Annual Report
2013–14

National Bureau of Fish Genetic Resources
(Indian Council of Agricultural Research)
Lucknow
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Sustainable management of aquatic genetic resources for food and nutritional security of present and future generations is a long-term need. The aquatic genetic resources have value in terms of economic, ecological and social uses. Therefore, they need to be explored, characterized, managed and utilized judiciously on sustainable basis in the country. With this vision the NBFGR has taken up its research programmes for greater understanding of aquatic genetic resources of the country with special focus on conservation strategies for prioritized and endangered species.

During the reporting year (2013-2014), the Institute has strengthened its activities to foster the fish germplasm exploration, characterization and conservation. Two new fish species viz., *Plectranthias alcocki* and *Pempheris sarayu* were discovered from Arabian sea. A microsatellite enriched genomic library was constructed for *Silonia silondia*. A total of 65 marine finfish species and 27 finfish species of the north-eastern India were DNA barcoded. Complete taxonomic description of two new species (*Labeo icarae* and *Rita sp. nov.*) was accomplished. Progress in bioprospecting of genes and allele mining for abiotic stress tolerance, phylogenetic relationship of the fish species, development of baseline data on genetic variation in wild populations of selected commercially important fish species and several others have been quite significant. The current year saw the launch of a National Surveillance Programme on Aquatic Animal Diseases funded by National Fisheries Development Board which has been initiated in 14 states of aquaculture importance with involvement of 22 organisations, and is being coordinated by NBFGR, Lucknow. Inauguration of the advanced mini supercomputing hub for aquatic animals (ASHAA), by Dr. S. Ayyappan, Secretary, DARE and Director General, ICAR, New Delhi was another feather in the cap of NBFGR. The Institute organized a number of important workshops and training programmes of national and regional significance. I am confident that our hard work and commitment to research programmes will continue to provide research outputs for making effective strategies for sustainable use of fish genetic resources. Compliments go to all the members of NBFGR family for their commitment and dedication towards achievements of the Bureau.

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

National Bureau of Fish Genetic Resources (NBFGR) has grown into a premier institution for research on various issues related to fish germplasm resources of the country. Besides developing state-of-art facilities and expertise in several research areas including, development of fish databases, genetic characterization, genomics and proteomics, fish germplasm and habitat inventory, risks analysis of exotic species, diagnostics for OIE notified pathogens, aquatic microbes and other areas of germplasm conservation with special focus on threatened, prioritized and exotic fish species; the Institute is also catering to the needs of developing human resources in these areas by undertaking various specialized training programmes for different target groups. During the year under report, the research activities were conducted through 10 Institutional and 22 externally-funded research projects. Major achievements and activities of the Institute during the year 2013-14 are summarized below:

The existing database on finfish diversity of India was updated by adding information about 241 additional fish species (not included earlier) belonging to 149 genera under 73 families. The database now contains information on 2662 native finfishes reported from Indian waters belonging to 1019 genera under 246 families and 42 orders; and 291 exotic fishes.

Four genomic resource databases viz., Fish Microsatellite database (FishMicrosat), Fish EST database (FEST), Fish Ribosomal RNA database (FishRibo) and Fish Barcode Information System (FBIS) were updated with new records and these databases presently contains 4398, 4000, 1080 and 3424 records, respectively.

Fish Mitogenome (FMiR) was developed as a web-based delivery model of curated mitochondrial sequence database of fishes reported from Indian subcontinent. The database presently covers mitogenome sequences of 239 species belonging to 93 families.

Programme to develop baseline data on genetic variation in wild population of three Indian major carps *Labeo rohita*, *Catla catla*, *Cirrhinus mrigala* and catfish, *Clarias magur*, was continued. Microsatellite data of *L. rohita* (n=925), *C. catla* (n=495), *C. mrigala* (n=949) and *C. magur* (n=807) samples was genotyped for 21, 11, 16 and 18 polymorphic loci, respectively, and analyzed. The genetic differentiation was higher in *C. magur*. Indian major carps exhibited moderate level of differentiation. Total 547 samples of *Macrobrachium rosenbergii* were also genotypes for 20 polymorphic loci, which exhibited moderate level of differentiation. Total 2867 sequences for two mitochondrial genes Cytochrome b (307bp) and ATPase 6/8 (842bp) were analyzed in three Indian major carps and *C. magur*. The results indicate good genetic diversity with number of haplotypes ranged from 44 to 53.

A microsatellite enriched genomic library was constructed for *Silonía silonía* in which out of screening of 356 clones, 61 microsatellites were identified. In continuation to the work done during previous year in the great snakehead, *Channa marulius*, the microsatellite enriched genomic library was screened for tri, tetra, penta and hexa repeat containing regions of the genome using affinity capture approach.

A new collaborative programme to develop knowledge base on genome-wide variation and population structure of *Tenualosa ilisha* was undertaken to support breeding programmes for aquaculture and its natural stock management. Altogether, 23 locations were surveyed and a total of 613 samples of *T. ilisha* were collected from nineteen locations (13 from east coast and six from west coast), comprising 2 marine, 1 estuary and 3 riverine locations from west coast, and 5 marine, 2 estuary and 7 riverine locations from East coast. The study focused on development of species-specific *de novo* microsatellite (SSR) markers for genetic variation studies and genetic variability analysis using mitochondrial cytochrome b marker.

A total of 65 marine finfish species were DNA barcoded using mitochondrial COI gene.

Phylogenetic relationship of the fish species belonging to the family Rhinobatidae and family Sphyraenidae was studied.

Phylogenetic analysis was carried out in three mackerel species of Indian waters viz., *Rastrelliger kanagurta*, *R. faugni* and *R. brachysoma*.

Two new fish species viz., *Plectranthias alcoki* and *Pempheris sarayu* were discovered from Arabian sea.
Genetic stock structure analyses of silver pomfret along the Indian waters were done using genotyped data of twelve loci in populations from five locations (Veraval, Cochin, Chennai, Visakhapatnam and Kolkata).

Studies continued for bioprospecting of genes and allele mining for abiotic stress tolerance in *Clarias batrachus*. The results indicated that in brain, heart, liver, muscle, spleen and head kidney, respectively, 28S rRNA/TUB, 28S rRNA/TUB, RPL30/28S rRNA, RPL30/TUB, ELF-1A/28S rRNA and ELF-1A/TUB gene pairs were highly stable and were suitable as reference genes to study oxidative stress, while *ACTB* and *B2M* were the least stable genes in examined tissues under normoxic and/or hypoxic conditions. The observations suggested the consideration of tissue types and use of at least two reference genes, instead of one in accurate normalization of quantitative PCR data. Molecular characterization and transcriptome profile of heat shock protein genes under hypoxia in *C. batrachus* was also carried out.

Molecular cytogenetic studies were undertaken in a number of fish species including *Channa punctatus*, *Clarias batrachus*, *Cyprinus carpio*, *Glossogobius giuris*, *Heteropneustes fossilis*, *Barilius ngawa*, *Neolissochilus stracheyi* and *Garra* sp.

Under the programme on genetic characterization and DNA barcoding of fishes from north east India, 501 tissue samples along with voucher specimens were collected from 95 fish species belonging to various rivers of the Brahmaputra, Chindwin, Kaladan and Barak-Meghna-Surma drainages of north-eastern India and 103 DNA barcodes were prepared in 27 species.

A new collaborative programme on whole genome sequencing of two important fish species namely *L. rohita* and *C. batrachus* was initiated in collaboration with CIFA, Bhubaneswar; Anand Agricultural University, Anand and IASRI, New Delhi.

The study on identification and evaluation of reproductive traits and genetic structure of *Ompok bimaculatus* provided baseline data on reproductive patterns of *O. bimaculatus* from 24 rivers of India. It also provided new insights into the inter-population reproductive strategies and population structure which may be helpful for development of responsible management of the wild population.

The National Repository of Fish Cell line (NRFC) established at NBFRG, Lucknow for collection, deposition and distribution of cell lines to the research community, is currently maintaining 46 cell lines of 22 fish species developed and deposited by various research organisations across the country including NBFRG. All the cell lines submitted to NBFRG have been characterized with both cytogenetic and molecular markers and successfully cryopreserved at NRFC.

A new programme on exploration of the Western Ghats wetlands for indigenous fishes and extent of invasion of exotic fishes was undertaken in which exploratory surveys were conducted in two major river systems in Goa - Mandovi and Zuari, which yielded 40 species of freshwater fish belonging to 31 genera of 14 families, and 7 species of crustaceans. Notable observation were the collection of *Pangio goensis* from the type locality for the first time since it was described in 1972 by Tilak, collection of putative specimens of *Ompok goae*, and possible new species of *Amblyceps*, *Orechthys*, *Pethia*, *Danio* and *Schistura*.

Explorations were continued in the upper basin of River Mahanadi in the Chhattisgarh state, including its tributaries joining in this stretch. Nine sites of River Mahanadi and 10 sites of its 6 tributaries and sub-tributaries viz., Sheonath, Pairi, Sondur, Maniyari, Arpa and Lilagar, were explored for documentation of fish diversity in three seasonal explorations undertaken during pre-monsoon, post-monsoon and winter seasons. A total of 63 fish species belonging to 20 families and 8 orders were recorded from River Mahanadi and its tributaries.

Exploratory surveys also continued in various rivers/other waterbodies of three ecologically fragile districts Chamba (H.P.), Udaipur (Rajasthan) and Adilabad (A.P.). These explorations confirmed two species, which are new to science (*Labeo icarae* and *Rita sp. nov.*); putative possibility of finding three more new species (*Silonia sp. nov.*, *Glossogobius sp. nov.* and *Garra sp. nov.*), and new extended distribution of four species (*Tor tor*, *Garra orientalis*, *Labeo dero* and *L. dyocheilus*) in the rivers beyond the range known until now. The potential indigenous fish species were identified in the three districts, for
technological interventions, integration in production system with an aim to conserve as well as utilize these resources. Length weight relationship and condition factor of 57 fish species were also analyzed.

A National Surveillance Programme on Aquatic Animal Diseases (NSPAAD) funded by National Fisheries Development Board has been initiated in 14 states of aquaculture importance with involvement of 22 organisations including fisheries research institutes of ICAR, Colleges of Fisheries and other colleges, which is being coordinated by NBFRG, Lucknow. More than 100 districts have been identified to be covered for active surveillance and the remaining districts under passive surveillance.


A series of short-term training programmes on ‘Aquaculture in Wetlands’ were organized in which a total of 371 progressive fish farmers were trained. The Institute also participated in several exhibitions/aqua-fairs in different parts of the country.
INTRODUCTION

Brief History

Genetic resources has gained tremendous attention globally both from the policy makers and researchers during last over two decades. India is fortunate to possess vast and varied fish genetic resources in different aquatic ecosystems viz., freshwater, brackishwater and marine. However, our rich fish fauna is facing serious threats due to several anthropogenic and natural environmental changes. In view of this, the conservation of fish germplasm resources has assumed tremendous significance in the management perspective of our fishery resources. It is realized that scientific basis is necessary to document, understand and preserve the genetic resources which can be utilized for nutritional and environmental security of the mankind.

In the above perspective, 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, wet laboratories, public aquarium, guest house, staff quarters and above all, required experimental tanks and ponds have been created satisfying the need of research and other amenities. The 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 fascinating journey of NBFGR has seen it growing not only in terms of creation of infrastructure, but also expansion of research programmes by including important areas viz., whole genome sequencing, population genetics, functional genomics, molecular disease diagnostics, national surveillance programme for aquatic diseases, exploration of newer geographical areas and unexplored aquatic resources for assessment of fish diversity, etc., to name a few.

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.
Staff Position

The overall staff position as on 31st March, 2014 is given below:

<table>
<thead>
<tr>
<th>S. N.</th>
<th>Category of posts</th>
<th>Post created</th>
<th>Staff in position</th>
<th>Post vacant (out of created posts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Research Management (Director)</td>
<td>01</td>
<td>01</td>
<td>--</td>
</tr>
<tr>
<td>2.</td>
<td>Scientific</td>
<td>41</td>
<td>27</td>
<td>14</td>
</tr>
<tr>
<td>3.</td>
<td>Technical</td>
<td>38</td>
<td>38</td>
<td>--</td>
</tr>
<tr>
<td>4.</td>
<td>Administrative</td>
<td>21</td>
<td>19</td>
<td>02</td>
</tr>
<tr>
<td>5.</td>
<td>Supporting</td>
<td>20</td>
<td>19</td>
<td>01</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>121</td>
<td>104</td>
<td>17</td>
</tr>
</tbody>
</table>

Financial Statement

Allocation of funds and expenditure incurred during the year 2013-2014.

(Rs. in lakhs)

<table>
<thead>
<tr>
<th></th>
<th>Budget Allocation</th>
<th>Expenditure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plan</td>
<td>607.00</td>
<td>606.78</td>
</tr>
<tr>
<td>Non Plan</td>
<td>1026.62</td>
<td>1026.39</td>
</tr>
<tr>
<td>Total</td>
<td>1633.62</td>
<td>1633.17</td>
</tr>
<tr>
<td>Revenue generation</td>
<td>Target</td>
<td>Achieved</td>
</tr>
<tr>
<td></td>
<td>26.10</td>
<td>27.96</td>
</tr>
</tbody>
</table>
RESEARCH ACHIEVEMENTS

4.1 Cataloguing of Fish Genetic Resources of India

Project Title: Information base on Fish Genetic Resources of India

Project Period: April, 2012 - March, 2015


Funding Agency: Institutional

The collection and cataloguing of information on fish genetic resources of India is an important mandate of NBFGR. During the period under report, the Bureau continued its efforts towards collection and cataloguing of the fish genetic resources of India. Besides updating information on existing species, the existing database on finfish diversity of India was updated by adding information about 241 additional fish species belonging to 149 genera under 73 families which were not included earlier. A total of 981 fish species were screened for taxonomic and valid scientific names of which, 139 fishes were revised. Germplasm explorations were carried out in the selected water bodies of Chambal National Park, Uttar Pradesh and 45 fish species belonging to 32 genera under 14 families were recorded and their information added to the database. The updated database contains information on 2662 native finfishes reported from Indian waters belonging to 1019 genera under 246 families and 42 orders; and 291 exotic fishes. The habitat-wise distribution of fishes is as follows:

<table>
<thead>
<tr>
<th>Category of Fishes</th>
<th>Ecosystem</th>
<th>No. of Fish Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native fishes</td>
<td>Freshwater</td>
<td>877</td>
</tr>
<tr>
<td></td>
<td>Brackishwater</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>Marine water</td>
<td>1672</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>2662</td>
</tr>
<tr>
<td>Exotic fishes</td>
<td></td>
<td>291</td>
</tr>
<tr>
<td>Grand Total</td>
<td></td>
<td>2953</td>
</tr>
</tbody>
</table>

A web application of the master database with more appropriate design and coding of fishes was developed for facilitating the retrieval, search, editing and other options for selected species. This information system contains data on 2953 fishes occurring in India. A checklist of 462 species of prawns/shrimps belonging to 135 genus under 30 families was completed. A list of 3000 species of Molluscs belonging to 792 genus under 219 families reported from India was tentatively completed.

The web-based computer-aided automated identification system using shape categories and images was updated by including more shape categories and associated images. Further the identification database covering shape information and images was updated by adding information on additional 12 families. The web application of the database includes options for administration and management thereby enabling the database administrator to create, manage users and granting rights and privileges for updating the content of the database. The validation and testing of the identification system is continuing with the addition of information into the database (Figures 1 & 2).
Project Title: Establishment of National Agricultural Bioinformatics Grid in ICAR

Project Period: April, 2010 - March, 2014


Funding Agency: NAIP, ICAR

Updating of genomic resource databases

Four genomic resource databases viz., Fish Microsatellite database (FishMicrosat), Fish EST database (FEST), Fish Ribosomal RNA database (Fish Ribo) and Fish Barcode Information System (FBIS) were updated with new records and these databases presently contains 4398, 4000, 1080 and 3424 records, respectively (Fig. 3). The design and implementation technology of Fish Karyome database was improved and the database presently hosts karyological details of 161 fish species.

Development of FMiR database

Fish Mitogenome (FMiR) was developed as a web-based delivery model of curated mitochondrial sequence database of fishes reported from Indian subcontinent. The database presently covers mitogenome sequences of 239 species belonging to 93 families downloaded from RefSeq/NCBI (Fig. 4). FMiR has capability for finding SSR motifs, repeats orientation, and primer designing. The blast search algorithm has been implemented in the model for complete and individual components of genome for similarity searching and deducing variation across species. FMiR provides services for annotation and re-annotation of mitogenome sequences of fishes and explores the relative abundance of microsatellite repeats in the mitogenomes. Thus, FMiR can help the researchers immensely in cutting edge areas viz., population genetic studies, speciation, evolutionary studies, genetic diversity among the species and genetic improvement programs of important aquaculture species.

Screening of suitable compounds for inhibition of PmRab7 and VP28 complex formation in white spot disease

The formation of complex between shrimp receptor protein PmRab7 and viral envelope protein VP28 can be pivotal in initiation of White Spot Disease in tiger shrimp Penaeus monodon. Validation of amino acid residues of PmRab7 and VP28 sandwiched within the PmRab7-VP28 complex is mandatory step to predict a suitable inhibitor that could disrupt the PmRab7-VP28 complex formation. This validation was performed by docking PmRab7 and VP28 using ClusPro software. The results indicated that Leu73, Arg79 of PmRab7 and Arg53 of VP28 contributed in the formation of PmRab7-VP28 complex. Hence, targeting the bonds formed by these amino acids can disrupt PmRab7-VP28 complex which may, in turn, block possibility of entry of virus into the shrimp. Hence, a virtual screening of eight inhibitors namely, sodium hypochlorite, povidone iodine, benzalkonium chloride, sodium alginate, stigmaster, lupeol, bis(2-methylheptyl) phthalate and betulin was performed to screen the inhibitor that depicted more affinity of binding with PmRab7 and VP28 individually. The
AutoDock energy evaluation, Hex Etotal score and PatchDock complementarity analysis revealed that bis (2- methylheptyl) phthalate depicted optimum free binding energy and shape complementarity score both with PmRab7 and VP28. Hence, bis (2- methylheptyl) phthalate can be used as one of the suitable inhibitor that may inhibit complex formation between PmRab7 and VP28.

Mining SSRs in mitogenome of fishes and their analysis

Mitogenome sequences of 85 fish species belonging to Indian subcontinent were downloaded from NCBI (mostly Ref_Seq entries) and in silico approaches were applied for finding SSRs, their location, length and distribution. A total of 92 microsatellites in different nucleotide combinations were detected in 59 species. Twenty six interspersed SSRs, mostly poly (AT)n were found in the D-loop regions of Cyprinid species and 56 interspersed SSRs of 12 bp length were observed only in eight genes. Further, identical repeat motifs were found on the same location in ATP6 and ND4 genes and these were found biased towards particular habitat. The comparison of ATP6 and ND4 gene sets with other homologous sequences showed point mutations. The study explored the discovery and utility of mitochondrial SSR markers for species and population identification.

4.2 GENETIC AND BIOLOGICAL CHARACTERIZATION

Project Title: Outreach activity on Fish Genetic Stocks

Project Period: April, 2008 – March, 2014


Funding Agency: Institutional

The programme is intended to develop baseline data on genetic variation in wild population of three Indian major carps (IMCs) *Labeo rohita*, *Catla catla*, *Cirrhinus mrigala* and catfish, *Clarias magur*. The baseline data is aimed to identify genetic stocks, phylogeographic signals and population bottlenecks in the wild populations of these cultivable fish species. The research was based on molecular tools, polymorphic mitochondrial DNA sequences of partial Cytochrome b and ATPase 8 & 6 genes; polymorphic microsatellite markers and also description of morphological descriptors using truss network analysis.

Microsatellite data of *L. rohita* (n=925), *C. calla* (n=495), *C. mrigala* (n=949) and *C. magur* (n=807) samples was genotyped for 21, 11, 16 and 18 polymorphic loci, respectively, and analyzed. The
genetic differentiation was higher in C. magur. IMC’s exhibited moderate level of differentiation. Total 547 samples of Macrobrachium rosenbergii were also genotyped for 20 polymorphic loci, which exhibited moderate level of differentiation. Total 2867 sequences for two mitochondrial genes viz., cytochrome b (307bp) and ATPase 6/8 (842bp) were analyzed in three Indian major carps and C. magur. The results indicate genetic diversity with number of haplotypes ranged from 44 to 53. The haplotype network using cytochrome b and ATPase 6/8 genes indicating relatedness of different haplotypes, both shared and exclusive haplotypes is given in Fig. 5. Overall, genetic differentiation based on the two mitochondrial genes was concordant. Coefficient of genetic differentiation $F_{st}$ was computed in L. rohita (0.126), C. magur (0.681) and C. mrigala (0.078) also indicated low to moderate differentiation. In M. rosenbergii, analysis of 93 samples with ATPase 8/6 gene revealed 35 haplotypes. In C. magur populations, within population variation was less than that observed among populations, however, in case of IMCs, variation among the population was accounted by a smaller fraction than the within population variation. High genetic differentiation was seen in this species. Total 1184 truss morphometry images of L. rohita (528), C. catla (275) and C. mrigala (381) were analysed. The variation in relative fecundity in three IMCs from different rivers of Ganga river system is given in Fig. 6.

As a part of new technical activity, microsatellite enriched genomic library was constructed for Silonia silondia. Out of screening of 356 clones, 61 microsatellites were identified. The microsatellite identified, are in the process of analysis for designing of primers and their testing for polymorphism.

**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 to October, 2016

**Project Personnel from NBFRG:** Vindhya Mohindra (CCPI), Kuldeep K. Lal, Rajeev K. Singh, Saneee Mandal, and J. K. Jena

**Funding Agency:** NFBSFARA, ICAR

*Tenualosa ilisha* (Hamilton 1822), the anadromous hilsa shad, is a highly preferred fish for its taste and delicacy. It migrates from its marine environment to the freshwater rivers for spawning. In India, distribution of freshwater hilsa has been recorded from the rivers Hooghly, Brahmaputra, Ganga, Godavari, Mahanadi, Narmada and Tapti and marine locations in Arabian Sea and Bay of Bengal. As a result, the species is subjected to a range of climatic and environmental extremes throughout the region. Idea of presence of more than one race of hilsa has always evoked great interest among researchers. Based on the morphological differences, slender and broad morphotypes of hilsa have been identified in the Ganges, however, there are reports which suggest that the morphological variation in hilsa is due to the local environments. Attempts have been made on genetic characterisation and stock delineation in the past, however, the question, whether there is a single or multiple stocks, still remains to be answered. In this perspective, the objective of this work is to develop knowledge based on genome-wide variation and population structure of hilsa to support breeding programmes for aquaculture and its natural stock management.

**Collection of tissue accessions and truss morphological data for specimen**

Altogether, 23 locations were surveyed and a total of 613 samples of *T. ilisha* were collected from nineteen locations (13 from east coast and six from west coast), comprising 2 marine, 1 estuary and 3 riverine locations from west coast, and 5 marine, 2 estuary and 7 riverine locations from East coast (Fig. 7). Length, weight, scales, truss photography and gonadal stage, were taken for all the specimens. Tissue samples (blood, muscle, fin) were collected from all the specimens in 95% ethanol (Fig.8). Truss photographs of all the 613 samples from different populations were digitally saved. A total of 12 landmarks were recorded on the left view of each specimen and transformed truss data were generated.
Development of species-specific *de novo* microsatellite (SSR) markers for genetic variation studies

The present investigation aims at developing novel microsatellite markers in *T. ilisha* through enriched genomic library approach. Species specific *de novo* microsatellite (SSR) markers were identified through microsatellite enriched genomic library construction through screening using biotinylated oligos, followed by enrichment. A total number of 450 recombinant clones were picked up, presence and size of inserts were verified through colony PCR (Fig. 9). Two hundred sixty one clones with insert size 300bp and above were selected and sequenced. Out of 201 good sequences, 128 (63.68%) sequences contained microsatellite repeats.

A total of 129 microsatellite loci were identified with repeats of (GT)\(_n\), (CA)\(_n\), (CT)\(_n\), (GA)\(_n\), as well as, compound repeats (Fig. 10), for which primers were designed and tested for the polymorphism. A total of 16 loci were found to be polymorphic, while 22 were monomorphic. The number of alleles per locus ranged from 2 to 9. These polymorphic markers will be used...
for genotyping individuals of *T. ilisha* across the native distribution range to estimate the extent of genetic variability.

