SOUVENIR

WORKSHOP
ON
GENETIC IMPROVEMENT OF CARPS IN ASIA

JULY 26 - 29, 1997

ICLARM

BEST ICAR INSTITUTION
CIFA
≈1996≈

CENTRAL INSTITUTE OF FRESHWATER AQUACULTURE
(Indian Council of Agricultural Research)
Kausalyaganga, Bhubaneswar-751002, INDIA

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Credits

Dr. P.V.G.K. Reddy
Dr. Kuldeep Kumar
Ms. K. D. Mahapatra
Dr. A.D. Barat

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Phone : 501452, 515178

Dr. R.S. PARODA
SECRETARY
&
DIRECTOR-GENERAL

भाकृअप
ICAR

भारत सरकार
कृषि अनुसंधान और शिक्षा विभाग एवं
भारतीय कृषि अनुसंधान परिषद
कृषि मंत्रालय, कृषि भवन, नई दिल्ली ११० ००१

GOVERNMENT OF INDIA
DEPARTMENT OF AGRICULTURAL RESEARCH & EDUCATION
AND
INDIAN COUNCIL OF AGRICULTURAL RESEARCH
MINISTRY OF AGRICULTURE, KRISHI BHAVAN, NEW DELHI110 001
Tel. 3382629; Fax 91-11-3387293; E.Mail : rsp@icar.delhi.nic.in

MESSAGE

It gives me great pleasure to note that the International Centre for Living Aquatic Resource Management, Manila, Philippines is organizing an International Workshop on "Genetic Improvement of Carps in Asia" at the Central Institute of Freshwater Aquaculture, Bhubaneswar during July 26 - 29, 1997. Carps form the mainstay of not only Indian Freshwater Aquaculture but also that of Asian Aquaculture and it is a matter of great satisfaction that ICLARM is giving the importance that carp genetic improvement deserves for improving aquaculture productivity in the region.

Organization of the Workshop for planning the future strategies of carp genetic improvement at CIFA, Bhubaneswar is a recognition of the pioneering efforts made by the Institute in the aspect of selective breeding of one of the major Indian carps, rohu, in the recent years. Participation of Geneticists from China, Vietnam, Indonesia, Philippines, Bangladesh, Malaysia, Fiji, Thailand and India provides opportunities for international collaborative programmes in this direction. I am sure the proceedings of the Workshop would pave the way for new initiatives for cooperation among these countries in the region for carp genetic improvement and sustainable production in aquaculture.

I wish the International Workshop every success.

(R.S. PARODA)

DR. P.V. DEHADRAI
Deputy Director General (FY)

भारतीय कृषि अनुसंधान परिषद
कृषि मंत्रालय, डा. राजेन्द्र प्रसाद रोड
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भाकृअप
ICAR

INDIAN COUNCIL OF AGRICULTURAL RESEARCH
MINISTRY OF AGRICULTURE, DR. RAJENDRA PRASAD ROAD
KRISHI BHAVAN, NEW DELHI - 110 001
Phone : Off. 3382713, 3388991 Extn. 454, Res. 4672385
Fax : 0091-11-3387293
Telex : 031 - 62249 ICAR IN, E.Mail : PVD@icar.delhi.nic.in
Gram : AGRISEC

MESSAGE

It is highly satisfying that ICLARM has taken up the program on genetic selection of carps for the benefit of Asian region. ICLARM deserves appreciation and compliments for organising the International Workshop on the subject under the auspices of INGA at CIFA, Bhubaneswar. These activities of ICLARM would help the entire region in achieving a sizeable progress on genetic upgradation of the stock of the most popular carp species of Rohu and would set acceptable standards in collaborative programs for larger benefits.

It would be a pleasure to watch CIFA and ICLARM work together and all the more so when several countries of South East Asia region have joined the crusade for a common cause to upgrade the quality of life of those poor in the continent.

I wish all success to the International Workshop and best wishes to Rohu for its faster growth and disease resistance.

(P.V. DEHADRAI)

Dr. M.Y. Kamal
Assisstant. Director General
(Inland Fisheries)

INDIAN COUNCIL OF AGRICULTURAL RESEARCH
KRISHI BHAVAN, DR. RAJENDRA PRASAD ROAD
NEW DELHI - 110 001
Tel : 3384774, 3388991/205
Telex : 031 62249 ICAR IN
CABLE : AGRISEC
FAX : 91 11 3387293
E Mail : mykamal @ icar.nic.in.

MESSAGE

CIFA with a tradition of international linkages as a UNDP Centre and a lead centre of NACA is a natural choice by ICLARM, Manila (Philippines) for organising an International Workshop on "Genetic improvement of Carps in Asia". Having successfully completed the first phase of a three years project on selective breeding and genetic improvement of rohu in collaboration with NORAD, Govt. of Norway, is now embarking on the second phase of the project. Thus the participants to this Workshop will find CIFA an ideal location for deliberations on the subject.

I am sure interaction of International Scientists on genetic improvement of Carps will lead to greater improvement for development of aquaculture in the region.

I wish the Workshop a great success.

(M. YUSUF KAMAL)

Dr. S. Ayyappan
DIRECTOR

भाकृअप
ICAR

CIFA

Central Institute of Freshwater Aquaculture

(Indian Council of Agricultural Research)
Kausalyaganga, Bhubaneswar-751002, Orissa, India
Phone : 91-0674-430796 (R), 0674-463421, 463446, Ext. 216 (O)
Telex : 91-0675-6443 CIFA IN, Fax : 91-0674-463407
E-mail:cifa.@x400.nicgw.nic.in

MESSAGE

It gives me great pleasure to note that the International Centre for Living Aquatic Resources, Manila, Philippines is organising an International Workshop on 'Genetic Improvement of Carps in Asia' at this Institute during 26-29 July, 1997. Carp farming being the main component of Asian aquaculture deserves great R & D attention for improving aquaculture productivity. Research achievements in carp genetic improvement would have long reaching effects in the region.

Organisation of the workshop at this Institute is deemed as a recognition of the efforts being made by this Institute in the aspect of fish genetics, particularly selective carp breeding. The expertise at the Institute and experience in the project over past few years would enable offering consultancy on these aspects for the region. I am sure the International Cooperative endeavours in this field would be of great benefit for the region.

I wish the International Workshop every success.

(S. AYYAPPAN)

THE INTERNATIONAL NETWORK ON GENETICS IN AQUACULTURE (INGA)

MODADUGU V GUPTA
International Centre for Living Aquatic Resources Management (ICLARM)
MCPO Box 2631, 0718 Makati City, Philippines

Background

Fish forms the primary source of animal protein to about one billion people, mostly in developing countries, contributing as much as 70% of the animal protein intake in some of the countries. Population growth projections indicate that at a conservative estimate, the demand for food fish would be in the range of 110 million to 120 million tonnes (live weight) for the year 2010, as compared to 75 million to 80 million tonnes in 1994-95. Available information indicates limited possibility for increasing production from capture fisheries, as many of the fished stocks have either been over-exploited or have reached their maximum sustainable yields while aquaculture increased its contribution to world fisheries production and maintained its position as one of the fastest growing food production activities in the world. This scenario leads to the necessity for looking at various means of increasing production through aquaculture to bridge the gap between supply and demand.

