Progressive Agriculture 29 (3): 267-275, 2018 **ISSN: 1017 - 8139**

Progressive Agriculture
Journal homepage:http://www.banglajol.info/index.php/PA

Induced breeding of endangered spiny eel (*Mastacembelus armatus*) using PG extract

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Abstract

Mastacembelus armatus is an important freshwater spiny eel of Bangladesh. The experiment was initiated to establish an induced breeding technique of the species with two induced breeding trials. In trial I, four different doses viz. 20, 40, 60 and 80mg PGkg^{-1} body weight of the fish were used to standardize the PG dose to ovulate the female *M. armatus,* and in trial II, the best dose identified in trial I was injected to fish once (whole dose) or twice with divided dose of PG at the rate of 30% and 70% at 6h interval to observe the effect of the mode of hormonal injection. Among the four doses applied in trial I, best result was obtained from 40mg PGkg^{-1} body weight in respect of ovulation rate (100%) of females and fertilization (93.00±2.00%) and hatching rates (58.30±3.50%) of eggs. In trial II, the females treated once (with the whole dose) or twice with divided dose of PG equally responded and no significant difference ($p>0.05$) was observed between the modes of hormone injection in respect of ovulation rate of females and fertilization and hatching rates of eggs. As *M. armatus* is considered as endangered fish, this induced breeding technique will help to conserve the fish as well as to produce seed in commercial hatcheries.

Key words: *Mastacembelus armatus*, PG, induced breeding

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Introduction

Mastacembelus armatus, locally known as Baim, is a fish liked by many people of Bangladesh for its attractive flavour and relatively tough texture. Its lucrative size and high protein content increased its production potential to the aquaculturists. Eel flesh has high caloric value of as high as 303 cal/100g, while the average value of other fish flesh is 110 cal/100g (Nasar, 1997). According to IUCN (2015), 64 freshwater fishes are threatened of which 9 are critically endangered, 30 are endangered and 25 are vulnerable. *M. armatus* (Lacepede, 1800) has been categorized as an endangered fish species. So, it is necessary to take immediate measures to conserve the species.

M. armatus is a snake-like large elongated fish having no pelvic fins. Its anal and dorsal fins are elongated and are attached with caudal fin. The colour of the back is dark-beige while the head is silver-beige. The body looks dull brown while the belly is light brown. One to three darker longitudinal zigzag lines are found on the body which are confined to the dorsal two-thirds of the body. Brown stripes are also visible in the eyes (Butler, 2007). In wild environment the fish can get maximum 91cm in length but in captive condition they become 51cm (Butler, 2007) and attain a maximum weight of 500g (Huang *et al.,* 1982).

M. armatus are demersal and potamodromous fish (Riede, 2004). It is mostly found in freshwater and sometimes in brackish water. Generally, it lives in tropical climate where temperature and pH ranges from 22°C-28°C (Riehl and Baensch, 1991) and 6.5-7.5 respectively. It is native in Bangladesh including a number of South Asian countries (Jha *et al.,* 2006).

The fish are mostly found in canals, lakes and floodplains during flooding (Froese *et al.,* 2007). It is nocturnal in feeding habit foraging mainly on benthic insect larvae, worms and some submerged plant material **(**Rainboth, 1996). Male and female spiny eels can not be distinguished unless they are mature. The whitish milt and the brownish-yellow eggs come out if a gentle pressure is applied from anterior to posterior direction on the belly of ovulated female. The fecundity of the fish ranged from 3155 to 24684 at the size from 260 to 535 mm (Rahman *et al.,* 2006).

The reproductive biology of *M. armatus* was investigated under laboratory condition by Narejo *et al.* (2002) and Rahman *et al.* (2004). Mollah *et al.* (2013) conducted preliminary artificial breeding trial with domesticated *M. armatus* but did not observe any hatching of eggs though fertilization occurred. Besides these, no other mentionable works have been done to breed this species artificially through establishment of induced breeding and stockable sized seed production protocol which is the prerequisite for protecting any species from being extinct. So, this study was performed to build up an appropriate induced breeding technique of *M. armatus* that will help saving it from the present state of endangered situation and as such the biodiversity of this endangered fish will thus also be saved.

