

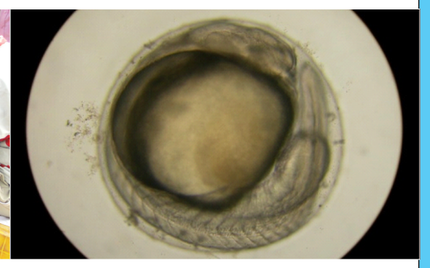

राष्ट्रीय मत्स्य आनुवंशिक संसाधन ब्यूरो
(भारतीय कृषि अनुसंधान परिषद्)
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NBFGR ANNUAL REPORT 2012-2013

हर कदम, हर डगर
किसानों का हमसफर
भारतीय कृषि अनुसंधान परिषद
Agrisearch with a human touch

ANNUAL REPORT
2012-2013



भाकृअनुप
ICAR

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(भारतीय कृषि अनुसंधान परिषद्)

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

Annual Report
2012–13

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CONTENTS

PREFACE

The aquatic genetic resources have been used by the human being for food, medicines and also various other services since ages, but, it is only in the past few years, that we have begun to recognize the limits of this gift of nature. With a vision to take integrated and holistic action to ensure conservation and sustainable use of fish genetic resources of the country, the NBFGR has taken up its research programmes for greater understanding of conservation strategies of prioritized and endangered species.

During the reporting year (2012-2013), the Institute has strengthened its activities to foster the fish germplasm exploration, characterization and conservation. The complete mitochondrial DNA sequence of three fish species was determined. Detailed taxonomic description of a new *Labeo* species from district Udaipur namely, *Labeo icar* was accomplished. Species-specific molecular signatures of eleven cultivable barbs of Peninsular India were generated to resolve taxonomic disputes. Progress in delineating genetically distinct natural populations of prioritized finfish species, development and maintenance of cell lines and several others have been quite significant.

It was a proud moment for the whole NBFGR family to receive the prestigious Sardar Patel Outstanding ICAR Institution Award for the year 2011. It reflects culmination of the efforts and dedication of all the staff over the years. The current year saw the foundation of the First National Fish Museum of the country at NBFGR laid by Dr. S. Ayyappan, Secretary DARE and Director General, ICAR, New Delhi. The Institute organized a number of important consultations and training programmes of national and regional significance. I am confident that our hard work and commitment to research programmes will continue to provide research outputs for making effective strategies for sustainable use of fish genetic resources. We at NBFGR intend to apply our vision, passion, discipline and conscience to take NBFGR into a new horizon of fish conservation research in the coming decade. Compliments go to all the members of NBFGR family for their commitment and dedication towards achievements of the Bureau.

I am deeply indebted to Dr. S. Ayyappan, Secretary, DARE and Director General, ICAR, New Delhi for his continued encouragements, guidance and support. I am grateful to Dr. B. Meenakumari, DDG (Fisheries), ICAR for her sincere advice and guidance. I place on record my sincere thanks to Dr. Madan Mohan, ADG (Marine Fisheries), Dr. S.D. Singh, ADG (Inland Fisheries) and other staff members of the Fisheries Division of ICAR for their cooperation and help in our endeavours. I also take this opportunity to thank Dr. L.K. Tyagi and Shri Amit Singh Bisht for their sincere effort and commitment in timely completion of the annual report.

(J.K. Jena)
Director

EXECUTIVE SUMMARY

National Bureau of Fish Genetic Resources (NBFGR) has developed state-of-art facilities and expertise in several research areas including, development of fish databases, genetic characterization, genomics and proteomics, 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 16 Institutional and 21 externally-funded research projects. Major achievements and activities of the Institute during the year 2012-13 are summarized below:

- The existing database on finfish diversity of India was updated by adding information of about 108 fish species reported from Indian waters. The database now contains information on 2553 native finfishes reported from India belonging to 999 genera of 255 families under 40 orders.
- Four genomic resource databases, *viz.*, Fish Microsatellite database, Fish EST database, Fish Ribosomal RNA database and Fish Barcode Information System were updated with 2570 microsatellite sequences, 4000 EST records, 353 ribosomal DNA records and 584 barcode records.
- Computational analysis of transcriptome was performed to identify genes that are involved in biotic and abiotic stresses and other biological processes in *L. rohita* which can be used for understanding the role of genes in expression of important traits.
- Genetic variation of three fish species, *Notopterus notopterus*, *Labeo calbasu* and *Silonia silondia* was documented from different riverine locations of the country, using molecular markers of mitochondrial and nuclear origin.
- The complete mitochondrial DNA sequence of *Channa marulius* was determined which was found to be of size16567 bp. The gene structure and order were comparable to those reported for other vertebrates.
- Whole mtDNA sequence of two ornamental barbs, endemic to the Western Ghats viz., *Puntius denisonii* and *P. chalakkudiensis* were generated jointly with CIFT, Kochi. The circular mtDNA of *Puntius denisonii* has a size of 16899 bp and that of *P. chalakkudiensis* has 16989 bp, showing a difference of 90 bp mainly in the control region.
- The work on characterization of the distribution of intra-specific variation in molecular, morphological and biological aspects in wild populations of cultivable fish species (Indian major carps and Indian catfish) across their native distribution range was expanded. In *Labeo rohita*, 345 sequences for two mtDNA genes, (cytochrome b and ATPase 6/8) and 1094 samples from 17 riverine localities were genotyped for 21 polymorphic microsatellite loci.
- In *Cirrhinus mrigala* accession (n=1102) were genotyped was for 22 polymorphic microsatellite loci and ATPase 6/8 (842 bp) was also analyzed for 446 individuals from 19 river localities. The results show a total of 26 haplotypes were observed out of which, five were shared, while 21 were unique to the location.
- In *Clarias batrachus*, the sequence of a 307 bp partial segment at the 5′ end of the cyt b and 842 bp of ATPase 6/8 mtDNA genes were determined for 403 individuals collected from 11 different locations. The patterns of genetic diversity and haplotype network clearly indicated population structuring from different locations.
- DNA barcodes for 32 species of marine finfish, totaling 165 samples, were generated and submitted to GenBank
- A total of 12 polymorphic microsatellite loci were identified in *Pampus argenteus* including 7 loci developed through cross-species amplification, from *P. cinereus*.
- Distinct genetic stocks of silver pomfret, *Pampus argenteus* (stock 1. Gujarat, Maharashtra, Kerala & Tamil Nadu; stock 2. West Bengal) were identified using complete (842bp) sequence data of ATPsynthase 6 and 8 genes.
- Species-specific molecular signatures of 11 cultivable barbs of Peninsular India were generated to resolve taxonomic disputes.
- Two genetically distinct stocks of *Coilia dussumieri* (stock 1. Gujarat, Maharashtra; stock 2. West Bengal, A.P.) were identified using complete (842 bp) sequence data of ATPsynthase 6 and 8 genes and 10 polymorphic microsatellite markers. The

level of genetic differentiation among groups (west and east coast) showed high levels ranging between 0.697 and 0.783 with ATPase 6/8 genes. The findings of the present study using combination of microsatellite and mitochondrial (ATPase 6/8 genes) markers would be helpful in developing stock specific management measures for conservation and sustainable utilization of the species.

- A novel transcript (Cystatin/monellin-like 2) was identified under hypoxic conditions from *C. batrachus* and the expression pattern of this novel transcript was studied in various tissues of *C. batrachus.*
- Genetic stock structure of *Tenualosa ilisha* was studied from different locations of the country and high haplotype diversity was observed in all the populations of *T. ilisha*, which may be attributed to migratory behaviour, large population size, environmental heterogeneity and life-history traits that favour rapid population increase.
- The karyotype analyses were done for the first time in 5 fish species endemic to the Indian region of the Indo-Burma biodiversity hot-spot *B. ngawa, D. aequipinnatus, D. yuensis, Mystus ngasep* and *P. meingangbii.*
- Under the fish germplasm exploration programme, 375 accessions from 44 fish species belonging to 30 genera of 16 families under 8 orders were collected from 7 sites of River Mahanadi and 5 sites of its 4 major tributaries during two explorations.
- Detailed taxonomic description of a new *Labeo* species from district Udaipur namely, *Labeo icar* was accomplished. A new species, *Rita sp. nov.* collected from a tributary of River Godavari was characterized and is in the process of validation.
- Rapid explorations of fish diversity were conducted in 12 main rivers, 7 tributaries, 12 reservoirs and four lakes/*'taals'* of U.P. and 124 native freshwater fish species belonging to 26 families were recorded.
- Three rivers viz., Ganga, Betwa and Gomti were assessed to determine the fish assemblage integrity index for prioritization of sites for conservation and to measure the ecological integrity. Potential areas were identified based on rarity index and origin index.
- Milt cryopreservation protocols were developed for two important freshwater fishes of the Western Ghats viz., *Osteachilichthys longidorsalis* and *Puntius sarana subnasutus,* and a *Labeo spp.* collected from District Udaipur (Rajasthan).
- Studies conducted to evaluate the effect of one of the widely used immunostimulant - vitamin C for increasing disease resistance against EUS indicated that dietary vitamin- C did not render protection against experimental infection with *Aphanomyces invadans*.
- Studies undertaken to determine the sequential changes in innate immune response of *L. rohita* during experimental infection with *A. invadans* indicated that this pathogen was able to modulate the immune response in *L. rohita* in advanced stages of infection.
- A biotin labeled DNA probe was designed from the highly conserved sequences of the 16S rDNA region and was used for detection of *Flavobacterium* species.
- Fish monoclonal antibodies (MAbs) MAbs were raised against purified serum immunoglobulins of *C. catla* and the results indicate that G10/1 MAb can contribute significantly to improved understanding of the architecture and functioning of the immune system in the candidate species.
- Ecological impact assessment of *Clarias gariepinus* was documented and its potential for resource competition analysed in U.P. Invasion of exotic fishes in various rivers of U.P. was documented.
- The Institute celebrated its Annual Day on 12 December, 2012. A Farm Innovators Day was organized on this occasion and Annual Institute Awards for the year 2011-12 were presented to the staff members of the Institute for their outstanding contributions and also to the selected progressive farmers/entrepreneurs of the country.
- The Institute celebrated "Agricultural Education Day" on 19 November, 2012. The day was celebrated as Open- House Day for the students and the visitors so that they could see various laboratories, fish farm and the Ganga Aquarium at the institute. Several programmes like 'Inter-school Arts Competition' and 'Quiz Competition' were organized for school children on themes "Aquatic Biodiversity" and "Fish & Environment".
- A number of technical workshops/trainings including, a National Workshop on 'Fish Cell Line:

Development and Storage' on 19 April, 2012; a National Consultation on 'Development of Surveillance Programme for Aquatic Animal Diseases' during 17–18 April, 2012; A workshop on 'Strategic action plan for exploration and characterization of fish germplasm resources and indigenous knowledge in North-Eastern region of India' during 5-6 May, 2012; a strategic workshop on 'Sustainable agriculture development and conservation of fish genetic resources in Uttar Pradesh' on 18 August, 2012; A National Consultation on 'Alien Fish Species in Aquaculture and Aquarium Trade: Issues and Perspectives' during 6-7 September, 2012; Two training programmes on application of genetic markers during 12-19 September, 2012 and 2-19 December 2012; A subject matter training on 'Tools for Functional and Comparative Genomics in Fisheries Domain' during 27 November – 7 December, 2012; a Agricultural Research & Development Conclave and Kisan Vigyan Sangam for Uttar Pradesh during 23-24 November, 2012; a Hindi Workshop on 'General administrative and financial rules' on 28 December, 2012; a Workshop on 'Strategic Action Plan for Exploration of Fish Germplasm Resources and Traditional Ecological Knowledge of Tribal People for Sustainable Development in Chhattisgarh State' during 22-23 February, 2013; a Training Programme on 'Co-management of Fisheries Resources for Sustainable Utilization' during 21-23 March, 2013 and A programme on 'Fish Conservation Awareness and Tribal Communities' on 25 March, 2013, were organized at NBFGR.

- A series of short-term training programmes on 'Aquaculture Technologies and Productivity Enhancement' were organized in which a total of 244 progressive fish farmers and 17 subject-matter specialists of KVKs were trained. A total of 512.5 lakhs of quality spawn of Indian major carps was produced under the ICAR Mega Seed Project and sold to the fish farmers. The Institute also participated in several exhibitions/aqua-fairs in different parts of the country.
- The Institute published 65 research papers in peer-reviewed journals, contributed 6 chapters to books and 10 popular articles in national magazines. Against a target of Rs. 22.0 lakhs, the Bureau generated revenue of Rs. 31.71 lakhs, during the year under report.

INTRODUCTION

Brief History

The concern for the loss of genetic resources worldover has necessitated policy developments for their management and sustainable utilization. This has not only brought shift in focus to the biodiversity rich regions of the world, but also technological investments to unearth the knowledge therein. It is realized that scientific basis is necessary to preserve the genetic resources which can be utilized for nutritional and environmental security of the mankind.

National Bureau of Fish Genetic Resources was established at the end of Sixth Five Year Plan, to provide scientific input for management of fish germplasm resources of the country under the aegis of Indian Council of Agricultural Research, by Govt. of India. Since its humble beginning at Allahabad in 1983, NBFGR has metamorphosed into a leading institution to address researchable issues related to conservation of fish resources. The Bureau has sprawling campus with administrative and laboratory infrastructure facilities including hatchery, wet laboratories, public aquarium, guest house, staff quarters and above all, required experimental tanks and ponds to fulfill the needs of a scientific organisation. The Bureau, over the years, has created infrastructure, state of the art facilities and in-house expertise in several research areas including, development of fish databases, molecular marker development, genetic characterization, *ex situ* gene banking, risks analysis of exotic species, diagnostics for OIE notified pathogens, aquatic microbes and other areas of germplasm conservation with special focus on prioritized fish agrobiodiversity species of indigenous and exotic origin.

VISION

Assessment and conservation of fish genetic resources for intellectual property protection, sustainable utilization and posterity.

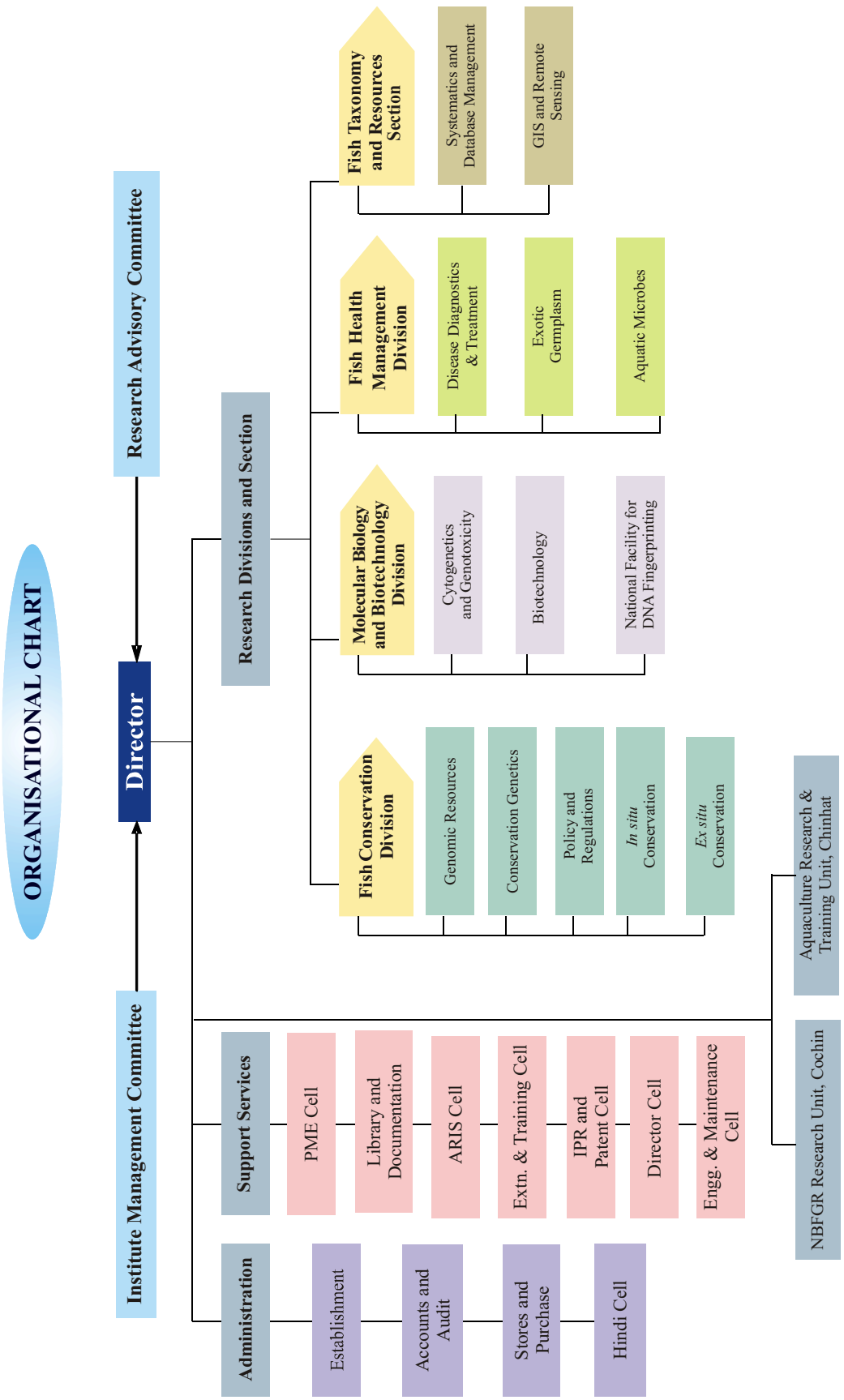
MISSION

Collection, cataloguing and documentation of fish genetic resources using operational strategies of partnership and cutting-edge technologies.

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, 2013 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	41	27	14
3.	Technical	38	35	03
4.	Administrative	21	19	02
5.	Supporting	20	19	01
	Total	**121**	**101**	**20**

Financial Statement

Allocation of funds and expenditure incurred during the year 2012-2013.

(Rs. in lakhs)

	Budget Allocation	Expenditure
Plan	755.00	754.92
Non Plan	918.50	918.23
Total		
Revenue generation	**Target**	**Achieved**
	22.00	31.71

REASEARCH ACHIEVEMENTS

4.1 CATALOGUING OF FISH GENETIC RESOURCES OF INDIA

Project Title: Information base on Fish Genetic Resources of India

Project Period: April, 2012 – March, 2015

Project Personnel: S.P Singh (PI), U.K. Sarkar, A.K. Pathak, R. Dayal, Reeta Chaturvedi and Ravi Kumar

Funding Agency: Institutional

The collection and cataloguing of information on fish genetic resources of India is an important mandate of NBFGR. During the period under report, the Bureau continued its efforts towards collection and cataloguing of the fish genetic resources of India. The existing database on finfish diversity of India was updated. The present database contains information on 2553 native finfishes reported from India belonging to 999 genera of 255 families under 40 orders. Besides, the database also includes information on 291 exotic fishes (Table 1). Information about 108 additional fish species reported from Indian waters. Database design was modified and coding of all 2844 fish species was revised. A total of 913 species were taken for taxonomic scrutiny and valid scientific names of 126 fishes were revised. Towards collection of primary data, germplasm exploration were carried out in the buffer zone and adjoining areas of Panna National Park in upper stretch of River Ken, and a total of 48 species of freshwater fishes belonging to 32 genera of 15 families under 4 orders were recorded.

Table 1. Fish diversity of India

Category of Fishes	Ecosystem	No. of Fish Species
Native	Freshwater	877
	Brackishwater	113
	Marine water	1563
	Total	**2553**
Exotic		291
Grand Total		**2844**

A list of 175 new fish species belonging to 80 genera of 40 families under 9 orders reported 1995 onwards from Indian waters was prepared after comparing with all the existing species and their synonyms. A user friendly software application for each database was designed and developed. For this purpose, MS ACCESS-2010, was used at backend and Dot.net at frontend. Crystal reports were developed and integrated with the software for on and off screen display of the result. A checklist of 141 commercially important species of prawns belonging to 29 genera of 9 families under 2 orders, reported from Indian waters was prepared. A revised checklist of catfishes of India from all ecosystems was prepared after checking the synonyms of each fish species from all available sources which contains 249 fishes belonging to 62 genera of 14 families. A revised checklist of fishes of Western Ghats region of India was prepared which now contains 358 fishes belonging to 138 genera of 39 families.

A web based computer aided shape-based automated system using images was designed for

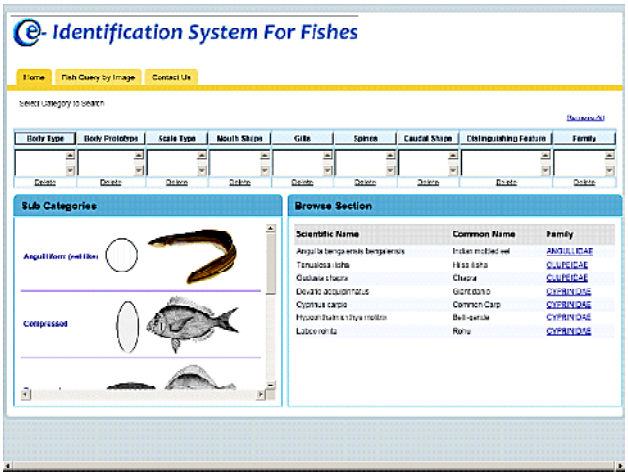


Fig. 1.a: Home page of shape based identification displaying the fishes under different families

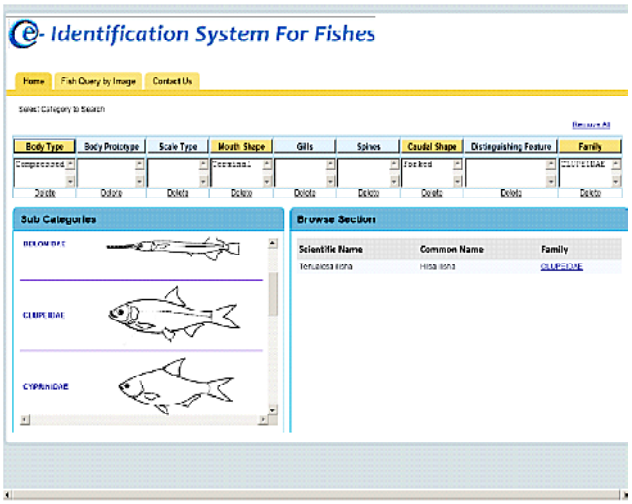


Fig. 1.b: A screen print of the result of the query on family and body type

identifying the fish species from the database. The system has been built to identify the fishes in Indian perspective using an ASP.NET website, the categories and respective sub-categories photographic information depicting morphological differences embedded in the quality images. The major categories included for the identification are: body type, body patterning, scale presence, mouth shape, gills, spine, caudal shape, family and other distinguishing features. The system is flexible, dynamic and controlled by its administrator. It provides option to administrator to add the user for uploading the data, editing and adding the information and add/edit the categories and subcategories of information. Proposed e-identification system is presently in testing phase for selected categories and family of the fishes.

Project Title: Establishment of National Agricultural Bioinformatics Grid in ICAR

Project Period: April, 2010 – March, 2012

Project Personnel: N.S. Nagpure (PI), S.P. Singh, A.K. Pathak, U.K. Sarkar and Mahender Singh

Funding Agency: NAIP, ICAR

Genome Resource databases

Four genomic resource databases, viz., Fish Microsatellite database (FishMicrosat), Fish EST database (FEST), Fish Ribosomal RNA database (FishRibo) and Fish Barcode Information System (FBIS) were updated with 2570 microsatellite sequences, 4000 EST records, 353 ribosomal DNA records and 584 barcode records, respectively (Fig. 2a&b). The design and implementation technology for 'Fish Karyome' was changed from ASP.Net platform to LAMP technology. A new database entitled "Fish Mitochondrial Genome Resource (FMiR)" was developed that contains mitochondrial genome sequence of 85 commercially important fish species from Asian region. The data was obtained from the Ref_Seq of NCBI and the database was designed using MySQL, PHP, Perl technology. Different type of analytical modules, like similarity search, microsatellite analysis, primer designing, genetic diversity estimation and phylogenetic analysis have been implemented in the database that might be helpful to researchers in species characterization, estimation of genetic diversity, phylogenetic analysis etc.

Computational methods to understand the molecular mechanism of white spot disease in *Penaeus monodon*

White Spot Disease is a devastating disease of shrimp *P. monodon* in which the shrimp receptor protein PmRab7 interacts with viral envelop protein VP28 to form PmRab7–VP28 complex that plays important role in initiation of the disease. We applied molecular modeling, molecular dynamics and docking for surface mapping of infective amino acid residues between interacting proteins. Our result showed that α-helix of PmRab7 interacts with β-sheets of VP28 and several H-bonds that contributed in the formation of PmRab7–VP28 complex (Fig. 3). Further studies on the amino acid residues and bonds may open the new possibilities for preventing PmRab7–VP28 complex formation and, thus, reducing chances of WSD.

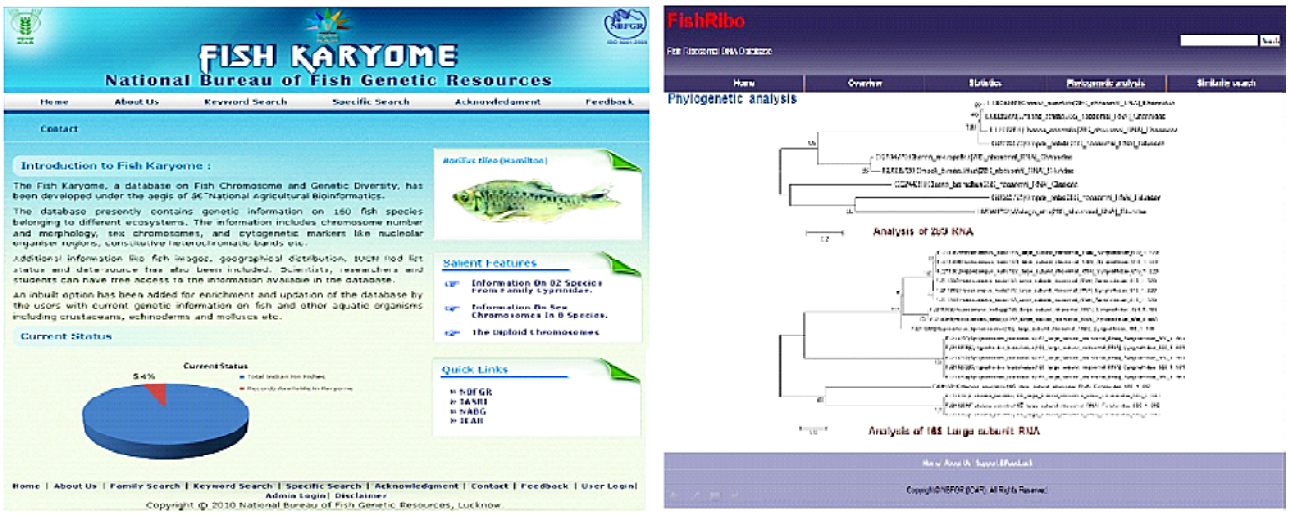


Fig. 2. Screenshots of (a) Fish Karyome and (b) FishRibo

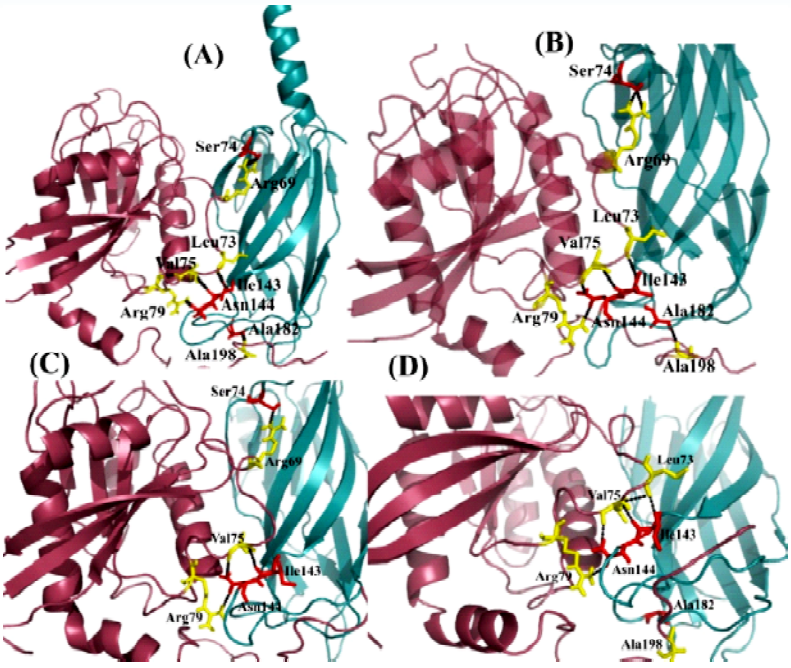


Fig. 3. Snapshots of molecular dynamics of PmRab7-VP28 complex during different time intervals, i.e. 3 ns (A) 7 ns (B) 11 ns (C) 13 ns (D). Protein-protein interaction are shown between viral envelop protein VP28 (deep teal cyan) and shrimp receptor protein in PmRab7 (raspberry red).

Fig. 4. Home page of website of NABG Fisheries domain.

Computational analysis of transcriptome of *Labeo rohita*

Computational analysis of transcriptome was performed to identify genes that are involved in biotic and abiotic stresses and other biological processes in *L. rohita*. The predicted genes can be used for understanding the role of genes in expression of important traits, like adaptability to various agro-climatic zones, disease control etc. in *L. rohita*.

Website for NABG Fisheries domain

A website of NABG Fisheries domain was designed and hosted on URL http://mail.nbfgr.res.in/nabg_nbfgr/index.html to provide access to the information related to research and development activities under the NABG project (Fig. 4).

4.2 GENETIC AND BIOLOGICAL CHARACTERIZATION

Project Title: Genetic approaches for conservation of prioritized Indian fish species

Sub-project I: Documentation of genetic diversity in prioritized Indian fish species belonging to groups featherbacks, carps, murrels and catfishes

Project Period: April, 2009 – March, 2013

Project Personnel: K.K. Lal (PI), Vindhya Mohindra, Peyush Punia, Rajeev Kumar Singh and Sangeeta Mandal

Funding Agency: Institutional

Genetic diversity is a critical component for long-term viability of natural populations. In the present study, the representative species from four evolutionary and commercially important groups of fishes were studied for genetic diversity in natural populations. The molecular markers of mitochondrial and nuclear origin were studied for polymorphism and analysed to document genetic variation and differentiation of three fish species, *Notopterus notopterus* (Order: Osteoglossiformes), *Labeo calbasu* (order Cypriniformes) and *Silonia silondia* (Order Siluriformes). A summary of the research work done in these species is given in Table 2.

Table 2. Summary of the genetic diversity analysis study for *Notopterus notopterus*, *Labeo calbasu* and *Silonia silondia*

Species	Populations		Total no. of samples sequenced		Microsatellite genotype
	ATPase6 /8	Cyto-b	ATPase6 /8	Cyto-b	Sample* Loci
N. notopterus	19		435		666*6
L. calbasu	11		215		
S. silondia	8 (5)	8 (5)	90	91	
Total			740	91	

Notopterus notopterus

The bronze featherback, *N. notopterus*, is widely distributed in Southeast Asia, Bangladesh, Cambodia, India, Laos, Burma, Nepal, Pakistan, and virtually all river basins of peninsular Thailand and Malaysia, Sumatra and Java. In India, this fish is widely distributed in Indus, Ganges, Brahmaputra, Mahanadi, Krishna, Cauvery and other river basins in peninsular India. *N. notopterus* is a teleostean fish represents prehistorical lineage and belonging to primitive order. Therefore, genetic diversity, phylogeographic and phylogenetic exploration through application of molecular markers in the fishes of this order are of considerable interest.

A total of 341 individuals of *N. notopterus* collected from 19 different locations were analysed for genetic variation using ATPase 6/8 mitochondrial region. Results revealed 45 different haplotypes defined by 54 divergent nucleotide sites, out of which 32 variables with parsimony informative. The overall F_{ST} of 0.611 was found to be significant (P<0.0001). Analysis of Molecular Variance (AMOVA) analysis indicated that 45.80% differentiation was contributed due to variation among groups, 15.32% was due to variation among populations within groups and 38.89% differentiation within populations.

A total of 666 individuals from 19 different natural populations were also analysed using six polymorphic microsatellite loci. The allele frequencies at these loci *Cch2**, *NN90-1**, *Cch18**, *Cch10**, *Cch20** and *Cch39** exhibited considerable variation in all the populations. The total number of alleles (Fig. 5) ranged from 8 to 34 with a size range of 84-243 base pairs (bp). Significant genotype heterogeneity (P< 0.0001) and the mean F_{ST} at all the loci and across all collections was 0.212 which indicated that the samples were not drawn from the same genepool.

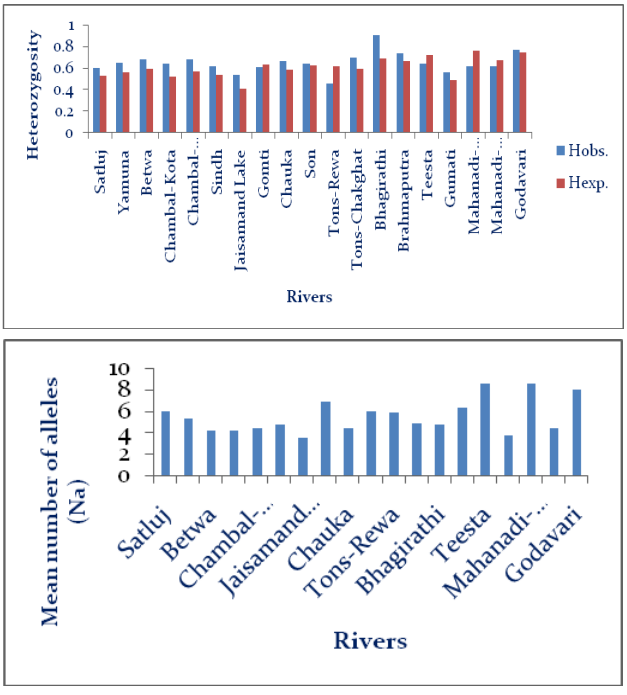


Fig. 5. Heterozygosity values and mean number of alleles for six polymorphic microsatellite loci in *N. notopterus*

Silonia silondia

A total of 91 individuals of *Silonia silondia* from five different rivers representing two different river basins (Ganges and Mahanadi river systems) were used for the study which were collected from eight different collection sites of rivers Chambal, two sites of Tons (Rewa and Chakghat), Son, Bhagirathi and three sites from Mahanadi (Hirakud dam, Tikarpada and Naraj Barrage). The population structure of *S. silondia* is revealed using cytochrome *b* and sequence analysis. In the cyt b region, 16 variable positions with 13 haplotypes were identified. AMOVA for cyt *b* sequences revealed that out of total variation, 23.17% variation was contributed by among population and 76.83% variation was contributed by within populations and the mean F_{ST} value was found to be 0.232 which was significant. In the ATPase 6/8 region, 12 variable positions with 11 haplotypes were identified. Results of AMOVA revealed 22.72% variation was contributed by among population and 77.28% variation was contributed by within populations and the F_{ST} value was found to be significant 0.227. AMOVA revealed high within population variation and low among populations variation for both cyt *b*, as well as, ATPase 6/8 region which is a characteristic of migratory fish species. With both the markers, haplotype network formed two distinct clusters for two distinct lineages. Population pair-wise F_{ST} values for cyt *b* region ranged from 0.000 to 0.661 whereas for ATP*ase* 6/8 region, it ranged from 0.000 to 0.671. The pattern of isolation by distance (IBD) was not supported when all the sampling locations were compared by Mantel test for cyt *b*, the IBD observed was non-significant (r = 0.131, *P* = 0.592). Similar results were obtained for ATP*ase* 6/8 where the IBD observed was non-significant (r = 0.116, *P* = 0.665).

Clarias batrachus

A total of 27 polymorphic EST-SSR loci were identified through transcriptome analysis of immune functions Out of 27 polymorphic loci, 15 loci were analysed to genotype samples from four geographically distinct populations for genetic variability, of which, 6 loci were found to exhibit high F_{ST} value and were considered to be the outlier loci.

Labeo calbasu

Two mitochondrial genes (ATPase 6/8 and cytochrome b) were amplified and sequenced to analyse the pattern of sequence divergence in a total of 206 individuals of *L. calbasu*, collected from 11 different river systems located in different geographical areas of India i.e. Ganga, Kosi, Ghaghra, Brahmaputra, Satluj, Bhagirathi, Gomti, Sharda, Mahanadi, Tons and Godavari. The analysis of combined length of 842 bp of ATPase 8 & 6 genes revealed the range of gene diversity from 0.125 to 0.817. AMOVA showed that the overall genetic variation within populations was much larger than the variation among groups. Haplotype network (Fig. 6) analysis revealed two distinct clades. The same numbers of samples have been optimised and amplified using whole gene of cytochrome b. The sequencing and analysis of these samples is under progress.

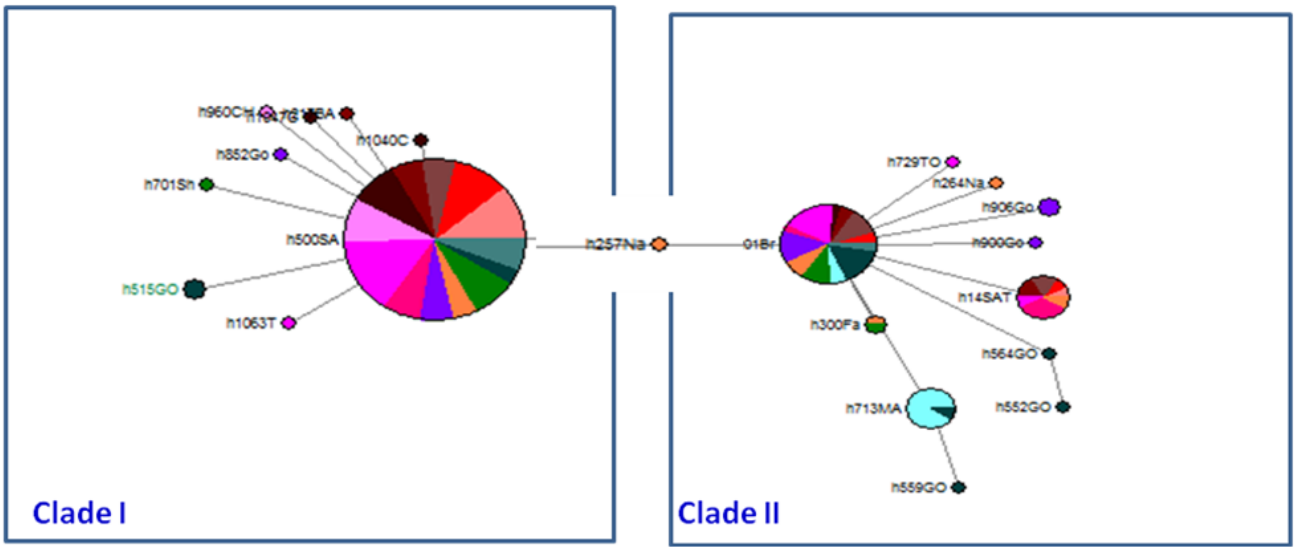


Fig. 6. Median Joining Network of ATPase 6/8 mtDNA gene in wild *L. calbasu* population

Sub-project II: Documentation of mitochondrial genomes in fishes from important taxonomic groups

Project Period: April, 2009 – March, 2013

Project Personnel: Rajeev Kumar Singh (PI), K.K. Lal and Vindhya Mohindra

Funding Agency: Institutional

Complete mitochondrial genome of great snakehead, *Channa marulius*

Mitochondrial DNA-derived sequences are considered as useful markers for evolutionary studies and their comparison can reveal the evolution of both the organism and their genome. For teleosts, the largest vertebrate group, about 1500 complete mtDNA sequences are available. The complete mitochondrial DNA sequence of *Channa marulius* was determined using a PCR-based method. The high quality genomic DNA was isolated by phenol–chloroform method extraction from the blood tissue. A total of 18 sets of primer pairs that amplify contiguous, overlapping segments of the entire mitochondrial genome were used. The overlapping mtDNA sequences were edited, assembled into larger contigs and analyzed.

The locations of the 13 protein-coding genes were determined by comparisons of DNA or amino acid sequences of bony fish mitochondrial genomes (Fig. 7). The 22 tRNA genes were identified by their proposed cloverleaf secondary structures and anticodon

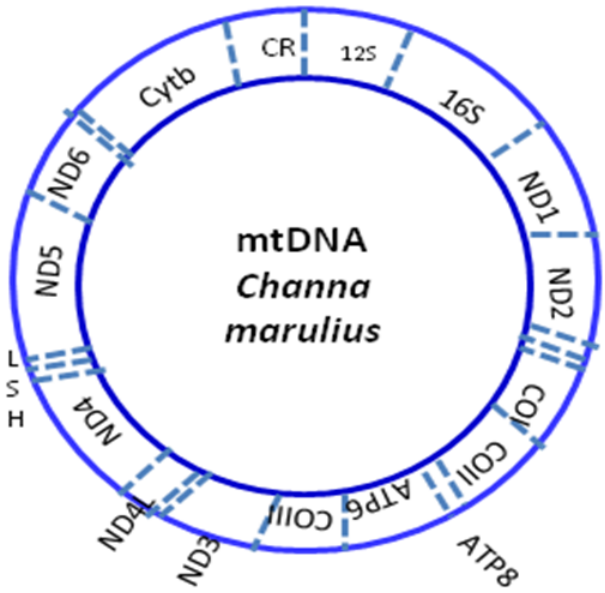


Fig. 7. Gene organization of mitochondrial genome of *Channa marulius*

sequences. The two rRNA genes were identified by sequence homology. Twelve of the thirteen protein coding genes were found to be encoded by the heavy strand in the order typically observed for vertebrate mitochondrial genomes, whereas only ND6 was located on the light strand. The complete mitogenome of *C. marulius* was found to be of size16567bp. The gene structure and order were comparable to those reported for other vertebrates.

Project Title: Outreach activity on Fish Genetic Stocks

Project Period: April, 2008 – March, 2013

Project Personnel: K.K. Lal (PI), A. Gopalakrishanan, P. Punia, Vindhya Mohindra, Rajeev Kumar Singh, U.K. Sarkar, M. Goswami and J.K. Jena

Funding Agency: Institutional

The work envisages to characterize the distribution of intra-specific variation in molecular, morphological and biological aspects in wild populations of cultivable species (Indian major carps and Indian catfish) across their native distribution range. The purpose is to document the genetic stocks of the species, along with their descriptors for valuation, registration and *ex-situ* conservation.

