Discussion Groups

Introduction

Three groups were selected to discuss: (i) strategies for genetic management and improvement; (ii) the role of institutions in genetic management and improvement; and (iii) the role of exotic cyprinids in aquaculture. Each group was selected to include participants with a variety of relevant experience. Participants were asked to bear in mind the main species and geographical focus of the workshop, i.e. Chinese carps in aquaculture in Bangladesh, but to broaden their discussion if appropriate (for example if experience from other species or countries was relevant, or if some points applied to indigenous as well as exotic species). All of the discussion was in the context of the main developmental goal, i.e. how can genetic management/improvement of fish stocks reach and benefit the livelihoods of the poor?

Discussion Group 1: Strategies for Genetic Management and Improvement

This group was asked to consider the technical aspects of genetic management and improvement, including aspects such as "top-down" v's "bottom-up" strategies, centralised v's decentralised strategies and different types of technologies, with the focus on the poor as the target group.

Group members:

MG Hussain (BFRI, chair)	GC Mair (AIT, rapporteur)
BK Barman (ICLARM/IOA/DOF)	A Bart (AIT)
TK Das (NFEP, DOF)	Hameed (BINA, BAU)
M Islam (NFEP, DOF)	MN Islam (FTEP, DFID)
MI Khan (private hatchery owner)	MY Mia (BFRI)
MS Shah (Kulna University)	S Sharia (BRAC)

Major issues:

- Lack of management in the hatcheries and understanding of genetics leads to inbreeding, introgression and negative selection (e.g. size biased selection of broodstock)
- There exists a need for shorter term and long term programmes for improvement and conservation of germplasm, including training
- Traits for selection should include growth and disease resistance

- There is a lack of feedback from farmers to seed producers so impact of current management practices are not known. Misconceptions about quality of seed are passed on to farmers through nursers
- Priority species for poor farmers and consumers, suited to low input aquaculture include:
  - Silver carp
  - Common carp
  - Silver barb
  - Tilapia

Hybridization issues:

- Scientific literature on hybridisation in carps suggests that it is unlikely that there are any major benefits from its application, particularly given the absence of heterosis for commercially important traits in most cases. The very limited incidences where hybrids have been adopted for commercial aquaculture appears to reinforce this view.
- Potential disadvantages of hybridisation relate to problems it creates in maintaining purity of broodstock and the loss of the benefits from distinct ecological niches occupied by pure species which forms the basis for composite fish culture (polyculture). Also the discrete nature of improvement may limit scale of impact (only those with access to parental strains would be able to produce improved hybrid seed).
- Hybridisation is done to address constraints existing within hatcheries, NOT in response to demand. Constraints include:
  - Male bighead carp mature later than the females (females mature in April, males in May in NW Bangladesh) meaning that males are not available early in the breeding season.
  - The demand is higher bighead carp as they grow faster. However silver carp females are more fecund so produce more fry. Hybrids are thus produce using silver carp females with bighead males but fry are often sold as bighead.
- Hybridisation should be discouraged and the following measures could be taken to achieve this.
  - Raise awareness of the issue through training and extension
  - Address constraints directly, e.g. through making cryopreserved sperm available early in the breeding season or including time of maturation as a trait in breeding programmes
  - Legislate through certification and application of genetic markers although doubts exist whether such legislation would be enforceable.

Genetic improvement approaches – longer term:

- Emphasis should be on selection, initially with mass selection as the most realistic technique
- Responsibility for quality should reside with research institutions, Government hatcheries and selected NGOs. There is a need to formulate policy on this issue (see Group 2 report)
- The risk remains that some hatcheries may remain outside of centralised systems of seed supply and this issue needs to be addressed, possibly through the greater inclusion of NGOs.
- Certification and monitoring systems are needed to ensure adequate quality control in the production and dissemination of improved fish breeds
- The selection environment should be representative of culture systems (particularly low-input systems) and ideally stakeholders (farmers) should be involved in setting breeding goals (and where feasible also in the process of selection)

Training needs:

- Formal training in technical aspects of broodstock management and development should be targeted at:
  - Selected hatcheries with the necessary resource base
  - Government employees within various agencies
  - Trainers of trainers (TOT) including DOF staff and extension agents
- Awareness of best practices in broodstock management should be raised thorugh a variety of media, focusing on hatchery operators, nursers and seed traders
- Cross-visits between scientists to different countries where advances in genetics are occurring would help researchers in maximizing the practicality and efficiency of breeding programmes.

Gene banking:

- It was reported by some participants that up to 90% of seed production of silver and bighead carps uses artificial fertilization, although this figure is much lower for other carp species. This creates the opportunity for the utilisation of cryopreserved sperm which requires artificial fertilization.
- There may be a demand from private hatcheries for cryopreserved sperm from good quality broodstock
- The potential appears to exist for using cryopreserved sperm to distribute quality germplasm and address constraints which give rise to the use and misuse of hybridisation
- It was thought by participants that use of cryopreserved sperm could be economically viable but this would need to be investigated.

- There may be some risk in the widespread use of cryopreserved sperm if sperm quality is not assured
- A pilot research study was advised and a proposal should be developed for such a study
- It was noted that there is also a demand for gene banking for conservation of threatened species

Alternative management/improvement technologies:
In a discussion on alternative and shorter term genetic improvement strategies, three priority research areas were identified.

- Continue with development and promotion of monosex silver barb
- Development of genetic markers for detection of introgression for all carps
- Respond to the growing demand for monosex tilapia through sex reversal and GMT technologies

Discussion Group 2: The role of institutions
This group was asked to look at how different institutions could work together to manage, improve and disseminate stocks of carps in ways which would reach and benefit the poor.

Group members:

MA Mazid (BFRI, chair)	BJ McAndrew (IOA, rapporteur)
MA Sattar (NFEP)	Y Basavaraju (UASB)
MG Rabbani (NFEP)	MR Azam
MTH Farhaji (MOFL)	M Hossain (BRAC)
F Rajts (4th FP)	

The attached figure shows a model devised by the group for the potential roles of different institutions in genetic management and breeding programmes for the different species of carps that are important in hatchery-dependant aquaculture and fisheries in Bangladesh. This centres around "*lead broodstock centres*" and addresses the role of different institutions in management of these stocks, dissemination, training and research. Staff in these lead broodstock centres would need to be "genetically aware", i.e. to have sufficient knowledge of genetics to manage these core stocks.

It should be noted that some of the elements of this already exist. For example, wild-origin stocks of Chinese carps are held at the NFEP campus in Parbatipur, BFRI maintains stocks of several species (including a selective breeding programme for silver barbs and landraces of major carps) and BRAC has a selective breeding programme for major carps (with scientific advice from BFRI). Realistically, it is beyond the capacity of any single institute to hold and properly manage stocks of all of the carp species that are important to aquaculture and enhanced fisheries in Bangladesh (an additional factor is that it is generally considered that separate hatcheries should be established to produce fish for aquaculture and restocking, since in the former it is desirable to select for improved performance in culture while in the latter the emphasis is generally on minimising any changes from wild characteristics).

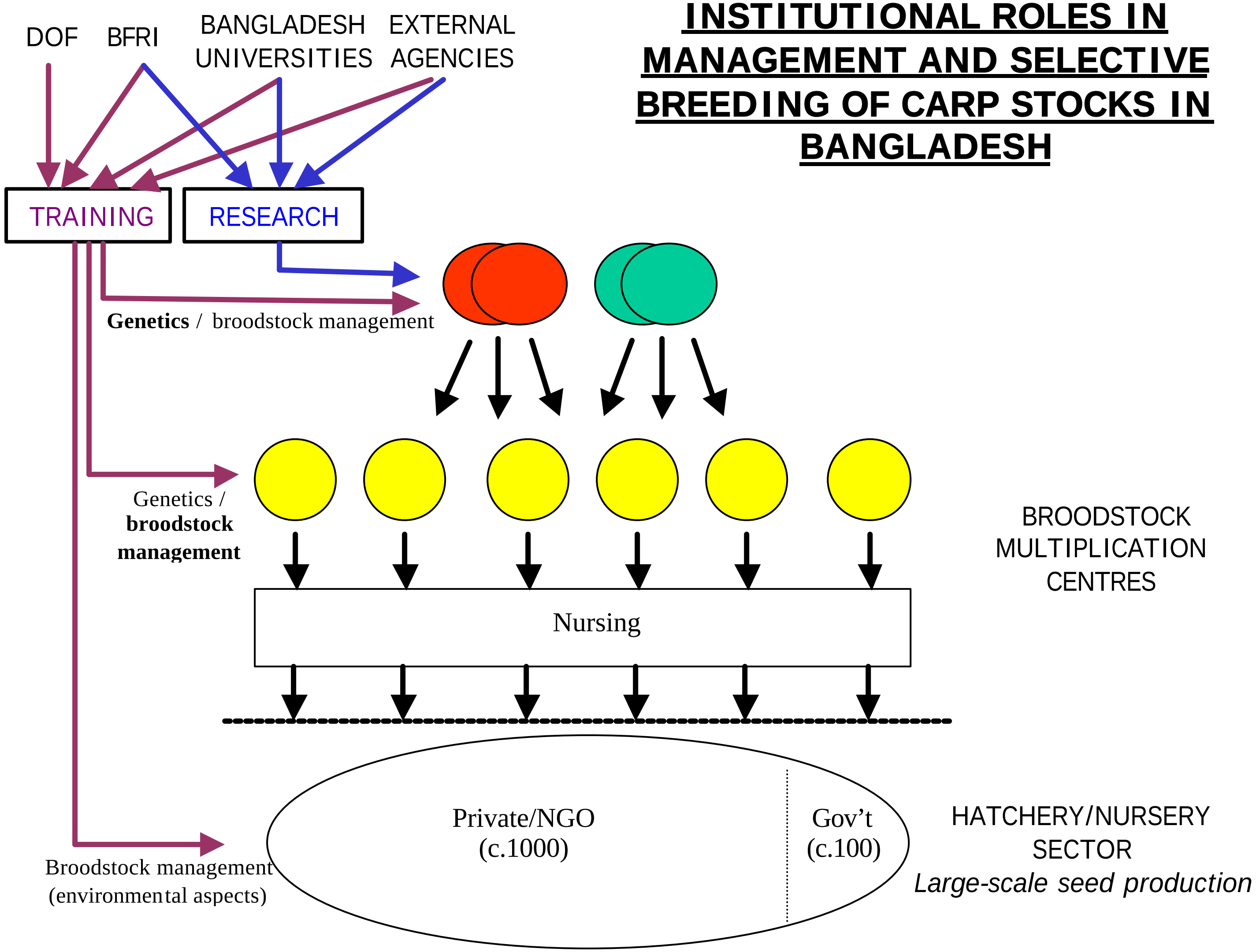
In addition to the lead broodstock centres, it would be necessary to have "*broodstock multiplication centres*" in different parts of the country to act as focal points for the multiplication and dissemination of quality broodstock from the lead centres in each geographical division of Bangladesh. These broodstock multiplication centres would need to have staff who were trained in appropriate aspects of genetic management of broodstock. These would probably be DOF hatcheries, although it is possible that NGO hatcheries could take up this role. There may be a need for additional nurseries to rear these seed to fingerling or larger stages before dissemination to the existing hatcheries (mostly private, also NGOs, some DOF) which account for mass seed production in Bangladesh.

The figure shows how different organisations would contribute to research and training. The policy for such a scheme would be largely determined by DOF and BFRI, and initiation would have to be taken up by the DG's of these two organisations. Training was identified as a key requirement: within the core of the scheme this would include 2-3 PhD's (for the lead broodstock centres), 4-6 MSc's and approximately 30 short course training places for hatchery managers.

The GEF may be able to organise some of the training (see Discussion following these presentations).

Note added during editing: cross-programme DFID funds have been allocated to study the role of genetic management in fisheries enhancement in Bangladesh (Ian Payne/BJM).

INSTITUTIONAL ROLES IN MANAGEMENT AND SELECTIVE BREEDING OF CARP STOCKS IN BANGLADESH



Discussion Group 3: the role of exotic carps

This group was asked to examine the role of Chinese and other exotic carps in aquaculture, fisheries and sustainable livelihoods in Bangladesh - species, trends, priorities and problems associated with the use of exotic species - and to consider whether the silver x bighead carp hybrid does have a role in aquaculture here.

Group members:

K Ahmed (BARC, chair)
MS Alam (BAU, rapporteur)
MS Islam (BFRI)
AHM Kohinoor (BFRI)
MA Rahman (BFRI)
M. Shazu (private hatchery owner)
AK Sarker (FFP)
A Woynarovich (MAEP)

Species and trends:

- Silver carp accounted for 25% of carp production in Bangladesh in 1999 according to FAO figures. Participants thought that this may be decreasing
- Bighead carp did not appear in the 1999 FAO figures – may be decreasing?
- Grass carp (10% of carp production in Bangladesh in 1999)
- Black carp (not listed in 1999 figures) – not widely accepted
- Silver barb (not listed in 1999 figures) – increasing, widely accepted
- Common carp (10% of carp production in Bangladesh in 1999) – stable

Priorities:

- A greater understanding of market-driven production is required. Production of different species changes very rapidly in Bangladesh in response to e.g. small changes in market prices, leading to rapid rises and falls in production levels (e.g. recent boom and bust in *Pangasius* production).
- Research supporting production, e.g. on broodstock propagation (including unplanned hybridization), nursery and table fish production.
- Training and extension.

Problems:

- Decreasing growth performance of silver and bighead carps is widely reported
- Competition exists between bighead carp and other zooplankton feeders (principally catla)
- Competition for farm resource allocation exists between cattle and macrophagous exotic fish species (grass carp, silver barbs)
- Disease susceptibility (e.g. silver barbs)
- Non-availability of required size fingerlings (e.g. silver barbs)

- Marketing of exotic species

The question of whether there is a role for the silver x bighead carp hybrid requires further evaluation and detailed study.

General discussion after summary of group presentations

This discussion took place following a presentation of a summary of the group presentations by D. Penman (the final version of this is included in the workshop Summary and Recommendations). The names of those who made the contributions to this discussion (below) were not recorded.

Main points raised in discussion:

- The genetic study on silver carp, bighead carp and hybrids should be broadened to look at intraspecific variation and morphological aspects such as length of gut (longer in silver carp), gill morphology and pectoral fin length (no overlap with pelvic fin base in silver carp, overlap present in bighead carp).
- Genetic studies should be broadened to indigenous species. This will not happen under the present project, but the human resource development suggested by this meeting should apply to all species.
- Aquaculture in Bangladesh has already benefited from movements of exotics (e.g. Chinese carps, silver barbs, Nile tilapia): this should be supported within a responsible framework (quarantine, etc).
- Hybridization could perhaps be avoided by using cryopreserved sperm when there is a lack of mature males to fertilise eggs?
- Although the present project focuses on exotic species (since it is more difficult to obtain wild or new stocks of such species), it was suggested that appropriate management/improvement measures should also be put into place for endemic species, before problems of availability of wild stocks arise.
- There may be a need for periodic upgrading of stocks of exotic species.
- Mechanisms and funding sources need to be identified which can support training and the upgrading of laboratory facilities.
- The role of different institutions in genetic management/improvement should be explicitly stated in the workshop recommendations.
- The project should be able to leave visible, sustainable outputs behind after it is finished. To do so, synergy between different projects, institutions, etc is needed for human resource development, upgrading institutional capacity, information networking, etc. It was pointed out that it is sometimes difficult to find out about all of the different projects in Bangladesh. The project organisers will attempt to

ensure maximum availability of the proceedings, both as a hard copy proceedings and by placing it on the DFID AFGRP website (**http://www.dfid.stir.ac.uk**).

- Silver carp and bighead carp contribute individually to aquaculture in Bangaldesh, but we need more information about what is happening in the field – especially hybridization.
- Market-driven production – Bangladesh has a very sensitive and rapidly changing market. Appropriate training, i.e. teaching farmers the principles of aquaculture, not simply "recipes", should allow them to adapt their aquaculture practices more rapidly to respond to changing trends. Training in genetic aspects would make farmers more capable of avoiding poor quality seed. It is best if such pressure can come from buyers (farmers), although certification could perhaps help.
- The role of exotics in fisheries was not discussed, since there was nobody in the group with the relevant expertise. This was put to the meeting for comments from outside of group 3. It was suggested that there is certainly a role for the silver carp in aquaculture in Bangladesh, since here are no suitable endemic species which consume phytoplankton. The silver carp does not do well in rivers during the rainy season due to suspended silt, and it is also highly migratory. A review of the open water stocking programme with silver carp did not support the yields initially claimed. The common carp may compete with endemic species, but does not appear to have established yet. The common carp does well in e.g. flooded rice stubble – it can grow to 2-3 kg after 5-6 months of the flood season, and has better growth potential than comparable endemic species e.g. mrigal (200-300 g over the same period). Turning to non-cyprinid exotic species, there seems to have been little establishment of the Nile tilapia in Bangladesh, and this may also be true of the African catfish, although this has not been studied in enough depth.
- It was suggested that the bighead carp should be banned from Bangladesh, due to its competition with catla and hybridization with the silver carp.

Future actions:

- The AFGRP cannot fund training (except in some cases as part of research projects), but recommendations from a workshop such as this may help to obtain such funding from other sources. The 4th Fisheries Programme may be able to provide some training, but this has not been decided yet.
- The Bangladesh Universities, IOA and AIT all expressed willingness to carry out training in genetics at various levels.
- There is a need for collaboration between the Bangladesh Universities, BFRI, DOF, etc on training of staff for developmental and implementation agencies.
- NGO's and the private sector should also be included in programmes such as that suggested by discussion group 2, where they can have competency. Different

institutions are already involved in genetic management/improvement of different species (NFEP, DOF – Chinese carps; BFRI – silver barbs, GIFT tilapia and others; BRAC – major carps)

- Trained staff also need to be given the appropriate opportunities to use their expertise. This is often a constraint. There is a need to use trained staff in effective ways.

Vietnam: Stock comparisons for polyculture and national breeding programmes

G.C. Mair[1] and P.A. Tuan[2]

1 Asian Institute of Technology, Bangkok, Thailand
2 Research Institute for Aquaculture No. 1, Hanoi, Vietnam

Mair, G.C. and Tuan, P.A. 2002. Vietnam: stock comparisons for plyculture and national breeding programmes. pp. 37-42. In: Penman, D.J., Hussain, M.G., McAndrew, B.J. and Mazid, M.A. (eds.). Proceedings of a workshop on Genetic Management and Improvement Strategies for Exotic Carps in Asia, 12-14 February 2002, Dhaka, Bangladesh. Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh. xxx p.

The government of Vietnam has formulated a Fisheries Master Plan to Year 2010. The national objective for fisheries and the fishing industry is to contribute effectively to the improvement of the national economy and the social and socio-economic conditions of the people. The Fisheries Master Plan includes a component on maximizing the economic contribution from aquaculture habitats. This component aimed to ensure that the natural resource potential for aquaculture would be realized in an effective and sustainable manner in support of economic development and growth and food security. One of the important broad based operational strategies for achieving this aim was to increase production of fish from freshwater aquaculture for both domestic and export markets. A major emphasis of aquaculture production is reflected in the Aquaculture Seed Development Programme, prepared by the Ministry of Fisheries (MOFI) in 1999. This Programme aims to promote the expansion of fish hatcheries, improving hatchery technologies in the commercial sectors to expand and improve the production of quality fish seed.

An important component of the Aquaculture Seed Development Programme is the formation of three National Broodstock Centers (NBCs), the proposed functions of which are shown in Box 1.

Box 1 The objective of the NBCs is the production of high quality broodstock and preservation of fish genes. This can be achieved through implementation of a number of key activities:

- The application of good broodstock management practices and genetic improvement programmes to maintain or improve the quality of commercially important cultured freshwater species
- The production and distribution of quality broodstock to hatcheries with initial emphasis on provincial hatcheries
- The production and distribution of fish seed to some growers
- The maintenance of gene banks for the most important freshwater fish species
- The development and demonstration of advanced hatchery technology
- The production of training and extension material and the training of fish seed producers

The Vietnamese component of the research project on the Genetic Status and Improvement of Exotic Carp for Low-input Aquaculture in Asia has the following objectives in support of the NBCs.

1. Development of methodologies for conducting on-station and on-farm stock comparison in carp polyculture illustrating effective optimisation of resources (compared to conducting comparisons of single species).
2. Rational choice of stocks for optimised production in low-input aquaculture systems, namely lowland and upland rice-fish culture, sewage-fed fish culture and integrated VAC systems.
3. Estimates of heterosis in crossbreds of these species, which would be expected to result in recommendations for the adoption under culture of one or more crossbred genotypes.
4. Quantifiable estimates of the potential production and economic gains (including in a livelihood context) of adoption of "improved" polyculture systems and of "best" stocks, compared to existing polyculture and traditional low input systems.

5. The formulation of recommendations on the choice of stocks, the management strategies and the further genetic improvement of these aquaculture stocks.
6. The creation of a live and cryopreserved (sperm) gene bank of base stocks available for carp aquaculture in Vietnam, duplicated at AIT.

Progress to date in the project includes the assembly of live gene banks of available stocks including the importation of new stocks of key species. Species on which the project focuses includes Chinese carp, silver carp and grass carp, the Indian major carp mrigal and rohu together with common carp and silver barb. In particular new introductions of silver and grass carp have been made from China and of Indian carp, rohu and mrigal, directly or indirectly from India. Protocols have been developed for the simultaneous evaluation of up to four stocks per species under polyculture in both on-station and on-farm environments (representing all of the aforementioned low-input aquaculture systems). Early results from the first year of growth trials indicate superior culture performance of newly introduced stocks of rohu (from India via Thailand) and grass carp (from China) compared to locally cultured stocks. However the opposite was true in the case of silver carp, possibly due to introgression of the introduced stock with an indigenous sub-species thought to have existed in Vietnam.

In addition, sperm cryopreservation protocols developed and optimized for common carp have been found to be effective for grass carp and mrigal and are likely to be effective across all the species on which the project is focused. Cryopreserved sperm gene banks have been initiated to supplement the live gene banks for grass carp and mrigal and these will be extended to include rohu and silver carp in the coming year.

The Vietnamese component of the exotic carp genetics project originally focused on the Indian and Chinese major carps and did not include any significant component on common carp. Since the project was initiated however, there has been a growing interest in the status and potential of “indigenous” common carp which are the mainstay of traditional rice-fish culture systems practiced by the indigenous people of the mountainous regions of northern Vietnam (extending into Laos and southern China). These mountain ranges have some of the poorest communities in the country due in part to their isolation from the mainstream economy (Little and Tuan, 1999). Under Programme Development funding from the (then) DFID Fish Genetics Programme, a survey was conducted of the role of indigenous common carp in these traditional culture systems, which included field research into the ancestry and genetic management of the stocks. The context in which the study was conducted was that government agencies, mainly the Research Institute for Aquaculture No. 1, were promoting and widely distributing an improved common carp strain derived from selection applied to a population derived from a native Vietnamese stock and stocks introduced from Hungary and Indonesia. Given the particular characteristics of the traditional upland rice-fish culture systems, it was not clear whether this improved strain would be most appropriate for these systems and furthermore, whether the indigenous stocks, that could be threatened by the widespread introduction of an improved strain, were in fact valuable genetic resources, particularly for these poor communities. The resulting outputs from this Programme Development research (Mair, 2000; Edwards *et al.*, 2000) painted an interesting picture. The outputs from this research, elements of which were subsequently integrated into the Vietnamese component of this project were:

- A report highlighting the major socio-economic and environmental impact issues related to the introduction of carp polyculture and in particular improved strains of common carp into regions where traditional rice-fish aquaculture is practiced.
- An article highlighting important issues from the report published in World Aquaculture magazine
- A live gene bank of common carp stocks available in northern Vietnam.
- The creation of a cryopreserved sperm gene bank of common carp stocks in northern Vietnam
- Initial recommendations on the appropriate policy and management of stocks of indigenous common carp in northern Vietnam

The main policy elements and recommendations derived from this study are shown in Box 2.

