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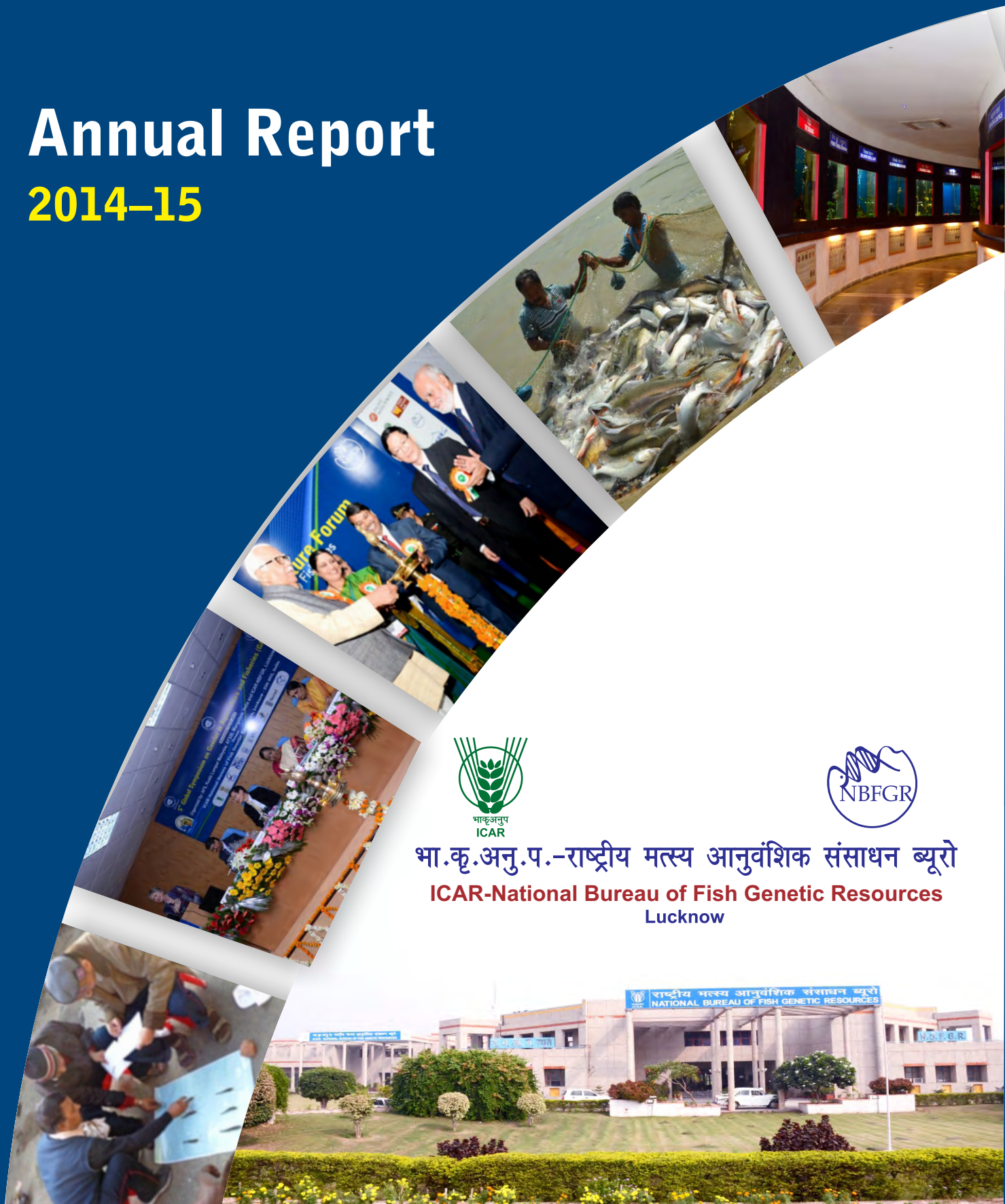
Annual Report
2014–15



भाकृअनुप
ICAR

NBFGR

भा.कृ.अनु.प.–राष्ट्रीय मत्स्य आनुवंशिक संसाधन ब्यूरो

ICAR-National Bureau of Fish Genetic Resources

Lucknow

Annual Report
2014–15

भाकृअनुप
ICAR

ICAR-National Bureau of Fish Genetic Resources

NBFGR

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PREFACE

The vast aquatic resources of India are source of rich biological wealth and provide immense opportunities for development of the fisheries sector in the country. Increased attention on aquatic organisms not only for food and ornamental values, but also on bioprospecting of the unique genes that they contain, combined with rapid biotechnological development has raised global concern for conservation of aquatic genetic resources. In view of this, the ICAR-NBFGR has taken up its research programmes to develop appropriate strategies for conservation and sustainable use of the aquatic biodiversity of the country for the benefit of the present and for posterity.

During the reporting year (2014-2015), the Institute has strengthened its activities to foster the fish germplasm exploration, characterization and conservation. Whole mitochondrial genome of Indian mottled eel, *Anguilla bengalensis bengalensis* was sequenced and whole genome sequencing data of *C. magur* was generated. DNA barcodes for about 100 marine and freshwater finfishes were generated. The genetic stock structure of Indian mackerel, *Rastrelliger kanagurta* was analysed from Indian waters under BOBLME/FAO project, which indicated low genetic differentiation among the mackerel populations distributed along Indian waters. Three species of finfishes from southwest coast of India were re-described. Genetic stock characterization of commercially important finfishes and shellfishes received greater impetus with initiation of 2nd phase of Outreach Project on Fish Genetic Stocks. Progress in exploration of fish genetic resources in prioritized areas, understanding of molecular pathogenesis of important pathogens, surveillance of aquatic animal diseases and several others have been quite significant.

It was a proud moment for the whole ICAR-NBFGR family to host several important events including, the 10th Indian Fisheries and Aquaculture Forum (10ifaf), the largest scientific triennial event in fisheries and aquaculture in India, the '5th Global Symposium on Gender in Aquaculture and Fisheries (GAF5)' and the 'International Workshop on Aquatic Animal Disease Surveillance at the premises during November, 2014. The current year saw several additions on infrastructure front, including the development of third generation advanced sequencing facility, a catfish hatchery and a fish farmers consultation center. The Kochi research unit of the Institute was upgraded into a full-fledged Center, named as Peninsular and Marine Fish Genetic Resources Center, which envisages to boost our research programmes in this important region. Eight newly recruited scientists also joined the Institute during the year and I am sure their young enthusiasm will further energize our research efforts. I am confident that our hard work and commitment to continuously innovate and improve upon our research programmes will continue to provide greater research outputs for making effective strategies for sustainable use of fish genetic resources. Compliments go to all the staff members of ICAR-NBFGR for their commitment and dedication towards the 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, Sr. Scientist, and Mr. Amit Singh Bisht and Mr. Ravi Kumar, Technical Officers of the Institute for their sincere effort and commitment in timely publication of the Annual Report.

25th June, 2015

(J.K. Jena)
Director

CONTENTS

EXECUTIVE SUMMARY

ICAR-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, germplasm conservation with special focus on threatened and prioritized species etc. During the year under report, the research activities were conducted through 14 Institutional and 17 externally-funded research projects. Major achievements and activities of the Institute during the year 2014-15 are summarized below:

The existing database on finfish diversity of India was updated by adding information of about 300 fish species reported from Indian waters. The database now contains information on 2868 native finfishes reported from India belonging to 993 genera of 236 families under 45 orders.

Work on documentation and development of passport information of exotic food and ornamental fishes in India was initiated and a check-list of over 400 exotic fish species available in India was developed through literature survey and collection of fish samples from different places of the country. Passport information of 45 exotic fish species was developed.

Under studies on genetic variability analysis in hilsa, *Tenualosa ilisha*, till date 842 individual samples have been collected from 23 sampling sites, covering both east and west coasts. Species specific 70 polymorphic EST-SSRs were developed from muscle transcriptome sequences. Analysis of genetic variability with ATPase 6/8 sequences (842 bp), from 361 samples of *T. ilisha* collected from six different locations of Hoogly, Padma (Farracka) and Bramaputra was undertaken and the haplotype network derived from ATPase 6/8 haplotypes resulted in only one clade.

The genetic stock structure of Indian mackerel, *Rastrelliger kanagurta* was analysed from Indian waters which indicated low genetic differentiation among the mackerel populations distributed along Indian waters, except moderate differentiation in the samples from Andamans.

Eleven microsatellite primers were developed in *Scomberomorus commerson* through cross species amplification and population genetic analysis using ATPase gene did not show any differentiation between five populations of *S. commerson.*

Under the collaborative programme on whole genome sequencing of two important fish species namely *Labeo rohita* and *Clarias magur*, whole genome sequencing data of *C. magur* was generated on multiple sequencing platforms viz., Roche 454, Ion Torrent and Illumina HiSeq. The *de novo* assembly of Roche 454 and Ion Torrent data resulted in 5,36,276 contigs with N50 contig size of 1,178 bp. The assembly also yielded whole mitogenome as the longest contig and mitogenome annotation revealed 13 protein coding genes, 22 tRNA, 2 rRNA and D-loop region. Further, gene prediction using 'Augustus' revealed 6,402 putative ORF/genes and their annotation yielded 1,100 well annotated putative ORF's. Microsatellite mining of the *C. magur* using MISA tool resulted in 14,548 microsatellites. The *de novo* assembly of Illumina HiSeq data using Abyss assembler revealed a reasonably good assembly at 85 hash length, the maximum contig size was ~89 Kb with N50 contig size of 6611.

Whole mitochondrial genome of Indian mottled eel, *Anguilla bengalensis bengalensis* was sequenced.

DNA barcodes for 62 marine finfish species were generated and haplotype sequences submitted to GenBank. Three species of finfishes from southwest coast of India, *Chelidoperca investigatoris, C. occipitalis* and *Chlorophthalmus corniger* were re-described.

Under the programme on genetic characterization and DNA barcoding of fishes from north east India, a total of 139 DNA barcodes based on COI gene region belonging to 37 species were prepared and submitted to NCBI GenBank. Cytogenetic profiles of eight fish species collected from North-Eastern part of India were prepared.

Explorations were continued in various parts of the country for documentation and characterization of the fish diversity. In Western Ghats, a total of 53 fish species were recorded from 10 sites of Sharavathi River in Karnataka and brought out first record of the species *Pterocryptis wynaadensis* from Sharavati River basin.

Exploration of fish species in Zuari, Mandovi and Chapora rivers, Goa yielded 13 finfish species and 6 species of crustaceans. In the upper basin of Mahanadi River, a total of 11 sites of Mahanadi and 12 sites of its six tributaries and sub-tributaries namely, Sheonath, Jonk, Hasdeo, Maniyari, Arpa and Lilagar, were explored and a total of 74 fish species belonging to 22 families of 8 orders were recorded.

Under ICAR-North East (NE) component, a participatory programme on 'Exploration and characterization of fish germplasm resources and indigenous knowledge in North Eastern Region of India' was implemented involving seven collaborators from various institutions of the NE region. A total of 24 rivers in seven states of the NE region were explored and fish diversity documented by the collaborating partners.

Studies carried out on molecular pathogenesis of epizootic ulcerative syndrome (EUS) in fish strengthened the understanding of molecular pathogenesis of *A. invadans* infection in *L. rohita*.

Under the National Surveillance Programme for Aquatic Animal Diseases which is implemented in 14 states of aquaculture importance with involvement of 23 national/state fisheries research institutions, infection with *Cyprinid herpesvirus*-2 was identified as the cause of large-scale mortality in goldfish in Hooghly District, West Bengal. Furthermore, a 'Diagnostic Manual for Aquatic Animal Diseases of National Concern' has also been prepared, which is aimed to provide a uniform approach for diagnosis of the aquatic animal diseases by the collaborating institutes of the surveillance programme.

NBFGR participated in the four rounds of Regional Proficiency Testing Program for Aquatic Animal Disease Diagnostic Laboratories in Asia-Pacific for improved aquatic animal disease laboratory diagnostic capability across Asia organised by NACA in association with DAFF Australia, CSIRO, Australian National Quality Assurance Program and OIE, and a total of 96 different virus samples were tested in all four rounds.

The Institute hosted the 10th Indian Fisheries and Aquaculture Forum (10ifaf), the largest scientific triennial event in fisheries and aquaculture in India under the aegis of Asian Fisheries Forum Indian Branch (AFSIB) during 12-15 November 2014. Over 700 distinguished scientists, technocrats, policy makers, members from financial institutions, NGOs, students, farmers and entrepreneurs from all over the country and overseas participated in the Forum.

The Institute also hosted two other international events: the '5th Global Symposium on Gender in Aquaculture and Fisheries (GAF5)' organized by the Asian fisheries Society (AFS), Kuala Lumpur in collaboration with Asian Fisheries Society Indian Branch and ICAR-NBFGR, Lucknow during 13-15 November, 2014 and an 'International Workshop on Aquatic Animal Disease Surveillance' organised in collaboration with the AFSIB on 14th November, 2014. Besides, a number of technical workshops/trainings were also organized by the Institute.

A series of short-term training programmes on 'Fish culture and Conservation' were organized in which a total of 304 fish farmers were trained.

INTRODUCTION

Brief History

The aquatic genetic resources are considered to be important biological wealth not only linked to the food and nutritional security of growing population of the country, but also for their ornamental and human utilities, and therefore need to be documented, characterized, managed and utilized judiciously on sustainable basis in the country. It was in this context that the Government of India approved establishment of the National Bureau of Fish Genetic Resources at the end of Sixth Five Year Plan to provide scientific input for conservation and sustainable management of fish germplasm resources of the country. Sanctioned in December, 1983 under the Indian Council of Agricultural Research, NBFGR started functioning as an independent institute initially at Allahabad, which later shifted to Lucknow during 1994. Since its humble beginning in 1983, NBFGR has evolved into a pioneering institution to undertake research on diverse issues related to conservation of fish diversity. The Bureau, occupied its magnificently built administrative and laboratory facilities during 1999. Since then several new infrastructure facilities including hatchery, wet laboratories, public aquarium, guest house, staff quarters and above all, required experimental tanks and ponds have been created, satisfying the need of research and other amenities.

The Institute has been growing not only in terms of creation of infrastructure, but also expansion of research programmes by including important areas viz., whole genome sequencing, population genetics, functional genomics, molecular disease diagnostics, national surveillance programme for aquatic diseases, exploration of newer geographical areas and unexplored aquatic resources for assessment of fish diversity, etc.

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

Institute Management Committee → **Director** ← **Research Advisory Committee**

- **Administration**
  - Establishment
  - Accounts and Audit
  - Stores and Purchase
  - Hindi Cell
- **Support Services**
  - Director Cell
  - PME Cell
  - Library and Documentation
  - AKMU
  - Extn. & Training Cell
  - IPR and Patent Cell
  - Engg. & Maintenance Cell
- **Research Divisions and Sections**
  - Molecular Biology and Biotechnology Division
  - Fish Conservation Division
  - Fish Health Management Division
- **Centre/Unit**
  - PMFGR-NBFGR Centre, Kochi
  - ARTU, Chinhat Unit, Lucknow

Staff Position

The overall staff position as on 31st March, 2015 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	34	07
3.	Technical	38	37	01
4.	Administrative	21	20	01
5.	Supporting	20	19	01
	Total	**121**	**111**	**10**

Financial Statement

Allocation of funds and expenditure incurred during the year 2014-2015 are as follows:

(Rs. in lakhs)

	Budget Allocation	Expenditure
Plan	630.00	629.92
Non Plan	1229.75*	1229.54*
Total	**1859.75**	**1859.46**

* Including loans and advances

RESEARCH 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 ICAR, 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. Besides updating information on existing species, the following major information was added to the database:

Additional 300 native finfish species reported from Indian waters were added into the main database after screening and comparing with synonyms and valid names of the respective fish species.

Additional 156 exotic fishes reported from India were added into the main database.

Total of 3159 fish species were taken for taxonomic study and valid scientific names of 362 fishes were revised.

Valid scientific names of 361 fishes were replaced in the database for updating. The old names of fishes were added in the list of synonyms accordingly.

Total of 2563 synonyms, 2021 references and 118 original photographs were added to update various lists maintained in the backend of database.

Germplasm explorations were carried out in river Son in Uttar Pradesh and 62 fish species belonging to 45 genera under 19 families were recorded and their information added to the database. The updated database contains information on 3315 finfishes, including 447 exotic fishes, reported from India. Total 2868 native finfishes belong to 993 genera under 236 families and 45 orders, and 447 exotic fishes belong to 283 genera under 98 families and 25 orders. The habitat-wise distribution of fishes are as follows:

Category of Fishes	Ecosystem	No. of Fish Species
Native fishes	Freshwater	877
	Brackishwater	113
	Marine water	1878
	Total	**2868**
Exotic fishes		447
Grand Total		**3315**

A database 'Molluscs of India' containing information on 3812 species of Molluscs belonging to 906 genera under 282 families reported from Indian territory was developed. This software application was developed using Visual basic enterprise edition (6.0) in frontend and MS ACCESS 2003 in the backend to manage the data.

Data on fish occurrence in seven different basins of Ganges system were collected from different published sources and a database on fish occurrences was developed. Presently the database covers records on fishes for Ganges, Ramganga, Gomati, Ghaghra, Sai and South Tons sub-basins. The database developed for fish occurrences of different basins of Ganges system was connected to the vector GIS map of basins of the Ganges system. A file geo-database named 'Ganges_subbasin_fishes' was developed covering the database on occurrence of fishes for seven different sub-basins of the Ganges system, vector GIS map of rivers and basins of the Ganges system by creating the relationship classes. This geo-database enables us to query on fishes and generate thematic maps for queries.

The electronic-based information system known as e-identification system for identifying the fish species reported from Indian waters using the shape descriptors was updated to include information on the shape descriptors of the species for Clupeidae (42 species), Notopteridae (02), Bagridae (49), Anguillidae (02), Belonidae (04) and Cyprinidae (28 species) families, besides updating various categories, sub-categories, data and image (Fig.1). The management of the database has been provided only to the authenticated user/users. Good quality photographs of fishes and photographs describing the different shape features for the species were also included into the database. The system hosted on the web is working well on the local server under testing and validation phase.

Fig.1. *e*-Identification System for Fishes

Project Title: Documentation and development of passport information of exotic food and ornamental fishes in India

Project Period: April, 2014 - March, 2017

Project Personnel: S. Raizada (PI), Peyush Punia, T.T. Ajithkumar, Rejani Chandran and Rajesh Dayal

Funding Agency: Institutional

A check-list of over 400 exotic fish species available in India was developed by procuring information from literature survey and availability at various places in the country. This check-list contains information on valid names, synonyms, common names, original distribution and IUCN Red-list status of the species. The passport information of 45 exotic fish species covering various important features like size, behaviour, breeding, water quality requirements, variants, market demand and price, etc. were developed. Besides species details, information on variants are also being collected. Specimens of over 100 exotic fish species were procured and preserved for the National Fish Museum being developed at ICAR-NBFGR.

Project Title: Establishment of National Agricultural Bioinformatics Grid in ICAR

Project Period: April, 2010 - June, 2014

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

Funding Agency: NAIP, ICAR

Development of Fish Karyome database

Fish Karyome (http://www.nbfgr.res. in/ fishkaryome/) is a database on karyological information of fishes and other aquatic organisms. The database was updated and presently covers 494 records for 422 species from 106 families (Fig.2). The upgraded version has modified user interfaces and flexibility for data browsing.

Fig.2. A screen print from Fish Karyome

Project Title: Centre for Agricultural Bioinformatics (CABin): Fisheries domain

Project Period: February, 2015 – March, 2017

Project Personnel: N.S. Nagpure (CCPI), S.P. Singh, Ravindra Kumar, Mahender Singh, A.K. Pathak and Murali S.

Funding Agency: ICAR - Indian Agricultural Statistics Research Institute

Updation of genomic resource databases

Four Genomic resource databases *viz.* Fish Microsatellite database (FishMicrosat), Fish Karyome, Fish Mitogenome Resource (FMiR) and Fish Barcode Information (FBIS) were updated with new records and the databases presently contains 11273, 494, 1302 and 16819 records, respectively. User friendly web based application modules were implemented in FBIS to increase the scope and usability. FBIS is an useful resource to support taxonomist and researchers working in molecular genetics to resolve the taxonomic ambiguities in species identification, population differentiation and analysis of genetic divergence across species at molecular level. The project activities of the fisheries can be accessed at URL http// mail.nbfgr.res.in/nabg_nbfgr.

4.2 Genetic and Biological Characterization

Project Title: Signatures of natural selection and genomic diversity in important freshwater fish species, *Tor putitora* and *Clarias magur*

Project Period: April, 2014-March, 2017

Project Personnel: Vindhya Mohindra (PI), Santosh Kumar and Trivesh Mayanker

Funding Agency: Institutional

Information on genetic diversity is of great importance for fisheries conservation and management. Presently, genetics-based studies use large number of neutral genetic markers, the variation influenced by mutational dynamics and demographic effects and not by selection. However, these approaches may be ineffective when populations are recently diverged, as this is not reflected at neutral loci. Recently, several methods have been developed for studying population structure using molecular markers, such as genomic and expressed sequence tag-derived simple sequence repeats (gSSRs and EST-SSRs) facilitating the measurement of genetic variation on a genomic scale. Out of these, non-neutral (outlier) loci are in association to environmental factors and could bias estimates of genetic structure, as selection affects the genome at specific loci by either reducing the genetic diversity in a specific region in favour of advantageous alleles (positive selection) or by maintaining similar levels of variation across populations (balancing selection). Nevertheless, these loci can better explain the adaptive genetic variation that is not accounted for by neutral loci and detecting the footprints of selection, since they occur in coding regions or the sequences that flank them. In this backdrop, a new work was undertaken to build genomic resources and to gain knowledge about functional biodiversity, that can be applied to sustainably manage the diversity of important freshwater fish species, *Tor putitora* and *Clarias magur*.

Analysis of transcriptome data for EST-SSR sequences in *Clarius magur*

A 2.88 gb transcriptome generated from muscle tissue of *C. magur* comprised of total 40,798 number of transcripts. Number of transcripts with significant BLASTX match was 18,404 (45.11%) and number of transcripts with UniProt annotation was 9,002 (22.06%). When Gene Ontology terms were identified for these transcripts, 1,826 Biological Processes (Fig.3.), 1,053 Molecular Functions and 474 Cellular Components could be obtained.

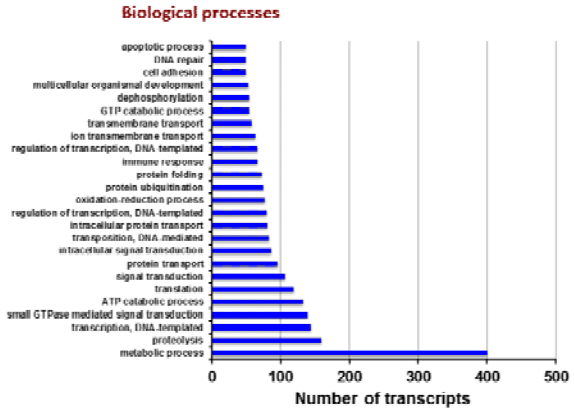


Fig.3. Categorisation of *Clarias magur* muscle transcripts under major biological processes

Out of the 18,404 annotated transcripts of *C. magur* muscle, SSRs were observed in 1632 annotated genes and for 795 gene-associated SSRs loci in annotated genes, sequences length was sufficient for designing primers. The primers were designed for 100 EST-SSR loci. The primers were synthesized and tested for primer amplification in 5 individuals and for polymorphism in 26 individuals from diverse geographic locations. A total 65 primers were amplified, 14 polymorphic microsatellite loci were obtained whereas 50 loci were monomorphic.

Project Title: Establishment of mapping and marker panel for first generation linkage map in Indian catfish, *Clarias magur*

Project Period: April, 2014 - March, 2017

Project Personnel: Rajeev Kumar Singh (PI), T.T. Ajith Kumar and Santosh Kumar

Funding Agency: Institutional

Among the catfishes, the air-breathing species *Clarias magur* is a popular cultivable fish in Asian countries, having several advantages over other species. The hardy nature and tolerance to adverse ecological condition enable its high-density culture with elevated production per unit area. ICAR-NBFGR has been constantly striving for holistic approach in research of this important species. Immune and hypoxia responsive genes have already been identified and characterized. Sequencing of whole genome is under progress. In such an effort, genetic linkage map, which is among the important genomic resources, is being constructed using nuclear markers, such as microsatellite and SNPs. Linkage maps have become

important tools in many areas of genetic research. Recently, genetic linkage maps have been generated for economically important species, such as salmon, tilapia, European sea bass, rainbow trout, Barramundi, catfish, grass carp, Japanese flounder and black tiger prawn. The objective of this work is to create marker panel consisting of SSR/SNP loci and assaying on reference families panel to generate first generation linkage map in *C. magur*.

A total number of 98 mature *C. magur* specimens were collected from five different places, transported to the Institute and acclimatized. Healthy mature fishes were selected for breeding purpose (8 pairs) and remaining were transferred to the earthen pond for further rearing. Captive breeding was attempted using the hormone Ovatide with success in three pairs. Three full-sib families were obtained (Fig.4) and the F1 progenies were shifted to the outdoor tanks and regular monitoring was done and progress of growth was noticed. Total genomic DNA was isolated from larvae from family 1 (n=110), family 2 (n=80) and family 3 (n=58). The quality of DNA was checked on 1% agarose gel. Testing of SSRs was done and EST-SSRs were used to know marker informativeness. A total of 67 polymorphic primers were tested on parents of three families, out of which, 15 were found informative (Table 1 and Fig. 5) in one or other pairs of parents.

Fig. 4. Captive breeding of *Clarias magur* for creating reference panel

Table.1. List of microsatellite loci exhibiting informativeness

S. No	Primer Name	Molecular weight (Range)	Status
1	EB24	133-204	Polymorphic
2	CHK130	65-75	Polymorphic
3	CB18NGS	121-152	Polymorphic
4	CHK116	182-200	Polymorphic
5	ES207NGS	147-168	Polymorphic
6	EB27NGS-1	105-122	Polymorphic
7	CBSPN1837	116-130	Polymorphic
8	CBSPN2339	87-129	Polymorphic
9	CBSPN4566	96-110	Polymorphic
10	EB121NGS-2	147-139	Polymorphic
11	EL225	193-203	Polymorphic
12	EM216	95-117	Polymorphic
13	CBSPN1335	108-137	Polymorphic
14	CBSPN4654	94-126	Polymorphic
15	CBSPN2617	113-143	Polymorphic

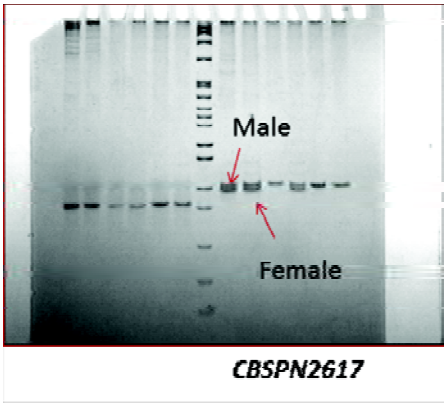


Fig. 5. Visualization of alleles on PAGE gel

Project Title: Stock characterization, captive breeding, seed production and culture of hilsa (*Tenualosa ilisha*): A network project coordinated by CIFRI, Barrackpore

Project period: November, 2012 - October, 2016

Project Personnel from NBFGR: Vindhya Mohindra (CCPI), Kuldeep K. Lal (Up to 18 August, 2014), Rajeev K. Singh, Sangeeta Mandal and J. K. Jena

Funding Agency: NFBSFARA, ICAR

Tenualosa ilisha (Hamilton 1822), the anadromous hilsa shad, is a highly preferred fish for its taste and delicacy. It migrates from its marine environment to the freshwater rivers for spawning. In India,

distribution of freshwater hilsa has been recorded from the rivers Hooghly, Brahmaputra, Ganga, Godavari, Mahanadi, Narmada and Tapti and marine locations in Arabian Sea and Bay of Bengal. As a result, the species is subjected to a range of climatic and environmental extremes throughout the region. Presence of more than one race of hilsa has always evoked great interest among researchers. Based on the morphological differences, slender and broad morphotypes of hilsa have been identified in the Ganges, however, there are reports which suggest that the morphological variation in hilsa is due to the local environments. Attempts have been made on genetic characterisation and stock delineation in the past, however, the question, whether there is a single or multiple stocks, still remains to be answered. In this perspective, the objective of this work is to develop knowledge based on genome-wide variation and population structure of hilsa to support breeding programmes for aquaculture and its natural stock management.

Collection of tissue accessions and truss morphological data for specimen

Samples from 228 individuals of *T. ilisha* were collected from 12 sites from west (Tapti and Narmada river systems) and east coasts (Hooghly, Baitrani and Godavari river system and Vellar Estuary), during the period under report. Till date 842 individuals of *T. ilisha* have been collected from 23 sampling sites, covering both east and west coasts (Figures 6 & 7). Length, weight, scales and images were taken for all the specimens. Tissue samples (blood, muscle & fin) were collected and preserved. Truss photographs of 814 samples are being analysed.

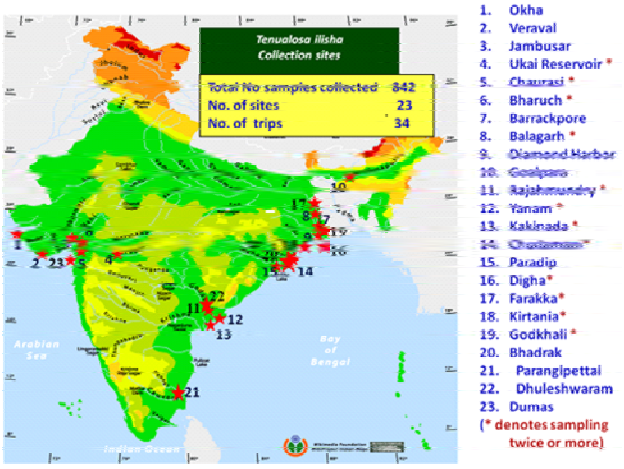


Fig.6. Collection sites for *Tenualosa ilisha* from its natural distribution for genetic variability studies

Development of microsatellite markers in *T. ilisha* for genetic variation studies:

Species specific gene associated microsatellite (EST-SSR) markers

The present investigation aims at developing EST-SSR markers in *T. ilisha* through transcriptome approach. Muscle transcriptome of 24,978 transcripts generated, with more than 150 bp and FPKM>=1.0, significant blastx match and Uniprot annotation were found for 9,516 (38.10%) transcripts, out of which 703 transcripts were found to be with repeat regions. The primers were designed and synthesised from suitable 271 transcripts. These were tested for amplification and polymorphism, a total 70 EST-SSRs were found polymorphic and 75 monomorphic.

Cross species amplification from related species

The microsatellite primers for 89 loci from 10 species belonging to family Clupeidae were tested for cross species amplification in *T. ilisha*. Out of 89 loci, four were found to be polymorphic and 14 were monomorphic (Fig. 8).

Genetic variability analysis in *T. ilisha* within Ganga and associated river systems using mitochondrial ATPase 6/8 marker

The complete ATPase 6/8 genes of 842 bp were amplified and sequenced from 361 samples collected from six different locations of Ganga and associated

Fig.7. Experimental fishing and collection of tissue accessions of *Tenualosa ilisha* at Hoogly River at Godhakhali, West Bengal

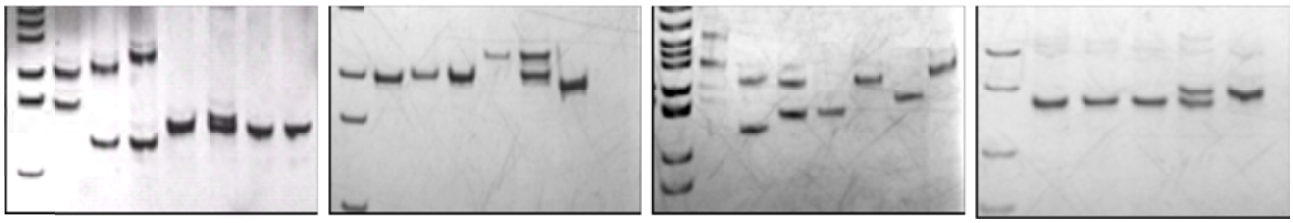
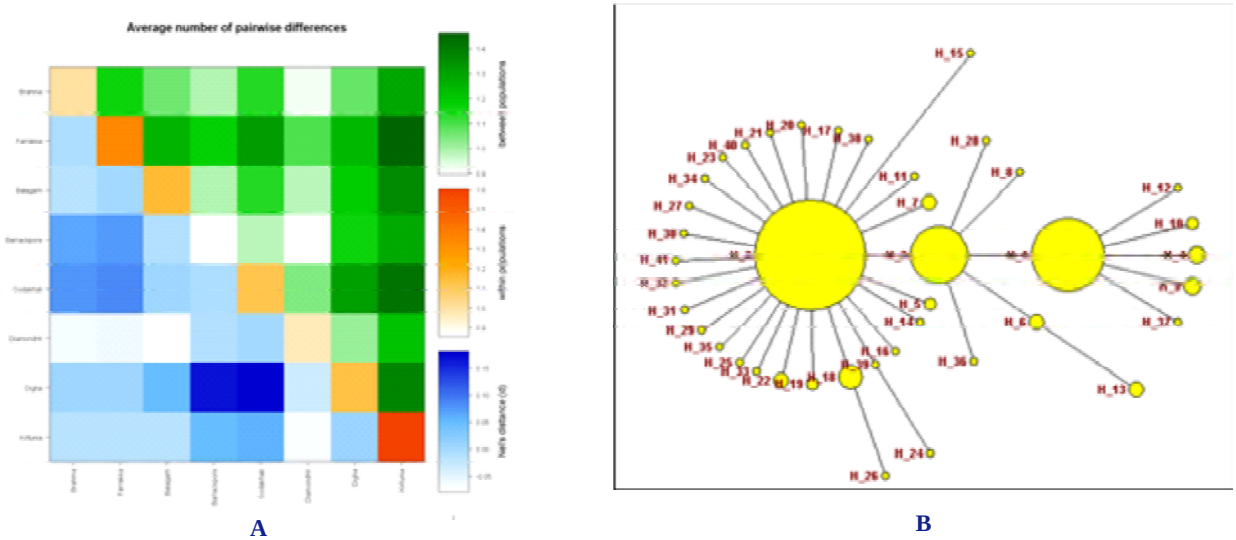


Fig.8. Polymorphic microsatellite loci identified in *T. ilisha*, namely, HIL51, HILE604771, D06(B), HILE607733-1

river systems, namely, Brahmaputra (Golpara) 65, Ganga (Farrakka) 79, Hoogly (Balagarh) 12, Hoogly (Barrackpore and surrounding areas) 46, Hoogly (Godakhali) 71, Hoogly (Diamond Harbour) 8 and two marine locations, Digha 44 and mouth of Subarnarekha 36. A total of 41 haplotypes were obtained with haplotype diversity (Hd) 0.6746. Out of 842 bases, 799 were conserved, 43 variables with 14 parsimony informative and an ti/tv ratio of 42.15. The average nucleotide frequencies were: T=28.0%, C=30.2%, A=26.0% and G=15.8%. In the samples from freshwater locations, the molecular diversity indices, i.e., gene diversities ranged from 0.5812 +/- 0.0498 (Brahmaputra) to 0.7141 +/- 0.0458 (Farrakka) and nucleotide diversity (average over loci) from 0.001014 +/- 0.000812 (Barrackpore and surrounding areas) to 0.001014 +/- 0.000812 (Farrakka). AMOVA results indicated that for ATPase 6/8, 4.88%, -0.47% and 95.58%, of the total variance, respectively, was attributed to differences among groups, among populations within groups and within populations, with total F_{st} value of 0.04415. For the six populations from above locations, the haplotype network derived from ATPase 6/8 haplotypes resulted in only one clade (Fig.9).

