

# **Genetic Status and Improvement Strategies for Endemic and Exotic Carps of Bangladesh**

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## **Abstract**

This paper highlights the present status and genetic improvement strategies for endemic and exotic carp species of Bangladesh, including future plans. In this country, both major and minor endemic and exotic (introduced) carps are the key species for aquaculture, particularly for polyculture. There are at least 13 endemic species of carps: among them 4 major carps (catla, rohu, mrigal and calbasu) are being used for most of the seed production in the hatcheries. Among the 8 introduced carp species, the 5 major Chinese carps (silver carp, grass carp, bighead carp, black carp, common carp) and silver barb are predominant. Although aquaculture production of these carps has increased significantly over the last several years, there are problems in hatchery populations due to poor brood stock management and inbreeding, while habitat degradation is leading to the loss of genetic diversity of some endemic carp species.

Bangladesh Fisheries Research Institute (BFRI) has, meanwhile, successfully initiated fish (including carp) genetics research programmes to generate better breeds and improved stocks for increasing aquaculture productivity. To avoid loss of genetic diversity and inbreeding depression problems in hatchery populations, the development of improved broodstocks through implementation of effective breeding plans and genetic stock improvement programmes for commercially important carp species has recently been identified as an important area of research for BFRI. A new dimension of this research has begun with the involvement of several international agencies, such as the DFID-AFGRP funded project which is the focus of this

workshop. Genetic conservation of some threatened carp species has also been started, with successful breeding of ten species.

## 1. Introduction

Bangladesh, a country of deltaic plains dominated by the main major river systems like Ganges, Brahmaputra and Megna, is endowed with unique water resources comprising both inland and marine waters. Along with potential water resources, the country is also rich in the diversity of various fish species. In regard to the wealth of water and fish genetic resources, Bangladesh is the 3<sup>rd</sup> ranking country in Asia after China and India.

In Bangladesh, artificial breeding of endemic carp seed has become a common practice since 1967 (Ali, 1967). Exotic carps have also been introduced. A large number of hatcheries in the private sector (estimated more than 700) have been established (Ali, 1998). These and a smaller number of state hatcheries presently contribute about 98% of the total spawn production, with the remaining negligible proportion of the spawn coming from natural sources, mainly rivers and their tributaries (Banik and Humayun, 1998).

Four endemic major carp species (principally *Catla catla*, *Labeo rohita*, *Cirrhinus cirrhosus* and *Labeo calbasu*) and six introduced or exotic species (*Ctenopharyngodon idellus*, *Hypophthalmichthys molitrix*, *Aristichthys nobilis*, *Mylopharyngodon piceus*, *Cyprinus carpio* and *Barbodes gonionotus*) are being used for major seed production in these hatcheries.

Polyculture of fish in seasonal or perennial ponds involving different species of carps is an age-old practice in Bangladesh. The rationale of using various carp species in the same pond ecosystem for culture is to maximize production, with the fish having different feeding habits. Endemic carps like catla, rohu, mrigal and calbasu are the first ranking fish of choice by farmers in aquaculture due to their fast growing nature and taste. Both endemic and exotic major carps have recently been used in polyculture systems to enhance fish production per unit area. Farmers use various combinations of 5-7 species of carps at a stocking density of 5000-7000 fingerlings/ha for polyculture in perennial ponds in this country. Production can reach 4000 – 5000 kg/ha/yr (Mazid, 2001).

The short cycle species like silver barb and mirror/common carp are being used in rice fields and similar seasonal water bodies due to their faster growth, consumer's preference and palatability.

Some other minor endemic carps like *L. bata*, *C. ariza* and *L. gonius* are also most suitable species which can easily be cultured in all sort of ponds to support additional income to the farmers. In the recent past, the natural stock of these species has become threatened by habitat destruction, etc, which is likely to cause a gradual loss of genetic diversity. The availability of hatchery-produced seed of these species in most parts of the country is still a big constraint.

Further, stock deterioration in hatchery populations due to poor brood stock management and inbreeding depression has been observed in recent years in Bangladesh. Retarded growth, reduction in reproductive performance, morphological deformities, increased incidence of diseases and mortalities of hatchery-produced seeds have been reported. As a result, deterioration of carp and barb seed quality has typically occurred. Introgressed hybrids of carps are being produced intentionally or unintentionally by the private hatchery operators and sold to the farmers and nursery operators. Presumably, large quantity of such seeds are being stocked in grow-out ponds or even in the open water bodies like floodplains, under the Government's massive carp seed stocking programme. There is widespread concern that mass stocking of such genetically poor quality stocks in the floodplains and related open water bodies might cause serious feral gene introgression into the pure wild stocks, that could adversely affect the government's planned aquaculture and inland open water fish production (Hussain and Mazid, 2001).

