

2008 - Silver Jubilee Year of NBFGR

वार्षिक प्रतिवेदन
Annual Report
2007-2008



राष्ट्रीय मत्स्य आनुवंशिक संसाधन ब्यूरो, लखनऊ
(भारतीय कृषि अनुसंधान परिषद्)

National Bureau of Fish Genetic Resources, Lucknow
(Indian Council of Agricultural Research)

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PREFACE

The issues of fish diversity, conservation and sustainable utilization have assumed great significance in recent years as there is global concern of biodiversity loss. The National Bureau of Fish Genetic Resources has been continuously providing sound empirical inputs towards aquatic germplasm conservation and management in the country and has emerged as a Centre of Excellence for research related to fish taxonomy, DNA barcoding, database development, genetic characterization, genomics, gene banking, registration of germplasm, *in situ* conservation, evaluation of exotic aquatic species and molecular diagnostics of exotic pathogens.

The year 2007-2008 has been eventful in several ways. The database on finfish diversity of India was updated by adding information on 47 more species. The work on developing an electronic database on the diversity of marine ornamental fauna and shellfish species of Indian waters was strengthened. A new programme on designing and development of digital fish taxonomic database for an easy identification of indigenous as well as alien fish species was undertaken. This work will help in proper identification of fish species which is required to assess the aquatic biodiversity and develop conservation strategies for facilitating scientific management of the resources.

Under the DNA barcoding programme of fish species, DNA barcodes were prepared for 180 species and a total of 395 DNA sequences of 110 species were submitted to NCBI Gene bank. Genetic divergence studies in prioritized marine finfish and shellfish species provided very encouraging results. A significant achievement was achieved by constructing microsatellite enriched genomic library for *Pangasius pangasius* to identify sequences containing microsatellite repeat regions.

The taxonomic ambiguity of the ornamental nandid fishes, endemic to the western ghats, namely *Pristolepis marginata* and *P. fasciata* was resolved using partial sequence information of 16S rRNA gene. Species-specific diagnostic molecular markers were developed to distinguish different species of *Trichodesmium* from Indian waters. A new research work was initiated to assess the diversity of seahorse species in the Indian waters and their molecular identification for conservation purposes. Cytogenetic characterization was completed in ten freshwater and marine fish species. The Amplified Fragment Length Polymorphism studies undertaken in *Tor putitora*, *T. tor* and *Rita rita* indicated that this technique has great potential for precise characterization and identification of fish species.

An interesting work was undertaken to investigate and evaluate the current status and changing scenario of freshwater fish diversity, richness, distribution and life history attributes of the threatened fishes, conservation status and priority habitat of the identified areas of Ganga basin. The outcome of the present study would be useful in developing criteria for planning fish and habitat restoration, management of potential aquatic zones and creating conservation areas within Ganges river basin and other large rivers. New size records of three species, *Ompok pabda*, *Cirrhinus reba* and *Glossogobius giuris* were recorded from river Tonse, a tributary of river Ganga and lower stretches of river Ganga at Farakka. A work programme for fish germplasm exploration, assessment, cataloguing and conservation in the North Eastern Region was strengthened through collaborations involving local universities, Institutes, NGOs, etc.

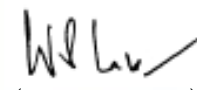
Under the *ex situ* conservation programme, a total of 401 tissue accessions were added and 200 voucher specimens from 135 marine teleosts were collected and preserved. In collaboration with Department of Fisheries, Guwahati, Govt. of Assam, a live gene bank was established at Ulubari Fish Seed Farm, Guwahati. A new study on the pathogenic fauna of selected marine and freshwater ornamental fishes was undertaken. This work aimed at exploring and documenting the pathogens of ornamental fishes and developing rapid molecular assays for identification of selected parasites for eventually helping in quarantine and health certification of ornamental fishes in the country. A programme was undertaken to elucidate the association of virus etiology in different diseases of koi carps so that capacity development can be done to fight the occurrence of future epizootics in the country. The diagnostics will be used for screening and health certification of the fish stock in freshwater aquaculture systems of the country. The work on targeted active surveillance of penaeids for three OIE-listed viruses namely, Monodon Baculovirus, White Spot Syndrome Virus and Yellow head Virus in selected maritime states of India and accurate and specific isolation and characterization of *Flavobacterium* spp. was strengthened.

The Institute organized the First International Training on “DNA Barcoding of Marine Life” in which 18 participants including foreign nationals from Kenya, Tanzania, South Africa, Canada and Australia participated. Besides, several other trainings and workshops including an ICAR-sponsored summer school on “Fish Biotechnology”, a training course on “Cellular and Molecular Approaches for Genotoxicity Assessment in Fishes”, a training on “Basic Tools in Molecular Biology Research” and four short-term training programmes for the benefit of the aqua-farmers, a workshop on “Fisheries Conservation and Enhancement: Linking Researchers and Stakeholders” at Guwahati, Assam and an awareness workshop on “Conserve Fish for Posterity” at Rajiv Gandhi University, Itanagar, Arunachal Pradesh, were organized.

A number of new facilities including a Fish Seed Production Unit were established under the Mega Seed Project of the ICAR and a new Cell Culture laboratory was developed at the Institute. The NBFGR promoted a concept of ‘State Fish’ for each state integrating conservation research and the stakeholders. The Institute signed a MoU with Babasaheb Bhimrao Ambedkar University, Lucknow to develop and share expertise and facilities at mutually agreeable terms and conditions. The Institute published 27 research papers in peer-reviewed journals and was able to generate a revenue of Rs. 13.15 lakhs against a target of Rs. 12 lakhs.

This report presents a summary of the achievements of team NBFGR during 2007-08 where all the scientists and staff of the Institute have strived hard towards achieving their targets.

I am deeply indebted to Dr. Mangala Rai, Secretary, DARE and Director General, ICAR, New Delhi for the visionary leadership and support. I am also grateful to Dr. S. Ayyappan, DDG (Fisheries), ICAR for the continued guidance and encouragement. I place on record my sincere thanks to Dr. V.V. Sugunan, ADG (Inland Fisheries) and other staff members of the SMD for their cooperation and help in our endeavours.


(W.S. Lakra)
Director

EXECUTIVE SUMMARY

The National Bureau of Fish Genetic Resources (NBFGR) was established in December 1983 in rented premises at Allahabad under the aegis of Indian Council of Agricultural Research to undertake research related to the conservation of fish germplasm resources of the country. The Bureau's permanent infrastructure was developed at Canal Ring Road, Telibagh, Lucknow, U.P. in 1999 comprising an administrative block, laboratories, farm and residential complex covering an area of 52 acres. The Bureau has created excellent infrastructure and expertise in several research areas including development of fish databases, genetic characterization, gene banks, fish germplasm and habitat inventory, risks analysis of exotic species, diagnostics for OIE notified pathogens, aquatic microbes and other areas of germplasm conservation with special focus on threatened, prioritized and exotic fish species. During the year under report, the research activities were conducted through 21 Institutional and 13 externally funded projects.

The existing database on finfish diversity of India was modified, restructured and updated by including additional information to the designated fields. The database now consists of 2243 finfish species. Besides, the information available on 291 exotic fish species reported from India was also included in the database. The information on 47 more species was added to the database, out of which, nine were newly reported species namely, *Pseudolaguvia ferula*, *Puntius ater*, *Puntius khugae*, *Channa aurantimaculata*, *Channa bleheri*, *Gerres phaiya*, *Psenopsis intermedia* and *Rita macracanthus*. During the period under report, 140 images of the fishes of Western Ghats and 10 images of the threatened fishes of India were added to the database. At

present the database contains a total of 5878 images.

Data on 30 new species of freshwater fishes reported from the Western Ghats were collected and added to the database. A consolidated list of the diversity of fishes from inland waters of Kerala was prepared which included 259 species. A new module was designed and added in the software in which all habitats of fishes have been categorized into freshwater, brackishwater and marine water. The state-wise revised lists of freshwater fishes for north-eastern, central and western states of India were prepared. Secondary data was collected on aquatic and fishery resources of Uttar Pradesh. Detailed primary data on fish distribution was also collected through extensive survey in 22 districts of the state. A total of 87 species were recorded during field survey in Uttar Pradesh.

The work on developing an electronic database on the diversity of marine ornamental fauna and shellfish species of Indian waters was continued. Checklists of 350 species of marine ornamental fishes, 1655 molluscan species, 923 crustacean taxa and 43 echinoderm species of the Indian waters were prepared. The checklist, prepared for crustacean included 424 species of crabs, 300 species of shrimps and lobsters, 155 taxa of hermit crabs and 43 species of stomatopods. The information pertaining to 450 crustacean species was collected. Information on the marine ornamental fish species, with details of taxonomy, nomenclature, image, bibliography and geographic information on occurrence and distribution etc. was incorporated in the database. Voucher specimens of ornamental fishes (50) and molluscs (60) were collected from Andaman and Lakshadweep Islands. The collected information was converted into digital

data format to develop information system using SQL server to facilitate information retrieval through websites.

A new work on designing and development of digital fish taxonomic database, to help in easy identification of indigenous as well as introduced alien fish species, was undertaken. This work will help in proper identification of fish species which is required to assess the aquatic biodiversity and develop conservation strategies for facilitating scientific management of the resources. The work was started with the fish species belonging to families under the order Clupeiformes (Clupeidae, Engraulidae) and Perciformes (Serranidae). The identification keys for these selected species were screened for choosing diagnostic features that would facilitate creating a path for digital identification. Such screened information was entered on trial basis in the SQL server 2000 database. Pathways were created for identifying selected species with the help of a unique feature or through sorting out cluster of features that tally with the profile in the back-end data.

A new work was initiated with an objective to develop the distribution and habitat preference model for sea cucumber species with special reference to *Holothuria scabra* for Gulf of Mannar.

Under the DNA barcoding programme of indigenous fish species, 925 tissue samples and voucher specimens of marine as well as freshwater fish species were collected from different parts of the country. The total DNA was isolated for 1427 samples of 410 species and PCR amplification was done for 733 samples of 280 species. DNA sequencing was done in 540 samples of 182 species. DNA barcodes were prepared for 180 species. A total of 395 DNA sequences of 110 species have been submitted to BOLD.

Under a programme for study of molecular

phylogeny of selected species of genus *Glyptothorax*, genomic DNA was isolated from 77 samples of three species. PCR amplification and DNA sequencing of cytochrome-c-oxidase-I and cytochrome-b gene region was done for 75 samples of three species. A total of 136 DNA sequences were submitted to NCBI GenBank (GenBank accession EU637404-EU637471 and EU637777-EU637844)

Genetic diversity analysis of *Labeo calbasu* was continued. A total of 105 primers of loci available for eight cyprinid species (resource species) were used for cross priming tests, with twelve specimens of *L. calbasu*. A total of 16 loci were found to be polymorphic, out of which nine polymorphic microsatellite loci were found suitable for assessing genetic variability across the natural range of distribution. A total of 45 samples of *L. calbasu* from Bhagirathi and Godavari were genotyped for each of the nine (*R1**, *R3**, *R12**, *Lr28**, *Lr 29**, *MFW11**, *Lro23**, *Lro 25** and *Lr 38**) microsatellite loci to assess genetic variation. No evidence of linkage disequilibrium was detected at any locus pair comparisons in any population. The results revealed that the identified loci are promising to genetic diversity analysis of wild populations of *L. calbasu*.

The work on taxonomic validation and phylogeny under the group Mahseer was continued using mtDNA markers. Allozyme studies were conducted for studying hybrid/morphotype of two Indian mussels, *Perna viridis* and *P. indica* along the Kerala coast. A total of 16 enzymes were used for initial screening. Eight of these enzymes, which were found to give scorable activity, were selected for extensive screening of green mussel, brown mussel and expected hybrid/morphotype. Three of the enzymes studied were polymorphic. An excess of homozygotes was observed at several loci in most of the individuals.

Microsatellite enriched genomic library was constructed for *Pangasius pangasius* to identify sequences containing microsatellite repeat regions. Out of the 25 microsatellite loci amplified, 9 were polymorphic, 3 monomorphic and 13 yielded unspecified products. A total of 42 individuals from Bhagirathi and Mahanadi were analysed with 9 polymorphic loci. Significant genetic heterogeneity ($P < 0.05$) was evident at three loci, Ppa02, Ppa05 and Ppa28.

In *P. pangasius*, slow evolving region of mitochondrial DNA, cytochrome oxidase I region was amplified using universal primers. Medium cytochrome b region of mtDNA was also investigated in *P. pangasius* to determine genetic variation. Gene diversity in samples of Bhagirathi river was 0.8583 ± 0.0626 while in Mahanadi samples it was 0.8889 ± 0.0910 and Nucleotide diversity (average over loci) were 0.004642 ± 0.003346 and 0.005248 ± 0.003877 , respectively.

Genetic divergence studies in prioritized marine finfish and shellfish species were continued. The genetic analysis of Bombay duck (*Herpedin nehereus*) from the northwest and northeast coasts of India using RAPD showed clear genetic differentiation with high overall G_{ST} (24.53%) and genetic distance (21.12%) values between both the populations. Five microsatellite markers were first developed in *H. nehereus*. Work on genetic characterization of a lobster (*Panulirus homarus*) with 80 samples using 8 polymorphic microsatellite loci and 10 Operon decamers indicated low genetic differentiation of the both species between east and west coasts. Successful cross-species amplification of 5 more microsatellite loci in *Holothuria scabra* using primers from Japanese sea cucumber (*Stichopus japonicus*) was achieved thus, developing a total of 10 polymorphic loci to be used in population genetic analysis of the species. Partial sequence information of 16SrRNA, CO I and Cyt b genes of three important endangered ornamental

species, *Gara surendranathanii*, *G. gotyla stenorhynchus* and *G. mullya* were generated. All the three genes exhibited species-specific genetic divergence values and sequence pattern. The sequences were submitted to NCBI GenBank.

The taxonomic ambiguity of the ornamental nandid fishes, endemic to the western ghats, viz. *Pristolepis marginata* and *P. fasciata* was resolved using partial sequence information of 16S rRNA gene. The work on genetic characterization of the harmful cyanobacteria, *Trichodesmium* spp. from Indian waters using molecular markers and scanning electron microscopy (SEM) was continued. Species-specific diagnostic molecular markers were developed to distinguish different species of *Trichodesmium* from Indian waters. All the samples studied were found to be *T. erythraeum*, based on partial sequence information of 3 genes and not the toxic species, *T. thiebautii*. A new work was initiated to access the diversity of seahorse species in the Indian waters and their molecular identification for conservation purposes

Cytogenetic studies were carried out in ten freshwater and marine fish species namely, *Rita rita*, *Aorichthys seenghala*, *Tor putitora*, *T. tor*, *T. khudree*, *T. mussullah*, *Nandus nandus*, *Arius subrostratus*, *Zanclus canescens* and *Thallasoma lunare*. RAPD studies were undertaken to identify genetic variations among three species of freshwater murrels namely, *Channa punctatus*, *C. striatus* and *C. orientalis*. The highest polymorphism was observed in *C. punctatus* followed by *C. orientalis* and *C. striatus*. The results showed a close genetic relationship between *C. striatus* and *C. orientalis*. Amplified Fragment Length Polymorphism (AFLP) studies were carried out in *Tor putitora*, *T. tor* and *Rita rita* using silver staining technique. The results indicated that this technique has potential for precise characterization and identification of fish species. Studies were undertaken to assess the genotoxic

potential of Quillaja Saponin, Chlorpyrifos and Rotenone and the ameliorative effects of turmeric (*Curcuma longa*) to reduce the DNA damage in fishes. The gene expression may get altered in response to pollutants and can be used as a biomarker of genotoxic exposure in fishes. Therefore, studies were initiated for cDNA synthesis of two genes *viz.* metallothionein and cytochrome p450 that play important role in detoxification of heavy metals and polycyclic aromatic hydrocarbons, respectively.

Under the *in situ* conservation programme, a new work was initiated to investigate and evaluate the current status and changing scenario of freshwater fish diversity, richness, distribution and life-history attributes of the threatened fishes, conservation status and priority habitat of the identified areas of Ganga basin. The outcome of the present study would be useful in developing criteria for planning fish and habitat restoration, management of potential aquatic zones and creating conservation areas within Ganges river basin and other large rivers. A total of 96 species belonging to 58 genera of 24 families were recorded from different stretches of river Ganga. New size records of three species (*Ompok pabda*, *Cirrhinus reba* and *Glossogobius giuris*) were recorded from river Tonse, a tributary of river Ganga and lower stretches of river Ganga at Farakka. New biogeographical shift of some of the species was observed in the upper stretches of river Ganga. These species were *Macrornathus aral*, *Cyprinus carpio*, *Cyprinus carpio*, var. *specularis*, *Aorichthys aor*, *Puntius sarana* and *Ompok pabda*. This shift may be due to rise in maximum temperature in upper Himalayan stretch making it a conducive habitat for the warm water fishes. Several threats to fish diversity and habitat were observed throughout the stretches of river Ganga.

A new work for fish germplasm exploration, assessment, cataloguing and conservation in north-eastern region was initiated in

collaborative mode involving local collaborators from the region. The study on status and role of temple sanctuaries in conservation of freshwater biodiversity was continued by covering six selected religious sites on the bank of river Gomti, Uttar Pradesh. It came out from this study that most of these religious sites with a temple management set-up, a history of protection and a deep pool, are ideal for conservation purposes. These sites need recognition by the state agencies as protected or religious waters for conservation of aquatic animals under custody of local institutions like Gram panchayats/Nyaya panchayats/or religious bodies or their cooperatives with proper terms and conditions.

Studies were continued to assess the potential of fishing cooperative societies to utilize them for conservation of fishery resources. The findings indicated that though the fishing cooperative societies existed at all the locations, they played important role and contributed in resource conservation at those locations and under those situations where some of the facilitating factors/conditions were present. These conditions were high level of orientation of fisherfolks towards resource conservation, effective internal functioning of societies, effective structural and functional linkages with and high perceived effectiveness of state fisheries agencies, strong controlling and regulatory powers held and exercised, and facilitating role played by the state fisheries dept.: effective linkages with, and regular support of, NGOs, and strong collective action and mass organization for common good. The policy makers, fishery administrators and conservation professionals can utilize these findings towards formulating strategies for harnessing organizational and institutional support for promoting fish conservation and resource enhancement programmes at the grass roots level.

Under the gene banking programme, the work on milt cryopreservation of the two selected

endangered fishes of the Western Ghats, *Horabagrus nigricollaris* and *Garra surendranathanii*, was continued by conducting more experiments and fertility trials. A total of 401 tissue accessions were made and 200 voucher specimens from 135 marine teleosts were collected and preserved. In collaboration with Department of Fisheries, Guwahati, Govt. of Assam, a new live gene bank was established at Ulubari Fish Seed Farm, Guwahati. Seed production of selected carps, catfish and endangered fish species was taken up at Aquaculture Research and Training Unit of NBFGR at Chinhat, Lucknow.

A new work to study the pathogenic fauna of selected marine and freshwater ornamental fishes was undertaken. This work aims at exploring and documenting the pathogens of ornamental fishes and developing rapid molecular assays for identification of selected parasites for eventually helping in quarantine and health certification of ornamental fishes in the country. Another study was undertaken to elucidate the association of virus etiology in different diseases of koi carps so that capacity development can be done to fight the occurrence of future epizootics in the country. The diagnostics will be used for screening and health certification of the fish stock in freshwater aquaculture systems of the country. Positive DNA controls of Koi Herpes virus (KHV) were used to develop diagnostic test using three sets of OIE referred primers. Serum samples from 28 koi carp were collected for detection of anti-KHV antibodies by ELISA. All the three primers resulted in sensitive and specific detection of the KHV in the control DNA samples. All the tested samples were negative for presence of Koi Herpes virus by PCR.

The work on targeted active surveillance of penaeids for three OIE-listed viruses namely, Monodon Baculovirus (MBV), White Spot Syndrome Virus (WSSV) and Yellow head Virus (YHV) in selected maritime states of India was

continued. Out of 238 shrimp and post-larvae samples, only 21 samples were positive for WSSV by PCR, 11 samples were positive for MBV, whereas none of the samples were positive for YHV complex. As the accurate and specific isolation and characterization of *Flavobacterium* spp. is important for fish disease diagnosis and health management, a study was undertaken to isolate and characterize *Flavobacterium* spp. from fish, sediment and pond water samples. Out of a total of 41 presumptive isolates of *Flavobacterium*, 7 isolates were confirmed as *Flavobacterium* spp. The study indicated significant genetic diversity between the isolates of *Flavobacterium*. Studies on development and characterization of cell lines from *Labeo rohita* and *Epinephelus merra*, and virus isolation studies on established cell lines of *Cyprinus carpio* undertaken during the year, gave encouraging results. Distribution of exotic fishes and their evaluation for reproductive performance in selected stretches of Ganga river system was studied.

The Institute organized the First International Training on “DNA Barcoding of Marine Life” in which of 18 participants including foreign nationals from Kenya, Tanzania, South Africa, Canada and Australia participated. Besides, an ICAR-sponsored summer school on “Fish Biotechnology”, a training course on “Cellular and Molecular Approaches for Genotoxicity Assessment in Fishes” and a training on “Basic Tools in Molecular Biology Research” were organized. The Bureau, at its Aquaculture Research and Training Unit, Chinhat organized four short-term training programmes sponsored by National Fisheries Development Board, Hyderabad on: “Quality Fish Seed Production and Hatchery Management”; “Integrated Aquaculture and Fish Disease Management”; “Aquaculture Diversification and Impact of Exotic Species” and “Freshwater Prawn Culture Technology”. These

programmes were organized for the benefit of the aqua-farmers of Uttar Pradesh. The Institute also organized a workshop on “Fisheries Conservation and Enhancement: Linking Researchers and Stakeholders” at Guwahati, Assam and an awareness workshop on “Conserve Fish for Posterity” at Rajiv Gandhi University, Itanagar, Arunachal Pradesh.

Dr. Mangala Rai, Secretary, DARE and Director General, ICAR, New Delhi visited NBFGR and inaugurated the newly constructed Fish Seed Production Unit at the Institute. This hatchery unit has been established under the Mega Seed Project of the ICAR with an aim to produce quality seed of commonly cultured fish species, especially Indian major carps and making them available to the fish farmers of the region. A new Cell Culture Facility was also developed at the Institute to strengthen the work on development of successful cell culture systems for selected fish species.

The meetings of Research Advisory Committee and Staff Research Council were successfully organized. A total of 16 guest lectures by various experts were organized at the Institute. Dr. W.S Lakara, Director was conferred with “Dr. M.S Swaminathan Best Indian Fisheries Scientist Award 2007”. A MoU was signed between NBFGR, Lucknow and Babasaheb Bhimrao Ambedkar University, Lucknow to develop and share expertise and

facilities at mutually agreeable terms and conditions. The NBFGR promoted a new concept of a ‘State Fish’ for each state linking and integrating conservation, research and the stakeholders.

The Institute published 27 research papers in peer-reviewed journals, contributed 19 chapters to books/proceedings and one popular article in a national magazine. Besides, NBFGR scientists also submitted 12 abstracts for various seminars and workshops in different parts of the country. The Institute also bought out 7 publications in the form of books. Against a target of Rs. 12 lakhs, the Bureau generated revenue of Rs. 13.15 lakhs during the year under report.

The NBFGR library added 439 documents comprising 216 books, 164 serials and 59 annual reports. The library subscribed to 34 international, and 59 Indian current journals. In addition to these, 46 current journals were received on gratis/exchange basis. The library also subscribed to 110 electronic journals of Blackwell Publishing.

A function was organized on September 14, 2007 to celebrate the Hindi Divas. The Institute also observed a Hindi Pakhwada during September 15-29, 2007 during which seven Hindi competitions were organized among the staff of the Institute to promote the use of Hindi in official work.

कार्यकारी सारांश

राष्ट्रीय मत्स्य आनुवंशिक संसाधन ब्यूरो की स्थापना, भारतीय कृषि अनुसंधान परिषद् के अन्तर्गत सन् 1983 में, देश के मत्स्य आनुवंशिक संसाधनों के विवेकपूर्ण प्रबंधन एवं संरक्षण हेतु वैज्ञानिक सूचनाएं उपलब्ध कराने के लिए की गई थी। ब्यूरो का स्थाई भवन कैनाल रिंग रोड, तेलीबाग, लखनऊ में 1999 में तैयार हुआ जिसमें एक प्रशासनिक खण्ड, प्रयोगशालाएं, फार्म तथा निवास खण्ड सम्मिलित हैं जिनका कुल क्षेत्रफल 52 एकड़ है। ब्यूरो ने बहुत से शोध क्षेत्रों में उत्कृष्ट ढाँचागत सुविधाएं तथा विशेषज्ञता सृजित की है जिनमें सम्मिलित हैं : मत्स्य डेटाबेसों का विकास, आनुवंशिक चरित्र-चित्रण, जीन बैंक, मत्स्य जननद्रव्य एवं वासस्थल सूचीकरण, विदेशागत प्रजातियों की रिस्क का विश्लेषण, ओआईई द्वारा चिन्हित रोगजनकों की डायगनोस्टिक्स, जलीय सूक्ष्मजीव तथा जननद्रव्य संरक्षण से जुड़े अन्य क्षेत्र। इस प्रतिवेदन की अवधि के दौरान 21 संस्थान द्वारा पोषित तथा 13 वाह्य संस्थाओं द्वारा वित्त-पोषित परियोजनाओं के माध्यम से शोध कार्य चलाए गए।

भारतीय फिनफिश विविधता डेटाबेस को परिवर्तित व पुनः संरचित करके उसका अद्यतन किया गया। डेटाबेस में अब 2243 फिनफिश प्रजातियों के बारे में सूचनाएं सम्मिलित हैं, इसके अलावा भारत से रिपोर्टेड 291 विदेशागत मत्स्य प्रजातियों की सूचनाएं भी डेटाबेस में सम्मिलित हैं। इस प्रतिवेदन की अवधि के दौरान 47 और प्रजातियों के बारे में सूचनाएं डेटाबेस में सम्मिलित की गईं जिनमें से 9 नई रिपोर्ट की गई प्रजातियां थीं। इसी अवधि में पश्चिमी घाटों की मछलियों के 140 चित्र तथा संकटग्रस्त मछलियों के 10 चित्र भी डेटाबेस में

जोड़े गए जिसके पश्चात् डेटाबेस में मछलियों के कुल चित्रों की संख्या 5878 हो गई। पश्चिमी घाटों से रिपोर्ट की गई 30 नई मीठाजल प्रजातियों के आंकड़े एकत्र करके डेटाबेस में सम्मिलित किए गए। केरल के अन्तर्स्थलीय जलों की मत्स्य विविधता की एक विस्तृत सूची तैयार की गई जिसमें 259 प्रजातियां सम्मिलित हैं। उत्तर-पूर्वी, केन्द्रीय तथा पश्चिमी राज्यों की मीठाजल मछलियों की संसोधित राज्य-वार सूचियां तैयार की गईं। उत्तर प्रदेश के जलीय एवं जलकृषि संसाधनों के बारे में द्वितीयक सूचनाएं एकत्र की गईं। उत्तर प्रदेश के 22 जिलों में विस्तृत सर्वेक्षण द्वारा मत्स्य वितरण पर प्राथमिक आंकड़े एकत्र किए गए। उत्तर प्रदेश में क्षेत्र सर्वेक्षण के दौरान 87 मत्स्य प्रजातियां अभिलेखित की गईं।

भारतीय जल की समुद्री सजावटी मत्स्य प्रजातियों तथा शैलफिश प्रजातियों का एक इलेक्ट्रॉनिक डेटाबेस बनाने का कार्य जारी रखा गया, जिसके अन्तर्गत समुद्री सजावटी मछलियों की 350 प्रजातियों, 1655 मोलस्कन प्रजातियों, 923 क्रस्टेसियन प्रजातियों तथा 43 एचिनोडर्म प्रजातियों की चेकलिस्ट तैयार की गईं। कुल 450 क्रस्टेसियन प्रजातियों के बारे में सूचनाएं एकत्र की गईं। समुद्री सजावटी मत्स्य प्रजातियों के वर्गीकरण, नामकरण, चित्रों, संदर्भों, वितरण इत्यादि से सम्बन्धित सूचनाएं डेटाबेस में सम्मिलित की गईं। सजावटी मछलियों तथा मोलस्कस के क्रमशः 50 व 60 वाउचर स्पेशीमेन्स अन्डमान तथा लक्षद्वीप द्वीपों से एकत्र किए गए। इन सभी सूचनाओं को डिजिटल फार्मेट में परिवर्तित किया गया।

देशज तथा बाहर से प्रवेश कराई गई विदेशागत

मत्स्य प्रजातियों की पहचान करने में मदद हेतु, डिजिटल फिश टेक्सोनोमिक डेटाबेस की डिजायन व विकास का एक नया कार्य आरम्भ किया गया। आरम्भिक प्रयास में क्लूपीफारमेस तथा पर्सीफारमेस गणों के चुने हुए परिवारों की प्रमुख विशेषताओं पर एकत्रित सूचनाओं का प्रयोग किया गया है। कुछ चुनी हुई मत्स्य प्रजातियों हेतु पहचान कुंजियों को चिन्हित किया गया।

भारत की मत्स्य प्रजातियों के डीएनए बारकोडिंग कार्यक्रम के अन्तर्गत, देश के विभिन्न भागों से मीठाजल एवं समुद्री मत्स्य प्रजातियों के 925 ऊतक नमूने एकत्र किए गए। कुल 410 प्रजातियों के 1427 नमूनों का डीएनए पृथक किया गया, 280 प्रजातियों के 733 नमूनों का पीसीआर एम्प्लीफिकेशन किया गया, 182 प्रजातियों के 540 नमूनों का डीएनए सीक्वेंसिंग किया गया तथा 180 प्रजातियों के डीएनए बारकोड तैयार किए गए। ग्लिटोथोरेक्स वंश की चुनिदां प्रजातियों की फायलोजेनी का आण्विक अध्ययन करने के लिए 77 नमूनों का जीनोमिक डीएनए पृथक किया गया, तीन प्रजातियों के 75 नमूनों का पीसीआर एम्प्लीफिकेशन व डीएनए सिक्वेंसिंग किया गया तथा 136 डीएनए सिक्वेंस एनसीबीआई जीनबैंक को सम्मिलित किए गए।

लैबियो कलबासु का आनुवंशिक विविधता विश्लेषण जारी रखा गया। आठ साइप्रिनिड प्रजातियों के लोसाई के कुल 105 प्राइमर्स को, लै. कलबासु के 12 नमूनों के साथ क्रॉसप्राइमिंग परीक्षणों हेतु प्रयोग किया गया। कुल 16 लोसाई पालीमारफिक पाए गए, जिनमें से 9 पालीमारफिक लोसाई, प्राकृतिक वितरण में आनुवंशिक विविधता का निर्धारण करने हेतु उपयुक्त पाए गए। भागीरथी तथा गोदावरी नदियों से लै. कलबासु के कुल 45 नमूनों को, प्रत्येक 9 माइक्रोसेटेलाइट लोसाई द्वारा जीनोटाइप किया गया। किसी भी जनसंख्या में किसी भी लोकस जोड़े पर लिंकेज डिसइक्युलिब्रियम का कोई

प्रमाण नहीं मिला। इन परिणामों से पता चला कि पहचान किए गए लोसाई इस प्रजाति के प्राकृतिक जनसंख्याओं में आनुवंशिक विभिन्नता का अध्ययन करने हेतु उपयोगी होंगे।

माहसीर समूह की प्रजातियों में वर्गीकरण, पुष्टीकरण तथा फायलोजेनी पर कार्य जारी रहा। केरल तट से एकत्र किए गए, भारत के दो मसैल्स, पर्ना विरडिस तथा पर्ना इंडिका के हायब्रिड्स/मारफोटाइप का एलोजाइम द्वारा अध्ययन किया गया। पंग्गसियस पंग्गसियस में माइक्रोसेटेलाइट रिपीट रीजनसक्युम्त सिक्वेन्स की पहचान करने हेतु माइक्रोसेटेलाइट एनरिचर्ड जीनोमिक लाइब्रेरी निर्मित की गई। कुल 25 एम्प्लीफाई किए गए माइक्रोसेटेलाइट लोसाई में से 9 पालीमारफिक, 3 मोनोमारफिक तथा 13 से अस्पष्ट पदार्थ प्राप्त हुए। भागीरथी तथा महानदी से इस प्रजाति के 42 नमूनों का 9 पालीमारफिक लोसाई द्वारा विश्लेषण किया गया जिसमें तीन लोसाई ($P_{pa}02$, $P_{pa}05$ तथा $P_{pa}28$) पर सार्थक ($P < 0.05$) आनुवंशिक विविधता पाई गई। इस प्रजाति के भागीरथी तथा महानदी से प्राप्त नमूनों में साइटोक्रोम आक्सीडेज I रीजन तथा माइटोकान्ड्रियल डीएनए के साइटोक्रोम बी रीजन का भी अध्ययन किया गया। भागीरथी नदी से प्राप्त नमूनों में जीन विविधता $0.8583+/-0.0626$ तथा महानदी से प्राप्त नमूनों में यह $0.8889+/-0.0910$ थी जबकि न्युक्लियोटाइड विविधता क्रमशः $0.004642+/-0.003346$ व $0.005248+/-0.003877$ थी।

प्राथमिकता – प्राप्त समुद्री फिनफिश व शैलफिश प्रजातियों में आनुवंशिक विविधता अध्ययन जारी रखे गए। उत्तर-पश्चिम तथा उत्तर-पूर्व तटों से प्राप्त बाम्बे डक (*हरपेडिन निहेरस*) के नमूनों में आरएपीडी द्वारा आनुवंशिक विश्लेषण करने पर दोनों में स्पष्ट आनुवंशिक दूरी प्रदर्शित हुई। एक लोब्टर प्रजाति *पेन्युलिरस*

होमारस के पूर्व व पश्चिम तटों से प्राप्त 80 नमूनों का 8 पालीमरफिक माइक्रोसेटेलाइट लोसाई तथा 10 ओपेरान डिकेमर्स द्वारा आनुवंशिक चरित्र-चित्रण करने पर दोनों तटों के नमूनों में निम्न आनुवंशिक भिन्नता का संकेत मिला। होलोथूरिया स्केब्रा में जापानी प्रजाति स्टाइकोपस जेपोनिकस से प्राइमर्स का प्रयोग करते हुए 5 माइक्रोसेटेलाइट लोसाई का सफलतापूर्वक क्रास एम्प्लीफिकेशन किया गया। तीन महत्वपूर्ण संकटग्रस्त सजावटी प्रजातियों, गारा सुरेन्द्रनथाणी, गारा गोतयला स्टेनोरहायनकस तथा गारा मुल्या के 16S rRNA, COI तथा Cyt b जीन्स की आंशिक सिक्वेन्स सूचनाएं उत्पन्न की गईं। सभी तीनों जीन्स ने प्रजाति-विशेष आनुवंशिक विभिन्नता मान तथा सिक्वेन्स पैटर्न प्रदर्शित किए। सभी सिक्वेन्स एनसीबीआई जीन बैंक को भेजे गए।

पश्चिमी घाटों की सजावटी नैन्डिड समूह की प्रजातियों प्रिस्टोलेपिस मारजिनाटा तथा प्रिस्टोलेपिस फासिंग्टा में 16S rRNA की आंशिक सिक्वेन्स सूचनाओं का प्रयोग करते हुए वर्गीकरण अस्पष्टता का निवारण किया गया। भारत के जलों से प्राप्त हानिकारक सायनोबैक्टीरिया, ट्रायकोडेसिमियम प्रजाति के आनुवंशिक चरित्र-चित्रण पर कार्य जारी रहा जिसके अन्तर्गत प्रजाति-विशिष्ट नैदानिक आण्विक चिन्हक विकसित किए गए ताकि ट्रायकोडेसिमियम की विभिन्न प्रजातियों की पहचान की जा सके। अध्ययन किए गए सभी नमूने टी. एटीथ्रीयम के पाए गए, न कि विशैली प्रजाति, टी. थीबाँटी के। भारत के जलों में सी हार्स प्रजातियों की विविधता का अध्ययन करने तथा संरक्षण उद्देश्यों हेतु उनकी पहचान करने हेतु एक नया कार्य आरम्भ किया गया।

दस मीठाजल तथा समुद्री मत्स्य प्रजातियों में कोशिकानुवंशिकी अध्ययन किए गए, इनमें सम्मिलित

थीं : रीटा रीटा, एओरिक्थिस सिंघाला, टौर प्युटीटोरा, टौर टौर, टौर खुद्री, टौर मुसल्ला, नैन्डस नैन्डस, एरियस सब्रोस्ट्रेटस, जेन्क्लस केनेसेन्स तथा थालासोमा लुनारे। मीठाजल मुरैल्स की तीन प्रजातियों; चन्ना पंकटेस, चन्ना स्ट्रेटस व चन्ना ओरिएन्टेलिस में आनुवंशिक विभिन्नताओं का आरएपीडी द्वारा अध्ययन किया गया। परिणामों से चन्ना स्ट्रेटस व चन्ना ओरिएन्टेलिस में निकट आनुवंशिक सम्बन्ध प्रदर्शित हुआ। टौर टौर, टौर प्युटीटोरा तथा रीटा रीटा में सिल्वर स्टेनिंग के प्रयोग द्वारा एएफएलपी अध्ययन किया गया जिसके परिणामों से पता चला कि यह तकनीक मत्स्य प्रजातियों की सही पहचान तथा चरित्र-चित्रण हेतु उपयोगी है। क्युइलजा सेपोनिन, क्लोरपायरीफास और रोटेनन के मछलियों पर आनुवंशिक-विशाक्तता प्रभावों, तथा मछलियों में डीएनए क्षति कम करने हेतु हल्दी के प्रभावों का अध्ययन किया गया। प्रदूषकों के कारण प्रतिक्रिया-स्वरूप जीन की अभिव्यक्ति परिवर्तित हो सकती है और यह मछलियों में आनुवंशिक-विषाक्तता के अध्ययन में एक जैवचिन्हक (बायोमार्कर) के रूप में प्रयोग हो सकती है।

इन सीटू संरक्षण कार्यक्रम के अन्तर्गत, गंगा बेसिन में मीठाजल मत्स्य विविधता की वर्तमान स्थिति तथा बदलते परिवेश में प्रजाति-प्रचुरता, वितरण, वासस्थल स्थिति, जीवनवृत्त विशेषकों, संरक्षण स्थिति, इत्यादि के अध्ययन हेतु एक नया कार्य आरम्भ किया गया। इसके अन्तर्गत 24 परिवारों के 58 वंशों की 96 प्रजातियां, गंगा नदी के विभिन्न हिस्सों से अभिलेखित की गईं। गंगा नदी की एक सहायक नदी टोंस से, तथा फरक्का के पास गंगा के निचले हिस्से से, तीन प्रजातियों (ओमपोक पाबदा, सिरहाइनस रेबा तथा ग्लोसोगोबियस ग्युरिस) के आकार के नए रिकार्ड प्राप्त हुए। गंगा नदी के सभी भागों में मत्स्य विविधता के लिए बहुत से खतरों का भी पता चला।

उत्तरी-पूर्वी क्षेत्र में मत्स्य जननद्रव्य अन्वेषण, मूल्यांकन, सूचीकरण तथा संरक्षण हेतु स्थानीय सहयोगियों के साथ, एक नया कार्यक्रम आरम्भ किया गया। उत्तर प्रदेश की गोमती नदी में मत्स्य संरक्षण में मन्दिर विहारों की स्थिति एवं भूमिका पर अध्ययन जारी रहा। मत्स्य संसाधनों के संरक्षण में मत्स्यजीवी सहाकारी समितियों की स्थिति एवं क्षमताओं का अध्ययन जारी रहा। इसमें पाया गया कि यद्यपि अध्ययन किए गए सभी क्षेत्रों में ये संस्थाएं थीं किन्तु मत्स्य संरक्षण एवं प्रबन्धन में इन्होंने केवल उन्हीं स्थानों पर प्रभावी भूमिका निभाई जहाँ पर कुछ विशेष कारक विद्यमान थे।

जीन बैंकिंग कार्यक्रम के अन्तर्गत, पश्चिमी घाटों की दो संकटग्रस्त प्रजातियों *होरबैग्रस निग्रीकोलारिस* तथा *गारा सुरेन्द्रनथानी* हेतु शुक्राणु हिमपरिरक्षण प्रोटोकाल विकसित करने के लिए और परीक्षण किए गए जिनमें निषेचन के परीक्षण भी सम्मिलित थे। कुल 401 ऊतक संग्रह एकत्र किए गए तथा 135 समुद्री टेलिओस्टस प्रजातियों के वाउचर स्पेशीमेन्स परीरक्षित किए गए। असम सरकार के राज्य मात्स्यिकी विभाग के सहयोग से गुवहाटी में एक नया लाइव फिश जीन बैंक स्थापित किया गया। ब्यूरो की चिनहट इकाई पर चयनित कार्प व कैटफिश प्रजातियों का बीज उत्पादन किया गया।

चुनिंदा समुद्री तथा मीठाजल सजावटी मछलियों के रोगजनकों के अध्ययन हेतु एक नया कार्य आरम्भ किया गया जिसका प्रमुख उद्देश्य सजावटी मछलियों के रोगजनकों का अन्वेषण व अभिलेखन करके चुनिंदा रोगजनकों की पहचान हेतु त्वरित आप्ठिक ऐसेज का विकास करना है। कोई कार्प मछलियों के रोगों में वायरस इटायोलोजी की सम्बद्धता का अध्ययन भी आरम्भ किया गया ताकि देश में इस दिशा में भावी आवश्यकताओं हेतु क्षमताओं का विकास किया जा सके। इस नैदानिक क्षमता का उपयोग देश की मीठाजल

जलकृषि तंत्रों में मत्स्य स्टाकों की स्क्रीनिंग तथा स्वास्थ्य प्रमाणीकरण हेतु प्रयोग किया जाएगा।

