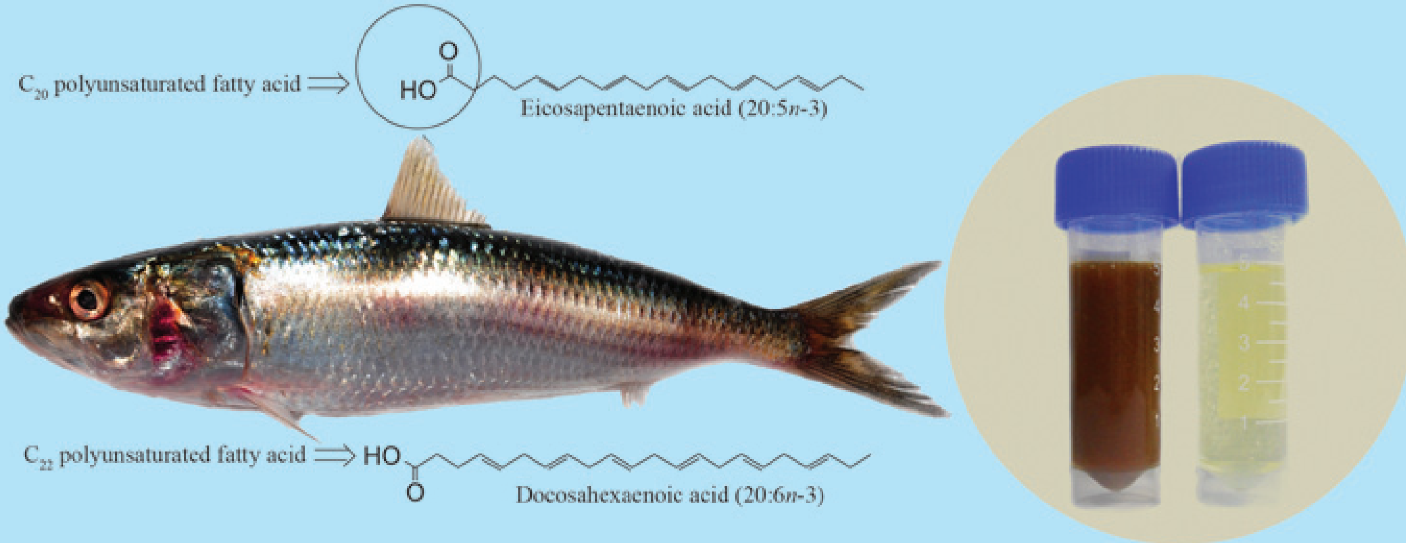
ISSN 0254-380 X

No. 223 & 224, January - June 2015

Marine Fisheries Information Service

Technical and Extension Series



Indian Council of Agricultural Research
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
P.B. No. 1603, Ernakulam North P.O., Kochi - 682 018, Kerala, India

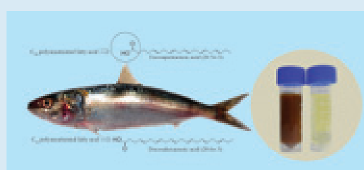
Marine Fisheries Information Service

PUBLISHED BY

Dr. A. Gopalakrishnan

Director

ICAR-Central Marine Fisheries Research Institute, Kochi



Front Cover : *Indian Oil Sardine, a small pelagic fish rich in PUFAs*

Back Cover : *Oil sardine landings*

The Marine Fisheries Information Service *Technical and Extension Series* envisages dissemination of information on marine fishery resources based on research results to the planners, industry and fish farmers, and transfer of technology from laboratory to field.

From the Editorial Board.......

Warm greetings to all

The omega 3 fatty acids found in oily fish are believed to have a beneficial effect on health in humans.Hence nutraceuticals or diet supplements prepared from fish oils have commanded attention and demand for them in our country is mainly met by imported brands. An indigenous *n*-3 PUFA emulsion developed from the locally available and affordably priced small pelagic fish *Sardinella longiceps* is highlightedin this issue. The Short-neck clam fishery of the Ashtamudi Lake in Kerala recently achieved the global standards of fishery certification for sustainable exploitation,through the MSC, UK.The observations made during the regular assessments of these clam stocks by the institute is presented in this issue, which can aid policy makers in the scientific management of the clam fisheries in the future also. An article on the various strains of potentially useful bacteria isolated from marine finfish and shellfish, that may find application in agriculture or related sectors, besides notes on emerging trends in fish landings, marketing and marine biodiversity are also included in this issue.

Marine Fisheries Information Service

No. 223 & 224, January - June 2015

Abbreviation - *Mar. Fish. Infor. Serv., T & E Ser.*

CONTENTS

Long chain *n*-3 polyunsaturated fatty acid enriched oil emulsion from sardine oil

Kajal Chakraborty, Vijayagopal, P., Iyyapparajanarasimapallavan, G., Dexy Joseph, Fasina Makkar, Vamshi Krishna Raola and Minju Joy
ICAR-Central Marine Fisheries Research Institute, Kochi

Dietary fats are used to build every cell in the body and cell membranes are made of a variety of individual fatty acids which are carboxylic acids with long hydrocarbon chains (usually C_{12-22}). The essential fatty acids from marine fish have protective mechanisms against coronary heart disease, which became apparent in the investigations of the health status of Greenland Eskimos who consumed diets very high in fat from seals,whales, fish etc, and yet had a low rate of coronary heart disease. This paradox was explained by the fact that the Eskimos diet contained large quantities of the very-long-chain and highly polyunsaturated fatty acids with C_{20-22} carbons and 5-6 olefinic bonds, which are abundant in marine fish, but scarce or absent in terrestrial animals and plants. Fatty acids with two or more double bonds are termed as polyunsaturated fatty acids (PUFAs) which are broadly divided into two major families, *n*-3 and *n*-6 PUFAs. The long chain C_{20-22} *n*-3 fatty acids are found abundantly in marine fish and phytoplankton. These affect many physiological processes including cognitive function, visual acuity, immunosuppressive and anti-thrombic activities along with a major role in glucose and lipid metabolism. Research on exploring sources for long-chain C_{20-22} PUFAs (LC-PUFAs) such as Eicosapentaenoic acid (EPA, 20:5 *n*-3) and Docosahexaenoic acid (DHA, 22:6*n*-3) for nutrition have received considerable attention (Fig. 1). Since these PUFAs are usually low in abundance in humans, but regarded as essential they have to be supplied in the diet. The importance of PUFAs in human nutrition has been extensively investigated during the past 20 years. DHA one of the important PUFAs maintains structural and functional integrity in larval cell membranes in addition to the neural development and function, while Arachidonic acid (AA, 20:4*n*-6) and EPA are involved in the production and modulation of eicosanoids respectively. DHA is a vital component of the phospholipids of cellular membranes, especially in the brain and retina, and necessary for their proper functioning. An imbalance in *n*-3/*n*-6 ratio can accentuate *n*-3 fatty acid deficiency state, as shown by earlier studies. The ratio is found to have increased in industrialized societies because of increased consumption of vegetable oils rich in *n*-6 fatty acids, *ie*, linoleic acid (18:2*n*-6) and reduced consumption of foods rich in *n*-3 fatty acids. Another important feature of *n*-3 fatty acids is their role in the prevention and modulation of certain diseases that are common. A partial list of diseases that may be prevented or ameliorated with *n*-3 fatty acids is given below.

