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Induced Breeding and Larvae Rearing of Critically Endangered Riverine Catfish *Rita rita* (Hamilton)

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Abstract

The paper reports the induced breeding of *Rita rita* using carpPG (collected pituitary gland from carp fish) extracts, ovaprim and the effects of stocking density on growth and survival rates of larvae. Three doses of carpPG extracts viz. 90, 100 and 110 mg·kg^{-1} body weight of female were used while males were treated with 40mg·kg^{-1} body weight in all cases. The dose of 100 mg·kg^{-1} body weight was proved the most suitable in terms of ovulation (100%), fertilisation (82.50±6.46 %), and hatching rates (62.75±7.182%). In case of ovaprim, females were treated with 0.5 and 1.0 mL·kg^{-1} body weight and males received a dose of 0.5 mL·kg^{-1} body weight but both doses failed to ovulate the fish. The growth study was carried out using two stocking densities (1 larva and 2 larvae·L^{-1} of water) with 5 day-old larvae fed chopped tubificid worms *ad libitum*. Better growth and survival rates were found at stocking density of 1 larva·L^{-1}.

Introduction

Presently, aquaculture practices have increased dramatically to meet the protein demand of the increasing population in Bangladesh. Major fish species cultured in Bangladesh are the Indian major carps, Chinese carps, common carps and tilapia. But more indigenous fish species should be cultured to ensure the sustainability of the aquaculture industry. Inclusion of new species for aquaculture is a common practice in the USA and Canada (Pannell et al. 2001). Consistent efforts are needed to establish viable breeding and seed production techniques for the selected new species.

At least 55 species of freshwater catfishes belonging to 35 genera have been recorded in Bangladesh (Rahman, 2005). *Rita rita* (Hamilton, 1822) is one of them and it is popularly known as Rita in Bangladesh and also in India. Rita is a sluggish, bottom dwelling and carnivorous catfish which prefers muddy to clean water. Maximum total length (TL) of Rita is up to150 cm and standard length (SL) of mature specimens ranges from 20 to 30 cm (Froese et al. 2007). It is a tasty fish commanding comparatively high price in the market.

According to IUCN (2000), 54 fish species of the inland waters of Bangladesh are considered as either endangered or critically endangered. *Rita rita* is categorised as critically endangered. Once, this fish was very abundant in Afghanistan, Pakistan, India, Nepal, Bangladesh and Myanmar

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(Tripathi, 1996). Dubey (1994) also categorised *Rita sp*. among the declining indigenous fauna of India. The collection of fry of this riverine catfish from the river system is decreasing day by day due to the ecological changes of the breeding ground. As a result, breeding grounds are losing their suitability to be used by the species posing a great threat to extinction. Unavailability of stockable sized seed from the wild and dependable induced breeding techniques have been identified as the main impediments towards its commercial culture. Thus, the development of successful induced breeding technique and mass production of fry in commercial scale is crucial to protect this species from becoming extinct. A report on the preliminary success in inducing breeding in *Rita rita* using carpPG extract was published earlier (Mollah et al. 2008). However, the technique needs further refinement. Moreover, a suitable method for rearing larvae which is a prerequisite to ensure reliable and regular supply of fry needs to be established.

The aim of this study was to explore the effective doses of inducing agents (carpPG and ovaprim) on induction of ovulation and fertilisation and hatching of eggs of Rita and simultaneously to know the effects of stocking density on growth and survival rates of the larvae.

Materials and Methods

Broodfish collection and rearing

Healthy male and female *R. rita* broods were collected from the Old Brahmaputra River near the Bangladesh Agricultural University, Mymensingh. The collected broods were reared in the raceway system (11.42×6.14×1.2 m^3) for 1 year at the stocking density of 1kg·m^{-3} at the male:female ratio of 40:60. Shelters were made in the raceway with bricks to provide the natural condition. The fish were fed twice daily, *ad libitum*, with earthworms, tubificid worms, chopped and cleaned poultry viscera and flesh of different fishes like silver carp, *Hypophthalmichthys molitrix* (Valenciennes, 1844) and lata, *Channa punctatus* (Bloch).

Broodfish selection

Healthy and sexually mature broods were selected for breeding purpose. Mature male and female broods were identified on the basis of secondary sexual characteristics. The males were identified by their flat abdomens and long protruding genital papillae. The females could be easily recognised by their soft and swollen abdomen, and round and swollen urogenital papillae. The selected broods were kept in cisterns for about 6 hr for conditioning to adjust to the new environment. Male and female fish were kept in separate cisterns and constant water flow was maintained to ensure proper aeration.