Labeo rohita

A total 345 sequences (Fig. 8) were analyzed for both mtDNA cytochrome b and ATPase 6/8 region from 17 different localities of rivers Satluj, Brahmaputra, Mahanadi, Rapti, Son, Chambal, Tons, Ghaggar, Ghaghra, Banganga, Gomti and Bhagirathi. Results revealed distinct haplotypes in Cytochrome b (45) and ATPase 6/8 (53). For Cyt b and ATPase 6/8 region nucleotide sequences were AT rich and transition to transversion ratio (Ts:Tv) was 4.23 and 4.58, respectively. Haplotype diversity was found to be high, ranging from 0.189 (Gomti) to 0.857 (Son) and the nucleotide diversity was low and ranged between 0.0002 (Gomti) to 0.002 (Son) for ATPase 6/8 region, while for Cyt b gene h_d ranged from 0.089 (Ghaggar) to 0.857 (Son) and the nucleotide diversity was low ranging from 0.0002 (Ghaggar) to 0.01 (Son). Figure 8 illustrates the network of haplotypes using ATPase 6/8 gene in *L. rohita.*

A total of 1094 individual samples were genotyped from 17 different riverine localities and analyzed using 21 polymorphic microsatellite loci. The total number of alleles ranged from 15 to 39 with a size

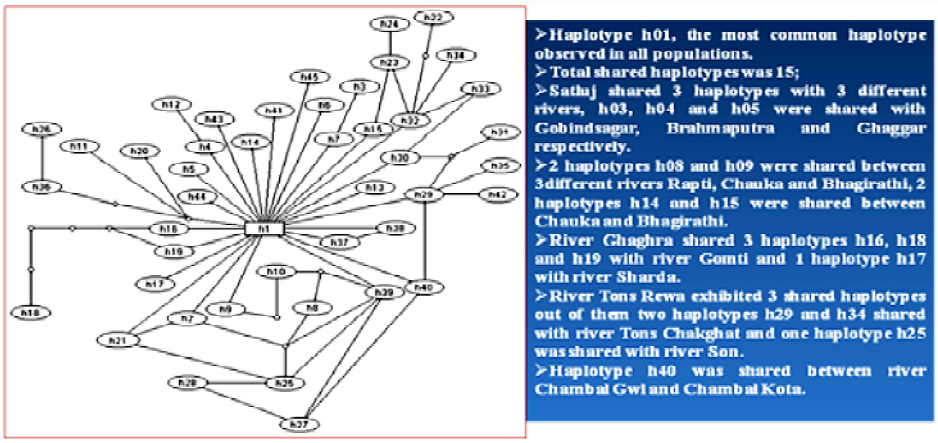


Fig. 8. Haplotype network for ATPase 6/8 region in *Labeo rohita* population in India

range of 60-251 base pairs (bp). The mean F_{ST} at all the loci and across all collections was 0.0477 indicating that 4.7% of the total variability was due to inter-population differences.

Catla catla

A total 245 sequences were analyzed using two mtDNA genes cytochrome b and ATPase 6/8 from fifteen different localities. For both the regions, nucleotide sequences were AT rich and transition to transversion ratio was 4.23 for cyto b and 5.1 for ATPase 6/8. Haplotype diversity was found to be high, ranging from 0.153 (Gobindsagar) to 0.645 (Bhagirathi) for ATPase 6/8 region. The AMOVA analysis revealed that 6.48% differentiation at ATPase 6/8 and 5.79% at Cyt b in Catla species was due to variation among groups.

In *C. catla* a total of 495 individuals were genotyped from 10 different riverine localities and analyzed using 11 polymorphic microsatellite loci. The total number of alleles ranged from 7 to 17. The mean F_{ST} at all the loci and across all collections was 4.09% which indicated that 4% of the total variability between the populations was due to inter-population differences.

Cirrhinus mrigala

Microsatellite genotyping was done for 1102 individuals of *C. mrigala* from 14 different riverine sites and one introduced population using 22 polymorphic microsatellite loci. Out of 22 microsatellite loci analyzed 18 had private alleles in samples from different riverine locations. Results from AMOVA analysis revealed that the pair-wise F_{ST} was lowest (0.01454) in Kali Sindh and highest (0.09907) in Gobindsagar. Deviations for P_{HW} and Pscore were evident at loci Lr28* (Gomti and Brahmaputra), Lr35* (Mahanadi), Lr36* (Satluj), Lr37* (Kali Sindh) and Lro26* (Chambal).

Complete region of ATPase 6/8 (842 bp) were amplified, sequenced and analyzed for 446 individuals collected from 19 different river localities. A total of 26 haplotypes were observed out of which, five were shared, while 21 were unique to the location. The overall F_{ST} was 0.098. AMOVA analysis indicated that 10.46% variability was among populations within group, whereas 90.16% was within populations. Analysis of partial cytochrome b from 348 individuals of *C. mrigala* sampled from 18 riverine sites demonstrated the overall F_{ST} to be 0.365. AMOVA analysis indicated that 10.34% variability was among populations within group, whereas 63.46 % was within populations.

Clarias batrachus

The sequence of a 307 bp partial segment at the 5' end of the cyt b and 842 bp of ATPase 6/8 mtDNA genes were determined for 403 individuals collected from 11 different locations. The mean F_{ST} value for overall populations, for cyt b and ATPase 6/8 (0.715 and 0.626, respectively) were found to be significant ($P<0.05$) and revealed high level of genetic structuring in the natural populations of *C. batrachus*. The patterns of genetic diversity and haplotype network (Fig. 9) clearly indicated population structuring from different locations. Mismatch distribution analysis and significant Tajima's D values of neutrality test were indicative of population expansion in Lucknow populations only inferred from both cyt b and ATPase 6/8 genes.

Amplification and genotyping of 807 individual samples of *C. batrachus* from 24 natural populations was done using 18 polymorphic microsatellite loci.

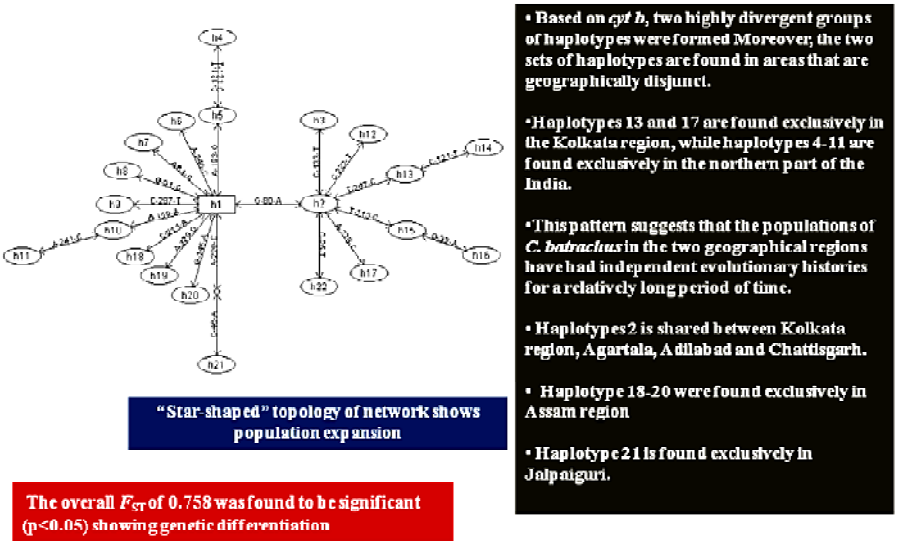


Fig. 9. Haplotype network for cyt b (A) and ATPase 6/8 (B) region obtained between eighteen different populations of *C. batrachus*

Biological characterization of wild populations

Reproductive parameters

The absolute fecundity of *L. rohita* ranged from 3.42 lakh (Ghagra) – 5.59 lakh (Betwa), for *C. mrigala* it ranged from 1.14 lakh (Ganga) – 4.41 lakh (Betwa) and for *C. catla* it was lowest for River Ken (2.48 lakh) and highest in River Betwa (16.29 lakh). In *C. batrachus* the fecundity was highest in River Rapti (0.19 lakh) and lowest in River Betwa (0.029 lakh). A significant ($P<0.05$) correlation was observed when fecundity of selected species was plotted against total length, total weight and ovary weight of fish. In case of *L. rohita*, it was noticed that with the increase in length of fish, the absolute fecundity increased and peaked at the maximum length whereas, the relative fecundity remained constant as the length of fish increased except in River Sharda. Relative fecundity in *L. rohita* was highest for River Sharda and lowest for River Tons. In *C. mrigala*, maximum relative fecundity was recorded in River Tons and minimum in River Ghagra, for *C. catla* the maximum relative fecundity was found in River Betwa and lowest in River Ken and in *C. batrachus* it was highest in River Rapti and lowest in River Gomti.

Age and Growth

Study revealed the age class of 1+ to 9+ for River Chambal and 0+ to 4+ for River Gomti in *L. rohita*. In *C. mrigala* the class ranged from 1+ to 8+ for Chambal and 0+ to 1+ in River Sharda and in *C. catla* it ranged from 0+ to 8+ and 0+ to 2+ for rivers Son and Gomti, respectively (Fig. 10).

Scanning Electron Microscopic structure of *Clarias batrachus* otolith was analyzed. The attempts was made to study the age and growth in *C. batrachus* (n = 350) by otoliths from three populations. The study indicated the distribution of age classes upto maximum of 2+ years. Length-weight relationships and condition factor were determined in *L. rohita*, *C. mrigala* and *C. catla*, while in *C. batrachus*, relationships between fish length-weight and otolith length-fish length were determined. The relationship between fish length (total and standard) and otolith length was linear, and was explained by a simple least-squares regression ($r^2 > 0.87$).

Project Title: DNA Barcoding of Marine finfishes and shellfishes

Project Period: November, 2012 – October, 2017

Project Personnel: A. Gopalakrishnan (PI), J.K. Jena and V.S. Basheer

Funding Agency: MoES - CMLRE, Govt. of India

Five to seven samples of 147 species of finfishes were collected [from the fish landings at Cochin, Trivandrum and Kollam in Kerala (41 species); Mangalore and Malpe in Karnataka (45 species); Dhiga, Diamond Harbour and Dariya in West Bengal (61 species)]. Tissue samples from each species were collected after recording the morphometric measurements, total weight, sex and gonadal condition of the specimens and preserved in 95% ethanol in screw cap vials. Total DNA was extracted and COI gene was amplified using the primers Fish F1–5'-TCAACCAACCACAAAGACATTGGCAC-3' and Fish R1-5'- AGACTTCTGGGTGGCCAAAGAATCA-3' (Ward et al, 2005). The PCR products were labeled using the Big Dye Terminator V.3.1 Cycle sequencing Kit (Applied Biosystems, Inc) and sequenced bidirectionally using an ABI 3730 capillary sequencer.

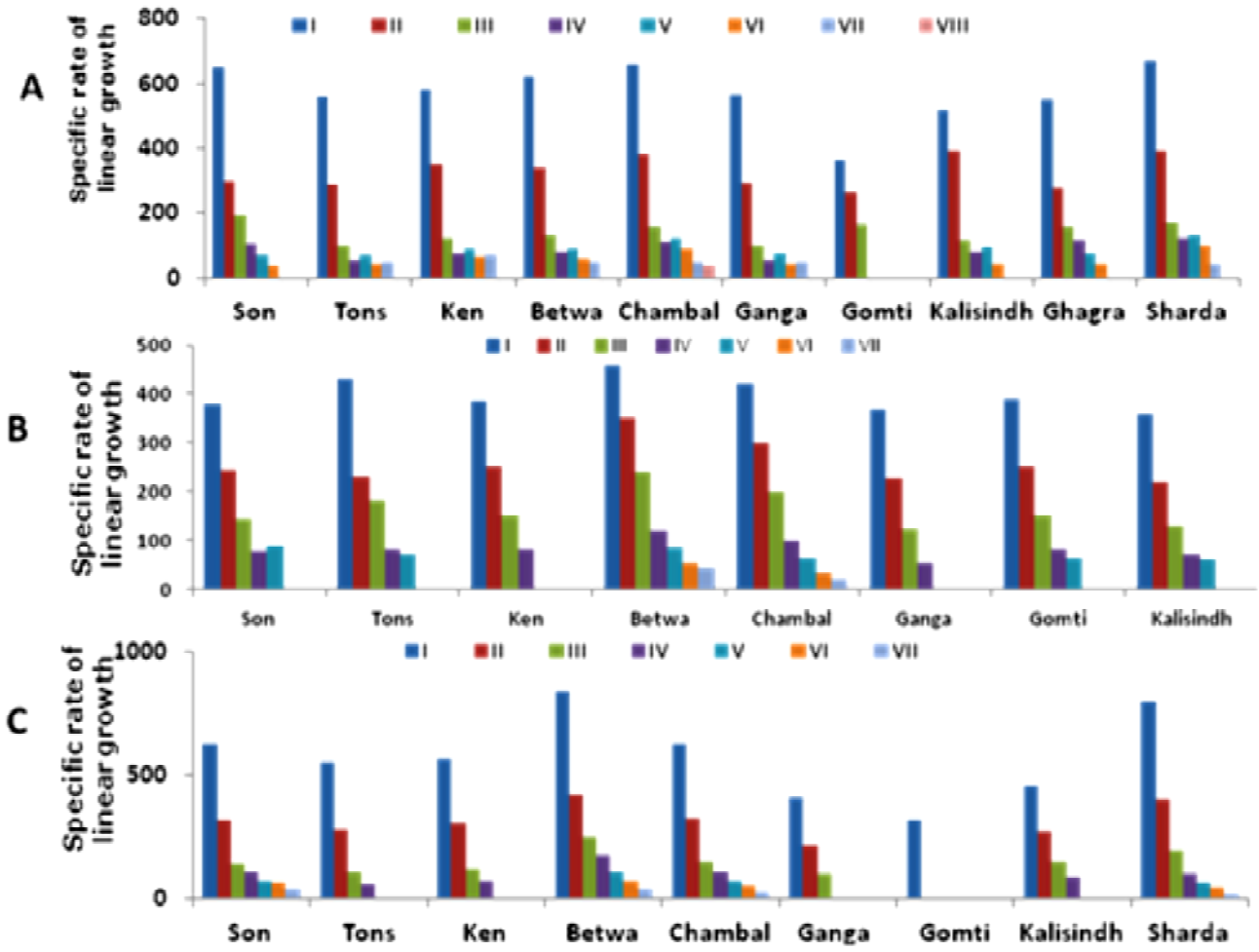


Fig. 10. Variation of specific rate of linear growth in different rivers for *L. rohita* (A), *C. mrigala* (B) and *C. catla* (C)

Table 3. Details of specimens for which Barcodes (mtDNA COI gene 655 bp sequence) have been generated

Sl. No.	Species	Location
1	*Alopias superciliosus, Himantura fai, Carcharhinus limbatus, Pastinachus atrus, Alepes djedaba, Selar crumenophthalmus, Carangoides fulvoguttatus, C. talamparoides, Alectis ciliaris, Trachinotus mookalee*	Off Cochin
2	*Carcharhinus amblyrhynchos, C. falciformis, C. amboinensis, Sphyrna zygaena, Carangoides caeruleopinnatus, C. arangoides equula, C. ferdau, Caranx hippos, C. sem, Sphyrna lewini, Manta birostris, Alepes Kalla, Seriolina nigrofasciata*	Off Kollam
3	*Carcharhinus sorrah, C. amblyrhynchos, Himantura jerradi, Taeniura meyeni, Mobula tarapacana, Carangoides armatus, C. chrysophrys, Caranx sexfasciatus, Gnathanodon speciosus*	Off Trivandrum

Sequences were aligned using ClustalW and submitted to GenBank. DNA barcodes for 32 species of finfish, totaling 165 samples, were generated and submitted to GenBank (Table 3). Sequencing of the COI gene produced an average of 655 base pairs per taxon.

Project Title: Microsatellite markers for genetic diversity analysis in natural populations of Cobia (*Rachycentron canadum*) and Silver pomfret (*Pampus argenteus*), candidate species for mariculture in India

Project Period: November, 2010 – November, 2013

Project Personnel: P.R. Divya (PI), A. Gopalakrishnan and V.S. Basheer

Funding Agency: DBT, Govt. of India

Sample collection of cobia was continued from Veraval (72 nos.), Cochin (81), Kolkata (81) and Chennai (64). Sample collection of silver pomfret was continued from Veraval (84), Cochin (85), Kolkata (79) and Chennai (87). Total DNA was extracted from all the collected specimens. A total of 12 polymorphic microsatellite loci were identified in *P. argenteus* including 7 loci developed through cross-species amplification (Table 4), from *P. cinereus* (Wei *et al.*, 2009). All the microsatellite sequences were submitted to the GenBank (Accs. No JX872231 - JX872237). PCR amplifications and genotyping was completed with 12 primers for 80 samples of silver pomfrets each from Kerala and Gujarat populations. Similarly for cobia, PCR amplificationand genotyping with 14 primers was completed for 70 samples of cobia each from Kerala and Gujarat populations. The alleles were separated using capillary electrophoresis on an ABI Prism 3730 genetic analyzer (Applied Biosystems). SSR fragment sizing was carried out using Gene Mapper software (Applied Biosystems).

Project Title: Genetic characterization of commercially important fishes from the Western Ghats and marine ecosystem using biotechnological tools

Sub-project I: Genetic characterization of commercially important fishes from the Western Ghats and marine ecosystem

Project Period: April, 2009 – March, 2013

Project Personnel: A. Gopalakrishnan (PI), V.S. Basheer, P.R. Divya, T. Raja Swaminathan and A. Kathirvelpandian

Funding Agency: Institutional

Amplification of ATPase 8/6 region in silver pomfret, *Pampus argenteus*

Pomfrets belong to the (i) family Stromateidae comprising of silver pomfret, *Pampus argenteus* and Chinese pomfret, *P. chinensis;* and (ii) family Carangidae which includes black pomfret, *Parastromateus niger* and these form about 2% of all India marine fish landings. Pomfrets are one of the most delicious food fish available along Indian coast and command high unit value. Fishery resources of pomfrets in Indian waters show a dwindling catch at alarming rates since the last few years due to over-exploitation and hence warrant measures of conservation. As the fishery along the north-west coast of the India collapsed during 1990s, restriction and regulation of gears were implemented to curb the recruitment over-fishing and growth over-fishing. Occurrence of high genetic variability has been reported in pomfrets from other

Table 4. Characterization of seven microsatellite loci amplified in *Pampus argenteus*

Locus	Accession number	Repeat motif	Primer sequences	Tm	Size range
PAP15-2	JX872231	(AC)n (TG)n	F: GCA AGC CTC TAA TTC ACT CC R: CTG CCT CTG TTT CTT CCT G	57	181-219
PAP72	JX872232	(GT)n	F: ACA CCC TAA ACA TGT CAG CAT C R: CAC AGC AGG AAT CAC TCA AAT A	53	204-308
PAP85	JX872233	(AC)n (CA)n	F: CGC ACA AAT CTC CAC CTA R: ATA CAG AGA CAG GGG AAG CCA A	50	93-147
PAP106	JX872234	(GT)n (TG)n	F: ATT CCA AAA CCG TGG CTA T R: GCA GAC ACC ATC CCA GAC T	52	237-275
PAP119	JX872235	(TG)n	F: CCC TCT ATC CTT CAA ACC CT R: TGA CTC TCA CCT CTG CCA TC	56	234-270
PAP189	JX872236	(GT)n	F:ATT CAA TAA CAA CTC CAC C R: TGT CTC ACC ACT CTT CAG C	56	138-190
PAP230	JX872237	(AG)n,(TG)n (GT)n	F:CCG TCC TCT TCC CTG TAA R: GCC AAG CAA GCC TCT AAT	57	153-183

regions of the world. Information genetic variability of the wild populations of pomfrets from Indian waters will help in evolving conservation and aquaculture strategies with long-term impact. Distinct genetic stocks of silver pomfret, *Pampus argenteus* (stock 1 - Gujarat, Maharashtra, Kerala & Tamil Nadu; stock 2 West Bengal) were identified using complete (842bp) sequence data of ATPsynthase 6 and 8 genes.

Complete ATPase 6/8 gene of 842 bp was sequenced in 108 silver pomfret samples. Out of a total of 842 bp of mitochondrial gene amplified, 168 bp fragments were of ATPase 8 and 684 bp of ATPase 6, with an overlapping region of 10bp from 159-168. ATG was the start codon in ATP 6/8 genes. TAA was the stop codon in ATPase 8 genes and incomplete stop codon of TA was found in ATPase 6 genes. A total of 24 haplotypes were identified among 108 individuals of four populations. Of the 842 bp in the mitochondrial sequences, 57 sites (6.7%) were variable among individuals. Among the 57 polymorphic sites observed, 7 were singleton variable sites and 50 were parsimony-informative. The pattern of nucleotide substitution was biased in favour of transitions over transversions in variable sites, including 18 transitions and 3 transversion changes. The overall transition/ transversion bias is R = 5.392. As expected, most of the changes occurred at the third codon, resulting in always synonymous substitutions. The A/T base contents were higher than the C/G base contents among the sequences examined and the mean number of nucleotide composition in the species was A = 29 %, T = 31 %, C = 27 %, and G = 13%. Estimates of the genetic divergence (P-distance) among haplotypes, based on Kimura 2 parameter ranged between 0- 0.057.

Samples from all four locations selected for this study were characterized by low values of nucleotide diversity (0.0025). Number of polymorphic sites, nucleotide and haplotype diversities for different populations were estimated using DnaSP 5.0 software. Mean haplotype diversity (Hd) was found to be 0.87 with variance 0.0054±0.023. Greatest genetic diversity with higher number of haplotype and nucleotide diversities was observed for samples from west coast (Arabian Sea) compared to east coast (Bay of Bengal). Sixteen haplotypes were specific to Arabian Sea and 6 specific to Bay of Bengal. Six were specific to Kerala, 8 to Gujarat, 2 to Tamil Nadu and the remaining 4 were unique to West Bengal. Mean number of polymorphic loci was estimated to be seven. All haplotypes representing ATPase gene were submitted to the GenBank (Accs. No JX 293025-293034) and (JX 460972 - JX460982, JX 944218-944220). Analysis of molecular variance of silver pomfret mtDNA ATPase 6/8 sequences in four different regions of the Indian sea was attempted using Arlequin ver 3.0. The significant pair-wise F_{ST} values and the AMOVA values between samples from West Bengal and other locations indicated the occurrence of distinct population structure in silver pomfret between north east (West Bengal) and remaining populations.

Collection of large barbs of Peninsular India from type localities and adjacent areas to resolve the taxonomic ambiguities & to develop species-specific molecular signatures

Species-specific molecular signatures of cultivable large barbs of Peninsular India were generated to resolve taxonomic disputes. Collections of the species were made from the type localities and other areas with a view to generate morphological and molecular signatures of the species, jointly with the ZSI-SRS. Partial sequence information of 16SrRNA, COI and RAG2 genes indicated validity of eleven species of large barbs of peninsular India (*Barbodes carnaticus, Puntius jerdoni, Puntius dobsoni, Puntius pulchellus, Gonoproktopterus curmuca, Gonoproktopterus kolus, Gonoproktopterus* (=*Hypselobarbus*) *kurali, Gonoproktopterus thomassi, Gonoproktopterus mysorensis, Gonoproktopterus periyarensis* and *Gonoproktopterus dubius*) (Table 5).

Table 5. Details of 11 species of barbs of peninsular India for which molecular signatures developed

S. N.	Species	No. of samples sequenced	Location and river basin	Molecular signatures developed
1	*Barbodescarnaticus* (Jerdon)	5	Srirangapatanam-Cauvery River & Chalakkudy River	✓
2	*Puntiusjerdoni* (Day)	5	Dakshina Kannada – Nethravathi River	✓
3	*Puntiusdobsoni* (Day)	5	Shimoga, & Chalakkudy River	✓
4	*Puntiuspulchellus* (Day)	5	Dakshina Kannada – Nethravathi River & Kalsa at Tungabhadra (Krishna River)	✓
5	*Gonoproktopteruscurmuca* (Hamilton)	5	Tungabhadra (Krishna River)	✓
6	*Gonoproktopteruskolus* (Sykes)	5	Deccan - Tungabhadra (Krishna River)	✓
7	*Gonoproktopterus* (=*Hypselobarbus*) *kurali* Menon & Rema Devi	5	Kumaradhara, Nethravathi River & Chalakkudy River	✓
8	*Gonoproktopterusthomassi* (Day)	5	Chalakkudy River	✓
9	*Gonoproktopterusmysorensis* (Valenciennes)	4	Srirangapatanam-Cauvery River	✓
10	*Gonoproktopterusperiyarensis* (Raj)	3	Periyar Lake – Periyar River	✓
11	*Gonoproktopterusdubius* (Day)	5	Kalakkad-Mundanthurai Tiger Reserve	✓

Mitochondrial genome sequencing of *Puntius denisonii* and *P. chalakkudiensis*

Whole mtDNA sequence of two ornamental barbs, endemic to the Western Ghats viz., *Puntius denisonii* and *P. chalakkudiensis* were generated jointly with CIFT, Kochi. The circular mtDNA of *Puntius denisonii* has a size of 16899 bp and that of *P. chalakkudiensis* has 16989 bp, showing a difference of 90 bp mainly in the control region. Both the species exhibited an overall divergence of 7.79% with the highest divergence of 11.3% in the control region and least in tRNA genes. The initial analysis of amino acid differences of 13 protein-coding genes of these two species in comparison with other *Puntius* spp. points out the need to revalidate the generic status of both *P. denisonii* and *P. chalakkudiensis*.

Population genetic analysis of Golden Anchovy *Coilia dussumieri* (Valenciennes, 1848) along the Indian coast

Coilia dussumieri (Teleostei: Clupeiformes: Engraulidae) commonly called as 'Golden Anchovy', is an important anchovy resource in Maharashtra and Gujarat along North West coast of India. As a fishery resource, it forms 1.2% of the fish catch of the country, whereas the species contributes about 10% of total anchovy landings. The species exhibits discontinuous distribution and constitutes a fishery also in West Bengal and Orissa along with other species *C. ramcarti*. Though the distribution of the species exhibits a clear–cut geographical isolation, the populations of *C. dussumieri* from north east and northwest coasts of India are considered as 'unit stock' for fishery stock assessment purposes. Two genetically distinct stocks of *C. dussumieri* (stock 1 - Gujarat, Maharashtra; stock 2 West Bengal, A.P.) were identified using complete (842 bp) sequence data of ATPsynthase 6 and 8 genes and 10 polymorphic microsatellite markers (developed through cross-priming from other clupeids/ engraulids). Altogether 244 samples were analyzed from four locations and the significant pair wise F_{ST} values and occurrence of population-specific haplotypes and private alleles indicated the genetic uniqueness North-West and North-East populations of golden anchovy.

Considering the importance of stock specific management, molecular markers like mitochondrial ATP synthase 6/8 genes and nuclear microsatellites were used to analyze its genetic stock structure. Samples were (n=70) collected from West Bengal, Andhra Pradesh (east coast) and Gujarat, Maharashtra (west coast) (Fig.11). For microsatellite analysis, a total of 32 primer pairs from three closely related species (resource species) belonging to the family Engraulidae were evaluated through cross-species amplification in *C. dussumieri*. Successful cross-priming was obtained with ten loci which were used as markers in stock identification studies. All the ten loci were found to be polymorphic and contained repeats. The numbers of alleles per locus ranged from 8 to 18, with a mean of 12.3. The mean observed and ex-pected heterozygosities across ten loci were 0.5880 and 0.6255, respectively. Some of the loci exhibited deviation from Hardy-Weinberg equilibrium and no significant linkage disequilibrium between loci pairs was detected. No null alleles were recorded. Significant pair wise F_{ST} values between populations of east and west coasts were observed [0.4001 (West Bengal and Maharashtra) to 0.4843 (Andhra Pradesh and Maharashtra)] and pair wise R_{ST} values were similar to that of F_{ST} in *C. dussumieri* varying significantly higher between populations of east and west coasts of India and lower within populations of each coast (Table 6). Mitochondrial ATPase 6/8 genes sequencing in *C. dussumieri* yielded 842 bp with 10 bp overlapping sequence between the genes. Comparison of the sequences revealed 34 different haplotypes out of 80 individuals from four different geographic locations with maximum (11 nos.) belonging to Gujarat. Haplotype diversity (*h*), within the four populations was found in the range of 0.7421 to 0.9368. Similarly, nucleotide diversity (π) was varied for the four populations from 0.0011 to 0.0025. AMOVA results indicated a high total variance of 72.66% between the populations of east and west coasts and 1.34% variance among populations within the coasts. The level of genetic differentiation among groups (west and east coast) showed high levels ranging between 0.697 and 0.783 with ATPase 6/8 genes. The findings of the present study using combination of microsatellite and mitochondrial (ATPase 6/8 genes) markers would be helpful in developing stock specific management measures for conservation and sustainable utilization of the species.

Table 6. Pair-wise F_{ST} values for between populations of *Coilia dussumieri* based on 842 bp ATPase 6/8 gene sequences (below the diagonal) and 10 microsatellite markers (above the diagonal).

	Coilia dussumieri populations			
	WB	**AP**	**GUJ**	**MAH**
WB	----	0.0032	0.4318*	0.4001*
AP	0.06097	----	0.4516*	0.4843*
GUJ	0.69695*	0.77180*	----	0.0104
MAH	0.70751*	0.78259*	0.04077	----

* P<0.001

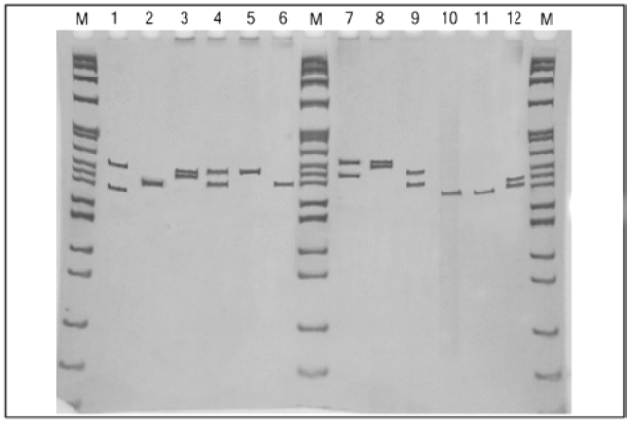


Fig. 11. Microsatellite pattern of locus *CDEJ4* in *C. dussumieri*
Lanes 1-3 samples from Gujarat; 4-6 Maharashtra; 7-9 West Bengal and 10-12 Andhra Pradesh. M- Molecular weight marker (pBR322 with *Msp*I cut)

Project Title: Development of novel microsatellites in *Channa* species (Channidae: Perciformes) from North East for conservation genetics

Project Period : April, 2012-March, 2015

Project Personnel: Rajeev Kumar Singh (PI), L.K. Tyagi and A.S. Barman (College of Fisheries, CAU, Lembuchera, Agartala)

Funding Agency: DBT, Govt. of India

Snakehead fishes or Murrels are old world fishes belonging to the order Perciformes, family Channidae (Nelson, 2006). These are native to Asia, Malaysia, Indonesia and Africa. Within the family Channidae, 25 of the 28 species are classified in the genus Channa, and are found in Southern and Eastern Asia. Among murrels, the great snakehead *Channa marulius* (Hamilton, 1822) is a commercially important fish of South East Asia valued as an esteemed table fish and also used for ornamental values whereas, the great snakehead, *C. marulius* has been assessed at lower risk-near threatened (LRnt) status (CAMP, 1998) due to declining abundance.

Simple sequence repeats (SSRs) are proven markers and have been used to characterize the natural populations in diverse groups of fishes. The *de novo* microsatellite loci were developed which can be used for stock characterization in *C. marulius*. The high molecular weight DNA from tissue samples was extracted and genomic DNA was double digested. Enrichment was performed with magnetic beads using biotinylated oligos, followed by cloning and selection of transformants (Fig. 12). Transformants were cultured in LB medium. The colony PCR and sequencing of the plasmids yielded 92 sequences that contained repeat motifs with sufficient flanking region for primer designing. The electrophenograms (Fig. 13) demonstrating the repeat motifs comprised of GT(32%), CA(27%), TG(18%) and GA (23%) and are given in (Fig. 14). Overall, the majority of microsatellite repeats found in *C. marulius* were perfect repeats (67%), with a few compound (21%) and imperfect repeats (12%).

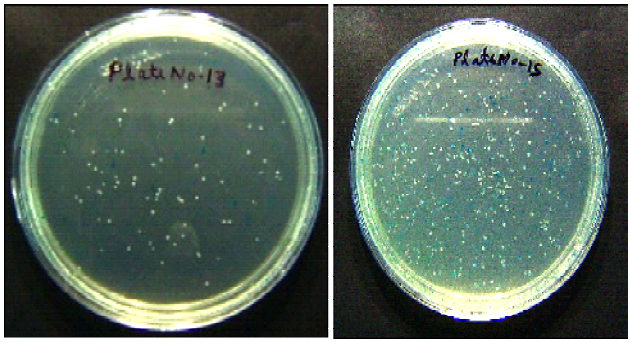


Fig. 12. Selection of bacterial colonies containing insert

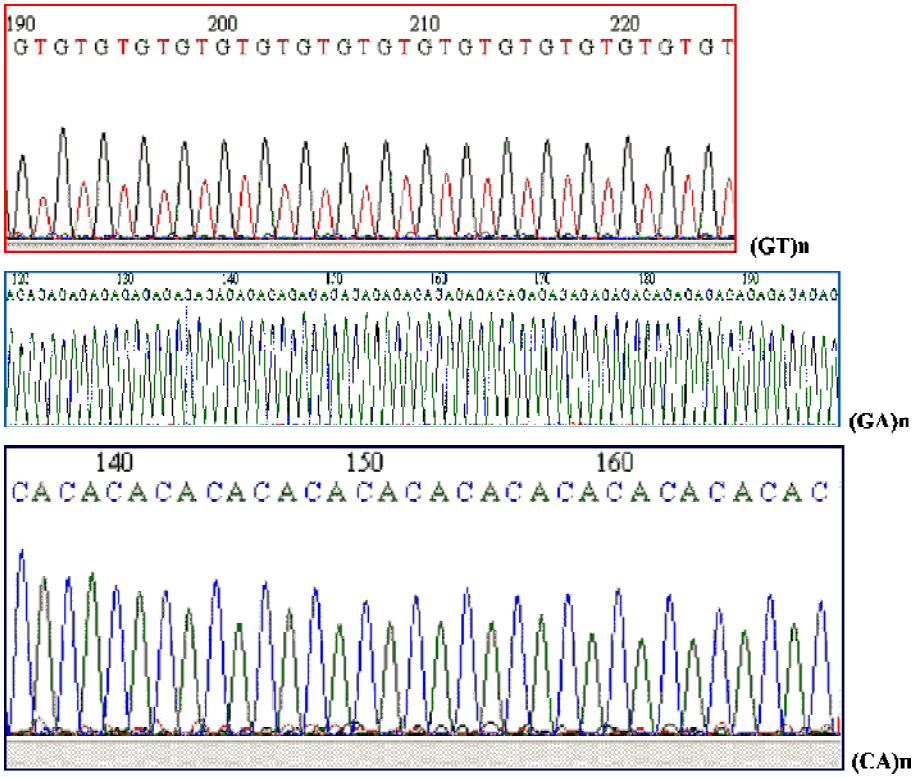


Fig. 13. Representative electrophenograms of perfect and imperfect repeats

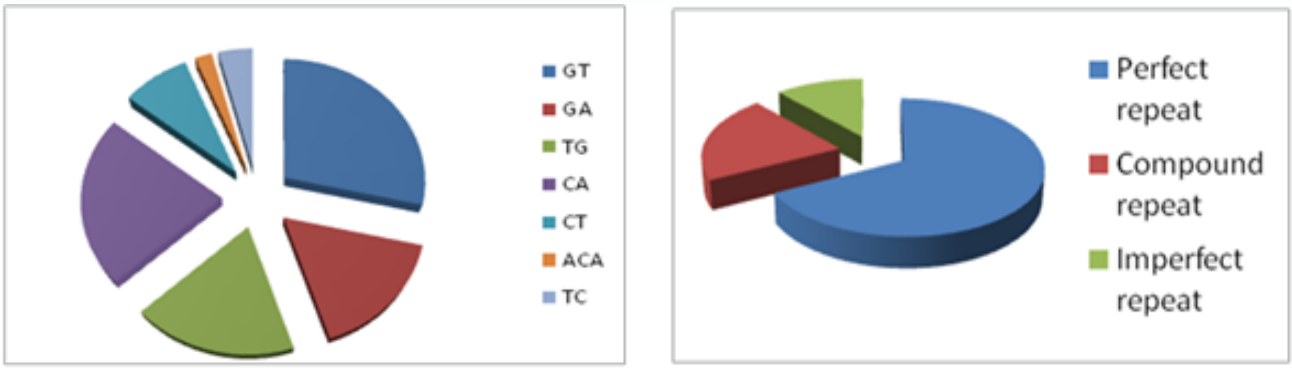


Fig. 14. Graphical view of microsatellite repeat motifs (a) Types of repeats and (b) Nature of repeats

Project Title: Bioprospecting of genes and allele mining for abiotic stress tolerance

Project Period: May, 2009 – March, 2014

Project Personnel: Vindhya Mohindra (CCPI) and Ravindra Kumar

Funding Agency: NAIP, ICAR

Characterization and expression analysis of signaling genes under hypoxia in catfish, *Clarias batrachus*

In *Clarias batrachus*, full length cDNA of six signaling specific genes, ATF-4 (accession no. KC633814), AXUD-1 (accession no. KC633815), SYND-4 (accession no. KC633816), SEPTIN-5 (accession no. KC633818), PPP1R-3C (accession no. KC633817) and PHKG-1 (accession no. KC633816) were characterized and their significant differential expression was found under short-term and long-term hypoxia exposure. It was found that short-term hypoxia exposure caused significant changes in the expression of signaling genes in all the examined tissues (brain, liver, muscle and spleen), however, after long-term exposure, liver and spleen tissues were highly affected (Table 7 and Fig. 15). The analysis indicated a positive role of signalling genes in hypoxia-tolerance in *C. batrachus*.

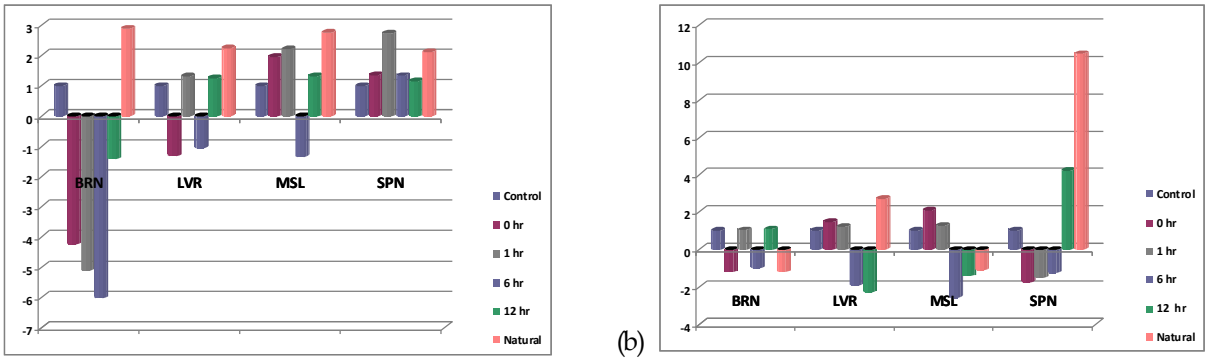


Fig. 15. Effects of hypoxia exposure on mRNA expression of a) ATF-4 and b) Septin-5 in different tissues of *C. batrachus* after 0, 1, 6, 12 h (short-term) and natural hypoxia exposure (long-term)

(*) Significant difference (P<0.05) in expression levels in comparison to normoxic control group

Table 7. Expression pattern of signaling specific genes in different tissues of *C. batrachus* after different periods of hypoxia

Gene name	Expression pattern*				
	0 hr	1 hr	6 hr	12 hr	Long-term
ATF-4	Brain (↓)	Brain (↓)	Brain (↓)	-	Liver (↑)
AXUD-1	-	Spleen and Liver (↑)	-	-	Liver (↑)
SDC-4	Muscle (↑)	Spleen (↑)		Muscle (↑)	Liver (↑)
SEPTIN-5	Muscle (↑)	-	-	Spleen (↑)	Spleen (↑)
PPP1R-3C	Muscle (↑)	-	Liver (↑)	Liver (↑)	Spleen and Liver (↑)
PHKG-1	Spleen and Muscle (↑)			Liver (↓)	Spleen (↑)

*↑ = up-regulated; ↓ = down-regulated

Characterization and expression analysis of hypoxia induced factor-α subunits (HIF1α, 2α and 3α) under hypoxia in catfish, *C. batrachus*

Characterization of complete CDS revealed that the full-length of HIF-1α of *C. batrachus* consisted of 2833 bp, which contained a 52 -untranslated region (52 -UTR, 311 bp), an open reading frame (ORF) of 2322 bp, encoding a polypeptide of 774 amino acid residues, and a 32 -untranslated region (32 -UTR, 200 bp). Similarly, HIF-2α cDNA was 4270 bp in length, which contained a 52 -UTR (204 bp), an ORF (2454 bp), and a 32 -UTR (1615 bp). The full-length cDNA of HIF-3α (3256 bp) contained a 52 -UTR (117 bp), an ORF (1884 bp), and a 32 -UTR (1255 bp) including a poly A signal sequence.

The mRNA expression of all the HIF's subunits during short-term hypoxia was not significantly different, while, following long-term exposure the expression of HIF-1α and -2α was found to be significantly upregulated in spleen (2.736 fold) and muscle (15.745 fold) tissues. However, the expression of HIF-3α was significantly down-regulated following long-term (1.869 fold) hypoxia in head kidney tissue (Fig. 16). These observations suggest that the differential expression of HIF-α subunits in *C. batrachus* was hypoxic time period dependent and may play specialized roles in adaptive response to hypoxia. HIF-2α, with its highly elevated expression in muscle tissues, can be a robust biomarker candidate for exposure to hypoxic environment.

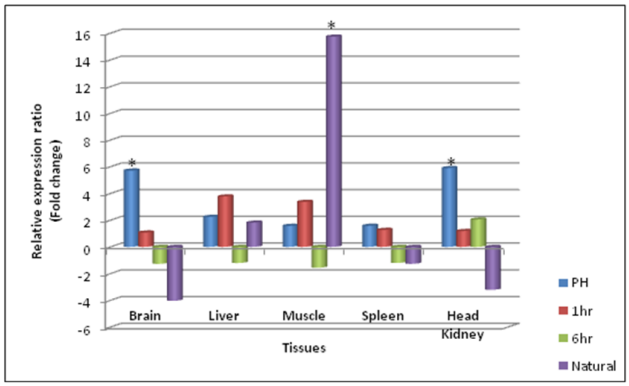


Fig. 16. Effects of short-term (PH, 1 and 6 hr) and long-term (Natural) hypoxia exposure on HIF-2α mRNA expression in *C. batrachus*. Y-axes represent the mean±SE (N=3, in duplicate). PH, progressive hypoxia upto 0.98 mg/l, dissolved oxygen, H1 and H6 (hypoxic time period, 1 and 6 hr), NTR, natural hypoxia exposure

(*) Significant difference (P<0.05) in the expression levels in comparison to normoxic control group

Identification and characterization of Novel transcript expressed under hypoxic stress in catfish *C. batrachus*

A novel transcript (Cystatin/ monellin-like 2) was identified under hypoxic conditions from *C. batrachus*. The expression pattern of this novel transcript was studied in brain, liver, spleen and head kidney tissues (Fig. 17). At transcriptional level, its expression was significantly up-regulated in response to short as well as long periods of hypoxia in liver, spleen and head kidney tissues, suggesting its positive association with oxygen concentrations lower than physiological concentrations.

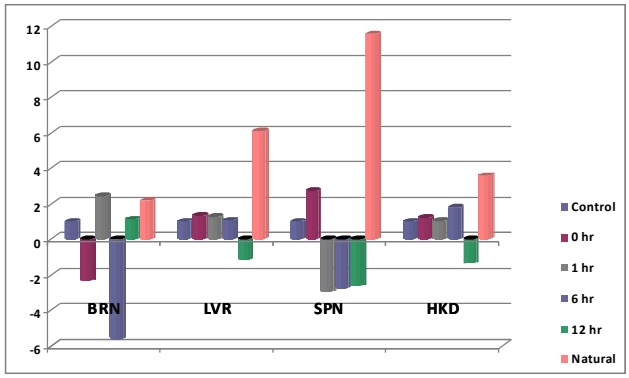


Fig. 17. Effects of hypoxia exposure on cystatin monellin-like 2 mRNA expression in *C. batrachus* after 0, 1, 6, 12 hr (short term) and natural hypoxia exposure (long term)

(*) Significant difference (P<0.05) in the expression levels in comparison to normoxic control group

Project Title: Genetic stock structure elucidation of *Tenualosa ilisha* and *Channa striatus* using mitochondrial DNA marker, microsatellites and molecular cytogenetic tools

Project Period: April, 2010 – March, 2013

Project Personnel: Mahender Singh (PI), N.S. Nagpure, Ravindra Kumar and Ajay Kumar Singh

Funding Agency: Institutional

Explorations of various populations of *Tenualosa ilisha* from Ganga River at Farraka, Hooghly River at Kolkata, Paradip port, Godavari River at Rajahmundry and Narmada River at Bharuch were done and tissue samples were collected (Fig. 18). DNA was isolated from all samples and the final concentration was adjusted to 100 ng/ ml. As universal primers were not working in *T. ilisha*, therefore, specific primers were designed for amplification of various regions of mitochondrial DNA. PCR amplification, sequencing and analysis of

cytochrome b (69 samples), control region (67 samples), ATPase 8/6 (63 samples) of mitochondrial DNA was completed in *T. ilisha* (Table 8). Similarly, PCR amplification, sequencing and analysis of cytochrome b (62 samples), ATPase 6/8 (63 samples), RAG1 (59 samples) and cytochrome c oxidase I (64 samples) was also completed in *Channa striatus* (Table 9).