पीनेइडस प्रजातियों की तीन ओआईईई द्वारा सूचीबद्ध विशाणुओं, मोनोडोन बैक्युलोवायरस (एमबीवी), व्हाइटस्पाट सिन्ड्रोम वायरस (डब्लूएसएसवी) तथा येलोहेड वायरस (वाईएचवी) हेतु, चुनिंदा समुद्री तट वाले राज्यों में, टारगेटिड एक्टिव सर्विलिएन्स पर कार्य जारी रहा। शिम्प व पोस्ट-लार्वा के कुल 238 नमूनों में से मात्र 21 नमूने ही पीसीआर द्वारा डब्लूएसएसवी के लिए पाजीटिव थे, 11 नमूने एमबीवी के लिए पाजीटिव थे जबकि कोई भी नमूना वाईएचवी के लिए पाजीटिव नहीं था। चूँकि मत्स्य रोग निदान एवं रोग प्रबन्धन हेतु फ्लैवोबैक्टीरियम प्रजातियों का सही एवं विशिष्ट पृथक्करण तथा चरित्र-चित्रण आवश्यक है, अतः मछलियों, सेडीमेन्ट तथा तालाब के जल से फ्लैवोबैक्टीरियम के पृथक्करण एवं चरित्र-चित्रण पर कार्य आरम्भ किया गया। फ्लैवोबैक्टीरियम के 41 संभावित आइसोलेट्स में से 7 आइसोलेट्स की फ्लैवोबैक्टीरियम के रूप में पुष्टि हुई। *लैबियो रोहिता* तथा *एपिनेफेलस मैरा* से सैल लाइन्स के चरित्र-चित्रण एवं विकास तथा *साइप्रिनिस कार्पियो* की स्थापित सैल लाइन्स से विशाणुओं के प्रथक्करण पर अध्ययनों से उत्साहित करने वाले परिणाम प्राप्त हुए। गंगा नदी तंत्र के चुनिंदा भागों में विदेशागत मछलियों के वितरण और रिप्रोडक्टिव परफारमेंस हेतु उनके मूल्यांकन पर अध्ययन किया गया।

संस्थान ने समुद्री जीवन की डीएनए बारकोडिंग पर पहला अन्तर्राष्ट्रीय प्रशिक्षण कार्यक्रम आयोजित किया जिसमें 18 प्रतिभागियों ने भाग लिया जिनमें आस्ट्रेलिया, कनाडा, दक्षिण अफ्रीका, तंजानिया तथा केन्या से आए प्रतिभागी सम्मिलित थे। इसके अलावा भा.कृ.अ.परिषद् द्वारा प्रायोजित मत्स्य जैवप्रोद्योगिकी पर एक समर स्कूल, 'सेल्युलर एण्ड मालीक्युलर एप्रोचेज़

फार जीनोटोक्सिसिटी एसेसमेन्ट इन फिशिज' तथा 'बेसिक टूल्स इन मालीक्युलर बायोलाजी रिसर्च' पर प्रशिक्षण कार्यक्रम आयोजित किए गए। संस्थान ने अपनी चिनहट, लखनऊ स्थित इकाई पर प्रदेश के मत्स्य पालकों के लाभ हेतु चार अल्पकालीन प्रशिक्षण कार्यक्रम आयोजित किए, जिनके विषय थे : 'उत्कृष्ट मत्स्य बीज उत्पादन एवं हैचरी प्रबन्धन'; 'समन्वित जलकृषि एवं मत्स्य रोग प्रबंधन'; 'जलकृषि विविधीकरण एवं विदेश गत प्रजातियों का प्रभाव' तथा मीठाजल झींगा पालन तकनीकी। ये चारों कार्यक्रम राष्ट्रीय मात्स्यिकी विकास बोर्ड, हैदराबाद द्वारा वित्त-पोषित थे। संस्थान ने 'फिशरीज कन्जर्वेशन एण्ड एन्हान्समेन्ट: लिंकिंग रिसर्च एण्ड स्ट्रेटेजी' पर गुवाहटी, असम में एक कार्यशाला तथा 'कन्जर्व फिश फार पोस्टेरिटी' पर इटानगर, अरुणाचल प्रदेश में एक जागरूकता कार्यक्रम आयोजित किया।

डा. मंगला राय, सचिव, कृषि अनुसंधान एवं शिक्षा विभाग, भारत सरकार तथा महानिदेशक, भारतीय कृषि अनुसंधान परिषद्, नई दिल्ली ने संस्थान का दौरा किया तथा नवनिर्मित मत्स्य बीज उत्पादन इकाई का उद्घाटन किया जिसका निर्माण परिषद् की मेगा बीज परियोजना के अन्तर्गत किया गया है। संस्थान में एक नई कोशिका संवर्धन प्रयोगशाला का भी उद्घाटन किया गया। संस्थान की शोध सलाहकार समिति तथा स्टाफ शोध परिषद् की बैठकें सफलतापूर्वक आयोजित की गईं। संस्थान में विभिन्न विशेषज्ञों के कुल 16 आमंत्रित व्याख्यान आयोजित किए गए।

संस्थान के निदेशक डा. वजीर एस लाकड़ा को

“डा. एम.एस. स्वामीनाथन बेस्टफिशरीज साइंटिस्ट एवार्ड 2007” प्राप्त हुआ। ब्यूरो तथा बी. आर. अम्बेडकर विश्वविद्यालय, लखनऊ के बीच एक एमओयू पर हस्ताक्षर किए गए। ब्यूरो ने 'राज्य मछली' (State Fish) की एक नई अवधारणा को प्रोत्साहित किया जिसका उद्देश्य संरक्षण अनुसंधान तथा स्ट्रेकहोल्डर्स को जोड़ना एवं उनमें समन्वय बढ़ाना है।

संस्थान के वैज्ञानिकों द्वारा कुल 27 शोधपत्र विभिन्न प्रतिष्ठित जर्नलों में प्रकाशित किए गए जबकि 19 अध्याय/लेख विभिन्न पुस्तकों में प्रकाशित हुए। इनके अलावा ब्यूरो के वैज्ञानिकों ने 12 शोध सारांश विभिन्न गोष्ठियों/कार्यशालाओं हेतु प्रस्तुत किए। संस्थान ने पुस्तक के रूप में 7 प्रकाशन प्रकाशित किए। संस्थान ने वार्षिक राजस्व लक्ष्य रु. 12 लाख के सापेक्ष रु. 13.15 लाख का राजस्व वर्ष के दौरान अर्जित किया।

ब्यूरो के पुस्तकालय में 439 अभिलेख सम्मिलित किए गए। पुस्तकालय में वर्ष के दौरान 34 अन्तर्राष्ट्रीय तथा 59 राष्ट्रीय जर्नल मंगाए गए जबकि 46 जर्नल विनिमय/ग्राटिस आधार पर पुस्तकालय में आए। पुस्तकालय ने ब्लैकवेल प्रकाशन के 110 इलेक्ट्रॉनिक जर्नल भी सब्सक्राइब किए।

संस्थान में 14 सितम्बर 2007 को हिन्दी दिवस कार्यक्रम मनाया गया तथा 15-29 सितम्बर 2007 के दौरान हिन्दी पखवाड़ा मनाया गया जिसमें, कार्यालय कार्य के हिन्दी के प्रयोग को प्रोत्साहित करने के लिए स्टाफ हेतु 7 हिन्दी प्रतियोगिताओं का आयोजन किया गया तथा विजेता प्रतिभागियों को पुरस्कृत किया गया।

INTRODUCTION

Brief History

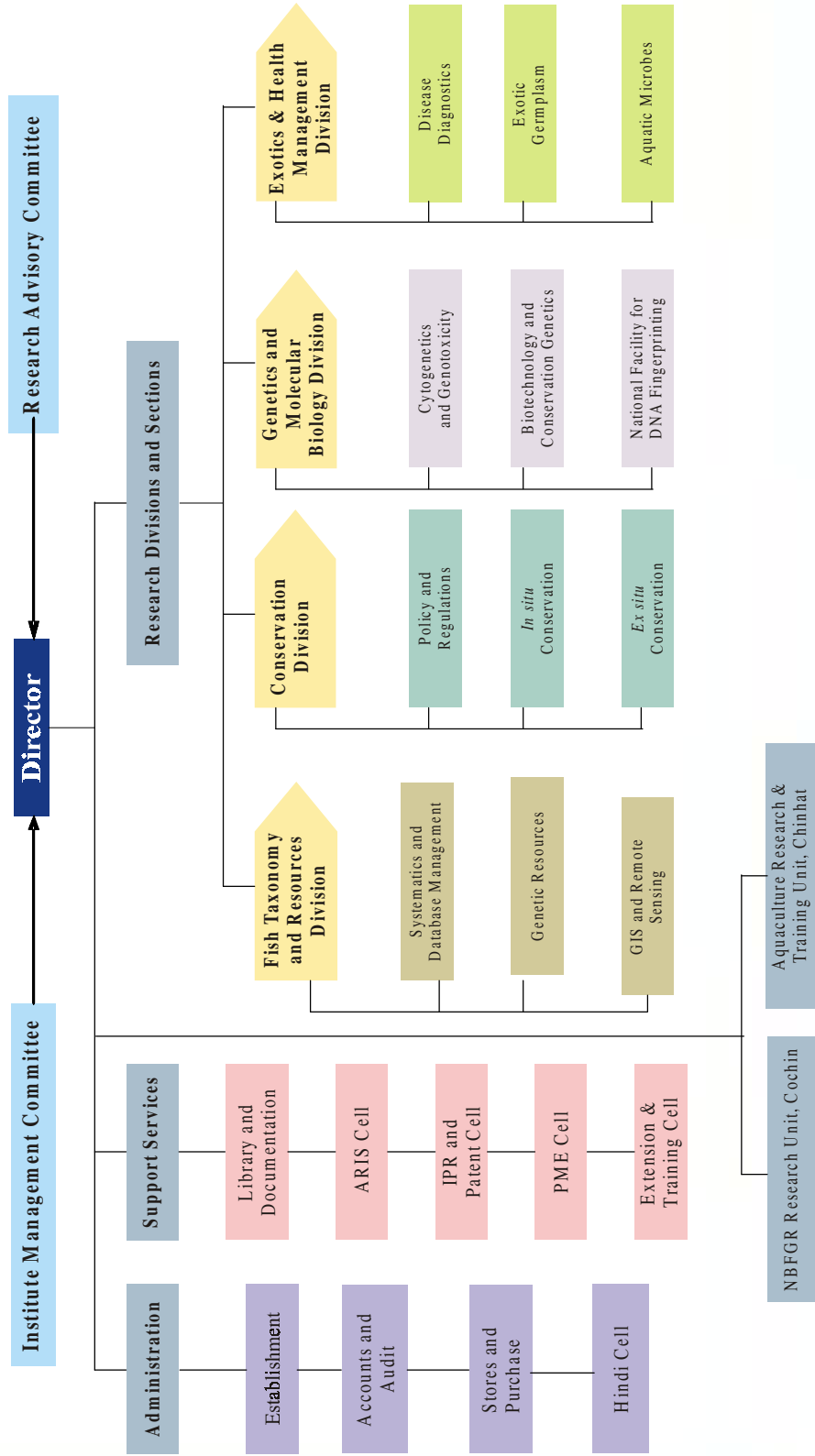
India is fortunate to possess rich and varied fish genetic resources in different aquatic ecosystems *viz.*, freshwater, brackishwater and marine. However, our rich fish fauna is facing serious threats due to several anthropogenic and natural environmental changes. In view of this, the conservation of fish germplasm resources has assumed tremendous significance in the management perspective of our fishery resources. In this scenario, the National Bureau of Fish Genetic Resources (NBFGR) was established in December 1983 in rented premises at Allahabad under the aegis of Indian Council of Agricultural Research to undertake research

related to the conservation of fish germplasm resources of the country. The Bureau's permanent infrastructure was developed at Canal Ring Road, Telibagh, Lucknow, Uttar Pradesh in 1999 comprising an administrative block, laboratories, farm and residential complex covering an area of 52 acres. The Bureau has created excellent infrastructure and expertise in several research areas including development of fish databases, genetic characterization, gene banks, fish germplasm and habitat inventory, risks analysis of exotic species, diagnostics for OIE notified pathogens, aquatic microbes and other areas of germplasm conservation with special focus on threatened, prioritized and exotic fish species.

MANDATE

- ❑ Collection, classification and cataloguing of fish genetic resources of the country.
- ❑ Maintenance and preservation of fish genetic material for conservation of endangered fish species.
- ❑ Evaluation and valuation of indigenous and exotic fish species.

ORGANISATIONAL CHART



Staff Position

The overall staff position as on 31st March, 2008 is given below:

S. N.	Category of posts	Post created	Staff in position	Post vacant (out of created posts)
1.	Research Management (Director)	01	01	--
2.	Scientific	40	29	11
3.	Technical	36	36	--
4.	Administrative	19	17	02
5.	Supporting	20	19	01
	Total	116	102	14

Financial Statement

Allocation of funds and expenditure incurred during the year 2007-2008.

(Rs. in lakhs)

	Budget Allocation	Expenditure
Plan	430.00	429.89
Non Plan	365.00	364.91
North East Component	50.00	50.01
Total	845.00	844.81

RESEARCH ACHIEVEMENTS

5.1 Cataloguing of Fish Genetic Resources of India

The Bureau has specific mandate for collection and cataloguing of information on fish genetic resources of India which is a prerequisite for sustainable management of fish germplasm resources. In view of this, NBFGR is engaged in collecting, maintaining and updating valuable information about fish diversity of India.

During the period under report, the existing database on finfish diversity of India was modified, restructured and updated by including additional information to the designated fields. The database now consists of 2243 finfishes (Table 1). The information on 47 more species was added to the database (Table 2), out of which nine were newly reported species (Fig.1) namely, *Pseudolaguvia ferula*, *Puntius ater*, *Puntius khugae*, *Channa aurantimaculata*, *Channa bleheri*, *Gerres phaiya*, *Psenopsis intermedia* and *Rita macracanthus*. Information available on 291

exotic fish species reported from India was also included in the database.

Digital images of live and freshly captured freshwater fishes were added to the database. The diagrammatic images are being supported by addition of the good quality original digital images. During the period under report, 140 images of the fishes of Western Ghats and 10 images of the threatened fishes of India were added to the database. At present the database contains a total of 5878 images.

Table 1. Finfish Diversity of India

Ecosystem	Fish species (No.)
Freshwater	765
Brackishwater	113
Marine	1365
Total	2243 (excluding 291 Exotic fishes reported from India)

Table 2. Details of fish species added into the database

S. No.	Family	Species	Author & year	Distribution	References
1	Aplocheilidae	<i>Aplocheilus parvu</i>	Sundara Raj, 1916	Madras, India	Menon, A.G.K., 1999
2	Tetraodontidae	<i>Carinotetraodon imitator</i>	Britz & Kottelat, 1999	Ernakulam, Kerala.	Gopalakrishnan, A. and A.G. Ponniah, 2000
3	Channidae	<i>Channa leucopunctatus</i>	Sykes, 1839	Known from Maharashtra; Mavanhalla, Mudumalai, Tamil Nadu; south India, Telangana area of Andhra Pradesh and West Bengal. Cultured in irrigation wells in South India	Arunachalam, M.,J.A. Johnson, A. Manimekalan, A. Sankaranarayanan and R. Soranam, 2000
4	Channidae	<i>Channa limbatus</i>	Cuvier, 1831	Pondicherry	Archarya, P. and M.B. Iftekhar, 2000
5	Tetraodontidae	<i>Cheolonodon patoca</i>	Hamilton, 1822	Chilka Lake	Gopalakrishnan, A. and A.G. Ponniah, 2000

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6	Cyprinidae	<i>Esomus thermoicos</i>	Valenciennes, 1842	Godavary and Krishna river systems to as far south as the tip of Peninsular India. Recorded from Kalakkad, Tirunelveli and Kerala; Chittar River basin, Tamil Nadu	Rema Devi, K. and T.J. Indra, 2000
7	Cyprinidae	<i>Garra periyarensis</i>	Gopi, 2001	Periyar drainage at Thanikkudy, Periyar Tiger Reserve, Kerala	Gopi, K.C., 2001
8	Heteropneustidae	<i>Heteropneustes longipectoralis</i>	Rema Devi & Raghunathan, 1999	Thirumurthi Dam, Anamalai Hills, Western Ghats, Tamil Nadu	Rema Devi, K. and Raghunathan, M.B. 1999
9	Balitoridae	<i>Homaloptera implicata</i>	Rüppell 1830		Gopalakrishnan, A. and A.G. Ponniah, 2000
10	Balitoridae	<i>Homaloptera menoni</i>	Shaji & Easa, 1995	Bhavani river, and Kerala	Shaji, C.P., P.S. Easa and A. Gopalakrishnan, 2000
11	Balitoridae	<i>Homaloptera pillaii</i>	Indra & Rema Devi, 1981	Silent Valley in Western Ghats, Kerala. Type locality, Kunthi River, Western Ghats, Kerala	Gopalakrishnan, A. and A.G. Ponniah, 2000
12	Balitoridae	<i>Homaloptera santhamparaensis</i>	Arunachalam, Johnson & Rema Devi, 2002	Panniyar Stream, Santhamparai Hills, Kerala, Western Ghats	Arunachalam, M.,J.A. Johnson and K.R.Devi, 2002
13	Clariidae	<i>Horaglanis alikunhii</i>	Subhash Babu & Nayar, 2004	India: Parappukara, Trichur dist., Kerala,	Subhash Babu, K.K. and Nayar, C.K.G. 2004
14	Cyprinidae	<i>Horalabiosa arunachalami</i>	Johnson & Soranam, 2001	Tributery of Panniyar stream, above Ponmudi reservoir at Santhamparai hills, Idukki district, Kerala,	Gopalakrishnan, A. and A.G. Ponniah, 2000
15	Syngnathidae	<i>Icthyocampus carce</i>	(Hamilton, 1822)	Throughout India. Found in Chilka Lake; Uttara Kannada, Karnataka	Dahanukar, N., R. Raut and A. Bhat, 2004
16	Synbranchidae	<i>Monopterus digressus</i>	Gopi, 2002	Homestead well at Kuthiravattom, a suburban locality of Calicut, Kerala	Gopalakrishnan, A. and A.G. Ponniah, 2000
17	Synbranchidae	<i>Monopterus roseni</i>	Bailey & Gans, 1998	Periyam village, North Kerala state, India	Shaji, C.P., P.S. Easa and A. Gopalakrishnan, 2000
18	Balitoridae	<i>Nemacheilus deninsoni pambaensis</i>	(Rema Devi & Indra, 1994)	Shabarigiri, Idukki Dist., Kerala; and Chinnar river	Shaji, C.P., P.S. Easa and A. Gopalakrishnan, 2000
19	Balitoridae	<i>Nemacheilus herrei</i>	Nalbant & Banarescu, 1982	India: Anaimalai hills, Valparai, Kerala.	Shaji, C.P., P.S. Easa and A. Gopalakrishnan, 2000
20	Balitoridae	<i>Nemacheilus ornatus</i>	Kottelat, 1990		Gopalakrishnan, A. and A.G. Ponniah, 2000

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21	Balitoridae	<i>Nemacheilus stigmofasciatus</i>			Gopalakrishnan, A. and A.G. Ponniah, 2000
22	Cyprinidae	<i>Oreochthys cosuatis</i>	(Hamilton, 1822)	Assam, West Bengal, Orissa, Madhya Pradesh, Maharashtra, Karnataka and Kerala	Shaji, C.P., P.S. Easa and A. Gopalakrishnan, 2000
23	Adrianichthyidae	<i>Oryzias dancena</i>	(Hamilton, 1822)	Tamil Nadu, Andhra, Pradesh and West Bengal	Shaji, C.P., P.S. Easa and A. Gopalakrishnan, 2000
24	Cyprinidae	<i>Osteobrama nelli</i>	(Day, 1873)	Bhavani river, Nilgiri Hills in South India, Maharashtra, Karnataka and Tamil Nadu; and Krishna river (Deccan) and Pennar river in Cuddapah, Andhra Pradesh. endemic to Western Ghats	Shaji, C.P., P.S. Easa and A. Gopalakrishnan, 2000
25	Schilbeidae	<i>Pseudeutropius taakree taakree</i>	(Sykes, 1839)	Krishna, Godavari and Jamuna rivers; Western Ghats, Kerala, Maharashtra and Karnataka	Shaji, C.P., P.S. Easa and A. Gopalakrishnan, 2000
26	Cyprinidae	<i>Puntius arulius tambraparniei</i>	Silas, 1954	Middle reaches of Tambraparni river basin which drains the eastern face of the Western Ghats mountains of southern peninsular India, into the Bay of Bengal	Arunachalam, M. and A. Sankaranarayanan, 2000
27	Cyprinidae	<i>Puntius kannikattiensis</i>	Arunachalam & Johnson, 2003	Ullar, a tributary of Tamiraparani river, above Karaiyar reservoir, Kannikatti region (Kalakad Mundanthurai Tiger Reserve), Tirunelveli, Tamil Nadu	Arunachalam, M. and A. Sankaranarayanan, 2000.
28	Cyprinidae	<i>Puntius muvattupuzhaensis</i>	Jameela Beevi & Ramachandran, 2005	Muvattupuzha river, Ooramana, Ernakulam, Kerala	Arunachalam, M. and A. Sankaranarayanan, 2000
29	Cyprinidae	<i>Tor malabaricus</i>	(Jerdon, 1849)	Balamore river, Western Ghats in Kanyakumari District, Tamil Nadu and Kallada River in Kerala	Silas, E.G., A. Gopalakrishnan, Lijo John and C.P. Shaji, 2005
30	Cyprinidae	<i>Cyprinus nicholsi</i>	(Mysers)	Manipur	Sen, N, 2000
31	Cyprinidae	<i>Garra gravelyi</i>	(Annandale)	Arunachal Pradesh	Sen ,N, 2000
32	Cyprinidae	<i>Gudusia variegata</i>	(Day)	Assam	Sen ,N,2000
33	Balitoridae	<i>Homaloptera modesta</i>	Vincigura 1890	Manipur	Sen ,N,2000
34	Sisoridae	<i>Nangra assamensis</i>	Sen & Biswas, 1994	India: Brahmaputra river. Dibrugarh, Assam	Sen, N, and Biswas, B. K. 1994

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35	Cyprinidae	<i>Osteobrama cotio cumna</i>	Day	Manipur, Tripura	Sen, N, 2000
36	Cyprinidae	<i>Psilorhynchus gracilis</i>	Rainboth	Assam, Mizoram	Sen ,N, 2000
37	Siluridae	<i>Silurus torrentis</i>	Kobayakawa	Arunachal Pradesh	Sen ,N,2000
38	Sisoridae	<i>Pseudolaguvia ferula</i>	Ng, 2006	India: Tista River in West Bengal,	Ng, H.H., 2006
39	Sisoridae	<i>Glyptothorax botius</i>	(Hamilton, 1822)	India: Ganges River drainage	Ng, H.H., 2005
40	Cyprinidae	<i>Puntius ater</i>	Linthoingambi & Vishwanath, 2007	India: Manipur, Khuga River (Chindwin Basin) at Churachandpur district	Linthoingambi, I. and W. Vishwanath, 2007
41	Cyprinidae	<i>Puntius khugae</i>	Linthoingambi & Vishwanath, 2007	India: Manipur, Khuga River (Chindwin Basin) at Churachandpur district	Linthoingambi, I. and W. Vishwanath, 2007
42	Channidae	<i>Channa aurantimaculata</i>	Musikasinthorn, 2000	Brahmaputra river basin	Musikasinthorn, P. 2000 Goswami M.M., et. al., 2006
43	Channidae	<i>Channa bleheri</i>	Vierke 1991	Assam	Vierke, J., 1991. Goswami M.M., et. al., 2006
44	Gerreidae	<i>Gerres phaiya</i> (Strong spined silver-biddy)	Iwatsuki & Heemstra, 2001	Western Indian Ocean: southwest coast of India; probably also in Bengal Bay and the Andaman Sea	Iwatsuki, Y. and P.C. Heemstra, 2001
45	Centrolophidae	<i>Psenopsis intermedia</i>	(Piontrovskiy, 1987)	Arabian Sea, Off Mangalore and Malpe	Piotrovsky, A.S., 1987. Sujitha Thomas and Prathiba Rohit, 2006
46	Bagridae	<i>Rita macracanthu</i>	Ng, 2004	Asia: Known from the Indus river drainage in Afganistan, Pakistan, and northwestern India	Ng H. H., 2004 Jayaram K.C., 2006
47	Bagridae	<i>Sperata aorella</i>	Blyth, 1858	Delta of river Ganga upto Bistrampur in Bihar	Blyth, E. 1858 Jayaram KC, 2006

Data on 30 new species of freshwater species reported from the Western Ghats were collected and added to the database. A consolidated list of the diversity of fishes from inland waters of Kerala was prepared which included 259 species. Besides, a list of 23 species, which have been reported as new additions to the freshwater fishes of Kerala, was also prepared. Out of these 282

species (259+23=282), 157 species (55.67%) are endemic to the Western Ghats hotspot (including Kerala) and 72 species (25.53%) are exclusive to Kerala.

As a part of the database project, a book entitled "Ornamental Fishes of the Western Ghats of India" was published containing colour images, identification characters, information on



Channa aurantimaculata Musikasinthorn (2000)



Channa bleheri, Vierke (1991)



Rita Macracanthus Ng, 2004



Gerres phaiya, Iwatsuki & Heemstra, 2001

Fig. 1 A few of the newly reported species added to the database

aquarium requirements and captive breeding techniques of nearly 150 indigenous ornamental fishes of the Western Ghats.

Information on 79 threatened finfishes of India was extracted from the database and a separate web page for this was developed using ASP/DHTML/HTML/VB Script technologies for Windows Server. This page was hosted on the NBFGR website under the Databases content hyperlinked to “Threatened finfishes of India” which can be browsed at the address www.nbfgr.res.in/threatened/matters/homepage/htm. A new module was designed and added in the software in which all habitats of fishes have been categorized into freshwater, brackishwater and marine water.

The state-wise revised lists of freshwater fishes for North-Eastern, Central and Western states of India were prepared in MS Access (Table 3). The administrative boundary along with

district boundary for these states was extracted from the digitized administrative boundary map of India at the scale of 1:250,000 in ARC INFO. Database on state-wise list of freshwater fishes was connected to the digitized administrative boundary map for selected states.

Table 3. No. of fish species in different states

State	No. of fishes
Arunachal Pradesh	158
Assam	197
Manipur	133
Meghalaya	159
Mizoram	49
Nagaland	67
Tripura	130
Delhi	87
Madhya Pradesh	123
Maharashtra	126
Rajasthan	91

Database on taxonomy and distribution of freshwater fishes of Uttar Pradesh

Under an AP Cess funded scheme, secondary data was collected on aquatic and fishery resources of Uttar Pradesh. Detailed primary data on fish distribution was also collected through extensive survey in the 22 districts of the state. A total of 87 species were recorded during field survey in Uttar Pradesh (Table 4).

River Ganga and Yamuna were surveyed in the districts of Allahabad, Mirzapur and Varanasi; river Tons at Katka (Meja) and river Ramganaga at Bareilly and Moradabad. Data was collected from experimental fishing, landing centres and fish markets. Two exotic fish species, *Cyprinus carpio* and *Oreochromis mossambicus* were recorded from the surveyed areas of U.P. *C. carpio* is found commonly in catches in river Ganga from Kanpur to Allahabad and in river Yamuna at Allahabad. *O. mossambicus* was reported in catches of river Yamuna at Allahabad.

The fishery of river Yamuna and Ganga which was dominated by the major carps has now drastically declined and has been replaced by the minor carps such as *Gudusia chapra*, *Gonialosa manmina*, *Salmostoma bacaila*, *Aspidoparia morar*, *Clupisoma garua*, *Eutropiichthys vacha* and *Ailia coila*. The percentage of *Channa marulius*, *C. striatus*, *Mastacembelus armatus* and *Macroganthus pancalus* has increased in the recent catches which were very less in the past.

Collected specimens were preserved in formaldehyde for reference and further identification. Photographs of specimens in fresh condition were taken and stored in computer (Fig.2). Draft copy of a "Guide for Identification of Freshwater Fishes of U.P." was prepared with description of key characters, morphology,

photographs, colouration, fin-formula, distribution, fishery information and other remarks.

The species distribution database for 16 districts of Uttar Pradesh on the presence and absence of fish species was prepared in MDB format. This distribution database contains the information on the occurrence of fish species district-wise on three confidence rating: confident, possible and probable. Thus, the distribution data on the presence and absence of fish species with these confidence ratings for 16 districts were arranged on the administrative boundary coverage base map of Uttar Pradesh. The species distribution database on other scales with these confidence ratings like watershed and drainage are in the preparation stage. In order to prepare the species richness map on the hydrological scales, four hydrological maps (river basin, catchments, drainage and watershed) were produced in ARC INFO. The species distribution database on the presence and absence of fish species with three confidence ratings for 16 districts of Uttar Pradesh was integrated with the district-wise administrative boundary base map of Uttar Pradesh.

Table 4. List of fishes recorded in Uttar Pradesh

S. No.	Species	Family
1	<i>Ailia coila</i>	Schilbeidae
2	<i>Anabas testudineus</i>	Anabantidae
3	<i>Aorichthys aor</i>	Bagridae
4	<i>Aorichthys seenghala</i>	Bagridae
5	<i>Aspidoparia morar</i>	Cyprinidae
6	<i>Bagarius bagarius</i>	Sisoridae
7	<i>Barilius barila</i>	Cyprinidae
8	<i>Barilius bendelisis</i>	Cyprinidae
9	<i>Botia Dario</i>	Cobitidae
10	<i>Botia geto</i>	Cobitidae
11	<i>Catla catla</i>	Cyprinidae
12	<i>Chanda nama</i>	Chandidae
13	<i>Chanda ranga</i>	Chandidae

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14	<i>Channa marulius</i>	Channidae
15	<i>Channa orientalis</i>	Channidae
16	<i>Channa punctatus</i>	Channidae
17	<i>Channa striatus</i>	Channidae
18	<i>Channa stewartii</i>	Channidae
19	<i>Chela cachius</i>	Cyprinidae
20	<i>Chela laubuca</i>	Cyprinidae
21	<i>Cirrhinus mrigala</i>	Cyprinidae
22	<i>Cirrhinus reba</i>	Cyprinidae
23	<i>Clarias batrachus</i>	Clariidae
24	<i>Clupisoma garua</i>	Schilbeidae
25	<i>Colisa fasciatus</i>	Belontiidae
26	<i>Crossocheilus latius latius</i>	Cyprinidae
27	<i>Cyprinus chagunio</i>	Cyprinidae
28	<i>Cyprinus gora</i>	Cyprinidae
29	<i>Esomus danricus</i>	Cyprinidae
30	<i>Eutropiichthys murius</i>	Schilbeidae
31	<i>Eutropiichthys vacha</i>	Schilbeidae
32	<i>Gagata cenia</i>	Sisoridae
33	<i>Garra gotyla gotyla</i>	Cyprinidae
34	<i>Gobius giuris</i>	Gobiidae
35	<i>Gonialosa manimina</i>	Clupeidae
36	<i>Gudusia chapra</i>	Clupeidae
37	<i>Heteropneustes fossilis</i>	Heteropneustidae
38	<i>Ilisha megaloptera</i>	Pristigasteridae
39	<i>Johnius gangeticus</i>	Sciaenidae
40	<i>Labeo bata</i>	Cyprinidae
41	<i>Labeo boga</i>	Cyprinidae
42	<i>Labeo boggut</i>	Cyprinidae
43	<i>Labeo calbasu</i>	Cyprinidae
44	<i>Labeo dero</i>	Cyprinidae
45	<i>Labeo fimbriatus</i>	Cyprinidae
46	<i>Labeo gonius</i>	Cyprinidae
47	<i>Labeo rohita</i>	Cyprinidae
48	<i>Lepidocephalus guntea</i>	Cobitidae
49	<i>Macragnathus aral</i>	Mastacembelidae
50	<i>Macragnathus pancalus</i>	Mastacembelidae
51	<i>Mastacembelus armatus</i>	Mastacembelidae

52	<i>Monopterus cuchia</i>	Synbranchidae
53	<i>Mystus bleekeri</i>	Bagridae
54	<i>Mystus cavasius</i>	Bagridae
55	<i>Mystus vittatus</i>	Bagridae
56	<i>Nandus nandus</i>	Nandidae
57	<i>Nangra itchkeea</i>	Sisoridae
58	<i>Notopterus chitala</i>	Notopteridae
59	<i>Notopterus notopterus</i>	Notopteridae
60	<i>Ompok bimaculatus</i>	Siluridae
61	<i>Ompok pabda</i>	Siluridae
62	<i>Osteobrama cotio</i>	Cyprinidae
63	<i>Pangasius pangasius</i>	Pangasiidae
64	<i>Puntius chola</i>	Cyprinidae
65	<i>Puntius conchoniis</i>	Cyprinidae
66	<i>Puntius sarana sarana</i>	Cyprinidae
67	<i>Puntius sophore</i>	Cyprinidae
68	<i>Puntius ticto</i>	Cyprinidae
69	<i>Rasbora daniconius</i>	Cyprinidae
70	<i>Rhinomugil corsula</i>	Mugilidae
71	<i>Rita rita</i>	Bagridae
72	<i>Salmostoma bacaila</i>	Cyprinidae
73	<i>Salmostoma boopis</i>	Cyprinidae
74	<i>Setipinna phasa</i>	Engraulidae
75	<i>Sicamugil cascasia</i>	Mugilidae
76	<i>Silonia silondia</i>	Schilbeidae
77	<i>Sisor rahabdophorus</i>	Sisoridae
78	<i>Tenualosa ilisha</i>	Clupeidae
79	<i>Tetraodon cutcutia</i>	Tetraodontidae
80	<i>Tor putitora</i>	Cyprinidae
81	<i>Tor tor</i>	Cyprinidae
82	<i>Wallego attu</i>	Siluridae
83	<i>Xenentodon cancila</i>	Belonidae
84	<i>Aborichthys elongatus</i>	Balitoridae
85	<i>Colisa sota</i>	Belontiidae

Exotic fishes collected from U.P.

1	<i>Cyprinus carpio</i>	Cyprinidae
2	<i>Oreochromis mossambica</i>	Cichlidae



Channa orientalis



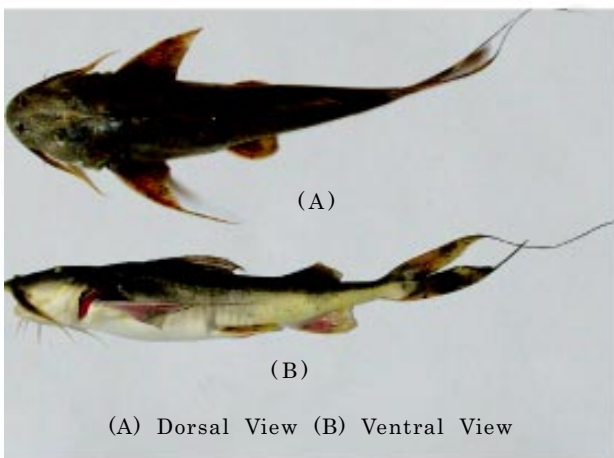
Silonia silonia



Channa stewartii



Tetraodon cutcutia



(A) Dorsal View (B) Ventral View

Bagarius bagarius



Chitala chitala

Fig. 2. A few of the fishes recorded in catches during field survey in Uttar Pradesh

Database development for marine ornamentals and shellfishes of Indian waters

The work on developing an electronic database on the diversity of marine ornamental fauna and shellfish species of Indian waters was continued during the year. The collection of information on ornamental fishes, molluscs and crustaceans (shellfishes) of Indian seas was carried out. The collected information was converted into digital data format to develop information system using SQL Server to facilitate information retrieval through websites. Data

entry fields were created for entering the data in SQL Server. Checklists of 350 species of marine ornamental fishes, 1655 molluscan species, 923 crustacean species and 43 echinoderm species of the Indian waters were prepared. Information on synonyms, distribution and key references on these species were collected. The checklist, prepared for crustacean, includes 424 species of crabs, 300 species of shrimps and lobsters, 155 species of hermit crabs and 43 species of stomatopods (Fig.3). The information pertaining to 450 crustacean species including its scientific name, synonyms, classification, distinguishing taxonomic features, biology, distribution and



Fig. 3. A sample (part) screen print of database on marine ornamentals and shellfishes

other key references was collected. For molluscs, the major part of information was collected for the species of Eastern India region totaling to 762 species, followed by Andaman and Western India regions, totaling 280 and 158 species, respectively. Information was also collected for 44 molluscan species of Tamil Nadu and 23 species from Kerala region.

The information on the marine ornamental fish species, with details of taxonomy, nomenclature (synonyms and common names), image, bibliography and geographic information on occurrence and distribution *etc.* was incorporated in MS-Access format and further the data, alongwith structure, were exported to SQL Server 2000 database. In addition, information on 14 species of seurchins, 9 species of sea-cucumbers, 12 species of seastars and 3 species of seafeathers were also collected for data entry in the SQL server. The images, photos taken in the field as well as from websites, were incorporated in the profiles of the species. The information on abundance per unit area of 330 species of marine ornamental fishes which were recorded in Andaman Islands through visual count by underwater observations in coral reef areas, was incorporated in the database. Voucher specimens of ornamental fishes (50) and molluscs (60) were collected from Andaman and Lakshadweep Islands. Images of molluscs (150) and ornamental fishes (80) were also included. The information on coral reef ecosystems (Kerala, Goa, Gulf of Kutch, Gulf of Mannar, Palk Bay and Andaman, Nicobar and Lakshadweep Islands) was also collected to provide habitat characteristics.

Development of digital information system on marine fish taxonomy

In India, more than 2500 fish species have been recorded including alien species. Proper identification of each species would help in

assessing the biodiversity and in developing conservation strategies to facilitate management and sustainable use of resources. The fish taxonomic inventory, characterization of population, mapping of distribution and evaluation of ecological interactions, correlated with environmental data, are the essential tools for successful conservation approaches. In fact, the inadequate knowledge in fish taxonomy may cause great limitation to biotechnology research also. Therefore, there is need for simplified identification systems in digital format for accurate identification of fish species, complemented with information on diagnostic features and phylogenetic relationship with other species and groups. Hence, a network on designing and development of digital fish taxonomic database to help in easy identification of indigenous as well as introduced alien fish species, was undertaken.

The work was started with the preparation of checklists of marine fishes; listing of morphometric, meristic and other diagnostic features; selecting available identification keys and storing of images. The various identification characteristics like standard measurements, body proportions, fin rays count, scutes count, lateral line scales with types, gill rakers, body colour, fin peculiarities and other special morphometric features were tabulated (Fig. 4 & 5). The identification keys for sharks, rays, skates, chimaeras and bony fishes were gleaned to work out suitable digital identification path from systematic position of Order to species level.

To begin with, the fish species belonging to families under the order Clupeiformes (Clupeidae, Engraulidae) and Perciformes (Serranidae) were selected. The identification keys for these selected species were screened for choosing diagnostic features that would facilitate creating a path for digital identification. Such screened information was entered on trial basis



Fig. 4. Fish search format of digital information system on marine fish taxonomy



Fig. 5. A sample (part) screen print from digital information system on marine fish taxonomy

in the SQL Server 2000 database. Pathways were created for identifying selected species with the help of a unique feature or through sorting out cluster of features that tally with the profile in the back-end data.

Spatial habitat preference and predicting model of sea cucumber (*Holothuria scabra*) using integrated approach of GIS and Artificial Neural Network

Despite their long history of consumption by Asian populations, sea cucumbers are a poorly understood coastal resource. The high demand for sea cucumbers has resulted in overexploitation in the main producing nations leading to expansion of the fishery into new fishing grounds as well as the development of sea cucumber aquaculture. Some studies have been conducted on sea cucumber in several countries in the Western Indian Ocean but there has been limited analysis of information relevant for fisheries management in individual countries and no attempt at a regional level. Therefore, a new work was initiated with an objective to develop the distribution and habitat preference model for sea cucumber species with special reference to *Holothuria Scabra* for Gulf of Mannar. Initially, references on environment, ecology, life-history and other biological attributes were collected. Two study areas Ramnathpuram and Tuticorin of Tamil Nadu in the Gulf of Mannar were identified for survey and sampling. The sea cucumber species selected for the study is now the banned species and for which the permission is required from The Principal Chief Conservator of Forests, Tamil Nadu State. Therefore, efforts were initiated to get the required permission from the concerned authority.

5.2 Genetic Characterization

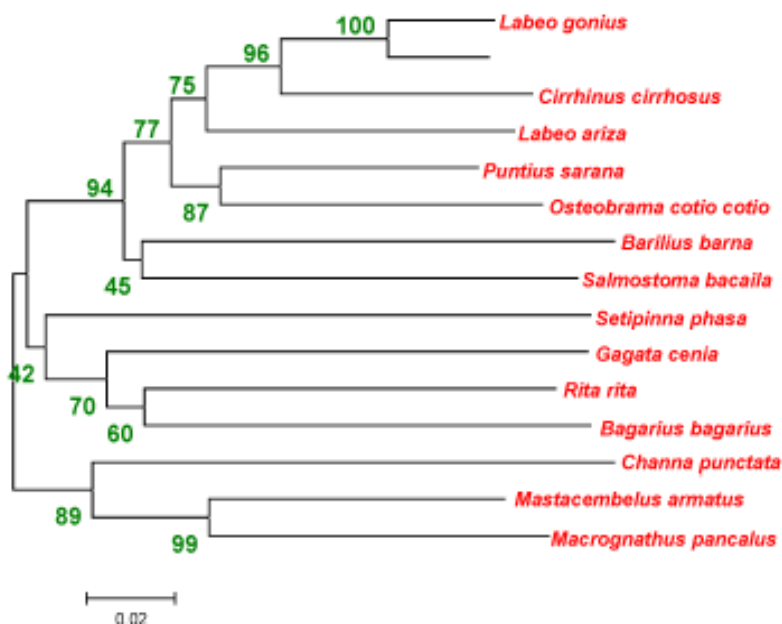
India has vast marine finfish and shellfish genetic resources and conservation of this natural genetic diversity is essential to maintain ecological as well as socio-economic equilibrium, besides, sustainable harvest of such resources. Documentation of genetic variation is of vital importance for evolving conservation and aquaculture strategies for long-term sustainability of the resources. Realizing this, NBFGR has been carrying out studies on population genetics of prioritized species so as to facilitate the conservation and management of different natural stocks of these species.

