MARINE BIOTECHNOLOGY DIVISION

Research Focus:

The research focus of the MBTD falls into four major areas

- **Fish Genetics, Genomics, Molecular Biology, & Fish Physiology**
- **Fish Nutrition**
- **Fish Health (Pathology, Microbiology, Immunology, Cell Culture & Molecular Diagnostics) and**
- **Biochemistry and Bioprospecting**

Scientist profile:

- Principal Scientists - 2
- Senior scientists - 5
- Scientists (SS) - 3
- Scientists - 4

The main objectives of this Division are to fill the technological gaps with reference to the research programmes in mariculture and fisheries resource management and also to conduct basic research for technological backstopping.

Theme: Genetics & genomics:

Projects

1. Genetics, genomics and biotechnological applications in mariculture and fishery resources management. *Project No: FISHCMFRISIL201202800028, Institute Project.*
2. Development of tissue culture technology for in-vitro production of pearls from the black lip pearl oyster *Pinctada margaritifera* and refinement of *in-vitro* pearl formation in *Pinctada fucata*. *FISHCMFRISIL201202900029. Institute Project.*
3. Bioprospecting of genes and allele mining for abiotic stress tolerance. NAIP *Funded Project*
4. Outreach Project on Fish Genetic Stocks
5. Genetic study on breeding stock of the Indian mackerel along the Indian coast. *FAO-BOBLME funded project*
6. Genetic stock structure analysis of Indian oil sardine using microsatellite markers - *NICRA funded project*
7. Molecular Approach to diet analysis in selected commercially important tunas – *DST funded project.*
8. National Surveillance Programme for Aquatic Animal Diseases – *NFDB funded project.*

Salient achievements:

A: Theme: Genetics & genomics:

➢ Barcoding of Indian Ocean tunas using mitochondrial cytochrome c oxidase gene

A phylogenetic analysis of nine species of tunas belonging to the family Scombridae (Tribe Thunnini) was carried out using partial sequences of mitochondrial cytochrome c oxidase gene. The sequences of *Auxis rochei*, *A. thazard*, *Katsuwonus pelamis*, *Euthynnus affinis*, *Thunnus albacares*, *T. tonggol*, *T. obesus*, *Sarda orientalis* and *Gymnosarda unicolor* were deposited to Bar code of Life Database (BOLD) and GenBank database of National Centre for Biological Information (NCBI). A maximum likelihood tree was constructed using the barcode generated by CMFRI and the sequences of similar species extracted from GenBank. The sequences of similar species clustered together as separate assemblage with significant boot strap values. All the 3 species of the genus Thunnus (*Thunnus albacares*, *T. tonggol* and *T. obesus*) also clustered together into a single clade. Average values of K2P distance which indicates genetic divergence between species was greatest (0.185) between *Katsuwonus* sp. and *Gymnosarda* sp. The genetic divergence values were lowest between *Auxis* sp. and *Euthynnus* sp. (0.097).

➢ Development of species-specific PCR primers for bivalves

Species-specific PCR primers based on the mitochondrial CO1 gene were developed for both green mussel (*Perna viridis*) and edible oyster (*Crassostrea madrasensis*) and its specificity was evaluated by PCR with the target species and the closely related organisms found in the same niche. The developed primers were found to be species specific with the target bivalve species.

➢ Genes involved in bio-mineralization process of pearl formation in *Pinctada fucata*

Expression analysis of four genes involved in biomineralization process of pearl formation viz. nacrein, prismalin-14, calreticulin and N19 following mabe implantation was continued during the year using semi-quantitative PCR. Housekeeping gene encoding glyceraldehydes-3-phosphate dehydrogenase (GAPDH) was selected as reference for the calculation of relative expression levels of target genes. Expression levels of nacrein, prismalin-14 and N19 showed a predominant up-regulation with slight variations among the different time periods.

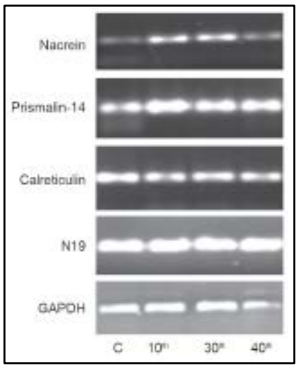


Fig.1. Agarose gel images of semi-quantitative PCR of Nacrein, Prismalin-14, Calreticulin and N19 genes carried out with cDNA samples from the mantle surrounding the mabe of implanted *P. fucata*. GAPDH was included as a positive control; lane: 1, PCR product of non-implanted control; lane: 2, 10th day; lane: 3, 30th day; lane 4, 40th day after implantation.

➢ Characterisation of functional gene Superoxide dismutase (SOD) from edible oyster *Crassostrea madrasensis*

Characterisation of the anti-oxidant enzyme gene, superoxide dismutase from *C. madrasensis* was carried out. The DNA sequence was analysed with the protein domain identifier software Inter Pro Scan. The analysis revealed the characteristic domains classifying the gene as Cu/Zn SOD. PCR trials to amplify the gene from both genomic DNA and cDNA revealed some peculiar features of this gene. PCR reactions using both genomic DNA as well as cDNA as template resulted in the amplicon of same size viz., 464 bp. This has clearly indicated the absence of introns in this gene. This is in contrast with SOD gene of other species where introns are present. It has been suggested that the lack of introns may help to circumvent the block of RNA splicing, allowing the rapid synthesis of proteins. This characteristic enables ready expression of these proteins during periods of stress.

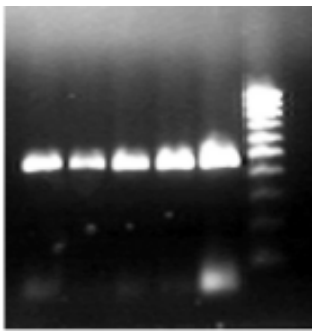


Fig.2 Amplified SOD gene segment from *C. madrasensis*

Laboratory experimental trials to study the expression of these genes on exposure to thermal stress was conducted. Thermal shock was given at sublethal temperature of 40^{o}C for one hour. Gills from control and test oysters were excised at regular intervals. Total RNA was isolated from the gill tissue and cDNA was synthesised to study quantitative gene expression. Transcription analysis through semi-quantitative PCR using cDNA have shown the regular upregulation of the SOD gene expression as time proceeds after the thermal shock.

➢ Microalgae and abiotic stress gene mining

Collection, isolation, purification and maintenance of 144 pure isolates of marine microalgae representing different habitats of Indian Coast. Phenotypic and genotypic identification of more than 100 isolates of marine microalgae. Gene mining (partial and complete characterization) of 9 important genes conferring tolerance to the survivability of marine microalgae under abiotic stressful conditions.

Allele mining, details of gene sequence information generated

Species	Stress	Gene(s) (sequence information)	Germplasm lines used	Sequence submission
Dunaliella salina & Dictyosphaerium ehrenbergianum	Salinity	4-Hydroxy-3-methylbut-2-enyl diphosphatereductase (HDR) (Complete CDS amplified, cloned and expressed in *E.coli*)	2	JQ762450 & JQ762456
Dunaliella viridis	Salinity	2-Cys peroxiredoxin (Prx2) (Complete CDS amplified, cloned and expressed in *E.coli*)	1	JQ762455
D. viridis and *Dictyosphaerium ehrenbergianum*	Salinity	Trehalose-6-phosphate synthase (TPS) (Complete CDS amplified and cloned)	2	JQ762453 & JQ762454
Dunaliella viridis	Salinity	Duplicated carbonic anhydrase (DCA1) (Complete CDS amplified and cloned)	1	JQ762452
Artrospira platensis	Salinity	Desaturase-D (des D)	1	JQ762449
Dunaliella sp.	Salinity	Phytoene synthase	1	JQ762451

HDR is an enzyme playing a key role in the regulation of isoprenoid biosynthesis and is required for tolerance to high salinity and nutritional depletion conditions. The full length (complete CDS from start codon to stop codon) HDR gene (~1960 bp) was amplified from cDNA using the primers HDR3Fw (5'TGATGTTGTCCAACAGCTTC3') and HDR4Rev (5'CCGGGTTGTGGATGATTTC GTTGGT 3'). This amplified product has been cloned into pJET1.2 cloning vector. The full gene sequence information has been generated from both ends using the vector specific forward and reverse primers.omplete characterization of HDR gene conferring tolerance to high salinity and nutrition depletion conditions

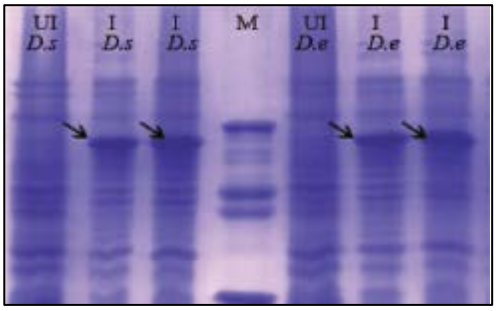


Fig.3. Expression profile of HDR gene on 10% SDS -PAGE

The completely characterised 4 - Hydroxy - 3 - methylbut - 2 - enyl diphosphate reductase (HDR) gene from *D. salina* has been recombinantly expressed in *E. coli.*

- **An abiotic stress gene for salinity tolerance from the microalgae, *Teraselmis indica***

An abiotic stress gene for salinity tolerance from microalgae *Teraselmis indica*, from a hyperhaline habitat has been identified using subtractive suppression hybridization (SSH)

method and characterized using RACE-PCR method. The hyperhaline microalgae isolated from the Pulicat hyper saline lake in southeast coast of India has the ability to tolerate wide range of salinity from 1M NaCl to 4M NaCl. This mined gene for salinity tolerance, FBP aldolase was 556bp and the sequence could be useful in the transgenic studies.