Materials and Methods

The experimental fish: The fish were collected from the Mithamoin *haor* of Kishoreganj district of Bangladesh and stocked in indoor cistern for domestication. The cisterns are of 2.33m × 1.34m in size each having the water exchange and aeration facilities. Special shelter of 0.91m long PVC pipe having a diameter of 10cm for breeding purposes was also provided in the cistern. The fish attained sexual maturity at the size of 32cm (total length) for female and 34cm for male both after their second year of life. The brood fish were fed with trash fish at the rate of 3 to 5% of body weight. Since the fish are nocturnal the feed was provided in the early morning and in the evening near the shelter made for the fish.

Selection of brood fish: Good looking and healthy broods were collected from the domesticating cistern and subsequently selected for breeding. Male and female broods were identified considering some external appearances. The females having swollen abdomen and round and swollen urogenital papillae were selected as probable broods. However, the mature males had their flat abdomens and long protruded genital papillae.

Conditioning of brood fish: Selected brood fish were weighed and kept in cistern to inject with PG extract. Separate cisterns having continuous water supply and aeration facilities were used for conditioning male and female broods.

Experimental procedure

Two trials were given to optimize the PG dose.

Trial I

Four treatments such as TI, TII, TIII and TIV each having three females were used. The females under each treatment were indicated as R_1, R_2 and R_3 and kept separately in cistern. Each female under TI, TII, TIII and TIV was treated with PG dose of 20, 40, 60 and 80 $mgkg^{-1}$ body weight, respectively. Two males were employed for each female and in total 12 females and 24 males were used in this trial.

Trial II

In this trial two treatments i.e., TI and TII each having 6 females were used. The females were treated with PG at the dose of 40$mgkg^{-1}$ body weight. Six females of TI received the whole dose at a time. On the contrary, the

dose was divided into two equals and administered to each of the 6 females of TII 6h apart.

Induction of ovulation

Source of pituitary gland (PG) and preparation of PG extract: For induction of ovulation, PG extract prepared from commercially available dry carp pituitary glands (PG, available in the market) was used. Requirement of PG was found out according to the total body weight of broods using the following formula:

Weight (mg) of required amount of PG (W_t) = $W_b \times P_t$

Where, W_b represents total body weight (kg) of all the fish to be injected and P_t represents the rate in mg of PG to be injected kg^{-1} body weight under a particular treatment.

The volume of extract needed was calculated using the following formula:
Volume of extract (ml) = $W_t \times 1.0$ [W_t = Weight of PG (mg)]

Where, 1.0 represents the volume of the extract in ml to be injected kg^{-1} body weight.

The required amount of PG was weighed and homogenized adding a little amount of distilled water. The homogenate was then taken very carefully in a centrifuge tube. The tissue homogenizer was washed several times by distilled water to ensure the transfer of suspension as much as possible. The suspension was centrifuged for 6min at 6000rpm. The clear supernatant on top was taken in a vial and pre-determined volume was obtained by adding distilled water.

Injecting the PG extract to fish: Based on the body weight of the gravid female the required volume of extract ($1.0mlkg^{-1}$) was taken in a graduated 1.0ml hypodermic syringe. Intramuscular injection was given to the dorsal side of the fish above the lateral line. In trial I, the dose was divided into two volumes (30% and 70%) and the females were injected at 6h interval. In trial II, the whole dose was injected to the females under TI at a time while the dose was divided into two volumes (30% and 70%) and injected to the females under TII at 6h interval. Each male was injected with a dose of $10mgkg^{-1}$ body weight when 2nd injection was given to the females.

Ovulation, collection of eggs and fertilization with milt: After treatment with PG extract both the males and females of each treatment were released in the same cistern. Close supervision was made during this period for their pairing or courtship behavior. Checking for commencement of ovulation was started after 18h of first injection by gently pressing their abdomen.

