J. Bangladesh Agril. Univ. 9(2): 305–310, 2011 **ISSN 1810-3030**

Effect of pituitary gland doses on artificial propagation of Guchibaim, *Mastacembelus pancalus* (Hamilton)

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Abstract

Artificial propagation of *Mastacembelus pancalus* was conducted in order to determine the optimum dose of pituitary gland (PG) hormone at the hatchery of the Field Laboratory Complex, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. The present study consisted of two trials and each trial had four treatments (T_1, T_2, T_3 and T_4, respectively) with three replications of each. In the experiment single dose and double dose had been used for T_1, T_2, T_3 and T_4, respectively to evaluate the efficiency on ovulation rate, fertilization rate, and hatching rate of *M. pancalus* eggs. The hatchlings were reared in aquarium up to 30 days and survival rate was determined. Treatment-3 (T_3) of double dose (170mg PG kg^{-1} and 60mg PG kg^{-1} body weight of fish for female and male respectively) showed better results in terms of ovulation rate (90.03±2.56%), fertilization rate (90±0.81%), hatching rate (80±1.84%), and survival rate (40.00±3.19%) was recorded than other treatments of single and double dose. The water temperature was recorded 27 to 31°C. In the present study, treatment-3 (T_3 in double dose) was the best findings in terms of ovulation rate, fertilization rate, and hatching rate which can be used in artificial propagation of *M. pancalus* for the development of hatchery production. May and June are the suitable months for artificial propagation of the said species.

Keywords: Artificial Propagation, PG, *Mastacembelus pancalus*

Introduction

Mastacembelus pancalus (Hamilton) is a freshwater spiny eel species, locally known as Guchibaim in Bangladesh. Like tropical cyprinids, it normally breeds in open waters (*beels*, rivers and floodplains). This fish is omnivorous and feeds mainly on algae, protozoans, diatoms, insects and crustaceans (Mustafa *et al.,* 1982 and Mookerjee *et al.,* 1986). This fish has high demand for its excellent taste with good market value. Unfortunately, availability of *M. pancalus* from our close and open water systems has declined drastically in recent years due to various ecological changes.

As such, this important freshwater species is going to be extinct due to lack of initiative of proper management and conservation. It is the high time to take appropriate measures for protection of this vulnerable species through artificial propagation before it is lost for ever.

The fish has enormous aquaculture potential and 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 and fingerlings. Thus the immediate goal is to protect this species from extinction and conserve fish biodiversity in nature. To the best of our knowledge no work has been done on artificial propagation of *M. pancalus* with PG hormone in Bangladesh. Therefore, the present work has been under taken with the objectives, *viz.,* (i) to determine the optimum dose of PG hormone for artificial propagation of *M. pancalus*, (ii) to determine the ovulation, fertilization and hatching rate, and (iii) to develop a protocol for its large scale breeding.

Materials and Methods

The experiment was conducted in the hatchery of the Field Laboratory Complex, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh during the period from May to July 2008.

Collection of fish

About 80 *Mastacembelus pancalus* both males and females were collected from different places of Mymensingh district. They were stocked in the ponds during the period from January to March, 2008 and reared up to full maturity.

Maintenance of brood stock

Proper rearing and maintenance of brood stock of both sexes to prime mature condition is a pre-requisite for successful artificial propagation. 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 by weight at 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., swollen abdomen and genital openings. Only healthy and uninjured fishes were selected for artificial propagation.

Experimental design

For artificial propagation broods were collected from the brood rearing ponds in the early morning. This experiment consisted of two trials and each trial had four treatments (T_1, T_2, T_3 and T_4) with three replications of each. A total of 24 female and 24 male were selected from the brood rearing ponds. To observe the effective dose for artificial propagation, the females and males were injected with different doses of PG extract. Both single dose and double dose had been used in treatments-T_1, T_2, T_3 and T_4. After ovulation, three females and three males from each treatment were selected for stripping.

Collection and preparation of PG

Locally available dry carp pituitary glands (PG) were collected from market in preserved condition in airtight vials and used as inducing agent. At first, the pituitary glands were gently removed from the vial with a pair of forceps and then weighed by an analytical electronic balance (College B204-s, Switzerland). The amount to be weighed out was calculated on the total of the body weight of all the fishes using the following formula:

$$\text{Weight of PG (mg)} = \frac{W_t \times P_t}{1000}$$

Where, W_t represents total body weight (g) of all the fishes to be injected and P_t represent the rate in mg PG to be injected/kg body weight under a particular treatment. The weighed PG was transferred to a tissue homogenizer for homogenization. The crushed PG was diluted in distilled water to dissolve it and centrifuged with a hand centrifuge for precipitation. The freshly prepared supernatant solution of hormone was then taken slowly in a 1 ml hypodermic syringe for injection.