DNA barcoding of fishes

Under a comprehensive DNA barcoding programme of indigenous fish species, 925 tissue samples and voucher specimens of marine as well as freshwater fish species were collected. Out of these, 409 samples of 166 marine fish species

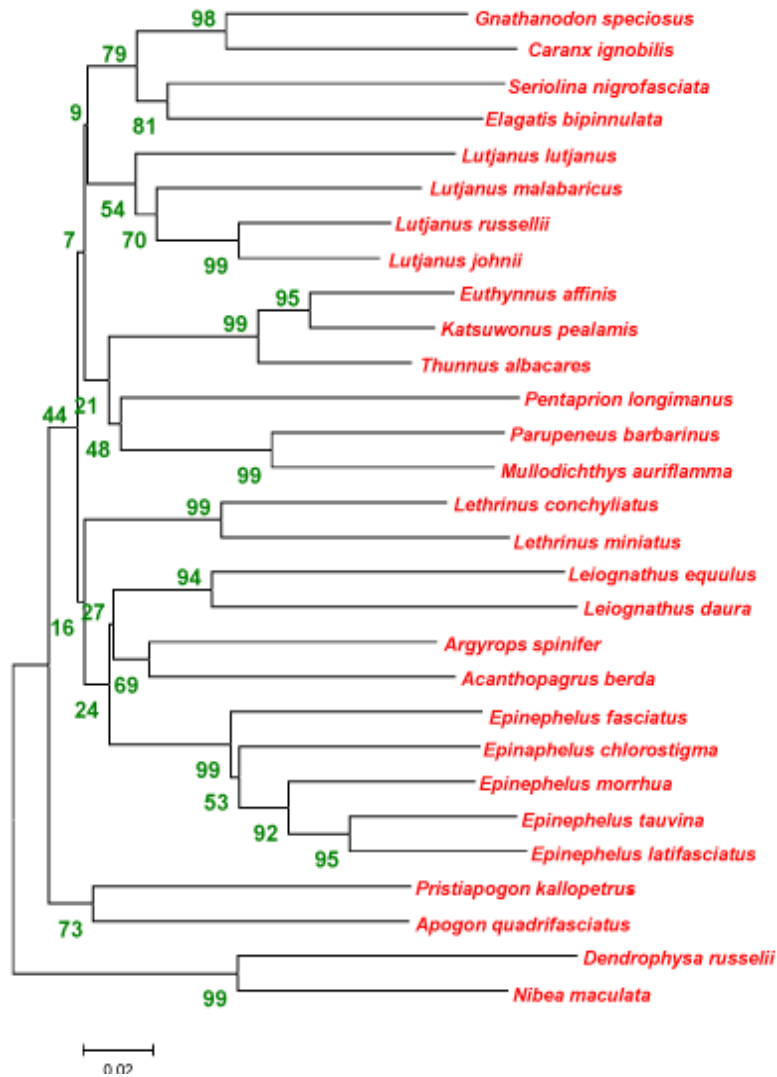
were collected from Lakshadweep, Chennai, Tuticorin, Gujarat Coast and Mumbai whereas 516 samples of 117 fish species were collected from Lucknow (river Gomti), Allahabad (Ganga), Kanpur (Ganga), Chakghat (Tones), Srinagar (Alakananda), Malda (lower Ganga), Deheradun (Yamuna), Maldevta (Song), Guwahati (Brahamaputra), Shillong (Umiam), Tatapani and Shimla (Sutlej). The total DNA was isolated for 1427 samples of 410 species and PCR amplification was done for 733 samples of 280 species. DNA sequencing was done in 540 samples of 182 species. DNA barcodes were prepared for 180 species (Fig.6 & 7). A total of 395 DNA sequences of 110 species have been submitted to BOLD.

The polymerase chain reaction (PCR) conditions have been standardized in diverse aquatic phyla viz. Crustaceans (shrimps, lobsters and crabs), sponges (Porifera), corals (black corals and stony corals), molluscs (bivalve) and fish parasites (cestodes and nematodes).



Neighborhood joining consensus tree

Fig.6 Phylogenetic relation among freshwater fishes based on mitochondrial COI region (bootstrap values with 1000 replicas)



Neighborhood Joining consensus tree

Fig.7. Phylogenetic relation among marine Perciformes fishes based on mitochondrial COI region (bootstrap values with 1000 replicas)

Molecular phylogeny of selected species of genus *Glyptothorax* in India

A total of 78 samples of four *Glyptothorax* species were collected from river Alakananda near Srinagar, Yamuna at Dakpathar near Deheradun, Song stream near Maldevta, Sutlej at Tatapani near Shimla and

Bhramputra at Guwahati. Genomic DNA was isolated from 77 Samples of three species. PCR amplification and DNA sequencing of cytochrome c oxidase I and cytochrome b gene region was done for 75 samples of three species (Fig.8). A total of 136 DNA sequences were submitted to NCBI GenBank (GenBank accession EU637404-EU637471 and EU637777-EU637844)

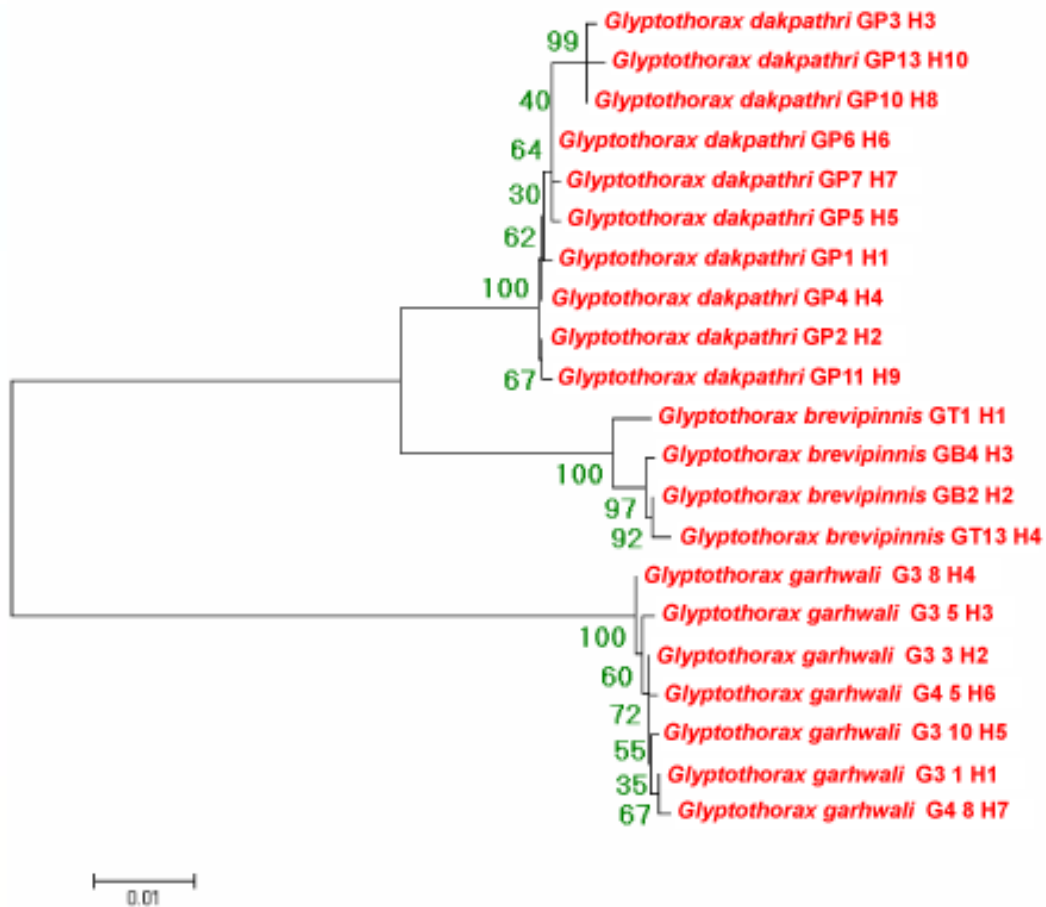


Fig.8. Phylogenetic tree of *Glyptothorax* species based on cytochrome b

Genetic diversity analysis in natural population of *Labeo calbasu* through polymorphic microsatellite markers

The work on genetic diversity analysis of *Labeo calbasu* was continued. A total of 105 primers of loci available for eight cyprinid species (resource species) that include *Cyprinus carpio*, *Barbus barbus*, *Pimephales promelas*, *Catla catla*, *Barbodes gonionotus*, *Labeo rohita*, *Carassius auratus* and *Camptostoma anamolum* were used for cross priming tests with twelve specimens of *L. calbasu*. Forty six primer pairs exhibited amplification in *L. calbasu*. A total of 16 loci were found to be polymorphic, out of

which nine polymorphic microsatellite loci were found suitable for assessing genetic variability across the natural range of distribution.

In continuation of the earlier work, more number of samples of *L. calbasu* from two rivers Bhagirathi (n=20) and Godavari (n=25) were genotyped for each of the nine (*R1**, *R3**, *R12**, *Lr28**, *Lr 29**, *MFW11**, *Lro23**, *Lro 25** and *Lr 38**) microsatellite loci to assess genetic variation. The mean number of alleles per locus was 7.33 in Bhagirathi and 8.11 in Godavari; and expected heterozygosity ranged from 0.795 (Bhagirathi) to 0.801 (Godavari). No evidence of linkage disequilibrium was detected at any locus pair comparisons in any population.

Deviation from Hardy Weinberg equilibrium was observed at loci *R1*, *R3*, *MFW11* and *Lr38* after the probability level was adjusted ($p < 0.003$) for sequential Bonferroni correction with likelihood of null alleles at locus *R3*. Significant genetic heterogeneity ($P < 0.05$) was evident at three loci, *Lr 29*, *Lro 25* and *Lro 23*. The results revealed that the identified loci are promising to genetic diversity analysis of wild populations of *L. calbasu*.

Taxonomic validation and phylogeny of fishes under the group Mahseer using mtDNA markers

The work on taxonomic validation and phylogeny of fishes under the group Mahseer was continued using mtDNA markers. Samples from 6 species of Mahseer i.e. *Tor putitora*, *T. tor*, *T. khudree*, *T. musallah*, *T. mosal mahanadicus* and *Neolissocheilus hexagonolepis* were analysed through the amplification of 5' prime region of the COI gene from mitochondrial DNA. The amplified product was approximately 710-bp region of the mitochondrial COI gene by PCR and archived 654-bp sequence. Of the 654 characters obtained (corresponding to the partial COI sequence), 495 (75.68%) were constant, 159 (24.31%) were variable and 117 (17.88%) were parsimony informative in comparisons of all the species analyzed. According to codon position, the most informative was the third (117 parsimony informative characters). The empirical percentages of the different nucleotides were T-28.9%, C-27.0%, A- 26.3% and G-17.7%. DNA of Mahseer fish estimated transition / transversions ratios ranged from 3.7 to 10.5. The ANOVA results for the specimen sequences also indicated that most diversity was due to interspecies differences (90.35% of the variation) relative to interpopulation variation (6.53%). The sequence divergence ranged from 0.180 to 0.990. Non-significant divergence values were observed between *Tor putitora* and *T. mahanadicus*.

Genetic diversity analysis of edible mussels *Perna viridis* and *P. indica*

Allozyme studies were conducted for studying hybrid/morphotype of two Indian mussels, *P. viridis* and *P. indica* existing along the Kerala coast. A total no. of 42 green mussels, 21 expected hybrid/morphotypes and 42 brown mussels were used for allozyme electrophoresis. A total of 16 enzymes were used for initial screening. Eight of these enzymes which were found to give scorable activity, were selected for extensive screening of green mussel, brown mussel and expected hybrid/morphotype.

The selected enzymes were Aspartate amino transferase, Esterase, Glucose phosphate isomerase, Malate dehydrogenase, Malic enzyme, Phosphoglucosmutase, Superoxide dismutase and Isocitrate dehydrogenase. Electrophoretic analyses of the tissue samples were standardized using Poly Acrylamide Gel Electrophoresis (PAGE) gels with 7% concentration. The buffer systems used for the present study was TBE (560 mM Tris, 650 Mm boric acid and 16 mM EDTA, pH 8.0). Three of the enzymes studied were polymorphic. An excess of homozygotes was observed at several loci in most of the individuals.

Population genetic structure in *Pangasius pangasius*

Microsatellite enriched genomic library was constructed for *Pangasius pangasius* to identify sequences containing microsatellite repeat regions. For the 29 sequences earlier found to contain microsatellite repeats, primers were designed and tested for amplification of microsatellite loci. Out of the 25 microsatellite loci amplified, 9 were polymorphic, 3 monomorphic and 13 yielded unspecified products.

In continuation of the earlier work, a total of 42 individuals from two rivers Bhagirathi

(n=22, Farakka, West Bengal, 24° 05' N; 88° 06' E) and Mahanadi (n=20, Cuttack, Orissa, 21° 58' N, 86° 07' E) were analysed with 9 polymorphic loci. Genotype data at each of the nine polymorphic microsatellite loci for samples collected from rivers, were analyzed to determine parameters of genetic variation. The mean number of alleles per locus was 5.22 (Bhagirathi) and 5.78 (Mahanadi). Expected heterozygosities were 0.567 and 0.578 and observed heterozygosities were 0.439 and 0.438, respectively for Bhagirathi and Mahanadi samples. After Sequential Bonferroni (SB) correction, linkage disequilibrium was not

detected for any pair of loci ($P > 0.005$) in individual sample or over all samples. There was evidence of significant deviation from Hardy-Weinberg expectations ($P > 0.003$) at 3 loci, Ppa2 (Mahanadi), Ppa14 (Bhagirathi) and Ppa28 (Bhagirathi and Mahanadi) after the probability level was corrected for SB correction. Analysis also revealed the possible signs of null alleles at these loci that could be responsible for the observed excess of homozygotes (+Fis). Null alleles were also indicated (Table 5) at loci Ppa14 (Mahanadi) and Ppa23 (Bhagirathi). Significant genetic heterogeneity ($P < 0.05$) was evident at three loci, Ppa02, Ppa05 and Ppa28.

Table 5. Characteristics of *Pangasius pangasius* microsatellite loci

Locus	River	Na	Size Range (bp)	He	Ho	P _{HW}	P _G
POLYMORPHIC							
Ppa01	Bha	3	135-145	0.210	0.231	1.000	0.9631
DQ 835618	Mah	5	135-145	0.281	0.313	1.0000	
Ppa02	Bha	6	109-123	0.590	0.500	0.032	0.007*
DQ 835619	Mah	9	111-135	0.839	0.471	<0.001**	
Ppa05	Bha	2	130-134	0.482	0.429	0.660	0.029*
DQ 835622	Mah	3	130-134	0.553	0.474	0.197	
Ppa14	Bha	8	136-152	0.790	0.550	0.001**	0.072
DQ 835631	Mah	7	136-150	0.750	0.444	0.004#	
Ppa17	Bha	7	113-131	0.626	0.441	0.007	0.311
DQ 835634	Mah	7	111-127	0.476	0.412	0.042	
Ppa18	Bha	5	110-122	0.370	0.449	0.666	0.406
DQ 835635	Mah	5	112-124	0.381	0.368	0.666	
Ppa22	Bha	4	112-120	0.611	0.564	0.027	0.066
DQ 835639	Mah	3	112-120	0.550	0.450	0.161	
Ppa23	Bha	6	095-125	0.636	0.646	0.008#	0.836
DQ 835640	Mah	6	103-129	0.364	0.600	0.820	
Ppa28	Bha	6	163-177	0.789	0.680	<0.001**	<0.001*
DQ 835645	Mah	7	161-173	0.471	0.412	0.003**	
MONOMORPHIC							
Ppa11	Bha	1	110	-	-	-	-
DQ 835628	Mah	1					
Ppa19	Bha	1	143	-	-	-	--
DQ 835636	Mah	1					
Ppa25	Bha	1	129	-	-	-	-
DQ 835642	Mah	1					

Na (alleles observed), H_e (expected heterozygosity), H_o (observed heterozygosity), Bha (Bhagirathi), Mah (Mahanadi), P_{HW} (Probability of conformity to HW expectations, * significant p<0.003, # possibility of null alleles), P_G (Probability of genetic homogeneity between the samples, * significant P<0.05).

Genotyping of individuals from natural populations with identified mitochondrial DNA markers

Identification of markers- Cytochrome oxidase I region

In *P. pangasius*, slow evolving region of mitochondrial DNA, Cytochrome oxidase I region was amplified using universal primers. Total length of the PCR amplified product was found to be 619 bp after sequencing, excluding the primers and the haplotypes were found varying at base 250 (C/T) and at 519 (A/G).

Population studies through Cytochrome b region

In *P. pangasius*, medium Cytochrome b region of mtDNA was investigated to determine genetic variation. Approximately 350 base pair fragments were amplified. The Cytochrome b region of individuals from two rivers, Bhagirathi (no=16) and Mahanadi (no=9) were amplified and sequenced. There were seven polymorphic sites and a total of 12 haplotypes were observed. Gene diversity in Bhagirathi was 0.8583 +/- 0.0626 while in Mahanadi 0.8889 +/- 0.0910 and Nucleotide diversity (average over loci) were 0.004642 +/- 0.003346 and 0.005248 +/- 0.003877, respectively.

Genetic divergence studies in prioritized marine finfish and shellfish

Documentation of genetic variation is of vital importance for evolving conservation and aquaculture strategies with long-term impact. Genetic variation can be directly assessed through genetically controlled molecular markers. These markers may involve assessment of variation directly at DNA level or through

phenotypic expression that can be protein or morphological variants. The use of more than one marker can help in enlarging the scope of utilization of such data. Therefore, the work on genetic divergence studies in prioritized marine finfish and shellfish was continued.

Bombay duck

Popularly known as 'Bombay duck' in English, *Harpadon (=Harpodon) nehereus* belongs to Order Aulopiformes and Family Harpadontidae. The species holds a pride of place in long established artisanal sector of the North West and North East coasts of India. It is an important fish for domestic use and also a valuable export item in dried or laminated form. Fresh extracts from Bombay duck is believed to have considerable medicinal properties. The fishery is supported by a single species *H. nehereus*. The landings of this species contribute about five percent of all India marine fish landings. The average annual landings have been estimated as 1.1 lakh tonnes by traditional and industrial sector along the NW (88%) and NE (12%) coasts of India. The population genetic structure of the stocks confined to north-east and north-west coast of India is unknown till date. In addition, occurrence of one more species *viz. H. squamosus* in the commercial landings in Visakhapatnam also needs to be ratified using molecular markers.

The genetic analysis of Bombay duck using RAPD [86 individuals each from the northwest and northeast coasts of India; 116 loci with 10 primers (OPA-07, 09, 11; OPAA-05, 12, 14; OPAC-05, 14; OPB-08, 09) in both the populations; 64 bands (55.17%) were polymorphic] showed clear genetic differentiation with high overall G_{ST} (24.53%) and genetic distance (21.12%) values between both the populations. Partial sequence information of 16SrRNA generated 2 stock-specific haplotypes; out of the two haplotypes observed, one was

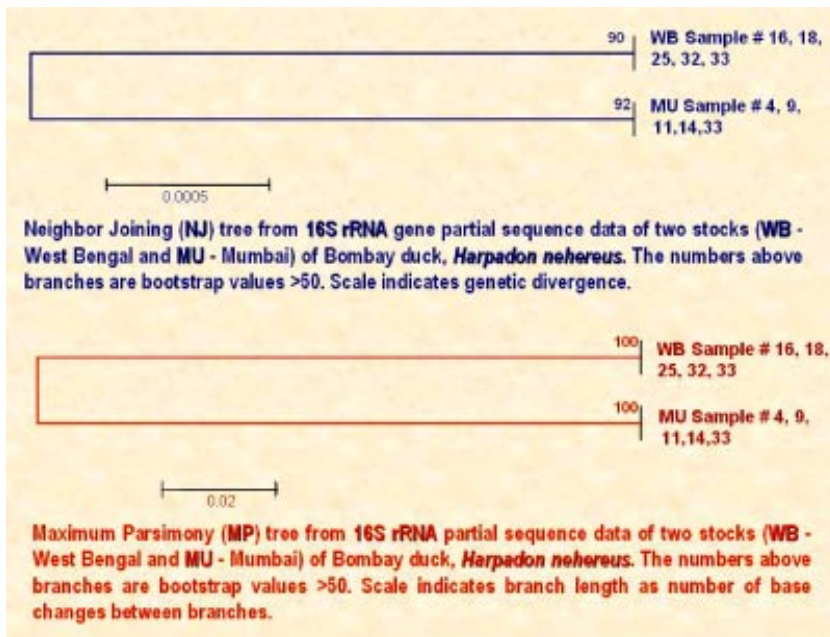


Fig.9. Genetic relatedness of north-east (West Bengal) and north-west (Mumbai) populations of Bombay duck based on partial sequence information of 16SrRNA gene indicating no sharing of haplotypes between the populations

specific for Mumbai stock and the other for West Bengal stock (Fig.9). The genetic divergence value between both the populations was 0.37% with 16SrRNA.

Five microsatellite markers were first developed in *H. nehereus* using enrichment procedure with biotin-labelled microsatellite probes (CA)₁₂ and (CT)₁₂ and streptavidin-coated magnetic beads. The percentage of positive clones containing microsatellite repeats were 32% and almost all the microsatellites contained (CA)_n repeats but no (CT)_n repeats. More microsatellite markers will be developed and these will be utilized to screen the genetic variation within and between the stocks of *H. nehereus* from east and west coasts of India.

The canonical standardized Discriminant Function Analysis (DFA) with additional samples (total 121 specimens each from both the coasts) confirmed the earlier results of population differences of Bombay duck between NE and NW

coasts, mainly due to the distance measures in the posterior region of the fish body behind the dorsal fin.

Lobsters

In India, the lobsters are an important export commodity. Heavy demand and attractive price for lobsters in the international market have resulted in increased exploitation of lobsters in recent years. Although the lobster fauna of commercial fishing grounds comprises 14 species of littoral and 6 species of deep-sea forms, only four littoral and one deep-sea forms contribute to commercial fishery viz. *Panulirus homarus* (scalloped spiny lobster), *P. polyphagus* (mud spiny lobster), *P. versicolor*, *P. ornatus* (ornate spiny lobster), *Thenus orientalis* (slipper or shovel-nosed lobster) and *Puerulus sewelli*. The genetic diversity of the lobster species from Indian waters is yet to be studied. In addition there are reports of occurrence of 3 sub-species of *P. homarus* namely, *P. homarus homarus*, *P. homarus rubellus* and *P. homarus megasculptus* from Indian waters which also needs to be confirmed using molecular markers.

In *Panulirus homarus*, work on genetic characterization with 80 samples each collected from Kollam (Kerala), Chennai (Tamil Nadu) and Visakhapatnam (Andhra Pradesh) and *Thenus orientalis* from Veraval (Gujarat), Chennai (Tamil Nadu), Visakhapatnam (Andhra Pradesh) and Kollam (Kerala) using 8 polymorphic microsatellite loci and 10 Operon decamers continued and initial analysis indicated low genetic differentiation of both the species between east and west coasts.



Fig. 10 : *Panulirus ornatus*



Fig. 11 : *Linuparus somniosus*

With a view to generate species-specific signatures of commercially important lobster species, partial sequence information of (>600 bp) 16SrRNA and Cyt-b of *P. homarus*, *P. versicolor*, *P. ornatus* (Fig.10), *P. longipes*, *P. polyphagus*, *P. penicillatus*, *Thenus orientalis*, *Linuparus somniosus* (Fig.11), *Puerulus sewelli* and *Petrarchus rugosus* (5 specimens each) were generated.

Sea cucumber (*Holothuria scabra*)

The sea cucumbers constitute an important marine living resources occurring in the seas around India. Nearly 200 species of sea cucumbers are known in the seas around India and most of them are found in deep waters, of which about 10 are of high commercial value including *Holothuria scabra* (Fig.12). They are captured indiscriminately for the preparation of highly priced export product 'beche-de-mer'. The resources gradually got depleted due to over-exploitation resulting in decline of export

quantity and reduction in size. Presently, there is a total ban on export of this item, though this has not prevented its clandestine export to Southeast Asian countries. For any endangered species, information on population genetics and life-history traits is highly essential to take appropriate management decisions. If genetic divergence exists between geographically isolated populations, there is a need to evolve separate management strategies for different populations.

Successful cross-species amplification of 5 more microsatellite loci in *Holothuria scabra* using primers from Japanese sea cucumber (*Stichopus japonicus*) was achieved, thus, developing a total of 10 polymorphic loci to be used in population genetic analysis of *H. scabra*.



Fig. 12 : *Holothuria scabra*

Species-specific mtDNA profiles for ornamental finfish species

The genus *Garra* Hamilton includes freshwater fishes belonging to the Order Cypriniformes and Family Cyprinidae. Most of the species inhabit rapid running waters and adapt to the substratum by means of the horizontally placed paired fins, especially the pectorals. Owing to their feeding of algae encrusted on aquarium walls, these peaceful species enjoy a good market as ornamental varieties in India and abroad. Aquaculture

technologies developed during recent decades have motivated private fish farmers in South India to take up ornamental fish culture of many local cyprinids. But no attempt so far has been made to popularize the *Garra* species to curtail their over-exploitation from the wild for ornamental trade. Owing to the highly restricted distribution, the Conservation Assessment Management Plan (CAMP) workshop in 1997 identified *G. surendranathanii* and *G. gotyla stenorhynchus* as 'endangered' as per the IUCN categorization. Despite the great deal of attention that has been focused on this group, we still know little about either the diversification of the Family or its placement within Cypriniformes. Partial sequence information of 16S rRNA, CO I and Cyt b genes of *G. surendranathanii*, *G. gotyla stenorhynchus* and *G. mullya* were generated (Fig.13). All the three genes exhibited species-specific genetic divergence values (Table 6) and sequence pattern; the Maximum parsimony (MP)

Table 6. Genetic divergence among *Garra surendranathanii* (Gs), *G. mullya* (Gm) and *G. gotyla stenorhynchus* (Gg), calculated based on sequence information of partial sequence information of mitochondrial 16S ribosomal RNA, cytochrome b and cytochrome C oxidase I genes.

16S rRNA	Gs	Gm	Gg
<i>Garra surendranathanii</i> (Gs)	----	0.0887	0.0628
<i>G. mullya</i> (Gm)		----	0.0730
<i>G. gotyla stenorhynchus</i> (Gg)			----
Cytochrome b			
<i>Garra surendranathanii</i> (Gs)	----	0.1610	0.1625
<i>G. mullya</i> (Gm)		----	0.1863
<i>G. gotyla stenorhynchus</i> (Gg)			----
Cytochrome C oxidase I			
<i>Garra surendranathanii</i> (Gs)	----	0.1348	0.1487
<i>G. mullya</i> (Gm)		----	0.1600
<i>G. gotyla stenorhynchus</i> (Gg)			----



Fig.13. Ornamental Cyprinid fishes of Western Ghats

and the Neighbor joining (NJ) analyses using Kimura 2 parameter yielded trees with identical topology with high bootstrap support values and the sequences were submitted to NCBI GenBank.

Taxonomic status of two ornamental nandid fishes confirmed

The taxonomic ambiguity of the ornamental nandid fishes, endemic to the western ghats viz., *Pristolepis marginata* and *P. fasciata* (Fig.14) were resolved using the partial sequence information of 16S14rRNA gene. The genetic divergence value was found to be 2.8% and the sequences were submitted in NCBI Genbank.



Fig. 14. Ornamental nandids of Western Ghats

Genetic characterization of *Trichodesmium* spp. from Indian waters

The work on genetic characterization of the harmful cyanobacteria *Trichodesmium* spp. from Indian waters using molecular markers and scanning electron microscopy (SEM) was continued. *Trichodesmium* spp. are a group of globally significant, most wide spread and abundant marine cyanobacteria and are believed to be an important source of fixed nitrogen in open oceans. Taxonomic ambiguity exists in *Trichodesmium* species and one of the five species—*T. thiebautii* is reported to secrete toxin similar to paralytic shellfish poison (PSP), hence, occurrence of this species in Indian Coast needs to be examined.

Diagnostic (species-specific) molecular markers were developed (sequence information of genes such as 16SrRNA, regulatory gene—*het R* and Internal Transcribed Spacers (ITS) region)

to distinguish different species of *Trichodesmium* from Indian waters.

All the samples provided by CMLRE and collaborators (two samples from Gulf of Mannar and one sample from Indian Ocean; two from North-west coast of India) were found to be *T. erythraeum* based on partial sequence information of 3 genes and not the toxic species, *T. thiebautii*.

Genetic diversity analysis in Indian seahorse

This new work was undertaken to access the diversity of seahorse species in the Indian waters and their molecular identification for conservation purposes. Survey was conducted along the coastal line of India covering Tamil Nadu, Kerala, Karnataka, Goa and Maharashtra. Eight species of seahorse belonging to one genus were recorded (Table 7). *Hippocampus kuda* and *H. spinosissimus* in Palk Bay and *H. trimaculatus* in the Gulf of Mannar are relatively more abundant. Few samples of *H. kelloggi* and *H. mohinikei* were reported from the Gulf of Mannar, *Hippocampus kuda* and *H. trimaculatus* from Kerala coast while *H. kuda* is the species available in the Karwar and Maharashtra waters. Seagrasses, seaweeds and dead corals were the preferred habitat of seahorses along the coastal region and in the deep sea while in the estuary water they live in mangrove area. *H. kelloggi* was recorded at 10 to 20 m depth while others were found at depths ranging from 1 to 4 m. Abundance of the seahorses was more in the Palk Bay followed by the Gulf of Mannar. Approximately 40% were *H. trimaculatus*, 30% *H. kuda*, 15% *H. spinosissimus* and rest of the species contributing only 15%.

Seahorses, including pipehorses, pipefishes and seadragons are members of the Family Syngnathidae under Order Syngnathiformes which includes 215 species under 52 genera. Seahorse characteristics of low fecundity, limited

mobility, structured mating patterns and site fidelity make them particularly vulnerable to heavy fishing pressure. Presently 7 species of seahorses are listed as 'vulnerable', one as 'endangered' and 25 species as 'data deficient' in the 2007 IUCN Red List of Threatened Animals.

In India, there has been considerable exploitation of the syngnathids in terms of quantity and value, especially for purposes of export. Skin divers, who collect molluscs and/or holothurians, also collected the seahorses stealthily. The Ministry of Environment and Forests, Govt. of India banned the export for all the syngnathids from the July 11, 2001 and kept them under Schedule I, Section 50 & 51 of the Indian Wildlife Protection Act, 1972.

Table 7. Species richness of seahorses in Indian waters

Present Status			
S. No.	Scientific Name	Complex of Species	Place
1.	<i>H. borboniensis</i>		Kochi/Palk*
2.	<i>H. fuscus</i>		Palk
3.	<i>H. histrix</i>	4	Expected
4.	<i>H. kuda</i>	10	Kochi/Palk
5.	<i>H. trimaculatus</i>	2	Kochi/Palk
6.	<i>H. spinosissimus</i>	p	Palk
7.	<i>H. kelloggi</i>	Not well described	Expected
8.	<i>H. mohani</i>		New 2007

Clear species identification will make it easier to: develop marking systems to distinguish aquacultured seahorses from specimens caught from wild, to modify fishing practices appropriately and to design protective marine reserves for assessment of captive breeding potential of seahorses (Fig.15). The extent and pattern of genetic diversity of seahorse from different geographic regions of India by using various mitochondrial Cytochrome b and Cytochrome c oxidase I gene segments and also fast evolving genes for intra-specific level studies was initiated.



Fig. 15 Pregnant male and female seahorses under laboratory breeding conditions

Cytogenetic characterization of freshwater and marine fish species

a. Freshwater catfishes

Rita rita (Hamilton, 1822)

The live specimens of *Rita rita* were collected from river Tons and the kidney tissue was processed for chromosome preparations. The species was found to possess diploid chromosome number of 54 and the karyotype formula was established as $20m+22s+6st+6t$. The C-banding technique revealed presence of heterochromatic bands on single pair of submetacentric chromosomes (Fig.16a). The nucleolar organizing regions (NORs) were found on short arm of 2nd submetacentric chromosome by both silver and Chromomycin A3 (CMA₃) staining (Fig.16b).

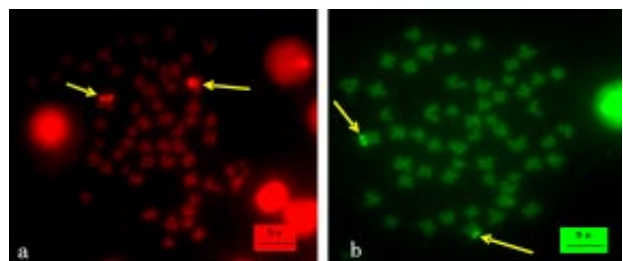


Fig. 16: Metaphase spreads in *Rita rita* showing. a. C-bands, b. CMA3 stained NORs. (Bar size- 5 μ m)

Aorichthys seenghala (Sykes, 1839)

The samples were collected from Allahabad for cytogenetic investigation. In this species, the diploid chromosome number was found to be 54. The karyotype formula established was 24m+18sm+2st+10t. The heterochromatic C-bands were observed on single pair of submetacentric chromosomes in *A. seenghala* (Fig.17a). The Ag-NORs were observed on short arm of one pair of submetacentric chromosome. Similarly, the CMA₃ bands were also observed on single pair of short arm of submetacentric chromosome (Fig.17b).

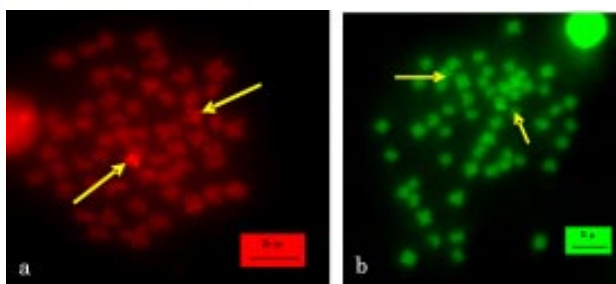


Fig. 17. Metaphase complement in *A. seenghala* showing. (a) C-bands and (b) CMA₃ stained NORs. (Bar size- 5 µm)

b. Marine fishes

Arius subrostratus (Valenciennes, 1840)

The samples of *A. subrostratus* were collected from Vypeen Island, Kochi, (Kerala). The diploid chromosome number was found to be 58 (Fig.18a) with karyotype formula of 22m+16sm+10st+10t. The NORs, as detected by CMA₃ staining, were present on three pairs of

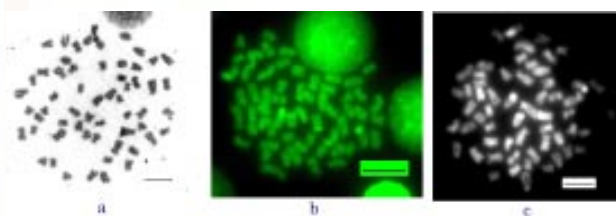


Fig. 18. Metaphase spreads in *A. subrostratus* (a) Giemsa stained, (b) silver stained NORs and (c) C-bands. (Bar length: 5 µm)

chromosomes (Fig.18b) The C-bands detected by propidium iodide staining were observed on seven pairs of chromosomes (Fig. 18c).

Zanclus canescens (Linnaeus, 1758)

Zanclus canescens, also locally called as Moorish idol, is an important ornamental species. The samples of this species were collected from Kovalam area of Kerala. In this species, the chromosome number was found to be 2n=48 and all the chromosomes were telocentric in morphology (Fig.19a). The CMA₃ staining revealed presence of intercalary NORs on one pair of chromosome near its centromere (Fig.19b) and there seems to be an association between C-band and CMA₃ stained region (Fig.19c).

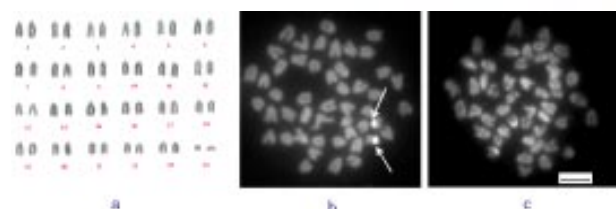


Fig.19. Karyological profile of *Z. cornutus* (a) Giemsa stained spread, (b) CMA₃ stained NORs and (c) spreads showing C-bands. (Bar size- 5 µm)

Thallasoma lunare (Linnaeus, 1758)

The samples of *T. lunare*, also known as Moon wrasse, were collected from Kovalam area of Kerala. The diploid chromosome number was found to be 48 with all acrocentric chromosomes (Fig.20a,b). The C-bands were present on several chromosomes at their centromeric positions (Fig. 20c).



Fig. 20. Karyological profile of *T. lunare* a. Giemsa stained metaphase spread, b. karyotype and c. spreads showing C-bands. Bar size- 5 µm

C. Mahseer species

The samples of *T. tor* and *T. putitora* were collected from river Tons at Chak Ghat in Rewa district, Madhya Pradesh and river Kosi at Manan in Almora district, Uttarakhand, respectively. The specimens of two species of Deccan mahseer, namely *T. khudree* and *T. mussullah* were collected from Tata Power Company, Lonavla, Maharashtra. In all the *Tor* species, a common chromosome number of $2n=100$ was observed.

Tor putitora

Based on the chromosome morphology, the karyotype formula was derived as $12m+22sm+14st+52t$ and fundamental arm number as 134 (Fig. 21a). The C-bands were found to be localized on several pairs of chromosomes (Fig. 21b). The 2nd metacentric; 6th, 9th and 10th sub-metacentric possessed small C-bands at their centromeric position. Most of the sub-telocentric chromosomes possessed C-bands at their centromeric position with the presence of very prominent heterochromatic block on largest sub-telocentric chromosome which could be used as a marker. The telocentric chromosomes also exhibited C-bands at their centromere position although the signals were less intense.

The silver nitrate (Ag) impregnation method technique revealed presence of NORs terminally on p arm of 2nd metacentric chromosome and 4th submetacentric chromosome (Fig. 21c). Staining

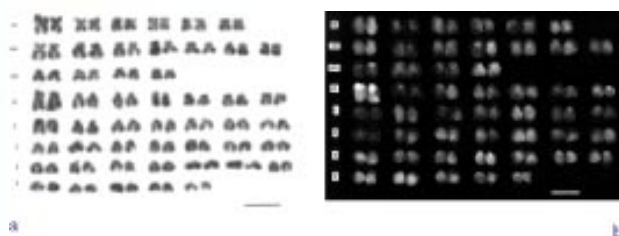


Fig. 21. Karyotype of *T. putitora* (a) Giemsa stained and (b) C-bands stained with propidium iodide. (Bar size: 5 μ m)

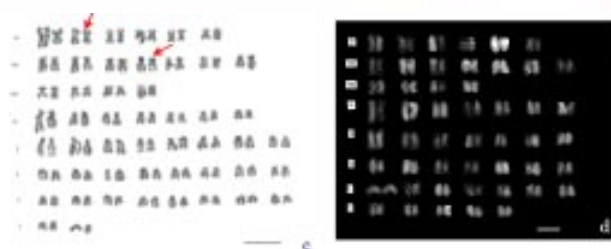


Fig. 21: Karyotype in *T. putitora* showing NORs (c) silver staining and (d) CMA_3 staining. (Bar size: 5 μ m)

with CMA_3 produced fluorescence in 2-3 chromosome pairs (Fig. 21d).

T. tor

The chromosome formula was derived as $20m+24sm+24st+32t$ with fundamental arm number (FN) as 144 (Fig. 22a). The C-bands were found to be localized on approximately 2 pairs of chromosomes (Fig. 22b). Both Ag-NORs were found to be localized on 4-5 pairs of chromosomes whereas the CMA_3 stained bands were found to be localized on 5 pairs of chromosomes (Fig 22c, d).

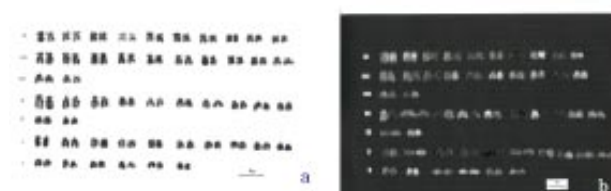


Fig. 22. Karyotype of *T. tor*. (a) Giemsa stained and (b) C-bands stained with propidium iodide. (Bar size: 5 μ m)

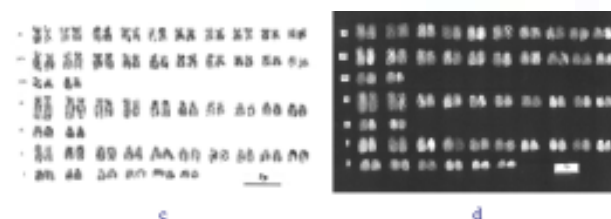


Fig. 22. Karyotype of *T. tor* showing NOR (c) silver staining and (d) CMA_3 staining. (Bar size: 5 μ m)

T. khudree

The chromosome formula was derived as 18m+16sm+44st+22t (FN=134) (Fig. 23a). The C-bands (Fig. 23b), Ag-NOR (Fig. 23c) and CMA₃ (Fig. 23d) were found to be localized on approximately 5 pairs of chromosomes.

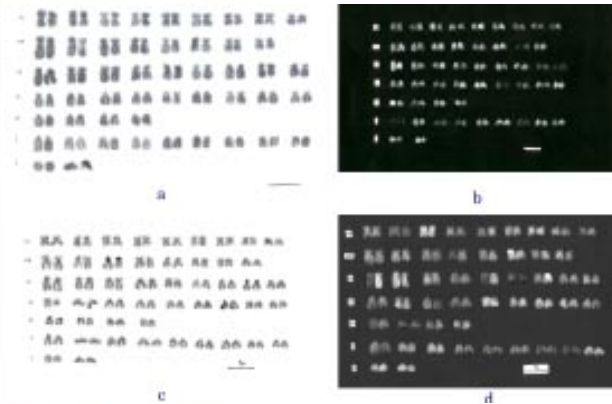


Fig. 23. Karyotype of *T. khudree* showing (a) Giemsa stained, (b) C-bands stained with propidium iodide, (c) silver stained and (d) CMA₃ stained NORs. (Bar size: 5 μm)

T. mussullah

The chromosome formula was derived as 22m+24sm+24st+30t (FN=146) (Fig. 24a). The C-bands were localized on 3 pairs of chromosomes (Fig. 24b). The NORs and CMA₃ stained bands were localized on 2 pairs of chromosomes in *T. mussullah* (Fig. 24c, d).

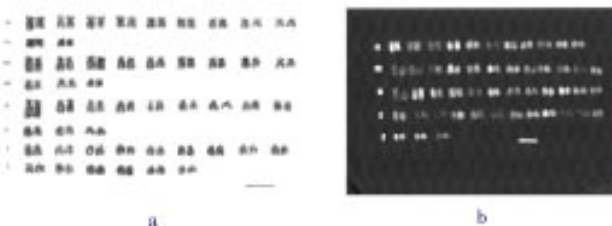


Fig. 24: Karyotype of *T. mussullah* (a) Giemsa stained and (b) C-bands stained with propidium iodide. (Bar size: 5 μm)

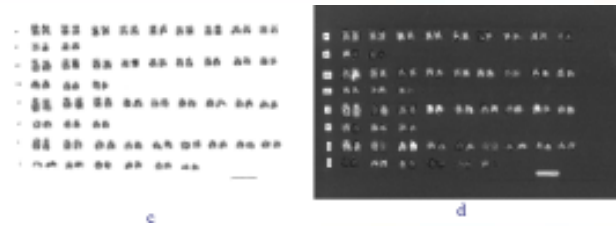


Fig. 24. Karyotype of *T. mussullah* showing NORs (c) silver stained and (d) CMA₃ stained. (Bar size: 5μm)

d. Other fishes

Nandus nandus (Hamilton, 1822)

Live specimens of *N. nandus* were collected from river Ghaghra near Gonda district of Uttar Pradesh for cytogenetic studies. The diploid chromosome number was found to be 48 and the karyotype formula was established as 6m+26sm+8st+8t (FN= 80) (Fig. 25).