Commencement of ovulation was confirmed when a few eggs extruded following a slight pressure on the abdominal region of the female. The ovulated females were immediately stripped and separate fertilization trays or petri dishes were used for incubation of eggs of each fish. Milt from males were also stripped out for fertilizing the eggs. If the milt is unavailable through stripping, the testes of male had to be dissected out from body cavity and subsequently macerated in 0.85% NaCl solution. The sperm suspension was mixed with eggs by gently stirring with a feather to ensure fertilization while a small amount of water was added to the egg- sperm mixture to have better activation of sperm for effective fertilization of eggs. Excess milt, blood and dirt if any were removed by washing the eggs several times with clean water.

Incubation and hatching of the fertilized eggs: The fertilized eggs were transferred to and spread as homogeneously as possible in plastic bowls for incubation. All the incubators were received water flow to ensure adequate aeration. Dead eggs were removed after every 3h and their number was carefully recorded. Upon completion of hatching, the number of hatchlings were also counted and recorded.

Indices of effectiveness of PG dose: Percent ovulation, percent fertilization and percent hatching were used as indices to ascertain the effectiveness of different PG doses:

The formula used for calculating percent ovulation was as below:

Percent ovulation was calculated using the following formula:

$$\% \text{ ovulation} = \frac{\text{No. of fish ovulated}}{\text{Total no. of fish injected}} \text{ X } 100$$

The following formulae were used for ascertaining the fertilization and hatching percentage:

$$\% \text{ fertilization} = \frac{\text{No.of fertilized eggs}}{\text{Total no.of eggs(fertilized + unfertilized)}} \text{ X } 100$$

$$\% \text{ hatching} = \frac{\text{No. of eggs hatched}}{\text{Total no. of eggs}} \text{ X } 100$$

For calculating percent fertilization about 50 eggs were randomly taken from each group and the counting of fertilized and unfertilized eggs was done using a microscope.

Data analysis: Several indices of effectiveness of PG dose were analyzed by Microsoft Excel (MS Excel) computer package as descriptive values such as mean and percentage. Data were analysed with the aid of the computer software SPSS version 11.5.

Results and Discussion

Maturity and induced breeding trials of M. armatus: Two trials were conducted for induced breeding of *M. armatus* during its breeding season (May-July). Broods reared in the cisterns showed full maturity and attained the state of ready to spawn. However, all of them did not seem to mature at a time.

Trial I

Female brood fish those injected with four different doses of PG viz. 20, 40, 60 and 80mgkg^{-1} body weight in treatments I, II, III and IV, respectively for standardizing the PG dose for ovulation are variably responded and the results are shown in Table 1. The ovulation rate showed spectacular difference so far as the effectiveness among four doses in inducing ovulation is concerned. PG doses of 20, 40, 60 and 80mgPGkg^{-1} body weight resulted in 0%, 100%, 100% and 100% ovulation respectively. The latency period i.e. time interval between the treatment with PG extract and ovulation varied between 30 and 42h of injection in all cases. The fish failing to ovulate within this time did not ovulate even after a period of 72h of PG treatment. The lowest dose of 20mg PGkg^{-1}body weight yielded no ovulation. On the other hand, although 100% ovulation was shown by the females treated with highest dose of 80 mg PGkg^{-1} body weight, the fertilization rate was very low and subsequent hatching rate was negligible. This is ascribable to the fact that the lowest dose of PG failed to induce ovulation while stripping of females treated with the highest dose of PG released quite high percentage of eggs including unripe ones.

Fertilization rate: The highest fertilization rate of eggs was obtained from females treated with 40mgPGkg^{-1} body weight followed by 60mg PGkg^{-1} body weight. Eggs obtained from females treated with 40mgPGkg^{-1} body weight showed 93.00±2.00% fertilization while those treated with 60 and 80mgPGkg^{-1} showed 29.00±3.61% and 16.67±1.53% fertilization respectively (Table 1). There was a significant ($P<0.05$) difference in fertilization rates for three doses of PG tested and the fertilization rate in treatment II was significantly ($P<0.05$) higher compared to others (Table 1).