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 PG extract was administered at the basal part of the dorsal fin. Needle was inserted at an angle of 45° with the body.

Single doses PG extract

Single dose from 140 to 180 mg PG/kg body weight was injected in the case of female while the male fishes were injected at the rate of 60 mg PG/kg body weight. Twelve male and twelve female fishes were injected. The injected male and female (1:1) were placed 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 a feather.

Double doses PG extract

In case of double doses, 45, 50, 60 and 65 mg PG/kg body weight was required for first injection, while second injection required 95, 100, 110 and115 mg PG/kg body weight of female. At the time of second injection of female, male fishes were injected at the rate of 60 mg PG/kg body weight. Twelve female and twelve male fishes were injected. The injected male and female (1:1) were placed in each spawning hapa. Then the fishes were carefully observed for any behavioral changes and also for spawning activity 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 a feather.

Spawning and fertilization

Spawning took place inside the breeding tank usually within 33 to 35 hrs after administration of 2^{nd} hormone injection. *Mastacembelus pancalus* generally breeds naturally inside the breeding tank. But 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 1.0 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, 5.0 g of tannin were added to 10 L of water and mixed, and then 1.0 L of this solution was added 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 10.0 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 33 to 35 hrs at temperature ranged from 27 to 31^0C. 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 analysed using statistical package through computer following Standard Methods (Zar, 1996).

Results and Discussion

Performance at single dose PG extract

The ovulation rate, fertilization rate, time of hatching and hatching rate following administration of PG single dose in a group of *Mastacembelus pancalus* have been shown in Table 1. The results revealed that single administration of PG extract at the dose of 140 mg/kg body weight in T_1 yielded no ovulatory response. When the dose of PG extract increased to 160 mg/kg body weight, partial ovulatory response was noted. Administration of PG extract at 170 and 180 mg/kg body weight were found to be effective in induction of spawning in *M. pancalus.* Best spawning occurred at the dose of 170 mg/kg body weight (T_3) in the case of female and increased dose of PG at 180 mg/kg body weight (T_4) and above, decreased fertilization and hatching rates. Partial spawning occurred only when PG was injected at the dose of 160 mg/kg body weight. But fertility and hatching rates were very poor at this dose. Ovulation occurred after 8-9 hrs of injection and hatchlings came out after 33 to 35 hrs of fertilization. Best fertilization and hatching rates were found to be 86±1.22 and 70±0.62, respectively in T_3 and thus, T_3 (170mg/ kg body weight) was the best dose. Significant variation ($P<0.05$) was observed in fertilization and hatching rate of *M. pancalus* eggs following administration of PG extract.

Table 1. Ovulatory response of *Mastacembelus pancalus* following administration of single dose pituitary gland (PG) extract

Treatment	Body weight (g)		Doses of 1st Injection (mg/kg)		Ovulation period (hr)	Fertilization rate (%)	Hatching period (hr)	Hatching rate (%)	Incubation temperature (°C)	Remarks
	Male	Female	Male	Female						
T_1	10.0±0.41^{ab}	13.0±0.82^{c}	60	140	8-9	0±0.00^{d}	0	0±0.00^{d}	27-31°C	No sign. of ovulation
T_2	9.0±0.61^{b}	14.0±0.32^{b}	60	160	8-9	40±0.81^{c}	33-35	45±0.61^{c}	27-31°C	Partial response of ovulation and hatching
T_3	8.0±1.02^{c}	16.0±4.08^{a}	60	170	8-9	86±1.22^{a}	33-35	70±0.62^{a}	27-31°C	Successful spawning was observed, fertilization and hatching rate was higher
T_4	11.0±0.41^{a}	17.0±0.89^{a}	60	180	8-9	75±1.02^{b}	33-35	65±0.82^{b}	27-31°C	Male and female produced milt and eggs early. Spawning and hatching rate was good.

The figure in common superscript in each column do not differ significantly ($P < 0.05$)

Nasim (2008) found better spawning performance of *Notopterus notopterus* at 12.0 and 4.0 mg PG/kg body weight for female and male, respectively. Chakraborty (2004) stated that the successful spawning performance was observed in *P. sarana* with 6.0 and 2.0 mg PG/kg body weight for female and male, respectively. Farid (2009) also recommended a single dose of 90.0 mg PG/kg body weight for spawning of *M. aculatus*.

