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## First Record of Artificial Spawning of *Nandus nandus* (Hamilton) in Bangladesh Using Carp Pituitary Gland: An Endangered Species Bred in Captivity

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**Abstract:** In view of domestication of the endangered species *Nandus nandus*, the artificial breeding of the fish with carp pituitary gland (PG) was trialed. The optimal dose of PG was found to be 150 mg kg<sup>-1</sup> of fish body weight. Best fertilization rate (92±5%) was obtained at 175 and 200 mg kg<sup>-1</sup> body weight PG application and best hatching rate (90±2, 90±5, 90±5%) was revealed at 150, 175 and 200 mg kg<sup>-1</sup> body weight PG application. Fertilization and hatching rate were 92 and 81% respectively in case of the experiment where only females were injected. Ovulation occurred after 10 to 14 h of injection and the eggs hatched out after 18 to 20 h of fertilization. The larvae started feeding after 56 h of hatching and best survival rate (16%) was achieved by supplying zooplankton as food. It was also found that larvae denied egg yolk and *Tubifex* worm as their first food.

**Key words:** *Nandus nandus*, artificial spawning, PG, ovulation, incubation, capacity

### INTRODUCTION

In Bangladesh seed production is limited to carps. But a large number of wilds endangered fish species, especially small indigenous species (SIS), are available in the natural water bodies of Bangladesh. The taste of those fishes are better than the exotic fishes to the people of this country. One such fish, *Nandus nandus*, is very common in the freshwater of Bangladesh. This fish can be cultured in shallow water body, such as rice field, in low oxygen level (Mustafa *et al.*, 1980). It is a carnivorous fish and feeds on small shrimp, small fish larvae and insect which are abundantly available in rice field as well as shallow water bodies. The fish is locally known as 'Nondoi', 'Meni' or 'Veda'. Successful technology of breeding and rearing will help to culture this fish in shallow waters like rice fields, that would play a substantial role in the overall nutrition of the people of Bangladesh especially the poor and lower middle class people of rural Bangladesh (Das and Zamal, 2000).

To evaluate the culture and reproductive potentials of *N. nandus*, information on the fecundity, reproductive biology, behavior and breeding season are considered as essential (Narejo *et al.*, 2002). The effectiveness of carp pituitary gland in induced breeding of different fish has been supported by various authors (Barua, 1990; Das *et al.*, 1992 and Rahmatullah *et al.*, 1993). The species under current study has not yet been tried to breed in the laboratory or in the hatchery and therefore, no information is available on its breeding technique. Only some work has been done on its rearing technique and predatory behavior in laboratory conditions by Akther (1999) and

Das and Zamal (2000). Successful breeding of 'Meni' in farm conditions would be tremendously helpful to develop its culture in the country. These efforts will prevent the fish from being extinct.

Therefore, artificial breeding of laboratory reared *N. nandus* has been tried to study the reproductive behavior, to detect the first feeding time of the larvae, to determine the dose of the carp pituitary for its induced breeding, to estimate the rate of fertilization and rate of hatching of its eggs and finally to develop a technique of artificial propagation of this species using carp pituitary gland.

### MATERIALS AND METHODS

In the present study eight glass aquaria having size of 60 × 35 × 30 cm, were used for brood rearing (Series A) and six glass aquaria having size of 35 × 25 × 20 cm were used as spawning tank (Series B) and subsequently as egg incubation chamber.

**Collection and selection of brood fish:** As a follow up experiment of Das and Zamal (2000) and Tarafder (2000), the present experiment was carried out with the fishes which were collected and reared in the laboratory and in the earthen pond. Forty eight brood fish were used in this experiment of which 22 were female and rests were male. Thirty two of those were reared as a group of four fish (unisex) in each aquarium in the laboratory and remaining brood fishes (16) were collected from the brood stock reared in an earthen shallow pond in the Fisheries Faculty compound of Bangladesh Agricultural University, Mymensingh.

Table 1: Characteristics of mature male and female brood fish of *Nandus nandus*

Male	Female
i. Smaller and slender in appearance	i. Relatively larger and robust in appearance
ii. Colour prominent, brightly reddish in colour with black strip.	ii. Female comparatively dull in colour
iii. Rough pelvic fin	iii. Smooth pelvic fin
iv. Eject white milt when light pressure was applied on the abdomen.	iv. Eject brown eggs following gentle pressure on the abdominal region.

During selection of *N. nandus* brood for induced breeding only healthy uninjured and well matured fishes were taken. The brood fish were selected based on the criteria presented in Table 1.