Variation in size-free landmark based morpho-metric traits trough TRUSS network analysis for shape analysis

To evaluate natural population structure of *T. ilisha* on landmark based morphological variations, 306 specimens of various size classes were used to study population differentiation from 7 locations, within Ganga and associated river systems, Brahmaputra (Golpara), Ganga (Farrakka), Hoogly (Barrackpore and surrounding areas), Hoogly (Balagarh), Hoogly (Godakhali) and two marine locations Digha and Kirtunia. The images were captured, TPS file generated and 13 landmarks were recorded on the left view of each specimen (Fig.10). A 13-landmark truss network system was used to generate 78 morphometric variables, which were transformed and standard length was excluded from the final analysis, retaining 77 variables. Transformed measurements were employed to univariate analysis of variance (ANOVA), principal component analysis (PCA) and discriminant function analysis (DFA). Univariate analysis of variance extracted significant differences in 73 transformed morphometric characters out of 77 studied. PCA



A B

Fig.9. (A) Pair-wise differences and (B) Haplotype network of ATPase 6/8 sequences showing one distinct clade of *T. ilisha* from within Ganga and associated river systems

extracted 14 principal components (eigen value >0.7) accounting for 96.83% variation. Forward stepwise discriminant function analysis produced 6 functions and 10 discriminating variables. DFA showed that 66.7% original grouped cases correctly classified; 61.4% of cross-validated grouped cases correctly classified (Fig.11).

Land marks refer to: 1. Anterior tip of snout at upper jaw; 2. most posterior aspect of neurocranium (begiA nning of scaled nape); 3. Origin of dorsal fin 4. end of dorsal fin; 5. Anterior attachment of dorsal membrane from caudal fin; 6. Posterior end of vertebrae column; 7. Anterior attachment of ventral membrane from caudal fin; 8. End of anal fin; 9. Origin of anal fin; 10. Insertion of pelvic fin; 11. Insertion of pectoral fin; 12. End of operculum; 13. Posterior end of eye.

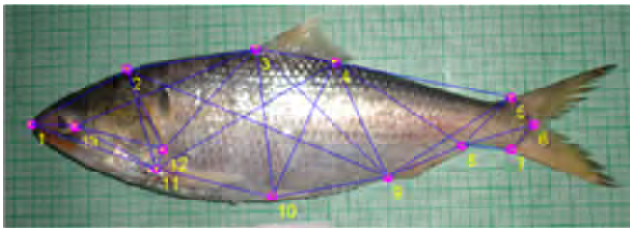


Fig.10. A TPS image of *T. ilisha* showing 13-landmarks used for truss analysis

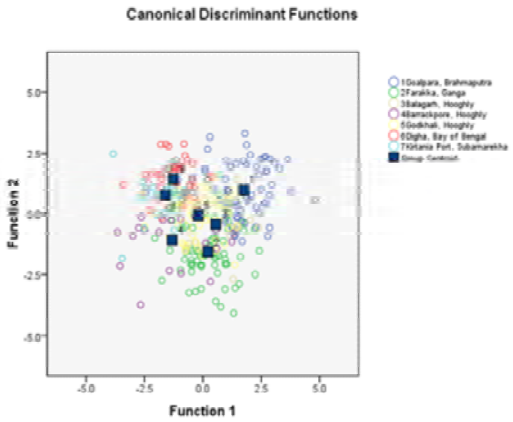


Fig.11. Discriminant analysis plot for morphometric variables of *T. ilisha*

Project Title: Genetic stock structure analysis of *Parapenaeopsis stylifera* and *Scomberomorus commerson* along the Indian coast using molecular markers

Project Period: April, 2013 - March, 2016

Project Personnel: P.R. Divya (PI), V.S. Basheer, A. Kathirvelpandian and Mog Lebrachai Chowdhury

Funding Agency: Institutional

Scomberomorus commerson (King seer) and *Parapenaeopsis stylifera* (Kiddi shrimp or Karikkadi) constitute important fishery across both the coasts of India and also forms important commodities in seafood export from India. Traceability of export items from India to the European Union has become mandatory that makes information on genetic stock structure essential. In addition, genetic variability analysis of these species from Indian waters could possibly be helpful for India to develop a regional management plan and strengthen national plan of action for management and conservation of genetic stocks of these two species. In view of this, work on identification of genetic stocks of above mentioned commercially important marine genetic resources has been undertaken.

Specimens of *S. commerson* and *P. stylifera* were collected from five different geographical locations along the Indian coast (Veraval, Mangalore and Kochi of West coast; Chennai and Vishakhapatnam of East coast). A total of 60 tissue samples of each species were collected from each collection location and stored in absolute alcohol. Total genomic DNA was extracted for all the samples collected from different locations across both the coasts of India.

Cross-species amplification of microsatellite loci in *S. commerson*

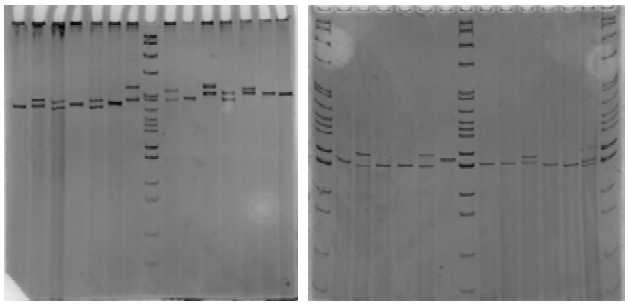
Total of 34 primer pairs from *Scomber australasicus* [12- Tang *et al*, 2009], *S. japonicus* [10-Cha *et al.*, 2010] *S. cavella* [6- Broughton *et al.*, 2010] and *Rastrelliger kanagurta* [6-Candy *et al.*, 2013] were tested for cross species amplification, out of which, 11 primers were successfully amplified. A total of 7 primers from *S. australasicus*, 2 from *S. cavella*, and one each from *R. kanagurta* and *S. japonicus* were standardized. The percentage of polymorphic loci was 81.8%. The two primers, Sca 37 and Ksj 26 were found to be monomorphic. The standardized loci were confirmed through sequencing. Details of the microsatellite primers developed are given in Table 2 & Fig. 12.

ATPase 6/8 gene analysis of *S. commerson*

A total of 842 bp sequence of ATPase 6/8 gene was obtained in 86 individuals from five different geographic locations along Indian coast. Two fragments, 168 bp fragment of ATPase 8 and 684 bp of ATPase 6, were obtained and used for detecting the genetic variation in *S. commerson*. An overlapping region (10 bp) between two genes was found from 159–168 bp. A total of 22 variable positions with 23 haplotypes including 9 parsimony informative sites and 13 singleton variable sites identified (Table 3). The average

Table 2. Characterization of eleven microsatellite loci amplified in *S. commerson*

S. No.	Locus	Repeat motif in resource species	Repeat motif in *S. commerson*	Primer sequences	Ta (°C)	Size range (bp)	Accession number
1	*Sa 2769* *Scomber australasicus*	$(AC)_{18}$	(AC)9	F-TTTTGCATTTTAAGCAGCTCAGT R-GTGGTGGACACACACAGATTCAT	56	221-259	KP120688
2	*Sa 2657* *S. australasicus*	$(CA)_{16}$	(CG)8 (AG)6	F-TGTCAGAGATGTAGCACATACGG R-AGCATTATCTGGTGCTGTAAGGA	56	240-328	KP120686
3	*Sa 2068* *S. australasicus*	$(GGA)_9$	(AG)8	F-CAAGACATGACAGTAGGACATTGAC R-AGATTGGGAGTTTGTAGGGGTAATA	56	146-176	KP120684
4	*Sa2770* *S. australasicus*	$(CA)_{13}(CCT)_3$	(AC)15 (CCT)4	F-AGAAATGAAAAGGGCTTTAAGGA R-ACTGAGCTGCTTAAAATGCAAAA	56	195-285	KP120689
5	*S ca44* *S. cavella*	(CTCG)2 CTAT $(CTGT)_5$	(TCTG)8	F-ATGGCCAAATGGCACATAATCA R-GGGCAGCTCCATGGGTCTGAGT	58	169-175	KP120692
6	*Sca-37* *S. cavella*	$(TG)_8$ AG $(TG)_4$ AG $(TG)4_3$	(GT)10	F-GCG CCGTGACTTTTTATTGCTC R-CAACAATTAGTCGCAGCCCTAG	58	154- 168	KP120692
7	*Raka -36* *Rastrelliger kanagurta*	$(AGTG)_{10}$	(AC)9	F-TGTGTCTACACAGACAGAGGG R-TAATCACTCTCGCTCGCTCG	58	101-153	KP120683
8	*Sa2873* *S. australasicus*	$(CA)_{17}$	(TC)11	F- TCACACTGTGCAATAATCACTCC R- TATTTGAGCAGCCTCAAGAAGAG	59	224-312	KP120690
9	*Sa2683* *S. australasicus*	$(TG)_{17}(GA)_{15}$	(GT)8 (TC)8	F-CTGAGACACAGTGATGTTTGTCC R- TGCATATAGCACGAAAAAGTCAT	52	223-297	KP120687
10	Sa2344 *S. australasicus*	(GT)51	(TG)20	F-CACAAAAGCTGCTTAACACACTCT R- TCACACTCAGCAAAATGAAGTTTC	48	118-180	KP120684
11	*KSj-26* *S. japonicus*	$(GT)_{13}AT\ (GT)_3$	(GT)7	F-GGAGCATTTGACAACACTTAC R-AGTCAGTTTTTGGTGGATGAG	55	174-196	KP120682



S. australasicus Sa 2683 (223-297 bp) Tzeng *et al.* (2009)
S. australasicus Sa 2344 (118-180 bp) Tzeng *et al.* (2009)

Fig.12. PAGE depicting microsatellite loci standardized in *S. commerson* through cross-priming

nucleotide diversity for all the samples of *S. commerson* were: A: 27.37%; T: 27.37%; C: 32.15%, G: 13.12%; Nucleotide sequences of ATPase 6/8 were A+T rich (54.74%) with transition transversion bias (R) was 12.02. All the haplotypes were submitted to NCBI GenBank (Accession numbers: KP281779 - KP281801).

Population variability and differentiation analysis of five populations of *S. commerson*

The haplotype diversity was found to be high and ranged between 0.782 - 0.844; whereas, the nucleotide diversity was low and ranged between 0.00179 - 0.00231. The number of polymorphic sites varied from six for the Veraval population and 10 for Chennai population. Population variability parameters are given in Table 3.

Table 3. Population variability parameters from five populations of *S. commerson*

Location	No. of polymorphic sites	Haplotype diversity	Nucleotide diversity
Veraval	6	0.833	0.00224
Kochi	7	0.805	0.00209
Mangalore	6	0.844	0.00179
Chennai	10	0.782	0.00231
Vishakha-patnam	9	0.842	0.00214

Co-efficient of genetic differentiation (F_{ST}) was calculated for pair-wise and overall populations of the species. Pair wise F_{ST} values did not show any differentiation between five populations of *S. commerson* (Table 4). The overall F_{ST} value was found to be -0.02074.

Table 4. Pair wise mean F_{ST} values between populations of *S. commerson*

	Veraval	Kochi	Manga-lore	Tamil Nadu	Visakha-patnam
Veravel	0				
Kochi	-0.02932	0			
Manglore	-0.04683	-0.00293	0		
Tamil Nadu	-0.03286	-0.00009	-0.02859	0	
Visakhapatnam	-0.04363	-0.02353	-0.03854	-0.01611	0

Identity of *P. stylifera* and *P. coromandelica* through molecular analysis

Morphomeristic parameters often fail to differentiate the species of *P. stylifera* and *P. coromandelica* except for telsonic armature. The reported information like rostral teeth is quiet overlapping and not possible to delineate the species. Both the species are found to co-exist in Indian waters. The morphological identification reported based on the number of telson spines was confirmed through mitochondrial cytochrome oxidase I (COI) gene analysis for differentiating both look-a-like species. A total of 655 bp partial COI gene was sequenced and analyzed. Analysis indicated the mean genetic divergence value of 7.3% between the two species (Fig.13).

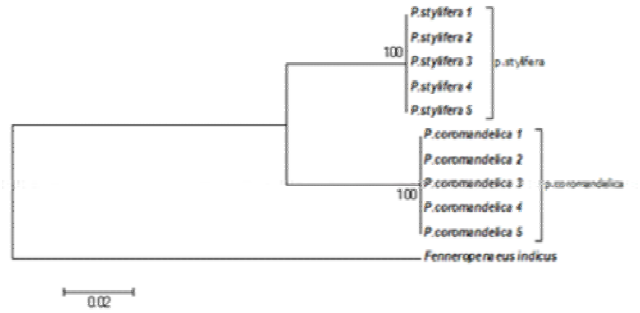


Fig.13. NJ tree depicting the evolutionary relationship of *P. stylifera* and *P. coromandelica*

Cross-species amplification of microsatellite loci in *P. stylifera*

Ten microsatellite primers were developed in *P. stylifera* through cross priming out of 30 microsatellite primers from *Litopenaeus vannamei*, *Fenneropenaeus chinensis* and *Penaeus monodon* and 9 primers from *Parapenaeopsis hardwickii*. The details of the primers developed are given in table 5.

Project Title: DNA barcoding of marine finfishes and shellfishes

Project Period: November, 2012 – October, 2017

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

Funding Agency: CMLRE - MoES, Govt. of India

Samples of 167 species of finfishes were collected from the fish landings at Cochin, Trivandrum and Kollam in Kerala; Mangalore, Malpe and Karwar in Karnataka; Mumbai in Maharashtra; Paradeep in Odisha; Vishakhapatnam in Andhra Pradesh and Port Blair in Andaman & Nicobar Islands. 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. DNA barcodes for 62 species of finfish were generated and haplotype sequences submitted to GenBank. Sequencing of the COI gene produced an

Table 5. Characteristics of ten microsatellite loci amplified in *P. stylifera*

S. No.	Locus	Resources species	Repeat motif	Primer sequences	Ta	Size range
1.	*FC04*	*Fenneropenaeus chinensis*	$(TTA)_{18}$	F- TGCTTTAATGGTTGCTG R- TACCAAGAATGGAGTG	55	199-238
2.	*FC10*	*F. chinensis*	$(TG)_{10}$	F- GGTCTTCGCCGACTCAGA R- CCCACCATCTCATCCACC	52	211-235
3.	*PmMS2G2*	*Penaeus monodon*	$(GACA)_{24}$	F- AGAGGTTTGCAGCCGAGCGAAAAG R- CGCTGATCCTGGCTTCTTGGAAAT	56	177-276
4.	*Lvan0510*	*Litopenaeus vennamei*	$(GT)_8$	F-GCCATTTGATTGCTCT R-TGACTTGGTCTTTGTTAG	50	242-308
5.	*MIph05*	*Parapenaeopsis hardwickii*	$(AC)_{16}$	F- CCTTCCACTTGGATTTTGG R-TGTCCAGGTGTGAGTTGA	58	240-270
6.	*MIph07*	*P.hardwickii*	$(TC)_{30}$	F-GTCCTTTCTCGAGGGCTA R-AAGTGGCCTAAAAAGTGT	50	182-256
7.	*MIph10*	*P.hardwickii*	$(TC)_{19}$	F-TTCAACCCACACCCTTTTT R-TCACACGTGACCCTGACC	57	186-226
8.	*MIph14*	*P.hardwickii*	$(TC)_{14}$	F-TCTCCTCCATAACCCTTTT R-CGCGGGAATTCGATTACTT	51	238-262
9.	*MIph18*	*P.hardwickii*	$(CA)_9$	F-ATTTAAATTATAAGCGTTG R-ACGAAAAAGGACTTCAAA	49	170-192
10.	*MIph21*	*P.hardwickii*	$(CA)_{18}$	F-ACCCGATCAAACAATGTAT R-GTGTAGAGGCCAAGGAAT	49	200-240

average of 655 base pairs per taxon. Three species of finfishes from southwest coast of India, *Chelidoperca investigatoris, C. occipitalis* and *Chlorophthalmus corniger* were re-described. A cruise was undertaken to south-west coast of India to explore deep-sea fish diversity and collected 35 species of deep-sea fishes.

Re-description of *Chelidoperca spp.*

Chelidoperca investigatoris (Alcock, 1890) was described from two specimens collected off the Ganjam coast, Bay of Bengal and *Chelidoperca occipitalis* (Kotthaus, 1973) was described from a single specimen collected off the Socotra Islands, Arabian Sea. Recently, many additional specimens of these two species were collected off Kollam, Kerala, south-west coast of India. For *C. occipitalis* report from south-western India forms a considerable extension of its known distribution range. *C. investigatoris* and *C. occipitalis* are re-described based on these new specimens.

C. investigatoris (Fig. 14 A) diagnosed with dorsal-fin rays X, 10, fourth spine largest (3.14-3.37 in HL) longer than 3rd spine; Body depth 26.23-27.82% SL; head length 41.95-48.71% SL; orbit length 8.85-10.06 in SL; 2.5 scales above lateral line to dorsal-fin origin; serrae on margin of pre-opercle 25-37; head and body bright pinkish in colour. A broad bright yellow band passes from the tip of the snout through the eye to the caudal fin (very clear in the snout region). Narrow pale red borderline on anal fin. Head and body pinkish in colour, belly and throat white. A broad bright yellow band passes from the tip of the snout through the eye to the caudal fin. Bright yellow markings on the cheeks,

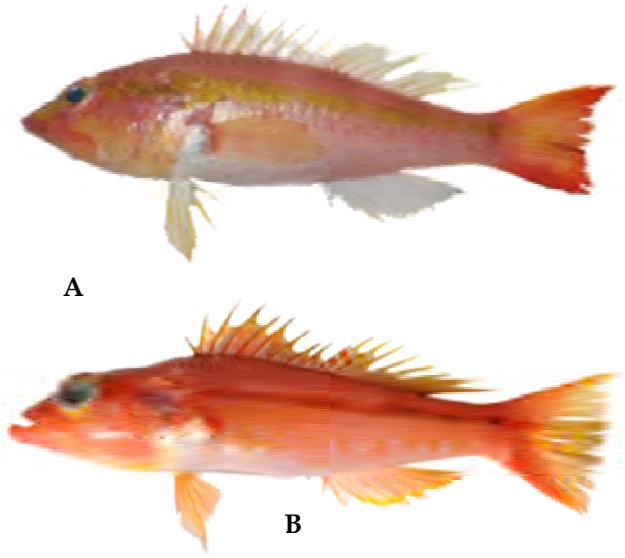


Fig. 14 A. *Chelidoperca investigatoris* and B. *Chelidoperca occipitalis*

opercles, dorsal, ventral and anal fins. Narrow pale red borderline on anal fin. Colour in formalin- Pale with four incomplete cross bands of grey.

C. occipitalis (Fig. 14 B) diagnosed with dorsal-fin rays X, 10, fourth spine largest (2.4-2.9 in HL) slightly longer than 3rd spine, all soft rays branched; anal fin rays III, 6; pectoral fin rays 15; Lateral-line scales 43-44; Gill rakers 7+12-8+13; Body depth 3.9-4.3 in SL; head length 2.2-2.4 in SL; orbit length 9.4-10.2 in SL; 3

Table 6. GenBank accession numbers of *Chelidoperca* spp.

Species	Code	Collection locality	COI	16S	Voucher reg. no
C. investigatoris	TK29	Tuticorin	KP009557		NBFGR CHN 3014
C. investigatoris	TK31	Tuticorin	KP009558		NBFGR CHN 3015
C. investigatoris	TK32	Tuticorin	KP009559	KF814973	NBFGR CHN 3016
C. investigatoris	K32	Kollam		KF814970	NBFGR CHN 3017
C. investigatoris	D19	Kollam	JX185305		NBFGR CHN 3018
C. investigatoris	H29	Kollam	JX185307		NBFGR CHN 3019
C. investigatoris	R30	Chennai	JX185312	KF814971	NBFGR CHN 3020
C. investigatoris	R21	Chennai	JX185310	KF814972	NBFGR CHN 3021
C. occipitalis	F25	Kollam	JX185306	KF814974	GB31.139.16.1
C. occipitalis	D18	Kochi	JX185304		GB31.139.16.2
C. occipitalis	R22	Kochi	JX185311	KF814976	GB31.139.16.3
C. occipitalis	R36	Kollam	JX185313	KF814975	NBFGR CHN 3001
C. maculicauda	K22	Kollam	JX185308	KF814976	GB31.139.14.5
C. maculicauda	K23	Kollam	JX185309	KF814978	GB31.139.14.5.1
C. maculicauda	K24	Kollam	JX262929		GB31.139.14.5.2

scales above lateral line to dorsal origin; circum-peduncle scales 14 and Serrae on margin of preopercle 28-38. Body pinkish in colour with a dark band along body, to caudal. Yellow spots on dorsal, caudal and anal fins and caudal rays prominent. Body pinkish-orange in colour with a prominent dark stripe running along body, from opercular spine to base of caudal fin. Ventral portion of trunk pale with 8-9 white bands on side. Colour in formalin pale with prominent black stripe along middle of trunk and caudal fin with pale spots.

Molecular charecterisation of *C. investigatoris* and *C. occipitalis* was also done based on COI gene and 16s rRNA gene. Accession numbers are given in Table 6 and phylogenetic tree based on COI gene is given in Fig. 15.

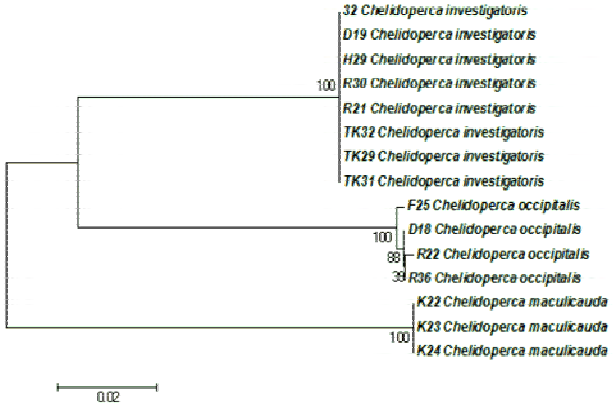


Fig. 15. Neighbour Joining (NJ) phylogenetic tree of *Chelidoperca* inferred from mitochondrial COI gene

Molecular identification of *Macolor macularis* and *M. niger*

The Midnight Snapper, *Macolor macularis* is often confused with *M. niger* since they can appear very similar, both as juveniles and adults. There are a number of diagnostic differences in meristics and coloration (Kishimoto *et al.*, 1987; Allen & Erdmann, 2012). Nevertheless, misidentifications are frequent.

The DNA barcode sequences for the two species are clearly different, providing a useful check on the identifications based on other features. *M. macularis* (Fig. 16 A) was compared with its only congener, the Black and White Snapper, *M. niger* (Fig. 16 B) both collected from the south-west coast of India. The examination of fresh specimens of the two species showed diagnostic anatomical and coloration differences and the DNA barcoding showed a genetic divergence of 3.51% between the species.

Fig. 16 A. *Macolor macularis*

Fig. 16 B. *Macolor niger*

Redescription of *Chlorophthalmus corniger*

Chlorophthalmus corniger (Fig. 17) is re-described on the basis of recently collected specimens. The species is redefined as a species of Chlorophthalmus with the lower jaw terminating in a distinctly projecting horizontal plate with strong, spine-like processes directed forward from the plate's corners; body silvery grey, with numerous minute black spots and traces of broad darker crossbars; base of anterior dorsal fin spines and distal parts of dorsal fins black; adipose fin tiny with numerous black spots; caudal fin black; 3 ·5 scales above lateral line; three rows of cheek scales; head very large, 34 ·3–40 ·1% standard length (LS); eye large, 29 ·8–40 ·8% head length (LH); pectoral fin long, extending to beyond dorsal fin base, 21 ·7–26 ·2% LS. *C. bicornis* is a junior synonym of *C. corniger* based on the examination of the type series of both species. It is confined to the northern half of the Indian Ocean, reliably recorded from Somalia and the Gulf of Aden to southern Java, Indonesia, at depths between 200 and 500 m. A lectotype and three paralectotypes were designated for *C. corniger*. DNA barcodes for Indian species of Chlorophthalmus were generated and phylogentic tree constructed to know the evolutionary position of the *C. corniger* (Fig. 18).

Fig. 17. Lateral views of *Chlorophthalmus corniger*: (a) lectotype ZSI 13713, 65·9 mm standard length (LS) and (b) GB.8.6.1.4.3, 120.7 mm LS, off Kollam, Kerala (fresh)

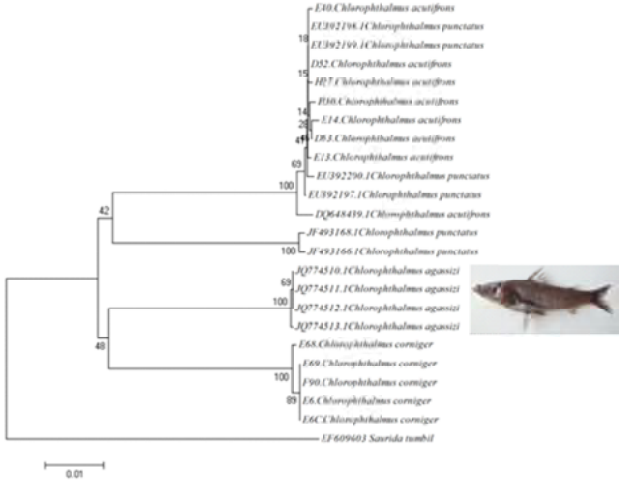


Fig. 18. Phylogenetic tree based on COI sequences data of *Chlorphthalmous* spp.

Project Title: Genetic stock structure analysis of the Indian mackerel, *Rastrelliger kanagurta* from Indian waters using microsatellite markers

Project Period: April, 2013 – February, 2015

Project Personnel: J.K. Jena (PI), Divya P.R. and V.S. Basheer

Funding Agency: FAO/ BOBLME, Bangkok, Thailand

In this Bay of Bengal Large Marine Ecosystem (BOBLME) funded project, Bangladesh, India, Indonesia, Malaysia, Maldives, Myanmar, Sri Lanka and Thailand were working together towards a coordinated programme of action designed to improve the lives of the coastal populations through improved regional management of the Bay of Bengal environment and its fisheries. The Indian mackerel, *Rastrelliger kanagurta*, is a species of great commercial importance in the BOBLME region. The stock status of various populations of this species was uncertain, which makes it difficult to accurately identify levels of sustainable harvest and formulate management plans. With this in mind, BOBLME included an ambitious programme on Indian Mackerel Genetic Stock Assessment in its Annual Regional Work Plan in 2012. ICAR-NBFGR was assigned to identify the population genetic structure of the Indian mackerel in the Indian region.

Collection of tissue samples of Indian mackerel (70 numbers/site) was completed from Mumbai, Calicut, Tuticorin, Nagapatnam, Kakinada, Paradeep, Port Blair and two additional locations Mangalore and Kollam. A total of 630 individuals collected from nine geographic locations were analyzed with 14 microsatellite loci. The alleles were separated using capillary electrophoresis on an ABI Prism 3730 genetic analyzer (Applied Biosystems). Electropherogram obtained in the form of .fsa files were analyzed using GeneMapper software (version 4.0; Applied Biosystems Inc., California, USA) to generate genotype calls for each marker. Size matching was evaluated for each sample using Size Match Quality Indicator. Fragment sizing were carried out using the default analysis settings in the software, by comparison with the internal size standard ROX 400 or LIZ 500. Preliminary data analysis of samples from Indian waters was completed using Genpop version 3.3, Bottleneck version 1.2.02 and GenAlex version 6.5 softwares.

Fourteen polymorphic microsatellite loci were identified for the study. There was no significant association indicative of linkage disequilibrium between any pair of microsatellite loci for any populations ($P<0.05$) indicating independence of 14 loci. Number of alleles ranged from 15 (SA 2068) to 72 (Ksj26) and the allele size ranged 74 to 436 bp. Over all microsatellite loci, the mean number of alleles for populations was 25. The genetic variability of all mackerel populations were high and were within the range of 0.79 - 0.82. Mean value of observed heterozygosity and expected heterozygosity for the populations were 0.70 (Hobs) and 0.80 (Hexp), respectively. Bottleneck analysis using softwares show that heterozygosity is high; hence, the null hypothesis of the Wilcoxon's test is accepted and it suggests that there is no satisfactory evidence for a bottleneck in the *R. kanagurta* population. The Mode-shift indicator test was also utilized as a second method to detect potential bottlenecks and the distribution pattern shows normal L-shaped form and this clearly indicates that the studied population has not experienced a recent bottleneck analysis of the genetic data revealed that population had high genetic variation; they are genetically stable or at random mating.

The co-efficient of genetic differentiation, F_{ST} ranged from 0.017 for the locus Raka 2 to 0.101 for the locus SC 8, with a mean of 0.061 ±0.008. The gene flow or migration rate (Nm) for each locus for overall population ranged from 2.2 (Sc 8) to 14.213 (Raka 2) with the mean value of 5.185. Pair-wise F_{ST} analysis of mackerel and gene flow Nm showed low genetic differentiation among samples collected from various locations of the Indian mainland. The pair-wise genetic distance was the highest between samples of Andamans and Mangalore (0.0543) and lowest

between Nagapattinam and Tuticorin (0.02). Specimens collected from Andaman were most distinct compared to all other locations. Hierarchical genetic structuring of the samples was carried out by analysis of molecular variance (AMOVA) and the result showed 81% was contributed by variation within individuals, whereas, only 13% and 6% variation was observed among the individuals and among the populations respectively (Fig. 19). Preliminary data analysis showed low genetic differentiation among the mackerel populations distributed along Indian waters, except moderate differentiation in the samples from Andamans. This implies the need for fisheries management of mackerel at international level to ensure the preservation of genetic diversity and sustainability of the regional fisheries.

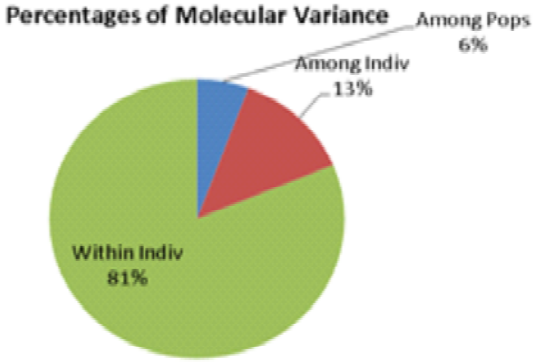


Fig. 19. Analysis of Molecular Variance (AMOVA) based on microsatellite alleles in *Rastrelliger kanagurta* distributed along Indian waters

Title of Project: Whole genome sequencing and development of allied genomic resources in two commercially important fish - *Labeo rohita* and *Clarias batrachus* (A collaborative project of NBFGR, Lucknow; CIFA, Bhubaneswar; Anand Agricultural University, Anand and ICAR-IASRI, New Delhi).

Project Coordinator: J.K. Jena

Project Personnel from NBFGR: N.S. Nagpure (PI), Basdeo Kushwaha, Ravindra Kumar and Mahender Singh

Project Period: October, 2013- October, 2016

Funding Agency: Department of Biotechnology, Govt. of India

Labeo rohita and *Clarias batrachus* (now *Clarias magur*) are two important and widely cultured food fishes in India and adjoining countries. Lack of genomic resources for these species are major constraints for genetic improvement of these species. Therefore, it is envisaged to undertake the whole genome sequencing in two economically important indigenous fish species, with the view to i) establish reference draft sequence of these two species, ii) generate associated genomic resources such as large insert libraries, SNPs, etc. iii) develop a platform for estimating and preserving genetic variability of indigenous populations of carp and catfish species with related marker information, and iv) develop human resource in frontier areas of fisheries research.

Genome size estimation

Information about the genomic size of organisms is important to study structure and evolution of genomes and for planning whole genome sequencing projects. In view of the paucity of information, genome size estimation has been carried out in rohu and magur using flow cytometry using three reference standards, viz., *Gallus gallus*, *Oreochromis niloticus* and *Homo sapiens*. The nuclear DNA content in *L. rohita* and *C. batrachus* was estimated to be 1.45 (~ 1.40 GB) and 0.98 (~ 0.95 GB) pg/ nucleus, respectively.

Multi platform sequencing

High quality genomic DNA was isolated from testes, liver and muscles of magur yielding approximately 736 µg (O.D. 260/280> 1.8 and O. D. 260/230 >1.8). Genomic DNA were mechanically sheared into short fragments, followed by ligation of specific adaptors. The sequences of these adaptors were specific for emulsion PCR primer and sequencing primer. Genomic DNA was sequenced via multi sequencing platforms viz., Roche 454, Ion Torrent and Illumina HiSeq using single end, paired end and mate pair approaches. The statistics of the data generated for magur is provided in the table 7.

Table 7: Data statistics of *C. batrachus* from different sequencing platforms

Sequencing Platform	Data statistics			
	Runs	Data (GB)	No. of reads (Millions)	Average read length
454	Run 1	0.50 GB	1.49	350.2
	Run2	0.56 GB	1.54	372.72
Ion Torrent	Run 1	0.89 GB	2.76	333.2
	Run2	0.99 GB	3.39	300.6
Illumina	PE_150-250	53.3 GB	363.92	150
	PE_350-450	48.9 GB	333.72	150
	PE_550-650	43 GB	293.95	150
	MP_4kb	24 GB	164.60	150
	MP_9-11kb	25.65 GB	175.14	150

De novo assembly of *C. batrachus*

The *de novo* assembly of data generated from four sequencing runs on Roche 454 and Ion Torrent was carried out using GS assembler. The input data comprised of ~10 million reads with 3,212,313,421 bp. Assembly resulted in 7,730,461 aligned reads comprising of 2,468,347,647 bp. The genome assembly generated 5,36,276 contigs comprising of 481,576,303 bp. The N50 contig size was 1,178 bp and the longest contig was 16,510bp (Table 8).

Table 8. Assembly statistics of *C. batrachus* using Roche 454 and Ion Torrent data

GS Assembler (Newbler)	
Input File No. of Reads	10,127,018
Input File No. of Bases	3,289,947,570
Total No. of Reads	10,153,852
Total No. of Bases	3,212,313,421
No. of Aligned Reads	7,730,461 (76.13%)
No. of Aligned Bases	2,468,347,647 (76.84%)
Large Contigs (Length >500bp)	
No. of Contigs	4,06,042
No. of Bases	442,250,392
Average contig Size	1,089
N50 Contig Size	1,178
Largest Contig Size	16,510
All Contigs	
No. of Contigs	5,36,276
No. of bases	481,576,303

The *de novo* assembly of data generated from three sequencing runs on Illumina HiSeq was carried out using Abyss assembler. The assembly was carried out on 4 different hash lengths in order to select the best assembly based on the assembly statistics. The best assembly was observed at 85 hash length with the maximum contig size of ~89 Kb. The total genome coverage as per the assembly stats was found to be ~891 Mb. A total of 18674 contigs were found to be greater than 10 Kb. N50 of the assembly contigs was found to be 6611 (Table 9).