Remarkable achievements have been made in the last four decades in increasing production of crops, livestock and poultry through genetic research and application of breeding programs. Even though the tradition of fish farming dates back thousands of years, efficient breeding and genetic selection programs have not been practiced till recent years, especially in the case of tropical aquaculture species. Studies undertaken in recent years in trout and salmon in Norway have clearly indicated the potential for increasing aquaculture production through application of quantitative genetics. Realizing the need for similar studies for tropical finfish such as carps and tilapia which are the main species for tropical aquaculture, the International Center for Living Aquatic Resources Management (ICLARM) in collaboration with AKVAFORSK in Norway and

Philippine research institutions and with financial support from the United Nations Development Program (UNDP) initiated a project "Genetic Improvement of FarmedTilapias (GIFT)" in 1988. This pioneering work since then has clearly demonstrated the possibility of enormous gains in economic performance of tropical finfish through a systematic selection program. GIFT strain Nile tilapia has shown 60-70% higher growth as compared to local strains.

Establishment of International Network on Genetics in Aquaculture (INGA)

Through a series of meetings among senior scientists/leaders from national institutions in Asia and Africa, sponsored by ICLARM and UNDP/DGIP during 1990 and 1992, the need for enhancing fish genetics research and develop collaborative linkages that could help the evolution of national breeding programs was recognized. The prospects for genetic improvement of tropical finfish as demonstrated by the GIFT project, and the success of an international networking approach for rice genetic improvement further strengthened the need for establishment of an aquaculture genetics network. These efforts culminated in the formation of the International Network on Genetics in cote d'Ivoire, Egypt, Ghana, India, Indonesia, Malawi, Philippines, Thailand and Vietnam. Subsequently. Fiji and Malaysia joined the network in 1996. ICLARM participates in INGA as a member. as well as its Coordinator.

The primary objectives of INGA are to :

- foster regional and international cooperation in aquaculture genetics research;
- assess needs and opportunities for application of genetics to aquaculture;
- strengthen the national research capacity for continued genetic enhancement of farmed fish;
- facilitate exchange of information, methods, boost efficiency and stimulate thinking;
- facilitate exchange of germplasm;
- contribute through collaborative research, to domestication of tropical finfish;
- evaluate performance of promising lines of tilapias and carps;
- assist in development of strategies for national fish breeding programs.

The network activities are governed by a Steering Committee, comprising of one senior scientist from each of the member countries and ICLARM. The activities are coordinated by a Research Coordinator based in ICLARM. The Steering Committee meets annually and ensures that the network research agenda is responsive to national needs and priorities. The first Steering Committee meeting was held in Bangkok, Thailand in 1994, the second in Hyderabad, India in 1995 and the third in Cairo, Egypt in 1996 - hosted by the governments of Thailand, India and Egypt, respectively.

Progress to date

Germplasm enhancement and breeding work involves a continuous activities, documentation and characterization of economically important fish genetic resources; utilization of these genetic resources in appropriate breeding programs; and evaluation of improved products for aquaculture targeted to resource-poor, small-scale producers. Tilapias and carps are the major species of importance for freshwater aquaculture and hence the network activities are focused initially on these two groups of fishes. In Asia, where freshwater aquaculture is well developed, the network research is targeted at improvement of breeds of tilapias and carps and evaluation of their performance, while in Africa, the emphasis is on characterization and evaluation of indigenous species for their aquaculture potential.

The progress made during the first four years of INGA include :

1. Enhancing the value of research carried out by various national programs as part of their own national research efforts and also through various bilateral and multilateral initiatives.

2. Development of quarantine and fish health protocols for safe exchange of fish among member countries (in consultation with FAO).

3. Coordination of germplasm exchange following strict quarantine protocols :

   (a) improved common carp (*Cyprinus carpio*) strain from Vietnam to Bangladesh, India and Thailand (funded by the Govt. of Vietnam);

(b) the GIFT strain Nile tilapia (*Oreochromis niloticus*) from the Philippines to Bangladesh, China, Indonesia, Thailand and Vietnam (funded by UNDP/ ADB/ICLARM);

(c) diverse stocks of silver carp (*Puntius gonionotus*) from Indonesia and Thailand to Bangladesh (funded by USAID);

(d) mrigal (*Cirrhinus mrigala*) from India to Vietnam.

4. Development of plans and strategies for implementation of national breeding programs in Vietnam for Nile tilapla, mrigal and silver carp and in Indonesia for GIFT strain Nile tilapia, common carp and milkfish (*Chanos chanos*) (in collaboration with AKVAFORSK and funding from the Government of Norway and ICLARM).

5. Initiation of collaborative research and training programs for :

(a) genetic improvement of carps in six Asian countries (funded by the ADB);

(b) characterization and documentation of tilapia genetic resources in four African contries (funded by IDRC);

6. Organisation of training programs in quantitative genetics (in collaboration with AKVAFORSK);

7. Coordination and assistance in preparation of research proposals for donor support;

8. Organization of annual Steering Committee Meetings (funded by UNDP, ICLARM and the governments of Egypt, India and Thailand);

9. Organization of national aquaculture genetics networks in India, Indonesia, Malawi, Malaysia, and the Philippines;

10. Publication of INGA news in *Naga, the ICLARM Quarterly*;

11. Continuous information dissemination among members.

Organization of Regional Aquaculture Genetics Research Programs

During the second Steering Committee Meeting of INGA held in Hyderabad, India in 1995, the members requested a collaborative research and training project for the genetic improvement of carps in Asia, while the African member countries in the network during the Third Steering Committee Meeting held in July 1996 in Cairo, Egypt, requested a collaborative project for characterization and documentation of tilapia genetic resources and their utilization in aquaculture. As a result of this, ICLARM prepared two proposals, one for carps genetics research in Asia and the other for tilapias in Africa. While the Asian Development Bank (ADB) provided funding for the carps project, the International Development Research Centre (IDRC) of Canada provided funding for the tilapia project. Six countries : Bangladesh, China, India, Indonesia, Thailand and Vietnam are participating in the carps project, while four countries : Cote d'Ivoire, Egypt, Ghana and Malawi are participating in the tilapias project.

The objectives of the "Collaborative research and training project for genetic improvement of carps in Asia" are : (i) to assess the current status of Asian carp genetic resources for their immediate use in aquaculture; (ii) bring together the existing technical skills and experience scattered around various parts of Asia and build strategic research partnerships and networking arrangements; (iii) develop criteria for prioritizing carp genetics research and set research priorities : species, farming systems and breeding goals; and (iv) identify potential genetic approaches and initiate location specific strategic research and training in carp genetics leading to the development of high yielding carp strains.

The work will start with a research priority exercise which will : (a) assess how and to what extent existing carp species/strains are valued by different groups. (b) estimate future demand by income groups; (c) analyze present and future importance of various carp-based farming systems and (d) assess the relative economic importance of various traits.

The project activities are starting with a Planning Workshop Scheduled for 26-29 July 1997, which the Central Institute of Freshwater Aquaculture (CIFA) is hosting at its headquarters in Bhubaneswar, India.

CIFA - HOME OF MODERN FRESHWATER AQUACULTURE

S. Ayyappan, Kuldeep Kumar, Bijoylaxmi Dhir & U. L. Mohanty
Central Institute of Freshwater Aquaculture
Kausalyaganga, Bhubaneswar- 751002, India

The Central Institute of Freshwater Aquaculture (CIFA), ICAR, Kausalyaganga, located at about 10 kms from Bhubaneswar on Bhubaneswar - Puri highway and base of Dhauli is the Premier Research Institute on Freshwater Aquaculture in the country. The Institute had its beginnings in the Pond Culture Division of the erstwhile Central Inland Fisheries Research Institute which was established at Cuttack, Orissa, in 1949. The Division was later upgraded as Freshwater Aquaculture Research and Training Centre (FARTC) established at Bhubaneswar in 1976 with UNDP/FAO assistance. Further, the Centre attained the status of an independent Institute during 1986 and the functional existence of the Institute came into effect on 1 April, 1987. The Institute presently has six regional centres at Rahara, West Bengal; Bangalore, Karnataka; Vijayawada, Andhra Pradesh; Akola, Maharashtra; and Ludhiana, Punjab along with the Krishi Vigyan Kendra (KVK) / Trainers' Training Centre (TTC) at Kausalyaganga, Orissa, as also ten ORP centres located in ten states of the country. The Headquarters of the Institute has nine sections, viz., Production Technology, Soil-Water Environment, Fish Genetics, Fish Nutrition, Fish Physiology, Fish Pathology, Aquaculture Engineering, Aquaculture Economics and Statistics, and Aquaculture Extension.

