ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2016; 4(5): 109-113

www.fisheriesjournal.com
Received: 17-06-2016
Accepted: 18-07-2016

Mst S Sharmin
Bangladesh Fisheries Research Institute (BFRI), Bangladesh

Md Golam Sarwer
Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, Bangladesh

David Rintu Das
Bangladesh Fisheries Research Institute (BFRI), Bangladesh

Md Mahmudur Rahman
Bangladesh Fisheries Research Institute (BFRI), Bangladesh

AK Jilani Chowdhury
Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh

Correspondence
Md Golam Sarwer
Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, Bangladesh

Induced spawning and larval rearing of endangered species *Nandus nandus* (Hamilton) in cemented cistern using pituitary hormone injection

Mst S Sharmin, Md Golam Sarwer, David Rintu Das, Md Mahmudur Rahman and AK Jilani Chowdhury

Abstract
The present study deals with brood development at different stocking densities, Standardization of induced breeding technique and larval rearing of *Nandus nandus*. To develop *N. nandus* broods, average body weight (Male 20.50 ± 3.7cm, Female 14.50 ± 4.0 cm) were reared 3 months at different densities (T_1= 40/deci T_2= 60/deci T_3= 80/deci). Feeding with fry, dead small fises and artificial feed contain 30% rice bran, 30% wheat bran, 20% fish meal, 15% MOC, 5% molasses two times daily@ 5-3% body weight was done. Brood was highest wt. (Male 34 ± 4.20gm Female 62 ± 5.10 gm) in case of density T_1= 40/deci. For standardization of breeding technique of *N. nandus* three different doses of PG were tested viz 4, 6, 8 mg/kg BW for female and 1.5, 2, 2.5 mg/kg BW for male. Sex ratio male: female =1:1 was maintained. PG treatment with 1.5mg/kg for male and 4 mg/kg for female revealed best fertilization (95 ± 5) % and hatching (88 ± 2) %. After 72 hours hatchlings were transferred to pond for larval rearing for 20 days. Average Length attained from (4.01 ± 0.05) to (23.02 ± 0.245) mm and weight (0.90 ± 0.003) to (370.00 ± 0.025) mg for 20 days rearing.

Keywords: *Nandus nandus,* Brood development, PG, Induced breeding, Larval rearing

Introduction
There are potentials for culture of small indigenous species (SIS) in Bangladesh. Small Indigenous Species (SIS) are generally considered to be those fishes which grow to a maximum length of about 25 cm or 9 inches [9]. There are 50-60 different SIS in Bangladesh. Some SIS has high nutritional value in terms of proteins, vitamins and minerals whice are not commonly available in other foods. These SIS are Koi. Taki, Vheda, Pabda, Tengra, Gulsa, Mola, Dhela, Puti, Shing, Magur, Chapila, Chela and Chanda etc which are available in smaller water bodies like drains, ditches, pond, lakes, beels, haor, baor, rivers, stream andother ephemeral water bodies of the inland and estuarine areas. Among the available SIS, *Nandus nandus* [8] locally called Meni or Bheda is an indigenous vulnerable small fish in Bangladesh [8]. This fish use the floodplains as its breeding, nursing and rearing grounds. It inhibits in fresh and brackish water [9]. The fish is exclusively bottom and column feeder [14]. It is a carnivorous fish which feeds on small shrimp, very small fish, fish larvae and insects [7]. It attains maturity at the first year of its life [6]. Breeding period of this fish is April to September [9]. Once the fish was caught in a large amount from floodplains, beels, haors, rice fields etc. But now-a-days its abundance has declined drastically due to indiscriminate fishing, unplanned construction of bridge & flood protection embankments, use up of low land water for irrigation, residual effect of pesticides/insecticides, destroying breeding ground etc. Therefore, the fish is going to be rarely available and now considered to be an endangered or threatened species. International union for Conservation of nature [10] has enlisted mottled nandus *(N. nandus)* in their red list of threatened fishes of Bangladesh as a vulnerable taxon. With a view to overcome the odd situations induce breeding and development of culture techniques of *N. nandus* became important which will ensure its conservation and rehabilitation through increasing its production. That's why the present study has been undertaken to standardize of artificial propagation of this species using pituitary hormone injection.

Materials and Methods

Study area

The experiment was conducted during June to August, 2015 in the cemented cistern having size 2.74×1.5×0.5 m of the floodplain sub-station, Bangladesh Fisheries Institute, Santahar, Bogra.