Genotyping was done in 151 individuals in *T. ilisha* and 172 individuals in *C. striatus* using eleven and seven microsatellite loci, respectively (Tables 8 & 9).

Fig. 18. Processing of samples for molecular & cytogenetic study in *Tenualosa ilisha*

Consensus sequences were made from forward and reverse strands for all samples and aligned using ClustalW for analysis of cyt b gene in *T. ilisha*. Sequences were submitted to NCBI with accession numbers KC816465 - KC816533. Analysis of 69 cytochrome b sequences showed that out of 1152 nucleotides, 23 (2%) positions were found to be variable with 16 parsimony informative sites that resulted in 18 haplotypes (Hap_1 to Hap_18). Out of 18 haplotypes, 7 haplotypes (38%) were shared among individuals of different populations and one haplotype Hap_2 was shared in all the populations. The haplotype Hap_5 was shared among Diamond Harbour, Hoogly, Paradip and Ganga populations; haplotype Hap_6 was shared among Diamond Harbour, Hoogly, Paradip port and Godavari populations; haplotypes Hap_7 & Hap_8 were shared between Diamond Harbour and Paradip port populations; haplotype Hap_12 was shared between Godavari and Ganga populations; and haplotype Hap_1 was shared between Tapti and Narmada populations. Rest eleven haplotypes were private, each confined to a particular population. The haplotype Hap_3 was observed only in Tapti population, Hap_4 in Diamond Harbour population, Hap_9 in Hoogly population, Hap_10 and Hap_11 in Paradip port population, Hap_13 in Godavari population, Hap_14 and Hap_15 in Ganga population, and Hap_16, Hap_17 and Hap_18 in Narmada population only.

As expected for a migratory species, high haplotype diversity was observed in all the populations of *T. ilisha*, which may be attributed to migratory behaviour, large population size, environmental heterogeneity and life-history traits that favour rapid population increase. Paradip population exhibited maximum numbers of 7 haplotypes with 0.911 haplotype diversity (h) and 0.00243 nucleotide diversity (π). Populations of Diamond Harbour, Hoogly, Ganga and Narmada populations had five haplotypes each, while Godavari and Tapti populations had 4 and 3 haplotypes, respectively. Haplotype network demonstrated formation of a single clade and all the haplotypes originated from the haplotype Hap_2 directly or through subsequent mutations (Fig. 19). The overall F_{ST} value was found to be significant 0.0993 ($p<0.001$) that indicate genetic structuring among populations of *T. ilisha*.

Table 8. Detail of molecular markers used in *Tenualosa ilisha* for population genetic analysis

Collection sites	No. of Samples	Molecular markers			
		ATPase 8/6	D-loop	Cyto b	Microsatellite (9 loci)
Ganga River, Malda,	28	7	7	7	26
Hoogly River, Farraka,	13	10	11	11	12
Diamond Harbour	26	8	9	11	25
Paradip port	10	10	9	10	10
Godavari River, Rajamundry	19	8	7	8	19
Narmada River, Bharuch	34	13	13	12	32
Tapti River, Nawapur	32	7	10	10	27
Total	**162**	**63**	**66**	**69**	**151**

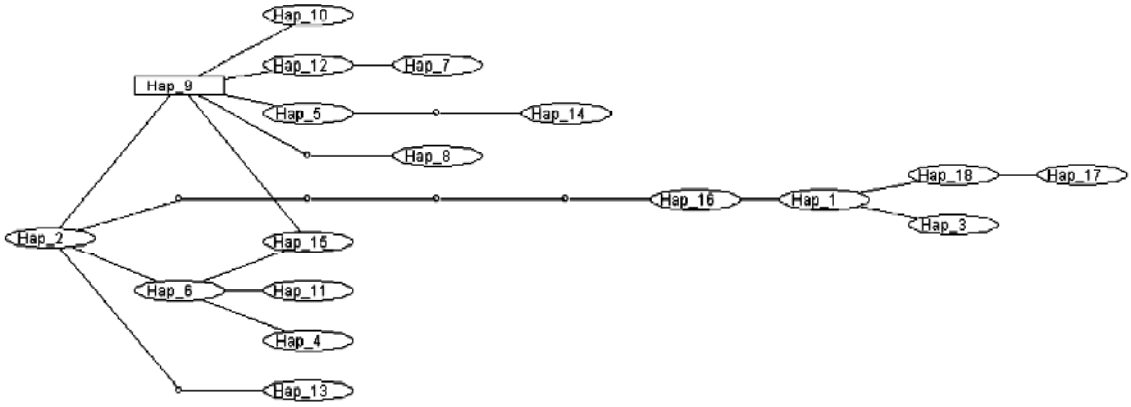


Fig. 19. Minimum spanning network of haplotypes in *T. ilisha*.

Table 9. Detail of molecular markers used in *Channa striatus* for population genetic analysis

Collection sites	No. of samples	Molecular markers				
		ATPase 8/6	RAG	COI	Cyto b	Microsatellite (7 loci)
Lucknow	60	10	9	10	9	35
Surat	4	4	4	4	4	4
Indore	35	9	9	10	10	35
Jhansi	35	10	10	10	9	35
Neelambur	8	4	3	4	4	4
Vijayvada	55	10	10	10	10	35
Udham Singh Nagar	18	10	10	10	10	18
Guwahati	6	6	4	6	6	6
Total	**221**	**63**	**59**	**64**	**62**	**172**

Project Title: Development of protocol for germ cell transplantation in fish

Project Period: April, 2011 – March, 2014

Project Personnel: B. Kushwaha (PI), Sudhir Raizada and Akhilesh Kumar Mishra

Funding Agency: Institutional

Germ cell transplantation has emerged as a potential biotechnological tool for fish breeding programmes. It can be very beneficial to a fish species of low reproductive capabilities in captivity. For transplantation of spermatogonial cells, a recipient fish in which endogenous spermatogenesis is naturally absent or experimentally depleted will be needed. In the present study, the optimization of depletion of endogenous spermatogenesis in male tilapia (*Oreochromis niloticus*) and common carp (*Cyprinus carpio*) were carried out. Mature male specimens of tilapia and common carp were maintained in two different water temperatures, i.e. 30 and 35°C, and treated with busulfan at rate of 15 and 18 mg/kg body weight by intra-peritoneal injection of two doses of busulfan at a two weeks interval for chemical depletion of spematogenesis. The effect of busulfan was assessed in the testes of treated specimens of both species by histological observations after one week of second treatment. The spermatogenesis was observed comparatively at a very low level after busulfan treatment in both species treated with 18 mg/kg body weight (Fig. 20)

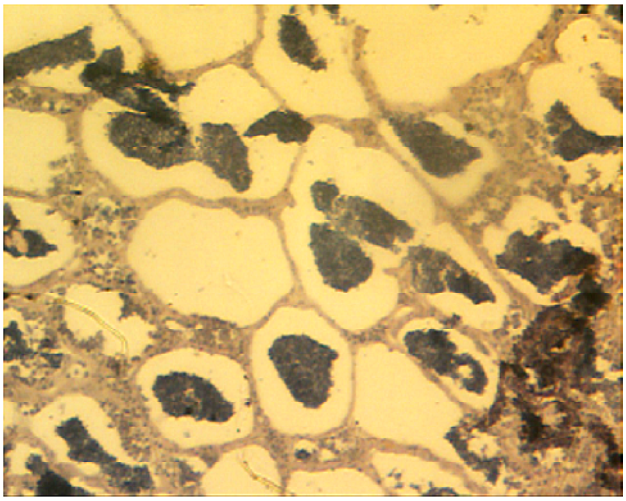


Fig. 20. Transverse section of testis of telapia after 21 days of 1st injection of busulfan

In order to obtain the enriched spermatogonial cells for transplantation, the optimization of isolation of spermatogonial cells from donor fish *L. rohita* was undertaken. The testes of male *L. rohita* was dissected out, cut into fine pieces and treated with collagenase 2 mg/ml in Dulbecco phosphate buffer saline (DPBS) for 60-75 min at room temperature for dissociation of tissue. The dissociated tissues were then incubated for 30 min in DPBS containing 0.25% trypsin, 1mM EDTA

and 2 µl/ml DNase. An equal volume of fetal bovine serum was added to stop activity of trypsin. The cell suspension was filtered with 60 µm nylon filter and centrifuged at 3,000 rpm for 30 min. The cells were isolated utilizing a different percoll gradients. The differential cell layers, resulted from the percoll gradient, were recovered by re-suspending the cell pellet in DPBS/MEM and centrifuged twice at 1,200-1,500 rpm for 5 min and examined under microscope. The layer formed in the middle of gradient was found rich in spermatogonial cells, whereas the lower layer at bottom was containing mostly blood cells and sperms (Fig. 21)

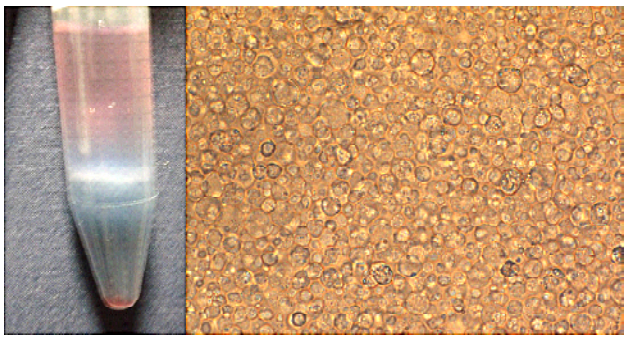
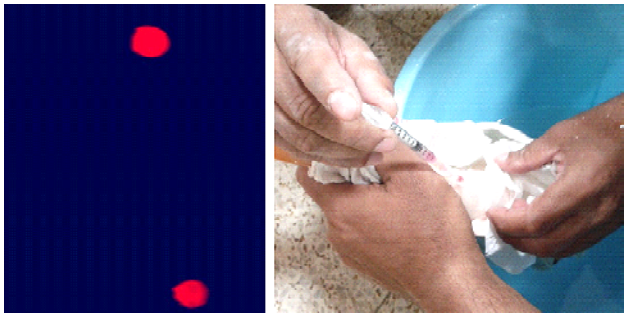


Fig. 21. Enriched spermatogonial cells of *L. rohita*

Effort was made for transfer of isolated enriched spermatogonial cells of *L. rohita*. The isolated cells were first labelled with red fluorescent cell linker PKH26, a lipofilic dye that intersperses between the cell membrane lipid bilayer and remains stable for several months, for identification and monitoring of proliferation of donor cells after transplantation. The transfer of labelled cells was done into the testes of sexually mature, busulfan treated tilapa and common carp through the uro-genital orifice using 1-ml syringe (Fig. 22a & b).



(a) (b)

Fig. 22. Photograph showing PKH 26 labeled cells (a) and transfer of cells in to testis of tilapia (b)

Project Title: Development of surrogate broodstock technology for commercially important fish species: implications for speedy propagation and conservation

Project Period: December, 2011–November, 2014

Project Personnel: B. Kushwaha (PI) and Ravindra Kumar

Funding Agency: DBT, Govt. of India

Two explorations were conducted during September - October, 2012 and March, 2013, for collection of samples of *N. hexagonolepis* from different locations in North Eastern States. A total of 131 samples (81 specimens from Jiabhoreli River near Bhalukpong, Arunachal Pradesh and 50 specimens from Umiam River near Barapani, Shillong, Meghalaya) were collected (Figures 23 & 24). Tissue samples of each specimen were taken and preserved in 90% ethanol for DNA extraction.

Fig. 23. Collection of samples in Jiabhoreli River near Bhalukpong, Arunachal Pradesh

Fig. 24. *N. hexagonolepis*

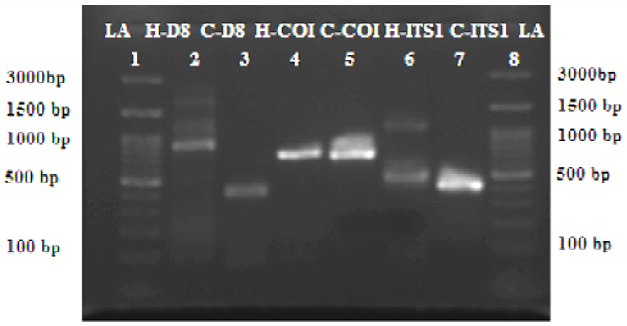


Fig. 25. Gel image showing amplified products. Lane 1 & 8= 100 bp DNA ladder, lane 2= D8 region of 28S in *N. hexagonolopes*, lane 3= D8 region of 28S in *Cyprinus carpio*, lane 6= ITS1 region of rDNA in *N. hexagonolopes*, lane 7= ITS1 region of rDNA in *C. carpio*.

Genomic DNA was extracted from muscles of the donor fish *N. hexagonolepis* and recipient fish *Cyprinous carpio*. A total of 12 primers were synthesized and used for amplification of 18S, ITS1, ITS2 and different regions of 28S (D1, D6, D7, D8, D9 and D11) of ribosomal DNA of *N. hexagonolepis* and *C. carpio* for screening of suitable DNA marker to distinguish the two fish species. All primers successfully amplified the targeted DNA region in both the species. Out of the amplified regions, primers of D8 domain of 28S produced a product of approx. 850-900 bp fragment in *N. hexagonolopes* and 450-500 bp in *C. carpio*. Primer for ITS1 region of rDNA produced amplification of approx. 500 bp fragment in *N. hexagonolopes* and approx.400 bp in *C. carpio* (Fig. 25). These two markers may be useful to distinguish between these two fish species.

Project Title: Genetic characterization of selected freshwater fish species endemic to Indian region of the Indo-Burma biodiversity hot-spot using advanced cytogenetic markers

Project Period: March, 2011 - March, 2015

Project Personnel: Ravindra Kumar (PI), B. Kushwaha (NBFGR, Lucknow) and Gusheinzed Waikhom (PI), T. Shantibala (IBSD, Imphal).

Funding Agency: DBT, Govt. of India

North-eastern region of India has been identified as a biodiversity hotspot with a high level of endemism. The fish diversity of this region has attracted many ichthyologists both from India and abroad. The rivers of Manipur belong to two different drainage systems, *i.e.* the Chindwin-Irrawaddy system, which drains eastern and central part of Manipur and Barak-Brahmaputra system, which drains northern and western hills of the state, emitting in to the Bay of Bengal. Fish samples were collected from Manipur during August - September, 2012. Samples of 9 indigenous species belonging to 7 genera, were jointly collected by NBFGR and ISBD teams from Loktak lake, Lokchao and Moreh rivers (Fig. 26) for cytogenetic and molecular studies. In addition, fish samples were also collected separately by IBSD team from the Iril, Imphal, Thoubal, Lokchao and Moreh rivers and Loktak lake of Chindwin basin and Tupul and Jiri rivers of Barak-Brahmaputra drainage. A total of 35 species, belonging to 22 genera of 13 families under 4 orders (Cypriniformes, Perciformes, Siluriformes and Synbranchiformes), were collected for cytogenetic and molecular studies.

Fig. 26. Sampling from Lokchao River in Chindwin basin

Chromosome spreads of metaphase stage were prepared from anterior kidney cells of *Barilius bendelisis, Channa gachua, D. aequipinnatus, Puntius bizonatus, P. chola, Glossogobius giuris* and *Tor tor* and karyotypes were prepared after staining the spreads with 6% Giemsa (Table 10 and Figures 27-29). The karyotype analyses were done for the first time in *B. ngawa, D. aequipinnatus, D. yuensis, Mystus ngasep* and *P. meingangbii*.

Table 10. Cytogenetic profiles of fishes collected from Manipur

S.N.	Name of species	Diploid chromosome number (2n)	No. of NORs	
			$AgNO_3$ stained	CMA_3 stained
1.	*Barilius bendelisis*	50	1 pair	1 pair
2.	*Channa gachua*	56	1 pair	1 pair
3.	*D. aquipinnatus*	50	1 pair	1 pair
4.	*Glossogobius giuris*	46	1 pair	1 pair
5.	*Puntius bizonatus*	50	-	-
6.	*P. chola*	50	1 pair	1 pair
7.	*Tor tor*	100	-	-

Genomic DNA was isolated from 5 species, namely *B. bendelisis, C. gachua, D. aequipinnatus, P. meinganbii* and *X. cancila*. Recombination activating genes 1 (RAG1) and Internal transcribed spacer2 (ITS

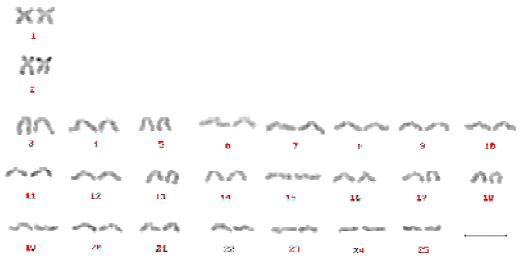


Fig. 27. Karyotype of *P. chola*

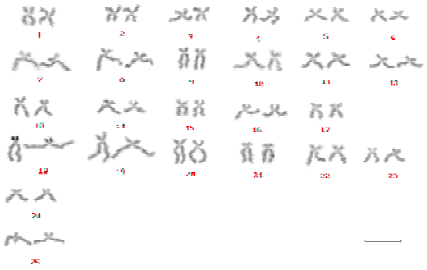


Fig. 28. $AgNO_3$ stained karyotype of *D. aquipinnatus* showing NOR

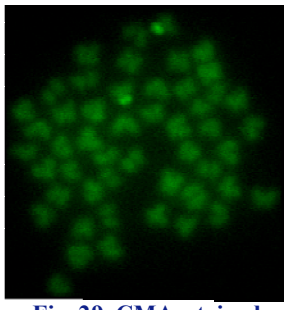


Fig. 29. CMA_3 stained spreads of Barilius bendelisis

2) region have frequently been used as molecular markers for characterization of fish species. RAG play an important role in the rearrangement and recombination of the genes related to immunity during the process of recombination. ITS 2 region of rDNA has been regarded as one of the candidate DNA barcodes because it possesses a number of valuable characteristics, such as, the availability of conserved regions for designing universal primers, the ease of its amplification, and sufficient variability to distinguish even closely related species. These DNA regions were amplified using suitable primers for assessing the variation in these DNA regions in the undertaken species. The primers amplified about 1500 base long fragment of RAG1 (Fig. 30) and about 645 base consensus lengths for analysis was found in these species. The amplicon sizes of ITS 2 varied in lengths.

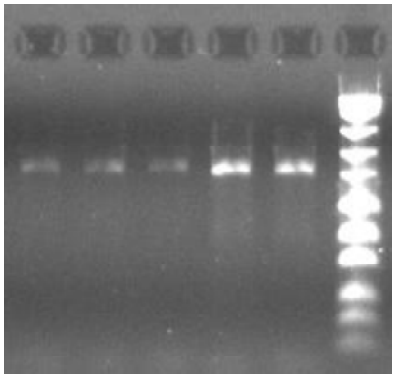


Fig. 30. Amplified product of RAG1

Project Title: Protein profiling of Indian major carps based on Mass Spectrometric Analysis (ESI)

Project Period: April, 2010 – March, 2013

Project Personnel: M. Goswami (PI), N.S. Nagpure, Ravindra Kumar and A. Srinivasan (AIIMS, New Delhi)

Funding Agency: Institutional

The sample preparation protocol was standardized for extraction and quantification of proteins from different tissue samples, like liver, heart, muscle, kidney and gill tissues of *L. rohita*. Live fish samples of *L. rohita*, weighing ~500 g were collected from various sites of Gomti River, Uttar Pradesh. Fish were sacrificed and the tissues were immediately collected and preserved at -80 °C for protein extraction. Total protein was extracted in buffer containing 7M Urea, 2M Thiourea, 4% CHAPS, 5 ml/ml IPG, 10 mM DTT and Proteasse inhibitor cocktail. After extraction, protein was quantified using Bradford's assay. The total protein extracted from different tissues were labeled and stored at -80 °C until use. Two-dimensional electrophoresis (2-DE) was carried out for liver, heart, muscle, kidney and gill tissue samples of *L. rohita* in triplicate. Gel images were captured with the help of BioRad CHEMIDOC XRS gel imaging system. The images were analyzed using PDQuest software from BioRad. Selected spots were picked from the 2-D gels (Fig. 31). In gel trypsinization was carried out before MS analysis and finally the proteins were identified with the help of MASCOT search. A total of 60 2-DE spots (Liver-20, Heart-10, Muscle-15 and Gill-15) were selected for protein identification. Proteins from liver, heart, muscle and gills were identified using MASCOT search. 2D reference image from various tissues of *L. rohita* were developed. This proteomic information generated from the project would be immensely useful as base line data for fish proteomic studies.

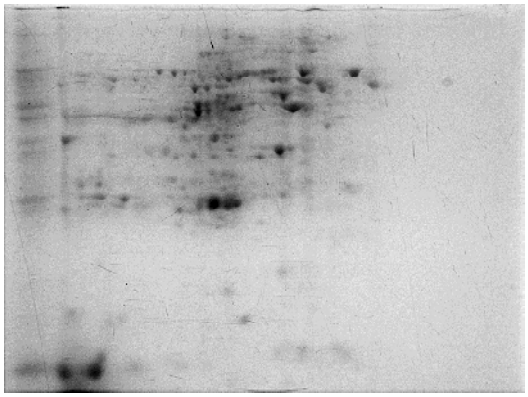


Fig. 31. 2-D Gel image of liver tissue sample of *L. rohita*

Project Title: Genetic characterization and conservation biology of economically important Siluroid fish *Ompok pabda* of Tripura

Project Period: March, 2011 – February, 2014

Project Personnel: U.K. Sarkar (PI), Mahender Singh and S. Banik (Tripura University)

Funding Agency: DBT, Govt. of India

Exploration of rivers and other water bodies in Tripura state of NE region was carried out for collection of fish and tissue samples for genetic and biological characterization of the wild populations of *O. pabda*. Out of 35 explorations in three major rivers *viz.*, Gomoti, Feni and Muhuri in Tripura were identified as important sites for sampling of *O. pabda*. A total of 480 tissue/fish samples were collected from three different populations from 23 sampling sites explored. Additional samples (n=70), from other hydrographic basin (Hoogly River from West Bengal and Gomti, Sai, Betwa and Ramganga rivers in Uttar Pradesh) were also collected for comparative analysis. Besides, collection of live individuals from different rivers of Tripura was carried out for breeding, linkage map preparation and further examination.

The detailed biological analysis of the three different populations was done using various attributes like length-weight relationship, age and growth, Gonadosomatic index (GSI), and fecundity. Out of 480 samples collected, the higher growth of fish in terms of length (27.5 cm) and weight (60 gm) was recorded in Feni River and the lower length (12.1 cm) and weight (48.75 gm) was recorded in Muhuri River. The size frequency depicted that 62 % of the individuals attain size >17.0 cm and a significant difference was recorded in the mean size (TL) among three populations from Gomoti (n=127), Mehuri (n= 139) and Feni rivers (n= 214) (t-test, $p < 0.05$). The length-weight relationship was recorded to be highly significant ($p<0.001$), with r value =0.997, $p<0.001$. Average value of condition factor (K) in different seasons ranged from 0.66 to 0.734 ($p < 0.001$).

The study revealed that the populations of females had higher weight (18.93- 45.2gm) and length (16.0-23.5cm) than males (7.3-9.1gm), (12.2-14.0 cm) and recorded to be in dominated over male populations with a ratio of 1:1.72 (M:F). The mature ovaries (stage IV) in *O. pabda* were observed during May - July and the first occurrence of ripe stage (stage V) was noticed during May - June and continued till August. Females were recorded 16.0 cm in length at the time of first maturity while it was 14.0 cm for male. Age and growth studies from otoliths of *O. pabda* estimated in between 1-2^{+}yr, of which 1^{+}old fish were the dominant age classes, and over 80% of fish were <2^{+}yr old. The higher age classes up to 2^{+} were recorded in Feni River, followed by Gomoti River.

Studies on GSI of the species revealed higher GSI (%) values (15.64) in females during June whereas, the lower values were recorded during January - March (4.648). In males, a very low GSI was recorded in January - March (0.406) which increased gradually upto a greater level (2.24) in June. Absolute fecundity varied from 2460 - 5986 eggs with mean value of 4330 ± 799 out of the 30 mature fishes. The fecundity per 100 g body weight ranged 10128 – 30838 with mean of 20228±5053 eggs. Fecundity increased with increase in length and weight of the fish. A linear relationship was observed between fecundity, total body length and body weight of the fishes (Fig. 32) with a positive coefficient of correlation (r = 0.99). In addition, a successful captive breeding of *O. pabda* with an average fertilization rate of 78% was carried out in Tripura. Survival and growth rate of the larvae in *ex situ* conditions was recorded significantly higher (92.86 %, r=0.984, P<0.0001) and the conditions to increase their survival potential was recorded.

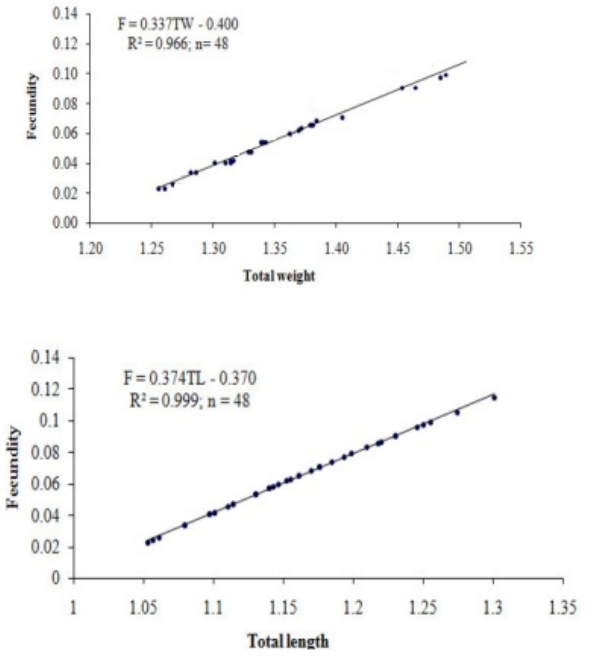


Fig. 32. Linear regression between total weight, total length and fecundity of *O. pabda*

In order to characterize the populations genetically, tissue samples from three different populations viz., Muhuri (n = 50), Gomoti (n = 30) and Feni (n = 25) were processed for DNA isolation

(Fig. 33). Microsatellite primers from closely related species were used for cross species amplification in *O. pabda* and thermal regimes for the primers were standardized. Microsatellite primers showing polymorphism among populations were selected based on PAGE and are being processed for labeling. Comparative population genetic studies were done by adding populations from other hydrographic basins. For linkage map preparation, small fin clip were taken non-invasively from male and female parents. One male and one female individuals of *O. pabda* were bred in one tank and offsprings of each pair are being reared in separate tanks.

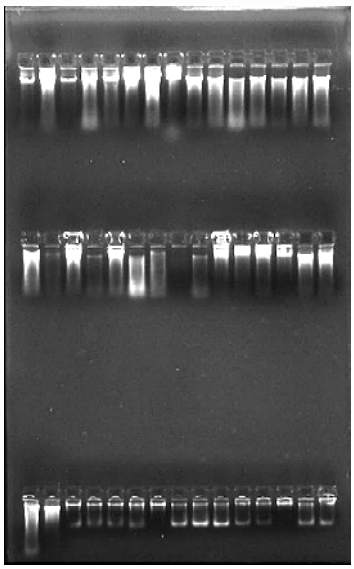


Fig. 33. Gel image showing DNA in *O. pabda*

The present study on various biological and molecular attributes of the different wild stocks of threatened *O. pabda* in Tripura provided valuable primary baseline information for their proper management and implementation of various conservation strategies.

Project Title: Identification and evaluation of reproductive traits and genetic structure of *Ompok bimaculatus* in India

Project Period: September, 2011 – September, 2014

Project Personnel: U.K. Sarkar (PI), Ravindra Kumar and Abha Mishra (BBA University, Lucknow)

Funding Agency: DBT, Govt. of India

The freshwater silurid catfish *Ompok bimaculatus* is a demersal, migratory fish occupying tropical freshwaters of the Indian sub-continent including Bangladesh, Nepal, Pakistan, Sri Lanka and Myanmar. In India, *O. bimaculatus* is highly popular due to its delicious and nutritious value. However, in recent years, the natural population of *O. bimaculatus* has declined drastically as a result of several anthropogenic factors like indiscriminate fishing, extensive habitat degradation and proliferation of exotic fish species. Due to its reduced abundance, the species has been listed in 'near threatened' category, as per IUCN (2010) criterion. In the present study, explorations were carried out in 22 rivers and 1392 fish specimen and 821 tissue samples were collected (Fig. 34). The largest specimen recorded was 410 mm in length, which was closer to the maximum reported size of 450 mm as per Fishbase. Out of 22 rivers, females were recorded to be dominating in 15 rivers (n= 971) with highest ratio of females in Krishna River 1:5 (M:F) and the lowest recorded in Cauvery River 1:0.42 (M:F).

In *O. bimaculatus*, females start to mature earlier than males and mean size at first maturity varied considerably between the male and female populations. Preliminary analysis indicated that in female the age at first maturity ranged from 156.33 - 333.0 mm while in male it was 215 - 380 mm. Earlier maturity (stage IV) of fish was recorded during March from Cauvery River (Krishnasagar dam) followed by, Narmada River while full maturity in other stocks was detected during April - August. The gonadosomatic index (GSI) of *O. bimaculatus* from 21 populations was studied to determine river-wise variation (Fig. 35). Comparative higher GSI in females was recorded in Brahmaputra River, followed by Krishna and Cauvery. The distribution of different maturity stages across different population was also recorded (Fig. 36). Length-weight relationship and condition factor of *O. bimaculatus* were assessed for 22 rivers. The fecundity from 14 populations showed considerable variation in different wild population and showed higher value (26686.41±5640.62) in Sharda River while it was minimum (3481.58±125.95) in Ganga River (Kanpur). Comparative breeding performances of the brooders of

Fig. 34. *O. bimaculatus* samples

Fig. 35. Tissue and gonadal data collection of *O. bimaculatus* in the laboratory

O. bimaculatus were determined under captivity in two separate geographical locations and patterns of variations were recorded (Fig. 37). Partial release of eggs (60-70%) was observed in both breeding trials. Reproductive potential and survivability of different populations were analyzed by egg weight and oocyte diameter. The highest mean egg-weight was observed in the populations of Krishna River and lowest in Krishnaraja Sagar dam. Maximum oocyte diameter of *O. bimaculatus* was recorded in Betwa River and minimum in Brahmaputra River.

DNA was isolated from over 225 samples of *O. bimaculatus* covering 20 populations. Mitochondrial cytochrome b (mtDNA Cyt b) marker was developed

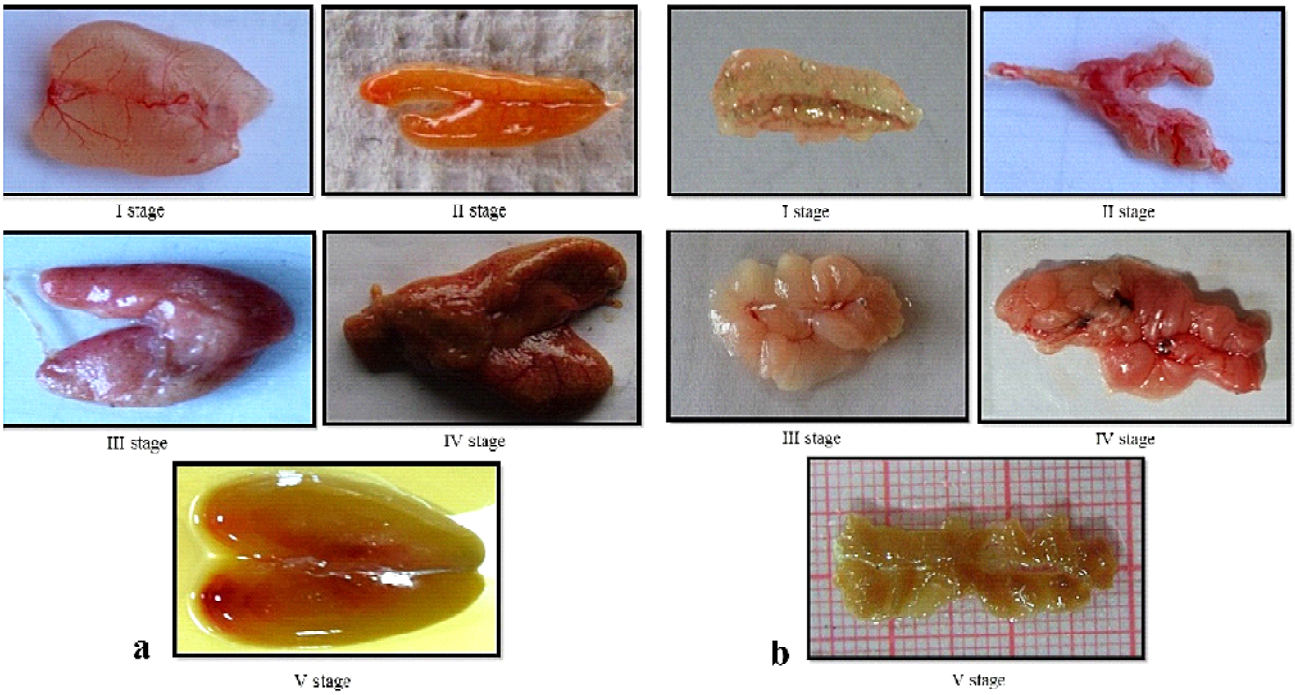


Fig. 36. Different maturity stages of (a) female and (b) male, *O. bimaculatus*

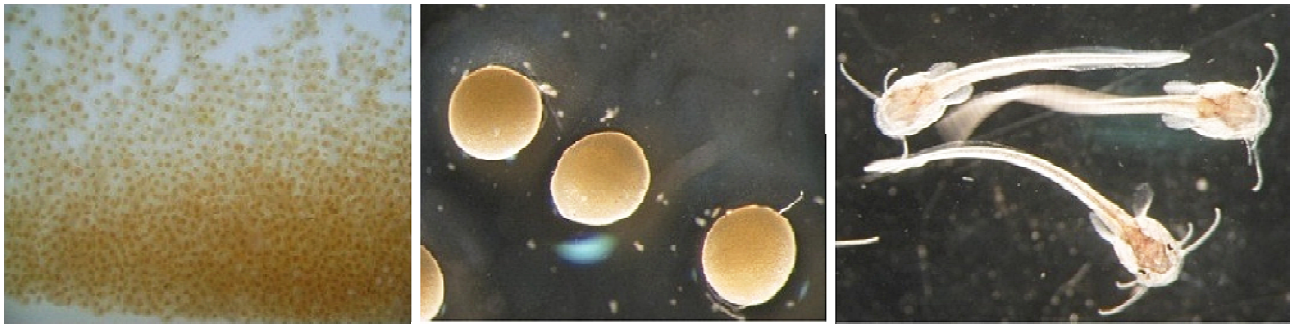


Fig. 37. Fertilized eggs and larvae of *O. bimaculatus*

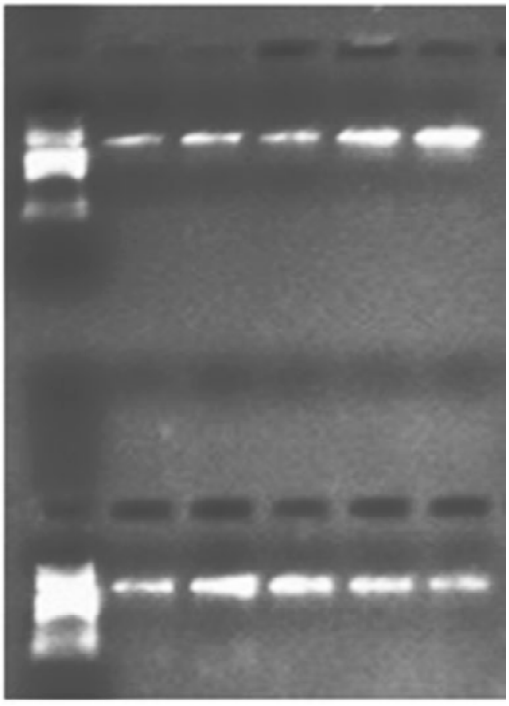


Fig. 38. Gel image showing PCR products of *O. bimaculatus*

with universal primers for screening the population-specific genetic variability. The DNA of six specimens from each population was used for amplification of mtDNA Cyt b gene. The amplicons of 120 samples from 20 populations were custom sequenced (Fig. 38). Sequencing data generated an average fragment length of approximately 1120 bp of mtDNA gene. Studies on protein level of fish ovary from different populations indicated that the protein level started increasing with the maturity of gonads, and the protein concentration varies according to its aquatic environment conditions. The initial studied showed fish ovaries from Cauvery and Mahanadi rivers had more protein concentration as compared to others. In the preparatory season, all the samples of different rivers showed gonad with immature gamete (oocyte). The study indicated that the local habitat play an important role in the species health that can affect their reproductive potential and also their survival.

4.3 EXPLORATION OF FISH GERMPLASM RESOURCES

Project Title: Exploration and assessment of fish diversity and traditional ecological knowledge in selected riverine and wetland ecosystems

Project Period: April, 2012 – March, 2015

Project Personnel: L.K. Tyagi (PI), A.K. Pandey, A.K. Pathak, Sangeeta Mandal, A.S. Bisht and Vikash Sahu

Funding Agency: Institutional

The Institute initiated a research programme for exploration and assessment of fish diversity and traditional ecological knowledge in selected riverine and wetland ecosystems. There are several areas and aquatic resources in the country which are rich in aquatic biodiversity but have not been explored adequately. Mahanadi river system and its important tributaries are among such rivers. Explorations were conducted in the upper basin of River Mahanadi starting from its origin and covering its entire length (approxemetely 450 km.) in the Chhattisgarh state, including its tributaries joining in this stretch.

Two seasonal explorations during post-monsoon and winter were undertaken in November, 2012 and February, 2013, respectively (Fig. 39). A total of 7 sites of River Mahanadi and 5 sites of its four major tributaries namely, Sheonath, Pairi, Mand and Jonk, were explored in each of the two explorations. A total of 44 fish species belonging to 30 genera of 16 families under 8 orders were recorded from River Mahanadi and its four major tributaries in initial explorations. Family Cyprinidae represented 45% of the fish diversity, followed by Bagridae (11%) and Mastacembelidae (5%) (Fig. 40). The highest species richness was recorded in lower stretch of Mahanadi at Pahanda near Sheorinarayan. Selected biological parameters of prioritized species collected during exploration and seasonal primary data on habitat parameters from selected sites were also recorded (Fig. 41). A digital base map of the Mahanadi river basin showing sampling sites was prepared.

A field survey of the Dah Lake (UP) was conducted to explore fish germplasm resources in which a total of 17 fish species belonging to 13 genera could be recorded from preliminary exploratory survey of the Dah Lake.

A total of 365 tissue samples (blood, muscle) and 75 samples of fish species recorded during explorations, were collected and deposited in tissue repository of the Institute. Preliminary surveys indicate possibility of recording of several more species and rich insights into distribution and abundance of fish species in this important river system of the country, as the explorations intensify.

Fig. 39. Exploration of fish diversity in River Mahanadi

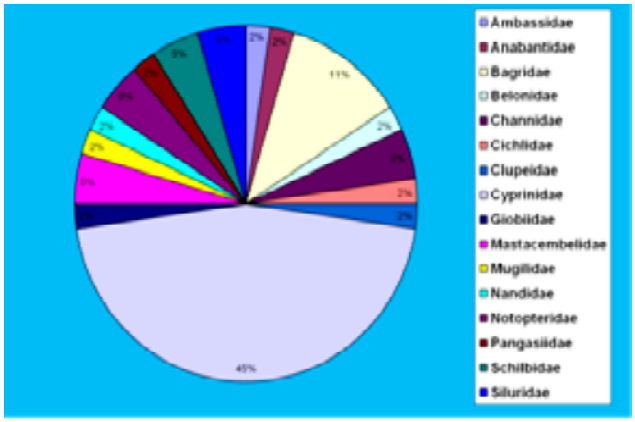


Fig. 40. Family-wise fish diversity in River Mahanadi and its tributaries

Fig. 41. Collection of tissue samples and biological parameters from River Mahanadi

Project Title: Harmonizing biodiversity conservation and agricultural intensification through integration of plant, animal and fish genetic resources for livelihood security in fragile ecosystem

Project Period: September, 2009-August, 2013

Project Personnel: K.K. Lal (CCPI), P. Punia, Vindhya Mohindra, L.K. Tyagi, Rajeev Kumar Singh, U.K. Sarkar, A.K. Pathak and J.K. Jena

Funding Agency: GEF/NAIP

Exploratory surveys were conducted in various rivers/streams/reservoirs/lakes of three districts Chamba (H.P.), Udaipur (Rajasthan) and Adilabad (A.P.) during April 2012-Jan 2013 (Fig. 42). In district Chamba, altogether, 22 sites were explored covering an altitude of approximately 1900 ft to 5700 ft in the main channel of River Ravi along its tributaries with 12 new rivers/streams namely Nainikhad, Khaziyar, Pukhari, Chanzunala, Teesa, Sahikhoti, Ki nala, Dali Khad, Mahla ki Kahd, Bhatri Khad and Machater Khad. In district Udaipur, explorations of fish germplasm were conducted for 6 rivers/streams (Beltz, Jhakham, Bari, Tidi, Madar ka naka and Sisorama) and Jaismand lake. In district Adilabad, 6 water bodies viz., Pranahita, Godavari, Kaddam, Satnala, Chityal and Kadam dam were explored. A total of 382 accessions from 10 species of genus *Nemacheilus, Tor, Garra, Glyptothorax, Glyptosternum, Schizothorax, Crossocheilus, Labeo, Puntius* and *Cyprinus* were collected from Chamba (H.P.). In district Udaipur, a total of 279 accessions of 37 species such as *Nemachielus, Schistura, Tor, Labeo gonius, Garra gotyla, Channa marulius*; some minor carps and catfishes including *Sperata seenghala, Mystus bleekeri* and *Xenantodon cancila,* etc., were collected. Altogether, 259 accessions from 54 species including *Tor sp., Labeo sp., Ompok sp., Gonopopterus sp., Silonia sp., Etroplus sp., Clupisoma sp.* and *Channa sp.* were collected across the study area in Adilabad (A.P.). Genetic characterization of fish species using 655 bp mitochondrial COI gene (n=99 accessions from Chamba, n=141 accessions from Udaipur and n=215 from district Adilabad) and 307

a. River Ravi at Chamba b. Pranahita River at Adilabad c. Tidi River at Udaipur

Fig. 42. Exploration of aquatic resources for fish germplasm in three identified regions

Fig. 43. Collection of sperm and running ripe oocytes for cryopreservation and fertility assessment for *Labeo icar* at Udaipur

bp mitochondrial Cytochrome b gene (44 accessions district from Chamba, 72 accessions from Udaipur and 91 accessions from Adilabad) has been accomplished during the study period. Detailed taxonomic description of a new *Labeo* species from district Udaipur (Rajasthan) namely *Labeo icar* has been accomplished. A new species, *Rita sp. nov.* collected from a tributary of River Godavari has been characterized and in the process of validation. The known endemic species from the region have conflicting descriptions in literature.