OBJECTIVES

The mandate of the Institute is :

a) to conduct research, more specifically in fish nutrition, physiology, genetics, pathology, pond environmental monitoring and aquaculture engineering for developing intensive and extensive warm freshwater fish farming systems for commercially important finfish and shellfish;

b) to conduct specialised training and extension programmes in freshwater aquaculture to enable economic utilisation of the cultivated and cultivable freshwater aquatic resources of the country; and

c) to act as a nodal agency to provide scientific information and technology transfer for freshwater aquaculture development.

INFRASTRUCTURE

The Headquarters of the Central Institute of Freshwater Aquaculture has a sprawling campus with an area of 147 ha. It is housed in a three-storeyed building with laboratory facilities for different specialisations in carps, catfish, prawn and pearl mussel, fish breeding and culture, fish genetics, fish nutrition, fish physiology, soil and water chemistry, aquatic microbiology, weed management, fish pathology and aquaculture engineering. The Institute has a computer centre, radio-isotope laboratory, tissue culture laboratories, central instrumentation laboratory and a workshop for plasticraft. The Institute has well equipped laboratories for researches on different aspects of freshwater aquaculture like production technology, aquatic environment, fish genetics, fish nutrition, fish physiology, fish pathology, aquaculture engineering, aquaculture economics and statistics, and aquaculture extension.

The fish farm with a total water area of about 50 ha comprises over 500 ponds of assorted sizes. The facilities in the farm include a carp hatchery with a production capacity of 50 million spawn, giant freshwater prawn, *Macrobrachium rosenbergii* hatchery of 10 million post-larvae, backyard hatchery for magur, (*Clarias batrachus*) and Indian river prawn (*M. malcomsonii*), a wet laboratory (2 units of 30m x 10m), facilities for running water fish culture, paddy-cum-fish culture, sewage-fed fish culture, Azolla and algal culture, integrated fish farming, yard facilities, feed mill, etc.

The Institute has a specialised library to serve different disciplines of freshwater aquaculture. The library possesses about 2000 volumes of books and subscribes to 20 foreign journals and 42 Indian journals. A CD-ROM facility for specialised aquaculture journals is also available at the Institute. The FAO has recognised the library as Depository Library for FAO publications.

ACHIEVEMENTS

Since its establishment at Cuttack in 1949, the Pond Culture Division had made epoch-making contributions to the development of fish culture especially in carp breeding, seed raising and table-fish production. In fact, two major technologies of induced carp breeding and composite carp culture that laid the foundations of the modern Indian aquaculture were developed at this Division. This has led to a virtual change in the scenario of fish seed production in the country from total dependence on riverine seed collection to production of over 15,000 million of farm-bred fry at present. The division had several credits in terms of development of specific packages of freshwater aquaculture technologies like sewage-fed carp culture, peninsular tank fisheries, integrated fish farming with agriculture and livestock, etc. As a reorganised Institute, CIFA has made several outstanding contributions such as multiple breeding of carps, gamete cryopreservation, intensive carp culture with production rates of 10 and 15 t/ha/yr, breeding and hatchery management of catfishes, *Clarias batrachus* and *Heteropneustes fossilis*, and freshwater prawns, *Macrobrachium rosenbergii* and *M. malcolmsonii*, production of cultured freshwater pearls through nuclei implantation in freshwater mussels, aquatic biofertilization with Azolla and utilisation of biogas slurry as organic farming practices, production of sterile triploid grass carp, formulation of diets for fish and prawn species (CIFACA-a commercial carp diet), formulation of CIFAX as a control measure for epizootic ulcerative syndrome, packages of practices for sewage-fed fish culture and cage culture, breeding and rearing of commercially important frog species etc. are a few to name. Ornamental fish breeding and culture is a new area that is being addressed to by the Institute in view of their high export potentials.

The Institute has hosted three UNDP/FAO programmes and the project on Centre of Advanced Studies (CAS) in Freshwater Aquaculture, in collaboration with the Orissa University of Agriculture and Technology, Bhubaneswar, provides for M.F.Sc. and Ph.D. degrees. Besides undertaking the Institute based research projects, the Institute has operated as many as 23 externally funded projects. The Institute has active research collaboration and extension linkages with many national and international institutions. The UNESCO-MIRCEN has recognised the RLCI as a Microbiological Resource Centre in Aquatic Microbiology.

IMPACT OF TECHNOLOGIES DEVELOPED AT CIFA

- The aquaculture technologies developed by this Institute have had a direct bearing on the production and economic aspects of the freshwater aquaculture sector.
- The intensive carp culture with production rate of 15 t/ha/yr is a quantum leap from the national mean productivity of 2 t/ha/yr.
- The Institute has presently undertaken the task of preparing a National Freshwater Aquaculture Development Plan on a District-basis, to serve as a blue-print for undertaking aquaculture development to raise the national mean productivity to 5 t/ha/yr.
- The technology of multiple carp breeding has not only prolonged the normal breeding season of the carp during monsoon but also resulted in quantitative increase in the seed production levels.
- Carp hatchery technology standardised by the Institute has been adopted all over the country.
- The development of hatchery technologies for catfish, magur and freshwater prawns, *M. rosenbergii* and M. malcolmsonii has opened the possibility of further grow-out culture systems.
- Production of cultured freshwater pearl, through the nuclei implantation in freshwater mussels, *Lamellidens* spp. has opened new avenues for diversification of aquaculture practices adding a new component to the produce. The Freshwater pearl culture also provides for import substitution in a big way, as India imports pearls and further exports after processing in the country.
- In the light of increasing environmental concerns and the need for suitable farming practices, Azolla biofertilization is a new component in freshwater aquaculture practices.
- The sterile triploid grass carp is apt for stocking in the reservoirs, for controlling aquatic vegetation. Commercial scale production of triploid grass carp would be an economic enterprise by itself.

- Fish feed manufacture is an industry in the country and the research at the Institute have provided technical support with regard to feed formulation and production of different types of feeds. The Institute has formulated a balanced feed for fish under the name of CIFACA.
- Researches with regard to fish diseases have provided technical support in disease management in intensive fish culture systems. The Epizootic Ulcerative Syndrome (EUS) that took a heavy toll of fish stock in the last few years was controlled by using a chemical mixture, CIFAX, formulated at the Institute.
- Standardised packages of practices for sewage-fed fish culture and allied practices like integrated fish farming provide for recycling of organic wastes.
- The productivity levels in carp culture ponds have gone up from 600-800 kg/ha/yr to about 2,000 kg/ha/yr in ponds under Fish Farmers' Development Agencies.
- Induced carp breeding, seed rearing and composite carp culture technologies have been well adopted in the country.
- The research, development, education and extension efforts with regard to freshwater aquaculture at the Institute have had a catalytic effect on the growth of the Industry. The economic impact of the findings in terms of production, growth, food and nutrition security, import substitution, export promotion and industrialization are perceptible.

