J. Agrofor. Environ. 3 (1): 195-197, 2009 ISSN 1995-6983

Development of artificial breeding technique of gutum, *Lepidocephalichthys guntea* (Hamilton, 1822) using carp pituitary gland

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Abstract: A study was conducted on artificial breeding of gutum, *Lepidocephalicthys guntea* (Hamilton, 1822) using carp pituitary gland. After rearing, the ready matured female fishes were injected at a rate of 20, 15 10 and 5 mg PG/kg body weight with two injections at 4 and 8, 6 and 10 and 8 and 12 PM. The female fishes were applied at first one-third and then two-third of the dose. The males were treated only with the second dose of injection. In the highest dose, all the fishes spawned naturally without stripping, but in other doses, partial natural spawning occurred that completed with hand stripping. Average fertilization rates (%) were 60.12±5.53, 62.18±2.22, 75.87± 5.66 and 78.60±3.21 and hatching rates (%) were 49.43±2.14, 50.5±4.21%, 60.32±3.43 and 65.49±5.23, respectively. Fertilization and hatching rates were significantly ($P<0.05$) higher in lower two doses. The incubation period of eggs observed 22-24 hrs at water temperature 28^{o}C, and the larvae started to feed within 72 hours after hatching. The survival rate of larvae found up to 50% and their average weight was 65 mg within two weeks of feeding with finely ground boiled chicken eggs. The successful dose for induced breeding of gutum was 5-10 mg PG/kg body weight and suitable time set of injection was 8 and 12 PM.
Key words: Gutum, induced breeding, weaning, fertilization, survival

Introduction

Aquatic resource is a vital tool for transforming Bangladesh into a nutritionally secured nation. Development of indigenous ornamental fish culture technique can bring both improvements of the socioeconomic status as well as financial security for poor livelihood with limited resources. Developing low cost technology in this sector and its proper dissemination can help Bangladesh to march towards prosperity as well as self sufficiency.

Ornamental fish keeping and its propagation has become an interesting activity for many, providing not only aesthetic pleasure but also financial openings. It has reached new heights in the recent past and has gained accelerated momentum, thus providing employment opportunities to many including unemployed youths, women and other unskilled masses. Bangladesh waterbodis possess a rich diversity of ornamental fishes with over 50 indigenous ornamental species. The Bangladesh ornamental fish market, however, is mainly dominated by imported fishes from Singapore, Thailand, Sri Lanka and many other European countries in the cost of millions of precious foreign currencies. On the other hand its expansion has been impeded seriously. It is necessary to adopt strategies for boosting commercial production of indigenous ornamental fishes includes innovation of low-cost breeding and rearing techniques, formulation and preparation of nutritious feed; health management and water quality management.

Gutum, *Lepidocephalicthys guntea* (Hamilton, 1822) is a very attractive fish and the hobbyists like this fish for their excellent color and behavior. Now it is listed as an endangered species (IUCN-Bangladesh, 2000). It is also very important from the nutritional point of view especially for the rural poor of Bangladesh. Thus, successful artificial breeding in the laboratory will help to take steps on the way to restore the previous status of the biodiversity of the fish in the natural habitat through the process of open water stocking or to develop a culture technique of the fish in shallow water bodies likes ditches, rice fields and also in aquarium. These efforts will prevent the fish from being extinct; at the same time rural people will get chance to take the taste of the fish and can have protein supply for their nutrition. The present study was aimed to develop techniques of induced breeding and larval rearing of *L. guntea*.

Materials and Methods

Study site: The experiment was carried out at the hatchery of the Department of Fisheries Biology and Genetics under the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh during April to September 2006.

Experimental Design: Thirty-seven pairs of gutum (*L. guntea*) used for this experiment were collected from the upazilla Modan under Netrokona district. The average weight of male and female fish was 8.3±1.8g and 9.7±2.2g, respectively. They were reared in two tanks of the size 35×30×30 cm^3 having twenty pairs and 35×25×20 cm^3 having seventeen pairs. During breeding time i.e., late June, 2006 one tank was used for rearing the brood fish and another tank was used for spawning and subsequent eggs incubation purpose. Both rearing and spawning tanks were provided with aerator (DAIVO PUMP NS 8200) for the aeration of water.

Brood fish rearing and maturity study: The brood fish were reared up to maturation for spawning in the hatchery by providing locally available pelleted feed to the rearing tanks. During breeding season (June-July), the body colors of the male fish were prominent with brightly reddish with yellow and greenish strip while the female fish were with comparatively less color. The abdomen of the female fish was swollen and soft. Brownish eggs would come out during gentle pressure on the abdominal region from anterior to posterior. From the above criteria the brood fish were selected for induced breeding.