Milt cryopreservation of *Labeo spp.* (45.5 ml) was carried out successfully from District Udaipur using two different types of extenders (NBFGR 7 & 7B) (Fig. 43). Fertility trials were undertaken which resulted in 40-50% fertility of eggs by cryopreserved milt and 65-75% fertilization by fresh milt.

Project Title: Germplasm exploration, assessment and documentation of the freshwater fish diversity of Uttar Pradesh

Project Period: May, 2011 – March, 2013

Project Personnel: U.K. Sarkar (PI) and A.K. Pathak

Funding Agency: UP State Biodiversity Board

Exploration and assessment of fish diversity

Rapid explorations of fish diversity were conducted in 12 main rivers, 7 tributaries, 12 reservoirs and four lakes/'*taals*' of Uttar Pradesh and information on the pattern of fish diversity, species composition, distribution and relative abundance were recorded. A total of 124 native freshwater fish species and belonging to 26 families were recorded from all the rivers explored (Figures 44-45). Overall high species diversity (90 species) was recorded in Ganga River, followed by 85 species in Ghaghara, 78 species in Gomti, 75 in Betwa, 68 in Sharda, 62 in Tons, 63 in Rapti, 60 in Chambal and 50 species in Sone river. Comparative evaluation of fish diversity in the different rivers were carried out using several indices like Shannon -Weaver index, similarity index, community dominance index and evenness index. The Shannon -Weiner biodiversity indices of 12 rivers were calculated of which Ganga River showed highest diversity of species (4.14),

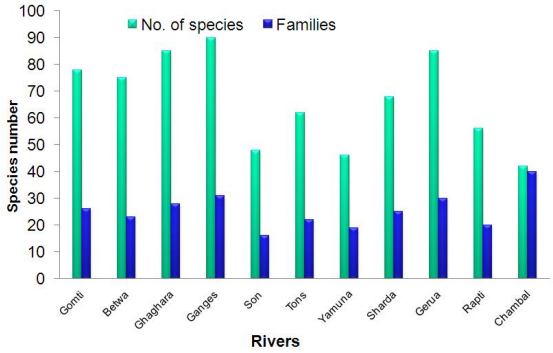


Fig. 44. Fish species and family richness in selected rivers of Uttar Pradesh

Fig.45. Field explorations and collection of fish samples for fish diversity assessment

followed by Gerua (4.17), Ghaghara (4.16) and Gomti (4.16) rivers. The similarity in species composition among 11 different rivers as per Bray–Curtis analysis revealed four groups with similarity cut-off value of 0.6 and the similarity between Sharda and Gerua rivers was found to be strong (Fig. 46). New biogeographical distribution of *Glyptothorax conirostris* and *Amblyceps mangois* (Family Amblycipitidae) was documented from Gomti, Ganga and Ramganga rivers (Fig. 47). The species described from different rivers and tributaries were assessed under various categories as per IUCN

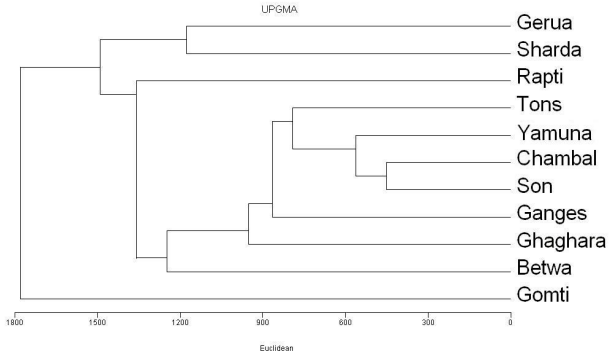


Fig. 46. Dendogram showing similarity between species of the selected rivers of UP

(2012). It was found that a total of 10 species were included as 'near threatened' (NT), 1 species as 'vulnerable' (VU), 78 species as 'least concern' (LC), 2 species as 'data deficient' (DD) and 34 species were under 'not evaluated' (NE) categories (Fig. 48).

Biological traits and GIS mapping

Analysis of data from different rivers revealed that the number of individuals collected from each river were directly proportional to the number of species in the respective rivers. The total fish catches was comprised of the major caps (*Labeo rohita, Catla catla* and *C. mrigala*) about 4%; minor carps (*L. calbasu, L. gonius, C. reba, L. bata, L. boggut,* etc.) 24%; catfishes (*Mystus cavasius. Wallago attu, M. tengara, Separata aor,* etc.) 54%; and small indigenous fishes contributed about 70%. Biological data of some important species collected from the rivers Gomti, Ghaghara, Betwa, Rapti and Ganga was estimated and represented according to their maturity levels (7-8). Length-weight relationship (LWR), size frequency, condition factor and reproductive potential of 25 species were also studied. The growth components b varied from 1.11 to 3.07 in Gomti River, 0.11 to 3.5 in Ganga River while 1.14 to 2.66 in Rapti River. Three large rivers of the basin viz., Ganga, Betwa and Gomti were assessed to determine the fish assemblage integrity index (FAII) for prioritization of sites for conservation and to measure the ecological integrity. Potential areas were identified based on indices like rarity index (RI) and origin index (OI) and value of each index between zones were compared.

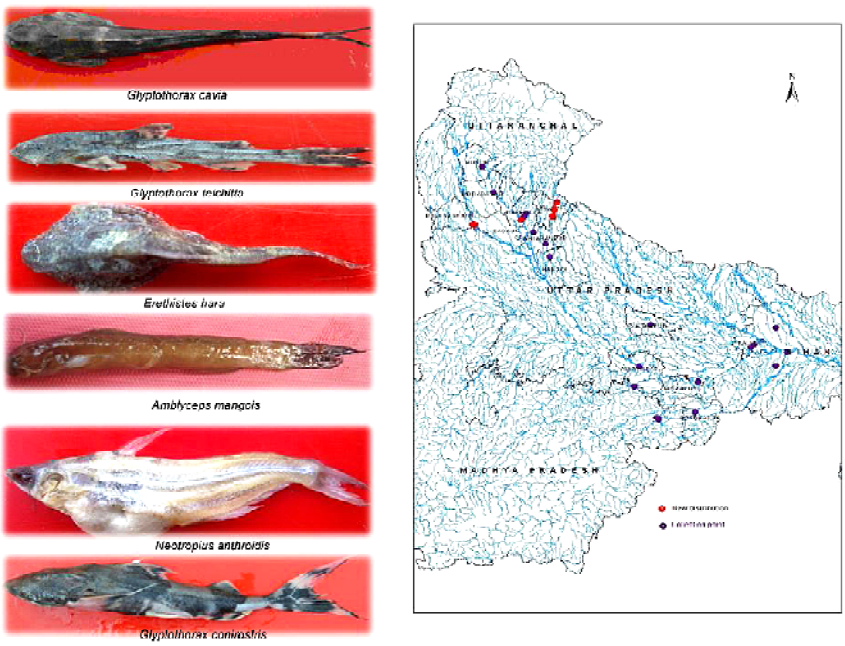


Fig. 47. New distribution records of fish species in UP

Fig. 48. Some rarely collected fish species from UP

Digital GIS base map comprised of vector layers of the water bodies, basins and administrative boundaries (districts and state) at the scale of 1:250,000 were prepared in shape file format using ARC GIS 10 desktop GIS software (Fig. 49). These GIS based digital base maps were used for depicting diversity index, distribution and abundance for different species and family occurrence in rivers and tributaries. GIS maps were prepared which included maps representing collection sites, sites representing the new distribution of species and their abundance, group-wise distribution and its abundance, representation of family and its abundance, and species and family-wise diversity and distributions. Fish habitat attributes like pH, dissolved oxygen, depth, water flow, substrate, etc., collected from the sampling sites, were arranged on the GIS base map and analyzed with respect to species abundance and geographically weighted abundance distributional thematic map was produced.

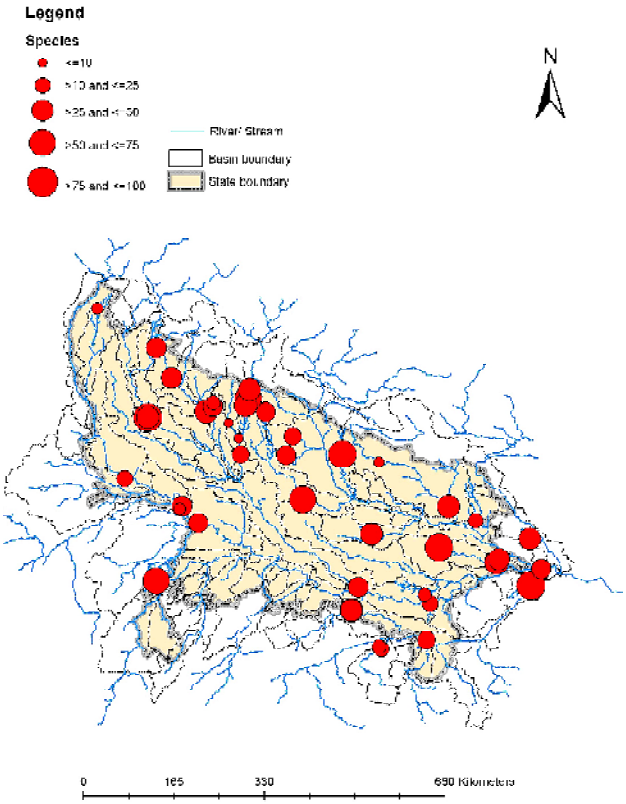


Fig. 49. GIS map showing sites of exploration and fish species distribution

Project Title: Fish diversity of Ramgarh and Bakhira Lake: comparison of present status with pristine data for conservation and sustainable utilization

Project Period: February, 2013 –March, 2015

Project Personnel: A.K Pandey (PI)

Funding Agency: UP State Biodiversity Board, Lucknow

Work was initiated in two important wetlands of Uttar Pradesh. Ramgarh Lake, a natural oxbow-lake formed by river Rapti, is situated to the south-east of Gorakhpur and covers an area of about 723 ha with the catchment area around 653 ha (Fig. 50). The condition of the lake has deteriorated in recent years as the water area reduced from 723 to 669 ha and depth from 4.5 to 3.8 m in 1998 and 2006, respectively. The fish catch in Ramgarh Lake was dominated by *Catla catla, Labeo rohita, L. calbasu, Cirrhinus mrigala, Notopterus notopterus, Gadusia chapra, Setipinna phasa, Pseudotropius atherinoides, Heteropneustes fossilis, Clarias batrachus, Mystus seenghala, M. vittatus, Channa punctatus, C. marulius, C. striatus, C. gachua,, Puntius ticto, P. sarana, Oxygaster bacaila, Chanda ranga, Wallago attu, Pangasius pangasius, Bagarius bagarius, Colisa fasciatus, Mastacembalus puncalus, M. armatus, Amphipnus cuchia* and *Xinantodon cancila* (Fig. 51).

Fig. 50. Ramgarh Lake, Gorakhpur, UP

Fig. 51. Fish catch of Ramgarh Lake

4.4 *EX SITU* CONSERVATION

Project Title: Genetic characterization of commercially important fishes from the Western Ghats and marine ecosystem using biotechnological tools

Sub-project III: Conservation of endemic freshwater fishes of the Western Ghats through milt cryopreservation and captive breeding

Project Period: April, 2009 – March, 2013

Project Personnel: V.S. Basheer (PI), A. Gopalakrishnan, P.R. Divya, T. Raja Swaminathan and A. Kathirvelpandian

Funding Agency: Institutional

Osteochilichthys longidorsalis

Osteochilichthys longidorsalis, locally known as modon, is an endemic and endangered fish to the Western Ghats (Fig.52). Its distribution is restricted to few rivers of Kerala and Karnataka. Having high growth potential, it can grow upto more than 5 kg in size. It is an excellent table fish and in younger stages it can be used as ornamental fish also.

Fig. 52. *Osteochilichthys longidorsalis*

Samples of *O. longidorsalis* (325-600 g; n=10) were collected from Chalakkudy River, Kerala. The mature male fish were given Ovaprim injection @ 0.2 ml/kg body weight and milt was collected from individual fish after 12 hours of the injection. The pooled milt showing high motility (above 60%) was used for cryopreservation. The duration motility of the sperm ranged from 55-90 seconds (Table 11). Spermatocrit of the milt ranged from 30.5-42% and sperm density from 7.0-8.5 x 10^8 SPZ (Table 12 and 13). The extender composition NBFGR 9B was selected to cryopreserve the milt. Dimethyl sulphoxide (DMSO) and Ethylene glycol at two rates viz., 5% and 10% were used as cryoprotectant. The ratio of milt, extender and cryoprotectant was kept as 1:3.5:0.5. The straws were filled with extender, milt, cryoprotectant mix and kept on ice for 10 minutes for bringing down temperature to 0°C, then 10 minutes on liquid nitrogen vapour phase for bringing down temperature to -80°C and then plunged in to liquid nitrogen for final freezing at-196°C.

Table 11. Duration of motility of fresh milt of *Osteochilichthys longidorsalis*

Sample	Motility of raw milt	Time (Vigourous) (Sec)	Oscillation (Sec)	Total (Sec)
1	5	65	95	160
2	5	65	90	155
3	4	60	90	150
4	5	55	80	135
5	5	55	80	135

Table 12. Sperm density of *O. longidorsalis*

Sample	Motility	Sperm density (per ml)	pH
1	5 (100%)	8.0× 10^8 SPZ	7.4
2	5 (100%)	8.0× 10^8 SPZ	7.4
3	5 (100%)	7.5× 10^8 SPZ	7.5
4	4 (80%)	7.2 × 10^8 SPZ	7.5
5	4 (80%)	7.2 × 10^8 SPZ	7.5

Table 13. Spermatocrit of *O. longidorsalis*

Sample	Height of total milt in haematocrit tube	Packed Cell Volume	Spermatocrit (%)
1	5.8	3.0	51.7
2	6.3	3.4	54.0
3	6.5	3.2	49.2
4	7.0	2.9	41.4
5	6.6	2.8	42.4

Viability and motility of the stored milt

After one day of the storage in liquid nitrogen, two straws from each extender used were taken out, thawed at 37°C and motility was checked using water as activator. Motility score of the stored milt was in the range of 2.0 -3.5 and the motility time was in the range of 25-55 seconds in DMSO, where as in ethylene glycol motility score range between 2.0-3.0 and the motility time was in the range of 22-30 seconds (Table 14).

Table 14. Motility score and time of cryopreserved milt of *O. longidorsalis*

Cryoprotectant	Motility score of cryopreserved milt	Time (Sec)
DMSO 5%	2.0	25
DMSO 10%	3.5	45
Ethylene Glycol 5%	2.0	20
Ethylene Glycol 5%	3.0	30

Fertility trial

The fertility trial was carried out using cryopreserved milt and fresh milt as control. Good quality of eggs obtained from two fishes were pooled for the experiment. A total of 200 eggs (200 ml) were fertilized with thawed milt from a single straw. Fertilization rate was calculated after eight hours. Highest fertilization was observed in DMSO 10% (60.4%), followed by ethylene glycol 10% (42.2%), DMSO 5% (38.7%) and ethylene glycol 5% (35.7%). Hatching percentage was also highest in DMSO 10% (40.4%), followed by ethylene glycol 10% (19.6%), DMSO 5% (16.3%) and ethylene glycol 5% (7.5%) (Table 15). From the experiment it is evident that, the cryoprotectant DMSO at 10% rate, which showed best result in compare to control, is the best extender for long term cryopreservation of the milt of *O. longidorsalis*.

Table 15. Fertility of cryopreserved milt of *O. longidorsalis* with different cryoprotectants

Cryoprotectant	Total eggs	Fertilization %	Hatchling %	% hatching as that of control
DMSO 5%	238	38.7	16.3	31.4
DMSO 10%	225	60.4	40.4	77.8
Ethylene Glycol 5%	224	35.7	7.5	14.5
Ethylene Glycol 10%	218	42.2	19.6	37.8
Control	290	63.8	51.9	100

Puntius sarana subnasutus

Puntius sarana subnasutus is a medium sized carp, having culture potential in the Peninsular states of India where as its juveniles have high ornamental value. It has a distribution throughout the Western Ghats. The fish is having good demand among local people. The population of this species is declining in many of its habitats. Hence this fish was selected for cryopreservation of milt.

The fishes were collected from wetlands. The mature male fish were given Ovaprim injection @ 0.2 ml/kg body weight. The pooled milt showing more than 60% motility was used for cryopreservation. The milt quality was checked using tap water as an activator and the duration motility of the sperm ranged from 50-120 seconds (Table 16). Spermatocrit of the milt ranged from 30-36% and sperm density from 7.5-8.4 x 10^8 SPZ (Tables 17 and 18). NBFGR 9B, which had given good results earlier, was used as extender for cryopreservation. Two cryoprotectants, DMSO and ethylene glycol, at two rates (5 and 10%) were used to study the efficacy of cryoprotectants.

Fig. 53. (a) *Puntius sarana subnasutus*, (b) Collection of milt, (c) Experiment on fertility trial of cryopreserved milt and (d) Development of *Puntius sarana subnasutus* produced using cryopreserved milt

Table 16. Duration of motility of milt of *Puntius sarana subnasutus*

No.	Quantity	Motility of raw milt	Time (Vigourous) Sec	Oscillation Sec	Total Sec
1	0.2ml	5	50	60	110
2	0.25ml	5	60	50	110
3	0.2 ml	5	50	65	115
4	0.2ml	5	55	65	120

Table 17. Sperm density of *Puntius sarana subnasutus*

Milt sample	Total volume of milt collected	Sperm density (per ml)	pH
1	0.2ml	7.0×10^8 SPZ	7.3
2	0.25ml	8.4×10^8 SPZ	7.5
3	0.2 ml	7.2×10^8 SPZ	7.4
4	0.2ml	8.0×10^8 SPZ	7.3

Table 18. Spermatocrit of *P. sarana subnasutus*

Milt sample	Height of total milt in haematocrit tube	Packed Cell Volume	Spermatocrit (%)
1	6.5	2.4	36.9
2	6.3	2.2	34.9
3	6	2.1	35
4	7	2.5	35.7

Viability and motility of the stored milt

After one day of the storage in liquid nitrogen, one straw from each cryoprotectants used were taken out, thawed at 37°C and motility was checked using water as activator. Motility score of the stored milt was in the range of 1.0 -3.5 and the motility time was in the range of 20-50 seconds in DMSO, where as in ethylene glycol motility score range between 0.5-2.0 and the motility time was in the range of 20-40 seconds (Table 19).

Table 19. Motility score and time of cryopreserved milt of *P. sarana subnasutus*

Cryoprotectant	Motility score of cryopreserved milt	Time (Sec.)
DMSO 5%	1.0	20
DMSO 10%	3.5	50
Ethylene Glycol 10%	2.0	40
Ethylene Glycol 5%	0.5	20

Fertility trial

The fertility trial was carried out using cryopreserved milt and fresh milt as control (Fig. 53). A total of 200 eggs (200 ml) were fertilized with thawed milt from a single straw. Fresh milt was used as control. Highest fertilization was observed in DMSO 10% (44.4%), followed by DMSO 5% (36.4%), ethylene glycol 10% (34.3%), and ethylene glycol 5% (7%) (Table 20). Hatching percentage was also highest in DMSO 10% (44.4%) followed by DMSO 5% (12.5%) and ethylene glycol 10% (7.2%) (Table 20). Hatching did not take place when ethylene glycol @5% was used as cryoprotectant. From the experiment it is evident that, the cryoprotectant DMSO at 10% is the best cryoprotectant for long term cryopreservation of the milt of *P. sarana subnasutus*.

Table 20. Fertility of cryopreserved milt of *Puntius sarana subnasutus* with using different cryoprotectants

Cryoprotectant	Fertilization %	Hatching %	% hatching as that of control
DMSO 5%	36.4	12.5	19
DMSO 10%	52.8	44.4	67.4
Ethylene Glycol 10%	34.3	7.2	10.9
Ethylene Glycol 5%	7	0	0
Control	62	65.9	100

Project Title: Genetic approaches for conservation of prioritized Indian fish species

Sub-project III: Development of breeding and sperm banking protocols for prioritized Indian finfishes belonging to groups catfishes, featherbacks, murrels and carps

Project Period: April, 2009 – March, 2013

Project Personnel: S. Raizada (PI), K.K. Lal, P.K. Varshney and A.K. Yadav

Funding Agency: Institutional

Breeding of *Ompok bimaculatus*

Ten pairs male and female of *O. bimaculatus* were subjected to induced breeding in indoor conditions using commercial preparation containing sGnRH anologue and dopamine antagonist at the dose rates ranging from 0.5-0.8 ml/kg body weight in case of females and 0.5 ml/kg in case of males. The most effective dose was found to be 0.7 ml/kg for females that yielded spawning of all the females naturally in the FRP tanks. No difference in breeding was observed with and without substratum (*Hydrilla reticulata*) and also when kept under translucent light (translucent covering) and shade (green netting) conditions. The breeding behaviour was recorded. The fertilization and hatching percentage was recorded to be 75-90% and 80-90%, respectively. The developmental stages of embryo were recorded and duration hatching was observed to be 21±1 h. Yolk-sac was completely

absorbed in 48 hours at the temperature 27-30^{0}C. High rate of cannibalism was noted and after 10 days of rearing, a survival of 10.4% was recorded. This work will provide the first baseline report for standardization of protocol of captive breeding of *O. bimaculatus* in hatchery condition without stripping (Fig. 54).

Fig. 54. Fingerlings of *O. bimaculatus* produced by induced breeding

Breeding of *Channa striatus*

After attaining success in induced breeding of C. *striatus* under pond conditions during 2011, the efforts were made to spawn the fish under indoor hatchery conditions at NBFGR, Lucknow during July, 2012. Three pairs comprising of 2 females (weight 450-550 g) and 3 males were kept in circular hatching pools of portable and cemented carp hatchery having flow-through system. The female fishes were given variable doses of commercial preparation containing sGnRH analogue and dopamine antagonist whereas, all males were given a dose @ 1.0 ml/kg body weight. Out of six females, two females spawned naturally. The eggs were pale yellow and floating, and settled all around the margins of central screen (Fig. 55). However, no embryonic development was recorded. One of the likely reasons could be non-synchronization of the sexes as these fishes are nest-builders. More work need to be done to overcome this problem particularly identification of maturity condition in male.

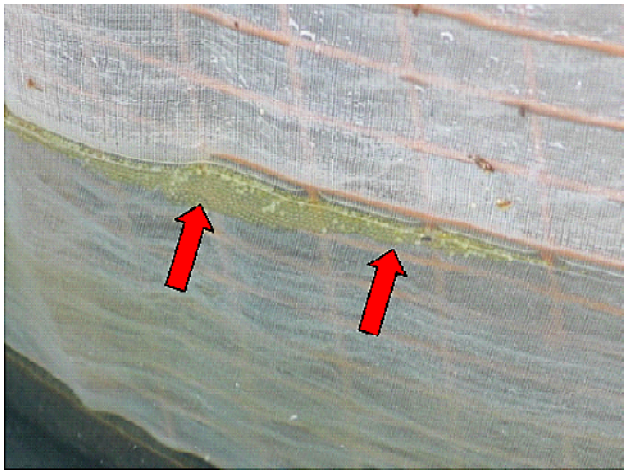


Fig. 55. Floating eggs of *C. striatus* in the carp hatching tank

Project Title: Neuroendocrine regulation of gonadal maturation in the golden mahseer, *Tor putitora* hypothalamus

Project Period: April, 2008 – March, 2013

Project Personnel: A.K. Pandey (PI), P.K. Varshney and A.K. Yadav

Funding Agency: Institutional

Hypothalamus

Hypothalamus in the vertebrates brain consists of groups of neurosecretory cells which mediate the endocrine responses of organisms in adjustments to the environmental changes via modulating the secretion of various releasing (-RH) as well as inhibiting hormones (-IH). Hypothalamus also contains specifically sensitive receptors to the hormone that regulate its activity through feedback mechanism. Recent evidences suggest that the hypophysial (pituitary) functions of the teleosts are also modulated by the hypothalamic neurohormones, neuromodulators and neuropeptides but the regulatory mechanisms are not yet clearly understood. An attempt was made to record changes occurring in hypothalamus and pituitary gland of the seabass, *Lates calcarifer* in relation to ovarian maturation. Brains (along with pituitary) of the female seabass were surgically removed and fixed immediately in Bouins solution. 5-6 µm thick paraffin sections of both the glands were stained in specific stains.

Hypothalamo-hypophysial system of the females *Lates calcarifer* consisted mainly of nucleus preopticus (NPO), nucleus lateralis tuberis (NLT) and their axonal tracts. NPO was a paired structure situated on either side of the third ventricle dorsal to the optic chiasma (Fig. 56). The dorsal portion of NPO comprised sparsely distributed neurons while the neurosecretory cells were closely packed in the vertical aspect of pre-optic area (POA). Based on size of the neurosecretory cells, NPO was further divisible into dorsal pars magnocellularis (PMC) formed of larger neuronal cells and ventral pars

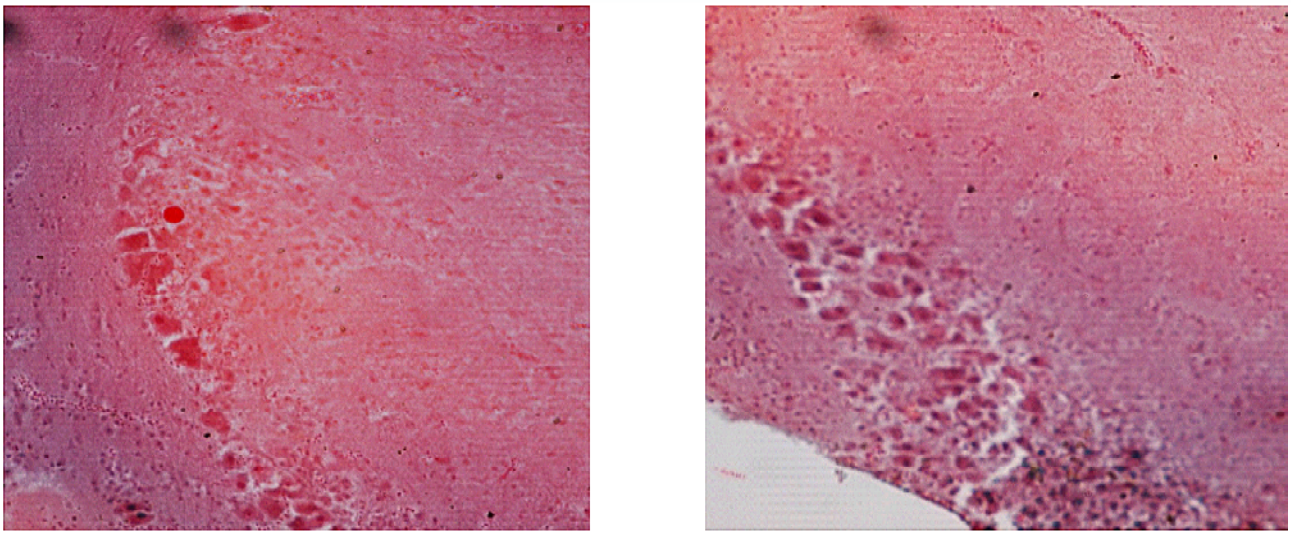


Fig. 56. NPO of (a) immature female and (b) maturing female *Lates calcarifer*

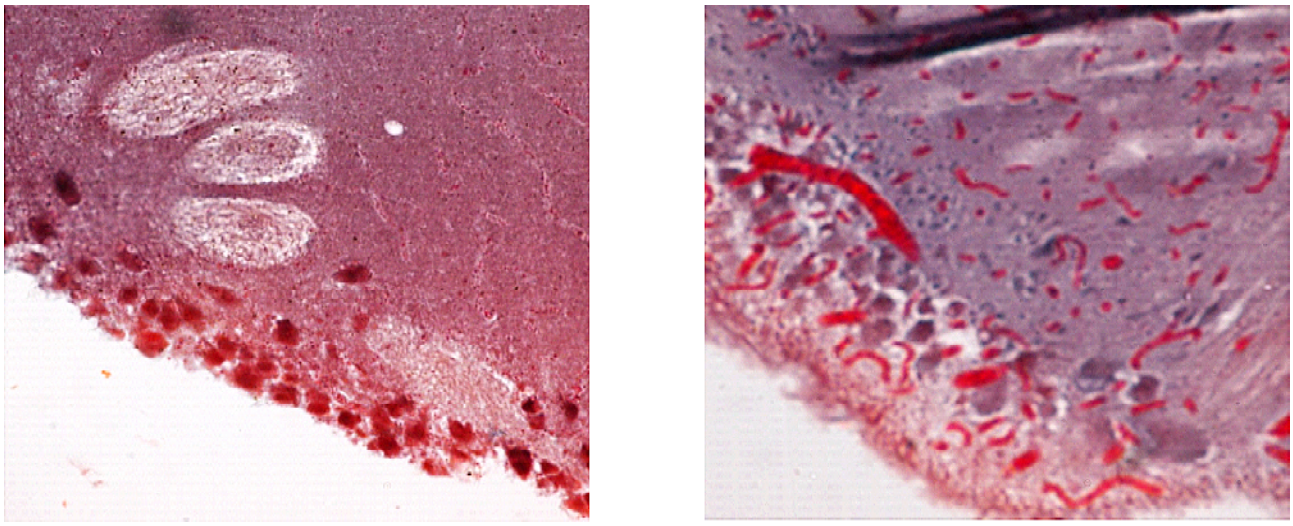


Fig. 57. NLT of (a) maturing female and (b) mature female *Lates calcaifer*

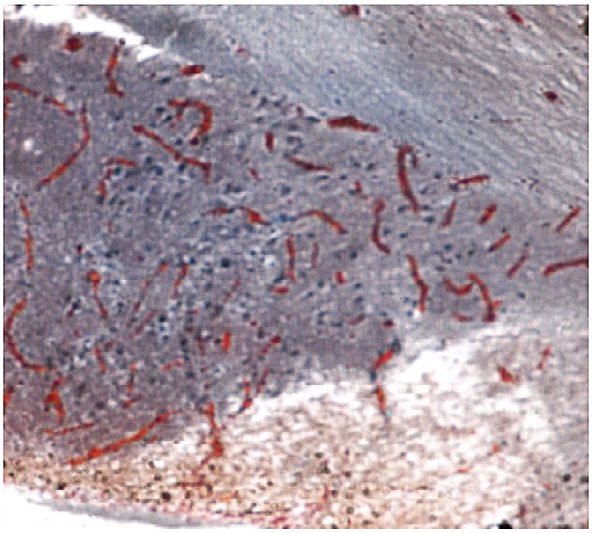


Fig. 58. NPP of immature female *Lates calcarifer* showing less active neurosecretory cells

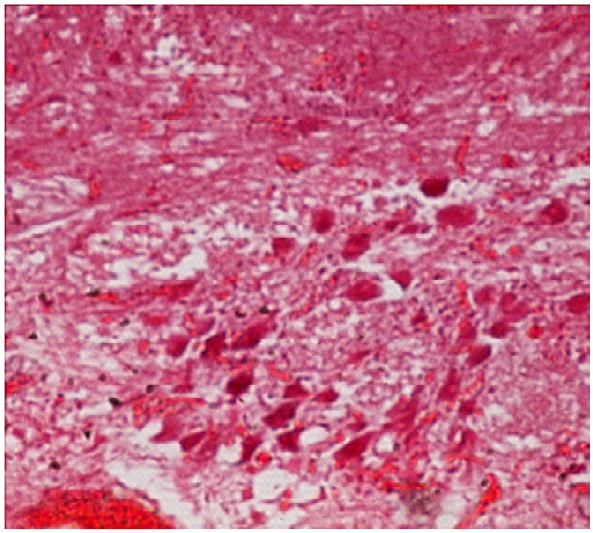


Fig. 59. NPBL of mature female *Lates calcarifer* exhibiting few neurosecretory cells

parvocellularis (PPC) comprising smaller cells. NPO was highly vascularized structure and its neurosecretory cells were positive to aldehyde fuchsin (AF) chrome-alum-hematoxylin-phloxine (CAHP) and acid fuchsin. Neurons of PMC and PPC were generally bipolar and contributed beaded axons to form left and right neurohypophysial main tracts. The neurosecretory cells in NPO of immature females (stage 2-3) were small but stained readily with acid fuchsin. Few acid fuchsin-positive globule-like materials were also encountered among the neurosecretory cells. Among the maturing specimens (stage 5), these were active and laden with the secretory material.

Nucleus lateralis tuberis (NLT) is the second prominent neurosecretory centre in the brain of teleosts. NLT cells of the female *L. calcarifer* were distributed in the ventral floor of the brain adjacent to the pituitary stalk (Fig. 57). They were negative to AF but stained readily with acid fuchsin and CHAP. These neurosecretory cells were variously shaped and their size ranged from very small to large with polymorphic nuclei. The neurons were usually bipolar but a few uni- or multipolar cells were also seen. Based on the distribution and size, NLT was divided into pars anterior, pars posterior and pars inferior. This structure was highly vascular and several neurons were seen in

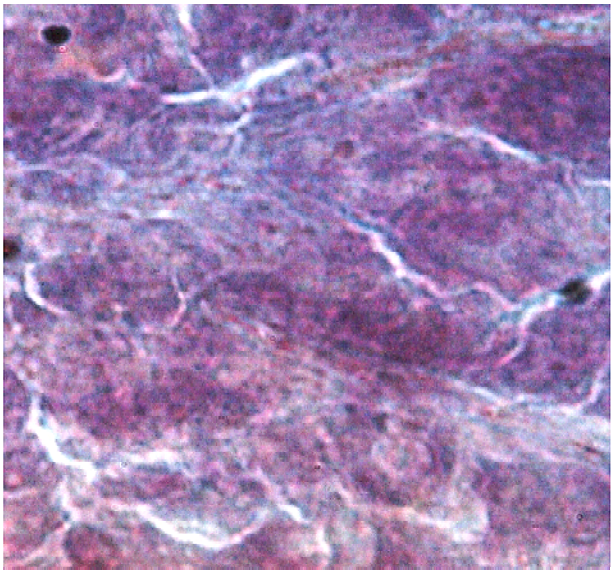


Fig. 60. Hypophysis of immature female *L. calcarifer* exhibiting inactive gonadotrophs

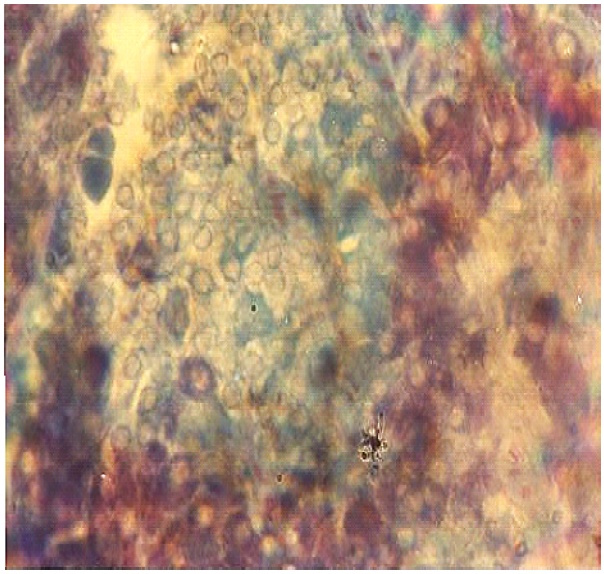


Fig. 61. Hypophysis of maturing female *L. calcarifer* depicting gonadotrophs and somatotrophs in PPD

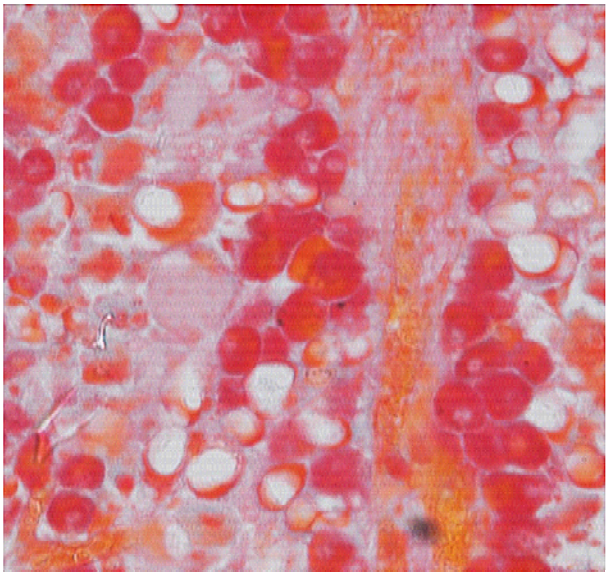


Fig. 62. Hypophysis of mature female *L. calcarifer* showing vacuolization of gonadotrophs. Erythrosine+ve cells (somatotrophs)

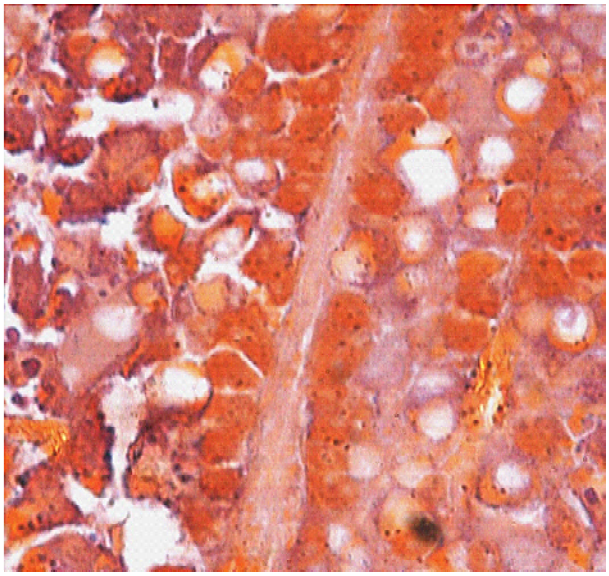


Fig. 63. Hypophysis of mature female *L. calcarifer* showing vacuolization of gonadotrophs. Orange G+ve cells (prolactin cells/lactotrophs)

close association with the blood vessels.. The neurosecretory cells of NLT in immature specimens (stage 2-3) were small but ladden with the secretory materials while they appeared active in mature females (stage 5-6) with enhanced vascularitzation. Some cells depicted vacuolization due to release of the secretory granules. The neuro-hypophysial tract entered the pituitary gland through infundibulum.

Besides the two neurosecretory centres, nucleus preopticus periventricularis (NPP) and nucleus basalis lateralis tuberis (NPBL) were also localized in the brain of *L. calcarifer*. NPP was located close to NPO in pre-optic area (POA) of the brain whereas NPBL, comprosing of a few neurosecretory cells, were located in the forebrain close to the optic lobe (Figures 58 and 59). Though function of NPP neurosecretory centre is not defined, NPBL is implicated in transduction of pheromonal stimuli to the higher centres of the brain.

Hypophysis

The pituitary gland of *L. calcarifer* had a distinct stalk. The three main components of the gland - rostral pars distalis (RPD), proximal pars distalis (PPD) and pars interrnedia (PI) were arranged serially one after the other along the antero-posterior axis (Figures 60-63). PPD of seabass comprised mainly of somatotrophs, lactotrophs (prolactin cells), gonadotrophs and thyrotrophs. Somatotrophs were found distributed among the gonadotrophs. They were acidophilic and stained positive erythrosine and predominated during non-breeding phases. Prolactin cells were also localized in PPD arranged around the sinusoids and stained positive with orange G. Gonadotrophs were cynophilic and stained positive with PAS, AB and aniline blue. Number of these cells increased considerably and became the major cell type of PPD as the maturity advanced. These cynophils exhibited hypertrophy and accumulation of secretory materials varying degrees of granulation as maturity advances while varying degree of vacuolization (degranulation) was observed in relation to ovarian maturation. Some cynophils which do not exhibit hypertrophy or degranulation in relation to maturity were identified as thyrotrophs. In anterior neurohypophysis (ANH), 3-5 acid fuchsin+ve varying sizes of Herring bodies (HB) were also encountered in the matured female specimens.

Project Title: Establishment of a National Repository for conservation and characterization of fish cell lines at NBFGR, Lucknow

Project Period: November, 2010 – November, 2013

Project Personnel: M. Goswami

Funding Agency: DBT, Govt. of India

A National Repository of Fish Cell Lines (NRFC) has been established at NBFGR, Lucknow for conservation and characterization of fish cell lines with all the infrastructural facilities. All the required forms for collection, deposition and distribution of cell lines have been finalized, i.e. NRFC Request form, NRFC Deposit form, NRFC Agreement and Registration form and NRFC Acknowledgement form. Currently, NRFC is maintaining 6 fish cell lines developed at cell culture facility at NBFGR, Lucknow. All the cell lines have been successfully cryopreserved at NRFC. These cell lines have been characterized with both cytogenetic and molecular markers. In addition, recently, two cell lines, namely KCF and HBF from Koi carp and *Horabagrus brachysoma,* respectively, have been submitted to the NRFC by NBFGR Kochi Unit. The facility is now fully functional for the collection, deposition and distribution of cell lines. Details of cell lines are given in Table 21. The cell lines will be authenticated after collection from the depositor and will be maintained in the NRFC. A website to facilitate collection and distribution of fish cell lines has also been developed. It contains all the detailed information and will be soon hosted for its usage.

Table 21. Details of cell lines maintained at NBFGR

S. N.	Name of Cell line	Fish Species	Organ	NRFC No.
1.	PCF	*Puntius chelynoides*	Fin	NRFC001
2.	SRF	*Schizothorax richardsonii*	Fin	NRFC002
3.	TTF	*Tor tor*	Fin	NRFC003
4.	CCF	*Cyprinus carpio*	Fin	NRFC004
5.	WAF	*Wallago attu*	Fin	NRFC005
6.	RF	*Labeo rohita*	Fin	NRFC006
7.	KCF	*Cyprinus carpio haematopterus*	Fin	NRFC007
8.	HBF	*Horabagrus brachysoma*	Fin	NRFC008

4.5 EXOTICS, QUARANTINE AND FISH HEALTH MANAGEMENT

Project Title: Exploration of protozoan and monogenean parasites among carps and catfishes

Project Period: April, 2010 - March, 2013

Project Personnel: Rehana Abidi (PI) and S.M. Srivastava

Funding Agency: Institutional

Carps and catfishes are the most important food fishes in freshwater systems of India. The parasites particularly Protozoans and Monogeneans are responsible for huge losses of eggs, fry and fingerlings of carps and catfishes. Collection of fish species namely *Labeo rohita, Catla catla, Cirrhinus mrigala, Cyprinus carpio, Ctenopharyngodon idella, Hypophthalmichthys molitrix, Carassius auratus, Puntius ticto, Clarius batrachus, C. gariepinus, Heteropneustes fossilis, Pangasianodon hypophthalmus, Mystus sp., Poecilia reticulata, P. sphenops, P. latipinna, Colisa lalia, Osphronemus exodon, Channa striatus* and *C. punctatus* was done from Allahabad, Kanpur, Fatehpur, Hosiarpur, Pratapgarh, Unnao, Raebareli, Barabanki, Faizabad and Lucknow in Uttar Pradesh.

A total 429 fishes were screed for isolation of parasites, out of which, 133 were infected/infested by the parasites. Thus, total prevalence of parasites in fishes was 31% (Table 22). Identification of isolated parasites was done by inspecting live parasites, temporary or permanent stained or unstained slides using compound microscope and NIS-E-B image analysis software.