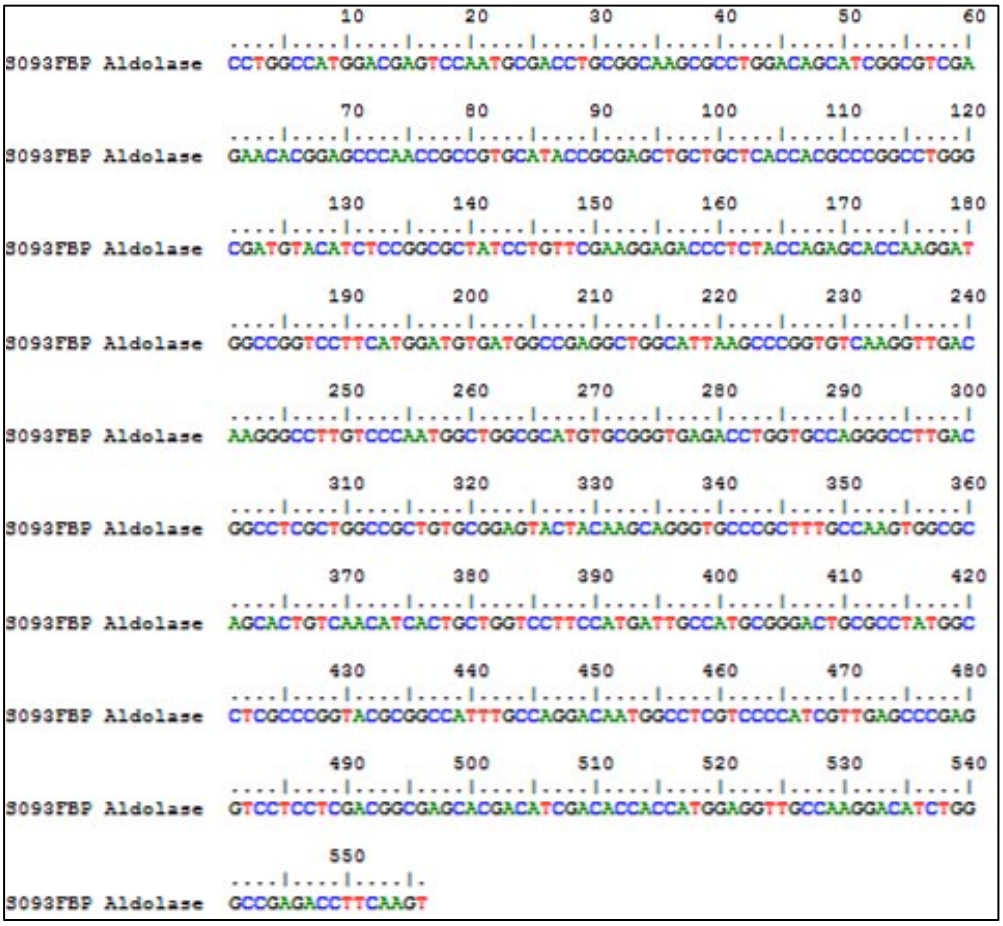


Fig.4. The full-length cDNA sequence of *Tetraselmis* FBP Aldolase derived through

- **An abiotic stress gene for acid tolerance genes from euryhaline microalgae *Dictyospherium ehrrenbergianum*.**

Micro algae, *Dictyospherium ehrrenbergianum* isolated from Cochin estuary strain has wide range of salinity tolerance from 0ppt to 40ppt and the saline acclimatized cells has the ability to withstand low pH (below4) compared to the cells grown at low salinity. Suppressive Subtractive Hybridization was carried out with the cells grown at pH 4 (Tester) and pH 7.5 (Driver) for the isolation and characterization of differentially expressed genes under low pH. Total RNA was isolated during the exponential phase of growth using TRI reagent and the cDNA was synthesized with 2µg mRNA purified from total RNA using mRNA purification Kit (Sigma). Differentially expressed genes were cloned and plasmids isolated from the positive clones were sequenced. Sequences edited and BLAST analyzed in NCBI.

➢ An abiotic stress gene for temperature tolerance from thermophilic microalgae, *Scenedesmus* sp.

Selected genes from the Suppressive Subtractive Hybridization library of thermophilic microalgae, *Scenedesmus* sp. were validated for their expression under temperature shock using Real Time PCR. A total of fifteen genes selected and gene specific primers were designed and PCR conditions were standardized. Normalization of the expression was carried out with 18S ribosomal reference gene. Total RNA isolated from Normal cells (grown under 22ºC) and Stressed cells (42ºC) were treated with RNase free DNase to eliminate genomic DNA contamination. Quantitative PCR data shown that all the selected genes has an upward expression under temperature shock except one gene (Metallothionin) which shown a downward regulation. The expression profile of the unknown genes (Sc331 and Sc281) and FKBP12 gene has higher expression compared to other selected genes under heat stress.

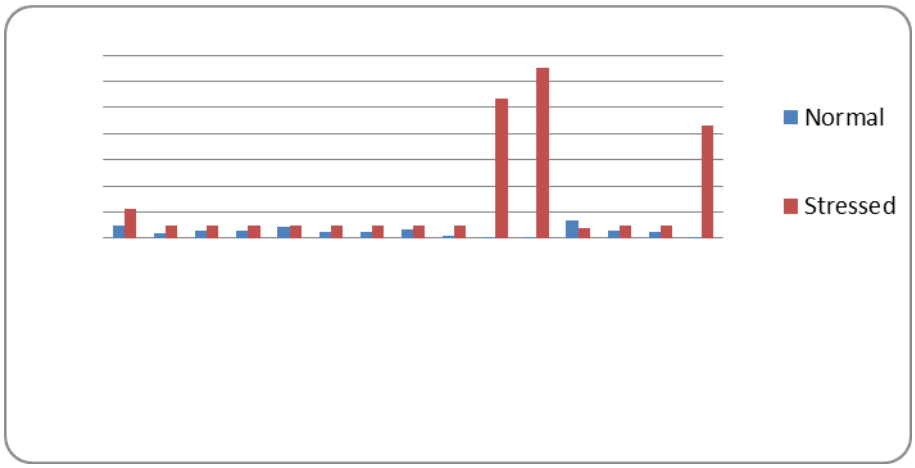


Fig.5. Quantitative gene expression profile of the selected gene fragments differentially expressed under heat shock.

➢ Selective breeding to develop small Artemia using native native *Artemia franciscana* strain

Mass selection was practiced to develop small nauplii strain (SNS) following the established method with suitable modifications. Selective breeding was carried out for fifteen generations (F1 to F15). Substantial reduction in naupliar size could be achieved. Naupliar length (first day length -FDL) could be reduced from 517 microns to 439 through selective breeding. Correlated response in other life history biometric traits such as third day length (TDL), sixth day length (SDL), length of male at sexual maturity (LMSM) and Length of female at sexual maturity (LFSM) are also calculated.

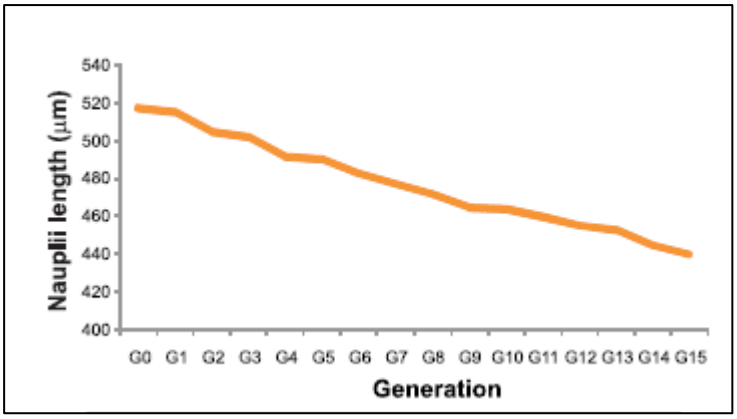


Fig.6. Nauplii length of the selected *Artemia* in different generations

➢ Species specific molecular markers for green mussel *Perna virdis* & edible oyster *Crassostrea madrasensis*

Species specific molecular markers were developed and standardized for the identification of the larval stages of these species using species specific PCR primer based on the CO1gene. Application of the species specific nested PCR for *P. viridis* and *C. madrasensis* in natural plankton samples was tested and evaluated. After validation this molecular method could be used for the larval detection of these two species, using natural plankton samples.

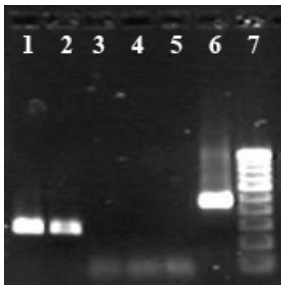


Fig. 7. Nested PCR assay showing species specific amplicons of *Perna viridis* (Lane 1&2) obtained from field collected plankton DNA. 18S rRNA positive control (Lane 6) also used to check the working quality of plankton DNA

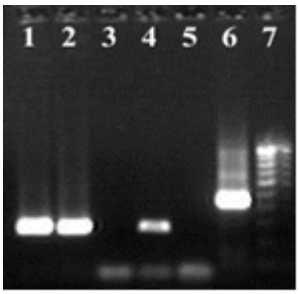


Fig. 8. Nested PCR assay showing species specific amplicons of *Crassostrea madrasensis* (Lane 1&2) obtained from field collected plankton DNA. 18S rRNA positive control (Lane 6) also used to check the working quality of plankton DNA

➢ Ornamental Transgenics

Trials were continued for the development of a transgenic fish expressing fluorescent proteins in their muscles. Transgenic larvae of *Danio rerio* expressing Green fluorescent protein was produced using electroporation. The integration of the gene was confirmed by PCR using specific primers. Green fluorescence expression was also visualized under fluorescent microscope. The transgenic larvae could survive only 1dph.

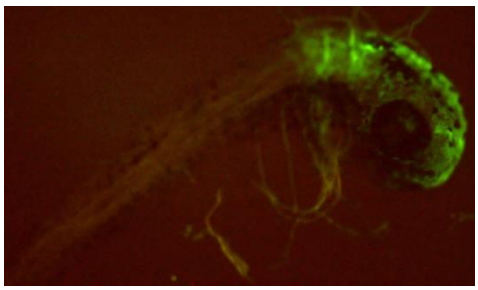


Fig.9. Transgenic Zebra fish *Danio rerio* larvae expressing Green Flourescent Protein

Transgenic *Etroplus maculatus* embryo expressing Red fluorescent protein was also produced using electroporation, the integration of the gene was confirmed using PCR with specific primers. The transgenic embryos were viable for 72 hours.