Mitogenome assembly and annotation

The various assembly approaches yielded mitogenome as the longest contig. The best mitogenome contig was annotated using MitoAnnotator and its sequence has been submitted in the NCBI (Acc. No. KM259918). Mitogenome annotation revealed 13 protein coding genes (PCGs), 22 tRNA, 2 rRNA and D-loop region (Fig. 20). The gene order and orientation were found to be similar with that of other vertebrates. The coding genes spanned over 69% of mitogenome followed by tRNA ~9.4%, rRNA ~15.9%, D-loop ~5.2% and intergenic region ~0.3%. A total of 11 noncoding intergenic regions were observed in the mitogenome of *C. batrachus*, the longest being 32 bp in length, which was found between tRNA-Asn and tRNA-Cys.

Table 9. Assembly statistics of *C. batrachus* using Illumina HiSeq data

Abyss Assembly				
Hash Length	**61**	**64**	**69**	**85**
Contigs Generated	270392	270018	265919	287999
Maximum Contig Length	59097	58997	65287	89416
Minimum Contig Length	200	200	200	200
Average Contig Length	3008.7	3045.7	3152	4191.5
Total Contigs Length	813531409	822382599	838189871	891793694
Total Number of Non-ATGC Characters	6628749	6182998	5465133	3424771
Percentage of Non-ATGC Characters	0.815	0.752	0.652	0.384
Contigs >= 200 bp	270392	270018	265919	287999
Contigs >= 500 bp	215724	214280	208309	211754
Contigs >= 1 Kbp	174852	174026	170275	173175
Contigs >= 10 Kbp	14408	14983	16427	18674
Contigs >= 1 Mbp	0	0	0	0
N50 value	5760	5893	6250	6611

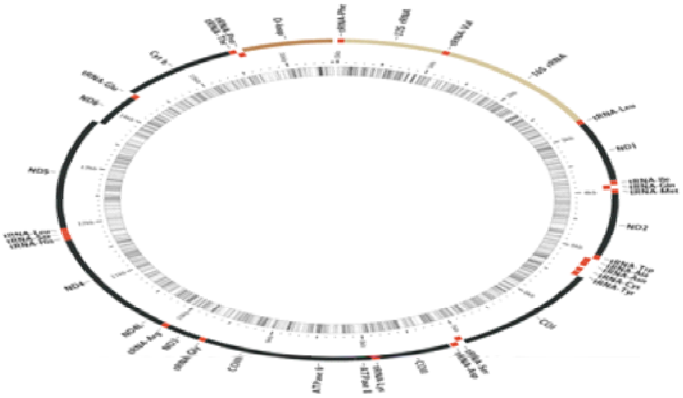


Fig.20. A complete mitogenome of *C. batrachus*

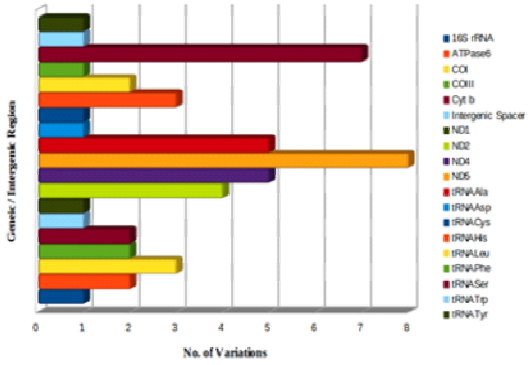


Fig.22. Histogram showing gene-wise single nucleotide variation

Gene wise coverage of mitogenome

The average coverage of the mitogenome obtained from pooled Roche 454 data, pooled Ion Torrent data and pooled all data were ~198X, ~87X and ~284X, respectively. Gene-wise coverage was computed for all the 3 data sets and the coding genes as well as the rRNA genes were found to assembly at higher coverage than the tRNA genes because of the large length of PCGs and rRNA (Fig. 21).

Phylogenetic analysis of catfish Mitogenome

Phylogenetic analysis was carried out using two data sets, one pertaining to the concatenated PCGs and other to the concatenated PCGs+tRNAs (Fig. 23). The phylogenetic relationship obtained from both the data sets yielded almost similar results and were mainly consistent to the previously reported phylogeny.

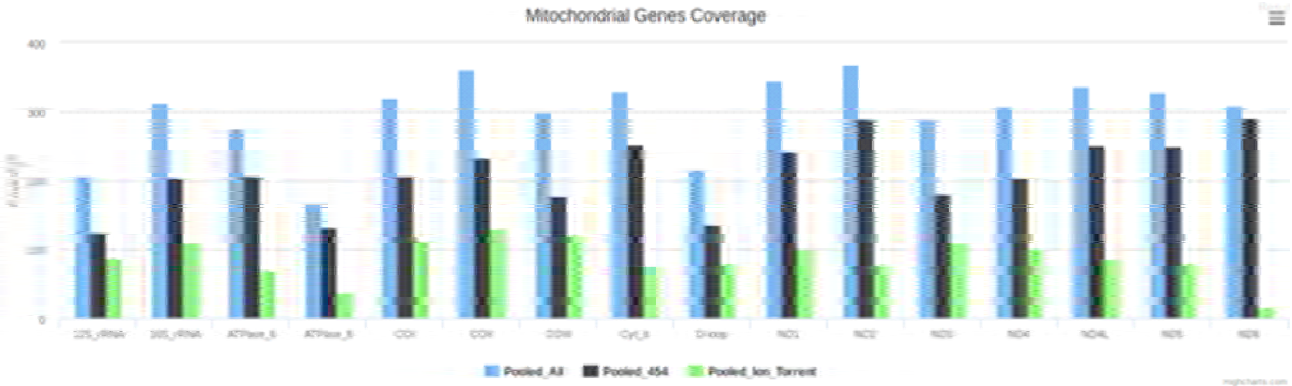


Fig.21. Gene-wise average coverage of mitochondrial genes

Variation analysis of mitogenome

Variation analysis was carried out between the *de novo* assembled *C. batrachus* mitogenome and the *C. batrachus* reference mitogenome available in NCBI. We observed 51 common single nucleotide variations (SNVs), out of which 49 were falling in the genic region. These SNVs were also verified at the gene level. Maximum number of variations were observed in ND5 (8), followed by tRNA-Ser (7). A total of 9 non-synonymous mutations were reported in PCGs, out of which 3 were in ND4 gene (Fig. 22).

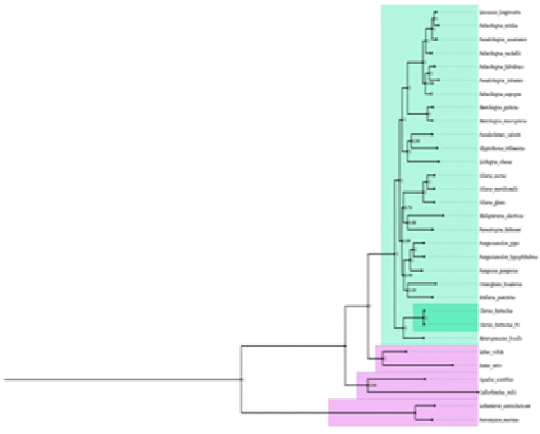


Fig.23. Coding DNA sequences based phylogenetic relationships among the catfishes

Gene prediction and annotation of *C. batrachus*

Gene prediction of the 454 and Ion Torrent assembled contigs was carried out using 'Augustus' which resulted in 6,402 putative ORF/genes. Annotation of these putative ORFs was carried out using Blastx against the database of reviewed proteins of all the fishes from Uniprot. The parsed blast output was further filtered out on 50% identity and 50% query coverage, which yielded 1,100 well annotated putative ORF's.

Microsatellite mining

Microsatellite mining of the *C. batrachus* assembled contigs was carried out using MISA which resulted in 28,956 microsatellites. After removing mono-repeats, the total number of microsatellite were reduced to 14,548. Using Primer3, flanking primers were designed for the SSRs and 1,670 unique amplifying primers were obtained.

WGS Website

The Institute developed the website 'Rohu and Magur Genome Sequence Consortium' and hosted onto the NBFGR Server (http://mail.nbfgr.res.in/RohuMagur_Genome/index.html) (Fig.24).

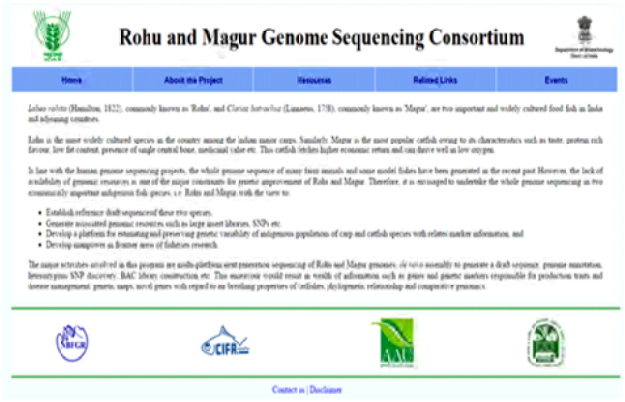


Fig.24. Snapshot of WGS Website on ICAR-NBFGR Server

Project Title: Population genomics of *Clarias magur* based on Restriction site Associated DNA (RAD) markers

Project Period: April 2014 - March, 2017

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

Funding Agency: Institutional

Samples of four populations were collected from Purva, Unnao District, Uttar Pradesh (20); Delang, Puri District, Odisha (39); Kaikaluru, West Godavari District, Andhra Pradesh (46) and Loktak, Bishenpur District, Manipur (40). Genomic DNA was isolated for all samples and DNA having absorbance ratios (260/280) between 1.8 to 2.0 was used for library preparation (Fig.25). The work of Illumina Highseq 2000 paired end RAD sequencing of Purva and Delang populations is in progress. PyRAD, a pipeline to assemble *de novo* RADseq loci was installed and configured. For SNP analysis of RAD-seq data, STACKS (http://creskoloab.uoregon.edu/stacks/) program was installed and test run.

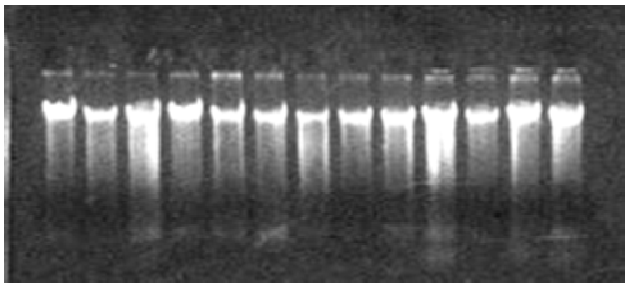


Fig.25. Gel picture of genomic DNA of *Clarias magur*

Project Title: Outreach activity on fish genetic stocks (Phase II). (A network project coordinated by NBFGR with other ICAR fisheries research Institutes

Project Period: April, 2014 – March, 2017

Project Coordinator: J.K. Jena

Project Personnel: Rajeev Kumar Singh (PI), Vindhya Mohindra, T.T. Ajith Kumar, Sangeeta Mandal, Santosh Kumar and J.K. Jena

Funding Agency: Institutional

Population studies in *Silonia silondia*

Microsatellite enriched genomic library was constructed for *Silonia silondia* through screening, using biotinylated oligos followed by enrichment. Enriched genomic libraries with di- and tri-repeat motifs were constructed and recombinant clones were detected via screening of blue/ white colonies followed by colony PCR technique. A total of 63 primers could be designed from the obtained sequences. Out of 63 primer pair, 22 primers yielded amplification, of which 12 (54.5%) were polymorphic, 8 (36.3%) showed monomorphic pattern and 2 (9%) generated poor amplification on a set of n=14 *S. silondia* genotypes. In *S. silondia*, 76 individual samples from four natural riverine populations (Chambal, Tons, Son, and Mahanadi) were genotyped using 12 polymorphic microsatellite loci for

identification of distinct genetic stocks in the native range of its distribution (Fig.26). The developed primers were cross primed in four related species, i.e., *Clupisoma garua*, *C. taakree*, *Ailia coila* and *Eutropiichthys vacha*.

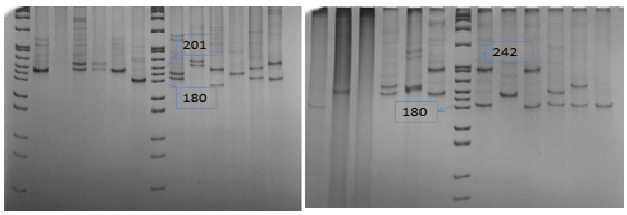


Fig. 26. Polymorphic microsatellite loci in *S. silondia* visualised by polyacrylamide gel electrophoresis

Identification of microsatellite loci in *Anguilla bengalensis bengalensis*

To identify microsatellite repeats in the genome of *Anguilla bengalensis bengalensis*, specimen collected from river Godavari was sequenced using C2 chemistry on 32 single-molecule real-time (SMRT) cells on the Pacific Biosciences (PacBio) RSII sequencing platform. A total of 4.76 gb data was produced by 32 SMRT cells. Assembly and polishing of sequences generated 4773 polished contigs yielding 12.6 mb data with high statistical confidence (QV score of almost 50, more than 99% accuracy).

Whole mitochondrial genome of *A. bengalensis bengalensis* sequenced

A. bengalensis bengalensis or Indian mottled eel is distributed in the rivers of Indo-Gangetic and Brahmaputra river system, ranging from Pakistan, India, Myanmar and Bangladesh. *A. bengalensis bengalensis* fetches high consumer price, however, at present species is not in the culture system. Complete mitogenome sequence for *A. bengalensis bengalensis* was generated through third generation sequencing platform. The 16.7 kbp mitogenome sequence contained 13 protein coding genes, 22 transfer RNAs, two ribosomal RNAs and a non-coding (control) region. The gene order was identical to that observed in most of the other vertebrates.

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, NBFGR), L.K. Tyagi and A.S. Barman (PI, College of Fisheries, CAU, Agartala)

Funding Agency: DBT, Govt. of India

De novo microsatellites developed in the great snakehead, *Channa marulius*

De novo microsatellites were developed in *Channa marulius* through construction of microsatellite enriched genomic library. A total number of 524 sequences containing repeat motifs were found, of which, perfect repeats were found in 324 (61%) sequences, 154 (29%) compound while 46 (8.7%) were imperfect repeats. Dinucleotide repeats, 383 (73%) were highest in number followed by tri 73 (13.9%), tetra 53 (10.1%), penta 9 (1.71%), hexa 4(0.76%) and octa 2(0.38%).

Primer designing and PCR amplification

The sequences having repeat motifs and flanked by sufficient nucleotides on both side were identified and the primers (n=212) were designed using online software, Primer 3 and custom synthesized. The secondary structural properties of primers were checked. Total 154 primers were amplified resulting in scorable band pattern, of which 27 primers were found polymorphic (Table 10).

Table.10 Characteristics of 27 polymorphic microsatellite loci in *C. marulius*

S. No	Locus	Repeat Motif	T_M (°c)	Size(bp)
1	CML-815	$(AGAC)_4$	53	190-200
2	CML-1551	$(GAT)_7$ $(TTA)_6$	51	140-152
3	CML-228	$(CA)_{15}$	59.4	200-210
4	CML-369	$(GT)_{14}$	55	151-161
5	CML-333	$(CA)_{15}$	56	200-214
6	CML-93	$(CA)_{12}$	61	107-140
7	CML-174	$(GT)_{14}$	49	195-200
8	CML-1410	$(ACT)_5$	47	142-155
9	CML-24	$((TG)_8$	56	190-200
10	CML-154	$(GT)_{17}$	55	184-194
11	CML-109	$(GT)_{14}$	54	110-180
12	CML-239	$(CA)_{23}$	65	112-150
13	CML-233	$(CA)_{14}$	59.4	228-234
14	CML-66	$(TG)_7$	55	140-152
15	CML-537	$(GT)_{11}$	55	120-130
16	CML-563	$(GT)_8$	57	110-120
17	CML-597	$(CA)_7$	51	150-165
18	CML-798	$(CA)_{12}$	55	145-155
19	CML-1122	$(GT)_8$	55.6	150-160
20	CML-1177	$((AC)_{11}$	55	160-175
21	CML-1474	$(TAA)_7$ $(TAC)_6$	54	120-130
22	CML-288	$(GT)_7$	57	140-155
23	CML-70	$(CA)_8$	54	187-195
24	CML-1143	$(GATA)_8$	52	155-165
25	CML-875	$(TAGA)_{12}$	50	140-155
26	CML-1586	$(TACTAC)_4$ $(TAA)_6$	55	160-192
27	CML-1631	$(TAGA)_7$	55	130-145

Testing the suitability of microsatellite marker for population genetics

The samples from distant geographic locations *viz.*, Godavari (n=19), Teesta (n=24) and Mahanadi (n=25) were selected for suitability analysis using 19 polymorphic microsatellite loci. Allele sizing of microsatellite bands was done comparing with the standard molecular weight bands MspI digested pBR322 using the BIOVIS-ID analysis software (Fig.27). Individual genotype data at each locus was analyzed using the software Genetix version 4.05. The genetic parameters of each locus such as, allele frequencies, allele size range, expected/observed hetrozygosity and polymorphic information content value (PIC) were calculated. PIC values showed that the developed microsatellites markers are promising for genetic variability assessment of the studied species.

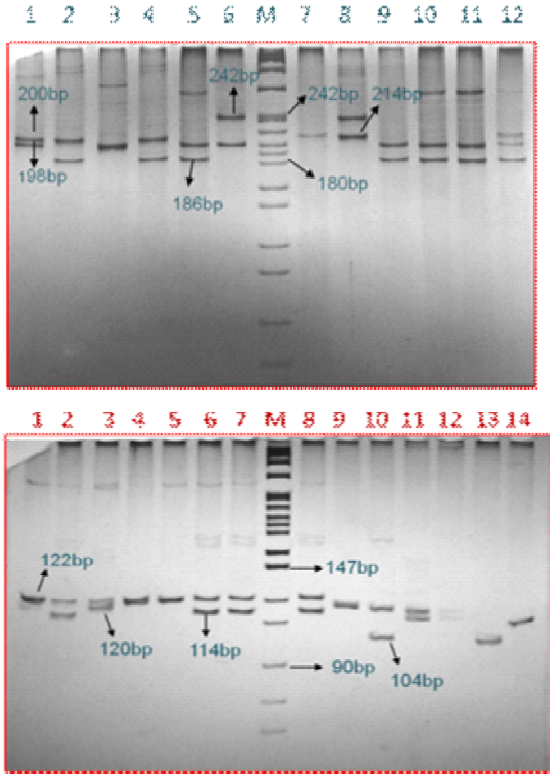


Fig.27 PCR amplicons visualised through polyacrylamide gel electrophoresis

Project Title: Characterization and DNA barcoding of endemic fishes of North east India

Project Period: November, 2012 – November, 2015

Project Personnel: Mahender Singh (PI, NBFGR), N.S. Nagpure and W. Vishwanath (PI, Manipur University, Imphal)

Funding Agency: DBT, Govt. of India

For collecting samples for DNA barcoding of fishes from north east India, exploration was carried out and 80 samples of 21 species were collected from Loktak Lake and Moreh in Manipur. The muscle tissue of 44 species were processed for DNA isolation. The universal set of primers were used to amplify the mitochondrial gene cytochrome c oxidase I (COI) and 16S rRNA genes. The edited sequences were blasted in NCBI for the nearest similar sequence matches. Total 139 DNA barcodes based on COI gene region belonging to 37 species were submitted to NCBI GenBank (Accn. Nos. KJ909336- KJ909474). Other sequin files of 351 sequences of 16S rRNA gene of 81 species and 73 COI sequences of 23 species were prepared for submission to NCBI GenBank. The phylogenetic analyses were performed by Neighbor-Joining, Maximum Parsimony (MP) and Maximum Likelihood (ML) methods in genus *Glyptothorax* (Fig.28) and *Clarias*. For amplification of nuclear marker Internal Transcribed Spacer region 2 (ITS-2), four sets of primers were tested and five new sets of primers were designed using PRIMER-blast software.

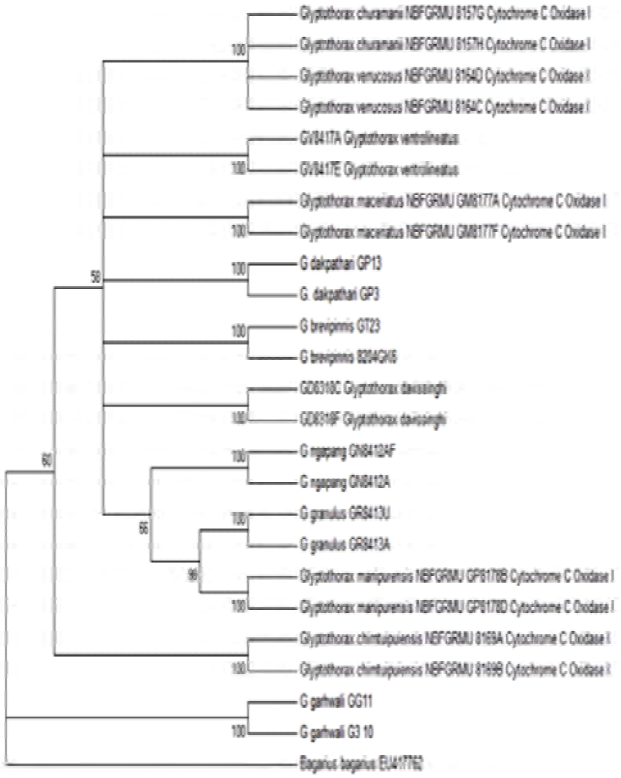


Fig.28. Phylogenetic tree of genus *Glyptothorax* based on COI sequence generated by Maximum Likelihood method

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 - June, 2014

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

Funding Agency: DBT, Govt. of India

North-east India is unique in providing a profusion of habitats, which features diverse biota with a high level of endemism. The rivers of Manipur - an important state of the region on Indo-Burma border, belong to two entirely different drainage systems *i.e.* the Chindwin-Irrawaddy and Barak-Brahmaputra. Fish sampling was done in Lokchao River near Lokchao village in Chandel District and in Tupul and Ijai rivers near Tupul in Tamenglong District of Manipur (Fig.29).

Fig.29. Fish sampling in Manipur

Cytogenetic studies

Live specimens collected from the rivers were transported to the laboratory, identified using morpho-meristic keys and were subjected to chromosomal analysis using kidney and gill cells, following the conventional hypotonic spreading method and stained with 6% Giemsa. The slides were made permanent to observe the metaphase spreads and the karyotypic analysis were performed following the rules of Levan *et al.* (1964).

For molecular cytogenetic studies, 5S (~200 bp size) and 18S (~1850 bp size) rDNA fragments were amplified and further purified with gel extraction kit. Purified rDNA amplicons were labelled with flourescien-12-dUTP and rhodamine-5-dUTP using nick translation protocol (Fig.30). Pellets of labelled 5S and18S rDNAs were dissolved in ultrapure water and checked on agarose gel with DNA ladder and the smear intensity of the probe ranged from 50- to 500-bp with higher intensity at 150- to 250-bp were considered good for Fluorescent *In Situ* Hybridization (FISH) work. The probes were stored at -20°C and used in FISH. Karyotype and other cytogenetic investigations were undertaken using giemsa/ silver nitrate/ chromomycin A_3 staining (Figures 31 & 32.). The details are presented in Table 11.

Fig.30. Molecular cytogenetic studies in progress

Table 11. Cytogenetic profiles of fishes collected from North-Eastern part of India

S.N.	Name of the species	Diploid chromosome number (2n)	No. of NORs		FISH	
			$AgNO_3$ stained	CMA_3 stained	18S	5S
1.	*Channa punctatus*	32	01 pair	02	01	01
2.	*Clarias magur*	50	01 pair	01 pair	-	01
3.	*Cyprinus carpio*	100	01 pair	01 pair	01	-
4.	*Glosogobius giuris*	46	1 pair	1 pair	01	01
5.	*Heteropneustes fossilis*	56	01 pair	01 pair	-	01
6.	*Neolissochilus stracheyi*	100	-	01	01	-
7.	*Barilius ngawa*	50	-	02	-	01
8.	*Tor tor*	100	02	02	-	-

Fig. 31. Karyotype of *Neolissochilus stracheyi*

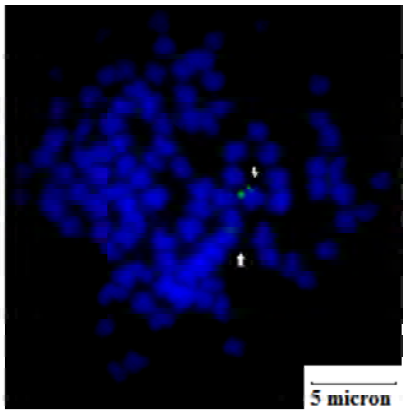


Fig.32. Fluorescein labelled 18S rDNA FISH signals in *Cyprinus carpio*

Project Title: Development of surrogate broodstock for propagation of valuable fish germlines

Project Period: April, 2014 - March, 2017

Project Personnel: S.K. Majhi (PI), Basdeo Kushwaha and S. Raizada

Funding Agency: Institutional

There is particular interest in developing surrogate broodstock due to the growing concern with dwindling fisheries stocks and loss of species/genetic biodiversity due to over exploitation and environmental degradation. In view of this, the work was initiated aiming at developing simplified method to create surrogate broodstock using intra-gonadal germ cell transplantation technique.

Common carp, *Cyprinus carpio* (var. Koi) and Gold fish, *Carassius auratus* intended to be used as recipient and donor, respectively, were procured from various sources and acclimatized in the wet laboratory conditions for three weeks prior to the experiment. For depletion of endogenous germ cells in common carp, four combinations of treatments involving Busulfan (20 and 40 mg/kg body weight) and temperature (25 and 30°C) were selected and named as B20T25, B20T30, B40T25, B40T30. A fifth group receiving only the vehicle (DMSO) at 25°C served as a control and was named as B0T25. The recipient common carp were intraperitoneally injected with four dosage of Busulfan at two week interval and constantly reared at 25°C and 30°C for 8 weeks. Samples (n=10) were drawn at two week interval from each treatment randomly and weight of fish and gonads were recorded. A small portion of collected gonad was preserved in RNA later solution (1ml RNA later solution/100 mg tissue) at –80 °C for vasa gene expression study and remaining gonad tissues were preserved in Bouin's fixative for histological analysis.

The histology of gonad tissues revealed that the treatment involving constant rearing of fishes at 30°C

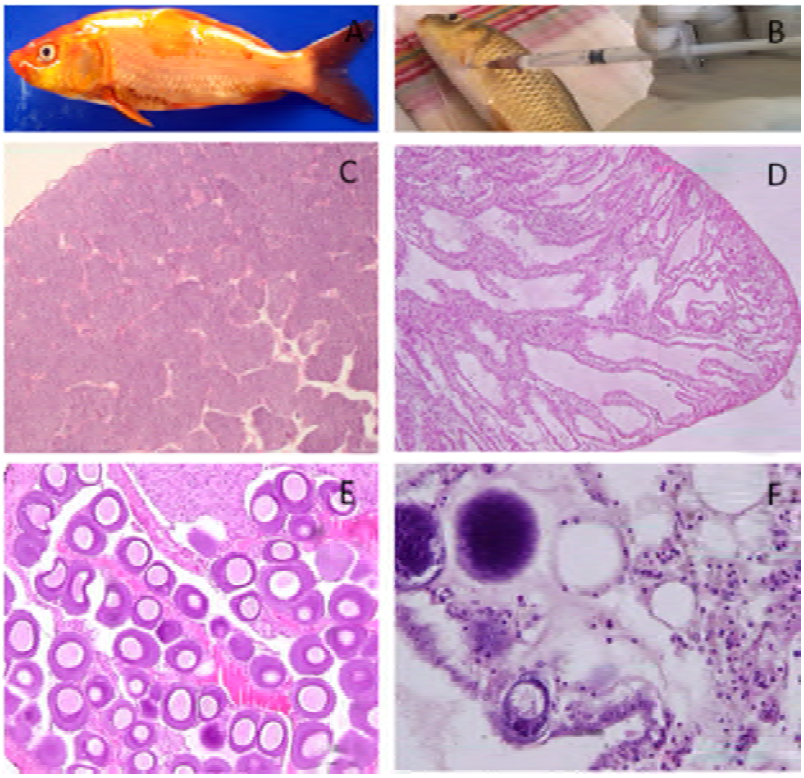


Fig.33. A) Common carp used as recipient. B) Intraperitoneal injection of Busulfan. C) Testis from a male at 0 weeks showing accumulation of spermatozoa in the efferent ducts. D) Testis from a B40T30 male at 8 weeks showing virtual lack of spermatogonia. E) Ovary of a control female at 0 week showing oocytes at various stages of development. F) Ovary from a B40T30 female at 8 weeks showing virtual lack of oogonia

and injection of Busulfan (40 mg/kg body weight; B40T30) at two week interval (total four dosage), for a total period of 8 weeks, resulted into significant loss of germ cells (Fig.33). The gonado somatic index (GSI) of samples from all groups, including the controls, decreased steadily in all the experiments between 0 and 8 weeks. However, the decreases were much more prominent in B40T30 group. The microscopic examination of the gonadal sections revealed that the gonads had severe germinal degeneration at the end of 8 weeks in B40T30 group oppose to active gametogenesis in the control animals, a fact that was strongly supported by the level of vasa transcript in B40T30 (0.02±0.01), which was 25-fold lower than control (B0T25; 0.5±0.07). Thus, the quantitative analysis of vasa expression indicated significant reduction between the treatments ($P<0.05$). The lowest level of vasa transcript was recorded in groups treated with 40 mg Busulfan/kg BW, which was 25-30 fold lower than control males and females. Columns with different letters vary significantly (Tukey's multiple comparison test, $P<0.05$) (Fig.34).

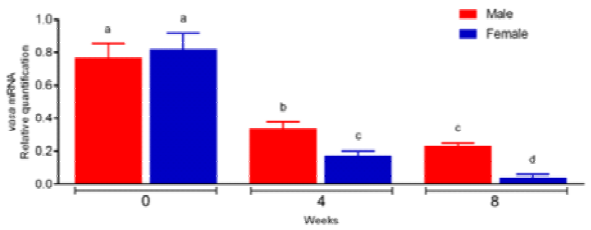


Fig.34. Quantitative analysis of vasa expression indicating significant reduction between the treatments (P<0.05)

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

Project Period: November, 2010 – November, 2014

Project Personnel: M. Goswami (PI) and N.S. Nagpure

Funding Agency: DBT, Govt. of India

The National Repository of Fish Cell line (NRFC) has been established at ICAR-NBFGR, Lucknow for the collection, deposition and distribution of cell lines to the researchers across the country. Four new fish cell lines were added to the NRFC during the year under report (Table 12) raising the total number of cell lines maintained at repository to 50. Out of these 17 cell lines are developed by ICAR-NBFGR while rest have been submitted by eminent scientists/researchers working on fish cell lines in the country. Several cell lines were distributed to the researchers working at reputed organisations in the country. An overseas demand of the fish cell lines was also received from Wageningen University, Netherlands and communication has been made to National Biodiversity Authority for seeking approval. The cell lines have been cryopreserved in liquid nitrogen and are revived routinely to check viability. The details of the available fish cell lines and forms for deposition, supply and material transfer agreement are available on NRFC website at *http://www.nbfgr.res.in.*

Table 12. List of new fish cell lines developed at ICAR-NBFRG and added to the NRFC

S. No.	Name of the Cell line	Accession No.	Source
1	WAM	NRFC047	Muscle tissue of *Wallago attu*
2	WAG	NRFC048	Gill tissue of *Wallago attu*
3	CPG	NRFC049	Gill tissue of *Channa puntatus*
4	DRM	NRFC0450	Muscle tissue of *Danio rerio*

Project Title: Development of an *in vitro* toxicity assessment system for aquatic pollutants

Project Period: April, 2014 - June, 2017

Project Personnel: Mukunda Goswami (PI), N.S. Nagpure, Sullip K. Majhi and Akhilesh K. Mishra

Fish cell line has been gaining importance in aquatic toxicology as an *in vitro* tool. Therefore, a new work to develop *in vitro* toxicity assay system in *Labeo rohita* using *L. rohita* gill (LRG) cell line was initiated. As gill of fish is the exquisite organ used for aquatic toxicity assessment, gill cell line (Accession no. NRFC023) developed from gill was used for the study. Four aquatic pollutants, Chlorpyrifos and Cypermethrin (synthetic pyrethroid) and Mercury and Chromium (heavy metals) were selected for the study. LRG cell line has been regularly sub-cultured and maintained for the study. The 96 hours LC_{50} values of Chloropyrifos, Cypermethrin and Mercury in *L. rohita* fingerlings for Chlorpyrifos, Cypermethrin and Mercury were estimated as 511, 1.73, 473 µg/l, respectively. *In vitro* toxicity of Chlorpyrifos was assessed based on MTT [3-(4,5-Dimethylthiazole-2-yl)-2,5-Diphenyltetrazolium Bromide] and Neutral Red (NR) assays. IC_{50} value of Chlorpyrifos was estimated as 1.66±0.04 mg/l based on MTT assay and 3.760 ±0.85 mg/l based on NR assay.

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

Project Period: September, 2011 – December, 2014

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

Funding Agency: DBT, Govt. of India

The freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794), is an important silurid fish species of high commercial importance and are widely distributed in the plains and sub-mountain regions and found in streams, rivers, canals, beels, jheels, reservoirs and tanks. In the present study, explorations were carried out covering different rivers basins across the states, *viz*. North East India, Andhra Pradesh, Jharkhand, Karnataka, Tamilnadu, Madhya Pradesh, Maharashtra, Odisha, Uttar Pradesh, Punjab, West Bengal, etc., for collection of samples of *O. bimaculatus*. Comparative analysis of reproductive parameters, concentrations of ovarian protein were assessed in *O. bimaculatus* along with the determination of genetic structure and phylogenetic relationships among the different populations.

Pattern of reproductive biology

Based on the results obtained from pooled analysis of 29 male populations, 8 populations attained L_{50} (length at which 50% of the individual reached to reproductive size) with mean TL ranged from 184 to 210 mm,17 population showed L_{50} with mean TL ranging 213-250 mm and 4 populations attained L_{50} at the range of 250-265 mm. In male, out of 29 populations, 2 populations showed L_{50} below 200 mm, 18 population attained L_{50} with TL ranged from 200-250 mm, 4 populations attained L_{50} with mean TL range from 245 to 253 mm,1 population attained L_{50} at 256 mm, whereas 3 populations attained L_{50} at 263-273 mm TL. Overall, the results indicated considerable variation in length at first maturity of both female and male across different rivers. The absolute fecundity and fecundity per 100 g BW showed significant variation ($p<0.05$) in different wild populations. Higher absolute fecundity (20,000-35,000) was recorded in 10 rivers, out of 29 rivers, that ranged from 21,202–35,410. Moderate absolute fecundity (10,000-20,000) was recorded in 14 rivers that ranged from 10,088–18,587. Lower absolute fecundity was recorded from 14 rivers that ranged from 2,427 to 6,041. The fecundity was better correlated with total length, followed by body weight and gonad weight.

Genetic characterization

Documentation of genetic differentiation among various populations of species can provide useful information that may play key roles in conservation of species and may have implications in breeding and in management plans. Genetic markers have extensively been developed and used to assess the levels of variation of different populations to compare and to determine the relationships with other populations. Therefore, the genetic structure and phylogenetic relationships of 24 populations of *Ompok bimaculatus* collected from Indian waters was examined using mitochondrial cytochrome b (cyt b) gene.