Fig. 25. Giemsa stained karyotype of *Nandus nandus*. (Bar size: 5 μm)

The NORs were found to be present on short arm of one pair of submetacentric chromosome by silver staining method, however, the CMA₃ staining technique revealed presence of NORs on three pairs of chromosomes which is an indication of inactivation of two pairs of NORs. C-heterochromatic bands were observed on 5 pairs of chromosomes with prominent one located on short of one pair of submetacentric chromosome.

Development of Fluorescence *in situ* hybridization (FISH) probes

Hybridization and localization of rDNA sequences on chromosomes of *Channa punctatus*

The 18S ribosomal DNA probe was utilized for studying localization of 18S rDNA sequences on *C. punctatus* chromosomes by FISH. In the beginning, the amplified 18S rDNA in the fish was labeled with tetramethyl-rhodamine-5-dUTP by nick translation. The labeled probe was then purified and tested on agarose before FISH. Metaphase spreads were prepared on the slide using air-drying technique and the chromosomes were aged by incubating the slides overnight at 37°C. FISH was carried out as per the procedure of Winterfeld and Roser (2007) with appropriate modifications. After post-hybridization washes, the slides were stained with DAPI counterstain and the slides were observed under microscope equipped with suitable filters. The positive hybridization signals were observed (Fig. 26) on two chromosomes of each metaphase complement as well as in all interphase nuclei indicating the success of the technique. In future, the FISH technique can

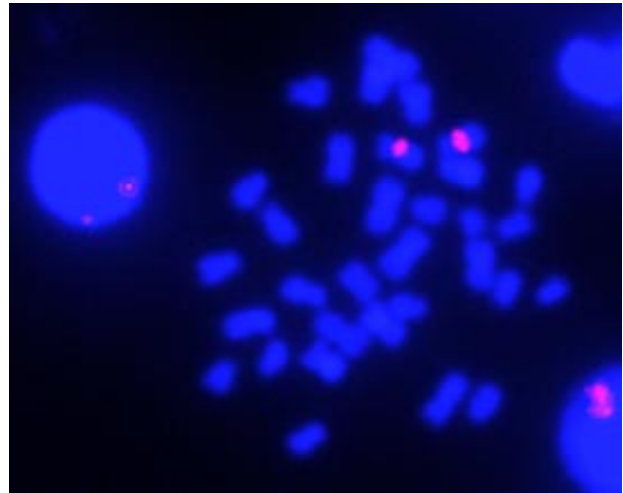


Fig. 26. Metaphase spread and interphase nucleus showing signals after hybridization with 18S ribosomal probe in *C. punctatus*

be used as indispensable tool for characterization of fish species.

Amplification and sequencing of rDNA regions

The different regions of rDNA gene was amplified and sequenced in four fish species with a view to identify and exploit the genetic variation for development of species-specific FISH probes. The sequences were submitted to the NCBI database (Table 8).

Table 8. Details of rDNA sequences submitted to NCBI database

S. No.	Accession No.	Definition	Length of fragment (bp)	Species
1.	EU442591	28S ribosomal RNA gene, partial D11	675	<i>T. tor</i>
2.	EU442592	28S ribosomal RNA gene, partial D11	677	<i>T. putitora</i>
3.	EU334837	28S ribosomal RNA gene, partial D9, D10	413	<i>T. putitora</i>
4.	EU327530	28S ribosomal RNA gene, partial D1	401	<i>T. tor</i>
5.	EU327531	28S ribosomal RNA gene, partial D1	400	<i>T. putitora</i>
6.	EU327532	28S ribosomal RNA gene, partial D3	570	<i>T. putitora</i>
7.	EU327533	28S ribosomal RNA gene, partial D6	551	<i>T. tor</i>
8.	EU327534	28S ribosomal RNA gene, partial D7	499	<i>T. tor</i>
9.	EU327535	28S ribosomal RNA gene, partial D7	501	<i>T. putitora</i>
10.	EU216546	ITS 1 région	588	<i>C. punctatus</i>
11.	EU216547	ITS 1 région	536	<i>L. bata</i>
12.	EU597006	18S partial (27.2.08)	1560	<i>T. putitora</i>
13.	EU597007	18S partial (27.2.08)	1565	<i>T. tor</i>
14.	EU621852	5S (05/08.04.2008)	201	<i>T. tor</i>
15.	EU621853	5S (05/08.04.2008)	201	<i>T. putitora</i>

Comparative RAPD profiling in *Channa* species

RAPD studies were undertaken to identify genetic variations among three species of freshwater murrels, namely *Channa punctatus*, *C. striatus* and *C. orientalis* using 20 decamer OPA primers. Out of the 20 primers tested, six primers (OPA-01, OPA-02, OPA-03, OPA-09, OPA-13 and OPA-18) generated polymorphic and detectable bands with good repeatability. The number of bands for each primer varied from 4 to 11 with an average of 6.83 bands per primer. The size of the amplified fragment ranged from 312 bp to 1915 bp. Among the *Channa* species, maximum number of bands were amplified in *C. punctatus* (18) followed by *C. orientalis* (17) and *C. striatus* (13).

Out of 41 major bands counted, there were 39 polymorphic bands with an overall polymorphism of 95.12% among the species and 2 monomorphic bands (Table 9). The percentages of polymorphic loci in each of three species varied between 84.62 and 88.89%. The highest polymorphism was observed in *C. punctatus* followed by *C. orientalis* and *C. striatus*.

Table 9. Percentage of polymorphic loci among three *Channa* species

<i>C. punctatus</i>	16		18	88.89
<i>C. striatus</i>	11		13	84.62
<i>C. orientalis</i>	15		17	88.24
Total	39	2	41	95.12

Among the three *Channa* species, inter-species genetic identity was largest between *C. striatus* and *C. orientalis* (0.7540) followed by between *C. punctatus* and *C. striatus* (0.6655) and between *C. punctatus* and *C. orientalis*

(0.5453). The results, based on the Nei's genetic identity and genetic distance, show a close genetic relationship between *C. striatus* and *C. orientalis*. The RAPD data provided evidence of a close relationship between the *Channa* species.

AFLP studies using silver staining

Amplified Fragment Length Polymorphism (AFLP) is a robust and reliable PCR based technique that is capable of producing high number of polymorphism per reaction than that revealed by RFLP or RAPD assay. AFLP has many advantages including cost effectiveness and speed for studying genetic divergence among the species or populations. The available methods for detection of AFLP bands are radio-labeling, fluorescence capillary electrophoresis, ethidium bromide staining, silver staining *etc.* The silver staining is relatively simpler with higher reproducibility and reliability. Therefore, AFLP studies have been initiated in a few fish species using silver staining technique.

Tor tor

The genomic DNA of *T. tor* was restricted by *EcoRI* and *MseI* followed by ligation of DNA fragments with adaptors. The known sequences of adaptors and restriction sites were used to serve as primer-binding sites for pre-selective and selective amplifications. The later was carried out using different *EcoRI* (*EcoRI*FACA, *EcoRI*FACC, *EcoRI*ACG, *EcoRI*ACT) and *MseI* primers (*MseI*CAC, *MseI*CTT, *MseI*CTG, *MseI*CTA) in 16 different combinations. The final PCR products were run on 8% denaturing polyacrylamide gel in 1X TBE buffer and the AFLP bands were detected with silver staining (Fig. 27). The results indicated that this technique has potential for precise characterization and identification of fish species.

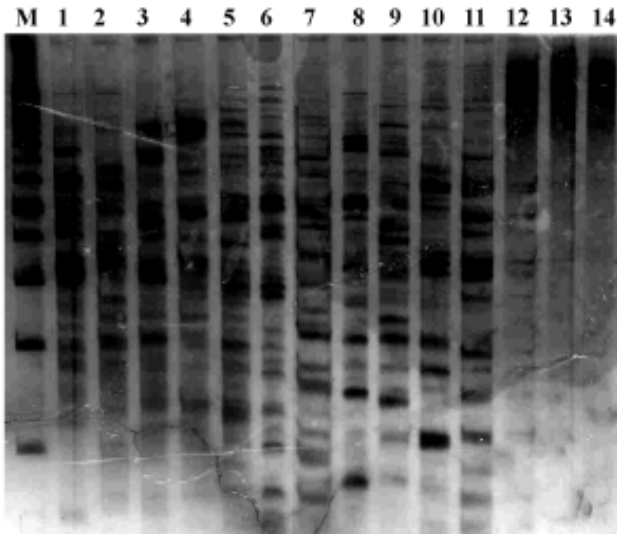


Fig. 27. AFLP bands in *T. tor* using silver stained gel, amplified with 14 primer combinations (M= 50 bp DNA marker)

Tor putitora

AFLP studies were also carried out in *T. putitora*. The bands amplified were resolved on 6% denaturing PAGE (19:1) on SQ3 manual sequencer. A modified technique using a mini PAGE denaturing gel in cold was used for resolving AFLP bands. Out of the 9 primer combinations utilized, 7 combinations produced bands whereas primer pair M-TT/E-CG and M-TC/E-CG did not produce any detectable bands. These 7 combinations produced variable number of bands, maximum numbers of bands were amplified with M-TA/ E-CT pair.

Rita rita

In *R. rita*, a total of 9 primer combinations were utilized for AFLP studies. Bands were resolved on 6% denaturing PAGE (19:1) on SQ3 manual sequencer. Primer pairs M-TT/E-CA, M-TA/E-CG, M-TA/E-CT and M-TA/E-CA did not produce any band. Maximum numbers of bands were amplified with M-TA/E-TT pair. The species specificity in the available AFLP band pattern was found between the species which could

further be used in the development of species-specific molecular markers.

Genotoxicity studies

Genotoxicity evaluation of Quillaja Saponin (QS) and ameliorative effects of turmeric

Plants containing saponin are being applied for fishing in natural water bodies like shallow streams and river stretches situated especially in the hilly areas. Incessant use of saponin can produce adverse effects on the existing fish diversity. As such, the information on these aspects is limited, hence, studies were undertaken to assess the genotoxic potential of QS and the ameliorative effects of turmeric (*Curcuma longa*) to reduce the DNA damage in fishes. At first, bioassay was conducted in static system to assess its acute toxicity to *C. punctatus* and the 96-h LC_{50} value was determined as 12.25 $\mu\text{g/g}$. The specimens were then administered with the sub-lethal doses *viz.* 3.06 mg/g and 1.53 mg/g by giving intra-peritoneal injection. Blood samples were collected after 24, 48, 72 and 96 h of exposure for performing the comet assay. The DNA damage in peripheral blood erythrocytes was assessed in terms of percentage of tail DNA and tail length. The overall comparisons between the DNA damage between treated and control group revealed a clear dose-dependent and time-dependent decrease in the induction of genotoxicity. The DNA damage in the erythrocytes was highest at 24h and 48h, respectively, for doses 3.06 mg/g and 1.53 mg/g and thereafter a gradual decrease in DNA damage was observed. The oral administration of crude extract of turmeric (3 $\mu\text{g/g}$) in combination QS (3.06 mg/g) resulted in reduction of the DNA damage after 24 h sampling, as compared to the specimens administered with QS alone (Fig 28). The results indicated that turmeric extract can reduce the DNA damage induced by QS.

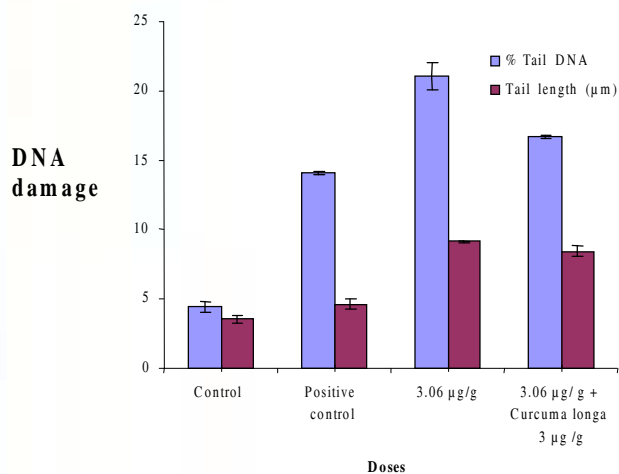


Fig. 28. DNA damage in erythrocytes by Quillaja Saponin and ameliorative effects of turmeric

Genotoxicity assessment of Rotenone in *C. punctatus*

Rotenone is a naturally occurring white odorless chemical obtained from roots of several tropical and subtropical plants with insecticidal, acaricidal and piscicidal properties. It is highly toxic and kills fishes within 24 to 36 h by inhibiting the process of oxidative phosphorylation. The present investigation was undertaken because scarce information is available regarding its toxicity and genotoxicity in fishes. The acute bioassay experiments were carried out in static system and the 96-h LC₅₀ value was determined as 0.45 ppm for *C. punctatus*. Based on this, the test concentrations of sub-lethal (SL) (1/8th of LC₅₀ = ~ 0.06 ppm) and non-lethal (NL) (1/16th of LC₅₀ = ~ 0.03 ppm) concentration were determined for studying its genotoxic effects using the comet assay.

The DNA damage was estimated in gill cells and whole blood cells in terms of percentage of tail DNA and significant effects of dose and duration of treatment on DNA damage were observed (Fig 29). In general, there was a dose-dependent increase in DNA damage which was

highest (15.44% and 11.86%) after 24 h exposure to sub-lethal dose (0.06 ppm) as compared to control values (3.59% and 5.59%) in whole blood and gill cells, respectively, followed by, a gradual non-linear decrease in DNA damage. The decrease in DNA damage with duration may be due to rapid breakdown of rotenone in water since its half-life has been reported to be 1-3 days in aquatic and terrestrial environments. The present study indicated that rotenone can induce genotoxic effect even at lower concentrations in fishes.

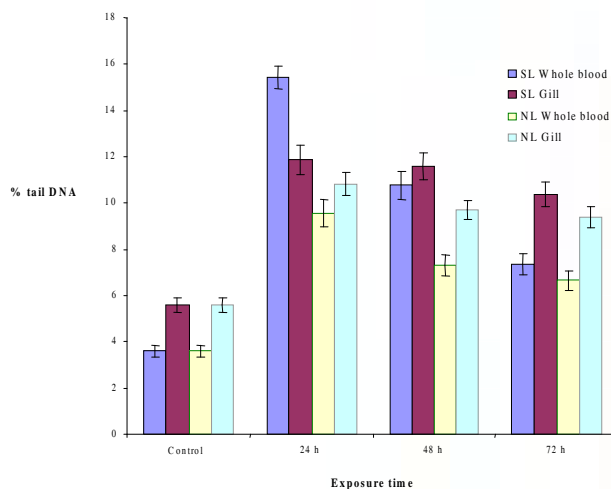


Fig. 29. DNA damage in different tissues by rotenone in *C. punctatus*

DNA damage induced by acute exposure of chlorpyrifos in *Channa punctatus*

Chlorpyrifos (O, O-diethyl O-3, 5, 6-trichloro-2-pyridylphosphorothioate) is one of the organophosphate pesticides widely used in agricultural practices. The information regarding the genotoxic and mutagenic nature of chlorpyrifos (CPF) in aquatic organisms is rare, especially the data pertaining to acute and long-term exposure to CPF in different tissues of fish. Therefore, genotoxicity of CPF was studied in freshwater teleost, *Channa punctatus* using

micronucleus assay (MN assay) and alkaline single-cell gel electrophoresis (comet assay).

Acute exposure

The fishes were exposed to three acute concentrations *viz.* 203, 406 and 609 $\mu\text{g/l}$ of CPF for 96 h and samplings were done at regular intervals for assessment of the MN frequencies and DNA damage. The DNA damage was measured in terms of the percentage of tail DNA in the lymphocyte and gill cells and significant effects from both concentrations and time of exposure were observed in exposed fishes. The lowest DNA damage was observed at 24 h. There was gradual non-linear increase in the DNA damage in all tissues with progression of the experiment and the highest DNA damage was observed on 96 h for all treatment groups (Fig. 30 & 31). The increase in DNA damage from 24

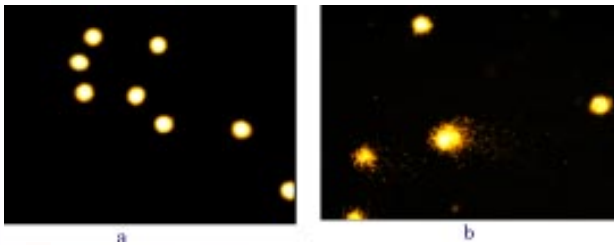


Fig. 30a. Normal DNA of lymphocytes, b. DNA damage in lymphocytes after exposure to chlorpyrifos

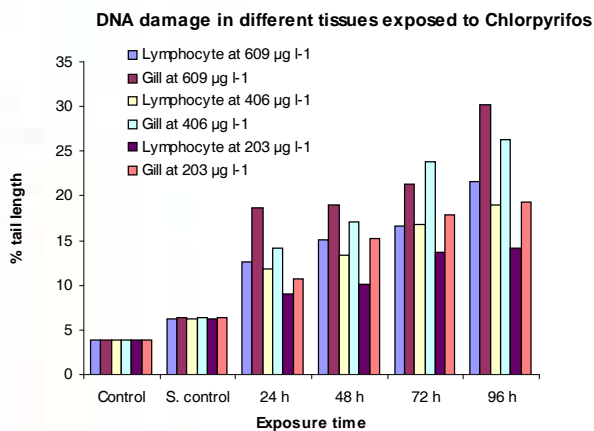


Fig. 31. DNA damage in different tissues due to Chlorpyrifos

to 96 h was significantly evident in all tissues except for the lowest dose in the gill cells. With regard to the variation in DNA damage between the tissues, the gill cells exhibited comparatively higher DNA damage than lymphocyte cells at most of the concentrations and durations. The micronucleus induction (MNI) was significantly higher in specimens due to exposure of different concentrations of CPF than the control group (Fig. 32). The MN frequency increased with the duration of exposure and was highest on 96 h at all concentrations in the peripheral blood.

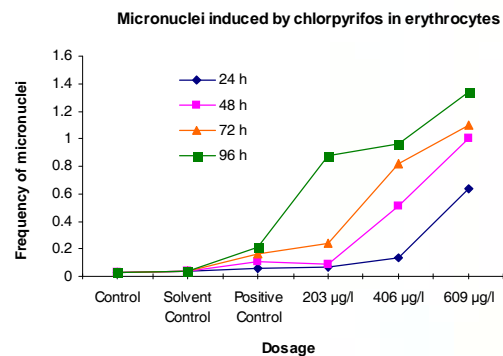


Fig. 32. Micronuclei formation in erythrocytes due to chlorpyrifos

Long-term exposure

Long-term genotoxicity investigation is an important approach for achieving greater insight into the organism's DNA repair ability and other protective mechanisms for excreting the toxic chemicals. For this study, the fishes were exposed to three test concentrations of chlorpyrifos *viz.* sub-lethal 1 ($1/4^{\text{th}}$ of $\text{LC}_{50} \sim 203.0 \text{ mg/l}$), sub-lethal 2 ($1/8^{\text{th}}$ of $\text{LC}_{50} \sim 102.0 \text{ mg/l}$) and non-lethal ($1/12^{\text{th}}$ of $\text{LC}_{50} = \sim 68.0 \text{ mg/l}$) in a semi-static system. The exposure was continued up to 35 days and tissue sampling was done at intervals of 1, 3, 5, 7, 14, 21, 28 and 35 days at the rate of five fishes per duration. The specimens, exposed to different concentrations of chlorpyrifos, exhibited significantly higher DNA damage in

their tissues than the control sample. The highest DNA damage was observed on 5 day and gradually there was a non-linear decrease in the DNA damage in all tissues as the duration of the experiment increased for all treatment groups (Fig. 33). With regard to the variation in DNA damage between the tissues, the gill cells exhibited comparatively higher DNA damage than lymphocyte cells at most of the concentrations and durations. Chlorpyrifos at different concentrations significantly induced MN in the fish specimens than the control group and the MN induction in the peripheral blood was highest on day 21 at 203.0 µg/l concentration of CPF (Fig.34). The present study indicated the

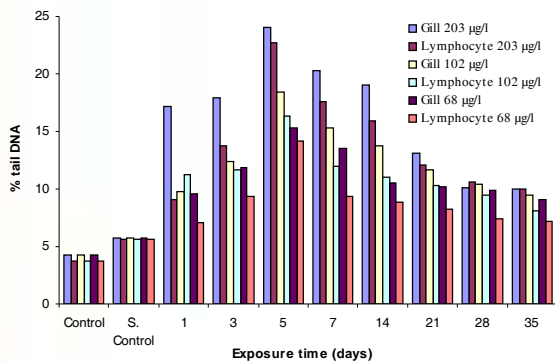


Fig. 33. DNA damage due to long term exposure of chlorpyrifos

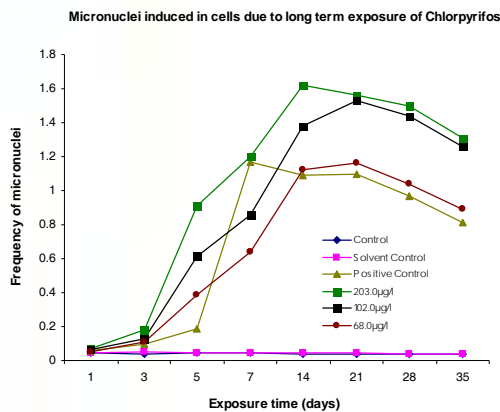


Fig. 34. Micronuclei induction due to long term exposure of chlorpyrifos

usefulness of combined use of micronucleus as well as comet assays for screening the genotoxic and mutagenic potential of xenobiotics in fish.

PCR amplification of selected genes for synthesis of cDNA

The gene expression may get altered in response to pollutants and can be used as a biomarker of genotoxic exposure in fishes. Therefore, studies were initiated for cDNA synthesis of two genes viz. metallothionein and cytochrome p450 that play important role in detoxification of heavy metals and polycyclic aromatic hydrocarbons, respectively. The total DNA was isolated from liver and kidney of *C. punctatus* using Tri reagent. The primers for these genes were designed from the sequence data available in the public domain and were commercially synthesized. The PCR optimization for amplification of genomic DNA was carried out (Fig. 35) and the same conditions were used for synthesis of first strand cDNA.

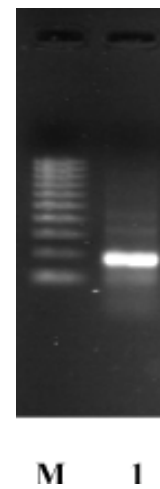


Fig. 35. Amplification of Metallothionein gene in *Channa punctatus*
M-100 bp ladder, 1-Amplified product *Channa punctatus* MT-3

Detection of DNA lesions using PCR based technique

The randomly amplified polymorphic DNA (RAPD) has been reported useful for preliminary assessment of eco-genotoxicity and detection of mutations. Therefore, this technique was utilized to study the genotoxic effects of acute concentration of heavy metals *viz.* arsenic trioxide and mercuric chloride. Genomic DNA was isolated from *L. rohita* fry exposed to arsenic trioxide (10 ppm) and mercuric chloride (1 ppm) and also the control specimens. RAPD was employed to detect the DNA damage using OPA 03 and OPA 09 primers and the amplified products were resolved on agarose gel. There was absence of bands in fry exposed to mercuric chloride whereas there was loss of bands and/or intensity in fry exposed to arsenic trioxide as compared to the control specimens (Fig. 36). Thus, the results indicated that RAPD can be used, along with other biomarkers, to evaluate the genotoxic nature of a chemical.

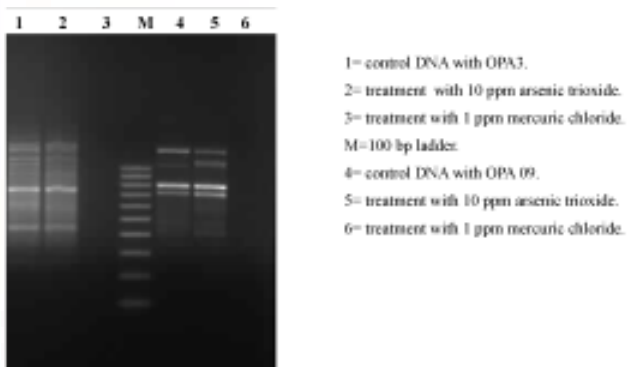


Fig. 36. RAPD profile of exposed *L. rohita*

In vitro induction of sister chromatid exchanges in *Channa punctatus*

Sister chromatid exchange (SCE) analysis is a sensitive indicator of chemically induced

chromosome damage and has increasingly been used for mutagenicity testing including aquatic models. Although the *in vitro* studies are very promising, the studies pertaining to *in vitro* induction of SCEs for assessment of genotoxicity in fishes are meager. Therefore, the present study was aimed at developing a suitable technique that uses peripheral blood lymphocytes (PBL) culture in *C. punctatus* for *in vitro* induction of SCE. Initially, sister chromatid differentiation (SCD) was standardized using different doses of Bromodeoxyuridine (BrdU) in cultured lymphocytes followed by induction of SCEs with varying doses of mitomycin C (MMC). An incubation period of 69 h was found to be optimum for getting higher mitotic index for PBL culture. The concentration of BrdU 10 $\mu\text{g}/\mu\text{g}/\text{ml}$ of culture medium was found to produce clear sister chromatid differentiation (Fig. 37). All concentrations of MMC induced SCEs in PBL and the frequency of induction was found to be concentration dependent (Fig. 38 & 39). The present technique could be utilized as a non-invasive and reliable *in vitro* genotoxicity assay using fish as a model for screening of environmental contaminants.

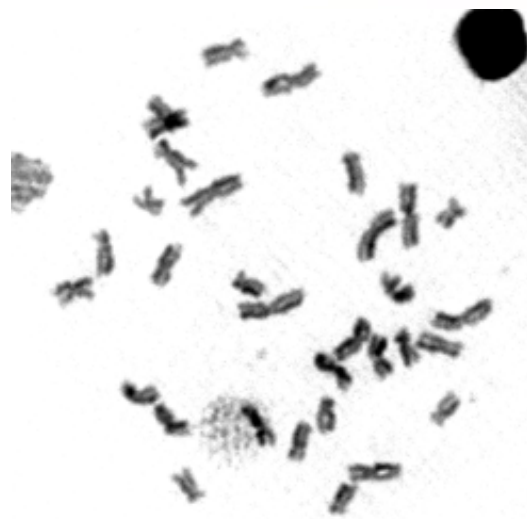


Fig. 37. Metaphase chromosomes showing SCD at 10 μg BrdU per ml of culture medium



Fig. 38. Metaphase chromosomes showing SCEs induced by MMC

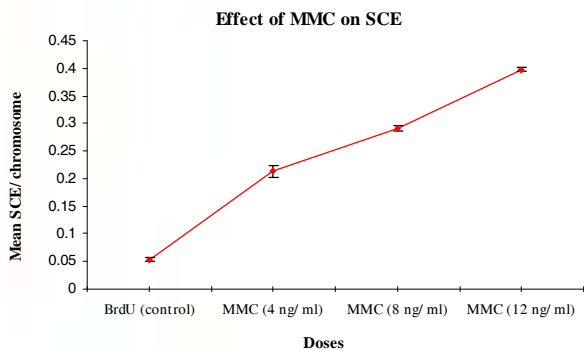


Fig. 39. Sister chromatid exchanges induced by mitomycin C

5.3 *In situ* Conservation

Evaluation and assessment of freshwater fish diversity of the river Ganga for conservation and management

The Ganges basin is located 70-80° 30' East and 22°-31' North. The total drainage area of the basin exceeds 1060000 km² and the basin is

the fifth largest in the world. The fish diversity is declining rapidly in Ganga basin due to various anthropogenic stresses. This emphasizes an immediate need for conducting research and actions for alternative management techniques to protect the fish diversity in freshwater aquatic resources. The detailed scientific information on the fish diversity, assemblage, occurrence and distribution and fish habitat of the target area are essential for undertaking conservation programme in any water bodies. Keeping this in view, a new work was initiated which envisages investigating and evaluating the current status and changing scenario of freshwater fish diversity, richness, distribution, life-history attributes of the threatened fishes, conservation status and the priority habitat of the identified areas of Ganga basin. The outcome of the present study would be useful in developing criteria for planning fish and habitat restoration, management of potential aquatic zones and creating conservation areas within Ganges basin and other large rivers. This work has been undertaken in collaborative mode with Wildlife Institute of India (WII), Dehradun (Uttarakhand) and Department of Zoology, Patna University, Patna (Bihar).

Preparation of drainage map

Topographical maps at the scale of 1:250,000 for some of the sampling areas were collected from Survey of India. Data on latitude and longitude for the collection points collected through GPS were overlaid on the above vector maps by creating the point vector file in ARC INFO. This point vector file shows place of collection where data on fish and habitat were collected. Maps with collection points were composed in ARC GIS. Vector maps of the drainage with administrative boundary at district level of Uttarakhand and Uttar Pradesh were created in ARC INFO from the existing Survey of India toposheets at the scale of 1:250,000. A multispectral satellite image

of IRS for Uttarakhand was collected and different collection sites were designated.

Germplasm exploration

The entire stretch of river Ganga has been divided into four sampling zones *viz.* (i) Upper (ii) Upper middle (iii) Lower middle and (iv) Lower, stretches for germplasm exploration. Upper stretch of river Ganga and its tributaries in Uttarakhand extends from Lakshar to Pauri Garhwal and Tehri Dam, covering about 350 km. Upper middle stretch of river includes Brijghat to Allahabad covering about 450 km., Lower middle stretch of river Ganga in Bihar include about 450 km area from Patna to Kahalgaon and Lower stretch of the sampling area in West Bengal include Mathurapur to Raghunathganj covering about 200 km. Across the stretches of river Ganga, four protected areas were also selected for exploration. These are Rajaji National Park, Dehradun, Uttarkhand; recently declared Ramsar site between Brijghat to Narora, Uttar Pradesh; Tortoise Sanctuary, Varanasi, Uttar Pradesh and Vikramshila Gangetic Dolphin Sanctuary, between Sultanganj to Kahalgaon, Bihar. Preliminary survey was carried out in all the identified stretches.

Fish diversity

Protected areas of river Ganga

Based on the germplasm exploration, maximum fish diversity was recorded from Turtle Sanctuary, Varanasi. A total of 59 fish species were reported from this sanctuary. A few of the threatened fishes found were *Chitala chitala*, *Eutropiichthys vacha*, *Pangasius pangasius* and *Cirrhinus reba*. In the Rajaji National Park, 40 fish species were recorded, about 50% of which are categorized as lower risk-near threatened. *Barilius dimorphicus*, a critically endangered species, was also recorded. Other important

species were *Chitala chitala*, *Ompok bimaculatus* and *O. pabda*. Altogether 41 fish species were recorded from Vikramshila Gangetic Dolphin Sanctuary, Bihar whereas 35 fish species were recorded from the Ramsar site (Brijghat to Narora stretch of river Ganga covering about 65 km all along the river stretch). *Salmostoma bacaila* was the dominant species followed by *Aorichthys seenghala*, *Aorichthys aor* and *Channa* spp. in this area. The study revealed that a good portion of fishes which are reported as threatened under different categories, were found in the protected and adjoining area indicating that protected area could be important for conservation of fish diversity of the Gangetic plains, especially for local and threatened species.

Fish diversity and abundance in the selected areas of river Ganga

A total of 96 species belonging to 58 genera of 24 families were recorded. Cyprinidae was the most dominant group (42.0%) followed by Bagridae (8.6%), Schilbeidae and Channidae (4.9%). Out of 96 species, 24 (25.0%) are considered as potential aquarium, fishes while 44 (45.83%) species as food fishes 6 (6.25%) species are potential sport fishes, 22 (22.91%) species are of miscellaneous category and 28 fishes are regarded of conservation importance (Fig. 40). Among all the species, 11 fish species (*Aorichthys aor*, *Bagarius bagarius*, *Catla catla*, *Channa striatus*, *Cirrhinus mrigala*, *Clupisoma garua*, *Labeo calbasu*, *Puntius sophore*, *Rita rita*, *Wallago attu* and *Xenentodon cancila*) were common in all the sampling zones. Maximum species richness (58 species) was recorded from lower zone followed by lower middle (50), upper middle (49) and upper zone (47). Significantly, exotic species *Cyprinus carpio* and *C. carpio* var. *specularis* were documented with higher relative abundance (> 4.0) in the upper stretch at Tehri Dam which is first distribution record in this site.

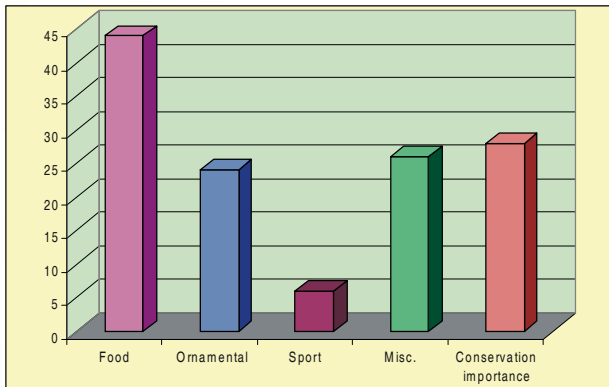


Fig. 40. Categorization of fishes in different zones of river Ganga

A comparative study on trophic level of fishes indicates that carnivorous group of fishes dominated in all the zones of the river, except upper stretches where omnivorous fishes were dominating (Fig. 41). Significant changes of overall fish diversity have taken place over the decade due to various causes. The change of overall fish diversity (%) in all the sectors of Ganga in comparison to earlier report is presented in Table 9.

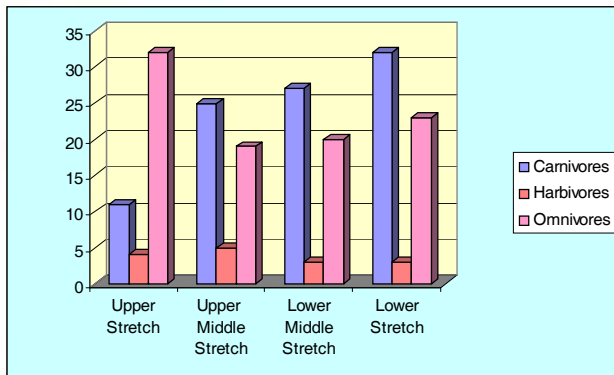


Fig. 41. A trend of shift in trophic position of fishes in river Ganga

The study indicated that a trend of swing of occurrence at various trophic levels has taken place with gradual dominance of less preferred species (*Barilius spp*, *Salmostoma bacaila*, *Puntius ticto*, *Johnius coitor etc.*) over the commercially important and conservation significant species (*Chitala chitala*, *Labeo rohita*,

Catla catla, *Aorichthys aor*, *Bagarius bagarius*, *Aorichthys seenghala*, *Clupisoma garua etc.*). It was observed that relative abundance of the conservation significant species was lower at all four sampling zones and less important species showed higher range of relative abundance (Table 10a & b). At species level, *Salmostoma bacaila* was dominant in upper middle and lower middle stretch while *Nandus nandus* was dominant in lower stretch.

Table 9 Changing pattern (%) of freshwater fish diversity of Ganga over the decade

Overall diversity	161	96	40.37
Upper stretches	58	47	18.96
Upper Middle	62	49	20.96
Lower Middle	56	50	10.71
Lower Stretch	80	58	27.5

Size distribution and new record size

The size range (total length;TL) of some conservation significant species was computed and it was found that *Aorichthys aor*, *Cirrhinus mrigala*, *Labeo calbasu*, *Labeo rohita* and *Wallago attu* were recorded with comparatively higher range of size group in upper middle zone whereas *Bagarius bagarius*, *Catla catla*, and *Salmostoma bacaila* were recorded with maximum size group in lower middle zone and *Clupisoma garua* in lower sampling zone. New size records of three species (*Ompok pabda*, *Cirrhinus reba* and *Glossogobius giuris*) were recorded from river Tonse, a tributary of river Ganga and lower stretches of river Ganga at Farakka (Table11, Fig. 42).

New extended distribution

New biogeographical shift of some of the species was observed in the upper stretches of river Ganga. These species were: *Macroglythys aral*, *Cyprinus carpio*, *Cyprinus carpio*-var.

Table 10a. Range of relative abundance (RA) of some conservation significant fish species

Range of RA (%)	Upper Stretch	Upper Middle Stretch	Lower Middle Stretch	Lower Stretch
0.1–1.5	<i>Aorichthys aor</i> , <i>Bagarius bagarius</i> , <i>Catla catla</i> , <i>Cirrhinus mrigala</i> , <i>Clupisoma garua</i> , <i>Labeo pangusia</i> , <i>Ompok pabda</i>	<i>Ailia coila</i> , <i>Aorichthys aor</i> , <i>Cirrhinus reba</i> , <i>Clupisoma garua</i> , <i>Labeo calbasu</i> , <i>Ompok pabda</i> , <i>Puntius sarana</i>	<i>Rhinomugil corsula</i> , <i>Puntius sararna</i> , <i>Mystus cavasius</i>	<i>Aorichthys seenghala</i> , <i>Anabas testudineus</i> , <i>Catla catla</i> , <i>Cirrhinus mrigala</i> , <i>Heteropneustes fossilis</i> , <i>Mystus cavasius</i> , <i>Chitala chitala</i> , <i>Glossogobius giuris</i> , <i>Labeo bata</i> , <i>Macrognathus aral</i> , <i>Mystus vittatus</i> , <i>Setipinna phasa</i> .
1.6–2.5	<i>Botia lohachata</i> , <i>Clarias batrachus</i> , <i>Channa striatus</i> , <i>Garra gotyla</i> , <i>Labeo bata</i> , <i>Nemacheilus beavani</i>	<i>Mystus tengara</i> , <i>Rita rita</i> , <i>Glossogobius giuris</i>	<i>Labeo calbasu</i> , <i>Labeo rohita</i> , <i>Botia dariio</i> , <i>Glossogobius giuris</i> , <i>Puntius sophore</i> .	<i>Glossogobius giuris</i> , <i>Labeo bata</i> , <i>Macrognathus aral</i> , <i>Mystus vittatus</i> , <i>Setipinna phasa</i> .
2.6–4.0	<i>Labeo gonius</i> , <i>Nemacheilus botia</i> , <i>Schizothorax richardsonii</i> , <i>Tor tor</i> , <i>Wallago attu</i>	<i>Chela laubuca</i> , <i>Puntius sophore</i> , <i>Rhinomugil corsula</i> , <i>Puntius ticto</i>	<i>Channa punctatus</i> , <i>Ompok pabda</i> , <i>Silonia silondia</i> , <i>Aorichthys seenghala</i>	<i>Amplypharyngodon mola</i> , <i>Eutropiichthys vacha</i> , <i>Gudusia chapra</i>
Above 4.0 %	<i>Barilius barila</i> , <i>Barilius bendelisis</i> , <i>Cyprinus carpio</i> , <i>Puntius sarana</i> , <i>Tor putitora</i>	<i>Eutropiichthys vacha</i> , <i>Salmostoma bacaila</i>	<i>Salmostoma bacaila</i> , <i>Xenentodon cancila</i> , <i>Macrognathus pancalus</i>	<i>Ailia coila</i> , <i>Puntius sophore</i> , <i>Puntius ticto</i> , <i>Nandus nandus</i>

Table 10b. Range of relative abundance in different sampling sites

Range of RA (%)	No. of species			
	Upper stretch	Upper Middle stretch	Lower Middle stretch	Lower stretch
0.1–1.5	19	39	26	45
1.6–2.5	12	3	10	6
2.6–4.0	10	4	8	3
Above 4.0 %	6	2	6	4
Total	47	48	50	58

Table 11. New size records of a few fishes

Name of the Species	Name of the river	Total length (cm)	Standard length (cm)	Body Weight (kg)	Earlier record (Talwar & Jingran)
<i>Glossogobius giuris</i>	Ganga (Farakka)	41	33	0.443	30 cm. (TL)
<i>Ompok pabda</i>	Tonse (Chakghat)	27.5	24.0	0.138	17 cm. (TL)
<i>Cirrhinus reba</i>	Tonse (Chakghat)	36.8	31	0.632	30 cm. (TL)

specularis, *Aorichthys aor*, *Puntius sarana* and *Ompok pabda*. This shift may be due to rise in maximum temperature in upper Himalayan stretch making it a conducive habitat for the warmwater fish species. According to IITM Pune, the maximum water temperature has increased by 6° C in this region within the last 30 years (1975-2005).

Life-history trait

Various biological parameters of some of the conservation significant fishes (13 species) were recorded during exploration which includes morphology, life stage and major reproductive

Glossogobius giuris



Cirrhinus reba



Ompok pabda



Fig. 42. Fish species for which new size records were recorded from Tonse, a tributary of river Ganga and lower stretches of Ganga at Farakka

parameters. During the survey in the month of November 07 to January 08, a total of 10 species were found to be gravid which might be due to changes in temperature and climatic variation of river system. Preliminary analysis of trophic niches of the available fish species indicated dominance of carnivorous species, except upper zone where omnivores species were found dominant (Table 12).

Table 12 Details of gravid fishes collected from different sampling sites of river Ganga

Name of fish species	Sampling station	Sampling period	Length (cm)	Weight (gm)
<i>Aspidoparia morar</i>	Patna, Munger and Bhagalpur	Dec 07	9.5 – 12.5	15 – 30
<i>Eutropiichthys vacha</i>	Patna	Dec 07	24	105
<i>Mystus tengara</i>	Munger	Jan 08	22	50
<i>Gudusia chapra</i>	Allahabad	Nov 07	14.2	19.5
<i>Mystus cavasius</i>	Munger	Jan 08	18	45
<i>Mystus menoda</i>	Munger	Jan 08	25	125
<i>Nangra punctata</i>	Munger	Jan 08	7.5	10
<i>Nandus nandus</i>	Allahabad	Nov 07	11.9	25.6
<i>Setipinna brevifilis</i>	Kahalgaoon	Jan 08	19	38
<i>Xenentodon cancilla</i>	Bhagalpur	Jan 08	18.5	25

Habitat attributes

There has been considerable changes in the aquatic environment due to decrease in rainfall and increase in siltation causing changes in the direction of water flow and turbidity of the Ganga river system over the years resulting in deterioration of fish habitat status. The impact of these changes would be felt on the fish diversity which needs to be correlated with fish conservation assessment. Therefore, major habitat parameters were recorded from each sampling sites of four zones and it is planned to collect more information for further analysis. The present study indicated that the water of river Ganga was slightly alkaline with a pH above 8 and conductivity in the range of 130.4 to 402.4 μ s. Variations in temperature was quite marked. In the upland zone (1082 – 2320' asl), temperature ranged from 18.8-22.7 °C during the month of October whereas it was 19.6 to 30.6 °C in the middle and lower zone of the river. However, in lower middle zone, the mean temperature was recorded 16.5 °C in Bihar during December. Higher value of total dissolved solids (209 ppm) was found in lower middle

stretch and turbidity was more (68 NTU) in lower stretch in comparison to other zones of the river.