Source of carpPG and ovaprim and preparation of carpPG extract

For hormonal induction of ovulation, commercially available acetone dried whole gland of carpPG (Bangladesh) and ready to use liquid ovaprim (India) were purchased in ampoules of 10 mL**.** To prepare the extract for injection, the required amount of carpPG (Collected Pituitary Gland from carp fish) was carefully weighed out in an electrical balance. The amount to be weighed out was calculated using the following formula:

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

where W_b represents total of the body weight of all the fishes injected and P_t represents the rate in mg of carpPG injected·kg^{-1} body weight under a particular treatment.

The total volume of the extract to be prepared was calculated by the following formula:

Vol. of extract (mL) = $W_t \times 0.5$

where 0.5 represents the volume of the extract in mL injected·kg^{-1} body weight.

The weighed carpPG was homogenised with a small volume of distilled water (1 mL·kg^{-1} body weight of fish) by using a tissue homogeniser and the mixture was centrifuged for 5 min at 5,000 rpm. The clear supernatant was used as carpPG extract.

CarpPG and ovaprim doses

The experiment was carried out on the effects of different doses of carpPG extract and ovaprim on breeding performance of *R. rita* under three and two different treatments, respectively, having four females and three males in each treatment. The females were treated with carpPG extract at the doses of 90, 100 and 110 mg·kg^{-1} body weight, respectively against a control group of fish (treated with 0.5 mL physiological saline·kg^{-1} body weight of fish). The dose was divided into two volumes (40% and 60%) and injected into the females at 6 hr interval. On the other hand, males in all treatments received 40 mg carpPG·kg^{-1} body weight in all cases during the second injection of female. In case of ovaprim, 0.5 and 1 mL·kg^{-1} body weight of female were used against a control (treated with same dose of physiological saline), whereas, males in both the treatments were treated with 0.5 mL ovaprim·kg^{-1} body weight at the same time interval.

Injecting the fish

For injection, the broods were taken out from the cistern by scoop net. Then the head region of the broodfish was wrapped by a wet, soft cloth while the fish lay on the foam. Based on the body

weight of the gravid female the required volume of extract was taken in a graduated 3.0 mL hypodermic syringe. The extract was injected intramuscularly on the dorsal side of the fish, above the lateral line. After injection of carpPG extract, the females were kept in a circular tank under close observation to monitor if they exhibited any change in behaviour. The females were checked every hour from 12 hr post-treatment with inducing agents, by gently pressing their abdomen to ascertain whether there was ovulation. A fish was considered to have ovulated when there was extrusion of a few eggs upon gentle pressure on the abdomen from anterior to posterior direction. The same procedure was followed in the case of ovaprim.

Ovulation, collection of milt and ovulated eggs and fertilisation

The females, upon ovulation, were immediately stripped and eggs from each fish were collected in separate fertilisation trays. For collection of milt, the testes from male fish were dissected out from its body cavity upon sacrificing the male and were macerated in 0.85% NaCl solution (milt and 0.85% NaCl solution at 1:1 ratio). Before sacrificing the male, the fish was anaesthetized following a standard protocol. The captured male was brought to the laboratory in a container (10 L capacity) with about 1 L of water. The water was then drained out from the container as much as possible. Prepared diluted MS222 (Sigma cat. # A-5040) solution (80 mg tricaine powder in 20 mL double distilled water) was poured into the container. Then the container was covered by a lid until the fish was completely anesthetised. Hand gloves and safety glasses were used during the anaesthetization process.

To ensure fertilisation, the sperm suspension was mixed with eggs by gently stirring with a clean and soft feather and water was added to the egg-sperm mixture to activate the sperms for fertilising the eggs.

Incubation and hatching of fertilised eggs

The fertilised eggs were transferred and spread as homogeneously as possible in separate mini circular hatchery tanks (50 L capacity) with constant water flow. Hatching took place 20 hr after fertilization, and this process lasted for 3 hr. After that, the number of unfertilised and fertilised eggs and hatchlings were counted and recorded in the notebook**.**

Although the hatchlings of Rita obtained nutrition from the yolk sac up to 3 days after hatching, the larvae started taking exogenous food from 36-42 hr post-hatching at ambient temperature of 28±1^{o}C. Hard-boiled chicken egg yolk was provided as first feed for the hatchlings up to satiation level.