**Genetic variability analysis using Mitochondrial cytochrome b marker**

The full length cytochrome b gene of 1141 bp was amplified and sequenced from 288 individuals collected from six different locations, namely from Brahmaputra (riverine), Hoogli (riverine) and Mahanadi (marine) in east coast and Mahisagar (estuary), Tapi (riverine) and Narmada (riverine) in west coast. Thirty four haplotypes were obtained and the haplotype (gene) and nucleotide diversities were 0.821 and 0.00369, respectively. Out of 1141 bases, 1106 were conserved, 35 variables with 17 parsimony informative and 18 singletons with an ti/tv ratio of 201.12. The average nucleotide frequencies were T=0.278, C=0.304, A=0.24 and G=0.178. For the six populations from east and west coast, most common haplotypes were different and one haplotype was found to be common in all the samples studied. Twenty eight haplotypes were observed to be exclusively in east coast samples, while five in west coast samples. The haplotype network derived from Cytochrome b haplotypes resulted in two distinct clades, representing east and west coast (Fig.11). In cyt b, haplotype diversity (*h*) within the geographical populations was high (from 0.3630 to 0.8555) and nucleotide diversity (*ð*) was low (from 0.001225 to 0.002020). AMOVA results indicated that for Cytochrome b, 58.81% and 41.19%, respectively, of the total variance was attributed to differences among and within populations, with total *F*<sub>ST</sub> value of 0.58807. When pairs of population samples were compared, *F*<sub>ST</sub> values ranged from 0.00995 to 0.77891.

![Fig.11: Haplotype network of cytochrome b sequences showing two distinct clades representing east and west coast *T. ilisha*](image)

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

Project Period: April, 2013 - March, 2016

Project Personnel: A. Gopalakrishnan (PI upto 31 July 2013), P.R. Divya (PI from August 2013), V.S. Basheer, and A. Kathirvelpandian

Funding Agency: Institutional

Seer fishes are one of the commercially important marine pelagic finfish resources of India contributing to 1.85% of (~60,000 tonnes) marine fish production in India. King seer *Scomberomorus commerson* forms a major fishery in Tamil Nadu, Kerala, Karnataka, Andhra Pradesh coasts. They are in great demand in the export market fetching good price due to its high grade meat quality. India contributes about 36,000 tonnes out of global production of about 0.25 million tonnes. Stocks of *S. commerson* are over-fished along both the coasts of India, warranting a 60% reduction in the exploitation rate to bring the fishery back to the Maximum Sustainable Yield (MSY).

Similarly, *Parapenaeopsis stylifera*, commonly called ‘kiddi shrimp’ or *Karikkadi* is one of the economically important penaeids. It possess abundance along the south-west coast of the country; constitutes 60% of the total marine shrimp landings (~ 2.1 lakhs) in India. The total catch of this species is around 62,000t in which Kerala ranks first with 26,000t followed by Gujarat with 16000t. *P. stylifera* constitutes around 95% of the shrimp catch in South west coast during south west monsoon. The species also constitutes an important shrimp resource from south-east coast of India. Considering the importance of the above species, population genetic analysis using microsatellite markers was initiated.

Cross-species amplification of microsatellite primers

Sample collection for *S. commerson* from Mumbai, Calicut, Cochin, Chennai, Kakinada and Andaman & Nicobar islands; and for *P. stylifera* from Mumbai, Cochin and Tuticorin waters, was carried out. DNA extraction was completed for all the specimens. Cross-species amplification of microsatellites from related fish species was initiated for both *S. commerson* and *P. stylifera*. In case of *S. commerson*, a total of six primers were standardized through cross-species amplification (Table 1 & Fig. 12).
### Table 1. Characterization of six microsatellite loci amplified in *S. commerson*

<table>
<thead>
<tr>
<th>Locus</th>
<th>Repeat motif</th>
<th>Primer sequences</th>
<th>Ta</th>
<th>Size range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sa 2769</td>
<td>(AC)_{18}</td>
<td>F-TTTTGCATTITAAAAGCAGCTCAGT R-GTGGGAGCAGACACATGATTCA</td>
<td>56</td>
<td>221-259</td>
</tr>
<tr>
<td>Scomber australasicus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sa 2657</td>
<td>(CA)_{16}</td>
<td>F-TGTCAGAGATGATGACATACAGG R-AGATTGGGAGTGGTACAGGTA</td>
<td>56</td>
<td>240-328</td>
</tr>
<tr>
<td>S. australasicus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sa 2068</td>
<td>(GGA)_{9}</td>
<td>F-CAAGACATGACATGACATGAC R-AGATTGGGAGTGGGTA</td>
<td>56</td>
<td>146-176</td>
</tr>
<tr>
<td>S. australasicus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sa2770</td>
<td>(CA)<em>{3}(CCT)</em>{3}</td>
<td>F-AGAATGAAAGGCCTTTAAGG R-ACTGAGCTGCTTTAATGAAAAA</td>
<td>56</td>
<td>195-285</td>
</tr>
<tr>
<td>S. australasicus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S ca44</td>
<td>(CTCG)2 CTAT (CTGT)5</td>
<td>F-ATGCCAAAATG TGG CAC ATA ATC A R-GGG CAG CTC CAT GGG TCT GAG T</td>
<td>58</td>
<td>169-175</td>
</tr>
<tr>
<td>S. cavella</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sca-37</td>
<td>(TG)8 AG (TG)4 AG (TG)4</td>
<td>F-GCG CC TG CTT GAT TTT ATT GCT C R-CAA CAA TTA GTC GCA GCC CTA G</td>
<td>58</td>
<td>154-168</td>
</tr>
<tr>
<td>S. cavella</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig.12: Cross-species amplification of microsatellite loci in *S. commerson*

### DNA barcoding of finfishes

**Project Title:** DNA barcoding of marine finfishes and shellfishes  
**Project Period:** November, 2012 – October, 2017  
**Project Personnel:** A. Gopalakrishnan (PI upto July 2013), V. S. Basheer (PI from August 2013) and J. K. Jena  
**Funding Agency:** MoES - CMLRE, Govt. of India

**DNA barcoding of finfishes**

A total of 147 finfish specimens were collected from fish landing centres [Cochin, Trivandrum and Kollam in Kerala (41 species); Mangalore and Malpe in Karnataka (45 species); Dhiga, Diamond Harbour and Dariya in West Bengal (61 species)]. Tissue samples from each specimen were collected after recording the morphometric measurements and preserved in 95% ethanol. Total genomic DNA extraction was carried out for all the samples and the partial sequence of COI gene was PCR amplified using universal primers.

COI sequences of 331 samples belonging to 65 species of finfish were generated with average length of 655 base pairs and were submitted to GenBank (Table 2). Raw sequences were edited and aligned using Bio Edit sequence alignment editor version 7.0.5.2

### Table 2. Fish species barcoded using mitochondrial COI gene

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Species</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Pseudanthias marcia</em></td>
<td>Serranidae</td>
</tr>
<tr>
<td>2.</td>
<td><em>Pseudanthias pillai</em></td>
<td>Serranidae</td>
</tr>
<tr>
<td>3.</td>
<td><em>Liopropoma randalli</em></td>
<td>Serranidae</td>
</tr>
<tr>
<td>4.</td>
<td><em>Chelidoperca investigatoris</em></td>
<td>Serranidae</td>
</tr>
<tr>
<td>5.</td>
<td><em>Chelidoperca occipitalis</em></td>
<td>Serranidae</td>
</tr>
<tr>
<td>6.</td>
<td><em>Chelidoperca maculicuad</em></td>
<td>Serranidae</td>
</tr>
<tr>
<td>7.</td>
<td><em>Brama dussumieri</em></td>
<td>Bramidae</td>
</tr>
<tr>
<td>8.</td>
<td><em>Psenes cyanophrys</em></td>
<td>Nomeidae</td>
</tr>
<tr>
<td>9.</td>
<td><em>Psenes arafurensis</em></td>
<td>Nomeidae</td>
</tr>
<tr>
<td>10.</td>
<td><em>Priacanthus blochii</em></td>
<td>Priacanthidae</td>
</tr>
<tr>
<td>11.</td>
<td><em>Priacanthus prolixus</em></td>
<td>Priacanthidae</td>
</tr>
<tr>
<td>12.</td>
<td><em>Pristignus refugens</em></td>
<td>Priacanthidae</td>
</tr>
<tr>
<td>13.</td>
<td><em>Pempheris malabaricus</em></td>
<td>Pempheridae</td>
</tr>
<tr>
<td>14.</td>
<td><em>Pempheris marcula</em></td>
<td>Pempheridae</td>
</tr>
<tr>
<td>15.</td>
<td><em>Pempheris shwenkii</em></td>
<td>Pempheridae</td>
</tr>
<tr>
<td>16.</td>
<td><em>Rachycentron canadum</em></td>
<td>Rachycentridae</td>
</tr>
<tr>
<td>17.</td>
<td><em>Pampus chinensis</em></td>
<td>Stromateidae</td>
</tr>
<tr>
<td>18.</td>
<td><em>Chlorophthalmus acutifrons</em></td>
<td>Chlorophthalmidae</td>
</tr>
<tr>
<td>19.</td>
<td><em>Chlorophthalmus corniger</em></td>
<td>Chlorophthalmidae</td>
</tr>
<tr>
<td>20.</td>
<td><em>Perulibatrachus aquilonarius</em></td>
<td>Batrachoididae</td>
</tr>
<tr>
<td>21.</td>
<td><em>Colletteichthys flavipinnis</em></td>
<td>Batrachoididae</td>
</tr>
<tr>
<td>22.</td>
<td><em>Colletteichthys dussumei</em></td>
<td>Batrachoididae</td>
</tr>
<tr>
<td>23.</td>
<td><em>Sphenanthias whiteheadi</em></td>
<td>Cepolidae</td>
</tr>
</tbody>
</table>
four recognized genera and 46 valid species, of which Rhinobatos is the largest genus with a total of 36 species. Nine Rhinobatid species are reported from the Arabian Sea, of which one belongs to the genus Glucostegus: *G. obtusus*. The species reported from Arabian Sea are *Rhinobatos punctinifer*, *R. annandale*, *R. granulates*, *R. halavi*, *R. salalah*, *R. ionatus*, *R. variegates*, *R. thouiniana* and *R. obtusus*.

Five species belonging to two genera (*Glucostegus* and *Rhinobatos*) in the family Rhinobatidae were characterized using mt COI gene (Fig. 13). The final alignments of COI gene sequenced in 20 individuals consisted of 637 bp. Twenty COI sequences were obtained in the present study and submitted to GenBank and Barcode of Life Database; accession numbers: *Glucostegus obtusus* (KF899437-KF899439), *Glucostegus thouin* (KF899440, KF899441), *Rhinobatos punctifier* (KF899664-KF899668), *Rhinobatos ionatus* (KF899669-KF899672) and *Rhinobatos variegatus* (HM467794, KF899673-KF899678). The Stripedose guitarfish, *Rhinobatos variegatus*, described from the Gulf of Mannar was distantly placed with other two *Rhinobatos* species. The genetic distance of intraspecies was ranged from 0.000 to 0.006, while it varied from 0.069 to 0.262 for interspecies. The average interspecies distance was found to be 15.5%. The intraspecific genetic distance was high in *Rhinobatos variegatus* (0.6%); perhaps warranting further taxonomic investigation.

**Phylogenetic relationship of the fish species belonging to the family Rhinobatidae**

The family Rhinobatidae (Chondrichthys: Rajiformes) comprises rays popularly known as guitar fishes or shovelnose rays. The family currently includes...
in the family Sphyraenidae. Globally, there are more than 22 species of barracuda with size range from less than 50 cm to nearly 2 meters in length. So far seven species have been reported from Indian waters viz., *S. barracuda*, *S. jello*, *S. putnamiae*, *S. genie*, *S. forsteri*, *S. obtusata* and *S. novaohollandiae*. *Sphyraena sp. A* represents the eighth known species in the Arabian Sea.

Seven species belonging to the genera *Sphyraena* were characterized using mt COI gene. The final alignments of COI gene sequences from 20 individuals consisted of 652 bp and submitted to the GenBank. Among the seven species examined, one species was confirmed as undescribed and designated as *Sphyraena sp. A*. The genetic distance of intraspecies was ranged from 0.000 to 0.007, while it varied from 0.111 to 0.273 for interspecies. Two specimens of *Sphyraena sp. A* clustered with, but well separated from (D=4.4%), the three specimens of *Sphyraena barracuda*. The COI sequences of *Sphyraena barracuda* *Sphyraena n. sp* showed a clear barcode split (4.4% divergence) congruent with morphological differences. The NJ tree revealed very distinct species clusters (Fig. 14).

**Phylogenetic analysis of three species of mackerel in Indian waters**

A total of 655 base pairs of aligned sequences of mt COI gene were studied in three mackerel species viz., *Rastrelliger kanagurta*, *R. faugni* and *R. brachysoma* (Fig. 15). Between the different mackerel species 582 sites were constant, 73 bases exhibited variation and 67 were parsimony informative. The average pair-wise ratio of transitions (Si) vs. transversions (Sv) was 5.92. Based on COI gene, the mean genetic divergence value between three mackerel species was 5%. The pair-wise divergence between *R. kanagurta* and *R. faugni* was 0.09 and with *R. brachysoma* it was 0.03-0.04. *R. kanagurta* samples between Indian mainland and Andaman waters showed a divergence level of 0.012.

**Fig. 15: Neighbour joining tree of mackerel species using mtDNA COI gene. RK1-5 represents *Rastrelliger kanagurta* from Andamans, RKP, RKM, RKK, RKG represents *Rastrelliger kanagurta* from Indian mainland, RB represents *R. brachysoma* and RF represents *R. faugni***.

Two new fish species discovered from Arabian sea

Two new fish species viz., *Plectranthias alcocki* and *Pempheris sarayu* were discovered from Arabian sea and the study were published in the international journals, *Zootaxa* and *Journal of the Ocean Science Foundation*.

**Plectranthias alcocki**

The serranid fish genus *Plectranthias* contains small benthic species found in tropical and subtropical seas on coral or rocky reefs at depths of 20 to 300 m, hence not often caught in trawls. They are poorly represented in museum collections and nearly half of the valid species are known from only one or two specimens. *P. alcocki* was described based on two specimens collected from deep-waters off Kollam (Fig. 16). The species is named in honour of W. Alcock, in recognition of his contribution to the taxonomy of deep-sea fauna of Indian seas.

*P. alcocki* can be distinguished from all other species of the genus *Plectranthias*, except *P. maugei* Randall, 1980 and *P. forsteri* Fourmanoir, 1977, by its unique colour pattern, consisting of dorsal fin with a black blotch at base of fourth to eighth spines, one at base of the last three spines, and two at base of soft
The pempherid fishes, or sweepers, of the genus *Pempheris* are represented in the seas of India by four species and *P. sarayu* is the 5th species. *P. sarayu* was described based on one specimen collected from Kovalam (Fig. ). The other four species are *P. flavicycla*, *P. malabarica*, *P. mangula* and *P. schwenkii*. These fishes are also unique in having a short-based, pointed dorsal fin and a very long, low anal fin. They are copper or bronze in color. Their short snout, strongly oblique mouth, protrusible upper jaw, and numerous long gill rakers are specializations for feeding on zooplankton.

*P. sarayu* seems most closely related to *P. rhomboidea*. The meristic data for *P. sarayu* are well within the range of counts for *P. rhomboidea*. The two species share the relatively large eye, the same prepelvic length, a slightly forked caudal fin with a faint dusky posterior margin, black pigment of the dorsal fin confined to the distal end, and no black spot at the base of the pectoral fins. *P. sarayu* differs from *P. rhomboidea* in having shorter pectoral fins (3.65 in SL, compared to 3.4-3.55 in *P. rhomboidea*), slightly greater body depth, and the dorsal surface of the tongue with very small, discrete papillae vs. transverse irregular rows of close-set, fleshy papillae. It is mainly copper-colored in life, compared to bronze in *P. rhomboidea*.

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

**Project Period:** November, 2010 - November, 2013

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

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

The project envisages the use of microsatellite markers for analyzing genetic variability in the natural populations of cobia and pomfret in Indian waters. Globally, cobia is considered as an ideal species for aquaculture (especially cage culture in marine waters) due to its fast growth (5-6 kg/yr) and low FCR (Food Conversion Ratio). Pomfrets are high valued food fishes due to its quality meat and good export potential. Information on genetic variation of natural stocks of pomfret and cobia along Indian coast can be used for resolving their population structure to define their management units and to develop suitable restocking strategies for the species.

**Population genetic structure of silver pomfret in Indian waters**

Silver pomfret samples were collected from five locations (Veraval, Cochin, Chennai, Visakhapatnam and Kolkata) to determine the population structure of the species along Indian waters. Eleven polymorphic microsatellites were identified in *P. argenteus* (Yang et al., 2006). Ten primers identified in *Pampus cinereus* (Wei et al., 2009) were tested for cross species amplification in 20 individuals of *P. argenteus*. Of the 10 primer pairs tested, seven provided successful amplification in *P. argenteus*. Out of the 21 primers available, population genetic analyses were done using twelve identified polymorphic primers, of which genotyped data using 12 primers are included in the analysis. The genotyped data were analysed using several softwares like Arlequin ver 3.0 and Genpop version 3.3. Possibility of null alleles were ruled out using software Micro-checker ver 2.2.3. Parameters estimated includes number of alleles, allelic frequencies,
percentage of polymorphic loci, 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. Polymorphic information content was calculated using online software (http://www.genomics.liv.ac.uk/animal/Pic1.html). Exact P-tests for conformity to Hardy–Weinberg Equilibrium (probability and score test) were performed using a Markov Chain approach in Genepop version 3.3d (Raymond and Rousset, 1998). Dendrogram was constructed using open source software Fig Tree 1.4 version (available from http://tree.bio.ed.ac.uk/) based on genetic distance estimates generated from microsatellite data of the silver pomfret populations.

Genetic stock structure analyses of silver pomfret along the Indian waters were done using genotyped data of twelve loci in five populations. The total no. of alleles per locus ranged from 28 (Par 20 and Par 06) to 67 (P 189) and the allele size ranged 94 to 444 bp. The mean no. of alleles of 20 was obtained across all the populations. Twelve microsatellite loci identified for this study were polymorphic. Mean value of expected heterozygosity (Hexp - 0.8756) for each population was high compared to observed heterozygosity (Hobs - 0.7559). Wright (1978) fixation index (F\textsubscript{IS}) is a measure of heterozygote deficiency or excess (inbreeding coefficient). Positive F\textsubscript{IS} indicating heterozygote deficiency was evident in all loci. Heterozygote deficiency can be interpreted as increased homozygote, which might be a result of increased inbreeding or decline in silver pomfret population in Indian waters. Null alleles with significant values were eliminated from analysis. Pair-wise fisher’ s Fst (u) between silver pomfret samples of 5 different population using microsatellite software differed significantly from zero for all the pairs of populations and were within the range of 0.026 (Fst value between Kerala and Gujarat) - 0.067 (Fst value between Gujarat and West Bengal). Significant pair wise Fst values between populations of east (Tamil Nadu, Visakhapatnam and West Bengal) and west coasts (Gujarat and Kerala) were observed. Based on the genetic distance, UPGMA dendrogram was constructed using open source software Fig Tree 1.4 (Fig. 18).

**Population genetic structure of cobia In Indian waters**

Genetic stock structure analyses of cobia along the Indian waters was done using genotyped data of 14 loci in four populations. All the 16 microsatellite loci analyzed were polymorphic. A total of 290 individuals (58 individuals/ location) were used in the study. The total no. of alleles per locus ranged from 7 (RCA1 A08) to 33 (RCA1B-D10) and the allele size ranged 104 to 315 bp. The mean no. of alleles was 15 for all populations. The average H\textsubscript{exp} and H\textsubscript{obs} of all populations were same 0.72. Out of the 14 primers in which we got genotyped results, two loci RCA 1-F 10 and RCA 1-D07 were not considered for analysis and genotyped data using 14 primers were used in the genetic analysis of cobia populations. Pair-wise Fst estimate between populations differed significantly from zero for all the pairs of populations (Table 3). The highest pair-wise genetic distance was 0.0398, between Andhra Pradesh and Gujarat and the overall pair-wise genetic distance was low within the range 0.024-0.039. Genotyping of samples from West Bengal are not included in the final analysis.

**Table 3. Pair-wise Fst between four populations of cobia (p<0.01)**

<table>
<thead>
<tr>
<th></th>
<th>Gujarat</th>
<th>Kerala</th>
<th>Tamil Nadu</th>
<th>Andra Pradesh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gujarat</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kerala</td>
<td>0.031</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tamil Nadu</td>
<td>0.035</td>
<td>0.034</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Andra Pradesh</td>
<td>0.039</td>
<td>0.031</td>
<td>0.026</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**Project Title:** Genetic Stock Structure Analysis of the Indian mackerel, *Rastrelliger kanagurta* from Indian waters using Microsatellite Markers

**Project Period:** April, 2013 – November, 2014

**Project Personnel:** J.K. Jena (PI), A. Gopalakrishnan (upto July 2013), Divya P.R. and V.S. Basheer

**Funding Agency:** FAO/ BOBLME
The Indian mackerel, *Rastrelliger kanagurta* (Family Scombridae, order Perciformes) is one of the most important pelagic fish resources of BOBLME region in the context of food security. The population genetic structure of the Indian mackerel is largely unknown in the entire region. Microsatellite primers were used for the genetic stock identification of Indian mackerel using primers developed from the resource species through Next Generation Sequencing (NGS) or identified using cross priming from related species. Fifteen primers were developed in *R. kanagurta* though NGS (Candy et al., 2013). Out of these fifteen loci, we have identified seven polymorphic loci which could be utilized for genetic stock analysis of Indian mackerel.

**Primer development through cross priming**

Seven microsatellite primers were developed in *R. kanagurta* through cross species amplification from other related species *Scomber australasicus*, *S. japonicus* and *Scomberomorus cavalla*. To confirm the occurrence of repeats, the cross-amplified polymorphic microsatellite loci were analyzed by sequencing. All the loci were found to contain the repeat sequences. All the microsatellite sequences were submitted to the GenBank (Accs. No KF668002- KF668008). These loci can be used for stock structure analysis of Indian mackerel in larger sample size from different locations. Details of polymorphic microsatellite markers developed through cross amplification are given in Table 4. Details of the primers developed and their standardized PCR conditions were communicated to all other seven BOBLME project participating countries for completion of population genetic analysis work.

A total of 70 mackerel samples were collected from each of the seven locations viz., Calicut (Kozhikode), Mumbai, Nagappattinam, Tuticorin, Kakinada, Paradeep and Port Blair. Genotyping of samples is being done to identify the genetic stocks of mackerel along Indian waters.

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

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

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

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

Identification of molecular markers is an essential pre-requisite for undertaking scientific management of a particular species. Microsatellite loci have been appropriately used for a wide range of applications and have remained the most robust marker system for studies of population structure in several important fish species. In continuation to the work done during previous year in the great snakehead, *Channa marulius* (Fig.19), the library was screened for tri, tetra, penta and hexa repeat containing regions of the genome using affinity capture approach.

![Fig. 19: Channa marulius](image)

The high molecular weight genomic DNA was digested briefly and ligated to *Mlu* adaptor. Enrichment was performed with magnetic beads using biotinylated oligos. Cloning was done and transformants were amplified with M13 universal primers. Plasmids were extracted through alkaline lysis method and sequenced.

### Table 4. Polymorphic microsatellite markers developed through cross amplification in Indian mackerel

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Locus</th>
<th>Resource Species</th>
<th>Repeats</th>
<th>T&lt;sub&gt;a&lt;/sub&gt;</th>
<th>Product Size</th>
<th>Work done at NBFRG, India in <em>Rastrelliger kanagurta</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(GT)&lt;sub&gt;17&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>KSJ 18</td>
<td><em>Scomber japonicus</em></td>
<td>(GT)&lt;sub&gt;10&lt;/sub&gt;</td>
<td>55</td>
<td>206-240</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>KSJ 26</td>
<td><em>Scomber japonicus</em></td>
<td>(GT)&lt;sub&gt;13&lt;/sub&gt; AT (GT)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>58</td>
<td>216-246</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Sa 2068</td>
<td><em>Scomber australasicus</em></td>
<td>GGA&lt;sub&gt;9&lt;/sub&gt;</td>
<td>55</td>
<td>155-167</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Sa 2657</td>
<td><em>Scomber australasicus</em></td>
<td>CA&lt;sub&gt;16&lt;/sub&gt;</td>
<td>56</td>
<td>238-312</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Sa2770</td>
<td><em>Scomber australasicus</em></td>
<td>CA&lt;sub&gt;13&lt;/sub&gt; (CCT)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>56</td>
<td>207-267</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Sca 8</td>
<td><em>Scomberomorus cavalla</em></td>
<td>CA&lt;sub&gt;13&lt;/sub&gt;</td>
<td>58</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Sca 30</td>
<td><em>Scomberomorus cavalla</em></td>
<td>GA/CA&lt;sub&gt;23&lt;/sub&gt;</td>
<td>59</td>
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</table>
using Sanger technology. Upon sequencing the inserts were searched with Tandem Repeat Finder. The electrophenograms depicting tri, tetra, penta and hexa repeats are illustrated in Fig. 20 a,b,c,d, respectively.