Cyt b gene was sequenced in150 individuals (GenBank acc. Nos. KJ646706-KJ646756, KJ646763-KJ646786, KJ646789-KJ646795, KJ646801- KJ646842, KJ646849-KJ646868 and KJ646876- KJ646881) from 24 rivers belonging to Ganges, Brahmaputra, Barak, Krishna and Narmada basins to assess their population structure and systematics. The pooled analyses of the phylogenetic information and the population structure suggested that the Indian *O. bimaculatus* consist of two distinct mtDNA lineages which exhibit high genetic variation and haplotypic diversity. We identified 32 haplotypes representing 24 populations (Fig. 35). Three distinct stars like clades were observed with cyt b mtDNA marker.

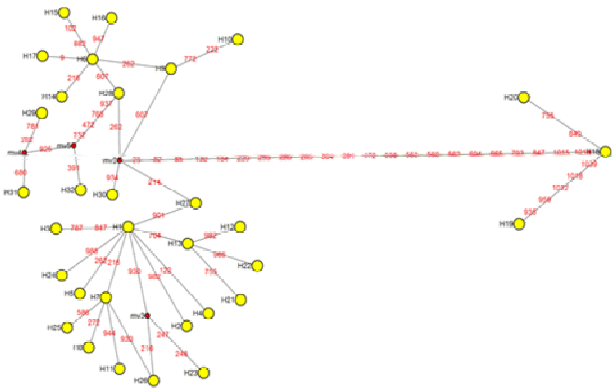


Fig. 35. Haplotype network of 32 haplotype representing 24 populations of *O. bimaculatus*

The high molecular divergence observed in *O. bimaculatus* mtDNA lineages and their phylogeographic structure indicated that these lineages have evolved independently from common ancestor. Analysis of molecular variance indicated that most of the genetic variation observed is found among the populations, suggesting restricted gene flow among the populations. Long-term interruption of gene flow was also evidenced by high overall F_{ST} values (0.82367) that could be favored

by the discontinuous distributions of the lineages. The significant correlation between the geographic and genetic distances provides support for importance of geographic discontinuity in shaping the genetic structure of *O. bimaculatus*.

Microsatellite loci were amplified and screened in individuals of *O. bimaculatus*. Out of the 42 loci tested, 10 loci amplified successfully. Among them, 9 loci showed polymorphisms, whereas one locus was monomorphic. The size range of the loci in the *O. bimaculatus* ranged from 100–300 bp. These loci may be useful in determining the genetic diversity in *O. bimaculatus* stocks.

Estimation of gonadal protein

During the different stages of reproduction, the protein concentration varies according to its aquatic environment conditions. In ovary, the protein concentration registered stage-wise increase reaching maximum in spawning phase which can be attributed to low metabolic activity and its correlation with fecundity. In spawning phase, due to maturity, the ovarian protein might be high as compared to pre-spawning season. During the study, it was observed that the protein level of fish ovary started increasing with the maturity of gonads and the protein concentration varied according to its aquatic environmental conditions. The ovarian protein ranged from 1.55±0.01 to 2.85±0.003 in the preparatory phase, 2.79±0.04 to 3.98±0.005 in pre-spawning phase and 3.58±0.01 to 7.98±0.0003 in spawning phase in major Indian rivers. The ovarian protein concentration showed a significant difference between different phases and sampling areas (preparatory phase: $F= 3820.68$, $p<0.001$; pre-spawning phase: $F= 822.95$, $p< 0.001$ and spawning phase: $F= 45.71$, $p < 0.001$). The samples collected from tributaries of major Indian rivers have ovarian protein concentrations ranged from 1.05±0.0067 to 3.03±0.003 in the preparatory phase, 2.02±0.003 to 4.62±0.001 in pre-spawning phase and 3.39±0.005 to 6.16±0.0007 in spawning phase. The ovarian protein concentration showed a significant difference among the different phases and tributaries (preparatory phase: $F= 472$, $p< 0.001$; pre-spawning phase: $F= 17.24$, $p< 0.001$ and spawning phase $F= 72.37$, $p< 0.001$). It was found that the samples collected from River Ganga (West Bengal) in preparatory season and sample collected from River Narmada in pre-spawning and spawning phase displayed a higher variation in ovarian protein concentration. Among the tributaries, samples from River Ghaghara had more protein concentration in preparatory phase and River Hooghly in pre-spawning and in spawning phase, as compared to others rivers. The protein concentration among the major Indian rivers showed that all major rivers are significantly different.

The present examination of fish ovary indicated that local aquatic habitat play important role in affecting the reproductive performance of the fish. This study provides a comprehensive quantitative account of the comparative reproductive potential, inter-population reproductive strategies and pattern of genetic variation of *O. bimaculatus* of the major rivers of India and their tributaries for the first time which can be useful for developing appropriate strategies for management of natural fish population.

4.3 Exploration of Fish Germplasm Resources

Project Title: Exploration of the Western Ghats Wetlands for indigenous fishes and extent of invasion of exotic fishes

Project Period: April, 2013 – March, 2016

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

Funding Agency: Institutional

Exploration of fish species in Sharavathi River, Karnataka

The Western Ghats along the west coast of Peninsular India are well known for their richness of biodiversity including freshwater species. The area has vast potential for endemic, cultivable and ornamental fish species. Of the 288 species of freshwater fishes in

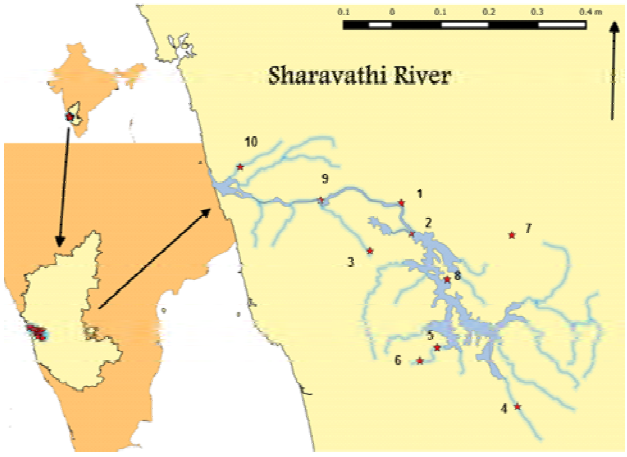


Fig.36. Sampling sites in Sharavati river

the Western Ghats, 68% are endemic to this region, 155 are considered ornamental fishes, of which 117 are endemic to the Western Ghats. As a part of exploration of the Western Ghats for fish species diversity studies, Sharavathi River, which flows through Shimoga and Uttara Kannada districts in Karnataka, was selected (Fig.36)

The headwaters of the river in Shimoga district flow into the Linganamakki reservoir and the outflow from the dam feeds Jog falls. Downstream of the falls, the Gerusoppa dam forms another reservoir, downstream of which the river flows in to the Arabian Sea at Honnavar. As part of our efforts to catalogue aquatic diversity in freshwaters, exploratory surveys were carried out in pre-monsoon and post-monsoon seasons at 10 different locations of the Sharavathi river namely, upstream of Jog falls, downstream of Linganamakki Dam, upstream of Dabbe falls, stream near Nagara, stream near Sampekatte, tributary of Nagodi river, Basava Hole dam, Sigandoor (Hole Bagilu), downstream of Gerusoppa dam and Sharavati river near Honnavar. Total 53 species belonging to 38 genus of 18 families were collected, including two exotic fishes (*Oreochromis niloticus* and *Cyprinus carpio)* collected from river Sharavathi, Karnataka. List of the species collected is given in table 1. Family cyprinidae dominates with 16 species followed by bagridae (3 species) and others (Fig. 37). Earlier studies conducted (ENVIS Technical Report: 52, November 2012; Sreekantha and Ramachandra, (2005) in this river basin recorded 41 and 49 freshwater fish species and in our studies we got 53 species (Figures 38 & 39). Endemic fish species like *Schistura sharavatiensis* and *S. nagodiensis* were recorded from Sharavati River basin along with two possibly new species of the Genus *Schistura*. *Pterocryptis wynaadensis* collected from upstream of Dabbae falls was the first record of the species from this region. Large numbers of pipe fish belonging to the Genus *Ichthyocampus* were observed in the middle and lower reaches of the Sharavathi River basin. Species specific molecular signatures were prepared using mitochondrial COI gene for 15 fish species belonging to the genus *Mystus, Schistura* and *Puntius*.

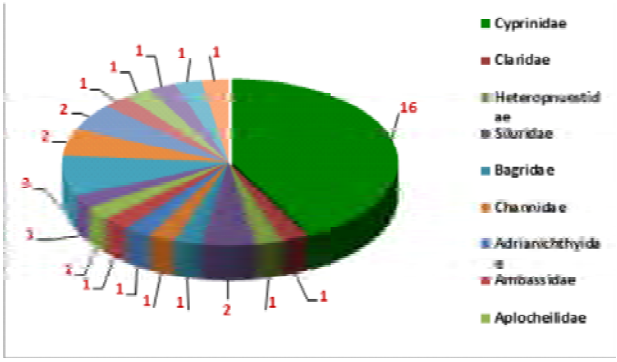


Fig. 37. Distribution of species under different families observed in Sharavathi river

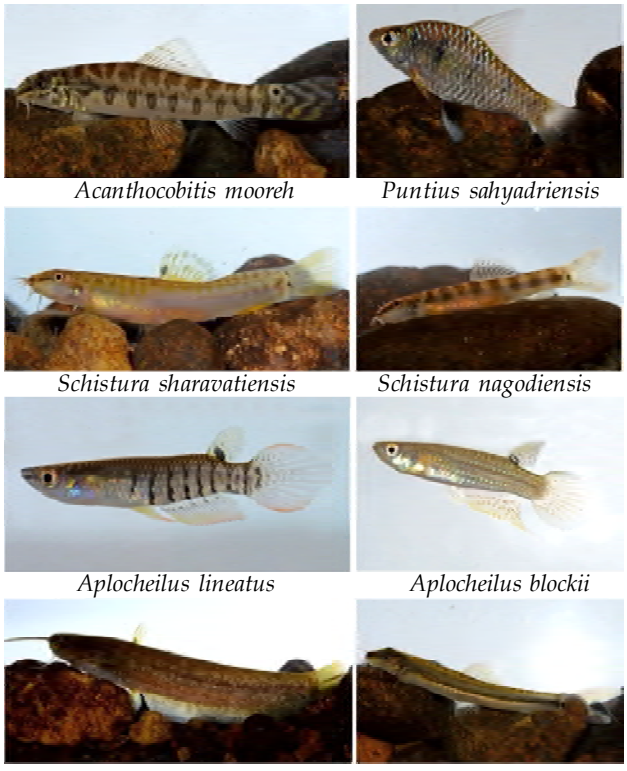
Evaluation of exotic fishes

Only two species of exotic fishes, *Oreochromis mossambicus* and *Cyprinus carpio* were recorded from the Shravathi river basin, in sampling station 5 (Basavahole) and 8 (Sigandoor). In other stations no exotic fish was recorded. Thus, from samplings of 10 stations, it is presumed that there is not much of invasion of exotic fishes in this river.

Fig.38. Sampling with (A) a dip net and (B) drag net

Exploration of fish species in Zuari, Mandovi and Chapora rivers, Goa

Sampling was conducted in lower reaches of the Zuari and Mandovi basins up to the estuarine zone at 5 locations namely, Mandovi at Ela, Mandovi at Ribander, Zuari estuary Carambolim lake and Chapora river. The sampling yielded 13 species of fish (*Puntius vittatus, Glossogobius giuris, Ophiocara porocephala, Yongeichthys nebulosus, Redigobius bikolanus, Mugilogobius tigrinus, Mugilogobius sp., Eleotris fusca,*



Acanthocobitis mooreh *Puntius sahyadriensis*

Schistura sharavatiensis *Schistura nagodiensis*

Aplocheilus lineatus *Aplocheilus blockii*

Pterocryptis wynaadensis *Nemacheilus anguilla*

Fig.39. Some of the fish species recorded during exploration

*Oryzias (Horaichthys) setnai, Aplocheilus kirchmayeri, Ichthyocampus carce, Microphis sp.*and *Pseuosphromenus cupanus*) (Fig.40), along with 6 species of crustaceans (*Macrobrachium canarae, Macrobrachium cf. gracilirostre, Macrobrachium sp., Caridina cf. gracilirostris, Caridina cf. babaulti* and *Caridina cf. hodgarti*), bringing the total number of fish species to 55 and crustacean to 13 from the Goa region of the Western Ghats.

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

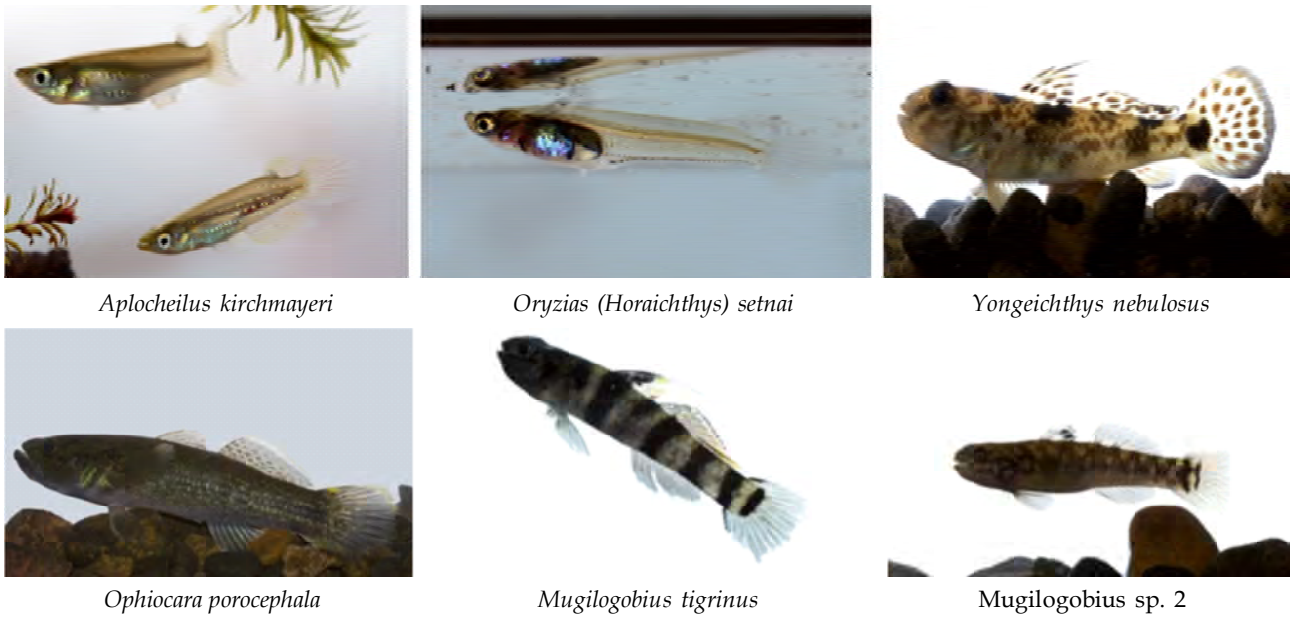
Project Period: April, 2012 – March, 2015

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

Funding Agency: Institutional

Explorations were continued in the upper basin of Mahanadi River starting from its origin and covering its entire length (approx. 450 km.) in the Chhattisgarh state, including its tributaries joining in this stretch. Two seasonal explorations were undertaken during pre-monsoon and post-monsoon seasons, for exploration and documentation of fish diversity (Fig.41). A total of 11 sites of River Mahanadi and 12 sites of its six tributaries and sub-tributaries namely, Sheonath, Jonk, Hasdeo, Maniyari, Arpa and Lilagar, were covered in these exploration studies.

A total of 74 fish species belonging to 22 families of 8 orders were recorded from Mahanadi River and its tributaries during explorations during the period under report. So far, a total of 82 fish species have been recorded from upper basin of Mahanadi River. Maximum species was richness recorded in the lower stretch of Mahanadi at Chandrapur ghat and Balpur in Janjgir-Chanpa district, and at Paiser ghat and Karamsend in River Sheonath. Data on total of 1761 individual fish samples were collected and 390 tissue samples (blood, muscle) were collected for further analysis from above explorations during the year under



Aplocheilus kirchmayeri *Oryzias (Horaichthys) setnai* *Yongeichthys nebulosus*

Ophiocara porocephala *Mugilogobius tigrinus* Mugilogobius sp. 2

Fig.40. Some of the fish species recorded from rivers of Goa

Fig. 41. Exploration of fish germplasm in Mahanadi river basin

Fig.42. Collection of tissue samples and biological parameters from River Mahanadi

report (Fig. 42). Analysis of seasonal abundance of fish diversity and biological parameters is in progress.

Exploratory survey of Dah Tal, Ballia, UP was conducted. Fish catch dominated by: *Catla catla, Labeo rohita, L. calbasu, Cirrhinus mrigala, Sperata seenghala, Mystus vittatus, Channa punctatus, C. marulius, C. striatus, C. gachua,, Puntius ticto, P. sarana, Oxygaster bacaila, Chanda ranga, Macroganthus puncalus, Colisa faciatus, Xinantodon cancila, Heteropneustes fossilis, Clarias magur, Pseudotropius atherinoides, Amphipnous cuchia, Wallago attu* and *Bagarius bagarius*.

A conceptual framework and specific proforma was developed for documenting the traditional ecological knowledge of fisherfolks about freshwater fish germplasm resources and their habitats, and integrating it with the fish germplasm exploration and resource management. Using the format, socio-economic survey of fishermen, interaction with fishermen/office-bearers of fishing cooperative societies and collection of information on management of fisheries resources in Mahanadi River and its tributaries, and traditional ecological knowledge was conducted in 46 villages along the selected sites on the bank of Mahanadi and its tributaries and a total of 375 people from fishing communities were interviewed on various socio-economic aspects and their perception on status of fish diversity and its conservation in the upper Mahanadi river basin (Fig. 43).

Fig.43. Interaction and group discussion with fishermen/ office-bearers of fishing cooperative societies in Mahanadi river basin

Special focus group discussions with the office-bearers and members of fishing cooperatives of tribal and fishing community people, including women fisherfolks in selected villages of Dhamtari, Balod and Kanker districts of Chhattisgarh were organised on functioning of fishing cooperative societies and various aspects of management of fisheries resources in Gangrel reservoir on Mahanadi by these societies. Nine traditional fishing gears & accessories used by the fishermen in the Mahanadi River and its tributaries were recorded along with information on their uses. These are: Pelna, Penna, Thenthri, Dholna, Bow and arrow, Dheer, Kathi jaal, Khalehi and Foi - Soal.

Project Title: Participatory programme on exploration and characterization of fish germplasm resources and indigenous knowledge in North-eastern region of India

Project Period: October, 2012 –March, 2017

Project Personnel: K.K. Lal (Coordinator till 18th August, 2014), L.K. Tyagi (Coordinator w.e.f. 19th August, 2014), Vindhya Mohindra and Rajeev Kr. Singh (Co-coordinators)

Funding Agency: ICAR-NE Component

NBFGR under ICAR-North East (NE) component, is implementing a participatory programme on 'Exploration and characterization of fish germplasm resources and indigenous knowledge in North Eastern Region of India' involving collaborators from various institutions of the NE region. The priority component of the programme is exploration, which consists of exploration for species richness, distribution, habitat and also the traditional ecological knowledge of the fisherfolks dependent upon such resources. Seven sub-projects were provided technical and financial support by the Institute and carried out by the collaborating partners under the programme (Table 13).

During the year under report, exploratory surveys were conducted by the project partners in identified areas and rivers in seven states of the NE region. In Maghalaya, two rivers Jinari and Dudhnoi (5 sites each) of Garrohills were explored and a total of 46 and 66 fish species were recorded from the two rivers, respectively. In Arunachal Pradesh, River Nargumri (5 sites) and its connected rivers namely, Dhansiri (3 sites) and Panchnoi (4 sites) in part of Assam, were explored and 18 fish species were recorded from each of the three rivers. Further in Assam surveys in three small but unexplored rivers namely, Diyung, Jatinga

Table 13. List of sub-projects supported under the NE component

S.N.	State	Principal Investigator	Title of Sub-Project
1.	Assam	Mr. Sarbojit Thaosen Department of Zoology Haflong Govt. College, (Affiliated to Assam University, Silchar), Haflong, Assam	Exploration and evaluation of fish faunal diversity and habitat ecology of the Diyung, Jatinga and Mahur rivers, Dima Hasao district, Assam.
2.	Sikkim	Dr. K.V. Radhakrishnan Assistant Professor College of Fisheries, CAU Agartala, Tripura	Inventorisation, characterization and documentation of local knowledge on fishes in the two Himalayan rivers: Teesta and Rangti in Sikkim state.
3.	Manipur	Mr. Y. Bedajit Singh Subject Matter Specialist (Fy.), KVK, Thoubal, Manipur	Exploration and characterization of fish germplasm resources and indigenous knowledge of the Chindwin drainage in Manipur
4.	Meghalaya	Dr. D. Sarma Associate Professor Department of Zoology, Gauhati University, Guwahati, Assam	Exploration and evaluation of fish faunal diversity of Dudhnoi and Jinari rivers of Garohills, Meghalaya.
5.	Arunachal Pradesh	Mr. Ratul Chandra Bharali Assistant Professor Dept. of Zoology Udalguri College, Udalguri, Assam	Exploration and evaluation of fish faunal diversity and their distributional pattern of River Nargumri West Kameng district of Arunachal Pradesh and its connected rivers Dhansiri and Panchnoi inUdalguri, Assam.
6.	Tripura	Dr. Pampa Bhattacharjee Assistant Professor College of Fisheries, CAU, Agartala, Tripura	Exploration of indigenous fisheries Knowledge and characterization of fish germplasm of major rivers of Tripura
7.	Mizoram	Dr. A.S. Barman Assistant Professor College of Fisheries, CAU, Agartala, Tripura	Exploration and inventorization of the fish germplasm from the rivers of Mizoram and its associated indigenous knowledge.

and Mahur (2 sites each) 41, 25 and 32 fish species, respectively, were recorded. In Sikkim, explorations in two rivers namely Teesta (26 sites, 14 fish species) and Rangit (15 sites, 14 fish species) revealed a total of 21 fish species. In Manipur, collection and characterization of fish genetic resources and indigenous knowledge was carried out for five rivers of Chindwin basin: Imphal (4 sites), Iril (4 sites), Thoubal (5 sites), Maku (4 sites) and Lokchao (4 sites) rivers. A Total of 33 fish species were recorded from these rivers during explorations. (Fig. 44). In Mizoram, 7 sites of three river systems namely, Koladyne, Tuichang and Mat were explored and a total of 41 fish species could be recorded. In Tripura six rivers: Gomati (8 sites), Manu (3 sites), Deo (3 sites), Dhalai (4 sites), Mahuri (7 sites) and Feni (9 sites) were explored and a total of 75 fish species were recorded. Besides, habitat parameters were recorded for each site explored and indigenous knowledge of the local fishing communities was documented. Threats to aquatic biodiversity were documented in the studied rivers and conservation significant areas were identified.

Fig.44. Exploration of fish germplasm in Mizoram

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

Ramgarh lake

Ramgarh Lake was surveyed extensively. Physico-chemical parameters of the water samples collected from the four sites of Ramgarh Lake were recorded during the period. The catch of the fishes from the lake during May - July 2014 was dominated (65-70%) by catfishes and contribution of the carps was minimum. After leasing out in May, 2014, the lake has been heavily stocked with *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala*, *Aristichthys nobilis*, *Hypophthalmichthys molitrix* and *Cyprinus carpio*. Since this lake is rich in nutrients, the advanced fingerlings of the bighead (*Aristichthys nobilis*) attained 1.25-1.75 kg weight in 5-6 months while the Indian major carp (*Catla catla*) was seen to grow only up to 650-750 g in the corresponding period. Hence, the catch from Ramgarh Lake at present is dominated (40-45%) by the bighead. The Matsyajivi Sahkari Samiti Limited, Parsahia Tola (Champa Park), Gorakhpur has purchased two Fish-Carrying Vans under Rashtriya Krishi Vikas Yojana (RKVY) with 50% subsidy which are being used to carry fish to the remote fish markets of Gorakhpur, Maharajganj, Kushinagar and Deoria districts. Information regarding species-wise catch composition of the fishes on yearly basis was collected. It is important to note that Ramgarh Lake is being developed under the National Lake Conservation Plan (NLCP) of the Ministry of Environment and Forests, Government of India, New Delhi. The details of the socio-economic status of 148 fishermen (28-68 age-group) involved in fishery-related activities of Maherva-ki-Bari (Ramgarh Lake), Saraiya Tola (near Tourist Bunglow), Budha Vihar and Parsahiya, Gorakhpur were recorded and analysed.

Bakhira Lake

Physico-chemical parameters of the waters collected from four sampling stations of Bakhira Lake were recorded and analysed. important fish species

recorded in Bakhira lake are: *Catla catla, Labeo rohita, L. dero, L. gonius, Cirrhinus mrigala, Aristichthys nobilis, Hypophthalmichthys molitrix, Ctenopharyngodon idella, Cyprinus carpio* var. *communis, Anabas testudineus, Channa punctatus, Colisa fasciata, Heteropneustes fossilis, Notopterus notopterus, Systomus sarana, Mystus vittatus, Macroganthus pancalus, Mastacembalus armatus, Nandus nandus, Osteobrama cotio, Oxygaster baicala, Glossogibius giuris, Xenantodon cancila* and *Sperata aor*.

Two Fisheries Co-operative Societies have been registered with the Uttar Pradesh Fisheries Co-operative Federation, Lucknow. These are - Matsya Sahkari Srmjivi Samiti, Mehdawal and Matsya Sahkari Srmjivi Samiti, Bakhira. Since Bakhira lake has been declared Bakhira Bird Sanctuary in 1990 under Sohagi Berava Wildlife Sanctuary, Maharajganj, members of the fisheries co-operative societies are encouraged/ given preference in leasing out the ponds above 2.0 ha area for fisheries-related activities. Socio-economic status of 88 fishermen belonging to Mehdawal and Bakhira Fisheries Co-operative societies was collected and analysed.

Histo-pathological studies of the target organs of fishes

Gill, kidney, intestine, gonad, brain and pituitary gland of the commercially important fishes were collected from Ramgarh and Bakhira lakes and processed for histo-pathological changes occurring in these vital organs following standard methodologies. Ovary of the commercially important fishes were in mature stage with oocytes in stage 5 and 6 of maturity during August-September depicting breeding period of the fishes. However, the ovarian activity is declined in the Indian major carps during last month of September 2014. Brain of *Colisa fasicata* and *H. fossilis* were processed, stained in specific stains and the two important endocrine centres like hypothalamus and pituitary gland were localized to correlate the season changes occurring in both the glands with gonadal maturation. In hypothalamus, nucleus preopticus (NPO) and nucleus lateralis tuiberis (NLT) were localized using aldehyde fuchsin (AF) and Mallory's triple stains.

In pituitary gland of both the species, gonadotrophs (GTH cells) were localized in proximal pars distalis (PPD) using alcian blue stain. Further, gill of *Labeo rohita* collected from both the lakes comprised primary and secondary gill lamellae, pilaster or pillar cells, chloride cells and epithelial lining cells. Blood sinusoids were also seen at the bases of gill lamellae. The gill of the carp appeared normal with wide interlamellar spaces facilitating respiration. However, secondary gill lamellae of the catfishes collected from sewage discharge points of Ramgarh lake depicted hyperplasia (increase in number of cells) and telengiectasis (aneurysms) in gill lamellae at few places. Liver, kidney and intestine of the selected fishes collected from sewage discharged sites exhibited varying degrees of cytoplasmic degeneration (vacuolization, pyknosis and karyolysis) while histological picture of these target organs of the fishes of collected from Bakhira lake did not exhibit much of deviation from the normal structure.

Project Title: Neuroendocrine regulation on ovarian maturation in the giant freshwater prawn, *Macrobrachium rosenbergii*

Project Period: August, 2013 - July, 2016

Project Personnel : A.K. Pandey (PI)

Funding Agency: UPCST, Lucknow

Quantification of the secretary activity of neurosecretory cells

On the basis of histological as well as histochemical characteristics, secretary activity of the neurosecretory cells (NSCs) in the female *Macrobrachium rosenbergii* was assigned quiescent or resting (Q), secretary (S) and vaccuolar (V) phases. When the NSCs in both brain and thoracic ganglia had comparatively less cell diameter, granulation and staining intensity, they were grouped under quiescent (Q) phase. When the cells were full of secretary granules, large number of nucleolus, migration of secretary granules towards periphery, high staining intensity and hypertrophy, they were grouped as active or secretary (S) phase. In vacuolar (V) phase, vacuoles were seen towards periphery due to release of the secretary materials. Interestingly, number of nucleolus in the NSCs were less during quiescent phase in brain and thoracic ganglia as compared to secretary as well as vacuolar phases.

Changes in secretary activity NSCs in relation to ovarian maturation

Changes in activity of different types of NSCs in brain and thoracic ganglia of the female *M. rosenbergii*

Table 14. Percentage of neurosecretory cells during different maturity stages of female *M. rosenbergii*

Maturity stage	Brain			Thoracic ganglia		
	Quiescent phase	Vacuolar phase	Secretory phase	Quiescent phase	Vacuolar phase	Secretory phase
Immature	62.1±0.40	30.1±0.44	7.6±0.45	77.8±0.29	12.7±0.15	9.6±0.26
Previtellogenic	60.2±0.35	23.5±0.34	16.4±0.33	42.3±0.39	24.5±0.52	33±0.61
Primary vitellogenic	50.1±0.45	25±0.42	24.9±0.45	39.9±0.27	28.2±0.72	31.6±0.49
Vitellogenic	29±0.57	34.9±0.50	35.3±0.7	27.5±0.68	34.2±0.46	38.3±0.55
Mature	20.2±0.35	30.4±0.26	49.4±0.52	12.8±0.44	31.6±0.61	55.6±0.68

in relation to ovarian maturation have been summarized in Table 14. As maturity advanced, number of S as well as V cells increased rapidly in brain and thoracic ganglia. In brain of immature female *M. rosenbergii*, 62.1±0.40% cells (GN+A+B) were in Q, 30.1±0.44% in S and 7.6±0.45% in V phase of the secretary cycle while in matured specimens 20.2±0.35% cells were in Q, 30.4±0.26% in S and 49.4±0.52% in V phase. In thoracic ganglia of immature prawns, 77.8±0.29% were in Q, 12.7± 0.15% in S and 9.6±0.26% in V phase as against 12.8±0.44% in Q, 31.6±0.61% in S and 55.6±0.68% in V phase of the matured individuals.

Effects of 5-HT and dopamine on ovarian maturation of *M. rosenbergii*

Recent studies have shown that biogenic amines, neurotransmitters (5-hydroxytryptamine; 5-HT and dopamine) and enkephalins play important role in the synthesis and release of the various neuropeptides from the neurosecretory cells affecting ovarian development and maturation of the crustaceans. In this direction, effects of exogenous 5-hydroxytryptamine (5-HT) and dopamine on administration on ovarian development of *M. rosenbergii* were studied. Intermolt females selected for the experiments were in previtellogenic stage of ovarian maturation. 5-HT administration induced ovarian development as gonadosiomatic index increased from 4.68±0.39 to 5.88±0.23 and ova diameter from 162±3.8 to 218.2±8.20 mm on day 28 of the experiment. Conversely, dopamine administration resulted in negative response as GSI decreased from 4..70±0.26 to 4.02±0.32 and oocyte diameter from 158±2.2 to 140.5±4.8 (mm) on day 28 of the treatment. Histologically, ovary of 5-HT treated prawns exhibited development from primary vitellogenic to vitellogenic stage on day 28 of the treatment, whereas ovaries of the dopamine treated group remained in previtellogenic stage but a few oocytes depicted the signs of degenerative changes too.

Other Research Output at ICAR-NBFGR

Research Group: S. Raizada and Vikas Sahu

Successful natural spawning of striped murrel, *Channa striatus* in indoor conditions

A study was undertaken to spawn *Channa striatus* by induced breeding in indoor conditions in two large and low height FRP tanks. Two sets of brooder (ratio 2 females to 3 males) raised at the NBFGR fish farm were subjected to induced breeding using sGnRH anolgue and dopamine antagonist in the catfish facility of NBFGR during August 2014. Successful natural spawning in one female was observed in each of the tank after 18-20 hrs of giving injection having 90% fertilization. The eggs were scooped out and reared separately in a around plastic tub for hatchery, where hatching occurred within 25±1 hr at 28°C with over 90% hatching rate (Fig.45). The study revealed that induced breeding of *C. striatus* may be undertaken in indoor conditions for its mass-scale seed production.

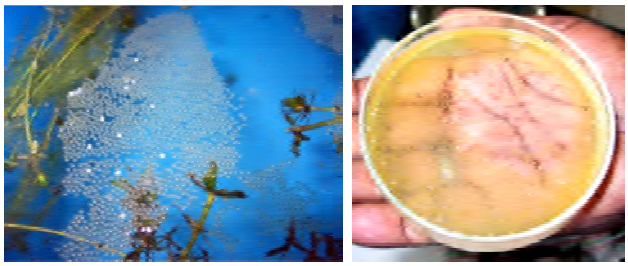


Fig. 45 Egg mass of *Channa striatus* (a) *in situ* and (b) in petri dish

Effect of fresh probiotics as feed supplement on the survival and growth of *Cirrhinus mrigala*

The effect of fish feed supplemented with four probiotics developed from the by-products of mango fruit industry (by ICAR-CISH), was studied on the survival and growth of *Cirrhinus mrigala* in a 60-days

trial. Four types of feeds having approximately CP 35% were developed by mixing commonly available feed ingredients and supplemented with fresh probiotics @ 1% on dry weight basis. The feeds were designated as Mango peel Lactobacillus (MPEL), Mango peel Saccharomyces (MPES), Mango stone wash Lactobacillus (MSWL) and Mango stone wash Saccharomyces (MSWS). The experiment was undertaken in 15 aquaria (size 4.0 x 2.0 x 1.5 feet) with three replicates for each treatment in the indoor conditions along with a control set. Each aquaria was filled with ground water, stocked with 25 test fishes (Mean length 6.36±0.58 cm, weight 2.0±0.57 g) and aerated continuously. The fishes were fed @ 3% body weight once during morning hours and water was partly changed twice a week. The survival and growth was assessed at every 30 days. No mortality was recorded during entire experiment period. MSWL showed highest gain (267.6%) in total biomass in 60 days followed by MSWS (230.9%), MPES (212.9%), Control (217.6%) and MPEL (182.5%). The mean length, mean weight, Ponderal index, length-weight relationship and coefficient of determination values indicated that fish fed with MSWL feed gave best results in terms of growth parameters. It is thus interpreted that Mango stone wash which is disposed off by the mango juice industry as a waste could be efficiently used as a feed supplement in fish diet for higher growth.

