

# Annual Report

## 2015-16



भा.कृ.अनु.प.-राष्ट्रीय मत्स्य आनुवंशिक संसाधन ब्यूरो  
**ICAR-National Bureau of Fish Genetic Resources**  
 Lucknow





*With best compliments from*

**Dr. Ravindra Kumar**  
Director (Acting)



**ICAR-National Bureau of Fish Genetic Resources  
Lucknow**







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## 2015-16



**ICAR-National Bureau of Fish Genetic Resources**

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# PREFACE

India is endowed with vast and diverse fishery resources which offer immense opportunities for income and employment generation in the country. Assessing the status and trends of the biological diversity is vital for developing strategies for sustainable development of the sector at all levels. The ICAR-National Bureau of Fish Genetic Resources (ICAR-NBFGR) is a nodal organization mandated for collection, characterization, cataloguing, maintenance and preservation of fish genetic resources at national level for their conservation and sustainable utilization aiming at benefit of the present and for posterity. Since its establishment in 1983, the Bureau has played an essential role in conservation and management of fishery resources of the country.



During the reporting year (2015-2016), the Institute has strengthened its activities to promote activities on fish germplasm exploration, characterization and conservation. Exploratory surveys were conducted in various water bodies of the country for documentation and characterization of the fish diversity. The existing database on 'Finfish Diversity of India' was updated periodically that presently contains information on 2,936 native finfish (belonging to 1,068 genera of 252 families under 44 orders) and 462 exotic fish species. A checklist of 3,827 molluscs species reported from India was also prepared. A database of 'Abiotic Stress Responsive Genes in Fishes' was developed that presently covering information on 250 abiotic stress related genes in 38 fish species. It also covers information on 250 proteins responsive for abiotic stress tolerance in fishes. Three important research programmes of the ICAR-Consortia Research Platforms (CRP) on Genomics, Agro-biodiversity and Vaccines & Diagnostics were undertaken at the Institute.

Under the whole genome sequencing, *de novo* assembly of *Clarias batrachus* reads were carried out using genome assembler. Whole genome sequencing of *Aphanomyces invadans* was undertaken to understand its mechanism of infection in fishes. Under the ICAR- Outreach Activity on Fish Genetic Stocks (Phase-II), species-specific molecular resources were generated for stock characterization of the targeted species. The findings on development of surrogate broodstock for propagation of valuable fish germplines gave encouraging results. Under the National Surveillance Programme for Aquatic Animal Diseases, awareness programmes were organized by the collaborating centers in which stakeholders including fish farmers and State Fisheries Officers participated and interacted. Training programmes were organized to strengthen the diagnostic capability of state fisheries officers.

Three newly recruited scientists also joined the Institute during the year. I am sure their young enthusiasm will further energize our research efforts. I am confident that our hard work and commitment to strive for improving 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. Trilochan Mohapatra, Secretary, DARE and Director General, ICAR, New Delhi and Dr. S Ayyappan, Former Secretary, DARE and Director General, ICAR, New Delhi for their continued encouragements, guidance and support. I am highly grateful to Dr. J K Jena, Deputy Director General (Fisheries), ICAR and Dr. B Meenakumari, Former DDG (Fisheries), ICAR for their sincere advice and guidance. I place on record my sincere thanks to Dr. P Pravin, ADG (Marine Fisheries); Dr. S Raizada, ADG (Inland Fisheries); Dr. Madan Mohan, Former ADG (Marine Fisheries) and Dr. S D Singh, Former ADG (Inland Fisheries) and other staff members of the Fisheries Division of ICAR for their cooperation and help in our endeavours. I take this opportunity to express my gratitude to Dr. J K Jena and Dr. Rehana Abidi, Former Directors of this institute for their kind supervision, support and help. I also express my sincere thank to Dr. L K Tyagi, Principal Scientist, Mr. Amit Singh Bisht and Mr. Ravi Kumar, Sr. Technical Officers and entire publication team of the Institute for their sincere effort and commitment in timely publication of the Annual Report 2015-16.

June 28, 2016

A handwritten signature in black ink, appearing to read 'Ravindra Kumar', with a horizontal line underneath.

**(Ravindra Kumar)**  
Director (Acting)



# CONTENTS

1. Preface	
2. EXECUTIVE SUMMARY	1
3. INTRODUCTION	4
4. RESEARCH ACHIEVEMENTS	7
4.1 Cataloguing of Fish Genetic Resources of India	7
4.2 Genetic and Biological Characterization	12
4.3 Exploration of Fish Germplasm Resources	35
4.4 Fish Health Management and Exotics	47
5. WORKSHOPS/ SYMPOSIA/ TRAININGS/ MEETINGS ORGANIZED	61
6. EXTENSION ACTIVITIES	70
7. AWARDS AND RECOGNITIONS	77
8. LIST OF PROJECTS	78
9. PARTICIPATION IN SEMINARS/ SYMPOSIA/ WORKSHOPS/ TRAININGS/ MEETINGS	82
10. PUBLICATIONS	87
11. LIBRARY AND INFORMATION MANAGEMENT	92
12. STAFF ACTIVITIES	93
13. LIST OF PERSONNEL	96
14. APPENDIX-I	100
15. APPENDIX-II	101
16. APPENDIX-III	102



## EXECUTIVE SUMMARY

ICAR-National Bureau of Fish Genetic Resources (NBFGR) is a premier institution carrying out research in several 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 19 Institutional and 17 externally-funded research projects. Major achievements and activities of the Institute during the year 2015-16 are summarized below:

- The existing database on finfish diversity of India was updated by adding information on 68 fish species reported from Indian waters. The database now contains information on 2936 native finfishes belonging to 1068 genera and 252 families.
  - A checklist of 3827 mollusks species reported from India was prepared by adding 84 more species. The existing database on mollusks was updated by adding information on taxonomy, habitat and other relevant parameters for 1550 species.
  - Passport information was developed for 200 exotic ornamental fishes.
  - A database of 'Abiotic Stress Responsive Genes in Fishes' was designed which presently covers information on 250 abiotic stress responsive genes of 38 fish species. It also covers information on 250 proteins responsive for abiotic stress tolerance in fishes.
  - Four genomic resource databases *viz.*, Fish and Shellfish Microsatellite Database (FishMicrosat), Fish Karyome, Fish Mitogenome Resource (FMiR) and Fish Barcode Information System (FBIS), were updated by including new records and now these cover 12111, 878, 1776 and 21011 records, respectively.
  - A content based fish genomic portal, named 'FishCABin', was developed and hosted on URL <http://mail.nbfgr.res.in/FishCABin/>. The portal provides ability to the user to work with all databases or selected databases, including the information on the activities under the fisheries domain.
- The portal also facilitates the access of the high performing computer (HPC) resources by the user.
- Under studies on genetic variability analysis in hilsa, *Tenuulosa ilisha*, altogether 25 sampling sites, covering both east and west coast (freshwater, brackishwater and marine locations) have been explored and tissue accessions from 1013 individuals of *T. ilisha* collected so far. A total of 411 samples from 10 locations were analysed using morpho-metric traits and results indicate existence of different stocks in the freshwater, marine and estuarine ecosystem of east coast. A total of 339 sample accessions were analysed with four mitochondrial markers, cyto b, ATPase6/8, 16S and 12S rRNA. A total of 71 polymorphic microsatellite loci developed, were used to generate a data set of 6816 individual genotypes involving with 96 samples to test the validity of microsatellite loci in deciphering the genetic diversity. The results indicated a high genetic variability and strong genetic differentiation especially from Narmada. The differentiation between the east coast rivers (Hooghly, Brahmaputra and Padma) was low. A total of 6 loci were identified as outlier loci that are putatively under selection for adaptive genetic variation and one locus under balancing selection.
  - Three major research programmes of the ICAR - Consortia Research Platforms *viz.*, ICAR-CRP on Genomics, ICAR-CRP on Agrobiodiversity and ICAR-CRP on Vaccines & Diagnostics, were undertaken at the Institute.
  - Under the ICAR - Outreach Activity on fish genetic stocks, species-specific molecular signatures (partial cytochrome c oxidase I) for five species belonging to the genus *Sillago* were generated and phylogenetic relationship among the species was determined on the basis of 628 nucleotides. *Mugil cephalus*, 37 species-specific microsatellite loci for *M. cephalus* were tested and twenty loci were found polymorphic. In green musel, *Perna viridis*, 37 polymorphic microsatellite loci were tested and twelve loci were found to be polymorphic which will be used for generating genotype data



for population genetics.

- Population genetic stock structure of *Scomberomorus commerson* using nuclear and mitochondrial markers revealed low and non-significant values of co-efficient of genetic differentiation for pair-wise populations indicating unit stock in Indian waters. The finding of the study indicates the species can be considered as a unit stock for fishery management in Indian waters.
- Analysis of genotyped data completed with 11 polymorphic microsatellite markers in *Parapenaeopsis stylifera* from five locations (Visakhapatnam, Chennai in the east coast; Veraval, Goa, Kochi in the west coast of India) revealed two stocks (1-West coast; 2-East coast) with low but significant variation ( $P < 0.001$ ). Phylo-genetic analysis indicated the closeness on *P. stylifera* and *P. coromandelica* in comparison to other three species under the genus.
- New work on developing DNA chip based on the species specific mitochondrial sequence probes for commercially important group of fish species of the country was initiated.
- Under the collaborative programme on whole genome sequencing of two important fish species namely *Labeo rohita* and *Clarias batrachus*, *de novo* assembly of the processed Illumina HiSeq data of *C. batrachus* was carried out using Abyss genome assembler at four different hash lengths, viz. 61, 64, 69 and 85. The assembly at hash length of 85 was observed comparatively better with the total number of contigs being 2,87,999. The *de novo* assembly of the quality filtered Illumina HiSeq data of *C. batrachus* was also carried out using CLC Genomics Workbench. This assembly resulted in 4,10,486 contigs.

A total of 44,505 putative genes were annotated at an identity greater than 50%. Gene ontology analysis of the annotated genes was done in order to infer its functional aspects. The transcriptome sequencing of *C. batrachus* was carried out for 4 tissues, viz. brain and gonads of male and female individuals. A graphical user interface (GUI) named 'WGSSAT- A high-throughput computational pipeline for mining of SSRs from whole genome sequencing data' was developed to annotate SSR from the whole genome assembly data.
- Under the programme on genetic characterization and DNA barcoding of fishes from north east India, a total of 283 sequences of 16S rRNA gene belonging to 71 species were submitted to NCBI GenBank. A total of 98 DNA barcodes based on cytochrome c oxidase I (COI) gene belonging to 27 species were prepared and submitted to NCBI GenBank.
- A new catfish species *Clarias serratobranchium* sp. nov. was discovered based on mitochondrial gene COI and morphological data of 11 individuals, collected from the wetlands of Moreh (Chindwin basin), a place in the Indo-Burma border. The study also confirmed that Indian *C. batrachus* (*C. magur*) is different from South East Asian *C. batrachus* as it formed a separate cluster with substantial divergence from *C. batrachus* of Malaysia, Philippines and Thailand.
- *Devario yuensis* was re-described based on morphological and molecular data of both 16S and COI markers.
- Work on development of surrogate broodstock for propagation of valuable fish germplines gave encouraging results. The results obtained in this study, besides recipient preparation in considerably short time for germ cell transplantation, will have implication for invasive fish species control in large open water bodies which are difficult to manage.
- Explorations were continued in various parts of the country for documentation and characterization of the fish diversity. In Western Ghats, a total of 66 fish species were recorded from Sharavathi river basin. Specimens of the miniature gobiid fish *Redigobius bikolanus* and *Schismatogobius deraniyagalai*, and minnows *Laubuka laubuka*, represent new records for this river basin. A total of 49 fish species were recorded from Valapattanam river system. Specimens of *Mesonoemachilus guentheri*, *Pethia pookodensis*, *Puntius bimaculatus* and *Pseudogobiopsis oligactis* represent new records for this river system. Survey in Chadragiri river in Kerala and Karnataka yielded a total of 55 fish species. Chaliyar river basin in Kerala yielded a total of 43 fish species. In the upper basin of Mahanadi river, a total of 7 sites of river



- Mahanadi and 30 sites of its six tributaries and sub-tributaries namely, Sheonath, Hasdeo, Mand, Maniyari, Arpa and Lilagar, were explored and a total of 92 fish species were recorded.
- A participatory programme on 'Exploration and characterization of fish germplasm resources and indigenous knowledge in North Eastern Region of India' was implemented under ICAR-North East (NE) component, involving six collaborators from various institutions of the NE region. Exploration programmes were carried out by the research partners in the selected rivers/water bodies of the NE region. A total of 44 fish species were recorded from Krisnai River of Garo Hills district in Meghalaya. Explorations from the Chindwin drainage in Manipur in 15 sites of 8 rivers of the basin yielded 82 species. Total 23 sampling sites of 16 rivers/tributaries of Kolodyne/Chimmtuipui and Barak drainage systems of Mizoram yielded a total of 70 fish species. Explorations in the Kameng river yielded 21 species where as a total of 44 fish species were recorded from nine sites of the four small rivers of Assam namely, Rubi, Abhung, Dihamlai and Dilama. Three exploratory surveys covering 72 locations conducted in Teesta river in Sikkim yielded 116 fish species.
  - Studies were carried out to evaluate the effect of  $\beta$ -glucan on the expression of immune-related genes in *Labeo rohita*, however, no significant difference in expression of immune genes compared to control could be observed.
  - New work on deciphering *Aphanomyces invadans* genome to understand its mechanism of infection in fishes was undertaken. Transcriptome of *A. invadans* zoospores was sequenced and a total of 84,286,058 clean paired-end reads were generated. Gene expression estimation resulted in the identification of 39,979 transcripts having expression  $\geq 1$ . The genomic sequence data is being analyzed for the generation of draft assembly of *A. invadans* genome.
  - Molecular characterization of six protozoan parasites, namely *Myxobolus yaseenii* sp. nov., *M. cerebralis*, *M. arcticus*, *M. grassi*, *Ichthyophthirius multifiliis* and *Trichodina* sp. nov., was done and the phylogenetic trees were constructed.
  - Under the National Surveillance Programme for Aquatic Animal Diseases, which is implemented in 15 states of aquaculture importance with involvement of 24 national/state fisheries research institutions, a total of 67 awareness programmes were organized by the collaborating centres and more than 3600 stakeholders including fish farmers and State Fisheries Officers participated in these programmes. A total of 7 trainings were organised to strengthen the diagnostic capability of state fisheries officers in which 123 stakeholders including fisheries officers, farmers and research scholars were imparted materials in different trainings on fish disease diagnosis. Awareness materials in regional languages were also distributed to fish farmers. A total of 1799 farms were visited for sample collection for active surveillance by the collaborating centres and major pathogens reported during this period included infection with carp edema virus, infection with cyprinid herpes virus - 2, viral encephalopathy and retinopathy virus, *Aphanomyces invadans*, *Saprolegnia parasitica*, *Aeromonas* sp., *Flavobacterium* sp., *Argulus* sp., *Lernaea* sp., *Dactylogyrus* sp., *Trichodina* sp. and *Myxobolus* sp. in finfish; *Enterocytozoon hepatopenaei*, white spot syndrome virus, infectious hypodermal and hematopoietic necrosis virus, monodon baculovirus, hepatopancreatic parvovirus and *Vibrio* sp. in crustaceans and infection with *Perkinsus olseni*, *P. beihaiensis* in molluscs. Infection with Carp Edema Virus was reported for the first time in India from a fish farm in Ernakulum District, Kerala. Infection with EHP, a microsporidian parasite, was also reported. Studies on disease outbreak and mass mortality in farmed green cobra guppy, *Poecilia reticulata*, indicated that *Serratia marcescens* may also cause disease in ornamental fish. This is the first report describing *S. marcescens* as a pathogen of freshwater ornamental fish in India.
  - A series of short-term training programmes on 'Fish Culture and Conservation' were organized in which a total of 380 fish farmers were trained.

## INTRODUCTION

### Brief History

The scientific basis is necessary to preserve the genetic resources which can be utilized for nutritional and environmental security of the mankind. Realising this, 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 under the aegis of Indian Council of Agricultural Research. Since its humble beginning at Allahabad in 1983, NBFGR has metamorphosed into a leading 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, research laboratories, public aquarium, guest house, staff quarters and required experimental tanks and ponds have been created for satisfying the need of research and other amenities. The Bureau, over the years, has created excellent infrastructure, state of the art facilities and expertise in several research areas including, development of fish databases, genetic characterization, gene banks, fish germplasm

and habitat inventory, risks analysis of exotic species, diagnostics for OIE notified pathogens, aquatic microbes and other areas of germplasm conservation with special focus on threatened, prioritized and exotic fish species.

The Institute has evolved not only in terms of creation of infrastructure, but also expansion of research programmes by including important



areas *viz.* exploration of newer geographical areas and unexplored aquatic resources for assessment of fish diversity, whole genome sequencing, population genetics, functional genomics, molecular disease diagnostics, national surveillance programme for aquatic diseases etc.

### Vision

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

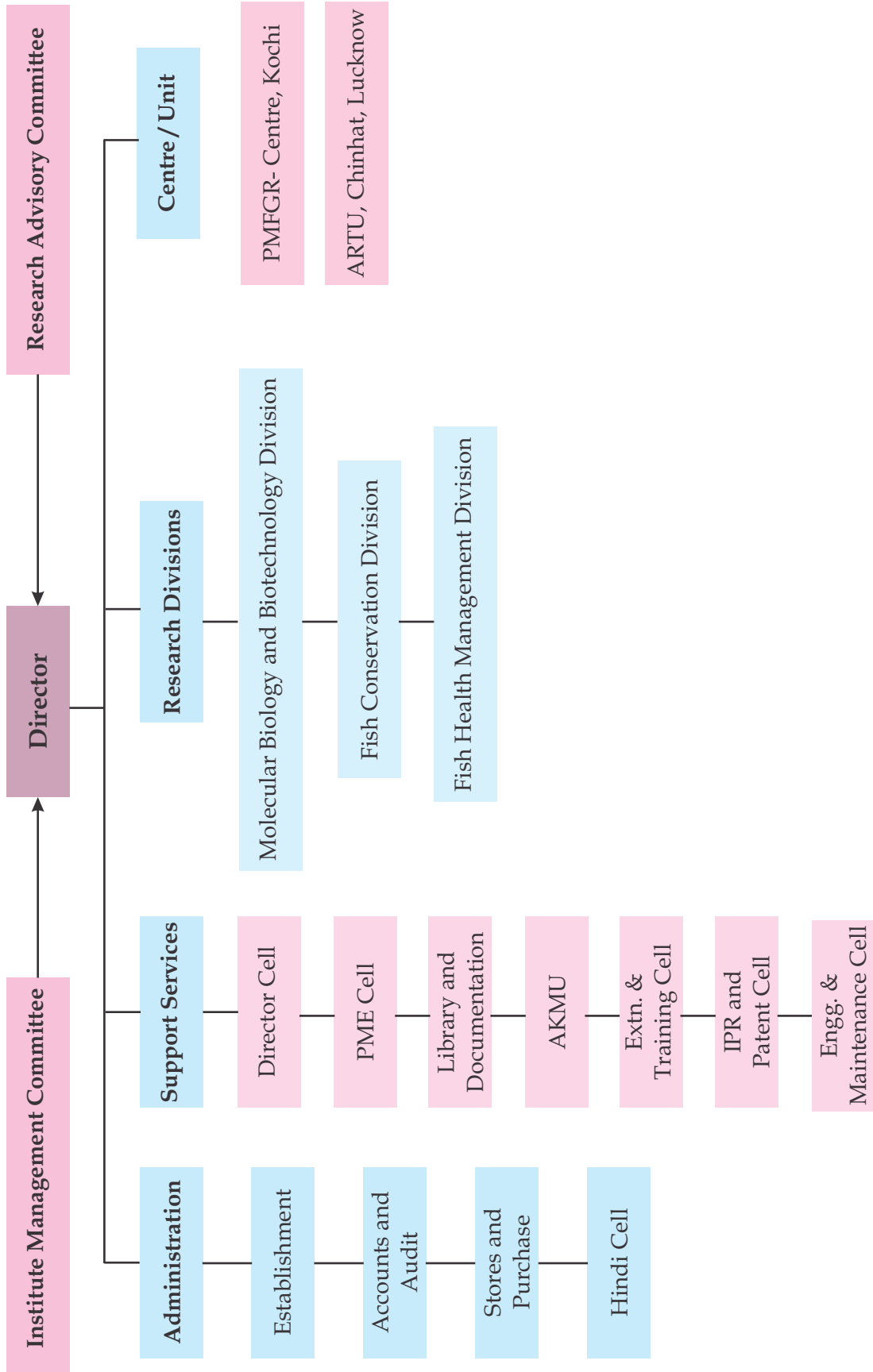
### Mission

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





## Staff Position

The overall staff position as on 31st March, 2016 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	32	09
3.	Technical	38	37	01
4.	Administrative	21	19	02
5.	Supporting	20	18	02
	<b>Total</b>	<b>121</b>	<b>106</b>	<b>15</b>

## Financial Statement

Allocation of funds and expenditure incurred during the year 2015-2016 are as follows:

(Rs. in lakhs)

	Budget Allocation	Expenditure
Plan	792.00	791.73
Non Plan	1257.60*	1255.48*
Total	2049.60	2047.21

\* 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, 2017

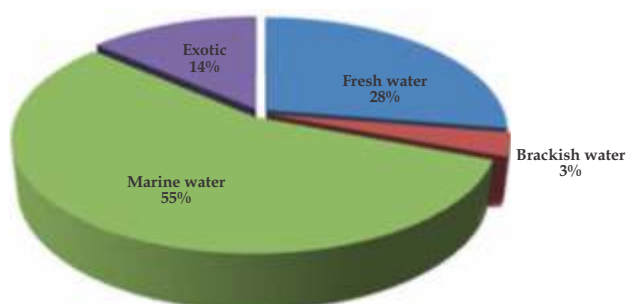
**Project Personnel:** S.P. Singh, PI (till 31.7.2015), S. Raizada, PI (01.08.2015 to 31.12.2015), Rehana Abidi, PI (w.e.f. 17.2.2016), T. T. Ajith Kumar, A. K. Pathak, Rejani Chandran, Rajesh Dayal, Reeta Chaturvedi and Ravi Kumar

**Funding Agency:** Institutional

During the period under report, Bureau continued its efforts towards collection and cataloguing the fish genetic resources of India. The existing database on finfish diversity of India was updated. Data on additional 68 native fin fish species reported from Indian waters was added in the main database after screening and comparing with synonyms and valid names of the respective fish species. These fishes belong to 6 orders and 20 families. The Information base now contains basic information on 3398 fin fishes including 2936 native species and 462 exotic fishes. These 2936 native fishes reported from India include 936 freshwater fishes, 113 brackishwater and 1887 marine species belonging to 44 orders, 252 families and 1069 genera (Table 1; Figures 1-3).

**Table 1. Ecosystem-wise present status of the Information Base on Fish Diversity of India**

Category of Fishes	Ecosystem	Additions in Year 2016	Total No. of Fish Species in 2016
Native fishes	Freshwater	59	936
	Brackishwater	-	113
	Marine water	09	1887
	<b>Total</b>	<b>59</b>	<b>2936</b>
Exotic fishes		15	462
<b>Grand total</b>			<b>3398</b>



**Fig. 1. Diversity of Indian fish genetic resources**

The existing checklist of 'exotic' fishes found in India was updated by adding information on 15 additional exotic species and the database on exotic fishes now includes information on 462 species. Scientific names of fishes were revised in the database and duplicate names were deleted. A total of 253 synonyms and 67 photographs were added during the period to update the database.



*Danio rerio*

*Pethia conchonius*



*Sahyadria denisonii*

*Dawkinsia filamentosa*

**Fig. 2. Few of the Indian freshwater ornamental fishes**



*Acanthurus leucosternon*

*Chaetodon vagabundus*



*Pomacanthus semicirculatus*

*Thalassoma lunare*

**Fig. 3. Few of the marine ornamental fishes**

The existing software application of the database known as "Indian Fish Information System" has been developed by applying ASP.NET 2008 technology for web interface and SQL server 2008 for database under the Windows operating 7.0 platform and hosted on the local intranet domain using IIS (Internet Information server) with limited access to the authenticated users. The file geo-database representing the spatial database of fishes of Ganga basin was updated by including data on fishes



**Fig. 4. A screen print of Indian fish information system**



**Fig. 5. A screen print of Indian fish information system**

of Sai and North Tons sub basins. An e-identification system for identifying fishes reported from Indian waters using shape characteristics was updated by adding data on shape characteristics of more species of family Cyprinidae (Figures 4 & 5).

**Database on Mollusk Species of India**

A checklist of 3827 mollusks species reported from India was prepared with 84 new additions. The existing database on mollusks was updated with addition of information on taxonomy, habitat and other relevant parameters for 1550 species. Species-specific information on each species of the mollusks was collected for the database on mollusks of India (Figures. 6).



**Fig. 6. Few Gastropod molluscs from Gulf of Mannar**

**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 Up to December 2015), T. T. Ajith Kumar (PI w.e.f. January, 2016), Rejani Chandran, Aditya Kumar and Rajesh Dayal

**Funding Agency:** Institutional

A check-list of 400 exotic fish species available in the ICAR-NBFGR database was revised. Additional 62 fish species including exotic food fishes were added in the check-list. The new check-list now comprises of 462 exotic fish species. Besides species, information on variants of some of new species was also collected. Documentation was done for 150 ornamental fish species/ variants from Southern parts of Tamil Nadu and Kerala. Passport information was developed for 200 exotic ornamental fishes. Documentation of exotic marine ornamental fishes and invertebrates was also initiated.

**Project Title:** Network Project on Agricultural Bioinformatics and Computational Biology' under CABIn Scheme: Fisheries domain

**Project Period:** February, 2015 – March, 2017

**Project Personnel:** N.S. Nagpure (CCPI upto December 2, 2016), Ravindra Kumar (CCPI w.e.f. December 3, 2016), Basdeo Kushwaha, Mahender Singh, A.K. Pathak and Murali S.

**Funding Agency:** ICAR - Indian Agricultural Statistics Research Institute

**Identification and validation of abiotic stress genes**

Genes responsive for temperature ammonia and salinity stresses were downloaded from

NCBI in respective format for annotation and sequences, respectively. The proteins of the respective genes were also downloaded from UniProt (<http://www.uniprot.org>) in the respective format for annotation and sequences, respectively. The survey of literature revealed that 36 genes are responsible for temperature, 90 for salinity and 124 for ammonia stress. Sequences of these identified genes responsive for temperature ammonia and salinity were retrieved from GenBank database (<http://www.ncbi.nlm.nih.gov/genbank/>) and downloaded in Fasta format. Similarly, the proteins sequences for the respective genes were retrieved from UniProt and NCBI protein database (in GenPept and FASTA formats) (<http://www.ncbi.nlm.nih.gov/protein>). The retrieved genes and respective protein sequences were validated through BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and other similarity search tools.

To design primers of selected abiotic stress responsive genes, the protein sequences of abiotic responsive genes of zebrafish (*Danio rerio*) were retrieved from UniProt. Thereafter, the putative

genes were predicted from the *de novo* assembled contigs of *Clarias magur* using Augustus (<http://augustus.gobics.de>) software. The homology of abiotic stress responsive proteins of zebrafish was checked against the putative proteins of *C. magur* using Blastp, which provided the stress responsive proteins in *C. magur*. Further, the putative gene sequence of the annotated protein was extracted with 50 bp flanking regions on both sides using a custom Perl script. Primer designing of the putative genes was done in an overlapping fashion using Primer Blast (<http://www.ncbi.nlm.nih.gov/tools/primer-blast>) (Tables 2 and 3). Primer designing of the putative genes was done and primers were checked for cross amplification in *C. magur* genome. For temperature, 19 and 4 sets of primers were designed for Park2 and Nupr1 genes, respectively. Similarly, for ammonia 4 set of primers were designed 4 for ammonium transporter gene, 2 for Aquaporin, 4 for Glulb and 8 for Slcl292. These designed primers were further checked for cross amplification in *C. magur* genome using Blast and custom Perl script.

**Table 2. List of few primers for identification of temperature responsive genes in *Clarias magur***

S. N.	Primer Name	Primer Length	Expected amplicon size (bp)
<b>E3 ubiquitin protein ligase parkin (Park2), RefSeqid: NM_001017635</b>			
1.	Park2 F1	TGAAAATGAGGAATGCAACTACTG	728
	Park2 R1	CAGACGTCCGATCAGGCAG	
2.	Park2 F2	TCGTTATGAAAATGAGGAATGCAAC	734
	Park2 R2	TTCAGACGTCCGATCAGGCA	
3.	Park2 F4	CAGTATGCCAGAGGCATGATCAGA	842
	Park2 R4	ACTTCTGGCCTGGAGTGGAT	
4.	Park2 F5	ATGGACCTGTCGGTTAATGCT	791
	Park2 R5	ACATCGGTCTGTTGAAACTCT	
<b>Nuclear Protein-1(Nupr-1), RefSeqid: NM_001281922.1</b>			
1.	Nupr F1	GAACTTAGGTTTCTCAAATGAGCC	510
	Nupr R1	GAAGCCAGTGTTTAGTGCAGAG	

Contd...



2.	Nupr F2	CTTAGGTTTCTCAAATGAGCCA CG	520
	Nupr R2	ACACTTTAGGTGGAAGCCAGT	
3.	Nupr F3	AACACTGGCTTCCACCTAAAGT	557
	Nupr R3	ACAGGGTGTTACTGCACCTC	

**Table 3. List of few primers for identification of ammonia responsive genes in *C. magur***

S. N.	Primer name	Primer sequence	Expected amplicon size (bp)
<b>Ammonium transporter Rhtype C -like 2(rhcg12), RefSeqid: NM_207082.1</b>			
1.	Ammo F1	ATTGTTCCCTTACCAGACTAAATGCT	771
	Ammo R1	TTTGCGTGGCTATCGCCTTT	
2.	Ammo F2	TCCACCTACAAGTCCAAAGGC	790
	Ammo R2	CCATCAACACGTACCTGTCCT	
3.	Ammo F3	GCTGCCAAGGACAGGTACG	781
	Ammo R3	GGACCCTGCAGATGGCAAGA	
4.	Ammo F4	AATCTTGCCATCTGCAGGGTC	652
	Ammo R4	GGCTGTTTGCTTTGTTTGGCA	
<b>Aquaporin 1 (aqp1a.1), RefSeq id: NM_207059.1</b>			
1.	Aquap F1	AAATTTCTGGAGGGCTGTCTTTG	769
	Aquap R1	ATTGCAAACGGAGCTACACTA	
2.	Aquap F2	TGTGCGTCCTAGCAACCAT	887
	Aquap R2	GGTTTATTTCCATCAGTGCATCA	
<b>Glutamine synthetase (glulb), RefSeq id: NM_182866.3</b>			
1.	Gluta F1	GGAATGAAGCTCAGTCCTCG	797
	Gluta R1	TGTCTACTCCATGGGTGCTAC	
2.	Gluta F2	CTGGAACTCCCACTGGAACA	605
	Gluta R2	CTCGGAACAGACGGTCATCC	
3.	Gluta F3	GACATAGACGCCGTACCCTG	511
	Gluta R3	GTGTCTGACTGGGACGAGTG	
4.	Gluta F4	TTTTCCACTCGTCCCAGTCAG	548
	Gluta R4	CATGCTCGCTGTGTGTTTGG	
<b>Sodium -potassium -chloride cotransporter 1 (slc12a2), RefSeq id: NM_001163654.1</b>			
1.	NaKCl F1	CGCGCCTGGATAAACCAC	781
	NaKCl R1	TGTTATTGCTATTAGTGGAGGTGT	



2.	NaKCl F2	ACTACCTTGGCTCTCTTGGCT	689
	NaKCl R2	TGACGCACAAAACTGTCCTT	
3.	NaKCl F3	GCCTTGATGTGACATATTTTGCG	569
	NaKCl R3	CAGCCAAACTTTACTGTGCC	
4.	NaKCl F4	CGAGGCTGCTCTAAAGGATGT	591
	NaKCl R4	AACCATTGGTGGTTATCGCTG	
5.	NaKCl F5	CCCAGCTCTGTCTTGTGCTATC	589
	NaKCl R5	TCCCATTCCATTCTGCCATAG	
6.	NaKCl F6	GTACGGCAGAGTGTAGATGCTC	593
	NaKCl R6	CTGGACCCATGTTCTCCATCAT	
7.	NaKCl F7	TGATGGAGAACATGGGTCCAG	559
	NaKCl R7	TGTTCTAAGATCCAGTGACTCCT	
8.	NaKCl F8	TCACAAAAAGGAGTCACTGGAT	441
	NaKCl R8	TGCAACATAACAATCCCGGTG	

For experimental validation, total DNA was isolated from muscle tissue of *C. magur* and the abiotic stress responsive primers were amplified for identification of the respective genes in the genome of *C. magur*. The amplified PCR products of desired sizes were exercised from the agarose gel and purified through purification columns. For validation of the PCR product, DNA sequencing was performed in the Applied Biosystem 3500 Genetic Analyser. The obtained nucleotide sequences, pertaining to temperature and ammonia responsive genes were assembled to achieve the complete gene for further analysis.

### Development of database on 'Abiotic Stress Responsive Genes in Fishes'

A database of 'Abiotic Stress Responsive Genes in Fishes' was designed for managing downloaded data (Fig.7). The web interface of the database was designed using PHP, JAVA, HTML and Perl scripting languages to provide ability to the user for searching browsing and data management activities included with analytical tools for sequence data analysis. Additionally, the interface includes analytical tools for sequence data analysis. The collected information (both sequence and annotation) of the identified genes responsible for temperature, ammonia & salinity stresses were populated in the database tables. The database presently covers information on 250 abiotic stress responsive genes of 38 fish species. It also covers information on 250 proteins responsive for abiotic stress tolerance in fishes.

The analytical tools include sequence similarity search, map viewer and phylogenetic analysis. The list of analytical tools was further strengthened by including protein sequence prediction tools, viz. ProtParam (<http://web.expasy.org/protparam/>), ProtScale (<http://web.expasy.org/protscale/>), ComputePI/MW ([http://web.expasy.org/compute\\_pi/](http://web.expasy.org/compute_pi/)), PeptideMas ([http://web.expasy.org/peptide\\_mass/](http://web.expasy.org/peptide_mass/)) and PeptideCutter ([http://web.expasy.org/peptide\\_cutter/](http://web.expasy.org/peptide_cutter/)).



Fig. 7. Screen shot showing the 'Abiotic Stress Responsive Database in Fishes'

### Updating genomic resource databases

Data was downloaded from NCBI using nucleotide search in FASTA and GenBank formats for sequence and annotation, respectively. Sequences of the fish species reported from the Indian subcontinent were extracted from the downloaded files with the help of Perl script. Perl parsing program was developed and applied to extract information from the files according to the database schema and extracted data was managed into the databases. This Perl program also includes functionality to update database based on new release of sequences in NCBI (<http://www.ncbi.nlm.nih.gov/>).

Four genomic resource databases *viz.* Fish and Shellfish Microsatellite Database (FishMicrosat), Fish Karyome, Fish Mitogenome Resource (FMiR) and Fish Barcode Information System (FBIS) were updated by including new records. FishMicrosat was updated by including 838 records of microsatellite sequences and Fish Karyome was updated by 390 records covering karyotype information and pictures along with other general information on fishes. Similarly, FBIS was updated by including 4186 barcode records and other general information on fishes. FMiR was updated by adding 474 records of mitogenome sequences. Thus, FishMicrosat presently covers 12111, Fish Karyome 878; FMiR 1776 and FBIS 21011 records.

A content based fish genomic portal, named 'FishCABin', was developed under the Linux operating system using PHP Perl and HTML scripting languages and hosted on URL

<http://mail.nbfgr.res.in/FishCABin/>. The portal provides ability to the user to work with all databases or selected databases and also includes the information on the activities under the fisheries domain. The portal also facilitates the access of the high performing computer (HPC) resources by the user through user registration process included in the portal. Centralized data browsing and search functionality was included into the portal to work with all or selected genomic resource databases.

The facility of Advanced Supercomputing Hub for Aquatic Animals (ASHAA) established under 'Centre for Agricultural Bioinformatics in Fisheries Domain', at the Institute for proteomic and genomic data analysis was extended to different labs and outside agencies for data analysis through remote log in process by sharing the hardware as well as software resources. The software resources for data analysis work include CLC Genomic, Discovery Studio and ABySS assemblers. All the jobs in HPC were managed using PBS Pro script and at present 09 users are registered with us to access the HPC resources remotely.

### 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 Kr. Yadav and Trivesh S. Mayekar

**Funding Agency:** Institutional

Information on the genetic diversity is of great importance for fisheries conservation and management. Therefore, the aim of this project is to build genomic resources and to gain knowledge about functional biodiversity, that can be applied to sustainably manage biodiversity of important fresh water fish species, *Tor putitora* and *Clarias magur*.

#### *Tor putitora*

A total of 33 individuals of *Tor putitora* were collected from Kosi River, Ramnagar, Uttarakhand during the period under report (Fig. 9). Length, weight, scales and images were taken for all the specimens. Tissue samples (muscle and fin), liver, kidney, gonad, heart, and brain were collected and preserved in liquid nitrogen. Separate tissue samples (muscle and fin) were preserved in ethanol.

Based on the lip structure of individuals collected, two morphological variants of *Tor putitora*



Fig. 8. Screen shot of 'FishCABin' portal



**Fig. 9. Sampling in Kosi river**

were identified (Fig. 10). The gDNA was isolated from all the individuals collected from muscle tissue and COI regions were successfully amplified and sequenced to differentiate different morphotypes. However the morphotypes could not be differentiated on the basis of COI sequences.

