (Version) and Duncan's Multiple Range Test (Zar, 1984) to determine whether there was a significant difference between treatment means.

RESULTS

Gonad somatic Index (GSI) and Fecundity

Any species' gonadal maturity and spawning season can be estimated using the species' gonadosomatic index. In this study, the GSI of male and female *A. mola* ranged from 0.14 to 0.35% and 0.20 to 0.71%, respectively. January through June was breeding season of the fish studied. The month of January had the lowest female GSI value (Fig. 2). It began to rise in February and reached its highest point in June (0.71%). Between July and September, the GSI began to decline; but, starting in September, it began to rise once more, reaching its second peak in October (0.66%). It continued to decline in November as well, hitting its lowest point once more in January (0.20%).

The month of January had the lowest GSI value (0.14%) for males (Fig. 2). It began to rise in February and reached its highest point (0.35%) in June. Between July and September, the GSI began to decline; but, starting in September, it began to rise once more, reaching its second peak in October. Then, from November to January, it similarly fell (0.24-0.18%).

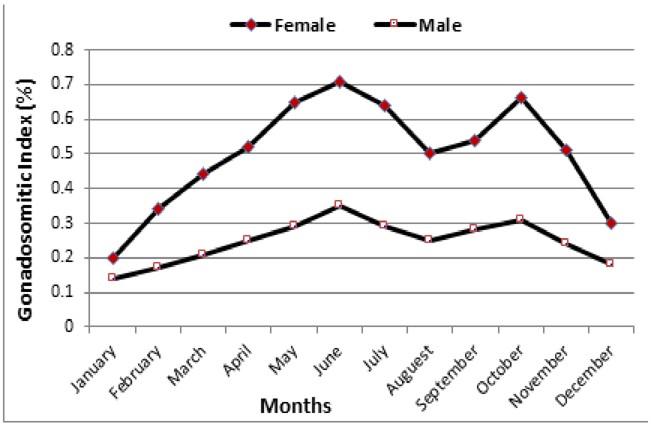

Fig. 2: Monthwise trend in the Gonadosomatic index of mola carplet.

Ovaries were extracted from *A. mola* individuals with known weights and lengths, which varied from 4.10±0.04 to 5.96±0.07 mg and 4.0±0.12 to 7.2±0.43 cm, respectively. According to Table 1, fecundity ranges from 2004.11±9.90 to 5218.81±33.82. Fish weighing 5.96±0.07g had the highest GSI value (12.75%) and fecundity (5218.81±33.82), while fish weighing 4.10±0.04g had the lowest GSI value (9.52%) and fecundity (2004.11±9.90).

Table 1: Gonadosomatic index and fecundity of mola carplet (*Amblypharyngodon mola*).

Body Length (cm)	Body wt. (mg)	Gonad wt. (mg)	GSI (%)	Fecundity (# Number)
4.0±0.12	4.10±0.04	0.42±0.04	9.52	2004.11±9.90
4.6±0.22	4.60±0.03	0.44±0.05	9.56	3141.06±11.08
5.1±0.26	5.00±0.05	0.48±0.06	9.60	3559.08±13.05
5.6±0.30	5.30±0.05	0.51±0.07	9.62	3888.38±14.20
6.1±0.38	5.74±0.06	0.56±0.08	9.75	4104.04±19.30
6.6±0.40	5.88±0.05	0.60±0.08	12.03	4518.26±27.16
7.1±0.38	5.90±0.06	0.72±0.09	12.20	4894.33±30.66
7.2±0.43	5.96±0.07	0.76±1.01	12.75	5218.81±33.82

In the case of females, GSI has demonstrated a strong positive correlation with gonadal length, total body weight, and total gonad weight. Male GSI has only been found to have a significant positive link with gonad weight; no significant relationship with total body weight, total length, or gonad length has been found.

Pituitary Gland (PG) extract

In the present investigation, in the months of early April to November, treatment with PG extract for females at doses of 3.5 and 4.0 mg/kg body weight resulted in ovulation, fertilization, and hatching. In the case of males, the amount of PG required to stimulate spermatogenesis was determined to be 1.5-2.0 mg/kg of body weight provided at the time of the second injection for females. Hatching occurred 16–18 hours after fertilization, and ovulation occurred 5-7 hours after the second injection (Table 1). With notable variations from other doses, the highest fertilization rate (98.04±3.12%) and hatching rate (87.12±2.40%) were obtained at the PG doses of 2.0 and 3.5 mg/kg body weight for the first and second injections, respectively. Consequently, for female *A. mola*, the PG dosages were tuned to 2.0 mg and 3.5-4.0 mg/kg body weight during the first and second injections, respectively, at a 6-hour interval.