Rearing of larvae

Five-day old larvae with more or less the same initial length (4.00±0.29 mm) and weight (5.60±0.28 mg) were stocked in bowls (21 cm deep having an internal diameter of 32 cm) to monitor the growth and survival rates of larvae for 28 days under two different stocking densities i.e 1 larva·L^{-1}, and 2 larvae·$L^{-1}$of water . Shelters were made with the broken parts of earthen pots locally known as "chara" which were placed in the bowls as the larvae had a tendency to cluster in the shelter. They were fed with chopped tubificid worms up to satiation level two times per day (7:00 am and 5:00 pm). Five larvae were randomly collected from each bowl by siphoning and the weight and length measured every 7 days. The weight (mg) and length (mm) were measured by using an electric balance and graph paper, respectively. Water quality parameters like temperature (^{o}C), pH and dissolved oxygen (ppm) content of rearing water were recorded weekly by Celsius thermometer, digital DO (Dissolved Oxygen) meter and portable digital pH meter respectively.

Statistical analysis

For statistical analysis of data, a one-way analysis of variance (ANOVA) was used. Significant results were tested by using Tukey's Multiple Comparison test to identify significant difference among the means. The statistical data analysis was carried out with the aid of the computer software SPSS version 11.5.

Results and Discussion

All the females treated with carpPG extract showed 100 % ovulation (Table 1). The time interval between the injection of carpPG extract and ovulation (latency period) varied between 18 and 24 hr of injection in all cases. There were significant difference (P<0.05) among the three treatments in terms of fertilisation and hatching rates of eggs. The highest fertilisation (82.50 ± 6.46%) and hatching rates (62.75±7.18%) were observed in the treatment where the females were treated with 100 mg carpPG·kg^{-1} body weight.

Both the doses of ovaprim failed to show any positive response in terms of ovulation in treated females and no further details are therefore provided for this experiment.

Five-day old larvae produced through the induced breeding technique were reared for 28 days under two different treatments (1 larva·L^{-1} of water and 2 larvae·L^{-1} of water) to monitor their growth and survival rates (Fig. 1 and 2). There was significant difference (P<0.05) between the two treatments in terms of growth parameters. The highest percent weight gain, specific growth rate, percent length gain were found to be 3,928.57±550.6, 13.17±0.51% and 636.67±7.64 respectively in the treatment stocked with 1 larva·L^{-1} (Tables 2 and 3). The survival was 68.33±7.64% and 43.33±5.77% where stocking density was 1 larva and 2 larvae·L^{-1} of water respectively (Table 3).

Table. 1. Rates of ovulation of females and fertilisation and hatching of eggs of riverine catfish *R. rita* when treated with different doses of carpPG extract.

Treatments	Weight of females (g)	Ovulation status of females		Latency period (h)	Average fertilization rate (%)	Average hatching rate (%)	Remarks
		Response	Average (%)				
90 mg carpPG·kg^{-1} body weight	900	+	100	21	22.00 ± 5.72^{a}	9.75 ± 2.5^{a}	Few larvae hatched
	500	+		19			
	800	+		20			
	700	+		20			
100 mg carpPG·kg^{-1} body weight	750	+++	100	20	82.50 ± 6.46^{c}	62.75 ± 7.18^{c}	Considerable no. of larvae hatched
	1000	+++		22			
	650	++		19			
	500	++		21			
110 mg carpPG·kg^{-1} body weight	800	++	100	22	64.25 ± 4.35^{b}	43.00 ± 4.76^{b}	Considerable no. of larvae hatched
	1000	+++		21			
	600	++		24			
	500	+		19			
Control (0.5ml physiological saline·kg^{-1} body wt. of fish)	900	-	-	-	-	-	-
	700						
	800						

+++ Profuse ovulation and yielded sufficient number of ripe eggs (80-90%) on stripping.
++ Considerable ovulation and yielded sufficient number of ripe eggs (80-90%) with considerable number of unripe eggs (10-15%).
+ Ovulation with preponderance of unripe eggs (60-70%)
- No response
Values in each column with different superscripts are significantly different at 5 % level of significance.

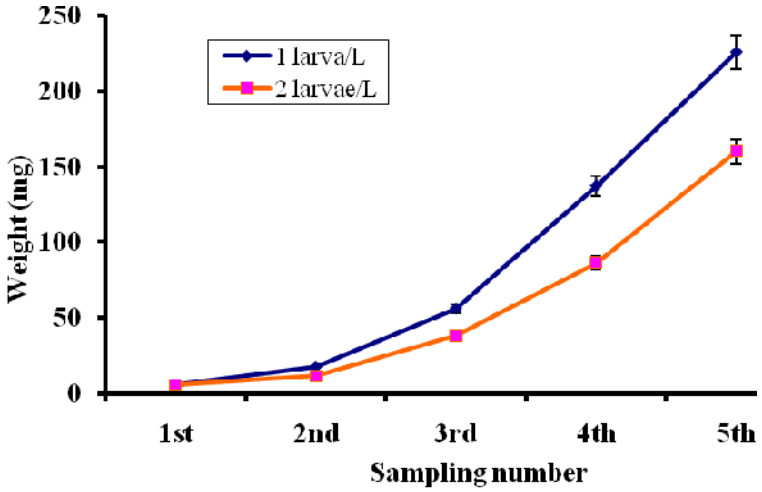

Fig. 1. Weight (mg) gain of larvae of *R. rita* during the 28 days experimental period (Mean ± SEM).

