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EFFECT OF PITUITARY GLAND (PG) DOSES ON ARTIFICIAL PROPAGATION OF ENDANGERED TARABAIM, *Macrognathus aculeatus* (BLOCH)

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ABSTRACT

Five hormone doses viz., 40, 60, 70, 85 and 90 mg of Pituitary gland (PG)/kg body endangered Tarabaim (*Macrognathus aculeatus*) were tested and they were designated as Treatments T_1, T_2, T_3, T_4 and T_5, respectively. Significantly ($p<0.05$) higher fertilization (86%) and hatching rates (50%) were obtained from T_5 (90 mg of PG extract/kg body weight) than those of other treatments. However, T_4 (85 mg of PG extract/kg, body weight) and T_3 (70 mg of PG extract/kg body weight) gave the highest (90%) ovulation rate. In May and June, the highest fertilization rate of 82% and 86% respectively and hatching rate 45% and 75%, respectively were obtained at the temperature ranged from 27.0 to 33.0°C. In conclusion, PG extract at a dose of 90 mg/kg body weight appeared to be the suitable dose for artificial propagation of *M. aculeatus*, May and June are the suitable months for its artificial propagation.

Key words : Artificial Propagation, PG, *Macrognathus aculeatus*

INTRODUCTION

Macrognathus aculeatus (Bloch) is an endangered species, locally known as tarabaim in Bangladesh. Like tropical cyprinids, it normally breeds in open waters including *beels*, rivers and floodplains. This fish is omnivorous and feeds mainly on higher aquatic plants, algae, protozoan, diatoms, insects and crustaceans (Mustafa *et al.,* 1982; Mookerjee *et al.,* 1986). This fish has high demand for its excellent taste with good market value. Unfortunately, availability of *M. aculeatus* has declined drastically in recent years due to various ecological changes in the aquatic ecosystem. Thus, this important freshwater species is going to extinct due to lack of proper management and conservation. It is therefore necessary to take appropriate measures for protection of this vulnerable species through artificial propagation before it is lost for ever.

This fish has enormous aquaculture potential and it could be easily grown in fish ponds along with other polyculture species. In order to do so, a huge quantity of fingerlings will be required which could be met through artificial breeding and successful rearing of fry

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and fingerlings. Therefore, the present study was conducted to find out optimal dose of hormones for successful breeding of large scale Tarabaim, *M. aculeatus* fry.

MATERIALS AND METHODS

The wild brood fish including Male and female of *Macrognathus aculeatus* was collected from the different places of Mymensingh district for breeding purpose. The collected brood fishes were stocked in Fisheries Field Laboratory complex, Faculty of Fisheries, Bangladesh Agricultural University during the period of one year from April 2004 to March 2005 or May 2004 to April 2005 and reared upto full mature stage.

Maintenance of brood stock

Proper rearing and maintenance of brood stock of both sexes to prime mature condition is a pre-requisite for successful induced breeding. The fishes were fed with a mixture of mustard oil cake, rice bran, wheat bran, fish meal, vitamin premix and di-calcium phosphate at the ratio of 20 : 33 : 25 : 20 : 1 : 1. Feeds were given at the rate of 4-5% of total body weight of fish per day.

The ripe male and female brood fish were selected based on physical and visual examination of secondary sexual characteristics i.e. abdomen and genital openings. Only healthy and uninjured fishes were selected for induced breeding.

Method of injection

One ml disposable syringe was used for injecting hormone to the recipient fish. The appropriate amount of diluted hormone stock solution was taken in the syringe. Then the fishes were caught very carefully from the spawning tank by net. A piece of clean, soft and wet cloth was used to wrap up the fish and kept lying on a table. The accurate dose of hormone solution was administered at the basal part of the dorsal fin. Needle was inserted at an angle of 45° with the body.