Some major properties of indigenous carp, largely derived from a genetic adaptation to these particular systems over many generations, included residence in the rice field (whereas other stocks often escaped), low incidence of disease and higher market price. It was however evident that the growth rate and productivity of these stocks was low, particularly when compared with the selectively improved RIA#1 strain. Based on information on the long-term management of the stocks of indigenous stocks, it was evident that most were maintained within farms, or groups of farms, as reproductively isolated populations and thus subject to genetic forces of inbreeding, genetic drift and unconscious selection. Inbreeding and genetic drift may have contributed to their relatively poor growth rates whilst unconscious selection may have acted as the mechanism behind the development of their advantageous adaptive properties.

Box 2. Policy implications and recommendations derived from a study of the role of indigenous common carp in traditional upland rice-fish culture systems of northern Vietnam

- There is a need for more detailed surveys of current practices and distribution of traditional rice /fish culture systems, and new species and culture systems, in particular their relevance for poor farming households
- There is a need for on-station and on-farm research into the biology (reproduction, nutrition) and growth of local common carp stocks in rice fields and ponds, with characteristics of relevance for the resource base of poor farming households
- Local common carp may have significant benefits for culture in traditional rice/fish systems despite what may be poor growth rates
- Undisturbed populations of local stocks should be identified and preserved through habitat conservation and gene banking
- The role of indigenous common carp stocks in traditional systems and their importance to poor households should be characterized before the introduction of "improved" strains or culture systems
- The inclusion of local stocks should be actively considered in genetic improvement programs developing strains for low-input rice/fish systems, which should consider such traits as retention in the fields, disease resistance and adaptation to shallow water

In accordance with the recommendations outlined above, stocks of indigenous common carp have now been included in farm trials and gene banking activities being carried out under the exotic carp genetics project (R 7590). Performance data, initially of the pure stock but at a later stage of crosses between them, will provide some useful data on which to base future management decisions. Results from data collected to date, after up to 120 days of grow out, show that survival of the indigenous stocks was superior to that of the RIA #1 selected carp during nursing on-station. However, growth of the selected strain was superior to all indigenous stock during nursing and subsequently during grow-out both on-station and in all four low input aquaculture environments. It was noted however that the superiority of the selected strain is lowest in the upland rice-fish culture systems to which the indigenous carps are adapted.

A supplementary project has now been proposed to extend the research work to include genetic characterisation of the indigenous carp stocks to provide further data to better inform future decisions on the management, exploitation and conservation of these potentially very important fish stocks. One potential application of these data is that if it shown that poor growth rates are associated with low levels of genetic variation within farm stocks, due primarily to inbreeding, it may be possible to improve growth performance of these stocks by outbreeding or crossbreeding them, whilst retaining their important adaptive properties. In the context of the current and future livelihoods of households operating these traditional systems such management decision could be of considerable importance.

References

Edwards, P., Hiep, D.D., Minh Anh, P. and Mair, G.C. (2000). Traditional culture of indigenous common carp in rice fields in northern Vietnam: does it have a future role in poverty reduction? World Aquaculture 31(4):34-40.

Little, D.C. and Tuan, P.A. (1999). Common carp Indigenous Ricefield based culture in Northern Vietnam. AARM Newsletter, 4(3):4-5.

Mair, G.C. (2000). Programme Development Research Completion Report: Socio-economic and environmental impacts of the introduction of genetically improved fish in Vietnam. Report to the DFID Fish Genetics Programme. 3p.

Genetic Status and Strategies for Improvement of Common Carp (*Cyprinus carpio*) in Karnataka, India - Evaluation of Stocks for the Development of a Breeding Programme

Y. Basavaraju[1], D.J. Penman[2] and G.C. Mair[3]

1 University of Agricultural Sciences, Fisheries Research Station, Hesaraghatta, Bangalore, India
2 Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland
3 Asian Institute of Technology, Bangkok, Thailand

Basavaraju, Y., Penman, D.J. and Mair, G.C. 2002. Genetic status and strategies for improvement of common carp (*Cyprinus carpio*) in Karnataka, India - evaluation of stocks for the development of a breeding programme. pp. 29-36. In: Penman, D.J., Hussain, M.G., McAndrew, B.J. and Mazid, M.A. (eds.). Proceedings of a workshop on Genetic Management and Improvement Strategies for Exotic Carps in Bangladesh, 12-14 February 2002, Dhaka, Bangladesh. Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh. 123 p.

Abstract

The importance of common carp (*Cyprinus carpio*) to aquaculture and enhanced fisheries in Karnataka state, southern India has steadily increased since its first introduction. According to the most recently available statistics, common carp now contributes 43% of the seed stocked by the Department of Fisheries (Basavaraju *et al*., 2000). Precocious maturation and unwanted reproduction have been identified as potential constraints on yields of common carp in aquaculture and culture-based fisheries in Karnataka (Basavaraju *et al*., 2002). Both males and females can attain sexual maturation well before reaching a marketable size. Even if fish do not spawn and produce fry, the gonadosomal index (GSI) can exceed 20% of the harvested weight of an individual fish. This early maturation poses a potential problem for culture, in that energy is likely to be diverted from somatic growth into gonad development and reproduction, and fry and fingerlings may compete with stocked fish for resources within the culture system. Various options such as induction of triploidy, production of monosex populations and evaluation of different stocks were

investigated under a research project funded by DFID FGRP, UK. The present paper discusses the results of the preliminary trials on evaluation of different stocks of common carp, and future strategies planned for the genetic improvement of common carp stocks.

Introduction

The common carp (*Cyprinus carpio*) is an important species in aquaculture and enhanced fisheries in Karnataka state, southern India. It is grown either alone or in polyculture, most commonly with catla (*Catla catla*) and rohu (*Labeo rohita*). The common carp presently grown in Karnataka originate from two introductions to India, in 1939 ("German" strain) and 1957 ("Bangkok" strain) (Jhingran, 1991). These have become mixed over many generations to give the current stock. This stock of common carp is characterised by early sexual maturation (at an age of approximately six months and sometimes at a weight below 100 g) and multiple spawning in a single year. This early maturation poses a potential problem for culture, in that energy is likely to be diverted from somatic growth into gonad development and reproduction, and fry and fingerlings may compete with stocked fish for resources within the culture system. Several local farmers, fisheries officials and scientists have cited this as a problem in the culture of common carp in the state, although the extent of this problem has not been quantified. The problem is unlikely to be as severe as that seen for tilapia but may nevertheless have significant negative impacts on yields.

The bulk of common carp seed production in Karnataka is by the State sector, with approximately 30 State controlled hatcheries producing seed of this species. Whilst there is a greater degree of gene flow in and out of some of these hatcheries than exists for Indian major carp stocks, most of these hatcheries nevertheless function as reproductively isolated units. Furthermore, hatchery managers are commonly focused on producing sufficient quantity of seed to attain prescribed targets, with little if any consideration for the genetic quality of the stocks. Inbreeding, negative selection and genetic drift are likely to have occurred during the long history of domestication of these stocks in such environments, although this is hard to verify (Basavaraju et al., 2000).

There are a number of potential solutions to problems of early maturation:

- Induce sterility by induction of triploidy or through hormonal treatments
- Produce monosex populations, which will not breed and may mature later (sexual maturation is commonly delayed in fish in the absence of the opposite sex).

- Evaluate other strains of the species in the hope of identifying later maturing strains.

The above options were investigated under research funded by DFID-AFGRP, UK.The present paper describes results of initial growth trials on several stocks of common carp and the development of a breeding programme for this species in Karnataka. Faster growing stocks of common carp may also mature later (at a larger size and/or greater age) and are likely to benefit those involved in producing and consuming common carp as long as genetic improvement is carried out in an appropriate way (e.g. selecting for improved performance in low input environments rather than increased appetite).

Identification and collection of different stocks of common carp

A number of stocks of common carp from different geographical locations outside India were identified as having potential for faster growth and delayed maturation. These were imported into India following appropriate quarantine, etc. Two local stocks of common carp were also included in the collections. These stocks are listed in Table 1.

Table 1. List of common carp stocks held by the project component in India and the abbreviations used for them.

Full Name	Abbreviations	Source
Wild Amur Carp	Amur	Hungary
P-3 (Selected Line)	P-3	Hungary
Selected Vietnamese	SV	Vietnam
Unselected Vietnamese	UV	Vietnam
Rajadanu	RJ	Indonesian
Local stock (FRSH)	L-FRSH	Fisheries Research Station, Hesaraghatta
Local stock (BRP)	L-BRP	BRP state hatchery

Evaluation of different stocks of common carp: trial I

The initial growth trials were conducted with the first two imported stocks (SV and UV) and with one local stock of common carp in mono and polyculture systems. For each pond, fingerlings of each stock were reared to a similar size before being communally stocked. Data is presented here from monoculture experiments conducted on station (at FRSH) and polyculture growth trials conducted in a farmer's pond. The fish were marked using PIT tags. The results of two monoculture trials are summarised in Tables 2 and 3, while those from the farmer's pond are shown in

Tables 4 and 5. It is clear from these results and other trials (not shown here) that the performance of the SV was superior to L-FRSH and UV in that it exhibited faster growth rate and lower GSI (gonadosomatic index).

Table 2. Stocking details and mean weight (g) attained by different stocks of common carp in monoculture at final harvest (after 36 weeks of rearing). Means within a column with different letter superscripts are significantly different from each other (P<0.05).

Stock	Pond 1		Pond 2	
	Initial wt	Final wt	Initial wt	Final wt
SV	13.41±0.16[a] (55)	238.79±45.97[a] (29)	13.39±0.12[a] (55)	181.6±9.13[a] (25)
UV	12.47±0.38[a] (55)	155.4±23.44[ab] (25)	13.03±0.46[a] (55)	160.38±8.03[a] (26)
L-FRSH	12.29±0.34[a] (55)	106.55±10.32[b] (29)	12.19±0.30[a] (55)	126.21±5.97[b] (29)

Table 3. GSI of different strains of common carp in monoculture at final harvest (after 36 weeks of rearing). Means within a column with different letter superscripts are significantly different from each other (P<0.05).

Stock	GSI - Pond 1		GSI - Pond 2	
	Male	Female	Male	Female
SV	8.50±1.00[a] (7)	22.22± 0.00[a] (1)	13.00±3.83[b] (2)	21.88±1.68[a] (6)
UV	10.01±1.23[a] (5)	22.29±1.51[a] (3)	11.76±1.45[b] (4)	18.69±6.58[a] (4)
L-FRSH	11.23±1.78[a] (4)	21.30±1.69[a] (4)	17.61±1.55[a] (5)	22.58±2.33[a] (3)

Table 4. Stocking details and mean weight (g) attained by SV and L-FRSH stocks of common carp in polyculture at harvest in on farm trial, Amareshwara camp. Means within a column with different letter superscripts are significantly different from each other (P<0.05).

Stocks	Initial No.	Initial Mean Wt. (g)	No. Harvested	Mean Wt. (g)	Survival (%)	Wt. Gain (g)
SV	50	6.78 $\pm0.10^{a}$	22	444.54 $\pm22.75^{a}$	44	437.76 $\pm22.72^{a}$
L-FRSH	50	6.69 $\pm0.14^{a}$	21	371.19 $\pm13.88^{b}$	42	364.45 $\pm13.90^{b}$

Table 5. GSI and Dressout percentage of SV and L-FRSH stocks of common carp in polyculture at harvest in on farm trial, Amareshwara camp. Means within a column with different letter superscripts are significantly different from each other (P<0.05).

Stocks	No.	GSI (%)	Dressout (%)
SV males	15	4.90 ± 0.74^{c}	88.33 ± 0.84^{a}
SV females	7	5.92 ± 1.75^{c}	87.24 ± 1.58^{a}
L-FRSH males	14	8.47 ± 1.35^{b}	81.97 ± 2.44^{b}
L-FRSH females	7	12.87 ± 2.00^{a}	82.27 ± 1.37^{b}

Evaluation of different stocks of common carp: Trial II

With the addition of more stocks, further trials were conducted during 2000-01 under different environments and farming systems. Table 6 shows the general design for this and subsequent trials. Figure 1 presents results from on-station environments in this trial as histograms, while Table 7 gives the mean rank of each stock across the different environments for a range of traits.

Table 6. Generalised trial design - environments and farming systems.

Location	Culture environment	No. of units
FRS	Pond polyculture – communal	1
	Pond monoculture – communal	1
	Concrete tanks monoculture – separate	5 x 2
BRP	Pond polyculture – communal	1
Farmer 1	Pond polyculture – communal	1
Farmer 2	Pond polyculture – communal	1

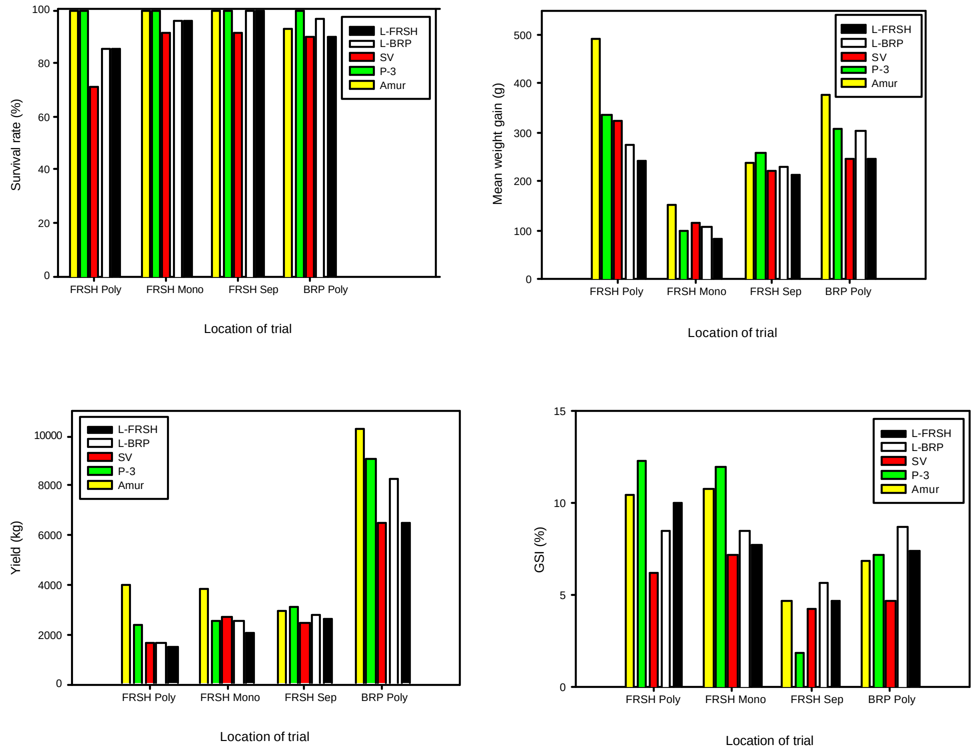


Figure 2. Histograms showing the relative culture performance (survival, mean weight gain, yield and GSI) of five strains of common carp cultured in four different on-station environments. Environments are (from left to right in each histogram) FRSH-Polyculture, FRSH-Monoculture, FRSH-Monoculture (separate stocking) and BRP-Polyculture (see also Table 6).

It is clear from these results that the Amur strain introduced from Hungary is superior for the majority of traits. The other Hungarian strain (P3) showed the highest survival rate during the trial. Interestingly, the SV strain, although performing relatively poorly for most traits, was the best for reproduction-related traits (GSI and dressout).

Table 7. Mean rank of different stocks for a range of traits across four culture systems (highlighted cells indicate highest ranking stock for each trait).

Trait	Amur	P-3	SV	L-BRP	L-FRSH
Survival	2.13	1.63	4.88	2.88	3.50
Weight Gain	1.25	2.25	3.50	3.25	4.75
Harvest Weight	1.25	2.25	3.50	3.25	4.75
Gutted Weight	1.25	2.75	3.00	3.00	5.00
Yield	1.25	2.00	4.00	3.25	4.50
Gutted Yield	1.25	2.25	3.50	3.25	4.75
GSI (%)	2.50	2.50	4.75	2.25	3.00
Dressout (%)	3.00	4.00	1.25	3.25	3.50
Seinability	2.13	2.75	2.75	3.63	3.75

Evaluation of different stocks of common carp and F1 crosses between stocks

The current series of trials focuses on completion of the evaluation of the different stocks and on evaluation of F1 crosses between the better performing stocks to determine if heterosis (superiority of crosses over parental stocks) is found and should be taken into account in future breeding plans. The L-FRSH and UV stocks have been dropped from this series of trials, but the most recent import, the RJ stock, has been added, making a total of five (Amur, P-3, SV, L-BRP and RJ). We are currently running one trial comparing pure stocks and one containing a mixture of F1 and pure parental stocks. Ranking of mean weights from the on-station ponds approximately three months after stocking gives the following overall rank order for the first of these (pure stocks): RJ > Amur > L-BRP > SV. The same analysis for the second trial (pure stocks and crossbreds) gives: Amur > L-BRP*Amur > L-BRP = L-BRP*SV > SV. This data is very preliminary, but does appear to suggest consistency of stock ranking between the two trials (not surprising given that they came from the same breeding sets) and an absence of heterosis in the crossbreds at this stage.

Future activities

The current series of trials will be completed in 2003, allowing us to determine which stocks and/or crosses are best for conditions in Karnataka. Heterosis has been observed in crosses between different stocks of common carp in other situations, and if large may be worth utilising. The exact nature of the breeding plan will be determined by the outcome of the current trials. For example, in the absence of significant heterosis, this may consist of a selective breeding programme based on a synthetic base population developed from the best stocks.

A socioeconomic survey is under progress to study the present status of carp aquaculture in Karnataka and also the perception of farmers and those involved in development of fisheries and aquaculture in the state. The survey will also provide information on the type and socioeconomic status of the farmers. Participatory studies are also expected to help us to prioritise traits for selection.

Cryopreservation will be used to set up a sperm gene bank from the founder stocks: this can be used in the future to replace any unexpected losses and to evaluate progress through selective breeding (eggs from a future generation of carp can be split and fertilised by milt from the future generation and milt from the base stock: comparison of the performance of the two groups should give us a measure of half of the genetic gain). Monosex female production is also being evaluated and may be incorporated into breeding plans.

References

Jhingran, V.G., 1991. Fish and Fisheries of India. 2nd Ed., Hindustan Publishing Corporation, New Delhi. 727p.

Basavaraju, Y., Mair, G.C. and Penman, D.J. 2000. Proceeding of the second workshop on Genetic Improvement of Carps. 14-16 February, 2000. UAS, Bangalore, 79p.

Basavaraju, Y., Mair, G.C., Mohan Kumar, H.M., Pradeep Kumar, S., Keshavappa, G.Y. and Penman, D.J. 2002. An evaluation of triploidy as a potential solution to the problem of precocious sexual maturation in common carp, *Cyprinus carpio,* in Karnataka, India. Aquaculture 204:407-418.

Private Hatchery Owners' Perspectives on Hatchery Management in Bangladesh

A.S. Sarder[1], M.R. Azam[2] and M.A. Hossain[3]

1 Arabpur fish farm, Puratan Kashba, Airport Road, Jessore, Bangladesh
2 MSc Student, Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna, Bangladesh
3 Research Associate, Bangladesh Aquaculture Research and Dissemination Service (BARDS), Puratan Kashba, Airport Road, Jessore, Bangladesh

Sarder, A.S., Azam, M.R. and Hossain, M.A. 2002. Private hatchery owners' perspectives on hatchery management in Bangladesh. pp. 77-81. In: Penman, D.J., Hussain, M.G., McAndrew, B.J. and Mazid, M.A. (eds.). Proceedings of a workshop on Genetic Management and Improvement Strategies for Exotic Carps in Asia, 12-14 February 2002, Dhaka, Bangladesh. Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh. xxx p.

Abstract
The paper is presented in a view to expose the overall management techniques that should be performed in a carp hatchery, such as pituitary gland collection and preservation, brood management, reconditioning of broods before induced breeding, pituitary extract preparation, hatching jar preparation, breeding techniques, multiple breeding of the same brood fish and post breeding management of broods. It also reflects on the genetic consideration for induced breeding to avoid inbreeding.

Introduction
Carp culture is an ancient practice in China and the emigrants from China introduced it into several southwest Asian countries. Indigenous system of Indian carp culture is seen to have existed in eastern parts of Indian subcontinent in the eleventh century AD (Pillay, 1990). Carp culture began in Bangladesh since 1926 (Nazmul, 2000). A king named Raja Gogendra Nath Roy, observed the collection of fingerling in the river of Hugli of Calcutta. The people started aquaculture and to mitigate the increasing demand of fingerlings, the establishment of hatchery became essential.

Collection and preservation of pituitary gland

The pituitary is collected by chopping off the root of the cranium, and exposing the brain. Then the brain is removed and the gland is collected with the help of forceps. All these should be done with a gentle hand. These glands may be preserved either in absolute alcohol or acetone. For the first time, in case of alcohol the old alcohol should be replaced after 24 hours of preservation and in case of acetone the old acetone should be replaced after 12 hours. These glands may retain their efficiency for up to two years.

Brood collection and management

Preferably 2-3 years old mature males and females should be collected from the natural sources (rivers, lakes, and reservoirs) as broodstock. Broodstock from culture ponds may also be of the same age. For the exotic species the culture ponds are the only source of broodstock.

Collected broodstock should be transferred into the brood stocking pond with aerator or with the facilities for water exchange in the month of October to December. The formulation of good brood diets is based on nutritional value: protein (28-30%), carbohydrates, lipid, and vitamins and improvements in feed presentation. Pond management schedules are to be followed strictly, involving weed clearance, removal of predatory and weed fishes, pond fertilization and application of supplementary feed, fish health care and monitoring of pond environment etc. Maximizing fecundity and milt yield from female and male respectively and to determine the multiple maturity and spawning (Kumar, 1992).

Netting of broodstock from the brood stock ponds and conditioning in the tank

In the breeding season the broodstock are netted from the pond for induced breeding. During netting it should be observed that either the broods are ready for spawning or not by examining the pectoral fin and/or the anus. Mature males ooze a milky fluid (milt), if the abdomen is slightly pressed near the vent. They are also characterized by the roughness of their pectoral fins. Mature females have a soft bulging abdomen with slightly swollen and reddish vent. The ready females and males are transferred to the rectangular tank for conditioning and during transferring of broodstock stress should be minimized. In the tank the broodstock should be kept for the time required. Then the weights are recorded.

Preparation of pituitary extract and injection to the fish body

After the selection of the broodstock, the injectable dosages of pituitary extract is calculated in term of milligram of pituitary gland per Kg body weight of the recipient fish. Then weighed gland is ground with a cell homogenizer in distilled water and centrifuged and the supernatant is separated. Females are given two injections at an interval of 6 hours while males are given only one injection at the time of the first injection of the females. The respected dose must be used. The dosage varies with the temperature, potency of the pituitary gland, gonadal maturity of the recipient and the prevailing climatic conditions (Jhingran and Pullin, 1988).

Hatching jar preparation

Before subjected the eggs in the hatching jar, it should be washed with disinfectant and rinsed several times with fresh water. It is also essential to dry the hatching jar properly. All the equipment that will be used must be disinfected and dried to reduce the microbial danger.