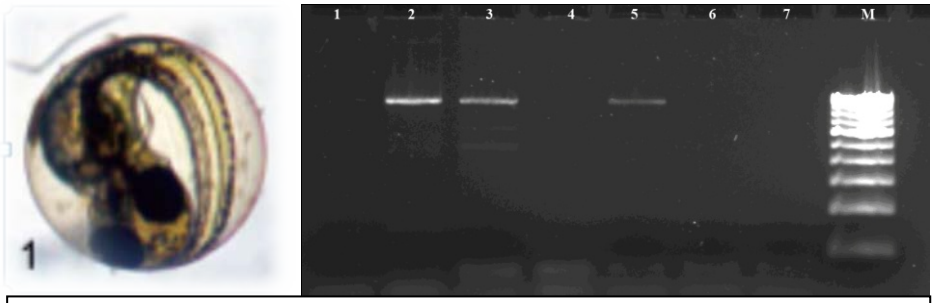


Fig.10. PCR confirmation of the presence of transgene in *E. maculatus* embryo. Lane 2,3,5 confirmed, lane 1, 4, 6 and 7 Negative. M-marker.

➢ Molecular cloning and recombinant expression of Gonad Inhibiting Hormone (GIH) from *Penaeus monodon*

Aimed at deriving an alternative way to induce growth and ovarian maturation without eyestalk ablation in *Penaeus monodon*, molecular and functional aspects of the Gonad Inhibiting Hormone (GIH) was investigated in the species, as a model for crustaceans. Primers were designed for directional insertion of mature peptide coding region encoding PmGIH into pET-28b Expression vector (Novagen, USA) for recombinant expression of PmGIH along with a fused 6xHIS tag at the C terminus. The recombinant pET-28b-PmGIH plasmid produced was used to transform *E. coli* ER2566 competent cells (NEB) and transformed colonies were selected for expression studies by IPTG induction. The recombinant PmGIH protein was affinity purified and eluted into denaturing elution buffer. The GIH was visualized and documented by 15% Tricine SDS polyacrylamide gel electrophoresis (Tricine SDS PAGE) under reducing conditions.

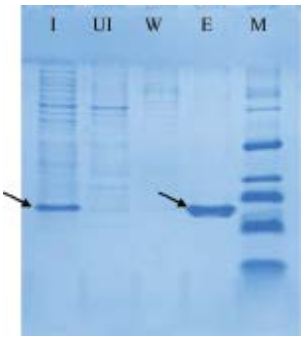


Fig.11. Tricine SDS-PAGE analysis of the rGIH expression

➢ Population genetic analysis of Indian oil sardine, *Sardinella longiceps* along the Indian coast using microsatellite markers

Population genetic structure of the Indian oil sardine, *Sardinella longiceps* was studied using six microsatellite markers developed using cross amplification. Studied the populations from Mumbai, Mangalore, Calicut, Trivandrum, Chennai and Visakhapatnam. Microsatellite genotyping was carried out on the ABI Prism genetic analyzer with primers labeled using 6FAM fluorescein dye and alleles were identified using allele calls in GENEMAPPER software. Alleles were mapped for 96 samples from each location. All loci were in Hardy-Weinberg equilibrium and none of them were in linkage disequilibrium ($P<0.05$). Maximum number of alleles was observed at locus 3 in all the analyzed populations. Average number of pair wise differences was highest between Mumbai and Chennai samples and lowest between Mangalore and Trivandrum. The values of genetic differentiation were also highest between Mumbai and Chennai samples and the lowest between Mangalore and Trivandrum samples. The analysis showed presence of well differentiated samples in all the locations. Calicut and Mangalore samples showed the highest allelic diversity. Calicut and Mangalore is located at the Malabar upwelling zone and may be providing the most ideal conditions for sardine populations to survive and reproduce. The pair wise genetic differentiation and pair wise genetic differences also showed high values between Mumbai and Chennai and Mumbai and Vizag.

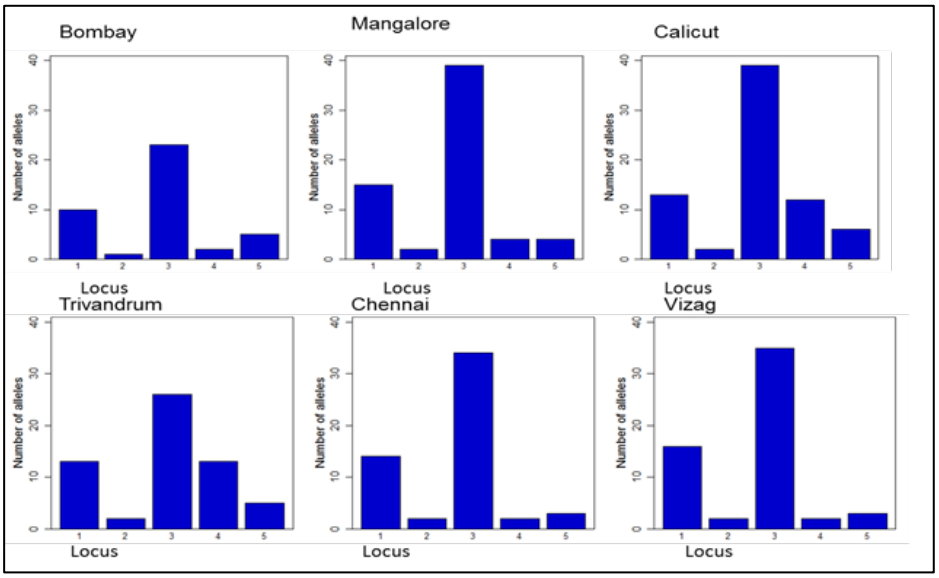


Fig. 12. Mean number of alleles in different locations

➢ Population genetic analysis of Indian oil sardine, *Sardinella longiceps* using mitochondrial DNA markers

Nucleotide diversity of mitochondrial cytochrome c oxidase gene from the Indian oil sardine, *Sardinella longiceps* was analyzed by taking samples from east and west coasts of India. *S. longiceps* collected from Veraval, Mumbai, Mangalore, Calicut, Kochi, Trivandrum, Chennai and Vizag were analysed for haplotype diversity, degree of genetic differentiation and nucleotide diversity. A total two hundred and fourteen sequences were analyzed from

the 8 sites and the number of segregating nucleotide sites was 94. Eighty unique mitochondrial haplotypes were detected with comparatively high haplotype diversity (Hd; 0.841) and nucleotide diversity values (PiT:0.004). Within-site haplotype diversity was high (Hd; 0.95) in *S. longiceps* collected from Chennai coast followed by Vizag coast (0.94). A maximum likelihood tree was constructed with a bootstrap value of 1000. *S. longiceps* collected from all the locations formed a single clade with insignificant bootstrap values. Kimura 2 parameter (K2P) distance values were the highest between *S. longiceps* collected from Chennai and Vizag (0.007) and the lowest between *S. longiceps* collected from Calicut and Veraval (0.002) and Kochi and Veraval (0.002). The K2P distance was not proportional with their geographic distances. The comparatively high values of haplotype and nucleotide diversity may be a reflection of the evolution taking place at genome level in response to environmental fluctuations. Recently, climate change studies have found extension of northern and eastern boundaries of oil sardine due to fluctuations in average annual sea surface temperature. The similarities in mitochondrial haplotypes collected from Kochi, Mangalore, Trivandrum, Chennai and Veraval may be due to this extension in boundaries of occurrence. Studies are in progress with more number of mitochondrial gene markers to ascertain whether different subpopulations/stocks of oil sardines exist in Indian waters.

- **Studies on the genetic stock structure of Indian mackerel, *Rastrelliger kanagurta* along the Indian coast.**

Fresh samples of Indian mackerel *Rastrelliger kanagurta* belonging to the breeding stock (Maturity Stage IV) were collected from 7 sampling sites, 1. Calicut. 2. Nagapatnam, 3. Paradeep, 4. Tuticorin, 5. Mumbai, 6. Kakinada and 7. Port Blair , with sample size of 30 nos each along the Indian coast for microsatellite analysis. Standard operating procedures were followed for tissue sample collection and preservation. 14 microsatellite primers were identified for genotyping the samples collected from each location. Microsatellite genotyping was carried out on the ABI Prism genetic analyzer with primers labeled using 6FAM fluorescein dye and alleles were identified using allele calls in GENEMAPPER software. Microsatellite genotyping has been completed in 30 samples of Indian mackerel, *Rastrelliger kanagurta* from Tuticorin, Calicut and Nagapatnam using the first 7 labeled primers, and further analysis is in progress.

- **Analysis of *Metapenaeus dobsoni* populations from Mangalore, Bhatkal and Goa**

Metapenaeus dobsoni collected from Mangalore, Bhatkal and Goa which had shown some morphological dissimilarities were tested for their species identity to resolve the taxonomic ambiguities from the region, using partial sequences of mitochondrial cytochrome c oxidase gene (CO1). Gene sequences for 650bp of the gene were generated for 9 samples from Mangalore, 4 samples from Bhatkal and 5 samples from Goa. The clustering pattern did not show any evidence of species level differentiation.

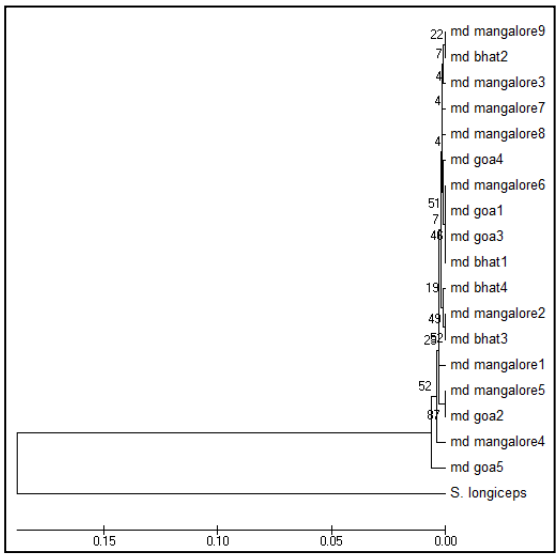


Fig.13. UPGMA tree generated using the mitochondrial cytochrome c oxidase gene sequences of *M. dobsoni* collected from Mangalore, Goa and Bhatkal coasts.

