Genetic Improvement and Conservation of Carp Species in Bangladesh

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carp seed are being stocked in grow-out ponds or even in open water bodies like floodplains under the government's massive carp seed stocking program. 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 in the pure wild stocks that would adversely affect the government's planned aquaculture and inland open water fish production.

Although the government is seriously making all out efforts to rehabilitate the inland fisheries, it has focussed its attention on aquaculture, which has tremendous potential in the country. In accordance with government objectives, the Bangladesh Fisheries Research Institute (BFRI), since its inception in 1986, has stressed fish genetics research under its Freshwater Station (FS), Mymensingh, breeding and stocking programs. To avoid loss of genetic diversity and inbreeding depression in hatchery populations, research has focused on the development of improved brood stocks through implementation of effective breeding plans for commercially important carps and other fish species. Such efforts are directed toward the goal of producing improved breeds for quality fish seed production in a large number of private and public hatcheries.

1.2 Potentials of fishery resources, species diversity and fish production target of the Fifth Five Year Plan (1997-2002)

Bangladesh, a country of deltaic plains dominated by the main major river systems like Ganges, Brahmaputra and Megna, is endowed with unique water resources comprised of both inland and marine waters (Figure 1 and Table 1). Inland water resources of the country cover an area of 4,339,694 ha of which 93% comprise open waters and 7% closed water bodies. The major inland open water capture fisheries are covered by floodplains (2,832,792 ha) ; rivers and estuaries (1,031,563 ha); beels and haors (natural depressions 114,161 ha), etc. The only reservoir, the Kaptai Lake, has an area of 68,800 ha. The inland closed-water fishery areas are comprised of ponds (146,890 ha), oxbow lakes (5,488 ha) and coastal shrimp farms (140,000 ha). In addition, marine water resources cover a total area of 16,606,600 ha in the form of internal water (2,515,100 ha); territorial water (906,470 ha); coastline (480 km); coastal polders (87,300 ha); continental shelves (8,515,300 ha) and 200 nautical miles of Exclusive Economic Zone (EEZ).

Because of the heavy seasonal river flow and monsoon rains, 34% of the country is considered wetlands that may be under water for at least 6 months of the year. The country has millions of small ponds, seasonal water-filled ditches, borrow pits,

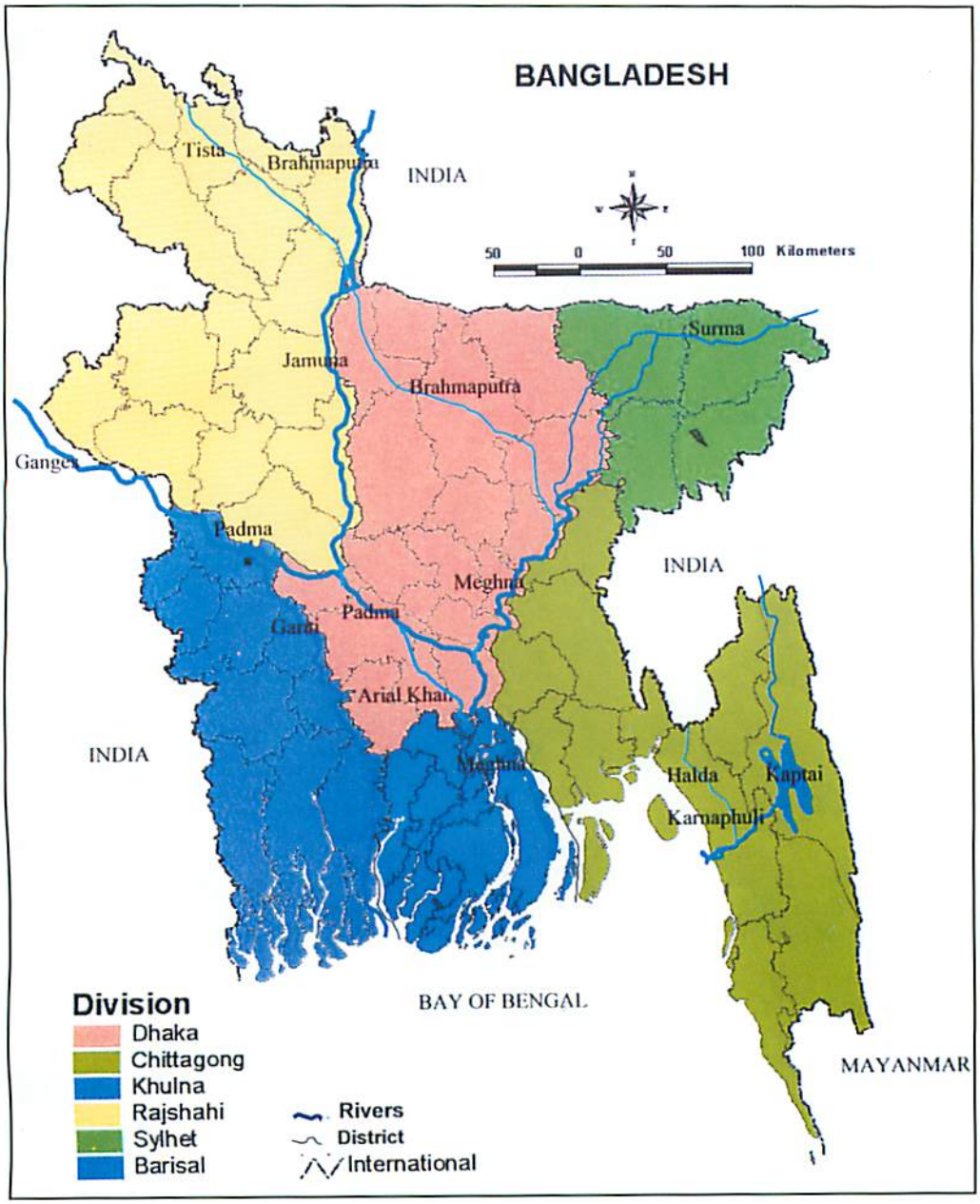

Fig. 1. Map showing main river systems of Bangladesh.

flooded paddy fields, and expansive flood plains that all have potential for producing increased yields of fish through managed aquaculture practices.

Along with extensive water resources, the country is also rich in diversity of fish species. It has approximately 296 fresh and brackish water fish species (including freshwater prawns) and 511 marine species (including marine shrimp). In 1996-1997 the per capita per day fish consumption was about 27 g, which was nearly 60% of all animal protein consumption. To increase the level of fish consumption to 34 g per capita per day by the end (2001-2002) of the Fifth Five Year Plan (FFYP) period, therefore, the fish production target has been set at 2.075 million mt (Table 1).

Table 1. Statement of water resources and fish production targets of Fifth Five Year Plan (FFYP) in Bangladesh

Water resources	Area (hectare)	Production in '000' mt		
		1996-97	1997-98	2001-2002 (targeted)
1. Inland fisheries				
a. Open water bodies				
Floodplains	2 832 792	362	378	751
Rivers & tributaries	1 011 563	169	164	180
Beels & haors	114 161	63	68	95
Kaptai Lake	68 800	6	6	9
Total open water bodies	4 047 316	600	616	1035
b. Closed water bodies				
Ponds	146 890	404	484	450
Oxbow lakes	5 488	3	3	27
Shrimp farms (coastal)	140 000	79	88	163
Total closed water bodies	292 378	486	575	640
Total inland water bodies (a + b)	4 339 694	1086	1191	1675
2. Marine fisheries				
Trawling		13	15	-
Artisanal		261	257	-
Total marine waters	16 606 600	274	273	400
Country total	20 946 294	1360	1464	2075

Source: DOF (1999)

1.3 Fish and aquaculture production trends

During 1988–98 the total fish production of the country increased from 841,000 to 1,464,000 mt (Table 2). Of the fish and shrimp production (1997-98), 42% comes from inland open water capture; 19% from marine capture and 39% from aquaculture (Figure 2). As in many other countries, aquaculture production in Bangladesh has rapidly expanded in recent years because of adoption of various improved aquaculture technologies (Figure 3). During 1988-1998, aquaculture production increased from 184,000 mt to 575,000 mt. Therefore, the contribution of aquaculture to total fish production has progressively increased from 22% in 1988-89 to 39% in 1997-98.

Table 2. Total fish and aquaculture production trends 1988-1998

(Production in '000' mt)

Year	Total Fish Production	Inland Open-water Capture	Marine Capture	Aquaculture (Fish + Shrimp)	Aquaculture Contribution (%)
1988-89	841	424	233	184	22
1989-90	856	424	239	193	23
1990-91	896	443	242	211	24
1991-92	952	479	246	227	24
1992-93	1021	533	250	238	23
1993-94	1091	574	253	264	24
1994-95	1173	591	265	317	27
1995-96	1249	600	270	379	30
1996-97	1360	600	274	486	36
1997-98	1464	616	273	575	39

Source: DOF (1999)

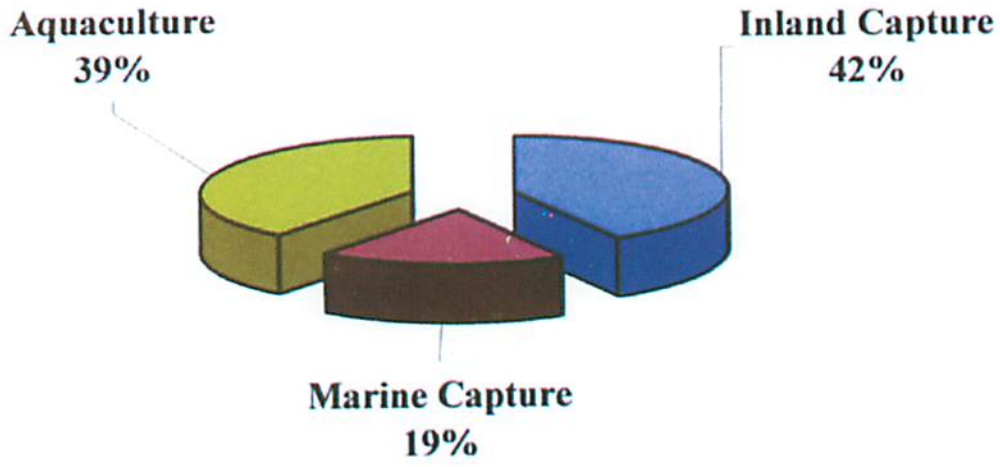

Fig. 2. Proportion of inland capture, marine capture and aquaculture production in Bangladesh (1997-98).

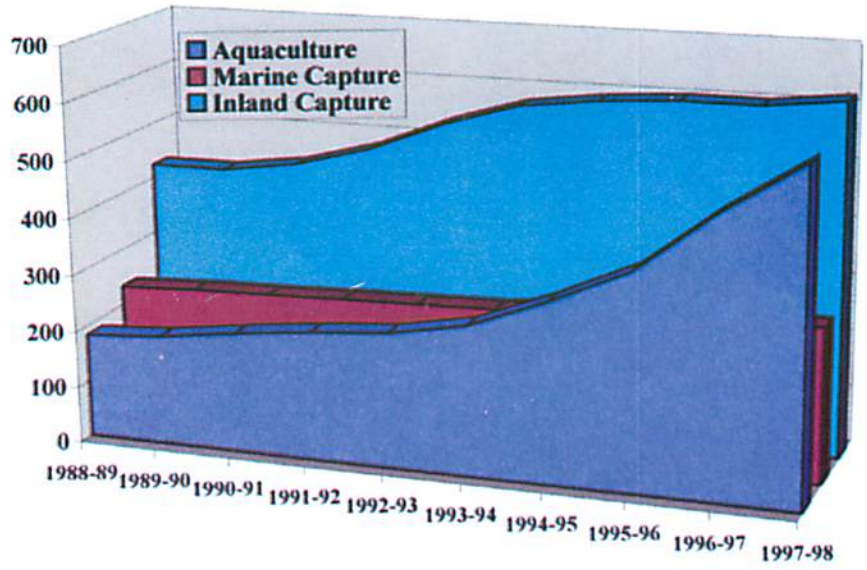

Fig. 3. The trend in inland capture, marine capture and aquaculture production in Bangladesh from 1988-1998.

CHAPTER 2

Status of Carp Genetic Resources

2.1 Background information on different species of endemic and exotic carps

2.1.1 Endemic carp species

The endemic carp species of Bangladesh can be divided into two sub-groups i) major carps (e.g., catla; rohu; mrigal; calbashu etc., and minor carps, e.g., bata; nandil; gonnia etc.). Most of the freshwater river systems and floodplains of the country are the natural breeding grounds for all the major and minor carps. All these carp species belong to the family Cyprinidae. The family includes the groups like carps, barbs and a large variety of minnows. All the major and minor carps and some of the barb groups are commonly called carp. There are at least 13 species of carps (belonging to carp and barb groups) under 6 genera in Bangladesh (Table 3).

Table 3. List of endemic carp and barb species of Bangladesh

Family	Species	Common name	Local name
Cyprinidae	*Labeo rohita*	Rohu	Rui
	Catla catla	Catla	Katla
	Cirrhinus mrigala	Mrigal	Mrigal
	Cirrhinus ariza	Reba	Bhangan
	Labeo calbasu	Calbashu	Kalibaush
	Labeo bata	Bata	Bata
	Labeo boga	Boga labeo	Bhangan
	Labeo gonius	Kuria labeo	Gonnia
	Labeo nandina	Nandina labeo	Nandil
	Bengala elanga	Bengala barb	Along
	Puntius sarana	Olive barb	Sarpunti
	Tor tor	Tor mahseer	Mohashol
	Tor putitora	Putitor mahseer	Mohashol

Source: Froese and Pauley (2000); Hasan (1990); Rahman (1985)

All the species belonging to the major carp sub-groups 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 available

in the Padma-Brahmaputra river system (i.e., Padma, Jamuna, Arial Khan, Kumar and Old Brahmaputra rivers) and in the Halda river system in Chittagong. Their favorite habitat is the deep pools of these rivers. During the time of monsoons they naturally breed in the inundated rivers and flowing waters.

Among the giant carps, mahseer, *Tor* spp., are the inhabitants of hilly streams of Mymensingh, Sylhet, Dinajpur districts and Kaptai Lake of Chittagong Hill Tracts. The natural spawning grounds of mahseer are sandy bottoms among pebbles and aquatic weeds, where temperatures are relatively high and dissolved oxygen is sufficient (Pathani 1982).

All the other species belonging to the minor carps are the natural inhabitants of small rivers and floodplains. Shallow freshwater zones of northeastern (Mymensingh, Netrokona and Mohanganj), southwestern (Faridpur and Jessore) and northwestern (greater Rajshahi area) floodplains are the natural habitats of the minor carps. They naturally breed there and new spawn grow to maturity within a short period of time (10–12 months).

2.1.2 Exotic carp species

Although Bangladesh is rich in endemic fish genetic resources, introduction of different varieties of fish species (mostly Chinese carps) has been occurring since 1960. But such introductions of exotic fish species have not been properly recorded. The only document available is a seminar paper entitled "Introduction of exotic fishes in Bangladesh" by Rahman (1985). Subsequently, the BFRI has maintained its record of new fish introductions for research purposes. A list of introduced species of carps is made on the basis of those records and furnished in Table 4.

Among the introduced carp species, the major Chinese carps (viz. silver carp, grass carp, bighead carp, black carp, common carp, etc.) and exotic barbs (silver barb and mahseer) are predominant. A brief history of their introductions in the country is given below:

Silver carp

Silver carp naturally dwells in the Chinese river systems (Yangtze, West River, Kwangsi and Kwangtung in south and central China) and in the Amur basin in the former USSR. In recent years, the species has been introduced in many countries of Asia and elsewhere. In Bangladesh, the fish was first introduced from Hong Kong in 1969. In 1970, a second consignment of 282 silver carp juveniles were imported from

Japan and transported to the Freshwater Fisheries Research Station, Chandpur (Rahman 1985).

Table 4. List of introduced or exotic carp and barb species of Bangladesh

Family	Species	Common name	Source	Year of introduction
Cyprinidae	*Ctenopharyngodon idella*	Grass carp	Hong Kong	1966
			Nepal	1979
			Japan	1970
	Mylopharyngodon piceus	Black carp	China	1983
	Hypophthalmicthys molitrix	Silver carp	Hong Kong	1969
	Aristichthys nobilis	Bighead carp	Nepal	1981
	Cyprinus carpio var. communis	Common carp	China	1960
			Vietnam	1995
	Cyprinus carpio var. specularis	Mirror carp	Nepal	1979
			Hungary	1982, 1996
	Barbodes gonionotus	Silver barb	Thailand	1987, 1994
	Tor putitora	Puntitor mahseer	Nepal	1991

Source: Hussain (1997); Hasan (1990); Rahman (1985)

Grass carp

The natural habitat of grass carp is the flatland rivers of China and the middle and lower reaches of the Amur River in the former USSR. Meanwhile, grass carp has been introduced into many countries of the world. In Bangladesh, the first batch of fingerlings was brought from Hong Kong in 1966 and reared in ponds of the Freshwater Fisheries Research Station, Chandpur. A second batch of 300 fry was imported from Japan in 1970. In 1979, a third batch of fingerlings and adults was introduced from Nepal and kept in Raipur Hatchery Complex, Laxmipur. According to Rahman (1985), these were successfully bred in 1980 and seeds were distributed to several seed multiplication farms and to the Aquaculture Experiment Station, Mymensingh, of the DOF.

Bighead carp

Bighead carp is the natural inhabitant of Chinese rivers like Yangtze, West River, Kwangsi and Kwangtung. This species has been transplanted into many countries for

aquaculture (Jhingran and Pullin 1985). In 1981, bighead carp fingerlings were imported from Nepal and reared in the Raipur Fish Hatchery Complex.

Black carp

Black carp is also an inhabitant of Chinese rivers. About 2000 fry were brought from Kwantung Province, China and introduced into Bangladesh by the DOF (Rahman 1985). These were distributed into several hatcheries including the hatchery of the former DOF's Aquaculture Experiment Station (presently Freshwater Station of BFRI), Mymensingh.