Breeding procedure

Six hours after the second dose, breeding takes place. Observing movements of the fishes the eggs and milt are to be collected from both female and male respectively by stripping the abdomen of the fishes with a gentle hand. The fish should be anesthetized by using MS222 (0.5g/50 ml water). The milt from the males should be diluted with normal physiological saline solution and mixed with the eggs, shaking for 5 minutes and then washed with fresh water, then put into the pre-prepared hatching jar with a continuous water flow to keep the eggs moving.

Hatching time is temperature dependent. Usually hatching takes place about 15-18 hours at the temperature range of 26-31^{0}C (Kumar, 1992). At lower temperature the hatching time is consequently longer. It takes 12-15 hours for the developing egg to hatch out in Bangladeshi conditions. Three days after hatching the fry are transferred to a cemented tank. Then the spawn are sold. While the eggs are in the hatching jar continuous supervision is essential for the estimation of hatching percentage, water temperature, dissolved oxygen and change the hatching jar after 12-14 hours of hatching.

Rearing of fry

Fry from the hatching jar are usually transferred to the tank by siphoning. The yolk sac is absorbed within 2-4 days after hatching and the fry are then fed with boiled yolk. It is suitable if possible to feed with natural feed (phytoplankton, zooplankton or zoobenthos). The newly hatched fry are acclimatized before release in the earthen ponds. For fry nursing special types of ponds are used, having low depth, small size and the pond should be pre-prepared. In these ponds fry are reared for three to four months (Kumar, 1992).

Post breeding management of Broods

It should always be borne in mind that spent carps are potential breeders for the next breeding season, so proper care should be taken to save them. For this, antibiotic and disinfectant treatment should be given.

Multiple breeding

Indian major carps generally breed once a year but recent years it has been possible to breed them twice in a year. They are induced to breed in the early part of the season, well cared and fed for the rest of the season, then they are again induced to breed in the late part of the season (Jhingran and Pullin, 1988).

Some specialization of induced breeding in case of exotic species

Exotic species are preferred to culture for their fast growth, short culture period, high production and availability of fingerlings in hatchery. For this, special information for the induced breeding of exotic species cultured in Bangladesh are given. A catheter is found to be quite helpful especially in case of silver carp and grass carp to identify their maturity. By inserting the catheter in the genital opening of a female spawner, some eggs are taken out and examined at the pond site in a petridish. Uniform size eggs of pale blue color in silver carp and brown or copper color in grass carp indicates proper maturation stage (Jhingran and Pullin, 1988). Silver carp and grass carp normally do not release eggs. In case of silver carp males, the quantity of milt is insufficient and hence extra males should also be injected to ensure maximum fertilization of stripped eggs (Kumar, 1992). For the brood management of Black carp, required calcium and high protein percentage is applied in their supplementary food. It takes five years or more for this species to mature.

Genetic considerations

At present, hatcheries produce slow growing spawn caused by inbreeding effects. 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).

Recommendations

- For choosing stock it should be borne in mind that the stock should have fast growth, good body conformation, disease resistance, high fecundity etc. to suit the hatchery owners and customers.
- Appropriate dosages for hormone injections of carp species should be followed for good quality seed production.
- It should be made sure that the hatchery owner have to have adequate facilities to keep stocks separate from each other, in sufficient numbers to avoid inbreeding and the means, either in his farm or through cooperation with other farmers, to undertake a sustained program of evaluation of stock performance.
- To develop breeding techniques and share knowledge of hatchery owners, workshops should be conducted yearly by FRI, DOF and Universities.
- Facilities and literature on latest technologies in potential areas should be made available to develop theoretical and practical knowledge of hatchery owners and technicians.
- To create skilled personnel, training programmes should be organized both in the country or abroad.
- Improved induced breeding method should be demonstrated for sustainable breeding.

References

Hussain, M. G. and Mazid, M. A. 2001. Genetic Improvement and Conservation of Carp Species in Bangladesh. Bangladesh Fisheries Research Institute and International Center for Living Aquatic Resources Management. 74 p.

Jhingran, V. G. and Pullin, R. S.V. 1988. A Hatchery Manual for the Common, Chinese and Indian Major Carps. Asian Development Bank, International Center for Living Aquatic Resources Management. 191 p.

Kumar, D. 1992. Fish Culture in Undrainable Ponds: A Manual For Extension. FAO Fisheries Technical Paper 325. Food and Agriculture Organization of the United Nations.

Tave, D. 1999. Inbreeding and brood stock management. FAO Fisheries Technical Paper No. 392. FAO, Rome.

Socioeconomic Aspects of Carp Production And Consumption in Bangladesh

M. F. Alam

ICLARM-The World Fish Centre, Dhaka, Bangladesh

Alam, M.F. 2002. Socioeconomic aspects of carp production and consumption in Bangladesh. pp. 83-93. In: Penman, D.J., Hussain, M.G., McAndrew, B.J. and Mazid, M.A. (eds.). Proceedings of a workshop on Genetic Management and Improvement Strategies for Exotic Carps in Asia, 12-14 February 2002, Dhaka, Bangladesh. Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh. xxx p.

Abstract

The paper describes the status of carp production and consumption in Bangladesh. The importance of the fisheries sector in general and carp species in particular is reviewed. Source-wise contribution of carp production has been highlighted. Consumption levels of different carp species have been focused on and socioeconomic factors affecting carp production and consumption have been identified. Finally, the need for fuller utilization of waterbodies has been emphasized, keeping in view the productivity of Bangladesh waters, potential of the species and its market acceptance.

1. Introduction

Fish and fisheries have been an integral part of life of the people of Bangladesh from time immemorial, and play a major role in employment, nutrition and foreign exchange earning and other aspects of the economy. Fish is a natural complement to rice in the national diet, giving rise to the adage *Maache-Bhate Bangalee* (a Bengali is made of fish and rice"): fish alone supplies about 60% of animal protein intake[1]. The fisheries sector provides full-time employment to an estimated 2 million fishermen, small fish traders, fish transporters and packers, etc. (World Bank, 1989), and another 10 million people are partly dependent on fishing, e.g. part-time fishing

[1] FAO statistics show fish providing 47% animal protein and 6% of total protein intake in 1993. Also, amongst UN-defined Low-Income Food Deficit countries, Bangladesh has an unusually low consumption of animal proteins.

for family subsistence (BFRRS, 1986). The sector contributes about 5.5% of GDP, 18% of Gross Agricultural product and 6.28% of export earnings. Fisheries exports comprise frozen shrimp, frozen frogs' legs, frozen fish, dry fish, salted and dehydrated fish, turtles, tortoises and crabs, shark fins and shark maws.

2. Fisheries Resources

Bangladesh is ideally suited for fish production, having one of the highest man-water ratios in the world, at 20 persons per ha of water area (Task Force, 1991). The fisheries are multi-species in nature: there are about 300 species of fish and 20 species of prawns in Bangladesh (Rahman, 1989). The most common species is Hilsha[2], which accounts for nearly half of the total marine catch, and about 18% of total fish production.

The fisheries sector of the country is generally classified as (i) inland open waters; (ii) inland closed waters; and (iii) marine waters (see Table 1):

- Inland open water bodies, where capture fishing is mainly carried out, comprise rivers and estuaries, *beels* (small lakes, low-lying depressions, permanent bodies of floodplain water, or bodies of water created by rains or floods that may or may not dry up in the dry season; in the wet season, *baors* or shallow lakes may be formed as smaller water bodies are joined up), Kaptai Lake (a man-made lake created for hydroelectricity), floodlands (annually flooded, low-lying areas associated with rivers) and polder/enclosures (regulated floodplains created mainly for irrigation purposes). The total area of inland open water bodies is 4.92 million ha (93.53 % of total inland areas), comprising 1.03 million ha of rivers and estuaries, 2.83 million ha of floodlands (53.63% of total inland water areas, 0.83 million ha of polder and enclosures (16.53% of total inland water) and small areas of *beels* and Kaptai Lake.

- Inland closed water bodies, where aquaculture (fish farming) at various intensities is carried out, include ponds, *dighis* (big ponds) and *baors* (oxbow lakes), and also some coastal waters. The 0.36 million ha of inland closed water areas include 0.11 million ha of ponds and *dighis* (4.07% of total inland water area), 0.14 Mha of traditional shrimp farms in coastal areas, small areas of *baors*, drains and ditches, and semi-intensive shrimp farms.

[2] Basically a marine diadromous species. For spawning, it migrates to rivers where a large number get caught. It is also caught in marine waters.

- Marine waters extend over 166,000 km^2 (16.6 Mha) of sea area, following the 1974 declaration of a 200-nautical mile Exclusive Economic Zone (EEZ), within which Bangladesh also has the right to exploit and manage living and non-living resources.

Table 1. Water areas for Bangladesh fisheries, 1999-2000 (source: Department of Fisheries, DOF, 2001; Saronika, Fish Week, 2001).

	Type of water body	Water areas (ha)	Water areas (%)	Fish production (metric ton)	Fish production (%)
A.	*Inland open waters:*				
	Rivers and estuaries (including	1,031,563	19.53	154,335	9.29
	Sundarban)			11,684	0.70
	Beels	114,161	2.16	72,885	4.38
	Kaptai Lake	68,800	1.30	6,852	0.41
	Floodlands	2,832,792	53.63	424,805	25.57
	Polder/Encloser	873,000	16.53		
	Total inland open water	4,920,316	93.15	670,465	40.36
B.	*Inland closed waters:*				
	Ponds and *dighis*	215,000	4.07	561,050	33.77
	Baors (Ox-bow lakes)	5,488	0.11	3,622	0.22
	Coastal shrimp farms	141,353	2.68	92,448	5.56
	Total inland closed waters	361,841	6.85	657,120	39.55
	Total inland waters (A+B)	5,282,157	100.00	13,27,585	79.91
C.	*Marine waters (Bay of Bengal):*	16,607,000			
	Industrial			16,304	0.98
	Artisanal			317,495	19.11
	Total marine waters			333,799	20.09
	Total waters (A+B+C)	21,889,157		1,661,384	100.00

3. Total Fish Production

Fish production of the country for the year 1999-2000 was 1.66 million metric ton, of which 79.91% came from inland waters and 20.09% from marine water. The contributions of inland open and closed waters are 40.36% and 39.55% respectively. Floodlands (including the regulated polders and enclosures) contribute the most from the inland open waters. Rivers and estuaries, although constituting large areas, contribute very little to the total fish production. On the other hand, ponds provide the most (about 38%) to the total production although water areas are much lower compared to the open waters. Artisanal fisheries contribute the most (19%) to the total production (Table 1).

3.1 Carp Production

There are two main production environments for carp species in the inland waters of Bangladesh. These are inland open waters, and inland closed waters. The different open waters growing carps are: (i) rivers and estuaries, (ii) beels, (iii) Kaptai lake, and (iv) floodplains. Inland closed waters producing carps constitute (i) ponds and *dighies* and (ii) *baors* (ox-bow lakes). The different carp species produced in different environments are shown in Table 2.

A few aspects of Table 2 merit attention. First, carp production in 1999-2000 constituted about 43 percent of the total inland fish production. Second, the share of production of carps in general to total inland fish production kept on increasing over the last decade. Third, production of major carps has been on the increase but production of minor carps has declined during the same period. Fourth, exotic carp production has been increasing gradually over the years. Finally, carps constitute very dominant species in the total inland fish production of the country.

Table 2. Production of carp species over time. Major carp = rui, catla and mrigal; minor carp = ghania, kalbasu and kalia; exotic carp = silver carp, common carp, mirror carp and grass carp. Data shown is metric tonnes, with percent contribution to total fish production in parentheses.

Year	Major carp	Minor carp	Exotic carp	All carp	All species
1990-91	120,178 (18.36)	16,473 (2.52)	24,858 (3.80)	161,509 (24.68)	654,397 (75.32)
1991-92	140,957 (19.95)	9,875 (1.40)	30,197 (4.27)	181,029 (25.62)	706,605 (74.38)
1992-93	144,877 (18.81)	8,735 (1.13)	31,293 (4.06)	184,905 (24.00)	770,162 (76.00)
1993-94	164,046 (19.58)	11,396 (1.36)	47,967 (5.73)	223,427 (26.67)	837,566 (73.33)
1994-95	173,774 (19.13)	10,481 (1.15)	54,916 (6.05)	239,171 (26.33)	908,218 (73.67)
1999-00	374,523 (28.21)	10,526 (0.79)	180,708 (13.61)	5,65,757 (42.61)	13,27,585 (57.39)

3.2 Contribution of different sources to carp production

Statistics indicate that major carp production of the inland open waters, particularly rivers and estuaries, has been declining (can be verified from different issues of the Fish Catch Statistics published by the Department of Fisheries). The overall increase however, is mainly due to the increasing acceptance and production of major and exotic carp species which are very popularly grown in the inland closed water environment such as ponds. The share of carp species in the closed water environments is expected to increase further as aquaculture expands because it is the main culture species. The details of shares of carp production by sources are displayed in Figure 1.

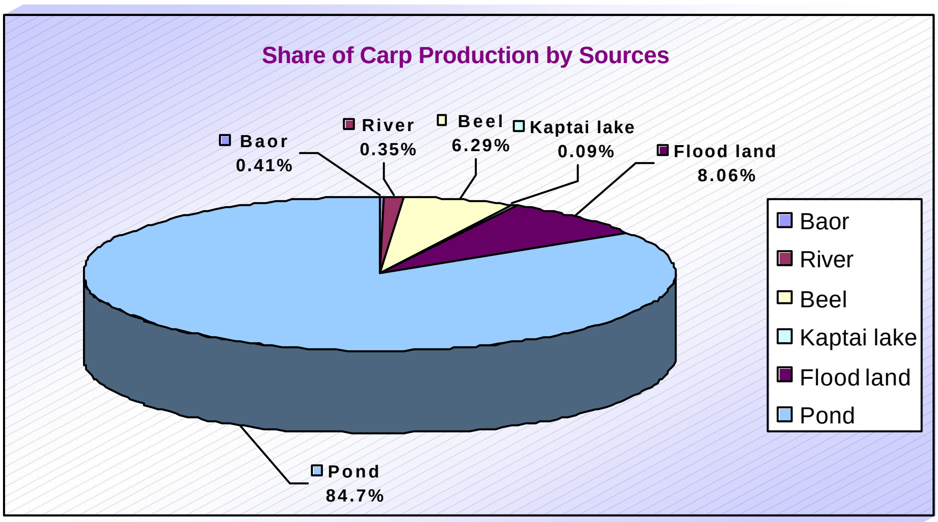


Figure 1. Contribution of different sources to carp production

The major contributor for carp production is pond, which accounts for 86 percent of all carp production in the country. The next important contributors are floodland, *beels*, rivers, *baors* and Kaptai lake. Floodlands and *beels* contribute respectively 8 and 6 percent to the total carp production. The contributions of rivers, baors and Kaptai lake to the carp production are very insignificant (less than 1 percent). It is to be mentioned that the share of carp production of rivers has reduced significantly over the past years. In 1990-91 rivers used to contribute 3.09 percent while this has gone down to only 0.35 percent in 1999-00. On the other hand, the share of floodlands to carp production has increased from 5.03% in 1990-91 to 8.05% in

1999-00. The increase of carp production from the floodland is due mainly to the positive impact of the stocking programme of the Department of Fisheries. The overall increase of carp production is due both to the expansion of pond fisheries and productivity increases.

3.3 Species for pond aquaculture :

Table 3 shows that Indian major carps such as rohu, catla and mrigal are the most dominant species in the pond aquaculture. Nearly 23% of the production of the cultured ponds is rohu. The share of the same for the culturable and derelict ponds is about 19 and 16 percent respectively. If the farmers are given the choice to select a single species for the pond, the farmer will certainly go for rohu. As the production statistics show, the most preferred species appear to be rohu, silver carp, catla, and mrigal for cultured and culturable ponds; and silver carp, rohu, catla, and mrigal for the derelict ponds. But the fact remains that the most dominant species for pond fish culture are indeed rohu, catla, mrigal and silver carp. Silver barb is, however, another potential candidate. Pangas got some importance in the mid-nineties but its geographical spread has been very limited.

Table 3: Species composition of pond catches, 1999-2000 (source: Fishery Statistical Yearbook of Bangladesh 1999-2000, DoF).

Species	**Percentage of weight of all pond production**			
	Cultured ponds	**Culturable ponds**	**Derelict ponds**	**All ponds**
Rui (*Labeo rohita*)	22.80	19.22	16.28	21.42
Catla (*Catla catla*)	20.98	14.70	9.85	18.60
Mrigal *(Cirrhinus mrigala*)	15.48	11.44	8.98	14.03
Kalbasu *(Labeo calbasu*)	1.92	0.71	0.13	1.50
Mirror carp *(Cyprinus carpio var. specularies*)	1.87	2.31	2.79	2.05
Silver carp *(Hypophthalmichthys molitrix*)	22.77	15.45	21.37	21.35
Grass carp *(Ctenopharyngodon idellus*)	2.44	0.21	0.02	1.78
Mixed carp	5.65	1.07	0.33	4.24
Non-carp species	6.09	34.89	75.36	15.03

The biases for the Indian major carps for pond freshwater aquaculture are due mainly to its relatively better taste and higher price. Studies such as Rahman and Ali (1986), Islam and Dewan (1987) and Alam (1995) showed that Indian major carps are the main cultured species for the fish farmers. A recent study published in 2001

(ICLARM, undated) indicates that order of consumer preferences for freshwater species in Bangladesh are: rohu, followed by catla, mrigal silver barb, common carp, mirror carp, silver carp, grass carp and kalbasu. Good taste, good appearance, easy availability and reasonable price are important reasons for choice of different species.

4. Pattern of Carp consumption in Bangladesh

Conflicting statistics available regarding fish consumption are often produced in Bangladesh. The production statistics published by the Department of Fisheries and the available population statistics of Bangladesh indicate that per capita annual fish consumption is to the tune of 12.51 kg in 1999-00 (Figure 2). One important feature of the per capita annual fish consumption statistics as shown in figure 2 is that the trend is increasing. The conflict is concerned not with the trend but with the magnitude. For example, The Household Expenditure Survey (HES) 1995-96 data (BBS, 1998) shows that per capita monthly fish consumption is 1.13 kg (15.98 kg per year). On the other hand the same source for the year 2000 indicates that annual per capita fish consumption is 14.03 kg. The Mid Term Review of the Fifth Five Year Plan (Planning Commission 2001) mentioned that the per capita per day fish consumption is 37.8 gm (13.79 kg per capita per year). These variations are mainly due to inflow of fish coming from neighboring countries, which is not represented in the published statistics. Since the HES estimate is based on samples, it takes into account the actual consumption. Taking the experiences of all these variations it can be safely concluded that the per capita annual fish consumption is between 13 to 14 kg in Bangladesh.

Data on fish consumption by species hardly exists on a representative basis. But it is believed that since carp constitutes about 43 percent of total inland fish production, its share in the consumption also is relatively higher compared to non-carp species. ICLARM (undated) while showing cross comparisons across different Asian countries showed that annual per capita fish consumption is to the tune of 19.92 kg for the urban producers, 21.36 kg for the rural producers and 18.36 kg for the non-producers.

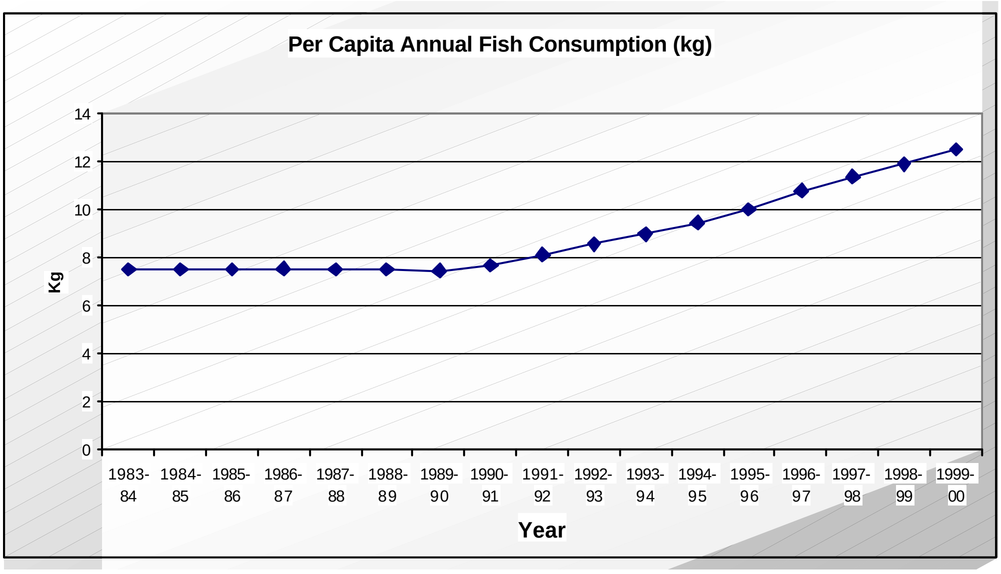


Figure 2. Trend of per capita annual fish consumption in Bangladesh

Table 4 shows the distribution of fish consumption by species. As single species the consumption of ruhu, catla, mrigal, silver carp, silver barb and river shad are most important. Table 4 provides information about the monthly as well as annual per capita fish consumption by species and income quartile groups. The mean annual per capita fish consumption of the selected households was found to be 19.95 kg. Of the total fish consumption, carp was found to be dominant. More than 50 percent of the consumption of fish is carp species. Of the carp species, rohu, catla, mrigal and silver carp are the most important. Consumers of different income quartile groups have different levels of fish consumptions. It is also clear that consumption of Indian major carp (rohu, catla and mrigal) is higher for higher income groups. The consumers of 1^{st}, 2^{nd}, 3^{rd} and 4^{th} income quartile groups have consumed 13.05, 17.88, 21.03, and 27.85 kg respectively. This clearly indicates that fish consumption in general is higher for higher income groups.

Table 4. Monthly and annual per capita fish consumption by species. Cons. = consumption.

Name of species	Mean		1st Quartile		2nd Quartile		3rd Quarter		4th Quartile	
	Cons. (kg)	% of Cons.	Cons. (kg)	% of Cons.	Cons. (kg)	% of Cons.	Cons. (kg)	% of Cons.	Cons. (kg)	% of Cons.
Rui	0.131	10.7	0.092	8.5	0.156	10.5	0.181	10.3	0.650	28.0
Catla	0.131	7.9	0.068	6.3	0.098	6.6	0.141	8.0	0.135	5.8
Mrigal	0.103	6.2	0.056	5.2	0.087	5.8	0.102	5.8	0.167	7.2
Silver carp	0.237	14.3	0.168	15.4	0.205	13.8	0.251	14.3	0.176	7.6
Other carp	0.127	7.6	0.063	5.8	0.110	7.4	0.127	7.2	0.107	4.6
Silver barb	0.102	6.1	0.047	4.3	0.082	5.5	0.114	6.5	0.166	7.2
Tilapia	0.045	2.7	0.037	3.4	0.050	3.4	0.050	2.9	0.053	2.3
Hilsha	0.185	11.1	0.123	11.3	0.188	12.6	0.183	10.4	0.260	11.2
Assorted	0.241	14.5	0.264	24.3	0.265	17.8	0.318	18.1	0.177	7.6
Live	0.207	12.5	0.108	9.9	0.155	10.4	0.157	9.0	0.260	11.2
High value	0.107	6.4	0.062	5.7	0.094	6.3	0.129	7.4	0.170	7.3
Total/ Month	1.663	100.0	1.088	100.0	1.490	100.0	1.753	100.0	2.321	100.0
Total/ Year	19.95		13.05		17.88		21.03		27.85	

5. Factors affecting carp production and consumption

Producers prefer to produce major carps (rohu, catla and mrigal) because of the expectation of higher prices while consumers prefer those species because of good taste. Easy availability and its good look are some important consideration for choosing major carps. Colour, size and shape also play good role for choosing carp species for consumption.

