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Domestication and Observation on Induced Breeding of Spiny Eel *Mastacembelus armatus*

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Abstract Spiny Eel, Mastacembelus armatus categorized as endangered is a highly potential species in Bangladesh. Two different experiments were conducted on domestication and induced breeding of Spiny Eel, M. armatus at mini hatchery cum breeding complex under the Department of Fisheries Biology and Genetics, Bangladesh Agricultural University. For domestication, fish were reared under three different treatments (treatment I, II and III) each having three replications (R_1, R_2, R_3) in nine indoor cisterns (2.33 m × 1.34 m area each) for six months and fed with three different feeds namely commercial supplementary feed (Mega feed), trash fish and chicken viscera. Highest growth performance was observed upon statistical analysis in treatment II fed with trash compared with treatment I and III. Subsequently four induced breeding trials were conducted using carp pituitary gland (PG) extract. Male and female broods were induced to breed using different doses (35-100 mg.kg^{-1} body weight of female and 5-10 mg.kg^{-1} body weight of male) of PG extract. PG dose of 35 & 40 mg.kg^{-1} body weight precipitated ovulation but successful fertilization (82.00 ± 2.21) was observed at the dose of 40 mg.kg^{-1} body weight. Data were analyzed using one-way analysis of variance (ANOVA).

Keywords: *Mastacembelus armatus, growth, pituitary gland, induced breeding*

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1. Introduction

The Spiny Eel (*M. armatus*) is a species of ray-finned, spiny eels belonging to the genus *Mastacembelus* of the family Mastacembelidae and is native to the riverine fauna of India, Pakistan, Sumatra, Sri Lanka, Thailand, Vietnam, Indonesia and other parts of South East Asia [10]. The other common names for this popular species are Zig Zag Eel, Spiny Eel, Leopard Spiny Eel and White-Spotted Spiny Eel [7]. *M. armatus* are nocturnal in habit that thrive in highland streams, lowlands, wetlands, still waters, coastal marshes and rivers with sandy or rocky riverbeds and heavy vegetation. They dwell in canals, lakes and other floodplain areas during the flood season. In Bangladesh, freshwater spiny eel is commonly found throughout the country, plenty in mud holes in shallow beels and boro-paddy fields particularly in Sylhet, Mymensingh and Tangail districts [22]. Being nocturnal carnivores, Tire Track Eel forage on benthic insect larvae, earthworms, blackworms and some submerged plant materials [19]. Narejo [16] reported that small dead fish and dead small shrimps are suitable food items for Spiny Eel. *M. armatus* can reach up to 36" (91 cm) in its natural habitat but does not usually exceed 20" (51 cm) in captivity.

The sex of eels cannot be recognized easily outside the spawning season except by dissection. During the breeding season the male become active while the female become pot-bellied. A gentle pressure on the belly of ripe fish causes the male to bring out the whitish milt and the female, the eggs. This coming out of milt and egg from mature male and female by gentle pressure was also observed by Singh *et al.* [26].

Spiny eel, a popular fish with very delicious meat is one of the highly demanded species in the market. Different biological aspects (mainly morphometric and ecological) of the species have been studied sporadically [6,12]. Very little information is available on the age and growth of freshwater eel, *Anguilla* sp [5], haematological study of freshwater eel *Amphipnous cuchia* [2] and comparative studies on the biology and culture of *Monopterus cuchia* and *M. armatus [16]*. To evaluate the culture and reproductive potentials of *M. armatus*, information on the fecundity, reproductive biology, behaviour and breeding season are considered essential [23]. Narejo *et al.* [18] and Rahman *et al.* [23] did some works on its biology and rearing technique under laboratory condition. However, no initiative seems to have been taken to breed this species artificially.

Therefore, *M. armatus*, endangered species needs protection from being extinct through the development of its culture technique. However, for developing culture techniques, biological studies of this species are indispensable and very little attempt has been made in Bangladesh to promote their breeding and culture. More comprehensive works need to be carried out if their

culture has to be popularized. Therefore, the present work was conducted to domesticate the species and to develop a suitable induced breeding technique of *M. armatus* that would not only be helpful in preventing the fish from being extinct but also in popularizing its culture.