**Collection of carp pituitary gland (PG) and preparation of PG solution:** A stock solution of 0.5 mg PG per ml of distilled water was prepared. To prepare this stock solution several pituitary glands were weighed on an electronic balance (Mettler Toledo, AB-204) with 0.1mg accuracy. Homogenized glands were then poured in a centrifuge tube and diluted with distilled water to obtain the desired concentration of the stock solution. The solution was centrifuged and the supernatant was stored in refrigerator.

**Period of induced breeding:** Induced breeding experiments were conducted during last week of June to middle of August, i.e. during the peak breeding season of *N. nandus* as reported by Mustafa *et al.* (1980) and Akther (1999).

**Range finding test for successful ovulation:** The first step to find out the response of any stimulus is the range finding test (Rand and Petrocelli, 1985; Das, 1995). For range finding test the brood fish were injected with a geometric series of doses viz. 0.2, 2, 20 and 200 mg PG extract per kg body weight of fish in duplicate. The amount of solution injected never exceeds 10 ml kg<sup>-1</sup>. If the fish did not respond properly, eggs and milt was obtained for fertilization by stripping.

**Definitive test with equally spaced lower doses of the lowest responded dose of the range finding test:** In case of range finding test fish responded at the dose of 200 mg kg<sup>-1</sup>. So, to identify the lowest possible dose, the brood was injected with five doses, viz. 100, 125, 150, 175 and 200 mg PG extract per kg body weight in duplicate.

**Induced breeding of *N. nandus* by carp PG injection into female only:** Based on the results of the previous experiment the best performed dose was used in this experiment. Two female fish were injected with the best dose of PG extract and kept in separate aquaria. Two male (without giving any injection) were released in each of the aquaria with female. Then the fishes were observed

thoroughly for any behavioral changes and also for spawning activity upto the following morning. If the fish did not respond to spawn, eggs and milt were collected for fertilization by stripping.

**Group breeding:** A group of four males and two females were kept in an aquarium without giving any injection, to see whether the fish respond to spawn without inducing agent in the laboratory conditions.

**Reproductive behavior:** To study reproductive behavior of *N. nandus*, each and every movements and activities were observed from the time of injection until next morning to the completion of spawning.

**Spawning and fertilization:** Both natural spawning and stripping methods were applied in the present experiment. After injection both male and female fish were kept in the same spawning tank and the eggs released by the female were allowed to fertilize automatically by the milt of the male in the spawning tank. Female fishes were stripped first and eggs were placed on an enamel tray. Then male was stripped and milts were mixed with the eggs. Fertilized eggs were washed by physiological saline solution (0.8% NaCl) and subsequently by tap water several times for cleaning. The fertilized eggs were then spread homogeneously on the spawning tank for incubation. Finally, fertilization rate was calculated from the number of transparent eggs to the total number of eggs, multiplied by 100.

**Incubation and hatching:** Eggs were incubated into incubation tank at room temperature with continuous supply of oxygenated water. Demersal eggs were spread in the aquarium to produce a uniform layer of eggs. Unfertilized eggs and egg shells were cleaned out of the tank within an hour of hatching to protect larvae from fungal infection. The rate of hatching was estimated from the number of hatchling to the number of fertilized eggs, multiplied by 100.

**Larval rearing:** Larvae from three different pair of parents were mixed in a bucket and a number of 50 larvae were placed in each of the tank for the rearing experiment for 3 weeks and rest of the larvae were released in the previously prepared nursery pond. Three types of food

such as hardly boiled chicken egg (the egg yolk mixed with water and screening through a cloth), *Tubifex* worm (as paste form by screening) and small sized zooplankton (caught from pond by plankton net having mesh size of 200 µm) were supplied for the larvae as starter in duplicate treatments. The larvae were found to search for food after 56 h of hatching. Survival and growth of the larvae were calculated at the end of the experiment.

**RESULTS AND DISCUSSION**

**Induced breeding**

**Range finding test for successful ovulation:** The doses of pituitary extract for range finding test were 0.2, 20 and 200 mg kg<sup>-1</sup> body weight both for male and female. Table 2 shows the results indicating the time of injection and ovulation, fertilization rate, time of hatching, hatching rate and incubation temperature. Spawning occurred only in case of the dose 200 mg kg<sup>-1</sup> body weight. Release of eggs occurred after 10-14 h of injection and the hatchlings

came out after 19-20 h of fertilization. Rate of fertilization and hatching were about 90±2% and 80±2% respectively. Thus it was suggested that dose of carp PG for induced breeding lies somewhere in between 20 and 200 mg kg<sup>-1</sup> body weight. Pathak (1982) reported that the lowest dose of PG was 40 mg kg<sup>-1</sup> body weight for female and 30 mg kg<sup>-1</sup> body weight for male in *Clarias batrachus*. Kohinoor *et al.* (1991) used 8-12 mg PG/kg body weight for successful induced spawning of *Anabus testudineus* which is similar to the present finding. In this experiment large number of hatchlings were obtained but no larvae survived for more than three days perhaps for the want of appropriate first food.