Carp fish farming is the most popular aquaculture practice in Bangladesh. Attitude of non-fish farmers to fish production has changed a lot over the years. For example, multiple ownership of pond was, for long time, a major constraint to carp production which is no longer believed to be a constraint for carp production in Bangladesh. Poaching/theft and marketing of produce are some other such examples. But factors like low farm gate price, high prices of supplementary feed, lack of technical knowledge, lack of quality fingerling and lack of fund etc. are currently the important constraints for carp production in the country. Some important factors affecting carp production are shown in Table 5.

Table 5. Factors affecting/influencing carp production and consumption:

Sl. No.	Production	Consumption
1	Lack of knowledge about practice	Income
2	Non-availability of good quality seed/fingerling	Price of fish
3	Tendency not to use purchased inputs	Availability/supply of fish
4	Lack of capital/credit	Colour, size and shape of species
5	High prices of fish feed and other inputs	Availability of cheaper and substitute species
6	Low farm gate price	Taste of species
7	Poor marketing opportunities/ facilities	
8	Breakthrough of disease	
9	Non-availability of water, seepage etc	
10	Multiple pond ownership	
11	Under utilization of resources potential (e.g. rice field, derelict ponds)	
12	Intensity of fish culture practice	

Income is perhaps the most important determinant for carp consumption as the carp species are relatively more costly compared to other species. Consumption of carp is also affected with the availability of substitute species. For example, silver carp production has increased dramatically because it is a cheap species and most poor people do have the access to buy it. The demand for major carp could have been much more had there been no existence of silver carps. Some other exotic carp species also compete with Indian Major carps. Colour, body size and taste are also important in influencing demand for carp species. Silver barb is getting good market due to its relatively cheap price, good look and body shape and taste.

6. Concluding remarks

Carp has a very good potential for production and consumption in Bangladesh. This is due to the existence of abundant waters conducive of production, choices and preferences of the producers and consumers and good existence of market demand. The economic profitability of the carp production practice is the most important reason for the expansion of aquaculture in Bangladesh. Many paddy lands are being converted for fish culture. Production of carps can be increased many fold in Bangladesh if aquaculture concepts are made clear to the fish farmers, unutilized waterbodies (ponds in particular) are brought into fish culture, practices of the underutilized waterbodies (ponds) are improved and productivity of the already cultured waterbodies (ponds) are improved.

References

Alam, S. 1995. M-AEP Rural Fish Market Survey 1994: Final Report, submitted to Mymensingh Aquaculture Extension Project.

Bangladesh Fisheries Resources Survey System, BFRRS. 1986. Water Area Statistics of Bangladesh. Fisheries Information Bulletin, vol. 1, Dhaka, Bangladesh.

Bangladesh Bureau of Statistics, BBS. 1998. Household Expenditure Survey 1995-96. Statistics Division, Ministry of Planning, GoB, Dhaka.

Bangladesh Bureau of Statistics, BBS. 2001. Preliminary Report of Household, Income and Expenditure Survey 2000. Statistics Division, Ministry of Planning, GoB, Dhaka.

ICLARM (undated). Genetic Improvement of Carp Species in Asia - Final Report. Asian Development Bank and ICLARM-The World Fish Center.

Islam, M.S. and Dewan, S. 1987. An Economic Analysis of Pond Fish Production in Some Areas of Bangladesh. Research Report No. 11, Bureau of Socioeconomic Research and Training, Bangladesh Agricultural University, Mymensingh.

Planning Commission. 2000. Mid Term Review of the Fifth Five Year Plan 1997-2002. Ministry of Planning, GoB, Dhaka.

Rahman, A.K.A., 1989. Freshwater Fishes in Bangladesh. Zoological Society of Bangladesh.

Rahman, M.L. and M.H. Ali (1986). A Study on the Credit and Marketing Aspects of Pond Fisheries in two Selected Districts of Bangladesh. Research Report No. 10, Bureau of Socioeconomic Research and Training, Bangladesh Agricultural University, Mymensingh.

Task Force 1991. Managing the Development Process: Bangladesh development Strategies, vol. 2. University Press Limited, Dhaka, Bangladesh.

World Bank 1989. Bangladesh Action Plan for Flood Control. 11 December.

Broodstock Management of Chinese Carps and Dissemination Strategy at NFEP, Parbatipur

M.A. Sattar[1] and T.K. Das[2]

1 Project Director, Northwest Fisheries Extension Project, Parbatipur, Dinajpur, Bangladesh
2 Hatchery Officer, Northwest Fisheries Extension Project, Parbatipur, Dinajpur, Bangladesh

Sattar, M.A. and Das, T.K. 2002. Broodstock management of Chinese carps and dissemination strategy at NFEP, Parbatipur. pp. 43-50. In: Penman, D.J., Hussain, M.G., McAndrew, B.J. and Mazid, M.A. (eds.). Proceedings of a workshop on Genetic Management and Improvement Strategies for Exotic Carps in Asia, 12-14 February 2002, Dhaka, Bangladesh. Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh. xxx p.

Abstract
Advancement of aquaculture in the Northwest region of Bangladesh is due to the success of induced breeding and development of public sector and private sector hatcheries and nurseries. During the recent years there has been a growing concern of the availability of good quality fish seed by the producers for sustainable fish production. Management of broodstock has, therefore, to be taken into consideration. This paper will firstly highlight the Passive Integrated Transponder (PIT) tagging technique and Genetically Improved Broodstock Replacement Programme in use at NFEP. A comparative growth trial of old, crossbred and new (imported from China) silver carp was conducted. The new silver carp stock demonstrated better performance in growth and survival compared to the old and crossbred ones. Secondly, dissemination strategies have been implemented for imported Chinese carp stocks at Parbatipur through provision of training and distribution of quality fingerlings to private and public hatchery operators from the project. A future plan of broodstock management is also highlighted for the development of more effective improved broodstock with newly approved project support.

Introduction

The Northwest Fisheries Extension Project (NFEP) was a bilateral project implemented by the Department of Fisheries (DoF), Government of Bangladesh and the Department for International Development (DFID) of the United Kingdom The overall objective of NFEP was to increase fish production and income of the poor farmers in the northwest region of Bangladesh. The following strategies were adopted to achieve these objectives:

- Development and execution of broodstock strategy for improved quality seed supply
- Testing and development of fish culture methodologies suitable for the low and middle income fish farmers in the locality
- Testing and development of effective extension and training methodologies appropriate for the needs of the poor
- Implementing Training of Trainers course to develop trainers in aquaculture in both public and private sectors

The existing Chinese carps populations in Bangladesh are all based upon 2 or 3 introduction of small numbers of fish from Nepal which were of unknown genetic quality. In 1994, in collaboration with the Network of Aquaculture Centres in Asia (NACA), NFEP imported Yangtze River silver, bighead and grass carp fry from China. 740 of those imported fingerlings were given away to 4 Government hatcheries (Kotchandpur, Kurigram, Ishwardi and Natore). The value of these fish has already been lost because the 4 farms mixed the pure Chinese fish with their old existing stock. With increasing international restrictions future importation will be difficult. It is vital therefore to at least maintain the genetic diversity and aquaculture performance of exotic stocks presently in the country.

The programme for broodstock development and dissemination started in 1994 after the importation of the fingerlings, with their management for making them as quality broodstock in keeping them in separate ponds. The breeding programme started when they matured in the 1997 breeding season and after that the dissemination programme started. The programme was conducted by using a tagging technique with support from a genetic advisor, Dr. Brendan MacAndrew of the University of Stirling. The advisor provided practical training to the hatchery staffs for successful implementation of the program. In between the hatchery officer also received overseas training at Stirling to make the programme more effective. Based upon gained knowledge, the hatchery staffs provided training to the private and Government hatchery operators on maintenance of quality broodfish in their farms for the production and supply of quality fingerlings.

This paper briefly highlights the overall methodology for effective management of broodstock and their replacement. Dissemination of broodstock management techniques and the distribution of imported quality Chinese carps fingerlings to private and public farms are described. Finally, the outcomes of on-station trials on the comparative performance of newly imported silver carp, old silver carp and cross bred fingerlings is mentioned.

Farm and Hatchery: physical and biological resources

Parbatipur farm complex covers an area of 50 acres and comprises a renovated old farm. This now contains a modern farm and hatchery with administration building, training room and library, dormitories, staff accommodation, fry traders temporary accommodation and a 175 decimal rice-fish trial plot facility (see Table 1). The total water area is 24 acres (10 hectares), with 42 ponds (1,801 decimals), 15 trial ponds (30 decimals), 3 borrow pits (555 decimals) and 2 drainage canals (125 decimals).

To attain sustained production by the producers, the availability of quality seed is vital. Realizing the importance of quality seeds for sustained fish production, NFEP has been selected as one of the brood development centres in the northwest. As stated earlier, NFEP imported pure strain fry of Silver carp, Bighead carp and Grass carp from the Yangtze River, China. The new stocks have been managed and reared separately to maintain pure strains of the imported Chinese carps. Table 2 shows the number and weight of Chinese carps kept and maintained by the NFEP to produce genetically improved seeds.

Table 1. NFEP physical and biological resources.

Pond Type	Number	Area (m^2)	Area (decimals)	Area (hectares)
Broodstock	13	280908	723	2.88
Broodstock replacement	5	23,662	592	2.37
Hatchling nursery	7	5,644	141	0.56
Fingerling nursery	14	25,758	644	2.58
Polyculture	1	4,280	107	0.43
Training Trial	3	3,104	78	0.31
Grand Total	60	95,452	2,386	9.54

Table 2. Pure broodstock of Chinese carps at NFEP.

Species	New broodstock (Number)	New brood stock Total Weight (kg)
Silver carp	486	625
Big head carp	399	950
Grass carp	155	387

Methodology of Broodstock Management and Replacement

It is important to note that as in other parts of the country, silver carp hatchlings are the major species produced by the NFEP. Due to rapid growth and better performance compared to native Indian major carp species, the demand for silver carp in particular in this region is high. It is also noted that the major production in the pond based aquaculture systems comes from silver carp. In addition, there is greater risk of hybridization between silver carp and bighead carps and eventually deterioration of the quality of the original strain. To maintain the genetic quality, maximisation of the effective breeding number (Ne) is necessary. (Ne = effective breeding number which is the number of parents that produce offspring that are represented in the next generation of breeders).

NFEP adopted the Trovan electronic tagging system to individually identify all genetically improved brood stock.. NFEP will implement techniques that will ensure the long term genetic viability of these stocks.

Tagging Technique

In 1997, NFEP piloted an electronic tagging system (PIT tagging) to address the problems encountered using external suture tags. For the technically minded the Trovan LID-500 system uses a passive transponder (the ID tag), consisting of a microchip connected to a small induction coil wrapped around a graphite antenna, sealed inside a glass capsule (1.5mm in diameter and 12mm long). Following anesthetisation of the fish in a Benzocaine solution, the tag is inserted intra-muscularly into a small scalpel incision between the lateral line and dorsal fin, or alternatively into the body cavity. The incision is then immediately sealed with a mixture of surgical adhesive and antibiotic powder. Since the glass case is biologically inert there is little risk of rejection or fouling. 200 fish can be tagged comfortably per day by a skilled team and tags can be re-used if dead fish can be retrieved.

A hand held tag reader emitting a low frequency electromagnetic field causes the tag (transponder) to give off a unique 10-digit ID code which is read by the reader .The hand held reader can collect and store over 1000 tag codes which can be downloaded to a computer data base. In addition the reader can be programmed to search for specified tag numbers which will be extremely useful for broodstock selection during the spawning season. This system is the most reliable method of identifying individual fish to date and will be invaluable to fish breeding centres (DFID Fisheries Bangladesh, 1997).

Broodstock Replacement Technique

A simple protocol was developed to ensure that all females contribute genetic material to the next generation of brood fishes. At the time of breeding, all tagged fish are mated in single pair crosses to ensure equal numbers of male and female fish, the PIT tag numbers are recorded for each pair. 10-20 grams of fertilised eggs from each pair are put it into a separate incubation jar while the rest of the eggs are put in the main production jar. This is done batch wise to maximise the effective population size (Ne: Tave, 1993) in the whole breeding season. All tag numbers and crosses are recorded in each cycle. It is important to ensure during the broodstock replacement programme that each pair of fish spawned contributes once only to the next generation: if spawned again the hatchlings are sold to the farmers and not added to the replacement pool.

For the replacement programme, each batch of hatchlings is put in a separate nursery pond, nursed for 4 weeks and 1000 fingerlings randomly selected (to minimise the negative selection) from each batch and put into the brood replacement pond. The main idea of this programe is to maximise the Ne as much as possible. Ne>50 gives inbreeding of less than 2%. By aiming to set Ne at 50-100, this should effectively limit inbreeding to a level which is acceptable. This is being done by having each pair of fish contribute an equal number of fingerlings to broodstock replacement. The total production % of pure strain Chinese carp fry/fingerlings at NFEP is presented in Fig. 1.

As part of the genetic improvement and maintenance programme, a number of trials were conducted. It was seen that the growth performance of new silver carps was significantly higher than that of the old and cross bred ones (Fig. 2: NFEP, 2000). However, the new stock fingerlings were larger at the start of this trial.

Fig : 1 % of production of pure strain Chinese Carps

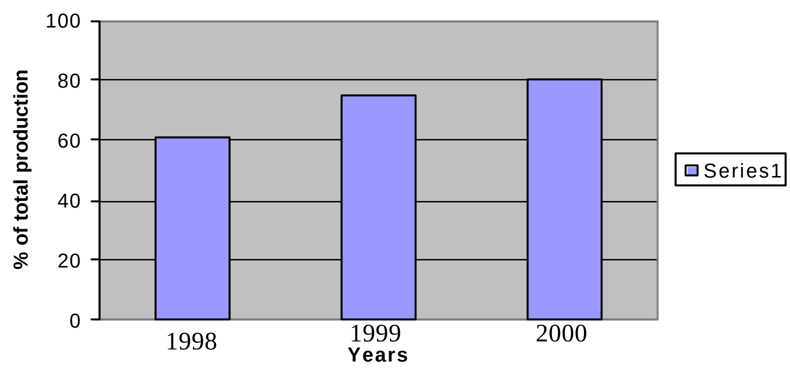
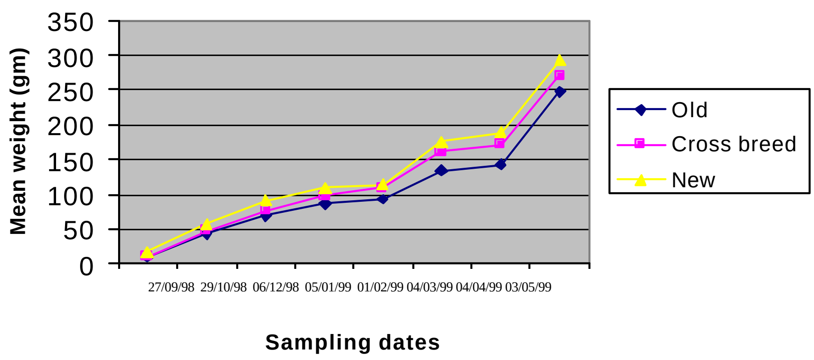


Fig. 2 **Mean weight (gm) of Old, Cross-breed & New Silver Carps against Time**



Dissemination Strategy

NFEP has been involved in the distribution and dissemination of pure broodstock in the northwest region, in particular. The project has been producing and distributing good quality fish seeds for future broodstock for both public and private sector. Since the implementation and maintenance of pure broodstock about 30,000 pure quality

Chinese carp seeds was distributed to 8 DoF farm, Bangladesh Fisheries Research Institute (BFRI), Mymensingh, Faculty of Fisheries, Bangladesh Agricultural University, and NGOs such as Bangladesh Rural Advancement Committees (BRAC), CARITAS, Grammen Matshya Foundation (GMF) and Rangpur Dinajpur Rural Service (RDRS), and private sector hatchery operators from Parbatipur for future broodstock development. Systematic monitoring is yet to be conducted to know about how the farms/hatchery operators are maintaining their broodfish. We have been informed that many of them are managing their stocks and producing quality fingerlings.

The project has also organised training programme on maintenance of genetically improved pure quality seeds and replacement of broodstock for those involved in fish breeding and fingerling production. The objective was to provide insight and practical skills so that they are able to maintain genetic pools for future development and maintenance of broodstock in their hatcheries. In addition, 12 private hatchery managers from Dinajpur, Rangpur, Gaibandha and Bogra district received 3-day intensive residential hands on practical training on genetic maintenance of broodstock for quality seed production and on broodstock replacement strategy.

Future plans

The Government of Bangladesh has approved a collaborative project (DoF and University of Stirling) for genetic improvement strategies for production of exotic carps for low input aquaculture. The project will continue till March 2004. In future, attempts will be made to maintain as large an effective number of fish in the hatchery to minimize inbreeding within the imported Chinese carp species. In addition, testing and development of broodstock management strategies will continue and appropriate broodstock management techniques will be recommended and disseminated.

Conclusions

Management of broodstock is providing two-fold benefits. Firstly good quality broods can be recruited and secondly higher production from the aquaculture system can also be achieved. Farmers /hatchery operators already reported better growth performance of fry/fingerlings of new strain Chinese carps. What NFEP did up to till now may serve as an initiative to solve the major problems related to quality seed production and supply. But to solve the problems throughout the country we should take long term initiatives in a planned manner and in that NFEP initiatives may be an example.

Recommendations

1 For maintenance of quality broodfish it is important to take long term programme with provision of financial as well as logistic support.

2 Similar programmes can be applied to other important species (Indian major carps of riverine source and tilapia) as well to maintain good quality broods.

3 Monitoring of the hatcheries which have received quality fingerlings for broodstock management is needed.

4 Legal support for quality seed producers in the form of licensing from the government should be initiated.

References

DFID Fisheries Bangladesh. 1997. Tagging Technology. Issue 2.

Hussain, M.G. 1998. Brood fish management to minimise genetic stock deterioration in hatcheries in Bangladesh. Fisheries Newsletter 6:2-4.

McAndrew, B.J. 1997. Consultancy report on fish genetics conducted in 30th Nov-9th Dec 1996. NFEP, Parbatipur, Dinajpur.

NFEP. 1998. The present status of status of brood stock management at the NFEP-2, Parbatipur. NFEP Paper No 15. 12p.

NFEP. 2000. A trial to compare the grow -out of old, hybrid and imported silver carp. NFEP Paper No 25. 7p.

Tave, D. 1993. Genetics for fish hatchery managers. Van Nostrand Reinhold, New York. 415 p.

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

M.G. Hussain and M.A. Mazid

Bangladesh Fisheries Research Institute, Mymensingh 2201, Bangladesh

Hussain, M.G. and Mazid, M.A. 2002. Genetic status and improvement strategies for endemic and exotic carps of Bangladesh. pp. 9-27. In: Penman, D.J., Hussain, M.G., McAndrew, B.J. and Mazid, M.A. (eds.). Proceedings of a workshop on Genetic Management and Improvement Strategies for Exotic Carps in Asia, 12-14 February 2002, Dhaka, Bangladesh. Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh. xxx p.

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

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

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

1. Introduction

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

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

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

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

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

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

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

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

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

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

2. Carp Genetic Resources of Bangladesh

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

2.1 Endemic carp species

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

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

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

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

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

2.2 Exotic carp species

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

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

Species	Common name	Source	Year of introduction
Ctenopharyngodon idellus	Grass carp	Hongkong	1966
		Nepal	1979
		Japan	1970
		China	1994
Mylopharyngodon piceus	Black carp	China	1983
Hypophthalmichthys molitrix	Silver carp	Hong Kong	1969
		China	1994
Aristichthys nobilis	Bighead carp	Nepal	1981
		China	1994
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
		Thailand	1994
		Indonesia	1994
Tor putitora	Mahseer	Nepal	1991

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

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

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

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

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

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

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

3.1.2 Evaluation of growth performances of F_1 crossbred and non-selected control groups

In 1997, the F_1 generation of progeny was produced from the mature parental base populations of each stock (B. I and T). For each of the reciprocal crosses, 5 to 8 pairs were mated separately and the best 3 progeny groups were selected, to make 18 full sib F_1 progeny lines, which were then communally stocked by mixing 125 larvae from each line and grown in nursery, rearing and grow out ponds. An 8 month growth trial was conducted during September'97 to May'98. The ponds were stocked with F_1 crossbred and non selected control (BxB) stock at the rate of 3 fish/m^3. The fish were sampled at monthly intervals to assess the growth performance and adjust feed ration. The data of monthly mean weight showed higher growth rate of the crossbred group all through the growth period, with final mean weights of 63.25±26.79 g (F1 crossbreds) and 58.88±14.26 g (BxB control), but no significant differences (P>0.05) were observed between these groups.

3.1.3 Evaluation of growth performance of F_2 crossbred and non-selected control groups

During the breeding season of 1998 when all the F_1 crossbred progenies had matured at the age of 10 months, the 20% largest and heaviest females and males were mass selected and held separately in earthen ponds until they were used to produce the next generation in the breeding programme. In the process of individual (mass) selection with respect to sexes, empirical assessment of the breeders was always followed on the basis of their large size, good health and shape and shiny colors. The production of F_2 generation of silver barb was carried out by pool mating of at least 150 pairs of mass selected breeders and 50 pairs of non selected control (BxB) breeders in separate spawning arenas. These mating operations of both selected and non selected breeders were completed within 2 - 3 days, involving 40 - 60 pairs per batch. A comparative growth performance trial of the F2 crossbred and non-selected control (BxB) groups was conducted in earthen ponds for five months. The stocking density was maintained at a rate of 1.5 fish/ m^3. There was no significant difference between the mean weights of the two groups of fingerlings at stocking.

At harvest, the F2 group were larger than the controls (77.41±28.23 g and 70.52±19.79 g respectively), but this was not significantly different. The sex ratios of both crossbred and control groups did not show any significant differences from 1:1.

3.1.4 Evaluation of growth performances of F_3 crossbred and nonselected control progenies

During the breeding season of 1999, further mass selection was carried out among all of the mature F_2 crossbred fish. The 15% best females and males were selected and used for producing the next generation. About 132 pairs of mass selected breeders and 50 pairs of non selected control (BxB) breeders were separately pool mated to produce the F_3 generation of progeny. An experiment was conducted for the evaluation of growth performances of F_3 crossbred and non-selected control (BxB) progenies for 6 months. The fish were sampled at fortnightly intervals to assess the growth performance and adjust feed ration.

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

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

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

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

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

The generation-wise additive genetic gain was estimated by weight and it was observed that 7.5% genetic gain in growth performance was attained by F_1 crossbred group over non-selected control group. The F_2, F_3 and F_4 selected groups attained respectively 2.3, 12.1 and 6.3% cumulative weight gain over three generations of selection. The weight gain values of the F_4 generation of the selected group compared with the non-selected control (BxB) group showed 28.2% superiority over the existing stock (BxB), and the average gain per generation across three generations of selection for growth performance in weight was estimated to be about 7.0%. The present findings suggest that this method for improvement of silver barb through several generations of genetic selection could develop a “Super Strain” of silver barb after several generations, and might be a useful technique for other carp species in Bangladesh and elsewhere in Asia (Hussain *et al*., 2002).