CONSULTANCY AND ADVISORY SERVICES

The Institute has the expertise to offer consultancies, advisory services and advanced training programmes in the following aspects of freshwater aquaculture:

- Carp breeding and hatchery management
- Intensive carp culture
- Catfish breeding and culture
- Freshwater prawn breeding and culture

- Freshwater pearl culture
- Pond fertilisation and management
- Sterile/monosex fish production
- Fish feed formulation and production
- Fish disease diagnosis and control
- Integrated fish farming including wastewater aquaculture
- Design and construction of fish farm and hatcheries
- Aquaculture project formulation and evaluation

EDUCATION / EXTENSION

The Institute is a Centre of advanced Studies in Freshwater Aquaculture offering Ph. D. and M. F. Sc. Degree programmes in collaboration with the Orissa University of Agriculture and Technology. The Institute is also recognised by several Indian Universities for Doctoral programmes.

The Institute is actively engaged in transfer of the developed techniques and technologies in different aspects of freshwater aquaculture through research publications in scientific journals, publication of annual reports, technology manuals and pamphlets, organisation of national and internatonal training programmes, organisation of national symposia and workshops, participation of the Institute and its staff members in national and internatonal symposia and exhibitions, demonstrations in farmers' ponds, etc. apart from KVK/TTC's exclusive on-campus and off-campus training programmes.

RECOGNITION TO THE INSTITUTE

- Regional Lead Centre for carp farming in India under the Network of Aquaculture Centres in the Asia-Pacific (NACA).
- Centre of Advanced Studies in Freshwater Aquaculture, offering post-graduate degree programmes.

- The library of the Institute is designated as a centre for FAO Depository Library.
- The UNESCO - MIRCEN have recognized the Institute as a Microbiological Resource Centre in Aquatic Microbiology.
- The Indian Council of Agricultural Research has recognized the Institute as a 'Centre of Excellence for Freshwater Pearl Culture' under the National Agricultural Technology Project Scheme.
- Adjusted the best Institution of the Indian Council of Agricultural Research for the year 1996.

The Central Institute of Freshwater Aquaculture, with its expertise and facilities is a potential international centre for training and education in different aspects of freshwater aquaculture for the AsiaPacific Region. Efforts are being made to establish an international centre of excellence in freshwater aquaculture at the Institute. The Institute is also a pioneer with regard to selective breeding of carps in the world, having launched the first international project on "Selective breeding of rohu, *Labeo rohita*". Organization of the present workshop, programme planning of the project on "Genetic improvement of carps in Asia" is expected to open new avenues for international collaboration in aquaculture research in general and fish genetics in particular.

Let us Grow Fish and Grow with Fish

CURRENT STATUS OF CARP GENETICS RESEARCH AND BREEDING PRACTICES IN INDIA

P.V.G.K. Reddy, R.K. Jana, Kanta Das Mahapatra, A. Barat, J.N. Saha,
H.K. Barman and P.K. Meher
Central Institute of Freshwater Aquaculture, P.O : Kausalyaganga,
Bhubaneswar, 751 002, Orissa (INDIA)

INTRODUCTION

The carp germplasm resources of India, consisting of a number of major and minor carps are very rich. The major carp species, viz, the catla (*Catla catla*), the rohu (Labeo rohita), the mrigal (*Cirrhinus mrigala*), the calbasu (*Labeo calbasu*) with their complimentary food habits are mutually compatible and grow relatively faster. These carps are thus readily a boon for the aquaculturists. Due to these qualities Indian major carps enjoy relatively wider distribution especially in the countries of South-East Asia.

Because of their mutually compatible food habits, a multispecies culture technology (intensive/extensive) which is popularly known as composite carp culture has been developed during seventies. Chinese grass carp (Ctenopharyngodon idella) and silven carp (Hypophthalmichthys molitrix) have formed a welcome addition to Indian aquaculture scenario due to their relatively faster growth.

The intensive carp culture technology is mainly based on species ratio and stocking density manipulations and other management practices. These include heavy feeding, manuring, aerating and also replenishing water from time to time. The average fish production of 0.6 t/ha year from culture fisheries in the country could be rised to as high as 10-12 t/ha/y by adopting this technology. Recently a production of 15-17 t/ha/y has been also demonstrated by taking multiple cropping system.

However, in view of economically sustained aquaculture production and the environment through the system, any further increase in the production with already

heavy inputs of feed and fertilizers, may not be perhaps economically viable. Under these circumstances, the fishery worker had to look for other means of enhancing production from aquaculture to make it an economically sustained profession.

Genetics is one of such potential means to provide improved varieties that may thrive and perform the best. Development of varieties through optimum exploitation of the genetic potentials of the cultivated species to provide quality seed will be a positive step and very much needed during the coming decade if aquaculture has to sustained.

Research for developing superior varieties of carps through genetic improvement methods has been already in progress as mentioned on some earlier occassions as the present one.

INSTITUTIONS INVOLVE IN ACTIVE CARP GENETICS AND BREEDING RESEARCH

The most actively involved Institute in carp genetics and breeding research is the Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, followed by the National Bureau of Fish Genetic Resources (NBFGR), Lucknow. Being a Premier Institute for freshwater aquaculture in the country, CIFA had initiated carp genetics research as early as late fifties as a Sub-station at Cuttack of the Central Inland Fisheries Research Institute (Barrackpore) West Bengal, with simple hybridization among Indian major carps belonging to three genera viz. Catla, Labeo and Cirrhinus.

1. RESEARCH COMPLETED

1.1 Hybridzation

Several interspecific and intergeneric hybrids have been produced and evaluated for their utility in aquaculture. Most of these hybrids whether interspecific or intergeneric, were found to be fertile and F2 generation was also produced. In some cases the F2 hybrids were also found fertile. Some of then have exhibited faster growth rate than the slow growing parent and possessed more fresh content

than the parent species. Several back-cross and also tripple cross hybrids have been produceed among Indian carp.

Intergeneric crosses between common carp (Cyprinus carpio var communism) and Indian major carps have yielded aneuploid sterile hybrids which may be very useful in aquaculture especially for resorvoirs. As they are sterile, unwanted reproduction can be easily checked.

1.2 Cytogenetics

Cytogenetic studies include simple chromosome number and Karyotype. About 250 India species of fishes have been studied, Chromosome number (2n) ranges from 16-100 with a peak of 48 and 50.

Comparative karyological studies of parent species and hybrids have been also made is relation to hybrid Viability and fertility.

Chromosome banding studies such as C,G and NOR-banding have been also attempted. C-banding was tried in about 20 species including some carp species to define heterochromatin blocks. Carp species showed very low response to C-banding probably due to lack of well defined heterochromation blocks in their genome.

NOR-banding studies have been made in about 50 species including Indian major carps. Most species show only small NORs on a single pair of chromosome. Multiple NOR site has been reported in catla catla and richardsonii.

Sister chromatid Exchange (SCE) assay has been standardized in *Channa Punctatus* to test the genotoxicity caused due to industrial effluents in aquaculture medium.

1.3 Genome manipulations (Chromosomal engineering)

Investigations have been successfully carried out to find out and standardize the optimum levels of UV irradiation for genetical inactivation of major Asiatic carps and the thermal and pressure stock regimes to induce diploid meiotic and mitotic gynogenesis and to induce polyploidy (triploidy/tetraploidy) in these carps.

Triploidy and tetraploidy have been also induced in tilapia, besides developing methods for the production of supermales of this species at Madurai Kamaraj University.

All male populations of tilapia could be also produceed through hormone treatment.