- **Characterisation of HSP 70 gene of *Crassostrea madrasensis***

The Open Reading Frame (ORF) of HSP 70 gene of *Crassostrea madrasensis* was deciphered. Amino acid sequence encoded by the complete coding sequences (CDS) of HSP70 gene was elucidated and the canonical domains in them were detected. BLAST search of the coding sequences have shown 93% identity with *C. gigas*, 92% with *C. ariakensis* and 92% with *Ostrea edulis*. Expression analysis of the stress management genes of the oysters were carried out. The up regulation of the stress management genes were found to result in enhanced thermo tolerance in the edible oyster, indicating higher stress tolerance ability.

- **Bivalve tissue culture experiments initiated at the newly developed tissue culture facility at Chennai centre of CMFRI, and also at CMFRI Tuticorin.**

In vitro culture experiments with mantle tissue of green mussel, *Perna viridis* and black-lip pearl oyster, *Pinctada margaretifera* were taken up using different tissue culture media including Leibovitz's L-15 medium, Medium 199 (M199) and also with sterile sea water. Cell cultures were assessed in terms of culture initiation, cell yield and susceptibility to contamination. After 8-10 days of culture, cell counts were made and cell size was measured for each treatment. Cells were observed to migrate from the periphery of the explant within 24 hrs after initiation of cultures and aggregate into groups. The liberated cells were mostly round and were either granulocytes or hyalinocytes. Fibroblast-like cells were also occasionally observed.

- **Muscle tissue culture:** Abalone is known for its multihued pearls and delicious meat. Abalone foot muscle attached to the shell region were excised and cut into small explants. The tissue was given antibiotic treatments and washed thoroughly before inoculation. The prepared explant was inoculated in cell plate of 6 well with one explant in

each well. Out of the 6 explants, one tissue gave promising result of tissue out growth. The growth comprises different types of cells characteristic of foot muscle. It included secretary cells, microvillous like cells etc. The tissue survived for one month duration. Experiment has to be continued for further studies and long term survival.

B: Theme: Fish Nutrition

Projects:

1. Aquatic feed biotechnology for aquariculture and mariculture. FISHCMFRISIL 201202700027 – *institute project*
2. ICAR Outreach Project on Fish Feeds
3. Polyunsaturated fatty acid enriched formulations from locally available low-value fish and fishery by-catch for use as nutraceuticals and aquafeed supplements - *DST-SERB funded*

Marine ornamental fish feed

- The **'Varna series'** of marine ornamental fish feed developed under the marine biotechnology division has been further refined in terms of packaging and colouring with natural colour sources. The feed has been field tested, showed wide acceptability among aquariculturists. The Cadalmin™ Varna Series of feeds are routinely used in CMFRI Centers for aquarium rearing facilities and sold through ATIC counter at CMFRI, HQ, Cochin. A Patent application entitled 'Formulated feed for marine ornamental fishes and a product therefore' No.32/ CHE/ 2010 has been filed. Efforts are underway for the commercialization the feed.

Fig.14. Packaged marine ornamental feeds

- A lobster fattening feed has been developed and tested. This feed is under further refining and evaluation.

- Formulated feeds (40% protein, 5% fat and 4 %< fibre) for fin fish mariculture using extrusion technology has been produced by the nutrition section of marine Biotechnology Division. A slow sinking nursery feeds (41% protein, 16% fat and 2% fibre) developed and used for nursery rearing provided to artisanal cage fish farms at Kottapuram under Kottapuram Integrated Development Society (KIDS) showed good acceptability in sea bass fingerlings.. Processes

Fig.15. Slow sinking pellet

optimized for sinking, slow sinking and floating feeds through twin-screw extrusion for fish rearing in different levels of water columns i.e., surface (floating), column (slow sinking) and bottom (sinking). On farm evaluation of slow sinking feeds on artisanal cages indicated good acceptability to use this feed for cage rearing.

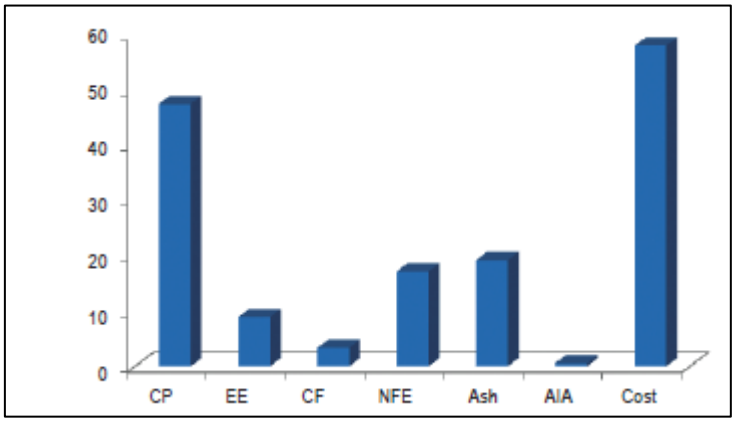


Fig. 16. Proximate composition (% dry matter basis) and cost (INR) of nursery feed for seabass

Fishmeal replacement studies

- Fishmeal replacement studies using leaf protein concentrate (LPC) prepared from locally available Tapioca (*Manihot esculenta*) in freshwater ornamental fishes (mollies and platys) through in an inter-institutional collaborative work with Central Tuber Crops Research Institute (CTCRI), Thriuvananthapuram, revealed that this product can be included up to 20% in the formulation, replacing fish meal. The potential of this product as a cost effective replacement for fish meal is promising.

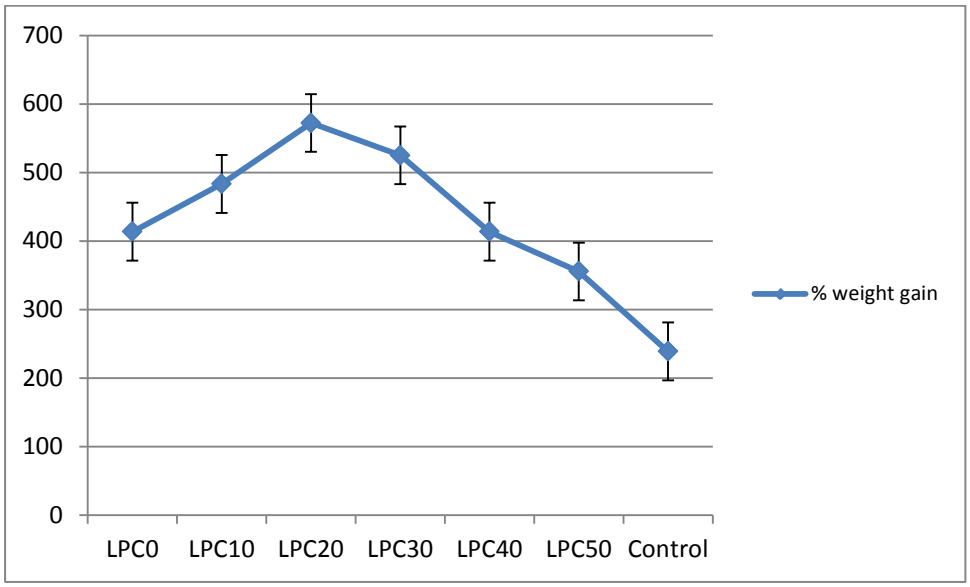


Fig. 17. Growth of black mollies on LPC substituted diets

New feed formulation for cage reared pompano

- New feed formulation for cage reared pompano (*Trachynotus blotchii*) using eight experimental feeds showed the formulation containing 50% protein and 9% lipid registered highest growth among the feed combinations.

Crude protein	30.22	41.32	50.71	60.36	32.94	41.45	50.74	60.72
Crude fat	15.58	12.31	9.48	6.34	6.46	9.05	12.06	15.70
Soluble carbohydrates	43.00	32.65	20.63	9.32	49.04	34.43	19.70	0.35
Crude fibre	0.63	0.70	2.25	1.19	0.73	1.82	1.45	1.01
Ash	9.67	11.69	14.93	19.63	9.87	11.96	14.32	18.86
Acid insoluble ash	0.90	1.33	1.99	3.16	0.97	1.29	1.74	3.36

Table.1. Proximate composition of feeds formulated with varying protein-lipid combinations for evaluation in pompano

A cost–effective feed formulation for *Etroplus suratensis*

- A cost–effective feed formulation for *Etroplus suratensis* (pearl spot – the state fish of Kerala) developed by MBTD is evaluated on farm with encouraging results. This feed is bulk produced through outsourcing and sold as a product by KVK of CMFRI. Further refinement of the formulation for higher FCR is contemplated.

Evaluation of enrichment emulsions in marine food fish larval rearing protocols and exploring development of a dry product either for enrichment of live feeds or for direct feeding of larval fish

- **Work done**
  - Developed an indigenous live feed enrichment emulsion with sardine oil, fish roe, emulsifiers and stablilizers
  - Standardized the enrichment protocols
  - Evaluated in marine ornamentals
  - Refined the product with some natural additives
- **Work proposed**
  - Evaluation if marine food fish larval rearing and comparing it with commercial products already available for cost effectiveness
  - Explore the possibility of developing a dry product which can be used as a stand-alone live feed diet

C: Theme: Fish Health and Marine Bioprospecting.

1. Health management in selected finfish and shellfish for mariculture and aquariculture and bioprospecting from marine resources. FISHCMFRISIL201202600026. *Institute project.*
2. Development of a library putative probionts from Marine environment belonging to the genus Pseudomonas, Bacillus and Micrococcus for application in mariculture systems. AMAAS Project 2020600004. *Funded Project*
3. Drugs from the sea: Development of antimicrobial, antiinflammatory and anticancer agents from the marine-organisms and micro-organisms – *MoES funded*
4. Characterization of bioactive compounds from marine macroalgae as defense metabolites against oxidative stress and inflammation. *DST-SERB funded*

First report of *Perkinsus* infection from Indian sub-continent

- Pearl oysters (*Pinctada fucata*) from the south east coast of India were found to be infected with *Perkinsus olseni,* an OIE listed protozoan parasite with a prevalence of 100%. This forms the first report on the existence of *P. olseni*, an OIE listed protozoan parasite in *P. fucata* from the Indian subcontinent and South Asia. Perkinsosis could be one of the major reasons for the decline of the natural pearl oyster beds along the Tuticorin coast during the past few decades.