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

Project Period: May, 2009 – May, 2014

Project Personnel: Vindhya Mohindra (CCPI), Ravindra Kumar and Rajeev K. Singh

Funding Agency: NAIP, ICAR

Identification of candidate reference genes for quantitative expression analysis by real-time PCR for hypoxic stress in *Clarias batrachus*

Selection of the most appropriate reference gene(s) is a crucial step in studies quantifying gene expression by quantitative real-time PCR (qPCR). As a need, reference gene(s) should be unaffected at transcription level by experimental conditions and/or tissue types. In this study, 11 candidate reference genes were tested in *C. batrachus* for their expression stability using the geNorm, NormFinder and BestKeeper statistical algorithms, and were compared in different tissues (brain, heart, liver, muscle, spleen and head kidney) and treatments (before and after hypoxia exposure). The results indicated that in brain, heart, liver, muscle, spleen and head kidney, respectively, 28S rRNA/TUB, 28S rRNA/TUB, RPL30/28S rRNA, RPL30/TUB, ELF-1A/28S rRNA and ELF-1A/TUB gene pairs were highly stable and were suitable as reference genes to study oxidative stress, while ACTB and B2M were the least stable genes in examined tissues under normoxic and/or hypoxic conditions. The observations suggested the consideration of tissue types and use of at least two reference genes, instead of one in accurate normalization of qPCR data.

A new candidate gene for hypoxia tolerance in Indian catfish, *C. batrachus*: Pleckstrin homology-like domain, family A, member 2

The pleckstrin homology-like domain, family A, member 2 (PHLDA2) gene was previously identified as an imprinted gene. The present study determined the structure of complete cDNA and the deduced protein of PHLDA2 and analyzed the changes in its mRNA expression in *C. batrachus* tissues under hypoxic conditions. The complete cDNA of *CbPHLDA2* gene consisted of 1009 nucleotides with an open reading frame of 417 nucleotides. The deduced *CbPHLDA2* protein of 139 amino acids shared high homology with *PHLD2A* of other fishes as well as vertebrates (Fig. 22). Importantly, a single amino acid (Asparagine/Lysine)
insertion was identified in the PH domain of CbPHLDA2 and other fishes, which was absent in that of other vertebrates studied. Under normoxic conditions, CbPHLDA2 was constitutively expressed with varying levels in analyzed tissues. Short and long-term hypoxia exposure resulted in significant changes in the expression of CbPHLDA2 in liver, spleen, head kidney, brain and muscle in time dependent manner (Fig. 23). The results suggested that CbPHLDA2 might play an important role for adaptive significance under hypoxia.

Molecular characterization and transcriptome profile of Heat shock protein genes under hypoxia in *C. batrachus*

Heat shock proteins (Hsps) are typically associated with stress response and tolerance. The molecular structure of three Hsps cDNA, CbHsc71, CbHsp90α and CbHsp10, and their transcriptional response in hypoxia tolerant Indian catfish, *C. batrachus*, under experimental and natural hypoxia were studied (Fig. 24). The deduced protein sequences of these Hsps had the characteristic signatures of their respective families. Comparative analysis revealed “leucine”, after the conserved “proline” residue as defined in the consensus sequence of chaperonins Hsp10/cpn10 family signature, has been replaced by “methionine”.

![Fig. 22: (A) Pleckstrin homology domain prediction in the CbPHLFA2 protein of *Clarias batrachus*. (B) Prediction of serine, threonine and tyrosine phosphorylation sites in CbPHLDA2 protein of *Clarias batrachus*. X-axis represents the position of sequence while, Y-axis represents the phosphorylation potential (cutoff 0.5)](image)

![Fig. 23: Differential expression of CbPHLDA2 mRNA estimated by qRT-PCR, after short-term (PH, 1, 6 and 12 hr) and long-term (NTR) hypoxia exposure in *C. batrachus* tissues. Y-axis represents the log2 ratio of CbPHLDA2 expression, mean±SE (N=3, in duplicate) as fold change. X-axis represents hypoxic treatments as PH, progressive hypoxia up to 0.98±0.1 mg/l, dissolved oxygen, H1, H6 and H12 (hypoxic time period, 1, 6 and 12 hr), NTR, natural hypoxia exposure. Significant differences (p<0.05) in the expression levels of CbPHLDA2 in comparison to normoxic control group are indicated by asterisks (*) above bars.)](image)

![Fig. 24: Effects of short-term (PH, 1, 6 and 12 hr) and long-term (NTR) hypoxia exposure on relative CbHsc71 mRNA expression in liver and muscle of *C. batrachus*. PH, progressive hypoxia up to 0.98±0.1 mg/l, dissolved oxygen, H1, H6 and H12 (hypoxic time period, 1, 6 and 12 hr), NTR, natural hypoxia exposure. Asterix (*) above bars represents significant difference in the expression levels in comparison to normoxic control group.)](image)
transcription level, these genes were found to be differentially regulated under hypoxia stress, in different tissues of *C. batrachus*. The *CbHsc71* and *CbHsp90α* were up-regulated after short and long-term hypoxia, whereas *CbHsp10* was significantly down-regulated after short-term hypoxia. The differential expression of these Hsps may play a role in protection and survival under hypoxia induced oxidative stress in *C. batrachus*.

**Activating transcription factor-4 gene in hypoxia-tolerant Indian catfish, *C. batrachus*: Molecular characterization and transcriptional response under oxidative stress**

Activating transcription factor-4 (ATF-4) is preferentially regulated during severe hypoxia and is an important mediator of the unfolded protein response (UPR). In the present work, ATF-4 cDNA was characterized and its transcriptional expression was studied in brain, liver, muscle and spleen tissues of *C. batrachus* after short (progressive hypoxia; PH, 1 hr, 6 hr and 12 hr) and long-term (natural) hypoxic stress. Sequence analysis identified the presence of "DpSGXXpS" and "PXSP" motifs for first time in fish ATF-4 protein and further showed that only three prolyl residues were conserved in oxygen dependent degradation domain of ATF-4. The expression level of ATF-4 transcripts in *C. batrachus* tissues was determined which showed that under normoxia, it was constitutively expressed in brain, liver and spleen tissue (negligible in muscle) with the varying levels (Fig. 25). After short-term hypoxia its expression was significantly (p<0.05) decreased in brain (progressive hypoxia, 4.25 fold; 1 hr, 5.11 fold; 6 hr, 6.02 fold). While, following long-term hypoxia, it was increased in liver (2.25 fold). No significant differences were observed in muscle and spleen. The findings of the present study suggest the possible role played by ATF-4 in *C. batrachus* for hypoxia adaptation.

**Full length cDNA characterization of differentially expressed genes under hypoxia**

Complete cDNA of 197 differentially expressed genes were identified and their ORFs were identified and deduced protein sequences were characterized. Average length of the full-length cDNAs was 1351 bp (ranged from 366 to 4651 bp) and average length of ORFs 741 bp (from 132 bp to 3297 bp). The *C. batrachus* Kozak motif, spanning the position -4 to +4 of the start codon was well represented by 8 bp sequences of AAACATGG (Fig. 26).

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**Project Title: Genetic Stock Structure Elucidation of *Tenualosa ilisha* and *Channa striatus* using mitochondrial DNA marker, microsatellites and molecular cytogenetic tools**

**Project Period:** April, 2010 – March, 2014

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

**Funding Agency:** Institutional
Population genetic studies of *Channa striatus*

Explorations and tissue sampling were carried out for analyzing the population structure in *Tenualosa ilisha* and *Channa striatus*. Samples representing four populations of *C. striatus* were collected from Vijayawada, Nilambur, Imphal and Agaratala. PCR amplification and sequencing of mitochondrial cytochrome b, ATPase 6/8, and cytochrome c oxidase I were completed in these four populations. The network of 23 haplotypes of ATPase 8/6 gene and 13 haplotypes of cytochrome b gene in nine populations of *C. striatus* is shown in Figures 27 & 28, respectively.

![Fig. 27: Haplotype network of mitochondrial gene ATPase 6/8 in *C. striatus*](image)

![Fig. 28: Haplotype network of mitochondrial gene Cyt b in *C. striatus*](image)

**Molecular cytogenetic studies in *C. striatus***

The probe of 18S rRNA gene was constructed and hybridized on metaphase chromosome of *C. striatus* using fluorescence *in situ* hybridization technique (Fig.29). The hybridization signals were found to be localized on two pairs of submetacentric/subtelocentric chromosomes. These markers could be utilized in documenting population specific variation in this species.

![Fig.29: 18S rDNA signals (red) on metaphase chromosomes of *C. striatus*](image)

Population genetic studies of *Tenuolosa ilisha*

Fish sampling of three populations of *T. ilisha* were undertaken from Ganga River, Farraka; Hooghly River, Kalyani, Kolkata and Brahmaputra River, Guwahati. PCR amplification and sequencing of mitochondrial cytochrome b, control region, ATPase 8/6 was accomplished for these populations. The genetic analysis of control region and ATPase 8/6 gene sequences were completed for all the eight populations. The haplotype frequency of ATPase 8/6 in different populations and the network showing relationship between the haplotypes is given in Fig. 30.

![Fig. 30: Network showing the haplotype relationships among populations of *T. ilisha* based on ATPase 8/6 sequence. Haplotypes separated by single lines are one mutation apart; small circles along lines represent missing haplotypes (not sampled or extinct)](image)

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

**Project Period:** April, 2011 – March, 2014

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

**Funding Agency:** Institutional

The germ cell transplantation is a very promising approach for *in situ* conservation. The technique originally devised for use in mammals, is based on testicular cell suspensions containing unknown numbers of spermatogonia derived from donor males is microinjected into sterilized male recipients, leading to establishment of donor-derived spermatogenesis. Over the years the technique has been efficiently used for the purpose of basic research, reproductive medicine and treatment of infertility. However, it is almost unexplored in fish, even though it has potential...
applications in conservation/propagation of species facing imminent extinction and/or large size species that are difficult to breed in captivity. In this study, depletion of endogenous spermatogenesis in mature male common carp (*Cyprinus carpio*) was optimized with minor modification of treatment protocol (normal water temperature and lower dose of cytotoxic drug in this case; oppose to elevated water temperature and higher dose). Briefly, mature male of common carp were injected intraperitonially with three dosage of Busulfan, an alkylating antineoplastic agent at 18 and 21 mg/kg body weight in two weeks interval for depletion of endogenous spermatogenesis (Fig. 31). It was found that fishes treated with higher dose i.e. 21 mg Busulfan/kg BW resulted in efficient depletion of endogenous green cells.

The isolation of spermatogonial cells from donor *Labeo rohita* by discontinued percoll gradient was standardized. The isolated and enriched spermatogonial cells from *L. rohita* were labeled with red fluorescent cell linker PKH26. These cells were then transferred into the testes of cytoablated common carp recipients through the urogenital orifice using 1 ml syringe. The 6 weeks post transplantation observations were encouraging as the transplanted donor cells were alive. This was characterised by retention of red fluorescent cell dye (PKH26), and found to be widely distributed inside the lobular region of recipient testes (Fig. 32).

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

**Project Period:** March, 2011 - March, 2015

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

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

**Cytogenetic studies**

Metaphase chromosomes were obtained from anterior kidney of *Cyprinus carpio, Heteropneustes fossilis* and *Clarias batrachus*. Karyotyping and other cytogenetic investigations were undertaken using Giemsa/ silver nitrate/chromomycin A3 staining (Table 5 and Figures 33-35).

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Name of the species</th>
<th>Diploid chromosome number (2n)</th>
<th>Number of NORs obtained after staining with</th>
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<tbody>
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<td>50</td>
<td>01 pair</td>
</tr>
<tr>
<td>2.</td>
<td>Cyprinus carpio</td>
<td>100</td>
<td>01 pair</td>
</tr>
<tr>
<td>3.</td>
<td>Heteropneustes fossilis</td>
<td>56</td>
<td>01 pair</td>
</tr>
</tbody>
</table>

**Table 5. Cytogenetic profiles of fishes**

Fig. 31: Transverse section of testis of common carp (a) control and (b) after treatment of Busulfan

Note: There was prominent depletion of germ cells in Busulfan treated group.

Fig. 32: Transverse section of testis of common carp showing wide distribution of proliferating PKH 26 labeled transplanted cells (red colored) post 90 days after the transplantation procedure.

Fig. 33: Karyotype of *C. carpio*

Fig. 34: AgNO3 stained metaphase complement of *C. batrachus*
Molecular cytogenetic studies

Molecular cytogenetic studies were undertaken in fish species, viz., C. punctatus, C. batrachus, C. carpio, G. giuris, H. fossilis, Barilius ngawa, Neolissochilus stracheyi and Garra sp. The 18S and 5S rDNA probes, labeled with biotin and tetramethylrhodamine-5-dUTP, were used for fluorescence in situ hybridization (FISH) Figures 36 & 37. In G. giuris and C. punctatus, hybridization signals for 18S and 5S rDNA were observed on one pair of chromosomes. In H. fossilis and C. carpio, hybridization signals for 5S rDNA and 18S rDNA were observed, respectively, on one pair of chromosomes.

Characterization of RAG1 gene in fishes

The consensus length of recombination activating gene 1 (RAG1) in 7 fish species, namely B. bendelisis, C. gachua, D. aequipinnatus, D. yuensis, E. danrichus, Puntius meinlangbii and X. cancila was found to be 645 bp in length. These partial sequences of RAG1 gene have been submitted in GenBank with Acc. Nos. KJ548106 - KJ548126. Further the phylogenetic studies among the fishes for these gene sequences is in progress.

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

Project Period: November, 2012 - November, 2015

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

Funding Agency: DBT, Govt. of India

For genetic characterization and DNA barcoding of fishes from north east India, 504 tissue samples along with voucher specimens were collected from 95 fish species belonging to various rivers of the Brahmaputra, Chindwin, Kaladan and Barak-Meghna-Surma drainages of north eastern India from the states of Assam, Manipur, Mizoram and Tripura (Table 6).
DNA isolation and PCR amplification of mitochondrial cytochrome c oxidase I (COI) of all 501 samples was completed and 103 DNA barcodes were prepared in 27 species using the universal set of primers FishF1 & FishR1 (Fig. 38). 16S rRNA gene region was amplified with primers, SARF & SBRR and DNA barcodes were prepared for 10 species (Fig. 39). PCR amplification of ITS 2 region was also carried out using ITSF4 and ITSR4 primers in 17 species. As a part of project, two JRFs from Department of Life Sciences, University of Manipur were also trained for three months in basic tools and techniques involved in DNA barcoding and bioinformatics software for phylogenetic analysis.

**Title of Project:** Whole genome sequencing and development of allied genomic resources in two commercially important fish- *Labeo rohita* and *Clarias batrachus*

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

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

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

A project on whole genome sequencing in two fish species namely *L. rohita* and *C. batrachus* has recently been initiated at this institute in collaboration with CIFA, Bhubaneswar; Anand Agricultural University, Anand and IASRI, New Delhi with the financial support from Department of Biotechnology, Government of India. This programme is aimed to: i) establish reference draft sequence of these two species, ii) generate associated genomic resources such as large insert libraries, iii) develop a genetic platform for estimating and preserving genetic variability of indigenous native populations of carp and catfish species with related marker information, and iv) develop human resource development in frontier areas of fisheries research. The major activities proposed in this programme include multi-platform next generation sequencing (NGS) of rohu and magur genomes, *de novo* assembly to generate a draft sequence, genome annotation and heterozygous SNP discovery. This endeavour would result in wealth of information such as genes and genetic markers responsible for production traits and disease management, BAC library, heterozygous SNP resource and genetic maps in these species, novel genes with regard to Indian carps and air breathing properties of catfishes.

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

**Project Period:** March, 2011 - March, 2014

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

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

The catfish *Ompok pabda* (Hamilton) is an indigenous small freshwater fish belonging to the family Siluridae (Fig. 40.) which is distributed in the plains and sub-mountain regions. However, the natural population of *O. pabda* is declining rapidly due to various anthropogenic factors and as a consequence
of that it has been categorized as ‘near threatened’ as per IUCN (2013). The objectives of this study was to generate baseline data on life-history attributes and development of microsatellite markers and linkage mapping. In the present study, fish samples were collected from three major rivers of Tripura viz., Gomoti, Mehuri and Feni (Fig. 41). The length of the sampled fish individuals were ranged from 12.2 cm to 23.5 cm.

![Fig. 40: Ompok pabda](image)

The age class estimated between 1-2+yr, of which 1+old fish were the dominant age classes, and over 80% of fish were <2+ yr old. The higher age classes upto 2+ were recorded in rivers Feni and Gomoti. Length-length relationship from otolith length and fish length indicates otolith increases in direct proportion with the increase in fish length ($r^2 > 0.95$).

![Fig. 41: A sampling site in river Feni, Tripura](image)

Altogether 76 fish individuals of mature females were induced bred in Tripura using synthetic hormone ovaprim and fertilized with the spermatozoan suspension collected from the dissected adult males. The average fertilization rate was 78%. Hatching time was recorded 22 – 24 hours after fertilization at 26 – 29°C. Absorption of yolk sack was noticed within 3 – 4 days of hatching. The development of mature eggs of *O. pabda* was determined during May-July with a diameter ranging between 0.544 to 0.826 mm. The diameter of immature eggs ranged from 0.22-0.548 mm and were developed during February-April. The mean fecundity per 100 g body weight was 20,442±6054 eggs.

To develop microsatellite markers for *O. pabda*, 105 samples were collected from three different rivers of Tripura state. Seventy nine sets of primers were screened and their respective annealing temperature was determined. Selected primers were used to amplify loci in male and female individuals of various populations to find out population specific variations and sex specific variations. These loci are being used for parental, F1 and F2 generations. For developing novel microsatellite markers, genomic DNA of *O. pabda* was digested with three blunt end restriction enzymes Atul (AGCT/TCGA), HaeIII (GGCC/CCGG), Rsat GTAC/CATG and one sticky and restriction enzyme Sau III 3AT (GATC/CTAG) to produce blunt end and sticky end fragments and run on agarose gel. Fragments ranging from 500-700 bp length were knifed out of gel and fragments eluted with gel elusion kit. Fragments were enriched with Biotin labelled dinucleotide repeats oligomers viz., (TG)$_{10}$, (AG)$_{10}$, (AC)$_{10}$ and (TC)$_{10}$ and dynabeads M280 with streptavidin (Fig. 42). The study establishes baseline data on reproductive patterns of *O. pabda* which may be helpful for development of responsible management strategies of the wild population in Tripura.
The freshwater butter catfish, *Ompok bimaculatus* (Bloch, 1794), is an important Silurid species of high commercial importance. It is widely distributed in the plains and sub-mountain regions and found in streams, rivers, canals, beels, jheels, reservoirs and tanks. In the present studies, explorations (Fig. 43) were carried out in 24 rivers covering different basins across different states, viz., North East, Andhra Pradesh, Jharkhand, Karnataka, Tamilnadu, Madhya Pradesh, Maharashtra, Orissa, Uttar Pradesh, Punjab, West Bengal, etc. Comparative analysis of reproductive parameters, viz., sex ratio, gonadosomatic index, size at first maturity, fecundity, percentage contribution of mature samples, contribution of different reproductive stages, oocyte diameter, egg weight, breeding traits and condition factor were determined.

In addition, genetic diversity assessments among different population of *O. bimaculatus* were also carried out using mitochondrial cyto-b partial gene and microsatellite markers. The results demonstrate the pattern of variation in reproductive traits and genetic structure. It was observed that females start to mature early than males and mean size at first maturity varied considerably between the populations. The reproductive period in most of the rivers of Ganges basin extended from April to August and in Southern rivers it was extended from March to July. Among the studied population, the female length at first maturity (L<sub>50%</sub>) was higher at Cauvery and lower at Mahanadi. Out of 19 female populations, 15 showed L<sub>50%</sub>, with mean total length ranged from 200 to 250 mm. The results indicated considerable variation in length at first maturity of both female and male across different rivers. The average fecundity also varied significantly between the rivers. Higher absolute fecundity was recorded in nine rivers, whereas moderate absolute fecundity was observed in ten rivers (Fig. 44). The best condition factor was recorded in female collected from river Cauvery and Krishna (Fig. 45).

The analysis of cyto b sequence data generated a consensus sequence length of about 1119 bp with the base composition of: A= 29.1%, C= 30.2%, G= 13.4% and T= 27.3%. In the analyzed sequence, there were 1077 conserved and 42 variable sites. The NJ tree topology suggested that Betwa and Rapti, Yamuna and Subarnarekha, and Godavari and Tapti are closely related to each other (Fig. 46). The cyto b sequence based study identified 14 haplotypes from the 20 populations. During spawning period, the gonadal protein concentration was high as compared to pre-spawning phase as per the gamete maturity and further the
protein concentration was varied according to the different aquatic environment conditions. The gonad (ovary), collected from Mahanadi, Cauvery, Ganga (West Bengal), Betwa, Tapti and Gomti rivers, had more protein concentration (>6 mg/ml/100 mg tissue weight) during spawning phase as compared to other rivers.

The study establishes baseline data on reproductive patterns of *O. bimaculatus* from 24 rivers. The differences in reproductive traits among populations may reflect environment induced phenotypic plasticity and/or genetic variations at macro-geographical scale. This also provides new insights in to the inter-population reproductive strategies and population structure which may be helpful for development of responsible management of the wild population.