Common carp

The homeland of common carp is probably China but the fish enjoys a worldwide distribution covering tropical and temperate regions and has been acclimatized to a wide range of habitats and environmental conditions. There are numerous strains of common carp with three general groupings based on different scale paterns, viz., i) scaled carp, *Cyprinus carpio* var. communis; ii) mirror carp, *C. carpio* var. specularis; iii) leather carp, *C. carpio* var. nudus. It has been reported that scaled common carp were first introduced in Bangladesh by DOF in 1960 (Rahman 1985). Recently, a batch of 1000 individuals of genetically improved common carp has been brought from Vietnam through ICLARM and transported to the FS, BFRI, Mymensingh. In 1979, the mirror carp was first introduced in Bangladesh from Nepal and reared in Raipur Fish Hatchery Complex. Francois Rajts (personal communication) reported that a second batch of pure strain carp was introduced from Hungary during the middle of 1980's and successfully bred in Jessore.

Mahseer

Mahseer is the natural inhabitant of hilly upland rivers and streams of Bangladesh, India and Nepal. A batch of 1000 fingerlings of *T. putitora* was transplanted from Nepal to the FS, BFRI, Mymensingh (Mahata et al. 1995).

Silver barb

Silver barb is an indigenous food fish of Thailand, locally called "rajputi' or "Thai shaputi" and in some other countries as "tawes". The favorite habitat of this species is rivers and floodplains. Silver barb has already been introduced in many Southeast Asian countries for its palatability and marketability and for its ease of breeding and high potential yield. In 1987, the fish was first introduced into Bangladesh from Thailand (Nuruzzaman 1988). Meanwhile, a successful induced breeding technique

for the species was developed in Bangladesh as in ASIAN countries (Hussain et al. 1987). During March 1994 two more broodstock for germplasm enhancement of silver barb were introduced from Thailand and Indonesia under the auspices of ICLARM for genetic stock improvement research at FS, BFRI, Mymensingh.

2.2 Habitat degradation and carp biodiversity in the natural ecosystems

In recent years natural fish stocks have declined because of natural and man-made degradation of environments and reduction of wetlands resulting in the loss of suitable habitats for a large number of riverine and floodplain endemic fish species. As a result many valuable indigenous fish species have been threatened or endangered.. Human effects on ecosystems have included water pollution from industrial, agricultural and municipal wastes; construction of embankments for flood control; and river dams for irrigation and hydroelectric generation. Over the past 15 years, the water flow into Bangladesh from upstream rivers has been reduced by 25% and the downward trend is expected to continue. All of these factors interfere directly or indirectly with fish migration, reproduction, and survival and ultimately reduce biodiversity within the aquatic ecosystems.

2.2.1 Impact on riverine ecosystems

Siltation in the upstream part of the major river systems is a serious problem, which reduces the rate of water flow and navigational capacity and causes habitat degradation, the loss of spawning and grazing fields of riverine fishes and the reduction of natural seed production in most of the river spawning grounds. Dumping of municipal wastes and disposal of industrial wastes has seriously threatened riverine ecosystems. Disposal of toxic wastes from sugar and paper mills, the tannery industry, and fertilizer factories into the Karnaphuli, Buriganga Rupsha and Shitalokkha river drainages has caused high levels of alkalinity and extensive eutrophication (Safiullah 1987). Industrial and other forms of pollution cause gradually detrimental changes in the genetic stock structure of riverine fish populations. Injudicious and destructive fishing practices are also responsible for the elimination of riverine stocks. The use of fine-mesh nets to catch juveniles and young wild fish, including carp, has become a common practice resulting in the reduction of the genetic base and natural stock structure of riverine fish populations.

2.2.2 Impact on floodplain ecosystems

Floodplains are the major part of inland open water ecosystems and may retain water for 4-6 months of the year in shallow seasonal wetlands. These floodplains are the

most suitable spawning and feeding grounds of many freshwater minor carp and barb species. Such wetlands have suffered drastically from the impacts of a burgeoning human population. In the Ganges-Brahmaputra floodplain alone, approximately 2.1 million ha of wetlands have been lost to flood control, drainage, and irrigation development (Khan et al. 1994). Most of these floodplains and the naturally connected deeper-water areas like beels and baors have been subjected to heavy siltation resulting from the construction of a large number of Flood Control and Drainage (FCD and Flood Control, Drainage and Irrigation (FCDI) structures. These projects have impeded the feeding and breeding migration of many important fish species of the floodplains and have had a negative effect on stock recruitment. The estimated total area underwater was formerly 6.3 million ha of which 0.81 million ha have now been recovered by flood control measures. Catch rates in areas affected by the FCDI projects have been reduced as much as 75% according to fisherman studies (Nishat and Bhuyan 1995). It is estimated that 2.0 million ha of floodplains with a yearly production of over 1.0 million mt of fish will be lost to development projects by the year 2005.

2.2.3 Impact on rice field ecosystems

Bangladesh has about 12 million ha of land in rice production, of which more than 2.5 million ha are subject to uncontrolled flooding of 1-3 months during the 3-5 months of monsoons. These rice fields are the natural habitats of many wild fish, including minor carps. Farming of high yielding varieties of rice (HYV), with the concomitant use of harmful insecticides , has resulted in the total disappearance of fish from many rice fields. The use of pesticides and fertilizers in rice fields is steadily increasing. Annually 4000-5000 mt of 242 different types of pesticides and 1.8 million mt of fertilizers are used in Bangladesh. The indiscriminate use of hazardous chemicals for crop production is extremely detrimental to resident fish and other aquatic fauna. A survey reveals that the killing of fish by pesticides mainly occurs through the use of improper dosages, the use of banned chemicals, and aerial spraying for mosquito control, etc. Organochlorinated pesticides are highly toxic to fish and other aquatic organisms.

2.2.4 Over all impact on fish spawning and migration

The reproduction, migrations, and availability for stocking of many fish species, including carp, have been threatened and endangered by alterations of aquatic ecosystems. Reproduction is a particularly vulnerable part of the life cycle and is regulated by many exogenous and endogenous factors. Fish species found in tropical

rivers, floodplains, and seas need suitable temperatures, acceptable ranges of salinity, adequate water depths and flows, optimum photo periods and adequate food supplies to achieve the gonad growth, regulated by hypothalamic and pituitary hormonal levels, necessary for the completion of gamete development. Fish migrations to suitable spawning habitat are also hampered by dams and channel modifications plus there is loss of suitable spawning substrate as a result of sedimentation, channelization and other physical modifications to the water systems.

2.3 Role of different carp species in fish production

Endemic and exotic major carps are the main aquaculture species in Bangladesh. Carp polyculture is an age-old practice that was formerly limited by lack of proper scientific technology. During the 1990's, production increased sharply as improved technology was adopted (Table 5). About ten years ago (1988/89) carp contributed 15% to total fish production and 69% to aquaculture production; while in recent years (1997/98), it contributed 35% to total fish production and 90% to aquaculture production.

Table 5. Yearly per cent of carp species in total fish production of Bangladesh

Year	Endemic carps	Exotic carps	Total
1986/87	12	1	1
1987/88	12	1	13
1988/89	14	2	16
1989/90	14	2	16
1990/91	15	3	18
1991/92	16	3	19
1992/93	15	3	18
1993/94	16	4	20
1994/95	16	5	21
1995/96	19	6	25
1996/97	23	8	31
1997/98	25	10	35

CHAPTER 3

Brood Stock Management and Artificial Breeding of Carp Species in Hatcheries

3.1 Biology of carp reproduction and artificial techniques for their seed production

Traditional subsistence fish culture was mainly based on the collection of unsorted fish seed from natural spawning grounds such as rivers, large canals, and floodplains. In most cases pond aquaculture programs are jeopardized by the use of such mixtures of desirable and undesirable seed. Over the last few decades, techniques of artificial or induced fish breeding have been developed for carps and other species, meeting the demand of a rapidly growing fish farming sector for quality seed. In addition to supplying fish seed for aquaculture production, artificial fish breeding techniques have made possible the genetic improvement of fish stocks through use of chromosome manipulation, hybridization, selective breeding, family line crossing methods; and have helped to conserve the "gene pool" of some critically endangered fish species.

3.1.1 Biology of fin fish reproduction

Fish reproduction is a rather complicated process, which in nature is controlled by environmental conditions such as water temperature, salinity, light, rainfall, substrate, availability of natural feeds, etc. Under favorable conditions the gonads respond to hormones produced by endocrine systems of the breeders so that viable ova and sperm are produced and mature and reproduction occurs.

Development of ova and sperm

Development of gonadal products (ova and sperm) is a long process. In the female fish, ovum development starts with the oogonia, then primary oocytes which give rise to secondary oocytes. During the preovulatory period, high-energy yolk (vitellogenin) deposition occurs in the growing oocytes. The secondary oocytes complete yolk deposition by the continuing process of vitellogenesis and remain as

mature ova in the ovary for variable periods of time until changes stimulate their final release or ovulation (Stacey 1985).

In the males, the process of sperm development starts with the spermatogonia, which develop into primary spermatocytes, which give rise to secondary spermatocytes and then to spermatozoa or mature sperm. Very similar to the mature ova, the sperm also remain dormant in the testes until the onset of environmental/hormonal changes which trigger the final process of spawning.

Environmental and hormonal regulation of the natural spawning of fish

In a normal course of gonadal development the ova or sperm remain in a dormant or resting phase in the gonads until the requisite internal and external environments stimulate a part of the brain known as the hypothalmus to secrete small peptide hormones called releasing hormones. These hormones pass a short distance to the ventral surface of the brain where they control the activity of gonadotropic cells of the pituitary gland. In turn these cells secrete gonadotropic hormones into the blood of the fish from which they control all structural and functional changes in the testes and ovary (Bromage 1992). As a result, the detachment of eggs from the ovary and release of sperm from the testicular cells occurs in most fin fish accomplishing natural spawning (Figure 4).

But in a situation where riverine or flood plain spawners are kept captive in closed environments like lakes, ponds, tanks; their gonadal cycles are arrested and the gametes remain dormant until they eventually breakdown and are resorbed. Under such unfavorable environmental conditions where their own gonadotropic hormone can not be released from the pituitary gland, gonadotropic hormone extracted from the pituitaries of donor fish can be injected into spawners to stimulate final ovulation and spawning. (Woynarovich and Horvath 1980; Hussain 1985, Hussain 1988).

3.1.2 Artificial fish breeding techniques

Propagation of common carp

The common carp has been recognized as a domesticated species for farming in a wide range of Asian aquaculture systems, including Bangladesh. This fish is preferred by farmers because of its fast growth and ease of reproduction.

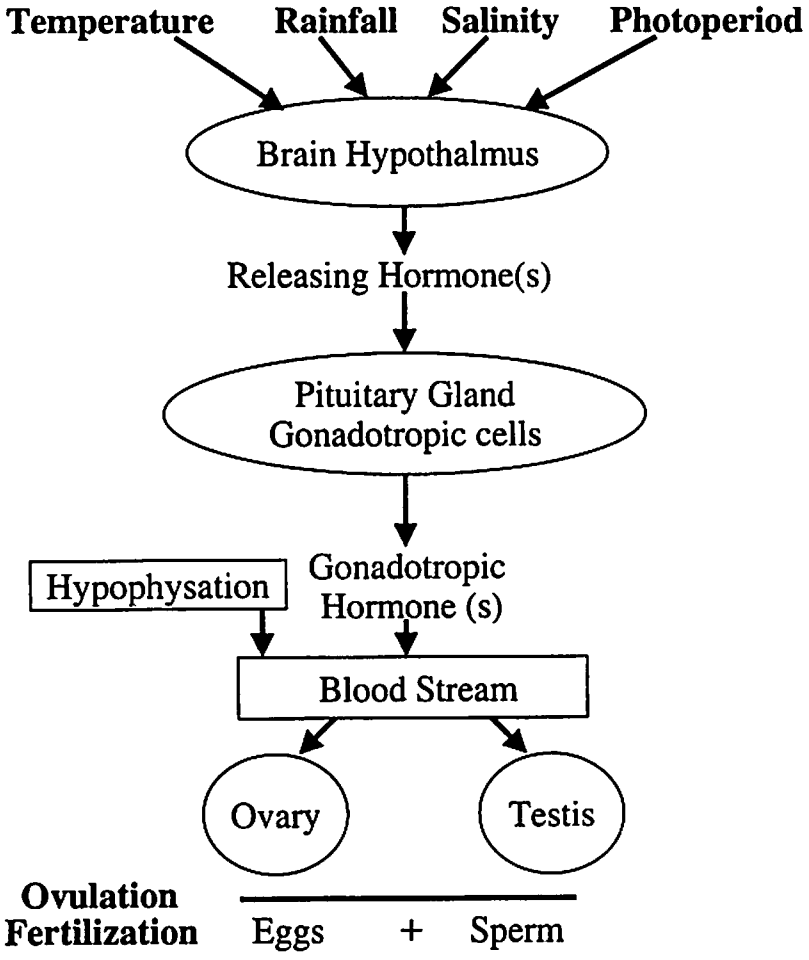

Fig. 4. Environmental and hormonal regulation of natural an artificial spawning of fish (modified after Bromage 1992)

Both scaled common carp and mirror carp females attain sexual maturity at the end of their second year of life, when they become 500 to 800 g in weight. The males mature earlier than the females. For spawning purposes, the best result may be expected from females weighing 3 to 4 kg and males 2 to 3 kg. Selection of ripe spawners is made on the basis of empirical observations of their health, abdomen and vent. A female common carp is considered sexually mature, when its belly is large but not swollen, not hard but not too soft, and the genital opening is swollen protruding and reddish in color. A male is considered ready for spawning when its milt (whitish sperm fluid) can be pressed out by applying a slight pressure with the fingers on the hind part of the belly near the genital opening.

The controlled natural propagation of common carp is carried out in net cages (size 5 m x 3 m x 1.25 m) or mini conbcrete tanks or earthen ponds, when water temperature is 20 to 24^{0}C. In each net cage or concrete tanks/earthen ponds, 2 to 3 sets of spawners per 15 m^2 area are transferred from brood ponds (it is best to keep the sexes in separate ponds) by selecting the best specimens. A set of spawners generally consists of one female and two males of similar size. For collecting eggs at the time of spawning, optimum quantity of aquatic grasses like Hydrilla, Najas, water hyacinth or floating kakabans (artificial egg collectors made of coconut fiber) are placed in the net cages or concrete tanks/earthen ponds. By the next day when spawning is finished, all the aquatic grasses or kakabans with attached eggs are thinned out from the net cages and distributed to a number of concrete cisterns/mini earthen ponds for hatching and provided with a continuous flow of clean and fresh water. The length of hatching period (60 to 150 hours) is inversely correlated with water temperature ranges between 17 to 25^{0}C. When hatching is finished, all egg collectors are removed from incubating cisterns/ponds and the tiny spawn are allowed to grow to the first feeding stage.

Similarly, by keeping the female and male spawners together, semi-artificial propagation of scaled common carp and mirror carp can also be initiated using a hypophysation technique (i.e., administration of pituitary extract or HCG solution by injection) where spawners do not breed naturally in a fluctuated temperature or any other environmental conditions (for PG dose see Table 6).

Artificial propagation of cultured carp species

Pituitary materials collection, preservation and preparation of extract solutions

Prior to beginning of artificial propagation of both endemic and exotic carps, sufficient number of fresh pituitary glands (PG) are mostly collected from live, as

well as dead but fresh and sexually matured common carp. Pituitaries are taken out from the head of fish with the help of a hand saw and a forceps. The fish head is cut by a saw between the nostrils and the top of the eye socket. and then the top of the skull is removed. The brain is then turned up and forward with a forceps to expose the pituitary gland. The pituitary gland is carefully removed from the area just below the brain with the aid of a needle and fine forceps. Immediately after collection, the glands are defatted by washing in acetone or absolute alcohol. After 2 to 3 washings, pituitaries are finally preserved in a vial filled with acetone or alcohol.

Just prior to hypophysation, the required quantity of pituitaries is thoroughly pulverized in a small porcelain mortar or a tissue homogenizer apparatus. Then the desired quantity of 0.7% physiological saline solution is added. The solution thus prepared is centrifuged using a manually operated centrifugal apparatus. The solution can be kept on the table for at least 30 min. Then the pale supernatant portion of the solution is withdrawn into a hypodermic syringe for injection.

Table 6. Optimum female hormone doses for the artificial propagation of different carp species

Species	Preliminary dose	Interval between two doses (hours)	Final dose	Ovulation (hours after final dose)
Labeo rohita	PG 2 mg/kg	6.0	PG 6 mg/kg	6.0
Catla catla	PG 1-2 mg/kg	6.0	PG 5-6 mg/kg	6.0
Cirrhinus mrigala	PG 2 mg/kg	6.0	PG 5-6 mg/kg	6.0
Ctenopharyngodon idella	PG 1-2 mg/kg	7.0	PG 4-6 mg/kg	6.0
Hypophthalmichthys molitrix	HCG 200 IU/kg	12.0	HCG 500 IU + PG 3 mg/kg	7-8
Aristicthys nobilis	HCG 200 IU/kg	12.0	HCG 500 IU + PG 3 mg/kg	7-8
Cyprinus carpio	-	-	PG 3 mg/kg	7-8
Barbodes gonionotus	-	-	PG 4-5 mg/kg	6.0

Induced breeding techniques

For artificial propagation, several sets of sexually matured spawners of cultured carp species are selected from brood stock ponds by choosing the best specimens. Set(s) of breeders are kept in separate concrete holding tanks prior to hypophysis or human chorionic gonadotropic (HCG) hormone injection. For injection, the breeders are

taken out of the water with a scoop net and then wrapped in soft towels. Intramuscular injection is always given by inserting the needle of the syringe at the base of the dorsal, pelvic or caudal region of the spawners . Hypophysis/HCG injection of female fish is always made by administering one or two doses (Table 6). Males are injected with a single dose of PG or HCG hormone solution and kept together with female partners in circular spawning arenas or suitable concrete tanks provided with a continuous flow of fresh clean water. The males take part in spawning just after the start of ovulation of the females; therefore, natural fertilization of eggs occurs in most of the carp. Prior to spawning a sufficient quantity of aquatic grasses or egg collectors should be placed in the breeding tanks of common carp or mirror carp to collect the sticky fertilized eggs.

For artificial fertilization of eggs, the spawners should be stripped immediately after they become ready for ovulation or spawning. First females are stripped into a plastic bowl. Then males are stripped over the same container. The ova and milt in the bowl should be mixed together with a strong feather for 1 min, then clean water is added to the mixture.. The eggs should be left in a wide bowl with more clean water for few minutes for hardening (in this stage eggs swell to several times their original size). Then the fertilized eggs should be washed carefully 3 to 4 times by changing the water and then removing broken, unfertilized eggs. Completely swollen eggs are transferred for hatching into a Chinese type circular incubating pool or a series of funnel type incubating jars, regulated and connected with a flow of clean water. The hatching of eggs is generally completed within 20 to 30 hours, if the water temperature of the incubating system remains 24 to 27^{0}C.