Survival and growth of *Cirrhinus mrigala* fed on Indian Gooseberry or Aonla (*Phyllanthus emblica*) pomace supplemented diets

A sixty-day feed trial was conducted to observe the effect of Indian Goosebery or Aonla (*Phyllanthus emblica*) supplementation in the diet of *C. mrigala*. Aonla Pomace generated as by-product in the Aonla juice industry is highly rich in vitamin C, stabilizing tannins and fibre and considered to have high antioxidant properties. Four types of feed comprising of 32% crude protein were prepared from locally available feed ingredients and mixed with 1% (T-1), 2% (T-2) and 3% (T-3) Aonla Pomace Meal (APM) on dry weight basis, whereas no APM was added in the control diet. The experiment was undertaken in triplicate set in 12 FRP tanks of 1125 litre in a CRD model. The tanks were filled with 600 litre water from a bore well and aerated continuously. Each tank was stocked with 50 numbers of *C. mrigala* fingerling (average length 5.0±0.25 cm, weight 1.05±0.06 g) and fed respective diets @ 3% body weight. The survival and growth was recorded at every 30 days. No mortality was recorded in any of the tank till completion of the experiment. The growth parameters indicated negative growth in all test diets (T1, T-2, T-3) with that of control at 30 days, whereas an spurt was observed in growth with all the test diets at 60 days period with that of control. The best performance in terms of growth i.e. FCR, SGR, LWR and R^2 was observed with 1% supplementation of APM.

Successful captive breeding of Asian needlefish, *Xenentodon cancila*

The captive breeding of the Asian needle fish (*Xenentodon cancila*) was successfully attempted at ARTU Unit of the ICAR-NBFGR at Chinhat, Lucknow. The juveniles of *X. cancila* were procured from River Gomti at Lucknow and reared in low height FRP tanks for one year. Five pairs of mature brooders comprising equal number of both sexes were transferred in another tank of similar type with 9" water depth and provided *Hydrilla verticellata* for the attachment of eggs. The female was found to lay eggs one-by-one and in a day around 10-20 eggs were laid by a single female. The spawning was continued by same female for 5-6 days during which more than 100 eggs were laid. The eggs were found hanging and swinging from the twigs of *H. verticellata* by long tendrils originating from the egg's surface with water movement. A fully swollen egg was recorded 3.2-3.5 mm in diameter. The fertilization was found over 90% and eggs hatched out in 5-6 days at 27±1°C. The hatchlings were tall and ranging in length from 10.5 to 11.8 mm in length (Fig.46). The yolk-sac was absorbed in 3 days. The larvae were fed a mixture of brine shrimp nauplii and plankton. High of cannibalism was observed when fry was only 3-4 days old and hence the survival was very poor. The study demonstrated that captive breeding of *X. cancila* could be undertaken in indoor condition without any hormonal application. However, feeding strategy needs to be developed as this fish is not only predatory but also observed great amount of cannibalism.

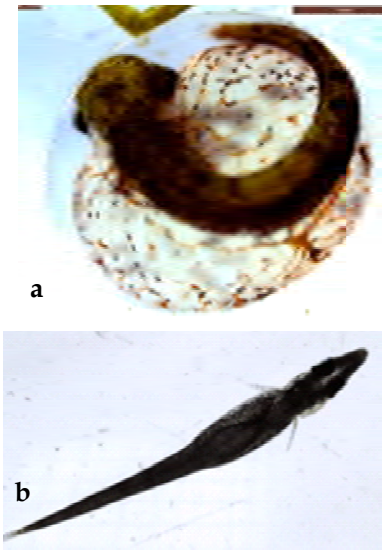


Fig. 46(a) Embryo and (b) hatchling of *X. Cancila*

4.4 Exotics, Quarantine and Fish Health Management

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 (ICAR-NBFGR); Chandan Debnath and Lopamudra Sahoo (ICAR Complex, Tripura)

Funding agency: DBT, Govt. of India

A macrophage cell line (CTM) previously developed from thymus of *Catla catla*, was employed as an *in vitro* model to evaluate the immunomodulatory properties of 12 extracts prepared from six different medicinal plants (*Tinospora cordifolia, Bacopa monniera, Nyctanthes arbor-tristis, Asparagus racemosus, Saponin* and *Quillaja saponaria*). The extracts were prepared in organic solvents. For evaluating the optimal dose of the above, CTM cells were incubated with varying concentrations (0.001, 0.01, 0.1, 1, 10, 100 µg/ml) of these extracts in L-15 medium and the effects on some of the important innate immunity parameters *viz.*, reactive oxygen species (ROS) production, nitric oxide production and lysozyme activity, were studied (Fig.47.). Some of the products (lipopolysaccharide, and â-glucan) having known immunomodulatory properties were used as control. All the plant extracts showed immunostimulatory properties and the maximum increase in ROS, nitric oxide production and lysozyme activity was observed at a concentration of 1 to 10 µg/ml. However, all the plant extracts showed less activity than β-glucan as well as LPS. Currently, using the results of the *in-vitro* studies, further experiment is in progress for evaluating the effect of β-glucan on protective effects against *Aphanomyces*

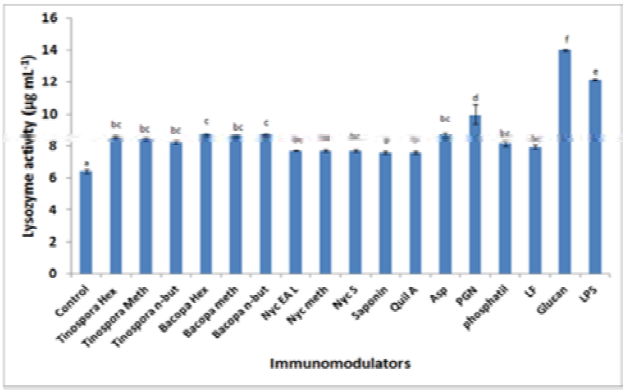


Fig.47. Effect of immunomodulators on lysozyme activity of CTM cells

invadans infection and expression of immune-related genes in *L. rohita*.

Sequential changes in expression analysis of immune genes (IL-1β, IL-10, TNFα, C3, CxCa, Lysozyme C and MHC II) of *L. rohita* following infection with *A. invadans* was studied. Expression analysis on immune genes indicated that most of the studied genes (except MHC II) were significantly up-regulated at advanced stages of infection i.e. 12 and 18 days post infection. On the other hand, in the case of MHC II, there was significant up-regulation of MHC II gene at one day post infection (Fig.48). However, along with progression of infection, the class MHC II gene was down-regulated with respect to 1 day post infection. Down-regulation of MHC molecules might be a mechanism of immune evasion by *A. invadans* because these molecules have a crucial role in the recognition of exogenous antigens, including those of bacteria and fungi. This, coupled with the inability of host macrophages to phagocytose *A. invadans*, might limit the host response, thereby providing increased susceptibility to this pathogen. The results obtained here may strengthen the understanding on molecular pathogenesis of *A. invadans* infection in *L. rohita*.

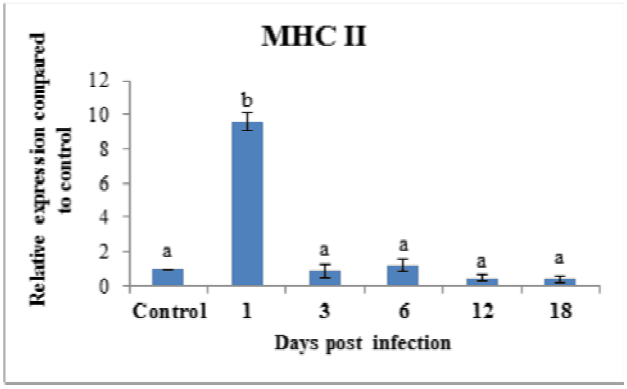


Fig.48. Expression of MHC II gene in muscle tissue of *A. invadans*-infected and uninfected (control) rohu at different days post-infection

Project Title: Characterization of *Aphanomyces invadans* from NE India to develop diagnostic techniques and control measures

Project Period: September, 2013 – September, 2016

Project Personnel: P.K. Pradhan and P. Punia (ICAR-NBFGR); Lopamudra Sahoo and Chandan Debnath (ICAR Complex, Tripura)

Funding Agency: DBT, Govt. of India

For identifying the virulence genes of *Aphanomyces invadans* through transcriptome analysis, *A. invadans*

isolated from one of the recent EUS outbreaks was confirmed following recommended OIE diagnostic procedures. For producing zoospores, agar plugs of actively growing mycelium were placed in a petri dish containing glucose pepone yeast broth and incubated for 4 days at 20 °C. Thereafter, the nutrient agar was washed out by sequential transfer through petri dishes containing autoclaved pond water (APW) and mats were left overnight at 20 °C in APW. After about 12 h, the motile secondary zoospores were collected and kept in - 80°C. After collection of zoospores, RNA was extracted and integrity of RNA samples was determined by measuring the RNA integrity number

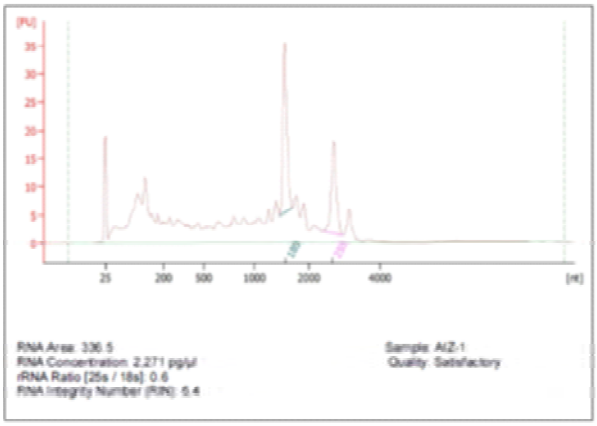


Fig.49. Analysis of RNA quality of the zoospores of *A. invadans*

through bioanalyzer (Fig.49.). The samples with satisfactory RIN value are being used for RNA sequencing.

Project Title: Development of an immune marker and understanding host-*Aphanomyces invadans* interaction using macrophage cell line

Project Period: April, 2014 - March, 2017

Project Personnel: Neeraj Sood (PI), P.K. Pradhan and M. Lini

Funding Agency: Institutional

With an aim to understand the early cellular and molecular mechanisms involved in pathogenesis of *Aphanomyces invadans*, efforts were made to develop a fish cell-line infection assay. A monolayer of catla blood macrophages (CCM cells) was challenged with *A. invadans* zoospores. Time course experiments revealed that the cells did not show chemotaxis, adherence or aggregation around zoospores or germinating hyphae. The oomycete hyphae were found to grow in tissue culture medium without any interaction with CCM cells. The cells remained viable up to 3 days post-challenge and there was no evidence of oomycete toxins. Subsequently, the culture conditions were

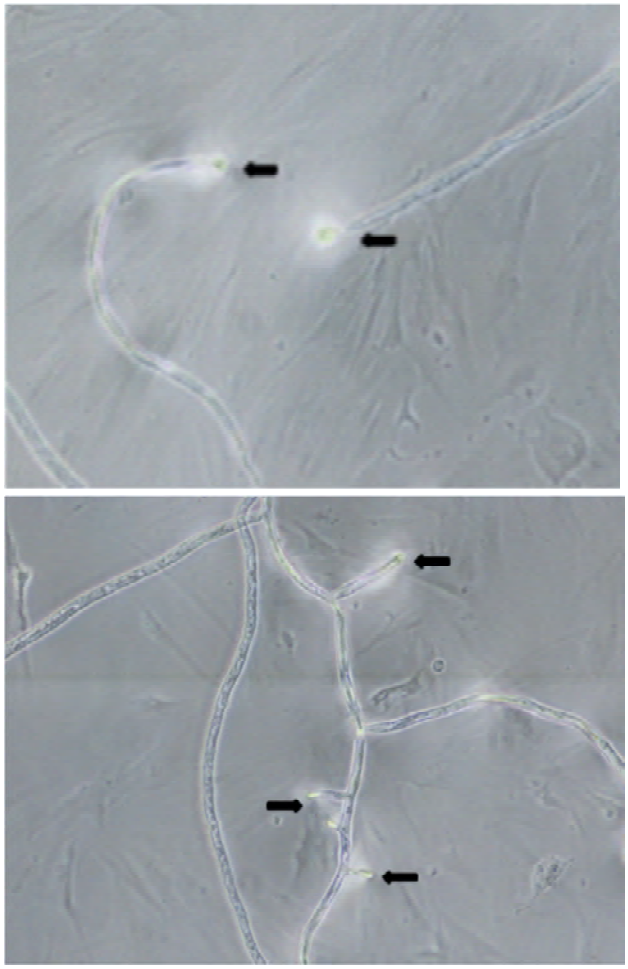


Fig.50. Microphotograph showing *A. invadans* hyphae interacting with the catla cell line (arrows)

optimized and close interaction could be observed between tips of hyphae and cells (Fig. 50). For studying the immune response of catla cells at the transcript level, a large set of primers for qRT-PCR were synthesized and PCR conditions were optimized.

Title of Project: Exploration of finfish parasites of river Gomti particularly protozoans and monogeneans through conventional and molecular techniques

Project Period: April, 2014 - March, 2017

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

Funding Agency: ICAR-NBFGR

River Gomti is a major tributary of the Ganga river system. It originates from a natural lake near Pilibhit town in Uttar Pradesh. The river is about 730 km large and crosses through the central and eastern part of

Uttar Pradesh before finally merging into the Ganga river near Varanasi. Total 56 fish species of 42 genera under 20 families are found in River Gomti. The fisher communities residing on the banks of River Gomti mainly depend on these fish species for their livelihoods. Indiscriminate catch, water pollution, siltation, changing land use pattern, decreased water discharge, fish diseases and exotic species invasion has threatened the fish diversity of Gomti. Recently several cases of fish mass mortality in Gomti have been found. Work was undertaken to understand the prevalence of parasites associated with fishes of Gomti. Based on the primary survey, four sites namely, Sitapur, Lucknow, Haidergarh and Sultanpur were selected for fish sample collection from Gomti River and during the period a total 335 fishes were collected from selected sites (Fig. 51 and table 15).

Fig. 51. Drainage map of river Gomti showing the sampling sites

Screening of fishes and isolation of protozoan and monogenean parasites

Screening of a total 305 fishes was done which included: *Labeo rohita, L. bata, L. calbasu, Cirrhinus mrigala, C. reba, Catla catla, Cypinus carpio, Ctenopharyngodon idella, Hypophthalmichthys molitrix, Carassius auratus, Ompok pabda, O. bimaculatus, Puntius ticto, Clarias magur, C. gariepinus, Heteropneustes fossilis, Rita rita, Channa punctatus, C. marulius, C. striatus, Mystus vittatus, M. aor* and *Wallago attu*. Total percentage prevalence of parasites in these fishes was 6.55%.

Protozoan parasites isolated from fish were characterised and identified. The identified parasites were: *Myxobolus arcticus, M. lucknowii* sp. nov., *M. gomtii* sp. nov., *M. deformis* sp. nov., *Myxidium tictoii* sp. nov., *Thelohanelus toyamai*, *Henneguya ritarei sp.* nov., *Pleistosphora cyprini* sp. nov., *P. rohitii* sp. nov., *Hexamita* sp., *Ichthyophthirius multifiliis*, *Trypanosoma* sp., *Ichthyobodo pyriformis*, *Chilodonella* sp., *Trichodina* sp., *Piscineoodinium* sp. and *Epistylis* sp. etc (Figures 52 & 53).

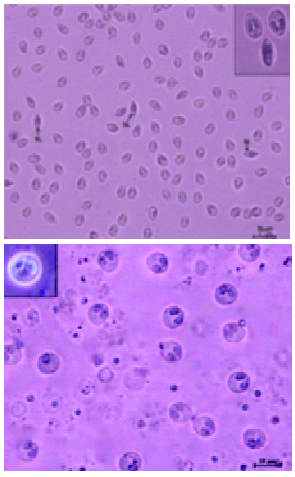


Fig. 52. Some *Myxobolus* species

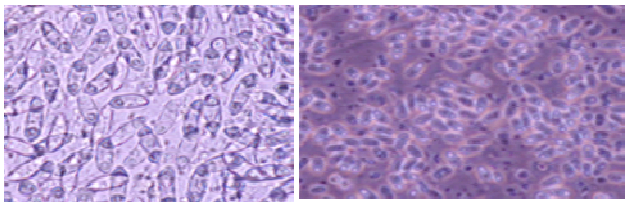


Fig. 53. Some protozoan species

Table 15. Sampling sites and their characteristics

S. N.	Sampling sites	Altitude (m)	Stream Type	GPS Coordinates	Land use patterns/Sources of pollution
1.	Sitapur	129	Up-stream	N27°31.885' E080°19.466'	Semi-urban, agriculture, domestic sewage
2.	Lucknow	94	Mid-stream	N36° 53.221' E080°54.027'	Urban, barrage, domestic sewage, beverage, distilleries, other industries
3.	Haidergarh	84	Mid-stream	N26°38.588' E081°31.768'	Rural, Agriculture
4.	Sultanpur	62	Down-stream	N26°17.454' E082°05.434'	Semi-urban area, domestic sewage, sugar mills, small scale industries, agriculture

Monogenean parasites observed in above fishes are: *Mizellius siamensis, Neodactylogyrus indicus, Ancyrocephalus* sp., two species of *Gyrodactylus*, three species of *Dactylogyrus*, two species of *Paradactylogyrus, Thaparocliedus* sp., *Bifurcohaptor* sp. etc. from screened fishes (Fig. 54). Besides, some Digenean larvae, Nematodes and Copepods were also observed (Fig. 55).

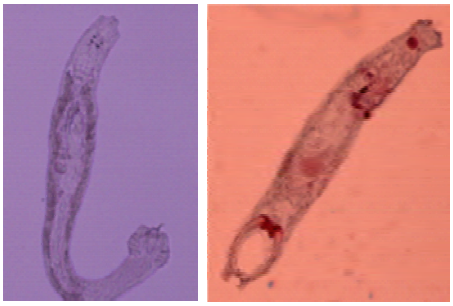


Fig. 54. Some monogenean parasites

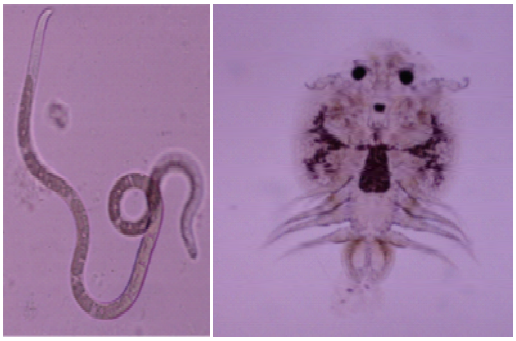


Fig. 55. Few metazoan parasites

DNA extraction and PCR amplification of parasites

DNA extraction from protozoan parasites *Myxobolus arcticus, Myxobolus* sp-1 and sp-2, *Thelohanelus toyamai, Trichodina* sp., *Ichthyophthirius multifiliis* and *Hennaguya* sp. was done through phenol: chloroform method of Sambrook *et al.* (1989). For DNA isolation of monogenean parasites, *Dactylogyrus, Gyrodactylus* and *Paradactylogyrus* sp., technique given in OIE dianostic manual (2003) and Qiagen's DNA isolation kits were used.

PCR amplification of 18SSu-rRNA genes (DNA) of protozoan parasites *M. arcticus, M. cerebralis, Thelohanelus toyamai, Trichodina* sp., *Ichthyophthirius multifiliis* and *Hennaguya* sp. was done using specific primers for each parasites through suitable PCR techniques. Specific primers were selected from the 18 SSU rDNA sequences through NCBI, designed with the help of software 'Primer3' and were synthesized by Sigma-Aldrich. The standard reaction volume was 50 µl containing 1x PCR buffer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 2.0U taq polymerase, 0.25 µM of each primer and 50 ng of the DNA template (Figures 56-5 7).

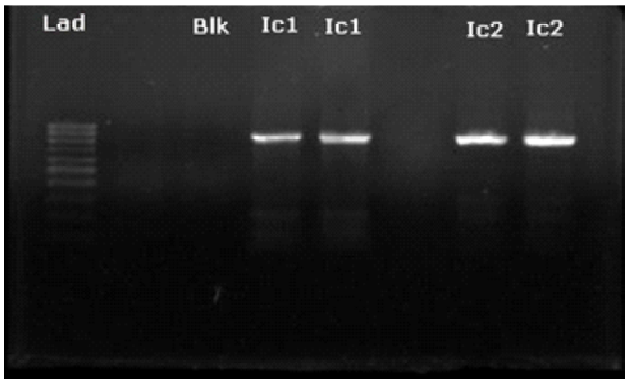


Fig. 56. 700 bp PCR product of *Ichthyophthirius multifiliis* from *Cyprinus carpio* (Ic1) 700 bp PCR product of *I. multifiliis* from *Labeo rohita* (Ic2). Blk : Blank

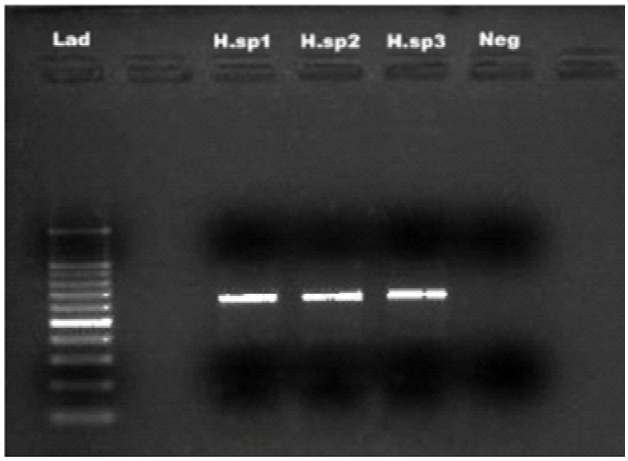


Fig. 57. A 650 bp large amplicon of *Hennaguya* sp.: Lad - Ladder 100 to 1500 bp, H1, H2, H3 - Replicates of *Hennuguya* template DNA, Neg. - Negative Control

Sequence analysis and molecular phylogeny

Sequences of 18SSu-rRNA genes of *Ichthyophthirius multifiliis, Myxobolus* sp., *Myxobolus arcticus, Trichodina, Thelohanellus toyamai* and *Henneguya* sp. were submitted to NCBI.

Molecular phylogenetic tree construction for above species was done using Maximum Parsimony and Maximum Likelihood methods (with bootstrap value of 500 -700) where similarity between two 18S rRNA gene sequences on scale 1 to 100 is measured (Fig. 58). To determine the phylogenetic positions of above species of protozoan parasites in relation to other geographically distant conspecific parasites and closely related species; 18S rRNA gene sequences of these parasites were compared to sequences of same; other closely related parasites and distant (out groups) parasites of other fish species. Phylogenetic analyses were conducted using software MEGA5.

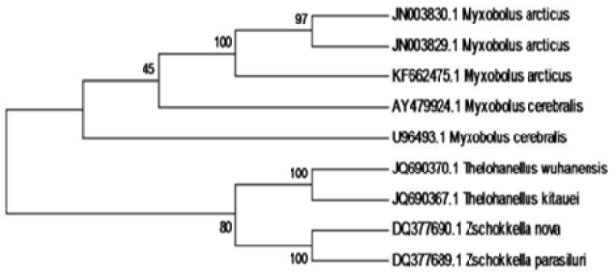


Fig. 58. Maximum Parsimony tree of the small subunit ribosomal DNA sequence of *M. arcticus* from *Clarias magur* with bootstrap values of 700.

(Bootstrap confidence values on the nodes of branches. GenBank accession numbers given before the species name)

Title of Project: National Surveillance Programme for Aquatic Animal Diseases

Project Period: April, 2013 – March, 2018

Project Coordinator: J.K. Jena

Project Personnel: Neeraj Sood (CPI), P.K. Pradhan, T. Raja Swaminathan, P. Punia and Rehana Abidi

Funding Agency: NFDB

The Institute is coordinating a 'National Surveillance Programme on Aquatic Animal Diseases (NSPAAD)' which is being implemented in 14 states of aquaculture importance with involvement of 23 national/state fisheries research institutions. The programme is funded by National Fisheries Development Board (NFDB), Department of Animal Husbandry, Dairying and Fisheries (DAHDF), Ministry of Agriculture, Government of India. During the reporting period under report, a total of 117 awareness meetings were organized to sensitize the stakeholders including state fisheries officers and fish farmers regarding the importance of disease surveillance as well as major diseases of aquatic animals. Awareness materials in different regional languages *i.e.* Bengali, Gujarati, Oriya, Malayalam, Tamil, Telugu, etc., and Hindi was also prepared by different participating centres and distributed to fish farmers for creating awareness. In addition, a manual 'Diagnostic Manual for Aquatic Animal Diseases of National Concern' containing information about etiological agent, susceptible host species, target organs, diagnostic methods, etc., for all the prioritized diseases was prepared with an objective to follow uniform protocol by all collaborating centres. Positive controls of finfish, crustacean and molluscan diseases were procured from OIE referral laboratories and are being provided to collaborating centres depending on their requirement.

The major pathogens reported during the reporting period include infection with *Aphanomyces invadans*, viral encephalopathy and retinopathy virus, cyprinid herpesvirus 2, *Aeromonas* sp., infestation with *Argulus* sp., *Ergasilus* sp. in finfish; white spot syndrome virus, infectious hypodermal and hematopoietic necrosis virus, hepatopancreatic parvovirus and infection with *Vibrio* sp. in crustaceans and infection with *Perkinsus olseni* in molluscs. The fish samples collected during active surveillance were negative for spring viremia of carp virus, koi herpes virus, infectious pancreatic necrosis virus and viral haemorrhagic septicemia virus, whereas, the tested shrimp samples were found to be negative for taura syndrome virus, infectious myonecrosis virus and yellow head virus. Information on aquatic animal diseases of national concern is being collected on quarterly basis from all the collaborating institutes and compiled at the nodal centre. This information has helped in strengthening quarterly reporting to OIE/NACA.

An Annual Review Meeting of Collaborating Centres under 'National Surveillance Programme on Aquatic Animal Disease Surveillance' was organized on 15 November, 2014 to review the progress of works of all the collaborating centres. An emergency response team comprising scientists from ICAR-NBFGR, Lucknow; ICAR-CIFA, Bhubaneswar and College of Fisheries, West Bengal was constituted following report of large-scale mortalities in goldfish, *Carassius auratus* in Hooghly District, West Bengal. The team collected samples from fish farms where goldfish mortality had been reported. The disease was identified to be goldfish haematopoietic necrosis caused by Goldfish Cyprinid Herpesvirus 2 on the basis of the PCR and sequencing of the amplified PCR products, virus isolation and histopathological findings as well as bioassay. The compiled report was submitted to DAHDF, Govt of India.

Sub-project: Surveillance of freshwater fish and shellfish diseases in Uttar Pradesh and Haryana

Project Personnel: P.K. Pradhan (PI), Neeraj Sood and Peyush Punia

Under the national surveillance programme, ICAR-NBFGR has the responsibility of surveillance in states of Uttar Pradesh and Haryana. Under the programme, awareness meetings were organized in Uttar Pradesh and Haryana. For Uttar Pradesh, along with National Fish Farmers Day celebration, an awareness programme on 'Fish Disease Surveillance'

was organised at ICAR-NBFGR, Lucknow on 10th July, 2014 (Figures 59 & 60). A total of seventy stakeholders including fish farmers, district fisheries officers and field extension officers from six districts of Uttar Pradesh *viz.*, Barabanki, Unnao, Bahraich, Kushinagar, Lakhimpur-kheri and Maharajganj participated in the programme. Similarly, for Haryana, an awareness programme on 'Fish Disease Surveillance' was organized in collaboration with Department of Fisheries, Government of Haryana on 9th September, 2014 at ICAR-National Bureau of Animal Genetic Resources, Karnal. Seventy one stakeholders including fish farmers along with district fisheries officers and field extension officers from five districts of Haryana *viz.*, Ambala, Hisar, Kaithal, Karnal and Rohtak participated in the programme. During the programmes, awareness brochures on fish disease surveillance (in Hindi) were distributed to the participants. In addition, awareness about disease surveillance was also created amongst all district fisheries officers of Uttar Pradesh and Haryana during one of their monthly meetings organised by respective State Fisheries Department. Furthermore, at Aquaculture Research and Training Unit, Chinhat of ICAR-NBFGR, Lucknow, 93 fish farmers were made aware of the importance of fish disease surveillance as part of the training programmes on fish culture and conservation.

Fig.59. Presentation by Dr. J.K. Jena, Coordinator, NSPAAD during National Fish Farmers Day at ICAR-NBFGR, Lucknow

Fig.60. Dr. P. K. Pradhan explaining the importance of fish diseases to the stakeholders during awareness meeting

During the reporting period, samplings were carried out twice in farms of selected districts of Uttar Pradesh and Haryana. The collected samples were screened for the presence of two viruses *viz.*, KHV and SVCV. All the samples were found to be negative for these viruses. During samplings, a case of large-scale mortality in *Oreochromis niloticus* in a fish farm in Talwandi-Rana, Hisar, Haryana was investigated and *Shewanella putrefaciens*, a Gram-negative bacterium was isolated from kidney as well as blood of *O. niloticus*. This bacterium has recently been reported to cause mortality in freshwater fishes *viz.*, goldfish, common carp and rainbow trout. Furthermore, outbreaks of infection with *Aphanomyces invadans* were observed in Maharajganj, Kushinagar, Unnao and Barabanki districts of Uttar Pradesh during samplings.

Sub-Project: Surveillance programme for aquatic animal diseases of ornamental fishes in the states Kerala and Tamil Nadu

Project Personnel: T. Raja Swaminathan (PI) and V. S. Basheer

Diseases are causing major setback for sustainability of the ornamental fish industry. The losses due to diseases are increasing day by day. All effective disease control measures need sound information on the distribution and nature of significant diseases. To substantiate the claim of freedom from diseases in the country, it is necessary to demonstrate that adequate system of active surveillance is in place to detect, diagnose and control aquatic animal diseases.

Under the national surveillance programme, Peninsular and Marine Fish Genetic Resources Centre, Kochi of ICAR-NBFGR has undertaken the work on surveillance of aquatic animal diseases of ornamental fishes in the states Kerala and Tamil Nadu. During the period under report, a total of 80 samples including 20 Koi carp (*Cyprinus carpio*), 25 gold fish (*Carassius auratus auratus*), 15 angel fish (*Pterophyllum scalarae*) and 20 Oscar fishes (*Astronotus ocellatus*) from five farms of Thrissur, Allapuzha and Ernakulam district of Kerala were collected.

The samples of the same species from the same farm were pooled together for active surveillance of koi herpes virus (KHV), spring viraemia of carp (SVC) and Iridovirus. A total of 20 pooled samples were checked for KHV, SVC and Iridovirus infection using PCR and found to be negative for all the viral infection. A total of 5 infected gold fishes with necrotic gill were collected

from a farm at Alappuzha. The microscopic examination of the infected gill revealed presence of *Myxobolus* sp. Homogenised gill samples were inoculated on agar medium and pure colonies of bacteria were isolated. Primary level biochemical tests and molecular identification using 16s rRNA was carried out. Based on the primary tests, pathogenic bacteria *viz., Aeromonas hydrophila, Klebsiella pneumonae, Citrobacter freundi, Vibrio cholerae* and *Flavobacterium* sp. were isolated from fishes. A total of 132 diseased ornamental fish samples collected from Kerala and Tamil Nadu were screened for important OIE listed pathogens *viz.,* KHV and SVC and all the samples were found negative.

Disease investigation on mass mortality in goldfish, *Carassius auratus* in Hooghly District, West Bengal

Large scale mortalities in goldfish, *Carassius auratus* and koi carp, *Cyprinus carpio* were reported in ornamental fish farms of Hooghly district, West Bengal by the Fishery Extension Officers of Department of Fisheries, Government of West Bengal to the Faculty of Fishery Sciences (FFSc), West Bengal University of Animal and Fishery Sciences, Kolkata. Considering the urgency of the situation, an Emergency Response Team (ERT) under the NSPAAD was constituted invloving scientists from ICAR-NBFGR, Lucknow; ICAR-CIFA, Bhubaneswar and College of Fisheries, West Bengal. As per the report of ERT in the affected farm, goldfish, koi carp and barbs were reared along with Indian major carps. However, no mortality was observed in the IMCs and barbs. The mortality rate was recorded as 1-5% daily in goldfish. The cumulative mortality rate was recorded above 90% during this period. The affected fish showed clinical signs such as sluggish movement, erratic swimming, anorexia, spinning, fin rot, focal cutaneous hemorrhages, reddish lateral line, protrusion and sloughing off the scales, exophthalmia, skin erosions, corneal opacity, etc.

Fig.61. Heap of dead goldfish thrown on the pond bund

Three farms in Piyarapur, Block Serampur (Uttarpara); District Hooghly showing large-scale mortality of goldfish were visited and samples collected for further investigation. It was observed that the dead fish were thrown on the sides of the farms all along the bunds (Fig. 61). Tissue samples were collected and whole moribund and dead fishes were also transported on ice and on transport medium for microbiological examination.

Affected three months old goldfishes showed protrusion of scales, dropsy, enophthalmia, hemorrhagic patches on the body surface particularly towards the base of the caudal fin (Fig. 62), sluggish movement and gulping of air followed by large scale mortality. All the affected fish (n=30) showed necrosis of the gills, splenomegaly with large white nodules, pale liver, swollen kidney with small white foci and the intestines were devoid of food (Fig. 63).

Experimental challenge of the healthy goldfish (n=10; Body weight = 25 gm) inoculated intraperitoneal with 0.22 µ filtered affected internal tissue homogenate produced 95% mortality after four days but not in koi carp. The fish showed typical clinical signs of gill necrosis and enlarged kidney and spleen as found in the natural cases. Further PCR examination of the pooled dead fish organs (gills, spleen and kidney) revealed positive for CyHV-2.

The viral inoculum (n=3; five fish from each farm were pooled as one sample) produced cytopathic effect (CPE) in three different fish cell lines namely, goldfish fin cell line, Koi carp fin cell line and *Pristolepis* fin cell line showing focal areas of granulation, cell vacuolization and appearance of rounded phase-bright cells typical to CyHV-2 infection (Fig. 64). The cell culture supernatant and affected cells from all three different cell lines found positive for CyHV-2 by PCR.

The samples (n=15; five fish from each farm) were found positive for Cyprinid Herpes Virus 2 (CyHV-2) (responsible for causing goldfish hematopoietic necrosis) by PCR (Fig. 65). All the samples were negative for KHV/CyHV-3 in both PCR by following standard OIE protocols. The nucleotide sequence of the target band (292 bp) was found to have 99% similarity to CyHV-2 available NCBI GenBank (Accession Numbers: DQ085628, DQ 085627, AY939863, KC841411, etc)

Histopathological lesions in kidney, spleen and gills of a goldfish with Goldfish Herpesviral Hematopoietic Necrosis Disease revealed the spleen sections showed area of necrosis and enlargement of nuclei with intranuclear inclusions and marginated chromatin and the kidney section with marginated chromatin in some of the cells. Section of the gills showed fusion of secondary lamellae and marginated chromatin in epithelial cells (Fig. 66).