Application of PG to the brood fish: In this experiment 24 pairs of the brood *L. guntea* were injected with PG at the doses of 20, 15, 10 and 5 mg PG/kg body weight for spawning. The female fish were administered two injections whereas the male fish were injected with two-third of the dose in a single injection at the time of second injection of female fish. Reproductive activities including nudging, dozing, circling, ovulation and finally, spawning

was observed in the rearing tanks and then transferred to the bowls.

Time interval between two injections: Three sets of time were used viz., 4 and 8 PM; 6 and 10 PM and 8 and 12 PM (designated as T_1, T_2 and T_3) at an interval of 4 hrs to identify the best time interval of injections. In all these treatments, the brood fish started to spawn after 8-10 hrs of second injection. After nudging and circling, the male bends its body around the female. The genital pore of male was brought nearer to the genital pore of female. Eggs were ejected, and at the same time male released the milt.

Statistical analysis: Comparison of treatment means were carried out using one-way analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was performed to compare the different treatment means at 5% level of significance. Statistical analysis was performed using SPSS software package.

Results and Discussion

Natural spawning was found successful at 20 mg PG/Kg body weight in a single injection and completed without hand stripping. It was observed that both the female and male fish ejected eggs and milt at the contemporary times when the male fish were treated with single injection. At doses 15, 10 and 5 mg PG/Kg body weight partial spawning was observed that completed by hand stripping There was significant (P<0.05) difference among four doses in terms of fertilization and hatchling rates. Fertilization and hatching rates were significantly (P<0.05) higher in lower two doses. Spawning largely depends on the synchronization of ova and sperm release (Hoar and Randall, 1969).

In this trial, the successful doses were 5 and 10 mg PG/kg body weight. The female fish were treated with two injections, firstly 3.3 and 1.65 mg PG/kg body weight and secondly 3.35 and 6.7 mg PG/kg body weight. The male fish were treated with a single injection (3.35 and 6.7 mg PG/kg body weight) at the time of second injection of the female fish. Kohinoor *et al.* (1991) applied single injection at a rate of 8-12 mg PG/kg body weight to the female *A. testudineus* and 4 mg PG/kg body weight to the male. Ovulation occurred in all the injected females. The dose applied was more or less similar except slightly higher in male which is reported by Kohinoor *et al.* (1991). This minor variation in doses might be due to the species variation. Thus, it may be said that every species is unique in their biological requirements.

No significant (P>0.05) difference was observed among T_1, T_2 and T_3 in the rate of fertilization and hatching (Fig.1). Therefore, the first PG dose for female fish should be at 8 pm and second dose should be at 12 pm, at an interval of 4 hrs. The male fish should be administered with single injection at the time of second injection to female fish. Because when the brood fish were treated with PG at the first two sets of time *i.e.* 4 and 8 PM; 6 and 10 PM, they ejected eggs and milt at late night which was not convenient for a person to observe and manage the breeding activities. However, when the brood fish were treated with PG at 8 and 12 PM, ovulation occurred at about 8 to 9 AM which was a very convenient time for a worker to observe and manage the breeding activities with charming mind and affinity to do work pleasantly

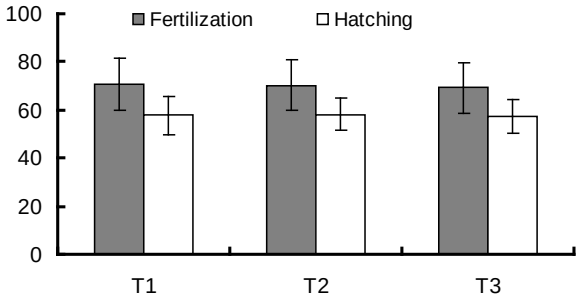

Fig. 1. Fertilization and hatching (%) at different time sets during study period

In all the treatments, the brood fish started to spawn after 7-8 hrs. of second injection. The male fish started moving around the female fish after 4 hrs. of second injection. After nudging and circling, the male bends its body around the female. The genital pore of male was brought nearer to the genital pore of female. Eggs were ejected, and at the same time male released the milt. Pal (2000) stated that the courtship behavior of *N. nandus* started after 4 to 6 hrs. of injection and spawned after 10 to 14 hrs. of injection. But it was found that the courtship behaviour of *C. fasciata* started after 5-6 hrs of injection and spawned after 7-8 hrs of injection. Ovulation was achieved when female fishes were treated with PG at the ambient temperature of 27-28°C. Pal (2000) performed breeding of *N. nandus* at ambient temperature of 28-29°C. Kohinoor *et al.* (1991) accomplished induced breeding of *A. testudineus* at the ambient temperature of 27-30°C. In Bangladesh, the normal water temperature retains 27-30°C during the month of June to September. In this temperature the most indigenous fish species of Bangladesh including *C. fasciata* performed their breeding activities.