- Coronary heart disease and stroke
- Diabetes
- Cancers of the breast, colon, and prostate
- Hypertension

The LC - PUFAs are also recognized to have beneficial therapeutic, physiological and nutritional effects on human health.

Imported products (PUFA supplement) include Seven Seas by a UK healthcare company producing PUFA rich Cod Liver Oil by Ocean Gold™ technology. A value-added PUFA concentrate named "fish oil-1000 natural omega 3®" containing 100 capsules manufactured by Healtheries of New Zealand Ltd. priced at about ₹ 1150 per pack, is currently being marketed in India by Perma Healthcare, Bangalore. "EPAX 1050 TG®", a marine omega 3 formula produced from selected marine oils and marketed by EPAXAS, Norway is an imported product containing

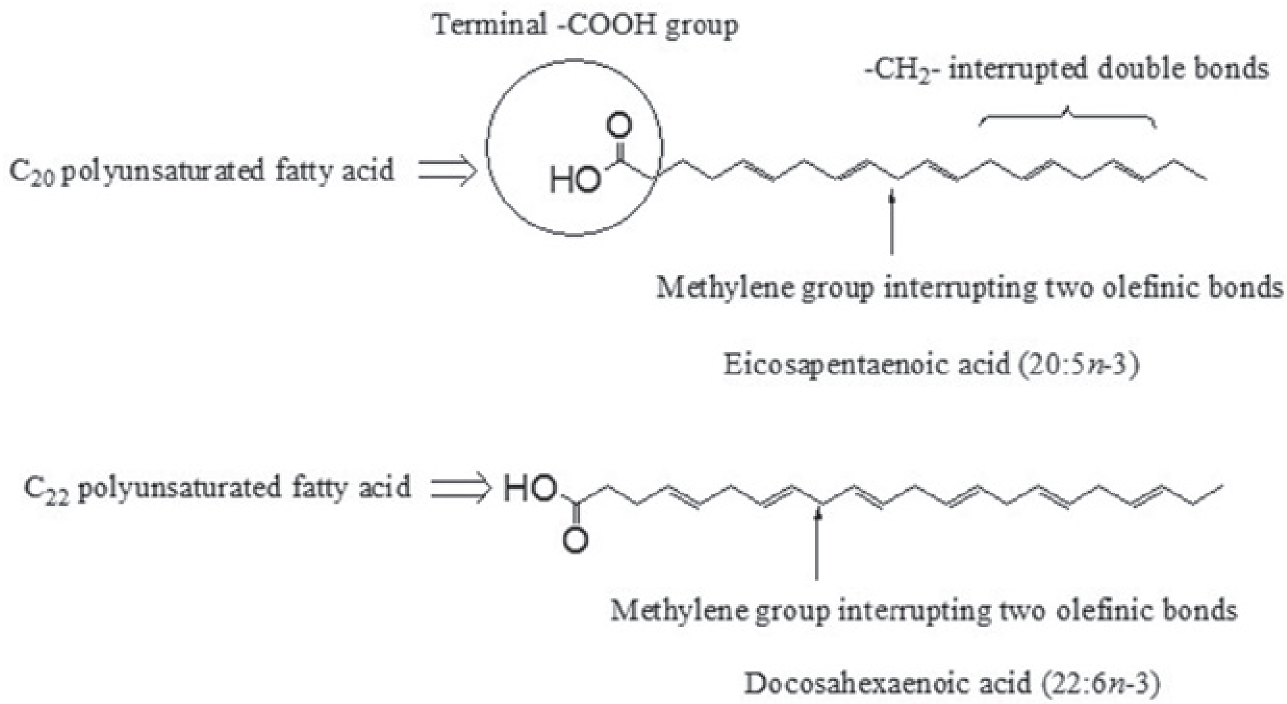


Fig. 1. C_{20-22} polyunsaturated fatty acids (Eicosapentaenoic and Docosahexaenoic acids)

17% EPA for use as fish feed supplement. OMEGA XL® is marketed by DeColores in a bottle containing 60 capsules of refined combination of omega-3's costing about $50 per pack, as a nutraceutical. Great Health Works manufactures a concentrate of PUFA from Blue Grenadier Fish that is also a costly PUFA supplement. DSM Nutritional Products, Switzerland manufactures and sells PUFA concentrate under the trade name ROPUFA® produced from refined vegetable and marine oils. These products are currently imported to meet the domestic demand of PUFA concentrate formulation for fish feed supplements and nutraceutical purposes. This underlied the need to develop an indigenous *n*-3 (PUFA) supplement for which the Marine Bioprospecting section of Marine Biotechnology Division screened locally available low-value fish for *n*-3 PUFAs, concentrating these essential fatty acids therefrom by chemical and/or enzymatic process.The aim was to develop an indigenous *n*-3 PUFA enriched formulation(s) comprising fatty acid concentrate and individual or combination of additives with potential antioxidant properties to form a stabilized and concentrated form of long chained *n*-3 PUFAs which may be a cheaper alternative to the imported PUFA supplements, and will be useful as nutraceuticals and in mariculture operations for fish larval nutrition.

It is believed that the optimal formulations for the first-feeding fish larvae should simulate the yolk composition and to some extent reflect the nutrient requirements and metabolic capacities of the pre-feeding fish. Dietary long chain PUFAs play an important role as vital sources of essential fatty acids, needed for normal growth and suvival. Larvae of many marine fishes require highly unsaturated fatty acids of the n-3 series, such as, DHA due to the absence of essential enzymes required for biosynthesis of C_{20-22} LC-PUFAs from their short chain analogues (Fig. 2). Some investigations have shown that DHA is superior to EPA for larval fish suggesting a different physiological function.

The important natural sources of *n*-3 LC-PUFAs are fishes such as mackerel, sardines, sharks; microalgae, polychaetes, etc. Among these, sardines are inexpensively available and contain considerable amount of PUFAs, particularly 20:5*n*-3. Hence sardine oil was preferred as the raw material to formulate