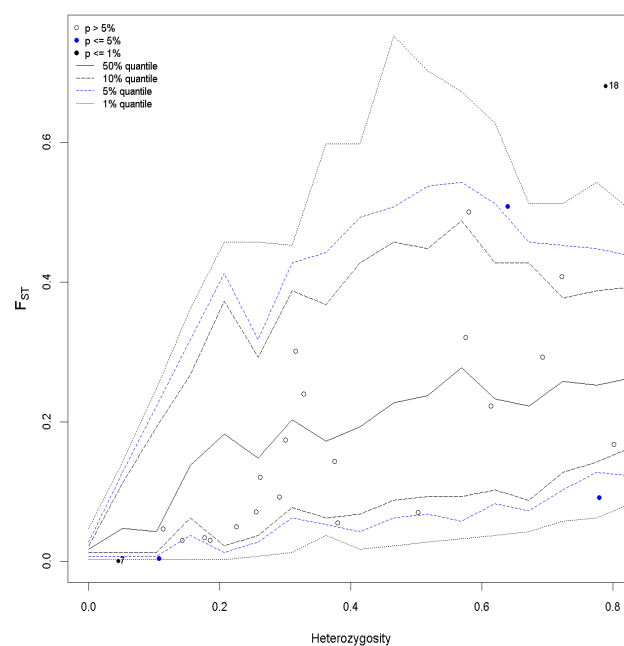
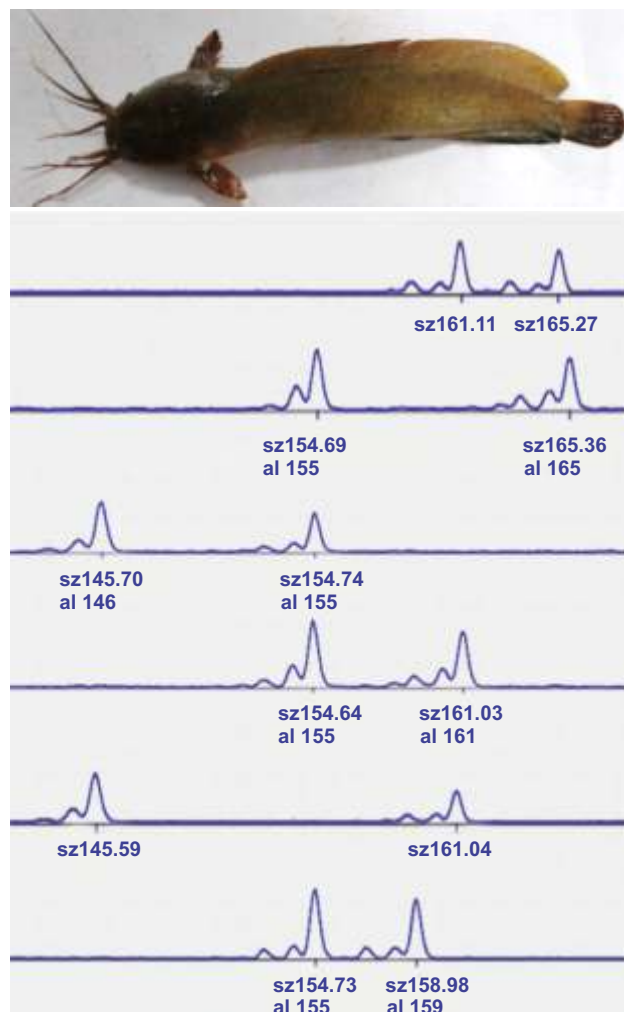


**Fig. 10. Morphotypes in *Tor putitora*.**

A-B *Tor putitora*: Wild type, C-D *Tor putitora*: Morphotype I and E-F *Tor putitora*: Morphotype II

### *Clarias magur*

In *Clarias magur*, 166 primers of EST-SSRs of annotated genes were tested on 24 individuals from eight different populations. Out of 166 primers, 128 primers amplified, 91 were monomorphic and 37 primers were polymorphic. Genotyping of 31 polymorphic primers were carried out for 96 samples from 4 different populations including Lucknow, Agartala, Kolkata and Adilabad. When analysed for outlier loci, three genes showed under positive selection, none for balancing selection and rest neutral effect (Fig. 11).



**Fig. 11. Detection of loci under selection from genome scans based on  $F_{st}$  in *Clarias magur***



**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 K. Singh (PI), T.T. Ajith Kumar, Santosh Kumar and Vikas Sahu

**Funding Agency:** Institutional

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 conditions enable its high-density culture with elevated production per unit area. NBFGR has been constantly striving for holistic approach for research on this important species. This work aims at creating marker panel consisting of SSR/SNP loci and assaying on reference family panel to generate first generation linkage map in *C. magur*.

A total of 166 *Clarias magur* individuals were collected from Uttar Pradesh (15), Madhya Pradesh (36), Jharkhand (45) and Bihar (70) and developed as brooders for captive family production (Fig. 12).



**Fig. 12. Brooders of *Clarias magur***

Using inter-population crosses, eight full-sib families were produced using individuals from Uttar Pradesh, Madhya Pradesh and Jharkhand. The commercially available hormone, *Gonopro* was injected in females @ 2 ml and males @ 1 ml per kg of body weight. After 16-



**Fig. 13. (a) Stripping of female magur (b) dissected out testes**

18 hours, the male was sacrificed to obtain sperm. The testes were dissected out, cleaned with saline solution and trimmed with scissors to remove excess tissue and blood (Fig. 13). The testes were chopped into smaller pieces and macerated with physiological saline and extract was used to fertilize eggs that were obtained through stripping of the female.

Following a brief incubation period for fertilization, the eggs were washed with water 2-3 times to remove unused seminal fluid (Fig. 14). The fertilized eggs were incubated in flow through system. The hatching took place after 20-22 hours.



**Fig. 14. Fertilized eggs of *Clarias magur***

After the absorption of the yolk sac, the zooplanktons and artemia were used to feed the juveniles. Up to the age of one month, the juveniles were reared family-wise in FRP tanks (Fig. 15). Progenies having attained size of minimum 5 grams were tagged with PIT tags and being reared communally in large cement tanks outdoor (Fig. 16).

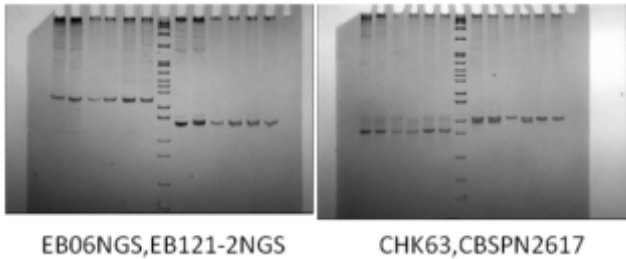


**Fig. 15. Rearing of juveniles**

A total of 100 tissue samples per family were collected and preserved in 95% ethanol for genomic DNA extraction. A total of 116 microsatellite loci were tested on parents of three families produced during previous year, of which 24 were found polymorphic in one or the other parent (Fig. 17).



**Fig. 16. PIT tagging of grown-up individuals**



**Fig.17. Images showing microsatellite amplification in parents of three families.**

**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- March, 2017

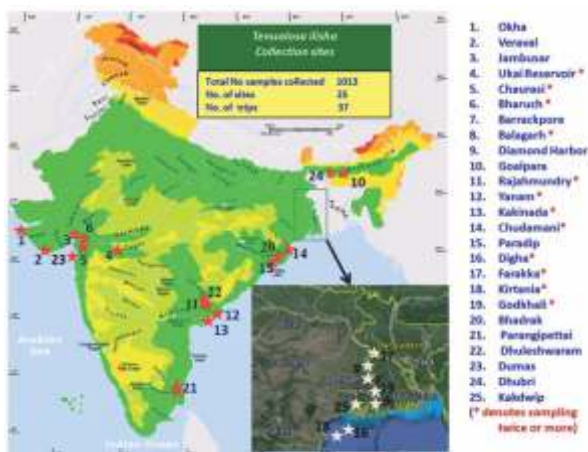
**Project Personnel from NBFGR:** Vindhya Mohindra (CCPI), Kuldeep K. Lal (Up to 18 August, 2014), Rajeev K. Singh, Sangeeta Mandal and J. K. Jena

**Funding Agency:** NASF, ICAR

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

A total of 233 samples of *Tenualosa ilisha*, comprising 104 from Hooghly river, 10 Brahmaputra river, 85 Ganga river and 34 Kakdwip were collected during the period under report. Altogether 25 sampling sites, covering both east and west coast (freshwater, brackishwater and marine locations) have been explored and tissue accessions from 1013 individuals of *T. ilisha* collected in this project so far (Fig. 18). Length, weight and truss images



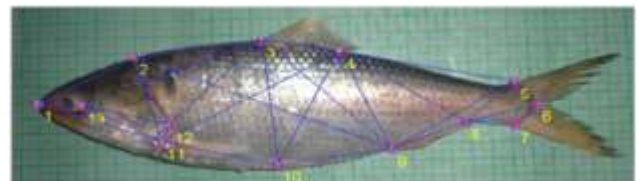
**Fig. 18. Collections sites for *Tenualosa ilisha* from natural distribution**

recorded for all the specimens, from which samples were collected (Fig. 19). Tissue samples (blood, muscle, fin) were collected from all the specimens in 95% ethanol.

### Population variation and diversity analysis through morphological and molecular markers

#### (i) Variation in size-free landmark based morpho-metric traits through TRUSS network analysis

A total of 411 samples from 10 locations of freshwaters: Padma (Farakka), Hoogly (Balagarh and Godkhali), Digha, Bay of Bengal (marine) Kirtania Port (marine), Chudamani (marine), Betrani (Bhadrak) freshwater, Dhavalesharam and Yanam (Godavari), Kakinada, Bay of Bengal, Parangipettai and Vellar estuary were analyzed to study stock structure. Mis-assignments within groups were observed between the samples of Farakka, Balagarh and Godkhali and between Digha, Kirtania and Chudamani. Mixing of samples was observed between the samples of Dhavaleswaram, Yanam and Kakinada. Therefore, results indicate existence of variation on the basis of TRUSS in the freshwater, marine and estuarine ecosystem of east coast.



**Fig. 19. Locations of 13 landmarks and TRUSS Network used for shape analysis**

Land marks refer to 1. anterior tip of snout at upper jaw 2. most posterior aspect of neurocranium (beginning 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 10. 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.

#### (ii) Genetic variability and divergence using molecular markers

Genetic divergence among four freshwater, three brackishwater and five marine ecosystems from East coast of India. A total of 339 sample accessions were analysed with four mitochondrial markers, cyto b, ATPase6/8, 16s and 12s rRNA.

#### Combined cyt b and ATPase6/8 (1986 bps):

A total of 73 haplotypes were observed, out of which 18 were shared haplotypes and 55 specific to their populations. Haplotype Diversity (h) ranged from 0.0000 +/-0.0000 (Rajahmundry) to 1.0000 +/-0.5000 (Paradip). Nucleotide diversity

( $\pi$ ) ranged from  $0.000000 \pm 0.000000$  (Rajahmundry) to  $0.002022 \pm 0.001150$  (Kirtunia). Hierarchical analysis of molecular variance (AMOVA) analysis revealed high within

population variation (80.12%), among populations within groups 14.91% and low among groups (4.97%). The fixation index was 0.1988 and pair wise  $F_{ST}$  ranged from 0.0000 to 0.8109 (Table 4).

**Table 4. Hierarchical analysis of molecular variance (AMOVA) of *Tenuulosa ilisha* for combined cyt b and ATPase6/8 regions**

Source of variation	Sum of squares	Variance components	% of variation	Fixation Index	P value
Among groups	45.700	0.08205	4.97	0.04972	0.22092 $\pm 0.01202$
Among populations	69.470	0.24607	14.91	0.15691*	0.00000 $\pm 0.00000$
Within populations	427.074	1.32221	80.12	0.19882*	0.00000 $\pm 0.00000$
Total	542.244	1.65034			

**Combined 16s and 12 s (951bps):** A total of 26 haplotypes were observed, out of which 8 were shared haplotypes and 18 specific to their populations. Haplotype Diversity (h) ranged from  $0.0000 \pm 0.0000$  (Rajahmundry, Kakinada\_1, Naraj\_old) to  $1.0000 \pm 0.5000$  (Paradip). Nucleotide diversity ( $\pi$ ) ranged from  $0.0000 \pm 0.0000$  (Rajahmundry, Kakinada\_1, Naraj\_old) to  $0.002103 \pm 0.002576$  (Paradip).

Mean number of pairwise differences ranged from  $0.0000 \pm 0.0000$  (Rajahmundry, Kakinada\_1, Naraj\_old) to  $2.000000 \pm 1.732051$  (Paradip). Hierarchical analysis of molecular variance (AMOVA) analysis revealed high within population variation (73.84%) and low among groups (5.42%). The fixation index was 0.26155 and pair wise  $F_{ST}$  ranged from 0.0000 to 0.85407 (Table 5).

**Table 5. Hierarchical analysis of molecular variance analysis of *T. ilisha* for combined 16s and 12srRNA regions**

Source of variation	Sum of squares	Variance components	% of variation	Fixation Index	P value
Among groups	16.842	0.02706	5.42	0.05416	0.21408 $\pm 0.01132$
Among populations	26.923	0.10362	20.74	0.21927	0.00000 $\pm 0.00000$
Within populations	118.806	0.36896	73.84	0.26155	0.00000 $\pm 0.00000$
Total	162.571	0.49965			

**Microsatellite analysis:** A total of 71 polymorphic microsatellite loci (16 anonymous + 55 EST-SSRs) developed, were used to generate a data set of 6816 individual genotypes involving with 96 samples to test the validity of microsatellite loci in deciphering the genetic diversity. The sample set was created from the collections from distant

localities covering four river locations from three river basins, Ganga (Hooghly and Padma), Brahmaputra and Narmada, so that the detectable genetic variability is available for testing the markers. The results indicated a high genetic variability and strong genetic differentiation especially from Narmada (16 to 20 %  $F_{ST}$ ). The



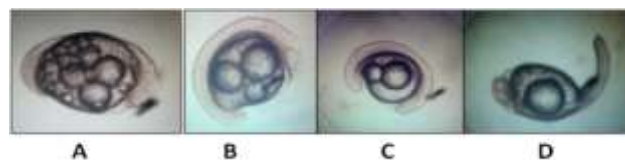
differentiation between the east coast rivers (Hooghly, Brahmaputra and Padma) was low ranging from (1.3 to 2.2 %). All the four populations indicated presence of private alleles NDM1 (18); BPT1 (40); HGL (14) and PDM1 (22). The observed heterozygosity ranged from 0.21 to 0.31 and expected heterozygosity was from 0.24 to 0.35. Mean number of allele per locus ranged from 2.6 to 3.4 with maximum of 26 alleles at the locus HIL Sd06. Most of the loci exhibited conformity to the Hardy-Weinberg expectations. A total of 6 loci were identified as outlier loci that are putatively under selection for adaptive genetic variation and one locus under balancing selection.

Forty three out of 71 loci, were found significant in determining genetic differentiation ( $p < 0.05$ ) which was used to generate 24381 reactions to genotype the 567 individual samples from 19 locations, comprising 22 collections from Ganga, Padma and Brahmaputra river systems.

### Cryopreservation of milt

Live, ripe milts of *Tenualosa. ilisha* (150 to 600 g) were obtained from commercial catches at Farakka barrage during November-December, 2015. Various extender compositions viz., TL7, TL7G, TL8 and TL8G were tried for cryopreservation of Hilsa milt. Milt was diluted both in 1:12 and 1:24 ratio for the purpose of cryopreservation. In fertility trial, fresh milt (control) was compared with frozen milt. Various trials were done and extender TL7 was found to be better than others. Extender TL7 gives better results than TL7G and using the extender TL7, three hundred fifty nine straws of 0.5 ml volume and nine cryo-vials of 2 ml volume were filled with milt extender in 1:12 ratio.

Fertility trial was carried out during March-April 2015 at Godkhali on river Hooghly. For three extenders tested, post-fertilized embryos developed cell division, survived upto 16-18 hrs and survival ranged from 37.4% to 51.9% after 16 hrs, which was due to poor egg quality. In November 2015 at Farakka on river Padma, a total four fertility trials were conducted and Extender TL 7 (1:12) gave best results. For extender TIL7, out of 212 and 167 fertilized eggs, 16.5% and 26.9% hatched out, respectively after 23 hours. For control, out of 706 and 650 fertilized eggs, 16.5% and 13.8% hatched out, respectively (Fig. 20).



**Fig. 20. Developmental stages of *T. ilisha* embryo.**

A. Early embryonic stage, B. 28 myotomes stage, C. 30 myotomes stage, D. 40 myotomes stage (vigorous twitching)

**Project Title:** ICAR-CRP- Genomics: *De-novo* genome sequencing of anadromous Indian Shad, *Tenualosa ilisha* (Hamilton 1822)

**Project period:** July, 2015 - March, 2017

**Platform Co-ordinator:** Dr. J. K. Jena

**Project Personnel:** Vindhya Mohindra (PI), Rajeev K. Singh, Basdeo Kushwaha, Trivesh S. Mayekar

**Funding Agency:** ICAR

The project aims to generate whole genome sequence information and allied resources in *Tenualosa ilisha*. The consolidated knowledge of sequenced genome will facilitate in understanding of genetic mechanisms influencing production traits in this potential aquaculture species.

### Flow cytometry analysis for *T. ilisha* genome size estimation

Genome size of *T. ilisha* was estimated to be 1.24 Gb, calculated using CEN (chicken erythrocyte nuclei) fluorescence and DNA content (2.5pg/nuclei) as standard (Fig. 21).

### *De novo* sequencing of Hilsa genome through third generation sequencer PacBio RSII

Genomic DNA was isolated from testes tissue of *T. ilisha* (Hilsa) for de novo sequencing using automated and phenol- chloroform methods. Quantitative and qualitative analysis was performed according to PacBio gDNA guidelines. Three 10 Kb libraries were prepared using DNA Template kit 2.0 and were sequenced using P4-C2 chemistry. In addition, seven 20 Kb libraries (after size selection with Bluepippin system) were constructed using SMRT Template kit 1.0 and sequenced using P6C4 chemistry. For 10 Kb libraries, a total of 86 SMRT cells were sequenced with P4C3 chemistry using 4 hr collection protocol. For 20 Kb libraries, a total of 60 SMRT cells were sequenced with P6C4 chemistry having 4 hr collection protocol. While, 96 SMRT cells were sequenced having 6hr collection protocol (Fig. 22). Through the SMRT cells for sequencing, a total of 182.8 Gb polymerase reads have been generated.

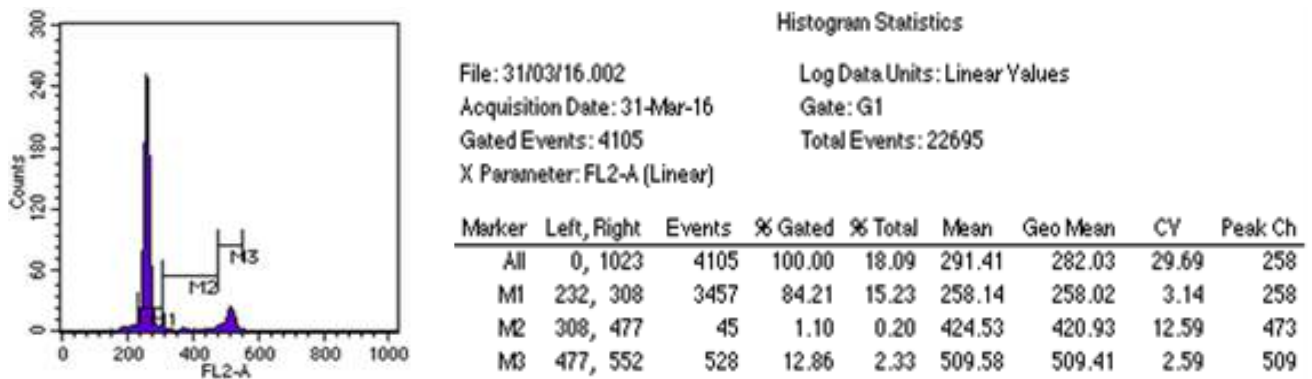


Fig. 21. Genome size estimation for *T. ilisha* through flow cytometry analysis

Read of insert analysis on SMRT portal using RS\_Read of Insert protocol for data generated from earlier 96 cells, showed mean read length of 7692 bp, having mean read quality of 95.99% and 4 number of mean passes showing high quality data.

*Chitala chitala* (n=45), *Mugil cephalus* (n=190), *Anguilla bengalensis* (n=5), *Silonia silondia*, (n=32), *Systomus sarana sarana* (n=285), *Sillago sihama* (n=362) and *Perna viridis* (n=231) were collected from different localities of river systems/estuaries and marine areas (Figures 23 & 24).

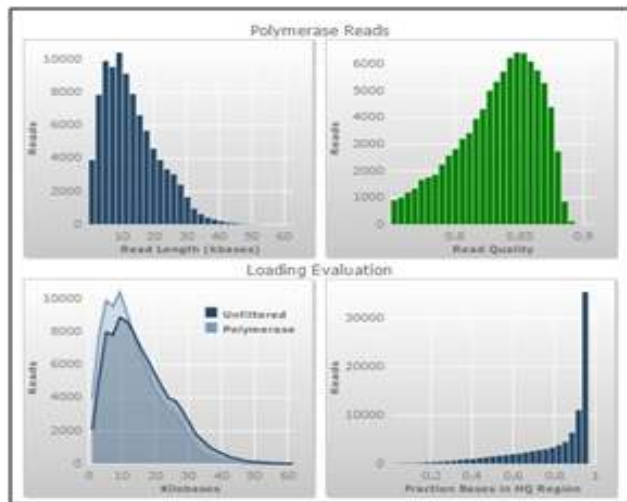


Fig. 22. Read length, quality and loading evaluation of 20 Kb library sequencing for *T. ilisha* on PacBio RSII

**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 K. Singh (PI), Vindhya Mohindra, T. T. Ajith Kumar, Sangeeta Mandal, Santosh Kumar

**PMFGR division:** V.S. Basheer, P.R. Divya, A.K. Pandian and Charan Ravi

**Funding Agency:** Institutional



Fig. 23. Map depicting collection sites of fish specimens/tissue samples

### Genetic Characterization

In featherback *Chitala chitala*, the microsatellite markers were identified through third generation sequencer PacBio RS II. The 1.5 Kb SMRT bell library of genomic DNA was sequenced to generate 2081.8 Mb polymerase read data. Primary analysis and removal of adapters generated Circular Consensus Sequence reads with >99% accuracy. The analysis of these reads resulted in identification of 2005 SSR motifs in 1164 sequences.

Tissue samples of target species, *Tor tor* (n=57),





**Fig. 24. Fishing/sample collection at riverine sites**

In mahseer *Tor tor*, genomic DNA was sequenced to mine microsatellites using the PacBio RS II sequencing platform. A single 1.5 kb library was sequenced using P6-C4 chemistry on two single-molecule real-time (SMRT) cells. The analysis of contigs and singletons produced a total of 19011 different SSR repeats in 8249 sequences. A total of 50 primer pairs were designed from the conserved flanking sequences and tested, of which 22 loci exhibited polymorphism.

In Olive barb, *Systomus sarana sarana*, primers of 65 microsatellite loci from three resource species, viz. *Labeo rohita*, *Camptostoma anomalum* and *Cyprinus carpio* were used to cross amplify the individuals. Twenty loci exhibited polymorphic banding patterns. These loci will be useful for their applications in population level studies.

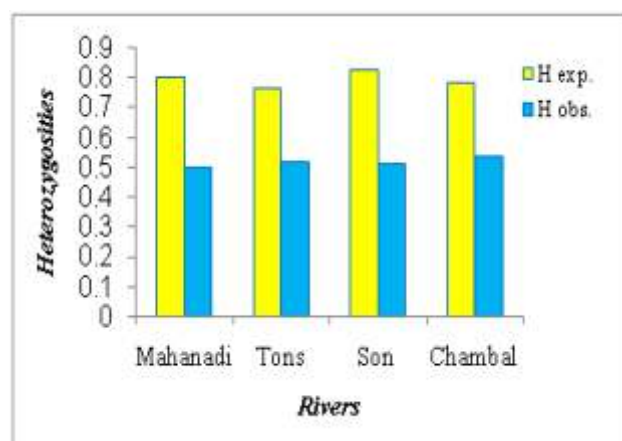
In green musel, *Perna viridis*, 37 polymorphic microsatellite loci (Cao *et al.* 2013, Lin *et al.* 2007, Ong *et al.* 2009) were tested. Twelve loci were found to be polymorphic with number of alleles ranging from 2 to 24. The repeat motifs were dinucleotide (9 loci) and tetranucleotide (3 loci). These validated loci will be used for generating genotype data for population genetics. In grey mullet, *Mugil cephalus*, 37 species-specific microsatellite loci (Xu *et al.* 2010 and Shen *et al.*, 2012) were tested and twenty loci were found polymorphic.

In *Sillago sihama*, a total of 20 polymorphic microsatellite loci (Yu-song guo *et al.* 2012) were tested in individuals from distant locations. Eight loci were selected for further analysis for genetic variation.

#### **Genetic diversity in four riverine populations of *Silonia silondia***

Genotyping data of n=76 individuals of *S. silondia* from four rivers, viz. Mahanadi (23), Son (24), Chambal (16) and Tons (13), was generated at 11 microsatellite loci to quantify the genetic

diversity parameters. No significant ( $P < 0.05$ ) linkage disequilibrium was detected in pairwise comparisons made for all loci over all the populations. Observed and expected heterozygosity values are presented in Fig. 25. The results indicated that Mahanadi samples displayed the highest number of alleles ( $N_a = 10.09$ ). The AMOVA analysis indicated significant genetic differentiation among riverine populations (overall  $F_{ST} = 0.075$ ;  $P < 0.0001$ ) with maximum variation (92.5%) within populations. Cross-priming tests resulted in successful amplification (35-38%) of heterologous loci in four related species viz., *Clupisoma garua*, *C. taakree*, *Ailia coila* and *Eutropiichthys vacha* (Table 6).



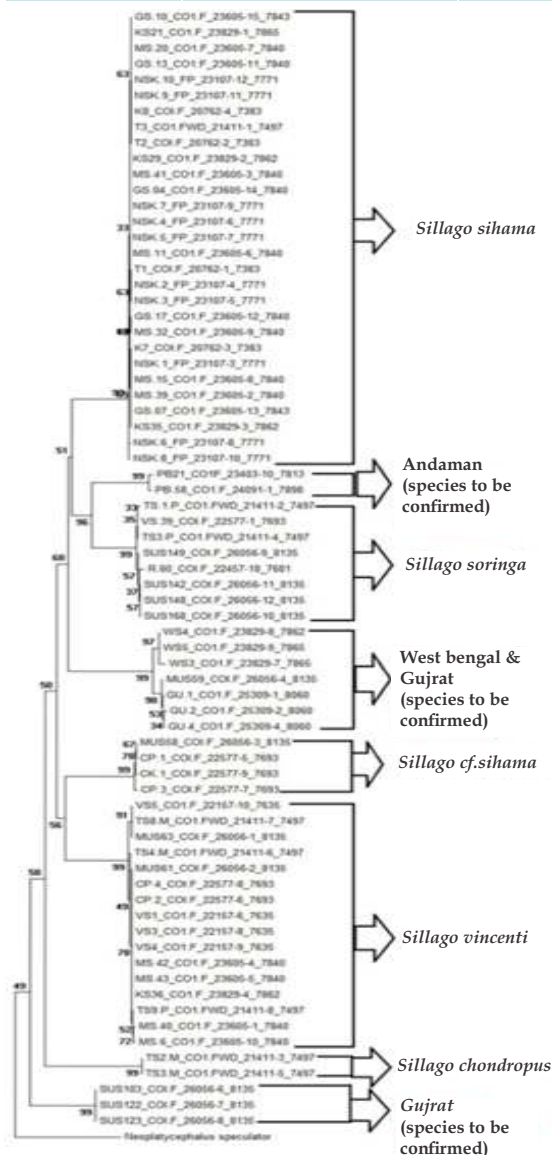
**Fig. 25. Heterozygosity values of four natural populations of *S. silondia* based on microsatellites**

#### **Species confirmation of *Sillago* spp. through mitochondrial COI sequence analysis**

With the purpose of resolving taxonomic ambiguity among fishes belonging to the genus *Sillago* in Indian waters, tissue samples were collected from eight locations of both the coasts of India and analyzed using both traditional and molecular tools. Species-specific molecular signatures (partial cytochrome c oxidase I) for five species belonging to the genus *Sillago* were

**Table 6. Cross-amplification of microsatellite loci for *Silonia silondia* in four other Schilbid catfishes**

Locus	Allele size ranges			
	<i>Clupisoma garua</i>	<i>C. takree</i>	<i>Ailia coila</i>	<i>Eutropiichthys vacha</i>
<i>Sis-1*</i>	236	228	-	232
<i>Sis-3*</i>	-	-	-	-
<i>Sis-18*</i>	186-194	126-138	124-136	180-186
<i>Sis-31*</i>	196	-	-	182-190
<i>Sis-79*</i>	192-204	202-276	-	-
<i>Sis-96*</i>	123-140	238-246	-	140
<i>Sis-111*</i>	186-218	-	-	186-274
<i>Sis-114*</i>	140-186	184	140	140
<i>Sis-115*</i>	-	-	-	-
<i>Sis-134*</i>	-	-	-	-
<i>Sis-142*</i>	-	-	-	-


**Fig. 26. Phylogenetic tree depicting evolutionary relationships of the species under genus *Silago* in Indian waters**

generated and phylogenetic relationship among the species was determined on the basis of 628 nucleotides. The genetic divergence values of intra-species ranged from 0.000 to 0.014, while 0.120-0.246 for inter-species in this study. The phylogenetic tree illustrating the taxonomic relatedness of *S. sihama* with other species under the genus is depicted in Figure 26.

### Length-weight relationships

Length-weight relationship is one of the important parameter for assessment of stock, production, biomass and environmental impact on biological and physiological conditions of fishes. Condition factor (K) and relative condition factor (Kn) were used to compare the 'condition' or 'well being' of the species as well as to indicate the physiological and nutritional status. The summary results are depicted in table 7 and Fig. 27.

**Table 7. Summary of results of Length-Weight relationships in four species**

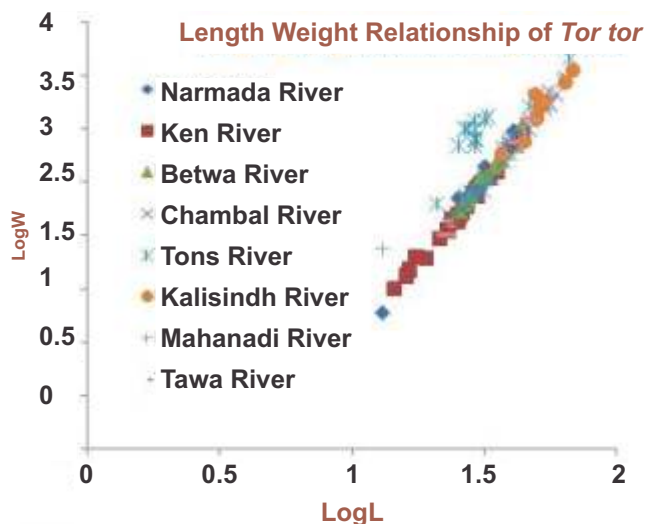
Species	Length-weight relationship	r <sup>2</sup>	K	Kn
<i>Chitala chitala</i>	LogW= 3.029LogX-2.197	0.935	0.57-0.87	0.9-1.09
<i>Systemus sarana sarana</i>	LogW= 3.314LogX-2.023	0.975	1.23-1.7	0.9-1.07
<i>Tor tor</i>	LogW= 2.914LogX-1.771	0.855	0.98-2.21	0.9-1.03
<i>Silonia silondia</i>	LogW= 2.505LogX-1.327	0.885	0.57-1.39	0.98-1.09

r<sup>2</sup>: Correlation coefficient; K: Fulton's Condition factor; Kn: Relative Condition factor

### Biological parameters of *Silago sihama*

In *S. sihama*, the parameters such as, length and weight, sex, maturity stages and gonad weight were recorded for all the collected specimens from Kochi, Mangalore and Goa. The estimates for specimens were: Goa (Total Length- 19.2 to 23.0;





**Fig. 27. Graphs illustrating the Length-weight relationships in *Tor tor***

Total Weight-50 to 80 g; Gonadosomatic Index- 2.3 to 7.5), Mangalore (TL- 17.1 to 28.1; TW-35 to 151 g; GSI -2.4 to 8.1) and Kochi (TL- 17.6 to 25.2; TW-30 to 115 g; GSI-2.2 to 7.0) (Fig. 28).

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

**Project Period:** April, 2012 – March, 2016

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

**Funding Agency:** DBT, Govt. of India

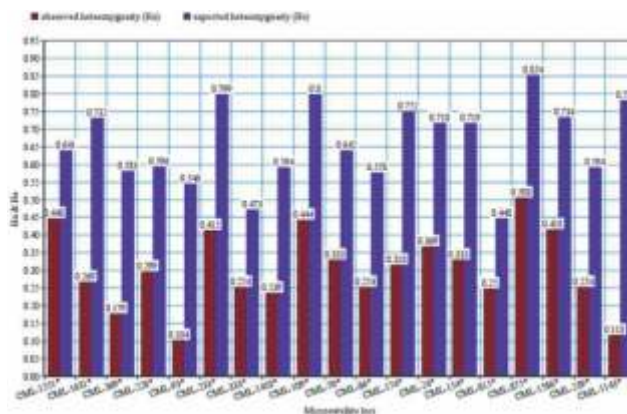
### Genetic variability studies in the great snakehead *Channa marulius*

Individual genotype data of 75 individuals of *Channa marulius* from three geographically distant locations, Godavari (25), Teesta (25) and Mahanadi (25) was generated for 19 polymorphic microsatellite loci, which were consistently amenable for scoring. The genotype data was analysed for genetic variation parameters. The genetic characteristics of each microsatellite loci such as repeat motif, allele size, annealing temperature, number of alleles, polymorphic information content (PIC) values and NCBI accession nos. are given in Table 8. PIC values suggested that the these microsatellite markers were promising for genetic variability assessment in *C. marulius*. The results did not reveal evidences of linkage disequilibrium between any pairs of loci tested for over all populations, which indicated that loci under study were genetically unlinked. The observed and expected heterozygosity values (Ho/He) are represented in Fig. 29. The coefficient

of genetic differentiation ( $F_{ST}$ ) between Godavari and Teesta was 0.0522, whereas, for Teesta-Mahanadi and Mahanadi-Godavari, 0.0567 and 0.0326, respectively.



**Fig. 28. Gonads of *S. sihama***



**Fig. 29. Bar diagrams depicting locus-wise observed (Ho) and expected (He) heterozygosity values**

The individual genotyping data is being developed for the amplification of microsatellite loci. Recently a total of n=280 genomic DNA samples of *C. marulius*, collected from rivers Godavari (61), Gomti (46), Kota/Chambal (47), Teesta (34), Mahanadi (22), and Rewa (70), are being genotyped at 19 loci to generate comprehensive dataset across its natural distribution range.

**Project Title:** Genetic stock structure analysis of two commercially important marine species *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 Labrechai Mog Chowdhury

**Funding Agency:** Institutional

**Table 8. Characteristics of 19 microsatellite loci developed for *C. marulius***

Locus	Repeat motif	Size (bp)	T <sub>a</sub> ( C)	Na	PIC	NCBI Accession no.
CML -1551*	(GAT)7(TTA)6	194	51	5	0.580	KT986492
CML -163 1*	(AAT)7	216	55	6	0.696	KT986495
CML -369*	(GT)8	180	55	4	0.531	KT986510
CML -228*	(CA)12	210	59.4	6	0.565	KT986500
CML -93*	(CA)12	105	61.5	4	0.453	KT986502
CML -233*	(CA)14	240	59.4	9	0.765	KT986501
CML -333*	(CA)9	180	56	3	0.366	KT986509
CML -1410*	(ACT)5	122	47	5	0.556	KT986494
CML -109*	(GT)10	112	54	9	0.768	KT986503
CML -70*	(TG)12	110	54	6	0.580	KT986507
CML -66*	(TG)6	178	55	3	0.505	KT986508
CML -174*	(GT)20	202	49	8	0.707	KT986505
CML -24*	(TG)13	210	55.5	8	0.675	KT986506
CML -154*	(GT)16	184	55	6	0.659	KT986504
CML -815*	(AGAC)4	124	55	5	0.415	KT986496
CML -875*	(TAGA)12	122	55	11	0.831	KT986497
CML -1586*	(TAC)8 (TAA)6	170	57	7	0.685	KT986493
CML -239*	(CA)19	100	65	6	0.556	KT986499
CML -1143*	(GATA)6	126	55	9	0.744	KT986498

*Scomberomorus commerson* and *Parapenaeopsis stylifera* constitutes 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 this 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. Therefore, work on identification of genetic stocks for the sustainable harvest of above mentioned commercially important marine genetic resources was continued.

#### ***Scomberomorus commerson***

Muscle tissues of *S. commerson* were collected from six different geographical locations along the Indian coast (Fig. 30) (Mangalore, Kochi and Veraval in the west coast; Chennai, Visakhapatnam and Kolkata in the east coast). A total of 60 tissue samples of each species were



**Fig. 30. *S. commerson***

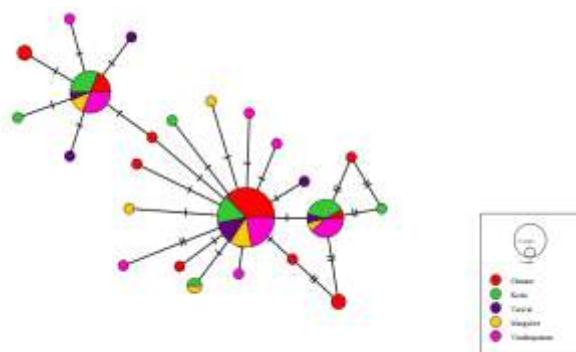
collected from each location and total genomic DNA was extracted for all the samples.

#### **Mitochondrial analysis**

Mitochondrial D loop region was sequenced for *S. commerson* samples collected from six sampling sites across both the coasts of India. A total of 427bp sequence of D loop was obtained. Pair wise F<sub>ST</sub> values obtained from D loop region analysis could not show any significant genetic differentiation of *S. commerson* between six sampling locations in Indian waters (F<sub>ST</sub>-0.02; P>0.05).