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

**Project Period:** November, 2010 – November, 2013

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

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

The National Repository of Fish Cell line (NRFC) is now fully equipped at NBFGFR, Lucknow for the collection, deposition and distribution of cell lines to the research community. The NRFC is currently maintaining 46 cell lines of 22 fish species developed and deposited by various research institutes across the country including NBFGFR, Lucknow (Table 7). These cell lines have been characterized using both cytogenetic and molecular markers. Among the 46 cell lines, NBFGFR has contributed 13 cell lines (Fig. 47). All the cell lines submitted to NRFC have been successfully cryopreserved at NRFC. A website containing all the information regarding the NRFC was designed to facilitate collection and distribution of cell lines (Fig. 48). A link of NRFC has also been made available on the website of NBFGFR (http://www.nbfgfr.res.in). Researchers can visit the website and check the list of available fish cell lines in the NRFC. Material Transfer Agreement Form along with Deposit Form and Request Form are available at the NBFGFR website for deposition and request for cell lines. Based on requests cell lines were supplied to CIFA, Bhubaneswar and Senguthar Arts and Science College, Tamil Nadu.
### Table 7. List of Fish cell lines available at NRFC, Lucknow

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<th>Organ</th>
<th>NRFC Code</th>
<th>Submitted by</th>
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<td><em>Tor tor</em></td>
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<tr>
<td>22.</td>
<td>ICF</td>
<td><em>Clarias batrachus</em></td>
<td>Fin tissue</td>
<td>NRFC022</td>
<td></td>
</tr>
<tr>
<td>23.</td>
<td>LGT</td>
<td><em>Labeo rohita</em></td>
<td>Gill tissue</td>
<td>NRFC023</td>
<td></td>
</tr>
<tr>
<td>24.</td>
<td>DT1CPEx</td>
<td><em>Dascyllus trimaculatus</em></td>
<td>Caudal peduncle explant</td>
<td>NRFC024</td>
<td>CMFRI, Kochi</td>
</tr>
<tr>
<td>25.</td>
<td>DT1F4Ex</td>
<td><em>Dascyllus trimaculatus</em></td>
<td>Fin explant</td>
<td>NRFC025</td>
<td></td>
</tr>
<tr>
<td>26.</td>
<td>DT1CPTTr</td>
<td><em>Dascyllus trimaculatus</em></td>
<td>Trypsinized caudal peduncle</td>
<td>NRFC026</td>
<td></td>
</tr>
<tr>
<td>27.</td>
<td>RC4H1Tr</td>
<td><em>Rachycentron canadum</em></td>
<td>Trypsinized heart</td>
<td>NRFC027</td>
<td></td>
</tr>
<tr>
<td>28.</td>
<td>CTM</td>
<td><em>Catla catla</em></td>
<td>Thymus (macrophage)</td>
<td>NRFC028</td>
<td>NBGR, Lucknow</td>
</tr>
<tr>
<td>29.</td>
<td>CTE</td>
<td><em>Catla catla</em></td>
<td>Thymus (epithelial)</td>
<td>NRFC029</td>
<td>NBGR, Lucknow</td>
</tr>
<tr>
<td>30.</td>
<td>EM2HTr</td>
<td><em>Epinephelus malabaricus</em></td>
<td>Trypsinized heart</td>
<td>NRFC030</td>
<td>CMFRI, Kochi</td>
</tr>
<tr>
<td>31.</td>
<td>EM2GEc</td>
<td><em>Epinephelus malabaricus</em></td>
<td>Gill</td>
<td>NRFC031</td>
<td></td>
</tr>
<tr>
<td>32.</td>
<td>EM3GEc</td>
<td><em>Epinephelus malabaricus</em></td>
<td>Gill</td>
<td>NRFC032</td>
<td></td>
</tr>
<tr>
<td>33.</td>
<td>EM4GEc</td>
<td><em>Epinephelus malabaricus</em></td>
<td>Spleen</td>
<td>NRFC033</td>
<td></td>
</tr>
<tr>
<td>34.</td>
<td>CBE</td>
<td><em>Catla catla</em></td>
<td>Blood (lymphocytes)</td>
<td>NRFC034</td>
<td></td>
</tr>
<tr>
<td>35.</td>
<td>PC1CpTr</td>
<td><em>Pomacentrus caeruleus</em></td>
<td>Trypsinized caudal peduncle</td>
<td>NRFC035</td>
<td>CMFRI, Kochi</td>
</tr>
<tr>
<td>36.</td>
<td>PC1F1Ex</td>
<td><em>Pomacentrus caeruleus</em></td>
<td>Fin</td>
<td>NRFC036</td>
<td></td>
</tr>
<tr>
<td>37.</td>
<td>PC1L1Tr</td>
<td><em>Pomacentrus caeruleus</em></td>
<td>Trypsinized liver</td>
<td>NRFC037</td>
<td></td>
</tr>
<tr>
<td>38.</td>
<td>HC2SpEx</td>
<td><em>Epinephelus merra</em></td>
<td>Spleen</td>
<td>NRFC038</td>
<td></td>
</tr>
<tr>
<td>39.</td>
<td>CFF</td>
<td><em>Pristolepis fasciata</em></td>
<td>Fin</td>
<td>NRFC039</td>
<td>NBGR, Kochi</td>
</tr>
<tr>
<td>40.</td>
<td>IEE</td>
<td><em>Etheostorus suratensis</em></td>
<td>Eye</td>
<td>NRFC040</td>
<td>FCRI, Tamilnadu</td>
</tr>
<tr>
<td>41.</td>
<td>IEK</td>
<td><em>Etheostorus suratensis</em></td>
<td>Kidney</td>
<td>NRFC041</td>
<td></td>
</tr>
<tr>
<td>42.</td>
<td>IEG</td>
<td><em>Etheostorus suratensis</em></td>
<td>Gill</td>
<td>NRFC042</td>
<td></td>
</tr>
<tr>
<td>43.</td>
<td>IEB</td>
<td><em>Etheostorus suratensis</em></td>
<td>Kidney</td>
<td>NRFC043</td>
<td></td>
</tr>
<tr>
<td>44.</td>
<td>RE</td>
<td><em>Channa straiatus</em></td>
<td>Eye</td>
<td>NRFC044</td>
<td></td>
</tr>
<tr>
<td>45.</td>
<td>CSK</td>
<td><em>Channa straiatus</em></td>
<td>Kidney</td>
<td>NRFC045</td>
<td></td>
</tr>
<tr>
<td>46.</td>
<td>CSG</td>
<td><em>Channa straiatus</em></td>
<td>Gill</td>
<td>NRFC046</td>
<td></td>
</tr>
</tbody>
</table>
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 Personnel: V.S. Basheer (PI), A. Gopalakrishnan (up to July 2013), T. Raja Swaminathan, P. R. Divya and A. Kathirvelpandian

Funding Agency: Institutional

Of the states in Peninsular India with rivers originating in the Western Ghats, Goa remains the least explored. As part of our efforts to catalogue the freshwater fish diversity of the Western Ghats, NBFRG Kochi Unit in collaboration with ICAR Research Complex Goa, conducted surveys of freshwater habitats in Goa during 2013-2014. Nine sampling locations covering two major river systems in Goa - Mandovi and Zuari, were sampled intensively using cast nets, drag nets and scoop nets. A total of 15 surveys were conducted in these river systems which yielded 40 species of freshwater fish belonging to 31 genera of 14
families, and 7 species of crustacean. Shanon-Wiener index (Table 8) shows that diversity of the fishes was fairly good in these river systems. Diversity index ranged from 1.411 to 2.717.

Notable observations were the collection of *Pangio goaensis* from the type locality for the first time since it was described in 1972 by Tilak, collection of putative specimens of *Ompok goae*, and possible new species of *Amblyceps*, *Oreithys*, *Pethia*, *Danio* and *Schistura*.

**New records and rediscovered species of freshwater fish from Goa**

*Hypselobarbus jerdoni*, a mid-sized carp endemic to the Western Ghats, was thus far known from West flowing rivers in Karnataka and Northern Kerala. Specimens collected during this survey from the Mandovi basin are the first records of this species from Goa (Fig. 49a). The genus *Carinotetraodon* is represented in the Western Ghats by two species, *C. travancoricus* in Kerala and *C. imitator* in Southern Karnataka. We collected specimens of a dwarf puffer species from the Mandovi river basin (Fig. 49b), which represents the first record of this genus from the northern Western Ghats. DNA barcoding using the CO1 gene suggests the specimens are *C. travancoricus*. *Pseudogobiopsis oligactis* is a small gobioi species previously known only from the East flowing Krishna and Cauvery basins in India. We collected a number of specimens from the Mandovi and Zuari basins in Goa, which represent the first records of this genus from the region (Fig. 49c.).

The cobitid loach *Pangio goaensis* was described by Tilak in 1972, based on a single specimen collected from the river at Colem (a tributary of the Mandovi). The species has remained elusive since then. Amongst the specimens collected in the survey of freshwater habitats of Goa was a specimen of *P. goaensis*. Thus, it may be considered as the species rediscovered after 40 years! (Fig. 49d).

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

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


**Funding Agency:** Institutional

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![Fig. 49: New records and rediscovered species from Goa: (a) H. jerdoni (b) C. travancoricus (c) P. oligactis (d) P. goaensis](image)

| Table 8. Shanon-Weiner diversity index for various sampling locations |
|-------------------------|---|---|---|---|---|---|---|---|---|
| Sampling locations      | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  |
| Total no. of specimens sampled | 83 | 97 | 92 | 110| 114| 157| 132| 79 | 27 |
| Shanon-Weiner diversity Index | 2.554| 2.429| 2.022| 2.566| 2.515| 2.717| 2.598| 2.276| 1.441 |
Several aquatic resources in the country are rich in aquatic biodiversity but have not been explored adequately. Mahanadi river basin including its tributaries are among such rivers. Explorations were continued in the upper basin of River Mahanadi starting from its origin and covering its entire length (approx. 450 km.) in the Chhattisgarh state, including its tributaries joining in this stretch. Three seasonal explorations were undertaken during pre-monsoon, post-monsoon and winter seasons, for exploration and documentation of fish diversity (Fig. 50). A total of nine sites of River Mahanadi and ten sites of its six tributaries and sub-tributaries namely, Sheonath, Pairi, Sondur, Maniyari, Arpa and Lilagar, were covered in these exploration studies. Besides, 12 new sites were identified in Mahanadi, Sheonath, Hasdeo, Jonk, Pairi, Mand, Kelo, Ghumaria and Sondur rivers in upper Mahanadi river basin.

A total of 63 fish species belonging to 20 families of 8 orders were recorded from River Mahanadi and its tributaries during explorations. Family Cyprinidae represented 40% of the fish diversity, followed by Bagridae (10%), Schilbidae (8%) and Channidae (6%) (Fig. 51). Maximum species richness was recorded in the middle and lower stretches of Mahanadi at Mudhena, Pahanda and Chandrapur ghat in Raigarh district, and at Nand Ghat and Paiser ghat in River Sheonath. Selected biological parameters of prioritized species were collected during exploration (Fig. 52) and seasonal primary data on habitat parameters from selected sites were also recorded (Fig. 53). Analysis of seasonal abundance of fish diversity and biological parameters is in progress. A total of 365 tissue samples (blood and muscle) were collected for further analysis.

Fig. 50: Exploration of fish diversity in Mahanadi river basin

Fig. 51: Family-wise fish diversity in River Mahanadi and its tributaries

Fig. 52: Collection of tissue samples and biological parameters from River Mahanadi

Fig. 53: Recording of habitat parameters from River Mahanadi
A field survey of the Dah Tal (UP) was conducted to explore fish germplasm resources. This lake is located at about 26 km from Ballia on Bansdih-Maniar Road. Fishermen of about 16 villages are involved in fishing activities of the lake with Halpur being the main village. Fishes are caught in gill-net, dragnet kulia jal, etc., (Figures 54-55). Capture fishery of the Tal is dominated by Cypriniformes (36%) of which the Indian major carps comprising 15-20% and minor carps 2-5%. Catfish and featherbacks constitute 35-40%, live fish 5-10% and forage fish 15-20%. Fish catches were dominated by Catla catla, Labeo rohita, L. calbasu, Cirrhus mrigala, Sperata seenghala, M. vittatus, Channa punctatus, C. marulius, C. striatus, C. gachua, Puntius ticto, P. sampa, Oxygaster bacaica, Chanda ranga, Mastacembalus armatus, Colisa fasciatus, Xenentodon cancila, Heteropneustes fossilis, Clarias batrachus, Pseudotropius atherinoides, Amphipnous cuchia, Wallago attu, Pangasius spp., Bagarius bagarius.

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

Project Period: September, 2009 - August, 2013


Funding Agency: GEF/ NAIP

Exploration and documentation of fish genetic resources was pursued under the given objective to enhance knowledge of fish species through characterization of available gene pool. The study areas represent distinct agroecosystems with specific fish bioresource components. Exploratory surveys were conducted in various rivers/streams/reservoirs/ lakes of three districts Chamba (H.P.), Udaipur (Rajasthan) and Adilabad (A.P.) (Fig. 56). In district Udaipur, explorations of fish germplasm were conducted for 6 river/streams (Beltz, Jhakham, Bari, Tidi, Madar ka naka and Sisorama) and Jaismand lakes. Godavari drainage system including river Godavari and its nine tributaries and reservoirs were explored in district Adilabad.
The fish biodiversity and habitat data were statistically analyzed and overlaid on GIS maps. The computation of different diversity indices indicated species abundance and richness pattern in different parts of the aquatic habitats and also preferred habitat characteristics with high diversity. In all, a total of 2966 accessions including multiple voucher accessions were collected and 991 accessions for 82 species were characterized through generation of 1960 barcode sequences and their subsequent analysis in this project (Table 9). Voucher accessions collected were also analyzed for 82 species, for morphological, biological and genetic characterization. DNA Barcoding (Cytochrome c oxidase I, Cytochrome b, and ATPase 6 & 8 mitochondrial DNA genes) was used to delineate species and to develop taxonomically reliable reference. These explorations confirmed two new species, which are new to science (*Labeo icarae* sp.nov. and *Rita* sp. nov. Fig. 57); putative possibility of finding four more new species (*Silonia* sp.nov., *Glossogobius* sp.nov., *Pangasius* sp.nov. and *Cara* sp. nov.) and new extended distribution of four species (*Tor tor, Garra orientalis, Labeo dero* and *L. dyocheilus*) in the rivers beyond the range known until now. The potential cultivable indigenous fish species were identified in three districts, for technological interventions, integration in production system with an aim to conserve as well as utilize these resources. Length-weight relationship and condition factor of 57 fish species were analyzed.

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

**Project Period:** February, 2013 –March, 2015

**Project Personnel:** A.K Pandey (PI)

**Funding Agency:** UP State Biodiversity Board

Ramgarh Lake, a natural oxbow-lake formed by River Rapti, is situated to the southeast of Gorakhpur (U.P.) and covers an area of about 723 ha with the catchment area around 653 ha, out of which 494 ha

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**Table 9: Summary statement of explorations, activities and new information generated in the three study areas**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Adilabad</th>
<th>Chamba</th>
<th>Udaipur</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rivers and other waterbodies explored for fish germplasm</td>
<td>Penganga, Godavari, Pranahita, Sriram, Dam and Kondapur, Chityal river, Wardha River</td>
<td>Satnala, Kadam, Seva, Syul, Dunali Chamera, Chandunala, Dali Khad, Bhatikkhad, Mahla ki Khad Nainikkhad, Shahikoti, and Tessa khad</td>
<td>Ravi, Sahoo, Bhandal, Chandan, Bijal, Ranjeet Sagar, Silargar stream, and, Dali Khad, Som, Mansi, Phalasia, Banas, Jaismand, Wakal, Fathesagar, Badi, Nadeshwar &amp; Tidi. Sukher Ka nala, Beltz, Sisorma, Kakdar, Daya, and Namanda Dam, Jhakham, Beltz, Oda</td>
</tr>
<tr>
<td>Sites of exploration</td>
<td>41 sites (Altitude 311-1118 ft)</td>
<td>35 sites (1800 - 8000 ft)</td>
<td>33 sites (900 -2709 ft)</td>
</tr>
<tr>
<td>No. of fish species and Accessions collected</td>
<td>63 sp; 1110 accessions</td>
<td>49 sp.; 993 accessions</td>
<td>19 sp.; 863 accessions</td>
</tr>
<tr>
<td>Parameters used for biological characterization</td>
<td>Morphometrics, Meristics, Truss Network Analysis, Length-Weight relationships, Condition factor, Maturity stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potential native species identified for captive propagation</td>
<td><em>Labeo finnibrians</em>, <em>L. calbasu</em>, <em>L. dero</em>, <em>L. dyocheilus, Clarias batarachus</em></td>
<td><em>Labeo gonius, L. icarae</em>. <em>Puntius sarana</em></td>
<td><em>L. dero</em></td>
</tr>
</tbody>
</table>
land is under Gorakhpur Development authority (GDA). Bichhia nullah (7 km), Gurdhuia nullah (10 km), Mohaddipur nullah (4 km), Daudpur nullah (12 km) and 10 numbers of mini-ducts (8 km) drain into this water body. Average depth of the lake is 2.4 meter which increases in rainy season.

Ramgarh Lake was surveyed and four stations were selected for recording physico-chemical parameters (Fig. 58). Fish catches were dominated by *Catla catla, Labeo rohita, Cirrhinus mrigala, Notopterus notopterus, Gadusia chapra, Setipinna phasa, Pseudotropius atherinoides, Heteropeustes fossilis, Clarias batrachus, Sperata seenghala, S. aor, Mystus vittatus, Channa punctatus, C. marulius, C. striatus, C. gachua, Colisa fasciatus, Wallago attu, Pangasius pangasius, Bagarius bagarius, Mastacembalus armatus, Amphipnus cuchia, Xinantodon cancila, Puntius ticta, P. sarana, Oxygaster bacaila, Chanda ranga and Chanda nama* (Figures 59-60).

Fishes are sold fresh locally in three main fish markets located near Kuraghat, Dharamsala and Padari Bazar. Small-sized fishes were sun-dried too. Bakhira Lake has good population of murrels, *Channa punctatus, C. striatus* and *C. marulius* though they have declined drastically in other freshwater bodies.

Gill, kidney, intestine and gonads of the commercially important fishes were collected from Ramgarh and Bakhira lakes, and processed for histopathological changes occurring in these vital organs. Preliminary observations revealed not much of deviation in these organs of the fishes inhabiting both the lakes except where the lake is receiving waste/sewage water and is infested with weeds (Fig. 61). In such stations, only air-breathing fishes like *Channa punctatus, Heteropeustes fossilis, Clarias batrachus, Anabas testudineus* and *Amphipnous cuchia* were observed.

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


Project Personnel: A.K. Pandey (PI)

Funding Agency: UPCST

Maturity States in *M. rosenbergii*

Female *M. rosenbergii* possessed paired ovaries located dorsally to the stomach and hepatopancreas in cephalothorax region. They gave paired oviducts.
which opened into gonopores on basal segment of the third pleopods. Morphologically, ovaries showed marked variations in relation to maturity. According to size of ova, position as well as size of nucleus, yolk deposition and distribution, maturity of the female M. rosenbergii was divided into the following five phases:

- (i) Oocyte in the first stage of meiotic prophase.
- (ii) Oocyte in the perivitellogenesis.
- (iii) Oocyte in the primary vitellogenesis.
- (iv) Oocyte in the secondary vitellogenesis and
- (v) Ripe eggs (Table 10). It was commonly observed that ovaries in each stage contained eggs of more than one stage but high proportion of ova were in a single maturity stage.

(i) Immature stage: At this stage, females could be recognized by clear, transparent ovary inside the rostrum. It was small in size just arising at the junction between carapace and first abdominal somite. Gonadosomatic index (GSI) of the animal was 0.63±0.26. Histologically, oocytes were in the first stage of meiotic prophase. They were small, round and irregular cells with large cytoplasm. Maximum size of oocytes was 19 mm and minimum 6 mm with an average of 11.54±0.25 mm and standard deviation 2.30 mm. Generally, weight of the animal in this stage was <20 g.

(ii) Previtellogenic stage: At this stage, animal could be recognized by development of colouration and increase in size of the ovary. Pigmentation (light orange or yellow) in the ovary was noticed inside the rostrum. The GSI of the prawn was 2.28±0.45. Development of ovaries towards rostrum was clearly visible. Histological observations showed that maximum and minimum size of oocytes were 70 and 30 mm, respectively with mean value of 55.52±1.89 mm and standard deviation 13.42 mm. Average weight of the animal in this stage ranged between 20-25 g.

(iii) Primary vitellogenic stage: At this stage, ovaries could be distinguished by deep orange colour. There was marked increase in size and extended anteriorly upto base of the rostrum. The GSI of the animal was calculated as 4.70±1.52. Maximum and minimum sizes of the oocytes were 160 and 100 mm, respectively. Mean ova diameter was 128.70±2.69 mm with standard deviation 14.99 mm. The cytoplasm was acidophilic at the periphery but was still basophilic around the nucleus. The animals weighed between 25-30 g.

(iv) Vitellogenic stage: This stage was recognized by considerable increase in the ovarian size as it extended upto first spine of the rostrum. It was clearly visible from outside as deep orange mass. GSI was computed as 6.62±1.08. Maximum and minimum sizes of oocytes were 400 and 250 mm, respectively. Mean value and standard deviation were recorded 347.0±5.29 and 39.27 mm, respectively. Heavy accumulation of yolk globules was observed in the oocytes. The cytoplasm of oocytes displayed acidophilic character. Weight of prawn during this stage ranged between 30-40 g.

(v) Ripe (mature) stage: In this stage, ovary was large, deep orange in colour, extended anteriorly upto base of second and third spine of the rostrum. Ovary could be recognized as bulky mass beneath the rostrum. GSI of the animal was computed as 6.79±1.10. Maximum and minimum sizes of the oocytes ranged between 700 and 180 mm, respectively. Average ova diameter was 544.0±15.29 mm with standard deviation 155.59 mm. The animal weighed > 40 g during this stage.

**Eyestalk X-organ sinus gland complex**

The optic peduncle within the stalked eye of M. rosenbergii consisted of three ganglionic formations such as medulla terminalis (MT), medulla interna (MI) and medulla externa (ME). Among the three, medulla terminalis contains a cluster of cells bodies called medulla terminalis X-organ or simply X-organ. A neurohaemal organ associated with the X-organ is the sinus gland located at the peripheral junction between the medulla externa and medulla interna. This gland is formed by the cluster of neurosecretory axonal processes which opened into gonopores on basal segment of the third pleopods. Morphologically, ovaries showed marked variations in relation to maturity. According to size of ova, position as well as size of nucleus, yolk deposition and distribution, maturity of the female M. rosenbergii was divided into the following five phases:

<table>
<thead>
<tr>
<th>State of Female</th>
<th>No. of Samples</th>
<th>GSI</th>
<th>Oocyte Measured (N)</th>
<th>Mean of Oocyte (µm)</th>
<th>Standard Error of Mean</th>
<th>Standard Deviation</th>
<th>Maximum Oocyte Size (µm)</th>
<th>Minimum Oocyte Size (µm)</th>
<th>Population Mean (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature</td>
<td>10</td>
<td>0.63±0.26</td>
<td>88</td>
<td>11.54</td>
<td>0.24</td>
<td>2.30</td>
<td>19</td>
<td>6</td>
<td>11.54±0.24</td>
</tr>
<tr>
<td>Previtellogenic</td>
<td>10</td>
<td>2.28±0.45</td>
<td>50</td>
<td>55.52</td>
<td>1.89</td>
<td>13.42</td>
<td>70</td>
<td>30</td>
<td>55.52±1.89</td>
</tr>
<tr>
<td>Primary vitellogenic</td>
<td>10</td>
<td>4.70±1.32</td>
<td>50</td>
<td>128.70</td>
<td>2.69</td>
<td>14.99</td>
<td>160</td>
<td>100</td>
<td>128.70±2.69</td>
</tr>
<tr>
<td>Vitellogenic</td>
<td>10</td>
<td>6.62±1.08</td>
<td>55</td>
<td>347</td>
<td>5.29</td>
<td>39.27</td>
<td>400</td>
<td>250</td>
<td>347.0±15.29</td>
</tr>
<tr>
<td>Mature</td>
<td>10</td>
<td>6.79±1.10</td>
<td>50</td>
<td>544</td>
<td>22.0</td>
<td>155.59</td>
<td>700</td>
<td>180</td>
<td>544.0±22.0</td>
</tr>
</tbody>
</table>
terminalis arising chiefly from the perikaria of the X-organ. Neurosecretory cells (NSCs) are modified neurons which in addition to usual characteristics (axons, dendrites, Niss substances, neurofibrils etc) show stainable material to specific dyes indicative of secretory activity. The neurosecretory cells occurring in eyestalk of *M. rosenbergii* along with the characteristics have been summarized in Table 11.

**Brain and Thoracic Ganglia**

Nervous system of *M. rosenbergii* consisted of a large supra-oesophageal ganglionic mass (brain) and a ventral nerve cord with a pair of ganglia corresponding to each embryonic somite. The ganglia were joined longitudinally by connectives and transversely by commissures. The nerve cord passing through thoracic region is known as thoracic ganglia. In brain and thoracic ganglia of female *M. rosenbergii* five categories of cells such as giant neuron (GN), A, B, C and D were encountered at different stages of maturity (Table 12).