Brood fish collection

Required number of healthy male and female *N. nandus* broods were collected from the Raktodahabeel in Sntahar, Bogra and stocked in the previously prepared brood rearing ponds.

Brood rearing

The collected broods were reared for two months in the brood rearing ponds at a stocking density of 40-45ecimal. Pond size was 10 decimal and water depth 1.0 meter. During rearing lime, fertilizer, and cow dung were properly applied. Fertilization with Urea and TSP at the rate of 150g/decimal and 75g/decimal respectively were applied at 15 days interval. Broods were fed with artificial feed (contain 30% ricebran, 30% wheat bran, 20% fish meal, 15% MOC, 4% molasses and 1% vitamin premix) two times daily at the rate of 5% body. Water quality parameter were recorded fortnightly during rearing period.

Brood selection

For successful induced breeding only healthy, uninjured and sexually mature broods were selected. Mature male and female broods were identified on the basis of secondary sexual characteristics. Maturity of fish was determined by following ways.

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

Male	Female
Smaller and slender in appearance	Relatively larger and robust in appearance
Color prominent, brightly reddish, in color with black strip.	Female comparatively dull in color
Rough pelvic fin	Smooth pelvic fin.
Eject while milt when light pressure was applied on the abdomen.	Eject brown eggs following gentle pressure on the abdominal region
Possessed normal mouth.	Possessed a more conical, pointed and upward bend mouth.

The selected broods were kept in cisterns with continuous water flow for induced breeding.

Conditioning of broods

Selected brood fish were kept in cemented cistern for about 6 hours for conditioning prior to injection with PG extract. Handling and carrying of fish was done very carefully to avoid possible injury and secondary infection. Males and females fish were kept in separate cistern and constant water flow was maintained to ensure proper aeration.

Preparation of PG solution

PG were collected from market in preserved condition in airtight vials and used as inducing agent. At first, PG were gently removed from the vial with a pair of forceps and then weighed by an analytical electronic balance. The amount to be weighed out was calculated on the total of the body weight of all the fishes using the following formula:

Weight of PG (mg) = $(W_t * P_t) /1000$

Where, W_t represents total body weight (g) of all the fishes to be injected and P_t represent the rate in mg PG to be injected/kg body weight under a particular treatment. PG was diluted in distilled water to dissolve it and centrifuged with a hand centrifuge for precipitation. The freshly prepared supernatant solution of hormone was then taken slowly in a 1 ml. hypodermic syringe for injection.

PG injection in to brood fishes

One ml disposable syringe was used for injecting hormone to the recipient fish. The appropriate amount of diluted hormone stock solution was taken in the syringe. Then the fishes were caught very carefully from the spawning cistern. A piece of clean soft and wet cloth was used to wrap up the fish and kept lying on a table. The accurate dose of PG extract was administered at the basal part of the pectoral fin. Needle was inserted at an angle of 45^0 with the body. For standardization of induce breeding of *Nandus nandus* three different doses of PG were tested.

Table 2: Different doses of PG used for induced breeding of *Nandus nandus*

Fishes	Doses of PG (mg/kg)		
	T_1	T_2	T_3
Male	1.5	2.0	2.5
Female	4.0	6.0	8.0

Spawning behavior

After hormone injection both male and female breeders were kept (male: female =1:1) in fine soft cloth made hapa in cemented cisterns and artificial shower of water was provided. After 6-8 hours of hormonal injection, fish started spawning activities. Male actively moved around the female started vent kissing. A male was observed to creep up to a female and to position himself to her near. Male bent its body with female and tried bring genital pore together. Play continuous for hours together finally female ejects brown colored egg and male ejected milt on the released egg. After releasing of eggs brood fishes were removed from the hapa.

Fertilization and hatching

The eggs released by the female were allowed to fertilize automatically by the milt of the male in the spawning cistern. Fertilization were also occurs with splashing movement. A total of 100 eggs from each hapa were transferred into a bucket and classified as fertilized or unfertilized under binocular microscope. The fertilization rate was calculated as the number of fertilized eggs divided by the total sampled number (n=100) of eggs. After 18-20 hrs of injection all fertilized eggs were hatched out. Unfertilized eggs and egg shells were cleaned out of the cistern within an hour of hatching to protect larvae from fungal injection. Hatchlings were fed with boiled chicken egg yolk after 36 hours. The rate of hatching was estimated from the number of hatchling to the number of fertilized eggs, multiplied by 100.