Double doses of pituitary gland (PG) extract

The female fish was good respondent fully to ovulation within 170 to 180 mg PG/kg body weight. In the case of male, amount of PG required to promote spermiation was found to be 60 mg PG/kg body weight administered at the time of application of second injection to the female. The time of injection and ovulation, fertilization rate, time of hatching, hatching rate and temperature were recorded and shown in Table 2. The best spawning occurred under dual hormonal regime at doses of 60 and 110 mg PG/kg body weight in the case of female. But administration of PG extract at the doses of 65 and 115 mg PG/kg body weight showed decrease in fertilization and hatching rates. Partial spawning took place at the doses of 50 and 100 mg PG/kg body weight. But fertility and hatching rates were very poor. No ovulatory response was noted when the females were injected with 45 mg PG/kg (initial dose) and 95 mg PG/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 8-9 hrs of 2nd injection and hatchlings came out after 33 to 35 hrs of fertilization. Best fertilization and hatching rate were found to be 90±0.81% and 80±1.84%,, respectively in T_3. Thus, doses of 60 and 110 mg/kg body weight in T_3 seemed to be the best dose of pituitary extract for artificial propagation of *M. pancalus.* Administration of higher amount of PG extract at 65 and 115 mg/kg in T_4 resulted in reduced success.

Table 2. Artificial propagation of *Mastacembelus pancalus* 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)		Ovulation after 2nd injection (hr)	Fertilization rate (%)	Hatching period (hr)	Hatching rate(%)	Incubation temperature (^{0}C)	Remarks
	Male	Female	Male	Female		Male	Female						
T_1	5.0±0.82^b	7.0±2.04^b	-	45	6	60	95	8-9	0±0.00^d	-	0±0.00^d	27-31^{0}C	No sign. of ovulation
T_2	6.0±1.22^b	8.0±0.41^b	-	50	6	60	100	8-9	40±2.04^c	33-35	40±0.20^c	27-31^{0}C	Partial ovulation was observed.
T_3	7.0±0.41^a	9.0±0.82^a	-	60	6	60	110	8-9	90±0.81^a	33-35	80±1.84^a	27-31^{0}C	Successful ovulation was observed. Hatching and fertilization rate were highest.
T_4	6.0±0.20^a	10.0±0.89^a	-	65	6	60	115	8-9	75±3.47^b	33-35	70±0.61^b	27-31^{0}C	Successful ovulation was observed. Hatching and fertilization rate were satisfactory.

The figure in common superscript in each column do not differ significantly (P < 0.05)

Ovulation rates were found 0%, 40%, 86% and 75% for single dose and 0%, 40%, 90% and 75% for double doses in T_1, T_2, T_3 and T_4, respectively. The hatching rates observed was 0%, 45%, 70% and 65% for single dose and 0%, 40%, 80% and 70% for double doses in T_1, T_2, T_3 and T_4, respectively.

In the present experiment, injection of pituitary extract at 170 mg PG/kg body weight of the *M. pancalus* showed better results in ovulation, fertility and hatchability. Further increase in the amount of hormone doses resulted in lower reproductive performances. Farid *et al.* (2008) found the better spawning performances of *M. aculeatus* at 90 mg PG/kg body weight. The doses of PG have been optimized to 60 mg and 110 mg/kg body weight at first and second injection, respectively for female of *M. pancalus* at an interval of 6 hrs, which was a higher dose than that required to breed *M. aculeatus.*

Significant variation was observed in fertilization and hatching rate of *M. pancalus* 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 170 mg PG/kg body weight of *M. pancalus* 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 performance. A very much similar trend in the breeding performance was also observed in fishes treated with other hormonal substances.

Successful induction of spawning in *M. pancalus* 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 artificial propagation 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 of fish at the proper stage of ovarian development and creation of congenial spawning conditions (Nash and Shehadesh, 1980). But for easy and success of breeding for higher output, it is very essential to use tannin solution to remove adhesive and sticky characteristics of the fertilized eggs of *M. pancalus.* Horvath *et al.* (1992) used tannin solution to get better result of fertilization and hatching rate of common carp, which is similar to this experiment.

Temperature was between 27.0 to 31.0^{o}C during the study period. Breeding of major carp was performed at an ambient water temperature from 27.0 to 29.0ºC. This range of temperature is suitable for breeding of most indigenous small fishes (Islam and Chowdhury, 1976 and Akhteruzzaman *et al.,* 1992). Temperature ranging from 26.5 to 35.0ºC is reported to be appropriate for spawning of major carps (Ibrahim *et al.,* 1968). *M. pancalus* being a small fish living in the similar habitats seemed to have similar environmental requirement with other Indian major carps.

Conclusion

It is evident from the findings that PG extract is effective in induction of spawning of *M. pancalus* under controlled hatchery condition. PG extract at the rate of 170 mg/kg body weight of brood fish appeared to be the suitable dose for its artificial propagation.The hatchery operators may use pituitary gland (PG) at this dose for artificial propagation of *M. pancalus.*

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