The first category of cells in brain and thoracic ganglia of *M. rosenbergii* was giant neuron (GN) having cell and nuclear diameter >80 and 20-40 mm, respectively. They were oval or round in shape with or without axon. These cells though very few in number were mostly confined to the peripheral region but also seen in the middle area. They displayed marked changes in cell size, number of nucleolus, granulation and vacuolization as well as migration of secretory granules towards axonal portion. Cell and nuclear diameters of the second group cells (A cells) ranged between 60 - 80 and 15 - 36 mm, respectively. They were oval or round in shape and located mostly in middle region of the brain. A type of cells are more in number than giant neurons in brain ad thoracic ganglia. They showed marked histological changes such as increase in size and number of nuclolus as well as

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Cell diameter (µm)</th>
<th>Nuclear diameter (µm)</th>
<th>Shape</th>
<th>Staining intensity</th>
<th>Other features</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>30-35</td>
<td>10-12</td>
<td>Round/oval</td>
<td>+++</td>
<td>Largest cells found in the eyestalk in medulla externa region, few in number, orange or red in Mallory’s triple.</td>
</tr>
<tr>
<td>B</td>
<td>20-25</td>
<td>6-10</td>
<td>Round/oval</td>
<td>++</td>
<td>Medium sized cells in medulla interna and medulla terminalis, few in number, orange or blue in colour.</td>
</tr>
<tr>
<td>C</td>
<td>15-20</td>
<td>&lt;5</td>
<td>Round</td>
<td>++</td>
<td>Small in size, abundant in number, found in all the regions in bunch, orange, blue or red in colour.</td>
</tr>
<tr>
<td>D</td>
<td>10-15</td>
<td>_</td>
<td>Round</td>
<td>++</td>
<td>Small in size, abundant in number, mostly in medulla interna and terminalis, orange, blue or red in colour.</td>
</tr>
<tr>
<td>E</td>
<td>&lt;10</td>
<td>_</td>
<td>Round</td>
<td>++</td>
<td>Smallest in size, nucleus not visible, abundant in number, orange or blue in colour.</td>
</tr>
</tbody>
</table>

**Table 11. Different types of NSCs in the eyestalk of the female *M. rosenbergii***

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Cell diameter (µm)</th>
<th>Nuclear diameter (µm)</th>
<th>Shape</th>
<th>Staining intensity</th>
<th>Other features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giant neurons</td>
<td>80-120</td>
<td>20-40</td>
<td>Oval or round</td>
<td>++ to ++++</td>
<td>Very few in number, giant cells having bigger nucleus, with or without axons, stain very deeply in Mallory’s triple.</td>
</tr>
<tr>
<td>A</td>
<td>60-80</td>
<td>15-36</td>
<td>Oval or round</td>
<td>++ to ++++</td>
<td>Large cells with comparatively large nucleus, with or without axons, granulated in secretory phase, more in number, generally red colour in Mallory’s stain.</td>
</tr>
<tr>
<td>B</td>
<td>40-60</td>
<td>15-25</td>
<td>Oval or round</td>
<td>+ to +++</td>
<td>High in number, with or without axons less secretory activity and granulation, red in colour in Mallory’s stain.</td>
</tr>
<tr>
<td>C</td>
<td>20-40</td>
<td>7-15</td>
<td>Oval to round</td>
<td>+ to +++</td>
<td>Very high in number, with or without axons, secretary activity and granulation is less, red in colour in Mallory’s stain.</td>
</tr>
<tr>
<td>D</td>
<td>5-20</td>
<td>2-10</td>
<td>Oval to round</td>
<td>+ to +++</td>
<td>Very numerous, with or without axons cytoplasm content is very less, nucleus prominent, secretory activity is very less, faint red in colour in Mallory’s stain.</td>
</tr>
</tbody>
</table>
staining intensity at different maturity stages. Migration of secretory granules towards axons has also been noticed in matured specimens of *M. rosenbergii*. The third group of cells (B cells) were more in number in comparison to giant neurons and A cells. They were oval or round in shape with or without axons. Their size ranged between 40-60 mm with bigger nucleus (15-25 mm). Histological changes were noticed in these cells in relation to ovarian maturation. The fourth group of neurosecretory cells (C cells) were having size between 20-40 mm and nuclear diameter 7-15 mm with scant cytoplasm. Cells were round with or without axon. They exhibited less secretory activity as compared to GN, A and B cells. Last type of cell (D cells) were axon. They exhibited less secretory activity as compared to GN, A and B cells. Last type of cell (D cells) were more in number with size range between 5-20 mm and nuclear diameter 2-10 mm. They were oval to round in shape and appear to show less secretory activity in relation to ovarian maturation.

4.4 Exotics, Quarantine and Fish Health Management

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

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

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

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

**Innate immune response of Indian major carp, Labeo rohita infected with oomycete pathogen Aphanomyces invadans**

In the present study, sequential changes in various innate immune parameters were monitored following experimental infection with *A. invadans* in *L. rohita*. One hundred eighty fishes were injected intramuscularly with 0.1 ml of spore suspension of *A. invadans* and distributed in FRP tanks (30 fish/tank). Similarly, 180 fish were injected with 0.1 ml autoclaved pond water and they served as control group. Furthermore, ten fish injected with same concentration of zoospore were kept in a separate tank for observing the mortality pattern. Five fish from each tank (both control and infected) were randomly selected and sampled on different days post infection (dpi). At each sampling day, 30 fish from control and infected groups were bled and an aliquot of the blood was heparinized (50 IU ml\(^{-1}\)) whereas, the remaining part was used for collecting serum and used for monitoring innate immunity parameters. Furthermore, at each sampling day, muscle tissue from five fish from control and infected group each were preserved in 10% neutral buffered formalin.

In experimentally infected fish, gross lesions characterized by slight swelling and redness were observed at the site of injection which progressed to severe swollen haemorrhagic areas on both sides of the body by 18 dpi. At the end of experimental period of 24 days, more severe gross lesions were observed and the fish were swimming inactively on the surface. The fish which were kept in separate tank for observing the mortality pattern showed 100% mortality by 25 dpi. However, no gross lesions and mortality were observed during the course of experiment in the control group. Histopathological examination of the infected fish indicated many aseptate hyphae at the injection site and floccular degeneration of the muscle fibres at 3 dpi (Fig. 62 b). At 6 and 12 dpi, severe myonecrosis of the large areas of the myotome was observed on the injected side and many hyphae had reached the non-injected side (Fig. 62 c, d). At the site of injection, although the hyphae were encapsulated by granuloma (Fig. 62 e), but no inflammatory response was observed around most of the hyphae away from site of injection (Fig. 62 f). At 18 and 24 dpi, there was extensive myonecrosis in large areas of myotome on both injected and non-injected sides and there was massive proliferation of hyphae (Fig. 62 e). These extensive pathological changes were always associated with severe swollen hemorrhagic areas. In the control group, no gross and histopathological lesions were observed during the course of experiment.

The respiratory burst activity of the infected group sampled at different dpi was higher from the control group and at advanced stages of infection, particularly from 12 dpi, this activity was significantly higher (*p*<0.05) from the control group (Fig. 63 a). The myeloperoxidase activity of serum of the infected group was higher from 6 dpi, compared to that of control group during the experimental period, however, the differences were not statistically significant (*p*>0.05) (Fig. 63 b). ACH50 activity of the infected group increased gradually along with progression of infection. The activity from 12 dpi was significantly higher (*p*<0.05) from that of the infected group at earlier stages of infection and also from the control group (Fig. 63 c). The total serum protein and albumin levels in the
infected group increased significantly ($p<0.05$) from 12 dpi in comparison with infected fish at earlier stages of disease and also from the control group (Fig. 63 d, e, f). However, no significant differences ($p>0.05$) in serum globulin levels were observed between the infected and control group during the course of infection.

From 6 dpi, the serum lysozyme activity of the infected groups was significantly lower than that of control group and infected group at 1 and 3 dpi (Fig. 63 g). The lowest lysozyme activity was observed at 24 dpi. Similarly, the antiprotease activity declined gradually with progression of infection in the infected group and activity was lowest at 24 dpi. From 6 dpi, the antiproteases activity of the infected group was significantly lower ($p<0.05$) compared to that of control group (Fig. 63 h). The activity of $\alpha$-2 M of the infected group at advanced stages of infection (18 and 24 dpi) was significantly ($p<0.05$) lower from the control group and also from the infected group of fish at earlier stages of infection (up to 12 dpi) (Fig. 63 i). No significant differences were observed in any of the non-specific immunity parameters in control group sampled at different dpi.
Project Title: Isolation and characterization of Flavobacterium species from fish and aquatic environment

Project Period: August, 2006 – March, 2014

Project Personnel: P.K. Pradhan (PI)

Funding Agency: NBAIM, ICAR

Freshwater common gold fish (average size 10.56±1.3 cm), showing ‘saddle back appearance’ and yellowish deposition on gills were collected (Fig. 64). Cytological examination of smears from skin and fins revealed typical bacterial haystacks. Representative skin and gills samples from affected fish were processed for histopathological examination. In the sections, long slender rods were observed. White deposits from skin surface were streaked on Shieh agar and incubated at 28°C for 72 h. Yellow rhizoid, adherent to agar surface colonies with spreading margins were sub-cultured and Gram stained to check purity. The isolate was identified as *F. columnare* strain (ING-1) on the basis of biochemical tests.

For the 16S rDNA based identification genomic DNA was amplified in PCR using the bacterial universal primers, 27F and 1492R. PCR product was purified and sequenced. The 16S rDNA sequence of ING-1 showed highest (e”99%) sequence similarity with *F. columnare* strains RDC-1. It also showed higher similarity with other published *F. columnare* strains isolated from different geographic locations. The sequence data were compiled, nucleotide substitution rate was calculated and a distance matrix tree was constructed by the neighbor-joining method. Phylogenetic tree based on the 16S rDNA sequences also showed the clustering of the strain ING-1 with the other *F. columnare* strains belonging to Genomovar II (Fig. 65).
Species-specific PCR confirmation was made by using *F. columnare* specific primer pairs. Both the primer sets (ColF/ColR & FvpF1/FvpR1) produced specific amplicons of 675 bp and 1193 bp, respectively. Furthermore, genomovar of the strain was confirmed by 16S rDNA PCR-RFLP (Fig. 66). The restriction profile of the strain ING-1 corresponded to Genomovar II which is the most virulent genomovar amongst 3 Genomovars of *F. columnare*. To determine the virulence potential of the isolate, bath immersion method was conducted. In bioassay, gold fish strain ING-1 was found to be pathogenic for rohu fingerlings in bath immersion challenge. *F. columnare* was re-isolated from the gills of moribund experimental fingerlings fulfilling Koch’s postulates, and it indicates that this pathogen was responsible for the mortality.

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

**Project Period:** April, 2011 - March, 2015

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

**Funding Agency:** Institutional

Biomarkers have been defined as “a change in biological response, which can be related to exposure or toxic effect of environmental chemicals”. Biomarkers are measurable and directly reflect the condition of the animal or the natural resources especially in rivers. Biomarkers can provide a better assessment of the physiological effects and bioavailability of xenobiotic compounds found in aquatic habitats. The present study was undertaken to develop biomarkers as an assessment tool of fish health status in river Yamuna to assess the effects of PCBs compounds which are known to exhibit carcinogenic, apoptogenic, mutagenic and endocrine-disrupting activities.

Fish samples in the form of muscle, gill, liver, spleen, gonads, blood and blood serum, along with length weight measurements, were collected from Auriya, Fatehpur and Sadiyapur in Allahabad. To understand the effect of exposure to PAHs, a set of fishes as control were exposed to known concentrations of PAHs and sampling was done. To estimate the effects of xenobiotics present in the river in comparison of control and experimentally exposed fishes, the cytochrome P450 family which metabolizes these effects, were targeted for expression studies. The expression of selected xenobiotics on cytochrome P450s (1A1, 1A2, 2B6) was found to be down regulated significantly in all the tested organs in the fishes collected from polluted zones of river Yamuna namely, Sadiyapur, Sultanpur and Auriya, when compared with the constitutive expression levels of CYPs in the

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**Fig. 64:** Diseased ornamental gold fish *Carassius auratus* (L.) showing saddleback appearance: A presumptive clinical sign of columnaris disease caused by *F. columnare*. Arrow indicates the dense mass of the bacteria

**Fig. 65:** 16S rDNA gene based phylogenetic analysis of *F. columnare* strain ING-1 showing phylogenetic relationship with other strains reported from India and abroad the dendrogram was generated by the MEGA v 5.2 using the Neighbour-joining method with 1000 replicate bootstraps

**Fig. 66:** Genomovar confirmation of the ornamental gold fish isolate of *F. columnare* strain ING-1 by restriction digestion of amplified 16S rDNA with *Hae* III enzyme

Lane M: Express DNA ladder (Fermentas); Lane 1: Strain ING-1 (genomovar II); Lane 2: Strain RDC-1 (genomovar II); Lane 3: reference strain LMG-10406 (Genomovar I)

by 16S rDNA PCR-RFLP (Fig. 66). The restriction profile of the strain ING-1 corresponded to Genomovar II which is the most virulent genomovar amongst 3 Genomovars of *F. columnare*. To determine the virulence potential of the isolate, bath immersion method was conducted. In bioassay, gold fish strain ING-1 was found to be pathogenic for rohu fingerlings in bath immersion challenge. *F. columnare* was re-isolated from the gills of moribund experimental fingerlings fulfilling Koch’s postulates, and it indicates that this pathogen was responsible for the mortality.
respective organs of control fishes. The expression of CYPs was maximum in gills, followed by liver, gonads and brain and the down regulation in the expression of CYPs was well correlated with the exposure levels at the collection sites of fish samples in Yamuna river (Figures 67-71).

The results showed well concurrence with laboratory data generated under both exposed and unexposed conditions. Unlike the hypothesis/anticipation, there was a significant reduction in the expression of all the tested CYPs, which was possibly the antagonistic effects of heavy metals/high concentration of pesticides, or any other environmental pollutant. It was also found that gills are more sensitive to xenobiotics than any other organs i.e., liver, brain, lungs, etc. Further, more samples are to be collected from more polluted sites viz, Mathura, Agra and Delhi and analysed to get results and correlate with the available results.
Endocrine disrupting compounds (EDCs) encompass a variety of chemical classes including natural and synthetic hormones, plant constituents and pesticides, compounds used in the plastics industry and in consumer products, other industrial by-products and pollutants. These ECDs ultimately find their way into the aquatic systems such as rivers causing their effects on fishes which are mainly carcinogenic, apoptogenic, mutagenic and endocrine-disrupting activities. These ECDs can, at the cellular level, induce endocrine disruption via a number of routes that involve steroid receptor binding (agonists), blocking steroid receptor binding (antagonists), or by disrupting the biosynthesis and or metabolism of steroids. Chemicals found in the waste outflows from pulp and paper mills and sewage treatment plants can affect reproduction and development in fish. Altered gonadal development, occurrence of ovotestis, induction of vitellogenesis in juvenile and male fish, reproductive abnormalities and reduced reproductive success are the main effects of such chemicals.

In the present study, the effects of endocrine disruption in the Gomti river, taking *Labeo rohita* as a test fish, was studied along with the assessment of presence of EDCs in the Gomti River. During the reporting period, the availability and abundance of *L. rohita* captured from Sitapur to down stream of Gomti river in Ghazipur was assessed. In total 12 sampling stations in Lucknow, two at Sitapur, three at Jaunpur, two at Ghazipur and three at Sultanpur were undertaken during the reporting period. The availability of *L. rohita* ranged from 7 to 12% of the total catch in data collected from sampling stations. In general, it was observed that the catch of *L. rohita* was very low as compared to earlier reports at different sampling locations along the river indicating that the fish is under stress. Different physico-chemical parameters viz., temperature, pH, DO, CO₂, alkalinity, hardness, conductivity and some heavy metals Cu, Pb, Cd, Ni, etc., of Gomti River water at selected sampling stations were also recorded. Biometrical parameters of 953 specimens of *L. rohita* were collected from the selected locations along the Gomti River (Fig. 72). The RNA/DNA ratio of specimens collected from different locations was determined to assess the health status of the *L. rohita* specimens exposed to EDCs (Fig. 73). The collected specimens were subjected to GSI estimations and histological examinations of gonads (Fig. 74). Hormonal assessment for testosterone, oestradiol and vitellogenin of the wild caught specimens of *L. rohita* was conducted and elevated level of serum vitellogenin was found particularly in males (Fig. 74).
**Project Title:** Risk and benefit assessment of an illegally introduced fish species *Piaractus brachypomus*, pacu in India

**Project Period:** October, 2013 - March, 2015

**Project Personnel:** A.K. Singh (PI upto 28 February, 2014), Peyush Punia (PI w.e.f. 28 February, 2014)

**Funding Agency:** NFDB

The present study was undertaken to study the risk and benefit assessment of illegally introduced *P. brachypomus* in India. To assess the present distribution and culture status of pacu, survey was carried out and data was collected in the states of West Bengal, Andhra Pradesh and Uttar Pradesh. Seven farms located in Lucknow, Kanpur, Azamgarh, Unnao, Barabanki, Akbarpur, and Kanpur Dehat, which are undertaking the culture of pacu, were visited and the culture system and production was observed. Pacu was found to be cultivated in monoculture as well as polyculture with *P. hypophthalmus*, *C. gariepinus* and Indian major carps *C. catla, L. rohita* and *C. mrigala*. Data was also collected on many important aspects like survival, pond conditions, growth, and feeding habits, etc., in pacu culture. The fish farmers obtained fish seed of pacu from West Bengal which is then locally stocked and distributed to the local farmers for culture. The bulk of the seed is produced in fish hatcheries/farms at Naihati in West Bengal which were visited for data collection. The information provided by the bulk producers revealed that every season 1-3 pacu truck seed is transported to Andhra Pradesh, Tripura and Assam for culture. The pacu is also having an ornamental fish trade and is also being sold in aquarium shops around the country. To understand the impact of this species on aquarium trade, 25 aquarium shops in Lucknow were surveyed for availability of pacu. It was found that all the aquarium shops in Lucknow were having pacu. These fishes were red belly pacu and black pacu, however, red belly pacu was available at majority (95%) of aquarium shops. In West Bengal, 11 aquarium shops in Howrah area were surveyed to find out availability of pacu on aquarium shops. It was recorded that pacu was available on all the aquarium shops visited in Howrah. This indicated that pacu is being sold in most of the aquarium shops as an ornamental fish and probability of this fish entering into natural water bodies is high due to escape or discarding of live fish.

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

**Project Period:** April, 2010 - March, 2014

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

**Funding Agency:** Institutional

**Molecular diagnostic assays for protozoan parasites**

*Ichthyophthirius multifilis*

Small pieces of heavily infected fins or gills (25–75 mg) preserved in 85% ethanol are suitable for DNA extraction from *Ichthyophthirius multifilis* infected tissue. DNA extraction can be done from *I. multifilis* isolates also. The phenol chloroform extraction technique is used for DNA extraction. PCR of the *I. multifilis* DNA was performed using following primers of the 18ssDNA:

- IMRIForward : AGTGACAAGAAATAGCAAGCCAGGAG
- IMRIReverse : ACCCAGCTAAATAGGCAGAAGTTCAA

PCR reaction is performed in a final volume of 50 µl, 30 µg/ml template DNA with 30 pmol of each primer, and 2.5 U *Taq* polymerase under the optimized amplification conditions. The PCR product is examined in 1% agarose gel through electrophoresis (Chen et al., 2008).

The sequencing of PCR product was done by outsourcing. The PCR product size was 200 bp. The aligned sequences were of 165bp and 175bp, respectively. The sequences were verified as *I. multifilis* by Genebank BLAST search. The determined sequences of *I. multifilis* were submitted to Genebank with following respective accession numbers; EF469202.1; U17354.1; KC512768.1. The 18S rRNA sequences for the outgroups *Ophryoglena catenula* and *Tetrahymena sp.* were retrieved from Genebank using respective accession numbers; U17355.1; HE820726.1; M26360.1; AF364041.1.

**Myxobolus sp. and Hennegua sp.**

DNA extraction of *Myxobolus sp.* isolated from gills and kidney of fishes was done. PCR amplification of DNA was done using primers the following primers:

- MeerForward: CCCCCTCGCTACTACCCGGAGT
- McerReverse: GATCTTCCCGCACGTTCAC
- McForward: AGACACTGGGAGGTGGTGAC
- McReverse: CACTGCGTGATCCAACTACG
- MyForward: TAATCCCGGTAACGAACGAG
- MyReverse: CGTCCTCGCAACAAACTGTA
The amplified product of *Myxobolus* sp. isolated from *Clarias batrachus* was of 200 bp size and showed 99% similarity with *Myxobolus articus* from 18s ribosomal RNA gene, partial sequence (Accession no. JN003830.1), and *Myxobolus articus* from Canada 18S ribosomal RNA gene, partial sequence (Accession no. JN003829.1).

DNA extraction and PCR amplification of *Hennegua* sp. was done in the same manner as above. The PCR product of 970 bp confirmed the *Hennegua* sp.

**Molecular diagnostic assays for monogenean parasites**

DNA isolation from *Dactylogyrus* and *Gyrodactylus* sp. was done through the technique given by Simkova et al (2004) and Cunningham et al. (2001). Isolated parasites were counted and preserved in 95-100% ethanol. For DNA extraction, lysis of parasites was done with lysis buffer. This lysate is used as the DNA template in PCR amplification, without further purification. PCR amplification of *Dactylogyrus* sp. DNA was done using various primers (Simkova et al 2003; Sinnappah et al 2001; Littlewood 1998). The PCR product was examined on 1% agarose gel and PCR products of 900 - 1100 bps confirmed *Dactylogyrus* spp.

Sequencing of PCR products was done by outsourcing. Following ribosomal gene sequences were deposited in NCBI database:

1. EU643632
   
   *Dactylogyrus inexpectatus* 18S ribosomal RNA gene and internal transcribed spacer, partial sequence; *Carassius auratus*
   
   gi|189916546|gb|EU643632.1|189916546

2. EU643633
   
   *Dactylogyrus ctenopharyngodonis* 18S ribosomal RNA gene and internal transcribed spacer, partial sequence
   
   gi|189916547|gb|EU643633.1|189916547

3. EU643634
   
   *Dactylogyrus eucalius* 18S ribosomal RNA gene and internal transcribed spacer, partial sequence
   
   gi|189916548|gb|EU643634.1|189916548

4. EU643635
   
   *Dactylogyrus ctenopharyngodonis* internal transcribed spacer 1, partial sequence
   
   gi|189916549|gb|EU643635.1|189916549

5. KF662475
   
   *Myxobolus arcticus* isolate INRA-Ma1 18S ribosomal RNA gene and internal transcribed spacer 1, partial sequence.

**Collection and screening of fishes, and isolation and identification of parasites**

Collection of selected fish species namely *Labeo rohita*, *Catla catla*, *Cirrhinus mrigala*, *Cyprinus carpio*, *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, *Carassius auratus*, *Puntius ticto*, *Clarias batrachus*, *C. gariepinus*, *Heteropneustes fossilis*, *Pangasius suchi*, *Mystus vittatus*, *Rita rita*, *Channa striatus* and *C. punctatus* was done from various ponds and river Gomti at Lucknow. Screening of fishes and isolation of parasites was done following the standard procedures.

The identified parasites were: *Ichthophthirius multifilis*, *Trichodina*, *Chilodonella*, *Hexamita*, *Plistophora*, *Myxidium*, *Hennegua*, *Ichthyobodo necatrix*, *Tetrhaumena*, *Epistylis*, *Heteropolaria colisarum*, *Oodinium* and various *Myxobolus* species (Fig. 76). Some new species of Monogeneans, *Dactylogyrus* and *Dactylogyrus* and other species like *Ancylodiscoidis Silurodiscoides*, *Bifurcohaptor*, etc., and adult and metacercarian digeneans viz., *Allocreadium*, *Diplostomum*; *Nematodes*; copepods like *Argulus*, *Lernaea*; cestodes and acanthocephalans and fungi were also observed in different organs of fishes.

**Title of Project: National Surveillance Programme for Aquatic Animal Diseases**

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

**Project Coordinator:** J.K. Jena

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

**Funding Agency:** NFDB
The Institute is coordinating a National Surveillance Programme on Aquatic Animal Diseases (NSPAAD) which is funded by National Fisheries Development Board (NFDB), Department of Animal Husbandry, Dairying and Fisheries (DAHDF), Ministry of Agriculture, Government of India. The programme is being implemented in 14 states of aquaculture importance with involvement of 22 fisheries research institutes of ICAR, Colleges of Fisheries and other colleges. More than 100 districts have been identified to be covered for regular monitoring and the remaining districts in the 14 selected states would be covered under passive surveillance by all the collaborating partners of the project.

A Technical Advisory Committee (TAC) under the Chairmanship of Jt. Secretary, DAHDF, Ministry of Agriculture, Govt. of India has been constituted by DAHDF for overall monitoring and supervision of the programme. The first meeting of the TAC was held in April 2013. The Project Launch Workshop was organised on 27-28 May, 2013 at NBFGR, Lucknow which was inaugurated by Dr. S. Ayyappan, Secretary, DARE and DG, ICAR. During the workshop, all the institutes presented the activities to be undertaken under the National surveillance programme. The issues of relevance viz., districts, species, diseases etc., to be covered by each collaborating partner, were discussed and finalized. The final project proposal along with budgetary allocation for all the collaborating partners was approved in 2nd TAC meeting in July, 2013.

For sensitization of all PIs and Co-PIs of all the collaborating centres, an Orientation Training Workshop was organised at NBFGR, Lucknow during 17-20 September, 2013 involving international resource persons Prof. Kenton Lloyd Morgan, University of Liverpool; Prof. C.V. Mohan, Research and Development Manager, NACA and Dr. Jiraporn Kasornchandra, Department of Fisheries, Thailand. During the workshop, different formats viz., baseline data collection of state and farms, sample collection and disease reporting were deliberated. The formats have been finalised and sent to all collaborating centres for collection of baseline information and sampling. The collection of baseline information is in progress. Quarterly reports on disease status of the selected states from partner institutes were compiled at the Nodal Institute and incorporated in the Quarterly Aquatic Animal Disease (QAAD) report of Network of Aquaculture Centres in Asia-Pacific (NACA) for the period October - December, 2013.