2. Materials and Methods

2.1. Experiment 1: Domestication of M. Armatus

To establish a suitable rearing technique of *M. armatus*, 270 baim were collected from natural habitat (haor region in Kishoreganj district) through the fishermen and were kept in nine indoor cisterns (2.33 m × 1.34 m area each) each having thirty fish. They were reared under three different treatments (treatment I, II and III) each having three replications (R_1, R_2, R_3). Cisterns were provided with all facilities including continuous water supply through porous plastic pipes for aeration, inlet and outlet, shelter where the fish were stocked and fed with 3 different supplementary feeds, i.e. commercial supplementary feed (Mega feed), trash fish and chicken viscera (treatment I, treatment II and treatment III respectively). As the fish has hiding tendency, two pieces of PVC pipe (0.91 m long having a diameter of 0.10 m) were used as shelter in each cistern.

In the treatment I, Mega feed (protein 33.72%, lipid 6%, ash 10% and dry matter 88.98%) was provided to the fish at the rate of 5 % of body weight. The fish of treatment II and III were fed with trash fish (protein 55.20%, lipid 10.80%, ash 21.60% and dry matter 22.40%) and chicken viscera (protein 47.58%, lipid 13.45%, ash 5.65% and dry matter 13.80%) respectively at the rate of 5 % (dry weight basis) body weight. As the fishes are nocturnal in habit, the feed were supplied in the early morning and in the evening. Monthly sampling was done on a regular basis. The weight (g) and length (cm) were measured by using an electric balance and measuring scale respectively for a period of 6 months from October 2011 to March 2012. Water quality parameters like temperature (°C), dissolved oxygen (ppm) and pH were also recorded weekly by Celsius thermometer, digital DO meter and portable digital pH meter respectively.

2.2. Experiment 2: Developing Induced Breeding Technique

Selection and conditioning of broodfish.

Mature male and female broods were identified by observing the secondary sexual characters. Males were comparatively large in size, dark in color and milt was available following stripping while mature females were comparatively small in size, light in color with soft and swollen abdomen. Selected broodfish were kept in cistern for about 6 hours for conditioning prior to hormone injection. Handling and carrying of fish were done very carefully to avoid injury and secondary infection. Male and female fish were kept in separate cisterns and constant water flow was maintained to ensure proper aeration.

Experimental design.

Four induced breeding trials were conducted for optimization of the dose of PG for M. armatus.

Trial I

In first trial, twelve pairs of mature male and female broods were selected for induced breeding. Females were treated with PG extracts at the doses of 100, 90 and 80 $mg.kg^{-1}$ body weight under three different treatments (I, II and III) with two replications for each. On the otherhand males in all treatments were treated with 5 $mg.kg^{-1}$ body weight during the second injection of female.

Trial II

Equal number of mature male and female broods as used in trial I were selected for induced breeding. In this experiment females were treated with 60, 55 and 50 mg $PG.kg^{-1}$ body weight under three different treatments (I, II and III) with two replications for each. Males were treated with the same dose as used for trial I.

Trial III

In trial III, twelve pairs of mature male and female broods were used for breeding. In this experiment slightly reduced doses from trial II were used. Females were treated at the doses of 45, 40 and 35 mg $PG.kg^{-1}$ body weight under three treatments (I, II and III) each having two replications. Males of all treatments were treated with 10 mg $PG.kg^{-1}$ body weight of fish.

Trial IV

In trial IV, two pairs of mature male and female broods were used for breeding. In this experiment only a single dose of 40 mg $PG.kg^{-1}$ body weight of female was used to confirm the PG doses on induction of ovulation of females. On the other hand, males were treated with 10 mg $PG.kg^{-1}$ body weight of fish.