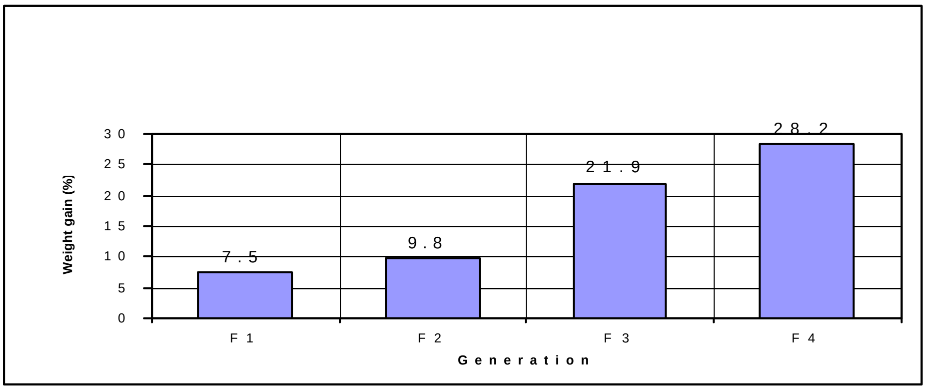


Figure 1. Additive genetic gain (%) by weight of crossbred group over control (BxB) group in different generations of *Barbodes gonionotus*.

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

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

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

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

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

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

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

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

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

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

In view of improving the stock of *L. rohita*, induction of mitotic gynogenesis and production of clonal lines was initiated during 1993/94 at FS, Mymensingh (Hussain *et al*., 1997). Efforts were made to interfere with the normal functioning of spindle apparatus during mitotic cell division of fertilized eggs using heat shock treatment, thereby leading to the induction of mitotic gynogenesis in F_1 generation (Table 5). Afterwards, putative mitotic gynogenetic alevins were reared as broodstock and a sexually mature female was used to obtain ovulated eggs which were fertilized later with UV irradiated milt. The UV irradiation was carried out for two minutes, applied 5 minutes after fertilization, to produce clonal lines (Table 6) which could be used in a breeding programme to improve stock performance.

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

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

Table 6. Production of F_2 clones in *L. rohita* through meiotic gynogens using F_1 brood from mitotic gynogens.

Trial No.	Parameters	Normal Control (%)	Haploid Control UV= 200 µWcm^{-2} (%)	Haploid Control UV= 250 µWcm^{-2} (%)	Meiotic Gynogens ie. Clones (%)
1	Fertilization	98	76	-	68
	Hatching	92	0	-	5
	Fry at one week age	84	0	-	66
2	Fertilization	87	62	-	64
	Hatching	82	2	-	2
	Fry at one week age	84	89	-	66
3	Fertilization	98	-	72	74
	Hatching	92	-	0	12
	Fry at one week age	84	-	0	66
4	Fertilization	87	-	67	62
	Hatching	82	-	0	2
	Fry at one week age	84	-	0	66

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

Hybrid introgression is thought to have occurred in some Chinese major carp populations, where hybrids are being produced both intentionally or unintentionally by the private hatchery operators and sold to the fish farmers and nursery owners. There is every possibility of hybrid introgression between silver and bighead carps, if F_1 hybrids are used as broodstoc k, that might have negative consequences on the overall performance of these stocks. In view of investigating this, fin samples from 106 silver carp and 47 bighead carp were collected from six private hatcheries at Mymensingh region and from Parbatipur hatchery (which has new, pure stocks of these species). Three microsatellite loci (*Hmo*1, *Hmo*3 and *Hmo*11) were found to distinguish between silver and bighead carp in the Parbatipur pure stocks. Agarose gel electrophoresis was used to visualize microsatellite alleles amplified from total genomic DNA extracted from fins.

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

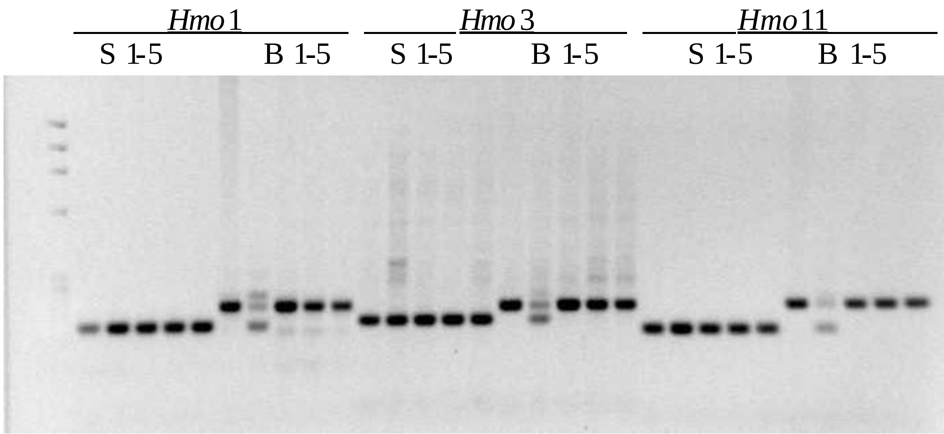


Figure 2. Three microsatellite loci (*Hmo*1, *Hmo*3 and *Hmo*11), with in each case 5 silver (S1-5) and 5 bighead (B1-5) carp samples. Bighead sample #2, from a hatchery, shows a hybrid genotype for all three loci.

3.6 Genetic Stock Improvement through Interspecific Hybridization and Chromosome Manipulation

3.6.1 Interspecific hybridization between endemic and exotic barbs

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

3.6.2 Radiation-induced heat shocked gynogenesis in rohu and mrigal

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

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

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

4. Genetic Conservation of Some Endangered Carp Species

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

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

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

4.1 Development of artificial propagation techniques

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

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

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

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

5. Future Genetic Research Plans on Carp Species

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

References

Ali, M.Y. 1967. Induced breeding of major carps in ponds by pituitary hormone injection. Agric. Inform. Serv., Dhaka, 3p.

Ali, M.L. 1998. Fishery resources development and management technique. Fish Week '98 Compendium. Department of Fisheries and Ministry of Fisheries & Livestock, Dhaka. pp.1-5.

Azad, M.A.K. 1997. A preliminary study on the sexual dimorphism for weight of silver barb, *Puntius gonionotus* Bleeker. M.S. Thesis, Bangladesh Agricultural University, Mymensingh, Bangladesh, 97p.

Banik, R.C.K. and Humayun, N. 1998. Fishery resources statistics. Fish Week '98 Compendium. Department of Fisheries and Ministry of Fisheries & Livestock, Dhaka. pp.100-102.

Begum, S. 1996. Studies on the hybridization between *Puntius sarana* and *Puntius gonionotus* and their embryonic development. M. S. Thesis, Bangladesh Agricultural University, Mymensingh, Bangladesh, 80p.

Hasan, M.R. 1990. Aquaculture in Bangladesh. pp. 105-139. *In* Mohan Joseph (ed.), Aquaculture in Asia, Asian Fisheries Society, Indian Branch, Bangalore.

Hussain, M.G. 1997. Current status of carp genetic research and breeding practices in Bangladesh. In: M.V. Gupta, M.M. Dey, R. Dunham and G. Bimbao (eds.), Proceedings of the Collaborative Research and Training on Genetic Improvement of Carp Species in Asia, 26-29 July 1997, Central Institute of Freshwater Aquaculture, Bhubaneswar, India. ICLARM Work Doc. 1 (Unpublished). 12p.

Hussain, M.G., Mahata, Rahman, M.S., Tanu, M.B., Mazid, M.A. and Islam, M.S. 1997. Induction of mitotic and meiotic gynogenesis and production of genetic clones in rohu, *Labeo rohita* Ham. Bangladesh J. Fish. Res. 1:1-7.

Hussain, M.G. and M.A. Mazid. 2001. Genetic improvement and conservation of carp species in Bangladesh. Bangladesh Fisheries Reserach Institute and International Center for Living Aquatic Resources Mangement. 74p.

Hussain, M.G., Islam, M.S., Hossain, M.A., Wahid, M.I., Dey, M.M. and Mazid, M.A. 2002. Stock improvement of silver barb (*Barbodes gonionotus* Bleeker) through several generations of genetic selection. Aquaculture 204:469-480.

Islam, M.S., Shah, M.S., Rahman, M.A. and Chowdhury, H.A. 1994. Induction of triploidy in rohu, *Labeo rohita*, by heat shock treatment and comparative growth with normal diploid. J. Aqua. Trop. 9:299-210.

IUCN Bangladesh. 2000. Red book of threatened fishes of Bangladesh. IUCN-The World Conservation Union. xii, 116p.

Jhingran, V.G. and Pullin, R.S.V. 1985. A hatchery manual for the common, Chinese and Indian major carps. ICLARM Studies and Reviews 11, Asian Development Bank, Manila, Philippines and ICLARM, Manila, Philippines. 191p.

Maclean, R.H. and Jones, R.W. 1995. Aquatic biodiversity conservation: a review of current issues and efforts. Ottawa, ON, SIFR.

Moyle, P.E. and Leidy, R.A. 1992. Loss of biodiversity in aquatic ecosystems: evidence from fish fauna. In: Fielder, P.L. and Jain, S.K. (eds.), Conservation of biology; the theory and practice of nature conservation, preservation and management. Chapman and Hall, New York.

Pongthana, N., Penman, D.J., Karnasuta, J. and McAndrew, B.J. 1995. Induced gynogenesis in the silver barb (*Puntius gonionotus* Bleeker) and evidence for female homogamety. Aquaculture 135:267-276.

Pongthana, N., Penman, D.J., Baoprasertkul, P., Hussain, M.G., Islam, M.S., Powell, S.F. and McAndrew, B.J. 1999. Monosex female production in the silver barb (*Puntius gonionotus* Bleeker). Aquaculture 173:247-256.

Rahman, A.K.M. 1985. Study on the exotic fishes in Bangladesh. Paper presented at a seminar on the Culture Need of Exotic Species in Bangladesh, organised by the Zoological Society of Bangladesh, Dhaka, 15 October 1984. 13p.

Schom, C.B. and Bailey, J.K. 1986. Selective breeding and line crossing to reduce inbreeding. The Progressive Fish Culturist 42:57-60.

Genetic management and improvement strategies for exotic carps in Asia: a project overview

David J. Penman[1], Graham C. Mair[2], Brendan J. McAndrew[1], M.G. Hussain[3], M.A. Sattar[4], Y. Basavaraju[5] and Pham Anh Tuan[6]

1 Institute of Aquaculture (IOA), University of Stirling, Scotland (djp1@stir.ac.uk; bjm1@stir.ac.uk).
2 School of Biological Sciences, University of Wales Swansea (UWS), Wales/ Asian Institute of Technology (AIT), Bangkok, Thailand (gcmair@ait.ac.th).
3 Bangladesh Fisheries Research Institute (BFRI), Mymensingh 2201, Bangladesh (fsbfri@bdonline.com).
4 Northwest Fisheries Extension Project (NFEP), Parbatipur, Dinajpur, Bangladesh.
5 Fisheries Research Station, University of Agricultural Sciences, Bangalore (UASB), India (ybraju@bgl.vsnl.net.in).
6 Research Institute for Aquaculture No. 1 (RIA 1), Hanoi, Vietnam (patuan@fpt.vn).

Penman, D.J., Mair, G.C., McAndrew, B.J., Hussain, M.G., Sattar, M.A., Basavaraju, Y. and Tuan, P.A. 2002. Genetic management and improvement strategies for exotic carps in Asia: a project overview. pp. 1-8. In: Penman, D.J., Hussain, M.G., McAndrew, B.J. and Mazid, M.A. (eds.). Proceedings of a workshop on Genetic Management and Improvement Strategies for Exotic Carps in Asia, 12-14 February 2002, Dhaka, Bangladesh. Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh. xxx p.

The project which is the focus of this workshop, which is funded by the DFID Aquaculture and Fish Genetics Research Programme (AFGRP: project R7590), was developed to address the common theme of management of exotic (non-indigenous) carp species in Asian aquaculture. It began in April 2000. In this brief overview, we address some of the key issues the project is dealing with. More information on specific project activities can be found in other papers in the workshop proceedings

(Hussain and Mazid; Sattar and Das; Mia *et al.*, Kohinoor *et al.*; Basavaraju *et al.*; Mair and Tuan) and in the project logical framework, which follows this overview.

Of the fish species cultured in Asia, it is most often carps and tilapias which make contributions to the livelihoods of poor people, through farming, consumption and associated activities. Carps feed low down in the food chain, making them suitable for low input aquaculture and for many species the market prices are fairly low, making them affordable as a source of protein for poor people.

Although the project is not promoting the introduction of non-indigenous species for aquaculture, realistically it is accepted that several such species do make important contributions to aquaculture in Asia and to the livelihoods of the poorer sectors of society. While the genetic management and improvement of stocks of all species in aquaculture is important to sustain and improve production, particular problems are presented in the case of non-indigenous species. The founder stocks may have been of poor, or unknown, quality. For example, some of the stocks of Chinese carps originally introduced into Bangladesh were secondary or tertiary transfers from countries other than China, although more recent introductions came straight from wild stocks in China. Small numbers of parent fish contributing to an introduction, or low survival/breeding rate among the introduced fish, may lead to poor performance through low genetic variation (bottlenecking) or a genetic makeup which is unrepresentative of the parent population (founder effect). For indigenous species, it is often possible, if necessary, to go back to local wild populations to replenish hatchery stocks, for example if a decline in performance is observed or suspected. For non-indigenous species, logistical and political factors may make this difficult, along with associated risks of introducing pathogens or other undesirable species. In tackling the particular problems of managing introduced species, we also hope to be able to promote the more general message of good genetic management of aquaculture broodstocks, which is often neglected.

Genetics is sometimes seen as a highly technical subject of little relevance to "real life". However, we believe that in the context of broodstock management, genetics is highly relevant to the livelihoods of stakeholders in aquaculture (hatcheries, nurseries, traders, on-growers, consumers), and that appropriate research in aquaculture genetics can play a strong role in sustaining and improving livelihoods. The breeding of fish in hatcheries is not isolated from the rest of aquaculture. The seed produced in hatcheries work their way right through the network of nurseries, traders, on-growers and markets to consumers, and changes in the genetic quality of those seed will likewise have effects throughout this network. Some networks may be local, while others are far reaching both in geographical terms and in terms of the different socioeconomic groups who are linked through the network. For example, large private carp hatcheries in Jessore in SW Bangladesh are the source of much of the fish seed supply to small-scale ongrowers in NW Bangladesh, one of the poorest regions of the country.

In addition to seed leaving hatcheries, there should also be a flow of information from the rest of aquaculture back to the hatcheries, to feed into the process of good broodstock management by, for example, defining important traits or identifying superior stocks. Too often, this link is missing. In the absence of good genetic management of hatchery stocks or where management considerations are entirely "internal", for example where a numerical seed target is the main objective and broodstock replacement is from leftover fish, changes in genetic quality of seed are likely to be negative (through inbreeding or negative selection). Good broodstock management, ideally taking into account information from performance during on-growing on farms, will result in sustained or improved quality of broodstock and seed produced for aquaculture (e.g. as a result of good stock choice, prevention of inbreeding, planned selection or monosex fry production). Inbreeding has negative effects on many traits of interest to aquaculture (growth rate, survival rate, deformities, etc). Planned selection, however, generally focuses on only one or a few traits. While "growth rate" is the most common trait initially targeted in selective breeding, this has to be more carefully defined. Does it mean faster growth on unlimited resources or faster growth on fixed resources? The latter (i.e. increases in production efficiency) is likely to be more important to resource-poor fish farmers. We are trying to involve such farmers in defining appropriate traits for selective breeding, and there is evidence of the benefits of such an approach from other agricultural crops.

While we can try to develop improved stocks for aquaculture and implement other changes at a local level, to have sustainable impact the project also needs to influence policy at state, national and international levels. This can mean supporting the implementation of improved broodstock management practices which will lead to sustainability of and improvements in seed quality at state or national level (as is already taking place for example with the Karnataka State Department of Fisheries in India). On a broader scale, links through international bodies such as INGA, the International Network for Genetics in Aquaculture, can help to spread knowledge and other project outputs more widely. INGA is coordinated by ICLARM, has member countries in Asia and Africa and also several associated Advanced Scientific Institute members. All of the institutes involved in the DFID project are members or associate members of INGA and participate in its activities.

In summary, we hope that through this project we will demonstrate that it is possible, in the medium to long term, to impact positively upon sustainable livelihoods through the appropriate application of genetics based methods focussed on basic broodstock management and on traits important to the target stakeholders. We believe this impact will come directly through application of knowledge and improved fish stocks and indirectly through influence on policy. Links to related initiatives such as the INGA-coordinated ABD carp genetics project and the AFGRP-funded seed quality in Asia project (R7052) will also be important in achieving impact and developing appropriate further research.

Acknowledgement
We would like to thank DFID for the funding of this project, through the Aquaculture and Fish Genetics Research Programme, which is managed by Prof. James Muir at the Institute of Aquaculture, University of Stirling.

Logistical Framework for DFID-AFGRP project R7590: **Genetic management and improvement strategies for exotic carps in Asia**

Narrative Summary	Objectively Verifiable Indicators (OVI)	Means of Verification	Important Assumptions
GOAL: Productive benefits of aquatic resources for poor people generated and sustained through improved knowledge of aquatic stocks and their selection, enhancement and culture.	By 2005, knowledge gains allow 500,000 poor people in S & SE Asia to improve food supply by 20% and income by 20%, based on yield increases related to better aquatic stocks, sustainable aquaculture and enhancement practices, and at least 100,000 people positively impacted by development activities incorporating programme outputs.	- National, FAO fisheries/aquaculture sector surveys and statistics, environment report - Evaluation of RNRKS and AFGRP - National reports to regional organisations.-reports of target institutions/key locations - Household and community surveys/ monitoring against base-line data.	Poor people invest benefits to improve livelihoods
PURPOSE: Strategies for genetic management and improvement of cultured exotic carp species in low input aquaculture systems developed, verified and recommended for adoption in Bangladesh, India and Vietnam and potential impact of improved fish on livelihoods demonstrated.	- Best stocks identified and strategies formulated by end of project - Upstream and downstream output to uptake pathways clearly identified at project end - Management tools and recommendations adopted and implemented by 2005 - Recommended and improved stocks widely adopted for aquaculture by 2007	Government and other official reports and statistics, project documentation and post project surveys.	- Enabling environments for widespread adoption of new technologies and strategies exist. - Climatic conditions remain favourable. Agencies responsible for dissemination of project outputs can appropriately target strategies at poor people.

OUTPUTS: 1. Strategies for genetic management and improvement of carp stocks developed and recommended	1. Workshops held at which the appropriate strategies are recommended to relevant national agencies by end of project.	Project reports and publications, based on research carried out in the laboratory, on-station and on-farm	Social, economic and political environment remains suitable for the adoption, implementation and sustainable utilisation of project outputs.
2. Superior stocks of carps for low input aquaculture systems identified and/or developed	2. Performance characterisation of available stocks of relevant carp species completed by the end of Year 3.		
3. Examples of improvements in production levels demonstrated in low-input aquaculture systems incorporating carps	3. Average of 20% improvement in yields of relevant species or systems demonstrated in trials by the end of the project.		
4. Preliminary assessment of potential impact of production and genetic improvements on livelihoods	4. Basic livelihood indicators assessed in all farm based trials		
5. Scientific tools incorporating genetic markers for sustainable genetic management of Common and Chinese carps	5. Genetic markers appropriate for monitoring genetic variation in hatchery broodstock of Chinese carps and some common carps developed and verified by the end of the project.		
6. Live and cryopreserved (sperm) gene bank of base strains for cultured exotic carps in Bangladesh, India and Vietnam.	6. Viable sperm banks developed in appropriate institutions by the end of year 3		

ACTIVITIES:			
1. 1.1. Development of appropriate broodstock management and dissemination strategies and formulation of recommendations based on characterisation of culture performance and genetic variation. 1.2. Recommendations presented and modified at workshop(s)	1. Recommendations formulated and presented at workshop(s) in the final year of the project	Project reports	- Cooperation between institutions is maintained and develops appropriately - Collection and local adaptation of newly acquired strains is successful - Work is not disrupted by natural disasters or personnel changes - Molecular markers can be developed in predicted timescale - Adequate information is available on social and economic aspects of dissemination and targetted beneficiaries
2. 2.1. Introduction, where necessary, of new carp strains 2.2. Optimise tagging and marking methods for strain identification in these species and develop protocols for researcher led and farmer led growth trials for strain comparisons 2.3. Evaluation of comparative culture performance and yield of carp stocks in on-station and on-farm trials including, where appropriate, assessment of heterosis and genotype x environment interactions	2. 2.1. New strains introduced by end of year 1 2.2. Methods developed and optimised by the end of year 1 (on-station) and year 3 (on-farm) 2.3. Comprehensive on-station and on-farm and trials completed by end of year 3		
3. On-station and on farm evaluation of superior and existing stocks in polyculture.	3. Production evaluated in minimum of two on-station and four on-farm trials in each country by end of project		

ACTIVITIES (contd.): 4. Participatory evaluation of impacts of superior stocks on livelihoods indicators of beneficiary farmers 5. Development and application of a suite of polymorphic DNA markers to Chinese carps and application of existing DNA markers to Vietnamese common carp. 6. Cryopreservation of sperm from base populations of all available strains and transfer of frozen samples to appropriate locations.	4. Assessment of livelihood indicators for all on-farm production trials. 5. 5.1. At least 5 markers with moderate to high polymorphism levels developed for each species of Chinese carps by end of year 2 5.2. Genetic variation in Bangladeshi & Vietnamese stocks of Chinese carps & Vietnamese common carp compared and applied to understanding genetic basis of performance differences by end of year 3 5.3. Verify the application of molecular tools to broodstock management and dissemination strategies by the end of the project 6. Cryopreservation of sperm from base populations of all available strains and transfer of frozen samples to appropriate locations by mid of year 3	Project reports	- Cooperation between institutions is maintained and develops appropriately - Collection and local adaptation of newly acquired strains is successful - Work is not disrupted by natural disasters or personnel changes - Molecular markers can be developed in predicted timescale - Adequate information is available on social and economic aspects of dissemination and targetted beneficiaries

Conservation of Fish Genetic Diversity: Need for Development of a Cryogenic Genebank in Bangladesh

A. Bart

Aquaculture and Aquatic Resources Management, Asian Institute of Technology, Bangkok, Thailand

Bart, A. 2002. Conservation of fish genetic diversity: need for development of a cryogenic genebank in Bangladesh. pp. 107-110. In: Penman, D.J., Hussain, M.G., McAndrew, B.J. and Mazid, M.A. (eds.). Proceedings of a workshop on Genetic Management and Improvement Strategies for Exotic Carps in Asia, 12-14 February 2002, Dhaka, Bangladesh. Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh. xxx p.

Background

Bangladesh is ranked 3rd largest in aquatic biodiversity in Asia behind China and India, with approximately 300 species of fresh and brackish water fish species (Hussain and Mazid, 2001). This species diversity has been attributed to one of the world's largest wetlands (Bengal Delta) and three large river systems (Brahmaputra, Ganges and Jamuna) that flow from the Himalayan mountains into the Bay of Bengal. Enormous freshwater fisheries resources feed millions of people living in the Delta. Recent acceleration of aquaculture production (700,000 tons of fish) in Bangladesh has begun to relieve fishing pressure from a few large rivers and water bodies. It is expected that the consumption demand for fish will reach over 2 million tons by the year 2002 (DoF, 1999). Unfortunately, overharvesting of fish with an increasing fishing population is likely to continue and place greater pressure on most small and large size water bodies. Rapid extraction of seed (for stocking) as well as broodfish (for seed production and consumption) from natural waters combined with destructive and unregulated fishing practices that use dynamite, cyanide, electrofishing and gillnets has led to the endangerment and possibly extinction of a number of rather valuable native species (Hussain and Mazid, 2001).