Although the government is making serious efforts to rehabilitate the inland fisheries, it has also focussed its attention on aquaculture, which has tremendous opportunity in the country. In consonance with the government objectives, since 1988, the Bangladesh Fisheries Research Institute (BFRI) has developed a fish genetics research programme under its Freshwater Station (FS), Mymensingh to generate better breeds and improved stocks for increasing aquaculture production as well as to minimize genetic stock deterioration in hatchery populations. In addition to institute's own programme, meanwhile, a number of international agencies viz. ICLARM, ACIAR/CSIRO and DFID-AFGRP (formerly FGRP) came forward to support some fish genetic research projects. During 1994-2000, a number of projects have been successfully completed. Among these were the ADB funded "Dissemination and Evaluation of Genetically Improved Tilapia in Asia" (DEGITA) for evaluation of the GIFT tilapia strain, "Genetic Improvement of Carp Species in Asia" (both under the auspices of ICLARM); "Production of all female silver barb" (DFID-FGRP) and "Hilsa Biology and Genetic Study" (ACIAR/CSIRO). Presently, one more project, entitled "Genetic improvement strategies for production in exotic carps for low input aquaculture in Asia" is being implemented with the technical assistance of the Institute of Aquaculture (IOA) under DFID-AFGRP funding.

Under BFRI core research and Bangladesh Agricultural Research Council (BARC) contract research funding, some other programs are in operation: i) Selective breeding of rohu, *Labeo rohita*; ii) further genetic selection and development of all male population of GIFT strain; iii) genetic manipulation study of shingi, *Heteropneustes fossilis*; and iv) genetic conservation of some endangered carp species, *Tor putitora*, *Puntius sarana*, *Cirrhinus ariza*, *Labeo bata*, *Labeo gonius* etc.

This report summarises the genetic status and improvement strategies of endemic and exotic carp species and future plans of such research in Bangladesh.

## **2. Carp Genetic Resources of Bangladesh**

Aquaculture in Bangladesh revolves around the cultivation of endemic and exotic carps. The commonly used fast growing major or minor carp species for seed production as well as for composite culture in the country are catla, rohu, mrigal, grass carp, silver carp, bighead carp, common/mirror carp and silver barb. The status of both endemic and exotic carp genetic resources of Bangladesh is briefly described below:

### **2.1 Endemic carp species**

Most of the freshwater river systems and floodplains of the country are the natural breeding grounds for all the major and minor carps. There are at least 13 endemic species of carps, from 6 genera, which are of interest to aquaculture in Bangladesh (Table 1).

All the species belonging to the major carp sub-group are the natural inhabitants of the freshwater sections of the rivers of Bangladesh, Burma, Northern India and Pakistan (Jhingran and Pullin, 1985). In Bangladesh, these species are mostly found in the Padma-Brahmaputra river system (i.e. Padma, Jamuna, Arial Khan, Kumar and Old Brahmaputra river) and the Halda river system in Chittagong.

On the other hand, all the other species belonging to the minor carps are the natural inhabitants of small rivers and floodplains. Shallow freshwater zones of North-East (Mymensingh, Netrokona and Mohanganj), South-West (Faridpur and Jessore) and North-West (greater Rajshahi area) floodplains in Bangladesh are the natural habitats of the minor carps.

**Table 1.** List of endemic carp species of Bangladesh. Sources: Hasan (1990), Rahman (1985), Hussain and Mazid (2001).

Family	Species	Common name	Local name
Cyprinidae	<i>Labeo rohita</i>	Rohu	Rui
	<i>Catla catla</i>	Catla	Katla
	<i>Cirrhinus cirrhosus</i>	Mrigal	Mrigal
	<i>Cirrhinus ariza</i>	Reba	Laachu, Bhangon
	<i>Labeo calbasu</i>	Calbashu	Kalibaush
	<i>Labeo bata</i>	Bata	Bata
	<i>Labeo boga</i>	Boga labeo	Bhangon
	<i>Labeo gonius</i>	Gonius	Gonnia
	<i>Labeo nandina</i>	Nandina labeo	Nandil
	<i>Bengala elonga</i>	Bengala barb	Along
	<i>Puntius sarana</i>	Barb	Sarpunti
	<i>Tor tor</i>	Tot mahseer	
	<i>Tor putitora</i>	Putitor mahseer	Mahashoal

## 2.2 Exotic carp species

Although Bangladesh is rich in endemic fish genetic resources, the introduction of exotic fish species (mostly Chinese carps) has occurred since 1960. However, such introductions of exotic fish species have not been properly recorded. The only document describing these introductions is a seminar paper entitled “Introduction of exotic fishes in Bangladesh” by Rahman (1985). Subsequently, Bangladesh Fisheries Research Institute (BFRI) maintained its record of new fish introductions for research purposes (Hussain, 1997). A list of different species of introduced species of carps, made on the basis of these records, is shown in Table 2. Additionally, stocks of Chinese carps (silver, bighead and grass carps) were obtained from wild populations in China and are held at the DOF hatchery in Parbatipur in NW Bangladesh.