Fry rearing

After 72 hours hatchlings were transferred to cistern in three replications (4 decimal each) for larval development. Before

stocking nursery pond, its roofed and encircled with fine mesh nylon net and pond was prepared by liming 1kg/deci, fertilizing (Urea 100gm/deci, TSP 75gm/deci, Cow dung 8kg/deci) for increasing primary with secondary productivity of pond. Cotton cloth was inserted in three side of the pond far from 2 ft of pond embankment to make shelter. Hatchlings were transferred to the pond near the cloth after proper conditioning in the evening two days after fertilizing. Feeding provided for three days (250 gm wheat flour with six pieces boiled egg yolk) three times in a day. From 4th to 6th day's 250gm wheat flour + six piece boiled egg yolk + Nursery brand feed was provided in two times in a day. The rest 14 days (500gm wheat flour +1kg mustard oil cake + 2 kg cow dung) was provided at alternative day in order to increase zooplankton production. Larval rearing was done for 20 days. To know growth of larvae, length and weight were recorded into 5 days interval. After harvesting survival rate was calculated.

Statistical analysis

Pertinent data were analyzed using Duncan's Multiple Range Test (DMRT) method and one- way analysis of variance (ANOVA).

Results

Table 3: Observation of growth performance of *Nandus nandus* at different stocking densities after three months rearing

Parameters		Treatment						
		T_1		T_2		T_3		
		Male	Female	Male	Female	Male	Female	Level of Significance
During stocking	Av Length(cm)	11.00 ±3.0	13.50 ±4.0	11.00 ± 3.0	13.50 ±4.0	11.00±3.0	13.50 ± 4.0	NS
	Av. wt (gm)	21.50 ±3.5	43.00 ±4.5	21.50 ±3.5	43.00 ±4.5	21.50±3.5	43.00 ± 4.5	NS
After stocking	Av. Length (cm)	13.50±2.5	16.00 ±3.8	12.50 ± 3.5	15.00 ±5.0	11.50±5.8	14.50 ± 6.5	NS
	Av. wt (gm)	34.00 ±4.2^{a}	62.00±5.1^{x}	25.00±4.4^{b}	60.00±7.3^{x}	23.00±5.2^{b}	54.00 ±6.9^{y}	*

NS= Means are not significantly different (P>0.05)

* Mean values with different superscript letters in the same row indicate significant difference at 5% significance level.

After rearing of *Nandus nandus* brood the final weight Male (34 ± 4.20) gm, female (62 ± 5.10) gm was highest in case of density with T_1= 40/decimal. Average weight after rearing of *Nandus nandus* brood showed significantly different (P<0.05). But length did not show different (P>0.05).

Table 4: Effect of different doses of PG on fertilization and hatching rate.

Treatment	Doses of PG mg/kg		No of induced		Av. Wt(gm)		Fertilization %	Hatching %
	Male	Female	Male	Female	Male	Female		
T_1	1.5	4.0	10	10	21±2.5	31± 3.5	95 ± 5^{a}	88 ± 2
T_2	2.0	6.0	10	10	22 ±3.5	30± 2.5	93 ± 3^{b}	86 ± 1
T_3	2.5	8.0	10	10	23±3.2	30±3.0	90 ±2^{b}	83 ±3
Level of significance					NS	NS	*	*

NS= Means are not significantly different (P>0.05)

* Mean values with different superscript letters in the same row indicate significant difference at 5% significance level.

Table 5: Water quality parameters during breeding

Water quality parameters	Mean values ± SD		
	Brooders tank	Breeding hapa	Larval hapa
Water temperature	30.22 ± 1.4	31.43 ± 2.2	31.32 ± 2.1
pH	7.6 – 8.4	7.2 -8.5	7.4 -8.7
Dissolve oxygen	7.5 ± 2.1	8.34 ± 2.6	8.21± 1.90
Alkalinity	118 ± 4	123 ± 5	128 ± 3

Table 6: Average measurement (Mean ± Sd) of hatchlings and post larvae of *Nandus nandus.*

Days after hatching	Length (mm)	Weight (mg)
At hatching	4.01 ± 0.05	0.90 ± 0.003
5 day old hatchings	8.45 ± 0.28	23.06 ± 0.035
10 day old hatchings	15.8 ± 0.175	45.50 ± 0.006
15 day old hatchings	20.00± 0.193	250.50 ± 0.045
20 day old hatchings	23.02 ± 0.245	370.00 ± 0.025

Discussions

This study showed lower stocking density increase body weight of *Nandus nandus.* It was an established fact that growth rate progressively increases as the stocking density decreases and vice-versa. This was because a relatively less number of fish of similar size in a pond could get more space, food and dissolve oxygen etc [4, 21]. was also similar to present study. *Barbonymus gonionotus* grew fast at high stocking densities was disagreed to present study [11]. It was occurred due to species variation and variation of feed supplying.