Threats

Several threats including excessive water extraction, illegal fishing, dams and embankments, water pollution *etc.* to fish diversity and habitat were observed throughout the stretches of river Ganga. There is a critical need to define the factors that maintain diversity and ecosystem services. In the upper stretch these were- i) reduced water flow due to commissioning of recently constructed Tehri Dam at Garhwal Himalaya, ii) illegal catch by using poison and dynamite and iii) infestation of exotics fishes in the upper stretch of the river. The major threats identified in the upper and middle lower to lower zone were- i) discharge of domestic and industrial sewage in the protected areas as well as other fished area, ii) high load of suspended sediment in river bed, particularly from Narora to Allahabad, iii) fishing by small mesh sized net, iv) high water abstraction and consequent reduced river flow, and v) agricultural farming in the river bed and use of insecticides. Some people also catch fish on the boundary area of protected area using cast net, trap nets and gill net. Nevertheless, these activities close to the sanctuary boundary may later disperse into and out of the sanctuary. Hence, appropriate management plans needs to be framed to mitigate the threats and to review the legislative and institutional arrangements for biodiversity conservation and protection of river Ganga.

Fish germplasm exploration, assessment, cataloguing and conservation in North-Eastern Region

The North-Eastern region of India, comprising the states of Assam, Meghalaya, Manipur, Arunachal Pradesh, Mizoram, Tripura, Nagaland and Sikkim possesses a unique potential of fishery resources and is considered as one of the hot-spots of freshwater biodiversity in the world. Under the broad mandate of conservation of endangered species and

maintenance and preservation of fish genetic materials, a new work for the North-Eastern region was initiated in collaborative mode involving local collaborators from the region. The objectives were- i) exploration of fish germplasm resources in selected water bodies in NE region and documentation, ii) identification of markers and characterization of prioritized fish species/taxonomic groups to describe genetic variation at inter-and intra-specific levels, iii) gene banking of fish and shellfish species and iv) to develop protocols of captive breeding for potential food and ornamental fishes. During the period under report, potential collaborators were identified and respective work components were formulated and initiated.

Inventory and phylogeny of the Family Sisoridae of NE region of India

This work was undertaken in collaboration with Manipur University, Imphal. Germplasm inventory was conducted in the upper Brahmaputra basin in Arunachal Pradesh. Specimens were collected at 14 different sites of 13 different rivers which are draining the 8 different districts of the Arunachal Pradesh. From these collections, 20 fish species under 11 genera of 3 families were documented. Of these 20 species, the species status of 17 taxa could be identified and 3 species of the genus *Glyptothorax* are yet to be identified.

Exploration and propagation of threatened chocolate mahseer (*Neolissochilus hexagonolepis*) in Meghalaya

This work was undertaken in collaboration with St. Anthony College, Shillong, Meghalaya. Extensive surveys were carried out for identifying sampling sites of chocolate mahseer (*Neolissochilus hexagonolepis*) and *Danio rerio*

from the rivers Umran, Umralleng and Umieram of Meghalaya. Chocolate mahaseer (N=50) and *Danio rerio* (N=50) were collected from the rivers as well as adjoining paddy fields. The live gene pool of these two species is being maintained for further study.

Exploration, diversity assessment and documentation of the fish of Pakhui Wildlife Sanctuary, Arunachal Pradesh

In collaboration with Rajiv Gandhi University, Itanagar, Arunachal Pradesh, germplasm exploration was carried out in Pakhui Wildlife Sanctuary (862 sq km). Arunachal Pradesh is traversed by five rivers namely, Pakke, Khari, Upper Dikroi, Doigurung and Nameri. Besides recording the hydrological parameters, 45 fish species were collected from the sanctuary, of which, 11 specimens possess taxonomic ambiguity indicating rich diversity, in spite of unauthorized fishing in certain pockets of the rivers.

Habitat mapping and development of spatial database for mahseers of North-East Region

This work was undertaken in collaboration with Assam Central University, Silchar, Assam. Fish diversity of river Barak of the region was studied and availability of mahseers in different rivers of the region and important microhabitat features were documented. Altogether 65 fish species were collected from Barak and Sonai. Among mahseers, *Neolissochilus hexagonolepis* and *Tor mosal* were reported.

Documentation of indigenous knowledge and policy issues related to fisheries in Arunachal Pradesh

In collaboration with Rajiv Gandhi

University, Itanagar, Arunachal Pradesh, survey was conducted among the Galo, Adi and Nyishi tribes of Arunachal Pradesh covering villages of Siang, East Kameng and Papumpare districts. In all three tribes villages, clan and individual ownerships of water bodies were found. Among the Galos, out of 22 different types of fishing techniques currently practiced, 12 are traditional. Similarly, among the Adis, out of 10 techniques 7 are indigenous and in case of the Nyishis 7 varieties of indigenous fishing techniques are traceable. Besides individual fishing, community fishing is also prevalent among these tribes.

Fishes have high cultural, aesthetic and religious values to the tribals of Arunachal Pradesh. Traditional believes of tribes can be utilized as an indirect mode of conservation of water resources and fish species. Their indigenous techniques are also sustainable for conservation of fishes. However, traditional village councils are playing very effective role through the use of customary laws to carve out the destructive fishing practices and to save their water resources for posterity.

Status and role of temple sanctuaries in conservation of freshwater biodiversity

The study on status and role of temple sanctuaries in conservation of freshwater biodiversity was continued in river Gomti, U.P. During the period under report, survey was conducted to document the-(i) detailed socio-economic profile of fishers of nearby villages for six selected religious sites and (ii) fish diversity nearby to protected water of four respective sites. Comprehensive inventory of 59 protected sites (religious set up) was prepared along with status of the site. Description of each site was divided into 9 sections - (i) location and description of site and temple, (ii) social and religious activity of temple, (iii) real prohibited

status of site, (iv) legal status of protected site, (v) fishing pattern and stakeholders, (vi) socio-economic profile of local fishers, (vii) trend of fish catch, (viii) fish species diversity and (ix) conclusion.

1. Protected site in front of Ramjanaki Mandir Dhopap, Sultanpur

Ramjanaki Mandir Dhopap, Sultanpur is a very old construction at a high place on the bank of river Gomti. This temple site was well-known for fish protection for 500 metres of distance in front of temple. Poaching was reported in the absence of *pujari* or during night by a few fishers. There was report of frequent confrontation between local temple management and poaching persons. The temple institution has made efforts to keep it protected for conservation of aquatic animals. Since last few years, this area of river was not given on lease for fishing by the government. As per law, it was assumed that no fishing will take place during closed fishing season in the entire stream but fishing was reportedly done illegally by different traditional means like rod, angling and small nets throughout year. Sample fishing was carried out by the NBFGR survey team nearby the protected part of river in which a total 30 fish species were recorded.

2. Bholenath Shiv Temple, Babhangawa, Lamhua, Sultanpur

Bholenath Shiv Temple is situated on the bank of river Gomti at Babhangawa Ghat in district Sultanpur. One km length in front of temple was claimed totally protected by *pujaris*. Maximum depth of the river stream in front of temple was 10 meters during summer. There was a cremation ground within 1 km distance from temple and a deep pool downstream which provides a good shelter to aquatic animals for safe habitat. A total of 27 fish species were recorded by the NBFGR team during survey at this site.

3. Ram Janki Mandir, Gular Ghat, Jaunpur

This is an ancient temple. (renowned Math) with association of twelve temples situated in a radius of 60 km. Since 2001, due to religious reasons 200 meters length of river area in front of temple was declared protected and prohibited for fishing by District Magistrate, Jaunpur. Head *pujari* of the temple takes measures towards protection of fish in the protected area. Temple management provides food (@10-15 kg. wheat flour per day) for fishes. Density of fishes was visibly higher in the protected area. The maximum depth of river near temple was 7-8 meters during summer. Specific strength of executive manager of temple, combined with religious faith of people, make a strong base to associate society and people in masses for fish diversity conservation and sustainable yield from the remaining river.

4. Ram Janki Temple, Suraj Ghat, Jaunpur

It was built in the fifteenth century and is situated in the outskirts of the city headquarter of Jaunpur. Fishing was totally prohibited for 500 meters length in front of temple. Maximum depth of the river in front of the temple was approximately 50 feet during summer. Thirty five percent of the respondents reported that the fish catch was slightly higher in the river near the protected water while 65% respondents reported no difference in their catch. Fish yield per year from this river was declining and exotic fishes constitute a major proportion of the fish catches. Thirteen fish species were recorded during sample fishing near the protected water.

5. Goddess Akhado Temple, Jamaitha, Jaunpur

People reported that before three decades, complete protection of fishes was observed at this site. However, presently, nobody from temple management looks after the protection of fishes in the river in front of the temple, though the

fishing is prohibited during day time at present in front of temple. Over half of the respondents reported that the fish catch was slightly higher in the river near the protected water. Eleven fish species were recorded during sample fishing near the protected water.

6. Shiv and Hanuman Temple, Ebrahimpur Ghat, Pratapgarh

At this site, 200 meters portion of river in front of temple was completely protected. There is a big deep pool near this old temple which provides a good refuge to aquatic animals. Seventy percent of the local fishers reported that they are not fishing inside the protected water area in front of the temple. Half of the respondents reported that the fish catch was slightly higher in the river near the protected water whereas remaining half reported no difference in their fish catch.

It came out during interaction with the local people that they support the religious activities but conservation part was limited to the temple management only. During closed fishing season, fishing was done illegally at most of the sites by different traditional means like rod, angling and small nets throughout year. Local fisherfolks informed that over a period of ten years drastic reduction in the fish catches has taken place. Most of these religious sites with a temple management set-up, a history of protection and a deep pool, are ideal for conservation purposes. These sites need recognition by the state agencies as protected or religious waters for conservation of aquatic animals under custody of local institutions like Gram panchayats/Nyaya panchayats/or religious bodies or their cooperatives with proper terms and conditions.

Studies on fishing cooperative societies with focus on their potential for conservation of fishery resources

Studies were continued to assess the potential of fishing cooperative societies to utilize

them for conservation of fishery resources. Detail primary data were collected from fishing cooperative societies from two mundals of U.P. namely, Faizabad and Jhansi. A total of 13 societies were covered from five districts namely, Faizabad, Sultanpur, Abedkarnagar, Jhansi and Lalitpur. Data were also collected from one large reservoir of U.P., namely, Matatila, where no fishing cooperative society is working and the fishing is done by way of open auctioning to contractors. Data were also collected from another selected site, Bergi reservoir in M.P., where a total of 15 societies were surveyed for primary data collection.

Profile of the fisherfolk

Profile of the fisherfolk members of the fishing cooperative societies was studied in terms of their age, education, socio-economic status, extension contact, cosmopolitanness awareness and involvement of members in government schemes. (Table 13). Majority of the members of the fishing cooperative societies studied were in the middle age category.

Table 13. Summary of the profile of the fisherfolk

Variables	Bergi, M.P. N=150	U.P. (Small reservoirs/Lakes) N=90	U.P. (Riverine) N=40	U.P. (No society) N=35
Av. age (Yrs.)	40.5	41.3	44.5	38.5
Socio-economic status	12.3	12.8	12.4	11.3
Extension contact	5.52 [16]	4.86 [16]	4.52 [16]	3.21 [16]
Cosmopoliteness	4.25 [11]*	7.36 [11]*	7.20 [11]*	2.35 [11]*
Awareness & involvement in Govt. schemes	7.89 [18*]	6.50 [24*]	6.10 [24*]	1.85 [24*]

[*] Maximum obtainable score

Perception of fisherfolk about selected structural variables

Perception of fisherfolk about selected structural variables was also studied (Table 14). Effectiveness of the state fisheries agencies was perceived as low at all the selected sites. Effectiveness of NGOs in the area was perceived as medium at Bergi reservoir while it was perceived to be low at all the sites of U.P. Effectiveness of Panchayats was perceived as medium at all the sites. The member fisherfolk had negative attitude towards the state fisheries agencies at Bergi (M.P.) and Matatila (U.P.) reservoirs whereas fisherfolk at other locations of UP had medium attitude towards the state fisheries agencies. Internal functioning of the fishing cooperative societies was perceived as very effective by the member fisherfolks at the Bergi reservoir, whereas, it was perceived as very low at all the sites of U.P.

Conservation orientation of the members and conservation performance of the selected fishing cooperative societies

The orientation the fisherfolk members of the selected fishing cooperative societies was

studied with the help of an index which consisted of 15 statements related to their awareness of the importance of and interest in fish conservation, perceived sense of responsibility and perceived sense of self-capability towards conservation. It is clear from the data (Table 15) that the conservation orientation of the fisherfolk members was on the higher side (mean score > 47 out of 60) at the Bergi reservoir whereas it was low at all the sites of U.P.

Performance of the selected societies, in terms of the resource enhancement and conservation measures undertaken, was documented and analyzed with the help of a specially prepared index. The data (Table 15) revealed that the conservation performance of the societies, as perceived by the fisherfolk members, was very low at all the sites of U.P., whereas it was very high (mean score > 18 out of 21) at the Bergi reservoir during the period when it was managed by the federation of the fishing cooperative societies. However, the fisherfolk also responded that in the post-fishing cooperative federation management period, the conservation performance of the societies has declined (mean score 7.5 out of 21). Similarly, the fisherfolk members of the societies at the Bergi reservoir also told that the societies used

Table 14. Perception of fisherfolk about the selected structural variables

Variables	Bergi, M.P. N= 150	U.P. (Small reservoirs/Lakes) N=90	U.P. (Riverine) N= 40	U.P.(No society) N=35
Perceived effectiveness of state fisheries agencies	3.3 [9*]	5.3 [9*]	4.5 [9*]	3.8 [9*]
Perceived effectiveness of NGOs in the area	2.5 [5*]	1 [5*]	1 [5*]	1 [5*]
Perceived effectiveness of Panchayats	4.0 [9*]	4.65 [9*]	4.42 [9*]	3.13 [9*]
Attitude towards state fisheries agencies	16.1 [40]*	25.9 [40]*	24.3 [40]*	18.4 [40]*
Internal functioning of societies	44.0 [52*]	24.9 [52*]	23.8 [52*]	NA

[*] Maximum obtainable score

Table 15. Conservation orientation and performance of the members and fishing cooperative societies

Variables	Bergi, M.P. N= 150	U.P. (Small reservoirs/ Lakes) N=90	U.P. (Riverine) N= 40	U.P. (No society) N=35
Conservation orientation of members	47.30 [60*]	36.18 [60*]	37.15 [60*]	22.50 [60*]
Rule enforcement by societies	3.7 (1.5)^ [4*]	0 [4*]	0 [4*]	NA
Conservation performance of societies	18.10 (7.5)^ [21]*	2.30 [15]*	3.10 [15]*	5.89 [15]*

[*] Maximum obtainable score

^ In post-fishing cooperative federation management period

to undertake monitoring and rule enforcement activities in their area to implement conservation measures during the period when it was managed by the federation of the fishing cooperative societies, however, these activities have declined in the post-fishing cooperative federation management period.

The above study, conducted in three states (M.P., H.P. and U.P.) and covered 58 fishing cooperative societies and a reservoir in U.P. (where no society is working), came out with field-based data about the status, current role played and future potential of the fishing cooperative societies in conservation and management of the fishery resources at the selected locations. The findings indicated that though the fishing cooperative societies existed at all the locations, they played important role and contributed in resource conservation at those locations and under those situations where some of the facilitating factors/conditions were present. These factors/conditions were- high level of orientation of fisherfolks towards resource conservation; effective internal functioning of societies; effective structural & functional linkages with, and high perceived effectiveness of, state fisheries agencies; strong controlling and regulatory powers held and exercised, and facilitating role played by the state fisheries department; effective linkages with, and regular support of, NGOs; and strong collective action

and mass organization for common good. The policy makers, fishery administrators and conservation professionals can utilize these findings towards formulating strategies for harnessing and promoting organizational and institutional support for promoting conservation programmes at the grass roots level.

5.4 *Ex situ* Conservation

Gene Bank

Milt cryopreservation of the selected fishes of the Western Ghats

The work on milt cryopreservation of the two selected fishes of the Western Ghats was continued by conducting more experiments and fertility trials.

Horabagrus nigricollaris

Horabagrus nigricollaris, Pethiyagoda & Kottelat is an endemic, cultivable yellow catfish belonging to Family Bagridae and found only in a single river (Chalakkudy River, Kerala) originating from southern part of the biodiversity hotspot – the Western Ghats, South India. This species enjoys a good market value as food and ornamental fish (Fig.43). The species has recorded a sharp decline in the catches due to over-exploitation and the workshop on



Fig. 43. *Horabagrus nigricollaris*

Conservation Assessment Management Plan (CAMP), held in 1997 to evaluate the status of freshwater species of India categorized this species as “critically endangered” based on IUCN criteria due to its highly restricted distribution, loss of habitat, over exploitation, destructive fishing practices and trade. Captive breeding and milt cryopreservation techniques have been developed in this species for the first time thus adding one more fish to the list of species in which *ex situ* conservation protocols have been developed by NBFGR.

H. nigricollaris, weighing 75-150g were kept in 1 tonne tank (filled with pond water) with aeration and hiding places for acclimatization. Eight fishes were given ovaprim injection @ 0.5 ml/kg body weight and milt was collected after 24 hours of ovaprim injection, by pressing the belly in a dry plastic box. Quantity of the milt was upto 1 ml and motility of the milt was checked separately for each specimen using tap water as activator. Sperm count ranged between $16.5\text{--}20.9 \times 10^8$ spz/ml and spermatocrit was in the range of 57-59%.

Five extenders (NBFGR 6, NBFGR 7, NBFGR 7B, Hank’s balanced salt solution (HBSS) and Modified HBSS) were used for cryopreservation with DMSO (10%) as cryoprotectant. The ratio of milt, extender and cryoprotectant was kept as 1:3.5:0.5. Straws were filled with the extended milt, sealed with PVA powder and kept over ice for 10 minutes, then

on liquid nitrogen vapour phase for 10 minutes and finally plunged into liquid nitrogen. The cryopreserved straws were kept in canisters and stored in cryocans. After 48 hours of cryopreservation, straws were taken out, waved in air for 25 seconds and immersed into water bath at 37°C for thawing. Motility of the milt was examined. Extenders HBSS and M-HBSS showed score 3 and 4, respectively, whereas extenders 6, 7, 7B showed motility score of 2 only.

Two fertility trials were carried out using cryopreserved milt and actual fertility percentage was in the range of 18.5 to 30.8 and hatching rate in the range of 22.4 to 31.8% (65.1 to 84.14% as that of control). Modified Hanks balanced salt (Table 16) solution (MHBSS) showed highest percentage of fertility over other extenders. This is the first time milt of this species has been cryopreserved which will help to conserve this highly endangered species. This *ex situ* conservation tool will be a boon to *in situ* conservation strategies like ‘propagation-assisted rehabilitation’ of this endangered fish.

Table 16. Fertility rate and hatching rate in fertility trial of *Horabagrus nigricollaris* using cryopreserved milt

Extender	% fertility	% Hatching	% Hatching as that of control
NBFGR 6	18.5±1.1	22.4±2.3	59.25
NBFGR 7	23.8±2.4	24.6±3.3	65.12
NBFGR 7B	23.9±0.8	27.3±3.6	72.23
M-HBSS	30.8±3.7	31.8±1.5	84.14
HBSS	28.6±2.8	29.1±2.5	77.00
Control	36±0.4	37.8±1.7	100.0

Milt cryopreservation of *Garra surendranathanii*

Popularly known as “stone suckers” or “kallotti” in local parlance, *Garra surendranathanii* (Teleostei: Cyprinidae) is endemic to the rivers in Kerala of the Western

Ghats. The species was described first in 1996 by Shaji, Arun & Easa and it is confined to Chalakkudy, Periyar and Pampa rivers in the State. This species is consumed by the tribal people and considered as a good table fish. *G. surendranathanii* grows to a maximum size of 25.0 cm and the algal browsing property makes it an ideal species for freshwater aquarium. Owing to the restricted distribution, over-exploitation for aquarium trade and habitat alteration, the species was considered as “endangered” as per IUCN categorization in the CAMP Workshop held at NBFGR, Lucknow in 1997. Experiments conducted to cryopreserve the milt of *G. surendranathanii* were continued to finalize the optimal extender-cryoprotectant combination for carrying out the large-scale milt cryopreservation of this species.

G. surendranathanii spawners collected from river were kept in fiber glass tanks initially. For experiments, fishes weighing 40-60 g were kept in a glass tanks for conditioning. Ten male fishes were administered ovaprim injection at the rate of 0.4 ml/kg kg body weight and after 12 hr milt was collected by gently pressing the belly. Individual collections were made for all fishes. Milt quantity ranged from 0.2-1.0 ml/fish. The collected milt was evaluated for motility time, pH, spermatocrit and sperm density. Motility of the milt was checked using tap water as activator and scored in the scale of 0-5. Motility time was in the range of 45-60 seconds. Spermatocrit was measured using a micro-capillary tube, drawn the milt in the tube, sealed the ends and centrifuged at 3000 rpm for 3 minutes. The spermatocrit values were in the range of 30-35%. Sperm count was done using a haemocytometer after diluting the milt with extender. The sperm count ranged from $8.5-9.6 \times 10^8$ spz/ml.

Five extenders (NBFGR 3, NBFGR 3B, NBFGR 6, NBFGR 7, NBFGR 7B) used previous year for screening, were employed for cryopreservation. Milt was collected after 12 hr

of ovaprim injection by pressing the belly in a clean and dry plastic box. Individual collections were made for all ten fishes. After collection the milt was kept on ice. Milt quantity ranged from 0.4-1.0 ml/fish. Five extenders, earlier screened, were used for cryopreservation. Ethylene glycol (10%), which gave positive result in previous year was used as cryoprotectant. The ratio of milt, extender and cryoprotectant was kept as 1:3.5:0.5. Cryoprotectant was added just before filling the straw to reduce the toxicity to milt. Straws were filled with the extended milt, sealed with PVA powder and kept on ice for 10 minutes, then liquid nitrogen vapour phase for 10 minutes and finally plunged into liquid nitrogen. The straws were then stacked in canister and kept in cryocans containing liquid nitrogen. After 24 hours of cryopreservation, straws were taken and thawed at 37°C. Straws were then cut open and checked for the motility of the milt. Extender 3, 3B and 6 showed score only 2 whereas extender 7 and 7B showed score of 3 in a 0-5 scale.

Six female fishes (50-80 g) were given ovaprim injection @ 0.4 ml/kg body weight and after 12 hr of injection fishes were stripped. Approximately 300 ml of eggs (~200 nos) were taken in a plastic tray and milt from a two straws used for fertilization. Fresh milt was taken as control. Milt was poured on the egg, mixed then added tap water, as an activator. It was thoroughly mixed and washed with water and kept for development. After 8 hr dead eggs were removed and fertilization percentage was calculated. Hatching percentage was calculated after 24 hr. Extender 7 showed 71.8% fertility and 73.35% hatching as that of control (Table 17). In a similar experiment in previous year, the fertility rates and hatching rates were 76% and 73% respectively. From the present experiment, it is clear that the extender NBFGR-7 along with ethylene glycol (10%) as cryoprotectant can be ideal for mass cryopreservation of this species.

Table 17. Fertility rate and hatching rate in fertility trials of *Garra surendranathanii* using cryopreserved milt

Extender	% Fertility	% Hatching	% Hatch as that of control
NBFGR-3	18.53±0.79	21.78±2.15	45.57
NBFGR-3B	16.92±0.90	22.65±4.79	47.38
NBFGR-6	19.54±0.36	24.44±4.31	51.14
NBFGR-7	26.13±1.22	35.06±3.01	73.35
NBFGR-7B	20.68±1.22	25.07±3.37	52.44
Control	36.36±0.95	47.80±3.22	100.00

Tissue Repository

During the year under report, 401 tissue accessions were made and 200 voucher specimens from 135 marine teleosts from Lakshadweep Island (Agatti) and Coromandal Coast (Chennai) that can be used as referral material and also for generating DNA barcodes. The muscle tissue and fin clips were collected and preserved in 95% ethanol/isopropyl alcohol and the voucher specimens were preserved in alcohol.

Live Gene Banks

In collaboration with Department of Zoology, Gauhati University, Assam, the concept of multispecies live gene bank was modified and single generic/species gene bank was initiated at Guwahati. The emphasis was given to murels and catfishes. Among murels, *Channa barca* and *C. aurantimaculata* and for catfishes, *Sperata seenghala*, *S. aor* and *Hemibagrus menoda* were prioritized. Habitat inventory was done and areas with significant distribution of these species were identified. The fishes were collected from potential areas of Pobitora Wildlife Sanctuary and Gogamukh to Dhemaji in the Lakhimpur district, Assam. Artificial habitat structures were prepared for murels in the live gene bank.

In collaboration with Department of Fisheries, Govt. of Assam, a new live gene bank was established at Ulubari Fish Seed Farm,

Guwahati. It was inaugurated by Prof. S.S. Baghel, Vice Chancellor, Assam Agricultural University on December 18, 2007. The species with conservation significance like *Chitala chitala* (n= 58), *Ompok pabo* (n= 178) and *Channa striatus* (n= 32) collected from Brahmaputra river basins were stocked in the live gene bank for further study and captive propagation.

Seed production of selected carps, catfish and endangered fish species

Seed production of selected carps, catfish and endangered fish species was taken up at Aquaculture Research and Training Unit of NBFGR at Chinhat, Lucknow. During the year under report, the quality seed of Indian major carps (catla, rohu and mrigal), exotic carps (silver carp, grass carp and common carp) and catfishes (magur and singhi) were produced and broodstock was maintained as per the details given in Table 18.

Table 18. Details of quality fish seed of selected carps and catfish produced at Aquaculture Research and Training Unit of NBFGR at Chinhat, Lucknow during 2007-08

S. No.	Species	Brooders quantity (kg)	Spawn produced (Lakhs)
1.	<i>L. rohita</i>	59.450	25
2.	<i>C. catla</i>	127.600	34
3.	<i>C. mrigala</i>	25.050	02
4.	<i>H. molitrix</i>	18.650	01
5.	<i>C. idella</i>	38.300	01
6.	<i>H. fossilis</i>	1.400	0.05
7.	<i>C. batrachus</i>	1.375	0.005
8.	<i>C. carpio</i>		15
	Total Spawn		68.055

The fish seed produced was sold to the aquafarmers of Uttar Pradesh. The details are given in Table 19. This programme will be expanded to include endangered species. The freshwater

prawn (*Macrobrachium rosenbergii*) culture activity was also taken up. A small pond measuring 0.1 ha was identified for this purpose. It has a central trench for the migration of the prawns during adversities. The pond was manured, limed and kept ready about a week prior to stocking. The seed PL-15 procured from Rohtak Center of CIFE on August 8, 2007 was stocked after acclimatization. The seed procured belonged to two batches. The survival was about 60% only during transportation which might be due to delay in the arrival of the train by 13 hours. The growth observed was satisfactory (Table 20). The water quality parameters were also monitored regularly. The Unit also provided technical inputs to the Lucknow Regional Center of the Central Soil Salinity Research Institute, Karnal in a collaborative research project on “Land modification for increasing water productivity by different farming systems in water logged sodic soils along the Sharda Sahayak Canal Command of U.P.”.

Table 19. Farm production and revenue generation at Aquaculture Research and Training Unit of NBFGR at Chinhat, Lucknow during 2007 -08

S. No.	Item	Quantity	Revenue (Rs.)
1.	IMC spawn	2.50 lakhs	1,250=00
2.	IMC fish fry and fingerlings	10.68 lakhs	87,280=00
3.	Fingerlings by weight	38 kg	7,600=00
4..	Total		96,130=00

Table 20. Details of the growth of *M. rosenbergii*

S. No.	Date	Mean length (cm)	Mean weight (gm)
1.	15.09.2007	6.24	2.32
2.	19.09.2007	9.98	9.50
3.	29.12.2007	12.60	24.05
4.	15.01.2008	13.00	28.00
5.	15.02.2008	13.50	31.00

Successful Captive Breeding of Threatened Bronze Featherback, *Notopterus notopterus* (pallas)

The oviparous fish Bronze Featherback (Knife fish), *Notopterus notopterus* (Pallas) is a popular food fish having ornamental value, as well. It thrives in a wide range of water bodies including freshwater rivers, ponds, lakes, etc; and is distributed all over India. It is a very hardy fish and can be easily reared in the aquarium, stagnant water and even in the aquaculture system on a variety of feeds. It breeds naturally during June to August in rivers and pond system in India. Over the years, however, the wild population of *N. notopterus* has been declining due to various reasons which, may lead to further reduction in the natural population of this important fish. Due to reduced population in the wild, the fish has now been categorized under the threatened category. However, there are scarce reports on induced breeding of this fish. Therefore, a new research programme was undertaken to breed this carnivorous fish in captivity by using Ovaprim. Wild brooders were collected from River Gomti at Lucknow and acclimatized for two months. The fishes were injected with Ovaprim at the rate of 0.5 ml/kg body weight and 1.0 ml/kg body wt.

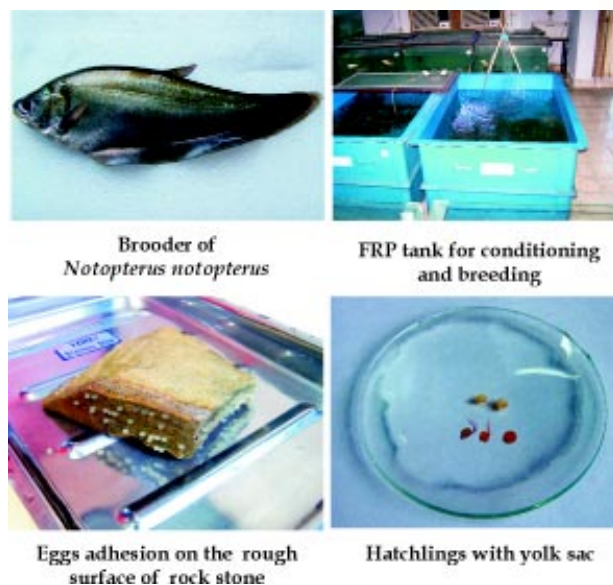


Fig. 44. Captive breeding of *Notopterus notopterus*

to male and female, respectively. A 100% breeding was recorded in the experimental fishes. The brooders showed aggressive and chasing behaviour after 10-12 hours of intra-muscular injection of Ovaprim and female released eggs in the night which attached on the rough surface of the cement tiles and rock stones, the male released its milt and eggs were fertilized externally. A parental care behaviour was observed particularly in females. The fertilization rate in experimental sets varied from 85 – 94%. The fertilized eggs were larger in size (3.5 + 0.5 mm). The survival was 80 – 85% and fecundity recorded was in the range of 2570 + 198/kg body weight of female fish. Hatchlings with yolk sac were transferred to other glass aquaria for further rearing up to next 15 days. The supplementary feed was provided in the form of paste and the fishes were fed *ad libitum*.

5.5 Exotics and Quarantine

Translocation and dissemination of pathogens and diseases to new regions world wide along with their hosts is well documented. Several pathogens may occur in asymptomatic carrier states and are difficult to detect. The introduction of such agents can cause immense damage to native fish germplasm resources and, therefore, needs special attention. Advances in live aquatic animal trade, facilitated by improved transportation efficiency, play a significant role in the introduction and spread of pathogens and diseases in many aquaculture systems. The serious socio-economic, environmental and international trade consequences arising from these exotic pathogens are quite evident.

Studies on pathogenic fauna of selected marine and freshwater ornamental fishes

Ornamental fish trade has a turnover of US\$ 5 billion globally and increasing rapidly with an annual growth rate of 8% per year. India's share in ornamental fish trade is merely US\$ 0.5

million. The current demand of ornamental exotics in domestic market is worth Rs. 30 crores which is growing @ 20% per annum. Presence of pathogens/diseases is one of the most important limiting factors in the ornamental fish culture sector. The increase in international trade of ornamental fish over the years poses a great challenge to national authorities in their vigilance against the potential establishment of exotic aquatic pathogens. This has prompted importing countries to impose more stringent health measures including certifications for specific pathogen free (SPF) fish consignment. In this scenario, as per SPS agreement, it is essential to have scientific evidence and documentation (inventory) of native pathogens/diseases as well as exotic pathogens of tradable ornamental fishes. Hence, a new work to study the pathogenic fauna of selected marine and freshwater ornamental fishes was undertaken during the year under report. This work aims at exploring and documenting the pathogens of ornamental fishes and developing rapid molecular assays for identification of selected parasites for eventually helping in quarantine and health certification of ornamental fishes in the country.

Sampling and collection of selected freshwater ornamental fishes namely, guppy (*Poecilia reticulata*), goldfish (*Carassius auratus*), koi carp (*Cyprinus carpio*), black molly (*Poecilia sphenops*), blue gaurami (*Trichogaster* spp.), yellow gaurami, puntii (*Puntius ticto*) and swordtail (*Xiphophorus* spp.) was done from Kochi, (Kerala), Rajghat, Karuna sangam, Thathra (Varanasi), Shanti Aquarium, Meja Road, Aasiwan, Unnao; Gau Ghat and Sadiapur (Allahabad), Butler Palace pond and selected fish markets and private aquarium houses of Lucknow. Among marine fishes, butterflyfish (*Chaetodon* spp.), angel fish (*Pomacanthus* spp.), cardinal fish (*Apogon* spp.), tang/surgeon fish (*Acanthurus* spp.) and damsels were selected.

A total of 160 samples of selected fish species, including 35 goldfish, 24 guppies, 25 koi carp, 25 blackmolly (*Poecilia sphenops*), 15 *Puntius* spp., 11 blue gaurami *Trichogaster*, 10 yellow gaurami and 15 swordtails were screened for isolation of monogenean and protozoan parasites. The prevalence of parasites was highest in the goldfish (57.14%), followed by guppy (*Poecilia reticulata*) (54.16%), koi carp (*Cyprinus carpio*) (48%), *Puntius* spp. (46.66%), black molly (*Poecilia sphenops*) (44%) yellow gaurami- (*Trichogaster*) (40%), swordtail

Xiphophorus spp. (40%) and lowest in blue gaurami (27.27%). The overall prevalence of parasites in selected fishes was 47 %.

The isolated parasites included *Trichodina*, *Tripartiella*, *Ichthophthirius multifiliis*, *Chilodonella* and *Tetrahymena* (Fig. 45). Among the protozoans and monogeneans, *Gyrodactylus* spp., *Dactylogyrus* sp. and some unidentified monogeneans were observed. *Ichthophthirius multifiliis* was in most of the fishes. *Tetrahymena* was isolated from guppy. *Chilodonella* and trichodinids were encountered in gills of black

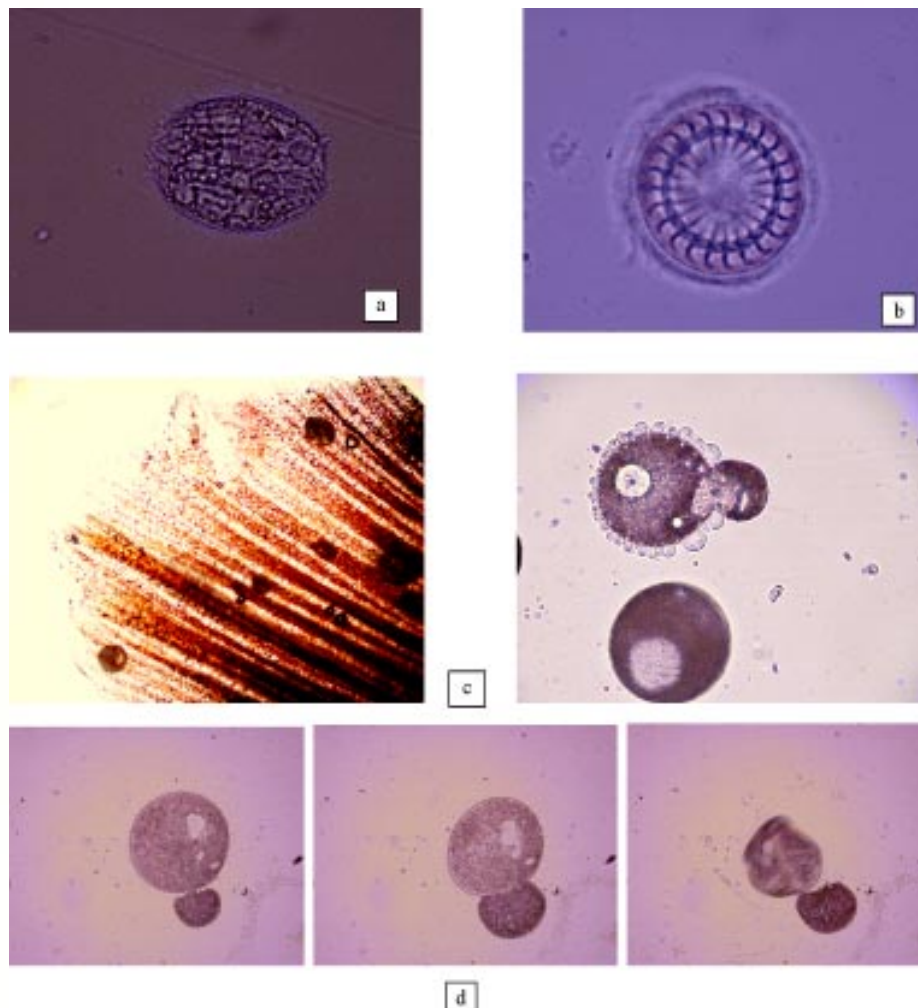


Fig. 45. A few of the parasites isolated from the selected fish species. (a) *Chilodonella* from black molly, (b) *Trichodina* from gold fish, (c) *Ichthyophthirius multifiliis* from guppy and (d) budding of *I. multifiliis* in blue gaurami

molly. Goldfish had *Gyrodactylus elegans indicus* Nordman, 1832, *Dactylogyirus intermedius* Nybelin 1924, trichodinids and *Ichthophthirius multifiliis*. Koi carp also had *Dactylogyirus intermedius* Nybelin, 1924, trichodinids and *I. multifiliis*. *Gyrodactylus* spp. was found in swordtail too. The intensity of monogenea was highest (more than 100) in a few samples of goldfish.

Detection of *Gyrodactylus* and *Dactylogyirus* species using PCR

For PCR amplification of internal transcribed spacer (ITS) region of *Gyrodactylus* DNA, the following primers were used:

GSF- 5'-TTT-CCG-GTG-AAC-CT-3'

GSR- 5'-TCC-TCC-TCC-GCT-TAG-TGA-TA-3'.

The amplified DNA was examined by agarose gel electrophoresis. Presence of a 1300 bp PCR product confirmed *G. salaris* and 1150 bp product confirmed *G. elegans*. DNA isolation from *Dactylogyirus* was done through the technique given by Simkova *et al* (2005) to develop molecular diagnostic assay. Following primers referred by Simkova *et al*, (2005) were used from 18 small subunit ribosomal RNA gene:

F-5'-GCATGGAATAATGGAATAGG-3'

R-5'-CCGTCAATTCCTTTAAGT-3'

PCR amplification of *Dactylogyirus* spp. DNA was also done using Simkova's primers. The PCR products of the 1050-1100 bps were observed in gels through agarose gel electrophoresis (Fig. 46).

With the outcome of this project, an inventory of marine and freshwater ornamental fish pathogens will be available. With development of PCR-based diagnostic assays, a new path will open for parasites identification and the taxonomic or phylogenetic ambiguities or wrong identification of parasite species could be

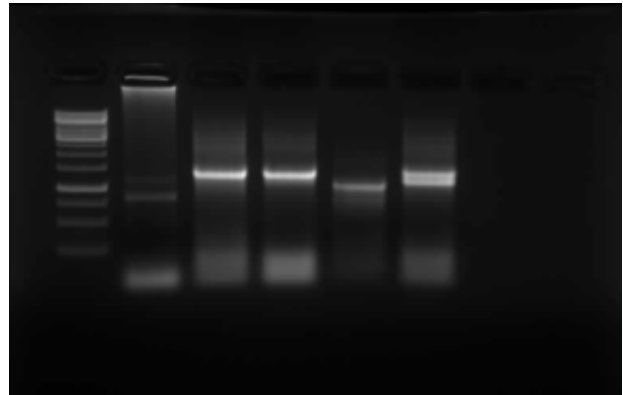


Fig. 46. PCR amplification of *Dactylogyirus* spp. from a few selected fish species

Lane 1: DNA marker 250 bp; Lane 2: *Dactylogyirus* spp. from gills of common carp; Lane 3, 4 and 6: *Dactylogyirus* spp. from gills of goldfish and Lane 5: *Dactylogyirus* sp. from gills of black molly

checked and corrected. It will help in quarantine and safe trans-boundary movement of ornamental fishes.

Studies on association of viral infections in diseases of koi carps by immunological and molecular techniques

Koi carp is an ornamental strain of the common carp, native to Asia and Europe. Internationally, Koi Herpes virus (KHV) has been regarded as an important pathogen causing severe disease outbreaks in koi carps. In India, increase in import of ornamental fish can lead to unintentional introduction of new pathogens into the aquaculture system. These pathogens affect fish production from both culture and capture fisheries but also endanger biodiversity as well as have wider socio-economic impact. So far, there is no report regarding incidence of viral diseases in any of the carp species of India except few sporadic attempts. Therefore, the present study has been undertaken to elucidate the association of virus etiology in different diseases of koi carps so that preparedness can be made to fight the

occurrence of future epizootics. The diagnostics will be used for screening and health certification of the fish stock in freshwater aquaculture systems of the country.

Collection of samples and isolation of DNA

A total of 62 samples of koi carps were processed for DNA isolation and detection of KHV by PCR. These samples were collected from different districts of Andhra Pradesh, Uttar Pradesh, West Bengal and Kerala. Gills, kidney and intestinal samples of each fish were collected in 95% ethanol and transported to laboratory for processing. Genomic DNA from fish tissues was isolated using following methods. Briefly, 50 mg of tissues were homogenized in 1 ml lysis buffer by using micro pestle and added 20 µl of proteinase K (10 mg/ml) and 100 µl of SDS (10%) in each sample. All samples were incubated at 37°C for 2 h. After incubation, equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added in each sample, mixed completely and centrifuged at 10,000 rpm for 15 min. Supernatants were removed in new eppendorf tubes. The DNA was precipitated with 2 volume of chilled ethanol and incubated at -20°C for 1 h.

Then, centrifuged at 10,000 rpm for 15 min at 4°C, removed the supernatant and washed the pellet with 1 ml of 70% ethanol, centrifuged at 10,000 rpm for 10 min at 4°C. Then, removed the supernatant and air-dried the pellet by leaving the tubes open on the bench for 15 min and re-suspended the pellet in 50 µl of T. E. buffer.