Parasites were identified as protozoans - *Ichthophthirius multifilis, Trichodina, Chilodonella, Epistylis, Tetrahymena* and *Heteropolaria colisarum;* Flagellates- *Ichthyobodo necator, Hexamita, Piscinoodinium; Microsporan* like *Pliestophora, Myxospora -Hennegua* and *Myxobolus* species. Some new species of monogeneans viz., *Gyrodactylus, Dactylogyrus, Silurodiscoides* and *Bifurcohaptor;* Digeneans adult and metacercaria - *Allocreadium, Diplostomum;* Nematodes and copepods *Argulus, Lernaea,* etc., were found in different organs of fishes (Figures 64 and 65). The intensity of monogenean parasites was highest (more than 200) in *C. auratus,* followed by *H. molitrix, L. rohita* and *C. carpio.*

Table 22. Prevalence of parasites in freshwater fishes

S. N.	Species	Number of fish screened	Number of fish infected	Prevalence %
1.	*Labeo rohita*	43	18	41.86
2.	*L. bata*	07	02	28.57
3.	*Cirrhinus mrigala*	05	02	40.00
4.	*Catla catla*	15	03	20.00
5.	*Cyprinus carpio*	107	24	22.43
6 .	*Ctenopharyngodon idella*	27	06	22.22
7.	*Hypophthalmichthys molitrix*	10	02	20.00
8.	*Carassius auratus*	70	41	58.57
9.	*Puntius ticto*	19	07	36.84
10.	*Clarius batrachus*	14	04	28.57
11.	*C. gariepinus*	17	02	11.76
12.	*Heteropneustes fossilis*	19	02	10.52
13.	*Pangasius suchi*	12	04	33.33
14.	*Channa punctatus*	09	02	22.22
15.	*C. striatus*	15	04	26.66
16.	*Osphronemus exodon*	12	03	25.00
17.	*Poecilia sp.*	27	07	25.92

Protozoan parasites were collected in 80% ethanol for DNA isolation. DNA was extracted by suspending the infected tissues or spores in lysis buffer and incubated at 55 degree Centigrade over night. The DNA content was estimated by agarose gel electrophoresis. Concentration was checked through nanodrop spectrophotometer. The optical density of DNA was 1.80 nm to 1.90 nm. Primers were designed through soft-wares Oligos and Primer3 and were synthesized by sigma-aldrich. Primers were selected on the basis of GC content and variable temperatures and were also taken from the literature. Primers selected for different species of family Myxobolidae are as follows:

S.N.	Primer	Code/Sequences
1.	Myx1Forward Myx1Reverse	CTAATCCCGGTAACGAACGA CGTCCTCGCAACAAACTGTA
2.	Myx2Forward Myx2Reverse	TAATCCCGGTAACGAACGAG CGTCCTCGCAACAAACTGTA
3.	Myx3Forward Myx3Reverse	TCGGTTACGGGGAGAGTATG TCGTTCGTTACCGGGATTAG
4.	Mcer1Forward Mcer1Reverse	CCCGTCGCTACTACCGAGT GATCCTTCCGCAGGTTCAC
5.	Mcer2Forward Mcer2Reverse	AGACACTGGGAGGTGGTGAC CACTGCGTGATCCAACTACG

PCR amplification of myxozoan parasite's DNA was done with above specific primers using four different concentrations of each primer (Figures 66 and 67). For standardization of protocol different concentrations of reaction mixture and variable temperature gradients and number of annealing cycles were applied for the amplification program. Amplified product was of 200 bp.

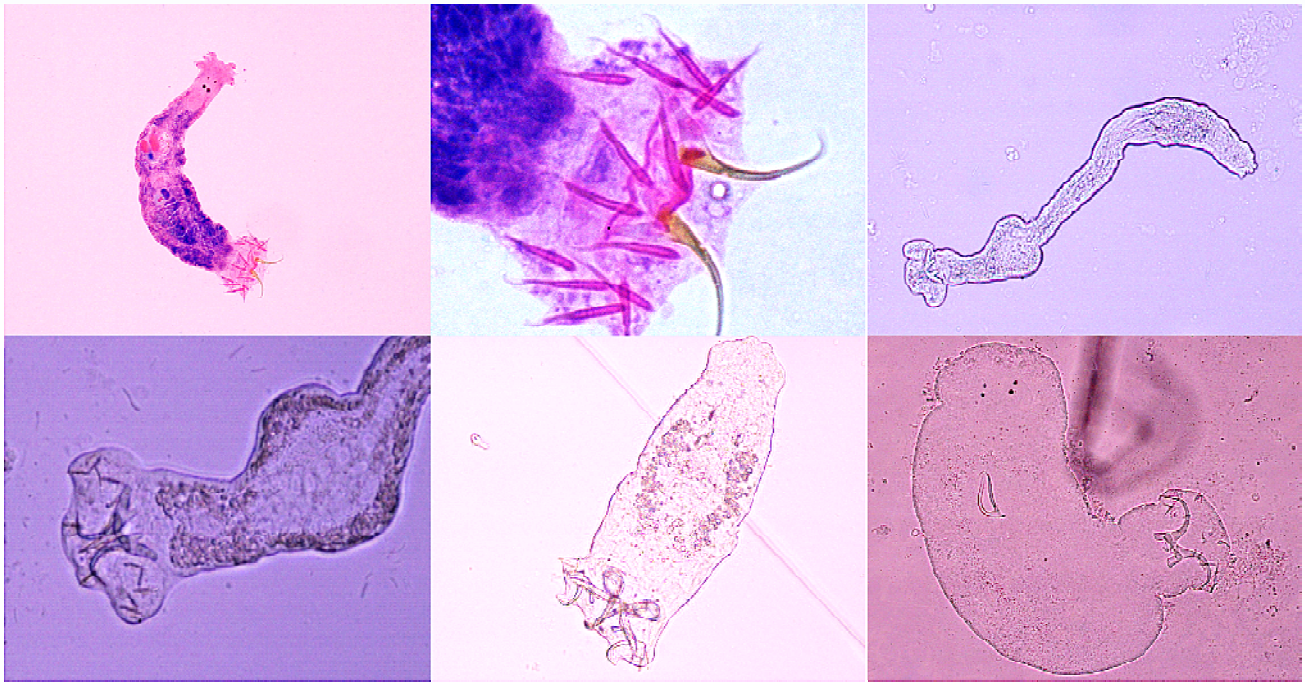


Fig. 64. Some monogenean parasites of freshwater fishes

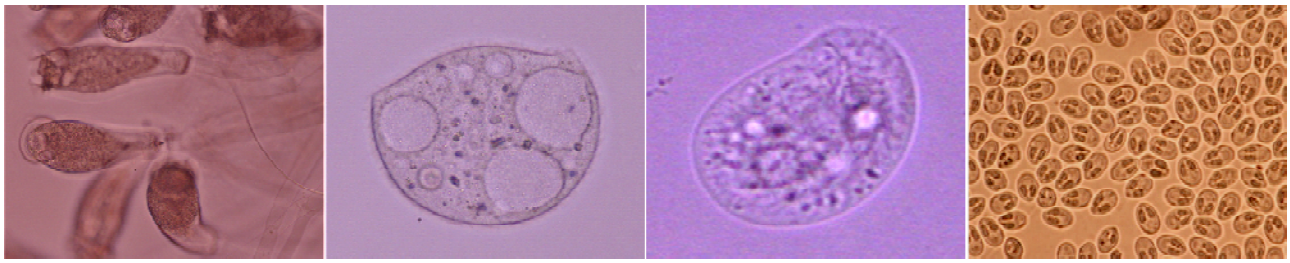


Fig. 65. Some protozoan parasites of freshwater fishes

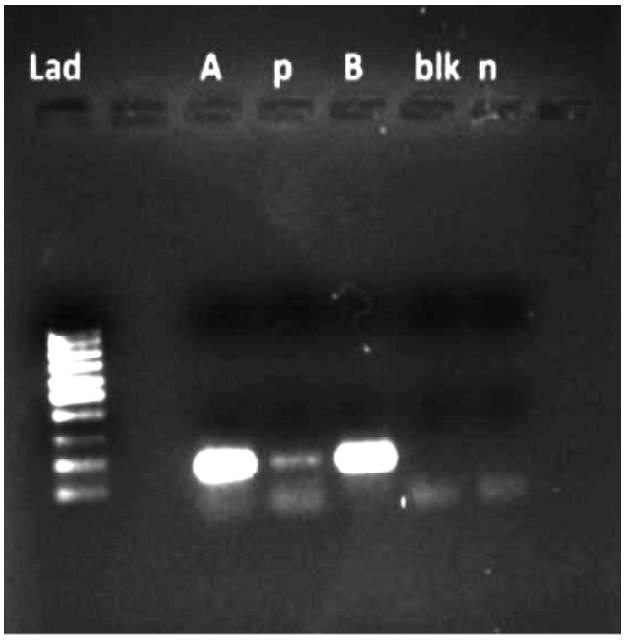


Fig. 66. (Lad) Ladder, (A) Amplified band of the *Myxobolus* sp. DNA sample A (p) Positive control of *Myxobolus cerebralis*, (B) Amplified band of *Myxobolus* sp DNA sample B (blk) blank (n) Negative

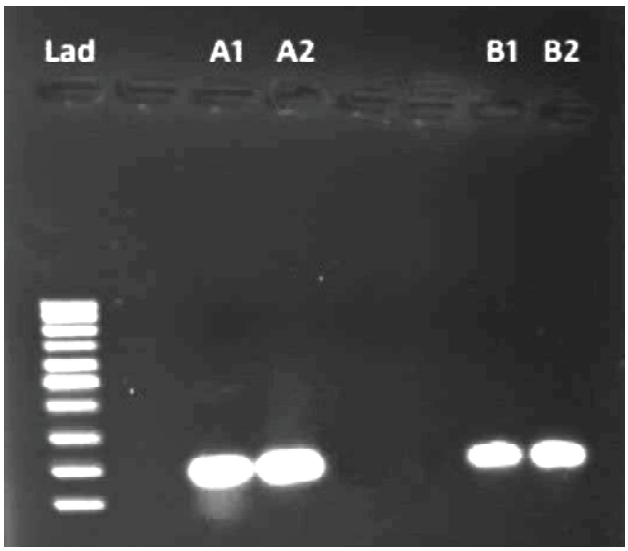


Fig. 67. (Lad) Ladder, (A1) Amplified band of the *Myxobolus* sp. DNA sample A at annealing temperature of 49^{0}C, (A2) Amplified band of the *Myxobolus* sp. DNA sample A at annealing temperature of 52^{0}C, (B1) Amplified band of *Myxobolus sp*. DNA sample B at annealing temperature of 49^{0}C and (B2) Amplified band of *Myxobolus* sp. DNA sample B at anealing temperature of 52^{0}C

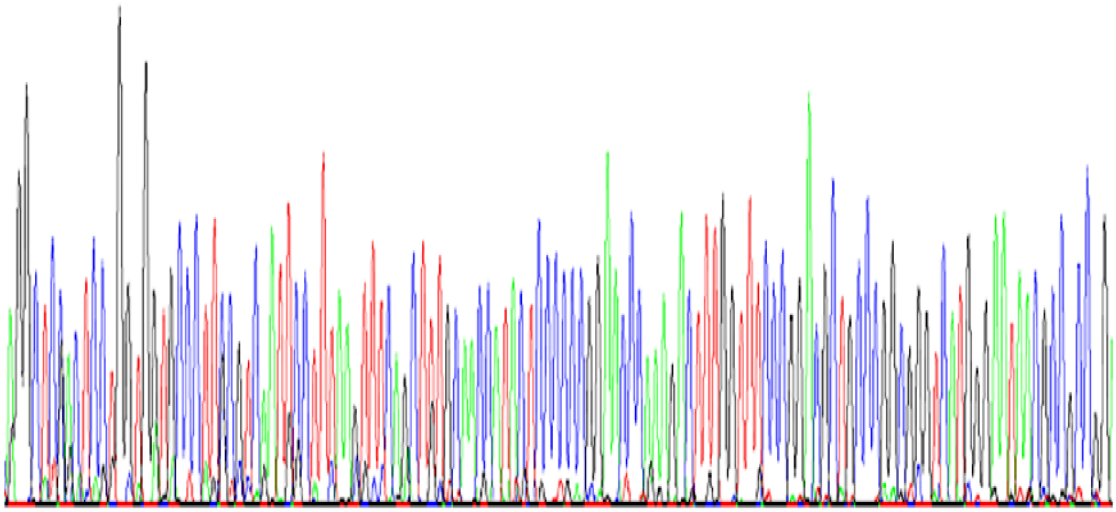


Fig. 68. Partial sequence of *Myxobolus cerebralis* 18 s Gene Clone

The amplified product of *Myxobolus sp.* A showed 100% similarity with *M. cerebralis* 18s ribosomal RNA gene partial sequence and complete sequence. (Accession no. EF370478.1, U96493.1, U96492.1, AF115254.1). The amplified product of *Myxobolus sp.* B showed 99% similarity with *Myxobolus articus* from 18s ribosomal RNA gene, partial sequence (Accession no. JN003830.1), and *Myxobolus articus* from Canada 18S ribosomal RNA gene, partial sequence (Accession no. JN003829.1) (Fig. 68).

Culture of protozoan parasites

For culture of protozoan parasite, *Cyprinus carpio* heavily infected with *I. multifillis* were anaesthetised in MS-222 (Ethyl-m-amino benzoate, methanesulfonate salt 80 mg/l) and killed by cervical dislocation. Fins were cut off and placed in petri dishes with sterile tapwater where free trophonts were allowed to escape from the fins. These trophonts were rinsed with 100 µg/ml streptomycin and transferred to fresh sterile tapwater (20°C) and then transferred to different culture media. Two media were used - Eagle's Minimal Essential Medium (EMEM with Earle's salt solution) and Leibovitz (L-15) supplemented with 5% foetal calf serum (FCS), pencillin (100 µg/ml) and streptomycin (100 µg/ml). EMEM media was used for the initial trials of culture (Fig. 69). Parasites were poured directly in the culture medium (without the cell line). Addition of streptomycin to the media had little effect on maintaining bacterial concentrations in culture. After 24 hours bacterial growth was heavy and there was no protozoan growth.

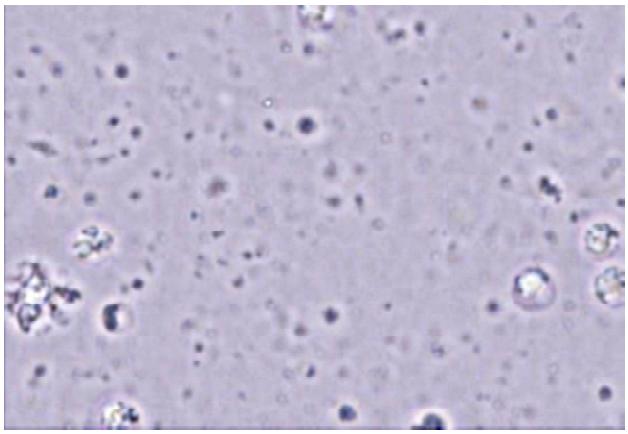


Fig. 69. *Ichthyophthirius multifilis* culture plate

Project Title: Molecular assay for detection of *Aphanomyces invadans* for surveillance and prediction of Epizootic Ulcerative Syndrome (EUS) outbreaks

Project Period: April, 2009 – March, 2013

Project Personnel: P.K. Pradhan (PI), Rehana Abidi, Gaurav Rathore, Neeraj Soood and T. Raja Swaminathan

Funding Agency: Institutional

Molecular detection of *A. Invadans*

An attempt was made to use a PCR-based method to detect EUS in healthy fishes (without typical EUS like gross lesions). Two fish farms, which were affected by EUS for past several years in the Lakhimpur district of Uttar Pradesh, were selected for the study. During the EUS seasons, one of the cultured Indian major carp,

Cirrhinus mrigala, which was found to be severely affected (with almost 100% prevalence), was selected as the test species. In the year 2011-12, although it was possible to detect *A. invadans* in fish having typical EUS like gross lesions, it was not possible to detect *A. invadans*, in healthy fish either during the outbreaks or during EUS off season. Therefore, further attempts were made in the year 2012-13, to collect tissue samples from healthy fishes collected only during EUS outbreak period. Since, it has been seen that in most of the infected fishes, the lesion site was mostly below the dorsal fin region, tissue samples were collected from below the dorsal fin region from 30 fish samples, including two other sites (caudal fin and below the pectoral fin regions). Interestingly, out of 50 healthy fishes, *A. invadans* was detected only in two fishes. In the next attempt, tissue samples were collected from infected fishes from the sites not having any gross lesion. However, out of 30 fishes tested, only one sample was positive for *A. invadans*. Hence, it can be concluded that the available PCR technique (as per OIE diagnostic manual) was probably not sensitive enough for detection of *A. invadans* in healthy fishes/ sites without gross lesions.

Effect of dietary vitamin-C on the disease susceptibility of *Labeo rohita* to experimental infection of *Aphanomyces invadans*

In the present study, an attempt was made to evaluate the effect of one of the widely used immunostimulant i.e vitamin C for increasing disease resistance against EUS. Two hundred advanced fingerlings of *L. rohita* (30-35 g) were randomly divided into two groups of 100 fish each and were fed with 1000 mg vitamin- C supplemented diet and non-supplemented diet. After 20, 40 and 60 days of experimental feeding, fish were challenged with *A. invadans* to test the level of protection. Each fish from experimental group was injected intramuscularly with 10 spores of *A. invadans*, whereas, the control fish group received 0.1 ml autoclaved pond water without spores at the same time and used as test fish. Interestingly, in both the control and experimental groups, there was 100% mortality. The mortalities had started by 18th day and by 24th day there was 100% mortality in both the groups. At the time of morbidity or death, 100% of the fishes had severe swollen hemorrhagic areas and tissue pathology of all the moribund fish was typical of a EUS condition. Both injected and non-injected sides and internal organs (kidney and liver) were extensively occupied by the mycotic lesions and there was severe myonecrosis in large areas of myotome. Hence, the results indicated that dietary vitamin- C did not render protection against experimental infection with *A. invadans*.

Project Title: Understanding of molecular pathogenesis of epizootic ulcerative syndrome (EUS) in fish and development of newer strategies to combat EUS

Project Period: May, 2012 – April, 2015

Project Personnel: P.K. Pradhan (PI), Neeraj Sood, Chandan Debnath and Lopamudra Sahoo (ICAR Complex, Tripura)

Funding Agency: DBT, Govt. of India

Immune response of *Labeo rohita* to *Aphanomyces invadans*

The study was undertaken to determine the sequential changes in innate immune response of *L. rohita* during experimental infection with *Aphanomyces invadans*. Fish used in the experiment were collected from the NBFGR fish farm with weight ranging from 25 - 32 g. *A. invadans* used in the challenge experiment was isolated from EUS affected fish farms of Lakhimpur district of Uttar Pradesh in the year 2010-11. Each experimental fish was injected intramuscularly with 100 spores of *A. invadans*/0.1 ml autoclaved pond water (apw) and the control fish group received 0.1 ml apw without spores. After experimental infection, blood samples were collected at regular intervals and on each sampling day, 12 fishes were used. An aliquot of the blood was heparinised and the remaining part was used for collecting serum. Results of the innate immune parameters indicated that although the superoxide production of the *A. invadans* infected fishes were higher than their respective controls at 1 dpi, as the infection progressed, no significant differences was observed between the control group and *A. invadans* infected fish groups (Fig. 70a). The myeloperoxidase content of serum of the *A. invadans* infected fishes was also not significantly different from the control except 24 dpi (Fig. 70b). Similarly, although, lysozyme and antiprotease content of the serum samples in the initial stages of infection did not show any clear trend, in the advanced stages of infection, the lysozyme content of the infected group of fishes at 18 and 24 dpi was significantly lower than their respective controls (Figures 70 c & d). Hence, it may be concluded that *A. invadans* infection was able to modulate the immune response in *L. rohita* in advanced stages of infection.

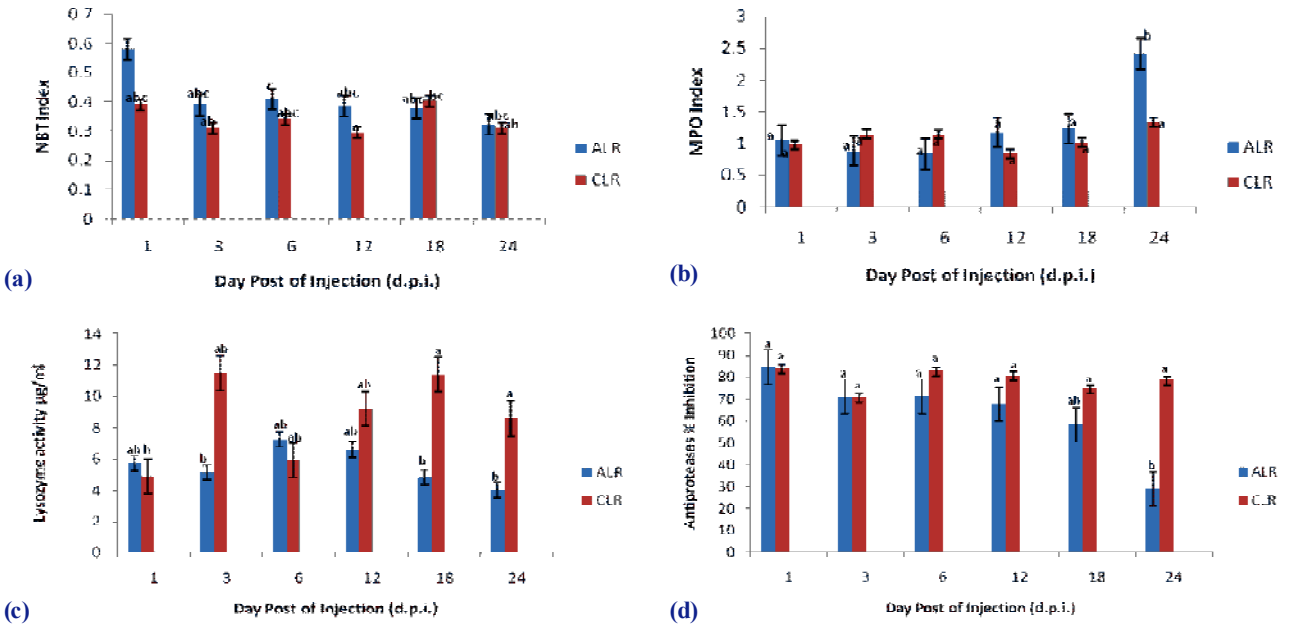


Fig. 70. Innate immune parameters of *L. rohita* infected with *A. invadans*

However, the results of the present experiment should be interpreted with caution, since number of fishes used in each sampling day was less.

Project Title: Isolation and characterization of *Flavobacterium* species from fish and Aquatic Environment

Project Period: August, 2006 – March, 2013

Project Personnel: G. Rathore (PI)

Funding Agency: NBAIM, ICAR

Development of a genus specific DNA probe for detection of *Flavobacterium*

A biotin labeled DNA probe was designed from the highly conserved sequences of the 16S rDNA region and was used for detection of *Flavobacterium* species. Available 16s rRNA sequences (23 nos.) of the type species of *Flavobacterium* were aligned by CLUSTAL W. A unique segment (26 bases) of conserved region of *Flavobacterium* sp. was selected for designing of probe. The probe has been commercially synthesized and labeled with Biotin at the 5′ end. The designed DNA probe was effective in hybridization with 10 reference *Flavobacterium* species. DNA Probe could detect 31 species of *Flavobacterium* from freshwater environment using EZ-taxon server. No significant similarity was found with other aquatic bacteria. PROBEMATCH program could successfully detect 709 nos. 16S rDNA gene sequences of *Flavobacterium* in RDP.

Intergenic Spacer Region (ISR) analysis of *F. columnare* isolated from diseased *C. catla*

ISR of RDC-1 was amplified by PCR and amplicon (~650 bp) was cloned and sequenced. A total of 18 published ISR sequences of *F. columnare* representing five different groups (IA, IB, IIA, IIB & III) of three genomovars were retrieved from GenBank. All the sequences were aligned using the multiple sequence alignment program CLUSTAL W and edited manually for ambiguous bases. tRNA genes within the ISR were predicted with tRNAscan-SE v.1.21. Determination of variable regions in the ISR and percent similarities between different groups were calculated through DNASTAR Lasergene software. Phylogenetic analysis was done by using MEGA v 5.0. The nucleotide sequence of ISR of RDC-1 was deposited to GenBank database with accession number JN825737.

All the analyzed strains contained tRNAIle and tRNAAla genes corresponding to ATCC 49512 positions 96-172 and 286-359, respectively. Comparison of the nucleotide sequences of the tRNAIle/tRNAAla genes revealed 100% identity between respective genes for all groups. A highly conserved domain of 10 nucleotides (GCTGTTAATT), located at positions 60-69, upstream of the tRNAIle, containing a putative box-A-like sequence was identified in all the genomovar groups. On the basis of sequence heterogeneity, three variable regions (V1, V2 & V3) were identified in all the groups. In the V1 and

V2 region of group IIA and IIB, block nucleotide additions and deletions were observed. V3 region was identified as the most hyper-variable among the three variable regions. Base composition within this region was highly variable and mean % GC content ranged from 20.69 to 63.75% among all the five groups. ISR of RDC-1 showed maximum 99.13% sequence similarity with group IIA and 90.4% with group IA strains of *F. columnare*. Mean percent similarities of RDC-1 with group IB, IIB and III were 84.16%, 89.6% and 80.86%, respectively. Phylogenetic analysis based on the V3 region, as well as, complete ISR indicated that strain RDC-1 formed a distinct cluster with *F. columnare* LP8 and EK28 strains belonging to group IIA, separate from the clusters of the other groups. The grouping of RDC-1 was supported by high bootstrap values of more than 90% in 1000 trials.

DNA–DNA hybridization of *F. columnare* isolated from diseased *C. catla*

In order to achieve DNA–DNA hybridization, the following type or reference strains were obtained from the BCCM™/LMG Bacteria Collection, University of Ghent, Belgium: *F. columnare* LMG 10406 (=ATCC 49512), *F. aquatile* LMG 4008, *F. hibernum* LMG 21424, *F. hydatis* LMG 8385, *F. johnsoniae* LMG 1341, *F. micromati* LMG 21919, *F. psychrophilum* LMG 13179 and *F. succinicans* LMG 10402. Genomic DNA relatedness of RDC-1 with *F. columnare* strains, as well as, other reference *Flavobacterium* species was examined using microplate quantitative DNA-DNA hybridization using colorimetric detection with streptavidin-alkaline phosphatase and pNPP as substrate. Genomic DNA from each of the reference *Flavobacterium* species or strains was briefly heat denatured and immobilized to microwells by simple dry-adsorption. Dry-adsorption was conducted by adding DNA solution (each 300 ng/ 50 µl) to micro-wells, evaporating at 37^{0}C overnight and heating to dryness at 60^{0}C for 2 h on polystyrene plates. Genomic DNA of RDC- 1 was sonicated and labeled with photobiotin following the manufacturers' protocol and used as a probe for hybridization with reference species or strains. The hybridization time and temperature was 4 hr and 42^{0}C in 50% formamide, respectively. The percentage DNA similarity was calculated as $100\{(I_{test} - I_{blank})/(I_{ref} - I_{blank})\}$; where I_{test} is the intensity of hybridization between the stain to be tested and reference strain, I_{ref} is the intensity of hybridization of the reference strain with itself and I_{blank} is the background hybridization. Strain RDC-1 was found to show highest 78% DNA homology to strain PB06-113, 53% homology with ATCC 49512 and 45% similarity with PB-172 strain of *F. columnare*. It showed less (48-32%) homology with other species of genus *Flavobacterium*.

Project Title: Development of monoclonal antibody-based marker for monitoring humoral immune response in *Catla catla*

Project Period: April, 2010 - March, 2013

Project Personnel: Neeraj Soood (PI), Gaurav Rathore, P.K. Pradhan and Peyush Punia

Funding Agency: Institutional

Fish monoclonal antibodies (MAbs) are crucial for developing sensitive and specific assays for detecting circulating antibodies to important fish pathogens and thereby, facilitate monitoring of sero-prevalence of pathogens in both captive and natural populations of the fish species. These MAbs can also be used for monitoring specific Ig levels and quantifying Ig+ cells in different lymphoid organs and blood, and therefore, are useful tools in evaluating efficacy of vaccines. In the present study, MAbs were raised against purified serum immunoglobulins of *C. catla*.

Flow cytometric analysis was carried out to quantify surface Ig+ (sIg+) cells in lymphoid organs as well as blood, using G10/1 MAb which revealed that a varying percentage of gated cells from kidney, spleen and blood had sIg (Fig. 71). The gated cells were presumed to be lymphocytes from their low scores of FSC and SSC in the dot plot. It was found that the percentage of sIg+ cells was highest in kidney, followed by blood and spleen. The lowest reactivity of G10/1 MAb was observed in catla thymus. This also implies that majority of thymocytes lack sIg and hence, can be presumed to be T-lymphocytes. Furthermore, the fishes were immunized with killed *E. tarda* and after 2 weeks, lymphoid organs namely; kidney and spleen, as well as, peripheral blood were collected. Mononuclear cells were separated by gradient centrifugation and percentage of Ig+ cells was determined by flow cytometry. An increase in Ig+ cells was observed in kidney, spleen and blood following inoculation of killed *E. tarda*. Therefore, G10/1 MAb can be a useful tool to study the kinetics of Ig+ cells following vaccination.

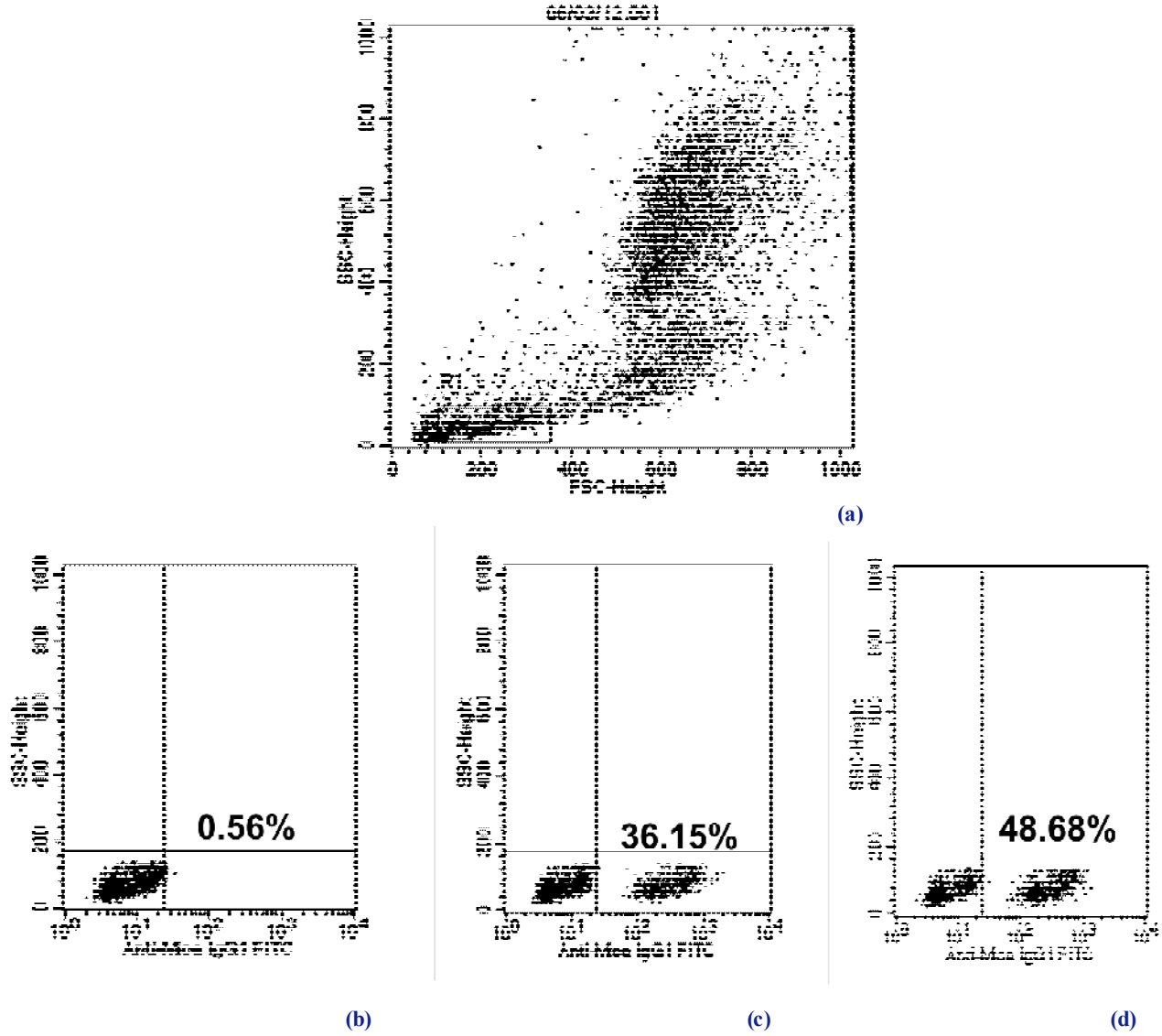


Fig. 71. Quantification of Ig+ cells in kidney of *Catla catla* by flow cytometry: (a) FSC/SSC dot plot of mononuclear cells of kidney showing gated lymphocytes, (b) Fluorescence histogram of gated mononuclear cells in kidney without MAb, (c) With G10/1 MAb in apparently healthy catla, and (d) After 2 weeks of inoculation of killed *Edwardsiella tarda*

Using G10/1 MAb as secondary antibody, the Fc receptors were demonstrated on surface of cells from a macrophage cell line (CCM) which was derived from adherent peripheral blood mononuclear cells of catla. This indicates that anti-catla IgM MAb can be useful reagent to study biological characteristics of immune cells. G10/1 MAb was applied in indirect immunoperoxidase test to demonstrate Ig+ cells in formalin-fixed paraffin-embedded tissue sections of lymphoid organs viz., kidney, spleen and thymus, as well as in blood smears. The Ig+ cells took DAB staining along the margins and there was substantial homogeneity of cellular size in different tissues. These cells were mainly scattered as single cells in kidney and spleen, and at few places, clumps of 2-4 cells were observed. In the kidney, the cells were observed in intertubular tissue whereas, in the spleen, the cells were observed in areas of white pulp. In blood smears, some of the lymphoid cells displayed marginal staining and these cells were assumed to be B lymphocytes whereas, non-reacting cells would be T lymphocytes and thrombocytes. G10/1 MAb did not show any reactivity with erythrocytes and granulocytes. However, no reactivity of the MAb was observed in thymus sections. Thus, the results indicate that G10/1 MAb can contribute significantly to improved understanding of the architecture and functioning of the immune system in the candidate species.

Project Title: Development of *in-vitro* cellular model for assessment of innate immunity parameters of *Labeo rohita*

Project Period: April, 2010 - March, 2013

Project Personnel: Gaurav Rathore (PI), Neeraj Soood, P.K. Pradhan and Peyush Punia

Funding Agency: Institutional

Long-term culture of peritoneal macrophages as *in-vitro* model

Mononuclear cells were harvested from the peritoneal cavity of *Labeo rohita*. Peritoneal macrophages were separated from other cells by density gradient centrifugation. The isolated mononuclear cells were washed with PBS by centrifugation and suspended in tissue culture medium (L-15 with 10% serum) at a concentration of 10^5 cell/ml. The isolated cells were seeded in the tissue culture flasks for attachment of macrophages. The cells were allowed to grow at 30^{0}C for 7 days to obtain confluency.

Confluent monolayer was sub-cultured by trypsinization to obtain first passage. A confluent monolayer was sub-cultured by treatment with one ml of trypsin solution (0.25% trypsin and 0.2% EDTA in PBS) after one wash with 5 ml PBS. The trypsin solution was allowed to act for one minute and then withdrawn. The cells were dislodged by vigorous shaking of flask. Once the detachment of cells was confirmed under inverted microscope, 5 ml of medium was added to the flask to arrest the activity of trypsin. The cell suspension was pipetted to make a single cell suspension after washing with PBS. The cells were seeded to a fresh 25 cm^2 and within 24 hours, attachment of nearly 80% of cells was visible. On day 4 of subculture, 50% of medium was replaced with fresh medium. A complete monolayer formation was observed in 7 days (1st passage). A cell count of 5 X 10^5 cells was sufficient to produce monolayer in 5-7 days after 10th passage. Confluent monolayer of peritoneal macrophages were regularly sub-cultured by trypsinization till a passage level of 40, since its initiation 24 months back.

Cryopreservation of cultured macrophages

After every 10 sub-cultures, the macrophages were cryopreserved at –196^{0}C. Cryopreservation of cultured cells was carried out by mixing equal volume of cell suspension (1x10^6 cells/ml) and cold L-15 medium containing 10% DMSO in cryovials. The cryovials were transferred in liquid Nitrogen (LN_2) after overnight storage at –80^{0}C. Cell viability was checked with trypan blue staining after 6 months and 1 year of storage in LN_2. Cultures at 20, 30 and 40 passage level showed an estimated viability of 76% ± 3.2 (SD) after more than six months to one year of cryopreservation.

Flow cytometry for quantification of phagocytic cells

The phagocytic activity of the cultured peritoneal/kidney macrophages was tested with fluorescent latex beads using flow cytometry. Macrophages (5×10^5 cells) were cultured for 24 h in tissue culture medium. Next day, the cultures were harvested as suspension and inoculated with bead suspension and incubated for 60 minutes in a humid chamber at 30^{0}C. After the incubation period, the cells were washed twice in PBS, followed by fixing with paraformaldehyde. Analysis was carried out on a FACS Caliber [Becton Dickinson] equipped with an argon-ion laser tuned to 480 nm. Ten thousand events were acquired from each sample and data were analyzed using software. Peritoneal macrophages were characterized and gated by their Forward Scatter (FSC) and Side Scatter (SSC) properties. Fluorescent cells of FSC vs FL1 dot plot were enumerated as percent of total events and considered positive for phagocytosis. The results indicate that 93.3% 4.9 SD of cultured peritoneal macrophages possessed phagocytic activity (Fig. 72).

Application of peritoneal macrophages for cytotoxicity testing

Neutral red (NR) uptake assay was carried out for testing the cytotoxicity of mercuric chloride to cultured peritoneal macrophages. Macrophages were grown in 96-well plates containing mercuric chloride in culture medium in different concentration (2 to and 128µg/ml). The lysosomal integrity of macrophages was affected mercuric chloride, evidenced by concentration-dependent decrease in uptake of NR by cells. Nuclear condensation was observed in cells incubated with medium containing 64 and 128 µg mercuric chloride/ml. This shows that peritoneal macrophages can be used as model for cytotoxicity screening of drugs or chemicals.

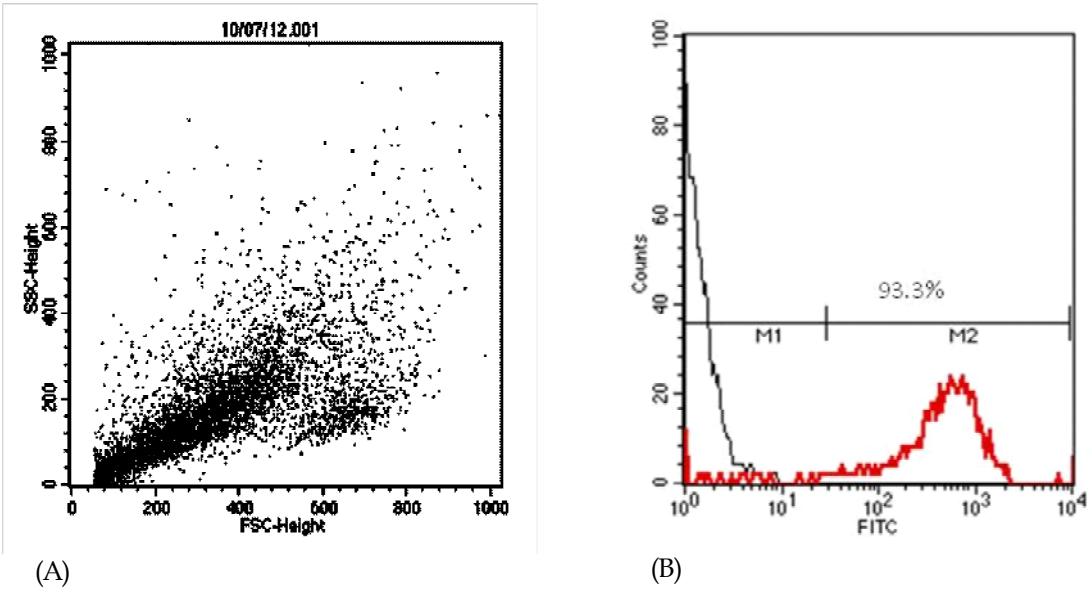


Fig. 72. Use of flow cytometry for quantification of cultured peritoneal macrophages of *L. rohita* exhibiting phagocytic activity: (A) FSC/SSC dot plot of cultured peritoneal macrophages at passage 40, and (B) Fluorescence histogram of cultured peritoneal macrophages showing shift in fluorescence due to phagocytosis of fluorescent latex beads (red line)

Project Title: Ontogeny of the digestive system of *Ompok bimaculatus* during larval development

Project Period: November, 2008 – May, 2012

Project Personnel: P.K. Pradhan (PI)

Funding Agency: International Foundation for Science, Sweden

Effects of different weaning strategies on growth in butter catfish, *Ompok bimaculatus*, larvae

In the present study, it was investigated how different feeding regimes (using different types of live food as a sole diet or co-fed with a microdiet through various weaning regimes), influenced the growth performance in *O. bimaculatus* under controlled laboratory conditions. In order to set up the most convenient feeding regime and weaning strategy, eight different feeding regimes with varying types of food (Artemia nauplii, zooplankton or microdiet) and the age at which those different food items were designed (Fig. 73). The larval performance was assessed at 7, 12 and 17 days post hatch (dph). At 7 dph, larvae fed with combination of artemia nauplii and zooplankton had highest growth and the poorest growth performance was observed in larvae group fed with micro-diet. At 12 dph, larvae fed with artemia nauplii had highest growth and similar to results at 7 dph, larvae fed with micro-diet had the minimum growth. At the end of the trial (17 dph), final larval mean growth in terms of BW was the highest in fish fed with artemia nauplii, followed by those fish fed with zooplankton. Larvae fed with micro-diet had minimum growth, and no significant difference was observed in the mean BW values between larvae from other feeding groups (except from larvae fed with artemia nauplii and/ zooplankton) (Fig. 74).