Injecting the PG extract to broods

Just prior to hypophysation, selected females and males were caught from the cistern using a scoop net. During administration of injection, the fish were wrapped by a soft cloth and kept lying on soaked foam. The PG solution was injected intramuscularly at the dorsal side behind the pectoral fin. The needle was inserted at about 45° angle during the time of injection and the spawners were handled very carefully. Total dose for females of each treatment was divided into two. The first dose (30 %) and the second dose (70 %) were administered 6 hours apart. On the other hand the males of all treatments were treated once at the time of 2nd injection of the females. Treated males and females were kept in same cistern covered with net and they were also provided with aeration through porous PVC pipes.

Stripping, collection of milt and ovulated eggs and fertilization.

Fish were checked every two hours following 6 hours of 2nd injection of the females and continued up to 20 hours. Immediately after ovulation, eggs were collected in a fertilization tray by applying gentle pressure on the abdomen from anterior to posterior direction. Milt was collected by same procedure as followed for egg collection. Fertilized eggs were washed several times with clean water to remove the excess milt, blood etc. The fertilized eggs were transferred to mini plastic circular hatchery (50 L capacity) for incubation.

Statistical analysis.

The data obtained in the present experiments were analysed statistically to see whether there was significant difference or not among the treatments. This was done by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at 0.05 probability level.

3. Results and Discussion

The initial average length in treatment I, II, III were 25.42 ± 2.39, 26.59 ± 2.04 and 24.81 ± 2.14cm and initial weight were 76.19 ± 1.36, 76.20 ± 2.53 and 75.93 ± 1.48 g respectively. After six months of experiment, there was significant difference (P<0.05) among the treatments fed with 3 different types of feed i. e. commercial supplementary feed (Mega feed), trash fish and chicken viscera (treatment I, treatment II and treatment III respectively). The highest length and weight gain and SGR (19.34 ± 3.12 cm, 67.70 ± 16.49 g and 0.15 ± 0.03 respectively) were observed in treatment II where trash fish was used compared to treatment I and III (Table 1 and Figure 1). Survival rate of fish was also higher in treatment II and III (96%) than treatment I (94%). It was observed that the fish easily took trash fish and chicken viscera as feed but the same thing was not happened when formulated feed was provided. Nutrition is very important for sound growth and maturation of gonads of the fish. The quality of eggs depends on the quality of feed provided. The observations reported on similar fishes like in *Clarias gariepinus* [15,21] and in *Pisodonophis boro* [18] with small dead fish are more or less similar with the present findings. Similar results are also obtained by Henken *et al.* [8] in case of *Clarias gariepinus* and Sanaullah *et al.* [24] in case of of *Clarias batrachus*. Nahar *et al.* [15] and Winfree and Stickney [27] found the best results using 58% protein containing feed. They mentioned that with the increment of protein concentration in feed the growth of fish also increased simultaneously. In the present experiment, protein percentages were 33.72% in formulated feed, 55.20% in trash fish and 47.58% in chicken viscera and the better growth performance was observed in treatment II where trash fish (55.20% protein) was supplied.

Table 1. Growth performance and survival rate of *Mastacembelus armatus* (baim) fed with Mega feed (Treatment 1), trash fish (Treatment II) and chicken viscera (Treatment III) during six months domestication period

Parameters	Treatment I	Treatment II	Treatment III
Initial weight (g)	76.19 ± 1.36^{a}	76.20 ± 2.53^{a}	75.93 ± 1.48^{a}
Final weight (g)	127.65 ± 10.72^{c}	143.90 ± 15.29^{a}	133.15 ± 6.26^{b}
Weight gain (g)	51.47 ± 10.97^{c}	67.70 ± 16.49^{a}	57.22 ± 6.42^{b}
Weight gain (%)	67.63 ± 14.93^{c}	89.28 ± 23.29^{a}	75.43 ± 8.95^{b}
Initial length (cm)	25.42 ± 2.39 ^{a}	26.59 ± 2.04^{a}	24.81 ± 2.14^{a}
Final length (cm)	39.78 ± 3.75^{c}	45.92 ± 1.69^{a}	41.67 ± 2.92^{b}
Length gain (cm)	14.36 ± 4.62^{c}	19.34 ± 3.12^{a}	16.86 ± 2.38^{b}
Length gain (%)	57.86 ± 22.03^{c}	73.92 ± 18.33^{a}	68.57 ± 12.56^{b}
SGR (%/day)	0.12 ± 0.02	0.15 ± 0.03	0.14 ± 0.01
Survival rate	94 %	96 %	96 %