ATPase 6/8 genes were amplified in 90 fish samples from five different geographical locations (n=90) along Indian coast (Mangalore, Kochi and Veraval in the west coast; Chennai and Visakhapatnam in the east coast). The amplification yielded 842 bp with 23 haplotypes across the sampling sites. Pair wise co-efficient of

genetic differentiation ( $F_{ST}$ ) values did not show any significant differentiation ( $p > 0.05$ ) between populations of *S. commerson* (Fig. 31). From these observations it is evident that the population of the *S. commerson* need to be considered as single stock along the Indian coast.



**Fig. 31. Minimum spanning network of ATPase 6/8 genes of *S. commerson***

### Microsatellite analysis

A total of 40 microsatellite markers were screened, out of which 12 polymorphic microsatellite loci were standardized through cross-species amplification. Genotyping was carried out using labelled primers from five locations ( $n=60$  each) using twelve loci. The genotyped data were analysed using Arlequin ver. 3.0, Genpop ver. 3.3, and GenAlex ver. 6.5. Parameters estimated included number of alleles, allelic frequencies, observed and expected heterozygosity, linkage disequilibrium, conformity of allele frequencies to that expected under Hardy-Weinberg equilibrium and estimates of population differentiation including F-statistics and gene flow, genetic similarity and distance.

Number of alleles ranged from 6 (J10Sc) to 33 (Sca30) and the allele size ranged 108 (Sca30) to 450bp (Sa2657). Considering all microsatellite loci, the mean number of alleles for populations was 15. There was no significant association indicative of linkage disequilibrium between any pair of microsatellite loci for any populations ( $P > 0.05$ ) indicating independence of 12 loci. Therefore, all 12 loci were considered for analysis. All the loci showing positive  $F_{IS}$  values in different populations were tested for presence of null alleles using MICRO-CHECKER. The estimated null allele frequency was not significant at all 12 loci of null alleles and false homozygotes. The values of observed heterozygosity ( $H_o$ ) ranged from 0.380 (J10Sc) to 0.920 (Sca30) and expected heterozygosity ( $H_e$ )

values ranged from 0.376 (J10Sc) to 0.944 (Sca30). Three of the loci showed significant deviation from HWE across all the populations.

**Table 9. Pair wise mean  $F_{ST}$  values between populations of *S. commerson***

	Veraval	Mangalore	Kochi	Chennai	Visakhapatnam
Veraval	0				
Mangalore	0.02256	0			
Kochi	0.01956	0.00229	0		
Chennai	0.03996	0.02202	0.01415	0	
Visakhapatnam	0.028	0.00993	0.0128	0.0166	0

The co-efficient of genetic differentiation,  $F_{ST}$  ranged from 0.009 for the locus Sca 44 to 0.053 for the locus 90 RTE, with a mean of  $0.023 \pm 0.004$ . Pair-wise Fisher's  $F_{ST}$  between *S. commerson* samples from five different locations was found to be low and non-significant (Table 9). Population genetic stock structure of *S. commerson* using nuclear and mitochondrial markers revealed low and non-significant values of co-efficient of genetic differentiation ( $F_{ST}$ ) for pair-wise populations indicating unit stock in Indian waters. The finding of the study indicates the species can be considered as a unit stock for fishery management in Indian waters.



**Fig. 32. *Parapenaeopsis styliifera***

Pleopod/muscle tissues of *P. styliifera* (Fig. 32) were collected from five different geographical locations along the Indian coast (Kochi, Goa and Veraval in the west coast; Chennai and Visakhapatnam in the east coast). A total of 60 samples from each location were collected and total genomic DNA was extracted for all the collected samples.

### Phylogenetic analysis of five species under the genus *Parapenaeopsis*

The morphological identification of *P. styliifera* is mainly based on number of telson

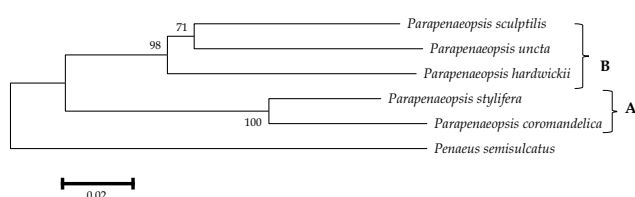


spines (One - *P. coromandelica*; 2 or more - *P. stylifera*). Mitochondrial COI gene analysis was carried out to differentiate the look-alike species. The analysis could differentiate *P. stylifera* and *P. coromandelica*, with a divergent value of 7.6%. Along with the above two species, another three species belonging to the same genus i.e. *P. uncta*, *P. hardwickii* and *P. sculptilis* collected from Visakhapatnam and Veraval, respectively, were also included for phylogenetic analysis using mtDNA COI gene (Table 10).

**Table 10. Genetic divergence values among five species of genus *Parapenaeopsis***

	<i>P. stylifera</i>	<i>P. coromandelica</i>	<i>P. hardwickii</i>	<i>P. sculptilis</i>	<i>P. uncta</i>
<i>P. stylifera</i>					
<i>P. coromandelica</i>	0.076				
<i>P. hardwickii</i>	0.19	0.20			
<i>P. sculptilis</i>	0.19	0.20	0.14		
<i>P. uncta</i>	0.19	0.21	0.14	0.12	

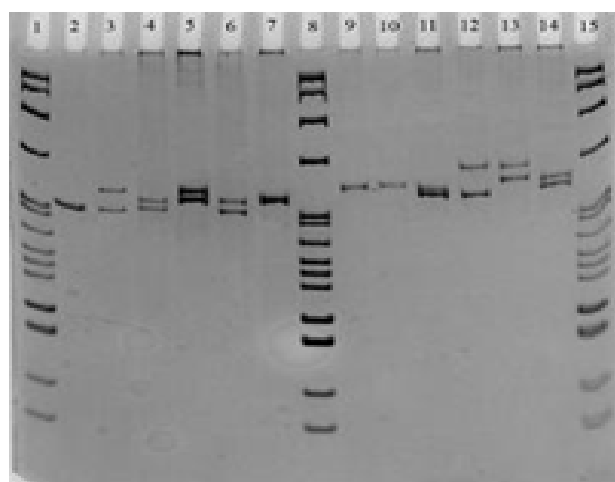
The phylogenetic tree constructed using Neighbor joining analysis based on Kimura 2 parameter model showed two distinct clades. *P. stylifera* and *P. coromandelica* formed a distinct branch in clade A, which was supported by high bootstrap value (100%) whereas, clade B comprised of *P. hardwickii*, *P. sculptilis* and *P. uncta* with high bootstrap values. The results indicate the closeness of *P. stylifera* and *P. coromandelica* in comparison to other three species under the genus (Fig. 33).



**Fig. 33. NJ tree depicting phylogenetic relationship of five species under *Parapenaeopsis* genus in Indian waters**

### Microsatellite analysis

Eleven polymorphic microsatellite primers were developed in *P. stylifera* through cross priming out of 40 microsatellite primers from *Litopenaeus vannamei*, *Fenneropenaeus chinensis* and *Penaeus monodon* and nine primers from *Parapenaeopsis hardwickii*. Genotyping was completed with 11 polymorphic microsatellite markers in *P. stylifera* from five locations (n=48 each) (Visakhapatnam, Chennai on the east coast; Veraval, Goa, Kochi on the west coast of India).



**Fig. 34. Amplification of microsatellite primer (AY500866) in *P. stylifera***

In the genotyped data of *P. stylifera*, the number of alleles ranged from 10 (AF360104) to 37 (AY500866) and the allele size ranged 90 (AF360104) to 484bp (CNMMG 365). Over all microsatellite loci, the mean number of alleles for populations was 13.7. There was no significant association indicative of linkage disequilibrium between any pair of microsatellite loci for any populations ( $P > 0.05$ ) indicating independence of 11 loci. Therefore, all 11 loci were considered for analysis. All the loci showing positive  $F_{IS}$  values in different populations were tested for presence of null alleles using MICRO-CHECKER. The estimated null allele frequency was not significant at all 11 loci suggesting the absence of null alleles and false homozygotes. The values of  $H_o$  ranged from 0.417 (AY500865) to 0.925 (CNM MG 362) and  $H_e$  values ranged from 0.424 (AY500865) to 0.918 (AF360098). The co-efficient of genetic differentiation,  $F_{ST}$  ranged from 0.023 for the locus AF360098 to 0.046 for the locus CNM MG365, with a mean of  $0.034 \pm 0.002$ . Pair wise fisher's  $F_{ST}$  between *P. stylifera* samples revealed two stocks (1-West coast; 2-East coast) with low but significant variation ( $P < 0.001$ ) (Table 11).

**Table 11. Pair wise mean  $F_{ST}$  values between populations of *P. stylifera***

	Veraval	Goa	Kochi	Chennai	Visakhapatnam
Veraval	0				
Goa	0.01311	0			
Kochi	0.01229	0.01937	0		
Chennai	0.03471*	0.02841*	0.02849*	0	
Visakhapatnam	0.03227*	0.02469*	0.02266*	0.00841	0

( $P < 0.001$ )\*

**Project Title:** Development of DNA chip for identification of commercially important fish species from Indian waters.

**Project Duration:** April 2015 – March 2018

**Project Personnel:** A. Kathirvelpandian (PI), Labrechai Mog Chowdhury, Murali S and Toms C. Joseph (ICAR-CIFT, Kochi)

**Funding Agency:** Institutional

DNA-based identification methods are established as powerful tools, exhibiting an unprecedented accuracy because of their inherently high resolution. Whereas most of the methods presently in use, such as PCR-based DNA amplification followed by sequencing techniques, allow to handle only single or a few species at the same time, DNA chips are believed to have the potential of identifying hundreds of species in parallel and to differentiate them against an even larger number of related species with reduced technical skill for analysis and interpretation. The customized chip can be used to identify fish and fishery products in reduced time, thus, making them useful for various fishery applications management, traceability and checking such as adulteration in export commodities. By considering the need, the proposed project aims at developing DNA chip

based on the species specific mitochondrial sequence probes for commercially important group of fish species of the country. The development of DNA chip will pave the way for further extending the same in identifying more number of fish species in the country.

The mitochondrial COI gene sequences for 16 species of scombrids were collected from NCBI database. Similarly, the mitochondrial COI sequences for 36 grouper species were generated in the lab and also collected from NCBI database. Mitochondrial COI sequences of over 650 of grouper and 550 of scombrids were modified as test data set in FASTA format. Bar-coding with Logic Formulas (BLOG-2.0) software was downloaded and configured for analysis of barcode information. A DNA barcode data set for fishes comprising of over 626 sequences created by BLOG was downloaded. Test data set of above species was analysed to identify characters or patterns or relationship specific to fish species using BLOG-2.0. A total of over 39 and 67 variable sites for differentiating species of scombrids and groupers were identified and degenerate probes were designed (Table 12). The identified species formula will be used to develop species specific probes for spotting in customised DNA chip for rapid identification of species belonging to scombrids.

**Table 12. Species formula analysed in Scombrids using BLOG-2.0**

Species	Species Formula						
<i>Auxis_rochei</i>	pos312=C	AND	pos357=C	AND	pos498=A		
<i>Auxis_thazard</i>	pos243=A	AND	pos312=T				
<i>Euthynnus_affinis</i>	pos276=G	AND	pos357=C				
<i>Gymnosarda_unicolor</i>	pos354=T	AND	pos576=C				
<i>Katsuwonus_pelamis</i>	pos579=T						
<i>Thunnus_alalunga</i>	pos435=C	AND	pos498=T	OR	pos516=G		
<i>Thunnus_albacares</i>	pos219=C	AND	pos354=A	AND	pos492=C	AND	pos576=A
	OR	pos312=T	AND	pos357=G	OR	pos575=T	
<i>Thunnus_obesus</i>	pos297=T	AND	pos312=C	AND	pos357=G		
<i>Thunnus_tonggol</i>	pos219=T	AND	pos498=T	AND	pos576=A	OR	pos354=G
<i>Rastrelliger_brachysoma</i>	pos516=T	AND	pos579=G				
<i>Rastrelliger_faughni</i>	pos297=C	AND	pos498=G				
<i>Rastrelliger_kanagurta</i>	pos222=T	AND	pos492=G				
<i>Sarda_orientalis</i>	pos579=C						
<i>Scomberomorus_commerson</i>	pos297=A	AND	pos498=G				
<i>Scomberomorus_guttatus</i>	pos315=C						
<i>Acanthocybium_solandri</i>	pos357=G	AND	pos492=T				

**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:** N S Nagpure (PI up to December 5, 2015) ; Basdeo Kushwaha (PI w.e.f. December 6, 2015), Ravindra Kumar and Mahender Singh

**Project period:** September, 2013 - September, 2016

**Funding Agency:** Department of Biotechnology, Govt. of India

### Generation of genome sequence data of *Clarias batrachus* using multiple sequencing platforms

**Table 13. Data statistics summary of Illumina MiSeq trimmed data**

Type of library	Read orientation	Maximum read length (bp)	Minimum read length (bp)	Data size (Gb)
150 -250 bp	R1	282	30	0.21
	R2	301	30	0.20
350 -450 bp	R1	301	30	1.87
	R2	301	30	1.46
400 -500 bp	R1	301	30	1.78
	R2	301	30	1.34
550 -650 bp	R1	301	30	0.45
	R2	301	30	0.33
4-6 Kb	R1	283	30	0.16
	R2	283	30	0.13
<b>Total data (Gb)</b>				<b>7.93</b>

Mate pair data (8 Kb and 20 Kb) for *C. batrachus* genome was generated on Roche 454 (Table 14). The linker sequences were removed,

Whole genome sequence data for *C. batrachus* generated on Illumina HiSeq using paired-end library (100 bp) for normal insert (150-250 bp, 350-450 bp and 550-650 bp) as well as mate-pair libraries (5 Kb and 10 Kb) was checked for quality and adaptor contaminations. The processed high quality data was used for further downstream analysis. Similarly, whole genome sequence paired end data (300 bp) for *C. batrachus* was generated on Illumina MiSeq for both normal insert (150-250 bp, 350-450 bp and 550-650 bp) and mate pair (4-6 Kb) libraries. The data processing was carried out using NGSQC Toolkit and the trimmed data statistics of Illumina MiSeq is depicted in Table 13. In addition, ~50 Gb paired end (100 bp) sequence data was also generated for *C. batrachus* on Illumina HiSeq from 5 mate pair libraries, viz., 2 kb, 4 kb, 8 kb, 12 kb and 20 kb, for the purpose of scaffold generation from the assembled contigs.

followed by length filtering and removal of low quality bases.

**Table 14. Data statistics summary of Roche 454 data**

Sequencing Platform	Runs	No. of reads (in millions)	Average read length	Data
454 GX FLX	MP_8 Kb	0.31	184.28	58.71 Mb
	MP_20 Kb	1.50	198.51	312.46 Mb
<b>Total data (Mb)</b>				<b>371.17 Mb</b>

### Assembly, gene prediction and annotation of *C. batrachus*

The *de novo* assembly of the processed Illumina HiSeq data of *C. batrachus* was carried out using Abyss genome assembler at four

different hash lengths, viz., 61, 64, 69 and 85. The assembly at hash length of 85 was observed comparatively better with the total number of contigs being 2,87,999. Maximum contig size was found to be 89,416 bp and N50 being 6,611. The



coverage of the assembled genome was found to be ~891 Mb (covering ~ 87% of the *C. batrachus* genome). The *de novo* assembly of the quality filtered Illumina HiSeq data of *C. batrachus* was also carried out using CLC Genomics Workbench. This assembly resulted in 4,10,486 contigs, with the maximum contig size of 56,074 bp and N50 being 4,184. The coverage of the

assembled genome was found to be ~822 Mb (covering ~80% of the *C. batrachus* genome). Further, refinement of the draft assembly will be made using other assemblers utilizing all the data generated from different sequencing platforms. The assembly statistics of the HiSeq data obtained from Abyss and CLC Genomics Workbench is presented in Table 15.

**Table 15. Assembly statistics of HiSeq data of *C. batrachus* obtained from Abyss and CLC Genomics Workbench**

Assembly parameters	Abyss assembler (hash length)				CLC genomics workbench
	61	64	69	85	CLC draft
Contigs generated	270392	270018	265919	287999	410486
Maximum contig length	59097	58997	65287	89416	56074
Minimum contig length	200	200	200	200	77
Average contig length	3008.7	3045.7	3152.0	4191.5	2004.5
Total contigs length	813531409	822382599	838189871	891793694	822806256
Number of non-ATGC characters	6628749	6182998	5465133	3424771	36237954
Percentage of non-ATGC characters	0.815	0.752	0.652	0.384	4.404
Contigs ≥ 200 bp	270392	270018	265919	287999	410281
Contigs ≥ 500 bp	215724	214280	208309	211754	281098
Contigs ≥ 1 Kb	174852	174026	170275	173175	198807
Contigs ≥ 10 Kb	14408	14983	16427	18674	9279
Contigs ≥ 1 Mb	0	0	0	0	0
N50 value	5760	5893	6250	6611	4184
Coverage	0.787	0.797	0.813	0.867	0.803

### Gene prediction from the assembled contigs of *C. batrachus* and functional annotation of the predicted genes

Gene prediction of the assembled contigs, obtained from Abyss assembly at 85 hash length, was carried out using Augustus gene prediction software (version 3.1). The output gff file was further parsed for the retrieval of protein, exonic and CDS nucleotide sequences. The total numbers of predicted genes were found to be 73,837.

The predicted genes were further annotated using Blast2GO. A total of 44,505 putative genes were annotated at an identity greater than 50%.

Gene ontology analysis of the annotated genes was done in order to infer its functional aspects. Most of the predicted genes were found to be involved in cellular processes, metabolic processes, biological regulation, developmental processes, growth and many more diverse processes. An attempt was made to get insight into the genes involved in sex determination. DMRT gene was found to have good identity and high query coverage revealed four DMRT genes, *viz.*, DMRT1, DMRT2, DMRT3 and DMRT5. The functional annotation summary of the putative genes in terms of gene ontology is depicted in Figure 35.

## GO Distribution by Level (2) - Top 20

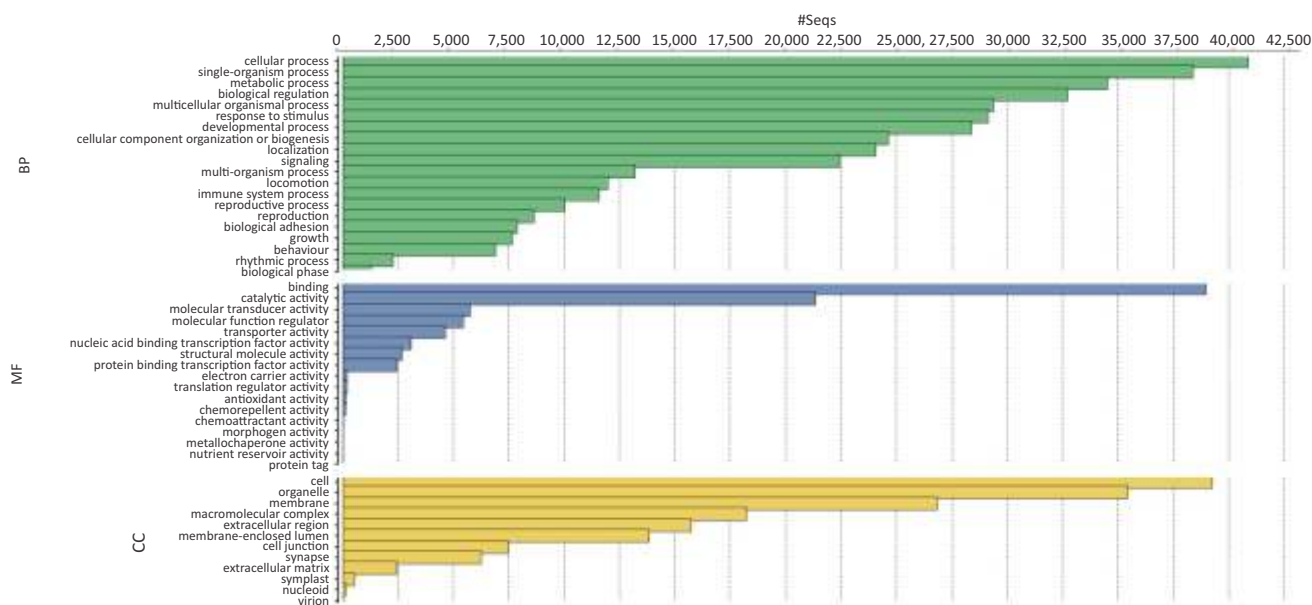


Fig. 35. Top hits of gene ontology distribution

 Transcriptome sequencing of *C. batrachus*

The reference data of expressed genes of a species is a very important resource for the functional annotation of any *de novo* genome analysis. In this context, the transcriptome data will aid in the annotation of the *C. batrachus* genes. The transcriptome sequencing of *C.*

*batrachus* was carried out for 4 tissues, *viz.* brain and gonads of male and female individuals. The transcriptome assembly was individually carried out for the 4 samples, along with the pooled data assembly, using Trinity software. The assembly statistics of the *de novo* assembled transcriptome is shown in Table 16.

 Table 16. Transcriptome assembly statistics of Illumina NextSeq data of *C. batrachus* obtained from Trinity assembler

Assembly parameters	Female brain	Female ovary	Male brain	Male testes	Pooled
Transcripts generated	101642	46535	93573	104786	185235
Maximum transcript length	18579	14450	26253	15440	30554
Minimum transcript length	224	224	224	224	224
Average transcript length	893.3	1,068.3	839.8	938.6	946.9
Total transcripts length	90796713	49715205	78584508	98349408	175403650
Number of non-ATGC characters	0	0	0	0	0
Percentage of non-ATGC characters	0	0	0	0	0
Transcripts $\geq$ 100 bp	101642	46535	93573	104786	185235
Transcripts $\geq$ 200 bp	101642	46535	93573	104786	185235
Transcripts $\geq$ 500 bp	48322	25985	43417	50937	84129
Transcripts $\geq$ 1 Kb	26464	15812	22742	29282	47751
Transcripts $\geq$ 10 Kb	36	10	28	37	194
Transcripts $\geq$ 1 Mb	0	0	0	0	0
N50 value	1564	1910	1401	1704	1864

### Mining of putative miRNAs in *C. batrachus*

An effort was made in order to mine the putative miRNAs from the assembled *C. batrachus* draft. Data was retrieved from miRBase (release 21), and was made into the non-redundant set for fishes and other organisms. This non-redundant data set was used as the raw data and was aligned onto the assembled genome draft to mine the putative miRs. In a similar fashion, an effort was made to mine the putative miRs from the EST sequences and in the BAC clones. Both forward and reverse BAC end sequences were assembled separately using CAP3 software. Hairpin sequences were separately aligned onto the assembled draft genome, EST and BAC end sequences. The miRNA precursor sequences were predicted and mined using mireap software. The predicted miRNA precursors were further filtered based on the MFEI values. A total of 137, 1, 2 and 2 putative miRNAs were mined using the assembled draft genome, EST sequences, forward BAC and reverse BAC end sequences respectively.

### BAC library construction and BAC end Sequencing

BAC library was constructed using high molecular weight (HMW) genomic DNA of *C. batrachus*. A total of ~55,000 BAC clones were developed, with an average insert size of 115 Kb. This will serve as a useful resource for genome finishing, gene characterization and other genome related studies in *C. batrachus*. A total of 3,686 BAC end sequences were generated from the BAC clones. The BAC end sequencing

included purification of plasmids using SPRI technology, DNA sequencing using BigDye Terminator v3.1, post reaction dye terminator removal using Agencourt CleanSEQ and sequence delineation on an ABI PRISM 3730xl with base calling and data compilation. The BAC end sequences were further trimmed for the removal of adaptors and were assembled using CAP3 software. These BAC end sequences will be used for scaffolding and gap closer of the draft genome of *C. batrachus*.

### Microsatellite mining

The short sequence repeat (SSR) prediction was carried out in *de novo* assembled contigs using MISA tool and only the SSRs belonging to the genic region were taken up for the downstream analyses. Primers for the amplification of genic SSRs and other microsatellites were designed using Primer3 tool. Cross amplification of these designed primers was checked against the *de novo* assembled contigs using Primer Blast. PCR amplification was carried out for all 30 loci, and only 14 loci showed amplification. These 14 amplified loci were further tested for polymorphism, and out of that nine were found to be monomorphic and five polymorphic (Table 17). For polymorphic loci, the number of alleles observed ranged from 3 to 5 with observed heterozygosity ( $H_o$ ) of 0.1972 (range from 0.038 to 0.526) and expected heterozygosity ( $H_e$ ) of 0.6146 (range from 0.434 to 0.784). Gene ontology study of the associated genes was done in order to infer their functional roles.

**Table 17. Characteristics of polymorphic microsatellite reported in *C. batrachus* (n=30)**

Locus	$N_a$	Observed allele size range (bp)	$H_o$	$H_e$	PIC
CB02*	4	231 -249	0.238	0.67	0.59
CB05*	3	187 -205	0.038	0.65	0.56
CB08*	3	251 -266	0.048	0.52	0.43
CB22	5	231 -252	0.526	0.78	0.78
CB23	3	195 -211	0.136	0.43	0.37

n= number of individuals;  $N_a$ = number of alleles;  $H_o/H_e$ = observed and expected heterozygosity; PIC= polymorphic information content; \*= Significant deviation from HWE after Bonferroni correction ( $P < 0.001$ ).

### SSR annotation pipeline

A graphical user interface (GUI) named 'WGSSAT- A high-throughput computational pipeline for mining of SSRs from whole genome sequencing data' was developed to annotate SSR from the whole genome assembly data using Java NetBeans and Perl script. The pipeline is featured

with the prediction of putative genes from whole genome data using 'Augustus'. Prediction of non-coding RNA genes, such as tRNA, rRNA, SnoRNA and miRNA, using both sequence based homology search method (BLAST against various RNA databases) and structure based homology search method (that includes tools like

tRNAscan, Infernal etc.). Repeats and transposable elements were predicted from whole genome assembly data using Repeat Masker. SSRs were predicted from assembled contigs using MISA tool. Mapping of the predicted SSRs onto the predicted genes, non-coding RNA, repeats and transposable elements were done followed by primer designing of mapped SSRs using Primer3. Cross amplification check of SSR's using Bowtie tool. Annotation of the predicted genes, as well the, genes containing SSRs is done by performing BLAST against UniProt database or other user specified databases. The annotation could be further enriched by gene ontology mapping, visualization of the predicted genes, as well as, SSRs using JBrowse. The development of this tool will not only ease the annotation process but will also help in identification of markers for linkage mapping and development of species or genus specific marker. A screenshot of the GUI based 'WGSSAT' is shown in Fig. 36.



**Fig. 36. A screenshot of the SSR annotation tool**  
**SSR mining in *C. batrachus* and its comparative analysis with *T. rubripes* (Fugu)**

SSR mining in *C. batrachus* and *T. rubripes* was carried out using 'WGSSAT' annotation pipeline and a comparative analysis was done based on SSR mapping onto the predicted genes, core proteins, non-coding RNA, repeats and transposable elements for magur and fugu genomes (Table 18).

**Table 18. Comparative statistics of SSR mapping between *C. batrachus* and *T. rubripes* genome**

Statistics	<i>C. batrachus</i>	<i>T. rubripes</i>
Genes reported	73836	46664
Proteins reported	73836	45129
RNA reported	20236	2080
Core protein reported	186	181
SSR reported in MISA	791423	177584
SSR amplified using Primer3	682164	172 101
Uniquely amplified SSR	605602	157761
SSR reported in gene	3420	3098
SSR uniquely amplified in gene	4	2709
SSR reported in exonic region	57	565
SSR uniquely amplified in exonic region	3	419
SSR reported in intronic region	3363	2533
SSR uniquely amplified in intronic region	1	2290
SSR reported in RNA	1	4
SSR uniquely amplified in RNA	1	4
SSR reported in core protein	4	10
SSR uniquely amplified in core protein	0	9
SSR reported in LINE	626	1242
SSR uniquely amplified in LINE	425	10 06
SSR reported in SINE	292	168
SSR uniquely amplified in SINE	223	155
SSR reported in LTR element	1508	2586
SSR uniquely amplified in LTR element	957	2295
SSR reported in DNA transposons	5648	6102
SSR uniquely amplified in DNA transposons	3865	5566



### Flow cytometric determination of genome size in freshwater fishes

The genome size of a large number of organisms, including fishes has been reported; however, such information is not available for the majority of fish species inhabiting in India. Estimation of the genomic DNA content and ploidy level in the blood cells of 52 economically important freshwater fish species (Table 19) was carried out following flow cytometry protocol, using blood cells of *Gallus domesticus* as a standard reference. The genome size in fishes in the present study ranged from  $0.58 \pm 0.03$  pg in banded gourami (*Trichogaster fasciata*) to  $1.92 \pm 0.04$  pg in scribbled goby (*Awaous*

*grammepomus*). Further, variations in nuclear DNA content were observed within and between the orders. Within the order, the DNA content varied from  $0.64 \pm 0.07$  to  $1.45 \pm 0.073$  pg in Cypriniformes,  $0.70 \pm 0.07$  to  $1.41 \pm 0.02$  pg in Siluriformes and  $0.60 \pm 0.05$  to  $1.92 \pm 0.04$  pg in Perciformes. Correlations between the genome size, chromosome number and organism complexity could not be recorded. In this study, the numbers of aneuploid cells were found to be higher in fishes collected from the wild, as compared to those collected from the ponds. New records on nuclear DNA content of 44 species were also generated and revalidated in 8 species in this study.

**Table 18. Nuclear DNA content in pg (average  $\pm$  SE), distribution (%), diploid phase (M1) and aneuploid phase (M3) in blood cells of freshwater fish species**

S. N.	Species	Family	Genome size in		2N	% cells in	
			pg	Mbp		M1 phase	M3 phase
<b>I. Commercially important fish species</b>							
1.	<i>Anabas testudineus</i>	Anabantidae	$0.72 \pm 0.03$	704.16	46	63.97	17.79
2.	<i>Anguilla bengalensis</i>	Anguillidae	$0.75 \pm 0.01$	733.5	42	42.85	7.80
3.	<i>Mystus gulio</i>	Bagridae	$0.70 \pm 0.07$	684.6	58	63.64	20.17
4.	<i>Mystus tengara</i>	Bagridae	$0.84 \pm 0.07$	821.52	54	70.15	16.50
5.	<i>Rita rita</i>	Bagridae	$0.77 \pm 0.03$	753.06	54	59.10	21.83
6.	<i>Sperata seenghala</i>	Bagridae	$1.26 \pm 0.07$	1232.20	50	81.35	12.12
7.	<i>Channa marulius</i>	Channidae	$0.94 \pm 0.03$	919.32	44	74.51	15.67
8.	<i>Channa punctata</i>	Channidae	$0.60 \pm 0.05$	568.8	32	81.35	13.05
9.	<i>Channa striata</i>	Channidae	$0.73 \pm 0.09$	713.94	40	54.39	23.97
10.	<i>Pseudotroplus maculatus</i>	Cichlidae	$1.14 \pm 0.04$	1114.92	-	73.28	19.23
11.	<i>Amblypharyngodon mola</i>	Cyprinidae	$1.22 \pm 0.02$	1193.1	50	82.70	7.44
12.	<i>Catla catla</i>	Cyprinidae	$1.22 \pm 0.03$	1193.1	50	89.37	2.60
13.	<i>Cirrhinus mrigala</i>	Cyprinidae	$1.37 \pm 0.17$	1339.8	50	66.45	20.03
14.	<i>Cirrhinus reba</i>	Cyprinidae	$1.07 \pm 0.07$	1046.4	48	42.65	23.99
15.	<i>Ctenopharyngodon idella</i>	Cyprinidae	$1.24 \pm 0.01$	1212.7	48	70.35	13.70
16.	<i>Cyprinus carpio</i>	Cyprinidae	$1.37 \pm 0.23$	1339.8	100	84.83	0.87
17.	<i>Garra gotyla</i>	Cyprinidae	$1.08 \pm 0.01$	1056.24	50	74.69	15.80
18.	<i>Labeo bata</i>	Cyprinidae	$1.36 \pm 0.11$	1330.0	50	85.92	5.89

Contd.....



19.	<i>Labeo calbasu</i>	Cyprinidae	0.95±0.09	929.1	50	41.93	18.03
20.	<i>Labeo fimbriatus</i>	Cyprinidae	0.64±0.07	625.92	50	45.52	18.00
21.	<i>Labeo gonius</i>	Cyprinidae	1.05±0.03	1026.9	50	90.64	4.68
22.	<i>Labeo rohita</i>	Cyprinidae	1.45±0.07	1418.1	50	80.83	4.05
23.	<i>Systomus sarana</i>	Cyprinidae	0.67±0.07	655.26	50	88.26	5.66
24.	<i>Puntius sophore</i>	Cyprinidae	0.71±0.09	694.38	48	62.78	17.95
25.	<i>Heteropneustes fossilis</i>	Heteropneustidae	1.15±0.18	1124.70	56	86.77	8.91
26.	<i>Mastacembalus armatus</i>	Mastacembelidae	0.69±0.06	674.82	48	63.61	20.14
27.	<i>Notopterus notopterus</i>	Notopteridae	1.00±0.01	978.0	42	85.78	8.99
28.	<i>Pangasius pangasius</i>	Pangasiidae	0.81±0.01	792.18	58	69.50	17.45
29.	<i>Clarias magur</i>	Siluridae	0.95±0.08	929.10	50	77.13	15.23
30.	<i>Ompok bimaculatus</i>	Siluridae	1.15±0.01	1124.7	42	54.58	36.39
31.	<i>Wallago attu</i>	Siluridae	0.79±0.07	772.62	86	79.12	14.52
<b>II. Threatened or vulnerable species</b>							
32.	<i>Horabagrus brachysoma</i>	Bagridae	1.21±0.06	1183.38	60	72.93	16.87
33.	<i>Mystus malabaricus</i>	Bagridae	1.18±0.01	1154.04	-	60.33	33.44
34.	<i>Sahyadria denisonii</i>	Cyprinidae	1.26±0.08	1232.28	-	61.78	18.77
35.	<i>Schizothorax richardsonii</i>	Cyprinidae	1.24±0.06	1212.72	98	74.62	12.49
36.	<i>Tor chelynoides</i>	Cyprinidae	1.23±0.01	1202.94	100	59.47	14.67
37.	<i>Tor putitora</i>	Cyprinidae	1.21±0.06	1183.38	100	70.37	6.20
38.	<i>Carinotetradon travancoricus</i>	Tetraodontidae	1.45±0.03		-	69.17	15.80
<b>III. Ornamental and Other fish species</b>							
39.	<i>Mystus vittatus</i>	Bagridae	1.41±0.02	1378.9	54	84.95	6.77
40.	<i>Botia dayi</i>	Botiidae	1.21±0.01	1183.38	-	55.77	24.36
41.	<i>Botia lohachata</i>	Botiidae	0.78±0.03	762.84	98	93.3	5.23
42.	<i>Barilius bendelisis</i>	Cyprinidae	1.22±0.07	1193.1	50	77.07	13.09
43.	<i>Barilius vagra</i>	Cyprinidae	1.22±0.01	1193.16	50	79.06	13.56
44.	<i>Devario malabaricus</i>	Cyprinidae	0.79±0.01	772.62	-	64.97	5.45
45.	<i>Pethia ticto</i>	Cyprinidae	0.70±0.03	684.6	50	71.43	16.09
46.	<i>Rasbora dandia</i>	Cyprinidae	0.85±0.01	831.3	-	82.58	12.39
47.	<i>Securicula gora</i>	Cyprinidae	0.68±0.03	665.04	50	90.55	5.26
48.	<i>Awaous grammepomus</i>	Gobiidae	1.92±0.04	1877.76	-	77.1	16.3
49.	<i>Macroganthurus pancalus</i>	Mastacembelidae	0.81±0.20	792.18	48	74.77	15.77
50.	<i>Trichogaster fasciata</i>	Osphronemidae	0.58±0.03	567.24	48	75.13	14.58
51.	<i>Glyptothorax pectinopterus</i>	Sisoridae	1.11±0.01	1085.58	52	89.93	7.85
52.	<i>Labeo rohita x Labeo calbasu (cross)</i>	Cyprinidae	1.22±0.01	1193.1	50	50.45	21.09

Mbp= mega base pair; 2n= diploid chromosome number

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

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

**Project period:** April, 2014 -March, 2017

**Funding Agency:** Institute funding

Tissue samples belonging to two populations of *Clarias magur* were collected from Unnao, Uttar Pradesh (18 nos.) and Kaikaluru, Andhra Pradesh (39 nos.). High molecular weight DNA of newly collected samples and selected samples of old collections was isolated to make the restriction site associated DNA (RAD) sequence data uniform for all populations. High quality genomic DNA having absorbance ratios (260/280) between 1.80 to 1.85 was used for library preparation. Genomic DNA from three populations (Unnao, Loktak Lake and Delang) was used for restriction digestion with different enzyme combinations (MluC1, Sph1, Sbf1 and EcoR1). About 10 µg of genomic DNA of 57 samples have been used for generation of paired-end RAD sequence data using on Illumina HighSeq 2000. The paired-end RAD data generated from three populations of *C. magur*, viz. Unnao (20 samples), Loktak Lake (10 samples) and Delang (4 samples), was analysed. The data was processed for reads quality and filtration of bad reads before the assembly of paired-end RAD data reads. After removal of barcode and restriction site (TAATCTTACA TGC) from 5' end from all 100 bp reads, a final sequence length of 88 bp of R1 sequences of all RAD sequences were generated. (Fig. 37). Similarly, 12 base barcode and restriction site part (CGACTGCGAATT) were trimmed from the 5' end of all 100 bp reads, which resulted in a final sequence length of 88 bp of R2 sequences of all RAD sequences.



**Fig. 37.** Raw 100 bp reads of R1 sequences of RAD sequences (CLC Genomics)

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

**Project Period:** November, 2012 - May, 2016

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

**Funding Agency:** DBT, Govt. of India

A total of 418 DNA sequences of 16S rRNA gene were generated for 98 species and the sequences were blasted in NCBI GenBank. After multiple sequence analysis, 283 sequences of 16S rRNA gene belonging to 71 species were submitted to NCBI GenBank (Acc. Nos. KT835291-KT835305, KT878041- KT878308). A total of 98 DNA barcodes based on cytochrome c oxidase I (COI) gene belonging to 27 species were prepared and submitted to NCBI GenBank (Acc. Nos. KT835306- KT835334, KT896674-KT896742).

