Conservation of endangered fish stocks through artificial propagation and larval rearing technique in West Bengal, India

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The sustainable utilization of genetic resources, including fish, is a vital part in improving the standard of living in a populous country like India. Concern over declining harvests and an obvious reduction in biodiversity of fish species has lead to a more holistic approach to fisheries management and research. About 11% (2,200) of the total world fin fish species (more than 20,000) have been recorded from the Indian subcontinent[1].

Unfortunately, many fish species are in decline and some have become endangered due to a combination of over-exploitation, pesticide and aquatic pollution, spread of disease, uncontrolled introduction of exotic fishes, and habitat modification due to industrialization, river-valley projects, excessive water abstraction and siltation due to clearing.

However, there is no comprehensive list of the threatened species of fishes critically in need of protection. This lack of information on threatened species of fishes and the general lack of identification manuals are barriers to the recognition and conservation of our vanishing fishes. An essential prerequisite to any broad programme of resource conservation is the proper taxonomic study of fish species occurring in the area concerned and a full checklist indicating the status of each species. Such a list would enable the IUCN to prepare an international list of endangered species, to be included in the Red Data Book.

We have identified 39 such local fish species that we believe are going to disappear from their natural habitat in West Bengal (Table 1).

The Department of Fisheries, Government of West Bengal is trying to conserve these species with the following objectives:

1. Brood stock management: Artificial breeding of threatened species for restocking in their natural habitat and to establish gene banks using cryopreservation techniques.

Table 1: List of 39 species of local fishes from West Bengal, India, that are likely to become locally extinct in their native habitat

Freshwater		
Endangered:	1.	*Ompok pabo*
Vulnerable:	2.	*Ailia coila*
	3.	*Anguilla bengalensis*
	4.	*Bagarius bagarius*
	5.	*Eutropiichthys vacha*
	6.	*Ompok bimaculatus*
	7.	*Puntius sarana*
	8.	*Semiplotus Semiplotus*
	9.	*Osphromenus nobiliis*
	10.	*Labeo diacanthyus*
	11.	*Anabas testudineus*
Threatened:	12.	*Notopterus chitala*
	13.	*Notopterus notopterus*
	14.	*Pangasiuspangasius*
	15.	*Balitora brucei*
	16.	*Gudusia chapra*
	17.	*Labeo fimbriatus*
	18.	*Labeo gonius*
	19.	*Mastocembelus armatus*
	20.	*Mystus tengara*
	21.	*Mystus aor*
	22.	*Rasbora rasbora*
	23.	*Setipinna phasa*
	24.	*Bengala elanga*
	25.	*Wallago attu*
	26.	*Nandus nandus*
	27.	*Amblypharingodon mola*
Cold Water		
Vulnerable:	28.	*Tor putitora*
	29.	*Tor tor*
	30.	*Raiamas bola*
	31.	*Barilus vogra*
Brackish & Marine		
Vulnerable:	32.	*Lates calcarifer*
	33.	*Mystus gulio*
Threatened:	34.	*Osteogeniosus militaris*
	35.	*Periophthalmus koelreutri*
	36.	*Etroplus suratensis*
	37.	*Plotosus canius*
	38.	*Tachsurus thalassinus*
	39.	*Polydactylus indicus*

2. To overcome disease problems in larval rearing tanks & culture ponds.
3. To generate income, self-employment and skill for interested farmers through demonstration & training.
4. To provide technical support to private hatchery owners to help them to maximize production of quality seed.

As little is known about the reproduction of many species, research is needed to develop and standardize techniques for their artificial propagation. This technology can then be used to help conserve threatened species through captive breeding programs and also to generate new employment opportunities for rural people.

We would like to share our findings with farmers and extension/conservation workers throughout the region with regard to the breeding techniques of two endangered species: 1) A freshwater fish Pabda, *Ompok pabo* and 2) a brackish-water fish, Tangra, *Mystus gulio*.

Gene banks

For conservation purposes, we have applied artificial reproduction techniques to establish an Endangered Fish Species Breeding Programme. The two main components of this programme are a) a live gene bank and b) gamete/embryo bank. In a live gene bank, the endangered species are reared in captivity and genetically managed to avoid inbreeding depression, domestication and unintended selection. In the gamete/embryo bank, 'adequate samples representative of the natural genetic variation of endangered species are held in a state of suspended animation, frozen under liquid nitrogen.

In the first phase of the programme, we have selected some freshwater fish species, reared them in captivity and attempted to artificially induce reproduction and rear the larvae. The results of some of our experiments on reproduction are given in table 2.