Standardization of polymerase chain reaction detection of koi herpes virus

The PCR is one of the best-known techniques used to detect several ornamental fish pathogens. Diagnostic capability has been developed to detect the Koi Herpes virus by PCR. Positive DNA controls of KHV were procured from Dr M. Kotler, Hebrew University, Israel and were used to develop diagnostic test using 3 primers.

PCR amplification was performed using all the three sets of OIE-referred primers in this study. The details of primers are given in Table 21. We used 10 ng of genomic DNA, 50 pmoles of primers, 10i moles of dNTP's mix, 1 unit of Taq DNA polymerase and 1.5 mM of MgCl₂. Samples were subjected to 35 cycles of

Table 21. Details of primers used for screening samples for Koi herpes virus

Name of Primer	Primer sequence	PCR product size	Reference
Kpn I	Forward 5'-GAC-GAC-GCC-CACAAG-TTC-AGT-CTG-TTC-CTC-AAC-3' Reverse 5'-CAC-AAGTTC-AGT-CTG-TTC-CTC-AAC-3'	484 bp	Gilad <i>et al</i> 2002
Sph I	Forward 5'-GAC-ACC-ACA-TCT-GCA-AGG-AG-3' Reverse 5' – GAC-ACA-TGC-TAC-AAT-GGT-CGC-3'	292 bp	Grey <i>et al</i> 2002 & Yuasa <i>et al</i> 2005
Bam HI	Forward 5'-TCG-CAT-GTG-AGG-GTT-CAT-GC-3' Reverse 5'-CAT-CAG-CGG-CAT-CAG-CAT-CG-3'	365 bp	Gray <i>et al</i> 2002

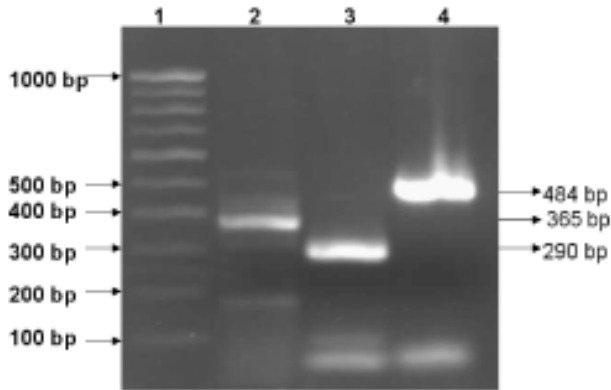


Fig. 47. PCR amplification of control DNA of Koi herpes virus using three different primers :

Lane 1 : 100 bp Genei Marker, lane 2: Bam HI digested (365 bp), lane 2: Saphi digested (290 bp) and lane 4 : KpnI/SacI digested (484 bp.)

amplification (94°C for 1 min, 55°C for 1 min and 72°C for 1 min on a Master cycler (Eppendorf). 10 μ l of the reaction mixture was then analyzed by submarine gel electrophoresis in 1.2% agarose (Fig.47).

Serum samples from 28 koi carps were collected for detection of anti-KHV antibodies by ELISA and were sent to CIFA, Bhubaneswar for testing. All the three primers resulted in sensitive and specific detection of the KHV in the control DNA samples (Fig46). All the tested samples were negative for presence of Koi Herpes virus by PCR.

Targeted active surveillance of penaeids for OIE-listed viruses in selected maritime states of India

The work on targeted active surveillance of penaeids for OIE-listed viruses in selected maritime states of India was continued. During the year, 238 adult shrimp and post-larvae samples were collected from Andhra Pradesh, Tamil Nadu and Kerala for screening of MBV WSSV and Yellow head virus (YHV) (Table 22). Screening of shrimp samples for WSSV was done by nested PCR as per the protocol described by Lo *et al.* (2003). A positive result in the first step of nested PCR implies a serious WSSV infection

whereas a positive result in the second amplification step only indicates a latent or carrier-state infection. For this, DNA extraction was carried out as per Lo *et al.* 2003 using CTAB. The PCR conditions used were as follows:

First step PCR reaction

1 μ l DNA template solution was added to a PCR tube containing 50 μ l of reaction mixture (10 mM Tris/HCl, pH 8.8, 50 mM KCl, 1.5 mM MgCl₂, 0.1% Triton X-100, 200 μ M of each dNTP, 100 pmol of each primer, 1.5 units of Taq DNA polymerase). The outer primer sequences were 146F1, 5'-ACT-ACT-AAC-TTC-AGC-CTA-TCTAG-3' and 146R1, 5'-TAA-TGC-GGG-TGT-AAT-GTT-CTT-ACG-A-3'. The PCR profile was one cycle of 94°C for 4 minutes, 55°C for 1 minute and 72°C for 2 minutes, followed by 39 cycles of 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes and a final 5-minute extension at 72°C. The WSSV-specific amplicon from this reaction was 1447 bp. The sensitivity was approximately 20,000 copies of a WSSV.

Second step of the (nested) PCR reaction

The same PCR amplification protocol was followed. 5 μ l of the first-step PCR reaction product to 45 μ l of a PCR cocktail with the same composition as above except that it contains the second (inner) primer pair: 146F2 (5'-GTA-ACT-GCC-CCT-TCC-TC-TCC-A-3') and 146R2 (5'-TAC-GGC-AGC-TGC-TGC-ACC-TTG-T-3'). The WSSV-specific amplicon from this reaction was 941 bp. The overall sensitivity of both steps was approximately 20 copies of a WSSV template. The PCR product was visualized on 1% agarose gel. Decapod-specific primers (143F 5'-TGC-CTT-ATC-AGCTNT-CGA-TTG-TAG-3' and 145R 5'-TTC-AGN-TTT-GCA-ACC-ATA-CTT-CCC-3' yielding an 848 bp amplicon) were used in control reactions to verify the quality of the extracted DNA and integrity of the PCR reaction.

Out of 238 samples, only 21 samples were positive for WSSV by PCR (Table 1). Detection of MBV was done by nested PCR as described below. Validation of the tests was carried out by using positive DNA controls of the MBV. DNA was isolated using hot phenol method. Nested PCR capable of detecting low concentrations of MBV was used. The two external and two internal primers used to detect MBV were as follows:

Primer	Sequence
MBV1F	5'-CGA-TTC-CAT-ATC-GGC-CGA-ATA-3'
MBV1R	5'-TTG-GCA-TGC-ACT-CCC-TGA-GAT-3'
MBV1NF	5'-TCC-AAT-CGC-GTC-TGC-GAT-ACT-3'
MBV1NR	5'-CGC-TAA-TGG-GGC-ACA-AGT-CTC-3'

PCR conditions for the first step PCR were 40 cycles of 94°C for 30 seconds, 65°C for 30 seconds, 72°C for 60 seconds and one cycle of 72°C for 7 minutes. The second step of the nested PCR was done with 0.5 µl of the primary PCR reaction used as template with the internal primers. The conditions for the second round of amplification were: one cycle of 96°C for 5 minutes; 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 60 seconds; and one cycle of 72°C for 7 minutes. Demonstration of the PCR products (533 bp first step and 361 bp second step) was accomplished by electrophoresis through a 0.8% agarose gel in TAE buffer.

Out of 238 samples, 11 samples were positive for MBV by PCR (Table 22). For validation of YHV diagnostic test, positive control from Dr. Jeff Cowley, CSIRO, Australia were used.

RT-nested PCR for detection of all currently known genotypes in the yellowhead complex

For juvenile or adult shrimp, lymphoid organ and gill tissues were used to detect YHV.

The collected tissues were preserved in RNA *later* and stored frozen at -20°C for total RNA preparation. A 10-20 mg lymphoid organ or gill tissue was disrupted in 1 ml of Tri reagent and total RNA was extracted according to the product manual. RNA was re-suspended in 25 µl DEPC (diethylpyrocarbonate)-treated water, heated at 55°C for 10 minutes and stored at -20°C until required. For cDNA synthesis, 2 µl of RNA (1.0 µg total RNA) was mixed with 1 µl (50 ng) of random hexamer and 12.0 µl of sterile DEPC-treated water, incubated at 70°C for 5 minutes and chilled on ice. Thereafter, 4.0 µl of reaction buffer was added along with 2.0 µl of 10 mM dNTP and 1.0 µl (40 U/µl) Rnase inhibitor and incubated at 25°C for 5 minutes. Then, 1.0 µl (40 U/µl) of M-MuLV RT was added and again incubated at 25°C for 10 minutes and then at 37°C for 60 minutes. The reaction was stopped by heating at 70°C for 10 minutes and chilled on ice. For the first PCR step, 1 µl cDNA was added to a total 50 µl reaction mixture containing 5 µl of 10× *Taq* buffer, 2 µl primer mix containing 25 pmol/µl of each primer pool YH-F1 and YH-R1, 1 µl 10 mM dNTP mix and 0.25 µl 5 U/µl *Taq* DNA polymerase. PCR amplification was done using denaturation at 95°C for 1 minute followed by 35 cycles at 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 40 seconds, followed by a final extension at 72°C for 7 minute. For the second PCR step, 1 µl of the first PCR product in the reaction mixture was used as above except substituting primer pools YH-F2 and YH-R2. PCR amplification was again done as described above. Amplified PCR product was visualized on 2% agarose gels containing 0.5 µg/ml ethidium bromide alongside a suitable DNA ladder (Fig. 48). None of the samples were positive for YHV complex (Table. 22).

YH-F1	5'-ATCGTCGTCAGYTAYCGYAAAYACYGC-3'
YH-R1	5'-TCTKCRYGTGTGAACACYTTCTTR-GC-3'
YH-F2	5'-CGCTTYCARTGTATCTGYATGCACCA-3'
YH-R2	5'-RTCDGTGTACATRTTKGAGAGTTTRTT-3'

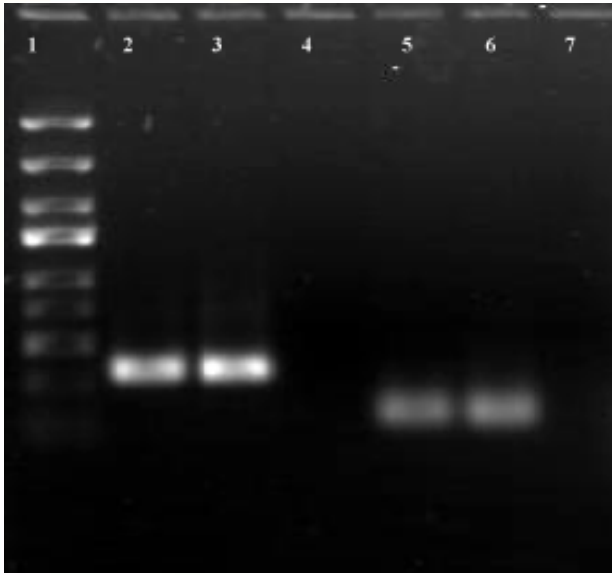


Fig. 48. Detection of YHV by nested PCR

Lane 1: Generuler express DNA ladder, Lane 2-3: 358 bp product

Lane 4: Negative control, Lane 5-6: 146 bp product, Lane 7: Test sample

PCR positive WSDV samples were used for purification on 10-50% sucrose density gradient and a band was obtained at interface of 30-40%. This band was subjected to SDS-PAGE analysis for confirmation of the peptides. For further confirmation, WSSV samples were submitted to CDRI for transmission electron microscopy. Rod shaped virions resembling whispovirus were detected in the purified samples. However, their concentration was very low. So a fresh attempt will be made for increasing the concentration of purified virions for further strategies.

Isolation and characterization of *Flavobacterium* species from fish and aquatic environment

Flavobacterium is an important genera causing diseases in the fishes. *Flavobacterium* is a potential fish pathogen which causes gill disease in fish. It is an opportunistic as well as primary

Table 22. Details of shrimp sample collection and their screening

S. No.	Sampling site	Number of samples	Type of samples	WSSV		MBV		YHV
				1st step	2nd step	1st step	2nd step	
Andhra Pradesh								
1	Kakinada shrimp farms	27	Adults (gills, pleopods & hepatopancreas)	2	3	-	-	N.D
2	Shrimp processing plant, Talareva, Kakinada	43	Adult-Cephalothorax	-	-	-	-	N.D
3	ITC Shrimp lab,	29	Post-larvae & adults- (gills& pleopods)	3	3	-	3	N.D
Tamil Nadu								
1	PAD labs, Chennai	14	Adults (gills & pleopods)	-	4	-	1	N.D
2	Marakkanam, Pondicherry	15	Adults- (gills, Pleopods)	-	2	-	-	N.D
3	Shrimp Farms, Sirkazhi, Nagapattinam	22	Post-larvae	-	-	3	2	N.D
Kerala								
1	Kalamukku Fish Harbour, Cochin	30	Adults- (gills, pleopods & hepatopancreas)	-	-	-	2	In progress
2	Cherrai Fish Farm, Cochin	30	Adults- (Gills, Pleopods & hepatopancreas)	In progress				
3	Aqua Plaza Shrimp Hatchery	28	Post-larvae & Adults (gills, pleopods & hepatopancreas)	3	1	-	-	-

N D= Not done

bacterial pathogen with very well known diversity than other bacteria. Therefore, accurate and specific isolation and characterization of *Flavobacterium* spp. is important for fish disease diagnosis and health management. Therefore, a study was undertaken to isolate and characterize the *Flavobacterium* spp. from fish, sediment and pond water samples. This study would provide technical information for prevention of fish diseases and help in health management.

Molecular identification of *Flavobacterium* spp. by 16S rDNA PCR

A total of 41 presumptive isolates of *Flavobacterium* were used for amplification of 16S rDNA. All these isolates were also characterized by biochemical tests. Approximately, 500bp of the downstream region of 16S rDNA gene fragment was sequenced from isolates. The 16S rDNA sequences were compared with sequences deposited in the Genbank database using the BLAST programme and Ribosomal Database Project II (RDP II) for molecular identification of bacteria. On basis of 16S rRNA sequencing, a total of 7 isolates were confirmed as *Flavobacterium* spp., 22 *Chryseobacterium* spp., 6 *Myroides* spp., 1 *Sejonia* spp., 1 *Weekesella* spp. and 4 *Flectobacillus* spp..

Phylogenetic analysis based on 16S rDNA sequences

All 41 16s rDNA sequences were aligned in

CLUSTALX 1.83. Pair-wise evolutionary distances were computed using the Jukes and Cantor equation implemented in the MEGA 3.1 program and a phylogenetic tree was constructed by the neighbor-joining method program package. A total of 7 16S rDNA sequences of *Flavobacterium* spp. were used for construction of the phylogenetic tree. A total of 100 bootstrapped values were sampled to determine a measure of the support for each node on the consensus tree.

Phylogenetic analysis showed 8 major clades, out of which five clades belonged to *Chryseobacterium* spp. *Flavobacterium* spp. and *Myroides* spp. were present in the same cluster but different clades indicating high degree of homology in 16S rDNA sequences. The complete 16S rDNA based phylogenetic tree is shown in Fig. 49.

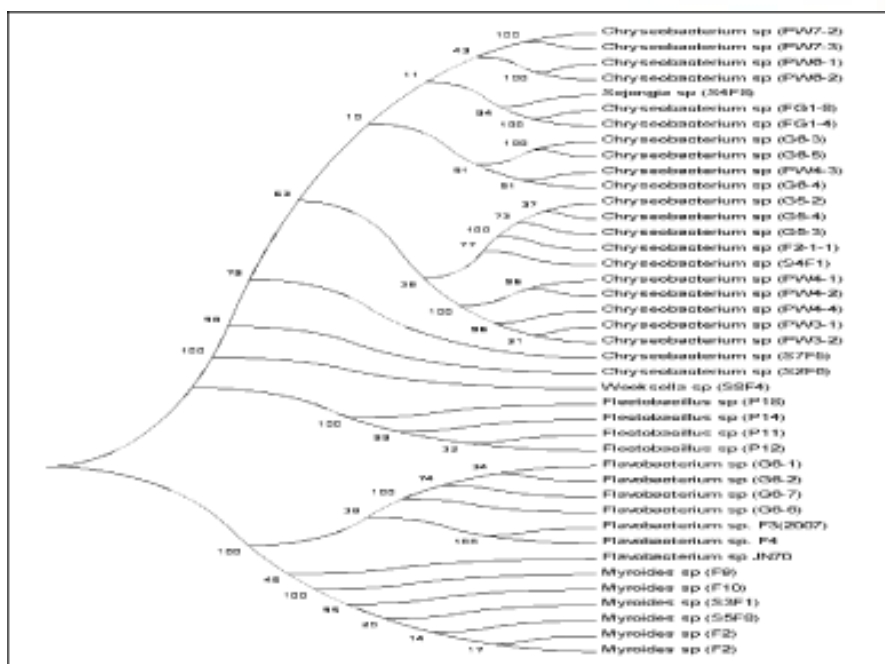


Fig. 49. Phylogenetic tree based on the 16S rDNA sequences of different isolates (41 no.) of *Flavobacteriaceae* & related family using neighbour-joining method and Jukes & Cantor algorithm. (The number indicates the bootstrap values of 100)

PCR amplification of 16S-23S ISR region

PCR primers were designed from the highly conserved sequences adjacent to the 16S-23S rRNA spacer region. Primers were designed to be complementary to conserved region near the 3' end of 16S and 5' end of the 23S region of the ribosomal operon based on a computer alignment of ribosomal RNA sequences available through Genbank. Standard PCR procedure was followed for amplification of ISR region using combination of newly developed forward and reverse primers.

Seven isolates of *Flavobacterium* spp. were used for the amplification of the ITS region. The different combination of forward and reverse primers used for the selection of *Flavobacterium* spp.

specific primers are given in Table 23. Out of these combinations, only 11 sets of ISR primers were specific to *Flavobacterium* species (Table 24). Among these 11 sets, AMSEF and AMSER combination resulted in amplification of all *Flavobacterium* spp. isolates. The PCR product ranged from 750-800 bp due differences in the copy number of tRNA encoding genes within the ISR region of *Flavobacterium* species (Fig. 50).

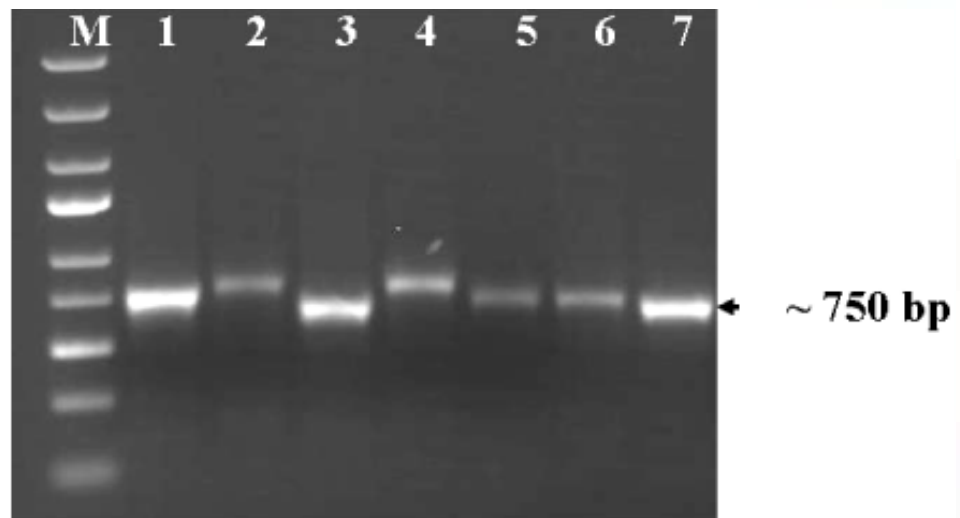


Figure 50. PCR Amplification of the ISR region in different isolates (Lane 1-7) of *Flavobacterium* sp using specific primer. M-Express DNA Ladder (Fermentas), Lane 1-JN 15, Lane 2-F 3, Lane 3-PW-16 (1), Lane 4-F4, Lane 5-G6-2, Lane 6-JN 9, Lane 7-JN 10.

Table 23. Details of testing the specificity of primers for amplification of 16S-23S ISR of *Flavobacterium* spp. with isolates of other related genus

Sets of ITS primer		<i>Flavo- bacterium</i>	<i>Chryseo- bacterium</i>	Myroides	<i>Coenonia</i>	<i>Sejongia</i>	<i>Flecto- bacillus</i>	<i>Empedo- bacter</i>	<i>Weekesella</i>
Forward	Reverse								
AMSAF	AMSAR	-	-	-	-	-	-	-	-
	AMSBR	-	-	-	-	-	-	-	-
	AMSCR	+	+	+	+	+	+	+	+
	AMSDR	+	-	-	-	-	-	-	-
	AMSER	+	+	+	+	+	+	+	+
	AMSER1	+	-	-	-	-	-	-	-
	AMSFR	+	+	+	+	+	+	+	+

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AMSBF	AMSAR	-	-	-	-	-	-	-	-
	AMSBR	-	-	-	-	-	-	-	-
	AMSCR	+	+	+	+	+	+	+	+
	AMSDR	+	+	+	+	+	+	+	+
	AMSER	+	-	-	-	-	-	-	-
	AMSER1	-	-	-	-	-	-	-	-
	AMSFR	-	-	-	-	-	-	-	-
AMSCF	AMSAR	-	-	-	-	-	-	-	-
	AMSBR	-	-	-	-	-	-	-	-
	AMSCR	+	+	+	+	+	+	+	+
	AMSDR	+	+	+	-	-	-	-	-
	AMSER	+	-	-	-	-	-	-	-
	AMSER1	+	+	+	+	+	+	+	+
	AMSFR	+	+	+	+	+	+	+	+
AMSDF	AMSAR	-	-	-	-	-	-	-	-
	AMSBR	-	-	-	-	-	-	-	-
	AMSCR	+	-	-	-	-	-	-	-
	AMSDR	+	-	-	-	-	-	-	-
	AMSER	+	+	+	+	+	+	+	+
	AMSER1	+	-	-	-	-	-	-	-
	AMSFR	+	+	+	+	+	+	+	+
AMSEF	AMSAR	-	-	-	-	-	-	-	-
	AMSBR	-	-	-	-	-	-	-	-
	AMSCR	+	+	+	-	+	+	+	+
	AMSDR	+	-	-	-	-	-	-	-
	AMSER	+	-	-	-	-	-	-	-
	AMSER1	+	-	-	-	-	-	-	-
	AMSFR	+	+	+	+	+	+	+	+
AMSGF	AMSAR	-	-	-	-	-	-	-	-
	AMSBR	-	-	-	-	-	-	-	-
	AMSCR	-	-	-	-	-	-	-	-
	AMSDR	-	-	-	-	-	-	-	-
	AMSER	-	-	-	-	-	-	-	-
	AMSER1	+	-	-	-	-	-	-	-
	AMSFR	-	-	-	-	-	-	-	-
AMSHF	AMSCR	+	+	+	+	+	+	+	+
	AMSER	+	+	+	+	+	+	+	+
	AMSFR	+	+	+	+	+	+	+	+
	AMSDR	-	-	-	-	-	-	-	-
	AMSBR	-	-	-	-	-	-	-	-

Table 24. Details of testing the specificity of newly designed ISR primers within *Flavobacterium* species isolates

Primer F	Primer R	PCR product size	Specificity within <i>Flavobacterium</i>
AMSBF	AMSER	1300 bp	+
AMSAF	AMSDR	1600 bp	+
AMSAF	AMSER1	1500 bp	+
AMSCF	AMSER	1200 bp	++
AMSDF	AMSDR	900 bp	+
AMSDF	AMSER1	1200 bp	+
AMSDF	AMSCR	1000 bp	+++
AMSEF	AMSER1	850 bp	+++
AMSEF	AMSDR	1000 bp	+
AMSEF	AMSER	750-800 bp	+++
AMSGF	AMSER1	1000 bp	++

+: Amplifying 2 isolates, ++: > 2 isolates, +++: All 14 isolates

PCR sensitivity for ITS primers

The genomic DNA (20 ng) of *Flavobacterium* spp. was diluted from 10⁻² to 10⁻⁶ in ten-fold dilutions and amplification by PCR was done using the diluted DNA template with standard conditions. Amplification of ISR (750-800 bp fragments) was observed in the template dilutions 10⁻¹ and 10⁻⁴. Above these dilutions, PCR product was not obtained. Therefore, the PCR detection sensitivity of ISR primers was calculated to be 20 pg of genomic DNA of *Flavobacterium* spp. (Fig. 51).

The cross-reactivity of *Flavobacterium* spp. specific ISR primers was also checked by NCBI-BLASTN and genomic DNA amplification of other genus of *Flavobacteriaceae* and other related family i.e. *Chryseobacterium* spp., *Myroides*, *Empedobactor*, *Weekseella* spp., *Flectobacillus* spp. and *Sejongia* spp. Cross-reactivity was seen with *Myroides* species due to high homology in the ribosomal operon.

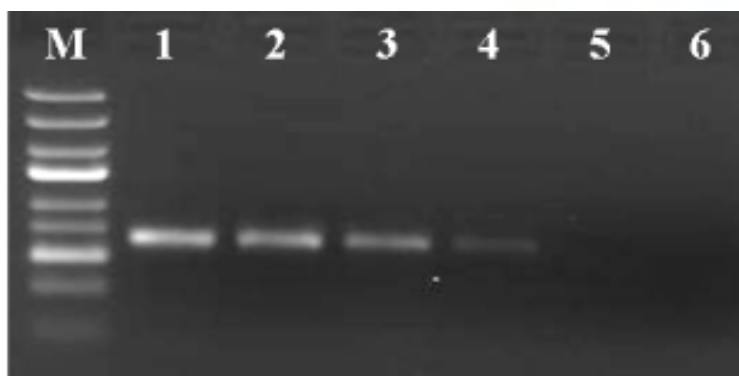


Figure 51. Demonstration of the PCR sensitivity of ISR primers using the genomic DNA of *Flavobacterium* sp JN 10.

Lane M : marker, Lane 1 : 20 ng, Lane 2 : 2nd, Lane 3 : 0.2 ng, Lane 4 : 20 pg, Lane 5 : 2pg and Lane 6: 0.2 pg.

PCR screening of *Flavobacterium* spp. from different aquatic environment using newly developed ISR primers

Three different environments were selected for the present study. A total of 290 presumptive yellow-pigmented Gram's negative bacterial isolates were isolated and taken for biochemical tests and almost all isolates gave similar

biochemical tests. Three different aquatic environments were screened for the presence of *Flavobacterium* spp. using the ISR specific primers (AMSEF/AMSER & AMSDF/AMSCR). The details are given in Table 24. Out of 260 isolates screened by PCR, only 14 isolates were positive for ISR amplification using *Flavobacterium* spp. specific primers. Surprisingly, no isolate from marine/brackishwater environment and alkaline lake water were positive for *Flavobacterium* species.

The identity of these 14 isolates was re-confirmed by amplification and sequencing of 16S rDNA as described above. All the isolates showed 100% homology to *Flavobacterium* sp. available at RDP-II. A representative phylogenetic tree of confirmed *Flavobacterium* isolates is given in Fig. 52. These results prove that the new primers designed for amplification of ISR are useful for highly specific and sensitive detection of *Flavobacterium* species.

Table 24. Screening of *Flavobacterium* spp. from the different environment

Sets of primers used for screening	Type of aquatic environment	Presumptive yellow pigmented isolates	Total identified <i>Flavobacterium</i> spp.
AMSEF/ AMSER	Freshwater fish isolates	90	6
	Fish pond water	50	6
AMSDF/ AMSCR	Freshwater sediment	60	2
	Alkaline lake water	45	0
	Marine isolates	45	0
Total		290	14

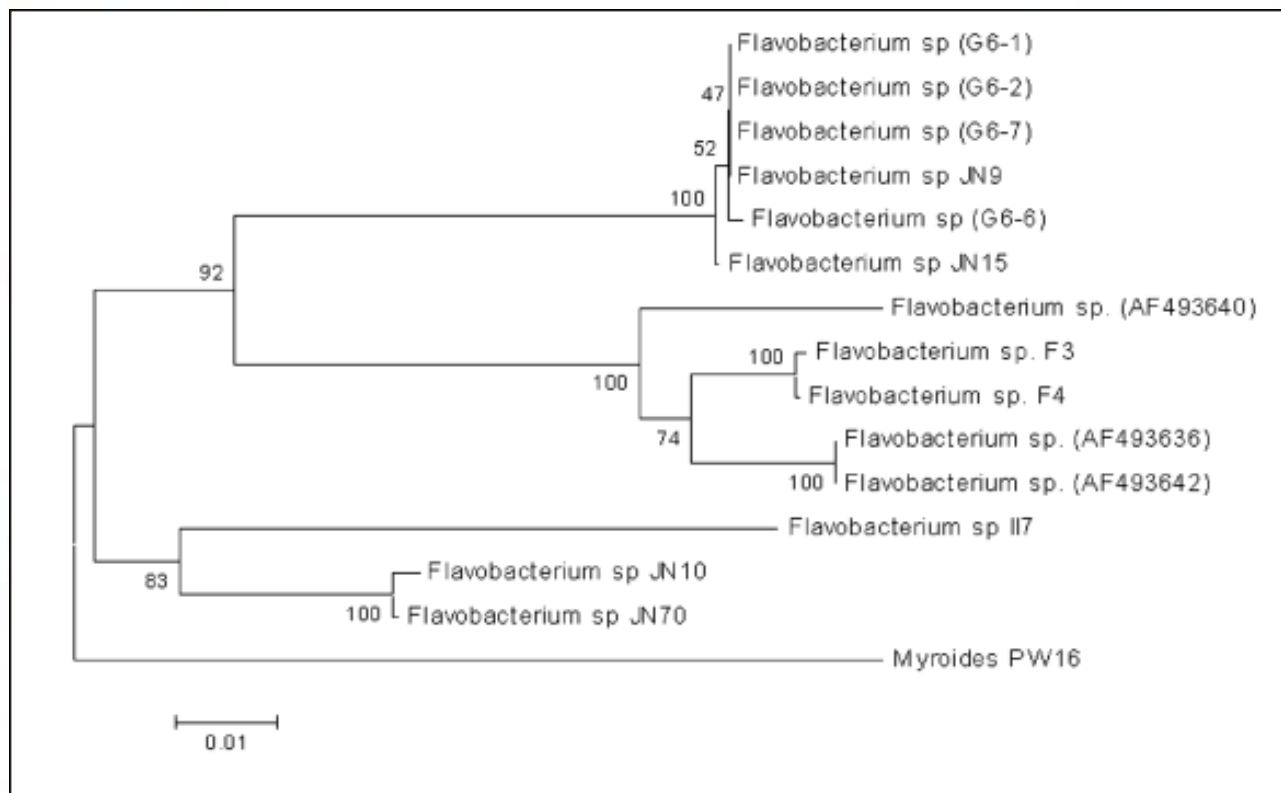


Figure 52. Phylogenetic tree based on the 16S rDNA sequences of different isolates of *Flavobacterium* species using neighbour-joining method and Jukes & Cantor algorithm (The number indicates the bootstrap values of 100. *Myroides* spp. is shown as an out-group)

Genetic diversity of *Flavobacterium* species

The Box-PCR was performed in 50 μ l reaction mixture using standard protocol. The DNA banding patterns of all isolates between the ranged from the 400-3000 bp. A common band of approx 400 bp of box element was observed in the most of the *Flavobacterium* spp. (Fig. 53). The results indicate significant genetic diversity between the isolates of *Flavobacterium* species.

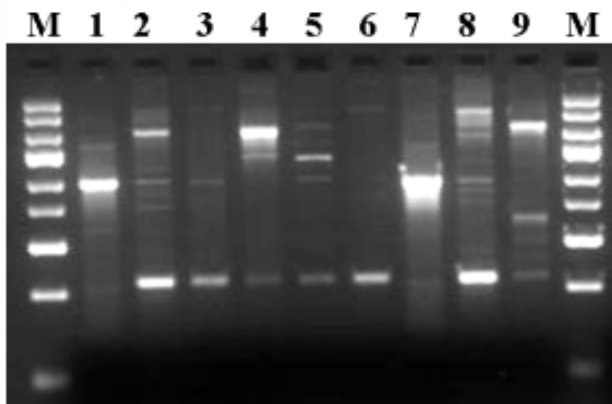


Figure 53. Diversity analysis of *Flavobacterium* spp by Box - PCR.

Lane M : DNA Market, Lane 1 : LkII-7, Lane 2: G6-6, lane 3: F4, Lane 4 : JN70, Lane 5: JN9, Lane 6 : LkII-2, Lane 7 : G6-7 Lane 8 : F3, Lane 9 : JN 15, Lane M : DNA Marker

Development and characterization of cell lines from *Labeo rohita* and virus isolation studies on established cell lines of *Cyprinus carpio*

Primary cultures

Primary cell cultures were initiated by aseptically collecting heart and tissue from juvenile *Labeo rohita* (body weight: 35 to 50 g, body length: 15 to 20 cm). Similar tissues were transferred to phosphate buffered saline (PBS) (Invitrogen), containing antibiotic and

antimycotic solution (1000U streptomycin ml/1, 1000ig penicillin ml/1 and 25ig amphotericin B ml/1) (Invitrogen). Tissue samples were minced with sterile dissecting blades and scissors at room temperature and washed four times with PBS containing antibiotic and antimycotic solution. Approximately, 25 tissue fragments (1 to 2 mm³) were individually explanted into 25 cm² tissue culture flasks (Nunc, Denmark) in 50 μ l of fetal bovine serum (FBS) (Invitrogen). After allowing the tissue to attach for 8 h at room temperature, 5 ml of Leibovitz-15 medium (L-15) containing 10% FBS was added to each flask.

Subculture and maintenance

Confluent primary cell cultures were trypsinized using 0.25% trypsin solution and 0.2% ethylenediaminetetraacetic acid (EDTA) in PBS. The subcultured cells were grown in fresh L-15 with 15% FBS medium. In the initial 10 subcultures, 50% culture medium was replaced with fresh medium. The concentration of FBS in the L-15 medium was reduced to 10% for subculture 11. An Olympus inverted microscope equipped with phase optics was used to observe and photograph living cell cultures every 2–3 days for primary cell cultures and subcultures. Morphologically, rohu heart (RH) is composed of fibroblastic-like cells.

Growth studies

Growth characteristics of the cell lines were assessed at selected temperatures, FBS concentrations, and with different growth media. Growth rates at 5 different incubation temperatures were compared: 18, 20, 24, 28 and 32°C over 7 d. A seeding concentration of 1×10^5 cells at passage 15 or a later passage was used in 25 cm² tissue culture flasks. On alternate days, 2 flasks from each temperature were trypsinized, and 8 counts (4 flask⁻¹) were performed using a hemocytometer. Analogous procedures were performed for the effects of

various concentrations of FBS (5, 10, 15, 20%) at 28°C on cell growth over 10 days. Rohu cells were able to grow at temperatures between 24 and 32°C. However, maximum growth was obtained at 28°C. Maximum growth in all cell lines occurred with 10–20% FBS, whereas no appreciable growth occurred with 5% FBS.

Development of cell lines from *Epinephelus merra*

Primary culture

The live samples of *Epinephelus merra* were anaesthetized in iced water and the caudal fin and heart tissue were collected aseptically from live specimens. The tissues were dipped in 20% Betadine for 10 min and then washed several times to remove the chemical with phosphate buffered saline (PBS) with 400 IU mL/1 penicillin, 400 mg mL/1 streptomycin. After washing, the tissue was minced with scissors and transferred to 25 cm² tissue culture flasks after adding two drops of fetal calf serum (FCS), the tissues were left at room temperature for 3–4 h for adherence. Care was taken to avoid over drying of the tissue. Most explants were attached to the plastic dish within 8 h of seeding. After ascertaining tissue adherence, the explants were fed with complete medium (L-15 with 15% FCS, 100 IU mL/1 penicillin, 100 mg mL/1 streptomycin). Microscopic observations were carried out everyday under an inverted microscope. Cell outgrowth from the explants was started on the tenth day of culture and the number of proliferating and migrating cells increased with time. Cells were in the growing phase for 5 to 8 days after plating and reached the peak number after 8 days. Most of the cells seemed to spread out from the explant. Morphologically, the cell radiated from fin and heart tissue is composed of fibroblasts and epithelial-like cells.

Subculture

In the second phase of the experiment, primary cultures were sub-cultured. When a complete monolayer had formed in primary culture, cells were washed with PBS. Subsequently, 0.25% trypsin solution was added and incubated until cells were dislodged from the flask surface. Cells were counted using a Neubour's chamber after trypsinization of the explants. The cells were pelleted at 1000 rpm for 10 min, and then resuspended in fresh complete medium. The cells were seeded into 25 cm² tissue culture flasks, containing 4 mL of L15, with 15% FCS (to a final concentration of 105 cells mL/1), and maintained at 28°C. During every subculture, 50% culture medium was replaced with fresh medium. Two types of adherent cells could be observed in culture – epithelial-like and fibroblast-like cells. Epithelial-like cells were predominant initially (about 80 to 90%) but during sub-culture, the cell phenotype changed into fibroblast like cells.

Distribution of exotic fishes and their evaluation for reproductive performance in selected stretches of Ganga river system

Under this new work undertaken during the year, data on fish diversity including exotic fishes were collected from different stretches of river Ganga at Allahabad, Varanasi, Mirzapur, Unnao and Kanpur. The presence of exotic fishes in the commercial catches at Ganga was recorded from Jhunsi, Naini, Soraon and also at Sadiyapur fish market in Allahabad. In Varanasi, similar information was collected from Saraimuhana, Rajghat and Ramnagar area and also from Dashswamedh fish market. The catch of local vs. exotic fishes was also recorded from Ganga at Adalhat area in Mirzapur, bridge area in Kanpur and Shuklaganj area in Unnao. In addition to sampling in river Ganga, information

was also collected from river Yamuna at Naini, Tons and Belan at Meja in Allahabad since these river stretches eventually merge with Ganga near Allahabad and Varanasi. The data on the commercial catch was divided primarily into five groups which were Indian major

carps (IMC), minor carps, catfishes, exotic fishes and miscellaneous fishes (Table 24). The details of catch composition, including contribution of exotic fishes, at different locations are presented in table 25 and figures 54-56.

Table 24. Fish diversity of river Ganga

Minor carps 5-7%	<i>Labeo bata</i> , and <i>Cirrhinus reba</i> (90- 250 mm and 150 - 1500g)
Catfishes 15-20%	<i>Aorichthys aor</i> , <i>Aorichthys seenghala</i> , <i>Wallago attu</i> , <i>Mystus gulio</i> , <i>Channa punctatus</i> , <i>C. marulius</i> , <i>Mystus cavasius</i> , <i>Clarias batrachus</i> , <i>Heteropneustes fossilis</i> , <i>Bagarius bagarius</i> , <i>Rita rita</i> , <i>Clupisoma garuua</i> (200 to 2000g)
Miscellaneous 10-15%	<i>Ganiolosa manmina</i> , <i>Chela bacaila</i> , <i>Glossogobius giuris</i> , <i>Ailia coila</i> , <i>Sciana coiter</i> , <i>Mestacembelus armatus</i> , <i>Anabas testudineus</i> , <i>Notopterus notopterus</i>
Exotic fishes 14-65%	<i>Cyprinus carpio</i> , <i>tilapia (Oreochromis niloticus)</i> , <i>African catfish (Clarias gariepinus)</i> , <i>Bighead (Aristichthys nobilis)</i> , <i>Ctenopharyngodon idella</i> , <i>Hypophthalmichthys molitrix</i> ,

Table 25. Average composition of fish catches at different locations of river Ganga

S. No.	Name of the District	Average percentage of indigenous and exotic fish species		Average percentage of different exotic fish species					
		Local	Exotic	Common carp	Grass carp	Silver carp	Bighead	African catfish	Tilapia
1	Allahabad	35.0	65.0	37.5	1.0	1.0	2.5	0.5	22.5
2.	Varanasi	59.2	40.8	28.3	nil	nil	1.0	0.5	11.0
3.	Mirzapur	65.2	34.2	19.5	1.0	1.0	nil	nil	12.7
4.	Unnao	85.6	14.4	14.4	-	1.0	-	-	-
5.	Kanpur	78.8	21.2	19.2	-	-	2.0	-	-

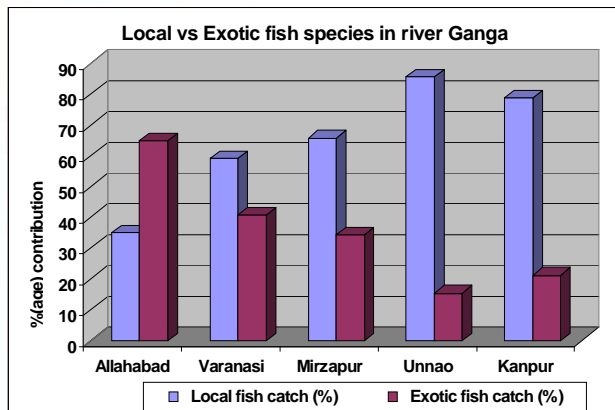


Fig. 54. Contribution of indigenous vs. exotic fish species at selected locations of river Ganga

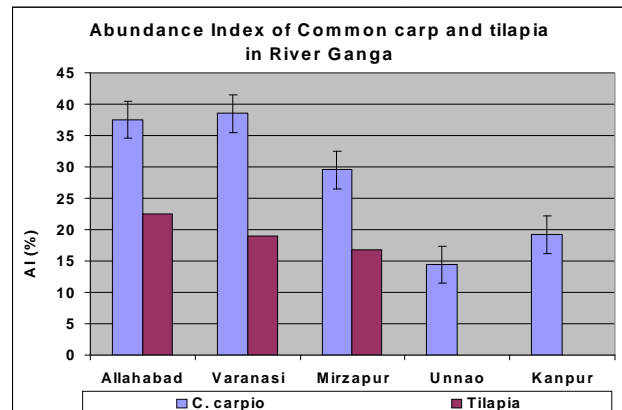


Fig. 55. Abundance index of common carp and tilapia at selected locations of river Ganga

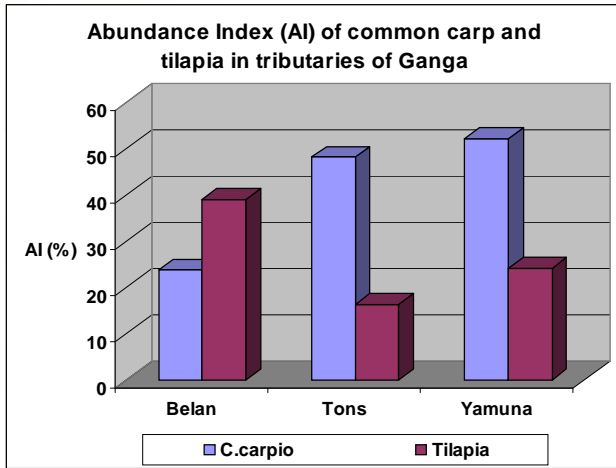


Fig. 56. Abundance index of common carp and tilapia in selected tributaries of river Ganga

Gut content analysis of exotic fishes

Guts of altogether 194 riverine samples of exotic fishes mainly, tilapia, common carp and African catfish, were collected from different sampling stations and preserved in 5% formalin for gut content analysis. The details of results in tilapia and African catfish are presented in figures 57 & 58. In tilapia (*Oreochromis*

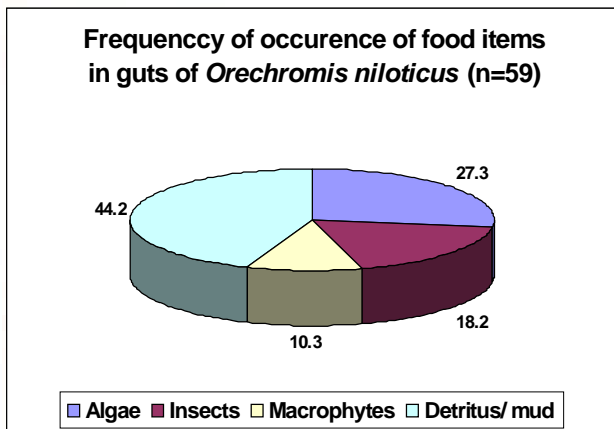


Fig. 57. Composition of different food items in guts of *Oreochromis niloticus*

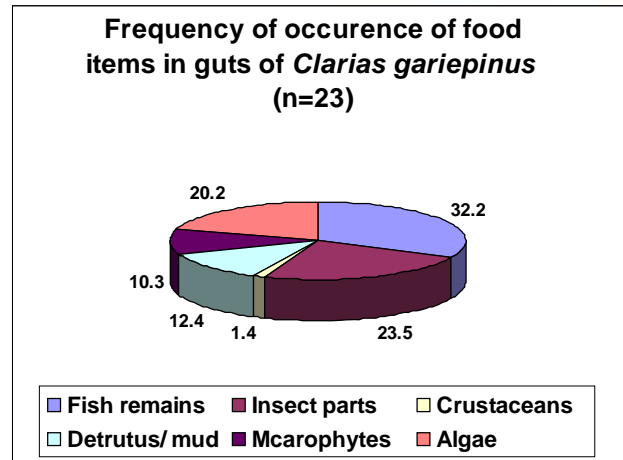


Fig. 58 Composition of different food items in guts of *Clarias gariepinus*

niloticus), detritus/mud, algae and insects constituted the major food while in *Clarias gariepinus*, the major items of the gut content were fish remains, followed by insects and algae.