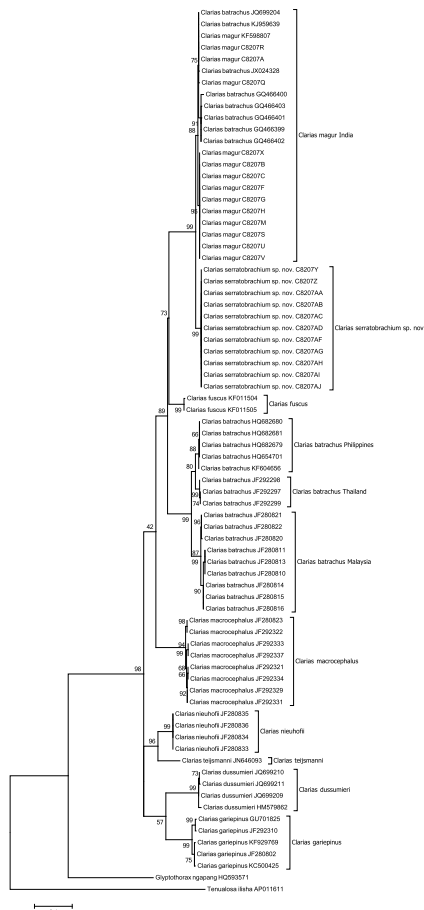
A new catfish species *Clarias serratobranchium* sp. nov. was discovered (Fig. 38) based on mitochondrial gene COI and morphological data of 11 individuals, collected from the wetlands of Moreh (Chindwin basin), a place in the Indo-Burma border. The study also confirmed that Indian *C. batrachus* (*C. magur*) is different from South East Asian *C. batrachus* as it formed a separate cluster (Fig. 39) with substantial divergence from *C. batrachus* of Malaysia, Philippines and Thailand.



**Fig. 38.** *Clarias serratobranchium*, a new species of Southeast Asian walking catfish

*Devario yuensis* was re-described based on morphological and molecular data of both 16S and COI markers. All the four species of *Devario* were well differentiated, which was evident by the clustering as four different clades formed using COI and 16S rRNA.





**Fig. 39. COI based molecular phylogenetic analysis of *Clarias* spp.**

**Project Title: Characterization and DNA barcoding of fishes from Mizoram**

**Project Personnel: Mahender Singh (PI)**

**Project period: December, 2014- December, 2017**

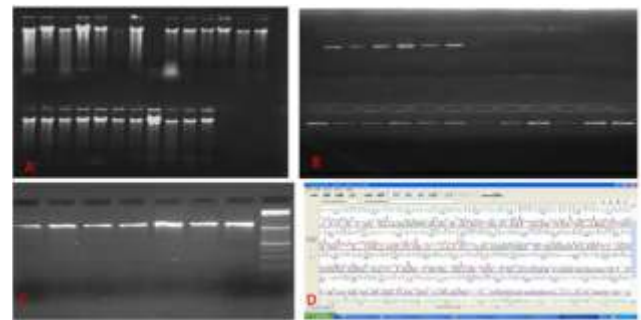
**Funding Agency: DBT, Govt. of India**

Mizoram is endowed with three drainage systems viz. Barak, Karnaphuli and Kaladan. In the first year of this work, the Barak drainage including major rivers and tributaries namely, Langkaih, Teirei, Tut, Tlawng, Tuirial, Tuivawl and Tuivai were explored for collection of fish samples for DNA barcoding (Fig. 40). Tissue samples were preserved in ethanol and voucher specimens were preserved in formaldehyde for further assessment in the laboratory. Samples from different collection sites (though belonging to the same species) were preserved separately in both alcohol and formalin for variation in DNA markers and morphology. Altogether 317 specimens of 76 species (based on morphology) were collected under 13 families and 5 orders. DNA Extraction was completed for all the tissues collected. PCR amplification was completed for 143 samples of 38 species for Cytochrome oxidase

I and Cytchrome b (Fig. 41). The double pass DNA sequencing and editing of both genes was completed in 94 samples of 23 species. The study resulted in the first ever report of the genus *Neonoemacheilus*, particularly *N. assamensis*, from Mizoram.



**Fig. 40. Fish collection at the Tam Dil reservoir lake near Saitual, Mizoram.**



**Fig. 41. A. Genomic DNA on 0.7% Agarose gel; B. PCR product of COI on 1.5% Agarose gel; C. PCR product of *cyt b* on 1.5% Agarose gel; D. Electropherogram of COI.**

**Project Title: Development of surrogate broodstock for propagation of valuable fish germplines**

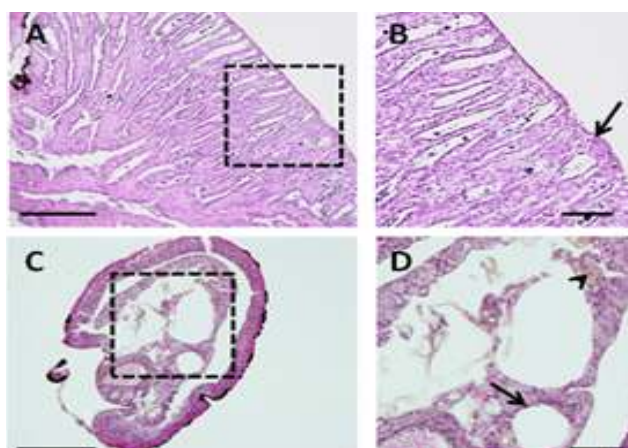
**Project Period: April, 2014 – March, 2017**

**Project Personnel: Sullip K. Majhi (PI), Basdeo Khuswaha and S. Raizada (upto 31<sup>st</sup> Dec., 2015)**

**Funding Agency: Institutional**

Germ cell transplantation (GCT), a powerful assisted reproductive technology, has the competence to increase the production efficiency of commercially important fishes that are difficult to breed in confinement and aid to the propagation and recovery of the endangered population. In this work, production of sterile adult common carp, *Cyprinus carpio*, gonads in months' time is reported, using the heat-chemical method, which would be used as recipients for GCT. We injected Busulfan (40mg/kg) intra-peritoneally at two weeks intervals (total 5 dosages) and constantly reared the animals in water temperature of 38°C between 1 to 10 weeks. The effectiveness of the

treatments was assessed by gonadal index, histology and *vasa* gene expression. At the end of 10<sup>th</sup> week, severe gonadal degeneration was observed in fishes treated with the heat-chemical combination as 100% male and female were found to be devoid of endogenous germ cells. However, high temperature alone caused minor gonadal degeneration (Fig. 42). The quantitative analysis of *vasa* gene transcription and change in colouration of gonads were found to be an additional useful tool to measure the degree of gonad sterility. The results obtained in this study, besides recipient preparation in considerably short time for germ cell transplantation, will have implication for control of invasive fish species in large open water bodies, which are difficult to manage.



**Fig. 42.** Image showing histological changes in the testes and ovaries of Busulfan-heat treated adult common carp. Panels on the right are high magnifications of insets inside the left panels. A, B-testis recovered from B40T38 (40mg/kg Busulfan at temperature 38°C) on 10<sup>th</sup> week (note the absence of spermatogonia and all other stages of spermatogenesis; arrow indicates a empty niche). C, D-Ovary recovered from B40T38 on 10<sup>th</sup> week (note the absence of oogonia and other types of germ cells (arrow) and occurrence of phagocytosis (arrow head))

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

**Project Period:** April, 2014 – March, 2017

**Project Personnel:** N.S. Nagpure (PI till 05.12.2015), Sullip K. Majhi (PI w.e.f. 05.12.2015), Murali S and Akhilesh K. Mishra

**Funding Agency:** Institutional

Fish cell line research has come a long way since the development of the first fish cell line from the gonad of rainbow trout and has been gaining importance in aquatic toxicology as an

indispensable *in-vitro* tool. Use of fish cell lines enable higher level of control of the experiment set up by reducing variability of *in vivo* responses, environmental conditions, etc. The *in vitro* toxicity of cypermethrin was assessed in *Labeo rohita* Gill (LRG) cell line using MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] assay. The LRG cells were exposed to 5 different test concentrations (i.e. 1.25, 2.50, 5.00, 10.00, 20.00 ng/ml) of the cypermethrin along with the control in L15 media in 96-well plate containing 10<sup>5</sup> cell per well for 24 h. Then L15 media containing test chemical was pipetted out and 10  $\mu$ l MTT solution (5mg/ml in PBS) was added to each well and incubated for 4-5 h. The MTT exposed cells were dissolved in 100ul DMSO followed by OD value estimation at 570-nm under micro plate reader. The OD observations were analysed for IC<sub>50</sub> estimation using GraphPad Prism software (Trial Version). The *in vitro* IC<sub>50</sub> value of cypermethrin was found to be 4.1 $\pm$ 2 ng/ml for LRG cell line.

### 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

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 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, Sharavathi River basin, Valapattanam River basin, Chandragiri River basin and Chaliyar River in the States of Karnataka and Kerala were explored for documenting fish diversity.

#### Exploration of Sharavathi River basin

Sharavathi, flowing through Shimoga and Uttara Kannada districts in Karnataka, is an important west flowing river in the central Western Ghats, with a total length of 128 KM, the River forms the iconic Jog falls near the border of the two districts. Explorations were continued in





### Exploration of Valapattanam River basin

Valapattanam River has a length of 110 km and a catchment area of 1321 km<sup>2</sup>. The River



Fig. 47. Valapattanam River basin showing sampling sites

originates in Coorg part of the Western Ghats in the Brahmagiri Reserve Forest in Karnataka and flows into the state of Kerala. Sampling was carried during pre-monsoon, monsoon and post-monsoon period covering 13 sampling locations (Fig. 47). The survey yielded a total of 49 fish species belonging to 38 genera of 17 families under 7 orders and 8 species of crustaceans. Specimens of *Mesonoemachilus guentheri*, *Pethia pookodensis*, *Puntius bimaculatus* and *Pseudogobiopsis oligactis*, represent new records for this river system (Fig. 48). Specimens of many species, including *Mystus malabaricus*, *Schistura semiarmatus*, *Garra mccllellandi* and *Sicyopterus griseus* were found to be gravid, suggesting they spawn there at the start of the monsoon.



*Pethia pookodensis*



*Puntius bimaculatus*



*Mesonoemachilus guentheri*



Gravid *Sicyopterus griseus*



Gravid *Garra mccllellandi*



*Pseudogobiopsis oligactis*

Fig. 48. Few rare specimens recorded from River Valapattanam

### Exploration of Chandragiri River basin

The River Chandragiri, also known as Payaswani River, originates from Patti Ghat Reserve Forest in Coorg district of Karnataka. It flows in a north-west direction covering a distance of 105 kms through the states of Karnataka and Kerala to join the Arabian Sea. Surveys were carried out in monsoon and post-monsoon seasons in 10 different reaches of Chandragiri River in Kerala and Karnataka (Fig. 49). The survey yielded a total of 55 fish species belonging to 44 genera of 22 families under 9 orders (Fig. 50 & 51). Collection includes *Schismatogobius deraniyagalai*, originally described from Sri Lanka and *Pangio ammophila*, originally described from the Nethravati River basin in South Canara. The exotic species *Oreochromis niloticus* was also recorded in the lower reaches of the river.

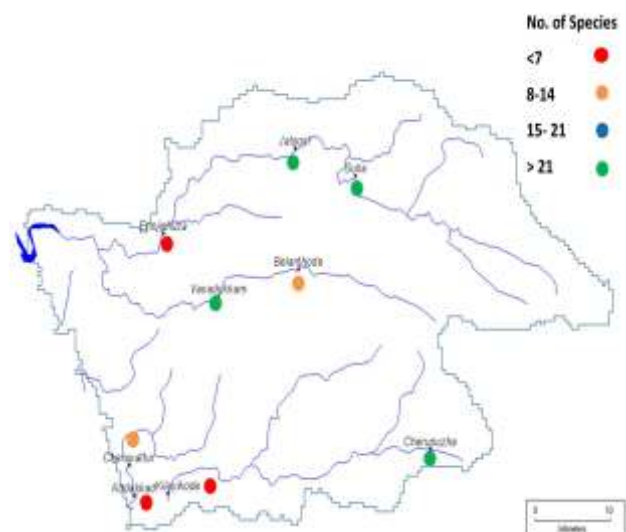


Fig. 49. Chandragiri River basin showing sampling sites





for exploration and documentation of fish diversity (Fig. 55). A total of 7 sites of river Mahanadi and 30 sites of its six tributaries and sub-tributaries namely, Sheonath, Hasdeo, Mand, Maniyari, Arpa and Lilagar, were covered in these exploration studies. Maximum species richness was recorded at Paudi Khurd in River Hasdeo whereas in River Mand, it was recorded at Aath-Pathra.



**Fig. 55. Exploration of fish diversity in Mahanadi river basin**

A total of 92 fish species have been recorded from upper basin of Mahanadi River (Fig. 56). Data on total of 3623 individual fish samples was collected from above explorations during the year under report (Fig. 57). Site-wise and season-wise abundance and distribution pattern of fish diversity in the whole upper Mahanadi basin was analysed.



**Fig. 56. Hauls of fish species recorded during explorations**



**Fig. 57. Collection of tissue samples and biological parameters from river Mahanadi**

Two awareness programmes on fish diversity of Mahanadi river basin and its conservation and management, and role of fishing communities in fisheries resource management were organised in villages located alongside the Mahanadi river and its tributaries in the Chhattisgarh state (Fig. 58). One awareness programme was organised at Pasaud village of Dhamtari distt. of Chhattisgarh right at the bank of Mahanadi river in its headwaters in Gagrel reservoir area in which 120 participants including fishermen, women, rural youths, office-bearers and members of fishing cooperative societies from Barbandha, Ur Putti, Mogra Ghan and Devi Nagaon villages participated. Another awareness programme was organised at Tansi village of Kanker distt. of Chhattisgarh in which 130 participants including fishermen, women, rural youths, office-bearers and members of fishing cooperative societies from Tansi, Teluguda and Koliyari villages participated.



**Fig. 58. Awareness programmes on fish diversity & its conservation**



Informal interaction with office-bearers of fishing cooperative societies and other fishermen were also had and information on various socio-economic aspects, their perception on status of fish diversity and its conservation in the basin and traditional ecological knowledge was collected from fishermen and fisherwomen along the selected sites on the bank of Mahanadi and its tributaries. Video documentation of the perception and experiences of fishing communities on status of fish diversity, its decline, issues, conflicts and possible measures for conservation and traditional ecological knowledge was also carried out.

The following threats to fish biodiversity and sustainability of fisheries emerged from field interactions:

- Indiscriminate fishing.
- Use of destructive fishing methods like electric current, poisoning.
- Loss of habitats due to several factors like silting of river beds, etc.
- Increased population pressure on the natural fisheries resources.
- Dams—for irrigation and drinking water supply purposes resulting in lack of synchronization of the availability and flow of water as per the requirements of fishes.
- Anthropogenic pressures on aquatic resources leading to loss and pollution of habitats and loss of natural breeding grounds of fishes.
- Climatic changes such as lack of/ inadequate rains, untimely rains, rising temperature, etc.
- Industrial pollution.

**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 August, 2014), L.K. Tyagi (Coordinator w.e.f. September, 2014), Vindhya Mohindra and Rajeev K. 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. During the period under report, six sub-projects were provided technical and financial support by the Institute and carried out by the collaborating partners under the programme (Table 20).

Exploration programmes were carried out by the research partners in the selected rivers/ water bodies of the NE region. Explorations included documentation and recording of fish diversity and indigenous knowledge (Fig. 59). The NBFGR team helped the research partners in prioritization and finalization of the technical programme, prepared and provided detailed formats for collection of data on exploration of fish diversity, habitat and indigenous knowledge, provided *hands-on* training in various aspects of exploration and collection of fish and tissue samples and conducted detailed periodic review of sub-projects and suggested modifications (if any). The technical as well as methodological support was provided for greater output.

Three exploratory surveys covering 72 locations were conducted in Teesta River (Sikkim). The species collected belonged to 65 genera under 25 families of 10 orders. These 49 species have ornamental and 38 on food values while 30 could be considered to have both. Five species, viz. *Semiplotus semiplotus*, *Schistura devdevi*, *Schistura rupecola*, *Laguvia shawi* and *Pseudocheneis sulcatus* are endemic to North East India. They represented restricted distribution and low abundance. *Schizothorax richardsonii* showed widest distribution and highest abundance in Teesta, reported from 51 of the total 72 locations surveyed. Interestingly, the exotic fish species, *Oncorhynchus mykiss* was restricted to the high altitude areas, above 1500 m. Details of 24 indigenous fishing gears/ practices were also documented from fisherfolks of the Teesta River. Development of Indices of Habitat Suitability (HSI) and Biotic Integrity (IBI) for fish and habitat conservation in Teesta River is under progress.

**Table 20. Details of sub-project under NE component.**

S.N.	State	Principal Investigator	Title of Sub-Project
1.	Assam	Mr. Sarbojit Thaossen Department of Zoology Haflong Govt. College, (Affiliated to Assam University, Silchar), Haflong, Assam	Exploration and Evaluation of Fish Faunal diversity, Distributional Pattern and Habitat Ecology of Rubi, Abhung, Dihamlai and Dilama River of Dima Hasao, Assam
2.	Sikkim	Dr. K.V. Radhakrishnan Assistant Professor College of Fisheries, CAU Agartala, Tripura	Development of Indices of Habitat Suitability (HSI) and Biotic Integrity (IBI) for fish and habitat conservation in Teesta River
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, Distributional pattern and Habitat Ecology of Krishnai River of Garohills, Meghalaya
5.	Arunachal Pradesh	Mr. Ratul Chandra Bharali Assistant Professor Dept. of Zoology Udalguri College, Udalguri, Assam	Ichthyofaunal diversity, distributional pattern and their habitat ecology of Kameng river, Arunachal Pradesh
6.	Mizoram	Dr. A.S. Barman Assistant Professor College of Fisheries, CAU, Agartala, Tripura	Exploration and Inventorization of the Fish Germplasm of Kolodyne Drainage System of Mizoram and its Associated Indigenous Knowledge


**Fig. 59. Explorations for documentation of fish diversity in various parts of north-eastern region**

A total of 44 fish species of 32 genera under 15 families were recorded from Krisnai River of Garo Hills district in Meghalaya, India covering an area of about 180 km of the entire stretch from its origin (near Nokrek Biosphere Reserve) to confluence point of River Brahmaputra (downstream).

Explorations from the Chindwin drainage in Manipur were conducted in 15 sites of 8 rivers of the basin, which yielded 82 species of 52 genera under 22 families of 8 orders. Two species are endangered, 11 species are vulnerable and 8 species are near threatened. Details on four indigenous fishing gears used in the surveyed basin were recorded.

Total 23 sampling sites of 16 river and/or tributaries of Kolodyne/ Chimmui and Barak drainage systems of Mizoram were explored. A total of 70 fish species from 12 genera belonging to 16 families were recorded from the selected basins. Genus *Garra* had highest number of species (10) followed by *Barilius* (05), *Channa* (05), *Puntius* (05), *Glyptothorax* (04) and *Neolissochilus* (03).

Kameng river system is the most important river basin of Arunachal Pradesh which comprises of the main Kameng River and its several tributaries. Explorations in the Kameng River yielded 21 species of 14 genera under 4 families. Out of these observed ichthyofaunal diversity of this river, presence of *Channa barca* in Bichom and Pichai tributaries of Kameng River was significant. The population of *Neolissocheilus hexagonolepis* is found to be restricted in the foot hills of the river while the population of *Tor putitora* is abundant in upstream of the River Kameng.

Explorations from 9 sites of four small rivers of Assam namely, Rubi, Abhung, Dihamlai and Dilama yielded a total of 44 fish species (32 genera under 15 families). Drainage and river-wise distribution and abundance data of all the documented fishes has been compiled. Information on indigenous knowledge on fishing methods was also collected through field survey and interaction with local fishermen from different parts of the region.

**Project Title:** ICAR-Consortium Research Project-Agro biodiversity (CRP-AB) Platform on.

**Sub-Project:** National Network of Germplasm Centre for prioritized finfishes of Ganga basin for Conservation aquaculture

**Project Period:** 2015 –2017

**Project Personnel:** Sudhir Raizada (CCPI Up to 31 December 2015), T.T. Ajith Kumar (CCPI w.e.f. 1st January, 2016), Santhosh K. Yadav L.K. Tyagi and Vikash Sahu

**Funding Agency:** ICAR

**Consortium Leader:** ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi.

**Lead Centre:** ICAR - National Bureau of Fish Genetic Resources (NBFGR), Lucknow

**Consortium Partners:** ICAR - Central Institute of Fresh water Aquaculture (CIFA), Bhubaneswar

ICAR - Directorate of Cold water Fisheries Research (DCFR), Bhimtal

The genetic resources are basic ingredient for enhancing food production. Therefore, incorporating strategies for enhancement and utilization of aquatic genetic resources with conservation efforts could be fruitful in harnessing potential of genetic resources to ensure sustained livelihood of masses. In such perspective, aquaculture development itself can be critically useful for conservation, as the availability of diversified species will satisfy consumer demand and reduce pressure on natural resources of such species. In this perspective, this work envisages developing stock specific broodstock of identified fish species in different regions as germplasm resource centers. The research can be carried to implement technological interventions for artificial propagation of species. The developed broodstock at such germplasm resource centres will conserve the native species on farm, can produce native seed (of similar genetic makeup) for stock enhancement in wild and also technology for regional level diversified aquaculture for livelihood enhancement.

During this 1st year of the project, brood stock of *Labeo calbasu*, *L. bata* and *L. dyocheilus* was successfully raised with wild specimens collected from Ganga tributaries and bred under captive conditions. An awareness camp on fish biodiversity and need for their conservation was organized at Dudhwa Tiger Reserve, Lakhimpur Khiri district, Uttar



Pradesh. About 50,000 fingerlings of above said three species were reared at Sharda River in Palia, Seth Ghat in Lakhimpur and Dudhwa Tiger Reserve. Total 2500 fingerling of *L. calbasu* were supplied to a farmer of Lucknow for rearing in polyculture pond. Exploratory surveys were conducted by the partner institutes (ICAR-CIFA, Bhubaneswar and ICAR-DCFR, Bhimtal) in their respective work areas and broodstock collected and reared for further project activities.

**Project Title: Techno-Legal Analysis of Policy issues and Patents for Strategic Management of Fish Genetic Resources**

**Plan Period:** July, 2015 - March, 2018

**Project Personnel:** Poonam Jayant Singh (PI), Rehana Abidi, A.K. Pandey, Amar Pal, Ravi Kumar and A.S. Bisht

**Funding Agency:** Institutional

**Analysis of Patent Documents related to Fish Biodiversity:** Issues in Governance, Access and Benefit Sharing for Innovation

Territorial flora and fauna have emerged from common heritage of mankind to Sovereign Right of a Nation, however, the aquaflore and aquafauna need clarity of regulation in connecting/shared waters. Though patents had come to existence to provide monopoly, but it also gives power to hinder innovation. The objective of the study is to understand issues related to fish patents, disclosure of origin and Access and Benefit Sharing with respect to indigenous fish germplasm nucleotides patented by countries without mentioning disclosure of origin. In this study, nucleotides were mined from patent documents related to fish genetic resources and categorised species-wise. Semantic Utilization of International Patent Classification Codes were used to extract information for fish genetic resource for exploring fish biodiversity through patent analysis for novel sequences for usable information retrieval. Out of 377,799 nucleotide sequences belonging to 10,020 fish species, 12,013 patented nucleotide sequences were retrieved from 181 fish species. The patents belonged to Gadiformes (26.45%), Cypriniformes (22.68%), Tetraodontiformes (11.74%), Anguilliformes (9.95%), Cichliformes (9.51%), Salmoniformes (6.44%), Belontiiformes (4.01%), Cyprinodontiformes (2.76%), Pleuronectiformes (2.24%), Scombriformes (1.17%) and others (2.45%).

Nucleotide sequences from Eukaryotes and Chordates were also retrieved for comparison. Year-wise distribution of nucleotide from NCBI Release 63 to 92 (1990-1995), Release 93-121 (1995-2000), Release 122 to 151 (2000-2005), Release 152-181 (2005-2010) and Release 182-211 (2010-2015), were studied for patent and non-patent nucleotides of Eukaryotes, Chordates and Bony fish. The patent and non-patent sequences were classified species-wise and sequence-wise (Figures 60 & 61).

**Implications for Access and Benefit sharing of pre-CBD and post-CBD sharing of Zebra fish (*Danio rerio*)**

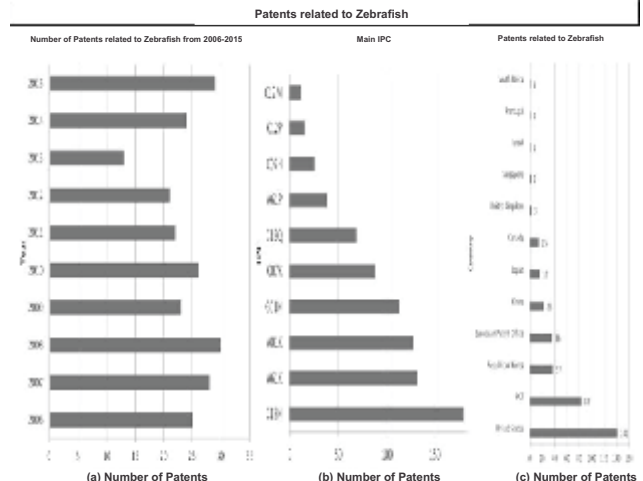
The model experimental fish initially procured from Asian freshwater streams/India is being studied as a case study for germplasm accruing its origin from India and biopiracy issues. Zebra fish has been used as a model experimental animal since 1960's that was a Pre-CBD (Convention on Biological Diversity) era, when biological material was considered common heritage of mankind. Its introduction to the western world was fuelled as an aquarium tradable commodity, later on becoming an instrument in the hands of biologist. In today's time line, it is not possible to ask for a retrospective sharing of benefits accruing through Zebra fish as an experimental model. For 64 food and forage crops, FAO through its ITPGRFA allows for multilateral sharing. For animal genetic resources, however, there is no parallel binding treaty from FAO, but CBD can take stock if, the nation is willing. Patent databases were mined to retrieve patents related to zebra fish to study Disclosure of Origin from 2006 to 2015 (Fig. 62). The information was categorized as per International Patent Classification Code-wise and country-wise to further study implications of Zebrafish - the model fish initially procured from India for Access and Benefit sharing issues. Zebrafish ranked second with most patented nucleotides among bony fish, belonging to C12N, C07K, C12P, A61K, C07H, G01N, C12Q, A01H, A61P and H04B IPC codes. Of the strains widely used in experiments, Wild Indian Karyotype (WIK) source of origin was screened. After enacting Biological Diversity Act and Rules, permission from National Biodiversity Authority (NBA), Chennai is mandatory for utilising any germplasm from Indian territory. Further, for any patent grant related to the germplasm, NBA approval is needed where Access and



Benefit sharing course is decided. The study is underway for access for WIK germplasm.

**Governance of Fish Genetic Resources: through a case study of traditional community fishing festival: Policy implication of negative traditional knowledge, Maund Mela**

CBD through NBA recognizes the role of local communities as holders of traditional knowledge related to biodiversity, however, some practices that are disastrous to the ecology of river needs intervention of local stakeholders and conservers. An ichthyotoxic Timur powder (*Zanthoxylum armatum*) is used by traditional communities during fishing festival called Maund, organized on a large scale in Aglar River, a tributary of Yamuna River, near Mussorie at Bandarkot, Uttarakhand. About 100 kg powder is thrown into the river as a festival having religious sentiments. Documentation of detailed information on this traditional fishing festival (known as Maund Mela), by medicinal plant (Timru), which is used as fish toxicants by the Jaunpuri

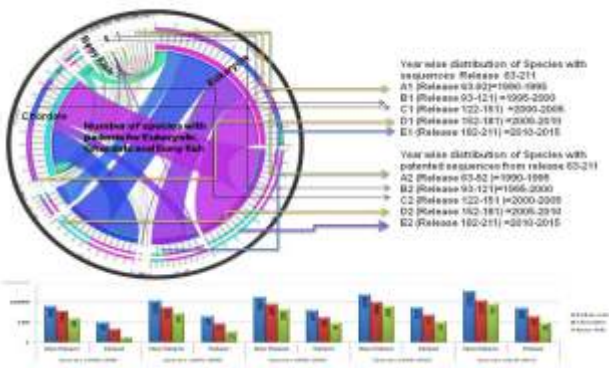


Number of Patents related to Zebrafish, (a) Year wise from 2006-2015, (b) Main International Patent Classification (IPC) Codes, (c) Country wise distribution

**Fig. 62. Patents related to Zebrafish (a) Year-wise 2006 to 2015, (b) International Patent Classification code-wise, (c) Country-wise patents**

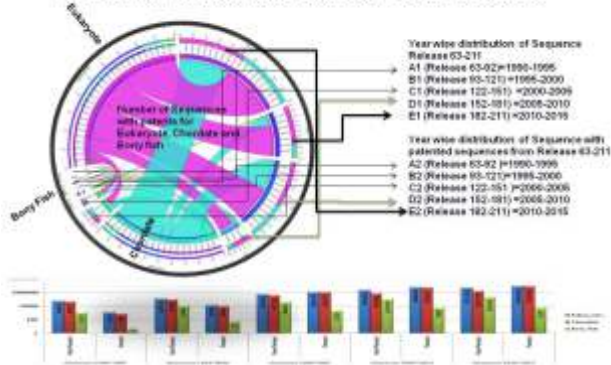
community and its conservation significance to fisheries in the Jaunpur tehsil (Tehri Garhwal) of Uttarakhand, India provided information on governance issues and beliefs of local people. For online awareness for biodiversity conservation, two short documentaries were prepared on Maund Mela entitled "Stunning Fish during Maund Mela: Raising awareness for Biodiversity Conservation" and "Maund Mela: A traditional Community Fishing Festival in the Himalayan River Aglar" Awareness programme through sensitisation and interaction with local community was conducted during the festival to create mass awareness among local community people who were participating in the fishing festival. The issue of Maund Mela having impact on ecosystem was discussed with the Chairman, State Biodiversity Board, Uttarakhand for conserving fish biodiversity and empowering Biodiversity Management Committee (BMC) in the region. Conservation awareness pamphlet in Hindi was also prepared and distributed to create public awareness in the region. The event was well covered by several local media in their news. The study identified a number of follow-up actions, initiatives for strategies for conservation of fishery resources within the Garhwal region by empowering BMC in the region and providing training with special orientation to fishery resources sustainability and impacts of destructive/ illegal fishing methods on fishes in order to develop and conserve natural resource (Fig. 63 a & b).

Twenty five years of Nucleotide submissions belonging to Fish Genetic Resources: A study of Patent and Non-Patent sequences categorised by species and Phyla



**Fig. 60. Patent and Non-patent sequences categorised by species and phyla**

Twenty five years of Nucleotide submissions of Fish Genetic Resources: A study of Patent and Non-Patent sequences categorised by species and sequences



**Fig. 61. Patent and Non-patent sequences categorised by species and nucleotide sequences**



(a)



(b)

**Fig. 63. Maund mela (Aglar-Yamuna valley, Uttarakhand): (a) Natives gathering at Aglar river; (b) Applying the Timur powder**

**Project Title:** Intellectual Property Management and Transfer/Commercialization of Agricultural Technology scheme (Up-scaling existing components i.e. Intellectual Property Right)

**Plan Period:** April, 2012 - March, 2017

**Project Personnel:** Poonam Jayant Singh (PI and Nodal Officer)

**Funding Agency:** NAIF, ICAR

Under the IPR Scheme of the Council, the Institute has undertaken various activities and skill development programmes for benefit of the fishing community and tribal people across the country. These activities are aimed to nurture grassroots innovations towards IP-driven income generation through handholding with grassroots innovators and help them develop skills in fisheries and aquaculture for initiating their own enterprises.

### **Traditional knowledge associated with fishing practices of Ramnagar (Uttarakhand)**

Traditional Knowledge (TK) is the practical knowledge based on tradition and experiences passed on from generation to generation. An intensive survey was conducted in Kosi River in Ramnagar. The majority of fish species reported were *Tor putitora* (locally mahseer), *Barilius bendelisis*, *Schizothorax* spp (locally Asela), *Garra gotyla*, *Acanthocobitis botia*, *Tor chylenoides*, *Labeo dero*, *Puntius* spp., *Mastacembalus armatus* (locally baam) and *Nemachelius* spp. (Figures 64 & 65). Kyashap community, the only fishing community in the Ramnagar region used indigenous techniques to catch fishes. Some of the fishing methods, mostly indigenous, were: *Gahan* (hammering), *Fass* (Knot Method), *Dola bujana* (Water Diversion

Method) (Fig. 66a), *Cast net* (Gher Jal) (Fig. 66b & c), *Kadiyali* (Bag net), *Mahajaal* (Gill net), *Herbs* and *Hooks*. This data regarding fishing methods associated with TK of coldwater fishes may be useful for developing non-destructive methods of fishing in hill stream fishes that can be used to sustainably consume the resources using traditional practices. Hence, there is need to explore more areas or methods related to traditional fishing practices.

### **Interactive postcard communication series for awareness to protecting grassroot innovations**

A postcard communication series was started for tribal community people since January, 2016 to interact with a large number of community people based across the country for answering their queries on various aspects of fisheries entrepreneurship, and related training interventions. In continuation of this series, a total of 130 training unit self-addressed postcards were distributed to the trainees from four districts of M.P. (Khandwa, Harda, Ashok Nagar, Betul) and one district of U.P. (Jhansi) during training programmes organised by the ARTU, Chinhat of the Institute (Fig. 67).

### **Promoting traditional knowledge based entrepreneurship in tribal communities of U.P and M.P.**

With a view to motivate and equip tribal community people with necessary knowledge and skill for arriving at innovation plans for setting their own enterprises, lectures were delivered to raise awareness during training programmes organised by the ARTU, Chinhat of the Institute. A total of 148 participants from four districts of M.P. (Khandwa, Harda, Ashok Nagar) and U.P. (Jhansi) participated in these



programmes. The lectures were intended to make tribal community people aware of TK associated with biodiversity and fishing practices and to explore indigenous knowledge system passed on by their ancestors in their tribes. Besides, interaction meetings followed by group discussion, was also organized with participants on 'Traditional cultural expressions of tribes of different districts' and Prior Informed Consent (PIC) related to Biodiversity was also collected. A questioner manual in Hindi was also prepared to collect inputs on agriculture products, food items, traditional fishing methods, water resources, fish availability and other resources available in their areas.

**Creativity assessment of researchers**

To promote creativity in the institute, a creative assessment framework was designed for comprehensive assessment of creativity and innovation capacity among different groups of scientific community. A total of 22 scientists from various disciplines from the institute participated in the exercise. In this series, a questioner manual on "Understanding Creativity" was designed consisting of three creativity exercises (Exercise 1: Creative Assessment Workout, Exercise 2: Creative During Research and Exercise 3: Creative Visualization: 2015-2025). The first exercise, includes 40 objective questions related to creative thinking, in which for each statement, researchers has to select the appropriate given responses (Agree, Undecided or Don't Know and Disagree). An initiative was taken for creative visualization, where researchers had to identify problems faced during research/ideation/developing an idea/proof of concept stage into research plan and visualization for present and future perspective of 10 years (Fig. 68).

**Awareness module for Researchers: 'Tool Box for innovation'**

In order to manage innovation in the Institute, an awareness module on innovation was prepared to create awareness among researchers to understand all tools needed for innovation and inspire researchers for creative thinking in lead areas to explore possibilities and information. In this version 1.1 focus was made on giving insights on latest patents related to fish genetic resources, creativity and on Global Innovation Index.



**Fig. 64. Sampling at Ramganga River, Ramnagar**



**Fig. 65. Few of the fish species caught during sampling**



**(a) Dola bujana**

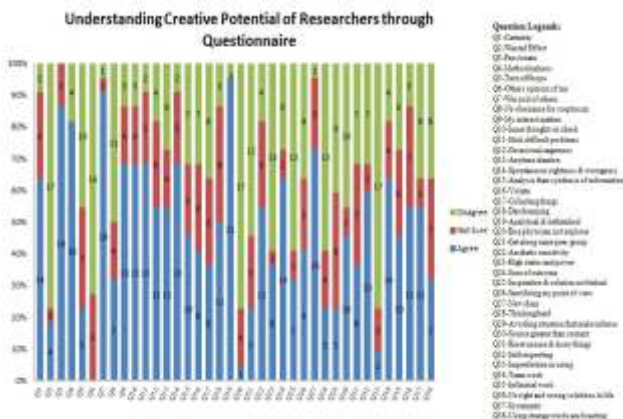
**(b) Cast net**

**(c) Cast net**

**Fig. 66. Few traditional fishing methods used for fishing in Ramnagar, Uttarakhand**



**Fig. 67. Fishing community people participated in postcard communication series at ARTU, Chinhat, Lucknow**



**Fig. 68. Understanding creative potential of researchers through questionnaire**

#### 4.4 Fish Health Management and Exotics

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

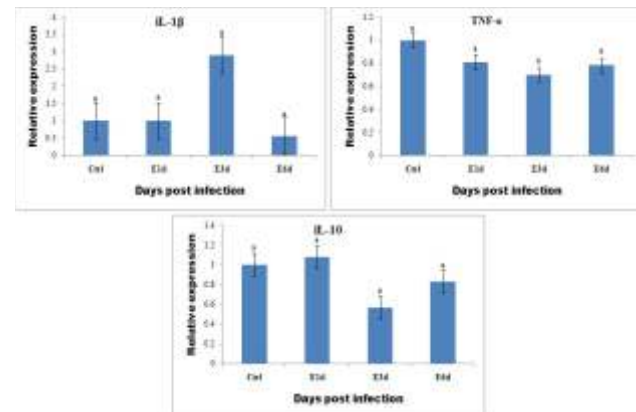
**Project Period:** May, 2012 – April, 2015

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

**Funding agency:** DBT, Govt. of India

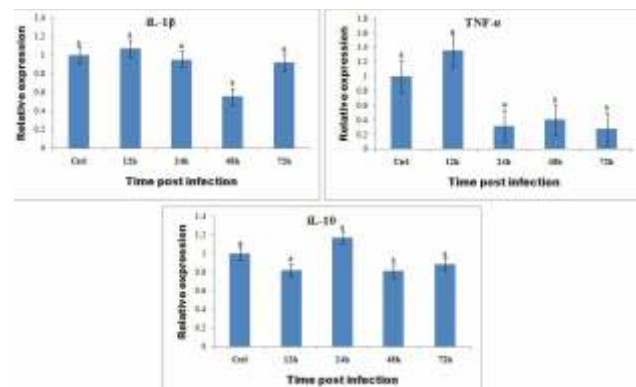
Two experiments were carried out to evaluate the effect of  $\beta$ -glucan on the expression of immune-related genes in *Labeo rohita*. In the first experiment, a single dose of  $\beta$ -glucan (500 $\mu$ g per fish) was administered to *L. rohita* through intraperitoneal route and head kidney

samples were collected after 1, 3 and 6 days post injection and analysed to see whether immunostimulation could enhance the expression of some of the important immunoregulatory genes *viz.*, (IL-1 $\beta$ , TNF- $\alpha$  and IL-10) (Fig. 69). However, no significant difference in expression of immune genes compared to control could be observed.