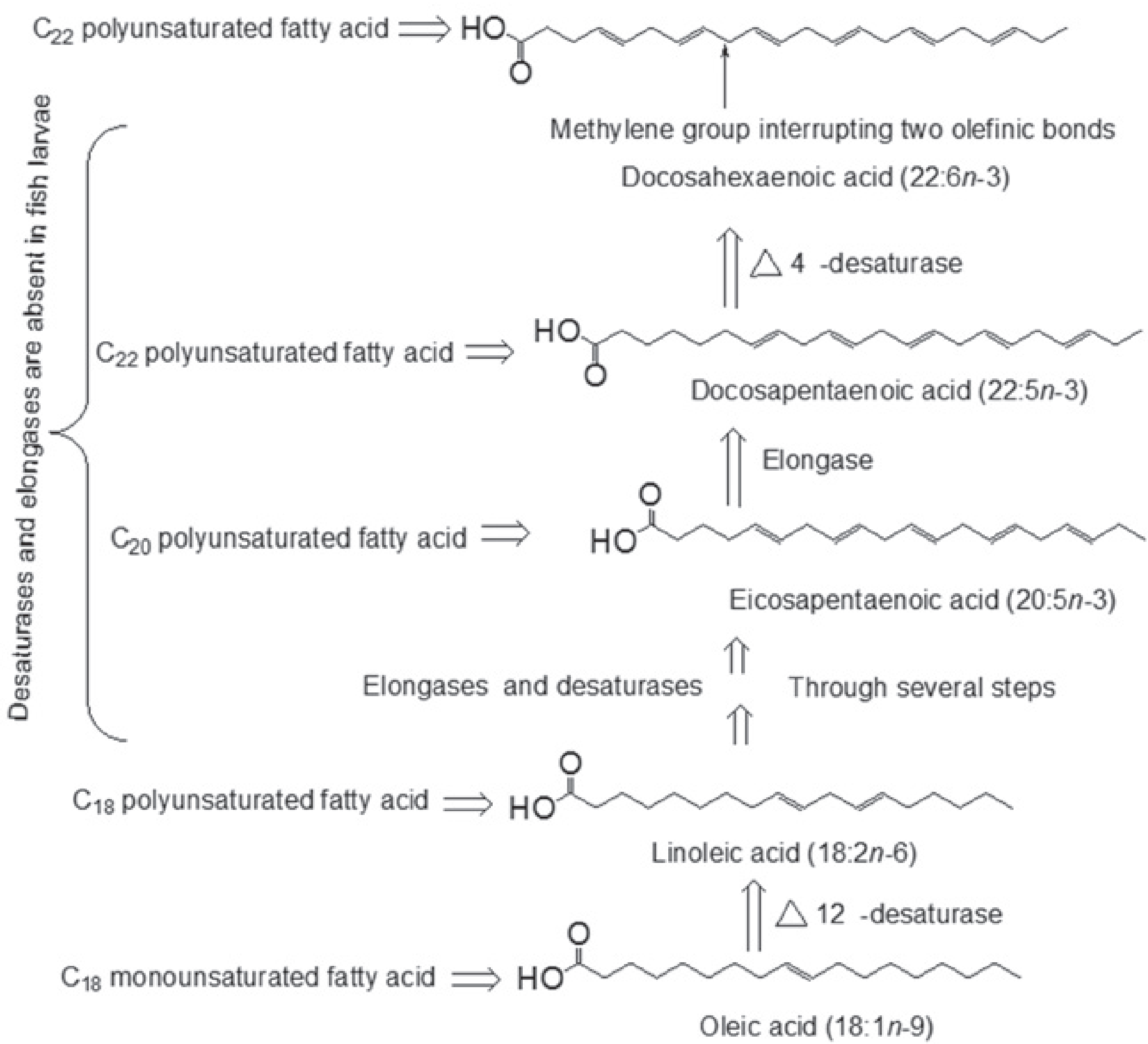


Figure 2. Biosynthetic pathways of the long chain polyunsaturated fatty acids (LC-PUFA)

the *n*-3 C_{20-22} LC- PUFA concentrates based on different physicochemical properties associated with the olefinic bonds in fatty acids and/or acyl chain length. The objective was to purify EPA and DHA from sardine oil by saponification of fish oil to derive free fatty acids, enrichment of PUFA content from the mixed fatty acid concentrate by amide fractionation and chromatography by utilizing the silica gel complexed with a d-block element.The unique substrate specificity of microbial triacylglycerol acyl hydrolases was utilized for the enhancement of PUFA content in triglycerides to further enrich the C_{20-22} LC PUFAs. Triacylglycerol acyl hydrolases specifically hydrolyse carboxyl esters of triglycerides into free fatty acids and partial acylglycerols. The unique characteristics of this group of enzymes such as positional and stereospecificity were utilized to selectively concentrate targeted fatty acids in triglycerides that can be readily absorbed into plasma triglycerides.

PUFA enrichment by different physicochemical procedures

The crude sardine oil was clarified by a sequential process of degumming, decolorization, and deodorization, and was found to contain LC-PUFAs, particularly 20:5 *n*-3 or EPA (17.80 ± 1.57%) of total fatty acids, TFA) and 22:6 *n*-3 or DHA (7.67 ± 1.50% of TFA) along with other *n*-3 and *n*-6 PUFAs like Linolenic acid (LA or 18:3 *n*-3; 4.47 ± 0.84% TFA), Linoleic acid (18:2 *n*-6; 0.71 ± 0.23% TFA), and Docosapentaenoic acid (DPA or 22:5 *n*-3; 1.14 ± 0.08% TFA) (Fig. 3). The *n*-6 fatty acids have a minor share of the total fatty acid content of sardine oil (0.81% TFA). The PUFAs containing C_{18}- C_{22} acyl chain length contributed a major share of the total fatty acids of the sardine oil (>30% TFA). Among the saturated fatty acids (SFAs), 14:0 was found to be predominant (7.04 ± 0.22% TFA), while 16:1 n-7 contributed the major share (31.56 ± 2.59% TFA) among the monounsaturated fatty acids (MUFAs). When fatty acids are required in free form for further analyses, lipids were hydrolyzed in alkaline medium for extracting the unsaponifiable material. Sardine oil was saponified with NaOH/Na_2EDTA to yield free fatty acids. Na_2EDTA appeared to form complex with traces of metal ions (Cu, Fe), which catalyze oxidation of unsaturated fatty acids during saponification, and subsequently removed by extraction with water thus hindering the interferences of metal ions during the course of further purification process. Relatively large volumes of *n*-hexane were added to the aliquot of the salt of fatty acid mixtures for better phase separation, thus removing the unsaponificable materials. Among saturated fatty acids (SFAs), 14:0 was found to be predominant (7.04% TFA), while 16:1*n*-7 contributed the major share among all individual fatty acids in the crude sardine oil (>31% TFA). EPA and DHA were found to be the major *n*-3 PUFAs contributing to 17.8% and 7.67% of TFA, respectively. The *n*-6 fatty acids have minor share in the total fatty acid content of sardine oil. Solvent extraction resulted in marginal increase of unsaturation (0.85%) in the fatty acid profile. The PUFA exhibited an increase of 6.49%, while MUFA and SFA reduced by 2.96% and 6.91% respectively. The *n*-3 fatty acids exhibited an increase of 5.73% in the solvent extract of fatty acids.

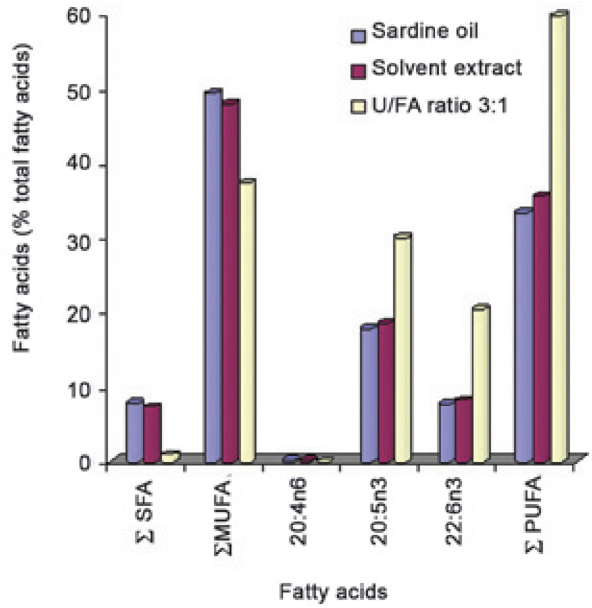


Fig. 3. Fatty acid composition of crude and solvent extracted sardine oil, and PUFA concentrate obtained by the amide fractionation (U/FA: amide/fatty acid ratio)

The free fatty acids derived from sardine oil were subjected to amide fractionation using methanol as solvent at three different temperatures and urea-fatty acid ratios to obtain PUFAs of high purity. The interfering SFAs and most of the MUFAs were removed in the form of amide inclusion compound. Further, as oxidized products do not form amide adducts, the peroxidation of *n*-3 PUFAs could be avoided during the extraction of free acids from fish oil triglycerides. Amide fractionation resulted in total reduction of SFAs (>95%) (14:0, 16:0, and 17:0), moderate reduction of MUFAs (>65%). The U/FA ratio of 4:1 (w/w) was found to be optimal for getting high-purity EPA (47.8%) while that at 3:1 (at 2^0 C) yielded higher content of DHA (>20 %) when crystallized at 2^0C. Based on these results, DHA obtained from sardine oil at 2°C temperature of amide-crystallization by using a U/FA ratio of 3:1 was selected for subsequent purification of C22 fatty acid DHA. It is likely that at the lower temperature (2^0C), the reaction kinetics to form amide-inclusion complex with SFAs and MUFAs was relatively lower resulting in higher DHA in the extract.