Fig. 62. Goldfish showing clinical signs and post mortem lesions: A) swollen abdomen and protruded scales and shrunken eyes, B) necrotic gills

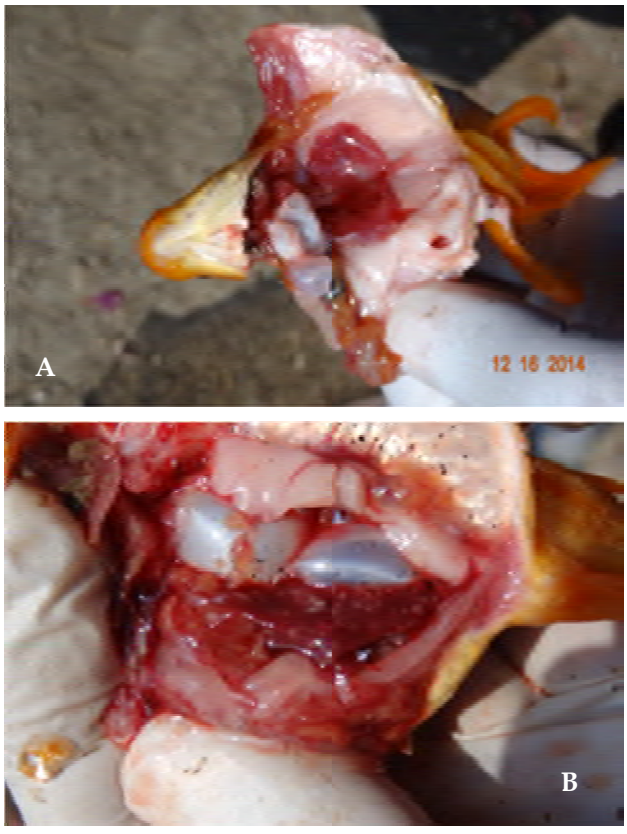


Fig. 63. Goldfish showing clinical signs and postmortem lesions: A) enlarged spleen with white foci, B) swollen and enlarged kidney with small white foci

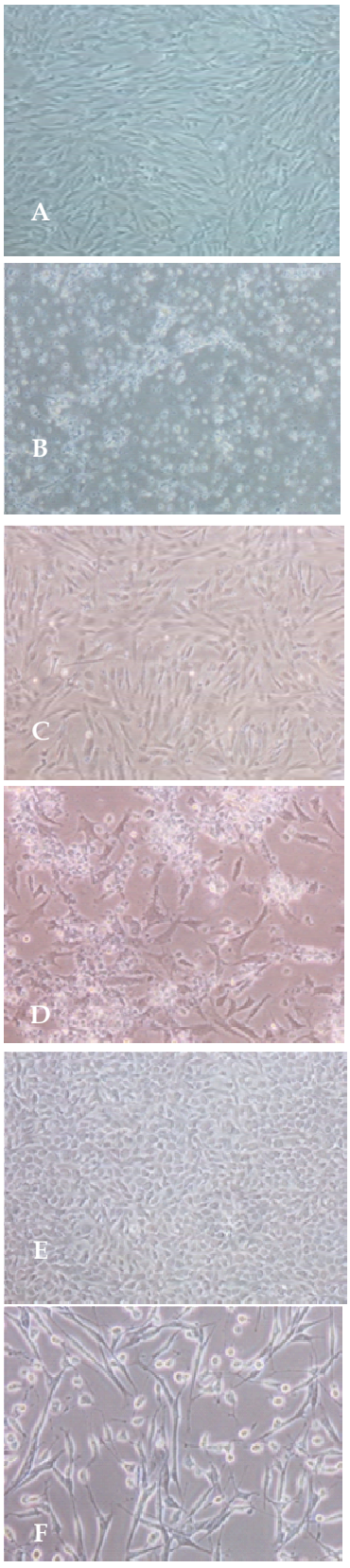


Fig. 64. The control cell lines (A, C and E) and CPE observed in those cell lines (B, D and F) infected with CyHV-2 suspension showing cytopathic effects (A and B: Goldfish fin cell line; C and D: CCKF cell line from Cyprinus carpio koi; E and F: CFF cell line from Pristolepis rubripinnis

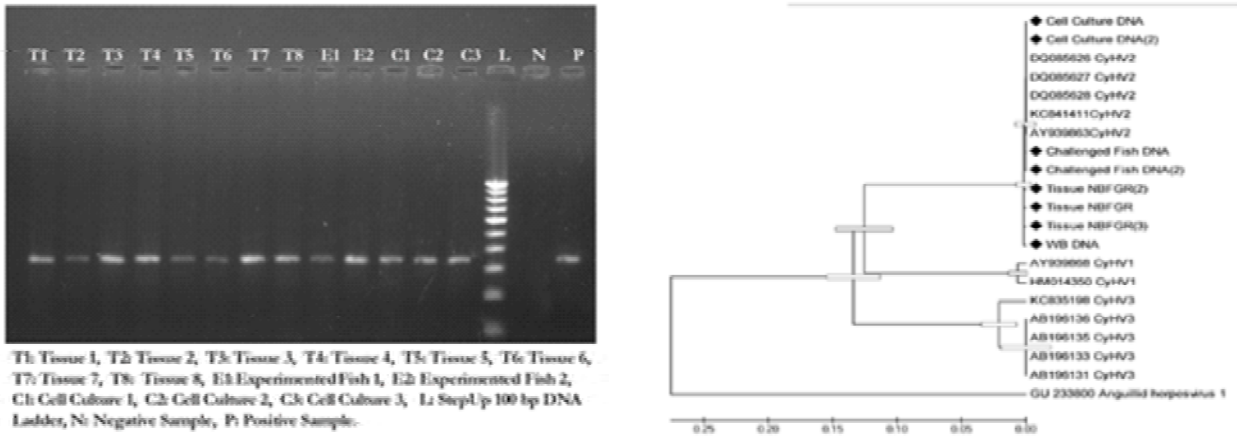


Fig. 65. Molecular diagnostics and phylogenetic analysis carried out for the diagnosis of CyHV-2 infection

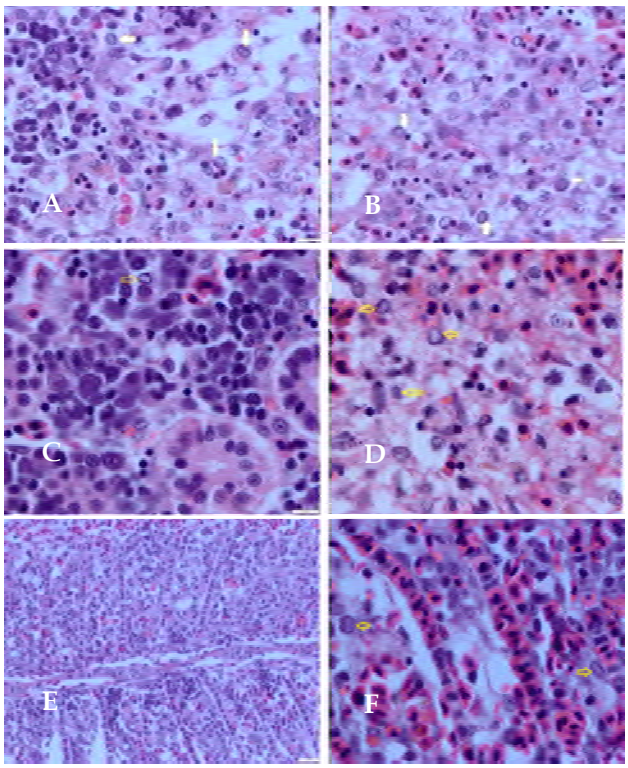


Fig.66. Histopathological lesions in Kidney, spleen and gills of a goldfish with Goldfish Herpesviral Hematopoietic Necrosis Disease: A) and B) Sections of spleen showing area of necrosis and enlargement of nuclei with intranuclear inclusions and marginated chromatin (arrows); C) and D) Section of kidney showing with marginated chromatin in some of the cells (arrows); E) Section of gills showing fusion of secondary lamellae and F) Section of gill showing marginated chromatin (arroes) in epithelial cells

PCR analysis, sequencing of the amplified PCR products from the naturally affected goldfish tissues, real time PCR analysis, CPE results from three infected cell lines and histopathological findings confirmed that the primary causative agent of the present disease outbreak to be *Goldfish Cyprinid herpes virus 2*. Further involvement of *Aeromonas hydrophila* might be playing as a secondary pathogen in the disease outbreak.

Establishment and characterization of fin-derived cell line from ornamental carp, *Cyprinus carpio* koi

Cyprinus carpio koi fin (CCKF) cell line was established and characterized from the caudal fin tissue of ornamental common carp, *Cyprinus carpio* koi. This cell line has been maintained in L-15 medium supplemented with 15% foetal bovine serum (FBS) and sub-cultured more than 52 times over a period of 24 months. The CCKF cell line consisted of epithelial cells (Fig.67) and was able to grow at temperatures between 22°C and 35°C with an optimum temperature of 28°C.

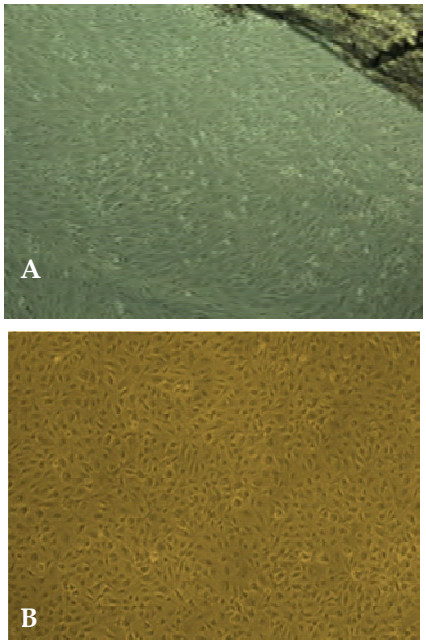


Fig.67. A) Explant of caudal fin of *Cyprinus carpio koi* showing radiation of cells (100X) and B) CCKF cell line at 45th passage (100X)

The growth rate of these cells increased as the proportion of FBS increased from 2% to 20% with optimum growth at the concentrations of 15% FBS. Karyotype analysis revealed that the modal chromosome number of CCKF cells was 2n=100. Partial amplification and sequencing of fragments of two mitochondrial genes 16S rRNA and COI confirmed that CCKF cell line originated from ornamental common carp. The CCKF cells showed strong reaction to the cytokeratin marker, indicating that it was epithelial in nature. The extracellular products of *Vibrio cholerae* MTCC 3904 and *Aeromonas hydrophila* were toxic to the CCKF cells and not susceptible to Viral Nervous Necrosis Virus (VNNV). These CCKF cells were confirmed for the absence of *Mycoplasma* sp. by PCR. Furthermore, 90% of viable cells could be effectively revived four months after cryopreservation from CCKF cell population, suggesting the possibility of long-term storage of the cells.

Regional Proficiency Testing Program for Aquatic Animal Disease Diagnostic Laboratories in Asia-Pacific

The programme was formulated by NACA in association with DAFF Australia, CSIRO, Australian National Quality Assurance Program (ANQAP), NACA and OIE for improved aquatic animal disease laboratory diagnostic capability across Asia [Laboratory proficiency testing (LPT)]. Forty-five participants attended the workshop hosted by NACA at Bangkok, Thailand during 25-26 July 2012, from 13 Asia-Pacific countries including Bangladesh, Cambodia, China, Hong Kong, India, Indonesia, Iran, Malaysia, Myanmar, Philippines, Sri Lanka, Thailand and Vietnam. The proficiency testing services (sending samples and analysis of results), which have a total of four rounds of testing was being provided by an accredited provider (the Australian National Quality Assurance Program; ANQAP) and with expertise from CSIRO Australian Animal Health Laboratory (AAHL). Overall, the LPT was offered for 10 diseases and testing was carried out according to OIE/international standards. All test samples (6 samples for each disease) that were sent to participating laboratories were inactivated, therefore, non-infectious. ICAR-CIBA, Chennai is the coordinating institute for receiving samples from ANQAP and distributing to labs in India. Testing of three pathogens *viz.*, KHV, TSV and YHV was carried out by the ICAR-NBFGR in 1st and 2nd round testing. Testing of five pathogens *viz.*, KHV, SVC, Iridovirus, TSV and YHV was carried out by the ICAR-NBFGR in 3rd and 4th round testing. A total of 96 samples were tested in all the four rounds. All the testing was done for three times (each pathogen) to confirm the results (Table 16).

Table 16. The details of the ICAR-NBFGR results in four rounds of the LPT testing

Round	Date		Pathogen	Result Score	Remarks
	Receipt	Results sent			
I	6th June 2013	22nd July 2013 (before due date)	KHV (DNA virus)	6/6	
			YHV (RNA virus)	4/6	Mild positive could not be detected
			TSV (RNA virus)	4/6	Mild positive could not be detected
II	6th June 2013	10th Sep 2013 (before due date)	KHV (DNA virus)	6/6	
			YHV (RNA virus)	6/6	
			TSV (RNA virus)	6/6	
III	26th May 2014	6th July 2014 (before due date)	KHV (DNA virus)	4/6	Mistake in coding of samples at our lab.
			Iridovirus (DNA virus)	6/6	
			SVC (RNA virus)	6/6	
			YHV (RNA virus)	6/6	
			TSV (RNA virus)	6/6	
IV	26th May 2014	10th Sep 2014 (before due date)	KHV(DNA virus)	6/6	
			Iridovirus (DNA virus)	6/6	
			SVC (RNA virus)	6/6	
			YHV (RNA virus)	6/6	
			TSV (RNA virus)	6/6	

Project Title: Risk and benefit assessment of an illegally introduced fish species *Piaractus brachypomus*, pacu in India

Project Period: October, 2013 - March, 2015

Project Personnel: Peyush Punia (PI)

Funding Agency: NFDB

The present study was undertaken to study the risk and benefit assessment of illegally introduced *P. brachypomus* in India. To assess the present distribution and culture status of pacu, survey was carried out and data collected from the state of West Bengal, Andhra Pradesh and Uttar Pradesh. Survey of hatcheries in West Bengal indicated that seed is produced and supplied to different parts of the country including, Andhra Pradesh, Tamil Nadu, Maharashtra and Uttar Pradesh (Fig.68). The breeding season of this fish starts in mid-April and brooders are mostly fed with rice bran, boiled chickpea and rice. Survey of fish markets to assess the availability of pacu indicated that pacu is available only in very few retail fish markets and it is not a preferred fish. The fish fetches a price of Rs. 100-200/kg depending on the size. As per the information received from the farmers, Pacu was introduced in 2006-07 and became popular. However, the demand for this fish slowly declined as the consumers resorted to Indian major carps again. To understand the impact of this species on aquarium trade, aquarium shops were also surveyed for availability of pacu. It was found that pacu (red belly) was available on all the aquarium shops. This indicated that pacu is being sold in most of the aquarium shops as an ornamental fish and probability of this fish entering into natural water bodies is high due to escape or discarding of live fish. Wet laboratory experiments to study the compatibility with Indian major carps indicated aggressive behavior of pacu and its fin nipping habit is harmful for other smaller sized fish species in the culture system.

Fig.68. (A) Production of pacu seeds in West Bengal and (B) Small quantity of pacu being sold along with other fishes in a market of West Bengal

WORKSHOPS/ SYMPOSIA/ MEETINGS ORGANIZED

ICAR-NBFGR hosts 10th Indian Fisheries and Aquaculture Forum

The 10th Indian Fisheries and Aquaculture Forum (10ifaf), the largest scientific triennial event in fisheries and aquaculture in India was organized under the aegis of Asian Fisheries Forum Indian Branch (AFSIB) in collaboration with the ICAR-National Bureau of Fish Genetic Resources, Lucknow during 12-15 November 2014 at the premises of ICAR-NBFGR, Lucknow. The 10ifaf with the theme "Towards Responsible Aquaculture and Sustainable Fisheries" was inaugurated on 12th November, 2014, by the Hon'ble Governor of U.P., Shri Ram Naik. Dr. S. Ayyappan, Secretary DARE and Director General, ICAR, New Delhi presided over the programme. Other dignitaries present on the dais were : Dr. B. Meenakumari, Deputy Director General (Fisheries), ICAR and Dr. Cherdsak Virapat, Director General, Network of Aquaculture Centers in Asia-Pacific (NACA), Bangkok as Guests of Honour; Dr. J. K. Jena, Convener, 10ifaf, Chairman, AFSIB & Director, ICAR-NBFGR; and Dr. B.A. Shamsundar, Secretary, AFSIB, Mangalore. Over 700 distinguished scientists, technocrats, policy makers, members from financial institutions, NGOs, students, farmers and entrepreneurs from all over the country and overseas participated in the Forum.

Dignitaries on the dais during inaugural function

Shri Ram Naik, the Chief Guest in his inaugural address while congratulating the organizers for selecting the present theme, which is timely, emphasized the importance of fisheries sector for ensuring nutritional security for the ever-growing populations of the country. He advocated establishment of a separate Union Ministry of Fisheries and proposed for concerted efforts in this direction. In order to provide fisheries due attention, he emphasised for economic

Chief Guest, Shri Ram Naik Hon'ble Governor of U.P. lighting the lamp

Inaugural address by the Chief Guest

issues like provision of interest rates on loans and subsidies at par with agriculture and policy issues like trans-boundary conflicts, safety at sea, etc. and expressed hope that the Forum would bring out recommendations for the welfare of fishers and fish farmers.

In his Presidential address, Dr. Ayyappan stressed upon the need to address on aspects towards sustenance of production and productivity of the sector and to meet the targeted demand of fish by 2020. He expressed concern on certain emerging issues like

Dr. S. Ayyappan, Secretary, DARE & DG, ICAR, delivering the presidential address

climate change, rise in sea-surface temperature, over-fishing, non-availability of certified fish stocks, gender issues, hatchery accreditation, drop in river fish production, food safety and quality control measures. He stressed that certain issues like integrated island fisheries management, impacts of exotics, stock improvement genetics and similar issues need to be addressed on wider scale.

Dr. B. Meenakumari, DDG (Fy.), ICAR in her address emphasized discussions on increased water productivity, impact of climate change and conservation of fish biodiversity. Speaking on the occasion, Dr. Cherdsak Virapat, Director General, NACA, Bangkok deliberated on the role of NACA in rural development in the Asia-Pacific region with

Dr. B. Meenakumari, DDG (Fy.) ICAR addressing the Forum

Dr. Cherdsak Virapat, DG, NACA, Bangkok addressing

emphasis on up-liftment of living standards of fishers of the region. He expressed that NACA would continue to cooperate and extend partnership with India. However, before that Dr. J.K. Jena, Convener of the 10ifaf and Director, ICAR-NBFGR, Lucknow welcomed the Chief Guest, guests and delegates to the forum.

The Abstract Book and Souvenir of the 10ifaf were released by the Chief Guest along with a Special Issue of Asian Fisheries Science "Gender in aquaculture and fisheries: Navigating change". Two progressive fish farmers of the area, Shri Parvez Khan of Jahagirabad, Barabanki, U.P. and Shri Nawaj Khan of Siddartha Nagar, U.P. were felicitated on this occasion. As a tribute to the fisheries veterans those passed away during last three years, namely Dr. Hiralal Chaudhury, Dr. P.V. Dehadrai, Dr. S.N. Dwivedi, Dr. M.C. Nandeesha, Dr. S.A.H. Abidi, Dr. K.C. Jayaram and Dr. C. S. Singh, the seven Seminar Halls used for parallel technical sessions were named after them.

Dr. J.K. Jena, Director, ICAR-NBFGR and Convenor 10ifaf giving welcome address

The inaugural session followed with two Key-note addresses by Dr. M.V. Gupta, World Food Prize Laureate and Former ADG, WorldFish, Malaysia on "Responsible Aquaculture: The context and Action Needed" and Dr. M.J. Modayil, Former Member, ASRB,

Dr. M.V. Gupta delivering the keynote address

New Delhi and Former Director, ICAR-CMFRI, Kochi on "Sustainable Fisheries". The Session was Chaired by Dr. S. Ayyappan, Secretary, DARE and DG, ICAR, New Delhi. There were two Lead Plenary Lectures delivered by international experts Dr. Cherdsak Virapat, DG, NACA, Bangkok and Dr. Chris O'Brien, Regional Coordinator, BOBLME, Thailand, which was Chaired by Dr. B. Meenakumari, DDG (Fy.), ICAR. Dr. Virapat in his lecture on "Indian Ocean tsunami early warning and mitigation systems: applications for

Dr. M.J. Modayil giving the keynote address

future sustainable aquaculture" gave an account of the recent tsunami's in the Indian Ocean and the devastating role played by it in the region. He stressed for establishment of ocean-based tsunami early

Dr. Chris O' Brien delivering the lead plenary lecture

A view of the distinguished participants

warning system in the region as a part of an overall multi-hazard disaster reduction strategy. Dr. O'Brien in his lecture on "Applying the ecosystem approach to fisheries management" pointed about the ecosystem benefit of Bay of Bengal, as an important critical marine habitat with high level of fish biodiversity, number of endangered species and four million fishers. An Evening Plenary Lecture was also delivered by Dr. Meryl J. Williams, Former DG, WorldFish who spoke on "Fish, food security and nutrition: A new analysis". She highlighted the issues of ecological sustainability, international fish trade and food security, small *vs* large-scale fish operators, need for effective governance & management reforms and international governance initiatives.

Dr. Meryl J. Williams delivering plenary lecture

The technical programmes of the Forum were conducted in six parallel sessions in which 208 papers were presented under different thematic areas namely, Fisheries Resource Management, Aquaculture-Breeding and Production Systems, Fish Genetics and Biotechnology, Fish Nutrition, Biochemistry and Feed Technology, Aquatic Animal Health, Fish Biology,

A view of the posters gallery

Toxicology and Environmental Health, Harvest and Post Harvest Technology, Social Sciences in Fisheries and Fisheries Poicy and Governance. Besides, seven lead lectures were also presented by leading experts of the country. A total of 149 Abstracts were also presented as Posters during the 10ifaf in three Sessions during 13-14 November, 2014, in a Poster Gallery developed for the purpose. Ten Best Posters presented during the Forum were Awarded with the Best Poster Award with Certificate and Cash prize of Rs. 2000/- each during the Valedictory Function.

As a parallel event of 10ifaf, an Exhibition with 32 stalls was also arranged for showcasing technologies developed by different Research Institutions, and products/publications of different industries/business firms and book publishers, which

Inauguration of the exhibition by Shri Ram Naik, Hon'ble Governor of U.P.

was inaugurated by the Chief Guest, Hon'ble Governor of U.P., Shri Ram Naik in presence of Dr. S. Ayyappan, Secretary, DARE and Director General, ICAR and other dignitaries on 12th November, 2014. Various award

View of the exhibition pavilian

presentations were also organised during the forum wherein the awardees presented their research work.

Dr. Raja Sekhar Vundru, IAS, Joint Secretary, DAHDF, Govt. of India was the Chief Guest of the Plenary and Valedictory Session and Dr. B.

Dignitaries on the dias during valedictory session

Meenakumari, DDG (Fy.), ICAR presided over the function. Dr. K.V. Devaraj, Former Vice-Chancellor, University of Agricultural Sciences, Bangalore; Dr. K. Gopakumar, Former DDG (Fy.), ICAR and Dr. Mohan J. Modayil, Former Member, ASRB were the Guests of Honour. Dr. J.K. Jena, Director, ICAR-NBFGR and Convenor of the 10ifaf welcomed the guest and the audience and gave a brief report of the 10ifaf. Dr. Vundru in his address congratulated the AFSIB for organizing such a mega-event on a very important theme.

Chief Guest, Dr. R. S. Vundru speaking during valedictory session

5th Global Symposium on Gender in Aquaculture and Fisheries (GAF5)

The Institute hosted the '5th Global Symposium on Gender in Aquaculture and Fisheries (GAF5)' organized by the Asian fisheries Society (AFS), Kuala Lumpur in collaboration with Asian Fisheries Society Indian Branch and ICAR-National Bureau of Fish Genetic Resources (NBFGR), Lucknow during 13-15 November, 2014, which was inaugurated by Dr. Leena Nair, Chairman, Marine Product Export Development

Chief Guest Dr. Leena Nair inaugurating GAF5 by lighting the lamp

Dr. Meryl J. Williams speaking during inaugural function

Authority (MPEDA), Kochi as Chief Guest. Dr. B. Meenakumari, Deputy Director General (Fy.), ICAR, New Delhi presided over the function. Dr. Meryl J. Williams, Former Director General, WorldFish, Malaysia and Director, Asia Pacific-Fish Watch, and Dr. Cherdsak Virapat, Director General NACA and Dr. J.K. Jena, Convener, 10ifaf and Director, NBFGR were the Guests of Honour on the occasion. This is the first time that an Asian Fisheries Society GAF event was held in conjunction with the Indian Fisheries

The GAF5 participants

Forum, the 10ifaf. Dr. Meryl J. Williams and Dr. B. Meenakumari were the Co-Chairs of GAF5.

The three day symposium was filled with around 50 stimulating contributions of several kinds which included 27 oral presentations, including 2 Plenary Lectures; 3 special workshops; 6 films and videos; 1 panel discussion; 9 posters and a special GAF Network Meeting. The forum witnessed the presence of many new researchers and development experts who work on gender within fisheries and aquaculture institutions and development agencies, especially non-government organisations (NGOs). Delegates from different parts of the world including India, Malaysia, Bangladesh, Indonesia, Lao PDR, Thailand, Vietnam, Cambodia, Japan, Philippines, Mozambique, Australia, Spain, France and USA participated in GAF5.

International Workshop on Aquatic Animal Disease Surveillance

An International Workshop on Aquatic Animal Disease Surveillance was organised by the Institute in collaboration with the Asian Fisheries Society Indian Branch (AFSIB) at ICAR-NBFGR, Lucknow on 14th November, 2014, which was inaugurated by Dr. Raja Sekhar Vundru, Joint Secretary (Fisheries), Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, Govt. of India. Dr. B. Meenakumari, DDG (Fy.), ICAR, New Delhi presided over the function. Other guests present on the dais were Prof. Kenton L. Morgan, University of

Chief Guest Dr. R. S. Vundru inauguration the workshop by lighting the lamp

Liverpool, United Kingdom and Dr. Eduardo Leano, Programme Coordinator, NACA, Bangkok as Guests of Honour; Dr. J.K. Jena, Convener and Director, ICAR-NBFGR, Lucknow and Dr. P. Punia, Co-coordinator, National Surveillance Programme on Aquatic Animal Disease (NSPAAD), ICAR-NBFGR, Lucknow.

Dr. Vundru, in his inaugural address, emphasized the necessity of aquatic animal disease surveillance in the context of huge economic losses due to diseases. He informed that Prevention and Control of Infectious and Contagious Diseases of Animal Act 2009 has been amended to include Aquatic Animal Diseases of National Concern and the State Governments have the responsibility to report about the occurrence of these diseases. He also expressed the hope that the National Surveillance Programme would be of immense help in view of proposed Blue Revolution in the country. Dr. Meenakumari mentioned that seed, feed and health management are three important components of aquaculture and she expressed her happiness that the third aspect is being taken care of through National Surveillance Programme initiated in the country under the leadership of ICAR-NBFGR with the support of NFDB and DAHDF.

The workshop included lead presentations on 'Aquatic Animal Disease Surveillance in Global Perspective' by Prof. Kenton, 'Aquatic Animal Disease Surveillance in the Asia-Pacific: Status and Gaps' by Dr. Eduardo and 'Does neoplastic transformation mimic speciation?' by Dr. Sen Pathak, distinguished Research Professor, Department of Genetics, University of Texas, Houston, USA. Prof. Kenton mentioned that in the case of any disease outbreak, it is important to take immediate action to prevent the spread of the disease and then diagnosis of the disease condition should be carried out. He also stressed on the importance of risk based surveillance. Dr. Eduardo mentioned that India is amongst few member countries of NACA which have initiated the National Surveillance Programme. He expressed the hope that with the available strength on fish health, in the days to come, India should be an example for other developing countries. Dr. A.G. Ponniah, Chairman of the session in his concluding remarks emphasized on the importance of system approach than individual

Dr. Eduardo Leano giving lead presentation

approach for the surveillance system to be effective and stressed that we should develop mechanism to respond as early as possible, to any new disease outbreaks in the country. Dr. Sen Pathak emphasized on several issues that could lead to successful transformation of disease surveillance programme as have been conducted in the developing countries.

Another important aspect of the Workshop was the Panel Discussions on 'Aquatic Animal Disease Surveillance in India' by several national experts on fish disease management in the country which was conducted under the Chairmanship of Dr. J.K. Jena. Over 200 delegates from India and overseas participated in this international workshop. The panelists stressed on developing standardized protocols for reporting new or exotic diseases, risk based surveillance, mandatory registration of farms with the government agencies, involvement of the state

Lead presentation by Prof. Kenton

fisheries department in the implementation of surveillance programme, establishing disease diagnostic laboratories in each state and uniform sampling strategy. It was also reiterated that though active monitoring of selected farms should continue, but the focus of the programme should be strengthened

Panel discusssion on Aquatic Animal Disease Surveillance

the passive disease surveillance in the country, so that each outbreak is reported and investigated. It was also suggested that monitoring of water quality parameters must be part of disease diagnosis in aquatic animals.

National Fish Farmers' Day celebrated

The National Fish Farmers Day was celebrated at the Institute on 10th July, 2014. Seventy fish farmers along with District Fisheries Officers and Field Extension Officers from six districts of Uttar Pradesh participated in the celebrations. On this occasion, an awareness programme on 'Fish Disease Surveillance in Uttar Pradesh' was organised under National Surveillance Programme on Aquatic Animal Diseases (NSPAAD) in collaboration with State Fisheries Department, Government of Uttar Pradesh. The programme was inaugurated by Dr. Pinki Jowel, Director of Fisheries, Govt. of Uttar Pradesh. Speaking on this occasion, Dr. J.K. Jena, Director, ICAR-NBFGR and National Coordinator, NSPAAD deliberated on potential of the state in increasing the fish production.

Dr. J.K. Jena addressing during National Fish Farmers' Day programme

He also highlighted the importance of the programme on disease surveillance and reiterated that the technical support will be provided by the Bureau in diagnosis of fish diseases in the state. Dr. Peyush Punia, Principal Scientist and Head, Fish Health Management Division, ICAR-NBFGR, Lucknow explained the importance of surveillance programme and contingency planning in reducing losses due to fish diseases. Dr. P.K. Pradhan, Senior Scientist, ICAR-NBFGR, Lucknow made a presentation on overview of establishing fish disease surveillance system in the state. Dr. M. Gajendragad, Principal Scientist, NIVEDI, Bengaluru presented an overview of the animal disease surveillance in the country and disease prediction models for forecasting diseases well in advance. He also explained about the need of fish disease surveillance programme, which can help in taking policy decisions for prioritizing research and developing control measures. The Chief Guest of the function, Dr. Pinki Jowel explained various schemes initiated by the U.P. State Fisheries Department for the welfare of the fish farmers. She also informed that Uttar Pradesh is the second state in the country, after Bihar, to give the status of agriculture to fish culture. She interacted with fish farmers and assured Department's commitment in addressing farmers' problems. She assured full support of the Department for developing fish disease surveillance programme in the whole state. Dr. Neeraj Sood, Principal Scientist and Principal Investigator of NSPAAD proposed vote of thanks.

Awareness programme on Fish Disease Surveillance in Haryana

The Institute organised an awareness programme on 'Fish Disease Surveillance in Haryana' in collaboration with Department of Fisheries, Government of Haryana on 9th September, 2014 at ICAR-National Bureau of Animal Genetic Resources (NABGR), Karnal. Seventy one fish farmers along with

Dr. Peyush Punia, speaking during awareness programme

District Fisheries Officers and Field Extension Officers from five districts of Haryana *viz.*, Ambala, Hisar, Kaithal, Karnal and Rohtak participated in the programme. The programme was inaugurated by Dr. R.K. Sangwan, Director, State Fisheries Department, Govt. of Haryana. Dr. A. Sharma, Director, ICAR-NABGR was the Guest of Honour of the programme.

In the introductory remarks, Dr. Peyush Punia, Principal Scientist and Head, Fish Health Management Division, ICAR-NBFGR, Lucknow explained the importance of National surveillance programme. Dr. A. Sharma stressed upon the importance of integration of aquaculture with animal husbandry and agriculture. He was optimistic that Haryana would emerge as a Model State in the field of aquaculture. The Chief Guest of the function, Dr. Sangwan explained about the need for the programme on disease surveillance in the state. He assured full support of the department for developing fish disease surveillance programme in the whole state.

View of the participants

Dr. P. K. Pradhan, Senior Scientist, ICAR-NBFGR made a presentation on Overview of Establishing Fish

Disease Surveillance System in the State. He mentioned that there is a need of better coordination between farmers, District Fisheries officers and scientists for successful implementation of the surveillance programme in the state. The NBFGR scientists interacted with fish farmers and assured Bureau's commitment particularly in the investigation of any fish disease outbreaks in the state and advised the farmers to take a serious note of disease problems in initial stages of their development and cautioned that small negligence can lead to disease outbreaks and result in severe losses. Dr. Neeraj Sood, Principal Scientist explained about the importance of contingency planning in reducing losses due to fish diseases.

Awareness Programme on National Surveillance Programme on Aquatic Animal Diseases in Kerala

An one day awareness programme for ornamental fish farmers and state fishery officials was conducted by PMFGR Centre of NBFGR, Kochi on 8th October 2014. The programme started with an invocation, followed by a welcome address by Dr. V. S. Basheer, Principal Scientist and Scientist Incharge, NBFGR Kochi. Dr. A. Gopalakrishnan, Director, ICAR-CMFRI, Kochi was the chief guest of the inaugural function. He inaugurated the function by lighting the ceremonial lamp and he reiterated the importance of disease surveillance in the event of trans-boundary movement of ornamental fishes. He also emphasized the importance of farmers, government officials and researchers joining together for successful completion of disease surveillance programme. Dr. Devika Pillai, Associate professor, KUFOS, Kochi, and Dr. M. S. Saju, General Manager, Kerala Aqua Venture International Ltd. (KAVIL), Kochi gave felicitation address in the inaugural session and shared their experiences in fish disease surveillance and ornamental fish trading respectively. A total of 61 participants from 5 selected districts of Kerala attended the awareness programme.

Awareness Programmes on National Surveillance on Aquatic Animal Diseases in Tamil Nadu

Two awareness programmes on 'Surveillance on Aquatic Animal Diseases' for ornamental fish farmers and state fishery officials were conducted by the PMFGR Centre, ICAR-NBFGR, Kochi in collaboration with the State Fisheries Department, Tamil Nadu. The first programme was organised on 7 February, 2015 at

Awareness programme on surveillance of aquatic animal diseases in Madurai

Madurai. The programme started with the welcome address by Smt. M. V. Prabhavathi, Assistant Director of Fisheries, Madurai. Mr. R.S. Ganesan, Deputy Director of Fisheries, Madurai, the Chief Guest of the function in his inaugural address emphasized upon the importance of farmers, government officials and researchers joining together for successful completion of disease surveillance programme. He also told that the Madurai district ornamental fish farmers were the pioneers in the ornamental fish breeding and rearing among the farmers in Tamil Nadu and advised them to take lead in the ornamental fish market. Mr. V. Ravichandran, DDF (Rtd) also shared his experiences in fish disease surveillance. He stressed upon the importance of having a society for proper marketing of their farm produce in the market and importance of cooperation among ornamental fish farmers. A total of 57 participants including 40 ornamental fish farmers and 17 serving and retired fishery officers from Madurai district of Tamil Nadu attended the awareness programme.

Inauguration of awareness programme at Chennai

Another awareness programme was conducted on 13 March, 2015 at Chennai. Mr. Lamek Jayakumar, Assistant Director of Fisheries, Ponneri, Thiruvallur,

Chief Guest of the function mentioned the importance of disease surveillance in this era of rapid and increasing movement of ornamental fishes. Mr. N.U.S. Veeramaindhan, President, Kolathur Ornamental Fish Farmer Co-Operative Society, Kolathur, Chennai also shared his experiences in ornamental fish culture. A total of 52 participants including 47 ornamental fish farmers and 5 state fisheries officials from Chennai and Thiruvallur districts of Tamil Nadu attended the awareness programme.