**Table 2.** List of exotic carp species of Bangladesh. Sources: Hasan (1990), Rahman (1985), Hussain and Mazid (2001).

Species	Common name	Source	Year of introduction
<i>Ctenopharyngodon idellus</i>	Grass carp	Hongkong	1966
		Nepal	1979
		Japan	1970
		China	1994
<i>Mylopharyngodon piceus</i>	Black carp	China	1983
<i>Hypophthalmichthys molitrix</i>	Silver carp	Hong Kong	1969
		China	1994
<i>Aristichthys nobilis</i>	Bighead carp	Nepal	1981
		China	1994
<i>Cyprinus carpio</i> var. communis	Common carp	China	1960
		Vietnam	1995
<i>Cyprinus carpio</i> var. specularis	Mirror carp	Nepal	1979
		Hungary	1982, 1996
<i>Barbodes gonionotus</i>	Silver barb	Thailand	1987
		Thailand	1994
		Indonesia	1994
<i>Tor putitora</i>	Mahseer	Nepal	1991

### 3. Present Status of Genetic Research in Endemic and Exotic Carp Species

#### 3.1 Stock Improvement of Silver Barb, *B. gonionotus*, using Selective Breeding and Line Breeding Techniques

##### 3.1.1 Development of base population and evaluation of growth performance of progenies derived from diallele crosses

The approach used was to obtain wild germplasms from different regions for developing a base population from which genetically improved strains were planned to be established for farming. A sequential breeding design was followed in this work. The diallele crossing of the present work was initiated to accumulate the characteristics of the wild germplasms to increase the magnitude of genetic variance in the progeny generations. Individual (mass) selection of broodstock in subsequent generations also ascertains the chances of genetic gains through accumulation of favorable alleles/traits with high genetic variability in the population (Schom and Bailey, 1986). The breeding programme was initiated involving two new wild germplasms, obtained through ICLARM from Thailand and Indonesia, and the existing local stock in Bangladesh. These three unrelated stocks were kept separately and maintained under intensive care in earthen ponds. Sexually mature fish were mated within their strain until the selection protocol was begun. These interstrain

crosses were termed as Thai x Thai (TxT), Indo x Indo (IxI) and Bangla x Bangla (BxB).

In 1996, parental base populations were made through a complete 3x3 diallele crossing experiment to produce nine different genetic groups. The three purebred (control) strains and six crossbred groups derived from these diallele crosses were stocked communally, using PIT tags, at the advanced fingerling stage (with an equal number of fish from each of the genetic groups) in each pond. A total of six ponds were selected on the basis of their overall ecological conditions (productivity, depth and other physical features) and categorized into “Good”, “Medium” and “Poor” ponds. Each of the test environments had two replicated ponds having the same stocking density. The fish in all the test environments were fed twice daily with a standard formulated feed per day at 2–4% of their biomass. Sampling was performed at monthly intervals to adjust their feed and monitor growth performances. Growth performance of individual genetic groups was evaluated until their maturity and harvest (8 months after stocking). Table 3 shows the mean growth performance data of the nine genetic groups. The only significant differences in final weights were that the TxI and BxI groups were significantly larger than the IxB group. With so many groups in this experiment, it was hard to maintain the homogeneity in stocking size, and the initial weight was significantly different among some of the groups. However, there was no significant correlation between mean initial weight and final weight for the different groups in each pond, so initial weight was not the determinant for final weight. The sex ratios of all the groups did not significantly differ from 1:1.

**Table 3.** Evaluation of growth performances of nine genetic groups derived from diallele crosses in *Barbodes gonionotus*. 1-5 = different sampling dates (1 = initial stocking; 5 = final harvest). Means within each column with different superscript letters indicate significant differences at 5% level.