**Fig. 69. Expression of immuno-regulatory genes *viz.* (IL-1 $\beta$ , TNF- $\alpha$  and IL-10) following single injection of  $\beta$ -glucan**

In the second experiment, to evaluate the effect of multiple injections of  $\beta$ -glucan on expression of immune genes,  $\beta$ -glucan was administered twice (on 1st day and 5th day) and head kidney was sampled after 12, 24, 48 and 72 hours of second injection of  $\beta$ -glucan and analysed for expression of immune genes (Fig. 70). However, like first experiment, no significant difference in expression of immune genes, compared to control, could be observed.



**Fig. 70. Expression of immuno-regulatory genes *viz.* (IL-1 $\beta$ , TNF- $\alpha$  and IL-10) following multiple injections of  $\beta$ -glucan**



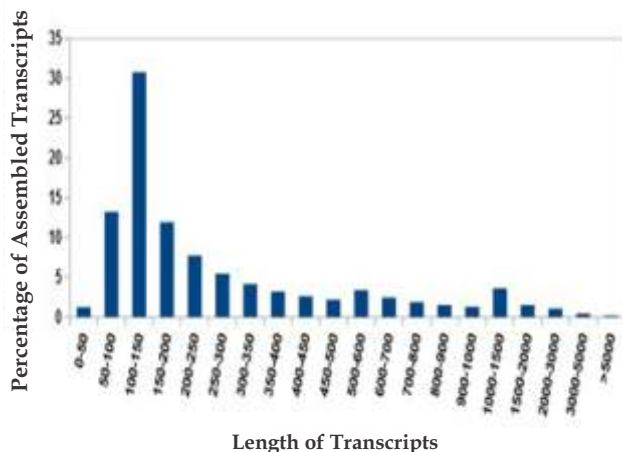
**Project Title:** Characterization of *Aphanomyces invadans* from NE India to develop diagnostic techniques and control measures

**Project Period:** October, 2013 – October, 2016

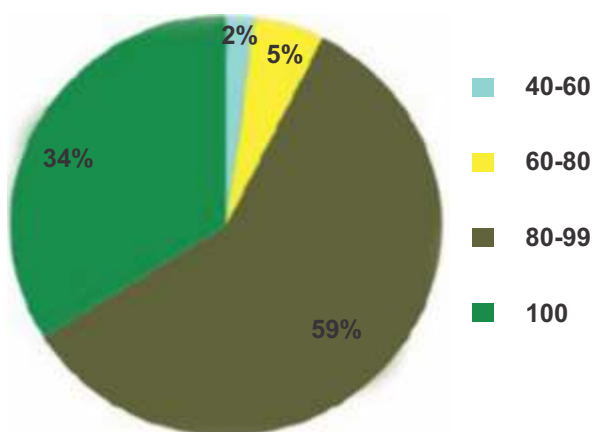
**Project Personnel:** P.K. Pradhan and P. Punia (Upto 28 April, 2015) (ICAR-NBFGR); Lopamudra Sahoo and Chandan Debnath (ICAR Complex, Tripura)

**Funding Agency:** DBT, Govt. of India

Transcriptome of *A. invadans* zoospores was sequenced and a total of 84,286,058 clean paired-end reads were generated. The *de novo* assembly was performed using multiple programs and steps resulting in 73,980 high quality transcripts (Fig. 71). Furthermore, about 30,305 (41.1%) of the assembled transcripts had at least one hit in NCBI database and around 71% of the transcripts had confidence level of at least  $1-E^{-50}$ . About 98% of the assembled transcripts had more than 60% similarity at protein level with the existing proteins at NCBI database (Fig. 72).



**Fig. 71.** Distribution of assembled transcripts of *Aphanomyces invadans*



**Fig. 72.** BLASTX similarity score distribution of *A. invadans*

Gene expression estimation using FPKM resulted in the identification of 39,979 transcripts having expression  $\geq 1$ . Functional annotation was carried out by comparing major databases resulting in 28,984 transcripts having significant match in NCBI database and 28,823 transcripts with UniProt annotations. The gene ontology (GO) annotations for transcripts were executed by Blast2GO program which resulted in the assignment of 1,621 GO terms for biological processes; 2,291 GO terms for molecular functions and 816 GO terms for cellular components. Further analysis of the RNASeq data is in process.

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

**Project Period:** April, 2014 to March, 2017

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

**Funding Agency:** Institutional

For development of monoclonal antibodies against macrophages, BALB/c mice were immunized with CTM cells emulsified in Freund's complete adjuvant, as well as, incomplete adjuvant. In all, six booster injections were given by intraperitoneal route, each at an interval of 10 days. The spleen from the immunized mice was collected and a fusion was carried out with myeloma cells. The hybridomas were selected using HAT medium and screening of hybridoma producing antibodies of choice is under progress.

Macrophage cultures of *Catla catla* were incubated with zoospores of *A. invadans* for understanding host-*A. invadans* interaction. During the study, a close interaction was observed between tips of hyphae and cells (Fig. 73). A total of eight experiments were carried out. The cells were collected at different time intervals during the course of interaction. However, expression analysis of immune genes did not reveal any consistent pattern in transcripts of the *C. catla* macrophages following incubation with *A. invadans* zoospores, in comparison to controls. Further studies are under progress.



**Fig. 73.** Interaction of *A. invadans* zoospores with *C. catla* macrophages

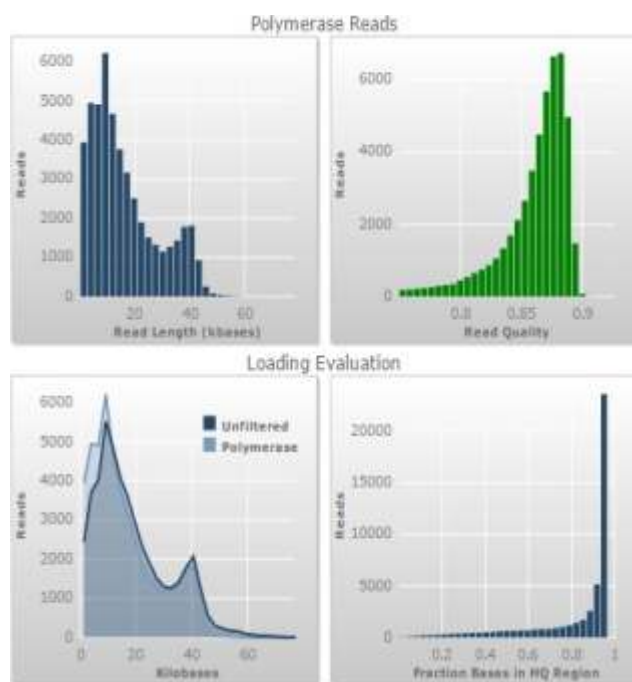
**Project Title:** Deciphering *Aphanomyces invadans* genome to understand its mechanism of infection in fishes

**Project Period:** April, 2015- March, 2017

**Project Personnel:** P.K. Pradhan (PI), Vindhya Mohindra, Neeraj Sood and Rajeev Kumar Singh

**Funding Agency:** Institutional

Genomic DNA of *Aphanomyces invadans* was isolated from 100 mg of overnight grown mat, using Phenol-Chloroform method. It was further purified and qualitative analysis was performed, according to PacBio gDNA guidelines. High quality genomic DNA was used for the construction of 20 Kb libraries using SMRT Template kit 1.0 and subsequently size selected for more than 7 Kb fragments. A total of 19 SMRT cells were used for sequencing in PacBio RS II using P6-C4 chemistry (Fig. 74).



**Fig. 74.** Read length, quality and loading evaluation of 20Kb library sequencing for *A. invadans* on PacBio RS II

Sequencing resulted in the generation of 20.6 Gb polymerase read data. Sub-read analysis on SMRT portal using RS\_Subread.1 protocol for data generated from 16 cells, showed: N50 Read length: 20,272bp; Mean read length: 13,219bp; Mean read quality score: 0.85 and Number of reads: 1,345,756. The genomic sequence data is being analyzed for the generation of draft assembly of *A. invadans* genome.

**Project Title:** ICAR-Consortia Research Platform on Vaccines & Diagnostics.

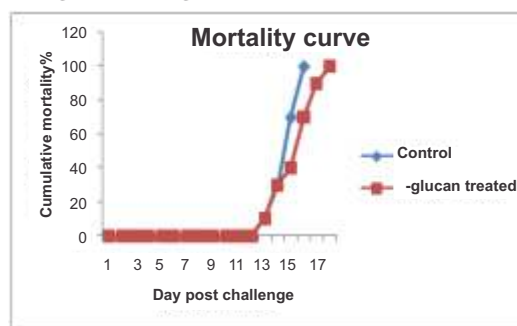
**Subproject:** ICAR-NBFGR Component: Evaluating the effect of immunization on protection against infection with *Aphanomyces invadans*

**Project Period:** August, 2015- March, 2017

**Project Personnel:** P.K. Pradhan (PI), Neeraj Sood and Chandra Bhushan Kumar

**Funding agency:** ICAR, New Delhi

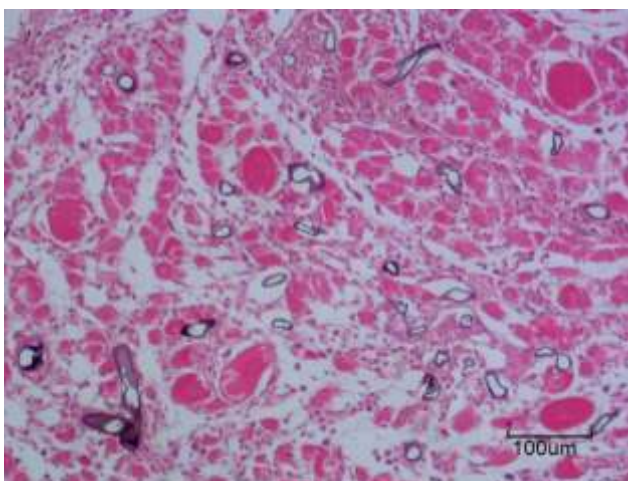
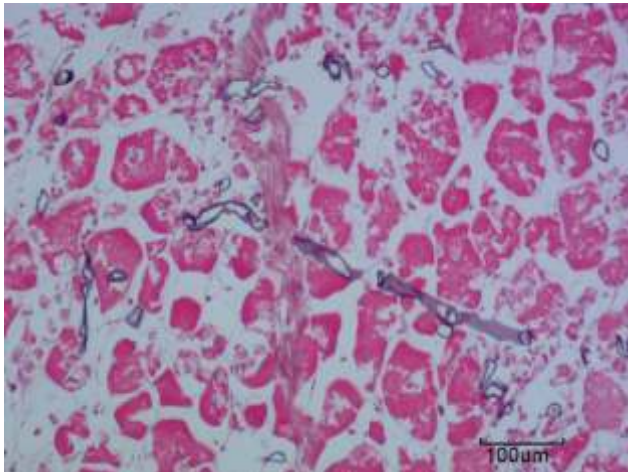
Infection with *Aphanomyces invadans* is recognized as one of the most destructive diseases of freshwater and brackishwater fishes and till today, there are no effective control and treatment measures available for this disease. Immunization could be one of the health management strategies to increase resistance against *A. invadans* infection. In addition, the observation that infection usually occurs during the winter months when the host immune system is suppressed, raises the possibility of improving resistance by stimulating the innate immune response through immunostimulation. During the reporting period, two experiments were conducted to evaluate the suitability of  $\beta$ -glucan as an immunostimulant in rendering protection against *A. invadans* infection. In the first experiment, a single dose of  $\beta$ -glucan (500 $\mu$ g per fish) was administered to ten numbers of *Labeo rohita* (average body weight of 40 $\pm$ 0.3 g) through intraperitoneal route and equal number of fish injected with PBS served as control. After one week of administration of  $\beta$ -glucan, the experimental, as well as, control group of fish were challenged intramuscularly with 100 zoospores of *A. invadans* in 0.1 ml of autoclaved pond water and mortality pattern was observed. The cause of the mortality was confirmed through histopathology, as well as, PCR. Importantly, in both the control and experimental groups, 100% mortality was observed between 11-19 days after challenge and no differences, either in mortality pattern or gross lesion development, were observed between both the groups (Fig. 75).



**Fig. 75.** Cumulative mortality of test (injected with  $\beta$ -glucan) and control groups of *L. rohita* challenged with zoospores of *A. invadans* after one week



At the time of morbidity or death, all the fish had severe swollen hemorrhagic areas and tissue pathology of all the moribund fish was typical of *A. invadans* infection. Both injected and non-injected sides were extensively occupied by the mycotic lesions and there was severe myonecrosis in large areas of myotome (Fig. 76).



**Fig. 76. Histopathology of moribund fish of both control and  $\beta$ -glucan treated fish after challenge with *A. invadans* zoospores, showing extensive necrosis of muscle tissue due to invading *A. invadans* hyphae (Grocott's methenamine silver-Haematoxylin and Eosin staining)**

In the second experiment, attempt was made to evaluate, if multiple (two) injections of  $\beta$ -glucan would render protection. For this,  $\beta$ -glucan @ 500 $\mu$ g per fish was injected to fish twice i.e. on day 1 and 5, and the fish were challenged with *A. invadans* on day 7. However, no protection was observed and there was 100% mortality in both the cases.

**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, Amar Pal and Ranjana Srivastava

**Funding Agency:** Institutional

In this study, a total of 474 fish samples were collected from four sites, viz. Sitapur, Lucknow, Haidergarh and Sultanpur of river Gomti which constitutes one of the major fishery resources of Uttar Pradesh.

### Screening of fishes and isolation of protozoan and monogenean parasites

Screening of the samples of *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 batrachus*, *C. gariepinus*, *Heteropneustes fossilis*, *Rita rita*, *Channa punctatus*, *C. marulius*, *C. striatus*, *Mystus vittatus*, *M. aor* and *Wallago attu* for protozoan and monogenean parasites revealed 28.73% prevalence (Table 21).

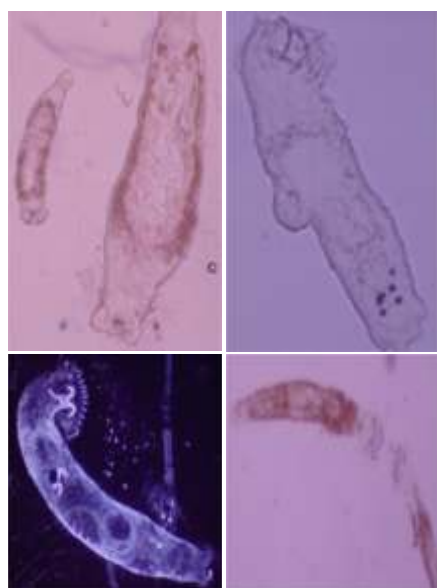
### Characterization and identification of parasites

The isolated parasites were identified and characterized and using microscopy, morphometry and image analysis. The detected protozoan parasites were: *Myxobolus yaseenii* sp. nov., *M. chauhanii* sp. nov., *M. awadhii* sp. nov., *M. macrolepi*, *M. gomtii* sp. nov., *M. arcticus*, *M. deformis* sp. nov. (Fig. 77), *Myxidium tictoii* sp. nov., *Henneguya aslamii* sp. nov., *Trypanosoma baigulensis*, *Ichthyophthirius multifiliis*, *Ambiphyra* sp., *Ichthyobodo pyriformis*, *Chilodonella* sp., *Trichodina* sp., *Piscineoodinium* sp., *Thelohanelus toyamai*, *Henneguya ritareii* sp. nov., *Pleistophora cyprini* sp. nov., *P. rohitii* sp. nov., *Hexamita* sp and *Ambiphyra* sp..

Monogenean parasites observed in screened fishes were: *Mastacembelocliedlius hetranchorus*, *Thaparocliedus* sp., *Dactylogyrus formosus*, *Mizellius siamensis*, *M. indicus*, *Dogielus catalius*, *Paradactylogyrus indicus*, *Bifurcophaptor indicus*, three species of *Gyrodactylus*, three species of *Dactylogyrus*, two species of *Paradactylogyrus*, etc.. Besides some Digeneans, Nematodes and Copepods like *Argulus* and *Lernaea* sp. were also observed (Fig. 78).

**Table 21. Prevalence of parasites in various fishes**

Name of Fish	Fish Screened	Fish having Parasites	Prevalence (%)
<i>Labeo rohita</i>	43	18	41.86
<i>L. bata</i>	16	5	31.25
<i>L. reba</i>	21	4	19.04
<i>Cirrhinus mrigala</i>	20	8	40.00
<i>Catla catla</i>	17	3	17.64
<i>Cyprinus carpio</i>	54	13	24.07
<i>Ctenopharyngodon idella</i>	27	7	25.92
<i>Hypophthalmichthys molitrix</i>	30	7	23.33
<i>Carassius auratus</i>	30	17	56.66
<i>Puntius ticto</i>	19	8	42.10
<i>Ompok bimaculatus</i>	16	2	12.50
<i>O. pabda</i>	11	1	9.09
<i>Channa punctatus</i>	12	4	33.33
<i>C. striatus</i>	15	4	26.66
<i>C. marulius</i>	08	2	25.00
<i>Clarias magur</i>	14	5	35.71
<i>C. gariepinus</i>	17	3	17.64
<i>Heteropneustes fossilis</i>	19	2	10.52
<i>Pangasius suchi</i>	12	4	33.33
<i>Mystus vittatus</i>	12	3	25.00
<i>M. aor</i>	09	1	11.11
<i>Rita rita</i>	13	4	30.76
<i>Wallago attu</i>	07	2	28.57


**Fig. 77. Some species of protozoan genus *Myxobolus* infecting fishes of River Gomti**

**Fig. 78. Monogenean parasitic species from fishes of River Gomti**

### Histopathology of tissues infected with parasites

Histopathology of infected tissues like gills, skin, kidney, liver and heart was done. The affected fish tissues were fixed, washed, wax embedded and blocks were made. Further microtomy was done and tissue sections were stained and imaging is done. Cell necrosis and myxosporan spores were clearly visible (Fig. 79).

### DNA extraction and PCR amplification

DNA extraction from tissues, cysts and directly from the following protozoan and monogenean parasites was done: *Myxobolus cerebralis*, *M. yaseenii*, *M. grassi*, *M. gomtii*, *M. macrolepi*, *Trichodina cirrhinii*, *Thelohanelus* sp., *I. multifiliis*, *Henneguya* sp.; and monogeneans *Dactylogyrus formosus* and *Paradactylogyrus indicus*. Specific primers were designed for PCR amplification of DNA from 18SSu-rRNA genes and ITS-1 region of *Myxobolus* species, *Ichthyophthirius multifiliis* and *Trichodina* sp. PCR amplification of DNA templates of six protozoan parasites namely, *Myxobolus yaseenii* sp. nov., *M. cerebralis*, *M. arcticus*, *M. grassi*, *Ichthyophthirius multifiliis* and *Trichodina* sp. nov. was done. Amplicons were checked and PCR products were quantified using spectrophotometer.



### Gene sequencing and analysis

DNA sequencing was done by using Sanger's dideoxy chain termination method. The sequences' alignment was done through Clustal W and Mega 5. The other analogous sequences available in 'GenBank' were searched by 'BLAST' to compare and verify the parasites sequences. Comparison of sequences was done on the basis of multiple hosts and parasites sites of infection.

Sequences of *Myxobolus yaseenii* sp. nov., *M. cerebralis*, *M. arcticus*, *Ichthyophthirius multifiliis* and *Trichodina* sp. nov. were submitted to NCBI. The accession numbers obtained this year from NCBI are given below:

- KU945825- *Myxobolus yaseenii*, 18S rRNA gene partial sequence (*Ctenopharyngodon idella*)
- KP268649- *Myxobolus grassii*, 18S rRNA gene partial sequence (*Ctenopharyngodon idella*)
- KP293877- *Trichodina cirrihunii* 18S rRNA gene partial sequence (*Cirrhinus mrigala*).
- KM870913 - *Ichthyophthirius multifiliis*, 18S rRNA partial sequences (*Labeo rohita*).
- KM822612- *Ichthyophthirius multifiliis*, 18S rRNA partial sequences (*Cyprinus carpio*).
- KM671790- *Myxobolus cerebralis*, 18S rRNA gene partial sequence (*Cyprinus carpio*).
- KJ701267 - *Myxobolus cerebralis*, 18S rRNA gene partial sequence (*Clarius batrachus*).
- KF662475- *Myxobolus arcticus*, isolate INRA-Ma1 18S rRNA gene & ITS1, partial sequence (*Clarius batrachus*).

### Construction of molecular phylogenetic tree to ascertain parasite's position

Molecular phylogenetic tree construction for protozoan parasite 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. Phylogenetic analyses were conducted using software MEGA5. To determine the phylogenetic position of protozoan parasite species 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 (outgroups) parasites of other fish species (Figures 79-83).

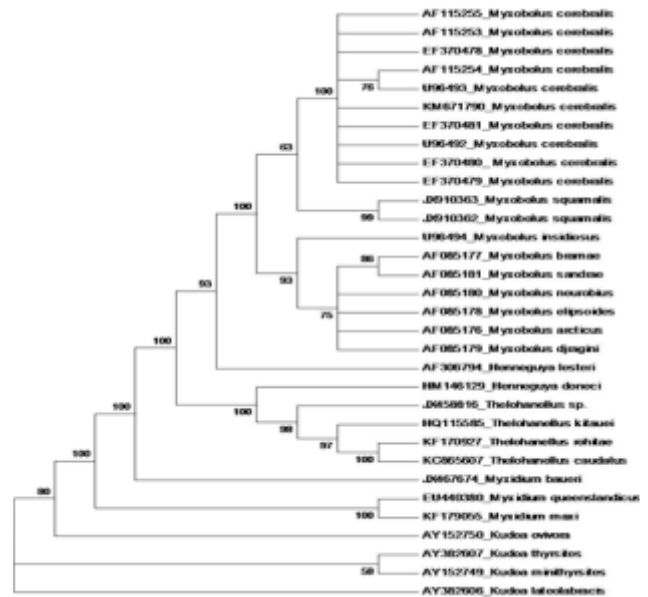


Fig.79. Maximum Parsimony tree of the small subunit ribosomal DNA sequence of *M. cerebralis* from *C. carpio* with bootstrap values of 700 on the nodes of branches. GenBank accession numbers given before the species name

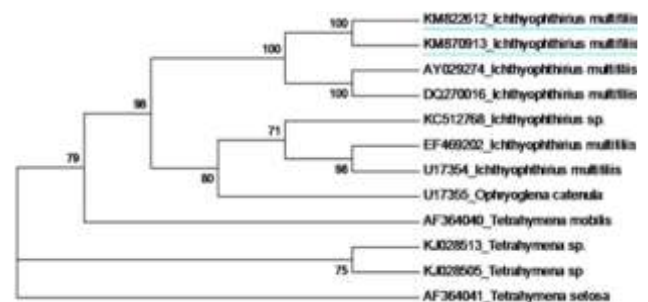


Fig. 80. Maximum Parsimony tree of the small subunit ribosomal DNA sequence of *I. multifiliis* from *Cyprinus carpio* and *Labeo rohita* with bootstrap values of 700 on the nodes of branches. GenBank accession numbers given before the species name

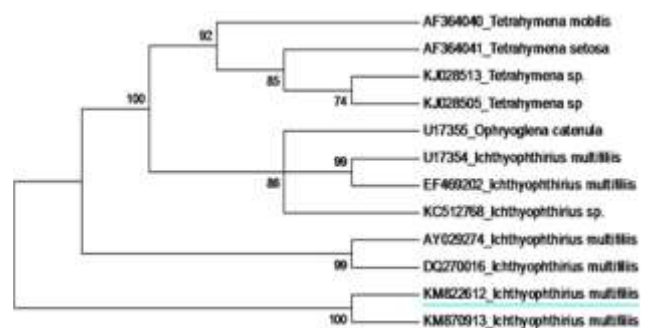
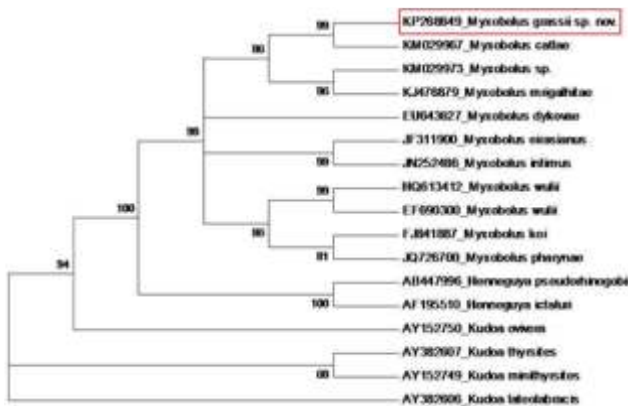
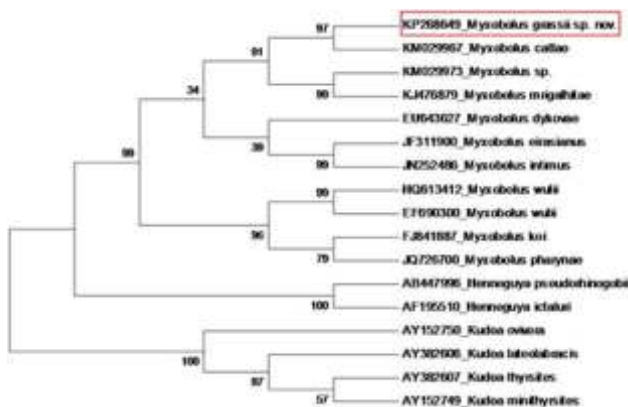


Fig. 81. Maximum Likelihood tree of the small subunit ribosomal DNA sequence of *I. multifiliis* from *Cyprinus carpio* and *Labeo rohita* with bootstrap values of 700 on the nodes of branches. GenBank accession numbers given before the species name



**Fig. 82. Maximum Parsimony tree of the SSu-R DNA sequence of *Myxobolus grassii* sp. nov from *Ctenopharyngodon idella* with bootstrap values of 700 on the nodes of branches. GenBank accession numbers given before the species name**



**Fig. 83. Maximum Likelihood tree of SSu-R DNA sequence of *Myxobolus grassii* sp. nov from *Ctenopharyngodon idella* with bootstrap values of 700 on the nodes of branches. GenBank accession numbers given before the species name**

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

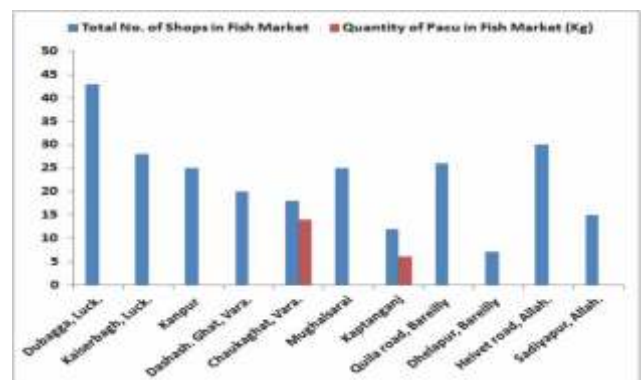
**Project Period:** August, 2013 - October 2016

**Project Personnel:** A.K. Singh (PI up to October 2014); Peyush Punia (PI November, 2014 - April, 2015); P.K. Pradhan (PI w.e.f. April, 2015); V.S. Basheer, Aditya Kumar and S.M. Srivastava (ICAR-NBFGR, Lucknow); P.P. Chakrabarty, ICAR-CIFA Regional Research Centre (RRC), Kalyani and S.S. Giri, ICAR-CIFA RRC, Vijayawada

**Funding Agency:** NFDB

A survey of the major fish markets (15 numbers) of Uttar Pradesh was undertaken during December 2015 to March, 2016, and most of the markets were visited consecutively for 2 to

3 days. The places which were surveyed included Lucknow, Kanpur, Allahabad, Varanasi, Mughalsarai, Deoria, Bareilly, Gorakhpur, Agra, Aligarh and Meerut. In the 15 markets, there were a total of 317 fish shops; out of which pacu was available only in 18 shops of 3 fish markets (Fig. 84-85). In most of the markets, the fish sellers mentioned that pacu comes very rarely and that too in less quantity. Importantly, only in Gorakhpur, one of the major fish markets of Uttar Pradesh, out of the 30 fish shops, pacu was available in 15 shops and sellers informed that there is good demand for pacu in that region. The price of pacu in most of the shops, where it was available, varied between Rs. 90-120/kg. From the above study, it was inferred that, there is very less availability of pacu in most of the fish markets. Furthermore, as mentioned by majority of the fish sellers in the surveyed fish markets, there is very less demand of pacu.



**Fig. 84. Survey of fish markets of Uttar Pradesh for availability of pacu.**



**Fig. 85. Shops in Gorakhpur fish markets showing availability of pacu**

**Project Title:** All India Network Project on Fish Health

**Project Period:** July, 2015 - March, 2017

**Project Personnel:** P.K. Pradhan (PI), Chandra Bhushan Kumar and Aditya Kumar

**Funding agency:** ICAR, New Delhi

During the reporting period, all the District Fisheries Officers of the State Fisheries Department, Govt. of Uttar Pradesh were contacted for knowing details about the distributors of drugs and chemicals used in aquaculture in their



respective districts. Based on the information received, visits were made to different shops/dealers/distributors located in Lucknow, Barabanki and Maharajganj and the information on some of the products of companies, like Virbac, Neospark and VxL, was collected and entered into the format provided by the lead centre.

For estimating the economic losses due to fish diseases and making the farmers aware about the need of reporting, two awareness programmes on 'Fish Health Management' were organized at Barabanki and Lakhimpur-Kheri districts in collaboration with State Fisheries Department, Uttar Pradesh on December 17 and 21, 2015, respectively (Fig. 86). A total of 199 fish farmers participated in the awareness programme at Barabanki, whereas, at Lakhimpur-Kheri, 162 fish farmers participated. The farmers were informed about the clinical signs of important, as well as, emerging fish diseases in freshwater aquaculture in these programmes. The farmers were advised to report all disease outbreaks in the initial stages, so that losses due to diseases can be minimized and economic loss can be estimated.



**Fig. 86.** Awareness programme on 'Fish Health Management' at Lakhimpur-Kheri

**Project Title:** Development and characterization of SSCs from endemic fishes of the Western Ghats

**Project Period:** April, 2015 – March, 2018

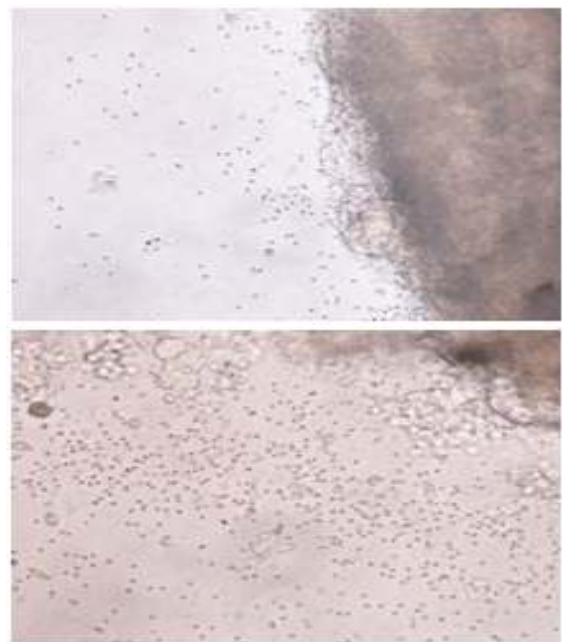
**Project Personnel:** T. Raja Swaminathan (PI) and Charan Ravi

**Funding Agency:** Institutional

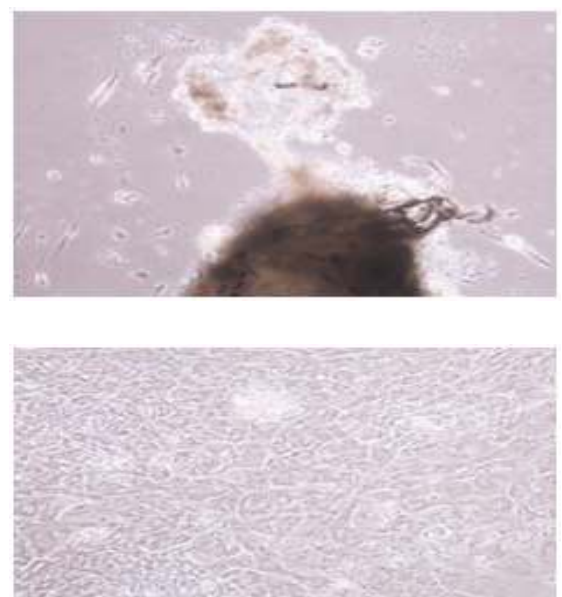
*Sahyadria denisonii* was procured locally and maintained in glass aquaria to obtain the maturity status for the preparation of testes explant preparation. Primary culture was initiated from testes tissues and cells were cultured at 28°C in L-15 medium (pH7.0), supplemented with 20% fetal bovine serum, basic

fibroblast growth factor, penicillin and streptomycin (Fig. 87). A total of ten trials of testes tissue explants of *S. denisonii* were carried out and radiation of cells reported from the explants, but monolayer could not be obtained (Fig. 88).

Different primers were designed and synthesized for identifying the SSCs; Germ cell markers, viz. *Oct4*, *Dmrt1* and *Vasa*; Sertoli cell markers, viz. *WT1*, *Foxl2* and *Sox9a* and Housekeeping gene, viz. - *GAPDH* and  $\beta$ -actin.



**Fig. 87.** Explants of testes tissue of *S. denisonii* and motile sperms in the tissue culture flask from the mature testes tissue



**Fig. 88.** Fibroblast like cells radiating from the testes explant of *S. denisonii*

### Project Title: National Surveillance Programme for Aquatic Animal Diseases

**Project Period:** April, 2013 – March, 2018

**Project Coordinator:** J.K. Jena, Deputy Director General (Fisheries Science)

**Project Co-coordinator:** Director, NBFGR

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

**Funding Agency:** National Fisheries Development Board

The National Surveillance Programme for Aquatic Animal Diseases (NSPAAD) is currently getting implemented in 15 states of aquaculture and fisheries importance through 24 collaborating partners. During the year 2015-16, one more state i.e. Bihar was included under the National Surveillance Programme where the surveillance is being carried out by ICAR-Research Complex for Eastern Region, Patna. The emphasis of the programme has been on creating awareness among stakeholders regarding the disease surveillance and training of officers of State Fisheries Departments for strengthening their diagnostic capability. The major activities of NSPAAD during 2015-16 have been as follows:

#### Organization of awareness/training programmes:

During the reporting period, a total of 67 awareness programmes were organized by the collaborating centres, and more than 3600 stakeholders including fish farmers and State Fisheries Officers participated in these programmes. The awareness meetings were conducted in collaboration with the respective state fishery department, in order to familiarize the stakeholders about the surveillance programme and discuss basic concepts on fish health management. In addition, awareness materials in different regional languages i.e. Bengali, Gujarati, Oriya, Malayalam, Tamil, Telugu, etc. are being distributed to fish farmers for creating awareness about aquatic animal diseases, as well as, importance of disease surveillance. Furthermore, in order to strengthen the diagnostic capability of state fisheries officers, a total of 7 trainings were organised in which 123 stakeholders including fisheries officers, farmers and SRFs were imparted training on fish disease diagnosis.

#### Sampling of farms for active surveillance

A total of 1799 farms were visited for sample collection during 2015-16, by the collaborating centres. The major pathogens

reported during this period included carp edema virus, cyprinid herpesvirus-2, viral encephalopathy and retinopathy virus, *Aphanomyces invadans*, *Saprolegnia parasitica*, *Aeromonas* sp., *Flavobacterium* sp., *Argulus* sp., *Lernaea* sp., *Dactylogyrus* sp, *Trichodina* sp. and *Myxobolus* sp. in finfish; *Enterocytozoon hepatopenaei*, white spot syndrome virus, infectious hypodermal and hematopoietic necrosis virus, monodon baculovirus, hepatopancreatic parvovirus and *Vibrio* sp. in crustaceans and infection with *Perkinsus olseni*, *P. beihaiensis* in molluscs. Importantly, the fish samples collected during active surveillance were found to be 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 negative for taura syndrome virus, infectious myonecrosis virus and yellow head virus.

#### Organization of Re-echo seminar on Acute Hepatopancreatic Necrosis Disease (AHPND) and round-table discussion on AHPND National Action Planning

A seminar on AHPND was organized at ICAR-NBFGR, Lucknow during September 14-15, 2015 on behalf of DADF in collaboration with Food and Agriculture Organization (FAO) of the United Nations (Fig. 89). Six international experts from FAO were the principal resource persons for the seminar. The seminar was attended by thirty-six selected personnel, including scientists associated with NSPAAD and officials from CAA, MPEDA, State Fisheries Department as well as, representatives from shrimp industry. This was followed by a round table discussion on AHPND National Action Plan on September 16, 2015 and a core committee was constituted under NSPAAD with ICAR-CIBA, Chennai as the lead centre, for preparing AHPND National Action Plan.