Seminar on Fish Conservation Organized

One-day Seminar on 'Fish Biodiversity of Ramgarh Lake and its Conservation' was organized on 15 June, 2014 at Meherwa-Ki-Bari, just besides the Ramgarh Lake, Kuraghat, Gorakhpur, U.P. The seminar was aimed at enlightening the fishermen community regarding recent developments in fishery-based technologies and various programmes for the benefits of the persons involved in fishery-related activities. While inaugurating the seminar the Chief Guest, Dr. M. Sinha, Former Director, ICAR-Central

Seminar on fish conservation being inaugurated by Dr. M. Sinha

Inland Fisheries Research Institute, Barrackpore gave an overview of Ramgarh lake and its fisheries during early sixties and emphasized the need for conservation of the lake for utilization of fisheries resources in sustainable manner. Dr. A.K. Pandey, Principal Scientist and Organizing Secretary, ICAR-NBFGR, Lucknow gave an overview of the work done on fish and fisheries of Ramgarh lake in the past. Dr. Radheyshyam, Former Principal Scientist, ICAR-Central Institute of Freshwater Aquaculture, Bhubneswar highlighted the importance of culture-based capture fisheries in such water bodies of eastern Uttar Pradesh while Mr. Sanjay Kumar Shukla, Deputy Director (Fisheries), Gorakhpur enumerated the government programmes for the upliftment of the fisher community and those involved in fisheries. Over 150 persons from fishing community participated in this seminar.

Initiation Meeting-cum-Workshop on Outreach Activity on Fish Genetic Stocks (Phase II)

An Initiation Meeting-cum-Workshop of 'Outreach Activity on Fish Genetic Stocks' (Phase II) was held at ICAR-NBFGR, Lucknow on 8 January, 2015. The meeting was attended by scientists from collaborating ICAR fisheries institutes: Central Marine Fisheries Research Institute, Kochi; Central Institute of Freshwater Aquaculture, Bhubaneswar; Central Inland Fisheries Research Institute, Barrackpore; Directorate

Meeting-cum-workshop on Outreach Activity on Fish Genetic Stocks (Phase-II)

of Coldwater Fisheries Research, Bhimtal; Central Institute of Brackishwater Aquaculture, Chennai and Central Institute of Fisheries Education, Mumbai. In the meeting, working strategies such as, sampling, tissue preservation, marker development and biological investigation were deliberated. The research plan and sample collection locations for target species by different partner institutes was presented and discussed.

NBFGR celebrated its '31st Foundation Day' and 'Farm Innovators Day'

The Bureau celebrated its '31st Foundation Day' and 'Farm Innovators Day' on 12 December, 2014. The programme was inaugurated by the Chief Guest of the occasion Dr. S.L. Goswami, Former Director, ICAR-NAARM, Hyderabad where as Dr. S. Solomon, Director, ICAR-IISR, Lucknow and Dr. P.C. Mohanta, Former Director, ICAR-DCFR, Bhimtal were the Guests of Honour. In his welcome address, Dr. J.K. Jena, Director, ICAR-NBFGR apprised about the salient achievements of the Institute. A total of 20 progressive aqua-farmers and entrepreneurs participated in the

Inaugural address by the Chief Guest Dr. S. L. Goswami during Foundation Day Function

programme and shared their innovative and profitable farming practices. The Chief Guest and the Guests of Honour addressed the staff and the farmers on this occasion and congratulated them for their achievements. Lectures were delivered on different aspects of aquaculture and fish conservation by the NBFGR scientists. A question answer session was also organized to solve the problems of the aquafarmers. The farmers also visited NBFGR farm and aquarium facilities.

On this occasion, Annual Institute Awards for the year 2013-14 were presented to the NBFGR staff members for their performance in various categories. Selected fish farmers from various districts of Uttar Pradesh were also awarded on the occasion for obtaining better fish production from their ponds after taking training from ARTU Unit, Chinhat of ICAR-NBFGR. 'Fish Genomics Section of the Conservation Division' was awarded the Best Unit/Division Award. Other awardees in various categories were: Dr. P.K. Pradhan, Sr. Scientist, Best Scientist; Mr. Ravi Kumar, Technical Officer and Dr. Vikash Sahu, Technical Assistant, Best Technical Staff; Mr. Swapan Debnath, Sr. Clerk and Mr. Sandeep, Jr. Stenographer, Best Administrative Staff; Mr. Anwar, SSS, Best Support Staff; Mr. K.K. Singh, appreciation award.

Farm Innovators Day Celebration

Annual Institute Awards presentation

Research Advisory Committee (RAC) Meeting

The RAC meeting of the Institute was held during 2-3 March, 2015 under the Chairmanship of Dr. W. Vishwanath, Professor, Department of Life Sciences, Manipur University, Imphal. Dr. (Mrs.) Usha Goswami, Scientist 'F' (Retd.), NIO, Goa; Prof. Bechan Lal, Professor, Department of Zoology, Banaras Hindu University, Varanasi and Dr. Madan Mohan, Assistant Director General (Marine Fisheries), ICAR, New Delhi participated as expert members of the RAC. Dr. J.K. Jena, Director, ICAR-NBFGR, Lucknow apprised the RAC about the Institute's achievements in various fields during last one year. The Heads of the Divisions and In-charges of Kochi center and Chinhat unit also gave

RAC meeting being chaired by Prof. W. Vishwanath

presentations on the significant achievements under different projects of the respective divisions/units. The RAC reviewed progress of all the ongoing research programmes of the Institute and provided significant inputs to improve the research programmes.

Annual Institute Research Committee (IRC) meeting

The annual Institute Research Committee (IRC) meeting for the year 2013-14 was held at ICAR-NBFGR, Lucknow during 9-11 April, 2014 under the Chairmanship of Dr. J.K. Jena, Director. Member Secretary, IRC, Dr. K.K. Lal welcomed the Chairman and Members of IRC. In his introductory remarks, the IRC Chairman greeted the new scientists who have joined the Institute and observed the good opportunity of learning, available to them during the course of meeting. He appreciated that many important workshops/consultations/ trainings and other academic activities were organized at the institute during the year 2013-14. After chairman's address, progress reports of projects were presented by the respective PIs. After presentation of progress report of on-going research projects, the new concept proposals

Annual IRC meeting being chaired by Dr. J.K. Jena

for the research projects were presented. After each project presentation, peer review reports compiled and commented upon by PME cell were also put up before the house. In principal the projects were accepted for further formulation incorporating the suggestions and full presentation in the follow-up IRC. Another meeting of the IRC was held on 12 May, 2014 for final presentations of the new project proposals. Detailed discussions were held on the proposals and seven new project proposals were approved.

New NGS facility established at ICAR - NBFGR, Lucknow

Dr. S. Ayyappan, Secretary, DARE and Director General, ICAR inaugurated 'ATGC' - Advanced Genomics and Transcriptomics Sequencing facility on 29 June, 2014 at the Institute. This facility houses third

Inauguration of the new NGS facility by Dr. S. Ayyappan, Secretary, DARE and DG, ICAR

generation sequencer (Pac Bio) and was financially supported by NAIP. Dr. S. Solomon, Director, ICAR-Indian Institute of Sugarcane Research, Lucknow; Dr. Vishal Nath, Director, ICAR-National Research Centre for Litchi, Muzaffarpur; Dr. J.K. Jena, Director, ICAR-NBFGR; and scientists and staff of ICAR-NBFGR were present at the occasion. Dr. Jena highlighted that the high throughput sequencing facility established at ICAR-NBFGR is a single-molecule, real-time

Dr. S. Ayyappan seeing the functioning of the NGS facility

Inauguration of new infrastructure facilities by Dr. B. Meenakumari, DDG (Fy.), ICAR

sequencing system and provides extra-long read lengths, that simplify and improve genome assembly.

In his address, Dr. Ayyappan said that the Council has identified genomics as one of priority areas to be addressed during the XII Five Year Plan. He also added that the demand for high-value commodities including fish is increasing faster than food-grains and emphasized that their future increase in production has to come through mainly productivity enhancements, that will require technological innovations and applications. He applauded the efforts taken by the Institute to set up this state of art facility and added that this can be useful for not only fish genomic studies, but for researchers working in other fields of agriculture including plants, animals, micro-organisms, insects, etc. On this occasion, Dr. Ayyappan also released various institute publications.

Catfish Hatchery, Fish Farmers Consultation Center and other infrastructure facilities established

A catfish hatchery and a fish farmers consultation center, were established at the Institute. Dr. B. Meenakumari, DDG (Fisheries), ICAR, New Delhi inaugurated these facilities at the ICAR-NBFGR farm complex on 13 August, 2014. Dr. Meenakumari while congratulating the ICAR-NBFGR expressed that the newly established facilities are a timely addition to the Bureau's infrastructure and will help the Institute in benefiting the ultimate stakeholders. On this occasion, Dr. J.K. Jena, Director, ICAR-NBFGR, Lucknow briefed the DDG on various activities undertaken by the NBFGR for the benefit of fish farmers.

An Education centre on ornamental fishes was established for spreading awareness about ornamental fish diversity of the country to students, farmers and general public. Green-house cover was developed over 12 cement tanks at the Institute fish farm.

Celebration of Independence Day

The Institute celebrated the Independence Day with full fervor and gaiety. Dr. J.K. Jena, Director, ICAR-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 and sought whole-hearted support from the staff members. The occasions were followed with cultural programme in which large number of children of the NBFGR family participated.

Independence Day Celebration

Hindi Pakhwada observed

The Institute observed a Hindi Pakhwada during 15-29 September, 2014 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 in a function. On this occasion Dr. Subhas Pani was the Chief Guest and

Hindi Pakhwada function

two reputed poets of the city were Guests of Honour who spoke on importance of Hindi language and recited their poems. Mr. Ram Sakal, Personal Assistant won the prize for the Best Hindi Competitors – 2014.

Republic Day Celebrated

A flag hoisting ceremony was observed on the Republic Day on 26 January, 2015. 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 ICAR-NBFGR during the year 2014 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. The sports meet was also

Republic Day Celebration

organized for the staff and their children in which several events were held. The winners were given trophies by the Director, Dr. J.K. Jena.

Swachh Bharat Abhiyan

The staff of the Institute actively participated in the cleaning drive initiated by the Hon'ble Prime Minister of India. In this drive, the ICAR-NBFGR staff cleaned unwanted vegetation and debris both from outside and inside of the ICAR-NBFGR campus. Two large fields of the campus were turfed with good quality lawn grass after leveling and cleaning of wild vegetation. The campus of the Institute was provided with good number of dust-bins both in open and covered areas of the buildings.

TRAINING AND EXTENSION ACTIVITIES

Training Workshop on Freshwater Fish Taxonomy in North-Eastern Region

NBFGR under its participatory programme on exploration and characterization of fish germplasm resources and indigenous knowledge in North-Eastern Region of India, oganised a training workshop on freshwater fish taxonomy in collaboration with Manipur University, Manipur at Imphal during 22-26 September, 2014. The programme was inaugurated by Prof. H.N.K. Sarma, Vice-Chancellor, Manipur University, Imphal where as Dr. J.K. Jena, Director, ICAR-NBFGR, Lucknow was the Guest of Honour. Prof. N. Irabanta Singh, Dean, School of Life Sciences, Manipur University, Imphal presided over the inaugural function. A total of 50 participants including NBFGR's collaborating research partners from the North east region, scientists from NBFGR and other ICAR fisheries institutes and research scholars of Manipur University participated in the programme. The training workshop was led by Prof. W. Vishwanath, Department of Life Sciences, Manipur University, Manipur and his team who have developed internationally renowned expertise in freshwater fish taxonomy. The programme included theoretical lectures, on concepts, methodologies/ protocols of fish germplasm exploration and identification and preservation, as well as, demonstrations and hands-on experience on taxonomic identification of freshwater fishes. On this occasion, a manual was also brought out by Prof. Viswanath and his team on freshwater fish taxonomy for identification of fishes of north-east India.

Prof. H.N.K. Sarma, Vice-Chancellor, Manipur University delivering the inaugural address

DFAT-PSLP Training Workshop on Safe Water

A DFAT-PSLP Training Workshop on 'Safe Water' was organized by ICAR-NBFGR; CSIR-IITR, Lucknow and CSIRO, Australia at ICAR-NBFGR Lucknow during 23-27 March, 2015. The workshop was organized to build capacity of the scientists involved in environmental and toxicological research for safeguarding availability of clean water for the future. The workshop was inaugurated by Dr. Jenny Stauber, Chief Research Scientist, CSIRO Land and Water, Australia. Dr. J.K. Jena, Director, ICAR-NBFGR, Lucknow while welcoming the guests and the participants, opined that the workshop is important for the existence of aquatic organisms and is directly related to global fisheries production. Dr. Stauber emphasized that major challenge in the present scenario is to secure water and maintain its quality by protecting it from industrial and domestic discharges to defend our environment. Dr. Anu Kumar, Principal Research Scientist and Project Leader, CSIRO, Land and Water, Australia expressed that the collaboration would be of mutual interest for both the India and Australia. Dr. Rai Kookana, Senior Principal Research Scientist, Dr. Jason Kirby, Senior Research Scientist from CSIRO, Australia were also present during the

Participants of DFAT-PSLP training workshop

occasion. A total of 23 participants from across the country joined the program. The 5-days training-workshop included lectures, demonstrations and hands-on practicals.

Short Course on Molecular Markers and Population Genomics

An ICAR sponsored short course on 'Recent Advances in Molecular Markers and Population Genomics' was organized by ICAR-NBFGR during 10-19 March, 2015. The course was inaugurated by Dr. S. Bandopadhyay, Member, ASRB, New Delhi. A total of 20 participants from various disciplines including fisheries, zoology and biotechnology attended the course. The training was intended to give theoretical as well as practical insights into different techniques used in modern biology and population studies. The lectures from eminent researchers in the areas of molecular markers/genomics/ bioinformatics

Chief Guest Dr. S. Bandopadhyay and other dignitaries releasing the training manual

were organised. The course included the development of microsatellite and mitochondrial markers for assessing the extent of genetic diversity present in natural genetic resources, gene expression analysis, genome sequencing on PacBio RSII, microbial diversity and bioinformatics tools for genetic diversity quantification. The demonstrations and hands on sessions were organized to substantiate information gained. The programme aimed to develop trained man-power in the field of biotechnology and population genetics/genomics. The course also exposed participants to recent advances in sequencing technology. A training manual was also released by the dignitaries.

Training programme on Development of Fish Cell Lines for Viral Disease Diagnosis

A training programme on 'Development of Fish Cell Lines for Viral Disease Diagnosis' was organized during 23-28 February, 2015. A total of 15 participants

Chief Guest Dr. A. Gopalakrishanan inaugurating the training by lighting the lamp

from various research organizations from all over India were participated in the training programme. Dr. A. Gopalakrishnan, Director, ICAR-CMFRI, Kochi the Chief Guest of the inaugural function mentioned the importance of fish cell culture for viral diagnosis and other applications. The training programme covered various aspects like different cell culture methods, characterization of cell lines using molecular and cytological means and virus isolations using cell lines and its storage and retrieval. The training also included the molecular approaches for viral pathogen identification and characterization.

Training programme on Molecular Markers

A training programme on 'Molecular Markers' used in fisheries research was organized during 9-14 February, 2015. A total of 18 participants from various research and academic institutions across the country participated in the programme. The training programme covered both the theoretical and practical exposure to various markers used in fisheries research and included topics like fish sample collection, DNA isolation, PCR standardization for various markers,

A practical training session on molecular markers

electrophoresis, as well as, the softwares used for genetic data analysis.

Training programme on 'Soil and Water Quality Management in Aquaculture Ponds'

A six-days training programme on 'Soil and Water Quality Management in Aquaculture Ponds' was jointly organized by the ICAR-NBFGR, Lucknow and ICAR-CSSRI Regional Research Centre, Lucknow during 17-22 February, 2015. Twenty six officers of the State Fisheries Department, Uttar Pradesh attended the programme which was sponsored by the Department of Fisheries, Govt. of U.P.

Inaugural function of the training

Tribal Sub-Plan (TSP) Scheme Activities

TSP Team: L.K. Tyagi (Nodal Scientist), Sharad Kr. Singh, A.K. Yadav, S.K. Singh and A.S. Bisht

Under the Tribal Sub Plan scheme of the Govt. of India, the Institute has undertaken a variety of extension programmes and activities for the socio-economic development of tribal people in various areas of the country. These activities are aimed at facilitating tribal development through fisheries-based enterprises by providing scientific inputs and are coordinated by a team of scientists and technical officers.

The activities include: (i) Imparting training to tribal farmers on fish culture and conservation, (ii) Establishing and popularising fish seed production enterprises among tribal areas by installing portable FRP carp hatcheries at tribal people's places in various states and (iii) Organising awareness programmes in tribal areas on fish conservation. During the year under report, the following activities were undertaken:

(i) Training of Tribal Farmers on 'Fish Farming and Conservation'

The Institute, at its Aquaculture Research & Training Unit (ARTU), Chinhat, organized ten short-term training programmes, under the TSP scheme, for tribal fish farmers. The participants were from seven districts of U.P. (Allahabad, Sonbhadra, Jhansi, Lalitpur), M.P. (Tikamgarh, Sagar) and Uttarakhand (Udhamsinghnagar) states. A total of 282 tribal fish farmers were imparted training on 'Fish culture and Conservation' in these training programmes (Table 17). 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 arranged to expose the trainees on various fisheries activities.

Table 17. Details of training programmes organised for tribal and other fish farmers

S. N.	Topic	Duration	Participants
1	Fish culture and conservation for tribal farmers of Lalitpur (U.P.)	24-26 June 2014	30
2	Fish culture and conservation for tribal farmers of Sonbhadra (U.P.)	05-07 August 2014	22
3	Fish culture and conservation for tribal farmers of Udhamsinghnagar (Uttarakhand)	19-21 August 2014	30
4	Fish culture and conservation for tribal farmers of Jhansi (U.P.)	17-19 Sept 2014	31
5	Fish culture and conservation for tribal farmers of Tikamgarh (M.P.)	28-30 October 2014	29
6	Fish culture and conservation for tribal farmers of Sonbhadra (U.P.)	10-12 December 2014	28
7	Fish culture and conservation for tribal farmers of Udhamsinghnagar (Uttarakhand)	15-17 January 2015	19
8	Fish culture and conservation for tribal farmers of Lakhimpurkhiri, (U. P.)	17-19 March, 2015	30
9	Fish culture and conservation for tribal farmers of Sagar (M.P.)	23-25 March, 2015	28
10	Fish culture and conservation for tribal farmers of Sonbhadra (U.P.)	26-28 March 2015	35
11	Aquaculture Technologies & Productivity Enhancement for farmers of Gopalganj (Bihar)	09-13 February, 2015	22
Total			**304**

Group of tribal women trainee farmers with Director and staff of ICAR-NBFGR

A tribal training farmer sharing experiences

Besides, a training programme on 'Aquaculture Technologies & Productivity Enhancement' was also organised for progressive fish farmers of Bihar state under the Agriculture Technology Management Agency scheme in which 22 farmers were trained at ARTU, Chinhat, Lucknow.

(ii) Establishing and popularising fish seed production enterprise among tribal areas

With the objective of facilitating tribal development through fish (carps) seed production enterprise, the Institute has initiated the task of establishing portable FRP carp hatcheries at tribal people's places in various states as a technological intervention. The hatchery unit once installed at the identified tribal people's/group's place would involve other tribal people of the respective villages and would serve as a means for creation and enhancement of livelihood opportunities and income of tribal people in the identified areas. Under the scheme, the Institute has undertaken to provide and install a FRP carp hatchery unit alongwith its accessories at the indentified sites in tribal areas and give demonstration and both on-campus and on-site training to the tribal beneficiaries of the village/area on operating the hatchery unit. The Institute also take commitment to provide overall technical guidance for any technical problem faced by the beneficiaries in running the unit.

During the year under report, the Institute TSP team visited and surveyed 11 villages in U.P. (Sonebhadra and Lalitpur districts), M.P. (Sagar district) and Chhattisgarh (Dhamtari and Kanker districts). Three locations were identified for installation of the fish seed production unit. The tribal people from the selected locations and nearby villages were imparted on campus training at the Institute's Aquaculture Research & Training Unit (ARTU) at Chinhat, Lucknow. On-site technical instructions were also given to the identified beneficiaries by the Institute team during field visit for making necessary initial arrangements at the site for installation of hatchery units. After installation of the hatcheries, on-site demonstration and training in operating the hatchery unit will be imparted to the tribal beneficiaries in the coming breeding season.

Field visit by ICAR-NBFGR team in tribal areas to identify sites for establisihing fish seed production unit

(iii) Awareness Workshop on Fish Conservation

A workshop on fish conservation awareness was organized in Chalakudy, Thrissur District Kerala, on 30th December, 2014 by PMFGR Center, Kochi of ICAR-NBFGR in collaboration with the World Wildlife Fund for nature (WWF). The workshop featured presentations on indigenous fish resources, conservation of habitats and opportunities for livelihood enhancement through

Awareness workshop on fish conservation at Chalakudy, Kerala

small scale aquaculture projects. The workshop was attended by 30 residents of 8 tribal hamlets within the Vazhachal forest division, Chalakudy district, Kerala. Range Officer of the division inaugurated the event and emphasised the importance of conserving freshwater biodiversity and the role tribal communities play in this. Mr. Tiju Thomas, co-ordinator, WWF-India, stressed on the need for conservation and the role of aquaculture in providing sustainable livelihoods. Dr. V.S. Basheer, ICAR-NBFGR provided an overview of the freshwater fish resources of the Western Ghats, summarized the threats facing freshwater biodiversity and outlined NBFGR's efforts to conserve this natural wealth. Dr. C.P. Shaji, former Principal Scientific Officer of the Kerala Biodiversity Board and a renowned expert on fish taxonomy spoke at length on freshwater fish diversity in the Chalakudy River. The participants expressed an interest in developing programmes for captive breeding, culture and ranching of important indigenous species for conservation and sustainable utilization.

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 2014-15:

Exhibition organised on the occasion of the 10th Indian Fisheries and Aquaculture Forum at Lucknow during 12-15 November, 2014.

Science & Technology Pavillion at Lucknow Mahotsava during 26 November - 7 December, 2014.

International Symposium, MECOS-2 held during 2-5 December, 2014 at Kochi, Kerala organized by Marine Biological Association of India, Kochi.

Dr. E.G. Silas, Former Director, ICAR-CMFRI, Kochi and Former Vice Chancellor, Kerala Agricultural University visiting NBFGR stall during Aqua Aquaria India 2015

Aqua Aquaria India held during 20-22 February, 2015 organized by MPEDA, Kochi at Vijayawada.

National Biodiversity Expo organized during 23-27 February 2015 organized by Kerala Biodiversity Board, Kerala.

Agro-Tech 2015 at ICAR-IISR, Lucknow during 12-14 March, 2015.

Fish Seed Production

The production of quality fish seed of carps was continued and a total of 417.0 lakh spawn of Indian major carps and exotic carps were produced. Revenue of Rs. 4,07,255/- was earned from the fish seed sale.

AWARDS AND RECOGNITIONS

Dr. N.S. Nagpure, Principal Scientist and Head, MBB Division was conferred with the Honorary Fellowship 2014 Award by the Indian Academy of Environmental Sciences, Haridwar. Dr. Nagpure was also conferred with the Dr. D.K. Belsare Medal 2014 by the Zoological Society of India, Bodh Gaya.

Dr. J.K. Jena, Director, ICAR-NBFGR receiving the Second Prize awarded to Institute Hindi Magazine, Matsya Lok

NBFGR Annual Hindi Magazine 'Matsya Lok' 2014 was awarded the Second Prize by the 'Nagar Rajbhasa Karyanvayan Samiti, Luck now, amongst the magazines brought out by central government offices located at Lucknow. It was co-edited by Dr. Akhilesh K. Mishra, Technical Officer and Dr. L.K. Tyagi, Sr. Scientist.

Dr. A.K. Pandey, Principal Scientist was Conferred with the Eminent Scientist Gold Medal of the Society of Life Sciences, Satna, Madhya Pradesh.

Dr. L.K. Tyagi, Sr. Scientist was conferred with the Young Scientist Award 2014 of the Indian Society of Extension Education, New Delhi on 28 February, 2015 at Gwaliar. Dr. Tyagi was also conferred with the Shanti Prasad Goel Award 2015 of the Society for Community Mobilization for Sustainable Development, New Delhi on 9 January, 2015 at Jammu.

Dr. L.K. Tyagi, Sr. Scientist receving the Young Scientist Award 2014 of the Indian Society of Extension Education, New Delhi

Drs. Mahender Singh and M. Goswami, Sr. Scientists were conferred with the Fellowship Award 2015 by the Bioved Research Society, Allahabad.

Dr. Mahender Singh, Sr. Scientist receving the Fellowship Award 2015 of Bioved Research Society, Allahabad

Dr Ajay Kumar Singh conferred the Dr. S.R. Bhargava Medal 2015 by the Bioved Research Society, Allahabad.

Dr. K.K. Bineesh, A. Gopalakrishnan, V.S. Basheer, Akilesh, N.G.K. Pillai and J.K. Jena received Best Poster Award for the poster titled 'Assessing Marine Fish Diversity of India - A Molecular Approach' during Swadeshi Science Congress hosted by M.G. University, Kottayam, Kerala.

LIST OF PROJECTS

Institutional Projects

S. No.	Project Title	Personnel	Period
Molecular Biology & Biotechnology Division			
1	Population genomics of *Clarias magur* based on Restriction site Associated DNA (RAD) markers	Mahender Singh (PI), N.S. Nagpure, Ravindra Kumar, Ajey Kumar Pathak, Murali, S. and Ajey Kumar Singh	April 2014 - March, 2017
2	Development of surrogate broodstock for propagation of valuable fish germlines	S.K. Majhi (PI), Basdeo Kushwaha and S. Raizada	April, 2014 - March, 2017
3	Development of an in vitro toxicity assessment system for aquatic pollutants	Mukunda Goswami (PI), N.S. Nagpure, Sullip K. Majhi and Akhilesh K. Mishra	April, 2014 - June, 2017
Fish Conservation Division			
4	Outreach activity on Fish Genetic Stocks (Phase II)	J.K. Jena (Coordinator) Rajeev Kumar Singh (PI), Vindhya Mohindra, T.T. Ajith Kumar, Sangeeta Mandal , Santosh Kumar and J.K. Jena	April, 2014 – March, 2017
5	Signatures of natural selection and genomic diversity in important freshwater fish species, *Tor putitora* and *Clarias magur*	Vindhya Mohindra (PI), Santosh Kumar and Trivesh Mayanker	April, 2014 –March, 2017
6	Establishment of mapping and marker panel for first generation linkage map in Indian catfish, *Clarias magur*	Rajeev Kumar Singh (PI), T.T. Ajith Kumar and Santosh Kumar	April, 2014 –March, 2017
7	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 and Sanjay Kr. Singh	April, 2012 – March, 2015
8	Documentation and development of passport information of exotic food and ornamental fishes in India	S. Raizada (PI), Peyush Punia, T.T. Ajithkumar, Rejani Chandran and Rajesh Dayal	April, 2014 - March, 2017
9	Participatory programme on exploration and characterization of fish germplasm resources and indigenous knowledge in North-eastern region of India	K.K. Lal (Coordinator till August, 2014), L.K. Tyagi (Coordinator w.e.f. September, 2014), Vindhya Mohindra and Rajeev Kr. Singh (Co-coordinators)	October, 2012 – March, 2017
Fish Health Management Division			
10	Development of an immune marker and understanding host-*Aphanomyces invadans* interaction using macrophage cell line	Neeraj Sood (PI), P.K. Pradhan and M. Lini	April, 2014 –March, 2017
11	Exploration of finfish parasites of river Gomti particularly protozoans and monogeneans through conventional and molecular techniques	Rehana Abidi (PI), S.M. Srivastava and Amar Pal	April, 2014 – March, 2017
Fish Taxonomy and Resources Section			
12	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

Peninsular and Marine Fish Genetic Resources Centre, Kochi			
13	Genetic stock - structure analysis of *Parapenaeopsis stylifera* and *Scomberomorus commerson* along the Indian coast using molecular markers.	P.R. Divya (PI), V.S. Basheer, A. Kathirvelpandian and Mog Lebrachai Chowdhury	April, 2013 –March, 2016
14	Exploration of the Western Ghats Wetlands for Indigenous fishes and extent of invasion of exotic fishes.	V.S. Basheer (PI), T. Raja Swaminathan, P.R. Divya, A. Kathirvelpandian and Charan Ravi	April, 2013 – March, 2016

Externally funded Projects

Sl. No.	Project Title	Personnel	Funding agency	Period
1	National Surveillance Programme for Aquatic Animal Diseases Sub-project: Surveillance of freshwater fish and shellfish diseases in Uttar Pradesh and Haryana Sub-Project: Surveillance programme for aquatic animal diseases of ornamental fishes in the states Kerala and Tamil Nadu	J.K. Jena (Coordinator) Neeraj Sood (CPI), P.K. Pradhan, P. Punia and Rehana Abidi P.K. Pradhan (PI), Neeraj Sood and Peyush Punia T. Raja Swaminathan (PI) and V. S. Basheer	NFDB NFDB NFDB	April, 2013 – March, 2018 April, 2013 – March, 2018 April, 2013 – March, 2018
2	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 – June, 2014
3	Centre for Agricultural Bioinformatics (CABin): Fisheries domain	N.S. Nagpure (CCPI), S.P. Singh, Ravindra Kumar, Mahender Singh, A.K. Pathak and Murali S.	ICAR -IASRI	February, 2015 – March, 2017
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	Establishment of a National Repository for conservation and characterization of fish cell lines at NBFGR, Lucknow	M. Goswami (PI) and N.S. Nagpure	DBT, Govt. of India	November, 2010 – November, 2014
6	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 Gusheinzed Waikhom (PI), T. Shantibala (IBSD, Imphal)	DBT, Govt. of India (Twining Project for NE)	March, 2011 – June, 2014
7	Characterization and DNA barcoding of endemic fishes of North east India	Mahender Singh (PI, NBFGR), N.S. Nagpure and W. Vishwanath (PI, Manipur University, Imphal)	DBT, Govt. of India (Twining Project for NE)	November, 2012 – November, 2015
8	Identification and evaluation of reproductive traits and genetic structure of *Ompok bimaculatus* in India	U.K Sarkar (PI), Ravindra Kumar and Abha Mishra, BRAU, Lucknow	DBT, Govt. of India	September, 2011 – December, 2014

9	Development of novel microsatellites in *Channa* species (Channidae: Perciformes) from North East for conservation genetics	Rajeev Kumar Singh (PI), L.K. Tyagi (ICAR-NBFGR) and A.S. Barman (College of Fisheries, CAU, Lembuchera, Agartala)	DBT, Govt. of India	April, 2012 – March, 2015
10	DNA Barcoding of Marine finfishes and shellfishes	V.S. Basheer (PI) and J.K. Jena and	MoES- CMLRE, Govt. of India	November, 2012 – October, 2017
11	Understanding of molecular pathogenesis of epizootic ulcerative syndrome (EUS) in fish and development of newer strategies to combat EUS	P.K. Pradhan (PI), Neeraj Sood, Chandan Debnath and Lopamudra Sahoo (ICAR Complex, Tripura)	DBT, Govt. of India	May, 2012 – April, 2015
12	Stock characterization, captive breeding, seed production and culture of hilsa (*Tenualosa ilisha*)	Vindhya Mohindra (CCPI), Kuldeep K. Lal (Up to August, 2014), Rajeev K. Singh, Sangeeta Mandal and J. K. Jena	NFBSFARA, ICAR	November, 2012 – October, 2016
13	Genetic Stock Structure Analysis of the Indian mackerel, *Rastrelliger kanagurta* from Indian waters using Microsatellite Markers	J.K. Jena (PI), Divya P.R. and V.S. Basheer	FAO/ BOBLME	April, 2013 – March, 2014
14	Whole genome sequencing and development of allied genomic resources in two commercially important fish- *Labeo rohita* and *Clarias batrachus*	J.K. Jena (Coordinator) N.S. Nagpure (PI), Basdeo Kushwaha, Ravindra Kumar and Mahender Singh	DBT, Govt. of India	October, 2013 – October, 2016
15	Fish diversity of Ramgarh and Bakhira Lake: comparison of present status with pristine data for conservation and sustainable utilization	A.K Pandey (PI)	UPSBB	February, 2013 –March, 2015
16	Neuroendocrine regulation on ovarian maturation in the giant freshwater prawn, *Macrobrachium rosenbergii*	A.K Pandey (PI)	UPCST	August, 2013 - July, 2016
17	Risk and benefit assessment of an illegally introduced fish species *Piaractus brachypomus,* pacu in India	Peyush Punia (PI)	NFDB	October, 2013 – March, 2015

PARTICIPATION IN SEMINARS/ SYMPOSIA/ WORKSHOPS/ TRAININGS/ MEETINGS

Abroad

Dr. J.K. Jena, Director visited Trondheim, Norway as a Member of the Indian delegation of Department of Animal Husbandry, Dairying and Fisheries, Govt. of India to attend 'Nor Fishing 2014' and 'Joint Technical Committee Meeting on Fisheries' during August 18-22, 2014. He also participated in the Meeting to discuss 11th Asian Fisheries and Aquaculture Symposium at Bangkok, Thailand during 19-22 January, 2015.

Dr. J.K. Jena, Director; Dr. V.S. Basheer, Principal Scientist and Dr. Divya P.R., Sr. Scientist participated in the BOBLME Indian Mackerel Genetics Data Analysis Workshop at Phuket, Thailand during 17-18 February, 2015.

Dr. V.S. Basheer, Principal Scientist & SIC PMFGR Center, Kochi underwent eight weeks training programme under the AusAID Project 'Safe water for the future through the Indo Oz Network' at CSIRO, Australia during 20 May - 19 July, 2014.

Dr. N.S. Nagpure visited CSIRO Land and Water laboratories at Adelaide and Sydney, Australia during 12-19 July, 2014 under the AusAID project 'Safe water for the future through Indo-Oz Network'.

Dr. T. Raja Swaminathan, Senior Scientist underwent 45 days training programme on 'Stem cell research (Fisheries)' at IPS core facility, University of Nantes, France, during 01 March to 15th April 2014.

In India

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

- Meeting of the 'Fish Genomics' project at ICAR - IASRI, New Delhi on 15 April, 2014.
- 74th Meeting of the Executive Council of the West Bengal University of Animal & Fishery Sciences, Kolkata on 26 April, 2014.
- Interactive Conference of the Vice Chancellors and Directors' convened by Secretary, DARE & DG ICAR at NASC Complex, New Delhi on 28 April, 2014.
- Participation in the State Level Workshop on the Draft Comprehensive Fisheries Policy for Odisha State at Bhubaneswar on 30 April, 2014.
- Review Meeting of the project 'Outreach Programmes on Fish Feed Development, Fish Genetic Stock Characterization and Nutrient Profiling of Fishes' on 9 May, 2014 at New Delhi.
- Workshop on 'Impact of Capacity Building Programs' under NAIP during 6-7 June, 2014 at NASC, Pusa, New Delhi.
- 5th Meeting of the National Advisory Board on Management of Genetic Resources on 16 June, 2014 at ICAR-NBPGR, New Delhi.
- XXIIth Meeting of the ICAR Regional Committee - II on 27 June, 2014 at ICAR-CIFRI, Barrackpore.
- Technical Advisory Committee (TAC) Meeting for the National Disease Surveillance Programme for Aquatic Animal Disease held on 30 June, 2014 at DAHDF, New Delhi.
- Directors' and VCs' Conference at NASC Complex, New Delhi on 29-30 July, 2014.
- Meeting at NCAP of the Directors of all the ICAR Bureaus with regard to Development of Performance Indicator for the Institutes on 31 July, 2014.
- Brain Storming Session on 'Insects Related to Veterinary and Fisheries Sciences' at ICAR - NBAII, Bangalore and Chaired the session on Insects related to Fisheries on 2 August, 2014.
- Brainstorming Workshop on "Prioritization of Animal Bioresources Research Areas: Fish and Insect" at IBSD, Imphal on 6-7August, 2014.
- Review Meeting of the Project on 'National Surveillance Programme for Aquatic Animal Diseases' and gave an invited presentation on 'Aquatic Biodiversity Management and Role of NBFGR' to the students of B.F.Sc. and M.F.Sc. and other interactions at WBUA&FS, Kolkata on 8 August, 2014.
- 29th Meeting of the Executive Council on Access and Benefit Sharing at NBA, Chennai on 1 September, 2014.

