Original Research

CONSERVATION OF AN ENDANGERED CARNIVOROUS FISH *RITA RITA* THROUGH INDUCED BREEDING

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Abstract: *In present study the Effects of different doses of ovaprim on induced spawning activities of Rita rita were observed with an aim to standardize the dose of ovaprim for successful breeding. In the trial experiment, the females of Rita rita were treated with ovaprim at the rate of 0.5, 0.8 and 1.0ml/kg body weight and males of Rita rita in all the cases were treated at the rate of 0.4ml/kg. The doses of ovaprim at the rates of 0.5, 0.8, 1.0 ml/kg body weight resulted in 0%, 100% and 100% ovulation respectively. In case of injection of ovaprim at the rate of 0.8ml/kg, the fertilization rate was observed to be about 70% and hatching rate was 58%, which occurred within 20-25 hours after fertilization at water temperature of 27^{0}C-29^{0}C. In case of injection of ovaprim at the rate of 1.0ml/kg, 18% fertilization rate and 5% hatching rate was observed. The doses of ovaprim at the rates of 0.8ml/kg and 0.4ml/kg body weight for female and male Rita rita respectively was found to be satisfactory for overall breeding performance and the commercially successful production of this fish. Hatchlings from over all experiment were reared up to fingerlings size to about 2 month and were finally stocked into the Chashma lake.*

Keywords: *Rita rita*, Induced breeding, Chashma Barrage, Conservation

Introduction

Sustainable utilization of fish play very important role in improving the living standard of human population of the Pakistan. It has been observed that 184 native freshwater fish species, including 37 catfish species from order siluriformes are present in Pakistan. *Rita rita* is a food fish and distributed in Afghanistan, Pakistan, India, Nepal, Bangladesh and Myanmar (Mirza, 2003). Fry of *Rita rita* are both surface and column feeder, fingerlings are marginal and bottom feeder while it's adult are bottom-dwelling carnivore and feed on mollusca, small fishes, crustaceans, and insects as well as on decomposing organic matter. *Rita rita* also feeds on aquatic algae, insects, crustaceans and rotifers (Gupta, 2015; Shrestha, 1990). Among the catfishes, *Rita rita* is commercially important and a well esteem food fish due to having good taste and affluent protein meat. Recently *Rita rita* has been regarded as ornamental fish. Many authors from different countries have documented it as indigenous ornamental fish (Jalbani et al., 2016; Rafique and Khan, 2012). It is locally called as "Khagga" in Pakistan. This fresh water specie found in streams, rivers, canals, ponds and occurs mainly in shallow waters. Young fishes are greenish brown above and on flank and brownish white on abdomen. It is extremely slimy when captured (Rahman, 1989). Pakistan is a land of high potential as far as its water resources are concerned. 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 should explored and domesticated for large-scale seed production, which will eventually cover the way for aqua culturists to grow more fish. It is important to mention that culture fishery is the single best contributor to the total production of fish in the country. The main barrier toward this venture is the non-availability of required number of stock able 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.

In spite of good taste and popularity, the *R. rita* fetching high price in the market, is now in endangered condition. Up to now the induced breeding techniques for only few commercially important fish species of Pakistan have been established. Until now the fry of *R. rita* can only be collected from the river systems where they breed naturally. The population of carps is gradually decreasing due to destruction of nesting and hatching sites and excessive hunting. Increasing toxicity due to fertilizers, pesticides in agro-forest land, gradual decrease in water level due to drought also led to reduction in its population size. Due to the change in ecology of breeding grounds, obliteration of nesting and

destruction of sites for hatching imposed negative impact on the fish survival. Some of the basic biology of *R. rita* has been studied (Amin et al., 2008; Molla et al., 2008). One of the possible explanatory measures that can be taken against the extinction of this fish is to initiate a domestication and breeding programme where by mass production of quality seeds can be ensured. In view of the above, the present experiment was undertaken to develop induced breeding technique of the cat fish *R. rita.*

Materials and Methods

An experiment was conducted on the induced breeding of *Rita rita*, during end of July 2015 in circular breeding tanks at Fish Biodiversity Hatchery Chashma Barrage, Mianwali, Pakistan. Brood stock was collected from Chashma reservoir, District Mianwali about one year prior to this experiment in the month of April 2014 and this brood stock was reared in earthen pond. The stock was reared on supplementary feeding having about 45% animal protein level in addition to the natural food in the pond. All the collected stock was checked for maturity at the time of collection. Most of the male stock was matured but no female was found to be ready for spawning. Periodical examination of the stock was carried out to check the spawning possibility. To determine the suitability of female brood for spawning, firstly few eggs were drawn from the posterior region of the ovary using a catheter. Then eggs were immersed in a solution containing 70 % acetic acid and 30% alcohol. After about five minutes nuclei position was examined by using microscope. The acentric or peripheral location of nuclei indicates readiness of fish for spawning, while the central location of nuclei indicates unsuitability of fish for breeding. In last week of July 2015, brood stock was checked and was found to be ready for spawning.