Just after hatching, the carp larvae normally face a very critical period. During that time a high rate of mortality may occur because of inadequate aeration or any other poor condition of the incubating system.. The incoming water should be free of plankton which may contain "Cyclops" (a zooplankter belonging to Copepod group), that may kill the larvae/spawn during early development. Transfer to well-prepared nursery ponds or suitable cement cisterns should be done within 4 to 5 days when the early fry (as newly hatched fish are called) begin swimming.

3.2 Fish hatcheries in public and private sectors

During the 1970's the public sector of the country began producing quality fish seed through artificial breeding techniques for government fish farms by establishing a number of fish hatcheries. During the middle 1980's basic training in fish breeding and hatchery operation and management was undertaken, initially by DOF, and later by BFRI. The private sector was instrumental in building a good number of

hatcheries. During 1988 the public and private sectors established 77 and 162 hatcheries, respectively. The number rapidly increased during the last ten years to 776 with the private sector predominating in the development of fish hatcheries in the country (Table 7).

Table 7. Number fish hatcheries and spawn production in Bangladesh

Year	No. of hatcheries		Spawn production (kg)	
	Public sector	Private sector	Natural sources	Hatcheries
1985			19 362	4 962
1986			13 222	6 287
1987			22 008	8 339
1988	77	162	12 533	6 849
1989	84	185	12 236	5 664
1990	89	204	5 128	14 773
1991	89	218	6 855	24 683
1992	89	222	9 342	35 851
1993	102	256	5 069	48 964
1994	102	389	5 872	72 536
1995	141	502	9 144	88 272
1996	141	600	2 399	103 615
1997	141	631	2 824	115 888
1998	141	635	2 885	117 700

3.3 Brood stock management scenario in the hatcheries

Recent regional advances of aquaculture, in particular carp polyculture and other related freshwater fish farming of rice fields, seasonal ditches, canals and perennial ponds, have resulted from the introduction of induced breeding or artificial propagation techniques during the late 1950's. In Bangladesh, artificial breeding of endemic carp seed has become a common practice since 1967 (Ali 1967). Meanwhile, Chinese carps, exotic barbs, and catfishes have been introduced and a large number of hatcheries in the private sector (estimated at about 635) have been established (Ali 1998). These hatcheries are presently contributing 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).

In this country, as in India, most hatcheries rear their own brood stock and usually do not recruit individuals from natural sources or exchange breeders between farms. Each hatchery, therefore, can be considered as an isolated, self-sustaining and genetically closed unit (Eknath and Doyle 1990). Some hatcheries are careful about maintenance of their brood stock but many may not. The result can be an accumulation of genetic effects from generation to generation that lead to severe permanent genetic changes in the stocks. In genetically closed hatchery systems, potential selective pressures exerted on finite and often small culture populations by selection of founder stock, the number of breeders maintained, the method of replenishing brood stock, stocking density, feeding methods, etc., can result in 'indirect' or 'negative' selection and inbreeding and genetic drift (Doyle 1983).

A study based on the data on various carp species from several fish hatcheries located in Jessore, Comilla and Mymensingh showed that most of the hatchery operators had no basic knowledge of simple brood stock management (Hussain and Mazid 1997). They did not follow any scientific principle on guideline in selecting adequate sized breeders, injecting proper hypophysation dosage, or mating unrelated male and female spawners, etc. Such ignorance of hatchery operators leads to unconscious negative selection from the use of undesirable size and left over brood stock and generation after generation mating of breeders from closely related or finite populations. As a result, stock deterioration in hatchery populations, mainly carps and barb species, has generally occurred.

3.4 Problems identified with existing fish stocks and hatchery operations

Because of a concern about probable genetic deterioration in hatchery stocks due to inbreeding, a team of scientists from BFRI (Dr. M.G. Hussain) and ICLARM (Dr. E. Eknath and Dr. M.V. Gupta) visited Jessore and Comilla during the last week of March 1994 to investigate and gather information pertaining to the practices followed by the hatcheries for brood stock handling and management. A workshop, "Broodstock management and opportunities for genetic improvement of cultured fish species," was organized during March 30-31, 1994, at BFRI, Mymensingh, attended by a large number of managers of government and private hatcheries, scientists, and planners. During March 1995 similar workshops were organized by BFRI and DOF in Rajshahi, Jessore and Faridpur. June to August 1999 additional training workshops were organized by BFRI and DOF in five main divisions, viz., Dhaka, Chittagong, Rajshahi, Khulna and Barisal, involving a large number of hatchery/nursery operators from both private and public sectors.

The BFRI/ICLARM team's investigation and the extensive discussions in the BFRI/DOF's workshops concluded that the stock deterioration in hatchery populations might be caused by poor brood stock management (i.e., unconscious negative selection or use of breeders of undesirable size), inbreeding depression (possibly from sibling or parent vs. offspring mating) and the ignorance of private hatchery managers regarding overall hatchery maintenance and operation.

3.4.1 Brood stock replacement and handling method followed by govenment and private hatcheries

The majority of fish hatcheries in Bangladesh replenish brood stock from either: i) internal sources (from the hatcheries themselves or from nurseries that received spawn from that particular hatchery) or ii) external sources (fingerlings/breeders collected from grow-out ponds that had received fingerlings from other hatcheries or from natural riverine sources). A source of unconscious 'negative' selection in some hatcheries has been the collection of fish for brood stock replacement from the smaller fish left in grow-out ponds after relatively bigger (and hence faster growing) individuals had been sold. Negative selection also results when hatcheries inadvertently use left-over fingerlings (after selling faster growing, hence 'good' fingerlings) from nursery ponds, for brood stock raising. In a given spawning season, it is generally the bigger breeders that are induced to spawn during the early part of the season and relatively smaller individuals that are spawned during later part of the season. If the fish seed produced early in the spawning season from bigger brood fish (probably faster growing) are sold and seed produced in the later part of the season from smaller brood fish (probably slow growing) are retained to build up hatchery brood stock, the ultimate result will be 'indirect' or 'negative' selection (Figure 5). Negative selection is one of the major causes of reduced growth potential and loss of other positive performance traits of fish farmed in Bangladesh.

3.4.2 Use of undesirable size of breeders

In the aforementioned workshops, an important concern raised by the hatchery operators, from Jessore and Mymensingh was the use of very small size brood fish, even less than 500 g each in weight and whether this would have any effect on the quality of hatchery seed production. Later, this problem was documented through survey and farm interviews for most of the fish species normally used for artificial seed production (Table 8). It was observed that the use of undesirably small breeders was a common practice in most of the private hatcheries and that this caused severe stock deterioration problems resulting in poor growth and survival in grow-out systems.

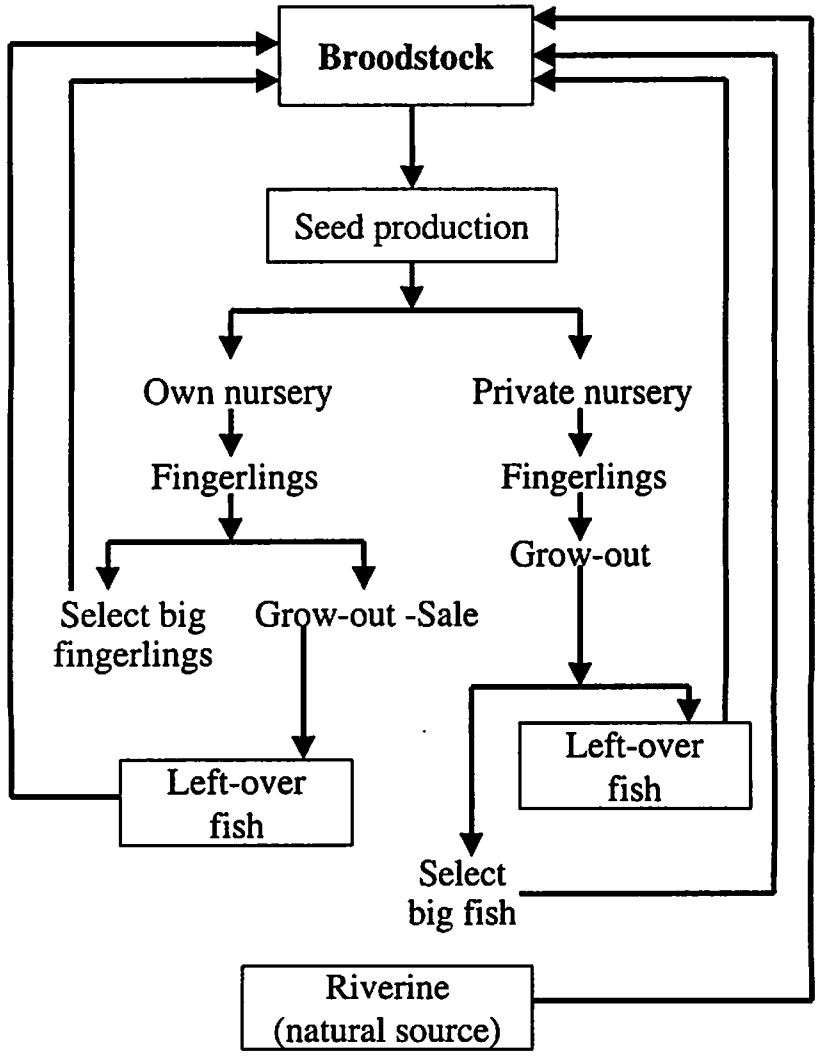

Fig. 5. Brood stock replacement practices followed by hatcheries in Bangladesh

Table. 8. Undesirable size grade of brood stock normally beinused in many private hatcheries in Bangladesh

Group	Common name	Weight (kg)	Remarks
Carp	Rohu	0.5 – 0.8	+++
	Mrigal	0.5 – 0.8	+++
	Catla	1.5 – 2.0	++
	Silver carp	0.8 – 1.0	++
	Bighead carp	0.8 – 1.5	++
	Grass carp	1.0 – 1.5	++
	Mirror carp	0.6 – 1.0	+
	Common carp	0.5 – 0.8	+
Barb	Silver barb	0.1 – 0.15	++
Catfish	Pangas	1.0 – 1.5	++
	African catfish	0.4 – 0.7	+
	Asian cat fish	0.1 – 0.15	+

Note: +++ = Extremely undesirable, ++ = Moderately undesirable; + = Fairly undesirable;

3.4.3 Inbreeding depression in hatchery stocks

With the recent rapid expansion of fish culture, farmers have come to depend upon private and public hatcheries for fish seeds. There is a likelihood of inbreeding in most small hatcheries where female and male breeders are chosen from finite (small) populations and there is a greater chance of sibling (brother-sister) or closely related fish crossing. This is the reason why most farmers may have experienced a decline in fish growth rate in more recent times. It has been suggested that the breeding programs used by most fish farmers will produce inbreeding of 3 to 5% per generation. If this occurs, inbreeding depression could begin to affect productivity and profits after only 3 to 5 generations (Tave 1999). Moav and Wohlfarth (1976) state that a single full sib mating of a particular fish might result a 10 to 20% depression of growth rate and a considerable proportion of individuals might show physiological abnormalities. Effects of inbreeding on rainbow trout *Oncorhynchus mykiss* populations were intensively studied by Kincaid (1976; 1983) where he found that one generation of brother sister mating produced inbreeding of 25%; two generations produced inbreeding of 37.5%; three generations produced inbreeding of 50%; and four generations produced inbreeding of 59.4%. In general, inbreeding studies with fish have shown that inbreeding decreased production phenotypes such as growth rate, fecundity, and survival, while increasing the number of deformed offspring (Tave 1999). Because of generations of inbreeding and accumulation of unfavorable alleles from close mating, genetic deterioration of the existing cultured

stocks might make them less suitable for culture. An inbred or homozygous population normally loses its general vigor. That means all performance traits like growth and survival rate, fertility; reproductive performance, disease resistance, etc., are reduced. The observed reduction in such performances of inbred population is termed as 'inbreeding depression', which might have a severe effect on aquaculture production.

3.4.4 Genetic introgression because of interspecific hybridization of carps

Interspecific hybridization in some carp species has recently been reported in this country. Either out of scientific interest or because of shortage of adequate hatchery populations (i.e., brood stock), introgressed hybrids are being produced intentionally or unintentionally by private hatchery operators and sold to farmers and nursery operators. These hybrids ultimately are being stocked, knowingly or unknowingly, either in grow-out ponds or in open water bodies like floodplains under the government's massive carp seed stocking program. There is wide spread concern that mass stocking of such hybrids in the floodplains and other related open water bodies might cause a serious genetic introgression problem that could adversely affect aquaculture and inland open water fish production. There is every possibility of segregation of the genes with the result that some of the fish carrying the introgressed genes could not be easily distinguished from the pure species.

Hybrid introgression in major carp species is very likely to have negative consequences as a result of loss of distinct feeding strategies of the pure species, which are the basis of successful carp polyculture systems (Mair 1999). If introgressed hybrids reproduce in natural water bodies or are used as brood stock in hatcheries, they will not be true breeders; therefore, collection of carp seed from the pure species/strain will be difficult.

3.4.5 Ignorance of private hatchery operators

A study based on the thorough investigation of a number of hatcheries located at Jessore, Commilla, Bogra and Mymensingh revealed the following:

- Hatchery operators have very poor knowledge of brood stock handing and management; viz., the need to recruit new breeders at regular intervals, stocking density of breeders, balanced feeding, basic disease identification and control, and water quality maintenance in stocking ponds, etc.

- Hatchery operators do not follow scientific principles and guidelines in maintaining record keeping systems for the management of brood stock and spawning operations. Problem areas include the selection of brood fish of undesirable sizes, injection of incorrect hypophysation dosages, the mating of related female and male breeders, and stocking of improper quantities of fertilized eggs into hatching jars/pools/units, etc.

There is no doubt that such ignorance of hatchery operators might lead to negative selection, hybrid introgression, random use of undesirable size of brood stock and generation after generation mating of breeders from the finite population. As a result, deterioration of hatchery produced seed quality has occurred.

3.5 Short and long term plans for brood stock development in hatcheries

A hatchery must have short and long term strategies to avoid the problems of negative selection and inbreeding and ultimately to build up its own genetically improved brood stock. The following steps should be followed in brood stock replenishment to minimize 'indirect' or 'negative' selection:

- The base population should be collected from natural open waters or known sources. Records should be kept at each hatchery including: location of collection, date of transfer, species, size and weight of the stock, and number of individuals stocked into each nursery/rearing pond;

- Selection of fast growing and best looking individuals should be initiated just before or immediately after maturation. The best procedure is to select a few individuals from as many brood stock sources as possible.

- Selected brood stock should be isolated and the hatchery manager/operator may also consider marking (e.g., Alcian blue) or tagging (e.g., numbered plastic tags or more sophisticated PIT tags) of individuals of different year classes.

- Further selection can be made by choosing the healthiest and most mature breeders.

- Hatchery produced spawn/seeds from different selected stocks should be stocked separately or in a pool by taking equal numbers from each lot. Records should be kept of the number of breeders used in each lot with their tag numbers, if

possible; date of spawning; date of hatching; date of stocking; and the number of individuals stocked in each nursery/rearing pond.

Stock deterioration caused by accumulated inbreeding can be avoided in the following ways:

- Brood stock should be collected and produced as much as possible in a hatchery. A medium type hatchery should keep at least 3000 to 5000 breeders per species, to allow selection of the best performers in terms of size, maturation and breeding efficiency.

- Pedigree records should be carefully maintained in each hatchery to reduce or avoid the chance of mating between closely related breeders and the accumulation of unfavorable alleles causing 'inbreeding depression.'

- Minimize inbreeding by the exchange of brood stock among hatcheries. Care should be taken that the stock exchanged should not be from the same collection source (except the base population from a river or natural source).

- An "effective population size (Ne)" should be maintained in a hatchery for a good breeding program. Ne should be maximized to minimize loss of genetic variation. This can be achieved by retaining equal numbers of individuals from all spawning lots or sets for future use as brood stock and maintaining a 1:1 ratio of contributing males and females. Such measures will allow the chance of family size variation to be controlled and the additive genetic gains in each generation to be equalized. The recommended number of Ne may vary from 50 to 1000, but inbreeding will rapidly occur if Ne is regularly below 50, as in many hatcheries (Mair 1999).

- A well planned selective breeding and line crossing program should be adopted in most hatcheries to improve desirable traits. Collection of wild broodstock germplasm and use of well designed breeding plans could increase the proportion of favorable alleles while maximizing heritability and genetic variability and minimizing the level of inbreeding.

Further suggested guidelines

i. The government. should impose restrictions on minimum size and age of fish to be used for breeding to control indiscriminate use of poor and inferior

quality brood stock for seed production by the hatcheries. Desirable size grade of different carp, barb and other species (particularly the female breeders) should be: rohu wt.= >1.0 kg, age=2 yr; mrigal wt.=>1.0 kg, age=2 yr; catla wt.=>3.0 kg, age=3 yr; silver carp wt.=>1.5 kg, age=2 yr; bighead carp wt.=>2.0 kg, age=2.0 yr; grass carp wt.=>2.0 kg, age=2.0 yrs mirror carp/common carp wt.=>1.0 kg, age=1.0 yr; silver barb wt.=>0.2 kg, age=1.0 yr; pangas (*Pangasius sutchi*) wt.=>2.0 kg, age=2.0 yr; African catfish (*Clarias gariepinus*) wt.=0.8 kg, age=1.0 yr; Asian catfish (*C. batrachus*) wt.=>0.2 kg, age=1.0 yr.

ii. For better seed production in hatcheries appropriate dosages for hormone injections of carp species should be followed.

iii. DOF lead hatcheries, FS, Mymensingh and Riverine Station, Chandpur, of BFRI should function as a permanent and central 'brood bank' where wild germplasm will be preserved and maintained, and thereafter genetically improved brood stock will be developed through a rotational line crossing scheme under the supervision and guidance of highly experienced fish geneticists/hatchery biologists.

iv. DOF should develop at least 25-30 sub-centers of 'brood banks' in its seed multiplication farms to cover the whole country. These centers would collect improved brood stocks from central 'brood banks' and distribute them to private hatchery operators.