Change in fatty acid composition as a function of microbial triacylglycerol acyl hydrolase-catalyzed hydrolysis of fatty acids from sardine oil

The free fatty acids were esterified by using a mixture of ethyl alcohol and 0.1 (N) H_2SO_4 resulting in the ethyl ester of the fatty acids. An extracellular triacylglycerol acyl hydrolase derived from *Bacillus subtilis* isolated from marine macroalga, *Turbinaria conoides*, was used to prepare C_{22} *n*-3 polyunsaturated fatty acid concentrates from the ester fraction. The enzyme was purified 132-fold with specific activity of 386 triacylglycerol acyl hydrolase units/mg. The urea fractionated fatty acyl esters were hydrolyzed with enzyme purified from the bacterium *Bacillus subtilis* and the TFA content of fatty acyl esters after lipase hydrolysis were analyzed. SFA levels showed a reduction to 0.05% after 3 hours (h) of hydrolysis. The decrease in the content of SFAs and MUFAs in the fatty acyl ester mixture with the progress of hydrolysis suggested that SFAs and MUFAs were more easily hydrolyzed by the lipase than those in esterified fatty acids that contain DHA, resulting in the enrichment of the latter in the ester fraction.

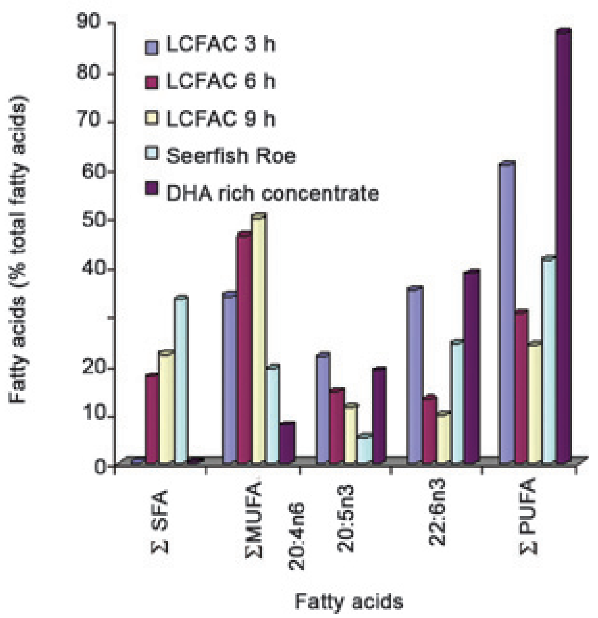


Fig. 4. Fatty acid composition of urea concentrated fatty acids, triacylglycerol acyl hydrolase-catalyzed fatty acid concentrate, seer fish roe, and DHA rich concentrate

The variations of PUFA content of sardine oil triglycerides as a function of time during the microbial triacylglycerol acyl hydrolase-catalyzed hydrolysis are illustrated (Fig. 4). The total DHA of fatty acyl ester fraction increased with time up to 3 h of enzyme-catalyzed hydrolysis (35.27% TFA), beyond which it slowly decreased (9.81% TFA after 9 h). The purified triacylglycerol acyl hydrolase was able to enrich DHA with 35.27% 22:6 *n*-3 after 3 h of hydrolysis. The results also suggested that the esteritic bonds of C_{22} acyl chain lengthened *n*-3 PUFAs are resistant to hydrolysis by the lipase. However, after prolonged hydrolysis (>9 h), when only a few target fatty acid ester bonds (*n*-6 fatty acyl ester bonds and esters other than C_{22} *n*-3 fatty acids) were available in the enzyme hydrolysate that were susceptible to hydrolysis by the bacterial hydrolase, the microbial triacylglycerol acyl hydrolase could cleave bonds highly resistant to hydrolysis, i.e., DHA. It can therefore be concluded that it is possible to separate and concentrate C_{22} PUFAs with *n*-3 double bonds like DHA using lipase from seaweed associated bacteria like *Bacillus subtilis*.

Separation of phospholipid fraction from the seer fish roe and preparation of DHA rich oil emulsion was also done.The total lipids of seer fish roe were separated into different lipid classes by silicic acid column chromatography. The lipid fractions were qualitatively analyzed by Thin Layer Chromatography (TLC) for identifying triglycerides, glycolipids and phospholipid components. Fatty acid methyl esters (FAMEs) of the total lipid and the individual lipid classes were prepared by transesterification process. The DHA enriched fatty acid concentrate was enriched through biochemical and microbiological procedures to formulate enrichment emulsions which contained roughly, 90% DHA enriched fatty acid concentrate and 10% phospholipids fraction extracted from seer fish roe. The aggregate content of DHA in fish roe phospholipidic fraction was recorded as 24.3% DHA along with 5.2% EPA. The polyunsaturated fatty acid concentrate of the fish body oil after adding fish roe was found to contain greater than 35% DHA with significantly lesser content of saturated fatty acids (0.22%) and monounsaturated fatty acids (~7% of total fatty acids).

Use of emulsifiers to increase the stability of enriched PUFA emulsion

An emulsion is a mixture of two or more liquids that are normally immiscible and an emulsifier is a substance that stabilizes an emulsion by increasing its kinetic stability. Emulsion should be used when both the dispersed and the continuous phase are liquids. An experiment was conducted to understand the effect of emulsifier to stabilize the DHA concentrate. The emulsifier used was Tween 20 (or Polysorbate 20), which was able to contain the stability of the preparation for an extended time period (Figs. 5 & 6). Longer chain TAGs such as polysorbate 20 are more hydrophobic and therefore have higher oil/water interfacial tension than shorter chain ones. The stability of the PUFA concentrate (as such without Polysorbate 20) decreased after 15 minutes whereas the same appended with Polysorbate 20 was able to maintain the stability for an extended period of time due to increased kinetics (increased Brownian movement)

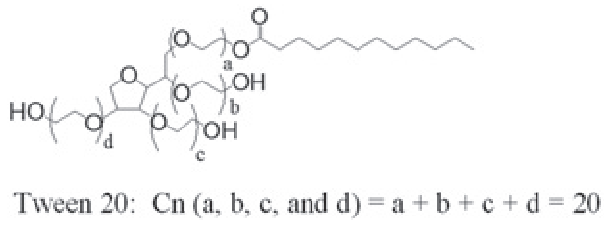


Fig. 5. Polyoxyethylene derivative of sorbitan monolaurate (or Polysorbate 20)