Figure 1a) Nandos broodstock, Nandus nandus

Captive breeding of Pabda and Tangra

Pabda, *Ompok pabo* and Tangra, *Mystus guillo* are Indian major catfishes belonging to the family siluridae (eel-tailed catfish). Both are important components of riverine and brackish-water fisheries in the Indian sub-continent. Little information is available on the biology and culture practices of these two species[2,3]. Pabda dwells and breeds in the rivers and reservoirs and in connected water sheds during floods. Tangra are found in seas and estuaries throughout the India. Tangra dwells and breeds in the estuaries during the monsoon. There are no prior reports of captive breeding in either of these two species.

Table 2 : Experiments on some threatened fish species

Fish species	Hormone used	Sex ratio M: F	Dose of hormone used per kg body weight	Percentage hatching
Pabda *Ompok pabo*	Pituitary extract Ovatide	2:1 2:1	• 16 mg (in female only) • 3.0 ml (in female only)	50% 70%
Chital *Notopterus chitala*	Pituitary extract	3:1	• 11 mg (in female only)	60%
Pholni *Notopterus notopterus*	Pituitary extract	2:1	• 4 mg (in female) 2.5 mg (in male)	70%
Indegenous tangra *Mystus vittatus*	Ovatide	2:1	• 8 ml (in female)	80%
Saral punti *Puntius sarana*	Pituitary extract	2:1	• 4 mg (in female) • 2.5 (in male)	70%
Indigenous magur C/art us batrachus	Ovaprim	1:1	• 2.5 ml in female • 1.0 ml in male	50%
Nuna tangra *Mystus gulio*	Ovaprim	2:1	• 2.5 ml in female • 1.0 ml in male	80%
Mourala *Amblypharingodon mola*	Pituitary extract	2:1	• 4 mg (in female) • 2.5 (in male)	80%

Brood Stock Management

Pabda

Pabda, juveniles were collected from the river Punarvava, in the vicinity of Malda District, West Bengal, India. They were stocked in a polyculture tank with Indian Major Carp in a 0.5 ha pond. Along with regular liming the fish stock were fed with conventional feed consisting of mustard oil cake and rice bran in the ratio of 1:1. Pabda were maintained throughout the carp culture period. The brooders (Fig. la) attained maturity after one year and the average body weight was 85g Both male and female broodstock were found to be mature in May.

Matured males have a rough first ray in the pectoral fin at the lower side and have a narrow and rather pointed genital papilla, which releases white milt if slight pressure is applied to the abdomen. Females have a smooth pectoral fin and the genital papilla has a thick muscular round opening.

Figure 1b) Pabda broodstock, Ompok pabo

Tangra

Adult Tangra, (Fig. 1c) were collected from the local bheri at Digha, Midnapore and were stocked in a round cement tank in the hatchery for a period of 15 days for acclimatisation. Along with regular water exchange the fish stock were fed with conventional feed consisting of mustard oil cake, rice bran and dry fish in the ratio of 1:1:5.

Males had a muscular, conical reddish-pink genital papilla. In the case of females, the genital papilla was found to have a thick muscular ring round the opening.

Figure 1c) Tangra broodstock, Mystus gulio

Induced breeding techniques

Pabda

Gravid females were identified with a simple cathet ring device[4]. Free oozing males and ripe females were used for breeding in the ratio of 2:1.

Breeding was carried out in a bundh. These are special type of impoundments where riverine conditions are simulated during monsoon months. The bundhs, after a heavy shower, receive large quantities of rainwater from their extensive catchment and provide a large spawning ground. Broodstock were kept in a hapa in the bundh and conditioned for 24 hours.

Two techniques were trialed to induce breeding, one with carp pituitary extract and second with a synthetic hormone 'ovatide' (Hemmo pharma, Mumbai, India). For each experiment six males and three females were kept in a separate breeding hapa. Single doses of hormone were administered to females only. The dose of hormone used, as pituitary, was 16mg/kg body weight and as Ovatide 3.0 mg/kg body weight. The synthetic hormone was diluted with double distilled water before use and the main water quality parameters of the tanks were recorded regularly (Table 3) following the method given by APHA[5].

Figure 2a) Induced breeding in Mystus vittatus

Tangra

Broodstock were selected in June 2001. Free oozing males and ripe females were used in the ratio of 2:1 respectively for breeding, which was conducted in a in 200 liter round earthen cistern (60cm in diameter), filled with saline water (salinity 20 ppt), vigorously aerated.

For the breeding operation (Fig. 2b), a synthetic hormone 'ovaprim' was used. Double doses of hormone were administered to the female. Male fish were injected with a single dose at the time of final dose to the females. The dose of hormone used was 2.5 ml/kg body weight in females and 1.0 ml/kg body weight in males.

The first batch of eggs (about 20%) was released about 10 hours post-injection and a second batch (about 35%) were released at 12 hours post-injection. The remaining eggs (45%) were released in a third batch 15 hours post-injection.