Table 1. Comparative data of fertilization rate and hatching rate at different doses of carp PG

Dose of PG (mg/Kg)	Average weight of fish (g)		Fertilization rate (%)	Hatching rate (%)
	Female	Male		
20	9.23	8.36	60.12±5.53b	49.43±2.14b
15	8.93	7.97	62.18±2.22b	50.50±4.21b
10	9.11	8.83	75.87±5.66a	60.32±3.43a
5	8.91	8.27	78.60±3.21a	65.49±5.23a

Values are mean ± standard deviation; values of same column with same superscripts do not differ significantly at 5% level

Average fertilization rates (%) were 60.12±5.53, 62.18±2.22, 75.87± 5.66 and 78.60±3.21 and hatching rates (%) were 49.43±2.14, 50.5±4.21%, 60.32±3.43 and 65.49±5.23, respectively (Table 1). Pal (2000) found that fertilization and hatching rate of (*N. nandus*) were above 90% and above 80% respectively. Kohinoor *et al.* (1991)

found that fertilization and hatching rate of *A. testudineus* was 82±2% and 73±3%. Temperature is very important factor for the incubation of eggs. The development of embryo and the variability of hatching time in fertilized eggs and their viability of most of the fish generally are influenced by the temperature of water (Hoar and Randall, 1969; Jhingran, 1983 and Rahman, 1975). Temperature is inversely proportional to the time of hatching (Alikunhi *et al.* 1962; Mollah, 1983; Rana, 1990) and hatching success (Hoar and Randall, 1969). In the present study the incubation period of eggs was 20-24 hrs. after fertilization at 28°C water temperature. Although no comparable data is available from elsewhere several authors (Chakrabarty and Murty, 1972; Thakur, 1980; Haque, 2007) observed incubation period of fertilized eggs of some fishes lies between 18-32 hrs. Pal (2000) and Tarafder (2000) found that the incubation period of *N. nandus* was 18 to 20 hrs. after fertilization at 29±1°C water temperature and Haque (2007) also found the incubation period of Colisa fasciata with in 18-22 hrs. at water temperature 27-28°C in the same laboratory.

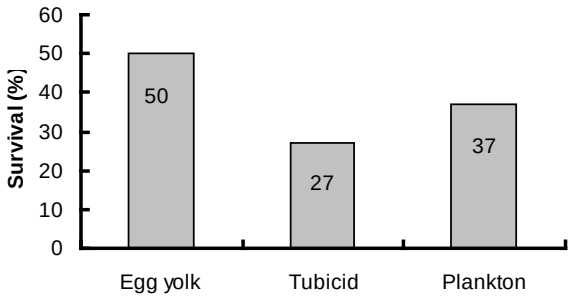

Fig. 2. Larval survival (%) with different feed types during study period

Weaning is of great importance for any larvae of fish. Because, the survival rate of any larvae completely depends on the starter food. Therefore, the appropriate food particle size and food density are very important for the survival of larvae. The larvae of gutum started to feed after 70-72 hrs. of hatching. The larvae were fed with three types of feed viz, chicken egg yolk, tubificid worm and zooplankton. The height survival rate of larvae (Fig.2) was up to 50% when their average weight was 65 mg by providing mixed zooplankton as starter food for 21 days, the larvae of this fish species can easily be reared in a well prepared nursery pond where zooplanktons are available. The availability of zooplanktons in a pond can be increased through proper fertilization.

The lowest successful dose for the induced breeding of female gutum was 5-10 mg PG/kg body weight applying at time 8 and 12 PM at an interval of 4 hrs. between two injections. The fertilization and hatching rates of the fish were good and specific zooplankton for larval rearing should be applied for higher survival and growth of larvae by regular fertilization and manuring. Further experiments to identify specific zooplankton for larval rearing should be addressed for higher survival and growth of larvae and the successive culture of food plankton to make the culture of *L. guntea* as a sustainable technique is essential.

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