A 'gene bank' should also be established and maintained separately or under the DOF/BFRI central hatcheries so that gametes (particularly the sperm of improved brood stock) could be preserved through cryopreservation techniques. Such preserved sperm later could be distributed to public and private hatcheries for fish breedin

v. Genetic stock improvement through inter-generic or inter/intra specific cross breeding/hybridization of cultured fish species should be initiated under well-designed breeding plans at research institutes and lead central hatcheries under the guidance of fish breeding specialists/biologists. Many private hatchery operators in this country hybridize fish without knowledge of fish breeding science and genetics that may cause deterioration of hatchery populations. Therefore, the government should immediately ban the unplanned fish hybridization practices being carried out by the private hatchery operators.

vi. Periodic assessments of hatchery conditions should be made throughout the country by a team of senior fish breeding scientists/specialists.

vii. DOF lead center hatchery staff/hatchery managers of sub-centers, NGO and private hatchery operators should regularly be trained on simple brood stock management, breeding plan development and stock improvement aspects at BFRI's lead research station(s) and Fisheries Academy at Savar, Dhaka.

viii. A manual on 'Brood stock Management Guidelines' should be formulated, edited, printed and widely circulated among all hatchery operators throughout the country.

ix. Periodic regional workshops/seminars should jointly be organized by DOF and BFRI involving participants from GO/NGO hatchery managers, DOF's regional officers, Thana fishery officers, private hatchery operators, etc.

CHAPTER 4

A Breeding Plan for Cultured Minor Carp Species

4.1 A breeding program and the genetic improvement of silver barb

Background information

Silver barb (Figure 6a) has become a popular culture species because of its rapid growth, good market demand and ease of culture under a variety of different conditions.. The fish was first introduced into Bangladesh from Thailand in 1977. But management practices for its culture were not widely available and circulated until the late 1980s based on the efforts of BFRI and the NGO BRAC (Gupta and Rab 1994). The technology has been extended to rural farmers as a low-cost and small-scale homestead aquaculture enterprise and silver barb now show up in most carp polyculture systems across the country including rice cum fish systems. As *B. gonionotus* gains popularity and farmers mainly depend on private and public hatcheries for stocking material, the likelihood of inbreeding is great because female and male spawners are chosen from finite populations for mating. This close inbreeding causes homozygosity to increase rapidly with a typical loss of genetic vigor. Such genetic deterioration of hatchery populations of *B. gonionotus* all over the country has been reported (Hussain 1998). Silver barb have also been susceptible to diseases, particularly Epizootic Ulcerative Syndrome (EUS), that particularly occurs at the onset of the winter cold season, perhaps because of temperature stress to this tropical species (Lilley et al. 1998). Good genetic management and selection has the potential to help overcome both of these problems.

In 1994 ICLARM provided BFRI with two wild lines of *B. gonionotus* from Thailand and Indonesia. The existing strain that was introduced from Thailand in 1977 is also being used for the designated breeding program. The scientists of FS, BFRI, have been trying to develop a genetically improved strain of female silver barb using these three founder stocks through selective breeding and chromosome manipulation techniques (Hussain 1997). The genetic improvement of this fish has quickly drawn the attention of farmers in this country because of its survival and growth capabilities and its potential to benefit aquaculture production. Now founder stocks with potentialities of superior growth, survival, fertility and disease resistance need to be developed through further genetic research to replenish the next generations of brood stocks to ensure the availability of quality and healthy seeds to the farmers (Figure 6b). Recent results have been encouraging (Hussain et al. 2000).

a

b

Fig. 6. Silver barb. **a.** *Barbodes gonionotus;*
b. Genetically improved breed of silver barb.

General and spawning biology

Silver barb grows well on low protein diets, whether feeding on certain aquatic plants or given supplementary feeds, and can tolerate a wide range of environmental conditions (Bentsen et al. 1996). The suitable range of water quality parameters under which the fish can survive well are: water temperatures 25 to 30^{0}C; pH 7.0 to 8.0; dissolved oxygen 2.0 to 8.0 mg/l; salinity 0 to 7 ppt. In tropical productive pond conditions, silver barb can grow from 15 to 250 g in 5 to 6 months and 500 to 800 g in 10 to 12 months. Females grow 20% faster than males (Pongathana 1997). The male:female ratio in natural populations remains 1:1.

In Bangladesh, silver barb breeds April to July when water temperature remains around 25 to 28^{0}C. Artificial breeding is mainly carried out by injecting females and males with PG extract hormones. Females are given a single injection at a decisive dose of 4-5mg PG per kg body weight and the males are injected with a half dose at the same time. Both sexes are kept for spawning in circular concrete tanks with clean flowing water. Natural spawning normally occurs within 5 to 7 hours after the decisive injection. The number of eggs resulting is estimated at about 250,000 to 350,000 per kg body weight. The hatching of fertilized eggs is normally completed within 16 to 20 hours at a water temperature of 26-28^{0}C. The larvae/spawn attain the first feeding stage within 3 to 4 days after hatching. In some cases, controlled natural spawning of silver barb is carried out by water flashing, but such practice is not very common in Bangladesh. Brood stocks are normally used for 2 to 3 breeding seasons and spawning may be repeated 4 to 5 times with an interval of 30 to 45 days in a single season (Bentsen et al. 1996).

4.1.1 Stock improvement of the silver barb using selective breeding and line crossing techniques

A most important objective is to improve the growth rate of the silver barb. The additive genetic variation of growth rate has not yet been studied in Bangladesh, unlike other neighboring countries. If the variation is similar to that of other fish species, the growth rate may easily be improved by individual (mass) selection. A breeding program should be designed to avoid loss of genetic variation and to avoid rapid accumulation of inbreeding (Bentsen et al. 1996). Such a breeding plan requires a large effective population size with a large number of breeders (at least 50 to 100 pairs) to be used in each generation.

Collection of the base population

At least 3 to 4 unrelated stocks (strains) need to be gathered from different locations to form a base population for the planned breeding program. As mentioned earlier, there are currently three unrelated strains of silver barb (two wild obtained from Thailand and Indonesia and the existing local stock of Bangladesh) available for selective breeding and line crossing programs in hatcheries.

Initiate the breeding program

Breeding of silver barb can be initiated in April/May. The three unrelated strains should be mated using 3 by 3 complete crossing design (i.e., diallel crossing scheme) to produce 9 genetic groups as shown in Table 9. The rationale behind this cross breeding scheme is to form a heterogeneous, out bred population.. The 6 crossbred and 3 purebred groups would be separately reared in suitable earthen ponds to maturity. In case of pond limitations, communal stocking of these groups could be made at the advanced fingerling stage (20 to 30 g in weight) using digital PIT tags or numbered plastic tags.

Table 9. A mating design for the production of a base population of silver barb (*B. gonionotus*) through 3 X 3 diallele crossing pattern

♀ from different locations	♂ from different locations		
	1	2	3
1	-	X	X
2	X	-	X
3	X	X	-

Location 1: Thai stock; Location 2 : Indonesian stock; Location 3: Existing Bangladeshi stock

For each of the reciprocal crosses, 5 to 10 pairs (sex ratio female to male 1:1) are mated separately, producing about 50 full sib progeny families. All matings are to be

performed within 1 to 2 days. The fertilized eggs are incubated in a series of funnel jars or units and hatchlings are kept separately in 50 hapas (fine-mesh net suspended in the water) until the first feeding stage. As nursing of all the sibling groups in separate hapas or nursery ponds might not be possible, 250 larvae could be counted from each of the 50 hapas (from each of the sibling groups), and communally stocked in a nursery pond until the fry could be transferred to a communal rearing pond and later on to a grow-out pond (Figure 7).

For communal stocking in rearing ponds, there should be about 5000 fry (assuming about 60% mortality rate in nursery ponds), stocking density could be maintained at 10 fry/m^2. In rearing ponds, the expected mortality rate of the fingerlings would be about 40%, therefore, about 3000 fingerlings would be available for stocking in grow-out ponds. For brood stock development, fingerlings could be stocked at the rate of 1 to 2 fish/m^2. During all phases of the growing period, the fish should be fed with protein rich feeds and at the age of 10 mon, at least 2400 to 2500 breeders would be ready for individual (mass) selection.

Selection of sexually matured breeders

During the months of April/May when all the communally stocked fish are sexually mature (10 mon of age), 10% of the largest females and males may be selected and kept separately in earthen ponds until they are used for the planned breeding program. Individual selection of the breeders is followed based on empirical assessment of best size, good growth and shiny appearance.

At the time of selection of the largest breeders, 50 sexually mature females with average female body weights and 50 sexually mature males with average male body weights should also be selected and kept separately to provide a control group, essential to a well-designed breeding plan.

Production of the next generation(s) and evaluation of growth performance

The production of the next generation of silver barb can be carried out by pool mating of at least 150 pairs of mass selected breeders and 50 pairs of average non selected breeders in a spawning arena. Such a mating operation of both selected and non selected breeders should be completed separately within 2 to 3 days involving 40 to 50 pairs per batch. Larvae obtained under such a pool mating operation are assumed to contain an equal share of progenies from each pair of breeders. For evaluation of growth performance, 100 to 150 larvae/m^2 of best selected breeders and non selected breeders are stocked separately in at least three replicated nursery ponds for one month. At this stage the early fry can be fed with a finely sieved mixture of

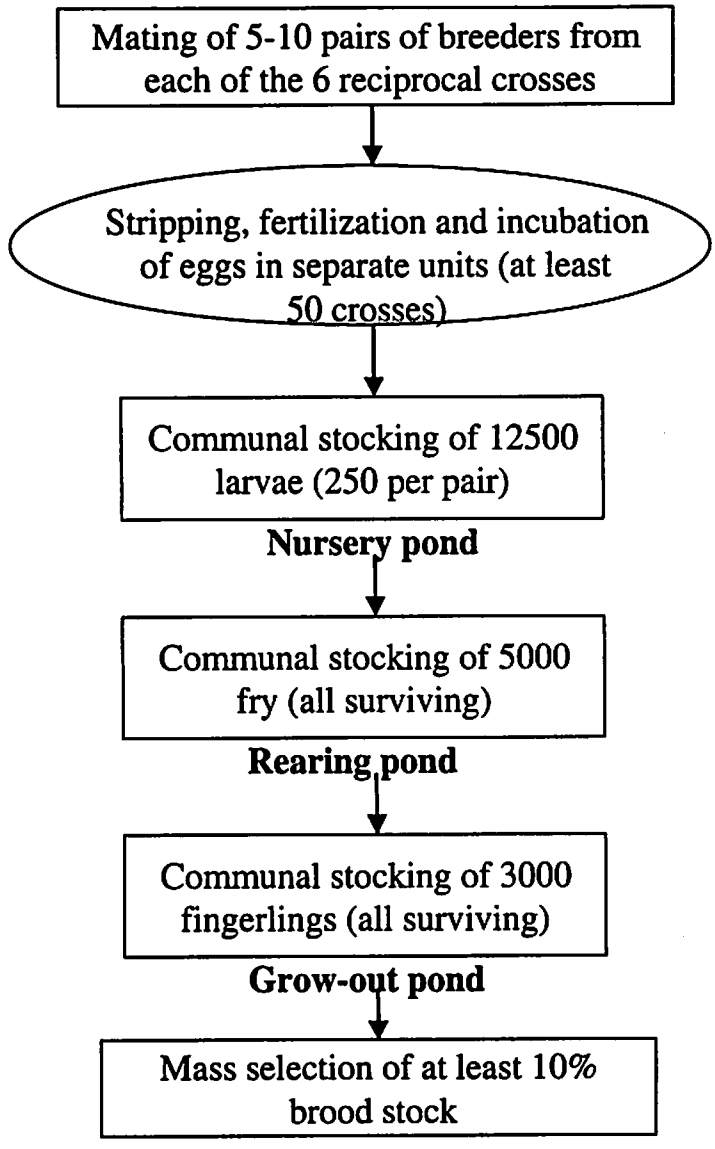

Fig. 7. Design for mating and reproduction of parental brood stock to produce F_1 progeny of *B. gonionotus*, communal rearing of progenies (in nursery, rearing and grow-out ponds) and mass selection of brood stock.

rice bran (30%), wheat bran (30%), mustard oil cake (30%) and fish meal (10%) or commercially available fry feeds at the rate of 10 to 12% of estimated body weight. The fry are to be sampled at weekly intervals to assess their growth performance and adjust the feed ration. All surviving fry after the nursing period are stocked in replicated rearing ponds at a rate of 10 fry/ m^2 and reared for 50 to 60 days until fingerling size and then transferred to replicated grow-out ponds for growing and maturation (Figure 8). The stocking density in grow-out ponds should be maintained at a rate of 1 to 2/ m^2. The fish should be fed with protein rich supplementary feeds at 3 to 5% and 2 to 3% per estimated bio-mass respectively in rearing and grow-out ponds. Growth sampling in both cases is performed at 15 to 30 days intervals.

Genetic enhancement of silver barb can further be continued generation after generation following the same selection protocol. According to Bentsen et al. (1996) an assumed heritability for body weight of about 0.3 and assumed coefficient of variation of 30%, as shown in many fish species, the expected genetic gain in the progeny should amount to about 15 to 17% compared to the mean of the parent generation (ie., 15 to 17% genetic gain per generation). In such circumstances, breeder selection and mating should be carried out at 10 to 12 mon of age to maintain a generation interval of about 1 year.

Distribution and dissemination of genetically improved breeds to fish farmers

The excess seeds of the best-selected breeders may be distributed directly to the fish farmers from the hatcheries. If the demand for improved seeds exceeds this level, the breeders may be spawned repeatedly and the spawn/fry/fingerlings produced can be sold. There is no doubt that these fry will all be the progeny of the best-selected breeders. After grow-out trials, the surplus mature breeders can be distributed to interested private hatchery operators.

4.1.2 Stock improvement of silver barb using chromosome manipulation and sex inversion techniques

Like other cyprinids, females of silver barb grow faster than males, therefore, mass production of an all female population could be of significant advantage. Direct hormonal feminization is not a viable technique for producing such fish, as this would not integrate easily with fingerling production techniques and might have adverse environment impacts or consumer reaction.

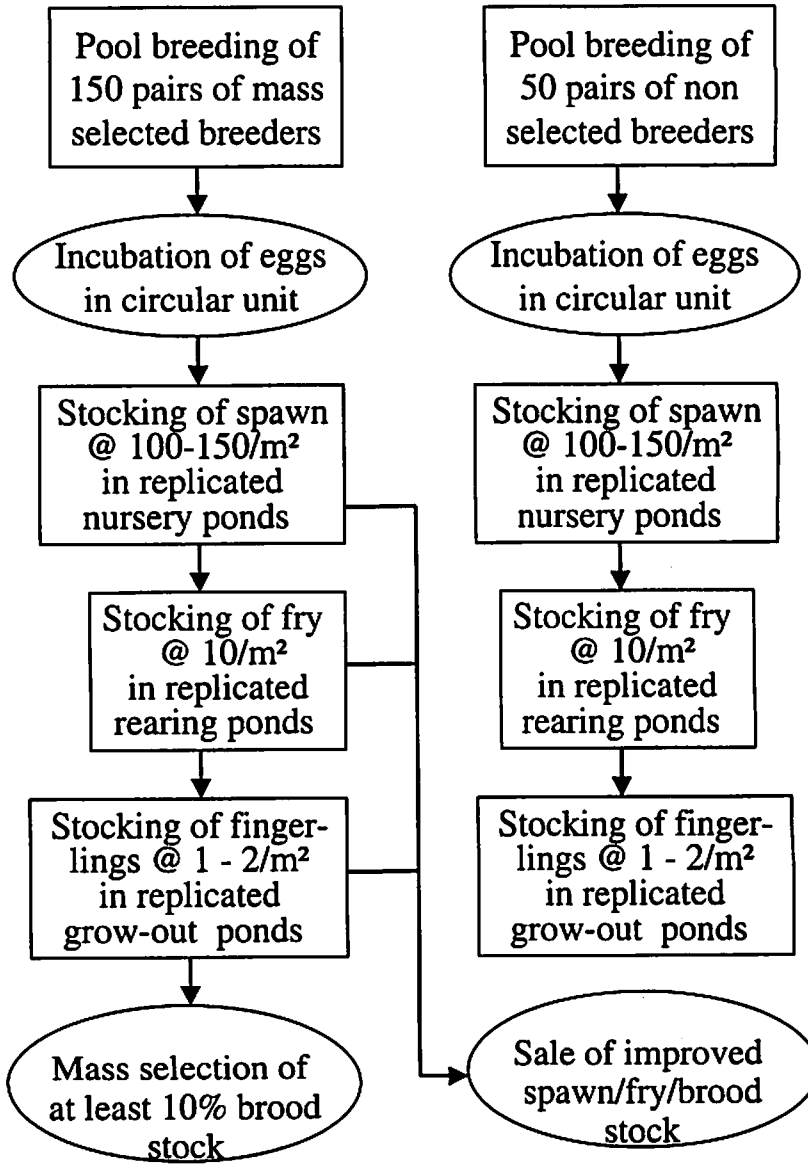

Fig. 8. Design for mating and reproduction of parental brood stock to produce F_2 generation. Rearing of improved and control progenies (in nursery, rearing and grow-out ponds), sale and distribution of improved stocks.

Pongathana et al. (1999) described a method for the production of all female silver barb using both genetic manipulation and sex inversion techniques. In their method the meiotic gynogens of the fish to be produced through chromosome manipulation would be all females and thus, the female homogamety (bearing XX genotype) could be confirmed in the species. The protocol for such approach was to produce neomale (phenotypic male having XX genotype) through feeding 17α-methyletestosterone (MT) 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. Monosex female fish production commercially has been demonstrated in some species, e.g. rainbow trout (Bye and Lincoln 1986) and grass carp (Shelton 1987), using hormonally masculinized XX genetic females (neomales).

Gynogenesis and hormonal masculinization techniques

In the process of meiotic gynogenesis in silver barb, eggs are fertilized with UV irradiated (UV dose 200 μWcm^{-2} for 1 min). Sperm are then are exposed for 1.5 min after fertilization to cold shock (temperature 2^0C) for 10 min that suppresses the anaphase stages of the second meiotic division by disruption of metaphase spindles, resulting in artificial diploidization of the maternal chromosome complement (retention of 2^{nd} polar body) of eggs (Pongathana et al. 1995).