Hatching rate: Hatching rate of eggs collected from females treated with 40mgPGkg^{-1} body weight was the highest followed by 60mgPGkg^{-1} body weight. Hatching rates shown by the eggs obtained from females treated with PG dose of 40mgPGkg^{-1} body weight was 58.30±3.50% while those treated with 60 and 80mg PGkg^{-1} body weight showed 12.30±1.50% and 6.67±1.53% hatching respectively (Table 1). Hatching rate of eggs in treatment II was significantly ($P<0.05$) higher than all other treatments.

Table 1. Effect of different doses of PG on ovulation of females and fertilization and hatching of eggs of spiny eel *M. armatus*.

Trial I	Dose of PG ($mgkg^{-1}$ body weight)	Replication	Body weight of females (g)	Ovulation of females		Latency period (h)	Fertilization rate (%)		Hatching rate (%)		Comment
				Response	Average rate (%)		Individual female	Average	Individual female	Average	
TI	20	R_1	150	-	-	-	-	-	-	-	-
		R_2	100	-	-	-	-	-	-	-	
		R_3	170	-	-	-	-	-	-	-	
TII	40	R_1	130	***	100	42	95	93.00±2.00[a]	58	58.30±3.50[a]	Considerable no. of larvae hatched
		R_2	175	***		40	93		62		
		R_3	135	***		39	91		55		
TIII	60	R_1	145	**	100	36	32	29.00±3.61[b]	14	12.30±1.50[b]	Few larvae hatched
		R_2	150	**		38	25		12		
		R_3	135	**		37	30		11		
TIV	80	R_1	165	*	100	30	18	16.67±1.53[c]	8	6.67±1.53[c]	Very few larvae hatched
		R_2	170	*		32	15		5		
		R_3	180	*		31	17		7		

*** Profuse ovulation and yielded sufficient number of ripe eggs on stripping; **Considerable ovulation and yielded sufficient number of ripe eggs with considerable number of unripe eggs; *Ovulation with predominance of unripe eggs, - No ovulation; - Values in each column with different superscripts are significantly different ($P<0.05$).

Trial II

In trial II, the best dose i.e. $40mgPGkg^{-1}$ body weight obtained from trial I was used. In this trial the variation in mode of injection was as follows: i) administration of the whole dose at a time (treatment I) and ii) administration of the dose by two different injections (treatment II) (30% and 70%) at 6h interval. In treatments I and II, 100% and 100% ovulation, 91.67±1.63% and 91.33±2.34% fertilization, and 57.00±2.83% and 58.33±3.61% hatching were achieved respectively. Data representing the effects of variations in mode of administration of PG doses on ovulation of females and the rates of fertilization and hatching of eggs are presented in Table 2. Statistical analysis revealed no significant difference among the females treated once (with the whole dose) or twice with divided dose of PG at the rate of 30% and 70% at 6h interval so far as the ovulation rate of females and fertilization and hatching rates of eggs are concerned.

Table 2. Effects of different modes of administration of PG dose on ovulation of females and fertilization and hatching of eggs of spiny eel *M. armatus*.

Trial II	Dose of PG (mg kg^{-1} body weight)	Body weight of females (g)	Ovulation of females		Latency period (h)	Fertilization rate (%)		Hatching rate (%)		Comment
			Response	Average rate (%)		Individual female	Average	Individual female	Average	
T I	40	155	***	100	36	94	91.67 ±1.63	58	57.00 ±2.83	Considerable no. of larvae hatched
		165	***		38	90		60		
		170	**		37	91		53		
		180	***		38	92		55		
		176	***		38	93		56		
		168	**		36	90		60		
T II	40	176	***	100	41	93	91.33 ±2.34	58	58.33 ±3.61	
		180	***		40	92		62		
		178	***		42	89		55		
		172	***		40	92		58		
		182	***		41	94		54		
		177	***		42	88		64		

*** Profuse ovulation and yielded sufficient number of ripe eggs on stripping.

Discussion

Two breeding trials of *M. armatus* were performed using PG extract in the present study. Earlier artificial breeding trials have been conducted with a number of species for standardizing the dose of PG for achieving satisfactory rate of ovulation, but an ambiguity remains among the doses used by the scientists (Khan, 1972; Thakur and Das, 1974; Khan and Mollah, 2004; Mollah *et al.,* 2008; Taslima and Mollah, 2012). For induced breeding of *M. armatus,* selection of suitable inducing agents and optimization of doses are felt essential but much information on these aspects are not available in Bangladesh or elsewhere in the world except the attempt was taken by Mollah *et al.* (2013) to breed the fish using different doses of PG but it was ended without any success. Therefore, it is necessary to launch a research by which appropriate inducing agents and their suitable doses can be identified.