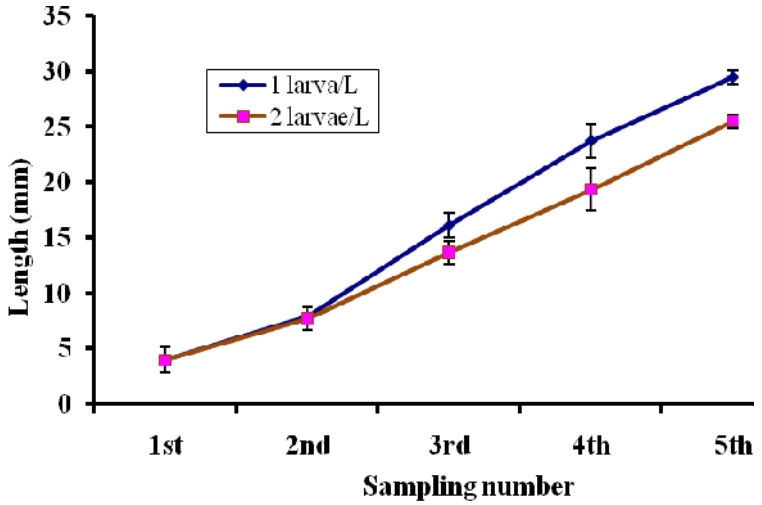

Fig. 2. Length (mm) gain of larvae of *R. rita* during the 28 days experimental period (Mean ± SEM).

Dose optimisation is an important factor for successful breeding programmes. Mollah et al. (2008) reported the preliminary success in inducing the breeding of *R. rita.* They used four doses (i.e. 80, 100, 120 and 140 mg PG·kg^{-1} body weight) of carpPG extracts with three females and two males to optimise the dose of pituitary gland extract in respect of ovulation of females and subsequently fertilisation and hatching of eggs. Maximum ovulation, fertilisation (71.66±7.64%) and hatching (48.33±7.64%) rates were shown by the females treated with 100 mg carpPG·kg^{-1} body weight. To bring further refinement in the choice of PG dose, the present experiment was conducted with three different doses of carpPG extract viz. 90, 100 and 110 mg·kg^{-1} body weight of female using four females and three males in each treatment. Females of all the three treatments showed 100% ovulation. The achieved fertilisation (82.50±6.46%) and hatching rates (62.75±7.18%) of eggs were higher than those reported by Mollah et al. (2008). Since, all the fish used were approximately of similar size and maturity and the experiment was conducted under the same environmental and management conditions, the difference in the result obtained was only due to the variation in PG doses. It is therefore confirmed that the dose of 100 mg carpPG·kg^{-1} body weight can be used as the most suitable dose for inducing ovulation in *R. rita.*

The ambient water temperature during incubation period was 28±1 $^{\circ}$C. Hatching occurred within 24 hr of post fertilisation. Higher temperature always shortens the incubation periods but affects the survival and hatching rates of the eggs of *Clarias macrocephalus* Guenther, 1864 (Mollah and Tan, 1982). They found that the eggs incubated at 20 $^{\circ}$C did not hatch but at 25 $^{\circ}$C hatching started after 34 hr of incubation and was completed by 58 hr. On the other hand, eggs incubated at 35 $^{\circ}$C started hatching after 22 hr of incubation and completed by 30 hr. The above discussion suggests that the incubation time is more species specific and inversely related to temperature.

Table. 2. Weight gain, percent weight gain and specific growth rate (% SGR) of larvae of *R. rita* during 28 days experiment under two stocking densities.