Group	Mean Weight at sampling dates (g)				
	1	2	3	4	5
IxI	36.01 <sup>a</sup> ±2.90	82.31 <sup>a</sup> ±15.28	105.92 <sup>a</sup> ±14.03	135.00 <sup>b</sup> ±16.06	166.53 <sup>ab</sup> ±15.04
BxT	31.75 <sup>b</sup> ±2.69	81.61 <sup>a</sup> ±15.15	115.32 <sup>a</sup> ±19.16	143.41 <sup>ab</sup> ±31.29	168.92 <sup>ab</sup> ±39.15
BxI	30.20 <sup>b</sup> ±0.81	79.26 <sup>ab</sup> ±13.07	111.58 <sup>a</sup> ±14.61	146.36 <sup>ab</sup> ±31.65	194.63 <sup>a</sup> ±54.86
BxB	29.84 <sup>b</sup> ±6.25	76.92 <sup>ab</sup> ±11.49	112.50 <sup>a</sup> ±18.27	145.00 <sup>ab</sup> ±20.43	187.37 <sup>ab</sup> ±43.84
IxB	27.73 <sup>c</sup> ±2.55	73.69 <sup>bc</sup> ±11.05	103.69 <sup>a</sup> ±16.40	130.86 <sup>b</sup> ±29.91	156.54 <sup>b</sup> ±38.54
TxT	26.90 <sup>c</sup> ±1.62	72.47 <sup>bc</sup> ±12.85	111.42 <sup>a</sup> ±10.66	147.11 <sup>ab</sup> ±20.22	181.43 <sup>ab</sup> ±35.65
TxI	23.52 <sup>d</sup> ±3.56	75.21 <sup>abc</sup> ±12.51	113.15 <sup>a</sup> ±11.58	161.07 <sup>a</sup> ±28.15	197.14 <sup>a</sup> ±37.08
IxT	23.35 <sup>d</sup> ±2.33	68.59 <sup>c</sup> ±14.12	102.57 <sup>a</sup> ±15.32	134.88 <sup>b</sup> ±16.37	169.49 <sup>ab</sup> ±24.27
TxB	17.33 <sup>c</sup> ±2.60	59.82 <sup>d</sup> ±10.49	90.91 <sup>b</sup> ±12.22	137.42 <sup>b</sup> ±24.51	179.61 <sup>ab</sup> ±44.04

### **3.1.2 Evaluation of growth performances of F<sub>1</sub> crossbred and non-selected control groups**

In 1997, the F<sub>1</sub> generation of progeny was produced from the mature parental base populations of each stock (B. I and T). For each of the reciprocal crosses, 5 to 8 pairs were mated separately and the best 3 progeny groups were selected, to make 18 full sib F<sub>1</sub> progeny lines, which were then communally stocked by mixing 125 larvae from each line and grown in nursery, rearing and grow out ponds. An 8 month growth trial was conducted during September'97 to May'98. The ponds were stocked with F<sub>1</sub> crossbred and non selected control (BxB) stock at the rate of 3 fish/m<sup>3</sup>. The fish were sampled at monthly intervals to assess the growth performance and adjust feed ration. The data of monthly mean weight showed higher growth rate of the crossbred group all through the growth period, with final mean weights of 63.25±26.79 g (F<sub>1</sub> crossbreds) and 58.88±14.26 g (BxB control), but no significant differences ( $P>0.05$ ) were observed between these groups.

### **3.1.3 Evaluation of growth performance of F<sub>2</sub> crossbred and non-selected control groups**

During the breeding season of 1998 when all the F<sub>1</sub> crossbred progenies had matured at the age of 10 months, the 20% largest and heaviest females and males were mass selected and held separately in earthen ponds until they were used to produce the next generation in the breeding programme. In the process of individual (mass) selection with respect to sexes, empirical assessment of the breeders was always followed on the basis of their large size, good health and shape and shiny colors. The production of F<sub>2</sub> generation of silver barb was carried out by pool mating of at least 150 pairs of mass selected breeders and 50 pairs of non selected control (BxB) breeders in separate spawning arenas. These mating operations of both selected and non selected breeders were completed within 2 - 3 days, involving 40 - 60 pairs per batch. A comparative growth performance trial of the F<sub>2</sub> crossbred and non-selected control (BxB) groups was conducted in earthen ponds for five months. The stocking density was maintained at a rate of 1.5 fish/ m<sup>3</sup>. There was no significant difference between the mean weights of the two groups of fingerlings at stocking.

At harvest, the F<sub>2</sub> group were larger than the controls (77.41±28.23 g and 70.52±19.79 g respectively), but this was not significantly different. The sex ratios of both crossbred and control groups did not show any significant differences from 1:1.

### **3.1.4 Evaluation of growth performances of F<sub>3</sub> crossbred and nonselected control progenies**

During the breeding season of 1999, further mass selection was carried out among all of the mature F<sub>2</sub> crossbred fish. The 15% best females and males were selected and used for producing the next generation. About 82 pairs of mass selected breeders



and 50 pairs of non selected control (BxB) breeders were separately pool mated to produce the  $F_3$  generation of progeny. An experiment was conducted for the evaluation of growth performances of  $F_3$  crossbred and non-selected control (BxB) progenies for 6 months. The fish were sampled at fortnightly intervals to assess the growth performance and adjust feed ration.