For hormonal masculinization, the first feeding fry of silver barb are treated orally with 17α MT hormone at a dose of 25 to 30 mg /kg of feed. The hormone treated feed is prepared by dissolving the appropriate amount of hormone in ethanol that is then sprayed onto the food in a shallow tray. The food is then mixed well and left in the sun to dry for several hours.

Hormone-treated feed can be offered to gynogenetic fry for 4 to 5 weeks in aquaria or concrete cisterns. At the end of the hormone treatment, the fry need to be transferred initially to earthen nursery ponds and finally to grow-out ponds to be grown to an age of 4 to 7 mon. For sexing, mature fish can be killed to check the gonads or immature fish gonads can be given a histological examination.

A schematic model for the commercial production of all female silver barb using genetic manipulation and sex reversal techniques is presented in Figure 9.

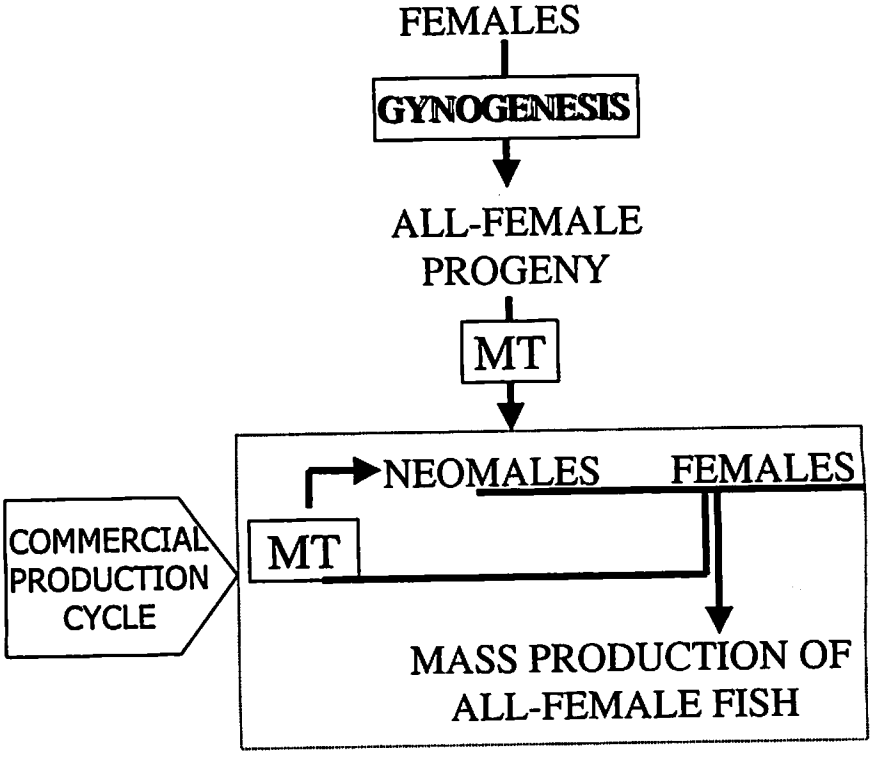

Fig. 9. A schematic model for commercial production cycle of producing all-female silver barb.

CHAPTER 5

A Breeding Plan for Cultured Major Carp Species

5.1 A Breeding program and the genetic improvement of major carps

Background information

Aquaculture in Bangladesh revolves around the cultivation of endemic and exotic major carps. The commonly used fast growing carp species for composite carp culture in the country are catla , rohu, mrigal, silver carp, grass carp and mirror/common carp. Figure 10 represents the six major carp species most commonly used in aquaculture. Induced breeding of mainly endemic major carps has been established as a dependable source of fish seeds since the mid 1960's (Ali 1967). Today hatchery-produced fry/fingerlings contribute significantly to the overall aquaculture production of the country. It has been observed that while some of the hatcheries are careful in selection and maintenance of their brood stock, practices followed by others result in inbreeding and genetic deterioration of stocks. Once a hatchery is constructed, the general practice is to stock with whatever materials that are easiest to acquire. This is a shortsighted and unscientific approach since the pedigree of a farmed stock can be an important determinant of its performance and therefore, of future profitability of the operation. Common practice in small hatcheries often involves the use of a small number of brood fish of a species with high fecundity like carps and their replacement by succeeding generations. Such uncontrolled inbreeding often leads to inbreeding depression with reduced growth rates, loss of fecundity, and poor survival (for details see Chapter 2). The problems that are produced by uncontrolled inbreeding are usually accompanied by loss of alleles via genetic drift. When a breeding program is used, genetic changes are planned and desired because they will lead to improved growth rate etc. When no breeding program is conducted, genetic changes are unplanned and random, and those caused by inbreeding and genetic drift can ruin the population (Tave 1999). Through implementing a breeding program, the hatchery operators have a chance to select superior brood stock and mate them accordingly. In this way, genetically superior individuals can be developed, heritability and genetic variability of all traits can be increased to a maximum level, and inbreeding depression can be kept at a minimum level. So, many fish breeding experts suggest selective breeding as the simplest, most promising and useful method to improve desirable traits in a founder stock with high genetic variability. Preservation of such genetic variability may ultimately be of benefit to fish breeding and aquaculture by increasing the growth rate, fecundity, survival and disease resistance of commercially important fishes like carps and other cultivable fish species.

General and spawning biology

General and spawning biology of the six most commonly used cultured major carp species briefly described below:

Catla

Among the endemic carps catla is the fastest growing species in Bangladesh. In culture conditions, it grows well in large and deep fertilized ponds, lagoons, lakes and other closed water bodies, whether feeding on natural foods, mainly zooplankton (Reddy 1999), or artificial feeds, and it can adapt to various tropical environmental conditions. It feeds on the surface or in the upper zone of the water body and attains a length of 30 to 40 cm (1.0 to 1.5 kg) in the first year and grows up to a length of 90 to 92 cm (10 to 14 kg) by the fifth year. It can grow to over 1.5 m in length. The longest size recorded was 96.7 cm (19.8 kg) in Bangladesh (Rahman 1989).

Catla attains sexual maturity in the third year. The breeding season of catla goes from May to August with a peak at the monsoon months (June and July). Artificial spawning can be induced by hypophysation. The females are given two injections with PG extract hormone. The dose of priming or first injection is 1.0 to 2.0 mg/kg body weight followed by the second injection of 5.0 to 6.0 mg/kg body weight. The males are injected with a half dose at the time the second injection is given to the females. Both sexes are kept in a circular spawning arena provided with a continuous flow of water (temperature 26 to 28 °C). Spawning occurs within 5 to 7 hours after the decisive or second injection. The fecundity of catla is estimated at 150,000 to 240,000 eggs/kg body weight of the female. Funnel type hatching jars or circular incubators are used for hatching eggs in most of the hatcheries. About 16 to 20 hours are required for complete hatching of eggs at water temperature 26 to 28 °C. A water hardened and fully swollen egg of catla has a volume of approximately 0.08 ml and a hatchling just after yolk sac absorption weighs approximately 0.0025 g (Jhingran and Pullin 1985). Under well-managed hatchery conditions, catla can be spawned repeatedly every 2 mon for a maximum of 2 to 3 times in a season.

Rohu

Rohu is the most popular among carp species for food fish in Bangladesh. It is a fast growing fish in rivers and even in ponds and other closed water bodies but has a comparatively slower growth rate than catla. Under both wild and culture conditions, the fish is a column feeder and feeds mostly on periphytonic forms found attached to submerged vegetation and other objects occupying the water column (Reddy 1999)

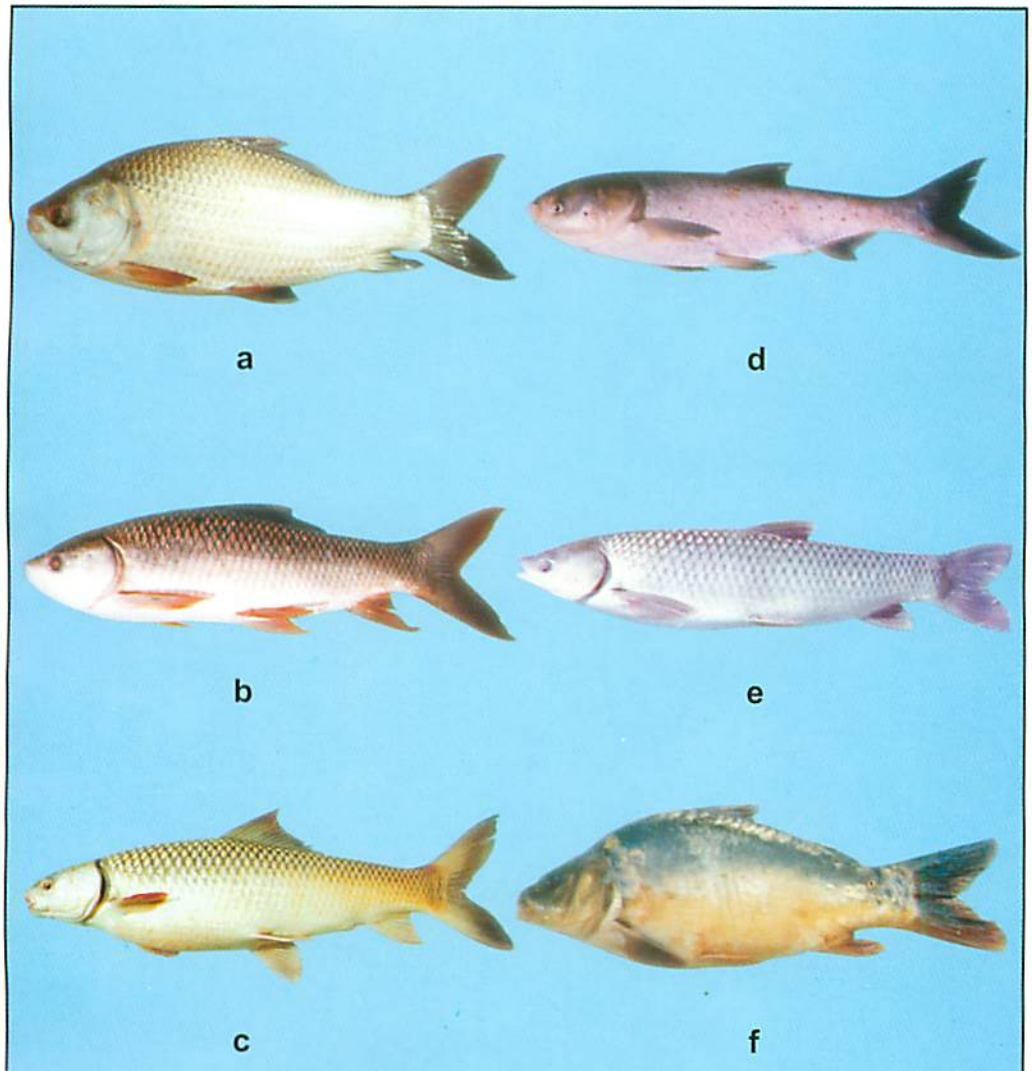

Fig. 10. Six endemic and exotic major carp species. **a.** *Catla catla;* **b.** *Labeo rohita;* **c.** *Cirrhinus mrigala;* **d.** *Hypophthalmicthys molitrix;* **e.** *Ctenopharyngodon idella;* **f.** *Cyprinus carpio.*

and on decaying plant matter; it also thrives on artificial feeds. In productive ponds, rohu attains the length of 35 to 45 cm (0.7 to 0.9 kg) in the first year and up to 75 to 80 cm (6 to 7 kg) by the fifth year. It grows over 90 cm and the longest specimen ever recorded in Bangladesh was 94.0 cm (12.5 kg) (Rahman 1989).

In ponds, both female and male rohu attain sexual maturity in the second year of life. Males are found to mature earlier than females during the spawning season (May - August). Climatic conditions and intensity of feeding are trigger factors for sexual maturation in both the sexes. Artificial spawning of rohu through hypophysation is a common practice in hatcheries of Bangladesh. The female is injected with PG hormone at an initial dose of 1.5 to 2.5 mg/kg body weight followed by a second dose of 5 to 7 mg PG/kg body weight after an interval of 6 hours. The males are given a dose of 3 to 4 mg/kg body weight at the time of second injection of the females. Both the injected females and the males are kept in a spawning arena where the spawning is completed within 6 hours of the second injection. The hatching of fertilized eggs is done in a series of hatching jars or circular incubators and is completed in 16 to 22 hours (temperature 26 to 28 °C). The fecundity of female rohu is recorded between 115000 - 303000 eggs/kg body weight. The hatchlings attain a first feeding stage within 3 to 4 days after hatching. It is recorded that a fully swollen, water hardened egg of rohu has a volume of approximately 0.078 ml and a larvae/spawn after absorption of yolk sac weighs approximately 0.0021 g (Jhingran and Pullin 1985). In hatcheries, rohu can also be used for multiple spawning (2 to 3 times) in a single breeding season.

Mrigal

In polyculture, mrigal is another important species next to catla and rohu. The fish feeds at the bottom on bottom biota such as tubifex and other blood worms (Reddy 1999) as well as on decayed vegetation. Under wild conditions in riverine waters, the fish grows fastest during the early years of its life (Jhingran and Khan 1979). Under conditions of culture, mrigal attains an average weight of 0.6 to 0.8 kg during the first year; 1.5 to 2.5 kg during the second year and 3 to 4 kg by the end of the third year. It can grow to over 10 kg, with a maximum size recorded in Bangladesh of 8.8 kg (84.0 cm; Rahman 1989).

The fish attains sexual maturity during the second year like rohu. Its spawning season runs from April to September. For induced breeding, the females are injected with a stimulating dose of 1.5 to 2.0 mg PG/kg body weight followed by a second dose of 5.0 to 6.0 mg PG/kg body weight after 6 hours. Males are given a single dose at the time of second injection of the females and left with the females in spawning arena.

Ovulation occurs within 5 to 7 hours after second injection. Similar incubating systems and hatchery conditions are used for hatching of eggs like catla and rohu. The fecundity is estimated to range between 50,000 to 250,000 eggs/kg body weight of the female. The volume of a fully swollen egg is approximately 0.11 ml with the average weight of spawn just after yolk sac absorption about 0.0025 g (Jhingran and Pullin 1985).

Silver carp

Silver carp is the fastest growing species among exotic carps. The fish feed high in the water column mainly on phytoplankton and competes with catla in polyculture systems. Under culture conditions, it attains a body weight of 0.6 to 8.0 kg during the first year,1.8 to 2.5 kg during the second year, and 4.0 to 4.5 kg during the third year with its growth rate declining sharply after that (Jhingran and Puulin 1985). Under natural conditions silver carp can attain a length of 98 cm.

In China and Japan silver carp breeds naturally during April-July in some rivers. Under hatchery conditions in Bangladesh, sexually mature breeders are available for artificial breeding during March to July. Fully ripe females are given two injections at a 12 hour interval.. The first injection is 200 IU HCG/kg body weight and the second injection is 500 IU HCG + 3 mg PG/kg body weight. Males are injected with 200 IU HCG at the time of second injection of females. Injected females and males are kept separately in cemented holding tanks flushed with fresh water of 22 to 25 °C. Ovulation takes place within 6 hours after second injection of females. Ovulated females are stripped to collect eggs and then fertilized with collected milt of the injected males. Incubating systems similar to those used for catla and ruhu are used for hatching the fertilized eggs. The average fecundity of a silver carp female is estimated at about 116,00 to 194,000 eggs/kg body weight (Islam 1999). Ova diameter ranges from 1.07 to 1.36 mm, a fully swollen egg has a volume of about 0.07 ml, and yolk sac absorbed hatchlings weigh approximately 0.0031 g (Alikunhi and Sukamaran 1965).

Grass carp

Biological characteristics of grass carp that make it valuable for aquaculture and weed control are its rapid growth, good flavor and voracious consumption of macrophytes. In China, India, Bangladesh and other Southeast Asian countries where polyculture systems are growing in importance, these two characteristics of grass carp are considered vital. It is reported that particularly in Chinese polyculture, the

grass carp is used as a second species to stock with other types of major Chinese carps. Because the droppings of grass carp are rich in undigested plant fibers, they may help to develop planktonic food for other fishes (FAO 1983). Jhingran and Pullin (1985) reported that in Chinese ponds, grass carp attains a weight of 0.23 to 0.68 kg in the first year, 1.2 to 2.3 kg in the second, 2.7 kg in the third and 3.8 kg in the fourth year. The fish can grow to more than 20 kg.

Under tropical and sub-tropical conditions, grass carp become sexually mature at the end of second year. Like other cyprinids, the males mature earlier than females in a spawning season (May to August). The fish does not breed in ponds; therefore, for spawning and seed production, induced breeding is essential. According to the standardized technique of Hussain (1988), females are given two PG hormone injections at a 7 to 9 hour interval: as a preparatory dose (25%); of the average 2 mg/kg body weight, and as a decisive dose (75%), of the average 6 mg/kg body weight. Males are injected with 2 mg PG/kg body weight. Both injected females and males are left in the spawning arena for natural spawning with a continuos flow of clean fresh water (temperature 25 to 26 °C). Spawning occurs within 4 to 6 hours after the decisive injection. Incubating systems similar to those of catla and ruhu are used for hatching the fertilized grass carp eggs. Fecundity of grass carp has been estimated between 60, 000 - 110,000 /kg body weight. The average diameter of an egg varyies between 1.19 and 1.37 mm. A water hardened swollen egg has a volume of approximately 0.0022 ml and the yolk sac absorbed hatchling weighs about 0.0022 g (Alikunhi and Parameswaran 1963).

Common carp

Common carp is the most extensively cultured species of carp in the world. It enjoys global distribution in tropical and temperate regions and has been acclimatized to a variety of habitats and extremes of environment (Alikunhi 1966). A large number of strains of these types of common carp, particularly scaled carp and mirror carp, have been developed throughout Asia and Europe by cross breeding and techniques of genetic manipulation. The colorful koi carp originated in Japan for ornamental purposes is one variety of common carp.