#### Development of diagnostic capability



**Fig. 89. Dr. J.K. Jena, Coordinator, NSPAAD addressing the participants during seminar on AHPND**



Under the programme, diagnostic capability for OIE-listed and delisted diseases of finfish, crustaceans and molluscs has been developed. Furthermore, diagnostic capability for the emerging pathogens is continuously being upgraded and during the reporting year, the same was upgraded for *Vibrio parahaemolyticus* AHPND strain using AP4 primers and covert mortality nodavirus. The positive controls are being shared with the collaborating centres.

### Finalization of data entry modules

Formats for entering information about aquatic animal diseases have been finalized in consultation with a software firm, for preparing a Database on Aquatic Animal Diseases.

### Strengthening of disease reporting

Information on aquatic animal diseases from all the collaborating institutes is being compiled at the nodal centre. This information is being used for Quarterly Aquatic Animal Disease (QAAD) reporting to international organisations viz., OIE and NACA. Importantly, all the diseases are getting reported on the basis of level 3 diagnosis.

### First report of infection with carp edema virus

A disease outbreak in adult koi from a fish farm in Ernakulum District, Kerala, India was reported in June 2015, with cumulative mortality of 100% within 8 days of stocking, by PMFGR Centre, Kochi of ICAR-NBFGR, Lucknow. The disease was confirmed to be koi sleepy disease, caused by carp edema virus on the basis of PCR, sequencing of PCR amplicon, bioassay and electron microscopy. Screening of koi showing symptoms similar to sleepy disease from different locations revealed that CEV infection was widespread. This forms the first report of infection with CEV in the country. Since the disease was reported for the first time from the country, a report has been submitted to DADF, Govt. of India.

### Infection with *Enterocytozoon hepatopenaei* (EHP)

Infection with EHP, a microsporidian parasite, is associated with severe growth retardation and white faeces syndrome in shrimps. It is considered to be a critical threat to shrimp aquaculture. The disease was reported by RGCA, Chennai and ICAR-CIBA, Chennai during the year under report, and considering its potential to adversely affect shrimp aquaculture, a targeted active surveillance in coastal states has

been initiated under NSPAAD, by involving collaborating institutes involved in shrimp disease surveillance. Presently, the pathogen has been reported from Tamil Nadu, Andhra Pradesh, Odisha, West Bengal and Maharashtra.

### Widespread occurrence of cyprinid herpesvirus-2 infection

Consequent to first report of herpesviral haematopoietic necrosis disease in Hooghly District of West Bengal during the year 2014, samples of goldfish are being screened for cyprinid herpesvirus-2. The studies indicate that the virus is quite widespread in goldfish populations.

### Sub-project 1: Surveillance of freshwater fish and shellfish diseases in Uttar Pradesh and Haryana

**Project Personnel:** P.K. Pradhan (PI), Neeraj Sood, Rehana Abidi, Mary Lini, Aditya Kumar and Chandra Bhushan Kumar

One of the major emphases of the programme has been to strengthen the passive surveillance system, so that each disease outbreak is reported and investigated. In this direction to create awareness among the stakeholder, two mass awareness programmes on 'Fish Disease Surveillance' were organized at Barabanki and Lakhimpur-Kheri in collaboration with State Fisheries Department, Uttar Pradesh on December 17 and December 21, 2015, which were attended by a total of 199 and 162 fish farmers, respectively (Fig. 90).

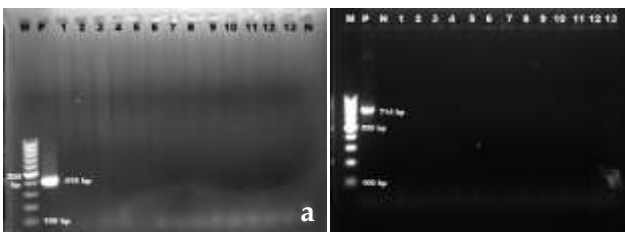


**Fig. 90. Mass awareness programme on 'Fish Disease Surveillance' at Barabanki, UP**

In addition, about 100 fish farmers in the Maharajganj district of UP were made aware about fish disease surveillance programme in the training programme organized by the State Fisheries Department, Government of Uttar Pradesh. Furthermore, 268 farmers under different training programmes conducted by

Aquaculture Research and Training Unit of the Institute were made aware about fish disease surveillance.

Under active monitoring, sampling was carried out in selected fish farms of 6 districts of Uttar Pradesh, viz. Bahraich, Barabanki, Kushinagar, Lakhimpur-Kheri, Maharajganj, Unnao and 5 districts of Haryana, viz. Ambala, Hisar, Karnal, Kaithal and Rohtak covering 154 fish farms. The samples consisted of mainly Indian major carps and common carp. All the samples were screened for two important viruses viz., SVCV and KHV and found negative for both the viruses (Fig. 91-93).



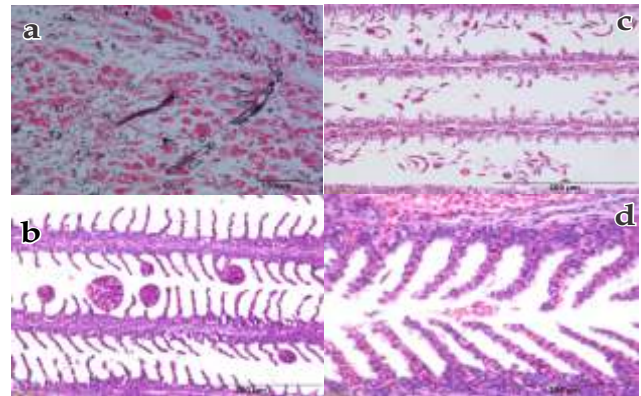
**Fig. 91. a & b. Screening of samples from Barabanki, Uttar Pradesh for KHV & SVCV**



**Fig. 92. a & b: Disease outbreak due to infection with *Aphanomyces invadans* and Fig. c & d: mortalities in fish farm of Haryana due to higher ammonia level**

In the context of detection of a new viral disease i.e. Herpesviral haematopoietic necrosis caused by Cyprinid herpesvirus-2 virus (CyHV-2) in ornamental fishes, a targeted active surveillance was carried out in ornamental fish shops in some of the major cities (Lucknow, Gorakhpur, Kanpur, Agra, Lakhimpur-Kheri, Meerut) of Uttar Pradesh, screened for CyHV-2 infection and confirmed in NSPAAD referral sub-project.

In addition, to strengthen the diagnostic capability of fisheries officers of the State Fisheries



**Fig. 93. a: Histology of *Aphanomyces invadans* infected muscle of *Cirrhinus mrigala* collected from Ambala; b: *Trichodina* sp. infection in the gill tissue of *Pangasianodon hypophthalmus* collected from Barabanki; c & d: Hematomas and necrosis of epithelial cells in the gill lamellae of fish collected from fish farm from Kaithal with total ammonia level more than 5 ppm**

Departments of Uttar Pradesh and Haryana, a Hands-on Training Programme on 'Fish Disease Diagnosis and Treatment' was organised during February 1-6, 2016 at the Institute (Fig. 94). A total of 18 fisheries officers including 5 officers from Haryana and 13 officers from Uttar Pradesh participated in the training programme. During the training programme, the participants were familiarised with level I, level II and level III diagnostics. During the training programme, experts from ICAR-CIFA, Bhubaneswar and Faculty of Fishery Sciences, WBUFAS, Kolkata were also invited for giving insights into latest developments in aquaculture and pond management, as well as, treatment of fish diseases. A field trip was also arranged to an intensive pangas culture farm in Barabanki district, U.P. The officers were made aware about the surveillance programme particularly about information that needs to be collected and reported, so that the passive surveillance system is strengthened and each disease outbreak is reported.



**Fig. 94. Training programme for fisheries officers of Uttar Pradesh and Haryana**



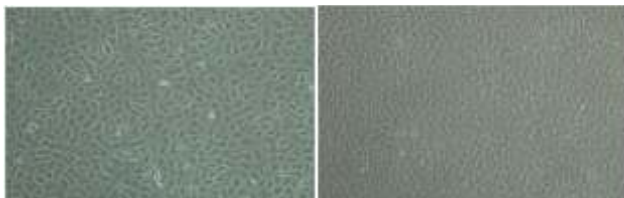
**Sub-Project: Surveillance of ornamental fish diseases**

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

Under the national surveillance programme, Peninsular and Marine Fish Genetic Resources Centre, Kochi of ICAR-NBFGR has undertaken the work on surveillance of ornamental fish diseases in the states of Kerala and Tamil Nadu. During the period under report, a total number of 50 ornamental fish farms in Kerala and 20 ornamental fish farms in Tamil Nadu were covered to document the national fish diseases of concern. In addition, a total of 300 Koi carp, *Cyprinus carpio* koi and 260 gold fish, *Carassius auratus* were screened for KHV (Koi Herpes Virus), SVCV (Spring Viraemia of Carp Virus) and Iridovirus infection.

**Fish cell lines developed**

A highly permissible cell line was developed and characterized from caudal fin of goldfish, *Carassius auratus* to isolate CyHV-2. In addition, two cell lines were developed from caudal fin of two commercially important ornamental fishes, Angel fish, *Pterophyllum scalare* and Oscar fish, *Astronotus ocellatus* for virus isolation (Fig. 95 & 96).



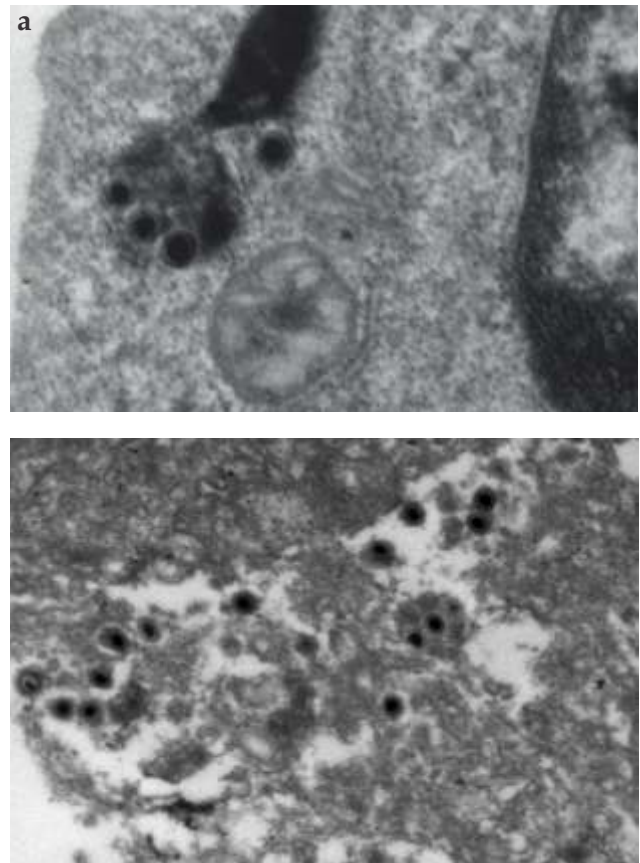
**Fig. 95. Angel fish cell line**

**Fig. 96. Oscar fish cell line**

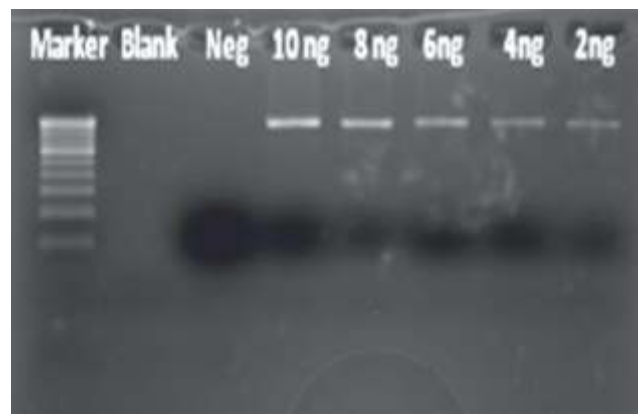
**Sensitive primer designed for the rapid detection of cyprinid herpesvirus-2 (CyHV-2)**

Goldfish haematopoietic necrosis herpes virus or Herpesviral haematopoietic necrosis (HVHN) is a fatal disease of goldfish leading up to 100% mortality and is caused by cyprinid herpesvirus-2 (CyHV-2). It was detected from goldfish in a disease outbreak and confirmed using molecular methods and histopathology. The virus had been isolated using the cell line, GFF developed from caudal fin tissue of goldfish, *Carassius auratus* at the Institute. The mature viral particles (approximately 90 - 120 nm in diameter) was demonstrated in cytoplasm of CyHV-2 infected gills cells of goldfish, *C. auratus* by transmission electron microscopy (TEM) which confirms the viral infection in goldfish (Fig. 97). A highly sensitive primer pair was designed to

amplify 932 bp partial sequence of major capsid protein (MCP) gene of CyHV-2 from its whole genome sequence (NC019495) and these primers could detect up to 2 ng of genomic DNA of CyHV-2 infected tissues of goldfish (Fig. 98). A GenBank BLAST search on the sequence revealed a high identity to CyHV-2 isolate SYC1 strain (KM200722, 100%) and CyHV-2 isolate STJ1 strain (JQ815364, 100%).



**Fig. 97. Mature hexagonal shaped virus particles demonstrated in (a) spleen and (b) gills of CyHV-2 affected goldfish in TEM**



**Fig. 98. Amplification of 932bp fragment of major capsid protein gene of CyHV-2 using newly developed primers**



### Mass mortality in ornamental fish, *Cyprinus carpio koi* caused by a bacterial pathogen, *Proteus hauseri*

Moribund koi carp, *Cyprinus carpio koi*, from a farm with 50% cumulative mortality were sampled with the aim of isolating and detecting the causative agent. Three bacterial species viz. *Citrobacter freundii* (NSCF-1), *Klebsiella pneumoniae* (NSKP-1) and *Proteus hauseri* [genomospecies 3 of *Proteus vulgaris* Bio group 3] (NSPH-1) were isolated, identified and characterized on the basis of biochemical tests and sequencing of the 16S rDNA gene using universal bacterial primers. Challenge experiments with these isolates using healthy koi carp showed that *P. hauseri* induced identical clinical and pathological states within 3 d of intramuscular injection. The results suggest *P. hauseri* (NSPH-1) was the causative agent. In phylogenetic analysis, strain NSPH-1 formed a distinct cluster with other *P. hauseri* reference strains with  $\geq 99\%$  sequence similarity. *P. hauseri* isolates were found sensitive to Ampicillin, Cefalexin, Ciprofloxacin and Cefixime and resistant to Gentamycin, Oxytetracycline, Chloramphenicol and Kanamycin. The affected fish recovered from the infection after ciprofloxacin treatment.

### Disease outbreak and mass mortality in farmed green cobra guppy, *Poecilia reticulata* associated with *Serratia marcescens* in Kerala, India

Isolation and characterisation of *Serratia marcescens* (NPSM-1) from diseased freshwater ornamental fish, green cobra guppy, *Poecilia reticulata* was reported. A pathogenic strain of *S. marcescens* was isolated from green cobra guppy with clinical signs of fin rot and was confirmed by biochemical tests and 16S rRNA sequencing (Fig. 99). The phylogenetic tree constructed using sequences of 16S rRNA gene indicated that they were related to *S. marcescens* (Fig. 100). The ECP of the bacteria exhibited marked cytotoxic activity *in vitro* on CFF cell line and the *in vivo* challenge studies of the isolate confirmed that these were highly pathogenic to fish when the fishes were injected with 500  $\mu$ l of  $1 \times 10^5$  CFU per ml. The bacterial isolate was tested to determine sensitivity against 16 antibiotics and was found sensitive to only 5 antibiotics viz., cefixime, chloramphenicol, ciprofloxacin, gentamycin and cefixime/ clavulanic acid. The study indicates that *S. marcescens* may cause disease in ornamental fish. As this pathogen is also associated with human infections, therefore,

caution should be taken in handling such infected fish. This was the first report describing *S. marcescens* as a pathogen of freshwater ornamental fish in India.

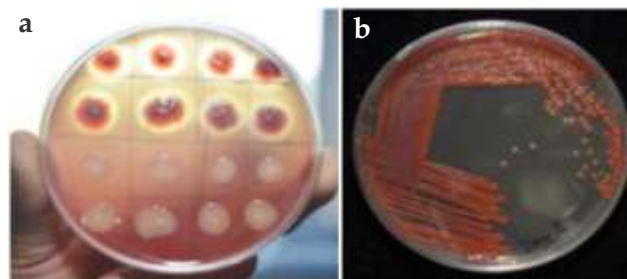


Fig. 99. *Serratia marcescens* isolated from green cobra guppy, *Poecilia reticulata*. These bacteria produce red colonies on nutrient agar (a) and hemolysis on blood agar (b)

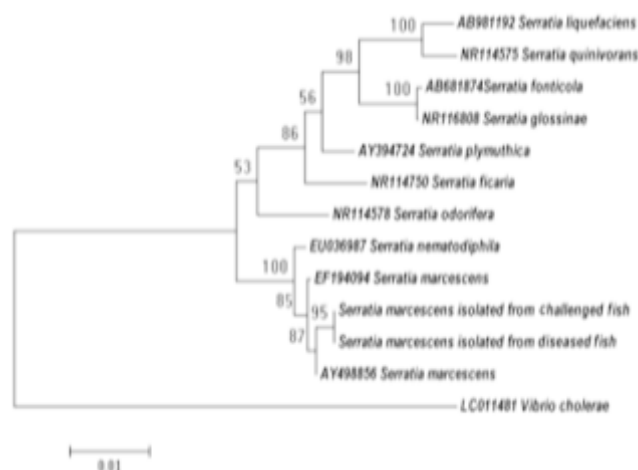


Fig. 100. Molecular phylogenetic analysis for the confirmation of *Serratia marcescens*

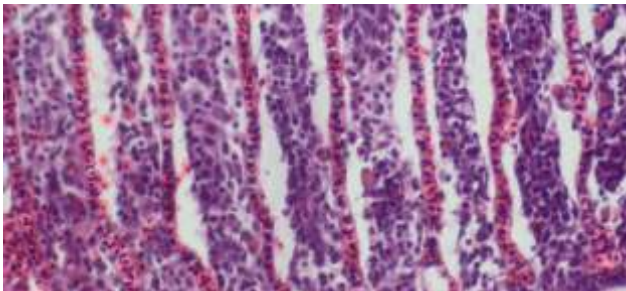
### Investigation of disease outbreak associated with carp edema virus

A disease outbreak was reported in adult koi from a fish farm in Ernakulam District, Kerala, India during rainy season. The clinical signs were observed only in recently introduced adult koi, whereas, existing population of koi carp, goldfish and Indian major carps did not show any external signs or mortality. Microscopic examination of wet mounts from the gills of affected koi revealed minor infestation of *Dactylogyrus* sp. in a few koi, and only opportunistic bacteria were isolated from the gills of affected fish in bacteriological studies. However, bacteria could not be isolated from the spleen and kidneys of affected fish. The histopathological examination of the affected fish revealed necrotic changes in gills of affected koi and importantly, virus particles were demonstrated in cytoplasm of gill epithelial cells. The tissue samples from affected koi were negative for SVCV, cyprinid herpes viruses, KHV, KRIV

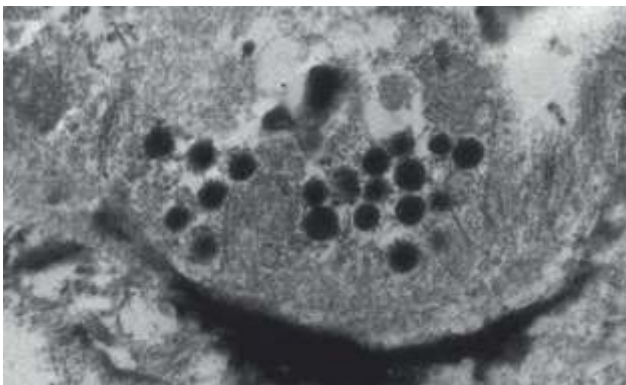
and iridovirus in PCR screening. However, gill tissue from affected koi carps was positive for CEV in 1<sup>st</sup> step of nested PCR, and sequencing of PCR amplicon confirmed infection with CEV. No cytopathic effect (CPE) was observed in koi carp fin (CCKF) cell line and 5 other fish cell lines. In bioassays, the symptoms could be reproduced by inoculation of filtrate from gill tissue homogenate of CEV-positive fish. Screening of koi showing symptoms similar to sleepy disease from different locations revealed that CEV infection was widespread (Figures 101-103). This forms the first report of infection with CEV in India.



**Fig. 101. Enophthalmia, swollen body and gills in koi due to infection with carp edema virus**



**Fig. 102. Hyperplasia of epithelial cells at the tips of secondary lamellae (arrows) and Extensive infiltration of mononuclear cells with few eosinophilic granular cells (bold arrows) and erythrocytes (line arrows) in the interlamellar region in gills of koi affected with koi sleepy disease**



**Fig. 103. Transmission electron photomicrograph of a koi gill epithelial cell containing large intracytoplasmic spheroid CEV virions**

### ***Aeromonas hydrophila* associated with mass mortality of adult Goldfish, *Carassius auratus* in ornamental farms of Kerala**

*Aeromonas hydrophila* was identified as the causative agent of a mass mortality in goldfish, *Carassius auratus* in ornamental fish farms of Kerala. Four different bacterial isolates viz., *Plesiomonas shigelloides* (NPPS-1), *Aeromonas hydrophila* (NPAH-1), *Citrobacter freundii* (NPCF-1) and *Acinetobacter* (NPA-1) were obtained from the affected fish, and their identity was confirmed with 16S rRNA gene sequencing. Experimental challenge resulted in mortality of fish injected with *A. hydrophila* isolates while no mortality was observed in fish injected with the other bacterial isolates. Furthermore, *A. hydrophila* could be recovered in pure culture from the dead fish in the experimental challenge trials. The *A. hydrophila* isolate produced hemolysis on blood agar and the extracellular products of the isolate exhibited marked cytotoxicity on goldfish fin (GFF) cell line, suggesting the pathogenic potential of the isolates. The isolates possessed multiple virulence genes such as enterotoxins (alt), haemolytic toxins (aerA and hlyA) and outer membrane protein (aha1 and omp TS) as determined by PCR. An antibiotic sensitivity test revealed that *A. hydrophila* isolate was sensitive to Cefixime, Chloramphenicol, Ciprofloxacin and Kanamycin. The affected fish recovered from the infection after treatment with the suggested antibiotics.



## WORKSHOPS/SYMPOSIA/TRAININGS/MEETINGS ORGANIZED

### ICAR Consortium Research Platform on Genomics Launched at ICAR-NBFGR, Lucknow

The ICAR Consortium Research Platform (CRP) on Genomics was launched by the Secretary, DARE and Director General, ICAR, Dr. S. Ayyappan on 12<sup>th</sup> July, 2015 at the ICAR-NBFGR, Lucknow. The genomics platform intends to



**Dr. S. Ayyappan, Secretary, DARE and Director General, ICAR addressing the participants**

generate structural and functional genome level insights for several commercially important crops, animals, fish, insects and microbes. The ICAR envisages facilitating intensive genomics research through integrating all institutes possessing diverse capabilities across commodities and develop quality human resources in the country. Over 40 researchers from different institutes participated in the workshop. Addressing to the participants, Dr. Ayyappan stated that the genetics and breeding had served for long in improving agricultural productivity, and the current cutting-edge technologies could further enhance the production with added precision. During the technical session, scientists from the partner institutes made the presentations. Dr. J. K. Jena, Director, ICAR-NBFGR, Lucknow outlined the envisaged programme including the budget outlay.

### Seminar on Reducing and Managing the Risk of Acute Hepatopancreatic Necrosis Disease (AHPND) of Cultured Shrimp and Round Table Discussion on AHPND National Action Plan

The ICAR-NBFGR, Lucknow hosted a seminar on Acute Hepatopancreatic Necrosis Disease (AHPND) during 14-15 September, 2015 on behalf of Department of Animal Husbandry, Dairying and Fisheries (DAHDF) in collaboration with Food and Agriculture Organization (FAO) of the United Nations. Six international experts from



**Dr. J. K. Jena, Director, ICAR-NBFGR addressing the participants**

FAO namely, Dr. Melba B. Reantaso, Dr. Maria Victoria Alday-Sanz, Dr. Pornlerd Chanratchakool, Dr. Celia Lavilla-Pitogo, Dr. Iddya Karunasagar and Dr. Indrani Karunasagar were the principal resource persons for the seminar. Thirty six selected personnel including scientists associated with National Surveillance Programme for Aquatic Animal Diseases (NSPAAD), officials from key organisations viz. DAHDF, Coastal Aquaculture Authority (CAA), Marine Products Exports Development Authority (MPEDA), State Fisheries Department (SFD) and representatives from shrimp industry attended the seminar. There were four technical sessions and sixteen presentations in the seminar. Some of the key recommendations were: *Vibrio parahaemolyticus* causing AHPND should be included in screening of imported broodstock of *Litopenaeus vannamei* during quarantine; enrichment of samples is necessary for detecting *V. parahaemolyticus* causing AHPND using standard protocols; proper management of the ponds is essential as sediments with high nutrients (due to chitin and unutilized feed material) provide suitable condition for the growth of *V. parahaemolyticus*; avoid use of live feeds collected from the wild for broodstock; captive production of live feeds and use of separate nursery ponds for rearing the post-larvae to juvenile stage and then stocking the juveniles in grow-out ponds.

The seminar was followed by round table discussion for preparing AHPND National Action Plan on 16 September, 2015. During discussions, all the participants agreed for constitution of a core group under NSPAAD, with ICAR-CIBA, Chennai as the lead institute and officials from key organisations and representatives from shrimp industry as the members. The core committee would prepare AHPND National Action Plan and create awareness





### Round table discussion on AHPND

about AHPND among stakeholders, evolve policy decisions for recommendation to the Government and develop contingency planning.

### Training Programme on 'Fish Genomics and Proteomic Data Analysis with High Throughput Computing'

A training programme on 'Fish Genomics and Proteomic Data Analysis with High Throughput Computing' was organized during 19-24 November, 2015. The programme was sponsored by the Centre of Agricultural Bioinformatics (CABin),



### Participants with the Guests and staff of ICAR-NBFGR

ICAR-Indian Agricultural Statistics Research Institute, New Delhi. Fifteen participants representing various disciplines from across the country attended the training programme. The course dealt with use of application of bioinformatics approaches to understand software/tools, statistical techniques used in the genomics, transcriptomics and proteomics data analysis along with hands on experience on the test data sets. The genomics portion included different lectures on Fish Bioinformatics, Biological database, Genomic data analysis using CLC Bio, Genomic data analysis and metagenomics and NGS data analysis. All these lectures and their respective hands-on-sessions provided an insight into various open source tools and paid software CLC bio workbench used to perform next generation sequencing data analysis,



### A training session

different types of BLASTs, genome assembly, gene finding and annotation, etc. The next sessions included lectures and hands-on-sessions on R Bioconductor and data analysis using R Bioconductor.

The proteomics sessions included lectures on Homology modelling and Molecular docking by Discovery Studio. A lecture and hands on Molecular dynamics simulation was also by Ms. Sonali Gosh, Application Scientist, Apasara Innovation, Bangalore via WebEx meeting. Statistical analysis session included SAS and Jump genomic software. The participants were also provided a training manual.

### Short term Training Programme on 'Molecular Markers'

The Peninsular and Marine Fish Genetic Resources (PMFGR) Centre, Kochi of the Bureau organized a training programme on molecular marker for genetic analysis during 14-19 December, 2015. A total of 15 researchers from different research organizations across the country participated in the training programme. The programme covered different aspects of molecular markers, mtDNA-DNA barcoding, polymorphic microsatellite markers and single nucleotide polymorphism (SNPs). Apart from the above, Molecular diagnosis of fish diseases and various softwares used in genetic analysis were also taught in the training programme.



### Participants with the guests and staff of PMFGR Centre of ICAR-NBFGR, Kochi





**A practical session during training programme**

**Short term training programme on 'Introduction to Fish Taxonomy'**

The PMFGR Centre, Kochi of the Bureau organized a training programme on 'Introduction to Fish Taxonomy' during 15-20 February, 2016. A total of 15 researchers from various research organizations of ICAR and universities across the country participated in the training. The training programme covered aspects of fish taxonomy, teleost fish diversity, external and internal morphology of fishes, taxonomy of different groups like freshwater fishes, marine fishes, elasmobranchs, flatfishes, etc., The training programme also included hands-on practicals on aspects like fixing, storing and tagging of specimens, photography and documentation, identifying different group of fishes using morphomeristic tools and other parameters.



**Participants with guests and staff**



**A practical session**

**Hands-on Training Programme on 'Fish Disease Diagnosis and Treatment'**

A Hands-on Training Programme on 'Fish Disease Diagnosis and Treatment' was organised to strengthen the diagnostic capability of fisheries officers of the State Fisheries Departments of Uttar Pradesh and Haryana, during 1-6 February, 2016 at the Institute. A total of 18 fisheries officers including 5 officers from Haryana and 13 officers from Uttar Pradesh participated in the training programme. During the training programme, the participants were familiarised with level I, level II and level III diagnostics. During the training programme, experts from ICAR-CIFA, Bhubaneswar and Faculty of Fishery Sciences, WBUFAS, Kolkata were also invited for giving insights into latest developments in aquaculture and pond management, as well as, treatment of fish diseases. A field trip was also arranged to an intensive pangas culture farm in Barabanki district, U.P. The officers were made aware about the surveillance programme particularly about information that needs to be collected and reported, so that the passive surveillance system is strengthened and each disease outbreak is reported.



**Chief guest Dr. J. K. Jena, DDG (Fy. Sci.) delivering inaugural address**



**A practical session on 'Fish Disease Diagnosis and Treatment'**



### Training-cum-Workshop on 'Intellectual Property Rights for Innovation in Agricultural Research'

A training-cum-workshop on 'Intellectual Property Rights (IPR) for Innovation in Agricultural



**Dignitaries releasing the training manual**

Research' was organized on 29<sup>th</sup> March, 2016 for inculcating capacity building among researchers towards IPR and Innovation. A total of 53 participants from various disciplines and organizations across the country participated in the programme. The expert resource persons were: lawyer from Dr. Ram Manohar Lohiya Law University (Mr. Vikas Bhati), scientist from CSIR-IITR, Lucknow (Dr. K.C. Khulbe), scientist from ICAR-CISH (Dr. Neelima Garg) and IP attorney from Patracode Services (Mr. Gaurav Singhal). Deliberations were focused on Registration of IPRs, Understanding patent registration through PTC system, Boosting territorial protection, Value addition in food & vegetable for agri-products development and Patent drafting: understating technicalities. The training programme also covered sessions on IP for research management, IP for innovation research and IP for entrepreneurship, followed by a panel discussion by the experts and convened by Mrs. Poonam Jayant Singh, Nodal Officer, ITMU, ICAR-NBFGR, Lucknow.

### Training programme on 'Start-ups and Innovation for Agri-Entrepreneurship'



**Participants with guest and staff**

A training programme on 'Start-ups and Innovation for Agri-Entrepreneurship' was organised for creating awareness about the vision of start-ups and to create future agri-entrepreneurs, in collaboration with ICAR-Central Institute of Fisheries Technology, Kochi on 30<sup>th</sup> March, 2016.. A total of 56 participants from various disciplines and organization across the country participated in the programme. The training programme covered various aspects like Starting start-ups for agri-entrepreneurship, Creation for start-ups, Incubation, Writing a business plan, Understanding elevator pitch, seeking investment through angel funding, seed funding and venture capital in agricultural sector. The training also included the case studies of successful start-ups and success stories of young innovators. The programme was convened by Mrs. Poonam Jayant Singh, Nodal Officer, ITMU, ICAR-NBFGR, Lucknow.

### Review Meeting of North-east Research Programme



**Research partners of NE research programme with ICAR-NBFGR team**

A review meeting of the Institute's North East (NE) component participatory research programme on 'Exploration and characterization of fish germplasm resources and indigenous knowledge in North Eastern Region of India' was convened under the Chairmanship of Dr. J.K. Jena, Director, ICAR-NBFGR, Lucknow on 4<sup>th</sup> July, 2015 at Department of Zoology, Gauhati University Guwahati. All the project partners presented progress of the work done during 1<sup>st</sup> phase of the programme which were thoroughly discussed and reviewed by the Director and Scientists of ICAR-NBFGR, Lucknow in the meeting. Based on the discussions in the meeting, the sub-programmes were selected for providing technical and financial assistance during 2<sup>nd</sup> phase of the programme (2015-16 to 2016-17).

### Seminar on Fish Biodiversity of Bakhira Lake

A seminar on 'Fish Biodiversity of Bakhira





### Seminar on fish biodiversity

Lake and Conservation' was organized on 29<sup>th</sup> April, 2015 at Bakhira Bird Sanctuary, Jaswal, District Sant Kabir Nagar, U.P. which was inaugurated by Dr. J.P. Shukla, Head, Department of Zoology S.H. Kisan Post-Graduate College, Basti. Scientists of ICAR-NBFGR, Lucknow and other speakers deliberated upon various issues concerning the Bakhira lake and emphasized its ecological importance for the conservation of fish biodiversity as well as the wildlife for the posterity. Fishermen from Sanichara and Jaswal villages also expressed their views regarding fish and fisheries of the lake and suggested some measures to be adopted for conservation of the fish species. Students from fishermen community from both the villages also participated in the deliberations.

### National Fish Farmers' Day Celebrated

The Institute organized National Fish Farmers' Day on 10<sup>th</sup> July, 2015. Major highlight of the day was question-answer session between farmers of Chhattisgarh and Uttar Pradesh and resource persons of ICAR-NBFGR, Lucknow. More than 50 farmers participated in the deliberation. Dr. J. K. Jena, Director of the Institute highlighted the importance of National Fish Farmers Day celebration and highlighted the recent achievements of ICAR-NBFGR. Dr. V.P. Kamboj, Former Director, CSIR-CDRI, Lucknow, as the Chief Guest of the function, narrated importance of endocrinological regulation

in the animals with special reference to the welfare of mankind. He also gave overview of various revolutions taken place in the field of agricultural science and their impact on farmers and society. Dr. P.C. Mahanta, former Director, ICAR-DCFR, Bhimtal, Uttarakhand highlighted participation of fishing community in fisheries development. Dr. S.K. Singh, Joint Director, Department of Fisheries, Govt. of Uttar Pradesh spoke on the importance of fisheries and aquaculture in Uttar Pradesh and sought scientific input from ICAR-NBFGR for farmers' trainings and fish seed production. The celebration ended with felicitation of fish farmers and officials of Uttar Pradesh and Chhattisgarh for their contribution on disseminating scientific fish culture practices.

### Agricultural Education Day Celebrated

The Institute celebrated Agricultural Education Day on 19<sup>th</sup> November, 2015 to enhance awareness and knowledge on fish biodiversity and need for their conservation to



### Group photo of participating children with guests and staff

students of the schools. The day was marked as Open House Day for the school children of the city and the facilities of Ganga aquarium, labs, farm and hatchery were kept open for them where they were given necessary information on fish biodiversity and fisheries related aspects.



### National Fish Farmers Day Celebrations

Four events namely, Drawing & Painting Competition (Junior and Senior groups), Quiz Competition and Essay Writing Competition for students of various schools of Lucknow city were also organized on the day in which around 100 students of fourteen schools took part. The best three students or group of students of each event were given away the cash prize, a trophy and certificate of merit on the occasion of 32<sup>nd</sup> Foundation and Farm Innovators Day held on 12<sup>th</sup> December, 2015.

**NBFGR celebrated its '32nd Foundation Day' and 'Farm Innovators Day'**

The Bureau celebrated its '32nd Foundation Day' and 'Farm Innovators Day' on 12 December, 2015. The programme was inaugurated by the



**Dr. J. K. Jena giving the welcome address**

Chief Guest of the occasion Dr. Rakesh Kapoor, Director, SGPGIMS, Lucknow where as Shri Eashwar Anand, Managing Editor, Fishing Chimes, Vishakhapatnam was the Guest of Honour. In his welcome address, Dr. J.K. Jena, Director, NBFGR apprised about the salient achievements of the Institute. A total of 25 progressive aqua-farmers and entrepreneurs participated in the programme and shared their



**Chief Guest Dr. Rakesh Kapoor delivering the inaugural address**

innovative and profitable farming practices. The Chief Guest and the Guest 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 aqua-farmers. The farmers also visited NBFGR farm and aquarium facilities.



**A view of the audience during Farm Innovators Day function**

On this occasion, Annual Institute Awards for the year 2014-15 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 Health Management Division' was awarded the Best/Division Award. Other awardees in various categories were: Dr. Neeraj Sood, Principal Scientist, Best Scientist; Dr. S.M. Srivastava, Chief Technical Officer, Best Technical Staff; Mr. Shreelal Prasad, Sr. Clerk, Best Administrative Staff; Mr. Chhote Lal, SSS, Best Support Staff; Mr. Om Prakash, appreciation award.

**Jai Kisan Jai Vigyan week celebrated by ranching fish seed of important fishes at Dudhwa tiger range**

The Institute celebrated the 'Jai Kisan and Jai Vigyan Week' during 23-29 December, 2015. On this occasion, an Awareness Camp on 'Fish Biodiversity and Need for their Conservation' was organized on 29<sup>th</sup> December at the Dudhwa Forest Range, Lakhimpur district U.P., where large number of protected wetlands and river network exists. The programme was inaugurated by Shri Neeraj Kumar, District Forest Officer, South Kheri, Lahimpur and presided over by Dr. J. K. Jena, Director, ICAR-NBFGR, Lucknow. Shri A.K. Gupta, Assistant Director Fisheries,





### Annual Institute Awards

Lahkimpur district was the Guest of Honour. About 200 people including officials of Forest Department, Fisheries Department, NBFGR staff, fishermen, fish farmers and students of the area, attended the programme. The Chief Guest in his inaugural address said that in order to have sustainable fish production from our open waters, such awareness camps can play a leading role. He appreciated NBFGR team for initiating such activities and selecting Dudhwa as the venue of this programme. Dr. J.K. Jena, Director in his presidential address stated that the Indian Council of Agricultural Research has taken a new mega initiative under the Consortium Research Project on Agrobiodiversity (CRP-AB) for conservation of indigenous germplasm of crops, animals and fishes for their sustainable production. The NBFGR along with four more partner institutes, will lead research on conservation of fish biodiversity under this platform project. He emphasized that in order to augment production of selected fish species from open waters, the Bureau has taken initiative to produce seed of three fish species which are important from culture and conservation aspects namely, kalbasu (*Labeo calbasu*), bata (*Labeo bata*)

and kali rohu (*Labeo dyocheilus*) of the Ganga Basin for their conservation. He stressed that fish conservation is possible only with the help of all stakeholders in which govt. departments, NGO's



### Dr. S. Raizada giving overview of the agrobiodiversity programme

and largely fishermen and the fish farmers can play a leading role.