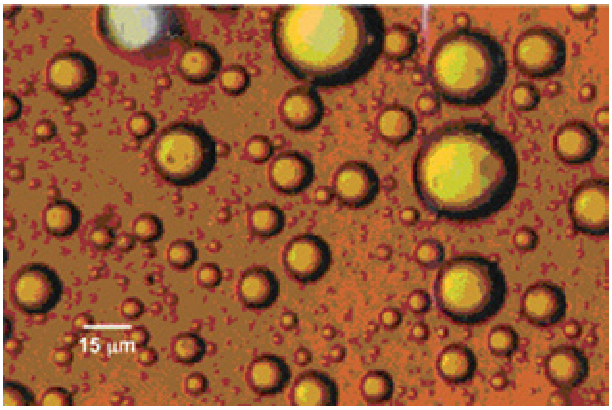


Fig. 6. Photomicrograph of a water-in-oil emulsion by using Tween 20

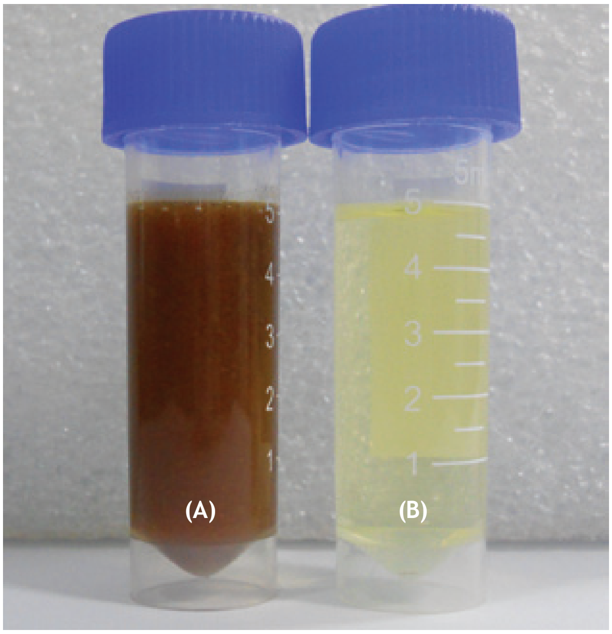


Fig. 7. Polyunsaturated fatty acid concentrate prepared from sardine oil. (A) Crude sardine oil (B) PUFA concentrate

Conclusions

A fatty oil from the livers of various fishes (as cod, halibut or sharks) used chiefly as a source of fatty acids, vitamin A and also of vitamin D is called fish liver oil. Fish oil is short for "fish body oil" and not the same thing as "cod liver oil" available in the market. Cod liver oil contains greater concentrations of vitamin A. Taking cod liver oil in the same amounts that are recommended for fish oil can be toxic, and even more so in people who have chronic renal failure (because vitamin A can build up to toxic levels). The $C_{20\text{-}22}$ *n*-3 polyunsaturated fatty acid concentrate prepared from the inexpensively available marine sources, such as sardine oil can be a potential substitute of the imported PUFA supplements as functional food product.The PUFA enriched formulation (Fig. 7) from low value "fish body oils" will also overcome the risks associated with hypervitaminosis (A, D) and exposure to environmental toxins (mercury, PCBs, dioxins etc.) associated with "liver oils" available in market. The indigenous *n*-3 polyunsaturatated fatty acids emulsion developed from the locally available low-value fish may also serve as a cheaper alternative to the imported fatty acid emulsions used in fish larval nutrition during mariculture operations.

An assessment of the Short-neck clam biomass in Ashtamudi Lake

Venkatesan, V., Vidya, R, Alloycious, P.S., Jenni, B., Sajikumar, K.K., Jestin Joy, K.M., Sheela, P.P., Abhilash, K.S., Gishnu Mohan and Mohamed, K. S.
ICAR-Central Marine Fisheries Research Institute, Kochi

Ashtamudi Lake (Lat. 8°45' - 9°28' N and Long. 76°28' - 77°17') supports more than 500 families who depend on the clam resources available here for their livelihood. Among clams, the Short neck clam (*Paphia malabarica)* is widely distributed and continuously exploited for local consumption as well as for export. The alarming increase in the exploitation of *P. malabarica* immediately after the initiation of frozen clam meat export during 1981, forced the local administration to impose a ban on clam fishing during the breeding season (December to February) and also place restriction on the mesh size of clam dredges in 1993, based on the recommendation of the Central Marine Fisheries Research Institute (CMFRI). The CMFRI has conducted Resource Abundance Surveys of clams in 1984, 1996, 2012, 2014 and 2015 and assessed the total stock. After the creation of the Fishery Management Plan (FMP) for the Ashtamudi Lake Clam Fisheries Governance Council (ACFGC) during 2013, the fishery became the first Marine Stewardship Council (MSC) certified fishery in the country in November 2014, indicating that the fishery is managed as per globally accepted standards.

Considering the fishing pressure on the clam resources of Ashtamudi Lake, two surveys were undertaken in the five clam fishing zones (Fig.1) during 2015 in February (Survey #1) and May (Survey #2) to assess the status of existing clam resources and potential for exploitation. The results of the two surveys are shown in the Tables 1a and 1b.

The study revealed that the estimated fishable biomass in both surveys of 2015 were considerably lower than the 10,438 tonnes (t) estimated in 2014. The Survey #1, was carried out during 9th to 11th February, 2015 i.e., before the fishery opened on March 1st 2015. Clams were present in all the five zones, but poor in Zones III, IV and V. Biomass was estimated as 5283 t which was 49% less than last

Fig. 1. Map of Ashtamudi Lake showing the five clam fishing zones (I-V) and the new clam bed

Table 1a. Zone-wise assessment of *P. malabarica* in February 2015

Zone and Area (in ha)	*P. malabarica*						*M. casta*	
	Spat		Juvenile		Large size-Adult			
	Mean density (million numbers/ zone)	Mean biomass (tonnes/ zone)	Mean density (million numbers/ zone)	Mean biomass (tonnes/ zone)	Mean density (million numbers/ zone)	Mean biomass (tonnes/ zone)	Mean density (million numbers/ zone)	Mean biomass (tonnes/ zone)
I (15)	9.36 (62)	0.84 (5.62)	16.08 (107)	28.46 (189.7)	68.64 (458)	5.38 (2461.9)	-	-
II (60)	286.08 (477)	37.19 (61.9)	22.08 (37)	13.25 (22.1)	146.88 (245)	1645.06 (2741.8)	-	-
III (50)	36 (72)	21.6 (43.2)	-	-	34.18 (68)	393.77 (787.5)	20.36 (41)	148.86 (297.7)
IV (31.7)	3.04 (10)	0.30 (0.96)	-	-	3.04 (10)	38.04 (120)	-	-
V (16.6)	23.15 (139)	5.55 (33.5)	-	-	28.84 (174)	203.29 (1224.7)	-	-
Total	357.63	65.48	38.16	41.71	281.58	2285.55	20.36	148.85

a) Total estimated number of *P. malabarica* (zone I-V) = 677.37 million
b) Total estimated actual biomass of *P. malabarica* (zone I-V) = 2392.75 t
c) Total estimated number of *M. casta* (zone I-V) = 20.36 million
d) Total estimated actual biomass of *M. casta* (zone I-V) = 148.9 t
e) Estimated mean harvestable size = 7.8 g
f) Estimated fishable biomass (shell-on weight) of *P. malabarica** = 5283.49 t

year. The presence of very small spat indicated that spawning had occurred late. Unlike previous years the clam beds in Zone III (main production zone) were much depleted. In the Survey #2, biomass survey was carried out during the fishing season and clams were observed in Zone I and II. Zone III was totally devoid of clams as 40% of the zone had become a sand bar exposed to air at most tides. The presence of small spat (< 10mm size) indicated further late spawning.