Both scaled carp and mirror carp are commonly used varieties for aquaculture in Southeast Asian countries including Bangladesh. Under culture conditions it has been observed that the growth rate of the scaled carp is comparatively slower than the mirror carp. Both types attain a weight of 0.6 to 0.8 g in first year; 1.0 to 1.5 kg in second; 2.0 to 2.5 kg in third and 3.0 to 3.5 kg in the fourth year. In tropical and sub-tropical conditions common carp is usually sexually mature at the age of 1 year

because of its fast growing characteristics. The males have highly developed testes and some have abdomens bulging as conspicuously as those of gravid females. Testes in some males may weigh as much as 20 to 30% of total body weight (Jhingran and Pullin 1985). In Bangladesh, the spawning season of common carp lasts from January to May at water temperatures ranging from 18 to 24 °C. It can easily be bred under controlled natural conditions in small ponds and cemented tanks using egg collectors made of aquatic grasses or coconut fibers. Besides natural spawning (see section 3.1.2), the common carp can also be propagated semi-artificially or fully artificially by hypophysation techniques. The fecundity of female common carp ranges between 90,000 to 140,000/kg body weight (Hussain 1982).

5.1.1 Stock improvement of major carp species using selective breeding techniques

Since 1994 BFRI in its Freshwater Station at Mymensingh has implemented a breeding plan and genetic stock improvement program to restore the genetic potential of endemic major carp species viz. rohu and catla through collecting the land races from the Halda, Jamuna and Brahmaputra river systems. Through this selective breeding model, inbreeding and loss of genetic variation has been avoided (Hussain and Mazid 1998). This chapter describes a simple technique which can be followed for most of the commonly used cultured major carp species.

Use of a large "effective population size" (Ne) supported by a large number of best-selected breeders (at least 75 pairs) in each generation will be the main principle of the proposed breeding plan. Mating between males and females of unrelated stocks will exclude crossing between siblings and pair wise separate incubation of the progeny groups will reduce the chance of genetic variation between pairs. A pool of fixed number of larvae (al least 125) from each pair will be formed and communally stocked for nursing, rearing and grow-out trials. According to Bentsen et al. (1996), this type of design is expected to result in a rate of inbreeding of less than 1% per generation.

Collection of the base population

At least 4 unrelated stocks (strains) are to be gathered from varied locations to form a base population for the proposed breeding plan. In the case of endemic major carps, stocks can be collected from different river systems viz. Halda, Jamuna, Brahmaputra, Dhoelshari, Arial Khan, etc. For exotic major carps, unrelated multi-locational strains/stocks can be collected. These stocks are to be reared in separate ponds by providing protein rich artificial feeds until sexual maturation is attained.

Initiation of the breeding program

During the peak breeding season of major carps, at least 4 unrelated stocks or strains of the selected carp species are mated using 4 by 4 complete diallelic crossing design to produce 16 genetic groups as shown in Table 10. The 12 crossbred and 4 purebred progeny groups are then stocked and kept separately for further use.

Table 10. A mating design for the production of base populations of major carps

♀ from different locations	♂ from different locations			
	1	2	3	4
1	-	X	X	X
2	X	-	X	X
3	X	X	-	X
4	X	X	X	-

For each of the 12 reciprocal crosses, 6 to 7 pairs (sex ratio female to male 1:1) are mated separately, producing at least 75 full sibling progeny families. All mating is to be performed within 1 to 2 days. The fertilized eggs are incubated in a series of funnel type incubating jars or units and hatchlings are kept separately in 75 hapas until the first feeding stage. For nursery rearing, 125 larvae are counted from each of the 75 hapas and communally stocked in an earthen pond until the fry can be transferred to a communal rearing pond and later on to a grow-out pond. This group will be the first batch of progeny. During the following week, the procedure should be repeated by mating another set of 75 pairs of breeders. For nursery rearing, a second pond should be used to grow the second communal batch of progeny. The two communal batches should be grown separately until their sexual maturity (Figure 11). Stocking densities for spawn/larvae, fry and fingerlings in nursery, rearing and grow-out ponds should be maintained respectively at 100, 10 and 2 individuals per square meter. Regular feeding in the respective culture systems should be done with a recommended formulation of artificial carp feeds (Table 11 and 12). To stimulate the

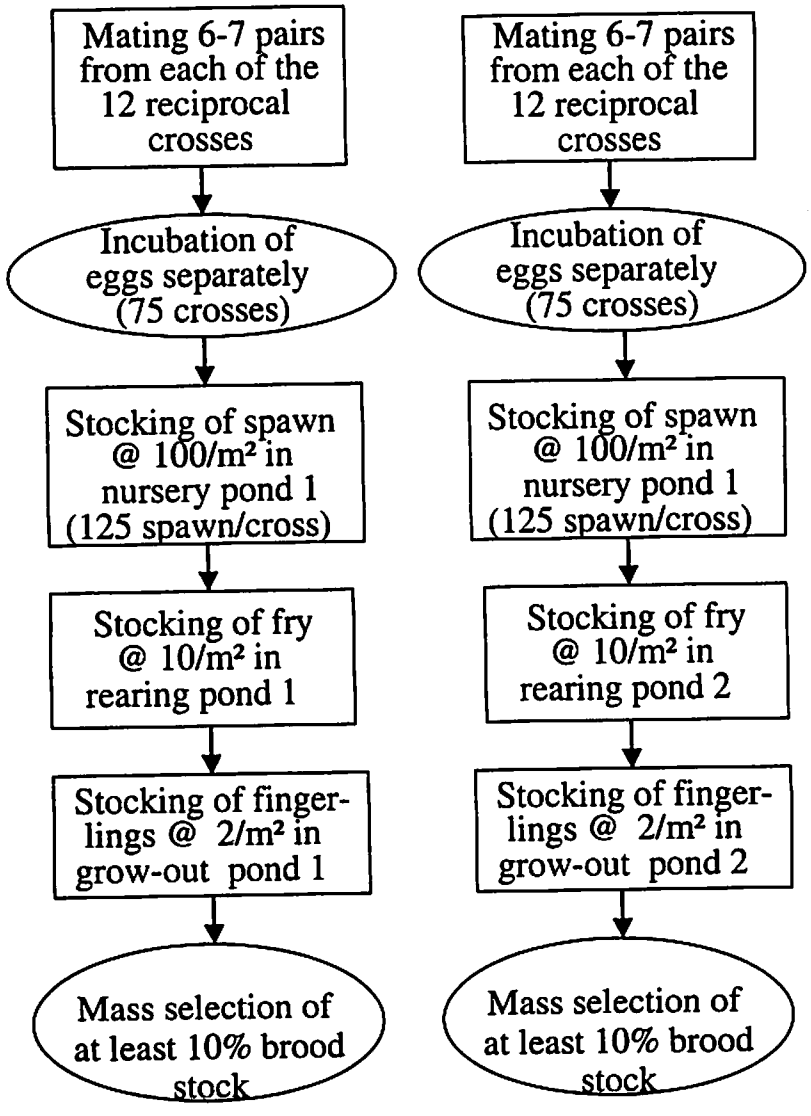

Fig. 11. Design for mating and reproduction of parental brood stock to produce F_1 progeny, communal rearing of progenies (in nursery, rearing and grow-out ponds) and mass selection of brood stock.

sexual maturity of fish in grow-out ponds, stocking density can further be reduced to 1 fish per m^2. At harvest, the survival rate of stocked fish in nursery, rearing and grow-out ponds is presumed to be 60, 80 and 90% respectively.

Table 11. Feed formulation for carp fry and fingerlings

Feed ingredients	Quantity (%)	Crude protein (%))
Fish meal	21	11.92
Mustard/sesame oil cake	46	13.28
Rice bran (auto mill)	18	02.00
Wheat flour	14	02.50
Vitamin and mineral premix	01	-
Total	100	30.00

Source: Hussain and Mazid (1998)

Table 12. Feed formulation for carp brood stock

Feed ingredients	Quantity (%)	Crude protein (%)
Fish meal	16.00	8.96
Mustard oil cake	10.00	3.03
Sesame oil cake	26.00	7.07
Rice bran	20.00	2.38
Wheat bran	19.00	2.38
Wheat flour	19.00	2.77
Molasses	5.00	0.22
Vitamin & mineral premix	1.00	-
Total	100.00	25.00

Source: Hussain and Mazid (1998)

The individual selection of brood stocks

At the beginning of the breeding season of a desired carp species, 10% of the largest females and males are selected from among all the F_1 sexually mature breeders in the grow-out ponds of each communally stocked batch and are kept separately in an earthen pond until use. In the process of selection with respect to sex, empirical assessment of the breeders should be done on the basis of best size, good growth and shiny appearance.

Production of the next generations

For the production of the next generation, 75 best-selected females should be chosen from the F_1 first communal batch and 75 best-selected males should be chosen from the F_1 second communal batch for single pair mating (Figure 11). Fertilized eggs obtained from each pair should be hatched in separate incubation jars and each full sibing group of spawn/larvae should be kept initially in separate hapas. From each hapa, 125 larvae should be counted and pooled together in a nursery pond to make a new batch 1. The mating should be repeated after 7 days, this time 75 best selected males should be chosen from the F_1 first communal batch and 75 best selected females should be chosen from the F_1 second communal batch, to make a new batch 2. Again the two F_2 communal batches should be grown separately until their sexual maturity (Figure 12).

The above selection protocol can be followed generation after generation for genetic improvement of both endemic and exotic carp stocks. As is mentioned in case of silver barb in Chapter 3, and experimentally shown in other fish species as well (e.g., Atlantic salmon and GIFT strain of Nile tilapia), species with a presumed heritability of 0.3 and a coefficient of variation of 30%, would be expected to show a 16 to 18% genetic gain per generation (Bentsen et al. 1996).

Evaluation of growth performance and estimation of the genetic gain in each generation

To evaluate growth performance and to estimate genetic gains, trials should be attempted in each generation with both crossbred and purebred groups. Purebred average groups (at least 20 non selected females and 20 non selected males), which are supposed to be derived from diallele crosses at the beginning of the breeding program, should be used for pool mating in each generation and compared with selected crossbred groups.

To evaluate growth and survival parameters at different stages of the life span, equal numbers of spawn, fry, and fingerlings should be obtained from best selected and average breeders and should be stocked separately in replicate nursery, rearing and grow-out ponds (using at least 3 replicate ponds). In each culture system, the test group of progeny should be reared under proper feeding and equivalent management conditions. At harvest, survival of stocked fish should be estimated and individual body weights of all the captured fish should be recorded for each of the two groups. An estimate of the realized response to selection in each generation can then be made.

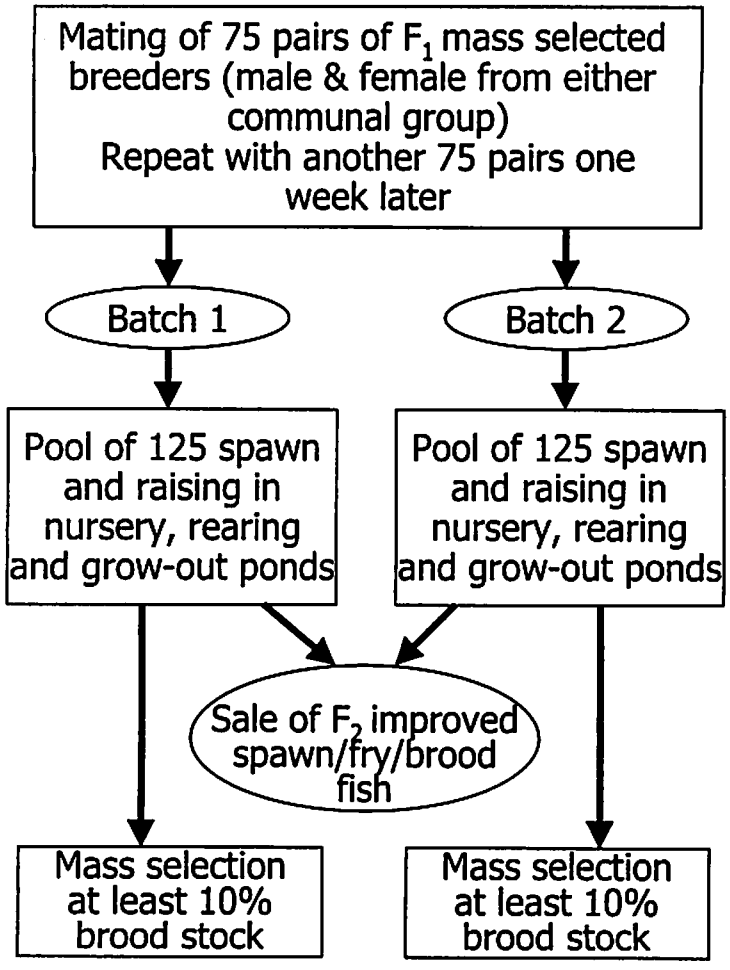

Fig. 12. Design for mating and reproduction of F_1 brood stock to produce F_2 generation, rearing of progenies (in nursery, rearing and grow-out ponds) and further mass selection of F_2 brood stock.

The sale and distribution of genetically improved breeds to fish farmers

From the second generation (F_2) onward, the excess seeds of the best-selected breeders (supposed to be genetically improved) may be distributed directly to the fish farmers from the hatcheries. If the demand for improved seeds exceeds their supply, the breeders may be spawned repeatedly to produce spawn/fry/fingerlings which can be sold. After grow-out trials, the surplus mature outbred brood stock can also be distributed to interested private hatchery operators.

5.1.2 A simple crossbreeding technique to avoid inbreeding in carp hatcheries

A simple crossbreeding technique can be followed to avoid carp inbreeding in hatcheries by mating two unrelated strains/stocks of the same species either collected from two different river systems or from two different locations. Crossbreeding can be combined with selection in this program to produce fish with no inbreeding generation after generation (Tave 1999).

Collection of stocks

A sufficient number (500 to 1000 per species) of wild stocks from two different riverine/natural sources or stocks from two different locations (may be from two distant hatcheries/farms) should be gathered prior to breeding season. Fish should be stocked separately, according to location of collection, at the rate of 1 fish per m^2 in individual ponds and fed regularly with protein rich artificial feeds as recommended in Table 12. If available ponds and limited, communal stocking of different brood stocks may be made using digital PIT tags or numbered plastic tags. For short time stocking marking may be done by alcian blue or fin clipping.

Breeding protocol

At least 50 sexually mature females should be selected from stock A and crossbred with at least 50 sexually mature males selected from stock B to produce the F_1 generation (Figure 13). A pool breeding technique should be followed, in which fertilized eggs are hatched in a series of incubating jars or in a large circular incubating system. Mating should take place within two days. The F_1 pool of progeny, will represent genomes from two unrelated stocks, therefore, there will be no chance of inbreeding (F = 0%). They should be grown together to sexual maturity.

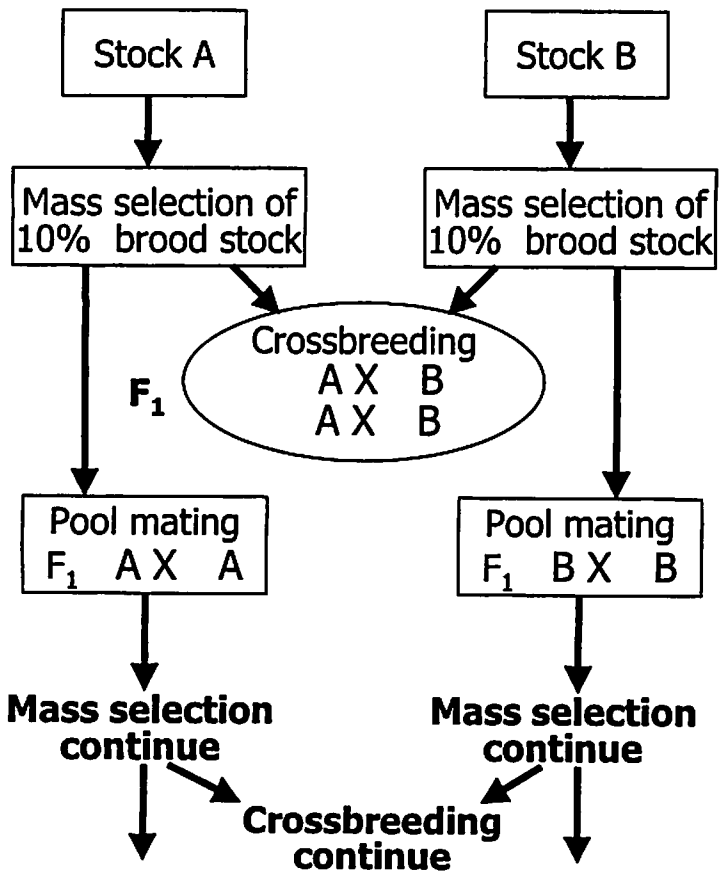

Fig. 13. Mating design for selection and simple crossbreeding of major carps.

Stocking densities for spawn/larvae, fry and fingerlings in nursery, rearing and grow-out ponds should be maintained respectively at 100, 10 and 2 individuals per m^2. Regular feeding in respective culture systems should be followed at recommended rates of artificial carp feeds in Table 12 and 13. To continue this breeding program, at least 50 pairs (sex ratio 1:1) of best selected (at least 10%) brood stocks should be used for pool mating from each of the pure stocks (both from Stock A and Stock B) thus maintaining purebred generations.

Sale and distribution of out bred stock to grow-out farmers and hatchery operators

The first generation of crossbred spawn/larvae of desired carp species can be sold and distributed at the first feeding stage to grow-out farmers. If demand for out bred seeds continues, the breeding program may be extended using more breeders producing additional spawn/fry/fingerlings for sale. Sale of non selected mature brood stock to farmers/hatchery operators should be made just before carp breeding season.

5.1.3 Genetic manipulation techniques to generate inbred clonal lines in carp species

Induced gynogenesis has been identified as a suitable technique for the rapid production of highly inbred strains of fish (Nagy 1987; Komen et al. 1991; Hussain et al. 1993). Gynogenesis achieved by the inhibition of the second meiotic division of eggs, will always produce a proportion of heterozygotes depending on the rate of recombination between non-sister chromatids during the first meiotic division (Purdom 1969; Nace et al. 1970; Hussain et al. 1994). This will not happen if gynogenesis is induced by the suppression of the first mitotic cleavage, in which case resulting progeny will be homozygous at every gene locus. Therefore, in subsequent generation(s) gynogenesis is of great advantage for producing inbred clonal lines. Clonal lines are supposed to be very valuable in the improvement of fish stocks (Han et al. 1991). Successful induction of diploid gynogenesis, both meiotic and mitotic, using methods of UV irradiation of sperm and application of various physical and chemical treatments has been reported by many authors (for reviews see Hussain 1996; 1998). The induction of mitotic gynogenesis and subsequent production of genetic clones in Asian carps has been reported by Reddy et al. (1993) and Hussain et al. (1997). Hussain et al. (1997) successfully produced mitotic gynogens in the first generation and developed inbred clonal lines in the second generation in rohu. Similar techniques can also be used for the production of homozygous inbred lines in other carp species.