Induced breeding by pituitary gland (PG) extract (Double doses)

During range finding test with double doses, it was found that PG extract between 35 and 45 mg/kg body weight was required for first injection, while second injection required 40 to 55 mg PG/kg body weight in the case of female spawners. Twenty female fishes were injected with PG extract and 10 female were placed in each of the one spawning hapas. At the time of second injection of female, male fishes were injected with PG extract at the rate of 35 to 40 mg/kg body weight. Then the fishes were carefully observed for any behavioral changes and also for spawning activity upto ovulation time. After 6 hrs of injection, the brooders were caught and the eggs from the female were stripped out and fertilized with stripped out milt which were mixed by feather.

Pituitary gland (PG) extracts (Single doses)

Single dose from 80 to 95 mg PG/kg body weight was injected in the case of female spawners. At the same time, the male fishes were injected at the rate of 35 to 45 mg PG/kg body weight and placed 10 male and 10 female fishes in separate hapas. Breeding behavior and spawning activities were observed upto ovulation time. After eighteen hrs of hormonal injection, the brooders were caught and checked. Then, the eggs from the female were stripped out and fertilized with stripped milt, and then mixed by feather.

Spawning and fertilization

Spawning took place inside the breeding tank usually within 36 to 40 hrs after administration of 2nd hormone injection. *M. aculeatus* generally breeds naturally inside the breeding tank. However, due to adhesive characteristic of fertilized eggs, it was preferably stripped for better fertilization. Male and female fishes were kept in the spawning hapa after injection. Dry method of stripping was followed as a routine procedure. At first, female fishes were stripped to collect eggs in an enamel tray. Milt from the male fish was collected by applying slight pressure on its abdomen. The eggs and milt were mixed thoroughly in the enamel plate with a soft and clean feather. A few drops of water were added in the tray and was shaken gently to ensure effective fertilization. To promote fertilization a special solution named "Tanick acid solution" was added in the fertilized eggs. This solution was prepared by using 200 g powder milk in 1 L of water. After the use of tanick acid solution to the eggs, it was stirred continuously for 10 minutes to mix homogeneously. To remove the stickiness, 5 g of tannin were added to 10 L of water and mixed, then 1 L of this solution was added to 12 L of swollen eggs and mixed thoroughly by hand. The eggs were allowed to settle for a few seconds and then the tannin solution was poured off. The eggs were washed several times with freshwater to eliminate the toxic effect of tannin to the eggs. The swollen eggs were transferred to hatching tray under continuous water showering circulating system. Each hatching tray with 10 L capacity contained 5000 eggs. The flow of water in the tray was regulated during the incubation period. Normally the rate of flow of water was maintained at 500 to 700 ml/min. The eggs hatched out within 36 to 40 hrs at temperature ranged from 27 to 33°C. During incubation period, dead embryos were removed to prevent fungal growth. Number of live eggs in each group was determined within 2 to 3 hrs of fertilization.

Estimation of fertilization and hatching rate

The fertilization and hatching rate was calculated by the following formula :

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

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

Statistical analysis

The data of fertility and hatchability were further tested following one way ANOVA to assess significant difference between treatment groups and then using Duncan's New Multiple Range Test (DNMRT). The data were analysis using statistical package through computer following Standard Methods (Zar, 1996).

RESULTS

Double doses of PG extract

During range finding test, it was noted that the female fish was good respondent fully to ovulation below 90 mg PG/kg body weight. Though partial response was observed but it was not considered satisfactorily. In the case of male, amount of PG required to promote spermiation was found to be 35 to 40 mg PG/kg body weight (35, 36, 40 and 45 mg PG/ kg body weight) administered at the time of application of second injection to the female. Pertinent data regarding the time of injection and ovulation, fertilization rate, time of hatching, hatching rate and temperature are shown in Table 1. Best spawning occurred under dual hormonal regime at doses of 40 and 50 mg PG/kg body weight in the case of female. But administration of PG extract at the doses of 40 and 55 mg PG/kg body weight showed decreasing fertilization and hatching rates. Partial spawning was happened at the doses of 40 and 45 mg/kg body weight. But fertility and hatching rates were very poor. No ovulatory response was noted when the females were injected with 35 mg/kg (initial dose) and 40 mg/kg (second dose). Fish showed some sort of courtship behavior after 1st injection. But after administering 2nd injection, male and female moved together in anti-clockwise direction and the female was hold by the male, later bending its body, rubbing, knocking and nudging her. Their bodies were twisted round each other and firmed with the fins. They were started to nudge themselves by snout in the mouth and ventral region of the female up to ovulation time. Ovulation occurred after 8 hrs of 2nd injection and hatchlings came out after 36 to 40 hrs of fertilization. Best fertilization and hatching rate were found to be at 86 ± 1.22 and 75 ± 0.62, respectively in treatment T_4. Thus doses of 40 and 50 mg/kg body weight in treatment 4 seemed to be the best dose of pituitary extract for induced breeding of *Macrognathus aculeatus.* Administration of higher amount of PG extract at 45 and 55 mg/kg in treatment E resulted in reduced success.