There were speculations regarding occurrence of the early mortality syndrome (EMS)/acute hepatopancreatic necrosis disease (AHPND) in the country during this period. To address this issue, an emergency response team was constituted with Director, Central Institute of Brackishwater Aquaculture (CIBA) as the Chairman, and involving experts from College of Fisheries, Mangalore; C. Abdul Hakeem College, Melvisharam; Fisheries College and Research Institute, Thoothukudi and College of Fisheries, Nellore as members under the surveillance programme. As a follow-up action, targeted surveillance was undertaken to confirm the status of the disease from the country. However, no positive results were obtained in shrimp samples tested by partner institutes. Furthermore, with concerted efforts by CIBA, a ‘Technical advisory on steps for first time confirmation of an exotic disease - A case study with EMS/AHPND’ has been prepared and hosted on CIBA website (www.ciba.res.in) (CIBA e-publication series no. 24). The progress of targeted surveillance for EMS/AHPND was reviewed during third meeting of the TAC in December, 2013.

It is expected that implementation of national aquatic animal disease surveillance programme will contribute immensely to improved disease diagnosis, better coordination amongst research institutes and provide reliable advice to aquafarmers.

Sub-Project: Surveillance Programme for Aquatic Animal Diseases of ornamental fishes in the states Kerala and Tamil Nadu

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

Under this sub-project, linkages were made by the NBFGR Kochi Unit with the State Fishery Officers of Kerala for disease surveillance and reporting in Kerala. List of ornamental fish farmers of Kerala (Ernakulam, Kottayam, Kollam, Alappuzha and Trissur district) and Tamil Nadu (Chennai and Madurai) were collected from department of fisheries, KAVIL (Kerala Aqua Ventures International Limited) and MPEDA (The Marine Products Export Development Authority).

A total of 42 diseased fish specimens belonging to eight species were collected from different fish farms of Ernakulam (six farms), Allappuzha (four farms) and Kolathur, Chennai (eight farms). Diseased Ornamental fishes viz., Angel fish (Pterophyllumsacare) from Allappuzha, Gold Fish (Carassiusauratus), Dwarf Gourami (Trichogasterlalius) from Kolathur, Neon Tetra...
(Paracheirodon innesi) from Ernakulam, X-ray Tetra (Pristellamaxillaris), Monoangel (Monodactylus sargenteus), Tiger shark (Pangasianodon hypophthalmus) from Kolathur and Filament barb (Dawkinsia filamentosa) from Ernakulam, were collected.

A total of 26 samples of Koi carp were collected from Wonder La, Kochi and screened for important OIE listed viral pathogens viz., KHV (Koi Herpes Virus) and SVC (Spring Viremia of Carp) and all the samples were found negative (Fig. 77 & 78). The samples were showing infection with bacteria.

Bacteriological analysis was carried out in collected specimens. Primary level biochemical tests and molecular identification using 16s rRNA were carried out. Based on the primary tests, pathogenic bacteria viz., *Pseudomonas* sp, *Aeromonas* sp, *Providencia* sp, *Citrobacteria* sp, *Myroides* sp, *Acinetobacter* sp, *Klebsiella* sp, and *Proteus* sp were isolated from fishes (Fig. 79).
IMPORTANT EVENTS AND MEETINGS

Quinquennial Review Team (QRT) Meeting

The Quinquennial Review Team (2008-2013) of NBFGFR visited the Institute headquarters, its units - Kochi Unit and Aquaculture Research & Training Unit, Chinhat during 5-8 February, 2014 under the Chairmanship of Dr. E.G. Silas, Former Vice-Chancellor, Kerala Agricultural University and Former Director, CMFRI, Kochi. The other members of the QRT were Dr. W. Vishwanath, Professor, Department of Life Sciences Manipur University, Imphal; Dr. K.G. Padmakumar, Associate Director (Retd.), Regional Agricultural Research Station, Kerala Agricultural University, Kumarakom; Dr. N. Ramaiah, Chief Scientist, National Institute of Oceanography, Goa; Dr. Chaitanya G. Joshi, Professor and Head, Department of Animal Biotechnology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand; Dr. K.N. Singh, Head, Forestry Division, Indian Agricultural Statistics Research Institute, New Delhi and Dr. K. K. Lal, Principal Scientist and Head, Fish Conservation Division, NBFGFR, Lucknow. The 1st meeting of the QRT was held at Lucknow during 5-6, February, 2014 in which Dr. J.K. Jena, Director, NBFGFR, Lucknow presented an overview of the Institute’s achievements during last five years. This was followed by a comprehensive presentation of research achievements of the Institute under different projects during the period of 2008 - 2013 by Heads of the Divisions. The 2nd meeting of the QRT was held at NBFGFR Kochi Unit during 7-8 February 2014 in which Dr. J.K. Jena, Director, NBFGFR, Lucknow; Dr. A. Gopalakrishnan, Director, CMFRI, Kochi and Dr. V.S. Basheer, Scientist-in-Charge, NBFGFR Kochi Unit also participated. Dr. A. Gopalakrishnan and Dr. V.S. Basheer presented the work done during 2008-2013 at the NBFGFR Kochi Unit. The review team appreciated the progress made by the Institute and suggested several measures for improvement.

Research Advisory Committee (RAC) Meeting

The RAC meeting of the Institute was held during 3-4 March, 2014 under the chairmanship of Dr. W. Vishwanath, Professor, Department of Life Sciences, Manipur University, Imphal. Dr. (Mrs.) Usha Goswami, Scientist ‘F’ (Retd.), NIO, Goa; Prof. Bechan Lal, Professor, Department of Zoology, Banaras Hindu University, Varanasi; Dr. M. H. Balkhi, Dean, Faculty of Fisheries, Sher-e-Kashmir University of Agricultural Sciences and Technology, Srinagar and Dr. Madan Mohan, Assistant Director General (Marine Fisheries), ICAR, New Delhi participated as expert members of the RAC. Dr. J.K. Jena, Director, NBFGFR, 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 and Chinhat units 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.
Some of the key recommendations of the RAC are as follows:

1. Considering the ecological and economical importance of exotic fishes, special efforts to be made for impact analysis of *Catla carpio* on aquaculture programme.

2. Taxonomic studies to be strengthened by way of:
   - Creation of minimum two scientific positions as fish taxonomists at Lucknow.
   - Conducting training programme with experts available in India and regular taxonomic workshops.
   - Taxonomic literature in library to be strengthened by subscription of *Zootaxa*, *Icthyological Explorations of Fresh Water*, *Zookeys*, etc., as well as collection of literature from other researchers of India.
   - Museum should be with voucher specimens with details of collection data and accession number. Specimens could be collected from other researcher with due acknowledgement.
   - Basin and eco-region-wise database should be created.
   - Available database to be made online in a phased manner after final verification.

3. Exploratory studies on fish biodiversity to be extended to western Himalayas.

4. Up-gradation of hatchery of ARTU, Chinhat to increase the seed production.

5. Vacant positions of scientists must be filled on priority.

6. Fish taxonomy and resources should be under Fish Conservation Division for effective output and coordination as per mandate.

7. Work initiated on one species should cover all aspects such as karyotyping/ barcoding/ biology/ functional genomics/proteomics/ diseases, etc.

8. Institute activities should be more publicised locally and in the whole country.

**Annual Institute Research Committee (IRC) meeting**

The annual Institute Research Committee (IRC) meeting for the year 2012-13 was held at NBFGGR, Lucknow on 17, 18, 20 and 23 April, 2013 under the Chairmanship of Dr. J.K. Jena, Director, NBFGGR. Member Secretary, IRC, Dr. K K Lal welcomed the chairman and members of IRC. In his introductory remarks, the Chairman, IRC emphasized the role of prioritization, monitoring and evaluation (PME) cell. He also invited the concerned personnel to speak and discuss on issues related to administration, finance, management services, etc. In his introductory remarks the chairman urged the members to discuss on the externally funded projects at equal level as those of the institutional projects in IRC meeting. He advised the members to avoid taking up external projects with meager funding just because of manpower. Dr. Jena also informed about the initiation of ICAR Consortium Research Platforms (ICRP) in 12th Five Year Plan, which can help building resources including manpower. He emphasized on the need for regular inputs on timely publication of newsletter, annual report, and preparation of other regular reports to be sent to Council. He advised PME Cell to discuss on mechanism of receiving such inputs and research publications. After Chairman’s address, Dr. P.K Pradhan, Sr. Scientist presented the RFD report of NBFGGR which was followed by presentation of progress reports of different projects by the respective Principal Investigators.

**Advanced mini supercomputing facility - ASHAA established at NBFGGR**

An advanced mini supercomputing hub for aquatic animals (ASHAA) established at NBFGGR, Lucknow under the NAIP funded project on ‘Establishment of National Agricultural Bioinformatics Grid in ICAR’, ‘ASHAA’, was inaugurated by Dr. S. Ayyappan, Secretary, DARE and Director General, ICAR, Government of India on 18th February, 2014. Dr. Ayyappan emphasized on utilizing this state-of-the-art facility by the scientists working in ICAR/
SAUs and stressed on creating awareness among the students and researchers regarding application of tools in omics research. It is one of the most advanced computational facilities in the National Agricultural Research and Education System dedicated to research in the fisheries sciences in the country. This facility has 16 Linux based cluster nodes with 96 GB internal memory, 126 TB storage capacity and three workstations. It is also adorned with several important application software packages such as CLC Genomics Workbench, Discovery Studio and other softwares useful for analysis of fish genome and proteome data and to discover genes in fish species. The supercomputing hub is expected to open up new vistas for molecular research in fisheries.

**NBFGGR Celebrated ‘Agricultural Education Day’**

The NBFGGR celebrated the ‘Agricultural Education Day’ on 19 November, 2013. The day was observed as an open house day for the school/college students and the visitors. The students as well as visitors were apprised of the activities of the NBFGGR. In order to generate awareness on fish biodiversity and its conservation needs among the students, four events comprising of Drawing and Painting Competitions (junior and senior students), Quiz Competition and Explore Ganga Aquarium Wizard Competition, were organized. Students from several schools of the Lucknow city participated in these events. The best three students in each event were presented with Trophies and the Certificates by Dr. J.K. Jena Director, NBFGGR, Lucknow.
NBFGC celebrated its ‘30th Foundation Day’ and ‘Farm Innovators Day’

The Bureau celebrated its ‘30th Foundation Day’ and ‘Farm Innovators Day’ on 12 December, 2013. Dr. S. Soloman, Director, Indian Institute of Sugarcane Research, Lucknow was the Chief Guest of the function where as Dr. P.C. Mahanta, Former Director, DCFR, Bhimtal was the Guest of Honour. Welcoming guests, fish farmers and staff present on the occasion, Dr. J.K. Jena, Director, NBFGC highlighted the significant achievements of the Institute for the year 2013. He also congratulated the staff members of the Institute for their continuous efforts in building the Institute. The Chief Guest Dr. Solomon in his address said that NBFGC has developed as a center of excellence in characterizing, cataloguing and conserving fishery resources of the country. He advocated to work in accordance with the challenges coming up in the future. Dr. Mahanta shared his experience on emergence of this institute since inception. The invited farmers also shared their experiences in developing aquaculture on their farm after obtaining training from NBFGC and interacted with the scientists to solve their technical problems facing in aquaculture.

On this occasion, Annual Institute Awards for the year 2012-13 were presented to the selected NBFGC 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 NBFGC. NBFGC Unit Kochi was awarded the Best Unit/Division Award. Other awardees in various...
categories were: Dr. N.S. Nagpure, Principal Scientist and Head of the Division, Best Scientist; Mrs. Reeta Chaturvedi, Sr. Technical Officer, Best Technical Staff; Mrs. Kaneez Fatima, Assistant, Best Administrative Staff; Mr. Indrajit Singh, SSS, Best Support Staff; Mr. D.K. Choudhary, JRF, Best Research Student; Mrs. Shashi Bala, Mr. Chakra Pal, Mr. Jai Kishan and Mr. Ram Ratan Rana, Best Fish Farmers. Mr. Raj Bahadur and Mr. Madan Lal, Technical Assistants were given Certificate of Appreciation.

**Hindi Pakhwada observed**

The Institute observed a Hindi Pakhwada during 14-28 September, 2013 during which seven Hindi competitions were organized among the staff of the Institute to promote the use of Hindi in official work. All the winners were given prizes in a function. On this occasion five reputed poets of the city were invited who spoke on importance of Hindi language and recited their poems. Mr. Ram Sakal, Personal Assistant and Dr. Mohit Tiwari, Pool Scientist jointly won the prize for the Best Hindi Competitors – 2013.

**Celebration of Independence Day**

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

**Republic Day Celebrated**

A flag hoisting ceremony was observed on the Republic Day on 26 January, 2014. Dr. J.K Jena, Director hoisted the National Flag in the presence of other staff members of the Bureau. In his address, the Director highlighted the achievements of NBFRG during the year 2013 and shared glimpses of upcoming programmes. Dr. Jena while complimenting the efforts of the staff members also reminded the staff about their rights and duties towards growth of the institute. The programme was followed with a small cultural programme in which large number of children of the NBFRG family participated.
Launch Workshop of National Surveillance Programme for Aquatic Animal Diseases

The two-day Launch Workshop of the project entitled ‘National Surveillance Programme for Aquatic Animal Diseases’ was organized during 27-28 May, 2013 at the Institute. The project, funded by the National Fisheries Development Board, Hyderabad, is being implemented by 8 ICAR fisheries research institutes, 12 fisheries colleges and other organizations in 14 selected states of aquaculture importance. The NBFGR has been taking the lead in coordinating this mega-project of national importance, with Dr. J K Jena, Director, NBFGR as National Coordinator, which is being monitored by Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, Govt. of India. The workshop included the Pre-Launch Workshop Consultative Meet on May 27, 2013 and the Launch Workshop on May 28, 2013, which was inaugurated and launched by Dr. S Ayyappan, Secretary, DARE and Director General, ICAR. The inaugural function was presided over by Dr. B Meenakumari, Deputy Director General (Fy.), ICAR and attended by Dr. Vishnu Bhat, Fisheries Development Commissioner, DAHDF; Dr. C.V. Mohan, Research and Development Manager, NACA, Bangkok and Dr. Madhumita Mukherjee, Executive Director (Tech.), NFDB as the Guests of Honour. More than 50 fish health experts from leading fisheries research institutions, fisheries colleges and other organizations participated in the workshop.

Dr. Ayyappan, in his inaugural address, emphasized the prospects of increasing agricultural productivity of the country by 30% through lessening the biotic and abiotic stresses. He cited the successful example of zoning concept of Foot and Mouth Disease free Haryana state through animal disease surveillance programme. He advised the scientists to take holistic approach in disease investigation instead of concentrating only on limited pathogen(s) of their area of interest. He congratulated Dr. J K Jena, Director, NBFGR and National Coordinator of the project for taking lead in initiating such programme of national importance in very short time-span. Dr. Meenakumari appraised the humble attempt of NBFGR for taking such a challenging task of Surveillance Programme on Aquatic Animal Disease to reach the goal. She stressed on the need of involvement of State Fisheries Departments for successful implementation of the programme. The participating organizations presented the activities to be commenced under the surveillance programme and the modalities of implementing the surveillance programme were finalized for both passive and targeted surveillance in the selected states. Institute publications were also released on this occasion.

International Workshop on ‘Indian Mackerel Genetics Harmonization’

NBFGR, in collaboration with Bay of Bengal Large Marine Ecosystem (BOBLME) project, organized an ‘International Indian Mackerel Genetics
Harmonization Training Workshop’ during 20-27 August, 2013 at NBFGR Kochi Unit, Kerala. A total of 16 delegates from eight participating countries i.e. Bangladesh, India, Indonesia, Malaysia, Maldives, Myanmar, Sri Lanka and Thailand participated in the workshop. The Indian Mackerel, *Rastrelliger kanagurta*, is a trans-boundary species, with the same population being harvested by different nations, making a collaborative effort critical to the success of any management plan. The BOBLME project, a collaborative international effort under the executive leadership of the Food and Agriculture Organisation (FAO), envisions a coordinated programme of action to formulate management plans and improve the lives of coastal populations. Standardized procedures to investigate the stock structure of the Indian Mackerel were finalized and the need for training programmes for capacity building was recognized at the Indian Mackerel Working Group meeting at Colombo in 2012, with NBFGR chosen to conduct the harmonization training workshop on Indian Mackerel Genetics.

The inaugural session of the workshop was held on 20 August, 2013. Dr. Rudolf Hermes, Chief Technical Officer, BOBLME was the Chief Guest and Dr. E.G. Silas, Former Director, CMFRI and Ex-Vice Chancellor, Kerala Agricultural University presided over the function. Dr. J.K. Jena, Director, NBFGR, Lucknow; Dr. John Candy, Technical Consultant BOBLME; Dr. A. Gopalakrishnan, Director, CMFRI, Kochi and Dr. V.S. Basheer, Officer-in-Charge, NBFGR Kochi Unit were present and spoke on the occasion. Over the seven days, participants at the workshop received hands-on training on various aspects of genetic characterization and population genetic structure analysis of Indian Mackerel. Dr. B. Meenakumari, Deputy Director General (Fy.) ICAR was the Chief Guest during the valedictory function held on 27 August, 2013 who distributed certificates to the participants. Dr. K. Vijayakumaran, Director General, Fisheries Survey of India and National Coordinator BOBLME; Dr. T.K. Srinivasa Gopal, Director, CIFT, Kochi; Dr. A. Gopalakrishnan, Director, CMFRI and Dr. J.K. Jena, Director, NBFGR, Lucknow were other dignitaries present.

**Expert Consultation on ‘Fish Genomics Research in India: A Way Forward’**

The Institute organized an Expert Consultation on ‘Fish Genomics Research in India: A Way Forward’ on 2 August, 2013 at Lucknow in collaboration with Asian Fisheries Society Indian Branch (AFSIB), Mangalore and Aquatic Biodiversity Conservation Society (ABCS), Lucknow. Dr. B. Venkatesh, Research Director, Institute of Molecular and Cell Biology, Singapore was the Chief Guest of the Consultation and Dr. B. Meenakumari, DDG (Fy.), ICAR presided over the meeting, whereas Dr. A.S. Ninawe, Sr. Advisor, Department of Biotechnology, Govt. of India was Guest of Honour on this occasion.

Dr. J.K. Jena, Director, NBFGR and Convener of the Expert Consultation while welcoming the dignitaries and participants, briefed on the objectives of the Consultation. He conveyed that to keep pace with
emerging challenges and global developments in genomics, NBFGGR is taking up a DBT-funded program on ‘Whole Genome Sequencing of Labeo rohita and Clarias batrachus’ in collaboration with CIFA, Bhubaneswar; Anand Agriculture University and IASRI, New Delhi. He also informed the participants that genomics program would also be taken up by different fisheries Institutes under ICAR Genomics Platform to be initiated during XII Plan period. Dr. Jena said that the Consultation is basically intended to develop a road map for at least a coming decade on various aspects of fish genomics including species to be undertaken for genome sequencing and functional genomics, utilization of genomic resources in production systems, traits to be selected for upgradation, manpower development, networking/linkages, infra-structure development and sources of funding for such research and development programmes.

Dr. Venkatesh emphasized the need to engage people who have passion in science and quoted the example of Beijing Genomics Institute. He delineated the development taken place globally in the area of genomics and emphasised on the necessity of greater thrust on a comprehensive subject like genomics in India. He also outlined the strength of India in the present context and possession of rich genetic resource, which need attention. Dr. Ninawe emphasized that genomics is need of the day for developing good traits, through adding values in nutrition, disease resistance, etc., in the future. He opined that DBT and ICAR can undertake genomics work through joint programmes.

Dr. Jena addressing the worshop participants

A view of the guests and participants

Orientation Training Workshop on ‘National Surveillance Programme for Aquatic Animal Diseases’

A four-days Orientation Training Workshop on ‘National Surveillance Programme for Aquatic Animal Diseases (NSPAAD)’ was organized at the Institute
Review-cum-Training workshop on ‘Exploration and Characterization of Fish Germplasm Resources and Indigenous Knowledge in North-Eastern Region of India’

NBFGR, under its NE component, has initiated 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 from NE region. During the year 2012-13, seven project proposals were provided technical and financial supports. In this connection, a review-cum-training workshop was organized involving collaborating partners and researchers for identified thematic work programmes at NBFGR, Lucknow during 14-16 May, 2013. The purpose of the workshop was to review the progress made in the identified projects and undertake capacity development programme for the collaborators from the NE region. A total of nine collaborating researchers from the region participated in the workshop. In his opening remarks, Dr. J.K. Jena, Director, NBFGR, Lucknow emphasized the importance of the biodiversity assessment and conservation in NE region, and appraised the collaborating institutions with regard to the initiative on exploration of fish diversity in network mode. He also conveyed thanks and appreciation to all the partners for their good work.

Prof. J.R. Dhanze, Dean, College of Fisheries, Central Agricultural University, Lembucherra, Agartala, Tripura and Prof. W. Vishwanath, Department of Life Science, Manipur University, Imphal participated in the workshop as resource persons and shared their experiences on fish germplasm explorations and fish taxonomy. Besides review of ongoing projects in NE region, the workshop also included demonstrations by the NBFGR scientists on...
the protocols for fish and tissue collections and preservation during germplasm explorations. New project proposals were also discussed during the workshop.

**Training programme on Bioinformatic Approaches in Genomics, Transcriptomics and Proteomics**

A subject matter training programme on Bioinformatic Approaches in Genomics, Transcriptomics and Proteomics was organized during 12-22 November, 2013 under NAIP funded project NABG in ICAR. The aim of the training programme was to acquaint researchers about application of bioinformatics in genomics, proteomics and transcriptomics and to apprise the participants about various resources and aids of bioinformatics tools. The programme was inaugurated by Prof. R.C. Sobti, Vice Chancellor, Babasaheb Bhimrao Ambedkar University, Lucknow. The 11 days training course covered various topics such as sequence alignment, comparative genomics, gene annotation, NGS data analysis by CLC Bio, molecular modeling, docking and dynamics of proteins, transcriptome data analysis, EST data analysis, database development and other bioinformatics tools and software. Twenty two participants from different universities/ institutes of the country attended the training programme. Dr. Shirish Ranade, Chief Scientist, NBRI, Lucknow gave the certificates to the participants during the valedictory function of the training.

**AusAID Ecotoxicology Training-Workshop on ‘Safe Water for the Future’**

A five-day AusAID Ecotoxicology Training Workshop under the CSIRO-IITR-NBFGR Project on ‘Safe Water for the Future’ was organized at the Institute during 2-6 December, 2013. This training workshop was organized for capacity building of the scientists involved in environmental and toxicological research to safeguard the availability of clean water for the future under the guidance of Dr. J.K. Jena, Director, NBFGR and Dr. Anu Kumar, Principal Investigator of the project. Various issues related to water pollution, and state of the art tools and techniques available to track them and manage them in our rivers, creeks and lakes were discussed during this workshop. The workshop was inaugurated by Dr. Jenny Stauber, Deputy Chief, CSIRO Land and Water, Australia. Dr. J.K. Jena, Director, NBFGR welcomed the guests, resource persons and trainee participants and said that the workshop on safe water is important for the existence of all living being including aquatic organisms, and is directly related to global fisheries production. He highlighted the fisheries research activities being undertaken at NBFGR, particularly genotoxicological studies in fishes using important biomarkers. Dr. Stauber emphasized that in the present scenario major challenge is to secure water and maintain its quality by protecting it from industrial and domestic discharges to defend our environment. Dr. Anu Kumar, Project Leader, Land and Water, CSIRO, Australia expressed that the collaboration would be of mutual interest for both the India and Australia. Dr. K.C. Gupta, Director, Indian Institute of Toxicology Research,
Lucknow stressed the need to develop advanced methods to analyze the effects of nano-materials which may potentially pollute the ecosystems. Dr. Merrin Adams, Project Leader, Ecotoxicology group, CSIRO and Dr. Peter Bain, Project Research Officer were also present during the occasion. Twenty-two participants from various research and academic organizations, cutting across the disciplines, from all over the country took part in the workshop. The course of 5-days training-workshop included lectures, demonstrations and hands-on practicals in *in-vivo* and *in-vitro* toxicity testing techniques in vertebrates and invertebrates as well as in plants and animals, case studies and a field trip to tanneries. On this occasion, an awareness programme on ‘Safe Water for Future’ was organized especially to the rural women, in which 60 fisherwomen participated.