Gonadal analysis of exotic fish species

Reproductive phasing in seven specimens of African catfish was carried out to study the reproductive development of this fish in riverine conditions of Ganga (Table 26). The reproductive phasing, gonado-somatic index (I_G) of pond reared African catfish revealed six reproductive phases and I_G for each stage was calculated in 131 fish specimens. The gonadal examination of common carp (n=132) and tilapia (n=95) was also carried out which revealed presence of all stages of its reproductive development and all stages of fish starting from juveniles to mature fish were caught from Allahabad. The examined range of gonado-somatic index and macroscopic features are given in table 26 & 27.

Table 26. Maturity scale to classify reproductive status of *C. gariepinus* from culture system as well as samples of river Ganga

Stages	Males	Females	Gonado-somatic Index (I _G)
I	A pair of thin thread like gonads, transparent sacs running along the dorsal wall of the body cavity	Sexes at early stage indistinguishable macroscopically	< 2.5 (n=11)
II	Testes semitransparent and flattened	Ovary reddish, smooth, small ova hardly visible in transparent matrix of follicular cells. Ova volumes smaller than matrix; exudes when lobes are cut and squeezed	2.0 - 7.5 (n=19)
III	Testes whitish, wider late and more or less flattened. No milt exudes when cut or squeezed	Ovary opaque yellowish small ova visible in transparent matrix of follicular cells.	8.0 - 15 (n=23)
IV	Testes with firm clear lobes less flattened. small amount of milt present	Ovary yellowish, fully swollen with translucent yellow ova pre-mature ova volume larger than matrix.	15.0 - 32.0 (n=32)
V	Testes with fully developed lobes. Readily produces milt when lobes are cut and squeezed.	Ovary yellowish very soft and swollen. Greenish yellow ova visible through superficial membranes, ova tightly packed. Little follicular matrix present	5.0 - 8.0 (n=32)
VI	Testes flattened having thin lobes but milt extrudes when cut and squeezed	Ova extrude from vent when pressure is applied from pectoral fin to vent.	3.0 - 10.0 (n= 14)

Note: The maturity stages shown in bold letters were found in the specimens collected from river Ganga (n=7)

Table 27. Macroscopic appearance and gonado-somatic index of ovary of *Cyprinus carpio* from river Ganga

S. No.	Stage	I _G	Description
1	Immature	< 1 (n=14)	Ovaries thin, transparent, circular (c.0.5-1 cm diameter) in cross section. Oocyte faintly visible upon rupture of tunica albuginea
2.	Immature developing/ re-mature developing (early)	0.5-5.0 n=23)	Ovaries opaque, granular and occupy less than a third of body cavity. Oocyte small, green yellow
3.	Immature developing/ re-mature developing (late)	5.0-20.0 (n=45)	Ovaries occupy less than two thirds of body cavity with abundant blood capillaries and large opaque oocyte (C.1 mm). Some oocyte appear translucent
4.	Ripe	15.0-30.0 (n=21)	Ovaries distinctly bulging and lobular in appearance. They fill the body cavity. Oocyte large (>1 mm)
5.	Spent	1-5 (n=18)	Body musculature is stretched and body cavity is flaccid. Ovaries are small, bloodshot and granular with scattered residual vitellogenic oocyte
6.	Regressing	3-10.0 (n=11)	Ovaries difficult to stage but appear blotchy with atretic oocyte in all developmental stages. Oocytes are variable in colour

IMPORTANT ACTIVITIES AND MEETINGS

Dr. Mangala Rai Visits NBFGR

Dr. Mangala Rai, Secretary, Department of Agricultural Research & Education, Govt. of India and Director General, Indian Council of



Fig. 59. Dr. Mangala Rai, Secretary, DARE and DG, ICAR visiting NBFGR

Agricultural Research, New Delhi visited NBFGR on June 23, 2007. The Director General inaugurated the newly constructed Fish Seed Production Unit at the Institute. This hatchery unit has been established under the Mega Seed Project of the ICAR with an aim to produce quality seed of commonly cultured fish species, especially Indian major carps and making them available to the fish farmers of the region. The seed production unit has 20 nursery and

rearing ponds with a targeted production capacity of 10 million fish seeds at a time. The DG appreciated the development of this facility and opined that supply of quality fish seed is the key to profitable fish farming. He also inaugurated a number of other newly developed facilities at the Institute including a Training Facility, a Tissue Repository and a Residential Block (Fig. 59-63).

In his thought provoking address to the scientists and staff of the Bureau, Dr. Mangala Rai emphasized upon the significance of issues and research related to Intellectual Property Rights (IPRs) and genomics in the fast changing global R&D and WTO scenario. He especially emphasized upon the urgent need for registration of



Fig. 60. Dr. Mangala Rai, Secretary, DARE and DG, ICAR inaugurating the Fish Seed Production Unit

National Bureau of Fish Genetic Resources



Fig. 61. Dr. Mangala Rai, Secretary, DARE, and DG, ICAR and Dr. S. Ayyappan, DDG (Fy.), ICAR, New Delhi visiting the Fish Seed Production Unit (A, B & C).

germplasm of the country. The DG called upon the scientists to pay greater attention to new ideas in research. He particularly stressed upon the need to move towards functional genomics through identifying economic traits and the genes. Dr. Mangala Rai also shared his views on future of gene banks. He opined that scientists need to work towards establishing real gene banks by identifying gene constructs. The DG while appreciating the achievements of NBFGR, opined that the scientists have to work even harder in the years to come as the challenges that Indian agriculture sector including fisheries is facing, are greater. On this occasion,



Fig. 61. A new Training facility inaugurated by Dr. Mangala Rai (D) and The DG planting a tree in front of the training facility (E).



Fig. 62. Dr. Mangala Rai inaugurating a new residential block (A)



Fig. 62. The newly constructed residential block (B)

Dr. S. Ayyappan, Deputy Director General (Fisheries), ICAR also appreciated the efforts of the Bureau and thanked the Director General for his active support and guidance to the

fisheries institutes of the Council. Dr. W.S. Lakra, Director, NBFGR expressed his gratitude to the DG for his visit, appreciation, support and guidance to the Bureau.



Fig. 63 Facilitation of Dr. Mangala Rai, Secretary, DARE and DG, ICAR, New Delhi at NBFGR by Dr. S. Ayyappan, DDG (Fy.), ICAR, Dr. W.L. Lakra, Director, NBFGR is also in the picture.

NBFGR signs MoU with Dr. B.B. Ambedkar University, Lucknow

A Memorandum of Understanding (MoU) was signed between NBFGR, Lucknow and Dr. Babasaheb Bhimrao Ambedkar University, Lucknow on February 22, 2008. The primary objective of this MoU is to develop and share expertise and facilities at mutually agreeable terms and conditions.

Meeting on Management of Agricultural Information and Dissemination

The Institute hosted a meeting on Management of Agricultural Information and Dissemination on August 18, 2007. The meeting was convened by the Directorate of Information & Publications of Agriculture (DIPA), ICAR, New Delhi to discuss issues related to improvement of the mechanisms of disseminating agricultural information to different stakeholders for greater impact. Dr. W.S. Lakra, Director, NBFGR; Dr. T.P. Trivedi, Project Director, DIPA; Shri Anil K. Sharma, Chief Public Relation Officer, ICAR and other staff of DIPA; Scientists and AF&AO of NBFGR, IISR, Lucknow; CISH, Lucknow; ICAR Zonal Coordination Unit IV, Kanpur and C.S.A. University of Agriculture & Technology, Kanpur participated in the meeting.

The Concept of a State Fish

The NBFGR promoted a new concept of a 'State Fish' for each state linking and integrating conservation, research and the stakeholders. The state governments were requested to declare and adopt an important food or threatened fish species of the state as the 'State Fish'. Interestingly, seventeen states have initiated steps to declare their state fish.

Hindi Day and Hindi Pakhwada observed

A function was organized on September 14, 2007 to celebrate the Hindi Day. The Institute also observed a Hindi Pakhwada during September 15-29, 2007 during which 7 Hindi competitions were organized among the staff of the institute to promote use of Hindi in official work (Fig. 64). Shri A.K Mishra, T-4 won the prize for the Best Hindi Competitor-2007. Dr. W.S. Lakra, Director, NBFGR distributed certificates and prizes to all the winners of these competitions.



Fig. 64. Dr. W.S. Lakra, Director, NBFGR addressing the staff on the Valedictory function of *Hindi Pakhwada*.

World Environment Day celebrated

The World Environment Day was celebrated at NBFGR, on June 5, 2007 with the theme “Global Warming and Biodiversity Conservation”. Dr. V.P. Kamboj, Former Director,

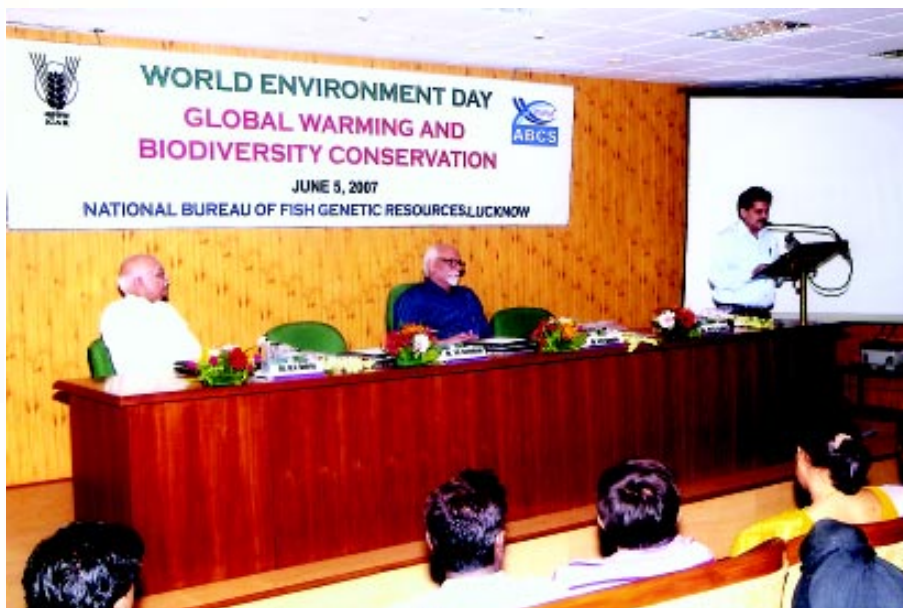


Fig. 65. A view of the World Environment Day Celebration

CDRI, Lucknow was the Chief Guest on this occasion whereas Dr. S.L Govindwar, Advisor, DBT, Govt. of India, New Delhi and Dr. R.C. Dalela, Founder President, Academy of Environment Biology were the Guests of Honour (Fig. 65). A large number of students from different schools of the city participated in this programme. A series of debates and lectures were organized for students and prizes were distributed to the winners.

National Festivals celebrated

National Festivals - the Independence Day and the Republic Day, were celebrated at the Institute on August 15, 2007 and January 26,

2008, respectively. The flag hoisting ceremony was observed on both the occasions. Dr. W.S. Lakra, Director hoisted the National Flag in the presence of other staff members of the Bureau. On these occasions, cultural programmes and drawing competitions for children of NBFGR staff were also organized.

New Cell Culture facility developed

A new Cell Culture facility was developed at the Institute which was inaugurated by Dr. C.D. Mayee, Chairman, ASRB, New Delhi on August 27, 2007. This facility has been developed to strengthen the work on development of successful cell culture systems for selected fish species

Mail Filtering Gateway Server developed

The ARIS Cell of NBFGR has developed a mail filtering gateway server with internet detection and protection mechanism to protect the mails from viruses/spywares/ spasm and to protect the server from hackers and intruders.

Participation in ICAR sports

NBFGR sports team participated in ICAR Zonal Sports meet held at National Dairy Research Institute, Karnal during September 26-29, 2007. Shri R.S. Patiyal, Technical Officer (T-6) won second prize in shot-put throw.

Staff Research Council (SRC) Meetings

The Annual Staff Research Council meeting of the Institute for the year 2008-09 was organized on April 8-9, 2008 under the Chairmanship of Dr. W.S. Lakra, Director (Fig. 66). In his introductory remarks, Dr. Lakra stressed upon the key trust areas indetified in the EFC document which are to be focussed



Fig. 66. A view of the SRC Meeting

during 11th Five Year Plan period. He emphasized to publish research results in high impact journals, develop international programmes and establish linkages with international groups of researchers. All the on-going projects, were critically reviewed and suggestion were given for their improvement. Seven new project proposals were also presented by the scientists. Each new presentation was followed by a detailed discussion and refinements/modification suggested therein.

A mid-term SRC meeting was conducted during December 6-7, 2007 under the chairmanship of Dr. W.S. Lakra, Director. Dr. P.V. Dehadrai, Former DDG (Fy.), ICAR, attended the meeting as a Special Invitee. Dr. W.S. Lakra,

during his introductory remarks, welcomed all the participants and highlighted the thrust areas for XI plan. Dr. Dehadrai in his remarks said that the mandate of the four Bureaus are more significant in biodiversity management. He suggested the scientists to improve the quality of research to global standards and work for the benefit of common people. He opined that endangered fishes could be focused for developing protocol for mass seed production and culture

practices. This was followed by presentations of the work done under different ongoing projects by the concerned principal investigators. The progress of all on-going projects was reviewed by the SRC and important suggestions emerged from the discussion. Dr. W.

S. Lakra expressed his gratitude to Dr. Dehadrai for attending the SRC and offering valuable guidance for improving the quality of research work. The meeting concluded with a formal vote of thanks by Dr. S.P. Singh, Sr. Scientist.

Research Advisory Committee (RAC) Meeting

The RAC meeting of NBFGR was organized during February 28-29, 2008 under the chairmanship of Padamshree Dr. Lalji Singh, Director, Centre for Cellular and Molecular Biology, Hyderabad (Fig. 67). The following members of the RAC and NBFGR attended the meeting:

Padamshree (Dr.) Lalji Singh, Director, Centre for Cellular and Molecular Biology, Hyderabad.	Chairman
Dr. M. Sinha, Advisor, Department of Fisheries, Govt. of Tripura	Member
Prof. M. S. Johal, Former Head, Department of Zoology, Punjab University, Chandigarh	Member
Dr. W. S. Lakra, Director, NBFGR	Member
Dr. R. Soundararajan, Principal Scientist, NBFGR	Member-Secretary
All Scientist and Technical Staff, NBFGR	Invitees



Fig. 67. A view of the RAC meeting being chaired by Padamshree Dr Lalji Singh, Director, CCMB, Hyderabad

Dr. W.S. Lakra, Director, NBFGR welcomed the Chairman, other members of the newly constituted RAC and all the participating staff of the Bureau. He expressed his appreciation and thanks to the RAC Chairman and members for sparing their time and made a presentation of over all achievements of the Institute. He referred to the 'Vision 2025' document of the Institute and mentioned about the current emphasis on

registration of fish germplasm. He expressed his hope that the present RAC would give new directions, focus and guidance for improvement of Bureau's research activities.

In introductory remarks, the RAC Chairman Dr. Lalji Singh told that the RAC's function was not making assessment of performance of the scientists but to play a catalytic role and help in improving the ongoing research activities of the Institute. He suggested that the scientists should not restrict their research programmes only to the issues at national level but address the global issues too. He emphasized that the scientists should target international

funding for their research programmes and harness the global opportunities. He urged the scientists to publish research papers in international journals with good impact factor. He suggested that NBFGR, as a Resource Centre, could develop zebra fish resource, since it is one of the model species for genomics research.

There were theme-wise presentations on the progress of work during year 2007-08 and brief

wok plan for coming year by respective Heads of Divisions/Sections/PIs of the projects. The chairman and members went through the Action Taken Report on the recommendations made by previous RAC meeting and appreciated the achievements. The chairman and members of the RAC also visited laboratories, Live Fish Gene Bank, hatchery complex and other facilities of NBFGR.

RAC made following general and specific key recommendations along with project-wise comments and recommendations.

General Recommendations

1. Applied research should be the prime motive of scientists.
2. Research publications should be aimed for high impact journals.
3. Feedback information from trainees may be obtained to refine the programmes.
4. Research programmes may be formulated in networking mode and to meet the global standards.
5. A Central Facility equipped with modern equipments and for application of proteomics may be created.
6. Scientists may develop a strong group on Genetics and Breeding and concentrate on the research work related to genetic characterization and selective breeding to develop strains with desired traits for faster growth, disease resistance, adaptability to changed environment and alike.
7. Breeding programmes on the zebra fish with network of the laboratories doing similar work on zebra fish may be initiated.
8. Linkages with first rate institutions may be established in complementary mode.

9. A document, justifying the need to strengthen the Cochin Unit as a full-fledged Research Centre of NBFGR may be prepared and submitted to ICAR endorsed with RAC recommendation.

Specific Recommendations

1. Complete sequencing of mitochondrial DNA and designing of universal primers from mitochondrial DNA may be taken up for identification of fish species.
2. All possible evidences regarding geographic, phenotypic and molecular characteristics may be collected which would become a very good database in long-term.
3. Different species of same genus as well as same species in different geographical locations may be targeted for characterization.
4. The farmers could be trained at all India level which would facilitate focused conservation.
5. Industry can be involved in breeding of endangered fish through training.
6. The exploration of the higher altitude areas may be extended with the help of the Indian Military Force.
7. Some scientists may be sent to CCMB, Hyderabad for training.

HUMAN RESOURCE DEVELOPMENT

Trainings Organized

First International Training on DNA Barcoding of Marine Life

The Institute organized the First International Training on “DNA Barcoding of Marine Life” during April 16-21, 2007 at Lucknow which was sponsored by Indian Council of Agricultural Research; Indian Ocean-Census of Marine Life (IO-CoML), Goa and Aquatic Biodiversity Conservation Society (ABCS), Lucknow (Fig. 68). A total number of 18 participants, representing various Universities, Colleges, and Research Institutes from different parts of the country as well as foreign nationals from Kenya, Tanzania, South Africa, Canada and Australia participated in the programme. The programme was aimed at

imparting hands-on training on the concept and techniques of DNA Barcoding which is expected to play a pivotal role in species identification and biodiversity conservation.

Dr. S. Ayyappan, Deputy Director General (Fisheries), Indian Council of Agricultural Research, New Delhi inaugurated the programme and highly appreciated the lead role of NBFGR in DNA Barcoding. Dr. W.S. Lakra, Director, NBFGR and Regional Chair of FISH-BOL, emphasized for increased international cooperation between the countries sharing water bodies and stated that this novel programme of NBFGR would lead to generation of the barcode data for all fish and shellfish species of the country in coming years towards registration of aquatic germplasm.

During the programme, Dr. Lalji Singh,



Fig. 68. A group photograph of the participants along with the Guests and faculty members of NBFGR



Fig. 69. A participant receiving certificate from the Chief Guest Dr. SAH, Abidi, Former Member, ASRB, New Delhi

Director, Center for Cellular and Molecular Biology, Hyderabad delivered an invited talk on “Science of Establishing Individual Identity: Past, Present and Future”. Dr. S.A.H. Abidi as Chief Guest on the valedictory day, advised the participants to look forward and adopt new technological advancements to complement the conventional taxonomy towards better insights into biodiversity issues (Fig. 69).

Summer School on Fish Biotechnology

The Institute organized an ICAR sponsored “Summer School on Fish Biotechnology” for scientists/researchers of various research institutes and state agricultural universities during August 07-27, 2007. A total number of 15 participants representing various ICAR institutes, State Agricultural Universities, Colleges and Research Institutes from various parts of the country participated

in the 21 days programme. The training was intended to give theoretical as well as practical insights into different techniques used in modern biology and biotechnology. Resource persons included scientists from NBFGR as well as invited experts from other institutes. Dr. V.V. Sugunan, Assistant Director General (Inland Fisheries), ICAR, New Delhi inaugurated the programme and highly appreciated the role of NBFGR in the field of human resource development in fish biotechnology.

The course contents included:- Biotechnology applications to aquaculture and fisheries, Techniques for development of molecular markers: Theory and Practice, Molecular cytogenetic markers and genotoxicity studies: Theory and Practice, Use of bioinformatics tools and softwares, Techniques for molecular diagnosis of fish diseases: Theory and Practice, Cell culture techniques: Techniques of fish gene banking: Theory and Practice and Marine biotechnology. A training manual on Fish biotechnology was also released on this occasion (Fig. 70). In the Valedictory Session Dr. C.D.



Fig. 70. Dr. C.D. Mayee, Chairman, ASRB, New Delhi releasing a training manual



Fig. 71. A view of the audience during Valedictory Session (A) and Chief Guest, Dr. C.D. Mayee, Chairman, ASRB, New Delhi addressing the participants & Staff (B)

Mayee, Chairman, Agricultural Scientists' Recruitment Board, New Delhi and Dr. S.A.H. Abidi, Former Vice Chancellor, CIFE Mumbai and Former Member, ASRB were present as the Chief Guest and Guest of Honour, respectively. Dr. W.S. Lakra, Director of the Institute while welcoming the guests stressed upon the need for adopting new biotechnological tools for various research programmes pertaining to conservation of the fish species, improvement in aquaculture production, disease diagnostics and control measures. In his address, Dr. Mayee appreciated the efforts of NBFGR for organizing such training programmes for the benefit of scientists from different regions of the country, especially from remote areas (Fig. 71). He also emphasized that techniques of transgenics, molecular markers and marker assisted selections have tremendous potential towards improving production of food fishes. The participants of the programme while expressing satisfaction about the content and utility of the training course, assured to use the new biotechnological tools in their research programmes.

Training Programme on "Quality Fish Seed Production and Hatchery Management"

The Bureau, at its Aquaculture Research and Training Unit, Chinhat organized a short-term training programme on "Quality Fish Seed Production and Hatchery Management" for the benefit of the aqua-farmers of Uttar Pradesh during Aug. 16-20, 2007 (Fig. 72). The programme was inaugurated by Hon'ble Shri Jamuna Nishad, State Minister of Fisheries, Government of Uttar Pradesh. Smt. Anita Mishra, Director, Fisheries, U.P.; Dr. C.S Singh, Former Dean, College of Fisheries, G.B Pant University



Fig. 72. A view of the inaugural function of a training programme

of Agric. & Tech., Pantnagar and Dr. S.C Pathak, Former Chief General Manager, National Bank for Agriculture and Rural Development, Mumbai were the Guests of Honour on this occasion. Twenty progressive aqua-farmers from nine districts of Uttar Pradesh, nominated through the Department of Fisheries, Uttar Pradesh, participated in the training. In the programme, major emphasis was given on practical demonstration and field-oriented activities. Apart from the theory classes, laboratory demonstrations and exercises on induced breeding and hatchery operations were conducted. Field visits to hatcheries around Lucknow and interaction with an ICAR awarded progressive fish farmer Shri Sultan Singh from Karnal (Haryana) were also arranged to provide the trainees exposure for commercial aquaculture. The training programme was sponsored by National Fisheries Development Board, Hyderabad

Training Programme on “Aquaculture Diversification and Impact of Exotic Species”

A short term training programme on “Aquaculture Diversification and Impact of Exotic Species” for the benefit of the aqua-farmers of Uttar Pradesh was organized during September 24-29, 2007 at Aquaculture Research and Training Unit, Chinhath of NBFGR, Lucknow. Dr. S.K. Singh, Joint Director, Department of Fisheries, U.P. was the Guest of Honour of the Inaugural function held on September 24, 2007 whereas Dr. W.S. Lakra, Director, NBFGR presided over. A total of eleven trainees participated in the training from

different districts of U.P. The training programme was sponsored by National Fisheries Development Board, Hyderabad and focused on practical aspects with major thrust on field work.

Training Programme on “Integrated Aquaculture and Fish Disease Management”

The Bureau, at its Aquaculture Research and Training Unit, Chinhath organized a short-term training programme sponsored by National Fisheries Development Board, Hyderabad on “Integrated Aquaculture and Fish Disease Management” for the benefit of the aqua-farmers of Uttar Pradesh during November 14-19, 2007. A total of nineteen progressive fish farmers of Uttar Pradesh participated in the programme. The programme was inaugurated by Shri Deo Dutt, Secretary (Fisheries), Govt. of U.P. (Fig. 73). The training was practical oriented with major thrust on field work and practical aspects. On successful completion of training, Dr. W.S. Lakra, Director, NBFGR, Lucknow gave certificates to the participants.



Fig. 73. Dr. W.S. Lakra, Director, NBFGR addressing the participants and Staff during training programme

Training Programme on “Freshwater Prawn Culture Technology”

A short-term training programme on “Freshwater Prawn Culture Technology” for the benefit of the aqua-farmers of Uttar Pradesh was organized during December 13-18, 2007 at Aquaculture Research and Training Unit, Chinhath of NBFGR, Lucknow. The programme was sponsored by NFDB, Hyderabad and inaugurated by Dr. B.N Singh, Former Deputy Director General (Fisheries), Indian Council of Agricultural Research, New Delhi. A total of fourteen trainees from nine districts of Uttar Pradesh participated in the programme. Besides lectures, demonstrations on identification of commercially important fishes and prawn, planktons, harmful aquatic weeds, identification of disease in fishes and analysis of important physico-chemical parameters were conducted. Visits to institute’s fish farm and hatchery as well as fish farms of private entrepreneurs were also arranged. The participants were given certificates by Dr. S.K Singh, Joint Director, Dept. of Fisheries, U.P.

Training Programme on “Basic Tools in Molecular Biology Research”

The NBFGR organized a training programme on “Basic Tools in Molecular Biology Research” during January 07-11, 2008. The participants were exposed to the baseline theoretical and practical insights in various types of tools used in molecular biology research. The training included DNA polymerase chain reaction and DNA sequencing. A total of 15 participants

belonging to different research organizations/ universities/national institutes of India, participated in the programme.

Training Programme on “Cellular and Molecular Approaches for Genotoxicity Assessment in Fishes”

A training course on ‘Cellular and Molecular Approaches for Genotoxicity Assessment in



Fig. 74. A group photograph of the participants along with the Guest and Faculty members of NBFGR

Fishes’ was organized during February 21-28, 2008. A total of 18 participants representing various Universities, Colleges and Research Institutes from different parts of the country participated in the programme (Fig. 74). The participants were given hands-on training on different techniques including chromosomal aberration test, sister chromatid exchanges, micronuclei detection, comet assay, random amplification of polymorphic DNA and ultrathin isoelectric focusing. These assays can be widely used for testing the impact of genotoxic agents

found in the environment whose presence may alter the integrity of gene pool of a wide range of aquatic organisms and human beings. Faculty members from renowned laboratories were also invited to enlighten the participants with recent developments in this field.

Lectures organized

A series of invited guest lectures by following leading experts were organized at the institute:

- Dr. C.S. Singh, Former Dean, Fisheries, G.B. Pant University of Agric. & Tech., Pantnagar on “Role of quality fish seed in the development of aquaculture”.
- Dr. S.C. Pathak, Former Chief General Manager, NABARD, Mumbai on “Financing and insurance in fisheries”.
- Shri Sanjay Shukla, Deputy Director, Department of Fisheries, U.P., Lucknow on “Schemes for the development of fisheries in Uttar Pradesh”.
- Dr. S.K. Singh, Joint Director, Department of Fisheries, U.P., Lucknow on “The role of the state fisheries in diversification of aquaculture”.
- Dr. R.N. Seth, Principal Scientist, Riverine Division, CIFRI, Allahabad on “The culture possibilities of *Mystus* species”.
- Dr. D.K. Sharma, Principal Scientist, Central Soil Salinity Research Institute, Regional Center, Lucknow on “The utilization of the waterlogged sodic soil for aquaculture”.
- Dr. A.K. Dutta, Chancellor, Dev Sanskrit Vishvavidhyalaya, Haridwar on “Stress management” on June 13, 2007.
- Smt. Sushma Sharma, Assistant Director of Fisheries, Uttar Pradesh on “Integrated aquaculture in U.P. and various schemes launched by the Department of Fisheries U.P. for the welfare of the aqua-farmers of the State” on November 18, 2007.
- Smt. Monisha Singh, Assistant Director Fisheries/CEO, FFDA, Unnao on “The economics of catfish culture and freshwater prawn farming”.
- Dr. S. Raizada, Senior Scientist, Rohtak Center of CIFE on “Breeding and hatchery management of the freshwater prawn” on December 14, 2007.
- Dr. A.K. Jain, Sr. Scientist, ICAR Research Complex, Patna on “The prospects of aquaculture diversification and aquaculture systems”.
- Dr. Krishna Gopal, Scientist E-II and Head, Aquatic Toxicology Division, Indian Institute of Toxicology Research, Lucknow on “Recent advances in aquatic toxicology” on February 22, 2008.
- Dr. Devendra Parmar, Head, Environmental Toxicology Division, IITR, Lucknow on “Cytochrome P450 expression” on February 25, 2008.
- Dr. B.N. Paul, Scientist E-II and Head, Immunobiology Division, IITR, Lucknow on “The application of gene expression profiling to ecotoxicology” on February 26, 2008.
- Dr. B.S. Khangarot, Scientist E-II, Ecotoxicology Division, IITR, Lucknow on “Use of aquatic test models in ecotoxicology” on February 27, 2008.
- Dr. Lalji Singh, Director, CCMB, Hyderabad on “What makes us human” on February 28, 2008.
- Shri A. K. Gupta, Lecturer/Assistant Director Fisheries, U.P. on “Schemes for the development of fisheries as well as for the welfare of the aqua-farmers of the state” on December 17, 2007.

AWARDS AND RECOGNITION

- Dr. W.S Lakra, Director has been conferred with “Dr. M.S Swaminathan Best Indian Fisheries Scientist Award 2007”.
- Dr. W.S Lakra, Director was conferred with the Fellowship of Inland Fisheries Society of India on December 14, 2007 at Central Inland Fisheries Research Institute, Barrackpore.
- Dr. A. Gopalakrishnan, Senior Scientist was nominated as a Member of the Task Force on Fisheries Research, Govt. of Kerala
- Dr. Rehana Abidi, Sr. Scientist was conferred with the Fellowship of Academy of Environmental Biology on October 26, 2007.

EXTENSION ACTIVITIES

Participation in Exhibitions

The Institute participated in the following exhibitions related to fisheries and aquatic resources during the year 2007-2008 in different parts of the country:

- National workshop-cum-exhibition on “*Parvatiya matsyaki paridrishya: Vikas, prabandhan evam sanrakshan*” (in Hindi) during April 6-7, 2007 at National Research Centre on Cold Water Fisheries, Bhimtal.
- Exhibition organized on the occasion of the Fourth Indian Fisheries Science Congress on “Ecology and fisheries of wetlands in India” at ICAR Research Complex, Patna during April 12 – 13, 2007.
- Exhibition organized on the occasion of the Foundation Day of Indian Vegetable Research Institute, Varanasi on Sept. 28, 2007.
- Exhibition organized on the occasion of the 8th Asian Fisheries Forum at Kochi during Nov. 20-23, 2007.



Fig. 75. Shri Sharad Pawar, Hon'ble Union Minister of Agriculture, Consumer Affairs, Food and Public Distribution, Govt. of India visiting NBFGR Pavillion at CMFRI, Kochi

- Exhibition organized on the occasion of All India Zoology Congress at Lucknow University, Lucknow during Dec. 07-09, 2007.
- “Matsya Utsav” organised by Central Inland Fisheries Research Institute and Inland Fisheries Society of India at Barrackpore during Dec. 14-16, 2007.



Fig. 76. Dr. Mangala Rai, Secretary, DARE and DG, ICAR discussing at NBFGR stall at CIFRI, Barrackpore



Fig. 77. Dr. W.S. Lakra, Director, NBFGR receiving Dr. S. Ayyappan at NBFGR Stall at the 8th Asian Fisheries Forum, Kochi.

- Exhibition organized on the occasion of the Diamond Jubilee celebrations of CMFRI, Kochi during January 3-5, 2008 at Kochi.
- International Aquashow organized at Kochi, Kerala during February 01–05 2008.
- Agri-Expo 2008 organized at Indian Institute of Sugarcane Research, Lucknow during February 16-19, 2008.
- Exhibition organized on the occasion of the National Science Day on February 28, 2008 by UPCST, Lucknow and Biotechnology Park, Lucknow at Biotechnology Networking Facility, Bakshi-Ka-Talab, Lucknow.

Advisory Services for Aquaculture and Conservation

During the year under report, the Institute at its headquarter, as well as, at its Aquaculture Research & Training Unit, Chinhat, provided advisory services to 172 visiting fish farmers and aqua-entrepreneurs on different aspects of aquaculture and fish conservation.



Fig. 78. Dr. A. Gopalakrishnan, Scientist-in-Charge, NBFGR Cochin Unit discussing with the tribal fisherwomen on endemic fish resources, while Dr. K. Dinesh, Asst. Professor, College of Fisheries, Kochi looks on

As a part of the “Training programme on aquaculture of indigenous ornamental and food fishes” organized by College of Fisheries, KAU, Kochi, 11 tribal fisherfolk (9 women and 2 men) from Vazhachal Forest Region (Chalakkudy River basin) visited NBFGR Cochin Unit and got acquainted with the fish conservation and cataloguing activities of the Bureau. Drs. A. Gopalakrishnan and V.S. Basheer conducted classes in local language (Malayalam) on the native ornamental and food fish resources of Kerala part of the Western Ghats and their sustainable utilization and management.

Technical lectures/ talks delivered

- Dr. W.S. Lakra, Director acted as a judge and delivered a Lead Lecture at Uttarakhand Academy of Administration, Nainital during Nov. 15-17, 2007.
- Dr. W.S. Lakra, Director gave an invited lecture at the All India Zoology Congress at Lucknow University on Dec. 8, 2007.
- Dr. A.K. Singh, Senior Scientist delivered a lecture on “Invasive fish species in North East India: Status and Impact” in National Seminar on Recent advances and Rebuilding of Fish and Fisheries in North east India, Organized by Department of Pisciculture, St. Anthony’s College, Shillong, Meghalaya during Aug. 22-23, 2007.
- Dr. U.K. Sarkar, Sr. Scientist, delivered a lecture as the resource person in a training programme of KVK staff of NEH region on “Sustainable development and conservation of fish genetic diversity with references to NEH states of India” during July 4-6, 2007 at CIFRI Center, Guwahati.

- Dr. Rehana Abidi, Sr. Scientist gave an invited lecture on “Fish diseases management” at the Department of Zoology, Allahabad University, Allahabad on Dec. 10, 2007.
- Dr. A. Gopalakrishnan, Sr. Scientist delivered a lecture on “Stress, Heat Shock Proteins and Biotechnological Interventions” in the Winter School on Impact of Climate Change on Indian Marine Fisheries, on February 06, 2008 at CMFRI, Kochi

Visits by development officials, students and farmers

The following batches of development officials, students and farmers visited different laboratories, hatchery and fish farm of the Institute during this period:

- A batch of 11 progressive aqua-farmers from district Meerut along with the CEO, FFDA, Meerut visited the NBFGR, Lucknow on May 22, 2007*.
- A group of Sr. Officers from sugar mills of various states on July 13, 2007.
- Sixty progressive fish farmers from district Bareilly, U.P. on Sept. 22, 2007*.
- A group of 16 students of M.Sc. from Institute of Science, Mumbai on Oct. 26, 2007.
- A group of 42 students from HRP Sardar

Patel Degree College, Barabanki on Nov. 12, 2007*.

- A group of 48 students from Desh Bharti Public Inter College, Rajajipuram, Lucknow on Dec. 11, 2007.
- A group of 50 students of B.Sc. from Dayanand Bachhrawan P.G College, Bachhrawan, Raebareli, UP on Dec. 12, 2007.
- A group of 60 progressive fish farmers along with two officials from Fish Farmers Development Agency, Lucknow on Dec. 14, 2007.
- A group of 14 students of B.F.Sc. from College of Fisheries, Ratnagiri on Dec. 22, 2007.
- A group of 75 students of M.Sc. along with faculty members from C.S.J.M University, Kanpur on Dec. 27, 2007*.
- A group of 22 students of M.Sc. and Ph.D from Baba Sahab Bhim Rao Ambedker University, Lucknow on Feb. 20, 2008.
- A group of 22 progressive fish farmers from Krishi Vigyan Kendra, Barielly on March 03, 2008.
- A group of B.Tech (Biotechnology) students from SRI Datia College, MP on February 19, 2008.
- A group of M.Sc students from Avadh University, Fazabad on February 18, 2008.

* Also visited ARTU, NBFGR Chinhat, Lucknow.

WORKSHOPS/SYMPOSIA ORGANIZED

Workshop on Fisheries Conservation and Enhancement

The NBFGR organized a Workshop on “Fisheries Conservation and Enhancement: Linking Researchers and Stakeholders” at Guwahati, Assam during December 18-19, 2007. The major objectives of the workshop were: 1) Prioritization of conservation research and programmes, 2) To assess the conservation status of all freshwater fishes of North-Eastern region for biodiversity management, 3) To develop conservation strategies on the recently proposed “State Fish” of various states, 4) To explore the status and potential of recent technological intervention for fisheries enhancement and biodiversity conservation in the NEH Region and 5) To strengthen linkages between researchers,



Fig. 79. Dr. P. Das, Former Director, NBFGR speaking during the inaugural function

stakeholders and policy makers for sustainable management of fisheries resources in the region. On this occasion, Dr S.S. Baghel, Vice Chancellor,



Fig. 80. A Group photograph of the Workshop participants at Guwahati



Fig. 81. Dr. S.S. Baghel, Vice Chancellor, Assam Agricultural University inaugurating the Regional Live Fish Gene Bank at Guwahati



Fig. 82. The dignitaries releasing fish in the gene bank

Assam Agricultural University unveiled a Live Fish Gene Bank at Ulubari Fish Farm, Guwahati which is a joint venture of Department of Fisheries, Govt. of Assam and NBFGR (ICAR), Lucknow. Two books published by NBFGR namely, *Fishes of North-East India* and *Ornamental Fishes of North-East India - An Atlas* were also released by the dignitaries on this occasion. A total of ninety participants including leading Professors, Eminent Scientists from

Institutes, Officers of the State Fisheries Departments from North-Eastern states, Non-governmental organizations and KVKs attended the workshop.

Awareness Programme on Fish Conservation

The NBFGR organized an Awareness Workshop on “Conserve Fish for Posterity” at the Rajiv Gandhi University, Itanagar, Arunachal Pradesh during December 20-21, 2007. The major objectives of the workshop were: 1) To create awareness among stakeholders for participating in fish conservation campaign, 2) To facilitate interaction, exchanges of ideas, sharing of knowledge and experiences of the end users of the aquatic resources of North-Eastern states; 3) To formulate and recommend strategic research plan for conserving ichthyo-fauna in diversified water bodies of Arunachal Pradesh and 4) To promote participatory research approaches for successful propagation of threatened wild fish fauna of the region. On this occasion, Mr. M. Pertin, Secretary (Fisheries), Government of Arunachal Pradesh spoke on the need and potential of fish conservation and enhancement in Arunachal Pradesh. A total of seventy five participants from the North-Eastern Region attended the workshop.



Fig. 83. A view of the awareness programme on fish conservation at Itanagar, Arunachal Pradesh.

LINKAGES

The NBFGR worked in collaboration with the following international and national organizations and agencies during the period under report.

National Organizations/Agencies

ICAR Institutes

- Central Institute of Freshwater Aquaculture, Bhubaneswar, Orissa.
- Central Inland Fisheries Research Institute, Barrackpore, West Bengal.
- Central Marine Fisheries Research Institute, Kochi, Kerala.
- Central Institute of Brackishwater Aquaculture, Chennai, T.N.
- Central Institute of Fisheries Education, Mumbai, Maharashtra.
- National Research Centre on Coldwater Fisheries, Bhimtal, Uttarakhand.
- National Bureau of Animal Genetic Resources, Karnal.
- ICAR Complex for NEH Region, Barapani, Shillong, Meghalaya.
- ICAR Center for Sikkim, Tadong, Gangtok, Sikkim.

International Organizations/ Agencies

- The WorldFish Centre, Penang, Malaysia.
- Network of Aquaculture Centers in Asia Pacific (NACA), Bangkok, Thailand.
- CSIRO Marine and Atmospheric Research, Hobart, Australia.
- Institute of Biodiversity, University of Guelph, Canada.