Table 2: Effect of different doses of Pituitary Gland (PG) on the spawning of mola carplet.

Hormone	Months	Body weight		Doses of 1st injection (ml/kg or mg/kg)		Doses of 2nd injection ((ml/kg or mg/kg)		Ovulation period (hr)	Fertilization rate (%)	Hatching period (hr.)	Hatching rate (%)	Incubation temperature (°C)
		Male (mg)	Female (mg)	Male	Female	Male	Female					
PG (Double dose)	April	2.05 ±1.55	4.10 ±1.62	-	2.0	2.0	4.0	6-7	82.02[c] ±3.32	18.0-24.0	72.02[c] ±2.66	26.5 -28.5
	May	2.60 ±1.51	4.60 ±1.54	-	2.0	2.0	4.0	6-7	94.40[a] ±2.18	18.0 -22.0	86.43[a] ±2.78	
	June	3.06 ±1.33	5.96 ±1.50	-	2.0	1.5	3.5	5-6	97.04[a] ±3.12	18.0 -21.0	86.02[a] ±2.10	
	July	3.04 ±1.20	5.90 ±1.62	-	2.0	2.0	4.0	6-7	90.11[b] ±2.88	18.0 -22.0	76.40[b] ±3.40	
	October	3.22 ±1.33	5.14 ±1.66	-	2.0	2.0	4.0	6-7	95.11[a] ±3.75	18.0 -23.0	80.32[b] ±3.33	26.0 -27.5

Figures with different superscripts in the same column varied significantly ($P < 0.01$).

Synthetic SGnRHa

Synthetic SGnRHa was a very effective agent for *A. mola* induced spawning. Fertilization and hatching rates were enhanced in April, May, June, July, and October whenever the hormone dosage was increased, i.e., between 0.45 and 0.50 ml/kg body weight. The optimum spawning happened in June when females received a dose of 0.044 ml/kg body weight and males received a dose of 0.12 ml SGnRHa/kg body weight. The quantity of SGnRHa needed to promote spermatogenesis in males was found to be between 0.12 and 0.15 milliliters per kilogram of body weight. After receiving a hormonal injection for 6.0–8.0 hours, ovulation took place, and hatchlings appeared 18–24 hours later. Fish injected with synthetic SGnRHa in June had comparatively higher fertilization and hatching rates (98.64±0.84 and 89.55±0.88%) (Table 2).

The size of the ovulated eggs of *A. mola* increased by about 0.12 mm when fertilized eggs were incubated in a hatchery; this could be connected to egg hydration. The fertilized eggs were found in a clutch among the eggs after they were incubated at the hatchery. The egg membrane separated, resulting in a uniform perivitelline area. The yolk sphere pushed toward the vegetal pole as the embryo's growth progressed. This could be due to more room for blastomere division at the animal pole.

As cleavage progressed to the 64-cell stage, blastomere purity in the 2-4 cell stage significantly decreased. The blastomere's distinctiveness vanished entirely during the morula and blastula phases. After 70 to 72 hours of hatching, the hatchlings began to travel horizontally, indicating the commencement of their first eating, once the yolk sacs had been completely digested. To achieve the dietary requirement, 200,000 hatchlings were fed chicken egg yolk emulsion at a rate of one egg per day. The alimentary canal was intended to be working before children were sent to the nursery (Price, 1983). The temperature of the water was the most important element for hatchlings and ovulation. Throughout the trial, temperatures ranged from 25.50 to 28.50 degrees Celsius.

DISCUSSION

The average fecundity of *A. mola* in this experiment ranged from 2004.11±9.90 to 5218.81±33.82, which is comparable to previous research (Mitra and

Table 3: Effect of different doses of Synthetic SGnRHa on the spawning of mola carplet.