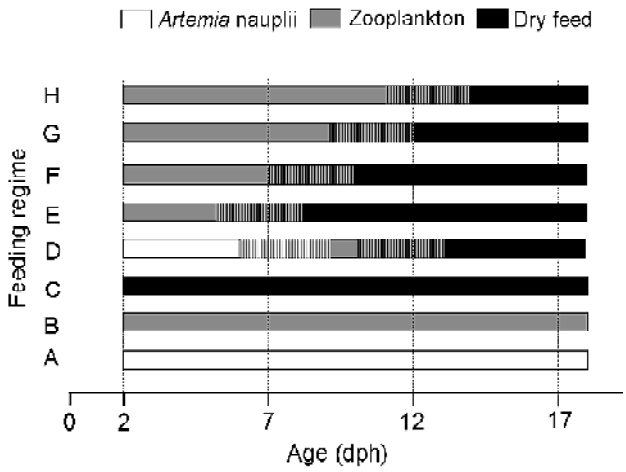


Fig.73. Different feeding regimes varying on the type of food (*Artemia* nauplii, zooplankton or microdiet) and the age at which those different food items were offered to larvae

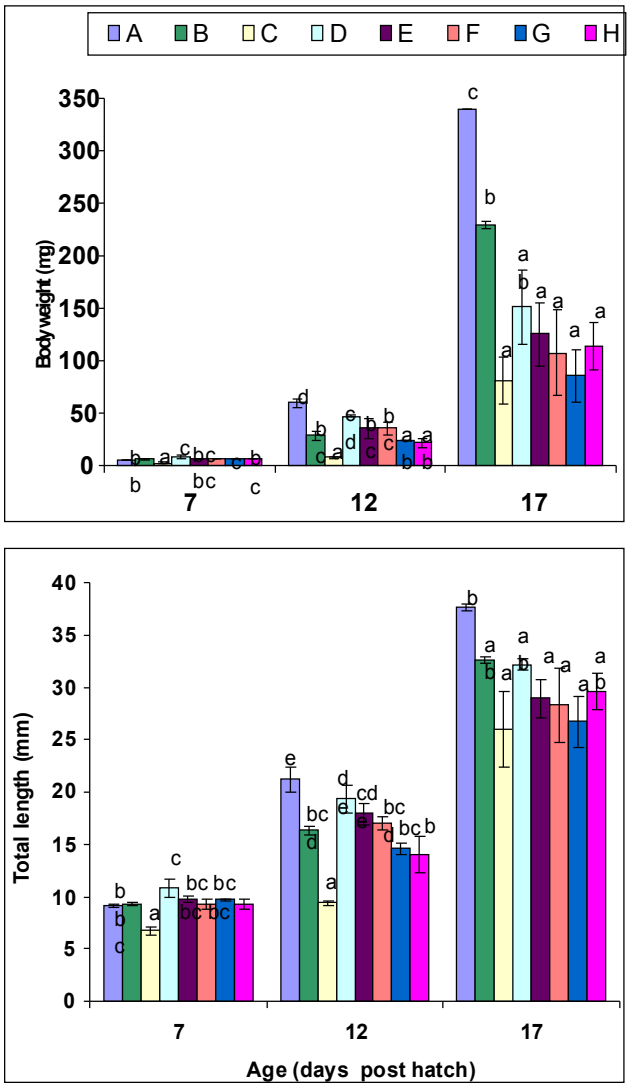


Fig.74. Effects of different feeding regimes on growth of larvae

Project Title: Production of monosex/ sterile common carp *Cyprinus carpio* using aromatase inhibitors

Project Period: April, 2009 - March, 2013

Project Personnel: A.K.Singh (PI)

Funding Agency: Institutional

The use of non-steroidal aromatase inhibitors (AI) on sex reversal in common carp, *Cyprinus carpio* and Nile tilapia, *Oreochromis niloticus* was studied in this study. Effect of different doses of tamoxifen, latrozole, anastrazole and fadrozole was evaluated on gonadal development and sex differentiation in sexually undifferentiated fingerlings of *C. carpio* (30 dpf) and *O. niloticus* (15 dpf) for 60 days, so as to know the efficacy and relative potential of AIs in manoeuvring the sex differentiation in these two species. Different haematological changes such as haemoglobin (g %), total erythrocytes count (REC), total leucocytes count (TLC), and biochemical changes such as serum protein, serum cholesterol, serum triglyceride; gonadal protein and gonadal lipid; were estimated after the treatment of different aromatase inhibitors (AIs) so as to make out how AIs influence the lipid and protein productions during gonadal development, besides haematological processes. It was observed that during gonadogenesis, there was hardly any change in the gonadal protein level, where as aromatase inhibitors reduced the cholesterol and triglyceride levels (Fig. 75). Lipid was recognized as a major form of energy stored in teleosts during gonadogenesis. It showed that stored lipid in *C. carpio* supplied the energy necessary for gonadal maturation. The survival, specific growth rate (SGR %), gonado-somatic index (GSI) and sex ratio were observed after the treatment of AIs to *C. carpio* and *O. niloticus*. The observed changes were compared from the control and between different treatment groups.

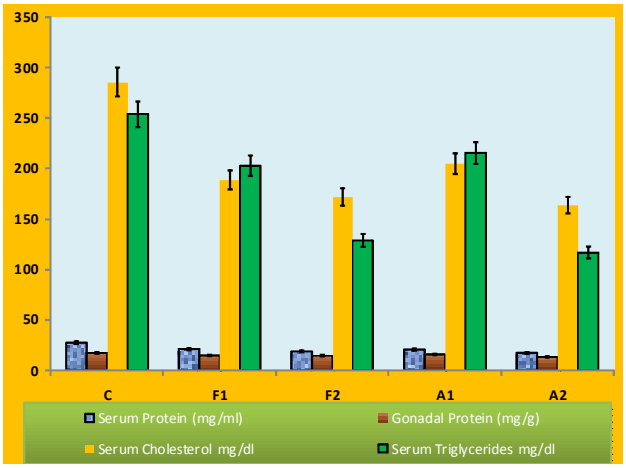


Fig. 75. Changes in lipid and protein levels after fadrozole and anastrazole treatments to *C. carpio*

Blood and gonadal hormonal levels of 17â estradiol (E_2) and testosterone (T) were assayed to know the aromatase induced changes in estradiol and testosterone productions (aromatase activity) in different treatment groups. The results showed that there was a sharp decline in the estradiol level while the testosterone level increased significantly (Fig. 76).

The hormonal level was also confirmed with the estimated aromatase activity (Fig. 77) and the same was correlated with the cellular changes in gonadal structure using histological techniques. The histological observations coincided with the hormonal changes. There was increased atresia while testicular structures were conspicuous elucidating the masculinising action of AIs (Fig. 78).

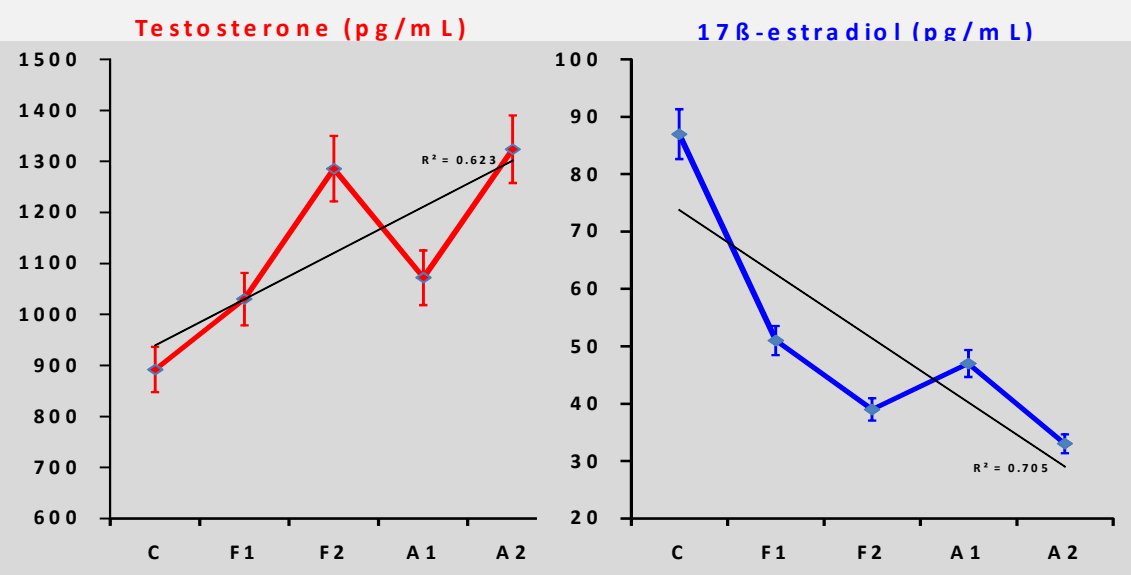


Fig. 76. Effect of fadrozole and anastrazole on the estradiol and testosterone levels in *C. carpio*

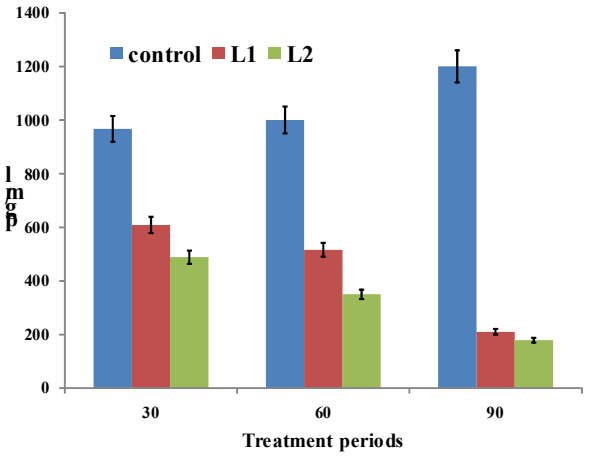


Fig. 77. Aromatase activity after letrozole treatment to *C. carpio*

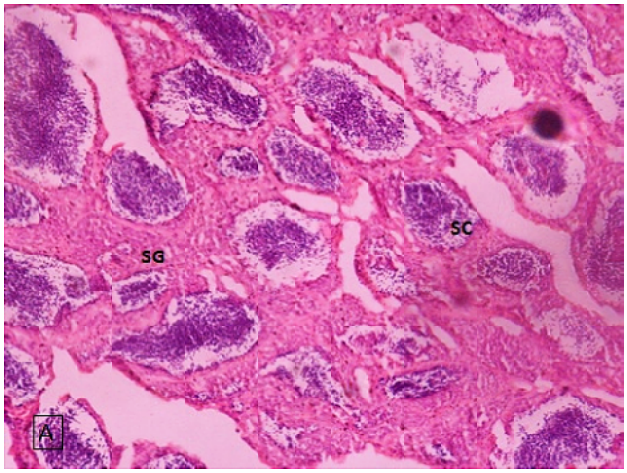


Fig. 78. Histological structure of letrozole induced masulinization *C. carpio*

Effect of exogenous melatonin (Sigma Co., USA) was studied on gonadogenesis and sex differentiation in sexually undifferentiated fry of *O. niloticus*. The melatonin treated fish was subjected to estimation of lipid and protein and also for histological examinations of testes and ovary. The results showed that exogenous melatonin suppressed SGR, GSI, ovarian cellular activity, protein lipid biosynthesis in tilapia suggesting that melatonin was useful in manipulating the gonadal maturity. The results of this study indicated that melatonin regulated metabolic activity of protein and lipid in *O. niloticus*. In *C. carpio*, the circulating melatonin was found depressed and was positively correlated with the inhibited action of aromatase (Fig. 79). The possible involvement of pineal melatonin during sex differentiation was the first report documented in this study.

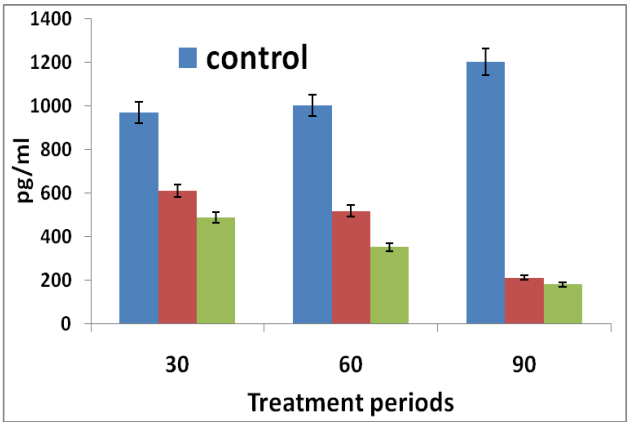


Fig. 79. Effect of letrozole on aromatase level in *C. carpio* (Significant level p<0.05)

Project Title: Ecological impact assessment of African catfish *Clarias gariepinus*: disease risks and potential for resource competition

Project Period: February, 2010 – February, 2013

Project Personnel: A.K. Singh (PI), Rehana Abidi and A.K. Pathak

Funding Agency: UPCAR

A benchmark survey was done for assessment of availability of African catfish, *Clarias gariepinus* in different fish markets of Uttar Pradesh. A total number of 80 fish markets located in 48 different district of Uttar Pradesh was surveyed on many occasions. The local fish species availability, abundance of C. *gariepinus* and its contribution to the total fish in the market was assessed. In all these fish markets, African catfish was present in large quantity. The contribution of C. *gariepinus* in different fish markets ranged from 10 - 80% at different places.

A survey conducted in Uttar Pradesh showed that the C. *gariepinus* was cultured in 32% of the rural ponds, tanks and other derelict water bodies. Data on the availability of C. *gariepinus* was generated from 419 ponds and the culture benefit economics was generated for few ponds. There was a large variation in production in different culture ponds (Fig. 80).

Food and feeding of *C. gariepinus* in culture farms of UP was studied and the data was analysed with respect to feed types and growth of the fish. An average growth of 1.63 mm/day was observed in managed grow-out ponds. The stocking rate varied from 30,000 – 85,400/ha. Size of fingerlings at stocking varied from pond to pond. Average weight of *C. gariepinus* fingerlings at stocking ranged from 4 - 50 g. The stocking density of fingerlings was very high.

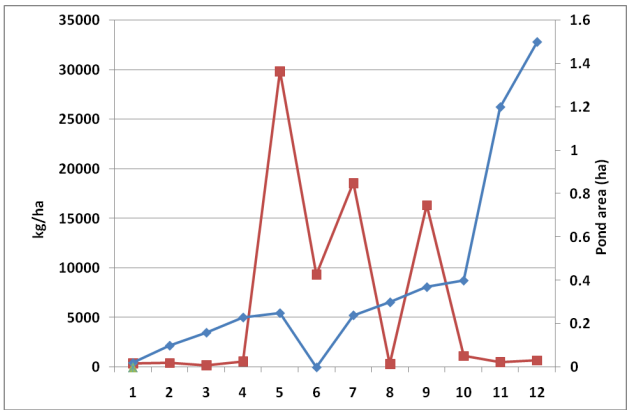


Fig. 80. Production response of *C. gariepinus* in different grow out ponds

C. gariepinus attained marketable size 500g - 1 kg in 6-7 months. The small fingerlings of about 4 g attained a marketable size of about 200 grams in just 12 weeks, allowing farmers to take three to four crops per year. Besides, it does not require pond preparation, water exchange or special feeding. As the fish is highly carnivorous, the fishermen generally used slaughter house waste and chicken waste as feed. *C. gariepinus*, when grown up to 200 - 400 g, looks similar to indigenous magur, *C. batrachus* and some time it is sold in the name of local magur, at a rate of Rs.200 -300/kg. In culture, *C. gariepinus* attacks fry, fingerlings or even adults of local fishes. There was a loss to the carps in the range of 38.2 – 46.3% when *C. gariepinus* was cultured under polyculture with carps.

Data collected from different rivers in UP showed that *C. gariepinus* was also present in riverine systems. Survey of rivers Ghaghra, Yamuna, Ganga, Sengar, Ool, Hindon, Varuna, Gomti and Gerua have shown that African catfish was available in the river stretches particularly in small streams and rivulet. The Yamuna River near Meerut, where Hindon River opens in the Yamuna River, was found as breeding ground of *C. gariepinus*. In Hindon River, more than 90% of the total catch was *C. gariepinus*. High abundance of *C. gariepinus*, with a size range of 0.5 kg to 18.5 kg was present in this river. In Ool and Sengar rivers, fisherman caught these fishes by applying some chemicals. Catch of *C. gariepinus* was observed at Ganga River at Sarai Mohan and Ramnagar area of Varanasi where 15-20 kg of *C. gariepinus* fish was caught every day. Hasanpur lake at Masoori in Ghaziabad was found to have an area of 2900 bigha harbouring *C. gariepinus* as 5-7 % of the total fish catch.

In general, diseases have not been found to be a serious problem within polyculture or monoculture operations of *C. gariepinus* at low stocking densities (up to 5 fish/m^2). Some fungal, parasitic and bacterial diseases were observed. However, more disease problems were encountered at higher stocking densities (over 10 fish/m^2), particularly when the pond environment deteriorated due to excessive use of slaughter house wastes as feed.

Project Title: Inventorisation, impact assessment and risk communication of invasive fish species in Uttar Pradesh

Project Period: April, 2011-March, 2013

Project Personnel: A.K. Singh (PI), Rehana Abidi, A.K. Pathak and S.M. Srivastava

Funding Agency: UP State Biodiversity Board

Occurrence of exotic fish species was recorded from seven rivers in Uttar Pradesh. Explorations of exotic fish species were done in different stretches of Yamuna, Gomti, Ram Ganga, Sharda, Gerua, Deva and Sone rivers covering 22 districts of UP (Fig. 81).

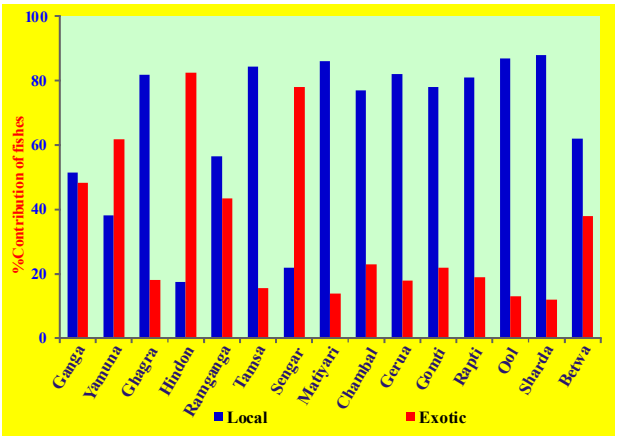


Fig. 81. Contribution of alien and local fish species in fishery of different River stretches

The fish species identified in different rivers of UP were: different strains of *Cyprinus carpio* (*Cyprinus carpio specularis*, *Cyprinus carpio nudus*, *Cyprinus carpio commnunis*, and koi), *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella*, *Aristichthys nobilis*, *Oreochromis mossambicus*, *O. niloticus*, *Clarias gariepinus*, *Pterygoplichthis disjunctivus*, *P. perdalis*, *Barbonemus gonionotus*, *Gambusia affinis* and *Poecilia reticulata*. The abundance index of exotic fish species was calculated at different sampling locations in Ganga and Yamuna rivers. The invasion index (II) at different locations was calculated to quantify the biodiversity loss due to invasions (Fig. 82). Data on food and feeding, as well as, reproduction and maturity, was also generated for different invasive fish species. Presence of exotic ornamental fish species in UP was listed and potential invasive ornamental fish species in the state were identified. A module to assess the risk level of exotic

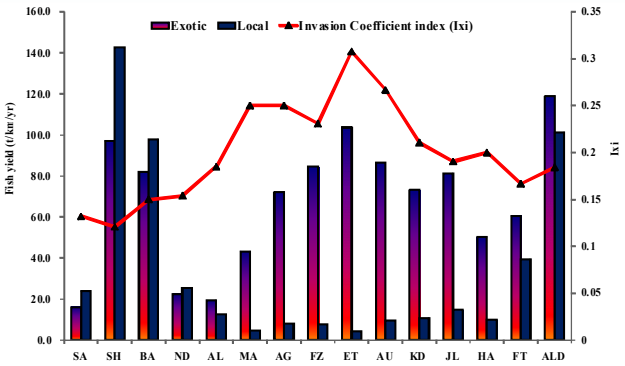


Fig. 82. Fish yield and invasion coefficient index of exotic fishes in the Yamuna River

fish species present in the country was developed as decision support to the policy makers (Fig. 83).

Further, the information was also collected on the presence of exotic species from four reservoirs and five lakes distributed in seven districts of UP in which seven exotic species were found to be present in these waterbodies (Table 23).

Table 23. Exotic fishes in natural lakes and reservoirs of UP

Water body	Exotic fishes present
Kartarniaghat	*Hypophthalmichthys molitrix, Aristichthys nobilis, Ctenophyrigodon idella, Cyprinus carpio, Clarias gariepinus, Oreochromis niloticus*
Derwn lake	*Ctenophayrigodon idella, C. carpio, Hypophthalmichthys molitrix, Aristichthys nobilis*
Surha tal	*Clarias gariepinus, Ctenophyrigodon idella, C. carpio, Hypophthalmichthys molitrix*
Hasanpur lake	*Hypophthalmichthys molitrix, Aristichthys nobilis, Ctenophyrigodon idella, C. carpio, Clarias gariepinus, Oreochromis niloticus*
Gujar tal	*Ctenophayrigodon idella, C. carpio, Hypophthalmichthys molitrix, Aristichthys nobilis*
Nawabganj	*Hypophthalmichthys molitrix, Aristichthys nobilis, Ctenophyrigodon idella, C. carpio, Clarias gariepinus, Oreochromis niloticus*
Ahrora	*Clarias gariepinus*
Rihand	*Ctenophyrigodon idella, C. carpio, Aristichthys nobilis, Clarias gariepinus, Oreochromis niloticus*
Dhandhrol	*Clarias gariepinus, C. carpio, Aristichthys nobilis*
Ramgarh tal	*Cyprinus carpio, Cyprinus carpio* (Koi Carp), *Aristichthys nobilis, Ctenophyrigodon idella, Barbonymus gonionotus*
Raipur lake	*Aristichthys nobilis, Hypophthalmichthys molitrix*

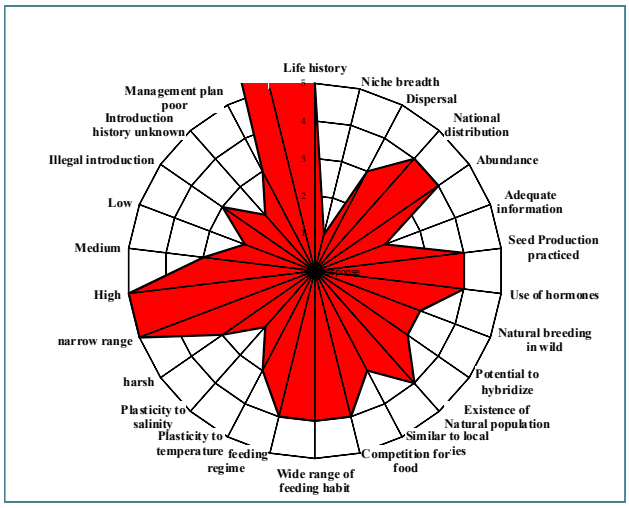


Fig. 83. Scoring system to determine the risk level of alien species

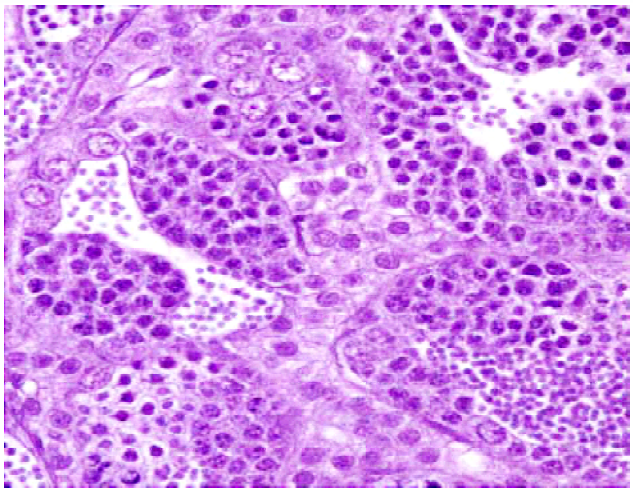


Fig. 84. Increased interstitial cells in male *Cyprinus carpio* with exposure of dioxin after 15 days

Project Title: Development of biomarkers as diagnostic tools for assessment of fish health status

Project Period: April, 2011 - March, 2014

Project Personnel: Peyush Punia (PI), P.K. Pradhan and Ranjana Srivastava

Funding Agency: Institutional

Fishes can be used as indicators of the water quality and the health of the aquatic ecosystem. The aquatic fauna in rivers are changing fast and are at risk due to habitat loss, pollution, introduction of exotic species, over-exploitation and other anthropogenic activities. Biomarkers provide information long before reactions in population or even ecosystems are occurring and responses of organisms living under unfavorable conditions which can be considered as early warning sentinels before populations or ecosystems are severely damaged in the river systems. In the present study, establishment of the diagnostic biomarker for fish fauna in River Yamuna for assessment of fish health was undertaken. Control fishes *Cyprinus carpio* and *Labeo rohita* were subjected to exposure of Dioxin for a period of 15 days and samples were collected for histological and hormonal studies to collect baseline data for comparison with the samples collected from Yamuna River (Figures 84 and 85). Samples of *C. carpio* were also collected from Allahabad region from River Yamuna which is considered as the diluted zone as water flow is more compared to middle region. Histological sections were prepared from different tissues and study is under progress. The fish weight and length were recorded for

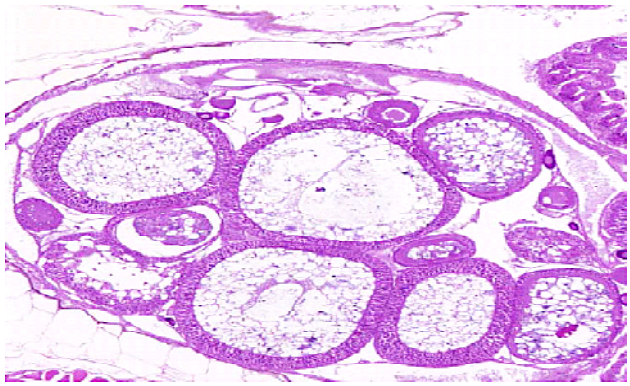


Fig. 85. Decreased Vitellogenesis in *C. carpio*

condition factor and haepatological parameters were also collected for haeptological index calculations. Blood plasma and tissue samples were analysed for hormonal and EROD assay to determine the effect of pollutants. Protocol for the EROD assay was standardized for the analysis of the collected samples. EROD assay of gill and liver showed three to four fold increased activity as compared to control (Figures 86 and 87). Blood serum was analysed for sex steroid hormones namely, 11-keto testosterone and 17 beta estradiol, in comparison to control. The test sample analysis showed higher levels of 11-keto testosterone in control samples followed by males and was observed to be low in the female fish samples (Fig. 88). Further, test analysis of 17 beta estradiol showed high levels in female fish as compared to males and control fish sample (Fig. 89). This indicates that the fish endocrine system is being disrupted due to exposure to the pollutants in the river. The results are under analysis with more numbers of samples for developing this assay as biomarker.

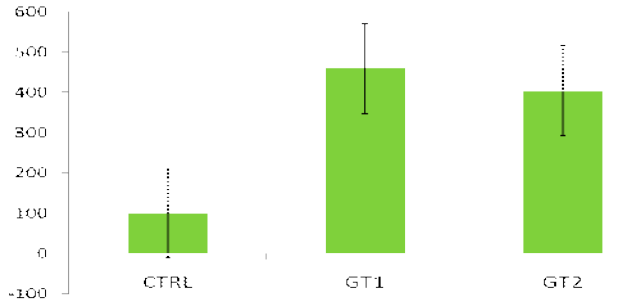


Fig. 86. EROD assay in gills of *C. carpio*

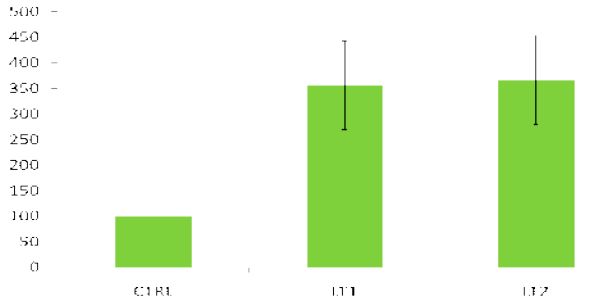


Fig. 87. EROD assay in liver of *C. carpio*

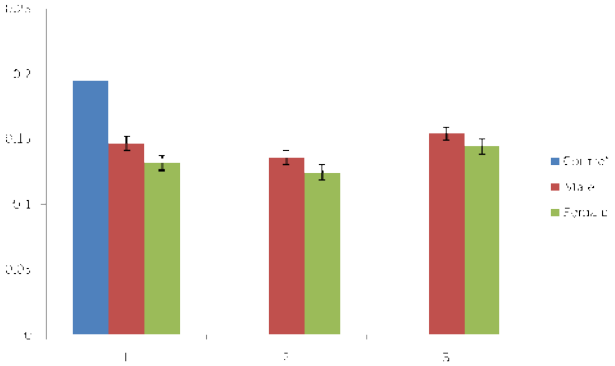


Fig. 88. Effect of sex steroid hormone, 11-keto testosterone, on *C. carpio*

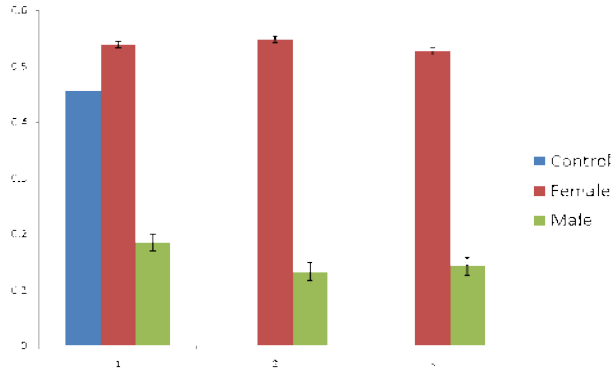


Fig. 89. Effect of sex steroid hormone, 11-keto testosterone, on *C. carpio*

Project Title: Assessment of aquatic health using recent cellular and molecular tools in endocrine research

Project Period: August, 2011 – August, 2014

Project Personnel: A.K.Singh (PI)

Funding Agency: UPCST

Survey of general fish diversity of Gomti river with particular reference to availability of *Labeo rohita* was conducted in Lucknow (four locations), Sultanpur (three locations, Jaunpur (three locations), Sitapur (three locations) and Ghazhipur (two locations) districts of Uttar Pradesh. Physico chemical analysis of waster was done for pH, DO, CO_2, alkalinity, total hardness and some heavy metals such as Cd, Pb, Al and Cu in Gomti river at Lucknow, Sultanpur, Jaunpur, Ghazhipur and Sitapur districts. 113 specimens of *L. rohita* was collected from different locations and were subjected to biometric analysis, GSI and also for histological examinations of gill, kidney, liver and gonads (Fig. 90).

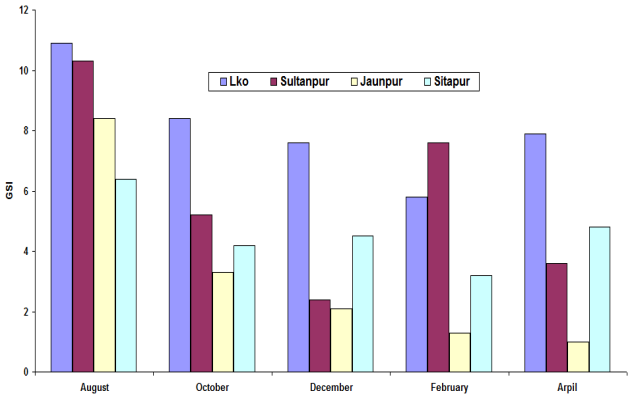


Fig. 90. GSI of *L. rohita* captured from Gomti River

The serum of the live specimens of wild and experimental *L. rohita* was collected and stored at -20°C for hormonal examinations particularly estradiol, testosterone, vitellogenin and melatonin levels using ELISA technique. Level of pesticide in water samples at Jaunpur, Lucknow and Sultanpur was determined and the bioaccumulated pesticide level such as pthallic acid ester, bisphenol A, HCH were estimated in the tissues of *L. rohita* both *in situ* and laboratory conditions and were found positive. The effect of identified EDCs was studied in experimental conditions in the laboratory. Effect of bisphenol A and HCH treatments on RNA/DNA ratio was determined in the muscle tissue where a decreased RNA/DNA ratio was observed in α HCH treatment (Figures 91 and 92).

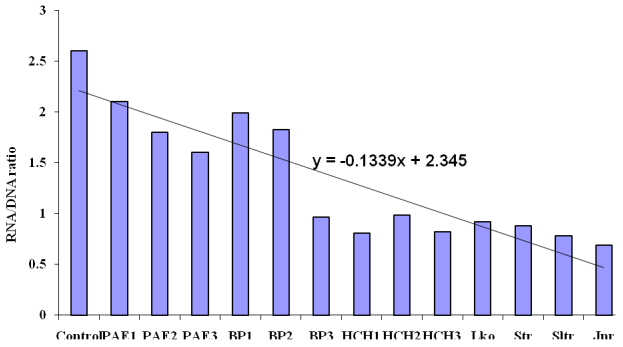


Fig. 91. Musicle RNA/DNA ratio of *Labeo rohita* of Gomti river and EDCs exposed experimental fishes

Total protein was isolated from control brain and gonads of normal as well as wild caught fish specimens and experimental fish tissues for studying the ER/ aromatase based expression in *L. rohita*. SDS Page gel revealed different banding pattern in ovary and testes of *L. rohita*. However, when compared with a standard protein marker, the band appeared in ovary and brain was very close to 43kd which was in conformity of published literature. This was further confirmed in SDG Page gel of control brain and bisphenol treated brain.

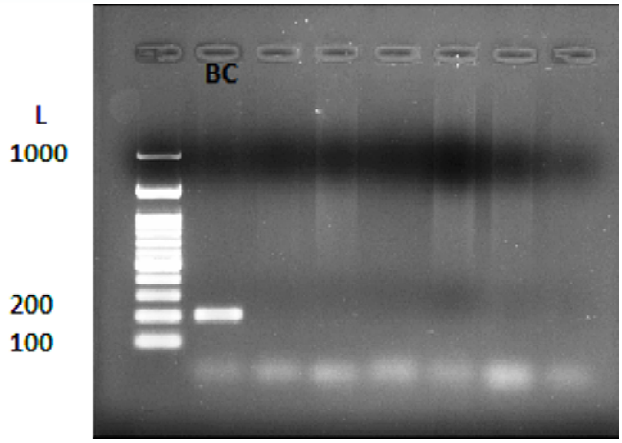


Fig. 93. CYP19 expression in brain of *Labeo rohita*

However, the band was absent in tamoxifen treated brain because tamoxifen inhibited aromatization process. Primer used for CYP19 expression was:

TACACATTCTGGAGAGTTTTATA F

GGAAGTTGTCTAGACTGAACTCAT R

CYP19 expression in *L. rohita* was observed (Fig. 93) and further investigation is in progress.

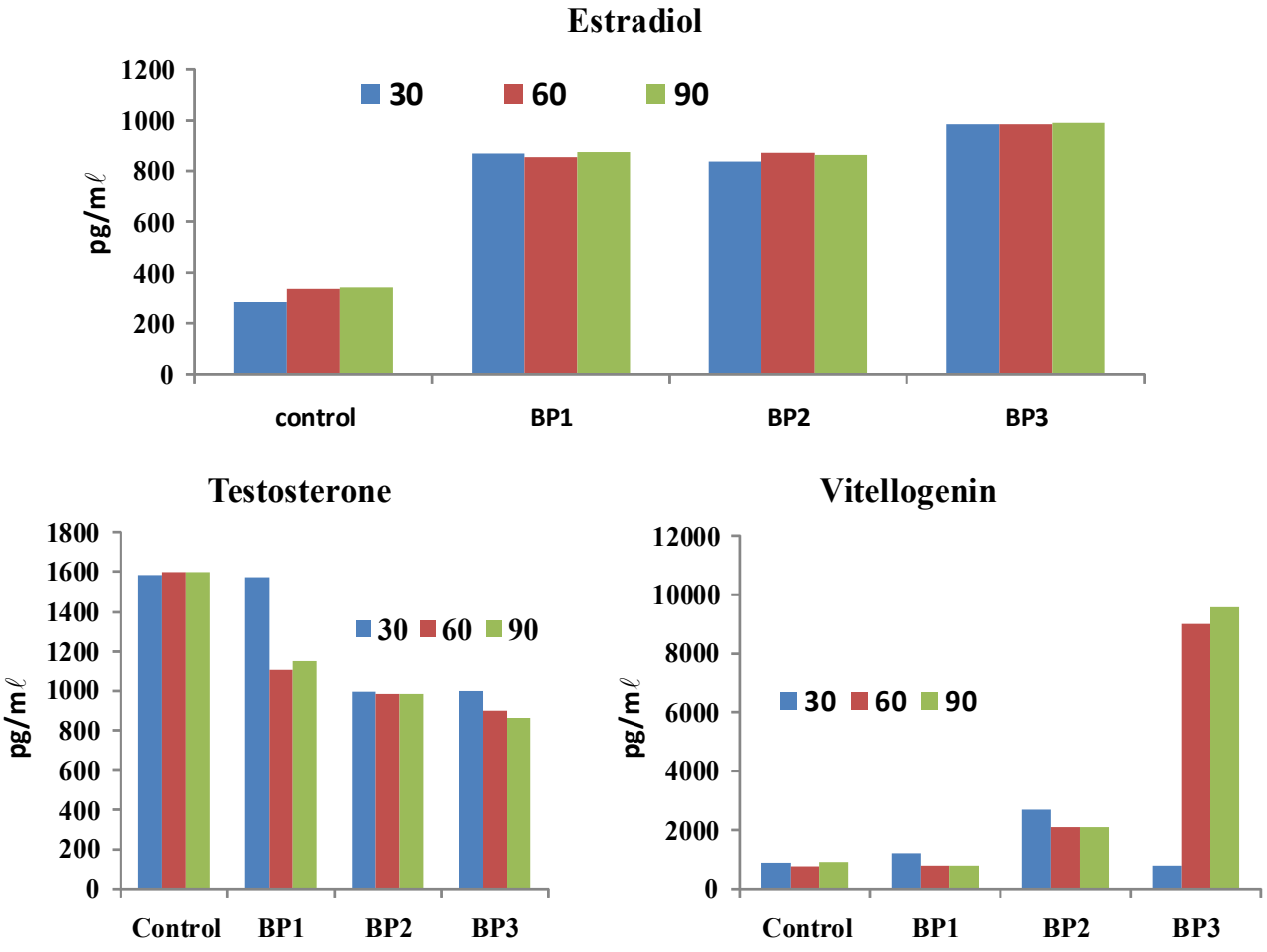


Fig. 92. Hormonal response of Bisphenol-A in *Laheo rohita*

Sub-project II: Disease surveillance of endemic and commercially important ornamental fishes of the Western Ghats

Project Period: April, 2009 – March, 2013

Project Personnel: T. Raja Swaminathan (PI), A. Gopalakrishnan, V.S. Basheer, P.R. Divya and A. Kathirvelpandian

Funding Agency: Institutional

Development of cell line from *Tetraodon travancoricus*

A total of 103 explants and 10 trypsinization of caudal fins were made in order to initiate development of cell culture from *Tetraodon travancoricus*. In more than 90% explants and trypsinization of caudal fin of *T. travancoricus*, monolayer could not be formed due to less number of explants (3-4 numbers) (Fig. 94) and cells (1 X 10^3 per ml) obtained from a fish in the respective methods. There was only less than 50% confluency monolayer formed when the enriched L-15 media (with 25% FBS, MEM vitamin mixtures (100X) and MEM non-essential amino acid mixture (100X), 0.1 mg of bFGF /ml and 1% glucose) was used in the development of cell culture from *T. travancoricus*. The sub-cultured cells could not attach to the surface of the flask in the subsequent passage and the monolayer could not form (Fig. 95). The details of the explant experiments are given in Table.1.

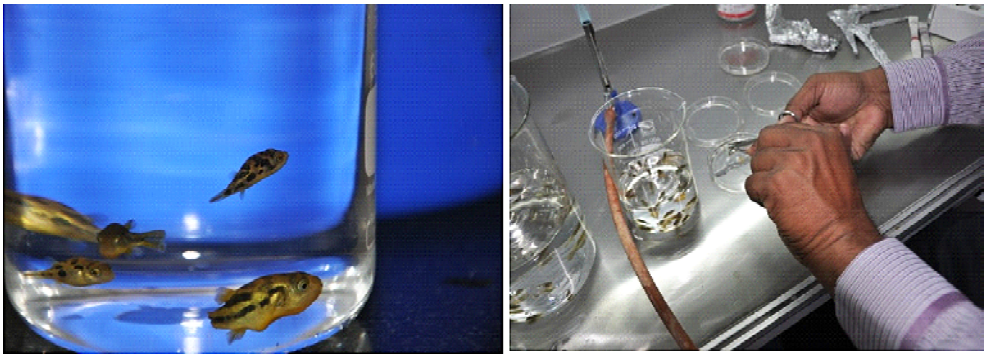


Fig. 94. Explant preparation from caudal fin of *Tetraodon travancoricus*

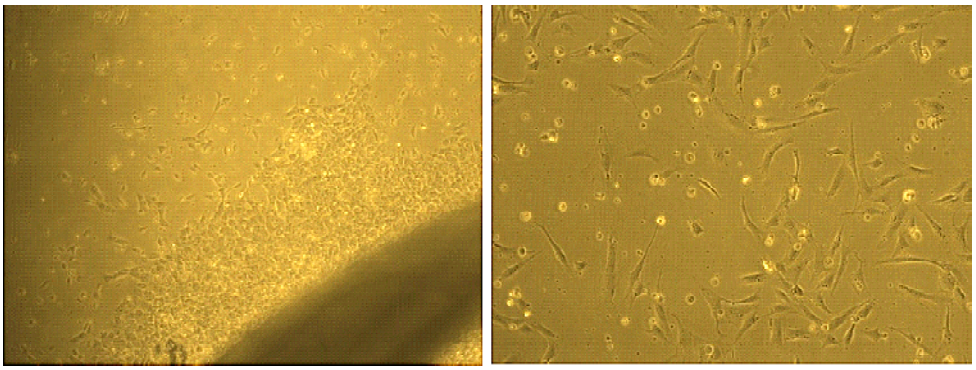


Fig. 95. Caudal fin explant of *T. travancoricus* showing radiation of cells and monolayer

Development of cell line from *Horabagrus brachysoma*

A total number of 45 caudal fin explants were taken aseptically, minced into small pieces (approximately 1 mm^3 in size) and washed three times in Phosphate Buffer Saline (PBS) containing antibiotics (500 IU/ml penicillin, 500 ìg/ml streptomycin and 2.5 ìg/ml Amphotericin B). The cells from primary explants showed both epithelioid and fibroblast-like morphology (Fig. 96), but fibroblastic cells were seen to dominate the culture. In the initial passages, fin cells were composed of a heterogeneous mixture of fibroblastic-like and epithelial-like cells. After 15 subcultures, both the fin cells were predominantly fibroblastic-like cells (Fig. 97). The details of the explant experiments are given in the Table 24.

Sub-culturing of the cell culture was done at 90% confluency in order to avoid the deterioration of the cells and increase the reseeding efficiency of the cells. After 15th sub-culture, the cells were sub-cultured at a ratio of 1:3 at 3-4 days interval, and FBS was reduced to 15% in the L-15 culture medium. One cell line was developed from caudal fin of the yellow catfish, *H. brachysoma* and the current passage level is 32. The morphology of the cells showed rapid deterioration at high cell densities and floating of the dead cells was also noticed. After 15th subculture, the cells were sub-cultured at a ratio of 1:3 at 3-4 d interval, and FBS was reduced to 15% in the L-15 culture medium. The fin cells of *H. brachysoma* have been sub-cultured 32 times since initiation.

The Extra Cellular Proteins (ECPs) from *Vibrio cholerae* MTCC3904 proved to be cytotoxic for fin cell lines. Cytotoxic effects could be observed within 10h after inoculation. The

morphological changes detected in these cell lines were rounding, detaching and finally monolayer destruction. Several fish cells have proven suitable for demonstrating the cytotoxic effects of fish pathogenic bacteria. The ECPs from *V. cholerae* MTCC 3904 and *Aeromonas hydrophila* proved to be cytotoxic for both cell lines. Cytotoxic effects could be observed within 10h after inoculation which included rounding, detaching and finally monolayer destruction.

Partial sequence information of mitochondrial genes viz., 16S rRNA (590 bp) and COI (655 bp) were used to further confirm the origin of PFF and CFF by PCR. The amplified PCR fragment was sequenced and this DNA fragment matched perfectly with the genomic sequence of 16S rRNA and COI previously reported for the respective fish.