**Training on High Performance Computing Administration**

The High Performance Computing (HPC) administration training was organized during 28–30 October, 2013 at NBFGR, Lucknow for the nominated scientists and technical officers of domain centers of sub-project NABG in ICAR under NAIP Component-1. The training was imparted for administering and managing HPC at the respective domain centers. Ten participants from NBFGR, Lucknow; NBAGR, Karnal; NBAIM, Mau and NBAII, Bangalore attended the training. The resource persons from Hewlett-Packard (HP) and APC imparted training on overview of the complete HPC, overview of the storage systems, as well as spread awareness about the usage of UPS. Interactive sessions were organised about administration and management of nodes, jobs and storages servers. Sessions on administration and management of infiniband and network switches were also demonstrated. The training also included a session on UPS operational management.

**Training programme on DNA Barcoding and Molecular Taxonomy**

A training programme on ‘DNA barcoding and Molecular Taxonomy’ was organized at NBFGR Kochi Unit during 16-21 December, 2013. Dr. A. Gopalakrishnan, Director, CMFRI inaugurated the training programme and highlighted the importance of the DNA barcoding and its role in fish taxonomy, conservation and forensic studies. The trainees received hands-on training on various aspects of DNA barcoding of fishes. Dr. B. Meenakumari, DDG (Fy), ICAR.

A total of 22 selected participants from Andaman & Nicobar Islands, Jammu & Kashmir, Andhra Pradesh, Tamil Nadu, Maharashtra and Kerala participated in the training. Dr. P.C. Thomas, In-Charge, HRD Cell, CMFRI, Chief Guest of the Valedictory function in his address stressed the need of developing human resources in the area of barcoding and taxonomy of fishes.

**Training course on DNA Barcoding**

A short course on ‘DNA Barcoding of Aquatic Organisms: A Tool for Molecular Taxonomy’ was organized during 5-14 February, 2014. The course, sponsored by the ICAR, was aimed to develop human
resource in National Agricultural Research and Education System. The course was inaugurated by Dr. E.G. Silas, Former Vice-Chancellor, Kerala Agricultural University and Former Director, CMFRI, Kochi. Hands-on training was imparted to the participants on various tools & techniques used in DNA barcoding including primer designing, PCR, sequence editing and submission, phylogenetic analysis, etc. It also provided exposure on different biological databases frequently required in DNA barcoding such as GenBank, EMBL, DDBJ, BOLD, iBOL, FISH-BOL, FBIS, etc. A total of 16 researchers from universities, research institutes of various part of the country attended the course. As Course Director Dr. Mahender Singh, Sr. Scientist, NBFGR coordinated the short course.

**Consultation on Promoting Farm Innovations in Uttar Pradesh**

A Consultation on Promoting Farm Innovations in Uttar Pradesh was organized at NBFGR on 30 December, 2013 by the Agri-Innovation Foundation, Lucknow. The Consultation aimed at identifying few impacting innovations, recognize them and identifying few challenging problems needing innovative solutions. A total of 45 participants including resource persons from various sectors, scientists, progressive farmers, representatives of NABARD and agro-industry, participated in the consultation. Dr. S.A.H. Abidi, Former Member, ASRB, New Delhi inaugurated the consultation where as Dr. A.K. Srivastava, Director, NDRI, Karnal presided over the inaugural session. Dr. J.K. Jena, Director, NBFGR, Lucknow welcomed the participants and explained about the research achievements and efforts made by the Institute to connect with various stakeholders for taking technologies to the end-users. Dr. Bengali Baboo, President, Agri-Innovation Foundation, Lucknow gave an overview of the challenges and opportunities for promoting farm innovations in UP.

**Awareness Workshop on ‘Challenges and Opportunities in Intellectual Property Management and Commercialization of Technologies in Agriculture and Fisheries Sectors’**

The NBFGR organised an awareness workshop on ‘Challenges and Opportunities in Intellectual Property Management and Commercialization of Technologies in Agriculture and Fisheries Sectors’ on 20 March, 2014. The workshop was aimed at enhancing the awareness of researchers on changing IP environment of the country and identify and discuss various issues related to development of IP enabled technologies with respect to agriculture and fisheries sectors of the country. The workshop was inaugurated by Dr. M.K.J. Siddiqui, Director, UPCST, Lucknow. Over 75 scientists and research scholars from NBFGR and other ICAR institutes participated in the workshop. The workshop programme included invited lectures on themes - Protecting Growing Innovation Ecosystem - Initiatives of ICAR; Copyright Law for Scientists and Researchers; Germplasm Exchange, Policy and related IPR Issues and Patent claims writing and interpretation delivered by scientists and faculty from ICAR institutes and Ram Manohar Lohia Law University, Lucknow. There was a open discussion and experience sharing session in which participants discussed several IP related issues involved in scientific activities.
AWARDS AND RECOGNITIONS

Dr. J.K. Jena receives Rafi Ahmed Kidwai Award

Dr. J.K. Jena, Director, NBFG, Lucknow was awarded with the prestigious Rafi Ahmed Kidwai Award of the ICAR. The award was presented to Dr. Jena for his outstanding research achievements in diversification of freshwater aquaculture. The work was carried out at CIFA, Bhubaneswar. Dr. Jena received the award from Shri Sharad Pawar, Hon’ble Union Minister of Agriculture & Food Processing Industries in presence of Shri Tariq Anwar, Hon’ble Minister of State for Agriculture and Dr. S. Ayyappan, Secretary, DARE and Director General, ICAR in a function on the Foundation Day of the ICAR at New Delhi on 16 July, 2013. The NBFG family congratulates Dr. Jena for this prestigious award and looks forward to his deep scientific insights and dynamic leadership in guiding research programmes of this Institute.

Dr. J.K. Jena, Director was conferred with the Bioved Ratna Award - 2014 of the Bioved Research Society, Allahabad on 22nd February, 2014 at Lucknow. Dr. Neeraj Sood, Principal Scientist was presented with ‘M.S. Swaminathan Award for the Best Indian Fisheries Scientist’ for the year 2013.

Dr. N.S. Nagpure, Principal Scientist and Head, MBB Division; Drs. P.K. Varshney and A.K. Singh, Principal Scientists were conferred with the Fellowship Award 2013; Dr. Rajeev Kr. Singh, Sr. Scientist was awarded the Young Scientist Award 2013; and Dr. L.K. Tyagi, Sr. Scientist and Mr. Rajesh Dayal, Chief Technical Officer were awarded with the Distinguished Service Award 2013 of the Society of Biological Sciences and Rural Development, Allahabad on 19 October, 2013.

Dr. N.S. Nagpure, Principal Scientist and Head, MBB Division was conferred with the Fellowship Award 2013 of the Academy of Environmental Biology, Lucknow on 19 December 2013.

Dr. A. K. Pandey, Principal Scientist was awarded Prof. J.S. Datta Munshi Gold Medal and Dr. U.K. Sarkar, Principal Scientist was awarded Dr. V.R.P. Sinha Medal, 2013 on the occasion of 24th All India Congress of Zoology & National Seminar on Biodiversity and its Management on 23 November, 2013.

Dr. M. Goswami was awarded Hiralal Chaudhary Young Scientist Award 2013.

Dr. N.S. Nagpure, Principal Scientist and Head, MBB Division was conferred with the Fellowship Award of The Zoological Society, Kolkata for the year 2012-13 and Fellowship Award - 2014 of the Bioved Research Society, Allahabad.

Dr. L.K. Tyagi, Sr. Scientist was conferred with the Distinguished Service Award - 2014 of the Bioved Research Society, Allahabad on 23 February, 2014.

Dr. P.K. Varshney, Principal Scientist was conferred with the ‘Smt. Snehalata Banarjee Gold Medal’ by the Academy of Environmental Biology, Lucknow.

Dr. U. K. Sarkar, Principal Scientist was conferred with Fellowship Award of Zoological Society of India, Kolkata in 2014.

NBFG was awarded “Best Freshwater Angelfish” display in 7th Indian International Aquashow (IIAS) held during 24-28 January, 2014 at Kochi, Kerala.

NBFG Annual Hindi Magazine ‘Matsya Lok’ 2013 was awarded the Third Prize by the ‘Nagar Rajbhasha Karyanvay Samiti, Lucknow, amongst the magazines brought out by central government offices located at Lucknow. It was co-edited by Dr. L.K. Tyagi, Sr. Scientist and Dr. Akhilesh K. Mishra, Technical Officer.

Linu Joy, C. Mohita, Raj Kumar, P.R. Divya, V.S. Basheer and A. Gopalakrishnan received Best Poster Award for the poster titled “Genetic Differentiation of Cobia in Indian Waters Inferred from Mitochondrial DNA ATPase 6/8 genes” during National Symposium on Emerging Trends in Biotechnology, 22-23 January, 2014 at Department of Biotechnology, CUSAT, Kochi, Kerala.

Bineesh K.K, A. Gopalakrishnan, V.S. Basheer, Akilesh, NGK Pillai and J.K Jena received Best Poster Award for the poster titled “Assessing Marine Fish Diversity of India - a Molecular Approach” during Swadeshi Science Congress at MG University, Kottayam, Kerala.
EXTENSION ACTIVITIES

National Fish Farmers’ Day celebration

The Institute celebrated the National Fish Farmers’ Day on 10 July, 2013. Dr. Saroj Kumar, Director, Department of Fisheries, Government of Uttar Pradesh was the Chief Guest of the occasion. In his welcome address, Dr. J.K. Jena, Director, NBFRG, Lucknow elaborated the role of NBFRG to Chief Guest and fish farmers who have come from Lucknow, Pratapgarh and Allahabad districts of U.P. He explained about the various aquaculture technologies available to the farmers that could help in the enhancing the aquaculture productivity and provide higher returns. He stated that although NBFRG is not a commodity institute but has taken lead in training the farmers under different programmes sponsored by NFDB, ATMA, DASP and through institutional programmes, and also through supply of quality fish seed to the farmers of Uttar Pradesh and adjoining states. Dr. Saroj Kumar in his address stated that the Fisheries Department of U.P. has taken several new initiatives in the recent past. He conveyed about the Mission Document that has been developed by the Department which includes several new technologies for dissemination. The action being taken by the Department for supply of quality fish seed to the farmers, development of brood bank for raising quality brood stocks and aerators at subsidized price for the farmers intending for crop intensification, were also stressed by Dr. Kumar. The inaugural programme was followed by a Technical Session, in which lectures were delivered by the experts, and the farmers’ queries were answered by the scientists of NBFRG.

Awareness Programme on Fish Conservation and Tribal Communities in Kerala

A one-day awareness programme on ‘Fish Conservation and Tribal communities’ was conducted at Kalpetta, Wayanad, Kerala by NBFRG unit Kochi in association with the Community Agrobiodiversity Centre of MS Swaminathan Research Foundation (CAbC-MSSRF) and Fisheries Research Station, Puthuvypu, KUFOS, Kerala on the occasion of World Biodiversity Day on 22 May, 2013. Shri Sasidharan, state award winning fish farmer from Wayanad, inaugurated the function. Shri Dattan and Smt Sosamma Kurien, entrepreneurs in ornamental fish aquaculture from the district made felicitation addresses. Dr. Anil Kumar, Director CAbC and Dr. C. P. Shaji, eminent fish taxonomist were the Guests of Honour. Over one hundred tribal community members, planners, educators, students and research scholars attended the programme.

Shri Sasidharan, in his inaugural address, said that this effort by NBFRG in highlighting the importance of conserving indigenous fish resources will give impetus to the conservation of freshwater ecosystems. Dr. Anil Kumar, in his presidential address, pointed out that the theme for the 2013 Biodiversity Day was Water and Biodiversity, which was reflected by the theme of the conservation programme. He applauded the work done by NBFRG, towards conservation of fishes, especially in the Western Ghats region and expressed willingness to extend all for the conservation programme.

The inaugural session was followed by a technical session. Dr. C.P. Shaji captivated the audience with a presentation on the indigenous fish resources of the region. Dr. K. Dinesh and Sarath of KUFOS conducted a session on setting up small aquaculture units for raising ornamental fish. After the technical session, there was an interaction with tribal people regarding the practices they used for conserving fishes in their
area. An exhibition and poster display, illustrating important indigenous species was also part of the programme.

**Awareness Programme on “Modern Fish Culture and Conservation” for Tribal Community in UP**

The Institute organized an awareness programme on “Modern Fish Culture and Conservation” for the tribal community of the district Sonbhadra at Renukot, Sonbhadra on 29 April, 2013. Smt.Anita Rakesh President, District Panchayat, Sonbhadra was Chief Guest on the occasion and the function was presided over by Smt Rubi Prasad, Hon’ble Member of Legislative Assembly, Dudhi. Shri Mahendra Singh, Chief Development Officer, Sonbhadra and Shri N. K. Dadhichi, HR Head, Aditya Birla Chemicals Private Limited, Sonbhadra were the Guests of Honour. Dr. P.K. Varshney, Principal Scientist, NBFRG welcomed the guests. Speaking on the occasion chief guest Smt. Anita Rakesh emphasized that such awareness programmes are of immense importance for the development of the district and the state in general. Smt. Rubi Prasad said that such programmes may bridge up the gap between tribal community and mainstream and will be helpful in their upliftment. The programme was attended by 235 fish farmers of Sonbhadra district. In the technical sessions the problems of aqua-farmers on various aspects of aquaculture were attended to and solutions were suggested by the experts. It was concluded that some progressive fish farmers will be selected and trained further at NBFRG, Lucknow.

**Seminar on Water and Biodiversity**

NBFRG Kochi Unit organized a seminar on ‘Water and Biodiversity’ in collaboration with CAbC-MSSRF, Waynanad and Koyilandi Muncipality at Koyilandi, Calicut, Kerala on 15 June, 2013. The programme was organized in connection with a month long celebration associated with the World Environment Day conceptualized by Koyilandi Muncipality at Koyilandi, Calicut and was aimed at creating awareness among local people and college students who attended the programme. The focal point of the seminar was Mangroves. The famous mangrove conservationist of Kerala, Mr. Kallel Pokkudan, gave lecture on his experience on mangrove farming and restoration of mangroves. Lectures on importance of mangrove biodiversity and its conservation were delivered during the programme. A total of 100 students and local people participated in the programme.

**Training on ‘Aquaculture technologies and productivity enhancement’**

The NBFRG, at its Aquaculture Research & Training Unit (ARTU), Chinhat, organized a series of short-term training programmes, sponsored by various agencies, for aqua-farmers and KVK subject-matter
specialists. A total of 14 training programmes were conducted during the period under report. A total of 371 progressive fish farmers from different districts of U.P. and Bihar were trained in these training programmes, as per details below:

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

<table>
<thead>
<tr>
<th>Period</th>
<th>Sponsored by</th>
<th>No. of trainees</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-12 April, 2013</td>
<td>NFDB, Hyderabad</td>
<td>34</td>
</tr>
<tr>
<td>22-26 April, 2013</td>
<td>-do-</td>
<td>33</td>
</tr>
<tr>
<td>6-10 May, 2013</td>
<td>-do-</td>
<td>22</td>
</tr>
<tr>
<td>20-24 May, 2013</td>
<td>-do-</td>
<td>23</td>
</tr>
<tr>
<td>17-21 June, 2013</td>
<td>-do-</td>
<td>20</td>
</tr>
<tr>
<td>8-12 July, 2013</td>
<td>-do-</td>
<td>38</td>
</tr>
<tr>
<td>22-26 July, 2013</td>
<td>-do-</td>
<td>26</td>
</tr>
<tr>
<td>14-16 May, 2013</td>
<td>Special training for Tribal farmers under TSP Programme, NBFG, Lucknow</td>
<td>16</td>
</tr>
<tr>
<td>26-28 June, 2013</td>
<td>-do-</td>
<td>25</td>
</tr>
<tr>
<td>2-4 July, 2013</td>
<td>-do-</td>
<td>30</td>
</tr>
<tr>
<td>14-16 May, 2013</td>
<td>-do-</td>
<td>19</td>
</tr>
<tr>
<td>26-28 June, 2013</td>
<td>-do-</td>
<td>42</td>
</tr>
<tr>
<td>21-25 January, 2014</td>
<td>ATMA, Gopalgarh, Bihar</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>371</td>
</tr>
</tbody>
</table>

Exhibition on the occasion of International Biological Diversity Day on 22 May, 2013 organised by the UP State Biodiversity Board at Lucknow.

7th Indian International Aquashow (IIAS) held at Kochi, Kerala during 24-28 January, 2014.

National Seminar on ‘Development of Fisheries in Water Deficient Regions’ which was organized by FTE in the campus of CIBA, Chennai during 25-26 February 2014.

Krishi Vigyan Sangam jointly organised at IISR, Lucknow during 16-17 February, 2014.

16th Indian Agricultural Scientists and Farmers’ Congress on Nanobiotechnological Approaches for Sustainable Agriculture and Rural Development on 22-23 February, 2014 at Integral University, Lucknow.


Outreach activities

Dr. S. Raizada, Principal Scientist visited the Kanpur Zoo for suggesting improvement in the public aquarium located inside the Kanpur Zoo. A detail up-gradation plan was submitted to the authorities of the Kanpur Zoo.

Dr. S. Raizada, Principal Scientist visited Chamba, H.P. on 1 March, 2014 and submitted a plan for the establishment of Interpretation Centre-cum-Museum at Dharwala (Churi) on Chamba-Bharmour Road in Himachal Pradesh to the Director-cum-Warden of Fisheries, Himachal Pradesh.
Fish Seed Production

The seed production of Indian major carps was continued under the ICAR Mega Seed Project. A total of 480 lakh spawn of Indian major carps and exotic carps, and 4 lakh spawn of minor carp *Cirrhinus reba*, were produced. Revenue of Rs. 7.19 lakh was generated from seed sale.

Dr. P. K. Varshney, Principal Scientist delivered a talk on ‘Matsya Beej Utpadan’ telecasted by Doordarsan Kendra, Lucknow in Krishi Darshan Programme on 5 July, 2013.

Dr. A.K. Singh, Principal Scientist delivered a Radio talk on Mastya Palan from AIR, Lucknow on 20 November, 2013.
### LIST OF PROJECTS

#### Institutional Projects

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Project Title</th>
<th>Personnel</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Molecular Biology &amp; Biotechnology Division</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Development of protocol for germ cell transplantation in fish</td>
<td>B. Kushwaha (PI), Sudhir Raizada and Akhilesh Kumar Mishra</td>
<td>April, 2011 – March, 2014</td>
</tr>
<tr>
<td>2</td>
<td>Genetic Stock Structure Elucidation of <em>Tenuilosa ilisha</em> and <em>Channa striata</em> using mitochondrial DNA marker, microsatellites and molecular cytogenetic tools</td>
<td>Mahender Singh (PI), N.S. Nagpure, Ravindra Kumar and Ajay Kumar Singh</td>
<td>April, 2010 – March, 2014</td>
</tr>
<tr>
<td></td>
<td><strong>Fish Conservation Division</strong></td>
<td></td>
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<tr>
<td></td>
<td><strong>Fish Health Management Division</strong></td>
<td></td>
<td></td>
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<tr>
<td>6</td>
<td>Exploration of protozoan and monogenean parasites among carps and catfishes</td>
<td>Rehana Abidi (PI) and S.M. Srivastava</td>
<td>April, 2010 - March, 2014</td>
</tr>
<tr>
<td>7</td>
<td>Development of biomarkers as diagnostic tools for assessment of fish health status</td>
<td>Peyush Punia (PI), P.K. Pradhan and Ranjana Srivastava</td>
<td>April, 2011 – March, 2015</td>
</tr>
<tr>
<td></td>
<td><strong>Fish Taxonomy and Resources Unit</strong></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td><strong>Kochi Unit</strong></td>
<td></td>
<td></td>
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<tr>
<td>9</td>
<td>Genetic stock - structure analysis of <em>Parapenaeopsis stylifera</em> and <em>Scomberomorus commerson</em> along the Indian coast using molecular markers.</td>
<td>A. Gopalakrishnan (PI upto 31 July 2013), P.R. Divya (PI from August 2013), V.S. Basheer, and A. Kathirvelpandian</td>
<td>April, 2013 - March, 2016</td>
</tr>
</tbody>
</table>
### Externally funded Projects

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Project Title</th>
<th>Personnel</th>
<th>Funding agency</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bioprospecting of genes and allele mining for abiotic stress tolerance</td>
<td>Vindhya Mohindra (PI), Ravindra Kumar and Rajeev Kumar Singh</td>
<td>NAIP, ICAR</td>
<td>May, 2009 – May, 2014</td>
</tr>
<tr>
<td>2</td>
<td>Genetic characterization and conservation biology of economically important Siluroid fish <em>Ompok pabda</em> of Tripura</td>
<td>U.K. Sarkar (PI), Mahender Singh and S. Banik (Tripura University)</td>
<td>DBT, Govt. of India</td>
<td>January, 2011– July, 2014</td>
</tr>
<tr>
<td>4</td>
<td>Microsatellite markers for genetic diversity analysis in natural populations of Cobia (<em>Rachycentron canadum</em>) and Silver pomfret (<em>Pampus argenteus</em>)</td>
<td>P.R. Divya (PI), A. Gopalakrishnan and V.S. Basheer</td>
<td>DBT, Govt. of India</td>
<td>November, 2010 – November, 2013</td>
</tr>
<tr>
<td>5</td>
<td>Establishment of a National Repository for conservation and characterization of fish cell lines at NBFGR, Lucknow</td>
<td>M. Goswami (PI) and N.S. Nagpure</td>
<td>DBT, Govt. of India</td>
<td>November, 2010 – November, 2014</td>
</tr>
<tr>
<td>7</td>
<td>Assessment of aquatic health using recent cellular and molecular tools in endocrine research</td>
<td>A.K. Singh (PI upto 28 February, 2014), Peyush Punia (PI w.e.f. 28 February, 2014)</td>
<td>UPCST</td>
<td>August, 2011 – August, 2014</td>
</tr>
<tr>
<td>8</td>
<td>Genetic characterization of selected freshwater fish species endemic to Indian region of the Indo-Burma biodiversity hot-spot using advanced cytogenetic markers</td>
<td>Ravindra Kumar (PI), B. Kushwaha (NBFGR, Lucknow) and Gusheinzed Waikhom (PI), T. Shantibala (IBSD, Imphal)</td>
<td>DBT, Govt. of India</td>
<td>March, 2011 – June, 2014</td>
</tr>
<tr>
<td>9</td>
<td>Characterization and DNA barcoding of endemic fishes of North east India</td>
<td>Mahender Singh (PI, NBFGR), N.S. Nagpure and W. Vishwanath (PI, Manipur University, Imphal)</td>
<td>DBT, Govt. of India</td>
<td>November, 2012 – November, 2015</td>
</tr>
<tr>
<td>10</td>
<td>Identification and evaluation of reproductive traits and genetic structure of <em>Ompok bimaculatus</em> in India</td>
<td>U.K Sarkar (PI), Ravindra Kumar and Abha Mishra, BRAU, Lucknow</td>
<td>DBT, Govt. of India</td>
<td>September, 2011 – September, 2014</td>
</tr>
<tr>
<td>11</td>
<td>Isolation and characterization of <em>Flavobacterium</em> species from fish and aquatic Environment</td>
<td>P.K. Pradhan (PI)</td>
<td>NBAIM, ICAR</td>
<td>August, 2006– March, 2014</td>
</tr>
<tr>
<td>12</td>
<td>Development of novel microsatellites in <em>Channa</em> species (Channidae: Perciformes) from North East for conservation genetics</td>
<td>Rajeev Kumar Singh (PI), L.K. Tyagi and A.S. Barman (PI, College of Fisheries, CAU, Lembuchera, Agartala)</td>
<td>DBT, Govt. of India</td>
<td>April, 2012 – March, 2015</td>
</tr>
<tr>
<td>Project Number</td>
<td>Title</td>
<td>Investigator(s)</td>
<td>Funding Agency</td>
<td>Start Date - End Date</td>
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</tr>
<tr>
<td>13</td>
<td>DNA Barcoding of Marine finfishes and shellfishes</td>
<td>A. Gopalakrishnan (PI), J.K. Jena and V. S. Basheer</td>
<td>MoES-CMLRE, Govt. of India</td>
<td>November, 2012 – October, 2017</td>
</tr>
<tr>
<td>14</td>
<td>Understanding of molecular pathogenesis of epizootic ulcerative syndrome (EUS) in fish and development of newer strategies to combat EUS</td>
<td>P.K. Pradhan (PI), Neeraj Sood (NBFG, Lucknow), Chandan Debnath (PI) and Lopamudra Sahoo (ICAR Complex, Tripura)</td>
<td>DBT, Govt. of India</td>
<td>May, 2012 – April, 2015</td>
</tr>
<tr>
<td>17</td>
<td>Whole genome sequencing and development of allied genomic resources in two commercially important fish- Labeo rohita and Clarias batrachus</td>
<td>N.S Nagpure (PI), Basdeo Kushwaha, Ravindra Kumar, Mahender Singh</td>
<td>DBT, Govt. of India</td>
<td>October, 2013 - October, 2016</td>
</tr>
<tr>
<td>18</td>
<td>National Surveillance Programme for Aquatic Animal Diseases</td>
<td>Neeraj Sood, P.K. Pradhan, P. Punia and Rehana Abidi</td>
<td>NFDB</td>
<td>April, 2013 – March, 2018</td>
</tr>
<tr>
<td>22</td>
<td>Characterisation of Aphanomyces invadans from North east India to develop diagnostic techniques and control measures.</td>
<td>P. K. Pradhan (PI), Dr. Peyush Punia (NBFG, Lucknow), Lopamudra Sahoo (PI) and Chandan Debnath (ICAR Complex, Tripura)</td>
<td>DBT, Govt. of India</td>
<td>September 2013 - March, 2016</td>
</tr>
</tbody>
</table>
PARTICIPATION IN SEMINARS/ SYMPOSIA/ WORKSHOPS/ TRAININGS/ MEETINGS

Abroad:

Dr. J.K. Jena, Director was deputed to:
- Bangkok and Singapore during 2-8 April, 2013 as a member of delegation led by Dr. Charan Das Mahant, Hon’ble Minister of State for Agriculture (A&FPI) alongwith other officials to study aquariums of global standards with a view to establish similar projects in India.
- Korea during 29 April - 4 May, 2013 for participation in the 10th Asian Fisheries and Aquaculture Forum and Final Meeting of the 10th Asian Fisheries Society Council.
- Australia during 16-25 June, 2013 for visiting CSIRO laboratories and interactions with the senior researchers and executive team for collaboration as a member of senior delegation under AUSAID-funded project on Safe water for future.