The brood fish were collected by reducing the water of the pond 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. Sexually mature males and females weighing approximately were selected for induced breeding. Selected broods were kept in circular breeding tanks with continuous water flow for about 6 hours for conditioning prior to injection of ovaprim. Male and female fish were kept in separate circular breeding tanks and constant water flow was maintained to ensure proper aeration. Twelve females were divided into three treatments and marked as T_1, T_2 and T_3 (Table. 1) having three females in each treatment. The females under each treatment were kept separately in different tank. The weight of selected female broods ranged from 1130g to 1300g whereas the weight of the males varied from 800g to 1100g. The females under treatment T_1, T_2 and T_3 were treated with ovaprim at the doses of 0.5, 0.8, and 1.0 ml/kg body weight respectively. While all the male under the treatment T_1, T_2 and T_3 were treated with ovaprim at the doses of 0.4ml/kg body weight respectively. Throughout the experiment the water temperature remained in range of 27^0C-29^0C.

On the basis of the body of brood, the required amount of ovaprim was injected intramuscularly to the fish on the dorsal side above the lateral line. After injection of ovaprim the females were periodically checked every hour after 12 hours post-treatment with inducing agent by gently pressing their abdomen. The females upon ovulation were immediately stripped and eggs from each fish were collected in separate fertilization trays. A male fish was stripped to obtain milt by using external pressure to the abdomen immediately. 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 fresh water to remove the excess milt, blood etc. The fertilized eggs were incubated in circular breeding tanks where they were in constant motion.

Percent ovulation, fertilization and hatching rates were recorded to observe the effectiveness of different ovaprim doses using following formulas.

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

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

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

For calculating percent fertilization 100 eggs were taken from each group and number of fertilized and unfertilized eggs was counted by observing 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.

Results and Discussion

In order to standardize the ovaprim dose for induced ovulation in female *R. rita*, three

different doses of ovaprim were used. Data representing the effects of ovaprim doses on ovulation of female fish, the rates of fertilization and hatching of eggs are presented in Table 2. The result shows marked difference in effectiveness among 3 doses in inducing ovulation, fertilization and finally hatching. The doses of ovaprim at the rates of 0.5, 0.8 and 1.0ml/kg body weight resulted in 0%, 100%, and 100% ovulation respectively. The time interval between the injection of ovaprim and ovulation varied between 18h 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 48 hours of ovaprim injection. Females treated with the ovaprim dose of 0.5ml/kg body weight showed no ovulation at all. The ovaprim dose of 0.5ml/kg weight was found to be very low to provoke ovulation (Amin et al., 2008; Rahman, 1989).

In case of ovaprim treatment at the rate of 0.8ml/kg, the average total number of eggs ovulated by individual female was estimated to be 14000/female. The fertilization rate was observed to be about 70%. Hatching rate was approximately 58%, which occurred within 20-25 hours after fertilization. In term of ovulation, fertilization and hatching rates, the dose of ovaprim at rate of 0.8ml/kg body weight showed the best performance. Thus fertilization and hatching rates of the eggs obtained from females treated with 0.8ml /kg were highest by body weight. In case of ovaprim treatment at the rate of 1.0ml/kg, 18% fertilization and 5% hatching rate was observed (Haylor and Mollah, 1995). The fish treated at the rate of 1.0ml/kg body weight, even if induced ovulation in 100% fish, but the fertilization rate and hatching rate of eggs was unsatisfactory. This lead to the conclusion that very large numbers of eggs obtained from the fish treated with relatively higher dose (1.0ml/kg body weight) were immature and highly saturated with blood. It may be regarded as the case abortion rather than normal ovulation. It resulted in very low rate of both the fertilization and the hatching (Molla et al., 2008), when the *Rita rita* was treated with PG. Low PG dose failed to induce spawning and thus resulted in 0% ovulation, while higher PG doses induced 100% ovulation in females but unexpected insufficient fertilization and hatching rates (Jalbani et al., 2016; Rafique and Khan, 2012).

Table 1. Different dosage of ovaprim practiced during experiment

Treatments	Sex	Ovaprim Dose(ml/kg)	Sex	Ovaprim Dose(ml/kg)
1	Male	0.4	Female	0.5
2	Male	0.4	Female	0.8
3	Male	0.4	Female	1.0

Table 2. Rates of ovulation, rate of fertilization and rate of hatching of eggs of endanger catfish *R. rita* belonging to three different Treatment doses of ovaprim

Treatment	Dose of ovaprim (mg/kg of body weight)	Weight of females (g)		% females ovulated	Average fertilization rate (%)	Average hatching rate (%)
		Individual	Average			
T_1	0.5	1240	1226	0	-	-
		1203				
		1236				
T_2	0.8	1130	1228	100	74	59
		1256				
		1298				
T_3	1.0	1170	1216	100	18	5
		1209				
		1270				

Conflict of interest

The authors declared the absence of any conflict of interest.

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