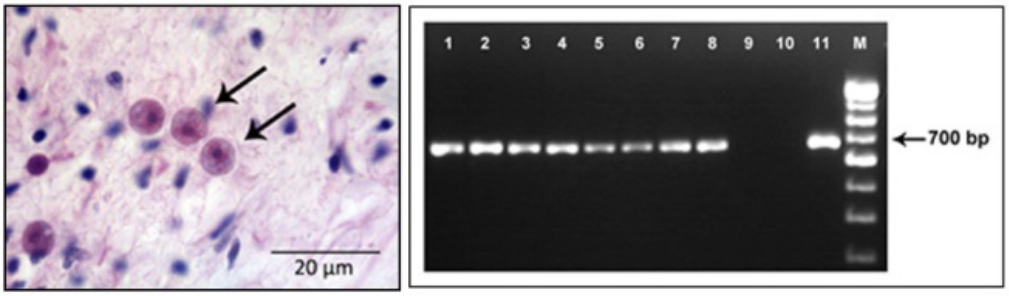


Fig. 18. Trophozoites of *P. olseni* in tissues; Agarose gel electrophoresis of the amplified products (ca 700bp) of the PCR.

- A DNA based diagnostic test using nested PCR was developed for the diagnosis of *P. olseni* infections in bivalves. This technique has high sensitivity and specificity and can detect infections which could not be diagnosed using conventional techniques like RFTM and first step PCR. This nested PCR could be suggested as the confirmatory test for *P. olseni* along with RFTM. A user friendly nested PCR kit is under development. Molecular diagnostic studies revealed that *Perkinsus olseni* enjoys a very wide host range and infects ten bivalve host species from the Indian subcontinent.

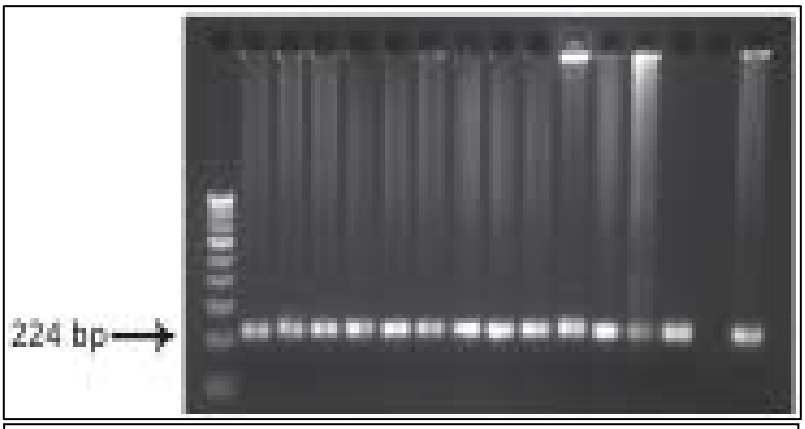


Fig.19. 224 bp nested PCR product specific to *P. olseni* Screening the bivalve population using the newly developed nested PCR clearly showed the advantage and efficacy of the technique over the conventional methods

Acanthocephalan (*Tenuiproboscis* sp.) infection in red snapper

- Pathogen profiling of red snapper *Lutjanus argentimaculatus* revealed the pathological manifestations of the acanthocephalan, *Tenuiproboscis* sp. The fish collected from Calicut, Cochin and Kannur harboured the acanthocephalan parasite, *Tenuiproboscis* sp. with up to 100% prevalence. Heavy infections with the parasites were observed in the posterior region of the intestine, almost blocking the lumen. At the site of parasite attachment, the surface of the intestine appeared thickened and the mucosal epithelium showed compression and abrasion. Intestinal folds were eroded along with thickening of lamina propria. The presoma of the parasites pierced the mucosal epithelium, lamina propria, muscle layers and serosa, reaching the peritoneal cavity, surrounded by a tunnel with collagenous fibers and granulocytes. Inflammation, granular tissue formation, connective tissue proliferation and associated host immune reactions were evident. Though the worms substantially damaged the architecture of the intestinal tissues, no apparent ill effects on the general health/condition of the fish were observed. The parasite was also recovered from *Epinephelus malabaricus* and *Lates calcarifer*. This is the first report of *Tenuiproboscis* sp. from L. *argentimaculatus*, *E. malabaricus* and *L. calcarifer*.

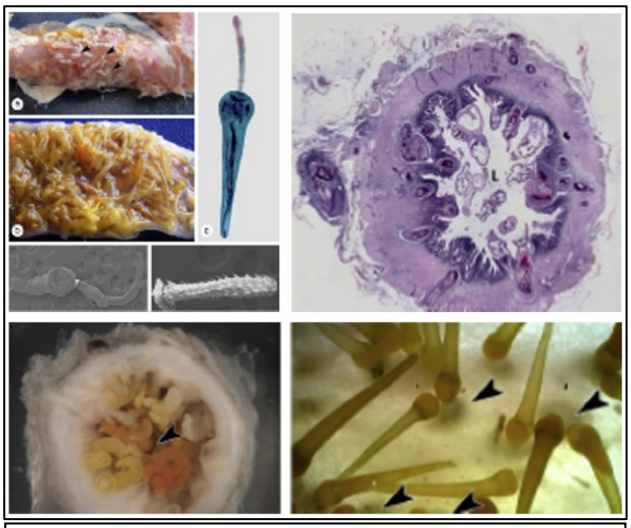


Fig.20. Outer view of the posterior intestine; b — intestine cut open to show the heavy parasite load; c — whole mount of the parasite; d — SEM of the parasite, e — SEM of the heavily armed proboscis; Cross section of the intestine showing the blocked intestinal lumen;. Cross section of the intestine showing the extent of tissue damage to the intestinal folds and muscle layers

First report of *Perkinsus beihaiensis* in *Crassostrea madrasensis* from the Indian subcontinent.

- Profiling study on the Indian edible oyster *Crassostrea madrasensis*, for the presence of *Perkinsus* spp. revealed the presence of *P. beihaiensis* for the first time in *C. madrasensis* populations from the Indian subcontinent and south Asia. Samples collected from the east and west coasts of India were subjected to Ray's fluid thioglycollate medium (RFTM) culture and histology which indicated the presence of *Perkinsus* spp. PCR screening of the tissues using specific primers amplified the product specific to the genus *Perkinsus*. The taxonomic affinities of the parasites on the ITS sequences showed 98 to 100% identity to *Perkinsus* spp. *(P. beihaiensis* and Brazilian *Perkinsus* sp.). The pairwise genetic distance values and phylogenetic analysis confirmed that 2 of the present samples belonged to the *P. beihaiensis* clade while the other 4 showed close affinities with the Brazilian *Perkinsus* sp. clade. The genetic divergence data, close affinity with the Brazilian *Perkinsus* sp., and co-existence with *P. beihaiensis* in the same host species in the same habitat show that the remaining 4 samples exhibit some degree of variation from *P. beihaiensis.* As expected, the sequencing of actin genes did not show any divergence among the samples studied. They probably could be intraspecific variants of *P. beihaiensis* having a separate lineage in the process of evolution.

- Fluorescent in situ hybridization (FISH) was used to confirm the presence of *Perkinsus* spp. in *C. madrasensis.*

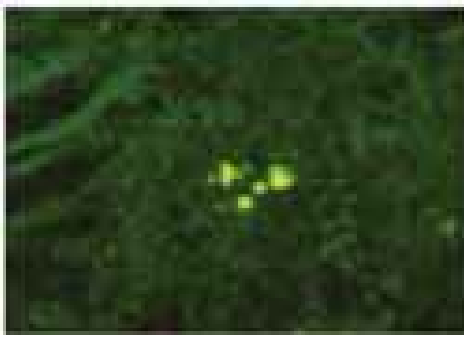


Fig. 21. Stages of *Perkinsus* spp. in the tissues

Vibrio alginolyticus infection in Asian seabass (*Lates calcarifer*) reared in open sea floating cages in India

- Asian seabass (*Lates calcarifer*) has been designated as a candidate species for open sea cage culture in India. A case of vibriosis in Asian seabass reared in open sea floating cages is reported. Haemorrhage and ulcer were observed grossly in the diseased fish. The pathogen, identified as *Vibrio alginolyticus* based on biochemical and molecular characterization, was isolated from liver, gill, kidney, brain and blood. Histological examination of the diseased fish showed congestion, haemorrhage and necrosis in vital organs. The LD50 studies showed that the organism was virulent to Asian seabass, and the LD50 value was 103.2 CFU g^{-1} fish.

