

## Induced breeding of the riverine catfish *Rita rita*

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### Abstract

The paper reports the first incidence of successful induction of breeding in the riverine catfish *Rita rita* using carp pituitary gland (PG) extract. A breeding trial using four PG doses viz. 80, 100, 120 and 140mg/kg body weight of fish was conducted to optimize the dose of pituitary gland (PG) extract in terms of induction of ovulation in female. The male received a dose of 40mg PG/kg body weight in all cases and was sacrificed for collection of milt. The best performance was shown by the fish treated with 100mg PG/kg body weight in respect of inducing ovulation in females and fertilization and hatching rates of eggs.

**Keywords:** Induced breeding, *Rita rita*, Ovulation, Fertilization rate, Hatching rate

### Introduction

Bangladesh is a land of high potential as far as its water resources are concerned. There are 260 freshwater fish species, 24 freshwater prawns, 475 marine fish species, 36 marine or brackish water shrimps and 16 exotic species available in this country (DoF, 2006). Fish supplies more than 70% of animal protein required by her population. Fish has become not only the most dependable source of animal protein but also a good means of employment. It is, therefore, essential that every inch of water body be properly utilized for fish production and new commercially important fishes are explored and domesticated for large-scale seed production, which will eventually pave the way for aquaculturists to grow more fish. It is important to mention that culture fishery is the single greatest contributor to the total production of fish in the country. The main impediment toward this venture is the non-availability of required number of stockable sized seeds of the concerned species on demand. Only a reliable induced breeding and fry rearing technique can ensure a steady supply of quality fish seeds.

According to IUCN (2000), 54 fish species of inland water are now in endangered to critically endangered situation in Bangladesh. In spite of good taste and popularity the riverine catfish *Rita rita* (popularly known as 'Rita' in Bangladesh and India) fetching high price in the market is now in endangered condition. Dubey (1994) also categorized *Rita* spp. among the declining indigenous fauna of India. The induced breeding techniques of few commercially important fish species of Bangladesh have been established. Until now the fry of *R. rita* can only be collected from the river systems where they breed naturally. But the ecology of breeding grounds are changing in such a way that the fish are unable to cope with. As a result the breeding grounds are losing their suitability to be used by the species posing a threat of extinction to existence of *R. rita*. Some of the basic biology of *R. rita* has been studied by Banarjee and Banarjee (1987), Devi *et al.* (1990, 1991, 1992), Mukhopadhyay *et al.* (1994), Pandey and Pandey (1997), Mollah (2005) and Amin *et al.* (2008). One of the probable mitigating measures that can be taken against the extinction of this fish is to initiate a domestication and breeding programme whereby mass production of quality seeds can be ensured. In view of the above, the present experiment was undertaken to develop a dependable induced breeding technique of the riverine catfish *R. rita*.

## Materials and Methods

The experiment was conducted in July 2008 in the “Mini hatchery complex” of the Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh. Before that more than 100 fish were collected from the river Old Brahmaputra in the month of March 2007. They were reared in cisterns and raceway system with water flow and fed with earthworms, tubificid worms and chopped and cleaned poultry viscera (protein 47.58%, lipid 15.78%, ash 6.74% and dry matter 16.71%) for domestication. The brood fish were collected by reducing the water of the raceway and the cisterns on the day of the breeding trial. Good and healthy broods were selected for breeding. Identification of male and female broods was done on the basis of some external features. The females could be easily recognized by their swollen abdomen and round and swollen urogenital papillae. On the other hand, the mature males were identified by their flat abdomens and long protruded genital papillae (Fig. 1). Sexually mature males and females weighing 650-1200g were selected for induced breeding. Selected broods were kept in cisterns with continuous water flow for about 6h for conditioning prior to injection with carp PG extract. Male and female fish were kept in separate cisterns and constant water flow was maintained to ensure proper aeration.