Clam Density

In survey #1,density of clams was highest (758 numbers/m^2) in zone II. A slightly lower density of 627 numbers/m^2 was observed in zone I. *P. malabarica* was the single species contributing to high density in all the zones. Density was as low as 19, 140 and 313 numbers/m^2 in Zone IV, III and V respectively. Spat and large clams were present in all the zones (I-V) while juvenile clams were present in Zone I and II. *M. casta* was present only in Zone III (41 numbers/ m^2). Total estimated densities in all zones were 561.7 million. In Survey # 2, low densities of 752 and 782 numbers/m^2 were observed in Zones I and II respectively, while in Zones III-V, clams were completely absent. Total estimated densities in the Zones I and II together formed 561.7 million.

Clam Biomass

In Survey#1, higher biomass of clams were observed in Zone I (2.7 kg/m^2) and II (2.8 kg/m^2). Very low biomasses were recorded in Zone V (1.3 kg/m^2), III (0.831 kg/m^2) and IV (0.121 kg/m^2). *P. malabarica* was present in all Zones contributing to biomass while *M. casta* was observed only in Zone III (0.298 kg/m^2) (Fig.2).

In Survey #2, higher biomass of *P. malabarica* were observed in Zone I (3.4kg/m^2) and Zone II (3.6 kg/ m^2) while in the rest of the zones, clams were completely absent (Fig. 2). Total estimated fishable biomass from all zones together was 4381 tonnes (Table 1b).

Ecology of clam bed

The water quality parameters recorded at each clam fishing zone in Ashtamudi Lake during February and May 2015 are given in Table 2a and 2b respectively.The surface salinity of Ashtamudi Lake showed a gradual reduction from Zone I to Zone IV

Table 1b. Zone-wise assessment of *P. malabarica* in May 2015.

Zone and Area (in ha)	*P. malabarica* Spat		Juvenile		Large size-Adult		*M. casta*	
	Mean density (million numbers/ zone)	Mean biomass (tonnes/ zone)	Mean density (million numbers/ zone)	Mean biomass (tonnes/ zone)	Mean density (million numbers/ zone)	Mean biomass (tonnes/ zone)	Mean density (million numbers/ zone)	Mean biomass (tonnes/ zone)
I (15)	3.40 (23)	0.58 (3.9)	22.60 (151)	27.79 (185.3)	86.8 (579)	482.93 (3219.5)	-	-
II (60)	1.53 (41)	0.61 (16.3)	193.92 (323)	252.09 (420.2)	253.44 (422)	1899.65 (3166.1)	-	-
III (50)	-	-	-	-	-	-	-	-
IV (31.7)	-	-	-	-	-	-	-	-
V (16.6)	-	-	-	-	-	-	-	-
Total	4.93	1.19	216.52	279.88	340.24	2382.58	-	-

a) Total estimated number of *P. malabarica* (zone I-V) = 561.69 million

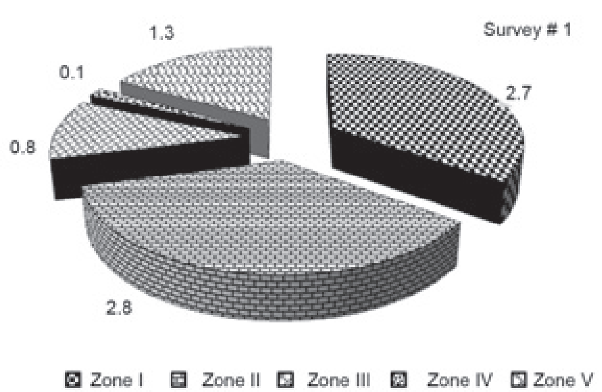
b) Total estimated actual biomass of *P. malabarica* (zone I-V) = 2663.65 t

c) Estimated mean harvestable size = 7.8 g

d) Estimated total fishable-size biomass (shell-on weight) = 4381.18 t

Fishable-size biomass is estimated by prospectively calculating of spat growth and mortality; Density and biomass values in parenthesis are in number/m^2 and gram/m^2 respectively; Areas of clam bed zones are given in parenthesis under each zone.

during both the surveys which was influenced by its proximity to the sea. During Survey #1, surface salinity in zones I-III (28.5ppt to 31.4ppt) were relatively higher than during Survey #2 (12.6 ppt to 17.9 ppt). Surface and bottom salinity exhibited relatively wider variations in zones III-V compared to I and II. Temperature showed normal variations in all zones with minima ranging from 27.9ºC to 31.3ºC during Survey #1 and maxima ranging from 30.3ºC to 31.8ºC during the Survey #2. Surface and bottom temperatures and pH also did not show much variations in all the zones during both the surveys. Dissolved oxygen (DO) (6.7-7.0 ml/L) never reached a limiting concentration in any of the zones in both the surveys. Nitrogen components such as Ammonia (NH_3), Nitrite (NO_2), and Nitrate (NO_3) are important water quality parameters that impacts health and well-being of clam and must be within threshold levels in an aquatic system. NH_3 excreted by clam and other aquatic animals or released from uneaten food is



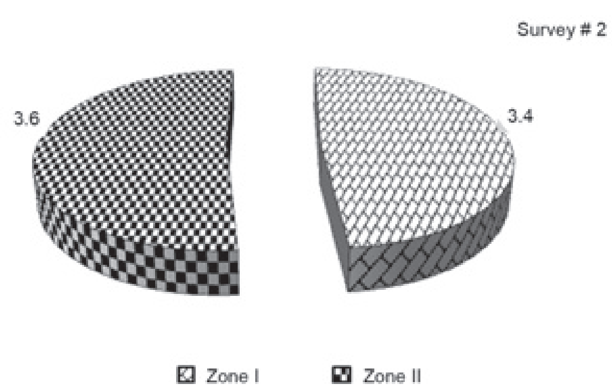


Fig. 2. Zone wise clam biomass (kg/m^2) during Survey #1 and Survey #2

Table 2a. Mean values of water parameters in each clam-bed zones during February 2015 (Survey# 1)

Zone	Salinity (ppt) Surface	Salinity (ppt) Bottom	Temperature (°C) Surface	Temperature (°C) Bottom	DO (ml/L) Surface	pH Surface	pH Bottom	Chl a (mg/m³)	TSS (mg/L)	PIM (mg/L)	POM (mg/L)	NH_3 (ppm)	NO_2 (ppm)	NO_3 (ppm)	PO_4 (ppm)
I	31.4 ± 1.85	ne	28.6 ± 0.26	ne	4.9 ± 0.3	6.7 ± 0.00	ne	0.036	95	ne	ne	0.002	0.004	0.003	0.016
II	31.3 ± 2.23	ne	27.9 ± 0.33	ne	5.2 ± 0.9	6.9 ± 0.3	ne	0.202	68	ne	ne	0.001	0.003	0.002	0.003
III	28.5 ± 1.32	ne	29.3 ± 0.63	ne	5.1 ± 0.1	7 ± 1.00	ne	0.116	71	ne	ne	0.006	0.002	0.005	0.006
IV	9.8 ± 2.63	ne	30.3 ± 0.28	ne	5.2 ± 0.9	6.7 ± 0.00	ne	0.261	75	ne	ne	0.004	0.001	0.002	0.004
V	17.2 ± 4.48	ne	31.3 ± 0.41	ne	4.9 ± 0.3	6.7 ± 0.00	ne	0.053	69	ne	ne	0.002	0.003	0.003	0.005

ne = not estimated

Table 2b. Mean values of water parameters in each clam-bed zone during May 2015 (Survey# 2)