Stocking density was an important parameter in fish culture operations, since it had direct effect on growth and survival and hence on production [3]. was similar to present study. During the experimental period, mean water temperature (*C), dissolved oxygen (mg/L), pH and total alkalinity (mg/L) were 29.34±1.75, 5.02±1.97, 7.57±0.21 and 167.00±9.84 respectively.

The spawning activity appeared to continue first after 6-8 hours post injection. This period was reported to be 3 hours in *Puntius gonionotus* [5], 16-20 hours in *Clarias batrachus* [2], 4 hours in *Mystus tengara* [1], 7-8 hours in *Mystus cavasius* [18] and 5 hours in *Mystus gulio* [13]. It was occurred due to species variation. Fish always spawned in pairs, while the male was observed to become active and to chase the female for 10-20 seconds. A male was observed to creep up to a female and to

position himself to her near. This way of chasing and resting continued for 6-10 minutes and took rest on the tank bottom by keeping them stationery in close association for a period of 21-25 minutes. This repeated swimming and resting continued for 2-3 times.

The present study showed (95 ± 5) % fertilization & (88 ± 2) % hatching by using PG with 1.5 mg/kg for male & 4mg/kg for female which was higher fertilization and hatching rate among all treatments. Lowest fertilization and hatching rate showed T_3. Water quality parameters of brooders tank, breeding hapa, and larval hapa was suitable for hatchery operation.

Male fish of *H. fossilis* release milt without giving any injection was disagreed to present study. *Nandus nandus* breeding was done by using inducing agent [20]. Induced breeding trials of *Nandus nandus* with three doses of PG showed significantly higher ($p<0.05$) at treatment 1,containing PG 1.5 mg/kg for male & 4mg/kg for female. Treatment 1 showed (95 ± 5) % fertilization & (88 ± 2) % hatching. S. Pal *et al.,* 2003 reported PG with 200 mg/kg for *Nandus nandus* provided (92 ± 7) % fertilization & (90 ± 5)% hatching rate which was disagreed to present study. This was occurred may be well maturity of brood, high quality of PG, water quality, different location, seasonal variation etc.

In case of *Clarias batrachus*, the fertilization and hatching rate of eggs were reported by 51-96% and 42-81%, 40-90% and 25-75% respectively which was similar to the present study [15, 17].

The lowest dose of PG was 40mg/kg, for female and 30 mg/kg for male in case of *H. fossilis* was reported [16] dissimilar to the present research. The fertilization and hatching rate of *A. testudineus* were 82 ± 2 % and 75 ± 3% respectively was near to the present study though species was different [18]. In case of *Mystus cavasius* fertilization and hatching rate were (70 ± 2) % and (60 ± 2) % was not near to author study due to dose and species variation. When *Nandus nandus* hatchlings were released it was4.01 ± 0.05 mm length and 0.90 ± 0.003 mg weight, after 20 days of rearing it attained 23.02 ± 0.245 mm length and 370.00 ± 0.025 mg weight. Survivality rate achieved 45.35%.

The larvae started wandering in search of food after 56 h of hatching but the present experiment observed after 36 h of hatching [20]. Survivality was found 50%. The main reason for the dissimilarity was first feeding time due to their cannibalistic characters. If first feeding time was increased mortality was increased due to want of food they showed their predatory behavior. We provided first feed after 36 h of hatching. The larvae of *C. batrachus* started feeding on Tubifex worm after four days of hatching [14]. The survival rate of *Mystus gulio* was varied from 55.5-67.3% was more or less similar to the present study [15, 17] observed 15 days old *M. gulio* larvae was (17.64 ± 2.11) mm length and (89.63 ± 4.52) mg weight which was dissimilar to the present study, the present study was (20.00± 0.193) mm length and (250.50 ± 0.045) mg weight in case of 15 days old *Nandus nandus* larvae. Dissimilarity was occurred due to species variation.

Conclusion

The present experiment was provided Standardization of Induced breeding and larval rearing of endangered Species Meni (*Nandus nandus).* This experiment will help to the endangered species (*Nandus nandus*) escape from extinction and thereby ensuring biodiversity conservation in nature. As this species is highly demandable further research is needed to start commercial seed production and culture of this species.