Fig. 1. Mature female (♀) and male (♂) of the riverine catfish *Rita rita*

Twelve females were divided into four treatments and marked as T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> having three females in each treatment in such a way that the average weight of 3 females under each treatment remained approximately similar. The females under each treatment were kept separately in different cisterns. The weight of selected female broods ranged from 650 to 1200g whereas the weight of the males varied from 750 to 1150g. The females under treatment T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> were treated with carp pituitary gland (PG) extract at the doses of 80, 100, 120 and 140mg/kg body weight respectively. Freshly prepared extract of commercially available acetone dried PG was used for inducing ovulation in fish. To prepare the extract for injection, required amount of PG was carefully weighed out in an analytical balance. The required amount was calculated on the basis of the total body weight of all the fish of a particular treatment using the following formula:

$$\text{Weight of PG (mg)} = W_b \times P_t / 100$$

Where, W<sub>b</sub> represents total of the body weight of all the fish to be injected and P<sub>t</sub> represents the rate in mg of PG to be injected/kg body weight under a particular treatment.

The required volume of the extract was calculated by the following formula:

$$\text{Volume of extract (ml)} = W_b \times 0.5$$

Where, 0.5 represents the volume of the extract in ml to be injected/kg body weight.

The weighed PG was homogenized with a small volume of distilled water and the homogenate was carefully transferred to a centrifuge tube by using distilled water to ensure complete transfer. The mixture was centrifuged for 5 min at 5000 rpm. The clear supernatant was transferred to a vial and was made pre-determined volume with distilled water. On the basis of the body weight of the gravid female the required volume of extract (0.5ml/kg) was taken in a graduated 5.0ml hypodermic syringe. The required amount of extract was injected intramuscularly to the fish on the dorsal side above the lateral line. Males of all treatments were injected at the dose of 40mg/kg body weight. After injection of PG the females were checked every hour from 12h post-treatment with inducing agent by gently pressing their abdomen to ascertain the ovulation.

The females upon ovulation were immediately stripped and eggs from each fish were collected in separate fertilization trays. A male fish however, had to be sacrificed since, with this species, it is impossible to obtain milt, even from a ripe male, by using external pressure to the abdomen because of the lobular structure of the testes. For collection of milt, the testes were dissected out from the body cavity and macerated in 0.85% NaCl solution. To affect and ensure fertilization the sperm suspension was mixed with eggs by gently stirring with a feather and water was added to the egg–sperm mixture to activate the sperms for fertilizing the eggs. Fertilized eggs were washed several times with clean water to remove the excess milt, blood etc. The fertilized eggs were incubated in mini plastic circular incubator where they were in constant motion.

Percent ovulation, fertilization and hatching rates were recorded as indices of the effectiveness of different PG doses using following formulae:

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

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

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

For calculating percent fertilization 50 eggs were taken from each group and number of fertilized and unfertilized eggs was counted under a microscope. The unfertilized eggs turned whitish and opaque few hours after incubation while the fertilized eggs remained transparent and showed distinct evidence of cell division of embryo when observed under microscope.

For statistical analysis of data, a one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at a significance level of  $P < 0.05$  was employed. The statistical data analysis was carried out with the aid of the computer software SPSS version 11.5.

## Results and Discussion

In order to standardize the PG dose for inducing ovulation in female *R. rita*, four different doses of carp PG extract were used. Data representing the effects of PG doses on ovulation of female fish and the rates of fertilization and hatching of eggs are presented in Table 1. The result shows marked difference in effectiveness among 4 doses in inducing ovulation. The doses of PG at the rates of 80, 100, 120 and 140mg/kg body weight resulted in 0%, 100%, 100% and 100% ovulation respectively. The time interval between the injection of PG extract and ovulation (latency period) varied between 18 and 24h of injection in all cases. The fish which did not ovulate within this time did not do so even after a period of 36h of PG injection. The fertilization and hatching rates of the eggs obtained from females treated with 100mg were highest followed by 120mg PG/kg body weight. Females treated with the PG dose of 100mg/kg body weight showed 71.66±7.64% fertilization and 48.33±7.64% hatching respectively while those treated with 120mg PG/kg fish showed 12.50±2.50% fertilization and 7.50±2.50% hatching respectively. However, there was no fertilization of eggs produced by the females treated with 140mg PG/kg body weight.