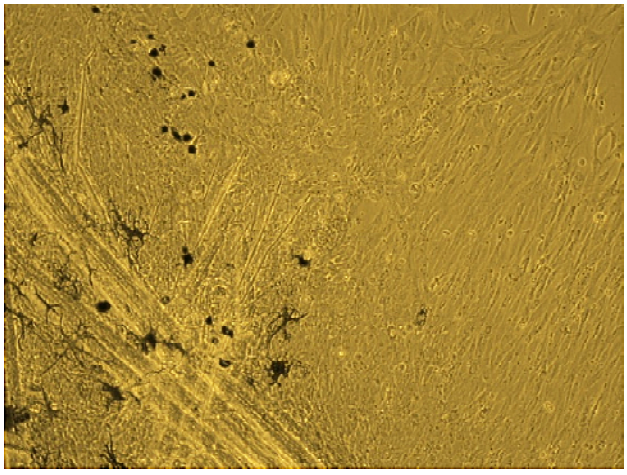


Fig. 96. Explants of caudal fin of *H. brachysoma* showing radiation of cells

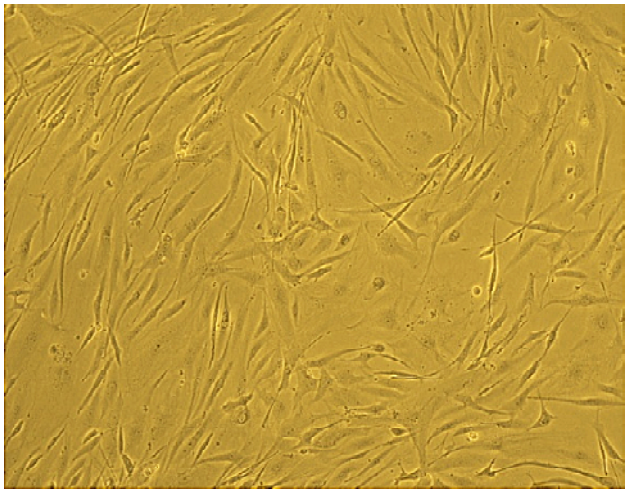


Fig. 97. Monolayer of caudal fin cells of *H. brachysoma* at different passage level

Table 24. The details of the cell line development from fins of *Tetraodon travancoricus* and *Horabagrus brachysoma*

Details	*Tetraodon travancoricus*	*Horabagrus brachysoma*
Number of explant	103	45
Contamination of the explant	85	32
Radiation of the explant	25	8
No radiation of the explant	3	5
Formation of monolayer	0	4
Contamination of the monolayer	0	3
Continuation of subculture	0	1
Passage level	0	**32**

Screening of *Puntius denisonii, H. brachysoma* and *T. travancoricus* for OIE listed diseases

A total of 85 fish samples consisting of *T. travancoricus* (42), *P. denisonii* (21) and *H. brachysoma* (22) were collected from different river systems along the Western Ghats and tested for presence of finfish viruses viz., Spring viraemia of carp (SVC), iridovirus and Koi herpes virus (KHV) through, either by cell culture isolation or polymerase chain reaction. No amplification of the target genes for important viral pathogens viz., SVC, iridovirus and KHV was detected by polymerase chain reaction. No cytopathic effect was detected in any of the cell lines even after 15 blind passages.

IMPORTANT EVENTS AND MEETINGS

NBFGR hosted 2nd Meeting of the National Advisory Board on Management of Genetic Resources

The 2nd meeting of the National Advisory Board on Management of Genetic Resources (NABMGR) was held at NBFGR, Lucknow on August 13, 2012. Dr. R.S. Paroda, Chairman, Dr. S. Ayyappan, Co-Chairman and other members of the Board attended the meeting. Dr. J.K. Jena, Director NBFGR welcomed the Chairman, Co-Chairman and other members of the Board. Dr. Paroda in his opening remarks while emphasizing the importance of genetic resources, mentioned that they are the building blocks for further progress in any sector. ICAR is unique in having a number of institutes catering to research on genetic resources. He opined that the NABMGR, constituted by ICAR, provides both coordination and convergence mechanism for creating required linkages and synergies. Capacity building is required in bio-safety issues. Dr. Paroda emphasized that cost effective conservation strategies and alternative methods of storage of genetic resources, like the one adopted by NORDIC Genebank need to be adopted suitably.

Dr. R.S. Paroda chairing the meeting

Dr. S. Ayyappan in his opening remarks emphasized the need for climate resilient germplasm in the context of severe drought situation in several states during this year. He expressed his concerns regarding divergent interpretations of provisions of BDA at different levels. He mentioned about other initiatives at ICAR like the Bio-informatics GRID for documentation of all information related to bio-resources. He expressed his concerns over the issue of movement of animals across neighboring countries which leads to serious concerns of trans-boundary

A view of the NABMGR meeting

diseases. He stressed the need of substantial efforts for tackling such issues. Dr. B. Meenakumari, DDG (Fisheries) thanked the Board members for organizing the meeting at NBFGR. She expressed that the conservation of aquatic genetic resources should be taken care of at the regional level by giving it utmost importance. The other members participated in the meeting were: Dr. K.M.L. Pathak, DDG (Animal Sciences); Dr. M. Mahadevappa, Former Chairman, ASRB; Prof. T.J. Pandian, Former National Professor, ICAR; Dr. Sushama R. Chaphalkar, Director, Vidya Pratishthan's School of Biotechnology, Baramati; Dr. C.S. Nautiyal, Director, NBRI; Dr. B. Senapathi, Former Vice-Chancellor, OUAT, Bhuvaneswar; Dr. D.J. Bagyaraj, Emeritus Professor, NASI; Dr. Ravindra Kumar, ADG (Coordination), ICAR; Dr. S. Mauria, ADG (IP&TM) ICAR; Dr. K.C. Bansal, Director, NBPGR; Dr. B.K. Joshi, Director NBAGR; Dr. S. Rajendra Prasad, Acting Director, NBAIM and Dr. B.S. Bhumannavar, Director, NBAII.

The Board confirmed the minutes of its first meeting held on December 13, 2011 at NBPGR. This was followed by the presentation of ATR by

Dr. S. Ayyappan addressing the meeting

Discussion on agenda items during meeting

Dr. K.C. Bansal, Director, NBPGR. Agenda items were discussed and important decisions taken by the Board. It was decided that a Core Committee to be identified with all the Bureaus representatives to finalize the guidelines document related to management of genetic resources including IPR issues and national/ international treaties. The Sub-Committee constituted by the Board would come up with specific guidelines for sharing germplasm with the private sector and develop required MTA for the purpose. The Board recommended that more taxonomists should be appointed at NBPGR and in other Bureaux. With respect to the fish germplasm resources, the Board recommended that research on indigenous ornamental fishes including their collection, documentation and conservation should be strengthened. The Board recommended for accrediting NBFGR as National Repository of Fish Genetic Resources. National policy guidelines on River/Reservoir should be formulated to address the issues of stock-based ranching programmes. It was agreed that collection and characterization of Indian major carps is of high priority for which state departments should also be involved as partners. Also, inter-institutional collaboration in this field be further strengthened. NBFGR should conduct regular training programmes on conservation of fish genetic resources, in partnership with best institutions abroad. There should be representation of Fisheries Institutions/NBFGR in the annual OIE meetings. Harmonization of biosafety procedures/regulations should be looked into by all the Bureaux.

Institute Research Council Meeting

The Annual IRC meeting of the Institute was conducted in three phases on 27-28 April, 2012, 17-19 May, 2012 and 25 July, 2012 under the chairmanship of Dr. J.K. Jena, Director. The progress of all ongoing projects was critically reviewed by the IRC and important suggestions emerged from the discussion.

IRC meeting in progress

Detailed presentations on all the projects were made by the respective Principal Investigators/Co-PIs. The new project proposals were also discussed and important ones were approved with suggested modifications.

Research Advisory Committee (RAC) Meeting

The RAC meeting of NBFGR was conducted during 19–20 February, 2013 at Kochi unit of NBFGR under the chairmanship of Prof. T.J. Pandian, Former National Professor, ICAR, Madurai. Other members of the Committee Dr. A.D. Diwan, Former ADG (Marine Fisheries); Dr. Y. Basavaraju, Prof. and Head, Fisheries Research and Information Center, Bangalore; Dr. George John, Senior Advisor, DBT, New Delhi; Dr. Bechan Lal, Professor, BHU, Varanasi and Dr. Madan Mohan, ADG (Marine Fisheries) attended the meeting.

Welcoming the RAC members Dr. J. K. Jena, Director, NBFGR made a presentation on the progress and achievements made by NBFGR during the year 2012-2013. Dr. Jena informed the Committee about the significant improvement made in number and quality of publications in recent years. After the initial opening remarks by the Chairman and Members, the Heads of

RAC meeting in progress

the Divisions presented salient achievements made under different identified research themes during the year. Reviewing the progress of the ongoing research projects of the Institute, the Committee conveyed their satisfaction on the progress of the Institute. They also provided significant inputs to improve the research programmes in coming years.

Dr. S. Ayyappan laid the Foundation stone of National Fish Museum at NBFGR

Dr. S. Ayyappan, Secretary DARE and Director General, ICAR, New Delhi laid the foundation of the First National Fish Museum of the country at NBFGR, Lucknow on 12 August, 2012 in the august presence of Dr. B. Meenakumari, DDG (Fisheries), ICAR, New Delhi; Dr. S. Solomon, Director, IISR, Lucknow; Dr. H. Ravishankar, Director, CISH, Lucknow; Dr. Madan Mohan, ADG (Marine Fisheries), ICAR, New Delhi; Dr. J.K. Jena, Director, NBFGR, Lucknow; and several other dignitaries from IISR, CISH, CSSRI, UP State Fisheries, CPWD and staff of NBFGR, Lucknow.

Dr. S. Ayyappan laying the foundation stone of National Fish Museum

Dr. S. Ayyappan, Secretary, DARE and Director General, ICAR visited NBFGR Kochi Unit

Dr. S. Ayyappan at NBFGR Kochi Unit

Dr. S. Ayyappan, Secretary, DARE & Director General, ICAR visited NBFGR Kochi Unit on 19 October, 2012. During his visit, he went around the laboratories and interacted with the Director, scientists and research scholars of NBFGR Kochi Unit about. Dr. G. Syada Rao, Director, CMFRI, Kochi was also present on this occasion

Celebration of Independence Day

The Institute celebrated the Independence Day with full fervor and gaiety. Dr. J.K. Jena, Director of NBFGR hoisted the National Flag in the presence of staff members of the Bureau and addressed the gathering. In his speech, he lauded the efforts made by the bureau in the past and proposed future plans for the bureau. The occasions were followed with cultural programme in which large number of children of the NBFGR family participated.

Dr. J.K. Jena addressing the staff on Independance Day

Republic Day Celebrated

A flag hoisting ceremony was observed on the Republic Day on 26 January, 2013. Dr. J.K Jena, Director hoisted the National Flag in the presence of other staff members of the Bureau. In his address, the Director highlighted the achievements of NBFGR during the year 2011 and shared glimpses of upcoming programmes. Dr. Jena while complimenting the efforts of the staff members also reminded the staff about their rights and duties towards growth of the institute. The programme was followed with a small cultural programme in which large number of children of the NBFGR family participated.

Hindi Day and Hindi Pakhwada observed

A function was organized on Sept. 14, 2012 to celebrate the Hindi Day. On this occasion five reputed poets of the city were invited who spoke on importance of Hindi language and recited their poems. The Institute

Hindi Day Programme

Agricultural Education Day Celebration

also observed a Hindi Pakhwada during 15-29 September, 2012 during which seven Hindi competitions were organized among the staff of the Institute to promote the use of Hindi in official work. All the winners were given prizes. Mr. Ram Sakal, Personal Assistant won the prize for the Best Hindi Competitor – 2012.

NBFGR Celebrated Agricultural Education Day

The Institute celebrated "Agricultural Education Day" on 19 November, 2012. The day was celebrated as Open- House Day for the students and the visitors so that they could see various laboratories, fish farm and the Ganga Aquarium at the institute. Several programmes like 'Inter-school Arts Competition' and 'Quiz Competition' were organized for school children on themes "Aquatic Biodiversity" and "Fish & Environment". On this occasion, Dr. J.K. Jena, Director, NBFGR apprised the students about the prospects of agricultural education and role of ICAR in agricultural development of the country through research, education and extension. Dr. H. Ravishankar, Director, CISH, Lucknow and Dr. S. Solomon, Director, IISR, Lucknow also delivered lectures on agricultural research and education. The best three participants of each event were honoured with cash prizes and the certificates by the honourable guests of the occasion. The Open House Day was visited by thousands of school children, teachers and others. Dr. S. Raizada, Principal Scientist, NBFGR coordinated the programme.

Agricultural Education Day Celebration

Agricultural Education Day Celebration

NBFGR Celebrated 29th Foundation Day

NBFGR celebrated its 29th Foundation Day on 12 December, 2012. A Farm Innovators Day was organized on this occasion to share the innovations developed by the farmers and also promote adoption of new technologies in freshwater aquaculture. On this occasion, Dr. S.A.H. Abidi, Former Member, ASRB,

Dr. J.K. Jena speaking on the Foundation Day

New Delhi; Dr. B.N. Singh, Former ADG (Fisheries), ICAR, New Delhi; Dr. S. Solomon, Director, IISR, Lucknow; Dr. H. Ravishankar, Director, CISH, Lucknow and Dr. V.K. Mishra, OIC, CSSRI Regional Research Station, Lucknow were the Guests of Honour. In his welcome address, Dr. J.K. Jena, Director, NBFGR apprised about the salient achievements of the Institute. A total of 25 progressive aqua-farmers and entrepreneurs participated in the programme and shared their innovative and profitable farming practices. NBFGR scientists also gave technical guidance to the participants.

On this occasion, the dignitaries also presented Annual Institute Awards for the year 2011-12 to the staff members of the Institute for their outstanding contributions and also to the selected progressive farmers and entrepreneurs.

Category of Award	Name of Awardees
Best Division of the Institute	Molecular Biology and Biotechnology Division
Best Scientist of the Institute	Dr. Sudhir Raizada, Principal Scientist
Best Technical Staff of the Institute	Shri Rajesh Dayal, Technical Officer and Shri Amit Singh Bisht, Technical Officer
Best Administrative Staff of the Institute	Shri P. K. Awasthi, Assistant Shri Rajan Kr. Malhotra, Junior Clerk
Best Supporting Staff of the Institute	Shri Dukhi Shyam Deo, SSS
Best Research Student/SRF/JRF/ RA of the Institute	Shri Ratnesh Kumar Tripathi, RA
Best Fish Farmer Award	Shri Rajesh Kumar Singh, Bihar Shri Santosh Kumar, Lucknow Shri Mohan Verma, Devaria

Presentation of Annual Institute Awards for the year 2011-12



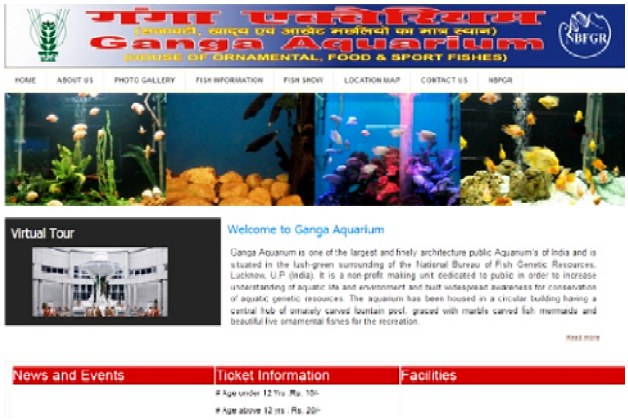

Presentation of Annual Institute Awards for the year 2011-12

Inauguration of new infrastructure facilities

A new training hostel was inaugurated by Dr. S. Ayyappan, Secretary, DARE and Director General, ICAR at Aquaculture Research and Training Unit of NBFGR at Chinhat, Lucknow on 12 August, 2012.

Dr. S. Ayyappan inauguration the Training hostel

A separate website for the Ganga Aquarium was inaugurated by Dr. S. Ayyappan, Secretary DARE and DG, ICAR, New Delhi on 12 August, 2012 in the presence of dignitaries and NBFGR staff. The website displays key features of the aquarium building and details of aquatic life being displayed in the aquarium.



Website of the Ganga Aquarium

Dr. B. Meenakumari inaugurating the Souvenir countar

It may be viewed through the website of NBFGR (http://nbfgr.res.in).

Dr. B. Meenakumari, DDG (Fisheries). ICAR, New Delhi inaugurated a Souvenir counter of the Ganga Aquarium at NBFGR, Lucknow on 12 August, 2012 which is intended to display and sale of different souvenirs, specially designed with the pictures of aquarium fishes and key-structures of the Ganga Aquarium. The counter also sells ornamental fishes, aquarium and accessories.

On this occasion Dr. Ayyappan and Dr. Meenakumari addressed the staff and research scholars of NBFGR.

Dr. S. Ayyappan addressing the staff

WORKSHOPS/ SYMPOSIA/ TRAININGS ORGANIZED

National Consultation on 'Development of Surveillance Programme for Aquatic Animal Diseases'

A National Consultation on 'Development of Surveillance Programme for Aquatic Animal Diseases' was organized at NBFGR, Lucknow during 17–18 April, 2012 in collaboration with DAHDF, Government of India; National Fisheries Development Board, Hyderabad and Aquatic Biodiversity Conservation Society, Lucknow. Forty experts including research scientists, development officials and policy makers involved in aquatic animal health from all over the country, as well as, an expert from Network of Aquaculture Centers in Asia-Pacific (NACA), Thailand participated in this meeting.

Dr. J.K. Jena welcoming the participants

Dr J.K. Jena, Director, NBFGR and Convener of the National Consultation welcomed the delegates and highlighted threats to the sustainability of the aquaculture sector due to new and emerging diseases. Dr. Jena opined that the present consultation would help in developing a roadmap for national surveillance programme for aquatic animal diseases and manage the disease risks associated with aquatic animal trade. Prof. C.V. Mohan, Research and Development Manager, NACA, Thailand shared his experiences on the global scenario of aquatic animal disease surveillance programmes. He appreciated the efforts of NBFGR for taking this initiative and assured support of the NACA in developing a national surveillance programme on aquatic animal diseases. In her presidential address, Prof. Indrani Karunasagar, Head, Department of Microbiology, College of Fisheries, Mangalore emphasized the need for level III diagnosis particularly metagenomics in detecting the causative agents of various aquatic diseases syndromes. In technical

Participants with Director and staff of NBFGR

sessions, status of diseases in finfishes and shellfishes was discussed by the participants. The development of implementation mechanisms for this surveillance programme was discussed by a panel of experts.

The consultation recommended that a "National surveillance programme for aquatic animal diseases" be initiated in 14 selected states of fisheries and aquaculture importance in the first phase. The consultation agreed on a national list of diseases to be included under national surveillance programme in selected 14 states in first phase for the purpose of implementing national disease control strategies and meeting international obligations.

Participants during a technical sessions

National Workshop on 'Fish Cell Line: Development and Storage'

A National Workshop on 'Fish Cell Line: Development and Storage' was organized at NBFGR, Lucknow in collaboration with the Department of Biotechnology (DBT), Govt. of India and Aquatic Biodiversity Conservation Society Lucknow on 19 April, 2012. A galaxy of scientists from Network of Aquaculture Centre for Asia-Pacific (NACA), Thailand; various institutes of ICAR and universities participated

Participants, dignitaries and staff of NBFGR

in the workshop. The workshop was inaugurated by Dr. S.A.H. Abidi, Former Member ASRB where as Dr. Dilip Kumar, Former Director, Central Institute of Fisheries Education, Mumbai presided over the function. Dr. J.K. Jena, Director, NBFGR and Convener of the Workshop highlighted the prospects of cell lines in treating fish diseases to enhance the fisheries production in India. Guest of Honor Dr. A.S. Ninawe, Advisor, DBT, Govt. of India, New Delhi stressed upon the need for developing a perfect protocol for characterization of the cell lines in order to ensure their utility. He stressed that over 50 fish cell lines have been developed from different species in India and to store these cell lines a cell line repository is urgently required. Keeping this in view DBT has funded a project to establish of a National Repository of Fish Cell lines at NBFGR, Lucknow. The participants deliberated on the status of fish cell lines, its application and storage in the repository. The workshop was aimed to prioritize the species to be used for cell line development and to make strategies for collection, characterization, storage and distribution of cell lines through a national repository of fish cell lines for R&D work.

Workshop on 'Strategic Action Plan for Exploration and Characterization of Fish Germplasm Resources and Indigenous Knowledge in North-Eastern Region of India'

A workshop on 'Strategic action plan for exploration and characterization of fish germplasm resources and indigenous knowledge in North-Eastern region of India' was organized by NBFGR, Lucknow, in collaboration with College of Fisheries (COF), Agartala during 5-6 May, 2012 at Agartala. The workshop was inaugurated by Prof S.N. Puri, Vice Chancellor, Central Agricultural University, Imphal.

Prof. S.N. Puri delivering the Inaugural Address

A total of 60 invited participants from different states of the North-eastern region, including scientists of NBFGR participated in the workshop. The objectives of the workshop were: (1) prioritize aquatic areas for exploration based on identified criteria like prior knowledge on biodiversity richness or endemicity of the region, recognized protected or conservation importance, dependence of tribal or native ethnic populations utilizing such aquatic resources, knowledge gap on aquatic bioresources of the region; (2) formulate work pogrammes under network and sub network mode, and (3) discuss operational strategy and capacity building needs of the perspective collaborators. The idea was to address researchable issues through combined use of expertise available at NBFGR and various organizations in the region for exploration of aquatic germplasm resources of the North Eastern Region of India. Participants from different North-eastern states presented their research proposals under four themes viz., exploration of fish germplasm resources, biological and genetic characterization, documentation of indigenous knowledge, and live gene banking and captive breeding.

Technical session during workshop

International Day for Biological Diversity

Uttar Pradesh State Biodiversity Board (UPSBB) and NBFGR, Lucknow jointly organized a Conference on 22 May, 2012 at Dr. Ram Manohar Lohia Law University, Lucknow and celebrated International Day for Biological Diversity with the theme 'Marine Diversity'. Dr. Syed Azmal Khan, Prof. Emeritus, Centre for Advanced Studies, Marine Biology, Annamalai University, Tamil Nadu was the Chief Guest of the function. The programme was attended by Shri R.K. Singh, President, UPSBB and Secretary, Ministry of Environment & Forest, Government of Uttar Pradesh; Shri Pawan Kumar, Secretary, UPSBB; Shri J.S. Asthana, Chief Conservator of Forest, Uttar Pradesh; Prof. B. Chauhan, Vice Chancellor, Dr. Ram Manohar Lohia National Law University, Lucknow; Dr. J.K. Jena, Director, NBFGR, Lucknow and a galaxy of eminent environmentalists, scientists, professors and teachers of various universities / institutions and students.

International Day for Biological Diversity

Workshop on ''Sustainable Agriculture Development and Conservation of Fish Genetic Resources in Uttar Pradesh''

The Institute organized a strategic workshop on ''Sustainable agriculture development and conservation of fish genetic resources in Uttar Pradesh'' for the benefit of officials of U.P. State Fisheries Department on 18 August, 2012 at NBFGR, Lucknow. The programme was inaugurated by Dr. Alok Ranjan, Agriculture Production Commissioner, Govt. of U.P. Dr. Ranjan lauded the support of NBFGR in the organization of this workshop and expressed that outcomes of this workshop would provide new dimensions for the development of fisheries in U.P. Dr. J.K. Jena, Director, NBFGR in his address gave brief account of various available techniques of freshwater aquaculture, prospects of species diversification for aquaculture, seed certification for quality assurance, multidisciplinary approaches for fish conservation and development of salt-affected land for aquaculture. Speaking on the occasion, Dr. Dilip Kumar, Former Director, CIFE, Mumbai gave an overview of the present status of fisheries in U.P. and the developments needed for the growth of fisheries sector in the state. He stressed that for effective development of fisheries in the state, close linkages among ICAR institutes, state fisheries department and the stakeholders need to be strengthened. Dr. A.P. Sharma, Director, CIFRI, Barrackpore gave an account of open water fisheries with regard to development of fisheries in rivers, reservoirs and wetlands and suggested measures for meeting burgeoning large-size seed demand for open water fisheries through cage and pen farming. Shri D.K. Singh, Director, Fisheries, U.P. highlighted about the programmes undertaken by the department

Dr. Alok Ranjan, APC, U.P. inaugurating the workshop by lighting the lamp

Workshop on sustainable agriculture development and conservation of fish genetic resources in UP

and briefed about the proposed action plan for the XII plan. Over 90 officials of state fisheries department, scientists of NBFGR and progressive farmers from various parts of the state attended the workshop.

National Consultation on 'Alien Fish Species in Aquaculture and Aquarium Trade: Issues and Perspectives

A National Consultation on 'Alien Fish Species in Aquaculture and Aquarium Trade: Issues and Perspectives' was organised at NBFGR, Lucknow during 6-7 September, 2012 in collaboration with Department of Animal Husbandry, Dairying and Fisheries (DAHDF), Government of India; National Fisheries Development Board (NFDB), Hyderabad and Aquatic Biodiversity Conservation Society (ABCS), Lucknow. Over 80 scientists, government officials, development officials, ornamental fish breeders, entrepreneurs, policy makers, academicians and representatives of NGOs participated in the consultation to discuss various issues related to fish introductions, aquaculture and aquarium fish trade development on sustainable basis. The Consultation

Shri Tarun Shridhar, Jt. Secretary (Fy.), DAHDF, inagurating the National Consultation by lighting the lamp

was inaugurated by Sh. Tarun Shridhar, Joint Secretary (Fisheries), DAHDF, Ministry of Agriculture, New Delhi. The other important dignitaries present on the occasion were Dr. R Paul Raj, Member Secretary, Coastal Aquaculture Authority; Dr. P Jayasankar, Director, CIFA and Dr. K Vijayakumaran, Director General, Fisheries Survey of India, Mumbai. Dr. J K Jena, Director, NBFGR and Dr. A K Singh, Principal Scientist, NBFGR coordinated the proceeding of the consultation. During the consultation, status and various issues pertaining to alien species in aquaculture and aquarium

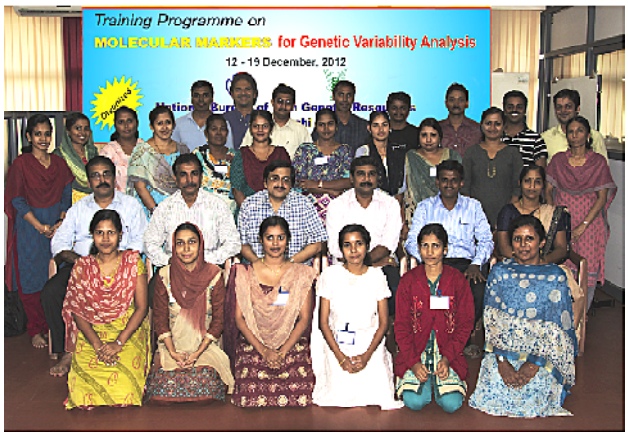
National consultation on alien fish species in aquaculture a aquarium trade

trade in the country were discussed. The consultation resulted in useful recommendations for follow up action which can support responsible trans-boundary movement and use of alien species in the country.

Participants during a technical session

Training programmes on application of genetic markers

Two training programmes on application of genetic markers entitled 'Molecular Markers in Fisheries Research' and 'Molecular Markers in Genetic Variability Analysis' were conducted at NBFGR Kochi Unit during 12-19 September, 2012 and 2-19 December



Training participants with staff of NBFGR, Kochi Unit

Training participants with staff of NBFGR, Kochi Unit

2012. The training programmes were aimed to impart theoretical and practical knowledge on various classes of molecular markers and on the recent developments in the field, with an ultimate goal to develop trained manpower in the field of DNA marker techniques.

Subject matter training on 'Tools for Functional and Comparative Genomics in Fisheries Domain'

A subject matter training on 'Tools for Functional and Comparative Genomics in Fisheries Domain' was organized during 27 November – 7 December, 2012. A total of 17 participants from various research institutes and universities of the country participated in the training programme. The training programme intended to provide a wide knowledge to the participants about utilization of various bioinformatics tools in comparative and functional genomics for extraction of biologically useful information. Eminent scientists from different institutes viz. NRCPB, New Delhi; IASRI, New Delhi; IITR, Lucknow; Amity University, Gurgaon and Lucknow; Biotech Park, Lucknow; BBA University, Lucknow and Shiv Nadar University Greater Noida delivered guest lectures during the programme.

Participants with Director and faculty of NBFGR

Agricultural Research & Development Conclave for UP

A two days Agricultural Research & Development Conclave and Kisan Vigyan Sangam for Uttar Pradesh was organized jointly by the ICAR institutes located at Lucknow viz., IISR, CISH, NBFGR and CSSRI Regional Station Lucknow at IISR premise during 23-24 November, 2012. The conclave was inaugurated by Professor R.B. Lal, Vice Chancellor, SHIATS, Allahabad. Multi-dimensional activities including brainstorming sessions, discussion sessions, demonstrations, *kisan gosthi*, exhibition, awareness lectures, etc., were organized in the conclave. Dr. S. Solomon, Director, IISR highlighted the

Glimpse of Agricultural research and development conclave for U.P.

importance of conclave while welcoming the guests and delegates. Professor Lal in his inaugural address said that agriculture is the source of livelihood for two-third population of the Uttar Pradesh. With huge yield gaps in the areas of agriculture and allied sectors there is immense scope for productivity enhancement by harnessing the potential of available technologies. Prof. Lal said that UP has a significant bearing on the national economy contributing more than 13% of agricultural GDP of the country. Dr. J.K. Jena, Director, NBFGR in his address highlighted that UP being a land locked state with vast freshwater resources, has immense potential in aquaculture sector. Dr. H. Ravisankar, Director, CISH said that the state with a share of 26% in the total horticultural production, has an important place in the horticulture sector of the country. Demonstration and explanation to development officials and farmers on technologies developed by various ICAR institutes, and *kisan gosthi* where farmers' queries were answered by the scientists, were major highlight of the programme.

Hindi Workshop on Administrative and Financial Rules

The Institute organized a Hindi Workshop on General administrative and financial rules on 28 December, 2012. All the staff of the Institute, as well as, other participants from the neighbouring ICAR institutes located at Lucknow participated in the Workshop.

Hindi workshop in progress

Workshop on Strategic Action Plan for Exploration of Fish Germplasm Resources and Traditional Ecological Knowledge of Tribal People

NBFGR organized a Workshop on Strategic Action Plan for Exploration of Fish Germplasm Resources and Traditional Ecological Knowledge of Tribal People for Sustainable Development in

Shri V.K. Shukla, Director, Fisheries, Chhittisgarh addressing the participants

Chhattisgarh State at College of Fisheries (Chhattisgarh Kamdhenu Vishwavidyalaya), Kawardha, Chhattisgarh during 22-23 February, 2013. The workshop was aimed at identifying potential locations and working strategy for taking up collaborative work programme for technological interventions for tribal development using fish germplasm resources. Over 150 participants participated in the workshop including fishermen/fish farmers/fishing cooperative societies office-bearers from tribal areas of Chhattisgarh state, faculty and students from College of Fisheries, Kawardha, Chhattisgarh; officials of Chhattisgarh State Fisheries Department and KVKs of Chhattisgarh

A view of the workshop

and scientists from NBFGR and other ICAR fisheries research institutes. Shri V.K. Shukla, Director, Fisheries, Chhattisgarh inaugurated the workshop, while Dr. J.K. Jena, Director, NBFGR, Lucknow presided over. Dr. H.K. Vardia, Dean, College of Fisheries, Kawardha, Chhattisgarh and Dr. B. Mal, Vice-Chancellor, Swami Vivekanand Technical University, Durg, Chhattisgarh delivered keynote lecture. Theme-wise open discussion sessions with the stakeholders were organized in the workshop.

Training Programme on Co-management of fisheries resources for sustainable utilization

The Institute organized a three days Training Programme on 'Co-management of Fisheries Resources for Sustainable Utilization' during 21-23 March, 2013 at NBFGR, Lucknow for middle level officials of the State Fisheries Departments and State Fisheries Development Corporations. A total of 15 participants from Assam, Bihar, Madhya Pradesh, Jammu and Kashmir, Punjab and Uttar Pradesh participated in this

Dr. P.C. Mahanta inaugurating the training programme by lighting the lamp

programme which was sponsored by the NFDB, Hyderabad. The training programme was aimed at enhancing the knowledge and skills of state fisheries officers to apply the co-management approach and participatory methodology in fisheries resources management for sustainable utilization of resources. The programme was inaugurated by Dr. P.C. Mahanta Former Director, DCFR, Bhimtal and ICAR Emeritus Scientist, NBFGR, Lucknow. In the valedictory function Dr. J.K. Jena, Director, NBFGR, Lucknow gave certificates to the participants.

Training participants with Guest and faculty of NBFGR

Dr. J.K. Jena giving certificate to a participant

Awareness programme on Fish Conservation and Tribal Communities at Agali, Attappadi, Kerala

A programme on "Fish Conservation Awareness and Tribal Communities" was conducted by NBFGR at Agali, Attappadi, Palakkad, Kerala in association with Attappadi Hills Area Development Society (AHADS), Integrated Tribal Development Project (ITDP), Govt. of Kerala and Fisheries Research Station, Puthuvypu, KUFOS, Kerala on 25 March, 2013. Smt. Usha Raja, Panchayath President, Agali Block was the Chief Guest of the function, Mrs. Sabira Ali, Agali Block Panchayath Standing Committee Chairperson, presided over the function. Mr. Radhakrishnan, ITDP Project Officer and other officials from ITDP and Panchayath were also present on this occasion. Over one hundred tribal people, planners, state and central government officials and research scholars attended the programme.

Dr. V.S. Basheer, Sr. Scientist speaking during awareness programme

AWARDS AND RECOGNITIONS

NBFGR Received the Sardar Patel Outstanding ICAR Institution Award for the year 2011

The NBFGR received the prestigious Sardar Patel Outstanding ICAR Institution Award for the year 2011 under small institute category. Dr. J.K. Jena, Director, received the award from Honb'le Union Minister for Agriculture Shri Sharad Pawar in presence of Shri Haris Rawat, Honb'le Minister of State for Agriculture and Dr. S. Ayyappan, Secretary, DARE and Director General, ICAR on the occasion of the Foundation Day of ICAR on 16 July, 2012 at New Delhi. The award comprised certificate, citation and cash prize of Rs. 10.0 lakhs. The NBFGR family takes pride in this achievement and vows to perform to the expectations of the Council and its stakeholders.

Dr. J.K. Jena, Director, NBFGR receiving the Award

Award of ISO 14001:2004 and 9001:2008 certificates to Ganga Aquarium

The Ganga Aquarium established at the NBFGR, Lucknow in 2010 for increasing public awareness about fish diversity and educating the children and general public towards its values, has been awarded with ISO 14001:2004 and 9001:2008 certifications. These certificates have been awarded to the aquarium for efforts of maintaining sound Environment Policy and Quality Management. Dr. J.K. Jena, Director, NBFGR,

Dr. J.K. Jena, Director receiving the ISO certificates

Dr. J.K. Jena, Director addressing the gathering on World Standards Day

Lucknow received the certificates in the presence of staff members, local guests and aquarium visitors in a function held on 14 October, 2012 on the occasion of World Standards Day.

Dr. P.R. Divya, Scientist, NBFGR Kochi Unit received the Kerala State Young Scientist award 2012 for the contributions in the field of Agricultural/ Fisheries Sciences, from the Kerala State Council for Science, Technology and Environment, Government of Kerala, Trivandrum. The award includes a citation, Rs. 50,000/- as cash and research grant up to Rs. 30 lakhs.

Dr. P. R. Divya receiving the Kerala State Young Scientist award for the year 2012

NBFGR Annual Hindi Magazine 'Matsya Lok' 2012 got the First Prize amongst the magazines brought out by the institutes/offices under central government located at Lucknow. It was awarded by the 'Nagar Rajbhasa Karyanvayan Samiti' at Hindustan Aeronautics Limited, Lucknow. The magazine was edited by Dr. L.K. Tyagi, Sr. Scientist and Shri Akhilesh Kumar Mishra, Hindi Officer.

Dr. J.K. Jena, Director, NBFGR receiving the First Prize awarded to 'Matsya Lok'

Dr. U.K. Sarkar, Principal Scientist was conferred with the Fellowship Award of the Inland Fishery Society of India, Barrackpore on 09 February, 2013 and Bioved Fellowship Award – 2013 during 14th Indian Agricultural Scientist and Farmers' Congress at Bioved Research Society, Allahabad..

Dr. A.K. Singh, Principal Scientist was awarded the Dutta Munshi Gold Medal Award of Zoological Society of India on 23 October, 2012 at Chennai.

Dr. A.K. Singh, Principal Scientist was conferred with the Fellowship Award of the Inland Fishery Society of India, Barrackpore on 10 February, 2013.

Dr. A.K. Singh receiving the (a) Dutta Munshi Gold Medal Award and (b) Fellowship Award of the Inland Fishery Society of India

Dr. L.K. Tyagi, Sr. Scientist was awarded the Best Paper Award for his oral paper presentation on 'Role and potential of grassroots institutions in sustainable fisheries resource management' in the National Seminar on Emerging Challenges and Paradigm for Sustainable Agri-Rural Development at Dr. Y.S. Parmar University of Horticulture and Forestry, Solan, H.P. during 18-20 December, 2012.

Dr. A. Gopalakrishnan, Principal Scientist & OIC, NBFGR Kochi Unit was elected as the Chairman, Genetics & Biodiversity of the E – Consultation of Task Force Members (TF) for Development of Network of Aquaculture Centers in Asia-Pacific (NACA), Bangkok Regional Thematic Work Programs (2012-2016).

Dr. L.K. Tyagi receiving the Best Paper Award from Dr. K.D. Kokate, DDG (Extension), ICAR

EXTENSION ACTIVITIES

Participation in exhibitions

The Institute participated in the following exhibitions related to fisheries and aquatic resources in different parts of the country during the year 2012-13:

- IAI Aquaculture Expo at IARI, Pusa New Delhi during 13 -15 November, 2012.
- COP-11 Biodiversity exhibition as a part of ICAR pavilion at High-tech City, Hyderabad organized by UN CBD and Ministry of Environment & Forests, Govt. of India during 1-19 October, 2012.
- Lucknow Mahotsav Festival during 25 November - 6 December, 2012.
- Exhibition in connection with Global symposium on Aquatic resources for eradicating hunger and malnutrition – opportunities and challenges at Mangalore during 2-7 December, 2012.
- XI Agricultural Science Congress Exhibition during 7-9 February, 2013 at Orissa University of Agriculture & Tech., Bhubaneswar.
- Exhibition on the occasion of Congress on 'Public-Private Partnership in Aquaculture and Culture Based Fisheries' at CIFRI, Barrackpore, Kolkata during 9-11 February, 2013.

Dr. S. Ayyappan and other dignitaries at the Institute's stall at CIFRI, Barrackpore

Outreach activities

- Dr. S. Raizada, Principal Scientist delivered a radio talk on Career opportunities in fisheries" from Akashvani, Lucknow on 20 June, 2012.
- Dr A.K. Singh, Principal Scientist delivered a Radio talk on "Matsya Palan" on 8 September, 2012
- Dr. Sudhir Raizada and Dr. P.K. Varshney, Principal Scientists of the Bureau visited Haridwar (Uttrakhand) for developing a strategic plan for development of fisheries in the district. The team visited 25 sites and made mass contact with the fish farmers and discussed various issues relating to poor performance of fisheries in the district. A report covering various problems and strategies suggested to the authorities. As a follow-up, quality carp fingerlings were supplied during September, 2012 for demonstration.
- Dr. S. Raizada, Principal Scientist delivered a talk on "Matsya Samayiki' telecasted on 17 December, 2012 at Lucknow Doordarshan.

Training on 'Aquaculture technologies and productivity enhancement'

The NBFGR, at its Aquaculture Research & Training Unit (ARTU), Chinhat, organized a series of short-term training programmes, sponsored by various agencies, for aqua-farmers and KVK subject-matter specialists. A total of ten training programmes were conducted during the period under report. A total of 244 progressive fish farmers from different districts of U.P., Bihar and Uttarakhand, and 17 subject-matter specialists of KVKs of UP and Uttarakhand were trained in these training programmes, as per details below:

The above training programmes were residential and field oriented hands-on trainings with practical demonstrations. Apart from theory classes, laboratory demonstrations and exercises were made. Field visit to the fish farms of the Institute were made to expose the trainees with various fisheries activities.

Period	Sponsored by	No. of trainees
5-7 July, 2012	NBFGR, Lucknow	29
11-15 September, 2012	ATMA, Gopalganj, Bihar	25
16-20 October, 2012	ATMA, Darbhanga, Bihar	20
21-25 November, 2012	NFDB, Hyderabad	26
18-22 December, 2012	NFDB, Hyderabad	31
12-16 February, 2013	NFDB, Hyderabad	42
25-28 February, 2013	ATMA, Bareily, UP	15
05-07 March, 2013	ICAR Zonal Project Directorate, Kanpur	17
12-16 March, 2013	NFDB, Hyderabad	35
18-20 March, 2013	NBFGR, Lucknow	21

Dr. J.K. Jena, Director giving certificate to a participant

Participants of a farmers training programme with staff of NBFGR

Fish Seed Production

The seed production of Indian major carps was continued under the ICAR Mega Seed Project. A total of 512.5 lakh spawn was produced. Emphasis was given on production of seed from riverine brood stock that was raised at the fish farm by stocking fingerlings collected from riverine sources. Revenue of Rs.5.21 lakh was generated from seed sale.