Loss of aquatic habitat due to siltation, dam construction (for irrigation, flood control and hydroelectric generation), and other anthropogenic activities has been one of the primary causes of species loss. Siltation in the upstream reduces water flow and water depth, impairing the opportunity of riverine fish to feed, navigate, and migrate and spawn. Construction of embankments for flood control and dams interferes directly or indirectly with fish migration, reproduction and ultimately survival of species. Deepening of channels by removing silt is a temporary and costly solution to improve habitat. While long term and effective measures are being sought, more effective and immediate measures are needed to protect and conserve threatened and endangered species.

Aquaculture practices (domestication, typical genetic manipulation: selection, sex reversal, hybridization and crossbreeding) and release of fry into natural water bodies also contribute to indigenous species degradation. The decade-long practice of stocking exotic and indigenous carp seed in the rivers and reservoirs already makes it difficult to determine the negative impact resulting from gene introgression and inbreeding. In other words, indigenous species in nature are probably contaminated to a level where it becomes difficult to compare performance against hatchery stock.

While little can be done to bring back lost species of Bangladesh, currently 56 species of freshwater species including 11 cyprinids are endangered or near extinction (IUCN, 1998), needing immediate measures to protect and conserve them. Establishment of *ex situ* gene banks, live and/or cryogenic, in the immediate term would ensure the maintenance of genetically pure stocks of fish while “buying us the time” necessary to improve habitat conditions for restocking.

Ex situ Preservation and its Importance to Bangladesh

The ideal strategy for conservation of threatened and endangered species is through *in situ* (conservation of the ecosystem or habitat to maintain them in their natural environment) protection/restoration of the native habitat of the species. Unfortunately, this is costly and requires a great deal of time as habitat restoration is clearly a slow process. One alternative is to maintain *ex situ* (conservation outside their natural environment) live or cryopreserved gene banks. Live gene banks for fish are also costly, requiring purpose built facilities, and being labor intensive are difficult to manage. Past attempts to maintain long-term live gene banks have often resulted in contamination of stocks.

There are fewer constraints to the establishment of long-term *ex situ* frozen gene banks, which are thought to ideally complement habitat conservation and in situ gene banks (Bart, 2001). There are several examples of cryogenic sperm banks for fish in Europe, and North and South America. They are comparatively less costly than live gene banks although some initial investment for equipment, maintenance and collection would be required. Cryogenic gene banking avoids the risk of contamination and requires little space and minimal facilities. While cryopreservation of sperm has been successful for many species, and the protocols have been well developed, cryopreservation of eggs and embryos has been successful only in oysters. Research into teleost egg and embryo is on going (Bart, 2000). Furthermore, it is possible to recover genotypes from cryopreserved sperm using androgenesis to produce viable diploid organisms with paternal only inheritance.

Ex situ conservation via cryopreservation has many potential practical applications. For example, maturation of males and females in a number of species is asynchronous. Bighead carp females come to seasonal maturity when sperm from males is not available. Hatchery producers of Bangladesh often use sperm from another species such as silver carp, which is less desirable as indicated by the lower price of this hybrid. Maintaining sperm in cold storage would facilitate artificial fertilization and subsequent seed production.

Many species of surviving brood animals have reached critically low levels, and this is likely to cause irreversible genetic bottlenecks in Bangladesh. Examples of these species in Bangladesh include 11 species of carps and barbs (Hussain and Mazid, 2001). Cryogenic preservation of sperm could facilitate dramatic increase in the effective breeding population in future restoration efforts.

Conclusions and Recommendations

Clearly, a cryogenic sperm bank has application to both aquaculture and conservation of species in Bangladesh. The Bangladesh Fisheries Research Institute (BFRI) has been charged with collection of indigenous species for propagation purposes. It has the necessary facilities, and pond and tank space to house a number of species and could naturally assume the responsibility of collection of samples from priority species, and the maintenance of collections and a database. Having established such a facility, it could join forces with academic institutions, the DoF and/or local NGOs such as BRAC to facilitate the education and training of hatchery operators and technicians on the use and distribution of cryopreserved sperm for hatchery use purposes.

References

Bart, A.N. 2000. New Approaches in Cryopreservation of Fish Embryos, In: Cryopreservation in Aquatic Species. Tiersch, T.R. and P.M. Mazik, Editors. World Aquaculture Society, Baton Rouge, Louisiana. Pp. 179-187.

Bart, A. N. 1999. Biodiversity and Ex Situ Fish Genetic Conservation. AARM Newsletter. Vol. 4(4) 6-8.

DoF. 1999. A Brief on Department of Fisheries, Bangladesh. DoF, Dhaka.

Hussain, M.G. and M.A. Mazid. 2001. Genetic Improvement and Conservation of Carp Species in Bangladesh. BFRI and ICLARM publication, Dhaka, Bangladesh.

IUCN Bangladesh. 1998. List of Threatened Animals of Bangladesh. Paper Presented in the Special Workshop on Bangladesh Red Book of Threatened Animals, 22 February 1998. Dhaka.

Evaluation of Different Stocks of Chinese Carps in Bangladesh: Design and Preliminary Results

A.H.M. Kohinoor[1], M.S. Islam[1], M.Y. Mia[1], M.A. Rahman[1], M.G. Hussain[1], M.A. Mazid[1], B.J. McAndrew[2] and D.J. Penman[2]

1 Bangladesh Fisheries Research Institute, Mymensingh 2201, Bangladesh
2 Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland

Kohinoor, A.H.M., Islam, M.S., Mia, M.Y., Rahman, M.A., Hussain, M.G. Mazid, M.A., McAndrew, B.J. and Penman, D.J. 2002. Evaluation of different stocks of Chinese carps in Bangladesh: design and preliminary trials. pp. 59-67. In: Penman, D.J., Hussain, M.G., McAndrew, B.J. and Mazid, M.A. (eds.). Proceedings of a workshop on Genetic Management and Improvement Strategies for Exotic Carps in Asia, 12-14 February 2002, Dhaka, Bangladesh. Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh. xxx p.

Abstract
New stocks of Chinese carps were introduced into Bangladesh from wild populations in China in 1994 and are being held at the DOF NFEP campus, Parbatipur. As part of the evaluation of the performance of these new stocks in comparison to the already existing stocks of these species in Bangladesh, trials are being conducted under the project which is the focus of this workshop. This paper describes the methodology being used for the first of these trials, which uses communal stocking of marked fingerlings from the different stocks, in a range of on-farm and on-station environments. Fin cauterization has been used for batch marking, based on research conducted elsewhere in common carp. The first of these trials has not yet been completed, but some preliminary data are presented.

Introduction

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, bighead carp, common carp and grass carp. 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 dominate the overall aquaculture production of the country. The exotic carps play a vital role in carp polyculture to boost fish production of inland waters in Bangladesh. In the last few decades exotic species, including the silver carp, grass carp, bighead carps, common carp and silver barb have become popular because of their quick growth with low cost feed in comparison to indigenous major carps. Due to low market price of both fingerlings and food fish, poor people can afford to eat species like the silver carp rather than the more expensive indigenous carps.

It has been observed that while some of the hatcheries are careful in selection and maintaince 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 broodstock 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 each species: the high fecundity of carps allows adequate seed production in this situation. However, over successive generations, low effective population sizes lead to inbreeding depression with reduced growth rates, loss of fecundity and poor survival.

Interspecific hybridization in some carp species has also recently been reported in this country. Either out of scientific interest or because of shortage of adequate hatchery populations (brood stock), hybrids are being produced intentionally or unintentionally by private hatchery operators and sold to nursery operators and farmers. There is widespread concern that mass stocking of such hybrids in the floodplains and other related open water bodies might cause a serious genetic introgression problems in indigenous species that could adversely affect aquaculture and inland open water fish production, while in some cases (e.g. silver carp x bighead carp hybrids) introgressive hybridization in hatchery broodstock is a potential problem. The case for the use of F1 carp hybrids in aquaculture on a positive basis (i.e. due to superior performance), as opposed to their production as a result of constraints (e.g. use of sperm from silver carp males to fertilise bighead carp eggs due to a shortage of bighead carp males), has not been made, despite widespread experimental hybridization trials (e.g. Reddy, 1999).

Hussain and Mazid (2001) summarise the earlier introductions of carps into Bangladesh. In 1994, stocks of silver carp, bighead carp and grass carp from the Yangtze River in China were introduced into Bangladesh, and are held and managed at the Northwest Fisheries Extension Project (NFEP) campus, Parbatipur. One of the aims of the present DFID-AFGRP funded project was to extend earlier trials on these new stocks to compare them to local stocks in different areas of Bangladesh, since the latter may have diverged in performance since introduction and dissemination (as a result of different management practices, etc). This paper describes the experimental design being used for these trials, and preliminary data from the first such trial, in which silver and bighead carps from the new stocks at Parbatipur and a local hatchery stock in the Mymensingh area were compared. F1 bighead x silver carp hybrids, obtained from a hatchery in the Mymensingh area, were also included to assess their performance relative to the parental species.

Materials and Methods

Trial design

The experimental design was based on communal stocking of the different groups of fish into a series of ponds representing different farming environments. This design allows direct comparison of different stocks in the same environment, and overcomes the problem of finding adequate numbers of "replicate" ponds for comparison in separate stocking (apparently similar ponds may actually differ in many environmental respects, particularly for complex ecosystems like polyculture). However, communal stocking does require reliable batch marking techniques to identify different stocks of the same species, and also requires that the different stocks of fish have very similar mean weights at the start of the trial. Significant differences in mean weight at the start of such trials can often be perpetuated and contribute to differences at the end. It is also possible that competition may exaggerate real differences in growth potential between test stocks, and thus the results may have to be interpreted cautiously in terms of the implications for the potential of the faster growing stocks.

Assessing different stocks of the same species in a variety of farming environments (e.g. different types of ponds, management practices, levels of inputs or species combinations) should allow assessment of genotype-environment interaction (put simply, changes in the ranking of different stocks across a range of environments implies significant GxE interaction, while consistency in rankings implies little or no GxE interaction). This has major implications for selective breeding (a lack of GxE interaction can be taken to imply that an improved line which has been developed in one type of environment should also perform well in the other types of environments, while strong GxE interaction may imply that different lines need to be developed for different environments). Assessing stocks under low input on-farm environments, rather than only in high-input on-station environments, is important in research aimed at testing, managing or genetically improving stocks for low input aquaculture.

Origin of stocked fish

The silver carp and bighead carp were collected from a private hatchery in the Mymensingh region and NFEP, Parbatipur. The other species (catla, rohu, mrigal, grass carp, silver barb and bighead x silver carp hybrids) were collected from local nursery operators in the Mymensingh region.

Fin Cauterization

Five groups of fish (two stocks of silver carp, two stocks of bighead carp and hybrids) were marked by fin cauterization. The fish weighed 4 to 11 g and were anesthetized in small groups (1-2 drops of clove oil added per litre of water). The blade of a small scalpel was heated using a gas heater until it became red hot. Then the chosen fin of each fish was spread on a flat surface of a plastic board and the red hot scalpel blade was used to cut off the fin at the base, as fast as possible to avoid the heat damaging the surrounding area. The scalpel blade and the body of the fish were angled in opposite directions away from each other. The Mymensingh silver and bighead carp were marked by cutting the right pelvic fin, the Parbatipur silver and bighead carps by cutting the left pelvic fin and the hybrid (silver x bighead) by cutting the anal fin.. The fish then were treated with 250 ppm solution tetracycline to avoid infection. The fish were also dipped in 10 ppm potassium permanganate. The fish were then kept in different on-station ponds to monitor recovery before stocking into growout ponds. Problems were experienced with the Parbatipur silver carp stock, which showed very high mortality after fin cauterization. This stock had to be replaced and added later to the trial ponds, with obvious implications for the performance comparison between the two stocks of silver carp. There were only sufficient fish from this group to stock the on-station ponds.

Stocking of fish

After one week, all the fish were stocked in prepared on-station and on-farm ponds according to design. Three on-station (400 m^2 each) and four on-farm ponds (320-480 m^2) were selected for conducting the experiment. The ponds were prepared through liming @ 250 kg /ha. Three days after liming, the ponds were fertilized with cowdung @ 1000 kg /ha, Triple Super Phosphate and Urea @ 50 kg and 25 kg, respectively. After three days, the ponds were prepared and filled with ground water. The species combination and stocking density of fish are illustrated in Table 1.

Post stocking management

***High input management*:** Experimental fishes in 3 on-station and 2 on-farm ponds were fed with a mixture of rice bran and mustard oil cake (3:1) at the rate of 3% of the estimated fish biomass. In addition, inorganic fertilizers were also applied monthly by following the preparatory doses.

***Low input management*:** Only rice bran was being applied to the rest two on-farm ponds at the rate of 3% of estimated fish biomass.

The ponds were fertilized with cowdung, Triple Super Phosphate and Urea, applied monthly at half of the initial dose. Ground water was added to only the on-station ponds at weekly intervals. Fifteen to twenty percent of fish of each species were sampled through seine netting at monthly intervals to assess their growth, health condition and to adjust the feeding ration.

Table 1. Details of stocking combination and ratio of different fish species in polyculture management under on-farm and on-station conditions. SC = silver carp, BH = bighead carp, Hybrid = bighead x silver cross, (P) = Parbatipur stock, (M) = Mymensingh hatchery stock.

Trial environment	Species stocked/ha										
	SC (P)	SC (M)	BH (P)	BH (M)	Hybrid (M)	Grass carp	Catla	Rohu	Mrigal	Rajpunti	Total
On-station	375	750	750	750	750	250	500	500	1000	1500	7125
On-farm	-	750	750	750	750	250	500	500	1000	1500	6750
Mean weight (g±S.D.)	4.1 ±1.2	10.0 ±2.2	10.1 ±1.7	6.9 ±0.9	11.4 ±2.1	12.2 ±2.3	15.8 ±3.1	14.9 ±2.5	6.5 ±0.8	7.1 ±0.8	

Results

Observation of fin cauterization

As the trial had not yet been completed before this paper was prepared, and thus data was not yet available on all of the fish, it was not possible to make an accurate assessment of the fin cauterization technique. From the monthly sampling, silver and bighead carp appeared to show approximately equal response to pelvic fin cauterization. In some individuals of both species, the cauterized fins had not regenerated. Others showed a shorter and/or fringed pelvic fin, which was also identifiable. There were also some fish that had normal fins, with no sign of marking. These fins appeared to have regenerated quickly, within a month, and the fish were not identifiable. The retention rates appeared to be higher in the second batch of Parbatipur silver carp (see M&M), with the improvement being due to greater experience with the technique. For some of the hybrid fish, the cauterized anal fin also regenerated within a month and looked like a normal fin, whereas some of them are also showing an identifiable cut-line. So in this event it is very difficult to identify the cauterized fin. However, the regenerated anal fin edges are softer and also smooth in comparison to the normal fin of hybrids.

Water quality parameters

The water quality parameters such as temperature, dissolved oxygen and pH of the ponds of on-station condition were recorded weekly in each experimental pond and the ranges were: temperature 18.8 to 30.20°C, dissolved oxygen 5.25 to 7.61 mg/L and pH 6.88 to 8.45. The observed water quality parameters are within the suitable range for fish culture.

Growth of fish

The sampling weights of the different species of fish after three months of culture in on-station and on-farm management are shown in Table 2. Given the significantly lower initial mean weight of the Mymensingh bighead carp compared to the Parbatipur stock, it is not surprising that the Parbatipur bighead carp are larger at this stage. Likewise, the delayed stocking and smaller initial size of the Parbatipur silver carp stock have resulted in these being smaller than the Mymensingh stock at this stage.

Comparison of the Mymensingh silver carp and the hybrids (which did not differ significantly in weight at the time of stocking) in the on-farm and on-station environments shows that the hybrids performed better than the silver carp in the on-farm ponds, but the situation was reversed in the on-station ponds (Fig. 1: Parbatipur bighead carp growth curve included for comparison as the initial mean weight of this group did not differ significantly from the other two groups shown here).

The ranking of the weight of the grass carp and silver barbs relative to the other species changed between the on-farm and on-station, being much higher in the on-station ponds and much lower in the on-farm ponds.

Table 2. Average sampling weights of different species of fish (g) in on-station and on-farm (high and low input) conditions after 3 months of culture period. SC = silver carp, BH = bighead carp, Hybrid = bighead x silver cross, (P) = Parbatipur stock, (M) = Mymensingh hatchery stock.

Trial environment	Average sampling weight of fish (g±S.D.)									
	SC (P)	SC (M)	BH (P)	BH (M)	Hybrid (M)	Grass Carp	Catla	Rohu	Mrigal	Rajpunti
On-station	49.5 ±10.7	121.9 ±23.9	145.3 ±12.4	92.1 ±20.4	77.5 ±18.2	323.0 ±63.3	114.2 ±7.1	101.9 ±29.0	72.4 ±12.4	140.7 ±12.2
On-farm (high input)	-	127.6 ±21.5	200.3 ±72.0	166.1 ±27.6	139.9 ±22.2	114.3 ±33.5	113.2 ±50.9	78.7 ±20.2	85.0 ±18.4	49.8 ±19.0
On-farm (low input)	-	96.4 ±18.7	152.3 ±18.0	105.5 ±31.8	112.3 ±13.0	58.0 ±22.6	98.9 ±35.2	86.1 ±14.0	68.0 ±33.9	34.0 ±10.5
On-farm mean	-	112.0	176.3	135.8	126.1	86.2	106.1	82.4	76.5	41.9

Discussion

Although the results of these trials will be compromised by the initial problems (caused by obtaining the fingerlings later than initially intended and thus not being able to equalise the size at stocking through careful nursing), the basic design appears to be adequate for the purpose of comparing the different stocks of Chinese carps (it has been used for several years for comparisons of different stocks of common carp in India – see Basavaraju *et al*., this volume). However, the performance of the hybrids relative to the silver carp changed from on-farm to on-station ponds. The stocking density of silver carp varied between these two sets of ponds (Parbatipur stock silver carp were absent from the on-farm ponds), and this may have been responsible for the difference in the hybrid performance. However, other factors also varied (e.g. water was exchanged on-station but not on-farm , and towards the last sampling period reported the water level was lower in the on-farm ponds), and the grass carp and silver barbs also showed changes in performance between the two situations (perhaps due to less access to bankside vegetation in the on-farm ponds).

Future trials will incorporate Chinese carps obtained from a wider variety of sources and include grass carp as well as silver carp and bighead carp. The fish will also be obtained as fry and reared on-station to ensure minimal size differences at the time of stocking.

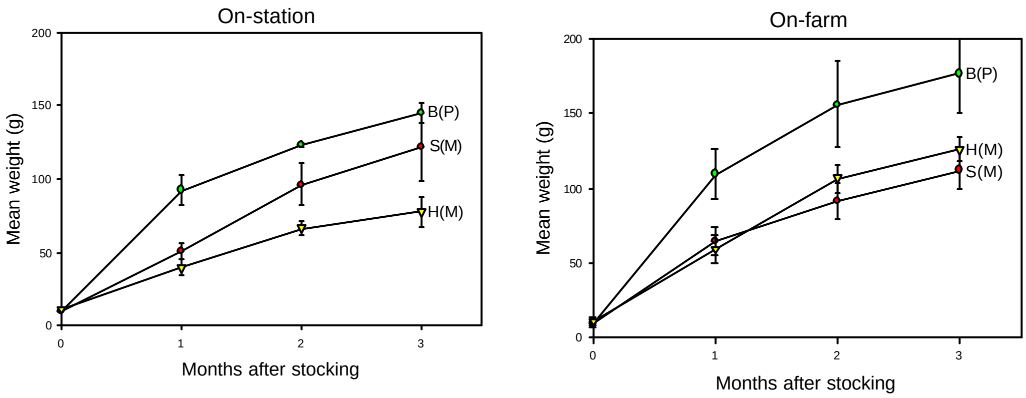


Figure 1. Growth of Mymensingh silver carp, S(M), and Mymensingh bighead x silver carp hybrids, H(M), under on-station and on-farm conditions (the latter shows the mean of high and low input on-farm conditions). The growth curve for the Parbatipur bighead carp, B(P), is also included for comparison (these three groups of fish did not show significant differences in weight at the start of the trial – see Table 1).

Acknowledgements
This research was supported by the DFID Aquaculture and Fish Genetics Research Programme (project R7590).

References

Ali, M.Y. 1967. Induced breeding of major carps in ponds by pituitary hormone injection. Agricultural Information Service, Dhaka.

Basavaraju, Y., Penman, D.J. and Mair, G.C. 2002. Genetic status and strategies for improvement of common carp (*Cyprinus carpio*) in Karnataka, India - evaluation of stocks for developing breeding programme. This proceedings.

Hussain, M.G. and Mazid, M.A. 2001. Genetic Improvement and Conservation of Carp Species in Bangladesh. BFRI, Mymensingh, Bangladesh and ICLARM, Penang, Malaysia. 74p.

Reddy, P.V.G.K. 1999. Genetic resources of Indian major carps. FAO Fisheries Technical Paper 387. FAO, Rome. 76 p.

Carp Brood Stock Management and Genetic Improvement Programme under Fourth Fisheries Project

F. Rajts[1], T. Huntington[1] and M. G. Hussain[2]

1 Aquatic Resources Development, Management and Conservation Studies Project of the Fourth Fisheries Project, Dhaka (rajts@gef-fish.com and tim@gef-fish.com)
2 Bangladesh Fisheries Research Institute, Mymensingh 2201 (fsbfri@bdonline.com)

Rajts, F., Huntingdon, T. and Hussain, M.G. 2002. Carp brood stock management and genetic improvement programme under Fourth Fisheries Project. pp. 95-106. In: Penman, D.J., Hussain, M.G., McAndrew, B.J. and Mazid, M.A. (eds.). Proceedings of a workshop on Genetic Management and Improvement Strategies for Exotic Carps in Asia, 12-14 February 2002, Dhaka, Bangladesh. Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh. xxx p.

Abstract
Bangladesh is rich in terms of globally important wetland ecosystems and associated aquatic biodiversity. The total area of inland waters is estimated at 4.3 million ha. These inland water habitats form the largest floodplains in Asia, widely considered to be one of the most important wetland complexes in the world. This rich biological diversity – including genetic, species, as well as habitat diversity - has been formed over millions of years. The evolution of habitat diversity has resulted from erosion of the Himalayas and tectonic movements, followed by successful adaptation of fish species to the slow changing of habitat, based on their genetic diversity. This ancient adaptive capacity of fish species has been seriously affected over just the last twenty years, not only through habitat degradation, but also through the use of inappropriate artificial reproduction and fish culture practices. The large-scale induced breeding operations by over 600 private hatcheries have raised concern about potential genetic degradation of cultured endemic and exotic species. Mainly due to a lack of technical knowledge and awareness of the potential ecological consequences, widespread inbreeding depression, negative selection and genetic introgression by hazardous hybridisations has and continues to occur. The increasing use of culture-based

fisheries in the open-water floodplains through the mass release of fingerlings has also endangered the genetic purity of wild endemic fish populations.

As this ecological issue is relevant to those in many other floodplain areas in the region, the Global Environment Facility (GEF) funded ‘Aquatic Resources Development, Management and Conservation Studies’ project is presently conducting studies to design a programme for genetic diversity improvement and the maintenance of appropriate broodstock lines. This paper is a brief presentation of the proposed studies which are just starting implementation.