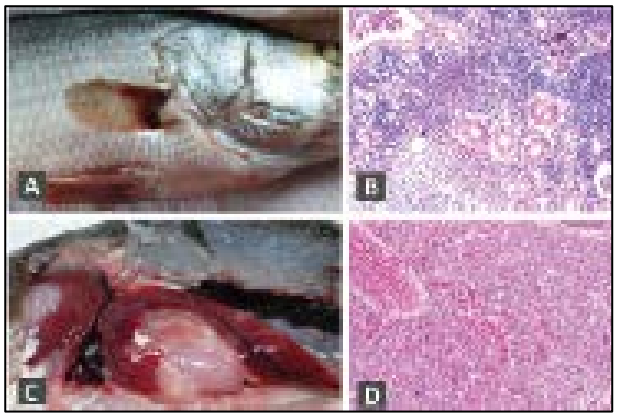


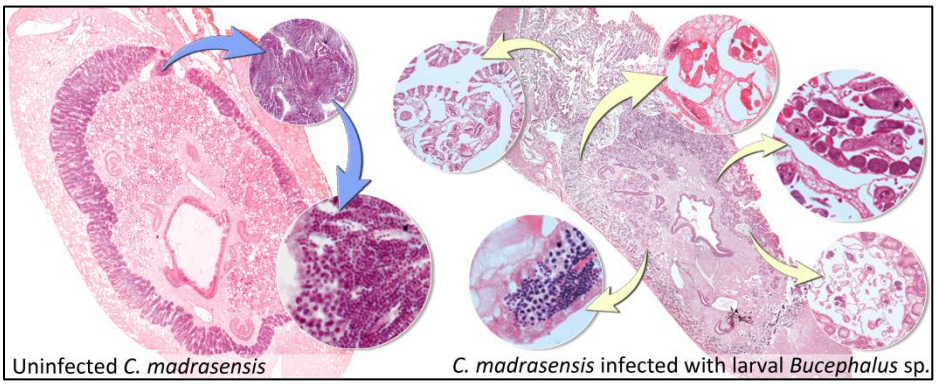
Fig.22. Pathological changes by *Vibrio* infections in seabass: A - Haemorrhage at the base of the pectoral fin; B - Haemorrhage and necrosis of the kidney parenchyma; C - Necrotic areas on the liver and congested kidneys; D - Severe haemorrhage in the liver

A disease outbreak caused by *Amyloodinium ocellatum*

- A disease outbreak caused by *Amyloodinium ocellatum* was reported from the gill tissues of pompano (*Trachynotus blotchii*) causing irritation, excess mucus secretion, respiratory distress leading to mortalities. Developmental stages including trophont & tomont were recovered. Treatment with 250mg Chloroquine diphosphate (Nivaquine-P) tablets with a dosage of 5mg/l for 10 days was able to control the disease.

Parasite induced reproductive dysfunction in *Crassostrea madrasensis*

- *Crassostrea madrasensis* samples collected from Tuticorin revealed the presence of larval Bucephalid infection in the gonads with a prevalence of 3.3%. The infected animal did not exhibit any apparent external manifestations but squash preparation indicated the presence of developmental stages of trematode infection in the gonadal tissues. Histopathological studies revelaed the massive replacement of gonadal tissues with sporocyst mass leaving only the remnants of gonadal tissues in small patches in the present study indicates the level of damage caused by the parasite. The altered tissue architecture as evidenced by the absence of typical acinar lumen and gonoducts and the drastic reduction in the number and volume of the gonadal acini appears to be insufficient to support any gametic release, resulting in gonadal dysfunction leading to parasitic castration of the host. An increase in the prevalence of Bucephalus infection in the region can seriously hamper the reproductive potential of the wild stocks and thereby limit the availability of the spat, affecting the viability of oyster farming in the region.



Sea lice (*Caligus* spp.) infestations

- Infestations with Sea lice (*Caligus* spp.) were recorded from marine ornamentals. Heavy infestation with *Lepeophtheirus* sp. was observed in the puffer fish *A. hispidus*. *Caligus* spp. infections were also reported from the skin and gills of bat fish and Koran angel. Life-cycle stages of *Lepeophtheirus* sp. were elucidated experimentally. The life-cycle of *Lepeophtheirus* sp. is as follows: adult parasite on the fish host, the eggs hatch into free swimming nuaplii which included two naupliiar stages and an infective copepodid stage. The copepodid seeks and settles on the host and undergoes moulting through a series of four different chalimus stages (chalimus 1-4). Sexual dimorphism is apparent by 4th stage and the chalimus passes through a young adult stage before they mature to adult stage.

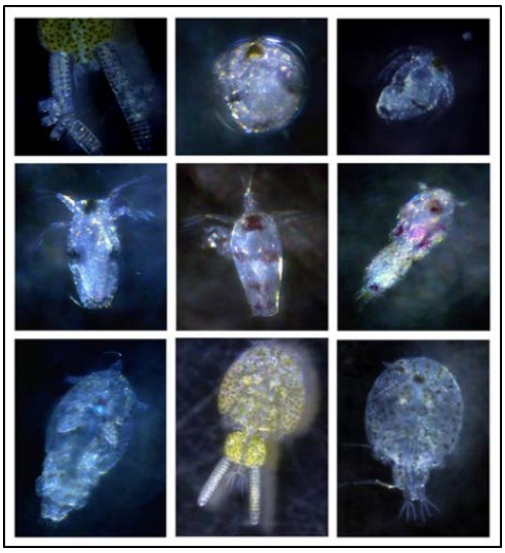


Fig. 24. Lif cycle of *Lepeophtheirus* spp.

- Infestation with the sea lice, *Calligus elongatus* was also recorded among the silver pompano (*Trachynotus blochii*) broodstock reared at Mandapam. The affected fishes showed frequent surfacing and anorexia. The parasites caused physical and enzymatic damage at the sites of attachment resulting in lesions/ulcers on the skin surface.

- Screening of cage reared finfishes, Snapper (*Lutjanus argentimaculatus*), Pearlspot (*Etroplus suratensis*), Mullet (*Mugil cephalus*), Asian Seabass (*Lates calcarifer*) and tilapia (*Oreochromis mosambicus*), revealed the presence of viral nervous necrosis (VNN), an OIE listed viral disease. This is being further studied using histopathology and molecular tools.

A novel Colony multiplex PCR (cmPCR), a molecular approach for the rapid detection of Bacillus and Pseudomonas genera—dominant antagonistic groups - from diverse ecological niches using colony has been developed.

- *Bacillus* and *Pseudomonas* are the dominant groups of bacteria known for their antagonistic potential against many plant and animal pathogens. Presently, exploration of these genera with antagonistic property for disease management of aquaculture system is gaining more importance to overcome the use of antibiotics and related resistance issues. Rapid screening and identification of these genera from diverse bacterial populations by conventional methods is laborious, cost-intensive, and time consuming. To overcome these limiting factors, in the present study, a colony multiplex PCR (*cm*PCR) method was developed and evaluated for the rapid detection of *Bacillus* and *Pseudomonas*. The

technique amplifies the partial 16S rRNA gene of *Bacillus* and *Pseudomonas* with a product size of ~1,100 and ~375 bp, respectively, using single forward (BSF2) and two reverse primers (PAGSR and BK1R). Reliability of the *cm*PCR method was confirmed by screening 472 isolates obtained from ten different eco-stations, of which 133 isolates belonged to *Bacillus* and 32 to *Pseudomonas*. The *cm*PCR method also helped to identify six different *Pseudomonas* spp. and 14 different *Bacillus* spp. from environmental samples. Of the total 472 isolates studied, 46 showed antagonistic activity, among which 63 % were *Bacillus* and 17.4 % were *Pseudomonas*. Thus, the newly developed molecular approach provides a quick, sensitive, and potential screening tool to detect novel, antagonistically important *Bacillus* and *Pseudomonas* genera for their use in aquaculture. Further, it can also act as a taxonomic tool to understand the distribution of these genera from wide ecological niches and their exploitation for diverse biotechnological applications.

The colony multiplex PCR (*cm*PCR) method amplifies the partial 16S rRNA gene of *Bacillus* and *Pseudomonas* with a product size of ~1100 and ~375 bp, respectively.

Lane 1: *Pseudomonas* sp.
Lane 2: *Bacillus* sp
Lane 3: *Pseudomonas* + *Bacillus*
Lane 4: *Vibrio* sp
Lane 5: Negative control
Lane 6: 100bp ladder

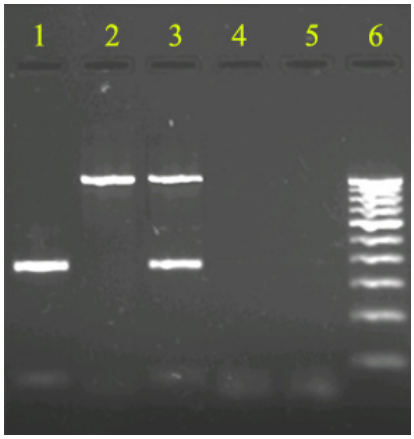


Fig.25. Agarose gel showing the PCR product of 400bp and 1000bp which were obtained by multiplex PCR reaction

Development of reverse transcriptase- Loop-mediated isothermal amplification for detection of beta noda virus:

- A Reverse transcriptase- Loop mediated isothermal amplification (RT- LAMP) was developed for detecting Beta noda virus infection. The RT- LAMP developed is capable of detecting single copy of the viral RNA. RT-LAMP is a novel approach to nucleic acid amplification which uses single temperature incubation, thus obviating the need for expensive thermal cyclers. Detection of the amplification product can be determined via photometry for turbidity or using SYBR green dye, a colour change can be seen without equipment, and can also be visualized in agarose gel electrophoresis. Primers were designed for detection based on conserved regions of RNA2 coat

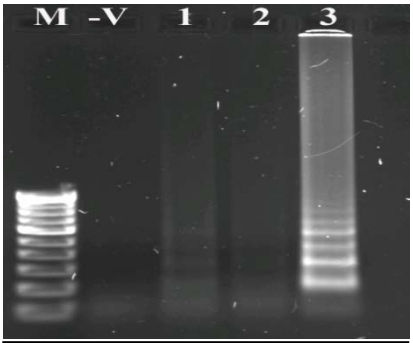


Fig: 26. RT-LAMP
M - Marker
-V - Negative Control
1 - 10 copies
2 - 5 copies
3 – sample from infected fish

protein of the four different forms of beta noda virus namely Striped jack nervous necrosis virus (SJNNV), Barfin flounder nervous necrosis virus (BFNNV), Tiger puffer nervous necrosis virus (TPNNV) and Red-spotted grouper nervous necrosis virus (RGNNV).The RT-LAMP is under validation to use as a novel diagnostic method for the detection of VNN in farmed finfishes.

- The health status of lobsters maintained in captive conditions before packing and exporting was monitored in different lobster holding facilities located around Kanyakumari. Infections in telson, appendages, inflammation in appendages and appearance of black spots in the abdomen were recorded as common problems in the live lobsters (species such as: Panuliurs homarus & P. versicolor) maintained in captive conditions. Bacterial examination of the infected lobsters revealed the association of 6 different isolates of which one exhibited high protease enzyme production. Results of antibiotic sensitivity revealed that chloramphenicol, tetracycline, erythromycin and ciprofloxacin in the decreasing order as effective inhibitory agents. During the sampling period, the microbial load in water varied from 4X106 to 5.3X108CFU/ml.