1.4 Selective breeding

Carp selection work has been first initiated at CIFA with rohu, Labeo rohita in Collaboration with the Institute of Aquaculture Research (AKVAFORSK), Norway in the year 1992. The project aims at improving the growth trait of rohu and develop a National breeding programme for the distribution of genetically improved seed of this species to the fish farmers throughout the country.

The first phase of the project has been successfully completed by March 1996. During this phase various experimental procedures for field and laboratory including tagging procedures have been developed and standardized.

The stocks of rohu procured from different river system viz. The Ganga, the Gomati, the Yamuna, the Sutlej and the Brahmaputra have been evaluated along with the farm (local) stock. It was observed that the differences between individuals of different stocks are narrower compared to individuals within the stock. The results showed a substantial additive genetic variation for growth in rohu.

An average selection response of about 13% has been observed in the first generations with a maximum of 24% in one of the replicates. The second please of the project has been also initiated.

A second project on selective breeding of catla (catla catla) has been taken up. This is a tripartite project among the University of Agriculture Science (UAS), Hasaraghatta, Bangalore (INDIA), University of Wales, Swansea (U.K) and University of sterling (U.K). This project mainly aims at investigating the present genetic status of domesticated catla in Karnataka State and design and indicate a suitable breeding programme for genetic improvement of this important species.

The National Bureau of fish Genetic Resources, lucknow, has taken into the fold of its mandate, the Collection, classification and evaluation of information on fish genetic resources of the country along with cataloguing of genotypes. The Bureau has also taken up the task of maintenance and preservation of fish genetic material in coordination with other geneticist for conservation of endangered species.

1.5 Molecular gentics/gene transfer

Earlier during late eighties experimental production of transgenic fish Labeo rohita, Cirrhinus mrigala) has been carried out of in-vitro recombinant DNA at the National Institute of Immunology, New Delhi.

Centre for Cellular and Molecular Biology (CCMB), at Hyderabad is at present concentrating on the production and evaluation of transgenics particularly carps and catfishes. It appears that the growth hormone gene of Labeo rohita and catla catla has been successfully isolated.

Other Institutions like Madurai Kamaraj University (Madurai) he also engaged in molecular genetics research.

The Department of Biochemistry in Bose Institute, Calcutta is working on developing methods for the identification of species specific genetic marker for various applications in aquaculture including screening hybrids.

2. RESEARCH IN PROGRESS

2.1 Genome manipulations

Assessment of the homozygosity level in mitotic gynogens of Indian major carps.

Evaluation of the performance of top-crossed progeny of gynogens for heterosis effect.

Inducing & production of allotriploids of carp hybrids (Catla x rohu and Calbasu x rohu) etc. Inducing tetraploidy in catla, calbasu common carp and grass carp to produce triploids of there species on large scale for experimental studies.

2.2 Selective breeding

Selective breeding of Indian major carps (Rohu and catla) is being continued to develop superior strains for aquaculture.

2.3 Molecular genetics

Cloning of isolated growth hormone genes and isolation of other like disease resistant genes, standardization of RAPD techniques etc.

3. BREEDING PRACTICES IN INDIA

Prior to the success of induced breeding of Indian major carps, the fish farmers used to depend mostly on riverine sources for the seed of these carps where they breed naturally. However, few farmers in the Midnapore and Bankura Districts of West Bengal used to follow traditional method of breeding the fish by simulating flooded riverine environment in a field having gradually sloping and undulating terrain for catchment of rainwater when the stocked brood fish of carps breed naturally. These are known as 'dry bundhs'. Later, this practice has been taken up by the state of Madhya Pradesh, followed by the state of Rajasthan. There are two types of Bundhs' viz. wet

and dry bundhs. The wet bundhs are usually connected to some streems or river sources for almost permanent supply of water. Thus during off season also these bundhs will be having some water. Unlike the wet bundhs the dry bundhs remain dry except during monsoon months.

Of late, many modifications have taken place in bundhs and one such popular bundh is 'Bangla bundh'. It's a oval cement pond-like structure in two compartments, a small shallower and a large deeper one, divided by a slightly raised wall is the middle, with a perforated pipe line all around for water circulation. After the administration of hormone the brood fish are released in the bundh and water is circulated for the precipitation of spawning.

The modern fish breeding technology is a gradual development by modification of the original hypophysation technique which was successful during late fifties at Cuttack (Orissa). The modern technology involves brood stock management use of inducing agents and environmental monitoring. The crude pituitary extrats containing several other hormones besides the gonadotropins, formed the basis for inducing spawning in carps which are now gradually replaced by many other products such as partially purified gonadotropins from carp and salmon (SG-G100), Human Chorionic Gonadotropin (HCG), Luteinizing Hormone-Releasing Hormone (LHRH) and its analogues. Salmon/ Murrel Gonadotropin Releasing Hormones (SGn RH and m Gn RH). etc.

However, the breeding practices at present followed by the Indian Hatchery Managers or those fish farmers who are able to produce seed by themselves, in the pursuit of profit making are only aiming at meeting their targets in quantity by neglecting quality. As such, do not show much concern to any genetic norms with regard to aspects like maintenance of minimum effective population number and other management practices. The same brood stock with a narrow gene pool is very often used repeatedly for quite a long period and subsequent brood fish raised from the same sibs. Many seed produces were also in the practice of mass producing seed by breeding all the species together (mixed breeding). This is a very dangerous practice in terms of genetic degradation as it many lead to unwanted and undesirable hybridization.

The authorities with the help of scientific community should take immediate steps to bringin some sort of awareness among the seed producers and convince them not to continue with such practices and avert the damage to the original gene pool, characteristic of the corresponding species.

4. FUTURE RESEARCH NEEDED

4.1 Hybridization

Hybridization between major and minor Indian carps and their evaluation for ploidy status and culture performance.

4.2 Genetic manipulation

Evaluation of allotriploids for disease resistance. Large scale production of triploids of common carp and grass carp after the successful induction and production of tetraploids of these species.

Investigation on the existance of different population if any in various species of Indian major carps.

Integrated approach to enhance aquaculture production by clubbing ploidy/ genome manipulation and selective breeding methods.

Production and evaluation of transgenics of carps and catfishes and study the socio-economic impact of genetically manipulated superior stocks of fish.

CONSERVATION OF CARP GENETIC RESOURCES OF INDIA

A.G. Ponniah
National Bureau of Fish Genetic Resources
351/28 Dariyapur, Talkatora Road
Lucknow - 226 004, U.P., India

Introduction

Carps as a group constitute an important component of India's rich fish biodiversity. Not only they support natural fishery substantially but Indian major carps, certain minor and exotic carps are major Indian aquaculture species. The break through in their polyculture gave the blue revolution to the country. Though Indian major carps, catla, rohu and mrigal are not endangered, other carps like *Labeo dero, L. dussumieri, L. fimbriatus and L. gonius* are on the endangered species list. Many of the endangered carps are from peninsular region and some are endemic only to India. Rohu, catla and mrigal might not be under threat as species to occupy the list of endangered species. However, they are prone to loss of genetic diversity and variability due to extinction of genetically distinct wild population, escape and ranching of farmed seeds and competition due to exotic carps. This paper highlights the importance of conservating individual species and the genetic diversity within the species. The paper has been divided into three major parts namely indigenous and exotic carp species in Indian, prerequisites for conserving and genetic threats faced by Indian carp resources.