Introduction

The Fifth Five Year Plan of Bangladesh is targeting 1.675 million tons of fish production from inland fisheries for the year of 2001/2002. The achievement of fish production in the year of 1999/2000 was 1.321 million tons, while the per capita annual fish intake was 12 kg instead of the required 18 kg. The aquaculture needs for fish fry are almost entirely (98.6% according to FRSS/DoF, 1999/2000) provided by hatcheries. Consequently the achievement of aquaculture targets in the immediate future will be determined by the genetic status of hatchery broodstock. In the recent past, inexperienced hatchery operators have damaged most of the broodstock through improper management, resulting in considerable genetic degradation. While induced breeding is practised, limited numbers of artificially selected breeders are involved; their success of reproduction is forced by hormone injections. The survival of the larvae and fry is promoted by providing highly artificial conditions, including the use of chemicals and selective insecticides to control predators. This results in the complete absence of natural selection, whose selective process in natural conditions eliminates traits of reduced vitality, slow growth rate, reduced resistance to diseases, etc. For this reason, the production potential of the Bangladeshi closed waterbodies and stocked floodplains are increasingly constrained.

The establishment of improved fish breeding systems is a considerable task, as the majority of hatcheries are affected by genetic degradation, with more than 4000 hatchery owners and nursery operators involved. Further training and establishment of an adapted legal framework is urgently required to improve genetic knowledge and increase awareness about the responsibility of hatchery operators. By improving and controlling the quality of broodstock and breeding activities in hatcheries, aquaculture productivity will be improved and the impacts on capture fisheries biodiversity reduced.

Under the Fourth Fisheries Project, the GEF financed ‘Aquatic Resources Development, Management and Conservation Studies’ will design and test appropriate methods for improvement and provide training. Based on these studies, the project will assist the DoF to design a strategy and Action Plan for ensuring the genetic integrity of hatchery stocks for aquaculture and culture-based open water stocking.

Study 1: Genetic Status of Endemic and Exotic Broodstock

1.1. Objectives

- Quantify the declining genetic pool of hatchery brood stock including inbreeding, negative selection, genetic drift, genetic introgression and decline of performance of improved strains.
- Identify likely genetic damage of the endemic population by mass stocking of floodplains by fingerlings coming from domesticated stocks.
- Assess the extent of damage caused by introgressed silver carp on national fish production.
- Assess findings to prepare a proposal for a framework to control hatchery brood stock management, breeding activities, nursery management, and quality control of hatchery products for pond culture, as well as for floodplain stocking.
- Report on genetic status of domesticated and wild stock and propose a framework for their control.

1.2. Declining Gene Pool of Cultured Aquatic Organisms

In Bangladesh there has been a long-term decline in genetic diversity through a reduction in habitat availability, but is more recently threatened by the rise in aquaculture production. In Bangladesh the large-scale breeding operations started by rapid expansion of hatcheries from 1980. The number of fish hatcheries in 2000 has reached 744, consisting of 113 public and 631 privately owned units. Their production of 4 to 5 days old fry was 184,343 kg (Fisheries Recourses Information of Bangladesh, 1999-2000), while the quantity of fry collected from natural sources was significantly reduced from about 20,000 kg in 1980 to 2,683 kg in 2000.

The significant increase in hatchery production has improved the availability of fry for culture. This has contributed to protection of wild stocks, as the price of hatchery-produced fry has dropped, while the availability of fry in the wild is reduced. Moreover, there is a serious concern about the genetic erosion of hatchery brood stock. Not less than 9 – 10 generations of Indian Major and Chinese carps and as many as 18 – 20 generations of common carps have been used for induced breeding from the private hatcheries. Proper selection has not been maintained, resulting in the use of undesirable small size breeders. Closely related stocks have been repeatedly used over many generations, and hazardous hybridization has been conducted in many hatcheries (Hussain and Mazid, 2001).

The danger of genetic degradation is extended to the wild stocks too. Although natural hybridisation accidentally occurs among Indian carps (Desai *et al*. 1970, Khan *et al*. 1989), these are usually eliminated through natural selection. On the other hand, a strong negative effect can be expected on the wild stocks by mass introduction of domesticated, genetically degraded stocks, as fingerling releases in floodplains, and large scale escapes of cultured stocks due to increased flooding.

1.3. Main cultured carp species in Bangladesh:

Catla ***(Catla catla):*** Catla is the fastest growth species among Indian Major Carps. It is an inhabitant of the rivers of Bangladesh, India, Pakistan and Myanmar (Jhingran and Pullin, 1985).

Rohu (*Labeo rohita):* Rohu is not as fast growing as catla, attaining only 500 g after one year under natural riverine conditions. “Red rohu” has been produced in some hatcheries in Jessore, by fertilizing rohu eggs with red common carp male. The phenotype of the hybrid is rohu, except the colour is red. [Shardar, A.S. personal communication 1999). A fertile hybrid of rohu-Calabash was also produced, as well as their F1 and F2 hybrids (Krishnaja and Rege, 1979).

Mrigal (*Cirrhinus cirrhosus):* The mrigal is a slow growing species, reaching approximately 500 g in the first year under natural conditions It is possible to produce a fertile hybrid of mrigal and rohu (Naseem, 1971).

Silver Carp (*Hypophthalmichthys molitrix):* First introduced in 1969, with initial great success in semi-intensive composite culture, as its main food is phytoplankton. Countrywide, the stocks are affected by bighead carp introgression.

Bighead Carp ***(Aristichthys nobilis):*** Bighead carp were first imported from Nepal in 1981. Affected by genetic introgression from silver carp. Strongly compete with the native Catla.

Grass Carp ***(Ctenopharyngodon idella):*** First imported from Hong Kong in 1966 (Hussain and Mazid 2001), [DP1], and from ………..Stocks are affected by negative selection and inbreeding.

Black Carp (*Mylopharyngodon piceus):* Introduced in 1983 from China. It is a mollusc eater, performs well in ox-bow lakes. Due to the fact that a limited number of females have been reproduced successfully in Bangladesh, the present stock of black carp is probably genetically closely related, representing a risk of inbreeding.

Silver barb *(Puntius gonionotus*): Silver barb was introduced from Thailand in 1977 (Hussain and Mazid, 2001).

Common Carp (*Cyprinus carpio*): Many common carp strains have been introduced to Bangladesh:

- The Chinese Big Belly**:** during its thousands years of culture, this variety has adapted to harsh culture conditions in still water, rather than being fit for living in rivers. This variety was introduced in around 1960 from China (Hussain *et al.* 2001). Other, unspecified scale carp varieties were introduced from India by farmers of Comilla and Jessore, in the early 1960s. A variety of scale carp, different from the Big Belly was found by the author in 1980 at Jessore farms.
- A genetically improved scale carp variety was imported from Vietnam in 1995 by BFRI Mymensingh through ICLARM (Hossain *et al.*, 2001). The origin of this variety is the geographically distinct Hungarian Scale carp strain of "Tata" (A. Woynarovich, personal communication).
- The date of introduction of Japanese red coloured carp *(Hi-Goi)* is not known, but the author observed this strain in Jessore private farms in 1980. This fish is red coloured, with some grey-black colour spread on dorsal scales just behind the head.
- Mirror carp *(Cyprinus carpio var. specularis)* was first introduced from Nepal in 1979 for the World Bank financed Raipur Fish Hatchery (Rahman, 1989). The origin of this mirror carp is Dinnyés, Hungary, from where the author sent fingerlings to an FAO project in Nepal, in 1973. In 1982 a high performance variety of mirror carp was imported for the World Bank's Oxbow Lakes-1 Project in Kotchanpur. This mirror carp was also developed in Dinnyés, by using German, Hungarian, Yugoslavian (Nasic) and Israeli strains. From this import only a few individuals were reproduced and spread to private hatcheries, which quickly resulted in inbreeding depression. In 1997, from the same origin, 700 mirror carp were reintroduced and are presently available in Parbatipur NFEP and in Natore FSMF. The mirror carp and the inbred local scale carp strain were maintained separately at Kotchanpur Central Hatchery Complex, to produce a heterosis hybrid, between 1983 and 1985. The hybrid had full scale covering, as it is a dominant characteristic. Its survival rate and growth rate was superior of that of the Mirror carp in the Oxbow lakes (baors).
- The Japanese Fancy carp *(Koi)* was introduced probably by the aquarium trade. This multiple coloured strain has been developed in Japan from the red coloured strain called *Hi goi*

Due to the fact that the Bangladeshi common carp stock is a result of recent mixing of many varieties by unskilled hatchery operators, it has a maximum genetic diversity in comparison with pure strains. For this reason the question of inbreeding does not arise in most of the cases, but stocks are affected by negative selection.

1.4. Issues

Genetic erosion of domesticated stocks: without appropriate broodfish management, the long-term decline of genetic quality was almost inevitable, allowing a high number of repetitions of management mistakes for each generation. In an unidentified number of hatcheries, improper management resulted in the decline of the genetic pools of reproduced populations of both endemic and exotic species as follows: (i) proper selection of breeders has not been maintained; (ii) closely related and small stocks have been repeatedly used generations after generations resulting in inbreeding, genetic drift and reduced resistance to diseases; (iii) negative selection was made for smaller sizes at sexual maturation; (iv) failure to follow selection criteria for improved varieties of Mirror carp, GIFT tilapia resulted in loss of improved performance, (v) hazardous hybridisations were made resulting in genetic introgression of several species.

Hatcheries without broodstock ponds: a recent practice is for hatcheries not to keep broodstock, but to procure early matured, undersized "breeders" from fishermen on the day of reproduction. The spent breeders are sold dead immediately after operation.

Lack of knowledge of hatchery and nursery operators: from the beginning of the introduction of the hatchery system, little training has been given to hatchery and nursery operators on genetic issues of fish breeding, resulting in lack of knowledge and genetic degradation of farmed stocks.

Lack of knowledge on effects of openwater stocking on wild stock: during the last decade mass release of hatchery produced endemic fish fingerlings has been conducted. The mass release of domesticated stocks (particularly of genetically degraded stocks) reduces vitality and may irreversibly alter the gene pool of wild stocks. This activity is continuing on an increasing level, not only by the DoF but also by NGOs and the public. It is important for the future to know the effect of stocking on wild populations.

Lack of knowledge on genetic status of hatchery stocks: there is an immediate need for establishment a framework, to control the genetic fitness of hatchery stocks (license, fitness, pedigree) in order to prevent further damage to farmed and wild stocks.

Genetic Introgression of silver carp: silver carp are the cheapest of the carps to produce, as it mainly feeds from a primary production level, saving the energy which is lost during transformation of food to secondary and tertiary levels for feeding other fish species. As a result it has become one of the dominant species in reservoir and semi-intensive fish culture systems in Europe and Asia. The species has been hybridised with bighead carp by many hatchery operators. This introgression of silver carp species has probably contaminated the whole silver carp stock of Bangladesh, as the hybrid is not sterile. A similar situation exists in the Mekong delta (personal observation). This so called “silver carp” has an intermediate number of gill rakers and an intermediate length of digestive tube. As a result it can not feed from phytoplankton of smaller size range as pure strain does, feeding more on zooplankton. Thus results in stronger competition to catla, in comparison with the pure silver carp. The loss in potential productivity probably equates to a substantial economic loss.

1.5. Outputs

The study will provide the following outputs:

- Monitoring model for hatcheries and nurseries.
- Model for quality control of hatcheries, broodstock, progenies and nurseries established.
- Genetic status of wild populations of Indian Major Carps (IMCs) identified.
- Geographically and genetically distinct strains of IMCs identified.
- Better understanding on quantitative contribution of pure silver carp to Bangladeshi carp polyculture system.

1.6 Methodology

General Approach

Firstly identification of the extent of genetic damage will be assessed through collection of information by enumerators. Information on degraded stocks will be confirmed by genetic analysis in BFRI/Stirling or other laboratories and field trials. Monitoring of hatchery and nursery farms will be made during the project period, in order to have data for preparation of standards on hatchery and nursery management techniques. This will help to formulate a draft Framework to control future activities of hatcheries and nurseries. Research will be carried out to investigate the genetic introgression of silver carp. A workshop will be organized to discuss the draft Framework, followed by preparation of final report on proposed framework which will be used for creation of the Action Plan.

Tasks and Activities

Task 1: Establish present genetic status and culture practices of domesticated and wild stocks

Select research institute(s) for collaboration to carry out trial rearing of domesticated and geographically distinct wild stocks to identify i) existing distinct strains, (ii) likely negative effect of stockings, genetic analysis of domesticated and wild stocks, collection of spawn from hatcheries, stocking of spawn in trial ponds for test rearing up to adult stage (tagging), to compare growth rate and other parameters of different hatchery stocks.

Conduct baseline survey.

- List hatcheries and nurseries in the country:
- Genetic status of hatchery stocks
- Status of hatchery and nursery techniques
- Genetic status of wild stocks

Genetic analysis of wild stocks. Genetic analysis will be made to identify genetic degradation of wild stocks due to mass fingerling release of domesticated stocks.

- Collection of spawn from Padma, Jamuna and Halda rivers.
- Stocking of spawn in trial ponds for test rearing up to adult stage (tagging).
- Compare growth rate and other parameters of different stocks.
- Compare gene pool of indigenous species, from different geographic origin to find likely existence of distinct geographic strains
- Compare genetic pool of different phenotypic species of different geographic regions, to control likely negative effect of floodplain stocking on wild populations.
- Compile results for guidelines for floodplain stockings and for controlling framework preparation.
- Results will be used for guidelines for improvement of breeders and for controlling framework preparation.

Monitoring of hatcheries and nurseries. Conduct survey and checking breeder condition, purity, age and size, density, feeding rates and food ingredients, hatchery design, water supply system, water quality, incubators type, density in incubators, system to eliminate deformed larvae, use of harmful chemicals, first feeding of spawn, packing/transportation techniques of spawn. Visiting private nursery operators to follow up the performance of produced spawn, their nursing techniques, feeding, harvesting, conditioning and transportation.

Report. Assessment of monitoring, which will help to design framework to control hatchery and nursery operations.

Task 2: Framework to control Hatcheries and Nurseries

Establish standards. Assess hatchery and nursery monitoring data to establish standards for controlling framework proposal preparation.

Design framework proposal including:

- Pond criteria, management.
- Breeder selection criteria, management, distinct stocks.
- Maintenance and control of genetic diversity of breeders.
- Hatchery design criteria.
- Hatchery techniques criteria species wise.
- Fingerling rearing criteria for aquaculture and for floodplain stocking.
- Certificate of fingerling origin.
- Hatchery license species wise.

Workshop. Workshop to discuss proposed framework and strategy

Action Plan Development. Report on proposed framework as basis for the Action Plan

Task 3: Genetic degradation of silver carp.

Develop 'brood bank'. Collection of pure species of Silver carp and Bighead carp. Research on morphological and nutritional characteristics in collaboration with Stirling University. Find differences of genetic markers to identify pure species from their hybrids.

Evaluation. Evaluate silver carp versus bighead carp, with special attention to their contribution to national fish production and food competition in carp polyculture system. The report will be used to prepare the Action Plan.

Study 2: Improvement of Broodstock Management Techniques

2.1. Objectives

Overall Objective

The overall objective of this study is contribution to establishment of improved fish breeding system by training and by providing guidelines for hatchery broodstock management, for the production of high quality brood stock in DoF and private hatcheries.

Specific Objectives:

The specific objectives of this study are:

- Designs and models for broodstock improvement of DoF and private hatcheries.
- Initiate testing of broodstock improvement models in selected farms.
- Training of nearly 100 trainers of DoF trained for genetic fitness maintenance of broodstock, hatchery and nursery management.
- Training of 631 hatchery owners and 3441 nursery operators by the trained DoF officers.

2.2. Outputs

This study will provide the following outputs:

1. Report on proposed framework for improved fish breeding system.
2. Testing of improvement models are initiated in selected hatcheries.
3. Selected hatcheries will demonstrate gene pool improvement and maintenance techniques.
4. 4000 hatchery and nursery operators trained in genetic diversity maintenance of cultured species.

2.3. Methodology

General Approach

The project will closely cooperate with the BFRI, Brood Bank project of the DoF and the development of 20 FSMFs under the FFP, as well as in Raipur Fish Hatchery and in private hatcheries in establishing improved quality broodstocks. Two types of broodstock will be initiated, one is a controlled domesticated stock of endemic and exotic species for aquaculture in closed waters, the other is wild broodstock of endemic species to produce fingerlings for flood plain stockings. The existence of geographically and genetically distinct wild populations of endemic species will be investigated and (if they exist) creation of broodstocks for each distinct strain will be proposed in order to provide appropriate quality of fingerlings for geographically distinct floodplain stockings. Selected hatcheries will be also used as models to test and demonstrate gene pool improvement and maintenance techniques. Based on findings of genetic analysis under the baseline survey, mating designs will be prepared for the selected hatcheries.

Tasks and Activities

Task 1: Select Model hatcheries

Select DoF and private hatcheries and train collaborators

Task 2: Mating designs

Line crossbreeding design, exchange of breeders, collecting milt from wild to increase gene pool of hatchery stocks and replacement of broodstock.

Task 3: Improvement of hatchery management techniques

Assess results from surveys, training of 631 hatchery operators and 3441 nursery operators

Task 4: Experimental production of monosex female common carp

For stocking common carp in floodplains, it may be possible to produce monosex female fingerlings. Female common carp grows approximately 15-30% faster than males do; in addition there is a reduced risk of its reproduction and establishment in the wild. Neomale fingerlings will be produced in collaboration with BFRI and distributed to selected FSMF's for all female fingerling production.

Task 5: Action Plan.

Prepare Action Plan on maintenance of genetic diversity in aquaculture and in Gangetic floodplain-riverine ecosystem.

References

Bardach, J.B., Ryther, J.H. and McLarney, W.O. (eds). 1972. Aquaculture: the farming and husbandry of freshwater and marine organisms. Wiley-Interscience, New York.

Desai, V. R. and Rao, K. J. 1970. On the occurrence of natural hybrid catla-rohu in Madhya Pradesh. J. Zol. Soc. India 22(1-2):35-40 .

Fisheries Resources Information of Bangladesh 1999-2000. Fisheries Resources Survey System, Department of Fisheries, Dhaka, Bangladesh.

Hussain, M.G. and Mazid, M.A. 2001. Genetic improvement and conservation of carp species in Bangladesh. Bangladesh Fisheries Research Institute and International Center for Living Aquatic Resources Management. 74 p.

Jingran, V.J. and Pullin, R.S.V. 1985. A Hatchery Manual for the Common, Chinese and Indian Major Carps. ICLARM Studies and Reviews 11. ADB and ICLARM, Manila, Philippines. 191 p.

Khan, H. A. and Kowtal, G. V. 1989. An account of naturally occurring and artificially produce cyprinid hybrids in India. In: Fish Genetics in India. P. Das and A. G. Jingran (eds.) Today & Tomorrow's Printers & Publishers, pp 155-162.

Krishnaja, A. P. and Rege, M. S. 1979. Genetic studies on two species of Indian carp, Rohu *(Labeo rohita)* and *Labeo calbashu*, and their fertile F_1 and F_2 hybrids. Indian J. Zool 17:3.

Naseem, H.K. 1971. Preliminary account of an intergenetic fertile hybrid between *Cirrhinus mrigala* and *Labeo rohita*. Annual Day Souvenir, C.I.F.E., Bombay. pp. 10-15.

Rahman, A.K. 1989. Freshwater fishes of Bangladesh. Zool. Soc. Bangladesh, Dhaka. 352 pp.

Suzuki, R. and Yamaguchi, M. 1980. Meristic and morphometric characters of five races of *Cyprinus carpio*. Jap. J. Ichthyol. 27:199-206.

Page: 98
[DP1]According to Hussain and Mazid (2001), grass carp were first introduced in 1966 from Hong Kong.

Development of DNA Microsatellite Loci in Chinese Carps and Application to Detection of Hybridization in Broodstock

M.Y. Mia[1], J.B. Taggart[2], A.E. Gilmour[2], T.K. Das[3], M.A. Sattar[3], M.G. Hussain[1], M.A. Mazid[1], B.J. McAndrew[2] and D.J. Penman[2]

1 Bangladesh Fisheries Research Institute (BFRI), Mymensingh 2201, Bangladesh
2 Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland
3 Northwest Fisheries Extension Project (NFEP), Parbatipur, Dinajpur, Bangladesh

Mia, M.Y., Taggart, J.B., Gilmour, A.E., Das, T.K., Sattar, M.A., Hussain, M.G., Mazid, M.A., McAndrew, B.J. and Penman, D.J. 2002. Development of DNA microsatellite loci in Chinese carps and application to detection of hybridization in broodstock. pp. 51-57. In: Penman, D.J., Hussain, M.G., McAndrew, B.J. and Mazid, M.A. (eds.). Proceedings of a workshop on Genetic Management and Improvement Strategies for Exotic Carps in Asia, 12-14 February 2002, Dhaka, Bangladesh. Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh. xxx p.

Abstract

Microsatellite DNA loci have been developed from silver carp (*Hypophthalmichthys molitrix*) DNA for use in broodstock management and research. Here we describe how three of these loci (*Hmo*1, *Hmo*3 and *Hmo*11) can be used to distinguish between silver carp and bighead carp (*Aristichthys nobilis*). These loci are being used in the analysis of samples collected from hatcheries in different regions of Bangladesh, in parallel with a survey of broodstock management practices. Analysis of samples from two of the hatchery areas revealed a fairly low proportion of hybrid genotypes among broodstock: this included fish which were identified as silver and bighead carp by the hatcheries. The genetic analysis suggested that while some of these fish might be F1 hybrids, others had more complex genotypes suggesting further generations of hybridization or introgression between the species. The study

is ongoing and will attempt to identify factors associated with hybridization in hatchery broodstock. The methodology could also be applied to detect hybrids in nursery or ongrowing stocks.

Introduction

Aquaculture now accounts for nearly 40% of total fish production in Bangladesh (Hussain and Mazid, 2001), with carps, produced in polyculture systems, dominating. The vast majority of the seed for aquaculture in Bangladesh is produced in private hatcheries, of which it is estimated that there are in excess of 600. There is much anecdotal evidence of genetic deterioration of the stocks of carps in Bangladesh, through inbreeding, negative selection and hybridization (Hussain and Mazid, 2001). Stocks of exotic (i.e. non-indigenous) species of carps are particularly vulnerable to such degradation, given that the opportunities to go back to wild populations for broodstock replenishment are very limited. Furthermore, anecdotal evidence suggests that hybridization between the silver carp and bighead carp is common, at least partly due to a shortage of mature bighead carp males towards the end of the breeding season. The silver carp accounts for 25% (126000 t) of reported major carp production in Bangladesh, while there was no reported bighead carp production in the same set of statistics (FAO, 2002), suggesting that the actual levels are low.

Several types of genetic markers have been developed which have potential application to fisheries and aquaculture. Used appropriately, these have the potential to differentiate between species, populations and individuals. Microsatellite DNA loci (Estoup and Angers, 1998) have a core of short, repeated units (generally 2-5 base pairs), flanked by unique sequence DNA. Primers for polymerase chain amplification (PCR) can be designed from the flanking DNA, ensuring specific amplification of single loci, with variation between alleles coming largely from variation in the number of repeat units in the central core region. Some microsatellite loci have very high numbers of alleles per locus (>20), making them very useful for applications such as parent-offspring identification in mixed populations, while others have lower numbers of alleles and may be more suited for population genetics and phylogeny (O'Connell and Wright, 1997; Estoup and Angers, 1998). Primers developed for one species will often cross-amplify microsatellite loci in closely related species (Estoup and Angers, 1998).

As part of the project which is the focus of this workshop, microsatellite loci were developed from a DNA library produced from the silver carp (unpublished data). Preliminary screening showed that some of these loci could be used as species-specific markers to distinguish between silver and bighead carp. Although allozymes can also be used to distinguish between these species, the simplicity of sample collection (biopsy – e.g. fin tissue or scales), preparation and transportation (fixation in 95% ethanol and transportation at room temperature) for PCR-based DNA techniques make sampling under field conditions much easier.

A survey was designed in which fin samples and information about hatchery management techniques were collected from several hatcheries in each of five major hatchery areas in Bangladesh. This paper reports initial analysis of samples from two of these regions.