Single dose of PG extract

The time of injection and ovulation, fertilization rate, time of hatching and hatching rate following administration of PG in a group of *M. aculeatus* have been shown in Table 2. Results indicate that single administration of PG extract at the dose of 80 to 82 mg/kg body weight in treatments 1 and 2 yielded no ovulatory response. When the dose of PG extract increased to 86 mg/kg body weight, partial ovulatory response was noted. Administration of PG extract at 90 and 95 mg/kg body weight were found to be effective in induction of spawning in *M. aculeatus.* Best spawning occurred at the dose of 90 mg/kg body weight (treatment 4) in the case of female. But further increase of dose of PG at and above 95 mg/kg body weight (treatment 5) decreased fertilization and hatching rates. Partial spawning occurred only when PG was injected at the dose of 86 mg/kg body weight. But fertility and hatching rates were very poor. Ovulation occurred after 7-8 hrs of injection and hatchlings came out after 36 to 40 hrs of fertilization. Best fertilization and hatching rates were found to be 86±0.81 and 75±1.84 respectively in treatment 4. Thus, treatment 4 (90 mg/Kg body weight) was the best doses.

Table 1. Induced breeding of *Macrognathus aculeatus* through administration of double doses pituitary gland (PG) extract

Treatment	Body weight (g)		Doses of 1st injection (mg/kg)		Interval of 2nd injection (hr)	Doses of 2nd injection (mg/kg)		Time of ovulation after 2nd injection (hr)	Fertilization rate (%)	Hatching period (hr)	Hatching period (%)	Incubation temperature (°C)	Remarks
	Male	Female	Male	Female		Male	Female						
1	20.0±0.82a	40.0±2.04b	-	35	6	35	40	7-8	0±0.00c	-	0±0.00d	27.33	No ovulation
2	22.0±1.22a	45.0±0.41a	-	36	6	36	42	7-8	0±0.00c	-	0.0±0.0d	27-33	Male and female responded. Partial ovulation took place
3	15.0±0.41b	27.0±0.82d	-	40	6	40	45	7-8	40.0±2.04b	36-40	46±0.20c	27-33	Availability of milt partial ovulation observed
4	16.0±0.20b	22.5±0.89d	-	40	6	40	50	7-8	86.0±0.81a	36-40	75±1.84a	27-33	Milt and eggs available, ovulation, fertilization, hatching were satisfactory.
5	20.0±1.83a	30.0±0.61c	-	45	6	40	55	7-8	80.0±3.47a	36-40	70±0.61b	27-33	Milt and eggs available, ovulation, fertilization, hatching were satisfactory.
LSD	4.428	4.591							7.5861		3.595		-

The figure in common letter in each column don't differ significant ($p<0.01$)

Table 2. Ovulation response of *Macrognathus aculeatus* following administration of single doses pituitary gland (PG) extract