Germplasm Resources

Indigenous Carps Species

Of the indigenous carp species, Indian major carps *Labeo rohita, Cirrhinus mrigala* and *Catla catla* are widely cultured than others. These have been introduced into peninsular India where they were originally not found and could have led to endangering endemic peninsular carps. Information in indigenous Indian carps is widely

scattered and some information from Jhingran (1984) on the world distribution, growth under farm and wild conditions for the important Indian carp species belonging to the genera *Catla, Cirrhinus* and *Labeo* are given below :

Catla catla (Hamilton) : Occurs in India, Pakistan and Burma; introduced into many countries and in peninsular drainages. Important component of composite fish culture. Faster growing of the Indian major carp. Grows to 38-45 cm and 900 g in first year and 4 -5 kg in the second year.

Cirrhinus mrigala (Hamilton) : Found in freshwater of India, Burma, Pakistan; more common in plains of North India. Attains maturity at +2 years (344 mm) in the wild and at one year in ponds.

Labeo rohita (Hamilton) : Occurs in freshwaters of Pakistan, Burma and India, where it occurs in Punjab, U.P., Bihar, West Bengal, Assam, Orissa, Madhya Pradesh and Ahmedabad. Maximum size recorded is 1030 mm.

Labeo calbasu (Hamilton) : Found in the streams of Punjab, West Bengal, Orissa and part of South India. It is more abundant in rivers above tidal reach and lakes.

Cirrhinus reba (Hamilton) : Found thoughout India. Males mature when 8 -13 cm long; females at 23 - 25 cm long in their first year of life. Grows to 24 cm in length in West Bengal; further south it grows to 38 cm or more in ponds.

Labeo dero (Hamilton) : Occurs in Assam, Darjeeling, eastern and western Himalayas, Punjab and Uttar Pradesh.

Labeo dyocheilus (McClelland) : Common in Assam and Nepal Himalayas and grows upto 91 cm in length.

Labeo fimbriatus (Bloch) : Commonly called as fringe-lipped carp. Occurs in freshwaters of E Punjab, U.P., Orissa, M.P., Madras, Deccan, Poona, Ahmedabad and Pakistan. It is cultivated in ponds alongwith other carps in India. Grows to about 23 cm and 450 g in one year and 31 cm in second year.

Labeo kontius (Jerdon) : Known as Cauvery carp. It occurs in rivers of South India. It is cultivated in ponds and grows to 23 - 30 cm and 330 g in one year. Being a hardy fish; it can tolerate polluted waters. Females mature at 30 - 36 cm and males at 27 cm.

Labeo bata (Hamilton) : Found normally in north India and Pakistan. It is cultured alongwith major carps.

Labeo gonius (Hamilton) : Freshwater of India, Pakistan and Burma. In Assam, it is cultured in ponds.

Exotic Carp Species

The Chinease silver carp, *Hypophthalmichthys molitrix* and grass carp *Ctenopharyngodon idella* are carps of Chinease river basins of south and central China and Amur basin of USSR that have been introduced into India and integrated into a composite fish culture practices with promising results. Silver carp has been found to grow to about 5 kg within the two years in reservoirs. Accidental entry of silver carp into Govindasagar reservoir during flood in 1971 led to the establishment of naturalized population and the decline in fishery of catla and rohu.

The prusion strain of common carp from Sri Lanka was introduced in 1935 in Nilgiris. The three varieties introduced were the mirror carp (*Cyprinus carpio* var *specularis*), scale carp (*C.carpio* var *communis*) and leather carp (*C. carpio* var *nudus*). The Bangkok strain of common carp was introduced from Bangkok in 1957 for culture in plains. At present there has been mixing of both strains and they are found as naturalized populations in many parts of India. In the hill region what is presently popularly called mirror carp is actually the scale carp. Mirror and leather carp occur only in few wild population. Introduction of common carp into Dal Lake in Kashmir has led to sharp decline in native populations of snow trouts.

Besides these, the big head carp (*Aristichthys nobilis)* mud carp (*Cirrhinus molitorella*) and snail carp (*Mylopharyngodon piecus)* have found unauthorized entry

into India. Since the feeding habits of these fishes clash with the native fishes, spread of these fishes can endanger indigenous carp species.

Prerequisites

The prerequisites for conserving carp germplasm resources of India are many. The institutional research support for such activity is available with NBFGR and CIFA. Prioritized requirements are given below to effectively utilize this research base.

Data Base

The conservation strategy for any aquatic species depends on its biology, genetic diversity within and between different populations and stresses that is modifying each population. One of the impediment to conservation of carp germplasm resources of India is the lack of database on the above aspects.

Present Status

Only for a few aquatic species like rainbow trout, tilapia and common carp there is substantial information on genetics. For others like Indian major carps, genetic information is inadequate and biological information from different geographic population of each species is limited. The importance of studying the different life history traits and population biology of Indian carps has not been adequately addressed and only limited information is available on these aspects. Differences between population can reflect adaptive response due to different habitats. Information on this coupled with genetics will form the basis on which prioritization of populations within a species can be undertaken for conservation. Also, this data base can be utilized in selection of founder stocks for genetic upgradation programmes. At present other than FAO synopsis and ICLARM Fish Base no detailed data base is available.

Future Projection

For conservation of germplasm resources of cultured carps what is required is the collection of information and building a data base on the pattern of trout brood

stock data base on registry for six species of trout. At National Bureau of Fish Genetic Resources (NBFGR) work is presently being carried out on cataloguing available biological and genetic information in a computer format which will ultimately be available online and CD-ROM. However, the non availability of published information is a limiting factor. There is need to give priority and adequate funding for the study of life history traits, ecology, population dynamics and genetics of each carp species.

Stock Identification

The description of the genetic diversity in a species is a basic requirement for management of its genetic resources. Initially, studies should be aimed at genetic confirmation of the species as described by morphological methods. Then quantification of genetic variation within a population and between geographic populations can lead to identification of discrete breeding units which have been variously termed as stock, strain etc.

Present Status

Genetic characterization can be carried out by cyto, biochemical and molecular genetic studies. Compared to biochemical and molecular genetic studies more number of Indian species are cytogenetically screened. Cytogenetic studies can be used to detect two types of chromosomal variations namely chromosome number and karyotype. At NBFGR, number of species including the Indian major carps have been cytogenetically investigated. Indian major carps have the same diploid number of 50 and no intraspecific variation in chromosomal number has been detected. Among the different banding techniques NOR, C, BrdU replication banding have been achieved at NBFGR. The chromosomal banding technique of NOR has been studied in 17 different endangered and commercial fishes. Of these, the carp species studied are *C. catla, L. rohita, L. calbasu* and *L. bata.* With the exception of catla which has two pairs of NOR, other Indian major carps have only one pair. In *Cyprinus carpio* var *specularis* from Bhimtal lake of Kumaon Hills (U.P.) NOR was observed in the terminal end of one pair of large size metacentric chromosome. While NOR polymorphism has been detected in *Schizothorax richardsonii,* no intraspecific variations have been

observed from both riverine and hatchery population of *L. rohita*. C band have been studied in *L. rohita* and *L. calbasu.*

Using limited number of isozyme systems, electrophoretic studies have been carried out in Indian major carps. At NBFGR, detailed biochemical genetic characterization using 25 enzyme system is being carried out in 15 prioritized commercial and endangered species. Among the carps, detailed studies have been carried out in *L. rohita, C. mrigala* and *C. catla* and limited studies in *L. calbasu* and *Cyprinus carpio.* Compared to the air breathing fishes, *Heteropneustes fossilis* and *Clarius batrachus* which reveal differences at more or less all the enzyme systems screened, the Indian major carps exhibit a few species specific markers indicating that it is a closely related genetic group.