Treatment	Replication	Initial weight (mg)	Final weight (mg)	Weight gain (mg)	Weight gain (%)	SGR (%)
1 larva·L^{-1}	R_1	5.60±0.51	192.00±2.54	186.40	3328.57	12.60
	R_2	5.60±0.51	232.20±9.85	226.60	4046.43	13.30
	R_3	5.60±0.51	252.60±6.96	247.00	4410.71	13.60
Mean ± SEM		**5.60±0.28**	**225.60±7.74**	**220.00±7.96^a**	**3928.57±142.28^a**	**13.17±0.002^a**
2 larvae.L^{-1}	R_1	5.60±0.51	160.80±1.24	155.20	2771.43	12.00
	R_2	5.60±0.51	157.40±1.50	151.80	2710.71	11.91
	R_3	5.60±0.51	162.20±1.42	156.60	2796.43	12.02
Mean ± SEM		**5.60±0.28**	**160.13±0.92**	**154.53±0.64^b**	**2759.52±11.39^b**	**11.98±0.02^b**

Mean values in the column with different superscripts are significantly different

Table. 3. Length gain, percent length gain, health condition and survival rate (%) of larvae of *R. rita* during 28 days experiment under two stocking densities.

Treatment	Replication	Initial length (mm)	Final length (mm)	Length gain (mm)	Length gain (%)	Health condition (mg/mm)	Survival (%)
1 larva·L^{-1}	R_1	4.00±0.54	29.40±0.25	25.40	635	6.53	75
	R_2	4.00±0.54	29.80±0.39	25.80	645	7.79	70
	R_3	4.00±0.54	29.20±0.20	25.20	630	8.65	60
Mean ± SEM		**4.00±0.29**	**29.47±0.16**	**25.47±0.08^a**	**636.67±1.97^a**	**7.66±0.28**	**68.33±1.97^a**
2 larvae·L^{-1}	R_1	4.00±0.54	25.80±0.38	21.80	545	6.23	50
	R_2	4.00±0.54	25.40±0.25	21.40	535	6.20	40
	R_3	4.00±0.54	25.20±0.20	21.20	530	6.44	40
Mean ± SEM		**4.00±0.29**	**25.47±0.17**	**21.47±0.08^b**	**536.67±1.97^b**	**6.29±0.03**	**43.33±1.49^b**

Mean values in the column with different superscripts are significantly different

The females treated with ovaprim at the doses of 0.5 and 1.00 $mL \cdot kg^{-1}$ body weight showed no ovulation. This indicated that the doses of ovaprim were insufficient for inducing ovulation. Recent study reported that in *Clarias batrachus* (Linnaeus, 1758) 1.00 mL of ovaprim$\cdot kg^{-1}$ body weight of female is the optimum for breeding performance resulting in fertilisation rate of 82.3 ±0.80% and the hatching rate of 55.3±1.54% (Sharma et al. 2010). Induced spawning of *C. punctatus* and *Heteropneustes fossilis* (Bloch, 1794) was carried out successfully by using ovaprim at the dose ranging from 0.3-0.7 $mL \cdot kg^{-1}$ body weight of fish (Haniffa and Sridhar, 2002). Zohar (1989) stated that the efficacy of ovaprim doses vary between species. Due to unavoidable reasons only two doses (viz. 0.5 and 1.0mL ovaprim$\cdot kg^{-1}$ fish) were used to carry out the present study. So there is scope for further experimentation with more doses of ovaprim to establish the appropriate ovaprim dose to induce the Rita fish to breed.

During the present study two stocking densities viz. 1 larva and 2 larvae$\cdot L^{-1}$ of water were used. The result suggested that there was significant level of variation between the two densities in terms of growth parameters and survival rate and the stocking density of 1 larva$\cdot L^{-1}$ of water showed better result. This result agrees with the other findings (Rowland et al. 2004 and Schram et al. 2006) where increased stocking density has a negative impact on growth and survival rate of fishes. Higher stocking density is considered to be chronically stressful to the animals (Sugunan and Katiha, 2004). Lower stocking density showed higher survival rate of Rita larvae which is similar to the findings in *H. fossilis* and *Clarias anguillaris* (Linnaeus, 1758) (Ita et al, 1989 and Narejo et al. 2005).

Tubificid worms are very popular live food used for feeding larvae of carnivorous and omnivorous fish species (Mollah and Nurullah, 1988). Tubificid worms were used as food for the larvae to keep the biasness as minimum as possible and to make sure that the difference in results obtained was due to the difference in stocking density. The water quality parameters were also recorded throughout the study period and found to remain within the suitable ranges that agree with other findings (Barua, 1990; Narejo et al. 2005).

Conclusion

It can now be clearly suggested that the dose of 100 mg carpPG$\cdot kg^{-1}$ body weight is a more precise and effective dose for inducing ovulation in female. Fertilisation and hatching rates of eggs of Rita were also higher at this dose. Stocking density of 1 larva$\cdot L^{-1}$ showed a better growth and survival rates of larvae. However further experimentation is necessary with wider dose range of ovaprim and higher stocking densities of larvae$\cdot L^{-1}$ of water to standardise the ovaprim dose for inducing ovulation in female and stocking density for larvae rearing respectively.

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