Fig.27. Telson rot conditions in live lobsters maintained in captive conditions

A new national project entitled 'National surveillance programme for aquatic animal diseases' (NSPAAD) has been initiated at CMFRI.

- A total of 6 districts were identified within the state of Kerala for regular screening of bivalves (*Perna virdis* and *Crassostrea madarsensis*) for listed diseases. Initial studies on farmed biavalves from Indian subcontinent using novel diagnostic methods indicate the presence of *Bonamia*, along with the already reported *Perkinsus* infection, diseases reported under OIE.

A novel nutraceutical from green mussel *Perna virdis*, the Cadalmin^{TM} GMe

- A novel nutraceutical from green mussel *Perna virdis* has been developed through unique biochemical engineering. This nutraceutical effective for the treatment of Cadalmin^{TM} GMe has sustained activity, no toxicity, less leachability. This is a green alternative to synthetic NSAIDs (viz., aspirin containing drugs having undesirable side effects). Cadalmin^{TM} GMe is designed to find a unique way to prevent the degradation by air, moisture, heat and light and to maximize the activity. The product is free from deleterious

Fig 28. Nutraceutical from green mussel – GMe

trans fatty acids, free radicals/free radical adducts, and low molecular weight carbonyl compounds. The product after patent has been subsequently commercialized through Amalgam Group of Companies at Kochi.

A novel nutraceutical from sea weeds, Green Algal extract (GAe) Cadalmin™

- From the the seaweeds belonging to different species were studied and bioengineered and developed a functional food material which contains useful immuno stimulating and anti-inflammatory ingredients, therby effective in the treatment of joint pain.. The active ingredients in GAe also suppress the build-up of uric acid in hyperuricemic patients. The GAe is a natural and 100% vegetarian product. With its therapeutic values, the green nutraceutical GAe is an import substitute with an international appeal, providing great market potential especially for the large vegetarian population in India and abroad. The product has been subsequently commercialized, through Celestial Biolabs, Hyderabad.

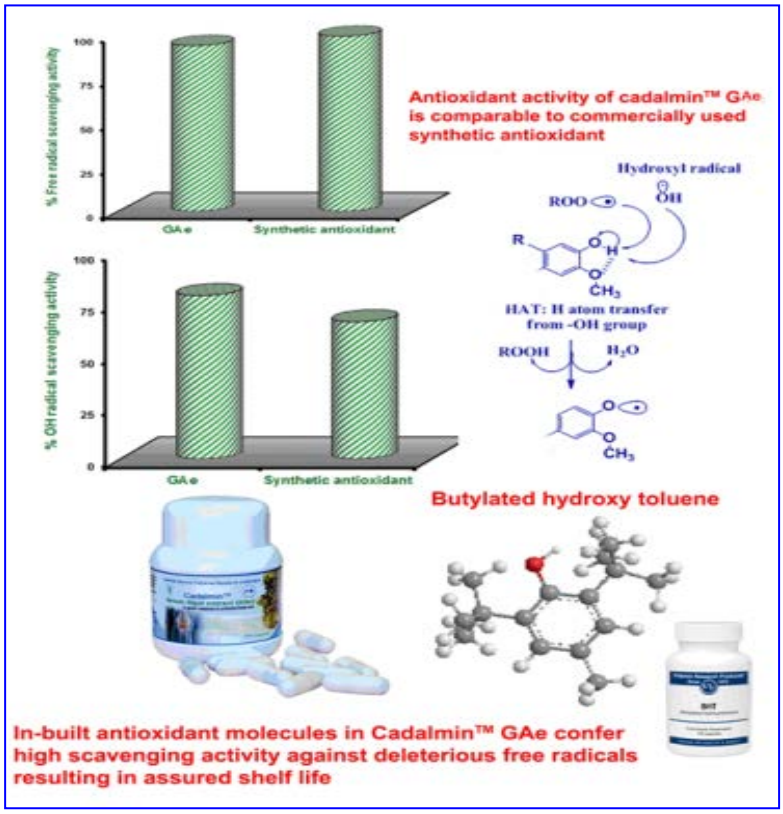


Fig 29. Nutraceutical product from seaweeds – GAe

Toxicity studies of Cadalmin™ Green Algal extract (Cadalmin™ GAe) for use against joint pain and arthritis

- Detailed evaluation using laboratory animal models proved that the Green Algal Extract, Cadalmin™ GAe could be safely taken without any side effects. The acute toxicity studies

and lethal dose of Cadalmin^{TM} GAe using Wistar rats was carried out to understand its effect on various parameters like mortality, weight change, food consumption, haematological function, liver function, renal function, serum electrolytes, and lipid profile. All the organs were examined visibly for any type of abnormalities in the structure. The results indicated that Cadalmin^{TM} GAe (1.5, 2.5 and 4.0 gm/kg body weight) given to experimental subjects (male and female) did not produce any clinical or behavior changes, change in food consumption, water consumption and body weights in rats, indicating that it has no toxicity to these animals. It did not produce any biochemical changes related to hepatic and renal function, in haematological parameters such as WBC, RBC, platelet, haemoglobin and differential count. This indicates that Cadalmin^{TM} GAe even at very high concentrations was not lethal to the mammalian subjects. Necropsy of the animals after sacrifice did not show any morphological changes in the tissues or any gross pathological abnormalities.

Preparation of eicosapentaenoic acid concentrates from sardine oil by *Bacillus circulans* lipase.

- An extracellular lipase derived from *Bacillus circulans*, isolated from marine macroalga, *Turbinaraia conoides*, was used to prepare n-3 polyunsaturated fatty acid (PUFA) concentrates from sardine oil triglycerides. The enzyme was purified 132-fold with specific activity of 386 LU/mg. The purified lipase was able to enrich sardine oil with 37.7 ± 1.98% 20:5n-3 and 5.11 ± 0.14% 18:3n-3 in the triglyceride fraction after 3 h of hydrolysis. Lower hydrophobic constants of n-3 fatty acids (18:3n-3log P = 5.65; 20:5n-3log P = 5.85, respectively) than n-6 (20:4n-6log P = 6.16) resulted in higher hydrolytic resistance of the former toward lipase, leading to their enrichment in the triglyceride fraction. Lipase-catalysed hydrolysis of sardine oil for 3 h, followed by urea complexation, provided free fatty acids containing 51.3 ± 4.65% 20:5n-3. The purified methyl ester of 20:5n-3 (68.29 ± 2.15%) from the urea concentrate was attained by chromatography on argentated neutral alumina.

Antibacterial labdane diterpenoids of *Ulva fasciata* from south western coast of the Indian Peninsula.

- Chromatographic purification of the dichloromethane-soluble fraction of alga, on neutral alumina, using increasing concentrations of ethylacetate/n-hexane as eluents, yielded seven labdane diterpenoids (1–7) as major constituents of green alga *Ulva fasciata*. Structures of these diterpenoids were established using extensive spectroscopic techniques. Antimicrobial assay showed that the compounds labda-14-ene- 3a,8a-diol (2) and labda-14-ene-8a-hydroxy-3-one (4) were inhibitory to the growth of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* with minimum inhibitory concentrations of 30 lg/ml by 2, and 40 lg/ml by 4, respectively against the former and 30 lg/ml by 2, and 80 lg/ml by 4, respectively, against the latter. Structure–activity relationship analyses revealed

that the compounds with electronegative hydroxyl or carbonyl group(s) exhibit greater activities, apparently by proton exchange reaction with the basic aminoacyl residue at the macromolecular receptor site of virulent enzymes of pathogenic bacteria. These might provide promising therapeutic agents against infections with multi-resistant Gram-negative fish pathogenic bacteria.

Microbial bioprospecting

A freeze dried microbial product (MP) from *Pseudomonas aeruginosa*, with antagonistic properties and high protein has been developed.

- This could be used in the development of larval feeds, and high health feeds, as feed additive. The antibacterial compound isolated from *Pseudomonas* sp was elucidated as octahydromethyl-dimethylphenazinecarboxylate.

- After mass production, heat inactivation was carried out and spray drying of heat inactivated broth was performed to make it into fine powder which can serve as microbial products (MPs')

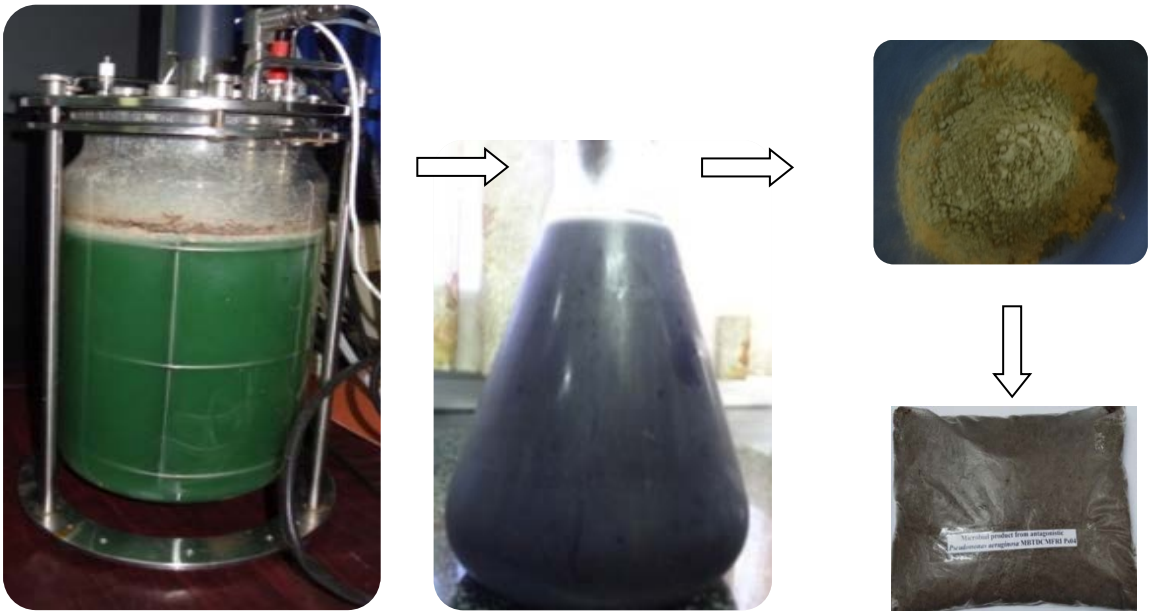


Fig.30. Mass production of Ps04 by biofermenter followed by cells was heat killed and spray dried. The spray dried form was estimated for activity and packed

Nutrient Profiling

- Nutritional content of the spray dried powder of *Pseudomonas aeruginosa* MBTDCMFRI Ps04 was evaluated to be used as feed additive.