All values were reported as mean standard deviation (± SD) of the mean
Values in the same row having different superscripts are significantly different (P < 0.05)

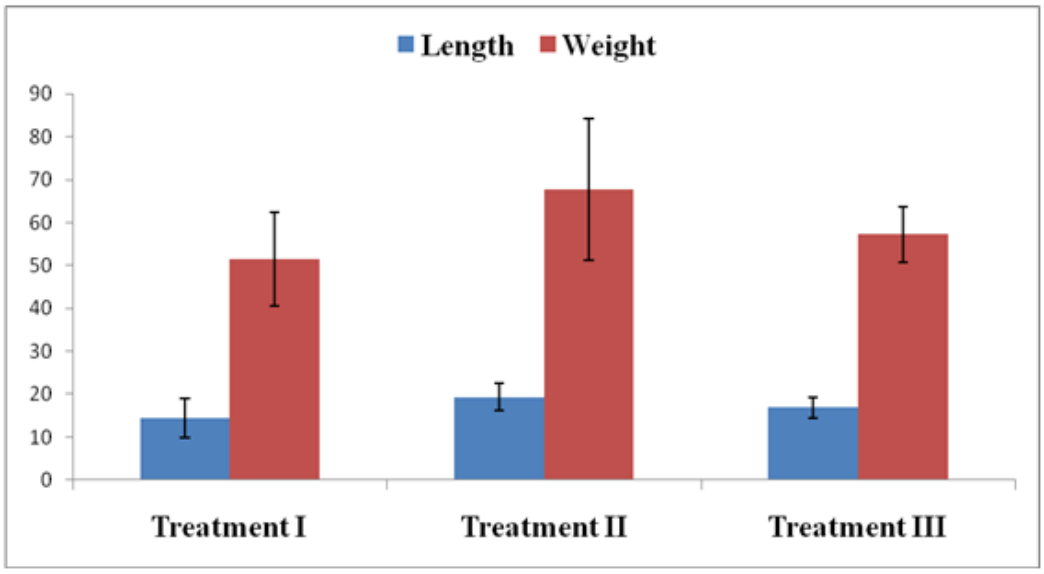

Figure 1. Variations in net gain in length and weight of *M. armatus* (baim) under three different treatments during six months domestication period

The freshwater eels having a burrowing habit often spend their day time hiding under crevices, stone and mud [19]. Artificial shelter has been used by various scientists for the better growth and survival in different fish species [16]. PVC pipes were provided as shelter in this experiment for better growth of *M. armatus*. Narejo [16] found the same results and stated that PVC pipes & water hyacinth are suitable shelters for the better growth and survival of *M. armatus*. Many researchers like Barua *et al.* [3], Shaima *et al.* [25], Kabir *et al.* [11] and Afroz [1] commented that female fish grow faster compared to male and attain larger size in a given period of time. Similar observations have been recorded for the spiny eel during the present study. The freshwater eel are long snake-like

fish with a smooth and slimy skin, the epidermis possess numerous mucus glands [13]. During the present study, handling of live eel proved very difficult. It might be due to the heavy secretions of mucus which made the fish slippery. The wriggling, serpentine and powerful movement of the fish might have also accounted for that.

Water quality parameters have significant role for the growth and survival of aquatic animals. Brown [4] reported that temperature altered the rates of metabolic processes and could be expected to have a considerable effect on the growth of poikilothermous animals. Nikolsky [20] observed that metabolic rates were most closely connected with changes in temperature of the surrounding water. The water quality parameters were also recorded throughout the study period and found to remain within the suitable ranges that agree with other findings [17].