According to Narejo *et al.* (2002) *M. armatus* shows only one breeding season which lasts from May to July having a peak in July. Similarly, Serajuddin and Pathak (2012) recorded high values of GSI for both male and female *M. armatus* during the months of May to July which pointed out their single breeding season. Considering the above information, the present experiment was designed to ascertain a suitable dose of PG for inducing ovulation in *M. armatus* during the months of May to July. Females injected with PG at 40mg PGkg^{-1} body weight showed best results in

ovulation, fertilization and hatching which was agreed with the findings of Mollah *et al.* (2013). Fish injected with lower dose, i.e. 20mg kg^{-1} body weight did not ovulate. On the other hand, fish injected with 60 and 80mg $PGkg^{-1}$ body weight showed 100% ovulation but fertilization and hatching rates of eggs were low. It is clear that higher doses of PG (60 and 80mg $PGkg^{-1}$ body weight) could not improve the breeding performance of *M. armatus.* Though the higher doses precipitated 100% ovulation but a high percentage of unripe eggs and presence of blood during stripping revealed the symptom of abortion rather than usual ovulation which might cause very low hatching rate. Khan and Mollah (2004) observed similar kind of response (100% ovulation) during induction of artificial breeding in *Pangasius pangasius*. In the present experiment, management and breeding protocol were maintained uniformly using more or less similar size and maturity of the fish. However, different results were obtained only due to the variation of PG doses. For incubation of fertilized eggs, plastic bowls (mini circular tank made by plastic bowl) were used where adhesive eggs required a sedentary situation like catfishes e.g. *Heteropneustes fossilis* and *Clarias batrachus*.

The male reproductive system of spiny eel was consisted of two lobed testis. They were elongated, tubular, situated on either side, ventral to the kidneys in the posterior region of the abdominal cavity and connected along the dorsal surface by mesorchium, from which they were suspended in the posterior ends into a short duct, which fused and led to the urogenital papilla. Milt came out through the urogenital papilla of *M. armatus* upon external pressure on the abdominal region of the fish. The tubular like projections of the testes at maturity might be the possible reason. Similar milt collection procedure was described by Khan (1997) in case of *Pangasius pangasius*. Milt collection from mature male *Notopterus chitala* by gentle pressure on the belly was also observed by Singh *et al.* (1980).

Although the present work generated some fundamental information on biology of the species, inducing agent and doses, more works need to be done to know the effectiveness of other inducing agents than PG for developing dependable induced breeding technique of *M. armatus*. However, it is hoped that these efforts will help to conserve the stock of endangered *M. armatus* from further deterioration and make them available in the natural habitat.

Conclusion

M. armatus is an endangered fish though it was abundant in natural water bodies few years ago. However, environmental and anthropogenic activities degraded the natural breeding and nursery grounds of the species and made them endangered. Therefore, it is essential to conserve the fish species otherwise, it will be extinct in near future. Development of induced breeding techniques and production of seeds is one of the ways to protect the species. Induced breeding of *M. armatus* has been possible using pituitary gland extract in this study and the larvae survived. So, this technique can be used to produce seed of the species in hatcheries in commercial scale and it can also be used for breeding of other endangered fish species of the country.

Acknowledgement

The authors would like to acknowledge the financial assistance of Bangladesh Agricultural Research Council -World Bank (NATP Phase-1) to the Sponsored Public Goods Research (SPGR) project entitled Gene banking of improved broodstocks of Indian major carps (catla, rohu and mrigal) and development of breeding technique of three threatened species (mohashol, bagair and baim).

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