LIST OF PROJECTS

Institutional Projects

Sl. No.	Project Title	Personnel	Period
Molecular Biology & Biotechnology Division			
1	Development of protocol for germ cell transplantation in fish	B. Kushwaha (PI), Sudhir Raizada and Akhilesh Kumar Mishra	April, 2011 – March, 2014
2	Genetic Stock Structure Elucidation of *Tenualosa ilisha* and *Channa striata* using mitochondrial DNA marker, microsatellites and molecular cytogenetic tools	Mahender Singh (PI), N.S. Nagpure, Ravindra Kumar and Ajay Kumar Singh	April, 2010 – March, 2013
3	Protein profiling of Indian Major Carpsbased on mass spectrometric Analysis (ESI)	M. Goswami (PI), N.S. Nagpure, Ravindra Kumar and A. Srinivasan (AIIMS, New Delhi)	April, 2010 – March, 2013
Fish Conservation Division			
4	Genetic approaches for conservation of prioritized Indian fish species		
	Sub-project I: Documentation of genetic diversity in prioritized Indian fish species belonging to groups featherbacks, carps, murrels and catfishes	K.K. Lal (PI), Vindhya Mohindra, Peyush Punia and Rajeev Kumar Singh	April, 2009 – March, 2013
	Sub-project II: Documentation of mitochondrial genomes in fishes from important taxonomic groups	Rajeev Kumar Singh (PI), K.K. Lal and Vindhya Mohindra	April, 2009 – March, 2013
	Sub-project III: Development of breeding and sperm banking protocols for prioritized Indian finfishes belonging to groups catfishes, featherbacks, murrels and carps	S. Raizada (PI), K.K. Lal, P.K. Varshney and A.K. Yadav	April, 2009 – March, 2013
5	Outreach activity on Fish Genetic Stocks	K.K. Lal (PI), A. Gopalakrishanan, P. Punia, Vindhya Mohindra, Rajeev Kumar Singh, U.K. Sarkar, M. Goswami and J.K. Jena	April, 2008 – March, 2013
6	Neuroendocrine regulation of gonadal maturation in the golden mahseer, *Tor putitora* Hypothalamus	A.K. Pandey (PI), P.K. Varshney and A.K. Yadav	April, 2008 – March, 2013
7	Captive propagation of indigenous ornamental fishes	P.K. Varshney (PI), S. Raizada, A.K. Pandey, A.K. Yadav, S.K. Singh and Vikas Sahoo	April, 2011 – March, 2014
8	Exploration and assessment of fish diversity and traditional ecological knowledge in selected riverine and wetland ecosystems	L.K. Tyagi (PI), A.K. Pandey, A.K. Pathak, Sangeeta Mandal, A.S. Bisht, Vikas Sahu	April, 2012 – March, 2015

Sl. No.	Project Title	Personnel	Period
Fish Health Management Division			
9	Exploration of protozoan and monogenean parasites among carps and catfishes	Rehana Abidi (PI) and S.M. Srivastava	April, 2010 - March, 2013
10	Development of monoclonal antibody based marker for monitoring humoral immune response in *Catla catla*	Neeraj Soood (PI), Gaurav Rathore, P.K. Pradhan and Peyush Punia	April, 2010 - March, 2013
11	Development of in-vitro cellular model for assessment of innate immunity parameters of *Labeo rohita*	Gaurav Rathore (PI), Neeraj Soood, P.K. Pradhan and Peyush Punia	April, 2010 - March, 2013
12	Production of monosex/ sterile common carp *Cyprinus carpio* using aromatase inhibitors	A.K. Singh (PI)	April, 2009 - March, 2013
13	Molecular assay for detection of *Aphanomyces invadans* for surveillance and prediction of Epizootic Ulcerative Syndrome outbreaks	P.K. Pradhan (PI), Rehana Abidi, Gaurav Rathore, Neeraj Soood and T. Raja Swaminathan	April, 2009 - March, 2013
14	Development of biomarkers as diagnostic tools for assessment of fish health status	Peyush Punia (PI), P.K. Pradhan and Ranjana Srivastava	April, 2011 – March, 2014
Fish Taxonomy and Resources Unit			
15	Information base on fish genetic resources of India	S.P Singh (PI), A.K. Pathak, U.K. Sarkar, R. Dayal, Reeta Chaturvedi and Ravi Kumar	April, 2012 – March, 2015
Kochi Unit			
16	Genetic characterization of commercially important fishes from the Western Ghats and marine ecosystem using biotechnological tools		
	Sub-project I: Genetic characterization of commercially important fishes from the Western Ghats and marine ecosystem	A. Gopalakrishnan (PI), V.S. Basheer, P.R. Divya, T. Raja Swaminathan and A. Kathirvelpandian	April, 2009 – March, 2013
	Sub-project II: Disease surveillance of endemic and commercially important ornamental fishes of the Western Ghats	T. Raja Swaminathan (PI), A. Gopalakrishnan, V.S. Basheer, P.R. Divya and A. Kathirvelpandian	April, 2009 – March, 2013
	Sub-project III: Conservation of endemic freshwater fishes of the Western Ghats through milt cryopreservation and captive breeding	V.S. Basheer (PI), A. Gopalakrishnan, P.R. Divya, T. Raja Swaminathan and A. Kathirvelpandian	April, 2009 – March, 2013

Externally – funded Projects

Sl. No.	Project Title	Personnel	Funding agency	Period
1	Bioprospecting of genes and allele mining for abiotic stress tolerance	Vindhya Mohindra (PI), Ravindra Kumar and Rajeev Kumar Singh	NAIP, ICAR	May, 2009 – March, 2013
2	Genetic characterization and conservation biology of economically important Siluroid fish *Ompok pabda* of Tripura	U.K. Sarkar (PI), Mahender Singh and S. Banik (Tripura University)	DBT, Govt. of India	March, 2011– February, 2014
3	Establishment of National Agricultural Bioinformatics Grid in ICAR	N.S. Nagpure (CCPI), S.P. Singh, A.K. Pathak, U.K. Sarkar and Mahender Singh	NAIP, ICAR	April, 2010 – March, 2014
4	Microsatellite markers for genetic diversity analysis in natural populations of Cobia (*Rachycentron canadum*) and Silver pomfret (*Pampus argenteus*)	P.R. Divya (PI), A. Gopalakrishnan and V.S. Basheer	DBT, Govt. of India	November,2010 – November, 2013
5	Development and characterization of cell lines from *Schizothorax richardsonii* and *Puntius* (Tor) *chelynoides*	M. Goswami (PI) and S.N. Bahuguna (HNB Garhwal University, Srinagar, Uttarakhand)	DBT, Govt. of India	November, 2009 –November, 2012
6	Establishment of a National Repository for conservation and characterization of fish cell lines at NBFGR, Lucknow	M. Goswami (PI)	DBT, Govt. of India	November, 2010 – November, 2013
7	Harmonizing biodiversity conservation and agricultural intensification through integration of plant, animal and fish genetic resources for livelihood security in fragile ecosystem	K.K. Lal (CCPI), P. Punia, Vindhya Mohindra, L.K. Tyagi, Rajeev Kumar Singh, U.K. Sarkar, A.K. Pathak and J.K. Jena	NAIP, ICAR	September, 2009 - August, 2013
8	Germplasm Exploration, Assessment and Documentation of the Freshwater Fish Diversity of Uttar Pradesh	U.K. Sarkar (PI) and A.K. Pathak	UPSBB, Lucknow	May, 2011 – March, 2013
9	Development of cryopreservation protocol for breeding and grow-out technology for giant snakehead *Channa marulius*	Peyush Punia (PI) and S. Raizada	UPCAR, Lucknow	January, 2010 – December, 2012
10	Ecological impact assessment of African catfish *Clarias gariepinus*: disease risks and potential for resource competition	A.K. Singh (PI), Rehana Abidi and A.K. Pathak	UPCAR, Lucknow	February, 2010 – February, 2013

Sl. No.	Project Title	Personnel	Funding agency	Period
11	Inventorisation, impact assessment and risk communication of invasive fish species in Uttar Pradesh	A.K. Singh (PI), RehanaAbidi, A.K. Pathak and S.M. Srivastava	UPSBB, Lucknow	April, 2011-March, 2013
12	Assessment of aquatic health using recent cellular and molecular tools in endocrine research	A.K. Singh (PI)	UPCST, Lucknow	August, 2011 - August, 2014
13	Genetic characterization of selected freshwater fish species endemic to Indian region of the Indo-Burma biodiversity hot-spot using advanced cytogenetic markers	Ravindra Kumar (PI), B. Kushwaha (NBFGR, Lucknow) and GusheinzedWaikhom (PI), T. Shantibala (IBSD, Imphal)	DBT, Govt. of India (Twining Pro. for NE)	March, 2011 – March, 2015
14	Development of surrogate broodstock technology for commercially important fish species: implications for speedy propagation and conservation	B. Kushwaha (PI) and Ravindra Kumar	DBT, Govt. of India (Twining Pro. for NE)	December, 2011 – November, 2015
15	Identification and evaluation of reproductive traits and genetic structure of *Ompokbimaculatus*in India	U.K Sarkar(PI), Ravindra Kumar and Abha Mishra, BRAU, Lucknow	DBT, Govt. of India	September, 2011 – September, 2014
16	Ontogeny of the digestive system during larval development of Indian butter fish, *Ompokbimaculatus*	P.K. Pradhan (PI)	Internal Foundation for Sciences, Swedan	November, 2008- May, 2012
17	Isolation and characterization of *Flavobacterium* species from fish and aquatic Environment	G. Rathore (PI)	NBAIM, ICAR	August, 2006-March, 2013
18	Development of novel microsatellites in *Channa*species (Channidae: Perciformes) from North East for conservation genetics	Rajeev Kumar Singh (PI), L.K. Tyagi and A.S. Barman (College of Fisheries, CAU, Lembuchera, Agartala)	DBT, Govt. of India	April, 2012-March, 2015
19	DNA Barcoding of Marine finfishes and shellfishes	A. Gopalakrishnan (PI), J.K. Jena and V. S. Basheer	MoES-CMLRE, Govt. of India	November, 2012 – October, 2017
20	Understanding of molecular pathogenesis of epizootic ulcerative syndrome (EUS) in fish and development of newer strategies to combat EUS	P.K. Pradhan (PI), NeerajSood, ChandanDebnath and LopamudraSahoo (ICAR Complex, Tripura)	DBT, Govt. of India	May, 2012 – April, 2015
21	Fish diversity of Ramgarh and Bakhira Lake: comparison of present status with pristine data for conservation and sustainable utilization	A.K Pandey (PI)	UPSBB, Lucknow	February, 2013 –March, 2015

PARTICIPATION IN SEMINARS/ SYMPOSIA/ WORKSHOPS/ TRAININGS/ MEETINGS

Abroad:

Dr. J.K. Jena, Director, participated in the Training programme on "Leadership Decision Making: Optimizing Organizational Performance" conducted by the Harvard Kennedy School, Executive Education, Cambridge, MA, USA during 28 October - 02 November, 2012.

Dr. L.K. Tyagi, Sr. Scientist participated in the International Conference on 'Design and Dynamics of Institutions for Collective Action' at the Utrecht University, Utrecht, The Netherlands during 29 November – 1 December, 2012.

Dr. A. Gopalakrishnan, Principal Scientist and SIC, NBFGR Kochi Unit attended the Bay of Bengal Large Marine Ecosystem (BOBLME) Project Working Party Meeting on Assessing stock structure of Indian Mackerel (Rastrelliger kanagurta) for Fisheries Assessment in Colombo, Sri Lanka, during 28 - 29 May, 2012.

Dr. P.K. Pardhan, Senior Scientist, attended two months training under International Foundation for Science at Institute of Research in Agriculture and Food Technology (IRTA), Sant Carlos de la Rapita, Spain during 20 April - 20 June, 2012.

In India

Dr. J.K. Jena, Director participated in the following:

National Consultation Workshop on 'Coastal & Marine Biodiversity: Gaps, Challenges and Opportunities' from 12-13 April, 2012 at Gandhinagar, Gujarat.

Inauguration of Office Complex of National Fisheries Development Board on 20 April, 2012 at Hyderabad.

Joint meeting of DARE/ ICAR and NBA on draft guidelines for access to genetic resources for food and agriculture and other related issues on 2 May, 2012 at NBPGR, New Delhi.

Meet on 'Higher Fisheries Education-Way Forward' on 14 May, 2012 at NASC, New Delhi.

National Consultation on "Setting National Biodiversity Targets" (2012-2020) at NASC Complex, New Delhi on 31 July, 2012.

Knowledge Meet, Meeting of State Agriculture Universities Vice-Chancellors and ICAR Directors at NASC Complex, Pusa, New Delhi during 21-22 Aug., 2012.

Meeting on State of the Word's Aquatic Genetic Resources: Preparation of Country Reports and Nomination of National Focal Points at Krishi Bhavan, New Delhi on 24 Aug., 2012.

Consultative Workshop entitled "Conclave on Aquaculture Development for Stakeholders of North Eastern Region" at Shillong on 11 September, 2012.

XXI Meeting of ICAR Regional Committee No. IV at ICAR - RCER, Patna during 21-23 Sep., 2012.

Expert Consultation on "Managing Trans-Boundary Diseases of Agricultural Importance in Asia-Pacific" at NASC from 10-12 October, 2012.

11th Meeting of the "Conference of the Parties to the Convention on Biological Diversity" (COP 11) during 17-18 October, 2012 at Hyderabad.

"Kisan Sammelan" at Bioved Research Institute of Agriculture & Technology on 11 Nov., 2012.

Indian Science Congress at CIFRI, Barrackpore, Kolkata during 3-6 January, 2013.

International Symposium on 'Genomics in Aquaculture' at CIFA, Bhubaneswar during 22-23 January, 2013.

National Symposium on 'Opportunities in Aquaculture Biotechnology' at Swami Ramanand Teerth Marathwada University, Nanded, Maharastra during 27-28, January 2013.

"Global Consultation on Use and Management of Agro-Biodiversity for Sustainable Food Security" at New Delhi during 13-14 February, 2013.

First Advisory Committee Meeting of the NFBSFARA project titled 'Stock Characterization, Captive Breeding, Seed Production and Culture of Hilsa (*Tenualosa ilisha*)' at New Delhi on 14 February, 2013.

3rd meeting of the 'National Advisory Board on Management of Genetic Resources' at NBAGR, Karnal on 5 March, 2013.

Meeting of the Directors and Head of Divisions of ICAR Institutes with Secretary, DARE & DG, ICAR

at NASC Complex, New Delhi on 15 March, 2013.

ICAR Directors' Conference at NASC Complex, New Delhi during 19-20 March, 2013.

Meeting on 'Fishpedia' chaired by DDG (Fy.) at ICAR, New Delhi on 21 March, 2013.

Dr. J.K. Jena, Director and Dr. K.K. Lal, Head, Fish Conservation Division participated in the Mid-term Review of NAIP-GEF project with DG, ICAR on 16 May, 2012 at New Delhi.

Dr. J.K Jena, Director; Dr. K.K. Lal, Head, Fish Conservation Division; Dr. P. Punia, Head, Fish Health Management Division; Shri A.K. Pathak, Scientist; Dr. L.K. Tyagi, Sr. Scientist and Shri A.S Bisht, Technical Officer (T-5) attended Workshop on Strategic Action Plan for Exploration of Fish Germplasm Resources and Traditional Ecological Knowledge of Tribal People for Sustainable Development in Chhattisgarh State at College of Fisheries, Kawardha, Chhattisgarh during 22-23 February, 2013.

Dr. J.K Jena, Director; Dr. A.K. Singh; Dr. U.K. Sarkar, Principal Scientists and Shri A.S. Bisht, Technical Officer (T-5) attended Congress on 'Public-Private Partnership in Aquaculture and Culture Based Fisheries' at CIFRI, Barrackpore, Kolkata during 9-11 February, 2013.

Dr. Sudhir Raizada, Principal Scientist participated in the following:

Meeting of the "Mission Document on Fisheries Development in U.P." on 01 August, 2012.

Workshop on "RTI Act 2005 for PIO's" at ISTM, New Delhi during 16-18 August, 2012.

Meeting of the Institute Management Committee of Zonal Project Directorate, Zone IV, Kanpur on 15 September, 2012.

Meeting of "Mass Media Support to Agriculture Extension" at Lucknow Doordarshan on 22 November, 2012.

Meeting of the Committee on Drafting guidelines and BMP's in hatcheries and nurseries for quality seed production of brackish water finfishes and shellfishes at CIBA, Chennai on 4 March, 2013 and for freshwater finfishes and shellfishes at CIFA, Bhubneshwar on 12 March, 2013.

Dr. A. Gopalakrishnan, Principal Scientist and Incharge, NBFGR Kochi Unit attended the following:

National Consultation on Coastal and Marine Biodiversity – Gaps, Challenges and Opportunities at Gandhi Nagar, Gujarat during 12-13 April, 2012.

Golden Jubilee celebrations of CIFRI, Barrackpore Centre and Interactive session on reservoir fisheries and aquaculture issues at CIFRI, Barrackpore centre on 14 April, 2012.

Indian Mackerel Workshop Group Meeting: Indian Mackerel and Genetics organized by the Bay of Bengal Large Marine Ecosystem (BOBLME) project at Colombo, Sri Lanka during 28-29 May 2012.

Foundation Day and Annual General Body Meeting of National Academy of Agricultural Sciences, New Delhi and received the NAAS fellowship during 4-5 June, 2012 at NASC complex, New Delhi.

23rd Regional Committee Meeting (Region VIII) during 15-16 June 2012 at Sugarcane Breeding Institute (SBI), Coimbatore during 15-16 June, 2012.

24th meeting of the SAC of Rajiv Gandhi Centre for Aquaculture on 25 March, 2013 at MPEDA, Kochi.

Dr. A. Gopalakrishnan, Dr. V.S. Basheer, Dr. T. Raja Swaminathan and Mr. A. Kathirvelpandian participated in the Global Symposium on Aquatic Resources for Eradicating Hunger and Malnutrition – Opportunities and Challenges at Mangalore during 2-7 December, 2012.

Dr. A.K. Pandey, Principal Scientist participated in the following seminars/ meeting/ workshops:

National Seminar on Advanced Biology: Emerging Trends in Biological Galaxy organized by Department of Zoology, C.M.P. College (University of Allahabad), Allahabad during 6-7 October, 2012.

National Seminar on "Mountain Fisheries: Challenges and Opportunity for Livelihood Security" at DCFR, Bhimtal during 5-6 November, 2012.

National Symposium on "Live Organisms and their Expression in the Environment" at University of Calcutta, Technology Campus, Kolkata during 26-28 November, 2012.

Global Meet of Biologists & Satellite Conference on "Vector Control and Management: Present Status and Future Strategies" at Osmania University & Indian Institute of Chemical

Technology, Hyderabad during 26-28 December, 2012.

32nd Annual Session of the Academy of Environmental Biology and National Seminar on "Emerging Pollutants and Pathogens: Challenges and Risk Reduction" at IITR, Lucknow during 20-22 September, 2012.

National Seminar on Emerging Pollutants and Pathogens due to Climate Change: Challenges and Risk Reduction at N.D.U.A.T, Kumarganj, Faizabad during 8-9 March, 2013.

National Symposium on Advances in Zoology & Environmental Science at Gorakhpur University, Gorakhpur during 13-14 March, 2013.

National Seminar on Faunal Diversity and its Conservation at D.A.V. Post- Graduate College, Muzaffarnagar during 19-20 March, 2013.

National Seminar on Changing Environment and Biodiversity at Department of Zoology, Navyug Kanya Mahavidya-aya, Lucknow on 24 March, 2013.

Dr. U.K. Sarkar, Principal Scientist participated in the following seminars/ workshops:

Symposium on Health and fisheries of the major river ecosystems of India with special emphasis on River Ganga at CIFRI, Barrackpore during 5-6 January, 2013.

Hindi Workshop on Fisheries Development of the Eastern and NE Region held at CIFE Kolkata Centre on 06 January, 2013.

National Conference of the Bioved Research Institute of Agriculture and Technology, Allahabad during 22-24 February, 2013.

Review meeting and Consultation of the NABG at IASRI, New Delhi during 19-21 July, 2012.

National Workshop on "Taxonomic keys and identification tools for different groups of flora and fauna" at Gurukula Kangri University, Haridwar, Uttarakhand on 01 November, 2012 and participated in the Expert consultation and review meeting of the National Agricultural Bioinformatics Grid, NAIP at NBAIM, Mau on 26 November, 2012.

Dr. P. K. Varshney, Principal Scientist participated in the following:

National workshop on "Technological Development in Fisheries" at CIFT, Kochi during 17-18 August, 2012.

Brainstorming session on "Present Agriculture Policy to Formulate the Future Plan in Uttar Pradesh" at U. P. Council of Agricultural Research, Lucknow on 7 August, 2012.

A Meeting on "Preparation of Mission Document for the Development of Fisheries in Uttar Pradesh" at Department of Fisheries, U.P, Lucknow on 31 July, 2012.

International Conference on "Food Processing and Development of Backward Regions: Preparing a Roadmap with special reference to Eastern Uttar Pradesh" at Department of Professional Studies, University of Allahabad during 27-28 December, 2012.

Dr. A.K. Singh, Principal Scientist Participated in the following:

Scientific Advisory Committee Meeting of KVK, Sitapur on 25 March 2013.

National Seminar on "Emerging Trends in Indian Aquaculture" at Thiruvanthpuram during 21-23 March 2013.

Satellite Symposium of Health and Fisheries of the Major River Ecosystem of India with emphasis on River Ganga at CIFRI, Barrackpore during 5-6 January, 2013.

23rd All India Congress of Zoology & National Conference on Conservation and Management of Faunal Resources for Sustainability at Guru Nanak College, Chennai during 3-5 October, 2012.

Dr. L.K. Tyagi, Sr. Scientist participated in the following:

Training workshop on "Institutional Innovations in Agri.-Extension for Inclusive Growth" at NAARM, Hyderabad during 1-7 August, 2012.

International Conference on "Governance of Commons and Livelihood Security" at XISS, Ranchi, Jharkhand during 17 - 18 August, 2012.

Meeting to prioritize areas for Human Resource Development at NFDB, Hyderabad as NBFGR representative on 09 October, 2012.

The CBD Conference of Parties 11 (CoP 11) including various side events and biodiversity exhibition at Hyderabad during 01 to 19 Oct., 2012.

Meeting of the Committee for "Formulation of Guidelines on Management of Genetic Resources"

at NBPGR, New Delhi. on 23 November, 2012.

National Seminar on "Emerging Challenges and Paradigm for Sustainable Agri-Rural Development" at Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, H.P.held during18-20 December, 2012.

Dr. V.S. Basheer, Sr. Scientist participated in the following:

CSIRO - IITR workshop on Safe water for the future, on 07 November, 2012 at IITR, Lucknow.

National Conference on Aquaculture: Fish for Billion in connection with CIFA silver jubilee celebration, during 16-17 March, 2012 held at Bhubaneswar.

15th Governing Council Meeting of the State Fisheries Resources Management Society (FIRMA), Thiruvananthapuram at Ernakulam on 9 April, 2012.

Workshop for "International Centre for Below Sea Level Farming in Kuttanad", on 1 March, 2013 held at Trivandrum.

Dr. T. Raja Swaminathan, Sr. Scientist participated in the following:

Consultation Meeting on Network on Fish Health and Platform on Diagnostic and Vaccine during 7-8 August, 2012 at CIBA, Chennai.

Workshop cum Training on Shrimp Pathology at Rajiv Gandhi Centre for Aquaculture at Karaimedu, Sirkali during 7-12 January, 2013.

Dr. Mahender Singh, Sr. Scientist participated in International Conference on "Statistics and Informatics in Agricultural Research" at IASRI, New Delhi during 18-20 December, 2012.

Dr. M. Goswami, Sr. Scientist participated in the International Conference on Stem Cells and Cancer at RML Hospital, New Delhi during 27-30 October, 2012 and Second National Conference on Fisheries Biotechnology, CIFE, Mumbai during 2-3 November, 2012.

Dr. P.R. Divya, Scientist participated in the following:

National Level Brainstorming Workshop on Gender Mainstreaming in Fisheries at CIFT, Kochi during 4 April, 2012.

State Level Seminar on Recent Trends in Molecular Taxonomy and delivered a talk on Barcoding of Fishes - Modern approach to resolve taxonomic ambiguities", on 28 February, 2013 at Maharaja's College, Ernakulam.

Kerala Science Congress at Kerala State Council for Science Technology and Environment, Trivandrum on 4 January, 2013.

Shri A.S. Bisht, and Shri S.K. Singh, Technical Officers (T-5) participated in IAI Aquaculture Expo at IARI, New Delhi during 13 -15 Nov., 2012 and COP-11 Biodiversity exhibition as a part of ICAR pavilion at Hyderabad during 01-19 October, 2012.

Dr. A.K. Singh; Dr. U.K. Sarkar and Dr. Basdeo Kushwaha, Principal Scientists participated in the 100th Indian Science Congress organized by Kolkata University at Kolkata during 3-7 January, 2013.

Dr. Ravindra Kumar and Dr. Basdeo Kushwaha, Principal Scientists and Dr. Mahander Singh, Sr. Scientist participated in International Symposium on 'Genomics in Aquaculture' at CIFA, Bhubaneswar during 22-23 January, 2013.

Dr. Sudhir Raizada and Dr. P.K. Varshney, Principal Scientists participated in the Meeting on "*Udyan evam khaad prasanskaran, pashudhan evam matsya sambandhi vishaion par sayunkta vichar ghosti*" (in Hindi) at Lucknow on 04 July, 2012.

Dr. P. K. Varshney, Principal Scientist, Shri A. K. Yadav, Technical Officer (T 7 & 8) and Shri S. K. Singh, Technical Officer (T-5) attended "Science Awareness Campaign" organized jointly by National Council of Science & Technology & Vigyan Jagrukta Samiti at Saket College, Faizabad on 02 March, 2013.

Dr. Sudhir Raizada, Principal Scientist; Dr. L.K. Tyagi, Sr. Scientist and Shri A.S Bisht, Technical Officer (T-5) attended "Science Expo 2013" at Regional Science City, Lucknow on 30 January, 2013.

Shri A.S. Bisht, Technical Officer (T-5) attended XI Agricultural Science Congress during 7-9 February, 2013 at Orissa University of Agriculture & Tech., Bhubaneswar.

PUBLICATIONS

INTERNATIONAL JOURNALS

Chowdhury, S., P. P. Srivastava, Suman Mishra, A. K. Yadav, R. Dayal. S. Raizada and J. K. Jena, 2012. Partial replacement of dietary animal protein with vegetable protein blend with different proportions of glucosamine on growth, feed efficiency, body composition and survival of fingerlings of Asian catfish (*Clarias batrachus*). *National Academy of Science Letters*, 35(4): 291–297.

Das, R., V. Mohindra, R. K. Singh, K. K. Lal, P. Punia, P. Masih, R. M. Mishra and W. S. Lakra, 2012. Intraspecific genetic diversity in wild *Catla catla* (Hamilton, 1822) population assessed through mtDNA Cytochrome b sequences. *Journal of Applied Ichthyology*, 28(2): 280-283.

Das, M. K., A. Basishya, U. K. Sarkar, W. S. Lakra and S. Bordoloi, 2012. Standard measurement and sexual dimorphism of a cobitid loach, *Lepidicephalichthys goalparensis* (Pillai & Yazdani, 1976). *International Journal of Science and Nature*, 3(4): 763-767.

Dayal, R., P. P. Srivastava, A. Bhatnagar, S. Chowdhary, W. S. Lakra, S. Raizada and A. K. Yadav, 2012. Comparative study of WLR of *Channa striatus* of fry, fingerling, grow-outs and adults of Gangetic plains. *Online Journal of Animal Feed Research*, 2(2): 174-176.

Dayal, R., P. P. Srivastava, A. Bhatnagar, S. Raizada, S. Chowdhary, A. K. Yadav and W. S. Lakra, 2012. Captive spawning of the striped murrel, *Channa striatus* (Bloch) using sGnRH, in Gangetic plains of India. *The Proceedings of the National Academy of Sciences, India, Section B: Biological Sciences*, 83(1): 65-70.

Dayal, R., P. P. Srivastava, A. Bhatnagar, S. Chowdhary, A. K. Yadav and J. K. Jena, 2012. Influence of different sources of dietary lipid on the growth, feed efficiency and survival of snakehead, *Channa striatus* (Bloch, 1793) grow-out. *National Academy Science Letters*, 35(6): 541–546.

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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 546 documents comprising 345 books, 142 serials and 59 annual reports. Now, the library has the total collection of 7920 books, 2254 bound volumes of journals, 3466 serials and 2261 reprints. The library has subscribed 23 international journals and 71 Indian journals. In addition to these, 38 journals were received on gratis/ exchange basis.

Library Automation

The NBFGR library is operating in fully automated environment. The various activities of library have beencomputerized 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 is under active process. Online Public Access catalogue is made available for the library users.

Information and Reference Services

The references from different databases using Internet were searched and arranged to suit the requirements of users. List of the books added to the library has also been brought out on quarterly basis. The users of the library extensively used the Consortium of E-Resources on Agriculture (CERA), to access the journals related to agriculture and allied sciences.

Technical Reports and Reprography Services

The library unit provided technical support to bring out departmental publications. The unit also attended to questionnaires on Bureau's infrastructure and other facilities. The unit continued active reprography services. Comb binding, spiral binding, electro-data binding and lamination facilities for departmental reports were also provided.

Exchange Services

The Library continued exchange relationship and resource sharing with 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 2011-2012 and NBFGR Newsletters to various institutions and organizations including, Universities, State Fisheries Departments, FFDAs, Krishi Vigyan Kendras, Entrepreneurs and Fish Farmers.

STAFF ACTIVITIES

1. Promotions

The following staff members were promoted to the next higher grade:

Scientists

Dr. Peyush Punia, from Sr. Scientist to Principal Scientist w.e.f. 01.01.2009

Dr. P.K. Varshney, from Sr. Scientist to Principal Scientist w.e.f. 01.01.2010

Dr. Ravindra Kumar, from Sr. Scientist to Principal Scientist w.e.f. 13.02.2010

Dr. Basdeo Kushwaha, from Sr. Scientist to Principal Scientist w.e.f. 20.10.2011

Dr. U.K. Sarkar, from Sr. Scientist to Principal Scientist w.e.f. 30.01.2012

Dr. Neeraj Sood, from Sr. Scientist to Principal Scientist w.e.f. 01.02.2012

Dr. Lalit Kumar Tyagi, from Scientist to Senior Scientist w.e.f. 25.01.2009

Dr. Rajeev Kumar Singh, from Scientist to Senior Scientist w.e.f. 29.10.2010

Dr. Mahender Singh, from Scientist to Senior Scientist w.e.f. 05.11.2010

Dr. T. Rajaswaminathan, from Scientist (RGP '7000/-) to Scientist (RGP '8000/-) w.e.f.29.10.2010

Dr. (Mrs.) Divya P.R., from Scientist (RGP '6000/-) to Scientist (RGP '7000/-) w.e.f.27.06.2009

Shri A. Kathirvelpandian, from Scientist (RGP '6000/-) to Scientist (RGP '7000/-) w.e.f.08.01.2012

Technicals

Shri Rajesh Dayal, from T(7-8) to T-9 w.e.f. 03.02.2012

Shri S.M. Srivastava, from T(7-8) to T-9 w.e.f. 06.02.2012

Shri Amar Pal, from T-6 to T(7-8) w.e.f. 01.01.2009

Mrs. Reeta Chaturvedi, from T-5 to T-6 w.e.f. 26.12.2011

Shri Ramashankar Sah, from T-5 to T-6 w.e.f. 30.08.2012

Shri A.K. Mishra, from T-6 to T(7-8) w.e.f. 24.02.2011

Shri Babu Ram, from T-6 to T(7-8) w.e.f. 24.02.2011

Shri S.P. Singh, from T-6 to T(7-8) w.e.f. 24.02.2011

Shri Gulab Chandra, from T-2 to T-3 w.e.f. 01.08.2011

Administrative

Shri Tej Singh Seepal, from Assistant to Asstt. Admn. Officer w.e.f. 01.01.2013

Mrs. Mamta Chakraborty, from Personal Assistant to Private Secretary w.e.f. 01.02.2013

2. Recruitment/Transfer

Shri Abhishek Rana joined as Administrative Officer, NBFGR, Lucknow on transfer from NBAIM, Mau on 23.01.2013.

Dr. Gaurav Rathore, Sr. Scientist appointed to the post of Principal Scientist at CIFE, Mumbai and relieved on 18.09.2012.

Shri Panchoo Lal, Asstt. Admn. Officer promoted to the post of Administrative Officer at IIPR, Kanpur and relieved on 31.12.2012.

3. Financial Up-gradation of Skilled Support Staff

Shri Chhote Lal, Skilled Support Staff PB-I '5200-20200+GP of '1900/-Granted 2nd MACP in PB-I with grade pay of '2000/- w.e.f. 09.07.2011

Shri Ram Lakhan, Skilled Support Staff, PB-I '5200-20200+GP of '1900/-Granted 2nd MACP in PB-I with grade pay of '2000/- w.e.f. 08.05.2011

Shri Suneet Kumar, Skilled Support Staff, PB-I '5200-20200+GP of '1900/-Granted 2nd MACP in PB-I with grade pay of '2000/- w.e.f. 20.07.2012

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. J.K. Jena, Director	-	Chairman
2.	Dr. N.S. Nagpure, Head of Division	-	Member
3.	Dr. (Mrs.) Vindhya Mohindra, Principal Scientist	-	Member
4.	Dr. Ravindra Kumar, Senior Scientist	-	Member
5.	Dr. Neeraj Sood, Senior Scientist	-	Member
6.	Shri Ravi Bhadra, Asst. Fin. & Accounts Officer	-	Member
7.	Assistant Administrative Officer	-	Member
8.	Shri A.K, Mishra, T-6 & In-Charge (Electrical)	-	Invitee-Member
9.	Shri Babu Ram, T-6 & In-Charge (Civil)	-	Invitee-Member

Staff side

1.	Shri Subhash Chandra, Technical Officer (T-5)	-	Secretary
2.	Shri. S.N. Srivastava, Assistant and Representative CJSC	-	Member
3.	Shri Om Prakash, T-4	-	Member
4.	Shri Santosh Kr. Singh, Jr. Clerk	-	Member
5.	Shri Balram Babu Bajpai, Skilled Support Staff	-	Member
6.	Shri Ashok Kumar, Skilled Support Staff	-	Member

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. N.S. Nagpure Head of Division	-	Chairman
2.	Dr. Rajeev Kumar Singh Sr. Scientist	-	Member
3.	Shri Ravi Bhadra AF & AO	-	Member
4.	Mrs. Mamta Chakraborty Personal Assistant (Lady Representative)	-	Member
5.	Shri Subhas Chandra, Tech. Officer (Secretary, IJSC)	-	Member
6.	Shri Chhote Lal, SSS (Group D representative)	-	Member
7.	Shri Panchoo Lal Assistant Administrative Officer	-	Member-Secretary

Women's Cell

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

1. Dr. (Mrs) Rehana Abidi - Head of the Cell
   Principal Scientist
2. Dr. (Mrs.) Vindhya Mohindra - Member-Secretary
   Principal Scientist
3. Mrs. Reeta Chaturvedi - Member
   T-5
4. Mrs. Mamta Chakraborty - Member
   Personal Assistant
5. Shri Anil Kumar - Member
   SSS

Grievance Cell

Grievance cell has been constituted at NBFGR, Lucknow with the following members:

Nominated members (official side)

1. Dr. J.K. Jena, Director - Chairman
2. Dr. U.K. Sarkar, Sr. Scientist - Member & Nodal Officer of Cell
3. Shri Babu Ram, Technical Officer (T-6) - Member
4. Administrative Officer - Member
5. Shri Ravi Bhadra, AF&AO - Member
6. Shri Panchoo Lal, Assistt. Admn. Officer - Member-Secretary

Elected members (staff side)

1. Dr. Ajey Kumar Pathak, Scientist (SG) - Member (Scientific)
2. Dr. Vikas Sahu, T-3 - Member (Technical)
3. Shri Sandeep, Jr. Stenographer - Member (Administration)
4. Shri Ashok Kumar, Skilled Support Staff - Member (Supporting)

Management Committee

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

1. Director, NBFGR : Chairman
2. Dr. Madan Mohan, ADG (Marine Fy.), ICAR, New Delhi : Member (ICAR)
3. Dr. V.V. Singh, Principal Scientist, CMFRI, Centre, Mumbai : Member
4. Dr. T. Mahapatra, Principal Scientist, NRCPB, New Delhi : Member
5. Dr. M.S. Tantia, Principal Scientist, NBAGR, Karnal : Member
6. Dr. P.C. Agarwal, Principal Scientist, NBPGR, New Delhi : Member

The 25th meeting of the Committee was held on 28 July, 2012.

DISTINGUISHED VISITORS

Dr. S. Ayyappan, Secretary, DARE and DG, ICAR, New Delhi

Dr. R.S. Paroda, Former Secretary, DARE and DG, ICAR, New Delhi

Dr. B. Meenakumari, DDG (Fisheries), ICAR, New Delhi

Dr. Alok Ranjan, Agriculture Production Commissioner, Govt. of U.P.

Sh. Tarun Shridhar, Joint Secretary (Fisheries), DAHDF, Ministry of Agriculture, New Delhi

Dr. K. M. L. Pathak, DDG (Animal Sciences), ICAR, New Delhi

Dr. S.A.H. Abidi, Former Member, ASRB, New Delhi

Dr. M. Mahadevappa, Former Chairman, ASRB, New Delhi

Dr. George John, Senior Advisor, DBT, New Delhi

Prof. T.J. Pandian, Former National Professor, ICAR

Dr. Dilip Kumar, Former Director, CIFE, Mumbai

Dr. B. Senapathi, Former Vice-Chancellor, OUAT, Bhuvaneswar

Dr. K. Vijayakumaran, DG, Fisheries Survey of India, Mumbai

Dr. B.N. Singh, Former ADG (Fisheries), ICAR, New Delhi

Dr. A.D. Diwan, Former ADG (Marine Fisheries), ICAR

Dr. Madan Mohan, ADG (Marine Fisheries), ICAR, New Delhi

Dr. Ravindra Kumar, ADG (Coordination), ICAR, New Delhi

Dr. S. Mauria, ADG (IP&TM) ICAR, New Delhi

Dr. A.S. Ninawe, Advisor, DBT, New Delhi

Dr. K.C. Bansal, Director, NBPGR, New Delhi

Dr. A.P. Sharma, Director, CIFRI, Barrackpore

Dr. B.K. Joshi, Director NBAGR, Karnal

Dr. Sushama R. Chaphalkar, Director, Vidya Pratishthan's School of Biotechnology, Baramati

Prof. C.V. Mohan, Research and Development Manager, NACA, Thailand

Dr. S. Solomon, Director, IISR, Lucknow

Dr. H. Ravishankar, Director, CISH, Lucknow

Dr. B.S. Bhumannavar, Director, NBAII, Bangalore

Dr. P. Jayasankar, Director, CIFA, Bhubaneshwar

Dr. R. Paul Raj, Member Secretary, Coastal Aquaculture Authority

Shri D.K. Singh, Director, Fisheries, U.P.

Dr. C.S. Nautiyal, Director, NBRI, Lucknow

Dr. S. Rajendra Prasad, Acting Director, NBAIM, Mau

LIST OF PERSONNEL

Research Management

Dr. J.K. Jena	-	Director

Scientific Staff

1.	Dr. N.S. Nagpure	-	Head of Division
2.	Dr. K.K. Lal	-	Head of Division
3.	Dr. Peyush Punia	-	Head of Division
4.	Dr. (Mrs.) Rehana Abidi	-	Principal Scientist
5.	Dr. A. Gopalakrishnan	-	Principal Scientist (NBFGR Cochin Unit)
6.	Dr. A.K. Pandey	-	Principal Scientist
7.	Dr. Sudhir Raizada	-	Principal Scientist
8.	Dr. S.P. Singh	-	Principal Scientist
9.	Dr. A.K. Singh	-	Principal Scientist
10.	Dr. (Mrs) Vindhya Mohindra	-	Principal Scientist
11.	Dr. P.K. Varshney	-	Principal Scientist
12.	Dr. Ravindra Kumar	-	Principal Scientist
13.	Dr. Basdeo Kushwaha	-	Principal Scientist
14.	Dr. U.K. Sarkar	-	Principal Scientist
15.	Dr. Neeraj Sood	-	Principal Scientist
16.	Dr. V.S. Basheer	-	Sr. Scientist (NBFGR Cochin Unit)
17.	Dr. Gaurav Rathore	-	Sr. Scientist (upto September, 2012)
18.	Dr. Mukunda Goswami	-	Sr. Scientist
19.	Dr. Parvata Kumar Pradhan	-	Sr. Scientist
20.	Dr. Lalit Kumar Tyagi	-	Sr. Scientist
21.	Dr. Rajeev Kumar Singh	-	Sr. Scientist
22.	Dr. Mahender Singh	-	Sr. Scientist
23.	Mrs. Poonam Jayant Singh	-	Scientist
24.	Shri Ajey Kumar Pathak	-	Scientist
25.	Dr. T. Rajaswaminathan	-	Scientist (NBFGR Cochin Unit)
26.	Dr. (Mrs.) Divya P.R.	-	Scientist (NBFGR Cochin Unit)
27.	Shri A. Kathirvelpandian	-	Scientist (NBFGR Cochin Unit)
28.	Ms. Sangeeta Mandal	-	Scientist

Technical Staff

1.	Shri Rajesh Dayal	-	Field Officer T-9
2.	Shri S.M. Srivastava	-	Field Officer T-9
3.	Shri A.K. Yadav	-	Technical Officer, T(7-8)
4.	Shri Amar Pal	-	Technical Officer, T(7-8)
5.	Shri A.K. Mishra	-	Electrical Foreman T(7-8)
6.	Shri S.P. Singh	-	Technical Officer T(7-8)
7.	Shri Babu Ram	-	Technical Officer T(7-8)
8.	Shri Ajay Kumar Singh	-	Field Surveyor (T-6)
9.	Mrs. Reeta Chaturvedi	-	Technical Officer (Comp. Operation) T-6
10.	Shri Ramashankar Sah	-	Technical Officer (T-6)

11.	Shri Mohd. Gayas	-	Driver (T-5)
12.	Shri Subhash Chandra	-	Technical Officer (T-5)
13.	Shri Ved Prakash	-	Technical Officer (T-5)
14.	Shri Akhilesh Kr. Mishra	-	Technical Officer (T-5)
15.	Dr. (Mrs.) Ranjana Srivastava	-	Technical Officer (T-5)
16.	Shri Ravi Kumar	-	Technical Officer (T-5)
17.	Shri S. K. Singh	-	Technical Officer (T-5)
18.	Shri Amit Singh Bisht	-	Technical Officer (T-5)
19.	Shri Satyavir Chaudhary	-	Technical Officer (T-5)
20.	Shri S. K. Upadhyay	-	T-4
21.	Shri R.K. Shukla	-	Sample Sorter (T-4)
22.	Shri B. N. Pathak	-	Gestetner Operator (T-4)
23.	Shri Samarjit Singh	-	Driver (T-4)
24.	Shri Om Prakash	-	Driver (T-4)
25.	Shri B. K Rao	-	Sample Sorter (T-II-3)
26.	Shri Rajesh Kumar	-	Laboratory Asst. (T-3)
27.	Shri Om Prakash-II	-	Driver (T-3)
28.	Dr. Vikash Sahu	-	Laboratory Technician (T-3)
29.	Shri Madan Lal	-	Farm Technician (T-3)
30.	Shri Raj Bahadur	-	Lab. Technician (T-3)
31.	Shri Gulab Chandra	-	Electrician (T-3)
32.	Shri K. K Singh	-	Jr. Field Asst. (T-2)
33.	Shri Sree Ram	-	Laboratory Asst. (T-2)
34.	Shri P. C. Jaiswar	-	T-2
35.	Shri Ram Bharose	-	T-2

Administrative Staff

1.	Shri Abhishek Rana	-	Administrative Officer
2.	Shri Panchoo Lal	-	Assistant Administrative Officer (upto December, 2012)
3.	Shri Navin Kumar	-	Assistant Administrative Officer
4.	Shri Ravi Bhadra	-	Assistant Finance & Accounts Officer
5.	Shri Tej Singh Seepal	-	Assistant Administrative Officer
6.	Smt. Mamta Chakraborty	-	Private Secretary
7.	Shri Jogendra Singh	-	Assistant
8.	Smt. Kaneez Fatima	-	Assistant
9.	Shri Swapan Debnath	-	Assistant
10.	Shri S. N. Srivastava	-	Assistant
11.	Shri P. K. Awasthi	-	Assistant
12.	Shri Ram Sakal	-	Personal Assistant
13.	Shri Sajivan Lal	-	Senior Clerk
14.	Shri Vinay Kumar Srivastava	-	Senior Clerk
15.	Shri Shreelal Prasad	-	Senior Clerk
16.	Shri Sandeep	-	Jr. Stenographer

17.	Shri Santosh Kumar Singh	-	Jr. Clerk
18.	Shri Ram Baran	-	Jr. Clerk
19.	Shri P.C. Verma	-	Jr. Clerk
20.	Shri Rajan Kr. Malhotra	-	Jr. Clerk

Skilled Supporting Staff

1.	Shri Laxman Prasad	-	Skilled Support Staff
2.	Shri DukhiShyam Deo	-	Skilled Support Staff
3.	Shri Anil Kumar	-	Skilled Support Staff
4.	Shri Indrajit Singh	-	Skilled Support Staff
5.	Shri Prahalad Kumar	-	Skilled Support Staff
6.	Shri Chhote Lal	-	Skilled Support Staff
7.	Shri Dinesh Kumar	-	Skilled Support Staff
8.	Shri Balram Babu Bajpai	-	Skilled Support Staff
9.	Shri Ashok Kumar Awasthi	-	Skilled Support Staff
10.	Shri Sidhnath	-	Skilled Support Staff
11.	Smt. Sabita Devi	-	Skilled Support Staff
12.	Shri Ram Lakhan	-	Skilled Support Staff
13.	Shri Sunit Kumar	-	Skilled Support Staff
14.	Shri Jai Narain Tiwari	-	Skilled Support Staff
15.	Shri Anwar	-	Skilled Support Staff
16.	Shri Sanjay Kumar	-	Skilled Support Staff
17.	Smt. Seema Devi	-	Skilled Support Staff
18.	Shri Ashok Kumar	-	Skilled Support Staff
19.	Smt. Raj Kumari	-	As a trainee

APPENDIX-I

NBFGR Cochin Unit

A Research Unit of the Bureau is functioning in the campus of Central Marine Fisheries Research Institute (CMFRI), Cochin, 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 : nbfgrcochin@vsnl.net
nbfgrcochin@eth.net

APPENDIX-II

Aquaculture Research & Training Unit, Chinhat

An Aquaculture Research & Training Unit of the Bureau is functioning at Chinhat, 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, Chinhat
Lucknow-227 105, U.P.
Telefax : 0522-2815848
E-mail : director@nbfgr.res.in

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