Hormone	Months	Body weight		Doses of 1st injection (ml/kg or mg/kg)		Ovulation period (hr)	Fertilization rate (%)	Hatching period (hr.)	Hatching rate (%)	Incubation temperature (°C)
		Male (mg)	Female (mg)	Male	Female					
Synthetic SGnRHa	April	2.50±1.50	4.60±1.42	0.15	0.52	6-8	81.40[d]±3.02	18.0-24.0	72.33[d]±3.56	26.5-28.5
	May	2.62±1.42	5.00±1.48	0.12	0.50	6-7	95.66[a]±3.84	18.0-21.0	87.05[a] ±4.88	
	June	3.33±1.34	5.88±1.66	0.12	0.44	5-6	98.48[b]±3.82	18.0-20.0	89.11[b]±2.68	
	July	3.30±1.50	5.94±1.80	0.15	0.45	6-7	90.83[c]±3.82	18.0-24.0	78.01[c]±3.08	
	Oct.	3.11±1.34	5.44±1.52	.15	0.50	6-7	91.44[a]±3.22	18.0-24.0	81.14[c]±3.22	25.5-27.5

Figures with different superscripts in the same column varied significantly ($P < 0.01$).

Jain,1985; Afroze and Hossain,1993; Azadi and Mamun, 2004; Saha *et al.,* 2009; Borah *et al.,* 2010; Gupta and Banerjee, 2013a; Mondal and Kaviraj, 2013). They investigated the fecundity of *A. mola* and discovered that there are different ranges of fecundity, ranging from 400 to 16,072, which is consistent with the findings of the study. According to the current study's fecundity statistics, it breeds twice a year, with two maxima in June and November, indicating a reproductive cycle that runs from April to December. Size, age, condition, and sample types are some of the variables that affect how many eggs a female produces (Lagler, 1956; Khan and Mukhopadhyay, 1975).

The recent experiment indicated that proper brood fish selection is crucial for the success of induced breeding (Borah *et al.,* 2010). The current experiment is highly accurate in establishing favorable spawning conditions and choosing appropriate recipient fish at the right stage of ovarian development, both of which are necessary for successful spawning (Nash and Shehadesh, 1980; Gupta and Banerjee, 2013b; Rahman *et al.,* 2018).

Gonad began to rise in February and peaked in June. It then fell in July before rising once more in September and hitting a second peak in October. In December, it also fell, and in January, it fell to its lowest value once more (Saha *et al.,* 2009; Borah *et al.,* 2010). For female *A. mola*, the PG and synthetic SGnRHa doses were adjusted to 2.0 mg and 3.5-4.0 mg/kg body weight at the first and second injections, respectively, and 0.50 ml/kg body weight at 6-hour intervals. This was roughly comparable to breeding Indian major carp (Nandeesha *et al.,* 1990), *Labeo rohita* and *Cirrhinus cirrhosus* (Peter *et al.,* 1988), and *Puntius sarana* (Chakraborty *et al.,* 2007).

The PG-treated fish (98.04±3.12 and 87.12±2.40%) did not differ substantially ($P>0.05$) from the synthetic SGnRHa-treated fish in terms of fertilization and hatching rates (2.0 mgPG/kg first dose and 3.50 mgPG/kg second dose). In comparison to PG, the Indian main carp treated with synthetic SGnRHa had higher rates of ovulation, fertilization, and hatching (Indira *et al.,* 2013). Taking into account the carp fishes' economic feasibility, farmer applications, ovulation, fertilization rate, and hatching rate, synthetic SGnRHa was suggested rather than PG hormone in carp breeding (Nandeesha *et al.,* 1988).

Results from the current investigation were comparable to those from the use of SGnRHa on *L. calbasu* by Jamroz *et al.* (2008). In a study on induced breeding of Silver carp (*Hypophthalmichthys molitrix*), SGnRHa was injected into each of the 30 female fish at a rate of 0.6 ml/kg body weight, and 100% ovulation was noted (Naeem *et al.,* 2005).

In 2023, *A. mola* was bred from April through July and October, with last June being the busiest month. This study's findings regarding the onset of the mating season for *A. mola* are in line with those of Borah *et al.* (2010); Chakraborty *et al.* (2007) and Indira *et al.* (2013). The ambient water temperatures used for A. mola breeding ranged from 25.50 to 28.50 °C. The majority of native tiny fish can be grown in this temperature range (Islam and Chowdhury, 1976). The temperature requirements of *A. mola* seemed to be comparable to those of Indian carps.

CONCLUSION

Mola carplet, a highly fecund fish, breeds twice a year, with a reproductive cycle from April to October, correlated with body length, weight, ovary weight, and ovary length. Lowest value of GSI has been observed in the month of January and reached the first peak in June and the second peak in October. Artificial propagation and nursery technology of *A. mola* was developed to protect this important highly nutrient mola carplet.

Therefore, the goal of the current study was to determine how simple it would be to artificially breed using both PG extract and Synthetic SGnRHa inducing agents under controlled hatchery condition.

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