In normal sodium dodecyl sulphate acrylamide (SDS-PAGE), only minor differences could be observed between catla and rohu. However, striking differences were observed in the gradient SDS-PAGE electrophoretic profile of catla and rohu with the catla having more number of bands. All the protein bands were observed within the range of 14,000 to 94,000 daltons. In rohu and mrigala studies to differentiate multi loci estrase system with seven substrates revealed very little substrate specificity. However, with five inhibitors it was possible to characterize the multi loci estrase system. For genetic characterization of endangered fish and valuable brood stock, non invasive and minimum invasive sampling have been carried out with *H. fossilis.* For non invasive sampling mucus has been used and for minimum invasive sampling, barbel, fin, gill and blood were screened. Majority of the enzyme systems could be screened using these tissues without killing the fish. This technique is also being standardized for Indian major carp.

Using ultrathin (0.1 mm) isoelectric focussing of eyelens protein (IEF-EL), species specific profiles of *L. rohita, L. bata, C. carpio, C. mrigala* and *C. catla* could be obtained. Though the over all number of bands did not very significantly (24 to 27), there were variations between species with regard to position of bands and even in those bands having common isoelectric focussing point, the intensity varied greatly giving an over all distinct pattern for each species. In *L. rohita* polymorphism could be detected in

natural populations from Rapti and Ganga river systems. Differences were observed in the common carp stocks of Ooty and West Bengal in their IEF-EL profile indicating that these two stocks are genetically different. Haemoglobin IEF of nine species including the carps rohu, mrigala and common carp revealed multiplicity of bands and a species specific pattern. Here, IEF has an edge over normal PAGE, which can resolve only one common haemoglobin band for three Indian major carps. This study indicate that haemoglobin IEF like IEF-EL can serve as good genetic marker for scoring intra population differences also. For identifying DNA markers in fish, Indian laboratories have started working with restriction fragment length polymorphism (RFLP) and PCR based DNA fingerprinting techniques.

Future Projection

With the successful development of many isozyme and isoelectric focussing markers NBFGR has initiated studies to genetically characterise different geographic wild populations of rohu. In collaboration with CIFA, the founder stocks used in the cross breeding and selection programmes of rohu are also being genetically typed. Having realised that chromosomal banding technique will require metaphase spread from cultured cells, facilities for cell culture have been established at NBFGR and studies have been initiated. For fish genetic characterization, the chromosome painting technique of FISH may need to be utilized. Under the genetic fingerprinting centre set up at NBFGR priority has been given to the study of Indian major carps.

Gene Banking

Present Status

At NBFGR sperm cryopreservation protocols have been developed for nine species including the three Indian major carps *L. rohita, C. mrigala, C. catla* and exotic *Cyprinus carpio.* The studies with nine species have that established the optimum requirements with respect of extender, activation media, dilution rate, activation period, sperm egg ratio differ between species. Through modification of the cryopreservation protocols it has been possible to enhance the hatching percentage from 10% in the initial years to 77% with rohu cryopreserved milt during recent trials. Stocks of *L.*

rohita milt cryopreserved in 1988 are still being maintained alongwith other species in a mini gene bank at NBFGR. Viable hatchlings have been produced from five year old rohu cryopreserved milt and from one year old milt of deccan mahseer, golden mahseer, common carp and rainbow trout thereby establishing the long term viability of the technique developed. The hatchlings did not exhibit any abnormality. For *Cyprinus carpio,* cryopreserved milt as a vehicle for transfer of wild genome into hatchery stocks. The cryopreservation methodology developed by NBFGR have been tested under the actual field conditions for cross breeding programme. Cross breeding of farmed common carp at Bilaspur (H.P.) with cryopreserved sperms collected from wild common carp stock of Ooty and Rewalsar were carried out, for determining the population which can outperform the local stock. Results indicate that Rewalsar stock is the superior performing population. Cross breeding of slow growing rainbow trout at Nilgiris (Tamil Nadu) with fast growing stock at Barot (H.P.) through cryopreserved sperm as a mean for transfer of germplasm has also been carried out. The ionic compositon of seminar and variation fluid of rohu and mrigal have been studied with the objective of providing basic information that can be used to fashion new extenders and spermatozoa motility inducing media. Experience at NBFGR, clearly establishes that gene banking through sperm cryopreservation can be viable option for long term storage of germplasm. It is possible to utilize such facilities to alleviate milt related problems, introducing genetic variability into hatchery stocks from wild and cross breeding between discrete and distant populations.

With respect to cryopreservation of embryos, cryoprotectant toxicities have been worked out in two embryonic stages of *Penaus monodon.* Success has been achieved in getting viable larvae of *P. indicus* frozen to - 40°C and of *P. monodon* that have been frozen to - 30°C. The thawed *P. monodon* larvae underwent further development stages without any abnormality. With embryos of common carp and rohu safe level of cryoprotectants have been determined and studies on vitrification and cryopreservation are in progress.

With the successful development of milt cryopreservation protocols on the one hand and successful embryo cryopreservation technique still at experimental level it has become necessary to develop methodology for retrieval of genome from cryopreserved spermatozoa. With the chromosomal engineering technique of

androgenesis, it would be possible to develop a full diploid individual from haploid spermatozoa without maternal genome contribution. Androgenetic experiment have been carried out with common carp and Indian major carps. With common carp, putative androgenetic individual have been obtained. With cryopreserved milt of common carp also, this experiment has been successful. In the case of Indian major carps, a high variability has been observed in the sensitiveness of eggs to UV radiation used for inactivation of maternal genome and consistant results have not so far been achieved.

Future Projection

With the successful development of protocols for long term cryopreservation it will be possible to upgrade the mini gene bank of NBFGR into a National facility for gene banking of fish gametes, embryos and DNA material. This will serve as a repository of wild stocks as well as improved strains. Research for expansion and improvement of gene banking technology will also be undertaken. Based on the Norwegean gene bank programme for Atlantic Salmon adequate samples will be collected from wild stocks of all cultivable carp species.

III Threats Faced

Aquatic resources face several stresses not only from development projects like dam construction, water diversion, pollution and physical degradation of habitat, but also from fishing and aquaculture industry itself like destructive fishery, over harvest and selective harvest, transplantations and introductions of non endemic species and escape/ranching of farmed stocks. Though a number of papers have discussed these stresses faced by Indian fish germplasm resources, the threat due to scape/ranching of farmed stocks have not been highlighted and this aspect is covered in this paper.
Impact of Hatchery Stocks on Wild Fish

In other countries serious concern has been felt about the negative effects of escaped farmed fish on wild stocks. Studies have clearly shown that due to hatchery breeding and rearing practices, the genetic variation and life history traits of farmed fishes differ considerably from wild fish. Through biochemical genetic markers, erosion

of genetic drift has been observed in hatchery stocks. Brood stocks maintained for hatchery operation are subject to artificial selection for production traits or unintended selection at the time of establishing founder stocks or during domestication. Selection for specific traits generally results in reduced genetic variance and fitness.

Due to massive introduction of cultured fish, displacement of wild stocks have been reported and high levels of introgression of the altered hatchery fish genome into wild stocks has also taken place Escape of farmed fish and inter breeding with wild population can result in decreased fitness of wild stocks. Releasing of genetically marked hatchery brown trout stock has clearly established introgression of hatchery stocks with wild stock and reduced survival of these stocks. Though outbreeding results in heterosis or hybrid vigour, if the genetic differences between two population is considerable it can result in outbreeding depression. One of the genetic mechanisms operating, is outbreeding depression which results in loss of local adaptation or break down of coadapted genes at different loci. Overall studies have clearly indicated that introgression of hatchery genome into wild stocks can have serious implication for long term survival of wild stocks.