Table 2. Nutritional content of the spray dried powder of Ps04

Sample	% Dry matter	% moisture	% crude protein	% crude fat	% crude ash	% crude fiber	% acid insoluble ash	% nitrogen free
Spray dried powder Ps04	100	NIL	14.63	0.403	73.68	0.48	0	11.29

Bioactive compounds responsible for antagonistic property in *Pseudomonas aeruginosa* P103 & Identification of bioactive compound

- The bioactive compounds obtained after bioassay guided purification from *Pseudomonas* sp. on spectroscopic analysis were found to be chromophore of phenazine substitution and the auxochrome belong the carboxyl ester moiety which is N-substituted methyl octahydro-1-phenazinecarboxylate and propyl 2-oxoacetate.

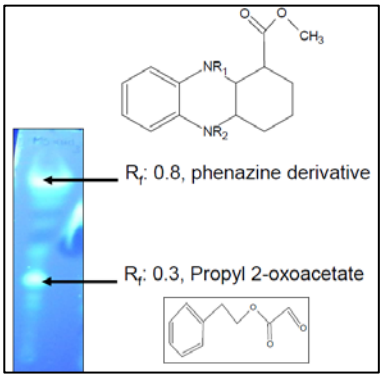


Fig 31. Thin layer chromatography showing the active fractions of P103.

- Structural characterization Ba37 by NMR spectroscopy revealed the bioactive principles responsible for antagonistic activity.

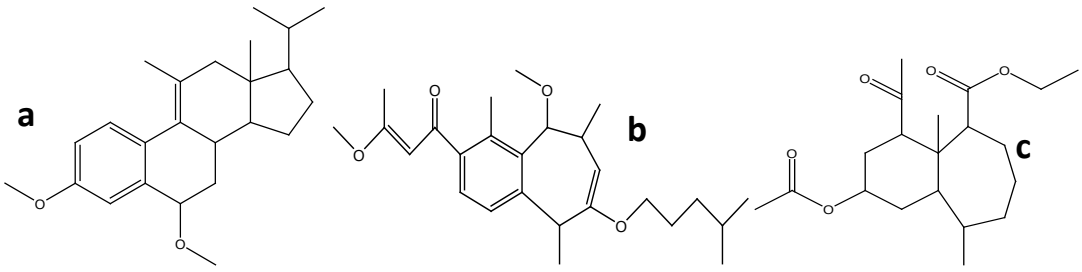


Fig. 32. The bioactive fractions isolated from Ba37 a. Octahydroisopropyl dimethyldimethoxy-cyclopentphenanthrene b. Methylpentyloxymethoxy-trimethylbenzoannulenyl-methoxybutenone c. Acetoxyeth-acetyl-dimethylbenzoannulenecarboxylate

Bioactive compounds from Bivalves

- Studies on the antioxidant composition of *Crassostrea madrasensis* revealed that among different purified components of this species CEM 1-7 showed 76-88% activity against DPPH free radical. The non-polar fractions too exhibited high DPPH activity (73-88%, 1mg/mL) and they are potentially active to deter lipid peroxidation in a model system (TBARS value <2 mMMDAEC/kg, 2 mg/mL).

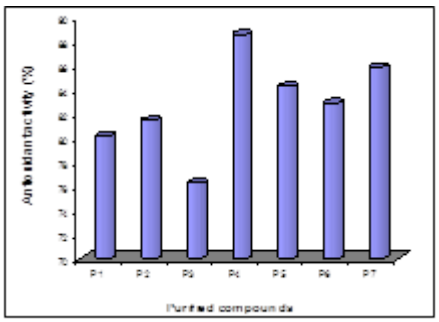


Fig. 33. Antioxidant activities from *C. madrasensis*

Evaluation of marine fish and mollusks for the micronutrient selenium in bioavailable form has been studied.

- Among different fish species, *Trichiurus lepturus* registered the highest Se content (0.95 mg/kg) followed by *Katsuwonus pelamis* (0.39 mg/kg) and *Leiognathus splendens* (0.36 mg/kg). Other fish species registered lower content of Se (<0.2 mg/kg). Among the molluscs, *Perna viridis* recorded Se content to the tune of 0.94 mg/kg and *Loligo duvacelli* 0.44 mg/kg.

Isolation and Characterization of Antioxidative and Anti-inflammatory Bioactives from Bivalves, *Villorita cyprinoides* and *Paphia malabarica* for using the resource as functional foods has been carried out.

- *Paphia malabarica* and *Villorita cyprinoids* are common sea foods in coastal regions of India, and utilize self defense systems to overcome free-radical induced oxidative stress diseases. *V. cyprinoides* was found to possess phenolics (3.50 mg/GAE) as antioxidant principles, which led to high antioxidative capacity (DPPH scavenging activity of 36.4%). The major bioactive compounds of which fatty acyl ester, methyl hexadeca-7,9-dienoate, 2-methyl-3-hydroxy-3,5-dien-hept-1-amine, phenolic compounds, furan analogues (phenyl-dihydrobenzofuranol) and keto derivatives (methyl phenylpropionylhexanoate) were identified. The free radical scavenging activity of *Paphia malabarica* showed higher activity (76% at 2000 ppm, ABTS) than those in black clam (58%, 2000 ppm, ABTS) due to higher total phenolic content (91%) than that in *Villorita cyprinoids* (74%). Anti-inflammatory biocatalysts such as cycloxygenase-II (>78%, 5000 ppm) and lypoxidase-V (80%, 5000 ppm) were significantly arrested by the solvent extracts of *Paphia malabarica* than that of *Villoritta cyprinoids* (65 and 78%, respectively in that order). These results indicated that *Paphia malabarica* and *Villorita cyprinoids* can be used to isolate bioactive molecules with potential medicinal properties.

List of publications

Research papers

International	66
Symposia/ Seminar papers	53
Popular articles	24
Others	12
Book Chapter	9
Book edited	1

Technologies developed, assessed and transferred

- **Varna series of Marine ornamental fish feed, presently produced at the CMFRI Laboratory extruder Facility and sold through ATTIC.**

Fig. 34: Packaged ornamental feeds

- **Cadalmin™ Green Mussel extract (Cadalmin™ GMe) has been commercialized with Accelerated Freeze Drying Company Pvt. Company of Amalgam Group of Companies, Kochi**

Fig. 35. Nutraceutical from green mussel – GMe

- **Cadalmin™ Green Algal extract(GAe) has been commercialized with Celestial Biolabs Limited, a Hyderabad based Pharmaceutical Company**

Fig.36. Nutraceutical from green mussel – GAe

Information on technologies assessed and transferred

- Nutraceutical - GMe
- NutrAceutical - GAe
- Formulated feed for *Etroplus surantensis* (A newly developed formulated feed for pearlspot Etroplus surantensis has been transferred to KVK, and the same has been tested in the cage culture programmes of KVK in the farmers field. This feed is under refinement and commercialization)

Honours/awards, recognition received during the period

- Dr. Vijayagopal received the ***"DBT Cutting Edge Research and Training Award"*** (2011-12) for one year in 2013 to work at the Temasek Polytechnic, Singapore.

Facilities created under the Marine Biotechnology Division

- Management & Maintenance of Genetic/Animal/ Database Resources/Facilities
- A new Tissue Culture laboratory has been developed at Chennai, CMFRI RC, under Marine Biotechnology Division.
- A new Fish Surveillance Lab has been developed for the National Surveillance project.
- Major bacterial and viral pathogens affecting the marine shellfish and finfishes, from the marine ecosystems are maintained at the CMFRI Fish Health Lab facility. Potential probiotic bacterial isolates are also maintained for the possible exploitation in the development of useful microbial products.
- A total of 144 pure isolates of marine microalgae representing different habitats of Indian Coast (isolated from 47 locations including the salt pans in Gulf of Kutch, Odisha, Andhra Pradesh, Tamil Nadu, Goa and hot springs in Himachal Pradesh) is maintained at the National Facility for Marine Micro Algae at CMFRI.
- *Artemia* cyst collection made from Indian Salinas and major strains from outside the country and selective-bred *Artemia* cyst of naturalized Indian strain of *A. franciscana* are maintained at Marine Biotechnology Division.

Resource Generation

- Through the commercialization of the nutraceutical GAe developed under the division, an income of Rs. 5,00,000 has been earned. Products developed under the division viz. 'Varna' ornamental feeds, has been made available for sale through institute, generating income and also awareness among the farmers and public.

HRD overseas trainings:

- Five scientists have been trained under NAIP in the areas of fish health, bioprospecting and genomics and one principal scientist in fish nutrition through DBT

Patents filed

32/CHE/2010	2010	Formulated feed for marine ornamental fishes and a process thereof	Examination request filed
2065/CHE/2010	2010	A process to concentrate anti-inflammatory principles from green mussel *Perna viridis* L. and a product incorporating these ingredients	Provisional patent filed
2066/CHE/2010	2010	A process to concentrate anti-inflammatory principles from green mussel *Perna viridis* L. and a process incorporating these ingredients	Provisional patent filed
2063/CHE/2010		A process to prepare *Artemia franciscana* from Indian sub-continent with high docosahexaenoic acid and trehalose for aquaculture applications	Provisional patent filed
2064/CHE/2010	2010	A process to prepare antioxidant and anti-inflammatory concentrates from from brown and red seaweeds and a product thereof	Provisional patent filed