Materials and methods

The microsatellite loci were developed using a slight modification of the library enrichment methodology of Kijas *et al.* (1994), from size-fractionated silver carp DNA (unpublished results). The names assigned to the microsatellite loci were derived from the Latin name of the species (*Hypophthalmichthys molitrix*) and a serial number. Four loci were initially identified as being potentially diagnostic for the silver and bighead carp, but one of these (*Hmo*5) was discarded as it gave an inconsistent multiple banding pattern in the bighead carp. The remaining three loci (*Hmo*1, *Hmo*3 and *Hmo*11) were used for this study.

Fin samples were collected from Chinese carps from the reference populations at NFEP, Parbatipur, from six private hatcheries in the Mymensingh region and five private hatcheries in the Jessore region. Each sample was removed from the edge of a fin using scissors, dried and placed into a microcentrifuge tube containg approximately one millilitre of 95% ethanol. The ethanol solution was discarded and replaced later the same day. The samples were then stored at ambient temperature during transportation and at 4-6^{o}C in the laboratory until DNA extraction and analysis.

DNA was extracted using protocols based on either phenol-chloroform or chelex. The three loci were amplified by the polymerase chain reaction (PCR) in separate reactions, and then run in 1.2% standard agarose gels for routine analysis, or 2% Metaphor agarose (Flowgen) to allow higher resolution and closer examination of the allele sizes.

Results

Table 1 summarises the samples analysed from the reference population and the two hatchery regions, with the data from the hatcheries within each region pooled. This shows that five fish with hybrid genotypes were found in the Mymensingh region, and none in the Jessore region. Two of these had been identified as silver carp at the time of sampling on morphological, while three were identified as bighead carp. Table 1 shows the genotypes of these five fish for each of the three microsatellite loci. Figures 1 and 2 show the appearance of some of these genotypes on agarose gels (in high resolution Metaphor and standard agarose respectively).

Table 1. Genotypes of broodstock from reference and hatchery stocks of silver and bighead carp in Bangladesh. The stocks at NFEP Parbatipur were imported from China in 1994 and served as reference samples for this study; the others were collected from hatcheries in the Mymensingh and Jessore areas (data pooled among hatcheries within each region). The identification of the hatchery stocks to species at the time of sampling was made on morphological grounds and for the purposes of this study was considered to be preliminary until the genotype was confirmed.

Region	**Species**	**Genotype**			**Total**
		Silver	**Hybrid**	**Bighead**	
Parbatipur (wild stocks)	Silver	30	0	0	30
	Bighead	0	0	30	30
Mymensingh	"Silver"	74	2 (2.6%)	0	76
	"Bighead"	0	3 (17.6%)	14	17
Jessore	"Silver"	122	0	0	122
	"Bighead"	0	0	12	12

Table 2. Genotypes of the five individuals considered to be hybrids on the basis of analysis using three microsatellite loci *Hmo*1, *Hmo*3 and *Hmo*11. S/S = homozygote for the allele found in the silver carp reference population; B/B = homozygous for the allele found in the bighead carp reference population; S/B = heterozygous, having one copy of each of these two alleles.

Hybrid		**Microsatellite locus genotype**		
		***Hmo*1**	***Hmo*3**	***Hmo*11**
"Silver"	1	S/B	S/S	S/B
	2	S/B	S/B	S/B
"Bighead"	1	S/B	S/B	S/B
	2	S/B	S/B	S/B
	3	S/B	B/B	B/B

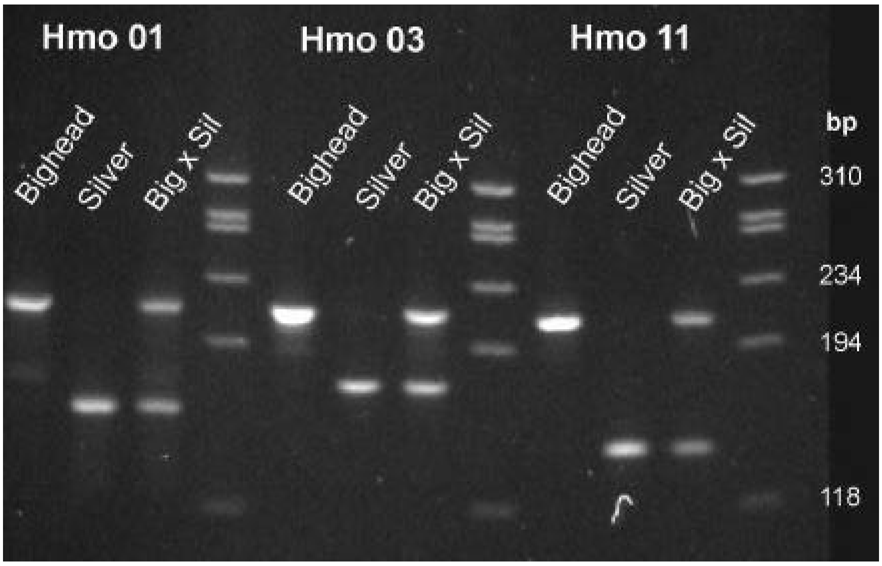


Figure 1. Electrophoresis of PCR-amplified microsatellite loci Hmo1, Hmo3 and Hmo11, showing an example of pure silver carp, pure bighead carp and hybrid (Big x Sil) genotypes for each locus. The right hand lane of each set contains molecular weight standards, with the sizes in base pairs (bp) given at the extreme right of the figure.

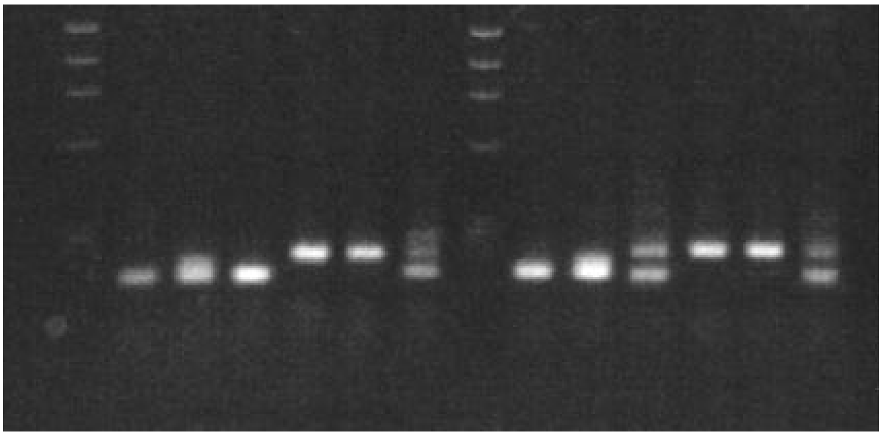


Figure 2. Electrophoresis of PCR-amplified microsatellite loci Hmo3 (left side of gel) and Hmo1 (right side of gel). For each locus, the order of the lanes is molecular weight standards, two reference silver carp samples, "silver" carp hybrid #1 (see Table 2), two reference bighead carp samples and "bighead" carp hybrid #1 (see Table 2).

Discussion

The methodology described in this paper appears to offer a rapid and simple procedure for the detection of hybridization and introgression between silver and bighead carps. Sample collection is quick and easy, and does not require that broodstock are killed. This would also be true for fish of only a few grams in weight, as the reliance on PCR allows even a tiny piece of fin to be used as the source of DNA. Sample storage is also easy, which is important during field work with limited facilities. The use of the chelex-based extraction method reduces the time taken for DNA extraction, and the analysis could be made even more efficient (although more expensive) if all three loci were amplified and analysed together in a "multiplex", where one of each pair of primers for each locus is labelled with a fluorescent dye, allowing separate detection of the alleles of each locus in a single sample in an automatic sequencer (e.g. Fishback *et al*., 1999).

Two of the five hybrid fish detected during this preliminary analysis can be concluded not to be F1 hybrids ("silver" #1 and "bighead" #3 in Table 2), since they displayed a mixture of both heterozygous and homozygous genotypes for the different loci. This would only be expected from backcrosses to one of the parent species, F2 hybrids or further generations of hybridization or introgression. The other three hybrids may have been F1s (heterozygous at all three loci) but this cannot be stated with any degree of certainty from analysis of only three loci. More detailed analysis would require larger numbers of loci.

The proportion of hybrids detected in this preliminary analysis was low (2.6% in silver carp and 17.6% in bighead carp in one of the two hatchery regions studied). It is intended to extend this survey to cover five major hatchery regions of Bangladesh and to attempt to correlate the presence of hybrids with factors such as geographical region, broodstock replacement strategy, presence of one or both parental species in the hatchery, or experience of hatchery manager. We also intend to survey nurseries for the presence of hybrid fingerlings. Anecdotal evidence suggests that F1 hybrids are produced towards the end of the bighead carp spawning season, when mature males are no longer available but mature silver carp males are.

Acknowledgements

This research was supported by the DFID Aquaculture and Fish Genetics Research Programme (project R7590). The authors would also like to thank the hatchery owners and staff who provided samples, information about hatchery management and hospitality during the hatchery survey.

References

Estoup, A. and Angers, B. 1998. Microsatellites and minisatellites for molecular ecology: theoretical and empirical considerations. pp. 55-86. In: Carvalho, G. (ed). Advances in Molecular Ecology. IOS Press.

FAO Fisheries Department, Fishery Information, Data and Statistics Unit. 2002. Fishstat Plus: Universal software for fishery statistical time series. Version 2.3. 2000.

Fishback, A.G., Danzmann, R.G., Sakamoto, T. and Ferguson, M.M. 1999. Optimization of semi-automated microsatellite multiplex polymerase chain reaction systems for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 172:247-254.

Hussain, M.G. and Mazid, M.A. 2001. Genetic Improvement and Conservation of Carp Species in Bangladesh. BFRI, Mymensingh, Bangladesh and ICLARM, Penang, Malaysia. 74p.

Kijas, J.M.H., Fowler, J.C.S., Garbett, C.A. and Thomas, M.R. 1994 Enrichment of microsatellites from the citrus genome using biotinylated oligonucleotide sequences bound to streptavidin-coated magnetic particles. *BioTechniques* 16: 657-662.

O'Connell, M. and Wright, J.M. 1997. Microsatellite DNA in fishes. Reviews in Fish Biology and Fisheries 7:331-363.

Taggart, J., Hynes, R.A., Prodöhl, P.A. and Ferguson, A. 1992. A simplified method for routine total DNA extraction from salmonid fishes. Journal of Fish Biology 40:963-965.

State of the System Report: Fish Seed Quality in Northwest Bangladesh

B.K. Barman[1], D.C. Little[2] and A. Nietes-Sataponvanit[3]

1 Post doctorate Research Fellow, ICLARM, House # 22B, Road # 7, Block - F, Banani, Dhaka-1213, Bangladesh.
2 Institute of Aquaculture, University of Stirling, FK9 4LA, Scotland, UK.
3 Integrated Coastal Zone Management Program, Asian Institute of Technology, Klong Luang, Pathum Thani -12120, Thailand.

Barman, B.K., Little, D.C. and Nietes-Sataponvanit. 2002. State of the System Report: fish seed quality in Northwest Bangladesh. pp. 69-75. In: Penman, D.J., Hussain, M.G., McAndrew, B.J. and Mazid, M.A. (eds.). Proceedings of a workshop on Genetic Management and Improvement Strategies for Exotic Carps in Asia, 12-14 February 2002, Dhaka, Bangladesh. Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh. xxx p.

Abstract
The production of freshwater fish based on stocking hatchery-produced seed has become increasingly important in Bangladesh, like many other countries in Asia, in the recent decades. Hatcheries, especially those producing carp seed, are well established in Bangladesh where both Government and private entrepreneurs are involved in the production and supply of fish seed. Increasingly networks of private producers and traders dominate the supply of seed to farmers and are important promoters of fish production. Although fish seed is abundant and cheap, a common emerging concern is that of poor quality. Fish seed that survive or grow poorly undermine both attempts to promote farming fish among new adopters and the consistency required by commercial farmers to produce low-cost fish for poorer consumers. A major issue is whether the poor performance of stocked fish is due to sub-optimal seed quality or simply inadequate management by the farmer after stocking. Further, if fish seed quality is to blame, is the major cause of poor quality genetic or management related? As fish seed stocked by farmers is frequently handled by many "actors" poor management may occur at many stages. It is important to understand the different aspects in fish seed quality, and its impact on fish production, if resources are to be best targeted and policy decisions on future

investment and management options improved. Conventional data collection, analysis and dissemination typically produce information that is too little and too late to inform such decisions. Participative methods involving collection and synthesis of information on current practice and opinion from a broad range of stakeholders are the basis of a concept of State of the System (SoS) reporting. Intensive fieldwork followed by initial analysis and presentation of results to stakeholders, is followed by revision and production of a concise, readable report (SoS report) in Bangla and English. The SoS report as described in this paper was based on a survey conducted in 8 districts of the Northwest region in 1998 and 1999. Additional data were also collected from nurseries and hatcheries in Adamdighi area of Bogra district, as they are also the major supplier of fish seed in the Northwest. Representative grow-out farmers (150), fry traders (122), nursery operators (37) and hatcheries (17) were interviewed using a short semi-structured questionnaire. In an SoS workshop held at CARITAS Center, Dinajpur in February 1999, the information was presented, discussed and reviewed with the stakeholders (fish farmers, fry traders, nursery operators, policy maker and extension personnel). Besides specific outcomes, research and implementation agendas were identified by the stakeholders which are incorporated in the SoS report. As a participatory method such an exercise may be an example to understand the existing situation and may be of help to develop future plans for overall development of quality fish seed production and distribution in the country.

Introduction

Successful fish culture depends on the availability and quality of fish seed to farmers. Most aquaculture in Bangladesh has been based on carps to date, supplied mainly by the private sector through fry trading networks of private producers and traders, delivering fish seed to even remote parts of the country. At present fish seed are abundant and cheaper than before but the issue of quality of fish seed has become an important issue. Poor quality seed results in low survival and slow growth of fish, thus creating negative impacts for farmers practising fish culture and discouraging new entrants. It is still not always clear whether poor performance of fish seed is due to the fish seed quality itself or results from inadequate management by grow-out farmers. Furthermore, if fish seed quality is a problem, is it due to poor genetic quality or related to management? Many actors handle fish seed from its source of production to delivery; poor management may occur at any stage. Understanding the stakeholders, their current practices, the constraints they face, and the experience gained as entrepreneurs in fish seed production, distribution and use is important. Identification of researchable constraints on fish seed quality, the genetic and non-genetic management factors and implications of strategies through policy implications will be useful for further development of aquaculture in Bangladesh.

This paper tries to explain briefly the methodology used for the study on ‘fish seed quality in northwest Bangladesh’ and the main outcomes of the study, with emphasis on the recommendations suggested for research and policy implications for improvement of quality seed production, supply and use in Bangladesh.

Research Methodology

The SoS methodology comprises several steps, such as identification of stakeholders, collection of primary information from stakeholders, analysis and synthesis of information and presentation of outcomes to the stakeholders in a participatory workshop for more discussion, followed by preparation and dissemination of reports in Bangla and English for wider use. Unlike more commonly used methods of data collection and analyses by survey using structured questionnaires alone, the SoS method provided more opportunities for participation of stakeholders and triangulation of the results within a short time frame.

Based on secondary information and working experience in the northwest, stakeholders related to fish seed production, distribution and use were selected. They included hatchery operators, nursery operators, fry traders and grow-out farmers. The government officials and NGO staff working on fish culture extension and development were also identified as stakeholders in the study. Information was collected from 150 grow-out farmers, 122 fry traders, 37 nursery operators and 17 hatchery operators for the study. Most of these stakeholders were located within the Northwest region but some hatchery operators were from Adamdighi, Bogra. This area produces a large proportion of the hatchlings used in the Northwest. Semi-structured questionnaires were used to collect the information. The information collected was analyzed, synthesized and presented in a two day workshop held at the CARITAS regional center in Dinajpur district town. The NFEP-2 extension personnel carried out all the activities for the studies with assistance from the local Department of Fisheries field level officials as part of their project activities. NFEP-2 was the local collaborator for the regional project on ‘Fish Seed Quality in Asia’ (FSQA) The different stakeholder groups assembled informally in different parts of the Caritas Center and information obtained from each of the stakeholder groups was presented to them in turn for comment and discussion. This allowed the different groups to assess the information independently and without the social pressures that typically occur in larger mixed groups. The method of presentation was tailored to each group, depending on literacy levels and delivery was pitched to ensure good communication and to stimulate discussion. This led in one instance to the research framework being critiqued and resulted in additional information being collected in the field after the workshop before its incorporation into the report. A further activity was the identification and prioritization of an agenda for both issues by the different groups of stakeholders. This was in two parts - issues that could be actioned immediately, i.e. did not require further research and those for which new knowledge was required i.e. a research agenda. Immediately after the workshop, project staff drafted a summary report in both English and Bangla and this was quickly disseminated to participants.

A more detailed report, again both in Bangla and English, entitled the 'State of the System on Fish Seed Quality in Northwest Bangladesh' has also been produced a draft of which was presented at the NFEP-2 end-of-project workshop in February 2001 in Dhaka, and the final report has now been completed and is being distributed to a wider audience.

Results and Discussion

In the northwest of Bangladesh, initially riverine hatchlings were the main source of seed. In the 1980s, hatchery hatchlings were produced and supplied from Jessore in SW Bangladesh. In recent times, besides hatchlings produced and supplied from government hatcheries (including NFEP) and a few private hatcheries within the region, a large amount of seed is supplied from Adamdighi, Bogra. The hatcheries surveyed in the northwest and in Adamdighi were classified into three categories based on the amount of hatchlings produced in a season: small (<500 kg), medium (500-750kg) and large (>750kg). Most hatcheries surveyed were small or medium in size, and all the operations in the Northwest were small.

The number of nurseries in the region has increased as demand for fingerlings increased. However, 70-100 million fingerlings are still imported from outside the region. Nursery operators normally nurse 8-9 species on average stocking 16-30g hatchlings per decimal in their ponds. Adamdighi nursery operators tend to fence their ponds with fine meshed nylon net to protect them from predators like frogs and snakes. Most nursery operators purchase hatchlings from private hatcheries but some operations both produce and nurse hatchlings. Nursery operators have observed that hatchlings produced in the middle of the season survived better in ponds and provided better outcomes compared to the early or late season hatchlings. The production period of nursery operators in the northwest region is short (April - August) because of limitations on the availability of water in ponds and the lower availability of quality hatchlings.

Most farmers in rural Bangladesh purchase their fish seed from itinerant traders carrying fry in open aluminium containers by foot, or increasingly by bicycle or rickshaw van. Traders are poor people but their numbers have increased substantially since hatchery seed has become available and aquaculture has expanded. The fry-trading network originated from Parbatipur railway station with the riverine hatchlings. The development of hatchery hatchlings and supply of fry/fingerlings from outside the region resulted in a longer trading season with increased involvement of middlemen and fry traders. Fry traders are the last link in the trading network, receiving the lowest benefits but bearing the greatest risks in their business. Traders reduce risk in several ways. For long distance transportation fry traders rest fry/fingerlings by keeping them in hapas in ponds or in nursery ponds before delivery to farmers. The fry traders also use different kinds of chemicals or additives to reduce mortalities.

The main season for fry trading extends from March to July. Early season trading based on over-wintered fingerlings appears to be increasing. On some occasions, fry traders suffer heavy mortality of their fry/fingerlings, and sometimes do not get the desired species most in demand from the nursery operators. Fry traders assess demand for fingerlings of different types from personal contacts within the daily market or during their visits for trading. Fry traders can transport fry/fingerlings for delivery to farmers even in remote areas, but typically risks are higher and benefits lower.

Almost 70% of farmers purchase fingerlings from fry traders at the pond side. The grow-out farmers in distant areas have limited choices to purchase fingerlings, as there are limited sources for them to purchase from. The stocking time of fingerlings for grow-out depends on the availability of water in their ponds. Of the species the farmers stock in their ponds for grow-out, Indian major carps and Chinese carps (especially silver carp) are most preferred.

A total of 17 policy recommendations and 15 research recommendations were developed by the stakeholders for implementation. Such policy recommendations may be of help to the policy makers to implement the activities with an objective to improve the production, supply and use of quality fish seed in the country. The research agenda, which was prioritised by a range of stakeholders should allow research organizations to ensure their research programme is needs driven [DP1]. For the stakeholders involved in these studies, this was an opportunity to share, to increase awareness on such emerging issues and may be of importance for development of their future activities. The policy and research recommendations are presented below.

Policy recommendations

1 Ban and monitor cross breeding and hybrid production.
2 Introduce cryopreservation techniques.
3 Develop and disseminate best practices for using anesthesia for transporting broodfish.
4 Improve information flow from government to farmers on broodstock quality and management.
5 Monitoring and certification of seed quality produced by private hatcheries by Government.
6 Dissemination of BFRI guidelines on size and age characteristics suitable for broodfish by species.
7 Set up a broodfish bank network – certain lead/regional centers to hold and keep broodfish, including introduction and importation of original strains from original sources.
8 Set up a dissemination programme on improved strains to private hatcheries.
9 Organise diploma-level training for seed producers.
10 Introduce training programmes for hatchery and nursery skill development.
11 Enforce controls on chemical uses including prohibition on the use of unsafe chemicals.
12 Develop and disseminate recommendations for nursing 5-day old hatchlings.
13 Develop and disseminate best practices for over-wintering different species.
14 Make facilities available to traders such as clean water during transportation.
15 Disseminate guidelines for using GIFT strains of Nile tilapia.

Research Recommendations

1 Factors caused growth deterioration of stocked fingerlings.

2 Information on good quality seed and its dissemination strategy.
3 Practical methods to monitor quality of fish seed before stocking.
4 Quality comparison of over-wintered and new season fingerlings.
5 Disease tolerance of different fish species stocked.
6 Use of additives/chemicals during transportation and their effects on quality and growth of fish.
7 Comparison of different transport containers based on cost and effect on seed quality.
8 Methods of testing of seed quality with traders during transportation.
9 Transport problems of more sensitive fish species.
10 Safer and cheaper alternatives for pesticides currently used in nursing.
11 Feasibility of nursing fry/fingerlings in polyculture.
12 Improved techniques for over-wintering carps.

13 Impact of multiple spawning on seed quality.
14 Possibility of broodfish exchange between hatcheries to improve seed quality.
15 Riverine and hatchery produced broodfish on their effects on seed quality and grow-out performance.

Conclusions/recommendations

The draft of the State of the System (SoS) report as distributed during the NFEP-2 end-of-project workshop has already elicited interest especially among researchers looking for suitable topics towards improvement of quality seed production, supply and use., some of which have received support from the SUFER (Support for University Fisheries Education and Research), [DP2] With wider dissemination in different research, academic, extension and development institutions, this report along with similar reports produced by the FSQA Project for other countries (Northeast Thailand, Northern Vietnam and Southern Vietnam) will enhance the lessons learnt across the Region and contribute to better policy and more needs-driven research.

References

AIT/DOF, 2001. Fish seed quality in Northwest Bangladesh. State of the System report. Aquaculture Outreach Programme, AIT, Bangkok. 37p.

AIT/RIA1, 2000. Fish seed quality in Northern Vietnam. State of the System report. Aquaculture Outreach Programme, AIT, Bangkok. 23p.

AIT/CAF, 2000. Fish seed quality in Southern Vietnam. State of the System report. Aquaculture Outreach Programme, AIT, Bangkok. 28p.

AIT/DOF 1, 2000. Fish seed quality in Northeast Thailand. State of the System report. Aquaculture Outreach Programme, AIT, Bangkok. 31p.

Page: 73
[DP1]Needs rewording
Page: 75
[DP2]Needs rewriting