In the Indian context specific studies to show the genetic consequences of hatchery stocks on wild fish is lacking. However, studies have clearly shown that Indian hatchery stocks differ greatly from wild stock. On screening of *L. rohita* fingerlings collected from hatchery and wild, biochemical genetic differences could be observed. These results indicate that the genetic structure of hatchery stocks and wild were different. Studies on hatchery stock of Indian major carps from Karnataka has clearly indicated that due to hatchery operations, inadvertant selection for certain traits can taken place in Indian hatcheries. A decline in fertility of hatchery bred rohu has also been reported. Inbreeding is Indian hatchery stocks has also been quantified.

Genetic introgression in farmed common carp stocks of Bilaspur (H.P.) has been detected using isozyme markers. The result pinpoint the contamination of common carp brood stock with gold fish genome, which could have been due to faulty breeding practices at the farm. Using three species specific markers it was possible to differentiate hybrids of rohu and mrigala from their parents at the fry stage. Varying percentage of

introgression has been observed in different hatchery stocks of the Indian major carps. Using RFLP markers found 10% introgression in one hatchery stock. Inadvertent hybridization (8.3%) of Indian major carps in Amethi hatchery due to mixed spawning has been reported.

The production and distribution of rohu hybrids called 'NATHAN' by private seed producers for aquaculture operation all over the country also adds to the risk of genetic contamination of wild stock due to such introgressed farm stocks. Many State Governments have ongoing programme of stocking reservoirs with hatchery bred seed of Indian major carps. Also many programmes for river ranching of Indian major carps is also being prepared. Studies carried out at NBFGR indicate that the level of introgression is different between hatcheries and ranching programme can threaten the genetic purity and variability of wild stocks. Also by stocking rivers with hatchery seed from brood stock originating from a different geographic area there is danger of homogeneisation of natural genetic diversity. The immediate requirement is developing a breeding strategy that can be easily applied in commercial hatcheries to avoid inadvertent selection, inbreeding and introgression. Using genetic markers developed by NBFGR it would be possible to screen out introgressed brood stock. Seed for ranching operation should be derived from wild stocks of that particular geographic area to avoid dilution of natural genetic variation.

Conclusion

Most of the conservation efforts are targeted at the level of ecosystem or species. Based on the genetic variation within and between population, a species has the capacity to adopt to varying environmental conditions. Also the genetic variability within a species forms the foundation on which genetic upgradation programmes can be based. Therefore, there is urgent need to fulfill the prerequisites outlined in this paper and also to take effective steps to safeguard against negative genetic consequence of escaped farmed fish.

FISH MOLECULAR BIOLOGY AND FISHERY GENETICS RESEARCH AT BOSE INSTITUTE : AN OVERVIEW

B.K. Padhi and **R.K. Mandal**
Department of Biochemistry, Bose Institute,
Calcutta -700 054, India

To develop genetic technology for improving the aquaculture productivity, it is imperative to have a basic knowledge on (i) the organisation of fish genome, (ii) finding out appropriate molecular markers for stock,species and hybrid identification, (iii) basic idea regarding gamete biology , (iv) the state of art of breeding and gamete/embryo preservation. I shall highlight the contribution of my laboratory on some aspects of fish molecular biology and fishery genetics, which are expected to further the cause of aquaculture genetics in India.

(a) Fish Molecular Biology

Being a biochemist by profession I was trying to address a basic question during 1970s using *H. fossilis* as a model organism : Does the catfish embryo transcribe its genes during early development or it depends on the maternal mRNA already present in the egg ?". Our investigatiion with *H. fossilis* embryo revealed that the developing embryoes depend on maternally acquired mRNA already present in the egg till late blastula . The changing pattern of RNA synthesis and RNA polymerase were also studied. Our other interest in those days was to use protamine - a basic protein, present in the chromatin of sperm for zoogeography and chemosystematic analysis. We studied about 26 species of fishes from freshwater esturine and marine habitat. The fishes living in saline water possess protamine in their sperm, while the exclusively freshwater species lack protamine. We proposed that fishes probably evolved in the freshwater and later radiated to the saline habitat.

In the early eighties molecular biology began to dominate biochemical research. People were interested in isolation of genes and study of their regulation. Since we were studying ribosomal RNA in *H. fossilis* embryo, we went for cloning its rRNA gene,which was characterized and mapped. The ribosomal RNA gene clone was used as a RFLP marker for systematics studies. Non-coding DNA sequences in the fish genome are of interest to understand about fish genome . Therefore, we also cloned a higly repetetive DNA sequence in *Cyprinus carpio* and studied its genomic organisation and quantified its genomic proportion. This DNA sequence can be used as a molecular weight marker. It was the first report of its kind in the world on fish.

(b) Fishery genetics

In 1990s our attention was drawn to some applied aspects. The decline of the populations of the Indian major carps (IMCs) in natural waters is a mattter of great concern. The direct impact is the loss of genetic diversity , which would hinder the future selective breeding programe. Thus,we undertook mitochondrial DNA polymorphism analysis for population characterization (stock identification) although in a limited scale. Since mitochondrial DNA has a small size (16-21 kb), show higher rate of mutation, inherited maternally without recombination, it is a good parameter for stock identification. Our limited collection from the riverine sources and fish farm showed polymorphism of mt-DNA in rohu at HindIII site.

Improper breeding practice like mixed spawning in carp hatchery is also a matter of concern. Since the hybrids of the closely related species of IMCs are fertile and are capable of interbreeding with their parental species, the genetic contamination due to genetic introgression is highly probable. We have demonstrated by nuclear DNA RFLP analysis that hybridization do occur in carp hatchery due to the practice of mixed spawning and estimated the approximate frequency of hybridization. Our concern of genetic contamination and loss of genetic diversity in the carps arise because the hatchery populations are recruited to natural waters and as such the natural habitat is also promoting greater hybridization rate due to environmental changes. In order to demonstrate that genetic introgression is taking place and quantifying the amount of introgression, we recently have found out a satellite DNA probe in *C. mrigala*. The *Mbol*

satellite DNA in this species was identified, cloned, characterized, sequenced and its genomic abundance analysed. It is 266 base pair in length (Genebank/EMBL database No.Y13109) having clusters of A's and T's and some direct and invereted repeats. This satellite DNA is present in high copy number (= 10^5) and is inherited uniparentally in the inter-generic hybrids of Indian major carps. We are now exploring its use as a marker for quantifying gene introgression in F2 crosses to demonstrate genetic introgression and as a marker for quantifying geneflow between these species.

We have produced reciprocal hybrids of catfishes, *H. fossilis* and *C. batrachus* by induced breeding. All the progeny of these reciprocal crosses closely resemble to one of the parents, *C.batrcahus*, in external morphology. But molecular and karyological analysis revealed that they were true hybrids, not developed by gynogenesis or androgenesis. Since these two species belong to two different families and are conspiciously distinguishable in their external morphology, anatomy and chromosome number, their reciprocal hybrids would be interesting models for genetic studies. Recently, we have cloned a MboI satellite DNA in *C. batrachus*, (Genebank/EMBL database No.Y13110) which was also found in a low dosage in *H. fossilis*. The F1 reciprocal hybrids inherit the MboI satellite biparentally. Since hybridization of distantly related species produce true hybrids along with gynogenetic offspring sometimes. This MboI satellite would be useful to score if gynogenetic offspring arise due to crossing between *C. batrachus* and *H. fossilis*.

We have developed a simple freezing protocol for spermatozoa cryopreservation in *H. fossilis* and *C. batrachus*. This methodology is simple,easy to adapt and can be useful in many ways:i) for the artificial propagation of these species by selective breeding,ii) ex situ germplasm conservation in a sperm bank and iii) would assure the availiability of milt during artificial breeding.