Protocol for production of gynogens and inbred clonal lines

Collection of brood stock

A sufficient number of good quality mature brood stock (both female and male) of desired carp species should be collected from known sources (rivers, hatcheries or farms) prior to the breeding season to obtain necessary gonadal materials, i.e., eggs and sperm. The breeders should be injected with a recommended dosage of hormones (viz. pituitay exytracts/HCG, etc.) and at the period of peak ovulation, ova and milt should be collected by stripping.

UV - irradiation of milt

The collected milt should be cooled to 4 °C for a few minutes and then diluted 1:100 to 1:200 with chilled physiological saline solution (Cortland Solution of pH 7.2 to 7.5; Wolf 1963) and samples from this solution should be checked for motility of sperm. Further dilution should be made to concentrate sperm at about 10^8 ml^{-1}. The diluted milt should be spread on a plastic petridish to form a film with a thickness of about 0.1 to 1.0 mm. This sperm solution should be exposed to ultraviolet (UV) irradiation under a short UV-lamp (Model UVBGL-58; Multiband-254/366 NM). The intensity of UV-irradiationshould be optimized at 200-250 $\mu W/cm^2$ applied for 2 min at 28±1°C and the irradiated sperm should be refrigerated at 4 °C. The irradiated sperm then should be used to inseminate the ova.

Induction of gynogenesis (both meiotic and mitotic)

To induce two types of gynogenetic diploids, collected eggs should be divided into three equal batches. The first and second batches of eggs should be fertilized separately with UV irradiated milt and exposed, respectively, to early heat shock treatment (temperature 40 °C; 3 to 5 min after fertilization for 2 min) for the production of meiotic gynogens and late heat shock treatment (temperature 40 °C; 25 to 30 min after fertilization for 2 min) for the production of mitotic gynogens (Hussain et al. 1997). For the induction of heat shock, a temperature controlled 50 liter water bath regulated by a heater/stirrer (Gallenkamp Ltd.) can be used. The third batch of eggs, fertilized with intact (non-irradiated) sperm from the same lot, is used as a control.

Production of inbred clonal lines

The F_1 putative mitotic gynogens are reared to sexual maturity in ponds. Eggs and milt collected respectively from the F_1 females and males are used for the production of F_2 meiotic gynogens following the protocol given in Figure 14. The meiotic gynogens derived in this manner will represent the inbred clonal lines.

Egg incubation and determination of ploidy

All the treated and untreated batches of eggs are incubated in separate funnel incubating jars. The temperature for incubation is maintained at 26±1 °C. The rates of fertilization, hatching and survival at first feeding stage are carefully recorded. The hatching of normal larvae is considered as the primary criterion for estimating the success of induced gynogenetic diploids. The ploidy of all treatment and control batches of newly hatched larvae is determined by the chromosome karyotyping technique described as below.

5.1.4 Chromosome karyotyping technique for carp species

Until recently, no reports have been available about chromosome karyotyping techniques of carp species in Bangladesh except Islam et al. (1994) and Hussain et al. (1997). They used such a technique to determine the ploidy status of genetically manipulated progeny groups of rohu. Shah et al. (1984) prepared the karyotypes at the embryonic stage (16 to 20 hours) following the methods of Kligerman and Bloom (1977) and Krasznai (1985). The embryos (approx. 10%) were placed in a 0.01% colchicine solution for 3 to 3.5 hours, then kept in 0.4% KCL for 20 to 30 min and then transferred to 3:1 methanol- acetic acid for 30 to 45 min before staining They were stained with 2 to 4% aceto-orcein stain (Krasznai 1985) and kept 24 hours refrigerated at 4^0C. Then embryos were taken out one by one and squashed on a slide with lactic acid. At least 10 preserved embryos were taken from each sample drawn out of a batch for ploidy determination (Islam et al. 1994).

Hussain et al. (1997) used the techniques as described by Hussain and McAndrew (1994). Their protocol is as follows: Embryonic tissues are collected from newly hatched or 1 day-old larvae of treatment groups. For each group (ca 100) 15 to 20% embryos are placed in a small petridish containing 8 to 10 ml of 0.002 to 0.005% colchicine solution (freshly prepared or stored for 4 to 6 hours at 28°C. Tissues are obtained from the embryos in a chilled 0.75% saline solution under a dissecting

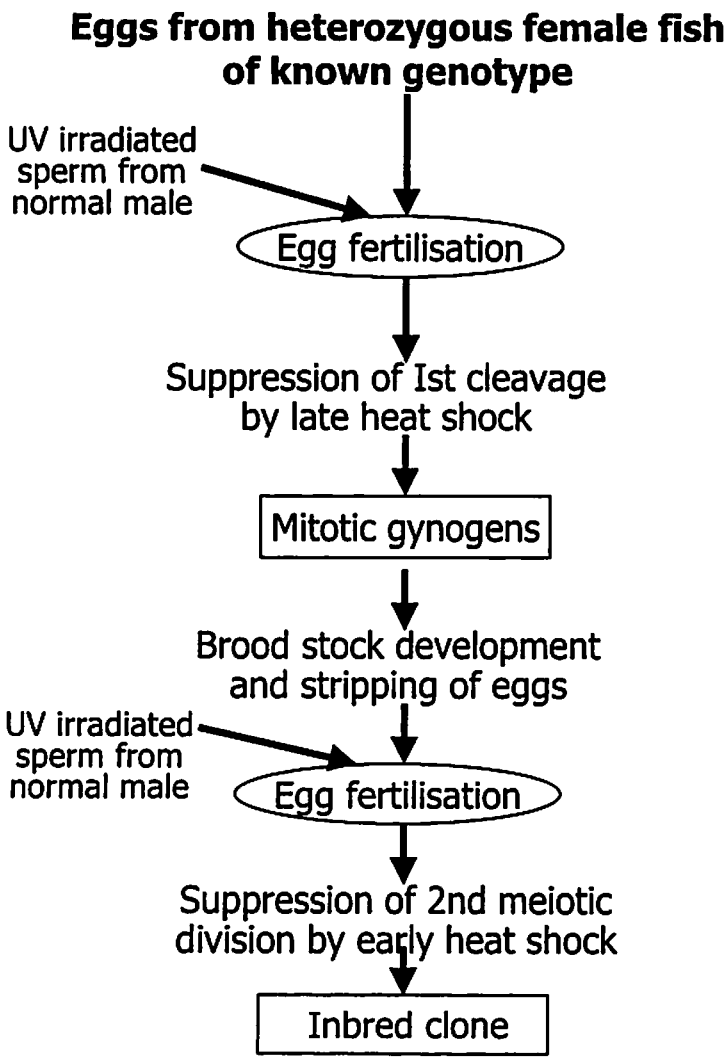

Fig. 14. A schematic model for the production of inbred clonal lines in major carps (adapted from Hussain *et al.* 1998).

microscope by removing their heads and yolk sacs and putting these in distilled water (hypotonic solution) for 8 to 12 min. The tissues are then immersed in a fixative of 4:1 methanol - acetic acid at 4°C. After two changes the tissues are stored in the fixative for 30 to 90 days. To prepare the slides, the tissues are removed from the fixative and, later blotting out the excess fixative, placed in the cavity of a perspex slide with two to three drops 60% glacial acetic acid and minced for 1 min with a glass rod to allow sufficient dissociation of epithelial cells. After 15 to 20 min, three to four drops of cell suspension are dropped from a height of 30 to 40 cm onto a clean glass slide on a warmed hot plate (44 to 48°C) and withdrawn within 8 to 12 s leaving a fine and clean ring of cells using a single micro-hematocrit dropper. Slides are air dried and stained with freshly prepared 10% Giemsa stain (preapared in 0.01-M phosphate buffer pH 7.0) for 15 to 20 min. The slides are rinsed in distilled water, air dried and mounted with DPX after 10 min of Xylene wash.

In both cases, metaphase spreads of chromosomes are to be checked and chromosome number noted by observing the slides under x400 and x1000 (oil immersion) magnifications, respectively, with a compound microscope. The karyological examination is carried out by counting the chromosomes of as many karyotypes as possible per slide. The haploid, diploid and triploid metaphases in *L. rohita* are composed of respectively one (n = 25), two (2n = 50) and three (3n = 75) sets of chromosomes.

It is also reported that both *C. catla and C. mrigala* bear similar numbers of chromosomes in their metaphases like *L. rohita* (Mr. S. Islam, personal communication).

CHAPTER 6

Breeding and Conservation of Endangered Carp Species

6.1 Biology and artificial breeding techniques

Background information

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 and their habitats 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 (Hussain and Hossain 1999). 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 (1998) has documented about 56 freshwater fish species critically or somewhat endangered including 11 cyprinid species (Table 13). Some important endangered carp and barb species are shown in Figure.15. There is a need; therefore, for development of artificial breeding and seed production techniques of such carp species for conservation of their "gene pool" and biodiversity.

General and spawning biology

A brief description of general and spawning biology of important endangered carp species of Bangladesh is as follows:

Bata

This minor carp is not commonly used for culture in ponds but is important as a food fish in Bangladesh because of its tastiness. Bata is found in most of the river systems

and in floodplains. It feeds on phytoplankton, algae and soft leaves of aquatic grasses. The fish attains maximum length of 60 cm.

Table 13. A list of endangered carp and barb species of Bangladesh.

Family	Scientific name	Common name	Critically Endangered	Endangered	Vulnerable
Cyprinidae	*Labeo nandina*	Nandina labeo	X		
	Labeo boga	Boga labeo	X		
	Labeo gonius	Kuria labeo		X	
	Labeo bata	Bata			X
	Labeo pangusia	Pangusia labeo		X	
	Labeo calbasu	Kalbasu		X	
	Cirrhinus ariza	Reba carp			X
	Puntius sarana	Olive barb	X		
	Puntius ticto	Ticto barb			X
	Tor tor	Tor mahseer	X		
	Tor putitora	Putitor mahseer	X		

L. bata does not normally breed in ponds. Under wild conditions, it breeds during monsoon months (April to July). The fish can easily be bred by hypophysation techniques. Fecundity of females is estimated around 23,000 to 81,000 per kg body weight of fish.

Olive barb

Olive barb is one of the most popular food fish among barb species and is widely distributed all over Bangladesh in rivers, beels and floodplains. Once the fish was abundant in these freshwater bodies but has sharply declined in recent years.

Therefore, it has been listed as one of the critically endangered species of fish in Bangladesh (IUCN, 1998). The fish is a plankton as well as detritus eater. It attains a maximum length of 40 to 42 cm and a weight of 1.0 to 1.5 kg (Rahman 1989).

P. sarana does not normally breed in confined waters but rather it breeds in the monsoon season (May to August) in running waters among boulders and vegetation (Talwar and Jhingran 1991). Females grow faster than males and become sexually mature at the age of one year. Induced breeding is successful for artificial spawning of *P. sarana* in Bangladesh since 1989 (Akhteruzzaman et al. 1992).

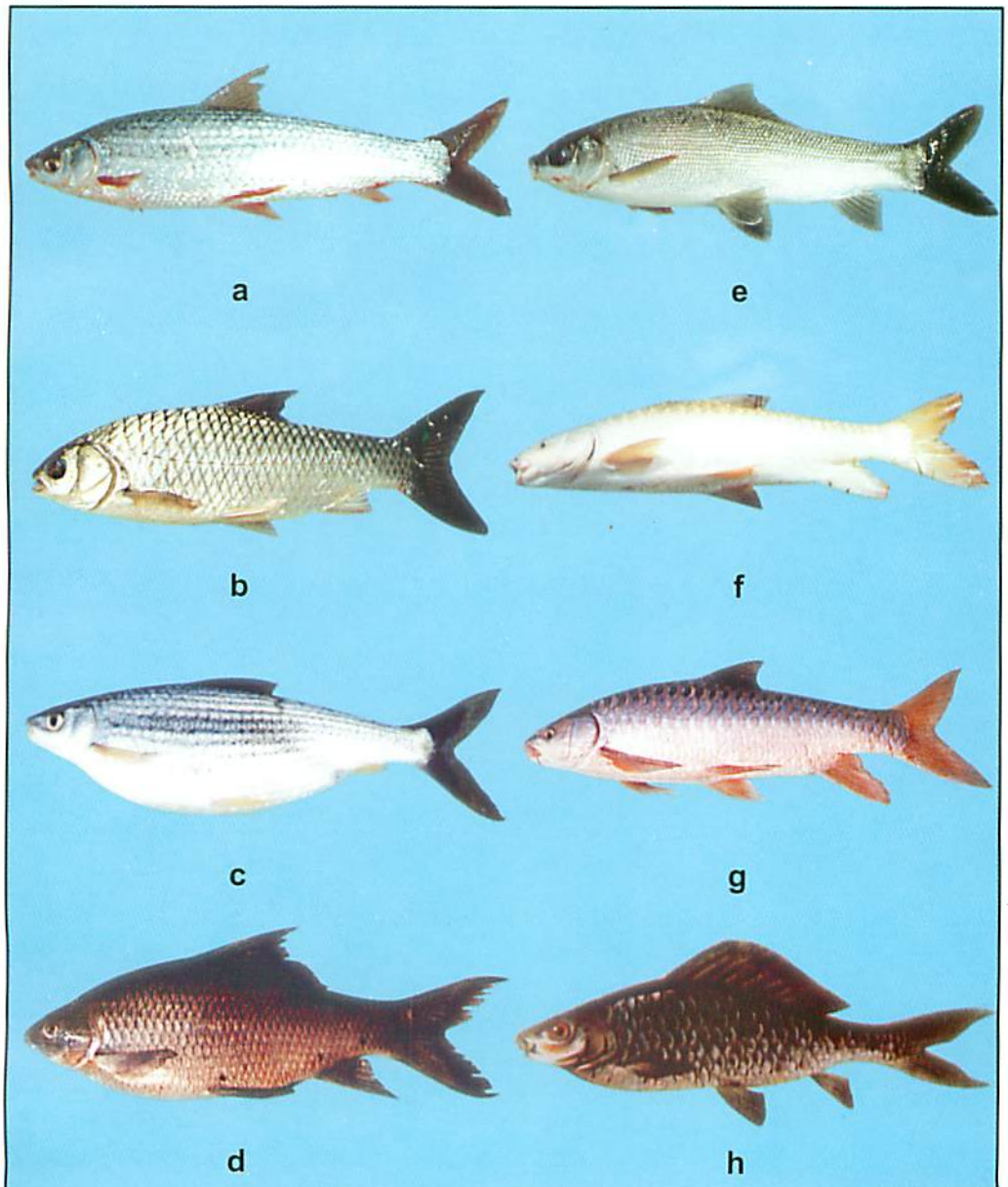

Fig. 15. Some important endangered carp and barb species of Bangladesh. **a.** *Labeo bata;* **b.** *Puntius sarana;* **c.** *Cirrhinus ariza;* **d.** *Labeo calbasu;* **e.** *Labeo gonius;* **f.** *Tor tor;* **g.** *Tor putitora;* **h.** *Labeo nandina.*

Reba carp

The natural distribution of reba carp included most of the rivers, small creeks and natural depressions (i.e., beels and haors) of Bangladesh. In recent times, the fish is not

commonly found in those water bodies. During the last 10 years, the genetic diversity of this species has been reduced remarkably because of the deterioration of aquatic habitats of suitable water bodies and wetlands. The fish grows slower than *L. gonius* and *L. bata* and attains a maximum body size of 40 to 50 cm. The body is covered with medium-sized scales, the upper body is deep bluish or darkish and the lower body is shiny and silvery. The lateral line has 38 or 39 scales, most of them appearing as a black mark elongated from the base margin of the operculum to the tail during the early stage of life. The black mark becomes shorter and remains between the operculum and at the end of the belly during the adult stage.

Males and females attain sexual maturity by the end of the first year. Its spawning season starts in April and ends in August with peak spawning occurring during the rainy months in flowing flood waters. A female can liberate 200,000 to 250,000 eggs/kg body weight.

Calbasu

Unlike catla, rohu and mrigal, once calbasu was another important species in polyculture because of its fast growing nature. The fish is marginally available in freshwater all over Bangladesh and is abundant in beels and haors of Mymnsingh and Sylhet. Recently, kalbasu has been listed as a threatened species in Bangladesh. The fish is a detritus eater and bottom feeder subsisting mainly on decomposed vegetation. Under wild conditions, it has been observed that the fish grows fastest during its early years and then its growth rate slows. Under natural conditions, kalbasu attains an average weight of 0.6 kg in the first year; 1.2 to 2.0 kg in the second year, and 2.5 to 3.5 kg in the third year. It can grow to over 6 kg with a maximum size recorded in Bangladesh of 5.5 kg at 71.0 cm (Rahman 1989). The fish attains sexual maturity in second year like mrigal and rohu. The spawning season runs April-August with natural breeding in flowing waters near the shelter of fallen trees and other bushes. The fecundity of a female 40 cm in length is estimated to be around 250,000 eggs. The volume of a fully swollen egg is approximately 0.10 ml and the average weight of spawn just after the yolk sac absorption stage is about 0.0023 g.

Kuria labeo

As one of the important members of the minor carp group, kuria labeo was once available in most of the freshwater bodies particularly in rivers, tributaries and floodplains of the greater Mymensingh and Sylhet districts. Presently the fish is considered a threatened species. The upper part of the body is covered with greenish and olive colored fine scales and the lower part of the body is covered with silvery scales. The lateral line has 75-80 scales. Under wild conditions, the fish feeds on algae, soft green grasses and zooplankton and grows up to 0.5 g in weight and 32 cm in length within one year. The fish can attain a weight of more than 2.0 kg, with a maximum size obtained of 1.4 kg and 61 cm (Rahman 1989).

The fish spawns once a year with the spawning period likely to be spread over 4 to 5 months from April to August. The fish matures in two years at a size of 300 to 500 g. The diameter of ova varies from 1.42 to 1.62 mm during spawning season. Fecundity is found to fluctuate widely from 42,925 to 1,22,895. The ova diameter and fecundity clearly indicate that early June to mid July is the spawning peak, correlating positively with ova diameter, fecundity, fertilization rate, hatching rate and survival rate of larvae (Mr. A. Hossain, personal communication).