During the initial 3 months the growth rate of the crossbred group was higher than the control group but did not show any significant differences ( $P>0.05$ ). During the next 3 months the crossbred group attained significantly ( $P<0.05$ ) higher growth rate than the control group. At harvest, the mean weight gain attained by crossbred group and control group were respectively 71.8 g and 58.9 g. In this experiment, the average sex ratios of all the replicated chambers in both the crossbred and control groups were not significantly different from the expected 1:1 ratio.

#### **3.1.5 Evaluation of growth performances of $F_4$ crossbred and non selected control progenies**

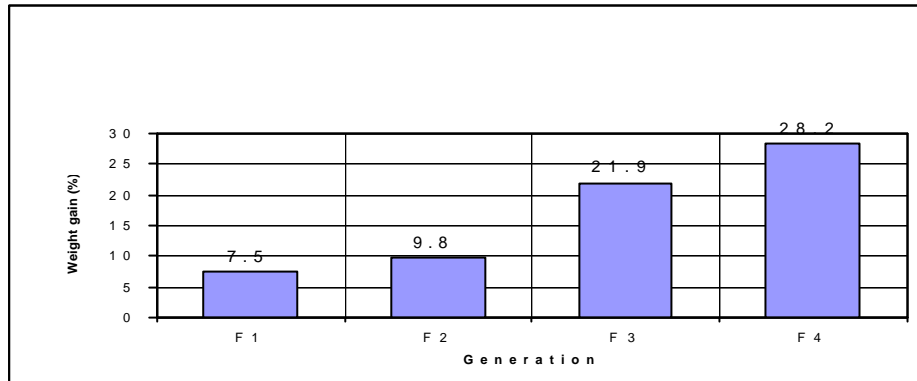
During the breeding season of 2000, mass selection was carried out among the mature  $F_3$  crossbred fish, and the 10% best females and males were selected for producing the  $F_4$  generation. About 165 pairs of mass selected  $F_3$  breeders and 86 pairs of corresponding non selected control (BxB) breeders were used separately in pool mating to produce  $F_4$  progeny. The fish in this growth trial were sampled at monthly intervals to assess growth performance and adjust feed ration.

The crossbred group attained significantly ( $P<0.05$ ) higher growth rate than the control group during four months of growth. The mean weight gains attained by the crossbred group and control group were respectively 112.11 g and 87.43 g.

#### **3.1.6 Generation wise additive genetic gain by crossbred group over non-selected control group**

The generation-wise additive genetic gain was estimated by weight and it was observed that 7.5% genetic gain in growth performance was attained by  $F_1$  crossbred group over non-selected control group. The  $F_2$ ,  $F_3$  and  $F_4$  selected groups attained respectively 2.3, 12.1 and 6.3% cumulative weight gain over three generations of selection. The weight gain values of the  $F_4$  generation of the selected group compared with the non-selected control (BxB) group showed 28.2% superiority over the existing stock (BxB), and the average gain per generation across three generations of selection for growth performance in weight was estimated to be about 7.0%. The present findings suggest that this method for improvement of silver barb through several generations of genetic selection could develop a “Super Strain” of silver barb

after several generations, and might be a useful technique for other carp species in Bangladesh and elsewhere in Asia (Hussain *et al.*, 2002).



**Figure 1.** Additive genetic gain (%) by weight of crossbred group over control (BxB) group in different generations of *Barbodes gonionotus*.

### 3.2. Stock Improvement of Silver Barb using Chromosome Manipulation and Sex Inversion Techniques

Female silver barbs (*B. gonionotus*) have better growth rate than males; therefore, mass production of all female population is desirable for aquaculture. FS, BFRI participated in a collaborative project with the University of Stirling, Scotland and the National Aquaculture Genetics Research Institute (NAGRI), Thailand to produce all female silver barb using the techniques of gynogenesis and sex reversal. It had previously been demonstrated that the meiotic gynogens of this species were all female, suggesting female homogamety (bearing XX genotype) (Pongthana *et al.*, 1995). The protocol for the next step in such an approach was to produce neomales (phenotypic male having XX genotype) through feeding androgen hormone treated feed to the gynogenetic fish. These neomales could then be crossed with normal females for mass production of all female seeds of silver barb.

Thus, for the production of all female silver barb along with large scale neomale, research was conducted using importing neomales from NAGRI, Thailand in view of mating them with females belonging to wild germplasm of Thai and Indonesian stocks. Moreover, production of additional batches of neomale through gynogenesis and sex reversal was initiated (Pongthana *et al.*, 1999).

A study was also carried out on the sexual dimorphism for weight in *B. gonionotus*. This was initially done through sampling of different populations from different culture systems. The sexual dimorphism index for weight ( $SDI_w$ ) was determined by the ratio of mean weight of females to the mean weight of males within one such sample. The  $SDI_w$  values for weight in *B. gonionotus* at the age of approximately one year were found to range between 1.1 to 1.7 in different populations and in different culture systems. Proximate composition of the carcass of female and male fish were also compared where insignificant ( $P > 0.05$ ) differences were found in the case of moisture, protein and ash content; however, fat showed a significant difference between the sexes ( $P < 0.05$ ) (Azad, 1997).