Universities and Colleges

- Cochin University of Science and Technology, Kochi.
- College of Fisheries, Kerala Agricultural University, Panangad, Kerala.
- College of Fisheries, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand.
- Regional Agricultural Research Station, Kerala Agricultural University, Kumarakom, Kottayam, Kerala.
- School of Industrial Fisheries, Cochin University of Science & Technology, Kochi.
- School of Life Sciences, NEHU, Shillong, Meghalaya.
- Department of Zoology, Gauhati University, Guwahati, Assam.
- School of Life Sciences, Dibrugarh University, Dibrugarh, Assam.
- Department of Life Sciences, Manipur University, Manipur.
- Department of Life Sciences, Assam University, Silchar, Assam.
- College of Fisheries, Assam Agricultural University, Assam.
- School of Life Sciences, R.G. University, Itanagar, Arunachal Pradesh.
- College of Fisheries, Central Agriculture University, Lambuchara, Agartala, Tripura.
- St. Anthony College, Shillong, Meghalaya.
- Department of Zoology, Patna University, Patna, Bihar
- University of Delhi, Delhi.
- HNB Grahwal University, Srinagar-Garhwal, Uttarakhand.

- Dr. BB Ambedkar University, Lucknow.
- Lucknow University, Lucknow.

Central Ministries/ Departments

- Ministry of Environment and Forests, New Delhi.
- Ministry of Agriculture, New Delhi.
- Ministry of Earth Sciences, New Delhi
- Department of Biotechnology, New Delhi

State Ministries/ Departments

- Department of Fisheries, Govt. of U.P., Lucknow.
- Department of Fisheries, Govt. of Himachal Pradesh, Bilaspur.
- Department of Fisheries, Govt. of Kerala.
- Department of Fisheries, Govt. of Punjab.
- Department of Fisheries, Govt. of Haryana.
- Department of Fisheries, Govt. of M.P., Bhopal
- Department of Fisheries, Govt. of Assam, Guwahati.
- Department of Fisheries, Govt. of Meghalaya, Shillong.
- Department of Fisheries, Govt. of Arunachal Pradesh, Itanagar.
- Department of Fisheries, Govt. of Tripura, Agartala.
- Department of Fisheries, Govt. of Nagaland, Kohima.
- Department of Fisheries, Govt. of Manipur, Imphal.
- Department of Fisheries, Govt. of Sikkim, Gangtok.
- Department of Fisheries, Govt. of Mizoram, Aizwal.
- Department of Forest, Govt. of U.P.

- Assam Fisheries Development Corporation Ltd., Guwahati
- Zoological Survey of India, Dehradun.
- National Informatics Centre, Delhi and Lucknow.
- U.P. Remote Sensing Application Centre, Lucknow.

Non-Government Organizations

- Several NGOs working in the North Eastern Region of India.
- Aquatic Biodiversity Conservation Society, Lucknow.

Other Organizations

- National Institute of Oceanography, Panaji, Goa.
- Central Drug Research Institute, Lucknow.
- Central Institute of Medicinal and Aromatic Plants, Lucknow.
- Kerala Forest Research Institute, Trichur, Kerala.
- Indian Institute of Remote Sensing, Dehradun.
- Sanjay Gandhi Post-Graduate Institute of Medical Sciences, Lucknow.
- Marine Products Export Development Authority, Kochi.
- Department of Limnology, Barkatullah University, Bhopal.
- North-Eastern Council, Shillong, Meghalaya.
- Indian Institute of Toxicology Research, Lucknow.
- Centre for Cellular & Molecular Biology, Hyderabad.
- Wildlife Institute of India, Dehradun.
- Fisheries Survey of India, Mumbai.

PUBLICATIONS

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Age and growth profile of Indian major carp, *Catla catla* from rivers of Northern India. *Acta Zoologica Sinica* 54 (1) : 136-143.

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Length-weight relationship and condition factor of endangered featherback *Chitala chitala* from different populations of Northern India. *J. Aqua. Biol.* 22(1) : 65-69.

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Leiomyoma of the vagina with recurrent

vaginal prolapse in a cow. *Indian Vet. J.* 85 : 93-94.

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Sood, N, G. Rathore, A. K. Singh and T. R. Swaminathan, 2008

Ballast water: a potential rout for introduction of non-indigenous aquatic organisms. *In*: W.S. Lakra, A.K. Singh and S. Ayyappan (Eds.). Fish Introductions in India: Status, Potential and Challenges. Narendra Publishing House, New Delhi. pp 191-200.

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Impact of exotic fish *Oreochromis mossambicus* on fish diversity of Jaisamand Lake, Rajasthan, India. Abstract No. BDP 005; Pp. 363

- 364, *In*: Bright Singh, I.S., Mohandas, A., Joseph, V., Pai, S.S., Paul, L., Philip, R., Paulraj, R., Mohammed, S.K. and Gopalakrishnan, A. (eds). *Fisheries and Aquaculture: Strategic Outlook for Asia – Book of Abstracts*, 8th Asian Fisheries Forum, November 20 – 23, 2007, Kochi, India.

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Assessment of genotoxicity of arsenic trioxide on gills of freshwater fish, *Channa punctatus* by comet assay. *In*: National Symposium on Biomarkers of Environmental Problems, organized by the Academy of Environmental Biology and Department of Zoology & Environmental Science, Ch. Charan Singh University, Meerut, October 26-28, 2007 p.5.

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Pillai, N.G.K., Mallia, J.V., Lijo John, A. Gopalakrishnan, B. Jabbar and A.I. Mohsin 2007

Population structure of yellow-fin tuna (*Thunnus albacares*) in the southwest Indian Ocean: A genomic view with mitochondrial cytochrome b gene. Abstract No. GMP 005; Pp. 324 - 325, *In*: Bright Singh, I.S., Mohandas, A., Joseph, V., Pai, S.S., Paul, L., Philip, R., Paulraj, R., Mohammed, S.K. and Gopalakrishnan, A. (eds). *Fisheries and Aquaculture: Strategic Outlook for Asia – Book of Abstracts*, 8th Asian Fisheries Forum, November 20 – 23, 2007, Kochi, India.

Sarkar, U.K., A.K. Pathak and W.S. Lakra, 2007

Conservation of freshwater fish diversity of India. *In*: Taal 2007: 12th World Lake Conference, Jaipur, October 28 - November 2, 2007. p.25.

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Use of GIS in developing freshwater conservation areas for management of endangered fish and aquatic habitat *In*: Workshop on Sustainability of Indian Aquaculture Industry (SUSTAIN-AQUA 07). Indian institute of Technology, Kharagpur, September 27-28, 2007.

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Management of fish genetic resources of North Eastern Region of India. *In*: Emerging Issues and Perspectives for Conservation, August 22-23, 2007. National Seminar on Rebuilding Fisheries in the North East, Shillong, Meghalaya. p.25.

Singh, A.K., A.K. Pathak, Salim Sultan, A. Mishra and W.S. Lakra, 2007

Distribution of African catfish and other exotic fishes in river Yamuna in Uttar Pradesh. *In*: 8th Asian Fisheries Forum November 20-23, 2007. Kochi, India. p.248.

Singh, A.K. and W.S. Lakra, 2007

Invasive fish species of northeast region: Status and impacts *In*: Proceedings of the National Seminar on Recent Advances and Rebuilding of Fish and Fisheries in North East India, August 22-23 2007, Department of Pisciculture, St Anthony's College, Shillong. p.1.

Swaminathan, T.R., G. Rathore, N. Sood, R. Abidi and W.S. Lakra, 2007

PCR cloning and partial sequencing of rtxA gene of non-O1/non-O139 *Vibrio cholerae* isolated from goldfish *Carassius auratus* in India. *In*: 8th Asian Fisheries Forum. Asian Fisheries Society & Indian Fisheries Society, Kochi. November 20-23 2007. p.29.

LIST OF PROJECTS

Institutional Projects

1. Development of database on marine ornamental and shellfishes of Indian waters. *R. Soundararajan, Rehana Abidi, V.S. Basheer, A.K. Pathak, Karan Veer Singh, M.S. Verma and P.R. Divya.*
2. Development of digital information system on marine fish taxonomy. *R. Soundararajan, A.K. Pathak and P.R. Divya.*
3. Digitized repository of Indian fish diversity. *D. Kapoor, A. Gopalakrishnan, V.S. Basheer, A.K. Pathak and R. Dayal.*
4. Spatial modeling the habitat preference of marine sea cucumber (*Holothuria scabra*) using GIS and neural network. *R. Soundararajan, S.P. Singh, U.K. Sarkar, V.S. Basheer and A.K. Pathak.*
5. Development of molecular markers and genetic divergence studies in Indian catfishes. *A. Gopalakrishnan, K.K. Lal, Vindhya Mohindra, Peyush Punia and Rajeev Kumar Singh.*
6. Genetic divergence studies in marine finfish and shellfish species. *A. Gopalakrishnan, P. Jayasankar (CMFRI, Kochi) and V.S. Basheer.*
7. Development of species-specific molecular markers for fluorescence *in situ* hybridization in selected fish species. *N.S. Nagpure, Ravindra Kumar, Basdeo Kushwaha, Poonam J. Singh, Rajeev Kumar Singh and Satish K. Srivastava.*
8. DNA Barcoding of Indian marine fishes. *W.S. Lakra, A. Gopalakrishnan, K.K. Lal, Vindhya Mohindra, Peyush Punia, M.S. Verma, and Karan Veer Singh*
9. Genotoxic studies of selected piscicides and xenobiotics in fishes using molecular assays. *N.S. Nagpure, Ravindra Kumar, Basdeo Kushwaha, Poonam J. Singh, and Satish K. Srivastava.*
10. Exploration of pathogenic fauna of selected marine and freshwater ornamentals. *Rehana Abidi, Neeraj Sood, V.S. Basheer, T. Raja Swaminathan and Amar Pal.*
11. Targeted active surveillance of penaeids for OIE-listed viruses in selected maritime states of India. *Neeraj Sood, T. Raja Swaminathan and Gaurav Rathore.*
12. Development of cell line from *Epinephelus meroa*. *T. Raja Swaminathan and A. Gopalakrishnan.*
13. Studies on metabolic utilization of dietary carbohydrate in *Catla catla* for reproductive performance. *P.P. Srivastava, Rajesh Dayal and S.M. Srivastava.*
14. Distribution of exotic fish and its evaluation for reproductive performance in selected stretches of Ganga river system. *A.K. Singh, A.K. Pathak and Amar Pal.*
15. Evaluation and assessment of freshwater fish diversity of the river Ganges basin for conservation and management: A new perspective. *U.K. Sarkar, A.K. Pathak, S.M. Srivastava, S.K. Paul and Reeta Chaturvedi.*
16. Fish germplasm exploration, assessment, cataloguing and conservation for north-eastern region. *W.S. Lakra and U.K. Sarkar.*
17. Status and role of temple sanctuaries in river Gomti towards developing policies for conservation and sustainable production. *S.P. Singh, L.K. Tyagi, S.M. Srivastava and Amar Pal.*
18. Studies on fishing cooperative societies with

- focus on their potential for conservation of fishery resources. *L.K. Tyagi, S.P. Singh, S.M. Srivastava and Amar Pal.*
19. Cryopreservation protocols and germplasm accession development for prioritized freshwater fishes. *V.S. Basheer, A. Gopalakrishnan and D. Noble (CMFRI, Kochi).*
 20. Quality seed production of selected carp, catfish and endangered species. *P.K. Varshney, A. K. Yadav, S. K. Upadhyay and S. K. Singh.*
 21. Network project on germplasm exploration, cataloguing and conservation of fish and shellfish resources of India.
6. Transcriptome analysis of *Clarias batrachus* spleen: Analysis of genes in immune function and type I markers. *Vindhya Mohindra and Neeraj Sood/* Funded by DBT, Govt. of India.
 7. Studies on selected endangered fish species using ecosystem scaling and habitat fingerprinting approach for tributaries of river Ganga. *U.K. Sarkar/* Funded by CST, Govt. of Uttar Pradesh.
 8. Studies on fish biodiversity and aquatic environment of Ken-Betwa river: An assessment prior to river inter-linking for conservation of aquatic bio- resources. *W.S. Lakra and U.K. Sarkar/* Funded by DBT, Govt. of India.

Externally funded Projects

1. Technology development for cryopreservation of embryonic stem cells in Indian catfishes, *Clarias batrachus* and *Heteropneustes fossilis*. *K.K. Lal and Peyush Punia/* Funded under APCESS- ICAR.
2. Database on taxonomy and distribution of freshwater fishes of India-U.P. *D. Kapoor and R K Tyagi/* Funded under APCESS- ICAR.
3. Development of molecular markers for fluorescence *in-situ* hybridization in genus *Tor*. *Ravindra Kumar, N.S. Nagpure and Basdeo Kushwaha/* Funded by DBT, Govt. of India.
4. Isolation and characterization of *Flavobacterium* spp. from fish and aquatic environmental samples. *Gaurav Rathore/* Funded by ICAR.
5. Studies on association of viral infections in diseases of cultured freshwater carps by immunological and molecular techniques. *Gaurav Rathore and T.Raja Swaminathan/* Funded by DBT, Govt. of India.
9. Development and characterization of cell lines from *Labeo rohita* and virus isolation studies on established cell lines of *Cyprinus carpio*. *W.S. Lakra, Gaurav Rathore and T. Raja Swaminathan/* Funded by DBT, Govt. of India.
10. Production of monoclonal antibodies to serum immunoglobulins of *Ophiocephalus striatus* and *Labeo rohita* and their application in immunoassays. *Neeraj Sood, Gaurav Rathore/* Funded by DBT, Govt. of India.
11. Risk analysis of exotic *Panagasius sutchii* in India. *W.S. Lakra and A. K. Singh.* Funded by Ministry of Agriculture DAHDF, Govt. of India.
12. Harmful algal blooms in the Indian EEZ. Genetic characterization of *Trichodesmium* spp. from Indian waters. *A. Gopalakrishnan/* Funded by Ministry of Earth, Govt. of India
13. Microsatellite markers for genetic variability studies in *Penaeus (Fenneropenaeus) indicus*. *A. Gopalakrishnan/* Funded by DBT, Govt. of India.

PARTICIPATION IN SEMINARS/ SYMPOSIA/ WORKSHOPS/ TRAININGS/ MEETINGS

Abroad

- Dr. W.S Lakra, Director attended the Inaugural Workshop of the Barcode of Life Initiative during June 17-20, 2007 at the University of Guelph, Canada.
- Dr. W.S. Lakra, Director participated in the Second International Barcode of Life Conference during September 18-20, 2007 at Taipei, Taiwan.
- Shri Amar Pal, Technical Officer (T-6) attended a Training Programme “Fish Health Master Class” during November 12-23, 2007 at Bangkok, Thailand.

In India

- Dr. W.S. Lakra, Director attended :
 - A meeting of the Directors of ICAR Fisheries Institutes during June 9–10, 2007 convened by the DDG (Fy), ICAR at Tirupati.
 - The Directors Conference held during July 16-18, 2007 at NASC, New Delhi.
 - A meeting of the Exotic Committee on July 19, 2007 at DAHDF, MOA, Krishi Bhawan, New Delhi.
 - A workshop on Reservoir Fisheries during July 27- 28, 2007 at Bhopal.
 - A workshop on “The India Portal” organized by National Knowledge Commission on Oct. 16, 2007 at New Delhi.
 - A regional workshop on “*Kendriya Rajyoo ka Aajivika Paridrishya Tatha Satatt Vikash Hetu Matasayaki Evam Jalkrishi Neeti*” during October 25-27, 2007 jointly organized at Patna by Animal Husbandry & Fisheries Resources Department, Bihar and Central Institute of Fisheries Education, Mumbai.
- An “International Conference on Loss of Biodiversity: Causes, Consequences and Conservation” on November 23, 2007 at Kolkata.
- A joint meeting of ICAR Bureaus regarding “Genomic Resources Conservation” on December. 27, 2007 at NBPGR, New Delhi.
- The Indian Science Congress during January 03-05, 2008 at Visakhapatnam.
- Dr. A.K Singh, Sr. Scientist, Dr. M. Goswami, CSIR Pool Scientist, Shri R.S. Patiyal, Technical Officer (T-6) and Shri A.S. Bisht, T-4 attended a National Workshop-cum-Exhibition on “Parvatiya Matsyaki Paridrishya: Vikas, Prabandhan Evam Sanrakshan” (in Hindi) during April 6-7, 2007 at National Research Centre on Cold water Fisheries, Bhimtal.
- Dr. U.K Sarkar, Sr. Scientist and Shri A.S. Bisht, T-4 attended Fourth Indian Fisheries Science Congress on “Ecology and Fisheries of Wetlands in India” during April 12–13, 2007 at ICAR Research Complex for Eastern Region, Patna.
- Dr. L.K Tyagi, Scientist (SS) attended an International Training Programme for the South Asian Researchers on “Property Rights, Collective Action and Environmental

Governance: The Links Between State, Community and Resources” during April 16-20, 2007, at Institute for Social and Economic Change, Bangalore.

- Dr. U.K. Sarkar, Sr. Scientist attended a meeting of the Nodal Officers of NEH programmes of ICAR Institutes held at North Eastern Regional Centre of CIFRI, Guwahati, Assam on April 21, 2007.
- Dr. U.K. Sarkar, Sr. Scientist attended the Task Force Meeting of the Department of Biotechnology on “Application of Biotechnology for Biodiversity Conservation and Environment” at Tata Energy Resources Institute, New Delhi on May 15, 2007.
- Mrs. Kaneez Fatima, Sr. Clerk attended a “Training Course on “Purchase Procedure” at ISTM, New Delhi during May 14-16, 2007.
- Mrs. Reeta Chaturvedi, T-4 and Shri Ravi Kumar, T-4 attended a “Workshop on Structural Bioinformatics” at Biotech Park, Lucknow during June 8–9, 2007.
- Dr. U.K. Sarkar, Sr. Scientist attended a “Workshop on Revision of Schedules under Wildlife Protection Act, 1972” organized by Wildlife Institute of India, Dehradun during August 6-7, 2007.
- Shri A.K. Pathak, Scientist (Sr. Scale) and Shri Ravi Kumar, T-4 attended a “Training programme on Intelligent Reporting System” during September 10-11, 2007 at IASRI, New Delhi.
- Shri Ravi Kumar, T-4 attended a “Refresher Course on Computer Based Multimedia Presentation” during June 20–July 10, 2007 at NAARM, Hyderabad.
- Dr. W.S. Lakra, Director; Dr. A.K. Singh and Dr. P.K. Varshney, Sr. Scientists attended “Farmers Fair-cum-Exhibition” on November 02, 2007 at KVK, Dhora, Unnao.
- Dr. W.S. Lakra, Director; Dr. R. Soundararajan, Principal Scientist; Dr. A. Gopalakrishnan, Dr. A.K. Singh, Sr. Scientists and Shri V.S. Basheer and Dr. T. Raja Swaminathan, Scientists (SS) attended the “8th Asian Fisheries Forum” during November 20-23, 2007 at Kochi.
- Dr. Rehana Abidi, Sr. Scientist participated and presented two papers entitled “Acanthocephalan parasites as potential sentinels of metal pollution on aquatic habitat” and “Molecular biomarkers for aquatic ecosystem health assessment through DNA microarray” in the “National Symposium on Biomarkers of Environmental Problems” held at Department of Zoology, C.C.S. University, Meerut during October 26-28, 2007.
- Dr. A.K. Singh and Dr. P.K. Varshney, Sr. Scientists acted as Jury members at the 15th National Children’s Science Congress on Biodiversity: Nurture Nature for Our Better Future at State level during Nov. 27-28, 2007 organized at Nehru Yuva Kendra, Lucknow.
- Dr. W.S. Lakra, Director; Dr. U.K. Sarkar; Dr. P.P. Srivastava, Sr. Scientists and Shri A.S. Bisht, T-4 attended a “National Symposium on “Ecosystem Health and Fish for Tomorrow” during December 14-16, 2007 organized by Central Inland Fisheries Research Institute & Inland Fisheries Society of India at Barrackpore.
- Dr. L.K. Tyagi, Scientist (SS) and Shri A.S. Bisht, T-4 attended the “All India Zoology Congress” at Lucknow University, Lucknow during December 07-09, 2007.
- Shri A. Kathirvelpandian, Scientist and Shri A.K. Singh, Technical Officer attended

a Winter School on “Biodiversity and Stock Assessment Methods for Fisheries Professionals” during November 14-Dec. 04 2007 at Fisheries College and Research Institute, Thoothukudi.

- Dr. L.K. Tyagi, Scientist (SS) attended the Indian Social Science Congress organized by the Indian Academy of Social Sciences during December 27-31, 2007 at SNDT Women’s University, Mumbai.
- Dr. L.K Tyagi, Scientist (SS), attended a “CAS Training Programme on Advances in Aquaculture Technologies” during January 8-28, 2008 at CIFE, Mumbai.
- Mrs. Poonam Jayant Singh, Scientist (SS) attended a “Training Programme on Patents” during January 16-17, 2008 at National Institute of Intellectual Property Management, Nagpur.
- Dr. A. Gopalakrishnan, Sr. Scientist attended an International “Workshop on Harmful Algae and Biotoxins” during January 21-25, 2008 organized by SIDA-FAO-UNESCO-MIRCEN at College of Fisheries, Manglore.
- Dr. V.S. Basheer, Scientist (SG) attended an “International Workshop on Ornamental Aquatic Species” during February 02-03, 2008 organized by Department of Fisheries, Govt. of Kerala and Ministry of Agriculture, Govt. of India at Kochi, Kerala.
- Mrs. Poonam Jayant Singh, Scientist (SS) attended a “Training programme on Intellectual Property Rights and World Trade Organization Related Issues” during February 18-22, 2008 organized by Department of Science and Technology, Govt. of India at Administrative Staff College of India, Hyderabad.
- Mrs. P.R. Divya, Scientist attended “ICAR Winter School on Impact of Global Warming on Indian Marine Fisheries” during January 18-February 07, 2008 at CMFRI, Kochi.
- Shri A.K Yadav, Technical Officer (T-6) attended a “Training Programme on Cellular and Molecular Approaches for Genotoxicity Assessment in Fishes” organized by NBFGR, Lucknow during February 21-28, 2008 at Lucknow.
- Shri A.S Bisht, T-4 attended a “Vigyan Kishan Mela” on the occasion of National Science Day on February 28, 2008 organized by Uttar Pradesh Council of Science and Technology at Biotechnology Networking Facility, Bakshi-Ka-Talab, Lucknow.
- Dr. P.K Varshney, Sr. Scientist attended a “Training-cum-Workshop on Awareness of Biodiversity” organized by Narendra Dev Institute for Development of Agriculture and Rural Upliftment Academy on March 19, 2008 at Pratpganj, Barabanki.
- Mrs. Poonam Jayant Singh, Scientist (SS) and Shri A. Kathirvelpandian, Scientist attended a training programme “Hands-on training on DNA Sequencing Using Own Samples” during March 27-29, 2008 organized by The Centre for Genomic Applications, Okhla, New Delhi.

LIBRARY AND INFORMATION SERVICES

The NBFGR Library and Documentation Unit acts as a repository of literature and information and provides latest information in the field of fish diversity conservation, fish genetics, fisheries and related aspects.

Resource Development

The library added a total of 439 documents comprising 216 books, 164 serials and 59 annual reports. Now, the library has the total collection of 5133 books, 2132 bound volumes of journals, 2703 serials and 2261 reprints. The library has subscribed to 34 international current journals and 59 Indian current journals. In addition to these 46 current journals were received on gratis/exchange basis. The library also subscribed to 110 electronic journals of Blackwell Publishing, related to Agriculture, Plant Sciences, Fisheries, Aquaculture and Ecology. The total expenditure incurred by the library during the year under report was Rs. 20, 42,403/-.

Library Automation

The NBFGR Library is operating in fully automated environment. The various activities of library have been computerized using integrated library management software Libsys. The record of books, journals, maps *etc.* were entered in the database. Barcoding of books, periodicals and maps for automated circulation was undertaken. Online Public Access catalogue was made available for the library users.

Readers and Reference Services

A total of 8337 documents were borrowed and 1345 users consulted 8337 documents in the library. A total of 232 scientists, professors and research scholars from other institutions

consulted the library. The references from different databases using Internet were searched and arranged according to suit the requirements of users by using the 'Reference Manager' and 'Biblioscope: Research Information Manager' software.

Information Services

Content lists of the selected journals were brought out periodically and circulated to all divisions and sent to fisheries institutions. List of the books added to the library was also brought out on quarterly basis. The Aquatic Sciences and Fisheries Abstracts (ASFA), International Information System for the Agricultural Sciences and Technology (AGRIS) and Fish and Fisheries Worldwide (NISC) CD-ROM databases were used extensively by the scientists and research scholars from NBFGR as well as other institutes and universities. Access to Blackwell E-Journals was provided to the scientists and research scholars through LAN at their desktop.

Technical Reports and Reprography Services

Technical reports on the progress and research activities of the Bureau were compiled and sent to various agencies. This unit also attended to Questionnaires on Bureau's infrastructure and other facilities. Review, research papers and abstracts of the scientists were communicated to various journals and symposia, seminar, conferences for publication and presentation. The unit provided technical support to bring out other departmental publications. The bio-data of scientists is also maintained and updated in this unit. The unit continued active reprography services. Comb binding, spiral binding, electro-data binding and

lamination facilities for departmental reports were provided. The reprography unit was equipped with a colour photocopier-cum-printer and one high speed duplicating machine.

Exchange Services

The Library continued exchange relationship and resource sharing with 72 leading national and international research institutes and development organizations. To keep abreast of the activities of the Bureau, the library sent the NBFGR Annual Report 2006-2007 to universities, research institutes, State Fisheries Departments, FFDA, entrepreneurs and fish farmers.

Institute Publications

The library facilitated in bringing out the following publications of the Bureau:

1. Freshwater Fish Diversity of Central India/
Edited by W.S. Lakra and U.K. Sarkar
2. Fishes of North East India/
W. Vishwanath, W.S. Lakra and U.K. Sarkar
3. Ornamental Fishes of North East India: An Atlas / *by S.P. Biswas, J.N. Das, U.K. Sarkar and W.S. Lakra*
4. Ornamental Fishes of the Western Ghats of

India/
T.V. Anna Mercy, A. Gopalakrishnan, D. Kapoor and W.S. Lakra

5. उत्तर पर्वतीय राज्यों की मत्स्य विविधता: संरक्षण एवं प्रबंधन (Fish Diversity of Northern Hill States : Conservation and Management)/*Edited by W.S. Lakra and L.K. Tyagi*
6. Genotoxicity Assessment in Fishes: A Practical Approach/*by N.S. Nagpure, Ravindra Kumar, Basdeo Kushwaha, Poonam Jayant Singh, Satish K. Srivasatva and W.S. Lakra*
7. Vision- 2025
8. NBFGR News, January-March, 2007; April-June, 2007; July-September, 2007, October-December, 2007 and January-March, 2008.
9. NBFGR Annual Report, 2006-07
10. Training Manual: Summer School on Fish Biotechnology
11. प्रशिक्षण मैनुअल: जलकृषि विविधीकरण एवं विदेशी मत्स्य प्रजातियों का प्रभाव
12. प्रशिक्षण मैनुअल: समन्वित जलकृषि एवं मत्स्य रोग प्रबन्धन
13. प्रशिक्षण मैनुअल: महाझींगा पालन तकनीकी

DISTINGUISHED VISITORS

The following distinguished personalities visited the Bureau and/or its Units at Kochi, Kerala and Chinhat, Lucknow during the year 2007-08:

- Shri Jumuna Nishad, Hon'ble Minister of State for Fisheries, Govt. of U.P.
- Dr. Mangala Rai, Secretary, DARE, Govt. of India and DG, ICAR, New Delhi.
- Shri A.K Upadhayay, Additional Secretary, DARE, Govt. of India and Secretary, ICAR New Delhi.
- Dr. C.D Mayee, Chairman, ASRB, ICAR, New Delhi.
- Dr. P.V. Dehadrai, Former Deputy Director General (Fy.), ICAR, New Delhi.
- Dr. S.A.H Abidi, Former Member, ASRB, New Delhi.
- Dr. E.G. Silas, Ex-Vice Chancellor, Kerala Agricultural University, Trichur & Chairman, RAC, NBFGR.
- Prof. (Dr.) Mohan Joseph Modayil, Member, ASRB, New Delhi.
- Dr. S. Ayyappan, DDG (Fy.), ICAR, New Delhi. #
- Dr. Dilip Kumar, Director, Central Institute of Fisheries Education, Mumbai. *
- Dr. V.R.P. Sinha, Former Director/Vice Chancellor, CIFE, Mumbai and Consultant, World Bank/F.A.O.
- Dr. S.D. Tripathi, Former Director, Central Institute of Fisheries Education, Mumbai.*
- Padmashree Dr. Lalji Singh, Director, CCMB, Hyderabad.
- Dr. B.N Singh, Former Deputy Director General (Fy.) ICAR, New Delhi.
- Dr. M. Sinha, Advisor, Department of Fisheries, Govt. of Tripura
- Shri Deo Dutt, Secretary (Fy.), Govt. of U.P., Lucknow.
- Shri H.C. Pathak, Director, Finance, ICAR, New Delhi.
- Dr. V.V. Sugunan, Assistant Director General (Inland Fisheries), ICAR, New Delhi.
- Dr. P. S. B. R. James, Former Asst. Director General (Marine Fisheries), ICAR, New Delhi & Former Director, CMFRI, Kochi. *
- Dr. P. Das, Member, RAC and Former Director, NBFGR, Lucknow. *
- Dr. V.P. Kamboj, Former Director, CDRI, Lucknow.
- Dr. S.L. Govindwar, Advisor, Dept. of Biotechnology, Govt. of India, New Delhi.
- Dr. R.L. Yadav, Director, IISR, Lucknow.
- Dr. B.M.C. Reddy, Director, CISH, Rehamankhera, Lucknow.
- Dr. S.C. Pathak, Former Chief General Manager, NABARD, Mumbai.
- Dr. C.S. Singh, Former Dean, G.B. Pant University of Agric. & Tech., Pantnagar.
- Prof. M.S. Johal, Former Head, Department of Zoology, Punjab University, Chandigarh.
- Dr. S.C. Agrawal, Former Director, Haryana State Fisheries and Advisor, National Fisheries Development Board, Hyderabad.
- Dr. D.P. Singh, Dean, School for

National Bureau of Fish Genetic Resources

Environmental Sciences, Dr. B.B. Ambedkar University, Lucknow.

- Dr. R.C. Dalela, Founder President, AEB, Lucknow.
- Dr. S.K. Singh, Jt. Director, Department of Fisheries, U.P., Lucknow.
- Shri Sanjay Shukla, Deputy Director, Department of Fisheries, U.P., Lucknow.
- Dr. George John, Adviser, Department of Biotechnology (DBT), Govt. of India, New Delhi. *
- Dr. A. G. Ponniah, Director, Central Institute

of Brackishwater Aquaculture, Chennai. *

- Dr. K.C. Majumdar, Scientist (F), CCMB, Hyderabad. *
- Dr. A.K. Rawat, Principal Scientific Officer, DBT, New Delhi. *
- Dr T. C. Santiago, Principal Scientist, CIBA, Chennai. *
- Prof. (Dr.) A. V. Saramma, Head, School of Marine Sciences, Cochin University of Science & Technology, Kochi. *

Also visited Kochi Unit of NBFGR.

* Visited only Kochi Unit of NBFGR.

LIST OF PERSONNEL

Research Management

Dr. W. S. Lakra - Director

Scientific Staff

1. Dr. R. Soundararajan - Principal Scientist
2. Dr. D. Kapoor - Principal Scientist
3. Dr. A.K. Pandey - Sr. Scientist
4. Dr. (Mrs) Rehana Abidi - Sr. Scientist
5. Dr. S. P. Singh - Sr. Scientist
6. Dr. A. Gopalakrishnan - Sr. Scientist (NBFGR Cochin Unit)
7. Dr. A. K. Singh - Sr. Scientist
8. Dr. N. S. Nagpure - Sr. Scientist
9. Dr. Kuldeep Kumar Lal - Sr. Scientist
10. Dr. (Mrs) Vindhya Mohindra - Sr. Scientist
11. Dr. Peyush Punia - Sr. Scientist
12. Dr. Ravindra Kumar - Sr. Scientist
13. Dr. U. K. Sarkar - Sr. Scientist
14. Dr. P. P. Srivastava - Sr. Scientist
15. Dr. P. K. Varshney - Sr. Scientist
16. Dr. V. S. Basheer - Scientist (SG) (NBFGR Cochin Unit)
17. Dr. Basdeo Kushwaha - Sr. Scientist
18. Shri Sanjeev Kumar Srivastava - Sr. Scientist
19. Dr. Gaurav Rathore - Scientist (SG)
20. Dr. Neeraj Sood - Sr. Scientist
21. Mrs. Poonam Jayant Singh - Scientist (Sr. Scale)
22. Dr. Lalit Kumar Tyagi - Scientist (Sr. Scale)
23. Shri Ajey Kumar Pathak - Scientist (Sr. Scale)
24. Shri Rajeev Kumar Singh - Scientist (Sr. Scale)
25. Shri Mahendra Singh Verma - Scientist (Sr. Scale)
26. Dr. T. Rajaswaminathan - Scientist (Sr. Scale)
27. Shri Karan Veer Singh - Scientist (Sr. Scale)
28. Mrs P. R. Divya - Scientist
29. Shri A. Kathirvelpandian - Scientist

Technical Staff

1. Shri Rajesh Dayal	-	Field Officer (T-6)
2. Shri S. M. Srivastava	-	Field Officer (T-6)
3. Shri R. S. Patiyal	-	Farm Manager (T-6)
4. Shri A. K. Mishra	-	Electrical Foreman (T-6)
5. Shri Amar Pal	-	Technical Officer (T-6)
6. Dr. S. K. Srivastava	-	Sr. Lab Technician (T-6)
7. Shri Babu Ram	-	Farm Engineering Asst. (T-6)
8. Shri A. K. Yadav	-	T-6
9. Shri S. P. Singh	-	T-6
10. Shri Ajay Kumar Singh	-	Field Surveyor (T-5)
11. Shri S. K. Paul	-	Field Surveyor (T-5)
12. Shri Mohd. Gyas	-	Driver (T-5)
13. Mrs. Reeta Chaturvedi	-	Computer Operator (T-4)
14. Shri Ramashankar Sah	-	Lab. Technician (T-4)
15. Shri Subhash Chandra	-	Library Asst. (T-4)
16. Shri Akhilesh Kr. Mishra	-	Lab. Technician (T-4)
17. Shri Amit Singh Bisht	-	T-4
18. Shri Ravi Kumar	-	T-4
19. Shri S. K. Upadhyay	-	T-4
20. Shri Satyavir Chaudhary	-	Senior Library Asst. (T-3)
21. Shri B. K Rao	-	Sample Sorter (T-II-3)
22. Shri R. K. Shukla	-	Sample Sorter (T-II-3)
23. Shri Ved Prakash	-	Library Attendant (T-II-3)
24. Shri S. K. Singh	-	T-II-3
25. Shri Samarjeet Singh	-	Driver (T-I-3)
26. Shri Om Prakash	-	Driver (T-I-3)
27. Shri B. N. Pathak	-	Gestetner Operator (T-3)
28. Shri Madan Lal	-	Farm Technician (T-2)
29. Shri Raj Bahadur	-	Lab. Technician (T-2)
30. Shri Gulab Chandra	-	Electrician (T-2)
31. Shri K. K Singh	-	Jr. Field Asst. (T-2)
32. Shri Rajesh Kumar	-	Laboratory Asst. (T-2)
33. Shri Sree Ram	-	Laboratory Asst. (T-2)
34. Shri Om Prakash	-	Driver (T-2)
35. Shri P. C. Jaiswar	-	T-2
36. Shri Ram Bharose	-	T-2

Administrative Staff

1. Shri Ashish Srivastava	-	Asst. Finance & Accounts Officer
2. Shri Panchoo Lal	-	Asst. Administrative Officer
3. Mrs. Mamta Chakraborty	-	Personal Assistant
4. Shri Navin Kumar	-	Assistant
5. Shri Tej Singh Seepal	-	Assistant
6. Shri Jogendra Singh	-	Assistant
7. Smt. Kaneez Fatima	-	Assistant
8. Shri Swapan Debnath	-	Sr. Clerk
9. Shri S. N. Srivastava	-	Sr. Clerk
10. Shri P. K. Awasthi	-	Sr. Clerk
11. Shri Sajivan Lal	-	Sr. Clerk
12. Shri Ram Sakal	-	Jr. Stenographer
13. Shri Vinay Kumar Srivastava	-	Jr. Clerk
14. Shri Sreelal Prasad	-	Jr. Clerk
15. Shri Santosh Kumar Singh	-	Jr. Clerk
16. Shri Ram Baran	-	Jr. Clerk
17. Shri P.C. Verma	-	Jr. Clerk

Supporting Staff

1. Shri Laxman Prasad	-	SSG-IV
2. Shri Dukhi Shyam Deo	-	SSG-IV
3. Shri Anil Kumar	-	Lab. Attendant, SSG-IV
4. Shri Indrajit Singh	-	Messenger. SSG-III
5. Shri Prahaldad Kumar	-	Lab. Attendant, SSG-III
6. Shri Chhote Lal	-	Fisherman, SSG-III
7. Shri Dinesh	-	Lab. Attendant, SSG-III
8. Shri Ram Lakhan	-	SSG-II
9. Shri Dash Raj	-	SSG-III
10. Shri Balram Babu Bajpai	-	Lab. Attendant, SSG-II
11. Shri Rajan Kumar Malhotra	-	Lab. Attendant, SSG-III
12. Shri Ashok Kumar Awasthi	-	Lab. Attendant, SSG-III
13. Shri Sidhnath	-	Fieldman, SSG-II
14. Shri Sunit Kumar	-	SSG-II (ACP)
15. Shri Jai Narain Tiwari	-	SSG-II (ACP)
16. Shri Mahesh Chandra	-	SSG-II (ACP)
17. Shri Anwar	-	SSG-II (ACP)
18. Shri Ashok Kumar	-	Lab. Attendant, SSG-I
19. Smt. Sabita Kumari	-	SSG-I

Staff Welfare Activities

Institute Joint Staff Council

The Institute Joint Staff Council with the members mentioned below, was operative at the Bureau during the period under report and considered the matters of common interest.

Official side

- | | | |
|---|---|--------|
| 1. Dr. R. Soundararajan, Pr. Scientist & HoO | - | Member |
| 2. Dr. (Mrs) Vindhya Mohindra, Senior Scientist | - | Member |
| 3. Dr. P.P. Srivastava, Senior Scientist | - | Member |
| 4. Sh. Ajay Kumar Singh, T-5 | - | Member |
| 5. Sh. Ashish Srivastava, AF&AO | - | Member |
| 6. Sh. P. Lal, Assistant Administrative Officer | - | Member |

Staff side

- | | | |
|---------------------------------------|---|-------------------------------|
| 1. Sh. Akhilesh Kumar Mishra, T-4 | - | Member |
| 2. Sh. Amit Singh Bisht, T-4 | - | Member |
| 3. Sh. S. N. Srivastava, Sr. Clerk | - | Member representative of CJSC |
| 4. Sh. Santosh Kumar Singh, Jr. Clerk | - | Member |
| 5. Shi. Anil Kumar, SSG-IV | - | Member |
| 6. Sh. Indrajit Singh, SSG-III | - | Secretary, IJSC |

Staff Welfare Fund Scheme

The Staff Welfare Fund Scheme with the following members was at the Bureau during the period under report and considered the matter for welfare of the staff.

- | | | |
|---|---|------------------|
| 1. Dr. D. Kapoor
Principal Scientist | - | Chairman |
| 2. Dr. S. P. Singh
Senior Scientist | - | Member |
| 3. Dr. P. P. Srivastava
Senior Scientist | - | Member |
| 4. Shri Ashish Srivastava
AF & AO | - | Member |
| 5. Mrs. Kaneez Fatima
Assistant (lady representative) | - | Member |
| 6. Shri Indrajit Singh, SSG-IV
(Secretary IJSC) | - | Member |
| 7. Mr. Rajan K. Malhotra, SSG III
(Group D representative) | - | Member |
| 8. Shri Panchoo Lal
Assistant Administrative Officer | - | Member-Secretary |

Management Committee

The Institute Management Committee was represented by the following members nominated by Director General, ICAR, New Delhi.

1. Dr. W.S. Lakra, Director, NBFGR - Chairman
2. Dr. A.D. Diwan, ADG (M.Fy), ICAR - Member (ICAR)
3. Dr. S.D. Singh, Principal Scientist, CIFE, Mumbai - Member
4. Dr. R. Soundararajan, Principal Scientist, NBFGR - Member
5. Dr. G. Rathore, Scientist (Sr. Scale), NBFGR - Member
6. Shri P. Lal, Asst. Adm. Officer, NBFGR - Member-Secretary

Women's Cell

The Women's Cell has been constituted at NBFGR, Lucknow with the following members:

1. Dr. (Mrs) Rehana Abidi
Senior Scientist - Head of the Cell
2. Dr. (Mrs.) Vindhya Mohindra
Senior Scientist - Member-Secretary
3. Mrs. Reeta Chaturvedi
T-4 - Member
4. Mrs. Mamta Chakraborty
Jr. Stenographer - Member
5. Shri Anil Kumar
SSG-III - Member

APPENDIX – I

NBFGR COCHIN UNIT

A Research Unit of the Bureau is functioning in the campus of Central Marine Fisheries Research Institute (CMFRI), Kochi, Kerala. This unit is carrying out research activities pertaining to genetic characterization, conservation and cataloguing of the vast fish genetic resources of marine and brackishwater ecosystems of the country as well as of endemic freshwater fish species from the Western Ghats – the megabiodiversity ‘hotspot’.

Address : Scientist-in-Charge
NBFGR Cochin Unit
CMFRI Campus
Post Box No. 1603
Ernakulam North P.O.
Kochi – 682 018, Kerala.
Telefax: 0484-2395570
E-mail: nbfgrochin@vsnl.net
nbfgrochin@eth.net

APPENDIX – II

**AQUACULTURE RESEARCH & TRAINING UNIT,
CHINHAT, LUCKNOW**

An Aquaculture Research & Training Unit of the Bureau is functioning at Chinhhat, Lucknow. This unit is carrying out human resource development activities including practical training programmes and fishery advisory services pertaining to fish culture, induced breeding, quality fish seed production, hatchery management and nursery pond management.

Address : Scientist-in-Charge
NBFGR Aquaculture Research & Training Unit
Malhore Road, Chinhhat
Lucknow - 227 105, U.P.
Telefax : 0522-2815848
E-mail : director@nbfgres.in

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