Acknowledgment

The authors would like to extend sincere gratitude to Director General Bangladesh Fisheries Research Institute (BFRI) and Chief Scientific Officer, Freshwater Station, BFRI, for their kind assistance and co-operation for allocating fund, providing different inputs directly or indirectly which contributed to the accomplishment of this work. The authors also wish to extend appreciations to all staffs of the Freshwater sub-station, Bangladesh Fisheries Research Institute, Santahar, Bogra.

References

1. Akteruzzaman M, Kohinoor AHM, Hossain MG, Shah MS. Observation on the induced of *Mystus cavasius* (Ham.) in Bangladesh. Bangla. J. Fish.1991; 14:101-105.
2. Alam MJ, Begum M, Islam MA, Pal HK. Spawning behavior and induced breeding of an estuarine cat fish, *Mystus gulio* (Ham.). Bangladesh J. Fish. Res.2006; 10(2):101-109.
3. Backiel TED, Le Cren. Some density relationship for fish population parameters. Biology of Fresh water Fish Production. S. D Gerking, Black well Scientific Publications, Oxford.1978, 279-302.
4. Brett JR. Marine fish Aquaculture in Canada. On H. R. Mac Crimmon, J. E Steward and J. R. Brett (Editors) Aquaculture in Canada. The practice and the promise. Department of the Environmental Fisheries and Marine Service, Ottowa, Canada, 1974, 55-84.
5. Bhuiyan AL. Fishes of Decca… Asiatic Soc. Pakistan.1964; 13:107-108.
6. Das M, ZamalN.Domestication of an endangered fish species *Nandus nandus* (Hamilton). I. Laboratory rearing of young fish up to sexual maturity. Bangla. J. Fish. Res.2000; 4:135-140.
7. Das MA, Islam, Mughul GU. Induced breeding and fry rearing of cat fish, *Clarias batrachus* (Lin). Bangladesh. J. Zool.1992; 20(1):87-95.
8. Hamilton F. Fishes of the Ganges. Archibald constable and company, Edinburgh.1822, 96.
9. Hossain MA, Afroze S. Small fish as a resource in rural Bangladesh. Fishbyte. 1991; 9(2):16-18.
10. IUCN. Red Book of Threatened of Bangladesh. IUCN-The world conversation union.2000, 9-24.
11. Kohinoor AHM, Akteruzzaman M, Hossain MG, Shah MS. Observation on induced breeding of Koi fish, *Anabas testudineus* (Baloch) in Bangladesh. Bangla. J. Fish.199122:125-128.
12. BegumM, Pal HK, Islam MA, Alam MJ. Embryonic and larval development of *Mystus gulio* (Ham.)2009.
13. AlamMJ. Spawning behavior and induced breeding of an estuarine catfish, *Mystus gulio* (Ham.) 2006.
14. Mustafa G, Ahmed ATA, Islam KR. Food, feeding habits and fecundity of a fresh water perch, Meni fish. Bangladesh J. Agric. 19805(4):205-210.
15. Najero NT, Rahmatullah SM, Mammur Rashid M. Reproductive Biology of fresh water spiny eel, *Mastacembelus armatus* (Lacepede) reared in the cemented cisterns of BAU, Mymensingh, Bangladesh. J. Aqua. Trop. 2002; 17:251-259.
16. Nesar MN, Shah MS, Barua G. Development of a new methodology on the artificial propagation of cat fish *Clarias batrachus* (Lin.) by influencing some physic-chemical parameters of the water. Bangladesh J Zool. 1990; 18:23-31.
17. Pathak SC. Project report of the fourth workshop of all

India coordinated research project on air breeding fish culture. Central inland fisheries research institute, Barrackpore, West Bengal, 1982, 60.
18. Saha DK, Akter N, Das M. Natural breding of Meni fish (*Nandus nandus)* in rice fields. In: Islam, Z. (ed), and proc. 2nd ANR Ag. Conf., CARE Bangladesh.2000, 118-119.
19. PalSH, Rashid MAK, Tarafder NT, Narejo, Das M. First record of artificial spawning of *Nandus nandus* (Hamilton) in Bangladesh using carp pituitary gland: An endangered species bred in captivity. Pak. J. Biological Sciences. 2003; 6(18):1621-1625.
20. Sarker PK, Pal HK, Rahman MM. Observation on the fecundity and gonado-somatic index of *Mystus gulio* (Ham.) in Brackish water of Bangladesh J Bio Sci 2002; 2(4):235-237.
21. Weatherly AH. Factors affecting maximization of fish growth. J. Fish Res. Bol. Can. 1976; 22:1046-1048.