### 3.3 Stock Improvement of Rohu, *L. rohita*, using Selective Breeding Techniques

A genetic stock improvement programme for *Labeo rohita* has recently been initiated. In view of this, land races of rohu, *L. rohita*, collected from different river systems of the country (Brahmaputra and Jamuna), were reared separately in ponds and screened by investigating differences in extrinsic genetic traits by means of morphological assessment. The two wild stocks, Jamuna and Brahmaputra, along with an existing domesticated hatchery stock, were mated to produce three crossbred lines through a 3 x 3 diallel design in 1999. The crossbred lines so produced using the three different stocks were the Hatchery x Jamuna, Jamuna x Brahmaputra and Brahmaputra x Jamuna. The aim was to upgrade the rohu stock for the establishment of a brood-bank in the country. These stocks of three purebred and three crossbred lines were screened by investigating differences in meristic characteristics in a preliminary trial. (Table 4).

**Table 4.** Differences in meristic traits of six genetic groups (purebred and crossbred) of rohu, *L. rohita*.

Name of Stocks	Number of dorsal fin rays	Number of Pectoral fin rays	Number of ventral fin rays	Number of anal fin rays	Number of Lateral line scales	Number of vertebrae
Hatchery	15-16	17-18	8-10	8	42-43	33-34
Jamuna	15	18	9	8	41-42	33
Brahmaputra	14-15	17-18	9	7-8	42	33
Hatchery E X	15	17-18	9	8	42	33-34
Jamuna Γ						
Jamuna E X	14-16	17-18	9	8	41-42	33
Brahmaputra Γ						
Brahmaputra E	15	16-17	9	8	42	33
X Jamuna Γ						

Genetic characterization and comparison of genotypes among various genetic lines will also be conducted through electrophoretic analysis. With all these lines, a selective breeding and line crossing programme will be continued, which will hopefully result in the development of a genetically improved strain having better cultivable traits. Genetic evaluation in terms of growth and other relative performances will be undertaken in nursery and grow out systems as per plan.

### 3.4 Stock Improvement of rohu, *L. rohita*, through Production of Mitotic Gynogens and Genetic Clonal Lines

In view of improving the stock of *L. rohita*, induction of mitotic gynogenesis and production of clonal lines was initiated during 1993/94 at FS, Mymensingh (Hussain *et al.*, 1997). Efforts were made to interfere with the normal functioning of spindle apparatus during mitotic cell division of fertilized eggs using heat shock treatment, thereby leading to the induction of mitotic gynogenesis in F<sub>1</sub> generation (Table 5). Afterwards, putative mitotic gynogenetic alevins were reared as broodstock and a sexually mature female was used to obtain ovulated eggs which were fertilized later with UV irradiated milt. The UV irradiation was carried out for two minutes, applied 5 minutes after fertilization, to produce clonal lines (Table 6) which could be used in a breeding programme to improve stock performance.

**Table 5.** Induction of mitotic gynogenesis in rohu, *L. rohita*.

Trial No.	Parameters	Normal Control (%)	Haploid Control (%)	Meiotic Gynogens (%)	Mitotic Gynogens (%)
1	Fertilization	85	85	87	80
	Hatching	46	2	5	7
	Viability at one week age	37	0	3.4	4.8
2	Fertilization	100	97	97	90
	Hatching	92	9	2.5	3.7
	Viability at one week age	35	0	1.67	2.5

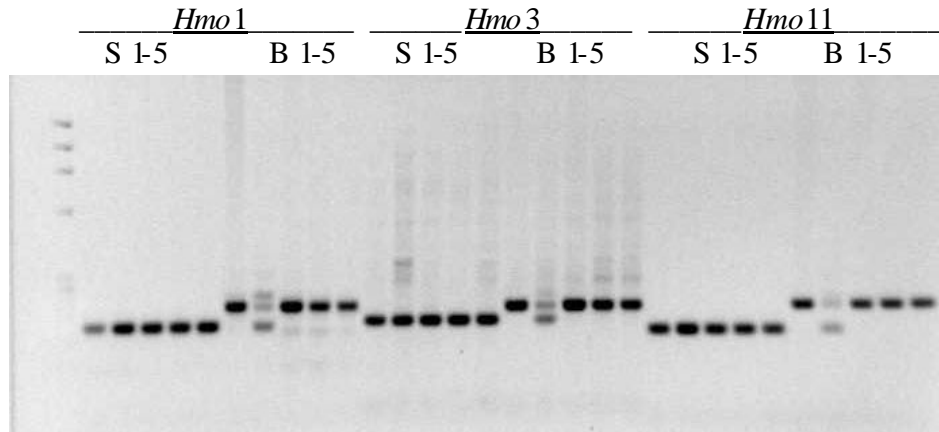
**Table 6.** Production of F<sub>2</sub> clones in *L. rohita* through meiotic gynogens using F<sub>1</sub> brood from mitotic gynogens.