Zone	Salinity (ppt) Surface	Salinity (ppt) Bottom	Temperature (°C) Surface	Temperature (°C) Bottom	DO (ml/L) Surface	pH Surface	pH Bottom	Chl a (mg/m³)	TSS (mg/L)	PIM (mg/L)	POM (mg/L)	NH_3 (ppm)	NO_2 (ppm)	NO_3 (ppm)	PO_4 (ppm)
I	14.3 ± 1.17	14.9 ± 1.09	31.6 ± 0.26	31.1 ± 0.27	4.9 ± 0.3	6.7 ± 0.00	6.7 ± 0.04	8.22	140	55	85	0.103	0.002	0.012	0.006
II	17.9 ± 2.53	18.9 ± 3.96	30.9 ± 0.37	30.8 ± 6.56	5.5 ± 0.9	6.6 ± 0.10	6.6 ± 0.09	3.05	92	57	35.3	0.028	0.001	0.007	0.003
III	12.6 ± 0.47	20.8 ± 0.53	31.8 ± 0.21	31.2 ± 0.33	5.0 ± 0.1	6.7 ± 0.00	6.6 ± 0.00	4.57	90	23	66.7	0.139	0.002	0.046	0.003
IV	9.8 ± 2.63	21.7 ± 7.26	30.3 ± 0.28	29.9 ± 0.77	5.2 ± 0.9	6.7 ± 0.00	6.6 ± 0.00	4.49	78	58	19.8	0.057	0.001	0.011	0.006
V	17.2 ± 4.48	29.8 ± 2.84	31.3 ± 0.41	31.0 ± 0.28	4.9 ± 0.3	6.7 ± 0.00	7.3 ± 0.64	2.51	68	41	26.8	1.028	0.025	0.098	0.013

ne = not estimated

converted to NO_3 via NO_2 through nitrification. High concentration of total ammonia concentration especially un-ionized form is toxic to aquatic life. Mean total NH_3 concentrations in all Zones were observed in normal range except zone V (1.028) in Survey #2. High NH_3 production in zone V might be due to increase in the amount of organic material undergoing decomposition. The NH_3 content in all the zones during Survey #2 (0.028 ppm to 1.028 ppm) was comparatively higher than Survey #1(0.001 ppm to 0.006 ppm). NO_2 is an intermediate product during nitrification process. The NO_2 content (0.001- 0.004 ppm) in the present study was found within the normal range in all zones during both the surveys except Zone V (0.025 ppm) in Survey #2. NO_3 is a nitrogen by-product of nitrifying bacteria in a substrate consuming nitrite. NO_3 is far less toxic than NO_2 and and NH_3. The NO_3 content (0.002 - 0.098 ppm) in all the zones was found to be in optimal range during both the surveys.

Phosphate is an important nutrient for primary production in aquatic ecosystems, and it is available in relatively small amount in most unpolluted waterbodies. If sufficient NO_3 is available, elevated concentrations of phosphates will lead to algal blooms. Phosphate content (0.003 - 0.016 ppm) in both the surveys were in the normal range in all the zones. Total suspended solids (TSS) during both the surveys ranged between 71 to 140 mg/L with maximum concentration at Zone I in Survey #2. High concentration of TSS can lower water quality by absorbing light, thereby increasing the temperatures and decreasing oxygen retention capacity of water. TSS values in all zones (68 to 140 mg/ L) were within the acceptable limits during the both surveys.

Chlorophyll a (Chl a) is essential for photosynthesis and used as an indicator of phytoplankton abundance. Chl a content in Survey #2 (2.51 - 8.22 mg/m^3) was found to be more than Survey #1(0.036 - 0.261mg/ m^3) with highest concentrations in Zone I during May. NO_3 is used for phytoplankton production. High Chl a content during Survey #2 might be due to terrestrial input of NO_3 mainly as land run off due to rainfall, resulting in lowered salinity. Higher content of NH_3 during survey #2 may have added NO_3 in the system through nitrification.

Length composition of clams

P. malabarica of 4.25 to 42.4 mm length were observed in the population. During Survey #1, the estimated total density of seed clams (<10 mm) in all zones together formed 40.9 % of total population while the rest was formed by juvenile (7.75%) and large-size clams (51.37%). Highest density of seed clams was observed in Zone II (25.66%) while the lowest was in zone IV (0.54%). Density of juvenile and harvestable size clams was found more in Zone I (30.39%) while the lowest was observed in Zone IV (0.54%).

In Survey #2, clams were present only in Zones I and II. Density of seed clams in all the zones together formed 4.16 % while the rest was formed by juveniles (30.8%) and adult clams (65.04%). Highest numbers of seed clams (2.66%) and adult clams including juveniles (48.41%) were observed in Zone II.

Conclusions

A lot of physical changes have taken place in the clam beds during the past year. The major clam fishing (Zone III) is now totally devoid of clams, and about 40% of this bed is always exposed to air. The fishing is mainly taking place in Zone 1 (under and west of Neendakara Bridge) which has been marked as a Clam Sanctuary and No-Fishing Zone as per CMFRI's Clam FMP. Fishing on the mother stock is detrimental to future harvests. Zones IV and V that were earlier poor in clams are even now devoid of clams because of excessive silt occurring in the clam bed.

A new part of Zone II (Mukkam, Aravana Kadavu) shows new clam settlement and fishery which has not formed part of our surveys. Part-time fishing to the tune of 15 canoes per day has been reported from this area. Although we are as yet unable to pinpoint the reasons for the decline in clam population, we believe that the water flow has been severely affected by the temporary bunds created under Dalawapuram Bridge during its construction. Another factor may be the changes in the monsoon pattern during 2014 and 2015.

Considering the results of the above study, the following is recommended to improve the standing stock of clams in Ashtamudi Lake.

a) Remove the temporary mud bunds created under the Dalawapuram Bridge ensuring tidal water flow between north and south regions of the Lake. This can also restore the most productive Zone III in the long run.

b) Strictly implement the no-take Sanctuary Zone in Zone I as per in CMFRI's clam FMP, through inspections and enforcements.

c) Strictly enforce the Minimum Legal Size (MLS) for clams as prescribed by the Government of Kerala notification G.O.(P) No. 40/15/F&PD dated 24th July 2015.

d) Encourage all concerned to preserve the ecological integrity and character of the Ashtamudi Lake ecosystem by proactively ensuring that encroachments and bunds are not allowed and no garbage or wastes are discarded into the lake.

A survey on functionally diverse bacterial strains from marine finfishes and crustaceans

Imelda Joseph, Hena Vijayan, Remya Sivanandan, Tijo Varghese, Susmitha, V., Anu Mathew and Asha Augustine

ICAR- Central Marine Fisheries Research Institute, Kochi

Prokaryotic microorganisms compromise a large portion of the organic biomass of the world's ocean and play an important role in the biogeochemical cycles and food webs of this ecosystem. Surface colonization by microorganisms is ubiquitous in marine systems with a large proportion of microbes occurring as complex communities. Despite their importance, comparatively little is known about the phylogenetic composition of this complex microbial population and the functional roles of their members. Living surfaces are ideal to explore colonization by microorganisms because eukaryotes are subject to a constant bombardment from the millions of microbial cells typically found in a millilitre of seawater. Alternatively, disease-causing microbes might already be present on fishes and their surroundings. So a survey and analysis of bacteria associated with marine fish can give an indication of the environment like water quality, feed availability, productivity and the presence of pathogens which cause havoc to the system or to the consumers. Also, many associated bacterial strains which find wide application in agriculture and allied sectors based on their biochemical/ physiological characteristics can be documented.