**Definitive test with equally spaced lower doses of the lowest responded dose of the range finding test:** To identify the lowest responded dose for induced breeding of *N. nandus*, another five doses of pg *viz.* 100, 125, 150, 175 and 200 mg kg<sup>-1</sup> body weight were used. Table 3 shows the results indicating the time of injection and

Table 2: Observation on induced breeding of *Nandus nandus* in range finding test injecting a geometric series of carp pituitary gland (PG) doses 0.2, 2, 20 and 200 mg kg<sup>-1</sup> body weight

Dose of injection (mg kg <sup>-1</sup> )	Average fish wt. (g)		Date and time of injection	Date and time of ovulation	Fertilization rate (%)	Date and time of hatching	Hatching rate (%)	Incubation temp. (°C)	Remarks
	Male	Female							
0.2	7.3	11.0	24-6-99 5 pm					29	Male and female did not show any sign of ovulation
2	6.1	12.3	24-6-99 5 pm					29	Male and female did not show any sign of ovulation
20	8.8	14.2	24-6-99 5 pm					29	Male and female did not show any sign of ovulation
200	6.7	10.5	24-6-99 5 pm	25-6-99 4-5 am	90±2	26-6-99 0-1 am	80±2	29	Female ovulated, male produced milt, successful spawning, high rate of fertilization and hatching

Table 3: Observation on induced breeding of *Nandus nandus* in definitive test with equally spaced lower doses of the lowest responded dose (200 mg kg<sup>-1</sup>) of the range finding test

Dose of injection (mg kg <sup>-1</sup> )	Average fish wt. (g)		Date and time of injection	Date and time of ovulation	Fertilization rate (%)	Date and time of hatching	Hatching rate (%)	Incubation temp. (°C)	Remarks
	Male	Female							
100	6.3	12.5	4-7-99 5 pm	-	-	-	-	28	No response
125	8.6	15.0	4-7-99 5 pm	5-7-99 8 am	90±5	6-7-99 2-4 am	80±8	28	Not ovulated normally, partial ovulation by stripping after 15 h of injection
150	10.2	13.3	4-7-99 5 pm	5-7-99 5-8 am	90±2	6-7-99 2-4 pm	90±2	28	Ovulated, partial natural spawning, successfully stripped
175	7.3	13.5	4-7-99 5 pm	5-7-99	6-7-99 4-8 am	92±5 2-4 pm	90±5	28	Partially spawned, than stripping after 15 h of injection
200	6.9	11.7	4-7-99 5 pm	5-7-99 4-8 am	92±7	6-7-99 2-4 pm	90±5	28	Partially spawned, than stripping after 15 h of injection

Table 4: Observation on induced breeding of *Nandus nandus* by carp PG injection into female only

Dose of injection (mg kg <sup>-1</sup> )	Average fish wt. (g)		Date and time of injection	Date and time of ovulation	Fertilization rate (%)	Date and time of hatching	Hatching rate (%)	Incubation temp. (°C)	Remarks
	Male	Female							
150	6.1	14.2	13-7-99 5 pm	14-7-99 5 am	90	15-7-99 1 am	80	28	Did not spawn naturally and successfully stripped.
	7.2								
150	8.6	13.7	13-7-99 5 pm	14-7-99 5 am	94	15-7-99 1 pm	82	28	Partial spawning occurred naturally and then stripped
	6.9								

Table 5: Larval rearing by supplying scrambled chicken egg yolk, pasted *Tubifex* worm and zooplankton

Treatment	Total no. of larvae reared	Larvae survived	Survival rate (%)	Comment
Chicken egg yolk	100	0	0	No larvae were survived
<i>Tubifex</i> worm	100	0	0	No larvae were survived
Zooplankton	100	16	16	Further study on feeding zooplankton is needed