Trial No.	Parameters	Normal Control (%)	Haploid Control UV= 200 $\mu$ Wcm <sup>-2</sup> (%)	Haploid Control UV= 250 $\mu$ Wcm <sup>-2</sup> (%)	Meiotic Gynogens ie. Clones (%)
1	Fertilization	98	76	-	68
	Hatching	92	0	-	5
	Fry at one week age	84	0	-	66
2	Fertilization	87	62	-	64
	Hatching	82	2	-	2
	Fry at one week age	84	89	-	66
3	Fertilization	98	-	72	74
	Hatching	92	-	0	12
	Fry at one week age	84	-	0	66
4	Fertilization	87	-	67	62
	Hatching	82	-	0	2
	Fry at one week age	84	-	0	66

### 3.5 Determination of the Extent of Genetic Introgression (Hybrid Introgression) in Chinese Carps using Molecular Genetic Markers

Hybrid introgression is thought to have occurred in some Chinese major carp populations, where hybrids are being produced both intentionally or unintentionally by the private hatchery operators and sold to the fish farmers and nursery owners. There is every possibility of hybrid introgression between silver and bighead carps, if F<sub>1</sub> hybrids are used as broodstock, that might have negative consequences on the overall performance of these stocks. In view of investigating this, fin samples from 106 silver carp and 47 bighead carp were collected from six private hatcheries at Mymensingh region and from Parbatipur hatchery (which has new, pure stocks of these species). Three microsatellite loci (*Hmo1*, *Hmo3* and *Hmo11*) were found to distinguish between silver and bighead carp in the Parbatipur pure stocks. Agarose gel electrophoresis was used to visualize microsatellite alleles amplified from total genomic DNA extracted from fins.

In Fig. 2, it can be seen that genotypes for all silver carp are faster (FF) and all bighead are slower (SS) except for bighead sample number two. The genotype of this fish was heterozygous (FS) for all three diagnostic loci. This fish, identified as a bighead carp by the hatchery owner, was concluded to be a hybrid between silver carp and bighead carp.



**Figure 2.** Three microsatellite loci (*Hmo1*, *Hmo3* and *Hmo11*), with in each case 5 silver (S1-5) and 5 bighead (B1-5) carp samples. Bighead sample #2, from a hatchery, shows a hybrid genotype for all three loci.

### 3.6 Genetic Stock Improvement through Interspecific Hybridization and Chromosome Manipulation

#### 3.6.1 Interspecific hybridization between endemic and exotic barbs

A study was conducted on the interspecific hybridization between two barb species, *P. sarana* and *B. gonionotus*, along with the comparative observation on the embryonic development of the reciprocal hybrids and control groups at FS, BFRI (Begum, 1996). Hybridization was attempted between two different species through fertilizing the eggs of each species with the heterospecific sperm. The rates of fertilization, hatching and survival of embryos of the reciprocal hybrid and control groups were observed. The rates of fertilization (55-62%) and hatching (19-25%) of reciprocal hybrids were found to be significantly lower ( $P < 0.01$  and  $P < 0.05$  respectively) than the rates of fertilization (73-81%) and hatching (49-56%) of the controls. Likewise, both the control groups produced significantly higher ( $P < 0.05$ ) percentage of normal embryos (52-59%) in comparison to both the hybrids (23-24%). No significant differences were found between the two controls and the two hybrid groups. The length frequency distribution of the newly hatched larvae of the control and hybrid groups showed that the hybrid, *B. gonionotus* female x *P. sarana* male was significantly smaller ( $P < 0.01$ ) than the controls and the reciprocal hybrid.

### **3.6.2 Radiation-induced heat shocked gynogenesis in rohu and mrigal**

Meiotic gynogenesis was induced by giving heat shock to eggs fertilized with UV irradiated sperm. Sperm was irradiated with a constant dose of UV-rays for 2 minutes from a distance of 13.5 cm. In rohu, 4 minutes after fertilization, when heat shock was applied at 40 °C for 2 minutes, gynogenesis was induced in 80 to 90% cases. This optimum heat shock regime was found to be similar for mrigal. A temperature of 39 °C was more effective and induction rate of gynogenesis was 80 to 100%. Survival of gynogens in both species was low (2 to 40%) compared to that of normal control (30 to 60%).