Different doses of PG extract were used as inducing agent in the breeding trials I, II, III and IV for females. PG doses of 100, 90, 80, 60, 55, 50, 45 mg.kg^{-1} body weight of fish did not show any response in female fish. But the dose of 40 mg PG.kg^{-1} body weight precipitated ovulation and successful striping of ovulated eggs was observed (Table 2). The time interval between the injection of carp PG extract and ovulation (latency period) varied between 21 and 28 hr of injection in all cases. Fertilization rate was 82.00±2.21 at the same dose and embryonic development was observed up to gastrula stage but hatching did not occur. On the other hand 35 mg PG.kg^{-1} body weight of female showed partial ovulation of female but no fertilization occurred. The PG dose of 35 mg.kg^{-1} body weight was proved low to induce ovulation. On the other hand, the fish treated with 50-100 mg PG.kg^{-1} body weight showed high doses to induce ovulation. So, dose optimization is very important to induce the fish to ovulate. Rahman *et al.*, [23] conducted induced breeding of *M. armatus* during breeding season. But they did not get the expected results. Hoar and Randall [9] stated that spawning largely depends on the synchronization of ova and sperm release which is only possible during peak breeding season. During this experiment, fertilized eggs were incubated in mini circular hatchery at the water temperature of 25°C to 28°C and no eggs were hatched out. During incubation temperature is the main determining factor that lead to the fertilized eggs to hatch. Mollah and Tan [14] conducted an experiment on the effect of temperature on incubation period and hatching of eggs of *Clarias macrocephalus* and found that higher temperature always shortens the incubation periods but affects the survival and hatching rates of the eggs. They found that the eggs incubated at 20°C did not hatch but at 25°C hatching started after 34 hr of incubation and was completed by 58 hr. On the other hand, eggs incubated at 35°C started hatching after 22 hr of incubation and completed by 30 hr. So incubation time is more species specific and inversely related to temperature.

Table 2. Observation of induced breeding trials of *M. armatus*

Trial	Treatment	Body weight (g) (Mean)		Dose of 1st Injection (mg.kg^{-1})		Interval of 2nd injection (hr)	Dose of 2nd Injection (mg.kg^{-1})		Ovulation period (hr)	Fertilization rate (%)	Hatching period (hr)	Incubation temperature (°C)	Remarks
		Male	Female	Male	Female		Male	Female					
Trial - 1	T_1	250.50 ± 6.04	260 ± 5.04	-	30.00	06	05	70.00	-	-	-	-	Male and female responded. No ovulation
	T_2	235.50 ± 4.66	250 ± 6.22	-	27	06	05	63	-	-	-	-	-do-
	T_3	230.50 ± 4.20	240 ± 5.14	-	24	06	05	56	-	-	-	-	-do-
Trial - II	T_1	263.50 ± 4.04	280 ± 6.04	-	18.00	06	05	42.00	-	-	-	-	No ovulation
	T_2	222.50 ± 8.66	250 ± 7.24	-	16.50	06	05	38.50	-	-	-	-	-do-
	T_3	224.50± 5.20	240 ± 6.15	-	15.00	06	05	35.00	-	-	-	-	-do-
Trial-III	T_1	221.50 ± 15.59	230 ± 9.25	-	13.50	06	10	31.50	-	-	-	-	No ovulation
	T_2	140.50 ± 12.12	150 ± 6.20	-	12.00	06	10	28.00	21-22	-	-	25 - 28	Successful ovulation but fertilization was not observed.
	T_3	126.50 ± 4.04	145 ± 8.14	-	10.50	06	10	24.50	15-16	-	-	-	Male and female responded. Partial ovulation took place.
Trial-IV	T_1	209.50 ± 10.97	240 ± 12.04	-	12.00	06	10	28.00	27-28	82.00 ± 2.21	-	26 - 28	Complete ovulation. Successful fertilization. Embryonic development was observed up to gastrula stage but hatching did not occur.

The experimental results revealed that the growth of *M. armatus* was better in confined environment when fed with trash fish than formulated feed and chicken viscera because of the high percentages of protein in trash fish. Breeding trials with 40 mg PG.kg^{-1} body weight of fish showed better performance. Therefore, there is scope for

further experimentation with different doses of different hormones applying single or double injections for perfection of artificial breeding technique of *M. armatus.*

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