ovulation, fertilization rate, time of hatching, hatching rate and temperature. In case of the dose 100 mg kg<sup>-1</sup> body weight, the fish did not show any courtship behavior and ovulation did not occur. The brood fish showed some courtship behavior such as moving together, male stroke at the female genital pore and bring their belly in the proximity but did not ovulate naturally at the dose of 125 mg kg<sup>-1</sup> body weight. Stripping was done to the fishes of this treatment on the next morning after 15 h of injection and a very small amount of eggs were collected. 90±5% eggs were fertilized and 80±8% eggs were hatched out for both pair of fishes at the dose of 125 mg kg<sup>-1</sup> body weight. The fish spawned successfully at the dose of 150 mg kg<sup>-1</sup> body weight. The fish showed courtship behavior actively after 4-6 h of injection. 90±2% eggs were fertilized and 90±2% eggs were hatch out. In case of 175 and 200 mg kg<sup>-1</sup> body weight application, fishes released eggs partially. For full release of egg and milt fishes were stripped on the next morning. Eggs were released after 11 to 14 h of injection where normal ovulation occurred and hatching was observed after 18 to 20 h of fertilization. Fertilization rate was above 92% and hatching rate was 90%. Therefore, the dose of 150 mg kg<sup>-1</sup> body weight *i.e.* lowest successful dose was selected for induced breeding of *N. nandus* in the laboratory for further experiments. Kohinoor *et al.* (1991) found that the fertilization and hatching rate of *A. testudineus* were 82±2% and 75±3%. In case of *Mystus cavasius* fertilization and hatching rate were 70±2 and 60±2% respectively (Akteruzzaman *et al.*, 1991). These results support the present findings.

**Induced breeding of *N. nandus* by carp PG injection into female only:** In this experiment only female fishes were injected with 150 mg PG per kg fish as selected from the result of the previous two series of tests. After 12 h of injection, partial spawning occurred in one case but other did not spawn naturally. Both male and female fish were stripped on the next morning, 15 h after injection and eggs

were fertilized and kept in incubation. Hatchlings came out after 18-20 h of fertilization in both replicates. Table 4 shows the results indicating the time of injection and ovulation, fertilization rate, time of hatching, hatching rate and temperature. 92% fertilization occurred in both cases and above 81% hatchling came out from the fertilized eggs. So, it was clear that male fishes released milt without giving any injection. Similar results were reported by Nayak *et al.* (2001) in *H. fossilis*.

**Group breeding:** A group of four males and two females were kept in an aquarium without giving any injection. They did not show any kind of courtship behavior. The fishes were examined on the next morning whether they were ready to ovulate or not. It was found that the female were not ready to ovulate without giving any inducing agent but the male were found ever ready to take part in courtship. Barua and Mollah (1987) reported same sort of courtship behavior in *Mystus tengara*.

**Reproductive behavior:** First 4 h after injection, fish exhibited normal movements and activities. The pre-spawning activities were observed after 4 to 6 h of PG injection. Male actively moved around the female and started to nudge with its snout at the ventral region of the female. After that the male bent its body with female and tried to bring its genital pore in the proximity of female's genital pore. The play continues for several hours and finally the female ejected its brown colored eggs and the male ejected milt on the released eggs and fertilization occurred. Fishes became calm and quiet after spawning and were found resting on the bottom of the tank and exhibited high rate of opercular movements. Similar results were observed in different fish species (Barua and Mollah, 1987; Mollah and Khan, 1999; Gheyas *et al.*, 2000).

**Larval rearing:** The larvae started wandering in search of food after 56 h of hatching. Fifty larvae were placed in

each of the six whole glass tank for larval rearing. Larvae were fed with scrambled egg yolk, pasted *Tubifex* worms and zooplankton in duplicate aquaria. Only 16 larvae were alive at the end of the experiment after 3 weeks in treatment where zooplankton were supplied as larval food. The survival rate was calculated 16% only by feeding zooplankton. No survival was observed among other food items (Table 5). Banu (1990) reported that the larvae of *C. batrachus* started feeding on *Tubifex* worm after four days of hatching. The reason that *N. nandus* did not feed on pasted *Tubifex* worm might be due to the predatory behavior of the fish.

We believe that the present work has practical implications on the context of biodiversity conservation and culture of the endangered species, *N. nandus*. The following package of recommendation and conclusion can be made based on the results of this work.

- *N. nandus* can be successfully induced to breed in captivity during the month of June-August.
- Carp pituitary gland extract at the rate of 150 mg kg<sup>-1</sup> body weight is the lowest successful dose for induced breeding of *N. nandus*
- First feeding of larvae starts after 56 h of hatching and zooplankton is the suitable diet as a starter.
- Further experiments on the artificial breeding of *N. nandus* by applying differential doses of PG in single and multiple injections should be addressed for the perfection of breeding technique of *N. nandus*.
- Larval rearing experiments applying different live food with differential densities should also be addressed for the purpose of successful fry rearing in view of domestication of this species.

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