### **3.6.3 Induction of triploidy in rohu by heat shock treatment and comparative growth with normal diploid**

Triploidy was induced in rohu, *L. rohita*, by applying heat shock to eggs fertilized with normal sperm at 40 °C for 2 min. starting 4 min. after fertilization (Islam *et al.*, 1994). Their ploidy status was determined karyologically, with triploid induction rates of 60%. The survival rate within the first five to seven days after hatching was recorded at 15% in heat-shocked group and 25% in the control. Growth in induced triploids after 18 weeks was significantly higher than in diploids (for weight,  $p < 0.01$ , and for standard length,  $p < 0.05$ ).

## **4. Genetic Conservation of Some Endangered Carp Species**

Because of natural and man induced phenomena occurring in aquatic ecosystems, the natural breeding and feeding grounds of some of the important floodplain and riverine fishes have been severely degraded. Open water capture fisheries are under great stress and their sustainability is in danger because of changing aquatic ecosystems, soil erosion, siltation, construction of flood control and drainage structures, dumping of agro-chemicals and industrial pollutants. In addition, indiscriminate and destructive fishing practices have caused havoc to the aquatic biodiversity. Although fish are the primary source of protein for over 1 billion people of the world, aquatic biodiversity remains a neglected issue (Maclean and Jones, 1995). Recent estimates suggest that worldwide 20% of all freshwater species are extinct, endangered or vulnerable (Moyle and Leidy, 1992). As a result, fish stocks particularly those dwelling in inland open water areas, have gradually become endangered. IUCN, Bangladesh (2000) has documented about 54 freshwater fish species critically or somewhat endangered including 11 carp and barb species (Table 7). There is a need, therefore, for development of artificial breeding and seed production techniques of such carp species for genetic conservation of their "gene pool" and biodiversity.

**Table 7.** List of endangered carp and barb species of Bangladesh.

Scientific name	Local name	Critically Endangered	Endangered	Vulnerable
<i>L. nandina</i>	Nandina	X		
<i>L. boga</i>	Bhangan	X		
<i>L. gonius</i>	Ghonia		X	
<i>L. bata</i>	Bata			X
<i>L. pangusia</i>	Ghora maach		X	
<i>L. calbasu</i>	Kalbasu		X	
<i>C. ariza</i>	Laachu, Bhangan			X
<i>P. sarana</i>	Sarpunti	X		
<i>P. ticto</i>	Tit punti			X
<i>T. tor</i>	Mahashol	X		
<i>T. putitora</i>	Mahashol	X		

#### 4.1 Development of artificial propagation techniques

Since 1990 BFRI, Mymensingh, has begun to conduct research on the conservation of fish biodiversity and has successfully developed a package of technology for artificial breeding and seed production of some important threatened carp and other fish species. For *Labeo calbasu*, *L. gonius*, *L. bata*, *Cirrhinus ariza*, *Puntius sarana* and *Tor putitora*, recommended breeding techniques were developed (Table 8).

The injected females and males are kept in spawning hapas where they can be spawned naturally or stripped. Ova of fully ripe female *T. putitora* can be stripped manually and a hormone injection is generally not required. The stripped ova can be fertilized with the freshly collected milt of males. Fertilized eggs are then left for incubation in incubation jars and pools at an ambient water temperature.



**Table 8.** Details of the artificial breeding technique of endangered carp and barb species.

Species	Preliminary dose (PG mg/kg)	Interval between two doses (hours)	Decisive Dose (PG mg/kg)	Ovulation (hours after decisive dose)	Hatching (hours after fertilization)
<i>Labeo calbasu</i>	Female 2.0	6	Female 6.0 Male 2.0	6 – 7	18 – 20
<i>Labeo gonius</i>	Female 2.0	6	Female 5.0 Male 2.0	7 – 8	16 – 18
<i>Labeo bata</i>	Female 1.0	6	Female 5.0 Male 1.0	7 – 8	16 – 18
<i>Cirrhinus ariza</i>	Female 1.0	6	Female 5.0 Male 1.0	7 – 8	14 – 16
<i>Puntius sarana</i>	-	6	Female 5.0 Male 2.0	6 – 7	14 – 16
<i>Tor putitora</i>	No requirement of hormone injection. Water flushing during spawning season induce female to be ripe which are stripped to collect ripe eggs.				72 – 80

### 5. Future Genetic Research Plans on Carp Species

- Genetic characterization of wild land races of catla and rohu;
- Initiation of genetic stock improvement of catla using selective breeding and line crossing techniques;
- Continuation of genetic stock improvement of rohu using selective breeding and line crossing techniques;
- Genetic evaluation and dissemination of Genetically Improved silver barb and continuation of selection;
- Continuation of project activities on 'Genetic improvement strategies for production in exotic carps for low input aquaculture in Asia';
- Continuation of genetic conservation of selected endangered carp species

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