**Table 1. Rates of ovulation of females and fertilization and hatching of eggs of riverine catfish *Rita rita* under different doses of PG**

Treatments	Dose of PG (mg/kg body weight)	Weight of females (g)	Ovulation status of females		Latency period (Hour)	Average fertilization rate (%)	Average hatching rate (%)	Remark
			Response	Average (%)				
T <sub>1</sub>	80	725	-	-	-	-	-	-
		750	-		-			
		1150	-		-			
T <sub>2</sub>	100	700	+++	100	22	71.66±7.64 <sup>a</sup>	48.33±7.64	Considerable no. of larvae hatched
		750	+++		24			
		1200	++		21			
T <sub>3</sub>	120	650	+	100	20	12.50±2.50 <sup>b</sup>	7.50±2.50	Few larvae hatched
		700	+		21			
		1150	+		18			
T <sub>4</sub>	140	725	+	100	20	-	-	-
		800	+		20.5			
		1125	+		19.5			

+++ 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 preponderance of unripe eggs

- No ovulation

Values in each column with different superscripts are significantly different ( $p>0.05$ ).

The ambient water temperature during incubation ranged between 23 and 24°C. The incubated eggs took 23-25h for hatching. At lower temperature of 20°C, incubation takes as long as 48 hours in *Clarias lazera* (Hogendoorn, 1979). Higher temperature shortens the incubation period and consequently affects the survival rate and hatching rate of the eggs of *Clarias macrocephalus* (Mollah and Tan, 1982). They found that the eggs incubated at 20°C did not hatch while those incubated at 25°C started hatching after 34h of incubation and hatching was completed by 58h. On the other hand, eggs incubated at 35°C started hatching after 22h of incubation and hatching was completed by 30h of incubation. Haylor and Mollah (1995) through a series of experiments showed how the increasing temperature within limit shortens the incubation period in *Clarias gariepinus*. According to these authors, hatching of *C. gariepinus* eggs took 57h and 15h when incubated at 20 and 35°C respectively. Above discussion indicates that incubation time is species specific and inversely related to temperature.

Usually catfishes do not spawn in the laboratory condition but readily respond to injection of fish and frog pituitary gland extract and to mammalian gonadotropins (Ramaswami and Sundararaj, 1958; Ramaswami, 1962, Barua and Mollah, 1987). Although many researchers (Thakur and Das, 1974; Khan, 1972) conducted experiments with an aim to standardize the dose of PG for successful ovulation in some species, there remains ambiguity among the doses reported by them. About the induced breeding of *R. rita*, no information is available in Bangladesh or elsewhere in the world regarding the dose optimization and suitability of the inducing agents. So, it was felt extremely essential to optimize a dose by which the induced breeding technique of the fish can be developed. However, very recently some of reproductive aspects were studied by Amin *et al.* (2008) where the highest gonado-somatic index was observed in the month of July. Having known this fact the present experiment was conducted to find out a suitable dose of PG for induction of ovulation in *R. rita* in the month of July. The fish treated with the dose of 100mg PG/kg body weight showed the best performance so far as the ovulation, fertilization and hatching rates are concerned. The PG dose of 80mg/kg body weight was proved to be too low to induce ovulation. On the other hand, the fish treated with 120 and 140mg PG/kg body weight although induced ovulation in 100% fish, the fertilization and hatching rates of eggs were insignificant. This is attributable to the fact that very high percentages of eggs obtained on stripping from the fish treated with comparatively higher doses (120 and 140mg PG/kg body weight) were unripe and at times blood used to come along with the eggs. This might be a case of abortion rather than usual ovulation. Consequently, the fertilization and hatching rates were unexpectedly low. Similar result was found when the *Pangasius pangasius* was treated with higher doses precipitating 100% ovulation in females (Khan and Mollah, 2004). Since fishes of approximately similar size and maturity were used under the same environmental and management conditions, the difference in the result obtained was only due to the variation in PG doses. The special feature unique for this species was the use of mini plastic circular hatchery where the incubated eggs were on constant motion unlike the eggs of other catfishes e.g. shing and magur especially needing a sedentary condition in trays during incubation period. This difference in incubation process was due to the non-sticky nature of the eggs of *R. rita*. Further study for testing the efficacy of other inducing agents toward ovulation and for establishing a suitable larvae rearing technique needs to be conducted to establish a breeding package to be released for the ultimate users.

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