Hatching and larval development

Pabda

22 hours post-fertilization, the embryos hatched. The newly hatched embryo were 5-6 mm in length with a small yolk sac attached After 24 hours, we transferred the larvae from the hatching hapa to a nursery tank for further development. Larvae were fed with formulated feed after three days of hatching, prepared by steam cooking a mixture of egg and mussel[6]. We mixed "Piscimix" powder into the prepared feed at the rate of 10mg/kg feed. We sieved the mixture and thoroughly washed it in tap water.

The synthetic hormone (ovatide) gave the best results, with the number of eggs released 80% higher than that from pituitary extract (Table 4). The number of egg released with the ovatide-induced hormone was 80% higher than that of pituitary extract. Hatchery percentage was higher in ovatide and 28% higher than the pituitary extract.

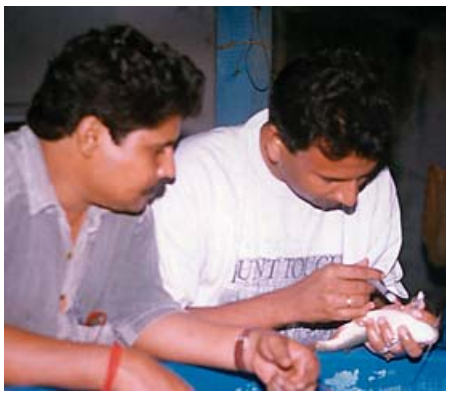

Figure 2b) Injecting hormone in Tangra

Table 3: Major water quality parameters managed during experiments

Pabda

Pond	Temp (C)	pH	DO (ppm)	NH_4N (ppm)	Alkalinity (ppm)	Hardness (ppm)
Brooders tank	29±1	7.5-8.0	6-6.5		130±10	110±15
Breeding tank	31±1	7.6-7.9	7-7.6	-	140±15	112±10
Larval rearing tank	29±1	7.5- 7.8	6.9-7.2	-	135±15	115±10

Tangra

Pond	Temp (C)	pH	DO (ppm)	NH_4N (ppm)	Alkalinity (ppm)	Salinity (ppt)
Brooders tank	29±1	8.0-8.2	7-7.5	-	130±10	20±3
Breeding tank	29±1	7.5-7.9	6.8-7.0	-	140±15	20±2
Larval rearing tank	30±1	7.9-8.0	6.5-7.0	-	135±15	20±2

Table 4a: Larval production of Pabda

Set	Hormone used	No. of fish	Dose of hormone (per kg)	No. of eggs released	Percentage hatching
I	Pituitary gland extract	Female 3 Male 6	16 mg	4,200	50%
II	Ovatide	Female 3 Male 6	3 ml	20,500	70%

Table 4b: Laval production of Tangra

Set	Hormone used	No. of fish	Dose of hormone (per kg)	No. of eggs released	Percentage hatching
I	Ovaprim	Female 9 Male 18	2.5 ml 1.0ml	30,000	80%

Figure 3a) Close up view of Chital eggs Notopterus chitala

Tangra

The embryos hatched after 20 hours of fertilization. Newly hatched embryos were 3.5 - 4.2 mm in length with a small yolk sac attached, which was consumed within 36 hours from hatching. We fed larvae with *Artemia* nauplii after complete absorption of yolk sac.

Larval rearing

Feeding is the most important factor in the larval period rearing, In Pabda, formulated feed with the addition of piscimix helps to combat mortality, enhances survival rates and allows larger growth. It also checks malnutrition and helps to maintain steady growth of bones and muscles.

The technique for induced breeding of Pabda and Tangra is comparable to the induced breeding of carp but special attention is needed in larval rearing as large mortalities occur mostly after 24 hours from hatching. Decomposing of eggshells can cause deterioration of water quality in the hatching hapa. However, after the initial mortality the rest population of survived to become fingerlings without additional problems.

Future work

In the near future we are going to try to breed some other locally endangered fish species such as Nados, *Nandus nandus*; Parse, *Mugil Persia*; Bhagan, *Mugil tade*; and Pangus, *Pangasius pangasius*. An improved breeding technique using bio-filtration in the hatchery and making suitable larval feeds is expected to help to increase larval survival.

With a view to making the conservation effort more effective, we are also attempting to store gametes and embryos in a liquid nitrogen gene bank as representative samples of the natural genetic variation of locally endangered species.

While a gamete/embryo bank is more cost effective in comparison to live gene bank, the present state of technology allows only cryopreservation of fish spermatozoa. Successful cryopreservation of fish eggs and embryo are yet to be achieved.

Figure 3b) Fertilized eggs of Pabda

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Fertilized eggs of Nados, Nandus nandus