Shri A.K. Gupta, Guest of Honour in his address explained to farmers the reasons of depleting of fish stocks in open waters, various methods required for their sustainable production





### Ranching of fish seed in Kakraha Wetland in Dudhwa Tiger Range

and the role to be played by different agencies and stakeholders towards augmenting production from the open waters. Speaking on the occasion, Dr. S. Raizada, Project Investigator of CRP-Agrobiodiversity sub-project Network of Conservation Aquaculture informed about the project and said that the process of conservation will be further strengthened in the coming years with addition of more number of species, seed volume and selection of more sites for ranching of fish seed. The programme ended with vote of thanks by Dr. L.K. Tyagi, Sr. Scientist. After the programme, around 50,000 fingerlings of kalbasu, bata and kali rohu were released at three places in Kakraha Wetland in Dudhwa Tiger Range, Sharda River and one Temple Pond in Lakhimpur Kheri district of U.P.

### Research Advisory Committee (RAC) Meeting

The RAC meeting of the Institute was held during 9-10 February, 2016 under the Chairmanship of Dr. W. Vishwanath, Professor, Department of Life Sciences, Manipur University, Imphal.



### RAC meeting being chaired by Prof. W. Vishwanath

Dr. (Mrs.) Usha Goswami, Scientist 'F' (Retd.), NIO, Goa; Prof. M.H. Balkhi, Dean, College of Fisheries, SKUAST, Srinagar; Prof. Bechan Lal,

Professor, Department of Zoology, Banaras Hindu University, Varanasi and Dr. P. Praveen, Assistant Director General (Marine Fisheries), ICAR, New Delhi participated as expert members of the RAC. Dr. (Mrs.) Rehana Abidi, Director (Acting), 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 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



### Annual Institute Research Committee meeting

meeting for the year 2014-15 was held at ICAR-NBFGR, Lucknow during 7-10 April, 2015 under the Chairmanship of Dr. J.K. Jena, Director, ICAR-NBFGR. Member Secretary, IRC, Dr. Ravindra Kumar welcomed the Chairman and members of IRC. In his introductory remarks, the IRC Chairman emphasized the role of prioritization, monitoring and evaluation cell. He appreciated the efforts of the scientists and other members of the Institute in organisation of several important workshops/consultations/trainings and other academic activities at the institute during the year 2014-15. After Chairman's address, progress reports of projects were presented by the respective PIs.

### Celebration of Independence Day

The Institute celebrated the Independence day with full enthusiasm. Dr. J.K. Jena, Director hoisted the National flag in the presence of staff members of the Bureau. In his address, he appreciated the efforts made by the Bureau in the past and highlighted future plans. On this



**Dr. J. K. Jena, Director addressing the staff on Independence Day**

occasion, a cultural programme was organised in which large number of the staff and children of the NBFGR family participated.

### Hindi Chetana Mas 2015

The Institute observed a Hindi Chetana Mas during 14<sup>th</sup> September-13<sup>th</sup> October, 2015. During the month long event, various competitions were organized among the staff of the Institute including the PMFGR Center, Kochi, to promote the use of Hindi in official work. All



**Hindi Chetna Mas celebrations**

the winners were given prizes on the closing day. On this occasion, noted poet of the Lucknow Shri Krishna Nandan Tiwari was the Chief Guest and a reputed poet Shri Gajendra 'Priyanshu' was the Guest of Honour. Both the guests reiterated importance of Hindi language and recited their poems. Mr. Subhash Chandra, Sr. Technical Officer won the prize for the Best Hindi Competitor - 2015.

### Republic Day Celebrated

A flag hoisting ceremony was observed on the Republic Day on 26 January, 2016. Dr. (Mrs.) Rehana Abidi, Director (Acting) hoisted the National Flag in the presence of other staff members of the Bureau. In her address, she highlighted the achievements of NBFGR during the year 2015 and shared glimpses of upcoming programmes. Dr. Abidi reminded the staff about their rights and duties towards growth of the Institute.



**Dr. (Mrs.) Rehana Abidi, Director (Acting) addressing the staff on Republic Day**

The programme was followed with a small cultural programme in which large number of children of the NBFGR family participated.



## EXTENSION ACTIVITIES

### Awareness programmes on 'Fish Disease Surveillance' in Uttar Pradesh

An awareness programme on 'Fish Disease Surveillance' was organized at Barabanki in collaboration with State Fisheries Department, Uttar Pradesh on 17<sup>th</sup> December 2015, under National Surveillance Programme for Aquatic Animal Diseases (NSPAAD). A total of 199 fish farmers from different parts of the district participated in the programme. In the programme, Dr. Sudhir Raizada, Principal Scientist informed the farmers about various aquaculture technologies for freshwater aquaculture. In addition to the carps, there are opportunities to include catfishes, freshwater prawn and ornamental fishes in freshwater aquaculture. He emphasized on the need of better management practices. He also stressed that the farmers could take advantage of such technologies to increase their productivity from the farm.

Dr. P.K. Pradhan, Senior Scientist mentioned that surveillance programme is currently getting implemented in 15 states through 25 collaborating centres in the first phase. He also explained about the clinical signs of important as well as emerging fish diseases in freshwater aquaculture. He advised the fish farmers to report all the disease outbreaks in the initial stages, so that losses due to diseases can be

minimized. Dr. S.K. Singh, Senior Scientist informed the fish farmers about various training activities being conducted by the Institute and assured all the help in providing scientific advice regarding fish culture/health management. Sh. Afaq Khan, Chief Executive Officer, FFDA, Barabanki informed about various government schemes and advised the farmers to take advantage of such schemes.



Awareness programme in Lakhimpur Kheri, U.P.



Awareness programme in Barabanki, U.P.

Another awareness programme on 'Fish Disease Surveillance' was organized at Lakhimpur Kheri in collaboration with State Fisheries Department, Uttar Pradesh on 21<sup>st</sup> December, 2015. A total of 162 fish farmers from different parts of the district participated in the programme. In the inaugural programme, Mr. A.K. Gupta, Assistant Director of Fisheries, State Fisheries Department, Lakhimpur Kheri advised the farmers to take advantage of the programme and thanked ICAR-NBFGR for organizing such a programme at Lakhimpur. Dr. Neeraj Sood, Principal Scientist and Consortium Principal Investigator, NSPAAD gave a brief outline of the National Surveillance Programme. He emphasized that main emphasis of the programme is to strengthen the passive surveillance system. Dr. Sudhir Raizada, Principal Scientist, Fish Conservation Division explained to the farmers



about various aquaculture technologies for freshwater aquaculture. He stressed on the need for diversification of the culture practices using some of the indigenous fish species to increase their productivity from the farm.

Dr. P. K. Pradhan, Senior Scientist explained about the fish disease surveillance programme and emphasized that farmers can play a crucial role in disease reporting. He also requested all the farmers to have a close vigilance on their fishes particularly during winter season and report the incidence of disease as early as possible. Speaking on the occasion, Dr. S. K. Singh, Senior Scientist explained about various training activities being undertaken by the Institute to the fish farmers and assured all the help in providing scientific advice regarding fish culture/health management.

#### **Awareness programme on aquatic animal disease surveillance at Takazhi, Allapuzha, Kerala**

An awareness programme on surveillance of aquatic animal diseases for ornamental fish farmers was conducted by PMFGR Centre, Kochi of the Institute at Takazhi, Allapuzha, Kerala on 14<sup>th</sup> April, 2015. A total of 30 ornamental



**Awareness programme on aquatic animal diseases surveillance at Takazhi, Kerala**

fish farmers attended the programme. Dr. T. Raja Swaminathan made a presentation with an overview of the National surveillance programme of aquatic animal diseases and explained the basic concepts on ornamental fish diseases and their management. The technical session was followed by the discussion with the farmers. Farmers shared their experience about ornamental fish culture followed by group discussion on fish diseases and their treatment. The participants were provided with a brochure on identification of important ornamental fish diseases and their treatment.

#### **Awareness programmes on fish diversity of upper mahanadi river basin and its conservation and management in Chhattisgarh**

Two awareness programmes on fish diversity of upper Mahanadi river basin, its conservation and role of fishing communities in fisheries resource management were organised in villages located alongside the Mahanadi river and its tributaries in the Chhattisgarh state. Extension literature was also distributed to fisherfolks. The Institute team comprised of Dr. L.K. Tyagi, Sr. Scientist, Mr. Amit Singh Bisht, Sr. Technical Officer and Mr. S.K. Singh, Technical Officer conducted the programmes involving local gram panchayat and fishing cooperative societies office-bearers, as guests.



**Awareness programmes on fish diversity of upper Mahanadi and its conservation in Chhattisgarh**

One awareness programme was organised at Pasaud village of Dhamtari distt. of Chhattisgarh right at the bank of Mahanadi River in its headwaters in Gagrel reservoir area in which 120 participants including fishermen, women, rural youths, office-bearers and members of fishing cooperative societies from Barbandha, Ur Putti, Mogra ghan and Devi Nagaon villages participated. Another awareness programme was organised at Tansi village of Kanker distt. of Chhattisgarh in which 130 participants including fishermen, women, rural youths, office-bearers and members of fishing cooperative societies from Tansi, Teluguda and Koliyari villages participated.



### Awareness programmes on fish diversity of upper Mahanadi and its conservation in Chhattisgarh

The following topics were included in the awareness programmes: General importance of fisheries & aquatic resources for livelihood generation of the fishing communities, overview of fish diversity and its importance for sustainable fisheries, role played by NBFGR in fish diversity conservation research and its utility for fishermen & fish-farmers, fish diversity of upper Mahanadi river basin and need for its conservation and sustainable management, role, potential of fishing communities and their institutions in conservation and management of fisheries resources of the basin, opportunities for fishermen and women in fish farming and other fish based enterprises, basic aspects of fish farming, NBFGR's activities under TSP scheme and experience and opinion sharing by participants and local guests. The programmes were covered by the regional press.

### Entrepreneurship development awareness programmes for tribal women in Madhya Pradesh

Three awareness programmes were organized for tribal women to create and enhance their awareness towards entrepreneurship from

agriculture utilizing traditional knowledge and skills by the IP & Patent Cell of the Institute. The 1st programme was organised in district Khandwa, M.P. on 16<sup>th</sup> March, 2016 in which 23 tribal women of three villages of district Khandwa namely, Matapur, Dabiya and Patalda participated. The programme covered both the interaction meeting followed by group discussion. The tribal women expressed their interest in traditional skill development towards entrepreneurship to strengthen their socio-economic status.

Another programme was organized in Betul district, M.P. on 17<sup>th</sup> March, 2016. The programme aimed at making tribal women aware about traditional knowledge associated with traditional culture expression, fishing methods and genetic resources for economic gain. A total of 25 tribal women of village Damjipura, district Betul participated in the programme. The third programme was organized in district Harda, M.P. on 18<sup>th</sup> March, 2016 which was attended by 19 tribal women of Jamaniya Khola district of Harda.

### Outreach activity on IPR for tribal kids at Harda, Madhya Pradesh

One day brainstorming session on IP was organised for tribal school kids of village Khirkiya, district Harda, M.P. on 18<sup>th</sup> March, 2016. Sixteen tribal school kids participated in the session which aimed at enhancing awareness and increased understanding of IP to



Group discussion and interaction sessions with tribal women in M.P.



Tribal school kids participating in creativity exercises in M.P.



promote creativity and innovation among rural kids. The IP & Patent Cell team of the Institute performed creativity exercise with the kids.

### Release of poster on endemic and economically important fish species

WWF (World Wildlife Fund for nature) in collaboration with PMFGR Kochi centre of ICAR-NBFGR prepared a poster on 'Endemic and economically important fish species of the Chalakudy river, Kerala'. An awareness programmes to release the poster was organised on 30<sup>th</sup> September, 2015, at Vazhachal, Chalakudy,



**Awareness programme on endemic fish species of Chalakudy River**

Kerala. The programme was presided over by Shri L. Chandrashekhar, IFS, Chief Conservator of Forest, Kochi and forest officials, representatives of NGOs and tribal people of the region participated. The poster featured important species known as indicators of habitat health and as a source of nutrition and livelihood to tribal communities. Published in English and Malayalam, the poster will be displayed at tribal hamlets and forest information centres in the region, thereby educating both residents and visitors on the fish wealth of the region.

### 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 training them (iii) Organising awareness programmes in tribal areas on fish conservation and (iv) Providing assistance for interested and trained tribal fish farmers in starting/strengthening fish farming enterprises. During the year under report, the following activities were undertaken in the scheme:

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

The Institute organized twelve (nine on-campus and three off-campus) short-term training programmes, under the TSP scheme, for tribal farmers. The participants were from nine districts of U.P. (Bahraich, Sonbhadra, Jhansi, Lalitpur), M.P. (Ashok Nagar, Khandwa, Harda, Betul) and Chhattisgarh (Dhamtari) states. A total of 380 tribal farmers including 72 tribal women were imparted training on 'Fish culture and Conservation' in these training programmes (Tables 22). All the on-campus 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.



**Participants with Director and staff of NBFGR**



**A tribal women sharing her experiences during training**



**Table. 22. Details of trainings conducted for tribal farmers under TSP scheme**

S.No	District	Type of Training	Duration	No. of Women	Total No. of Participants
1.	Bahraich, UP	On-campus at ARTU	15-18 May, 2015	15	29
2.	Dhamtari, Chhattisgarh	On-campus at ARTU	07-11 July, 2015	-	29
3.	Sonbhadra, UP	On-campus at ARTU	08-10 September, 2015	08	30
4.	Lalitpur, UP	On-campus at ARTU	26-29 Oct. 2015	-	30
5.	Khandwa, MP	On-campus at ARTU	26-29 Nov. 2015	-	30
6.	Ashok Nagar, MP	On-campus at ARTU	09-12 Dec. 2015	-	30
7.	Khandwa, MP	On-campus at ARTU	27-30 Jan. 2016	10	43
8.	Jhansi, UP	On-campus at ARTU	27 Feb - 01 March, 2016	04	31
9.	Harda/Khandwa, MP	On-campus at ARTU	09-12 March, 2016	02	16
10.	Sonbhadra, UP	Off-campus	02-04, August, 2015	03	12
11.	Betul, MP	Off-campus	17 <sup>th</sup> March, 2016	15	50
12.	Harda, MP	Off-campus	18 <sup>th</sup> March, 2016	15	50
<b>Total</b>				<b>72</b>	<b>380</b>


**Views of off-campus training programmes for tribal farmers**

### (ii) Establishing and popularising fish seed production enterprise in 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 in tribal areas 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 site 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 is also committed to provide overall

technical guidance for any technical problem faced by the beneficiaries in running the unit.

During the year under report, the Institute team installed FRP carp hatchery units at villages Kathaundhi and Jignahwa in Renukot Tehsil of Sonbhadra district of U.P. On-site demonstrations and training was also imparted to the beneficiaries


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

and other villagers in breeding and hatchery management. The TSP team surveyed and identified two sites for installation of the fish seed production units in Dhamtari district of Chhattisgarh state. The tribal people from the selected locations and nearby villages were imparted on campus training at the Institute's Aquaculture Research & Training Unit (ARTU), Chinhath, 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.



**A tribal fish farmer operating hatchery at his farm**

### **(iii) Awareness programme on livelihood opportunities for tribal farmers in fisheries enterprise and fish conservation**

An awareness programme was organised in Matapur village of Khandwa district of Madhya Pradesh to create awareness among tribal villagers about livelihood opportunities in fisheries based enterprises and also to promote



**Awareness programme for tribal farmers in M.P.**

fish conservation. About 200 tribal farmers and fishermen including tribal women and members of fishing cooperative societies participated in the programme. The TSP team of the Institute explained about the TSP scheme and various

activities undertaken by the Institute in tribal areas. The programme included a series of technical lectures by the team members, local guests and experience sharing by the tribal participants. The programme was organised on the bank of a large community pond in which fishing is done by the tribal people on lease, so that participants can easily relate to the information and knowledge imparted.

### **(iv) Providing assistance for interested and trained tribal fish farmers in starting/strengthening fish farming enterprises**

The TSP team also visited farms and fields of various tribal farmers in eight villages in Dhamtari and Kanker districts of Chhattisgarh;



**ICAR-NBFGR team providing technical guidance to tribal farmers in M.P.**

Sonebhadra district of U.P. and Khandwa, Harda and Betul districts of M.P. and provided technical advice on suitability of taking up or strengthening their fishing-based enterprises as a source of enhancing their livelihood opportunities.

### **Participation in exhibitions**

The Institute participated in several exhibitions related to fisheries and aquatic resources in different parts of the country during the year under report.

### **Fish seed production**

The production of quality fish seed of carps was continued and a total of 292.0 lakh spawn of Indian major carps and exotic carps was produced. A revenue of Rs. 4,43,329/- was generated from the sale of fish seed and table size fish.

### **Other farmer advisory services**

Various other technical guidance and advisory services were also provided to groups of clientele in aqua-farmers/college students, agri-clinic/ agri-business entrepreneurs etc. More than 297 clientele got benefited with above activities.



### Mera Gaon Mera Gaurav programme

The Institute undertook a number of activities under the Govt. of India programme 'Mera Gaon Mera Gaurav'. Bahuta village of Barabanki district and Samesi village of Lucknow district, U.P. were surveyed and farmers were

advised to grow different types of crops in case of drought driven pond bottom. A scientist-farmer interaction programme at Bahuta village, Barabanki district was conducted in which fish farmers of Bahuta, Lahi, Sahawal, Raili, Pokhara and Haidargarh villages participated.



Interaction with villagers under Mera Gaon Mera Gaurav programme



## AWARDS AND RECOGNITIONS

- Dr. Rajeev Kumar Singh, Sr. Scientist was conferred with 'Dr. Hiralal Chaudhary Best Young Scientist' award for the year 2014-15 on 31.08.2015 by ICAR-Central Institute of Fisheries Education, Mumbai.
- Dr. T.T. Ajith Kumar, Sr. Scientist was conferred with 'Prof. M. Aruchami award' for contributions to Indian marine ornamental aquaculture, especially on clownfish by Kongu Nadu Arts and Science College, Bharathiar University, Coimbatore on the occasion of National Conference on 'Perspectives and Prospects of Aquatic Research' held on 16 February, 2016.
- Dr. A. Kathirvelpandian, Scientist was awarded 'Best Ph.D. thesis award' by ICAR-CIFE, Mumbai for the year 2013-14.



**Dr. Rajeev Kumar Singh receiving 'Dr. Hiralal Chaudhary Best Young Scientist' award.**



**Dr. T.T. Ajith Kumar receiving 'Prof. M. Aruchami award'**

## LIST OF PROJECTS

### Institutional Projects

Sl.No.	Project Title	Personnel	Period
<b>Molecular Biology and Biotechnology Division</b>			
1	Development of an <i>in vitro</i> toxicity assessment system for aquatic pollutants.	M. Goswami (up to April 16, 2015), N.S. Nagpure (up to Dec. 05, 2015), S.K. Majhi (PI), Murali S. and A.K. Mishra	April, 2014 - March, 2017
2	Development of surrogate broodstock for propagation of valuable fish	S.K. Majhi (PI), B. Kushwaha and S. Raizada (up to Dec. 31, 2015)	April, 2014 - March, 2017
3	Population genomics of <i>Clarias magur</i> based on restriction site associated DNA (RAD) markers	Mahender Singh (PI), N.S. Nagpure (upto Dec. 05, 2015), Ravindra Kumar, A.K. Pathak, Murali S. and A.K. Singh	April, 2014- March, 2017

### Fish Conservation Division

4	ICAR-CRP on Genomics: <i>De-novo</i> gene sequencing of anadromous Indian Shad <i>Tenuulosa ilisha</i> (Hamilton, 1822) (ICAR-CRP)	Vindhya Mohindra (PI) Rajeev Kr. Singh, B. Kushwaha and Trivesh Mayekar	April 2015 - March 2017
5	Information-base on fish genetic resources of India	S.P. Singh (PI upto 31.7.15), S. Raizada (PI upto 31.12.15), Rehana Abidi (PI from 17 <sup>th</sup> Febuary 2016 ), T. T. Ajith Kumar, A. K. Pathak, Rejani Chandran, Bineesh K. K., R. Dayal, Reeta Chaturvedi and Ravi Kumar	April, 2012- March, 2017
6	Signatures of natural selection and genomic diversity in important freshwater fish species, <i>Tor putitora</i> and <i>Clarias magur</i>	Vindhya Mohindra (PI), Santosh Kumar and Trivesh Mayekar	April, 2014- March, 2017
7	Establishment of mapping and marker panel for first generation linkage map in Indian catfish, <i>Clarias magur</i> ( <i>batrachus</i> )	Rajeev K. Singh (PI), T.T. Ajith Kumar and Santosh Kumar	April, 2014- March, 2017
8	Outreach activity on fish genetic stocks-Phase II	J.K. Jena (Coordinator) Rajeev K. Singh (PI), Vindhya Mohindra, T.T. Ajith Kumar, Sangeeta Mandal and Santosh Kumar	April, 2014- March, 2017
9	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 K. Singh	April, 2012 - March, 2016
10	Participatory programme on exploration and characterization of fish germplasm resources and indigenous knowledge in North-eastern region of India.	L.K. Tyagi (Coordinator), V. Mohindra and Rajeev K. Singh	October, 2012- March, 2017
11	Documentation and development of passport information of exotic food and ornamental fishes in India.	Peyush Punia (up to April 28, 2015), S. Raizada (up to Dec. 31, 2015), T.T. Ajith Kumar (PI), Rejani Chandran, Aditya Kumar and Rajesh Dayal	April, 2014 - March, 2017
12	Techno-legal analysis of policy issues and patents for strategic management of fish genetic resources.	Poonam J. Singh (PI), Rehana Abidi, A.K. Pandey, Amar Pal, Ravi Kumar and A.S. Bisht	April, 2015 - March, 2018

### Fish Health Management Division

13	Exploration of finfish parasites of river Gomti particularly protozoans and monogeneans through conventional and molecular techniques.	Rehana Abidi (PI), S.M. Srivastava, Amar Pal and Ranjana Srivastava	April, 2014-March, 2017
14	Development of an immune marker and understanding host- <i>Aphanomyces invadans</i> interaction using a macrophage cell line.	Neeraj Sood (PI), P.K. Pradhan and Mary Lini	April, 2014 -March, 2017
15	Deciphering <i>Aphanomyces invadans</i> genome to understand its mechanism of infection in fishes.	P.K. Pradhan (PI) Vindhya Mohindra, Neeraj Sood and Rajeev Kr. Singh	April, 2015 - March, 2017

### Peninsular and Marine Fish Genetic Resources Centre, Kochi

16	Genetic stock - structure analysis of <i>Parapenaeopsis stylifera</i> and <i>Scomberomorus commerson</i> along the Indian coast using molecular markers.	P.R. Divya (PI), V.S. Basheer, A. Kathirvelpandian and Mog Lebrachai Chowdhury	April, 2013 -March, 2016
17	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
18	Development of DNA chip for identification of commercially important fish species of Indian waters.	A Kathirvelpandian (PI), Toms C. Joseph (CIFIT), L. Mog Chowdhury and Murali S.	April, 2015 - March, 2018
19	Establishment of spermatogonial stem cell line (SSC) from endemic fish of the Western Ghats.	T. Raja Swaminathan (PI) and Charan Ravi	April, 2015- March, 2018

### Externally Project Projects

Sl.No.	Project Title	Personnel	Funding Agency	Period
1	National Surveillance Programme for Aquatic Animal Diseases	J.K. Jena (Coordinator) Neeraj Sood (CPI), P.K. Pradhan, P. Punia (up to April 28, 2015), Rehana Abidi and T. Raja Swaminathan	NFDB	April, 2013 - March, 2018
	Sub-Project: Surveillance of freshwater fish and shellfish diseases in Uttar Pradesh and Haryana	P.K. Pradhan (PI), Neeraj Sood, Aditya Kumar, Chandra Bhushan Kumar and Mary Lini	NFDB	April, 2013 - March, 2018
	Sub-Project: Surveillance programme for aquatic animal diseases of ornamental fishes in the states Kerala and Tamil Nadu	T. Raja Swaminathan (PI) and V. S. Basheer	NFDB	April, 2013 - March, 2018
2	'Network project on agricultural bioinformatics and computational biology' under Centre for Agricultural Bioinformatics (CABin): Fisheries domain	N.S. Nagpure (CCPI, up to Dec. 05, 2015), S.P. Singh (up to 31 <sup>st</sup> July 2015, Ravindra Kumar (PI)	ICAR-IASRI	January, 2015 - March, 2017
	Sub-Project 1: Identification and characterization of genes associated with abiotic stress in commercially important catfish, <i>Clarias magur</i>	Basdeo Kushwaha, Ajey Kr. Pathak and Murali S.		
	Sub-Project 2: Omics information system for fish and other aquatic animals (ICAR-IASRI)	Ajey Kr. Pathak and Mahender Singh		



3	Stock characterization, captive breeding, seed production and culture of hilsa ( <i>Tenuulosa ilisha</i> )	Vindhya Mohindra (CCPI), Rajeev Kr. Singh, Sangeeta Mandal and J.K. Jena	NASF, ICAR	November, 2012-October, 2016
4	ICAR-CRP on Agrobiodiversity: National Network on Agrobiodiversity Management Sub-project: National network of germplasm centre for prioritized fin fishes of Ganga basin for conservation aquaculture	S. Raizada (PI up to Dec. 31, 2015), T.T. Ajith Kumar (PI), L.K. Tyagi, S.K. Srivastava, Santosh Kumar and Vikas Sahu	ICAR	April 2015 - March 2017
5	ICAR-CRP on Vaccines & Diagnostics: Evaluating the effect of immunization on protection against infection with <i>Aphanomyces invadans</i> .	P.K. Pradhan (PI), Neeraj Sood and Chandra Bhushan Kumar	ICAR	August, 2015 - March, 2017
6	Whole genome sequencing and development of allied genomic resources in two commercially important fish- <i>Labeo rohita</i> and <i>Clarias batrachus</i>	J.K. Jena (Coordinator) N.S. Nagpure (PI up to Dec. 05, 2015), Basdeo Kushwaha (PI), Ravindra Kumar and Mahender Singh	DBT	September, 2013 - September, 2016
7	Neuroendocrine regulation on ovarian maturation in the giant freshwater prawn, <i>Macrobrachium rosenbergii</i>	A.K. Pandey (PI)	UPCST	August, 2013 - July, 2016
8	Characterisation of <i>Aphanomyces invadans</i> from North east India to develop diagnostic techniques and control measures.	P.K. Pradhan (PI) and Peyush Punia (up to April 28, 2015)	DBT	September, 2013 - October, 2016
9	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	May, 2013- November, 2016
10	All India network project on fish health	P.K. Pradhan (PI), Aditya Kumar, Chandra Bhushan Kumar and S.M. Srivastava	ICAR	July, 2015 - March, 2017
11	Intellectual property management and transfer/ commercialization of agricultural technology scheme (Up-scaling existing components i.e. Intellectual property right)	Poonam J. Singh (PI)	NAIF	April, 2014 - March, 2017
12	Characterization and DNA barcoding of fishes from Mizoram	Mahender Singh (PI)	DBT	December, 2014-December, 2017
13	Characterization and DNA barcoding of endemic fishes of Northeast India	Mahender Singh (PI, NBFGR), N.S. Nagpure (up to Dec. 05, 2015) and W. Vishwanath (PI, Manipur University, Imphal)	DBT	November, 2012-May, 2016
14	DNA barcoding of marine finfishes and shellfishes.	V.S. Basheer (PI) and J.K. Jena	MoES- CMLRE, Govt. of India	April, 2012 - March, 2017

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	January, 2013- April, 2015
16	Risk and benefit assessment of an illegally introduced fish species <i>Piaractus brachypomus</i> Pacu in India	P.K. Pradhan (PI), Aditya Kumar and S.M. Srivastava	NFDB	October, 2013 - October, 2016
17	Development of novel microsatellites in <i>Channa</i> species (Channidae: Perciformes) from North East for conservation genetics	Rajeev Kr. Singh (PI), L.K. Tyagi and A.S. Barman (College of Fisheries, CAU, Lembuchera, Agartala)	DBT	April, 2012-March, 2016

## PARTICIPATION IN SEMINARS/ SYMPOSIA/ WORKSHOPS/ TRAININGS/ MEETINGS

### Abroad:

Dr. J.K. Jena, Director participated in the 46<sup>th</sup> Executive Council Meeting of Asian Fisheries Society and 2<sup>nd</sup> International Symposium on Aquaculture and Fisheries Education at Shanghai, China during 20-25 April, 2015.

### In India:

Dr. J.K. Jena, Director participated in the following meetings/ seminars/ workshops:

- 2<sup>nd</sup> International Symposium on Aquaculture and Fisheries Education as Co-ordinator of Technical Session-II (Fish Genetic Resource) on 27 April, 2015 and Plenary Session of the International Conference on 'Low Temperature Science and Biotechnological Advances' on 30 April, 2015 at NASC, New Delhi.
- 6<sup>th</sup> Meeting of Scientific Panel for Fish and Fisheries Product on 7<sup>th</sup> May, 2015 at New Delhi.
- Interactive Meeting of Vice-Chancellors of SAUs and ICAR Directors at NASC, New Delhi during 15-17 May, 2015.
- 22<sup>nd</sup> General Body Meeting and Silver Jubilee and the Foundation Day Programme of NAAS during 3-5 June, 2015 at New Delhi.
- 2<sup>nd</sup> Advisory Committee Meeting of the 'Centre of Excellence in Fisheries and Aquaculture Biotechnology (COE-FAB)' at College of Fisheries, Central Agricultural University, Lembucherra, Agartala, Tripura during 20-21 June, 2015.
- Live Programme 'Hello Kissan' for DD Kisan Channel at CPC Doordarshan, Khel Gaon, New Delhi on 25<sup>th</sup> June, 2015.
- Meeting on 'Outreach Project on Genetic Stock' organized at ICAR-CIFA, Bhubaneswar on 26<sup>th</sup> June, 2015 and 'Collaborative State Level Research-Industry-Farmer-Extension Interaction Workshop on Freshwater Aquaculture Development in Odisha' at ICAR-CIFA, Bhubaneswar during June 27-28, 2015.
- Review Meeting on the progress and achievements of 'Participatory programme on exploration and characterization of fish germplasm resources and indigenous knowledge in North-Eastern region of India' organized by NBFGR, Lucknow on 4<sup>th</sup> July, 2015 at Gauhati University, Guwahati.
- 87<sup>th</sup> ICAR Foundation Day and award ceremony and National conference of KVKs held on 25<sup>th</sup> July, at Sri Krishna Memorial Hall, Near Gandhi Maidan, Patna.
- Summer School on 'Aquaculture Diversification towards Boosting Pond Productivity and Farm Income' at ICAR-CIFA, Bhubaneswar on 28<sup>th</sup> July, 2015.
- Seminar on 'Genetics and Sustainable Aquaculture 2015' organized by Rajiv Gandhi Centre for Aquaculture (RGCA) at Chennai on 10<sup>th</sup> August, 2015.
- Review meeting of CRP-Genomics and in MoU signing meeting between ICAR-CIBA and private industry on Biofloc technology at CIBA, Chennai on 12<sup>th</sup> August, 2015.
- 7<sup>th</sup> meeting of the scientific panel on fish and fisheries products at FSSAI, New Delhi on 19<sup>th</sup> August, 2015.
- Workshop on 'Mahseer Fisheries' organized by ICAR- CIFE, Mumbai in collaboration with Tata Power Company Limited on 21-22 August, 2015 at Lonavala, Maharashtra.
- 82<sup>nd</sup> meeting of the Executive Council of the West Bengal University of Animal & Fishery Sciences on 2<sup>nd</sup> September, 2015 at WBUA & FS, Kolkata.
- Workshop on 'Drain Analysis and Water Quality Score Cards' organized by Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia and National Mission for Clean Ganga (NMCG) in New Delhi on 7<sup>th</sup> September, 2015.
- 2<sup>nd</sup> Meeting of Core Expert Group on Designated Repository of Genetic Resources at MoEFCC, New Delhi on 05<sup>th</sup> October, 2015.
- Meeting with Vietnamese Delegation and Presentation on Aquatic Disease Surveillance Programme of the Country at DADF, Ministry of Agriculture and Farmer Welfare, Krishi Bhawan on 09<sup>th</sup> October, 2015.
- CRP on Agro-biodiversity (CRP-AB)



Workshop at ICAR-NBPGR, New Delhi on 14<sup>th</sup> October, 2015.

- World Fisheries Day at NASC, Pusa, New Delhi on 21<sup>st</sup> November, 2015.
- 5<sup>th</sup> International Symposium on Cage Aquaculture in Asia (CAA5) organized by Asian Fisheries Society and Central Marine Fisheries Research Institute at Kochi during 26 – 27 November, 2015.
- 8<sup>th</sup> Meeting of Scientific Panel of Fish and Fisheries Products at Krishi Bhawan, New Delhi on 7<sup>th</sup> December, 2015.
- 4<sup>th</sup> Meeting of Steering Committee at National level to oversee and monitor the Tilapia seed and grow-out production at Krishi Bhawan, New Delhi on 14<sup>th</sup> December, 2015.

Dr. J.K. Jena, Director and Dr. L.K. Tyagi, Sr. Scientist participated in the 3<sup>rd</sup> Uttar Pradesh Agricultural Science Congress on 'Strategic Governance and Technological Advancement for Sustainable Agriculture' at Sam Higginbottom Institute of Agriculture, Technology & Sciences, Allahabad on 14<sup>th</sup> June, 2015.

Dr. Rehana Abidi, Director (Acting), participated in the following:

- ICAR Directors' and Vice Chancellors' meeting at NASC, New Delhi during 23-24 January, 2016.
- Meeting of the Directors of all ICAR Fisheries Research Institutes convened by DDG (Fy. Sci.), ICAR on 25<sup>th</sup> January, 2016 at KAB-II, New Delhi.
- 36<sup>th</sup> meeting of the Expert Committee on Access and Benefit Sharing (ABS) on 15<sup>th</sup> February, 2016 at NBA, Chennai.
- Working Group Meeting for Preparation of the Country Report (SoWAqGR) for FAO during 16-17 February, 2016 at Fisheries Division, ICAR, New Delhi.

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

- Management Development Programme on 'Scientific, administrative and research management organized by Administrative Staff College of India, Hyderabad during 17-28 August, 2015.
- 2<sup>nd</sup> review meeting of DBT project 'Whole genome sequencing and development of allied genomic resources in two commercially important fish- *Labeo rohita* and *Clarias batrachus*' held at ICAR-IASRI, New Delhi on 7<sup>th</sup> Jan, 2016.

Dr. A K Pandey, Principal Scientist participated

in the following:

- National Conference on Biotechnological Developments and Societal Benefits: Present Status and Future Prospects at Sky Institute, Lucknow during 08-09 April, 2015.
- OMICS Indo-Global Summit & EXPO on Veterinary on Indian (Indian Veterinary-2015) at HICC, Hyderabad during 26-28 October, 2015.
- National Conference on Climate Change and Sustainable Development: Emerging Issues and Mitigation Strategies (CCSD-2015) at B.B.A.(Central) University, Lucknow during 23-24 November, 2015.
- International Conference on Climate Changes and Sustainability at Thakur College, Mumbai during 21-23 December, 2015.
- National Seminar on Biodiversity Conservation and Sustainable Development at Brahmanand College, Kanpur on 20<sup>th</sup> January, 2016.
- 3<sup>rd</sup> Annual National Conference on Strategy for Human Welfare on Nature Conservation and Resource Management organized by Environment & Social Welfare Society at Khajuraho during January 31 to February 01, 2016.
- National Conference on Life-Styles and Chronic Diseases; A Threat to Sustainable Public Health organized by Sky Institute at Lucknow during 09-10 March, 2016.
- National Symposium on Recent Advances in Zoological Research, Department of Zoology, University of Lucknow, Lucknow on 30<sup>th</sup> March, 2016

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

- 32<sup>nd</sup> Executive Meeting on Access and Benefit Sharing held on 28<sup>th</sup> May, 2015 at National Biodiversity Authority of India, Chennai.
- 35<sup>th</sup> meeting of Expert Committee on Access and Benefit Sharing held on 28<sup>th</sup> November, 2015 at NBA Chennai.
- Working group meeting for preparation of the country report on Aquatic genetic resources for FAO during 16-17 February, 2016 at Krishi Anandhan Bhavan-II, Pusa, New Delhi.

Dr. V.S. Basheer, Principal Scientist, Dr. T. Raja Swaminathan and Dr. Divya PR, Sr. Scientists, Dr. Kathirvelpandian, Mr. Charan Ravi, Labrechai Mog Chowdhury and Mrs. Teena

Jayakumar T.K., Scientists and Dr. Rajool Shanis CP, Technical Assistant, attended Cage Aquaculture Asia (CAA5) Symposium at ICAR-CMFRI, Kochi during 25-28 November, 2015.

Dr. V.S. Basheer, Principal Scientist and Mr. Bineesh K.K., Scientist participated in Capacity Building Workshop CITES Appendix II listing of Sharks and Manta ray species at Kochi, Kerala during 15-16 December 2015 and attended 67<sup>th</sup> Inter-agency coordination meeting under the chairmanship of the APCCF (Protection), Tamil Nadu at Kendriya Bhavan, Kakkanadu, Kochi, Kerala on 29<sup>th</sup> December 2015. They also attended National Seminar on Seafood Safety, Trade & Management at Kochi during 9-12 March, 2016.

Dr. V.S Basheer, Principal Scientist and Dr. T. Raja Swaminathan, Sr. Scientist participated in the International Conference on Low Temperature Science and Biotechnological Advances (CRYO Biotech 2015) at New Delhi during 27-30 April, 2015.