Treatment	Body weight (g)		Doses of 1st injection (mg/kg)		Ovulation period (hr)	Fertilization rate (%)	Hatching period (hr)	Hatching period (%)	Incubation temperature (°C)	Remarks
	Male	Female	Male	Female						
1	19.0±0.81abc	39.5±0.90	35	80.0	7-8	0±0.00d	-	00.00±c	27.33	No ovulation
2	21.0±0.41a	42.3±0.89	36	82.0	7-8	0±0.00d	-	0.00±c	27-33	No ovulation
3	20.0±0.61ab	41.5±4.08	37	86.0	7-8	40.±081c	36-40	45±0.61b	27-33	Partial ovulation
4	18.0±1.02bc	39.4±0.32	40	90.0	7-8	86±1.22a	36-40	75±0.62a	27-33	Sufficient milt and ovulation egg high rate of fertilization. Moderate hatching rate
5	17.0±0.20c	38.2±0.82	45	95.0	7-8	60±1.02b	36-40	45±0.82b	27-33	Moderate fertilization. Slightly decrease in hatching
LSD	2.795	-				3.308		2.197		-

The figure in common letter in each column don't differ significant ($p<0.01$)

DISCUSSION

In the present study, it was found that *M. aculeatus* breeds from May to June which seems to be the peak breeding season. Commencement of breeding season for *Puntius sarana* was as observed in the present investigation agrees with the reports of Sobhana and Nair (1974); Sinha (1975); Talwar and Jhingran (1991). Breeding of major carp was performed at an ambient water temperature from 27 to 29^{o}C. This range of temperature is suitable for breeding of most indigenous small fishes (Islam and Chowdhury, 1976; Akhteruzzaman *et al.,* 1992). Temperature ranging from 26.5 to 35^{o}C is reported to be appropriate for spawning of major carps (Ibrahim *et al.,* 1968). *M. aculeatus* being a small fish seemed to have similar environmental requirement with other Indian major carps.

In the present experiment, injection of pituitary extract at 90 mg/kg body weight of the *M. aculeatus* showed better results in ovulation, fertility and hatchability. Further increase in the amount of hormone doses resulted in lower reproductive performances. Dose specificity of PG extract as observed in the present study is in conformity with the findings of Akhteruzzaman *et al.* (1992) who found better spawning performances of *P. sarana* at 6 mg PG/kg body weight. Kohinoor *et al.* (1995) found no ovulatory response of *P. Sarana and P. gonionotus* to PG extract below 4 mg/kg body weight of the female brooders. This single dose PG extracts is more or less similar in the case of *P. gonionotus* (Hussain *et al.,* 1987). The doses of PG have been optimized to 40 mg and 50 mg/kg body weight at first and second injection, respectively, for female of *M. aculeatus* at an interval of 6 hrs, which was higher than dose to breed *Cirrhinus reba* (Hossain, 2001).

Significant variation was observed in fertilization and hatching rate of *M. aculeatus* eggs following administration of PG extract. This apparent variation appeared to be related with the qualitative and quantitative nature of hormonal substance. Injection of PG extract at 90 mg/kg body weight of *M. aculeatus* showed better results in ovulation, fertility and hatchability of the treated fish. Further increase or decrease in the amount of hormone doses resulted in lower reproductive performances.

Successful induction of spawning in *M. aculeatus* indicated that spawners might have received hormone treatment at optimal breeding conditions. Khan and Mukhapadhyay (1975) pointed out that the success of entire operation of induced breeding depends largely on proper selection of brood fishes which has close similarity with the present experiment. Accomplishment of successful spawning depends on selection of suitable pair fish at the proper stage of ovarian development and creation of congenial spawning conditions (Nash and Shehadesh, 1980). However, easy and success breeding for higher output, it is very essential to use tannin solution to remove adhesive and sticky characteristics of the fertilized eggs of *M. aculeatus.* Horvath *et al.* (1992) used tannin solution to get better result of fertilization and hatching rate of common carp.

It is evident from the findings of the present study that PG extract is effective in induction of spawning of *M. aculeatus* under controlled hatchery condition. The hatchery operators may use PG for artificial propagation of *M. aculeatus.* Overall consideration, PG extract at

the rate of 90 mg/kg body weight appeared to be the suitable dose for artificial propagation of *M. aculeatus*.

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