- Training Programme for the New Members of Evaluation Committee under NPOP in Framing the Organic Aquaculture Standards at APEDA, New Delhi on 15 September, 2014.
- 2nd Coordination Committee Meeting of the NFBSFARA Project 'Stock Characterization, Captive Breeding, Seed Production and Culture of Hilsa' at Fisheries Division, ICAR, New Delhi on 18 September, 2014.
- Brainstorming Session on 'Reservoir Fisheries Development in India: Management and Policy Options' at NASC Complex, New Delhi on 19 September, 2014.
- 4th Meeting of the 'Scientific Panel of Fish and Fishery (SP-FFP)' under the Chairmanship of DG, ICAR, New Delhi at FSSAI, New Delhi on 19 September, 2014.
- Consultation Workshop on Impact Analysis of Agricultural Research, Education and Extension in U.P. during last 25 Years and chaired a session at IISR, Lucknow on 20 September, 2014.
- 79th meeting of the Executive Council and 9th Convocation of the West Bengal University of Animal & Fishery Sciences at WBUAFS, Mohanpur, Nadia on 29 January, 2015.
- 12th Agricultural Science Congress 2015 at ICAR-NDRI, Karnal during 3-4 February, 2015.
- 1st Research Council Meeting cum Scientific Workers Conference of Tamil Nadu Fisheries University at Fisheries College and Research Institute, Thoothukudi on 13 March, 2015.

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

- Meeting of the Management Committee of Kanpur Zoological Garden on 29 August, 2014.
- Delivered a talk on "Technical aspects of fisheries, adoption of new and innovative technologies for sustainable aquaculture to NABARD officials on 28 August, 2014.
- National Seminar on "Challenges for sustainability of natural resources and environment with emphasis on aquatic ecosystem for livelihood security" at the College of Fisheries, Pantnagar during 10-12 October, 2014 and presented a paper.
- International Symposium on Reproductive Biology & Comparative Endocrinology at the Zoology Department, BHU during 25-27 February, 2015 and presented a paper.
- Haryana Agri-Leadership Summit 2015 as aquaculture expert on 10 March, 2015 held at Gurgaon, Haryana.

Dr. N.S. Nagpure, Principal Scientist and Head, MBB Division participated in the National Seminar on 'Threats to Biodiversity: Impact of Developmental Projects and Climate Change' and 25th All India Congress of Zoology from 17th - 19th November, 2014 at Gurukula Kangri University, Hardwar.

Dr. Vindhya Mohindra, Principal Scientist participated in the Interaction Meeting of the NFBSFARA project on Stock characterization, captive breeding, seed production and culture of Hilsa convened by the Secretary DARE & DG, ICAR on 27 July, 2013 at CIFRI, Barackpore and Consultation on National Plan of Action for Hilsa on 8 August, 2013 chaired by Joint Secretary, DAHDF, Ministry of Agriculture at New Delhi.

Dr. V.S. Basheer, Principal Scientist participated/ delivered lectures in the following:

- NAAS silver jubilee national symposium on Indian Fisheries and Aquaculture during 21-22 October, 2014.
- 'Safe water for Indian Ocean RIM countries workshop at New Delhi during 18-20 November, 2014.
- 31st meeting of the Executive Council on ABS at National Biodiversity Authority, Chennai on 9 March, 2015.

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

- IUCN International Symposium on River Biodiversity; Ganga Brahmaputra Meghna river system at Patna University during 4 - 6 April, 2014.
- Conference on International Biodiversity Day organized by Uttar Pradesh State biodiversity Board on 22 May, 2014 at Lucknow.

Dr. Ravindra Kumar, Principal Scientist participated in the following:

- Workshop on 'Redefining the Priorities in the National Action Plan for Management of Genetic Resources' during 23-24 December, 2014 at NASC Complex, New Delhi.
- International Workshop on 'Fish Genomics' during 19-21 January, 2015 at ICAR- CIFE, Mumbai.
- Workshop on 'Training Need Assessment for HRD Nodal Officers of ICAR' at ICAR-NAARM, Hyderabad on 26 February, 2015.

Dr. Basdeo Kushwaha, Principal Scientist participated in the following:

- ICAR sponsored Short Course on "Metagenomics: Role of Next Generation Sequencing and Bioinformatics" held at Department of Animal Biotechnology, Anand Agricultural University, Anand during 6-15 October, 2015.
- XII Agricultural Science Congress at ICAR-NDRI, Karnal during 3-6 February, 2015.
- Workshop on 'Training Need Assessment for HRD Nodal Officers of ICAR' at ICAR-NAARM, Hyderabad on 26 February, 2015.

Dr. A.K. Pandey, Principal Scientist participated in the following:

- National Conference on Water, Demand, Sustainability and Future at Allahabad during 03-04 May, 2014.
- National Seminar on Island Biodiversity organized by Uttar Pradesh State Biodiversity Board at Lucknow on 22 May, 2014.
- National Seminar on Biodiversity Conservation for Human Welfare at Department of Zoology, Government Autonomous Post-Graduate College, Satna during 24-25 May, 2014.
- National Conference on Environmental Protection and Sustainable Development organized by Sky Institute, Lucknow during 05-06 June, 2014.
- Second International Conference on Animal and Dairy Sciences organized by OMICS Group at Hyderabad during 15-17 September, 2014.
- National Conference on Challenges for Sustainability of Natural Resources and Management with Emphasis on Aquatic Ecosystem for Livelihood Security at College of Fisheries, Pantnagar during 10-12 October, 2014.
- International Symposium on Biodiversity: Status, Utilization and Impact of Challenging Climate Conditions organized by Department of Applied Animal Sciences, Dr. B.R. Ambedkar (Central) University, Lucknow during 30-31 October, 2014.
- National Seminar on Current Trends in Biological Sciences: Advances and Challenges at Department of Zoology, Janta Post-Graduate College, Bakewar (Etawah) during 13-14 December, 2014.
- National Conference on Challenges of Biological and Environmental Sciences in 21st Century at Gorakhpur during 19-20 December, 2014.
- Satellite Symposium on Environment and Human Health organized in Department of Zoology, University of Lucknow, Lucknow on 17 January, 2015.
- National Conference on Environmental Degradation and Global Health organized by Environment & Social Welfare Society, Khajuraho (Madhya Pradesh) during 01-02 February 2015).
- 17th Indian Agricultural Scientists & Farmers Congress on Agri-innovation for Enhancing Production and Rural Employment at Allahabad during 21-22 February, 2015.
- International Symposium on Reproductive Biology & Comparative Endocrinology at Banaras Hindu University, Varanasi during 25-27 February, 2015.
- National Conference on Biotechnology and Human Welfare: New Vistas at the Department of Biotechnology, V.B.S. Purvanchal University, Jaunpur, U.P. during 21-22 March, 2015.

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

- Consultation Workshop on Impact Analysis of Agricultural Research, Education and Extension in U.P. during last 25 Years at IISR, Lucknow on 20 September, 2014.
- 5th Global Symposium on Gender in Aquaculture and Fisheries on 14 November, 2014 at Lucknow and presented a selected paper.
- National Seminar on 'Sustainable Rural Livelihood: Technological and Institutional Perspective' at SKUAST, Jammu during 8-10 January, 2015 and presented a paper.
- National Seminar on 'Extension Innovations and Methodologies for Market-led Agricultural Growth and Development' at RVS Krishi Vishwavidhyalaya, Gwaliar during 26-28 February, 2015 and presented an invited lead paper.

Dr. T.T. Ajith Kumar, Sr. Scientist delivered two invited lectures in the Summer school on "Technological advancements in the seed production of marine finfish and shellfish" held at Regional Rentre of Central Marine Fisheries Research Institute, Mandapam, Tamil Nadu on 25 -26 August, 2014.

Dr. Sharad K. Singh, Sr. Scientist participated in 4th National Seminar on Innovative Saline Agriculture in Changing Environment at RVSKVV, Gwalior during 12-14 December, 2014. He also attended "XIIth Agricultural Science Congress" of National Academy of Agricultural Sciences, New Delhi at NDRI Karnal during 3-6 February, 2015 and presented paper.

Ms. Sangeeta Mandal participated in the CAFT training programme on 'Stock Assessment of Marine and Freshwater Fishery Resources' during 2-22 September, 2014 at ICAR-CIFE, Mumbai.

Ms. Rejani Chandran participated in the CAFT training programme on 'Advances in Omics Data Analysis: Learning By Example' at ICAR-IASRI, New Delhi during 3-23 December, 2014.

Dr. A. Kathirvelpandian, Scientist attended a training programme on 'Metagenomics-role of next generation sequencing and bioinformatics' organized by department of animal biotechnology, Anand Agricultural University, Anand, Gujarat during 6-15 October, 2014.

Dr. T. Raja Swaminathan, Dr. V.S. Basheer, Dr. Divya P.R. and L. Mog Chowdhury, attended International Symposium, MECOS-2 – Marine Ecosystems-Challenges and Opportunities organized by Marine Biological Association of India, Kochi at Kochi during 2-5 December, 2014.

Dr. N.S. Nagpure, Principal Scientist and Mr. Murali, S., Scientist participated in the first meeting of the Steering Committee of Network Project on Agricultural Bioinformatics and Computational Biology at ICAR-IASRI, New Delhi on 14 March, 2015.

Dr. T. Raja Swaminathan and Dr. Divya PR, Sr. Scientist attended ICAR winter school on 'Bioinformatics and its emerging dimensions in agriculture' at Bioinformatics Centre, College of Horticulture, Kerala Agricultural University, Thrissur, Kerala during 12 January-1 February, 2015.

Mr. L. Mog Choudhuri, Scientist attended 'International workshop on fish genomics' organised by ICAR-CIFE, Mumbai during 19-21 January, 2015.

Mr. Charan Ravi, Scientist attended ICAR summer school on 'Recent advances in marine biodiversity conservation and management' organized by ICAR-CMFRI, Kochi, during 16 February- 8 March, 2015. He also attended a training programme on 'Introduction to GIS' at NRSC, Hyderabad during 16-27 March, 2015.

Shri Subhash Chandra, Senior Technical Officer participated in one day training & awareness workshop on J-Gate@CeRA on 29 September, 2014 at National Agricultural Science Centre, Pusa, New Delhi.

PUBLICATIONS

International Journals

1. Ayyappan, S., J. K. Jena and A. Gopalakrishnan, 2014. Molecular tools for sustainable management of aquatic germplasm resources of India. *Agricultural Research*, 3(1): 1–21.
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Book chapters

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symposium on river biodiversity: Ganges Brahmaputra-Meghna river system, ecosystems for life, A Bangladesh–India initiative, International Union for Conservation of Nature. pp. 193-199.

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LIBRARY AND INFORMATION MANAGEMENT

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 175 books. Now, the library has the total collection of 7026 books and 2655 bound volumes of journals. The library has subscribed 13 international journals and 64 Indian journals. In addition to these, 38 journals were received on gratis/ exchange basis.

Library Automation

The existing LSEase, Version 5.0 Library Management Software was upgraded to Web Centric LSEase Library Management Software Package, Version 7.0. The Library is operating in fully automated environment. The various activities of library have been computerized using integrated library management software Libsys. The record of books, journals, maps, etc. were entered in the database. Barcoding of books, periodicals and maps for automated circulation 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. The users of the library extensively used the Consortium for e-Resources in Agriculture (CeRA) to access the full text online journals related to agriculture, fish and fisheries and allied subjects.

Technical Reports and Reprography Services

The library and documentation unit provided technical support to bring out departmental publications. This unit also attended to Questionnaires on Bureau's infrastructure and other facilities. The unit continued active reprography services. 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 2013-2014 and Newsletters to various institutions and organizations including Universities, State Fisheries Departments, FFDAs, Krishi Vigyan Kendra's, Entrepreneurs and Fish Farmers.

Agricultural Knowledge Management Unit

Agriculture knowledge management unit (AKMU) at ICAR-NBFGR aims to spread the information and knowledge of fisheries domain generated through various research activities by applying multimedia information and communication technologies. The unit also facilitates the use of information technology and tools in research activities of the Institute. Recently established High Performance Super Mini Computing (HPC) facility at AKMU is extending its computing services to other researchers at the Institute for carrying out genomics, trancriptomics and proteomic works on fishes. Besides, AKMU is engaged in managing the data and information of other activities like PERMISnet (Personnel Management Information System), PIMS (Project Information Management System) and Central Procurement Portal (CPPP) for tender uploading and publishing for wide publicity. The unit also manages the communication network of the Institute. GIS facility maintained at the unit increases the multifaceted work structure and provides ability in mapping and modeling fish and fisheries resources.

STAFF ACTIVITIES

1. Promotions

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

Scientists

Dr. V.S. Basheer, Sr. Scientist Pay Band Rs. Rs. 37400-67000+ RGP Rs. 9000/- to Principal Scientist Pay Band Rs. 37400- 67000+RGP Rs. 10000/- w.e.f. 05.07.15.

Dr. Rajeev Kumar Singh, Sr. Scientist from Pay Band Rs. 15600-39100+ RGP Rs. 8000/- to Pay Band Rs. 37400- 67000+RGP Rs. 9000/- w.e.f. 29.10.2013.

Dr. Mahender Singh, Sr. Scientist from Pay Band Rs. 15600-39100+ RGP Rs. 8000/- to Pay Band Rs. 37400-67000+RGP Rs. 9000/- w.e.f. 05.11.2013.

Dr. T. Rajaswaminathan, Sr. Scientist from Pay Band Rs. 15600-39100 + RGP Rs. 8000/- to Pay Band Rs. 37400- 67000+RGP Rs. 9000/- w.e.f. 22.11.2013.

Dr. (Mrs.) Divya P.R., Scientist to Sr. Scientist Pay Band Rs. 15600-39100+ RGP Rs. 8000/- w.e.f. 27.06.2014.

Technicals

Mr. Ved Prakash from Technical Officer to Sr. Technical Officer w.e.f. 01.01.2014.

Mr. R.K. Shukla from Sr. Technical Assistant to Technical Officer w.e.f. 01.01.2014.

Dr. Akhilesh Kumar Mishra from Technical Officer to Sr. Technical Officer w.e.f. 16.07.2014.

Dr. (Mrs.) Ranjana Srivastava from Technical Officer to Sr. Technical Officer w.e.f. 27.07.2014.

2. Recruitment

The following new staff members joined the Institute during the year under report:

Dr. Sharad Kumar Singh, Sr. Scientist on transfer from ICAR-CSSRI, Karnal w.e.f. 01.12.2014.

Ms. Rejni Chandran, Scientist w.e.f. 07.04.2014.

Ms. Mary Lini R., Scientist w.e.f. 07.04.2014.

Ms. Priyanka C. Nandanpawar, Scientist w.e.f. 07.04.2014.

Mr. Labrechai Mog Chowdhury, Scientist w.e.f. 08.04.2014.

Dr. Santosh Kumar, Scientist w.e.f. 08.04.2014.

Mr. Aditya Kumar, Scientist w.e.f. 08.04.2014.

Mr. Charan Ravi, Scientist w.e.f. 09.04.2014.

Mr. Murali S., Scientist w.e.f. 09.04.2014.

Mr. Trivesh Suresh Mayekar, Scientist on transfer from ICAR-CMFRI, Kochi w.e.f. 05.11.2014.

Mrs. Sunita Kumari, Assistant on transfer from ICAR-IIHR, Bangalore 04.04.2014.

3. Transfer/ Deputation

Dr. K.K. Lal, Principal Scientist and Head, Fish Conservation Division, NBFGR, Lucknow was relieved on 19th August, 2014 to enable him to join as Programme Coordinator, Genetics & Biodiversity Programme, Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand.

Dr. U.K. Sarkar, Principal Scientist was relieved on 21.02.2015 to enable him to join as Head, Wetlands and Reservoirs Division, ICAR-CIFRI, Barrackpore.

Ms. Priyanka C. Nandanpawar, Scientist was relieved on 17.11.2014 to join on transfer at ICAR-CIFA, Bhubaneswar.

Mr. Avinash Rambhau Rasal, Technical Assistant was relieved on 27.12.2014 to enable him to join as Scientist for FOCARS training at ICAR-NAARM, Hyderabad.

3. Financial Up-gradation under MACP Scheme

Mr. Anwar, Skilled Support Staff PB-I '5200-20200+GP of '1900/- granted 2nd MACP in PB-I with grade pay of '2000/- w.e.f. 23.11.2013.

Management Committee

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

1. Dr. J.K. Jena, Director, ICAR-NBFGR, Lucknow : Chairman
2. Dr. Madan Mohan, ADG (Marine Fy.), ICAR, New Delhi : Member (ICAR)
3. Dr. A. Gopalakrishnan, Director, ICAR-CMFRI, Kochi : Member
4. Director, Fisheries, Govt. of U.P. : Member

5. Director, N.D. University of Agri.& Technology, Faizabad : Member
6. Dr. R.S. Kataria, Principal Scientist, ICAR-NBAGR, Karnal : Member
7. Dr. R.K. Tyagi, Principal Scientist, ICAR-NBPGR, New Delhi : Member
8. Dr. (Mrs.) Sherly Tomy, Senior Scientist, ICAR-CIBA Chennai : Member
9. Mr. Abhishek Rana, Administrative Officer, ICAR-NBFGR : Member Secretary

The 27th meeting of the Committee was held on 2nd June, 2014 and 28th meeting of the Committee was held on 15th October, 2014.

LIST OF PERSONNEL

Research Management

Dr. J.K. Jena - Director

Scientific Staff

1.	Dr. N. S. Nagpure	-	Principal Scientist & In-charge Head of Division
2.	Dr. K. K. Lal	-	Principal Scientist & Head of Division (Upto 18th August, 2014)
3.	Dr. Peyush Punia	-	Principal Scientist & In-charge Head of Division
4.	Dr. (Mrs.) Rehana Abidi	-	Principal Scientist
5.	Dr. A. K. Pandey	-	Principal Scientist& In-charge Head of Division
6.	Dr. Sudhir Raizada	-	Principal Scientist
7.	Dr. S. P. Singh	-	Principal Scientist
8.	Dr. (Mrs) Vindhya Mohindra	-	Principal Scientist
9.	Dr. Ravindra Kumar	-	Principal Scientist
10.	Dr. Basdeo Kushwaha	-	Principal Scientist
11.	Dr. U. K. Sarkar	-	Principal Scientist
12.	Dr. Neeraj Sood	-	Principal Scientist
13.	Dr. V. S. Basheer	-	Principal Scientist & In-Charge (PMFGR Center, Kochi)
14.	Dr. Mukunda Goswami	-	Sr. Scientist
15.	Dr. Parvata Kumar Pradhan	-	Sr. Scientist
16.	Dr. Lalit Kumar Tyagi	-	Sr. Scientist
17.	Dr. Rajeev Kumar Singh	-	Sr. Scientist
18.	Dr. Mahender Singh	-	Sr. Scientist
19.	Dr. T. Rajaswaminathan	-	Sr. Scientist (PMFGR Center, Kochi)
20.	Dr. T.T. Ajith Kumar	-	Sr. Scientist
21.	Dr. Sullip Kumar Majhi	-	Sr. Scientist
22.	Mrs. Poonam Jayant Singh	-	Scientist
23.	Shri Ajey Kumar Pathak	-	Scientist
24.	Dr. (Mrs.) Divya P.R.	-	Scientist (PMFGR Center, Kochi)
25.	Shri A. Kathirvelpandian	-	Scientist (PMFGR Center, Kochi)
26.	Ms. Sangeeta Mandal	-	Scientist
27.	Ms. Rejni Chandran	-	Scientist
28.	Dr. Santosh Kumar	-	Scientist
29.	Mr. Aditya Kumar	-	Scientist
30.	Mr. Charan Ravi	-	Scientist (PMFGR Center, Kochi)
31.	Mr. Labrechai Mog Chowdhury	-	Scientist (PMFGR Center, Kochi)

32.	Ms. Mary Lini R.	-	Scientist
33.	Mr. Murali S.	-	Scientist
34.	Mr. Trivesh Suresh Mayekar	-	Scientist

Technical Staff

1.	Shri Rajesh Dayal	-	Chief Technical Officer
2.	Shri S. M. Srivastava	-	Chief Technical Officer
3.	Shri A. K. Yadav	-	Assistant Chief Technical Officer
4.	Shri Amar Pal	-	Assistant Chief Technical Officer
5.	Shri A. K. Mishra	-	Assistant Chief Technical Officer
6.	Shri S. P. Singh	-	Assistant Chief Technical Officer
7.	Shri Babu Ram	-	Assistant Chief Technical Officer
8.	Shri Ajay Kumar Singh	-	Senior Technical Officer
9.	Mrs. Reeta Chaturvedi	-	Senior Technical Officer
10.	Shri Ramashankar Sah	-	Senior Technical Officer
11.	Shri Subhash Chandra	-	Senior Technical Officer
12.	Shri Ved Prakash	-	Senior Technical Officer
13.	Dr. Akhilesh Kr. Mishra	-	Senior Technical Officer
14.	Dr. (Mrs.) Ranjana Srivastava	-	Senior Technical Officer
15.	Shri Mohd. Gayas	-	Technical Officer
16.	Shri Ravi Kumar	-	Technical Officer
17.	Shri S. K. Singh	-	Technical Officer
18.	Shri Amit Singh Bisht	-	Technical Officer
19.	Shri Satyavir Chaudhary	-	Technical Officer
20.	Shri R.K. Shukla	-	Technical Officer
21.	Shri S. K. Upadhyay	-	Senior Technical Assistant
22.	Shri B. N. Pathak	-	Senior Technical Assistant
23.	Shri Samarjit Singh	-	Senior Technical Assistant
24.	Shri Om Prakash	-	Senior Technical Assistant
25.	Shri Rajesh Kumar	-	Senior Technical Assistant
26.	Shri B. K Rao	-	Technical Assistant
27.	Shri Om Prakash-II	-	Technical Assistant
28.	Dr. Vikash Sahu	-	Technical Assistant
29.	Shri Madan Lal	-	Technical Assistant
30.	Shri Raj Bahadur	-	Technical Assistant
31.	Shri Gulab Chandra	-	Technical Assistant
32.	Shri K. K Singh	-	Technical Assistant

33. Shri Rajool Shanis C.P. - Technical Assistant (PMFGR Center, Kochi)
34. Dr. E. Suresh - Technical Assistant
35. Shri Sree Ram - Senior Technician
36. Shri P. C. Jaiswar - Senior Technician
37. Shri Ram Bharose - Senior Technician

Administrative Staff

1. Shri Abhishek Rana - Administrative Officer
2. Shri Navin Kumar - Assistant Administrative Officer
3. Shri Ravi Bhadra - Assistant Finance & Accounts Officer
4. Shri Tej Singh Seepal - Assistant Administrative Officer
5. Smt. Mamta Chakraborty - Private Secretary
6. Shri Ram Sakal - Personal Assistant
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. Smt. Sunita Kumari - Assistant
13. Shri Sajivan Lal - Senior Clerk
14. Shri Shreelal Prasad - Senior Clerk
15. Shri Vinay Kumar Srivastava - 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 Dukhi Shyam 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	-	Skilled Support Staff

APPENDIX-I

PMFGR Center, Kochi

A Research Center of the Bureau is functioning in the campus of ICAR-Central Marine Fisheries Research Institute (CMFRI), Kochi, Kerala. This Center 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
Peninsular and Marine Fish Genetic Resources Center
ICAR-CMFRI Campus
Post Box No. 1603
Ernakulam North P.O.
Kochi – 682 018, Kerala.
Telefax : 0484-2395570
E-mail : nbfgrcochin@gmail.com
nbfgr_kochi@nbfgr.res.in

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
ICAR-NBFGR Aquaculture Research & Training Unit
Malhore Road, Chinhat
Lucknow-227 105, U.P.
Telefax : 0522-2815848
E-mail : director@nbfgr.res.in

APPENDIX-III

RESULTS-FRAMEWORK DOCUMENT FOR ICAR-NATIONAL BUREAU OF FISH GENETIC RESOURCES (2013- 2014)

RFD
Results-Framework Document
for
ICAR-National Bureau of Fish Genetic Resources
(2013- 2014)

Section 1
Vision, Mission, Objectives and Functions

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

Objectives

Collection, characterization and cataloguing of fish genetic resources

Maintenance and preservation of fish genetic material

Training for skill improvement of stakeholders

Functions

Explorations for genetic resources collection

Characterization of aquatic genetic and genomic resources

Updating/new databases for resource database/warehousing.

Ex-situ conservation of germplasm, maintenance and validation

Assessment of risk to genetic resources and disease diagnosis

Section 2
Inter se Priorities among Key Objectives, Success indicators and Targets

Sl. No	Objectives	Weight (%)	Actions	Success indicators	Unit	Weight (%)	Target/Criteria value				
							Excellent 100%	Very Good 90%	Good 80%	Fair 70%	Poor 60%
1.	Collection, characterization and cataloguing of fish genetic resources	71	Characterization of fish genetic / genomic resources	Fish population characterized	Number	20	40	35	32	28	24
				Fish species taxonomically characterized	Number	12	52	48	42	38	32
				Fish genomic resources generated	Number	15	105	95	85	74	64
			Explorations for fish genetic resources and cataloguing	No. of explorations made	Number	16	40	36	32	28	24
				New fish /shellfish species added in the database	Number	8	68	62	55	48	40
2.	Maintenance and preservation of fish genetic material	10	*Ex-situ* conservation of fish germplasm	Cryopreservation of sperm of new species and/or cell line developed	Number	4	6	5	4	3	2
				Cryopreserved sperm/ cell line maintained	Number	6	28	25	22	20	16
3.	Training for skill improvement of stakeholders	8	Trainings to stakeholders	Stakeholders trained	Number	8	320	300	260	220	200
	Efficient Functioning of the RFD System	3	Timely submission of Draft RFD (2013-14) for approval	On-time submission	Date	2	15/05/2013	16/05/2013	17/05/2013	20/05/2013	21/05/2013
			Timely submission of Results for RFD (2012-13)	On-time submission	Date	1	01/05/2013	02/05/2013	05/05/2013	06/05/2013	07/05/2013
	Administrative Reforms	4	Implement ISO 9001 as per the approved action plan.	% Implementation	%	2	100	95	90	85	80
			Prepare an action plan for Innovation	On-time submission	Date	2	30/07/2013	10/08/2013	20/08/2013	30/08/2013	10/09/2013
	Improving internal efficiency /responsiveness / service delivery of Ministry / Department	4	Implementation of Sevottam	Independent Audit of Implementation of Citizen's Charter	%	2	100	95	90	85	80
				Independent Audit of implementation of public grievance redressal system	%	2	100	95	90	85	80

Section 3
Trend Values of the Success Indicators

Sl. No	Objectives	Actions	Success Indicators	Unit	Actual Value for FY 11/12	Actual Value for FY 12/13	Target Value for FY 13/14	Projected Value for FY 14/15	Projected Value for FY 15/16
1.	Collection, characterization and cataloguing of fish genetic resources	Characterization of fish genetic / genomic resources	Fish population characterized	Number	58	32	35	40	45
			Fish species taxonomically characterized	Number	38	30	48	52	56
			Fish genomic resources generated	Number	92	39	95	105	115
		Explorations for fish genetic resources and cataloguing	No. of explorations made	Number	85	94	36	40	44
			New fish /shellfish species added in the database	Number	38	108	62	68	74
2.	Maintenance and preservation of fish genetic material	*Ex-situ* conservation of fish germplasm	Cryopreservation of sperm of new species and/or cell line developed	Number	6	8	5	5	5
			Cryopreserved sperm/ cell line maintained	Number	22	27	25	28	30
3.	Training for skill improvement of stakeholders	Trainings to stakeholders	Stakeholders trained	Number	263	338	300	320	350
	Efficient Functioning of the RFD System	Timely submission of Draft RFD (2013-14) for approval	On-time submission	Date	-	-	16/05/2013	-	-
		Timely submission of Results for RFD (2012-13)	On-time submission	Date	-	-	02/05/2013	-	-
	Administrative Reforms	Implement ISO 9001 as per the approved action plan.	% Implementation	%			95		
		Prepare an action plan for Innovation	On-time submission	Date	-	-	10/08/2013	-	-
	Improving internal efficiency /responsiveness / service delivery of Ministry / Department	Implementation of Sevottam	Independent Audit of Implementation of Citizen's Charter	%	-	-	95	-	-
			Independent Audit of implementation of public grievance redressal system	%	-	-	95	-	-

Section 4 (a)
Acronym

Sl. No.	Acronym	Description
1.	UT	Union Territory
2.	SAU	State Agricultural University
3.	ZSI	Zoological Survey of India
4.	DAHDF	Department of Animal Husbandry, Diary and Fisheries

Section 4(b)
Description and definition of success indicators and proposed measurement methodology

Sl. No.	Success indicator	Description	Definition	Measurement	General Comments
1.	Fish population characterized	A particular fish species comprises several genetic stocks which can be characterized through biological/genetic markers.	Fish population characterization refers to documentation of distinct population (intra-specific) from different riverine systems/ tributaries/rivulets from significant geographical locations	Number of populations of a targeted fish species	To identify genetic stocks which may help in species/stock conservation and management
2.	Fish species taxonomically characterized	Biological/molecular descriptions leading to characterization of new species or taxonomic validation of species	Fish species characterization refers to documentation and validation of inter-specific variation	Number of fish species	To characterize fish species
3.	Fish genomic resources generated	Development of validated molecular marker/gene	Molecular marker/gene is a segment of genome with a known location that can be used to identify stock/species	Number of validated molecular markers/genes	To identify/ characterize stock/species
4	No. of explorations made	Survey of different riverine systems/tributaries/rivulets/sites representing different habitat types or with significant distance or representing different seasons	Exploration of fish germplasm resources in the different aquatic ecosystems	Number of explorations	To determine the status of fish diversity
5.	New fish /shellfish species added in the database	Updation of the existing database by adding information of new species	Addition of new information in existing fish database	Number	For cataloguing of fish germplasm resources
6.	Cryopreservation of sperm of new species and/or cell line developed	Development of protocol for cryopreservation of sperm / cell line of new fish species	Preservation of fish sperm/ cell line	Number	For *ex-situ* conservation
7.	Cryopreserved sperm/ cell line maintained	Maintenance of the cryopreserved sperm/cell line	Long term storage of fish sperm/ cell line	Number	For breeding and in vitro characterization purposes
8.	Stakeholders trained	Training and awareness meets (farmers/researcher/academician/State fisheries department officers) / stakeholder meets/ workshops/ symposia/ exhibitions, extension materials (leaflets/bulletins etc.) generated	Number of trainings/ awareness meetings organized and extension materials/ documents prepared	Number	This activity is to train and aware stakeholders on various aspects of fish genetic resources conservation

Section 5
Specific performance requirements from other departments

Location Type	State	Organization Type	Organization Name	Relevant Success Indicator	What is your requirement from this organization	Justification for this requirement	Please quantify your requirement from this Organization	What happens if your requirement is not met.
River/ rivulets/ tributaries/ reservoirs/ wetlands/ marine/ coastal	All states/ UT	Govt./ Authorities/ Boards	Concern State Departments and Union Ministries	No. of explorations	Permission for sampling in protected areas	Sampling for research purposes	<5 %	The area remain un-explored and potential will not be known.
River/ rivulets/ tributaries/ reservoirs/ wetlands/ marine/ coastal	All State/ UT	Academic/ research institutions of State / Central Govt.	SAUs/ Universities/ State Fisheries Departments/ ZSI	New fish/shellfish species added in the database	For species validation and published information	Secondary information required to update the database.	45%	Database may not be adequately strengthened and updated.

Section 6
Outcome/ impact of activities of organization

Sl. No.	Outcome/ Impact of organization/ RCs	Jointly responsible for influencing this outcome/ impact with following organization(s)/ departments/ ministries	Success indicator (s)	Unit	2011-12	2012-13	2013-14	2014-15	2015-16
1.	Fish Genetic Resource management models for prioritized species	Ministry of Environment &Forests; Department of Animal Husbandry, Diary and Fisheries; State Agricultural Universities, State Fisheries Departments	Prioritized fish species/ genetically characterized populations maintained in live germplasm resource centre for conservation	Number	1	1	1	1	1

Performance Evaluation Report

Sl. No	Objective(s)	Weight	Action(s)	Success Indicator(s)	Unit	Weight	Target / Criteria Value					Achievements	Performance	
							Excellent 100%	Very Good 90%	Good 80%	Fair 70%	Poor 60%		Raw score	Weighted Score
1.	Collection, characterization and cataloguing of fish genetic resources	71	Characterization of fish genetic / genomic resources	Fish population characterized	No.	20	40	35	32	28	24	44	100	20
				Fish species taxonomically characterized	No.	12	52	48	42	38	32	54	100	12
				Fish genomic resources generated	No.	15	105	95	85	74	64	107	100	15
			Explorations for fish genetic resources and cataloguing	No. of explorations made	No.	16	40	36	32	28	24	43	100	16
				New fish / shellfish species added in the database	No.	8	68	62	55	48	40	82	100	8
2.	Maintenance and preservation of fish genetic material	10	*Ex-situ* conservation of fish germplasm	Cryopreservation of sperm of new species and/or cell line developed	No.	4	6	5	4	3	2	6	100	4
				Cryopreserved sperm/ cell line maintained	No.	6	28	25	22	20	16	29	100	6
3.	Training for skill improvement of stakeholders	8	Trainings to stakeholders	Stakeholders trained	No.	8	320	300	260	220	200	366	100	8
4	Efficient Functioning of the RFD System	3	Timely submission of RFD for 2013-14	On-time submission	Date	2	15/05/2013	16/05/2013	17/05/2013	20/05/2013	21/05/2013	15/05/2013	100	2
			Timely submission of Results for 2012-13	On-time submission	Date	1	01/05/2013	02/05/2013	05/05/2013	06/05/2013	07/05/2013	05/04/2013	100	1
	Administrative Reforms	4	Implement ISO 9001 as per approved action plan	% implementation	%	2	100	95	90	85	80	100	100	2
			Prepare action plan for Innovation	On-time submission	Date	2	30/07/2013	10/08/2013	20/08/2013	30/08/2013	10/09/2013	30/07/2013	100	2
	Improving internal efficiency/ responsiveness/ service delivery of Ministry/ Department	4	Implementation of Sevottam	Independent Audit of Implementation of Citizen's Charter	%	2	100	95	90	85	80	100	100	2
				Independent Audit of implementation of public grievance redressed system	%	2	100	95	90	85	80	100	100	2

भाकृअनुप
ICAR

NBFGR

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