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

- Management Development Programme on Leadership Development organized by ICAR-NAARM, Hyderabad during November 30 to December 11, 2015.
- 2<sup>nd</sup> review meeting of DBT project 'Whole genome sequencing and development of allied genomic resources in two commercially important fish- *Labeo rohita* and *Clarias batrachus*' held at ICAR-IASRI, New Delhi on Jan 7, 2016.
- 2<sup>nd</sup> International Symposium on Genomics in Aquaculture (ISGA-II) organized by ICAR-CIFA, Bhubaneswar during 28-30 January, 2016 and presented two papers.

Dr. Lalit Kumar Tyagi, Sr. Scientist participated in the Capacity Building Conclave' for fisheries sector during 25-26, February, 2016 at NFDB, Hyderabad. He also attended the 'National Dialogue on Innovative Extension Systems for Farmers' Empowerment and Welfare' organised by TASS, NAAS and ICAR during 17-18 December, 2015 at NASC, New Delhi.

Dr. Sharad Kumar Singh, Sr. Scientist participated in the International Day Seminar on Biological Diversity for Sustainable Development on 22<sup>nd</sup> May, 2015 at Lucknow. He also participated in the Scientific Advisory Committee Meeting of KVK, Lucknow on 13<sup>th</sup> April, 2015; KVK, Ambarpur, Sitapur on 17<sup>th</sup> November, 2015 and KVK, Unnao on 28<sup>th</sup> September, 2015.

Dr. Mahender Singh, Sr. Scientist participated in the following:

- Workshop on 'Molecular biology and bioinformatics techniques' at Dayanand Girls Postgraduate College, Kanpur on 11<sup>th</sup> September, 2015 and delivered invited lecture followed by hands-on session on 'Mitochondrial DNA and DNA barcoding'.
- 18<sup>th</sup> Indian Agricultural Scientists and Farmer's Congress on 'Prospects of Skill Development in Agriculture and Rural Development - A step towards make in India' organized during 20-21 February, 2016 by Bioved Research Institute of Agriculture & Technology, Allahabad. and delivered invited lecture on 'Role of biotechnology for sustainable agriculture in India'.
- 'FICCI India Innovation Growth Programme' on 19<sup>th</sup> February, 2016 organized by Federation of Indian Chambers of Commerce and Industry (FICCI) at Lucknow.

Dr. Rajeev Kr. Singh, Dr. T.T. Ajith Kumar, Sr. Scientists, Dr. A. Kathirvelpandian, Shri. Charan Ravi and Santosh Kumar, Scientists participated in the training programme on 'Generation and Analysis of Truss Morphometric Data for Aquaculture Species' at ICAR-CIBA, Chennai during 17-19 May, 2015.

Dr. Vindhya Mohindra, Pr. Scientist participated in the following:

- Review meeting of Megaprojects by the members of the Empowered Committee, Chaired by DG, ICAR and including Dr. Punjab Singh, Dr Rakesh Tuli experts from the field and Dr. K.K. Vaas on 20 April 2015 at the NASE Complex, New Delhi.
- Review Meeting of the NBFGR North East Research Programme being organised by the NBFGR at Gauhati University, Guwahati on 4<sup>th</sup> July, 2015 and survey.
- ICAR Consortium Research Platform on Genomics held on 12<sup>th</sup> July, 2015 at ICAR-NBFGR Lucknow.
- International Conference on 'Low Temperature Science and Biotechnological Advances' during 27-30 April 2015 at NASC Complex, Pusa Campus, New Delhi, Jointly conducted by ICAR-NBPGR and NAAS India in collaboration with Society for Low Temperature Biology (SLTB), UK and Royal Botanic Gardens (RBG), Kew, UK.
- 2<sup>nd</sup> International Symposium on Genomics in Aquaculture (ISGA-II) organized by the

AOA and AFSIB at ICAR-CIFA, Bhubaneswar, during 28-30 January, 2016, at ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar.

Dr. Rajeev Kr. Singh, Sr. Scientist participated in the following:

- International Conference on 'Low Temperature Science and Biotechnological Advances' during 27-30 April, 2015.
- Delivered lecture on occasion of Golden Jubilee Celebration Year at Gauhati University, Guwahati on 23<sup>rd</sup> November, 2015.
- 2<sup>nd</sup> International Symposium on Genomics in Aquaculture during 28-30 January, 2016 at ICAR-CIFA, Bhubaneswar.

Dr. T.T. Ajith Kumar, Sr. Scientist participated in the following:

- National Workshop on Lesser Known Marine Animals of India at Zoological Survey of India, Andaman and Nicobar regional centre, Port Blair during 11 - 13 June, 2015. Also delivered an invited lecture on 'Marine faunal diversity of Lakshadweep'.
- Summer School on 'Aquaculture diversification towards boosting pond productivity and farm income' held at ICAR - CIFA, Bhubaneswar during 8 - 28 July, 2015.
- National Workshop on Freshwater fish taxonomy held at the Kerala University of Fisheries and Ocean Studies, Kochi during 2-4, December 2015.
- Training Programme on Ornamental Fish Breeding and Culture held at the ICAR - CIFE, Kolkata centre on 15<sup>th</sup> March, 2016. Also delivered invited lectures on Hatchery production of Marine Ornamental fish & Problems and Prospects of Marine ornamental Aquaculture: Indian perspective.

Dr. T. Raja Swaminathan, Sr. Scientist attended the 6<sup>th</sup> Technical Advisory Meeting of NSPAAD held at New Delhi and also participated in the 18<sup>th</sup> National Committee on introduction to exotics aquatic species into Indian waters held on 22<sup>nd</sup> February, 2016 at DADF, New Delhi.

Mrs. Poonam J. Singh, Scientist participated in the following:

- 33<sup>th</sup> Meeting of Expert Committee on 'Access and Benefit Sharing' on 17<sup>th</sup> August 2015, at NBA, Chennai.
- 34<sup>th</sup> Expert Committee meeting on 'Access and Benefit Sharing' on 9<sup>th</sup> October 2015, at NBA, Chennai.

- Meeting of ITMC, IISR, Lucknow on IP awareness as a resource person on 'Understanding Creativity for Innovation in Research' for researchers on 16<sup>th</sup> November, 2015.

- Conference on 'Patinformatics for Technological Competitive Intelligence and Licensing' at CSIR-URDIP, Pune during 7-9 December, 2015.

- First Chevening Social Ventures Meeting at New Delhi on 12<sup>th</sup> December, 2015 as a Mentor.

- VIBCON 2015, XXII Annual Convention of Indian Society for Veterinary Immunology and Biotechnology and National Symposium on Immunomics and Proteogenomics in Livestock Health & Productivity, during 17-19 December, 2015 at ICAR-NRCE, Hisar. Also presented an oral paper.

- 'FICCI India Innovation Growth Programme' on 19<sup>th</sup> February, 2016 organized by Federation of Indian Chambers of Commerce and Industry at Lucknow.

- State level capacity building workshop on 'Economic valuation of Bio-resources for Access and Benefit Sharing' organised by Telangana State Biodiversity Board on 1<sup>st</sup> March, 2016 at Hyderabad. Also presented an oral paper.

- 'Boost Your Business with Facebook' event organised by Ministry of Micro, Small & Medium Enterprises (MSME) and Government of Uttar Pradesh in collaboration with Facebook at Taj Vedanta, Lucknow on 11<sup>th</sup> March, 2016.

- Annual meeting-cum-workshop 2016 organised by ZTMC, ICAR-CIFT, Kochi on 22<sup>nd</sup> March, 2016 at ICAR-NAARM, Hyderabad.

Dr. A. Kathirvelpandian, Scientist, participated in the following:

- Workshop on 'Genetics and sustainable aquaculture 2015' organized by RGCA at Chennai on 10<sup>th</sup> August, 2015.

- National seminar on 'Understanding biodiversity: progress and problems' at NSS College, Ottapalam, Kerala on 10<sup>th</sup> September, 2015. He also delivered an invited lecture in the seminar.

- Training programme on 'Agrobiodiversity conservation, sustainable livelihood and need for climate change adaptation' at Chennai during 21-25 September, 2015.

Dr. P.R. Divya, Senior Scientist participated and



delivered an invited lecture in the National seminar on 'Integrating taxonomy to DNA barcoding' at St. Xavier's College for Women, Aluva, Kerala on 11<sup>th</sup> September, 2015.

Shri Murali S., Scientist participated in the following:

- Workshop of Nodal officers on ICAR Research Data Repository for Knowledge Management held at NASC complex, New Delhi during 4-5 August, 2015.
- Workshop on Bio-entrepreneurship - 'Research to Business' at Biotech Park, Lucknow on 3<sup>rd</sup> November, 2015.

Mr. Mog Choudhary, Scientist participated in the following:

- AGRI SEARCH 2050 at ICAR NASC, New Delhi during 17-19 May, 2015.
- Winter school on 'Technological Advances in Mariculture for Production Enhancement and Sustainability' on 16<sup>th</sup> January, 2016 and delivered an invited lecture on 'Genome based Technologies in Mariculture'.
- National seminar on 'Fisheries and Aquaculture: Livelihood Security, Sustainability and Conservation' at Agartala, Tripura during 21-22 January 2016.

Mr. Charan Ravi, Scientist attended National Scientific Hindi Seminar on Mariculture, organized by ICAR-CMFRI, Kochi on 9<sup>th</sup> March, 2016.

Shri Bineesh. K.K., Scientist attended the 103<sup>rd</sup> session of Indian Science Congress, Mysore during 3-7 January, 2016 at Mysore University, Karnataka.

Shri Satyavir Chaudhary, Technical Officer attended National Conference of Agricultural Librarians and User Community on 'Integrating ICT in Agricultural Libraries in India: Policies, Issues and Challenges' during 17-19 June, 2015 at ICAR-IVRI, Izatnagar. He also participated in the National workshop on 'Effective management of E-resources in research Libraries' during 12-17 October, 2015 at ICAR-CMFRI, Kochi.

Shri Phoolchand Jaiswar, Senior Technician attended National Training Programme on 'Installation and Operation of FRP Carp Hatchery' during 07-10 July, 2015 at ICAR-CIFA, Bhubaneswar.

Dr. Neeraj Sood, Pr. Scientist; Dr. P.K. Pradhan and Dr. T. Raja Swaminathan, Sr. Scientists attended FAO Re-echo Seminar on Acute Hepatopancreatic Necrosis Disease (AHPND)

and Round-Table Discussion on AHPND National Action Planning held at NBFGR, Lucknow during September 14-16, 2015

Dr. Neeraj Sood, Pr. Scientist and Dr. P.K. Pradhan, Sr. Scientist participated in 'Orientation programmes on BMP's, Bio-security and Disease Management in Aquaculture to Aqua farmers and Field Technicians' at Guntur and Bhimavaram, Andhra Pradesh on August 13 and 14, 2015, respectively. Dr. P.K. Pradhan made presentations on 'Fish Disease Surveillance' in both the programmes.

Dr. Neeraj Sood, Pr. Scientist and Dr. P.K. Pradhan, Sr. Scientist participated in the meeting with Vietnamese Delegation at DADF, Krishi Bhawan on October 9, 2015.

Dr. Neeraj Sood, Pr. Scientist participated in the following:

- 9<sup>th</sup> Meeting of the Technical Committee constituted to oversee and monitor the functioning of the Aquatic Quarantine Facility held at CAA, Chennai on 3<sup>rd</sup> November, 2015
- 5<sup>th</sup> and 6<sup>th</sup> Technical Advisory Committee meetings of National Surveillance Programme on Aquatic Animal Diseases, held at Krishi Bhawan on May 28, 2015 and February 22, 2016, respectively

Dr. P.K. Pradhan, Senior Scientist participated in the following:

- Review meeting of NFDB funded Technology up-gradation projects on September 29-30, 2015 at NFDB, Hyderabad and made presentation regarding progress of research project entitled 'Risk and benefit assessment of an illegally introduced fish species *Piaractus brachypomus* Pacu in India'.
- 6<sup>th</sup> Technical Advisory Committee meetings of National Surveillance Programme on Aquatic Animal Diseases, held at Krishi Bhawan on February 22, 2016.
- 5<sup>th</sup> meeting of Steering Committee at National Level to oversee and monitor the Tilapia seed and grow-out production on January 27, 2016 at DADF, Ministry of Agriculture and Farmers Welfare, Krishi Bhawan, New Delhi and evaluated the proposal of Establishment of Tilapia seed hatcheries at places of Andhra Pradesh by Department of Fisheries, Government of Andhra Pradesh.

## PUBLICATIONS

### International

1. Agarwal S., N.S. Nagpure, P. Srivastava, B. Kushwaha, R. Kumar, M. Pandey and S. Srivastava, 2016. *In silico* genome wide mining of conserved and novel miRNAs in the brain and pineal gland of *Danio rerio* using small RNA sequencing data. *Genomics Data*, 7: 46–53.
2. Awasthi, A., G. Rathore, N. Sood, M. Y. Khan and W. S. Lakra, 2015. Establishment of a leukocyte cell line derived from peritoneal macrophages of fish, *Labeo rohita* (Hamilton, 1822). *Cytotechnology*, 67: 85-96.
3. Baisvar, V. S., R. Kumar, M. Singh, A. K. Singh, U. K. Chauhan, N. S. Nagpure and B. Kushwaha, 2015. ATPase 8/6 gene based genetic diversity assessment of snakehead murrel, *Channa striata* (Perciformes, Channidae). *Russian Journal of Genetics (Genetika)*, 51(10): 1007–1019.
4. Basheer, V.S., N. Vineesh, K.K. Bineesh, R.G. Kumar, C. Mohitha, S. Venu, A. Kathirvelpandian, A. Gopalakrishnan and J.K. Jena, 2016. Mitochondrial signatures for identification of grouper species from Indian waters. *Mitochondrial DNA*, DOI: 10.3109/19401736.2015.1137899.
5. Bineesh, K. K., A. Gopalakrishnan, J. K. Jena, V. S. Basheer, C. Mohitha, N. Vineesh, M. Joselet and N. G. K. Pillai, 2015. Molecular identification of Bigeyes (Perciformes, Priacanthidae) from Indian waters *Mitochondrial DNA*. Online, 1-5.
6. Bineesh, K. K., A. Gopalakrishnan, K. V. Akhilesh, K. A. Sajeela, E. M. Abdussamad, N.G.K. Pillai, V. S. Basheer, J. K. Jena and Robert D. Ward, 2016. DNA barcoding reveals species composition of sharks and rays in the Indian commercial fishery. *Mitochondrial DNA*, DOI: 10.3109/19401736.2015.1137900.
7. Basheer, V. S., C. Mohitha, N. Vineesh, P.R. Divya, A. Gopalakrishnan and J. K. Jena, 2015. Molecular phylogenetics of three species of the genus *Rastrelliger* using mitochondrial DNA markers. *Molecular Biology Reports*, 42: 873-879.
8. Chandra, S., L. K. Tyagi and P. J. Singh, 2016. Traditional Knowledge Associated with Fishing Practices of Ramnagar, Nainital District, Uttarakhand. *World Journal of Fish and Marine Sciences*, 8 (1): 22-29.
9. Das, S. P., A. Bit, S. Patnaik, L. Sahoo, P. K. Meher, P. Jayasankar, T. M. Saha, A. B. Patel, N. Patel, P. Koringa, C. G. Joshi, S. Agarwal, M. Pandey, S. Srivastava, B. Kushwaha, R. Kumar, N. S. Nagpure, M. A. Iquebal, S. Jaiswal, D. Kumar, J. K. Jena and P. Das, 2015. Low-depth shotgun sequencing resolves complete mitochondrial genome sequence of *Labeo rohita*. *Mitochondrial DNA*, 11:1-2.
10. Dey, A., R. Verma, M. Singh, and S. Barat, 2015. Evolutionary and taxonomic relationships in loach (Genus: *Botia*) through molecular characterization in a river of Terai region of West Bengal, India. *European Journal of Biotechnology and Bioscience*, 3 (9): 12-17.
11. Denis, V. L., R. Shukla, M. Singh and Lalramliana, 2015. First report of the genus *Neonoemacheilus* Zhu & Guo (Cobitidae: Nemacheilidae) from rivers of Mizoram, Northeastern India with a note on *N. assamensis* Menon. *Science Vision*, 15: 145-155.
12. Devassy, A., Raj Kumar, P.P. Shajitha, R. John, K.G. Padmakumar, V. S. Basheer, A. Gopalakrishnan, and L. Mathew, 2015. Genetic identification and phylogenetic relationships of Indian clariids based on mitochondrial COI sequences. *Mitochondrial DNA*, 27(5):3777-3780.
13. Dubey, A., M. Goswami, K. Yadav and D. Chaudhary, 2015. Oxidative stress and nano-toxicity induced by TiO<sub>2</sub> and ZnO on WAG cell line. *PLoS ONE*, 10(5): e0127493. doi:10.1371/journal.pone.0127493.
14. Hsuan-Ching Ho, M., R. Kumar and K. K. Bineesh, 2015. *Chaunaxmultilepis* sp. nov., a new species of *Chaunax* (Lophiiformes: Chaunacidae) from southern India. *Zootaxa*, 4103(2): 130-136.
15. Jeena, N. S., A. Gopalakrishnan, E. V. Radhakrishnan, J. K. Kizhakudan, V. S. Basheer, P. K. Asokan, and J.K. Jena, 2015. Molecular phylogeny of commercially important lobster species from Indian coast inferred from mitochondrial and nuclear DNA sequences. *Mitochondrial DNA*, 27(4):2700-2709.
16. Jeena, N. S., A. Gopalakrishnan, J. K. Kizhakudan, E.V. Radhakrishnan, R.

- Kumar and P. K. Asokan, 2016. Population genetic structure of the shovel-nosed lobster *Thenus unimaculatus* (Decapoda, Scyllaridae) in Indian waters based on RAPD and mitochondrial gene sequences. *Hydrobiologia*, 766(1): 225-236.
17. Joy, L., C. Mohitha, P. R. Divya, A. Gopalakrishnan, V. S. Basheer and J. K. Jena, 2015. Weak genetic differentiation in cobia, *Rachycentron canadum* from Indian waters as inferred from mitochondrial DNA ATPase 6 and 8 genes. *Mitochondrial DNA*, 27(4):2819-2821.
18. Kumar, R., B. K. Pandey, U. K. Sarkar, N. S. Nagpure, V. S. Baisvar, P. Agnihotri, A. Awasthi, A. Mishra and N. Kumar, 2016. Population genetic structure and geographic differentiation in butter catfish, *Ompok bimaculatus*, from Indian waters inferred by cytochrome b mitochondrial gene. *Mitochondrial DNA*, [http:// dx.doi.org/10.3109/ 19401736.2015.1137898](http://dx.doi.org/10.3109/19401736.2015.1137898).
19. Kumar, R., T. R. Swaminathan, R. Kumar, A. Dharmaratnam, V. S. Basheer and J. K. Jena, 2015. Mass mortality in ornamental fish, *Cyprinus carpio* koi caused by a bacterial pathogen, *Proteus hauseri*. *Acta Tropica*, 149:128-34.
20. Kumar, R., A. Gopalakrishnan, P. R. Divya, V. S. Basheer, R. K. Singh, V. Mohindra, K. K. Lal and J. K. Jena, 2016. Population genetic structure of *Macrobrachium rosenbergii* (Palaemonidae) from Indian waters using mitochondrial ATPase 6/8 gene, *Mitochondrial DNA Part A*. [http:// dx.doi.org/10.3109/24701394.2016.1149829](http://dx.doi.org/10.3109/24701394.2016.1149829).
21. Kushwaha, B., R. Kumar, S. Agarwal, M. Pandey, N. S. Nagpure, M. Singh, S. Srivastava, C. G. Joshi, P. Das, L. Sahoo, P. Jayasankar, P. K. Meher, T. M. Shah, A. B. Patel, N. Patel, P. Koringa, S. P. Das, S. Patnaik, A. Bit, S. Jaiswal, M. A. Iquebal, D. Kumar and J. K. Jena, 2015. Assembly and variation analyses of *Clarias batrachus* mitogenome retrieved from WGS data and its phylogenetic relationship with other catfishes. *Meta Gene*, 5: 105-114.
22. Lakra, W. S., M. Singh, M. Goswami, A. Gopalakrishnan, K. K. Lal, V. Mohindra, U. K. Sarkar, P. P. Punia, K. V. Singh, J. P. Bhatt and S. Ayyappan, 2015. DNA barcoding Indian freshwater fishes. *Mitochondrial DNA*, DOI: 10.3109/19401736.2015.1101540
23. Lalhlhimpaia, D. V., R. Shukla, M. Singh and Lalramliana, 2015. First report of the genus *Neonoemacheilus* Zhu & Guo (Cobitidae: Nemacheilidae) from rivers of Mizoram, northeastern India with a note on *N. assamensis* Menon. *Science Vision*, 15(3):145-151.
24. Mohindra, V., R. K. Singh, R. Kumar, R. S. Sah and K. K. Lal, 2015. Complete mitochondrial genome sequences of two endangered Indian catfish species, *Clarias batrachus* and *Pangasius pangasius*. *Mitochondrial DNA*, 26(5): 678-679.
25. Mohindra, V., R.K. Tripathi, P. Yadav, R. K. Singh and K.K. Lal, 2015. Hypoxia induced altered expression of heat shock protein genes (Hsc71, Hsp90 $\alpha$  and Hsp10) in Indian Catfish, *Clarias batrachus* (Linnaeus, 1758) under oxidative stress. *Molecular Biology Reports*, 42(7):1197-1209.
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2. Kumar, K. and A. K. Pandey, 2015. Effect of starvation and refeeding on certain biochemical and haematological parameters of the climbing perch, *Anabas testudineus*. *In: Gupta, V. K., A. K. Verma and G. D. Singh (Eds.). Perspectives in Animal Ecology and Reproduction. Vol. 10. Daya Publishing House, New Delhi. pp. 183-193.*
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  4. Chaturvedi, C. S., W. S. Lakra, R. K. Singh and A. K. Pandey, 2015. Successful induced breeding and larval rearing of *Pangasianodon hypophthalmus* under controlled conditions of Raipur (Chhattishgrah). *In: Souvenir International Day for Biological Diversity and National Conference on Biodiversity for Sustainable Development (May 22, 2015). Uttar Pradesh State Biodiversity Board, Lucknow. pp.106-112.*





## 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 369 books. Now, the library has the total collection of 7395 books and 2655 bound volumes of journals. The library has subscribed 17 international journals and 73 Indian journals. In addition to these, 38 journals were received on gratis/exchange basis.

### Library Automation

The Library is operating in fully automated environment. The various activities of library have been computerized using Web Centric LSEase Library Management Software Package, Version 7.0. The records 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 2014-2015 and Newsletters to various

institutions and organizations including Universities, State Fisheries Departments, FFDA's, Krishi Vigyan Kendras, Entrepreneurs and Fish Farmers.

### Agricultural Knowledge Management Unit

Agriculture knowledge management unit (AKMU) at ICAR-NBFGR, Lucknow is mandated to share and distribute the information and knowledge generated via various research activities by applying multimedia, information and communication technologies. The unit sensitizes and promotes the adoption of information technology and tools in the fisheries research. The operational facility of the High Performance Super Mini Computing (HPC) has been scaled up and it can be used by other researchers through a portal that can be accessed at URL: <http://mail.nbfgr.res.in/FishCABin/>. Besides, AKMU is involved in uploading and managing the data on the different web sources like PERMISnet (Personnel Management Information System), PIMS (Project Information Management System), KRISHI-Knowledge based Resources Information Systems Hub for Innovations in agriculture and Central Procurement Portal (CPPP). This year two computer servers procured under the project National surveillance programme for aquatic animal diseases was configured for operational use. The unit also engaged in providing support to employee in ICAR-ERP system and arranges training on different disciplines of ICT. The unit provided support in installation and configuration of the Biometric systems at the institute. The unit manages and maintains the web, mail, DNS, database, antivirus, internet servers along with the Unified Threat Management firewall system on regular basis. GIS facility included in the unit increases the multifaceted work structure and provides ability in managing existing GIS resources and creating and publishing GIS resources of fish and fisheries. In addition, the unit includes the Center for Fish Bioinformatics and provides facility for In silico works in fish omics. Beside facilitating the resources and activities, AKMU plays pivotal role in developing the display, training and bulletin materials of the institute.

## STAFF ACTIVITIES

### 1. Promotions

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

The following staff of the Institute were promoted

- Shri Shree Ram from Senior Technician to Technical Assistant w.e.f. 06.11.2014
- Shri A. K. Yadav from Assistant Chief Technical Officer to Chief Technical Officer w.e.f. 01.01.2015.
- Shri Ravi Kumar from Technical Officer to Senior Technical Officer w.e.f. 04.04.2015.
- Shri B.N. Pathak from Senior Technical Assistant to Technical Officer w.e.f. 12.04.2015.
- Shri Om Prakash from Technical Assistant to Senior Technical Assistant w.e.f. 01.05.2015.
- Shri Amit Singh Bisht from Technical Officer to Senior Technical Officer w.e.f. 26.06.2015.
- Dr. Vikas Sahu from Technical Assistant to Senior Technical Assistant w.e.f. 09.07.2015.
- Shri P.C. Jaiswar from Sr. Technician to Technical Assistant w.e.f. 11.07.2015.
- Shri Ram Bharose from Sr. Technician to Technical Assistant w.e.f. 11.07.2015.

The NBFGR family congratulates them for their achievements.

### 2. New Joining

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

#### Scientists:

- Mr. Bineesh K.K., Scientist (On probation) w.e.f. 04.04.2015.
- Mr. Chandra Bhushan Kumar, Scientist (On probation) w.e.f. 09.04.2015.
- Ms. Teena Jayakumar T.K. Scientist (On probation) w.e.f. 12.10.2015.
- Dr. S.K. Srivastava, Sr. Scientist on transfer from ICAR-DCFR, Bhimtal w.e.f. 16.01.2016.

#### Technicals:

- Shri Prem Chandra, Senior Technical Officer on transfer from ICAR-IVRI, Izatnagar w.e.f. 05.02.2016.

The ICAR - NBFGR family welcomes the new members.

### 3. Transfer/Deputation/Relieving

#### Scientists:

- Dr. Mukunda Goswami, Sr. Scientist was relieved on 16.04.2015 to enable him to join at ICAR-CIFE, Mumbai on transfer.
- Dr. Peyush Punia, Principal Scientist was relieved on 28.04.2015 to enable him to join at ICAR-IIFSR, Modipuram on transfer.
- Dr. N.S. Nagpure, Principal Scientist was relieved on 05.12.2015 to enable him to join at ICAR-CIFE, Mumbai on transfer.
- Dr. S. Raizada, Principal Scientist was relieved on 31.12.2015 to enable him to join as ADG (Inland Fishereis), ICAR, New Delhi.
- Dr. J.K. Jena, Director was relieved on 09.01.2016 to enable him to join as DDG (Fisheries Science), ICAR, New Delhi.

The ICAR-NBFGR family expresses heartfelt thanks for contributions of above colleagues to the growth and development of the Institute.

#### Technicals:

- Dr. E. Suresh, Technical Assistant was relieved on 28.04.2015 to enable him to join as Assistant Professor, Aquaculture at Tamil Nadu Fisheries University, Nagapattinam.

#### Skilled Support Staff:

- Mrs. Sabita Devi, Skilled Support Staff was relieved on 29.05.2015 to enable her to join at ICAR-CIFE, Mumbai on transfer.

#### Retirement

- Dr. S.P. Singh, Principal Scientist retired on superannuation from ICAR services on 31<sup>st</sup> July, 2015.
- Shri Jogendra Singh, Assistant retired on superannuation from ICAR services on 31<sup>st</sup> December, 2015.

The ICAR-NBFGR family expresses gratitude for their services to the Institute and wishes a happy retired life ahead.

#### Other activities:

#### 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 NBFGR staff undertook several activities both outside and inside of the NBFGR campus as well as its Center at Kochi and Unit at Chinhat.





**Staff of NBFGR headquarters & its center participating in Swachh Bharat Abhiyan**

**ICAR-NBFGR donated for victims of earthquake in Nepal**

In a devastating earthquake that occurred on 25<sup>th</sup> April, 2015, our neighboring nation 'Nepal' suffered massive loss of lives and property and the people there are still struggling for their survival. The ICAR-National Bureau of Fish Genetic Resource, Lucknow expressed profound sympathy for all the those who have

lost their near and dear ones in this devastating event. In order to convey the solidarity to the earthquake victims of Nepal, the staff members of ICAR-NBFGR, Lucknow donated one-day salary amounting to Rs. 2,21,000/- to Prime Minister's National Relief Fund vide Demand Draft No. 602161 dated 08.06.2015.

**Vigilance Awareness Week**

Vigilance Awareness Week was celebrated during 26-31 October, 2015. All the staff members took pledge on this occasion.



**Vigilance awareness programme**

**Sadbhavana Divas**

Sadbhavana Divas was celebrated during 20<sup>th</sup> August, 2015. All the staff members took pledge on this occasion.

**Participation in Sports Activities**

The Institute participated in Sports Tournament at ICAR-Indian Institute of Soil and Water Conservation, Dehradun during 18-21 April, 2015.

**Children's Day**

A colourful programme was organised on 14<sup>th</sup> November, 2015 to celebrate Children's Day. The programme included various activities for children, viz. quiz competition, drawing competition, singing, dancing, fancy dress competition etc.



**Children's Day celebration**





## Women's Day

International Women's Day was celebrated on 8<sup>th</sup> March, 2016 at the Institute in which a number of activities were organised in the Institute campus.



## Women's Day Activities

### Institute Management Committee

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

1. Director : Chairman  
ICAR-NBFGR, Lucknow
2. Asstt. Director General : Member (ICAR)  
(Marine Fy.), ICAR,  
New Delhi
3. Dr. A. Gopalakrishnan, : Member  
Director, ICAR-CMFRI,  
Kochi
4. Dr. R.S. Kataria, : Member  
Principal Scientist,  
ICAR-NBAGR, Karnal
5. Dr. R.K. Tyagi, : Member  
Principal Scientist,  
ICAR-NBPGR,  
New Delhi
6. Dr. (Mrs.) Sherly : Member  
Tomy, Senior  
Scientist, ICAR-CIBA  
Chennai

7. Director, Fisheries, : Member  
Govt. of U.P.
8. Director, Fisheries : Member  
(Commissioner)  
Govt. of Maharashtra  
Mumbai
9. Director Research : Member  
N.D. University of  
Agri. & Technology,  
Faizabad, U.P.
10. AF & AO : Member  
ICAR-NBFGR,  
Lucknow, U.P.
11. Shri Babu Ram Nishad : Member  
Lucknow, U.P.
12. Shri Manoj Kashyap : Member  
Shahjahanpur, U.P.
13. Administrative Officer : Member-  
ICAR-NBFGR, Secretary  
Lucknow, U.P.



### Institute Management Committee Meeting

The 29<sup>th</sup> meeting of the Committee was held on 6<sup>th</sup> April, 2015 and 30<sup>th</sup> meeting of the Committee was held on 16<sup>th</sup> November, 2015.

## LIST OF PERSONNEL

(As on 31.03.2016)

### Research Management

**Dr. (Mrs.) Rehana Abidi** - Director (Acting)

### Scientific Staff

1. Dr. Ravindra Kumar - Head, Molecular Biology and Biotechnology Division
2. Dr. (Mrs.) Rehana Abidi - Principal Scientist
3. Dr. A. K. Pandey - Principal Scientist and Scientist In-Charge, Fish Conservation Division
4. Dr. (Mrs.) Vindhya Mohindra - Principal Scientist
5. Dr. K.K. Lal - Principal Scientist (On deputation)
6. Dr. Basdeo Kushwaha - Principal Scientist
7. Dr. Neeraj Sood - Principal Scientist
8. Dr. V.S. Basheer - Principal Scientist & Scientist In-Charge (PMFGR Centre, Kochi)
9. Dr. Pravata Kumar Pradhan - Senior Scientist
10. Dr. Sharad Kumar Singh - Senior Scientist
11. Dr. Lalit Kumar Tyagi - Senior Scientist
12. Dr. Rajeev Kumar Singh - Senior Scientist
13. Dr. Mahender Singh - Senior Scientist
14. Dr. T. Raja Swaminathan - Senior Scientist (PMFGR Centre, Kochi)
15. Dr. T.T. Ajith Kumar - Senior Scientist
16. Dr. Sullip Kumar Majhi - Senior Scientist
17. Dr. S.K. Srivastava - Senior Scientist
18. Dr. (Mrs.) Divya P.R. - Senior Scientist (PMFGR Centre, Kochi)
19. Mrs. Poonam Jayant Singh - Scientist
20. Shri Ajey Kumar Pathak - Scientist
21. Dr. A. Kathirvelpandian - Scientist (PMFGR Centre, Kochi)
22. Ms. Sangeeta Mandal - Scientist
23. Ms. Rejani Chandran - Scientist
24. Dr. Santosh Kumar - Scientist
25. Shri Aditya Kumar - Scientist
26. Shri Charan R. - Scientist (PMFGR Centre, Kochi)
27. Shri Labrechai Mog Chowdhury - Scientist (PMFGR Centre, Kochi)

28. Shri Murali S. - Scientist
29. Shri Trivesh Suresh Mayekar - Scientist
30. Shri Bineesh K.K. - Scientist
31. Shri Chandra Bhushan Kumar - Scientist
32. Ms. Teena Jayakumar T.K. - Scientist

### **Technical Staff**

1. Dr. Rajesh Dayal - Chief Technical Officer
2. Dr. S. M. Srivastava - Chief Technical Officer
3. Shri A. K. Yadav - 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. Dr. 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 Ravi Kumar - Senior Technical Officer
16. Shri Amit Singh Bisht - Senior Technical Officer
17. Shri Prem Chandra - Senior Technical Officer
18. Shri Satyavir Chaudhary - Technical Officer
19. Shri Mohd. Gayas - Technical Officer
20. Shri S. K. Singh - Technical Officer
21. Shri R.K. Shukla - Technical Officer
22. Shri B. N. Pathak - Technical Officer
23. Shri S. K. Upadhyay - Senior Technical Assistant
24. Shri Samarjit Singh - Senior Technical Assistant
25. Shri Om Prakash - Senior Technical Assistant
26. Shri Rajesh Kumar - Senior Technical Assistant
27. Shri Om Prakash-II - Senior Technical Assistant
28. Dr. Vikash Sahu - Senior Technical Assistant





29. Shri B. K. Rao - Technical Assistant
30. Shri Madan Lal - Technical Assistant
31. Shri Raj Bahadur - Technical Assistant
32. Shri Gulab Chandra - Technical Assistant
33. Shri K. K. Singh - Technical Assistant
34. Shri Rajool Shanis C.P. - Technical Assistant
35. Shri Sree Ram - Technical Assistant
36. Shri P.C. Jaiswar - Technical Assistant
37. Shri Ram Bharose - Technical Assistant

#### **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. Smt. Kaneez Fatima - Assistant
8. Shri Swapan Debnath - Assistant
9. Shri S. N. Srivastava - Assistant
10. Shri P. K. Awasthi - Assistant
11. Smt. Sunita Kumari - Assistant
12. Shri Sajivan Lal - Senior Clerk
13. Shri Shreelal Prasad - Senior Clerk
14. Shri Vinay Kumar Srivastava - Senior Clerk
15. Shri Sandeep - Jr. Stenographer
16. Shri Santosh Kumar Singh - Jr. Clerk
17. Shri Ram Baran - Jr. Clerk
18. Shri P.C. Verma - Jr. Clerk
19. 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. Shri Ram Lakhan - Skilled Support Staff
12. Shri Sunit Kumar - Skilled Support Staff
13. Shri Jai Narain Tiwari - Skilled Support Staff
14. Shri Anwar - Skilled Support Staff
15. Shri Sanjay Kumar - Skilled Support Staff
16. Smt. Seema Devi - Skilled Support Staff
17. Shri Ashok Kumar - Skilled Support Staff
18. 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 brackish water 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 - 682018, Kerala.  
Telefax : 0484-2395570  
E-mail : [nbfgrcochin@gmail.com](mailto:nbfgrcochin@gmail.com)  
[nbfgr\\_kochi@nbfgr.res.in](mailto:nbfgr_kochi@nbfgr.res.in)



## APPENDIX-II

### **Aquaculture Research & Training Unit, Chinhat**

An Aquaculture Research & Training Unit (ARTU) 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-NBFG Aquaculture Research & Training Unit  
Malhore Road, Chinhat  
Lucknow-227 105, U.P.  
Telefax : 0522-2815848  
E-mail :director@nbfg.res.in

**Category wise trainings planned and implemented during 2015 - 16**
**A. Physical targets and achievements**

S. No.	Category	Total No. of Employees	No. of trainings planned for 2015-16 as per ATP	No. of employees undergone training during			% realization of trainings planned during 2015-16
				April-September 2015	Oct. 2015 - March 2016	April 2015 - March 2016	
1	2	3	4	5	6	5+6=7	7/4x100=8
1.	Scientist	31	21	5	8	13	61.9
2.	Technical	36	8	2	6	8	100
3.	Administrative & Finance	20	4	2	0	2	50
4.	SSS	19	4	0	0	0	0
	<b>Total</b>	<b>106</b>	<b>37</b>	<b>9</b>	<b>14</b>	<b>23</b>	<b>62.16 (Avg)</b>

**B. Financial targets and achievements (All employees)**

S.No.	RE 2015-16 for HRD			Actual Expenditure 2015-16 for HRD (Lakh Rs.)	%Utilization 2015-16
	Plan	Non plan (Lakh Rs.)	Total		
	1	4.15	0	4.15	4.15

**C. Number of trainings organised for various categories of ICAR employees including winter/ summer schools and short term trainings**

S. No.	Category	No. of trainings organised April 2015 -March 2016
<b>1</b>	<b>2</b>	<b>3</b>
1.	Scientist	3
2.	Technical	0
3.	Administrative & Finance	0
4.	SSS	0
	<b>Total</b>	<b>3</b>









## ICAR-National Bureau of Fish Genetic Resources

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Email: [director.nbfg@icar.gov.in](mailto:director.nbfg@icar.gov.in); [director@nbfg.res.in](mailto:director@nbfg.res.in)

Website: [www.nbfg.res.in](http://www.nbfg.res.in)