Nandina labeo

The fish nadina labeo belongs to the minor carp group and was once abundant in the rivers and natural depressions (i.e., beels and haors) of greater Mymensingh and Sylhet districts. It likes to inhabit deep stagnant and clean areas of suitable water bodies. The fish feeds mainly on decomposed vegetation. This carp grows faster than *L. gonius* and *L. calbasu* and can reach over 8.0 kg. A maximum size of 78 cm with weight of 10.0 kg was recorded from Sylhet district (Rahman 1989).The fish looks dark greenish above and lighter below. There are 41-43 scales on the lateral line including some scattered red-orange scales on the body.

Like *L. gonius* and *L. calbasu*, the fish becomes sexually mature at two years and naturally spawns in slow running and deep waters May to August. The fecundity is similar to *L. gonius*. Natural spawning and feeding grounds of *L. nandina* have been gradually lost to heavy siltation on the basin of rivers and other suitable water bodies. Therefore, the genetic diversity of this species has declined drastically during the last 10 years. Because this carp is no longer available in the landing centers and markets, it has been presumed recently that *L. nandina* is probably extinct in Bangladesh.

Mahseer

Mahseer, *Tor* spp. belong to the family Cypriniadae that is the most famous sport fish in Bangladesh, Nepal and India. For sport fishing these species are highly attractive to anglers particularly in Nepal and India. Many European anglers used to come in this part of the world to fish for this fantastic fish that inhabited hilly streams and reservoirs. In Bangladesh they were found in the hilly streams of greater Mymensingh (river Kangsha, Someswari of Netrokona), Sunamganj (river Para), Dinajpur (river Mahananda) and Chittagong Hill Tracts (Karnafully reservoir). For natural breeding mahseer need rocky and sandy grounds with low temperatures and high levels of dissolved oxygen. But their natural habitat has been degraded because of the gradual deterioration of aquatic ecosystems combined with the physical reduction of suitable wet lands, siltation and erosion of most the river basins. As a result, recently mahseer are considered one of the most endangered fish in Bangladesh.

Rahman (1974, 1989) reported only two species mahseer, *T. tor* and *T. putitora* in Bangladesh. In 1991, about 1000 fingerlings of *T. putitora* were transported to Freshwater Station, BFRI, Mymensingh for experimental breeding (Mahata et al. 1995). In natural waters, both the species feed on insects, mollusks and small fish (Pisolkar and Kramchandani, 1984). In experimental pond conditions, they were fed with formulated artificial feeds but their growth rates were not found to be encouraging (Islam 1999). In Bangladesh, *T. tor* has been caught recently maximum size 36 kg in Kangsha River. In case of *T. putitora*, the maximum size recorded from Cauveri River weighed 54 kg.

Both *T. tor* and *T. putitora* attain their sexual maturity in the second year. In this country, the spawning season of mahseer ranges from early November to late January. The fecundity of both species is very low in comparison to other carp species. Absolute fecundity in *T. putitora* was recorded between 8000 to 12000/kg body weight of female (Mahata et al. 1995; Hussain and Hossain 1999).

Artificial propagation techniques

Since 1990 BFRI under its Freshwater Station, 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 most of the threatened carp and barb species. The artificial breeding protocols of *T. putitora, L. calbasu, L. gonius, L. bata, C. reba* and *P. sarana* are as follows:

Brood fish collection and management

Brood fish should be collected at least 3 to 4 months before their breeding season which for *L. calbasu*, *L. gonius*, *L. bata*, *C. reba* and *P. sarana* runs from April to August, and for *Tor* spp. peaks from November to December. A minimum water depth of 1.5 m should be maintained in all brood fishponds during the growing or storing period. A regular exchange (at least once in a week) of water prior to the spawning period will help to enhance ovarian development as well as the growth of fish. Generally a conventional type of feed composed of 50% rice bran, 20% mustard oil cake, 20% wheat bran, 10% fish meal is given @ 4-5% of brood stock body weight. Inorganic fertilizers, both urea and TSP, are used @ 100 g per 40 m^2 per week. The next week organic fertilizers like cattle dung or poultry droppings can be applied @ 4 kg per 40 m^2.

Selection of brood fish

Swollen light reddish vent, bulging soft abdominal region, smooth pectoral fin of female and oozing of milt and rough pectoral fin of the male are the major criteria for selection of the female and male breeders.

Table 14. 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)
Labeo calbasu	Female 2.0	6	Female 6.0 Male 2.0	6 – 7	18 – 20
Labeo gonius	Female 2.0	6	Female 5.0 Male 2.0	7 – 8	16 – 18
Puntius sarana	-	6	Female 5.0 Male 2.0	6 – 7	14 – 16
Cirrhinus ariza	Female 1.0	6	Female 5.0 Male 1.0	7 – 8	14 – 16
Labeo bata	Female 1.0	6	Female 5.0 Male 1.0	7 – 6	16 – 18
Tor putitora	No need of hormone injection; fully ripe females are manually stripped to collect ripe eggs				72 – 80

Hypophysation and spawning

For *L. calbasu, L. gonius, L. bata, C. ariza* and *P. sarana* a recommended hypophysation technique is used (Table 14). 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.

6.2 Conservation and management measures

Habitat degradation recently has become a great concern in most aquatic ecosystems of Bangladesh. Remarkable changes have been observed in many natural fish populations, either in stock erosion or through loss of genetic diversity. Because of environmental modifications and man made interventions effecting spawning and feeding grounds, survival rates of many endemic fish species have been declining severely. In this situation, it is crucial that appropriate measures be taken to reduce habitat loss, to conserve aquatic ecosystems, and to protect the biodiversity of the carps as a national genetic resource.

i. In an effort to achieve conservation objectives, more new policies and strategies should be formulated to promote the rational and sustainable use of various endemic and highly productive ecosystems.

ii. The adverse effects of the construction of flood protection embankments and dams should be mitigated by proper modifications of engineering designs to ensure flood protection for the welfare of the people, and at the same time to protect spawning, migration and feeding grounds of various endemic fishes. To accomplish this goal, the hydraulic structures used in FCDI projects should be modified to make them "fish friendly" with fish passes for easy movement of brood fish and juveniles.

iii. Necessary environmental laws should be enacted for immediate implementation. Necessary guidelines for environmental impact assessment must be followed in all sectors concerned.

iv. Industrial pollutants should be treated economically so that they will not foul the receiving environment. Adequate measures should be adopted to provide wastewater treatment facilities to minimize the adverse effects of industrial

pollution on fisheries. A pollution monitoring system with a monitoring network should be established to obtain, process, and analyze reliable information by using modern technology.

iv. Use of harmful and hazardous pesticides should be banned and replaced with biodegradable insecticides. The gradual introduction of Integrated Pest Management (IPM) systems should be implemented.

v. Jute retting in ditches and other water bodies is a common practice that profoundly affects water quality and causes the deterioration of natural habitats of many fish species and other aquatic organisms. The red water flows in large quantities into canals, streams and inundated wetlands, and adversely effects the survival of larvae, fry, juveniles and brood fish of many fish species; therefore, jute retting should be limited to areas where fish do not spawn, feed and migrate.

vi. It is essential to ban indiscriminate and destructive fishing practices to protect young and juvenile fish, which are commonly harvested in all water bodies in Bangladesh. Catching should also be regulated to conserve the brood stocks of commercially important and threatened carp and other fish species. Existing laws and regulations (Fish Act) should be enforced properly to protect fish from destructive fishing.

vii. Proper research needs to be conducted to document threatened and endangered fish species in various ecosystems in Bangladesh. Assistance from international institutions/organizations can be sought for this effort.

viii. Conservation of existing gene pools of native fish species in floodplains, natural depressions, rivers and other related water bodies (*in situ* preservation of all natural habitats) is essential.

ix. When valuable endemic fish species including some carps are believed to be threatened with extinction, their germplasm needs to be preserved in an *ex-situ* condition. Gene pools of endangered fish stocks should be conserved through the establishment of gene banks. The objective of such banks is to preserve endangered fish germplasm either by keeping live species or by cryopreserving their gametes (sperm or ova) for further utilization and distribution among hatcheries through out the country. Subsequently, the development of artificial and mass seed production techniques of these fishes in hatcheries may save them from extinction.

x. A public awareness of the necessity of conservation of aquatic biodiversity and wise management of habitats needs to be created through mass media, viz., TV, Radio, Newspaper and Internet.

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Glossary

Alleles: Members of a pair of different hereditary factors that may occupy a given locus on a specific chromosome and that segregate in formation of gametes. Alternative form of gene.

Beel: Natural depression or floodplain lake whichthat may hold water permanently or seasonally (dry up during the winter season).

Blastodisc: A disc-shaped superficial layer of cells formed by the cleavage of a large yolky eggs such as that of a fish. Mitosis within the blastodisc products the embryo.

Breed: Group of animals having a common origin and identifying characters that distinguish them as belonging to a breeding group.

Brood stock: Parent (Female and Male) fish (female and male) cultivated to provide eggsova/milt or fry.

Chromosome set: A group of chromosomes representing a genome, consisting of one representative from each of the pairs characteristic of the somatic cells in a diploid species.

Chromosomes: Darkly staining bodies in cell nuclei whichthat carry the heredity material. They occur in pairs in somatic cells with the number of pairs being characteristic of the species.

Circular incubation unit: One of the principal forms of fish egg incubating or hatching facility; usually outflow in the center of a circular tank.

Cleavage: The process by which a egg cell gives rise to further cell divisions. In some species like fish, the pattern is lost after the first few cell divisions.

Clonal line: Individuals derived (both inbred or outbred clonal lines) by genetic cloning.

Clone: Individual genetically identical to another.

Crossbred: An animal produced by crossing two or more pure breeds, strains or lines. Crossbred individuals are from intraspecific matings.

Crossbreeding: Mating systems in which hereditary material from two or more pure breeds, strains or lines is combined.

Diallelic crossing design: Mating design for production of base population of fish (viz. carps) under a breeding scheme involving several unrelated stocks or strains of the same species having different alleles at a given locus.

Diploid: Cells with two members of each pair of chromosomes. This is termed the 2n condition and is characteristic of body of most fish species.

Effective Population Number or Size (Ne): The ideal population size in infinitely large, ensuring no loss of genetic variation which is a function of the total number of breeding

individuals, the sex ratio, the mating system employed, and the variance of family size in the production of fish that are used to produce the next generation.

F: The symbol of coefficient of inbreeding. It is a measure of the percent increase in homozygosity that has been created by inbreeding over the population average.

F_1: The hybrid offspring, crossbred offspring or first filial generation from a given mating.

F_2: Offspring of F_1 x F_1 matings or second filial generation.

F_3: Offspring of F_2 x F_2 matings or third filial generation.

F_4: Offspring of F_3 x F_3 matings or fourth filial generation.

Fecundity: Number of eggs produced; sometimes expressed as number per fish, sometimes per kg of fish.

Fertilization: Union of two gametes (ova and sperm) to form a zygote and initiate the development of an embryo.

Fingerling: More advanced stage of fish fry (8 – 10 cm size for most cyprinids).

Floodplain: The area which is largely being inundated by flood water during the moonson.

Fry: Very young stage of fish formed from egg.

Funnel type incubating jars or unit: The funnel shaped egg incubating system where water enters at the bottom and gently turns the eggs over maintaining them suspended in the waterflow; especially used for incubating cyprinid eggs.

Gamete: Reproductive or germ cell. In animals or fish, the male gamete is the sperm or spermatozoa and the female gamete is the ovum. Gametes carry the reduced, 1n or haploid number of chromosomes.

Gastrula: The stage of embryonic development when the gastrulation movements occur.

Gene: The classical term of the basic unit of heredity.

Genetic drift: Changes in gene or allele frequency in a population due tocaused by change variations in proportions of gametes whichthat are formed carrying specific genes or whichthat succeed in accomplishing fertilization. Genetic drift is caused by small reproducing population numbers. Genetic drift is inversely related to effective breeding number.

Genetic introgression: The incorporation of genes of one species into the gene pool of another. If the ranges of two species overlap and fertile hybrids are produced, they tend to back cross with the more abundant species. This process results in a population of individuals of most of whom resemble the more abundant parents but whichthat posses also some characters of the other species.

Genetic variance (V_G): The portion of phenotypic variance for a quantitative phenotype that is due tocaused by the genes.

Genotype: Genetic make up of a fish; that portion which is inherited. The complete genetic make up is also referred to as the genome.

Gonad: Fish organ in which either eggsova or sperm are produced; generally termed as ovary in female or testies in male.

Gonadotropin: Pituitary hormone which controls the production by the gonads (ovary and testies) of ovaeggs and sperm and also the gonadal hormones.

Gynogen: An individual whose DNA is all maternally inherited.

Gynogenesis: Gynogenesis involves fertilization of eggs with inactivated sperm, and prevents any contribution of the male genome to the embryo. As a result, embryonic development proceeds with the inheritance of only maternal chromosome sets.

Haor: Depression on floodplain, located between two or more rivers, which functions as a small internal drainage basin.

Hapa: Fine meshed rectangular or square structureenclosure suspended in water on which fertilized ovaeggs or larvae of fish are deposited after artificial spawning and hatching.

Haploid: Having half the normal number of chromosomes; most gametes are haploid so that when fertilization occurs the diploid condition is restored.

HCG: Human Chorionic Gonadotropin; commercially available, semi-purified hormone thatwhich is used to induce ovulation and spermiation, i.e. egg and sperm production in fish.

Hereditary: A condition controlled or influenced to some degree by gene action. This is in contrast to characters which are entirely controlled by environmental variables.

Heritability (h^2): An atribute of a quantitative trait in a population that expresses how much of the total phenotypic variation is due to genetic variation.

Heterozygosity: The condition of having one or more pairs of dissimilar alleles.

Homozygosity: The condition of having identical alleles at one or more loci in homologous chromosome segments.

Hormonal masculinization: Production of male progeny through sex reversal techniques using androgen hormones (i.e., 17 α- methyletestosterone hHormone).

Hybrid vigour: Increased vigour or productivity often observed in hybrid, crossbred, or crossline individuals as compared to that of the average of the parental types.

Hybridization: Crossing or fertilisation of one species by the sperm of another with the intention of producing progeny with the best characteristics of both parents.

Hypophysation: Injection with an extract of the pituitary gland used to induce ovulation and spermiation.

Hypothalmous: Part of brain whichthat controls many internal body functions and the activity of the pituitary gland; produces releasing hormones.

Inbreeding depression: Decreased performance in growth rate, fecundity, survival, etc., and in which an increased percentage of deformed/abnormal young may fish that occur due to inbreeding.

Inbreeding: A system of mating in which mates are more closely related than average individuals of the population to which they belong.

Indirect or negative selection: Selection of one trait by selecting a second trait.

Karayotype: The somatic chromosomal complement of an individual or species. The term is often used for photomicrographs of the metaphase chromosomes in a standard sequence.

Mass selection: Individual selection or empirical selection of the best male and female individuals from a population.

Meiotic gynogens: Gynogenetic diploids poduced by the suppression of meiotic cell division of fertilized eggs.

Milt: Common name for the milky male sex fluid containing sperm or spermatozoa.

Mitotic gynogens: Gynogenetic diploids produced by the inhibition of first cell division of fertilized eggs.

Monosex population: Production of either all-female or all-male fish through genetic manipulation or sex inversion.

Morula: An embryo that consists of a cluster of cleaving blastomeres.

Neomales: Hormonally masculinized XX genetic females whichthat can be crossed with normal females for mass production of all female seedsprogeny.

Oocyte: The cells that upon undergoing meiosis forms the ovum.

Oogonium: A mitotically active germ cell that serves as the source of oocytes.

Out breeding: A system of mating in which mates are less related than average individuals of the population being intermated.

Ova: Ripe eggs."Eggs" before fertilization.

Ovulation: Detachment or release of eggs from the ovary.

Phenotype: Physical appearance or characteristics of an organism.

Phenotypic variance (V_P): The total variance that is measured for a quantitative phenotype in a population. It is the sum of genetic variance, environmental variance, and genetic-environmental interaction variance; ie. $V_P = V_G + V_E + V_{G\text{-}E}$

Phytoplankton: Microscopic plant life usually found at surface of water.

Pituitary extract: Aqueous, alcoholic or acetone dissolved extract of pituitary gland used for hypophysation or the artificial induction of spawning.

Pituitary gland: Endocrine or hormone- producing gland found on the underside of the brain just behind the eyes.

Polar body: The minute cell produced and discarded during the development of an oocyte. A polar body contains one of the nuclei derived from the first or second division of meiosis, but has practically no cytoplasm.

Polyploid: Designating an individual having more than two sets of chromosome.

Releasing hormone: Peptide hormones whichthat control all aspects of the pituitary gland.

Selection: Any external influence in a population, either naturally or artificially imposed, which enhances opportunities of individuals of some genotypes to contribute genetic material to subsequent generations and thereby to change gene frequencies.

Sex reversal: Modification of sex using genetic or hormonal manipulation.

Sexual maturation: Condition of female and male individuals having ripe gonadal materials (eggsova and sperm).

Spawn: Post hatching stage of fish larvae up to first feeding.

Stocking density: Numbers or biomass of fish expressed per area or unit volume of tank or pond.

Triploid: An organism having three haploid sets of chromosomes in each nucleus. **capacity.**

Vitellogenesis: The formation of yolk in the developing oocyte.

Vitellogenin: A protein synthesized by vitellogenic females and incorporated into the yolk spheres of the developing oocyte.

Index

This manual is written for hatchery managers/operators, fishery biologists, aquaculturists, planners, and those who work practically with carp brood stock management and breeding programs in the hatcheries but have limited scientific background in fish breeding principles and simple genetics. The manual contains chapters on: status of carp genetic resources; brood stock management and artificial breeding of carps in hatcheries; breeding plan for cultured minor carp species; breeding plan for cultured major carp species; breeding and conservation of endangered carp species. Simple techniques to avoid the loss of genetic diversity and avoid inbreeding depression in hatchery populations and to develop genetically improved carp brood stocks through selective breeding and line crossing are explained. Finally, artificial breeding techniques of threatened carp and barb species of Bangladesh and conservation and management measures are discussed.