Dr. Neeraj Sood, Principal Scientist was selected for DBT Crest Awards 2011-12 and underwent training on ‘Cellular and Molecular Analysis of Immune Response in Fish’ at the Department for Innovations in Biological, Agro-food and Forest Systems, University of Tuscia, Viterbo, Italy from 2 October, 2012 to 15 September, 2013. During this period he also attended the ‘First International Conference on Fish and Shellfish Immunology’ at Vigo, Spain from 25-28 June, 2013.


Dr. P.K. Pradhan, Sr. Scientist, Fish Health Management attended three months training at University of Antwerepen, Groenenborgerlaan, Antwerp, Belgium under NAIP during 26 September – 24 December, 2013.

In India

Dr. J.K. Jena, Director participated in the following:
- International Workshop on ‘Status of good practices and lessons learnt in aquaculture in the SAARC region’ convened by CMFRI, Kochi and SAARC Coastal Zone Management Centre, Male, Maldives at Kochi on 4 June, 2013 and delivered an invited talk.
- Scoping Consultation on ‘National Architecture and Roadmap for CoMBINe (Coastal and Marine Biodiversity Integrated Network)’ at the National Centre for Sustainable Coastal Management, Chennai on 28 June, 2013.
- First Meeting of the ‘Scientific Panel on Fish and Fisheries Products’ chaired by the Secretary, DARE & DG, ICAR, New Delhi on 2 July, 2013 at New Delhi.
- Second Meeting of the Technical Advisory Committee for Overall Monitoring and Supervision of the National Surveillance Programme for Aquatic Animal Diseases (NPSAAD) held on 11 July, 2013 at New Delhi.
- Meeting of the Committee constituted under the Chairmanship of Joint Secretary (Fy.) to finalize the guidelines for setting up of Multiplication Centres (MCs) for SPF Litopenaeus vannamei and Penaeus monodon and make recommendation on policies to govern setting up and operation of MCs held at New Delhi on 6 September, 2013.
- First Advisory Committee Meeting of the ‘Centre of Excellence in Fisheries and Aquaculture Biotechnology’ at College of Fisheries, Lembucherra, Tripura on 14 September, 2013.
- Fourth Meeting of the National Advisory Board on Management of Genetic Resources (NABMGR) held on 10 October, 2013 at National Bureau for Agriculturally Important Insects, Bangaluru.

- 2nd meeting of Scientific Panel on Fish and Fisheries Products at New Delhi on 15 October, 2013.


- 3rd Meeting of the Sub-Committee for Studying the Potential and Viability of Culturing Endemic and Exotic Species’ held at CIBA, Chennai on 7-8 November, 2013.

- National Conference on Aquatic Toxicology, Biodiversity and Aquaculture at Acharya Nagarjuna University, Guntur on 15 November, 2013.

- “Consultative Meeting on Fisheries Development in the State of West Bengal: Research Extension and Development Support by the ICAR Fisheries Research Institutes” on 23 November, 2013 at CIFE Kolkata Center, Kolkata.

- Technical Advisory Committee (TAC) Meeting for the National Disease National Surveillance Programme for Aquatic Animal Disease at DAHDF, New Delhi on 9 December, 2013.


- Workshop on ‘Early Mortality Syndrome (EMS)/Acute Hepatopancreatic Necrosis Syndrome (AHPNS) of Cultured Shrimps’ at Chennai on 21 January, 2014.

- Launch Workshops of the Project ‘National Surveillance Programme for Aquatic Animal Diseases’ at CIFRI, Barrackpore on 21 February, 2014 and at CIFA, Bhubaneswar on 24 February, 2014.


Dr. J.K. Jena, Director and Dr. K.K. Lal, Principal Scientist and Head, Fish Conservation participated in the Annual Workshop and CAC Meeting of NAIP (GEF) sub-project ‘Harmonizing biodiversity conservation and agricultural intensification through integration of plant, animal and fish genetic resources for livelihood security in fragile ecosystems’ at NBPG, New Delhi on 7 May, 2013.

Dr. N.S. Nagpure, Principal Scientist and Head, MBB Division and participated in International Conference on Biosciences with special reference to Environmental Safety at Shivaji University, Kolhapur during 19-21 December, 2013.

Dr. K.K. Lal, Principal Scientist and Head, Fish Conservation participated in the following:

- First meeting of the National Repositories designated under Section 39 of the Biological Diversity Act, 2002 and Rules, 2004 convened by Chairman, National Biodiversity Authority, India for preparation of draft guidelines on repositories during April 2013 at Hyderabad.


- Workshop on Indo-German Research Cooperation in Support of the Management of Coastal and Marine Biodiversity in India organized by GIZ as a part of Indo-German Biodiversity Programme at New Delhi during 5-6 September 2013.

- 1st Steering Committee Meeting of NFBSFARA project on Stock characterization, captive breeding, seed production and culture of Hilsa at CIFRI Barrackpore on 16 August, 2013.

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

- International day for Biological Diversity programme on ‘Water and Biodiversity’ on 22 May, 2013 at UP Biodiversity Board, Lucknow.

Dr. N.S. Nagpure, Principal Scientist and Head, MBB Division; Drs. A.K. Pandey, P.K. Varshney, A.K. Singh, Principal Scientists; Drs. L.K. Tyagi and Rajeev Kr. Singh, Sr. Scientists and Dr. Rajesh Dayal, Chief Technical Officer attended the National Symposium on Innovative and Modern Technologies for Sustainable Agriculture & Rural Development held at Allahabad during 19-20 October, 2013.

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

Dr. S. Raizada, Principal Scientist participated in the following:
- Workshop on 'Water and Biodiversity' organized by the UP State Biodiversity Board at the Lohia University, Lucknow on 22 May, 2013.
- State Credit Seminar for Uttar Pradesh entitled 'Towards better farm productivity for sustainable growth' organized by NABARD on 24 January, 2014.


Dr. Peyush Punia, Principal Scientist and Head, Fish Health Management Division; Drs. Neeraj Sood and Vindhya Mohindra, Principal Scientists; Dr. Rajeev Kr. Singh, Sr. Scientist and Ms. Samgeeta Mandal, Scientist participated in Nextgen Genomics and Bioinformatics Technologies Conference at IGIB, New Delhi during 14-16 November, 2013.

Dr. Rajeev Kr. Singh, Sr. Scientist participated in the Workshop on Next Generation Sequencing at CCMB, Hyderabad, India during 18 - 27 November 2013.

Dr. A.K. Pandey, Principal Scientist participated in the following:
- International Conference on Faunal Biodiversity and their Conservational Strategies organized at University of Lucknow, Lucknow during 22-23 March, 2014.

Dr. B. Kushwaha, Principal Scientist participated in the Advanced workbench training of CLC-Bio software during 29 October - 1 November, 2013 at IASRI, New Delhi.

Dr. P.K. Varshney, Principal Scientist attended a Workshop on Corporate Compliance of Environmental Clearance Conditions: The Future Regime organized by The Academy of Environmental Biology, Lucknow on 28 October, 2013.

Dr. N.S. Nagpure; Dr. K.K. Lal and Dr. Peyush Punia, Heads of the Divisions; Dr. Neeraj Sood, Principal Scientist; Dr. P.K. Pradhan, Dr. L.K. Tyagi and Dr. Rajeev Kr. Singh, Sr. Scientists participated in the 16th Indian Agricultural Scientists and Farmers’ Congress on Nanobiotecnological Approaches for Sustainable Agriculture and Rural Development on 22-23 February, 2014 at Integral University, Lucknow.

Dr. Neeraj Sood, Principal Scientist attended 3rd Technical Advisory Committee Meeting of the National Disease National Surveillance Programme for Aquatic Animal Disease at DAHDF, New Delhi on 9 December, 2013 National Workshop on Early Mortality Syndrome (EMS) / Acute Hepatopancreatic Necrosis Syndrome (AHPNS) of Cultured Shrimps at Chennai during 20 – 21 January, 2014.

Dr. Neeraj Sood, Principal Scientist and Dr. P.K. Pradhan, Sr. Scientist participated in an Awareness Meeting on National Surveillance Programme on Aquatic Animal Diseases for District Fisheries Officers, State Fisheries Department, Uttar Pradesh on February 10, 2014.

Dr. V.S. Basheer, Principal Scientist participated/delivered lectures in the following:
- Seminar on ‘Water and Biodiversity’ at Koyilandi, Calicut, Kerala on 15 June, 2013.

- A lecture on ‘Genetic conservation of *Etroplus suratensis*’ in the Consultation on the Husbandry of Pearl Spot at Ernakulam on 10 July, 2013.

- A lecture on ‘DNA Barcoding and Molecular Taxonomy of Fishes’ at College of Fisheries, Mangalore on 28 November, 2013.

- A lecture on ‘Exotic Fish Culture: Indian experience’ in the customized training in mariculture for Maldivian officials at CMFRI, Kochi during 18 November – 14 December 2013.


- A lecture on ‘Exotics Fish Culture’ in the customized training in mariculture for Maldivian officials at CMFRI, Kochi on 10 December, 2013.


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

- National Conference on ‘Strategies for bridging the Yield Gap in Fisheries and Aquaculture’ during 24-25 March, 2014 at College of Fisheries, Mangalore and presented a paper.

- 1st and 2nd Technical Advisory Committee Meeting of the National Disease National Surveillance Programme for Aquatic Animal Disease at DAHDF, New Delhi on 16th April, 2013 and 11th July, 2013, respectively.

Dr. L.K. Tyagi, Sr. Scientist participated in the Expert Committee Meeting on Formulation of Guidelines for Management of Genetic Resources at NBPRG, New Delhi during 29 – 30, April, 2013.


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


- Delivered a lecture on ‘Genetics in Aquaculture – A brief overview’ in the customized training in mariculture for Maldivian at CMFRI, Kochi during 18 November – 14 December, 2013.


Sarkar U. K., W. S. Lakra, Vineet Kumar Dubey, Ajay Pandey, Madhu Tripathi, Rupali Sani and Abhishek Awashti, 2013. Freshwater fish


**Book chapters**


**Reviews**

Popular Articles


LIBRARY AND INFORMATION SERVICES

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

Resource Development

The library added a total of 554 documents including 360 books. Now, the library has the total collection of 6851 books and 2655 bound volumes of journals. The library has subscribed 21 international journals and 71 Indian journals. In addition to these, 38 journals were received on gratis/exchange basis.

Library Automation

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

Information and Reference Services

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

Technical Reports and Reprography Services

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

Exchange Services

The Library continued exchange relationship and resource sharing with leading National and International Research Institutes and Development organizations. To keep abreast of the activities of the Bureau, the library sent the NBFGR Annual Report 2012-2013 and NBFGR Newsletters to various institutions and organizations including, Universities, State Fisheries Departments, FFDAs, Krishi Vigyan Kendras, Entrepreneurs and Fish Farmers.
STAFF ACTIVITIES

1. Promotions

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

Scientists
Dr. L.K. Tyagi Sr. Scientist from Pay Band Rs. 15600-39100+ RGP Rs. 8000/- to Pay Band Rs. 37400-67000+RGP Rs. 9000/- w.e.f. 25.01.2012.
Dr. T. Rajaswaminathan, Scientist from Pay Band Rs. 15600-39100 + RGP Rs. 8000/- re-designated as Sr. Scientist Pay band Rs. 15600-39100 + RGP Rs. 8000/- w.e.f. 23.05.2012.

Technicals
Mr. Subhas Chandra from T-5 to Sr. Technical Officer w.e.f. 18.09.2013
Mr. Rajesh Kumar from T-3 to Sr. Technical Assistant w.e.f. 03.09.2012
Mr. K.K. Singh from T-2 to Technical Assistant w.e.f. 05.09.2012

2. Recruitment/ Transfer

Dr. A. Gopalakrishnan, Principal Scientist and Officer-in-Charge, NBFGR Kochi Unit was appointed as the Director, Central Marine Fisheries Research Institute, Kochi and relieved on 31.07.2013.
Dr. A.K. Singh, Principal Scientist was appointed as the Director, Directorate of Coldwater Fisheries Research, Bhimtal and was relieved on 18.02.2013.
Dr. T.T. Ajit Kumar joined NBFGR as Sr. Scientist w.e.f. 12.08.2013.
Dr. S.K. Manjhi joined NBFGR, Lucknow as Sr. Scientist w.e.f. 04.11.2013.
Mr. Avinash Rambhau Rasal joined NBFGR as Technical Assistant (Category-II) w.e.f. 12.08.2013.
Mr. Rajool Shanis C.P. joined NBFGR as Technical Assistant (Category-II) w.e.f. 22.10.2013.
Mr. E. Suresh joined NBFGR as Technical Assistant (Category-II) w.e.f. 28.10.2013.

3. Financial Up-gradation under MACP Scheme

Mr. Santosh Kr. Singh, Jr. Clerk PB-I ‘5200-20200+GP of ’1900/- granted 1st ACP in PB-I with grade pay of ’2000/- w.e.f. 01.11.2012.
Mr. Jai Narain Tiwari, Skilled Support Staff PB-I ‘5200-20200+GP of ’1900/- granted 2nd MACP in PB-I with grade pay of ’2000/- w.e.f. 19.05.2013.

Management Committee

The Institute Management Committee (IMC) was represented by the following members nominated by Director General, ICAR, New Delhi:
1. Dr. J.K. Jena, Director, NBFR: Chairman
2. Dr. Madan Mohan, ADG (Marine Fy.), ICAR, New Delhi: Member (ICAR)
3. Dr. A. Gopalakrishnan, Principal Scientist, NBFGR, Lucknow (Now Director, CMFRI, Kochi)
4. Dr. R.S. Kataria, Principal Scientist, NBAGR, Karnal
5. Dr. R.K. Tyagi, Principal Scientist, NBAGR, New Delhi
6. Dr. (Mrs.) Sherly Tomy, Senior Scientist, CIBA Chennai
7. Mr. Abhishek Rana, Administrative Officer, Secretary NBFGR, Lucknow

The 26th meeting of the Committee was held on 12 July, 2013.

NBFGR team participated in the ICAR Intra-Zonal sport meet at IIPR, Kanpur during 20-23 March, 2014. Mrs. Mamta Chakraborty, Personal Assistant won IInd Prize in Discuss throw (Women), and IIIrd Prizes in Shotput and Javelin throw’s (Women) while Mr K.K. Singh, Technical Assistant won IInd prize in Javelin throw.

Mrs. Mamta Chakraborty receiving IInd prize
DISTINGUISHED VISITORS

Dr. S. Ayyappan, Secretary, DARE and Director General, ICAR, New Delhi.

Dr. B Meenakumari, Deputy Director General (Fy.), ICAR, New Delhi.

Dr. E.G. Silas, Former Vice-Chancellor, Kerala Agricultural University and Former Director, CMFRI, Kochi.

Dr. B. Venkatesh, Research Director, Institute of Molecular and Cell Biology, Singapore.

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

Prof R.C. Sobti, Vice Chancellor, Babasaheb Bhimrao Ambedkar University, Lucknow.

Dr. Rudolf Hermes, Chief Technical Officer, BOBLME.

Dr. John Candy, Technical Consultant BOBLME.

Dr. Jenny Stauber, Deputy Chief, CSIRO Land and Water, Australia.

Dr. K. Vijayakumaran, DG, FSI, Mumbai and National Coordinator BOBLME.

Dr. A.K. Srivastava, Director, NDRI, Karnal.

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

Dr. C.V. Mohan, Research and Development Manager, NACA, Bangkok.

Sh. B. Vishnu Bhat, Fisheries Development Commissioner, DAHDF, New Delhi.

Prof. Kenton Lloyd Morgan, University of Liverpool, Neston, UK.

Dr. Jiraporn Kasornchandra, Department of Fisheries, Thailand.

Dr. T.K. Srinivasa Gopal, Director, CIFT, Kochi.

Dr. W. Vishwanath, Professor, Manipur University, Imphal.

Dr. K.G. Padmakumar, Associate Director, RARS, KAU, Kumarakom.

Dr. N. Ramaiah, Chief Scientist, NIO, Goa.

Dr. S. Solomon, Director, Indian Institute of Sugarcane, Lucknow.

Dr. Madhumita Mukherjee, Executive Director (Tech.), NFDB, Hyderabad.

Dr. A.S. Ninawe, Sr. Advisor, DBT, Govt. of India.

Dr. T. Mohapatra, Director, CRRI, Cuttack.

Dr. K.C. Gupta, Director, Indian Institute of Toxicology Research, Lucknow.

Dr. A. Gopalakrishnan, Director, CMFRI, Kochi.

Dr. M.K.J. Siddiqui, Director, UPCST, Lucknow.

Dr. Saroj Kumar, Director, Department of Fisheries, Government of Uttar Pradesh.

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

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

Dr. A.K. Sharma, Director, NBAIM, Mau.

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

Dr. P. Jayasankar, Director, CIFA, Bhubaneswar.

Dr. K. M. Shankar, Dean, College of Fisheries, Mangalore.

Dr. M. H. Balkhi, Dean, Faculty of Fisheries, SKUAST, Srinagar.

Prof. J.R. Dhanze, Dean, College of Fisheries, CAU, Agartala.

Dr. (Mrs.) Usha Goswami, Scientist ‘F’ (Retd.), NIO, Goa.

Prof. Bechan Lal, Professor, Department of Zoology, BHU, Varanasi.

Dr. Chaitanya G. Joshi, Professor and Head, Department of Animal Biotechnology, AAU, Anand.

Dr. Anu Kumar, Project Leader, Land and Water, CSIRO, Australia.

Dr. Merrin Adams, Project Leader, Ecotoxicology Group, CSIRO, Australia.

Dr. Peter Bain, Project Research Officer, CSIRO, Australia.

Dr. K.N. Singh, Head, Forestry Division, IASRI, New Delhi.
LIST OF PERSONNEL

Research Management
Dr. Joykrushna Jena - Director

Scientific Staff
1. Dr. N. S. Nagpure - Head of Division
2. Dr. K. K. Lal - Head of Division
3. Dr. Peyush Punia - Head of Division
4. Dr. (Mrs.) Rehana Abidi - Principal Scientist
5. Dr. A. Gopalakrishnan - Principal Scientist & In-Charge (Kochi Unit) (Upto July, 2013)
6. Dr. A. K. Pandey - Principal Scientist
7. Dr. Sudhir Raizada - Principal Scientist
8. Dr. S. P. Singh - Principal Scientist
9. Dr. A.K. Singh - Principal Scientist (Upto February, 2014)
10. Dr. (Mrs) Vindhya Mohindra - Principal Scientist
11. Dr. P. K. Varshney - Principal Scientist
12. Dr. Ravindra Kumar - Principal Scientist
13. Dr. Basdeo Kushwaha - Principal Scientist
14. Dr. U. K. Sarkar - Principal Scientist
15. Dr. Neeraj Sood - Principal Scientist
16. Dr. V. S. Basheer - Principal Scientist & In-Charge (Kochi Unit)
17. Dr. Mukunda Goswami - Sr. Scientist
18. Dr. Parvata Kumar Pradhan - Sr. Scientist
19. Dr. Lalit Kumar Tyagi - Sr. Scientist
20. Dr. Rajeev Kumar Singh - Sr. Scientist
21. Dr. Mahender Singh - Sr. Scientist
22. Dr. T. Rajaswaminathan - Sr. Scientist (NBFG Cochin Unit)
23. Dr. T.T. Ajith Kumar - Sr. Scientist
24. Dr. Sullip Kumar Majhi - Sr. Scientist
25. Mrs. Poonam Jayant Singh - Scientist (On Study Leave)
26. Shri Ajey Kumar Pathak - Scientist
27. Dr. (Mrs.) Divya P.R. - Scientist (NBFG Cochin Unit)
28. Shri A. Kathirvelpandian - Scientist (NBFG Cochin Unit)
29. Ms. Sangeeta Mandal - Scientist
Technical Staff

1. Shri Rajesh Dayal - Chief Technical Officer
2. Shri S. M. Srivastava - Chief Technical Officer
3. Shri A. K. Yadav - Assistant Chief Technical Officer
4. Shri Amar Pal - Assistant Chief Technical Officer
5. Shri A. K. Mishra - Assistant Chief Technical Officer
6. Shri S. P. Singh - Assistant Chief Technical Officer
7. Shri Babu Ram - Assistant Chief Technical Officer
8. Shri Ajay Kumar Singh - Senior Technical Officer
9. Mrs. Reeta Chaturvedi - Senior Technical Officer
10. Shri Ramashankar Sah - Senior Technical Officer
11. Shri Subhash Chandra - Senior Technical Officer
12. Shri Mohd. Gayas - Technical Officer
13. Shri Ved Prakash - Technical Officer
14. Dr. Akhilesh Kr. Mishra - Technical Officer
15. Dr. (Mrs.) Ranjana Srivastava - Technical Officer
16. Shri Ravi Kumar - Technical Officer
17. Shri S. K. Singh - Technical Officer
18. Shri Amit Singh Bisht - Technical Officer
19. Shri Satyavir Chaudhary - Technical Officer
20. Shri S. K. Upadhyay - Senior Technical Assistant
21. Shri R.K. Shukla - Senior Technical Assistant
22. Shri B. N. Pathak - Senior Technical Assistant
23. Shri Samarjit Singh - Senior Technical Assistant
24. Shri Om Prakash - Senior Technical Assistant
25. Shri Rajesh Kumar - Senior Technical Assistant
26. Shri B. K Rao - Technical Assistant
27. Shri Om Prakash-II - Technical Assistant
28. Dr. Vikash Sahu - Technical Assistant
29. Shri Madan Lal - Technical Assistant
30. Shri Raj Bahadur - Technical Assistant
31. Shri Gulab Chandra - Technical Assistant
32. Shri K. K Singh - Technical Assistant
33. Shri Avinash Rambhau Rascal - Technical Assistant
34. Shri Rajool Shanis C.P. - Technical Assistant (NBFG, Cochin Unit)
35. Shri E. Suresh - Technical Assistant
36. Shri Sree Ram - Senior Technician
37. Shri P. C. Jaiswar - Senior Technician
38. Shri Ram Bharose - Senior Technician
### Administrative Staff

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

### Skilled Supporting Staff

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

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

Address: Scientist-in-Charge
NBFG Cochin Unit
CMFRI Campus
Post Box No. 1603
Ernakulam North P.O.
Kochi - 682 018, Kerala.
Telefax: 0484-2395570
E-mail: nbfgcochin@vsnl.net
nbfgcochin@eth.net
Aquaculture Research & Training Unit, Chinhat

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

Address : Scientist-in-Charge
NBFRG Aquaculture Research & Training Unit
Malhore Road, Chinhat
Lucknow-227 105, U.P.
Telefax : 0522-2815848
E-mail : director@nbfrg.res.in