Sampling protocol

The survey was conducted for a period of eight years (2007-14) to assess the diversity of functionally important bacteria from selected marine fishes of Indian coast.The screening of bacteria was restricted to those from economically important finfish and shellfish species. The sampling sites selected were from the maritime states of Tamil Nadu, Puducherry (UT), Andhra Pradesh (East coast); and Kerala, Karnataka, Goa and Maharashtra (West coast). A total of 15 sites were covered for fish sampling. For stability of the data and its reliability for comparison, samplings were carried out only during early morning hours on sunny days (not in winter or rainy season). In majority of cases, only one time sampling was carried out during September to May months.During 2007-2014, 73 species of marine fishes, 7 species of shrimps, 2 species of crabs and one lobster species of commercial importance were collected. Finfishes collected live from shore seines were anaesthetized using clove oil (3-5 ppm) and were transported under sterile conditions to the laboratory for screening of the associated bacteria. The anaesthetized fishes were dissected and the alimentary canal was removed, cleaned, cut into pieces and slit open by longitudinal

Fig. 1. Sampling sites along the Indian Coast

incision. The pieces of the alimentary canal were homogenized with sterile saline and the homogenate thus prepared was used as inoculum for plating on culture media. Mucus extracts was swabbed aseptically for screening of skin isolates. For gill isolates, gills were macerated in normal saline and centrifuged and the supernatant was used as inoculum for microbial culture. Characterization of bacterial strains based on phenotypic data was done for all culturable strains. Characterization of bacterial strains using 16S rDNA sequence analysis was followed for selected strains that were of distinct functional properties.

Phenotypic Characterization :

Morphological tests like Gram staining and Motility were determined in 24-hours (h) cultures in nutrient broth. The morphology and size of colonies were examined in nutrient agar after 3 days incubation period. Fluorescence was examined in King's B medium after 24 - 48 h. Physiological tests for observation of growth at different salinity were determined by inoculating in nutrient broth with NaCl concentrations of 0, 5, 9, 12% (w/v). The growth at different pH was determined in nutrient broth with the pH adjusted to 5 and 8 or 9 and 10 using either 1N Hydrochloric acid (HCl) or sodium hydroxide (NaOH). Biochemical tests conducted for bacterial characterization include catalase, cytochrome oxidase, penicillin sensitivity, H & L glucose O/F, sugar fermentation tests, cellulose hydrolysis, gelatin liquefaction, starch utilization and phosphate solubilization test.

Genotypic Characterization

Selected strains with specific functional properties like salt tolerance, thermal tolerance, pigmented strains, starch and cellulose hydrolyzing strains were subjected to genotypic characterization. Pure bacterial cultures were cultivated in tryptone soya broth (TSB, Oxoid) for 2 days. Bacterial DNA was extracted using Genomic DNA Purification Kit (Genie, Bangalore, India). Identification of fluorescent strains was performed

Table 1: list of fishes sampled in various maritime states

Fish Species	States / UT							
	MAH	GOA	KAR	KER	TN	AP	OD	PUD
Finfishes								
Abalistes sp.				✓				
Acanthurus sp.				✓	✓			
Anodontostoma sp.	✓							
Arius maculatus						✓		
Arius sp.	✓							
Atule mate					✓			
Auxis rochei				✓	✓			
Auxis thazard				✓				
Carangoides ferdau					✓			
Carangoides sp.					✓			
Caranx sp.					✓			

Caranx tille					✓			
Cephalopholis sp.					✓			
Chanos chanos					✓			
Coilia sp.	✓							
Coryphaena equiselis					✓			
Cynoglossus bilineatus								✓
Cynoglossus sp.				✓	✓			
Daysciaena albida					✓			✓
Decapterus russelli			✓	✓		✓		
Dussumieria acuta				✓				
Epinephelus faveatus				✓				
Gerres filamentosus			✓					
Hemiramphus lutkei			✓					
Johnius amblycephalus						✓		
Johnius sp.	✓		✓	✓				
Lactarius lactarius		✓	✓					
Lagocephalus gloveri				✓				
Leiognathus sp.					✓			
Lepturacanthus savala			✓					
Liza macrolepis				✓				
Lutjanus johnii		✓						
Lutjanus lutjanus				✓				
Lutjanus sp.				✓				
Megalaspis cordyla				✓	✓			
Mugil cephalus		✓	✓					✓
Nematalosa nasus			✓		✓			
Nemipterus japonicus				✓	✓	✓		
Nemipterus randalli			✓					
Nibea sp.				✓				
Opisthopterus tardoore							✓	
Oreochromis sp.						✓		
Parascolopsis aspinosa			✓				✓	
Pempheris mangula				✓				
Polynemus sp.				✓				
Priacanthus hamrur			✓		✓			
Pseudorhombus arsius					✓			
Raconda russeliana					✓			
Rastrelliger brachysoma							✓	
Rastrelliger kanagurta				✓	✓	✓		✓
Rastrelliger sp.					✓			
Sardinella fimbriata						✓		
Sardinella gibbosa						✓		
Sardinella sp.					✓			
Saurida undosquamis				✓		✓		
Scomberoides tol					✓			
Secutor insidiator			✓					
Siganus sp.				✓	✓			
Sillago sihama								✓
Sillago sp.					✓			

Sphyraena obtusata				✓		
Sphyraena putnamae						✓
Sphyraena sp.			✓			
Symphurus sp.			✓			
Tetraodon sp.		✓				
Therapon sp.		✓	✓			
Thryssa sp.		✓				
Trachinocephalus myops		✓				
Trachinotus mookalee	✓					
Tylosurus crocodilus			✓		✓	
Upeneus moluccensis				✓		
Crustaceans						
Fenneropenaeus indicus		✓				
Litopenaeus vannamei				✓		
Metapenaeus dobsoni	✓	✓				
Metapenaeus monoceros	✓			✓		
Panulirus homarus		✓				
Parapenaeopsis stylifera		✓				
Penaeus monodon		✓				
Portunus pelagicus		✓				
Portunus sanguinolentus		✓				
Solenocera crassicornis				✓		

MAH: Maharashtra; GOA: Goa; KAR: Karnataka; KER: Kerala; TN: Tamil Nadu; AP: Andhra Pradesh; OD: Odisha; PUD: Puducherry

by sequence analysis of DNA coding for the 16S rRNA. Universal bacterial 16S rDNAs primers were used to amplify a fragment of 16S rDNA 760 bp in length. The PCR reactions were performed using standard procedures. For negative controls in PCR reactions, sterile distilled H_2O instead of DNA was used. The PCR product was bi-directionally sequenced using the forward, reverse and internal primers. The multiple sequence alignment program Clustal W was used to align the 16S rRNA sequence of the strains. For comparison, sequences of rRNA genes, were obtained from the NCBI GenBank and RPD data base. Evolutionary distance matrices were calculated by using the algorithm of the Kimura two-parameter model. A phylogenetic tree was constructed by using the Neighbour-Joining method with bootstrap re-sampling (data re-sampled 100 times) to assess the degree of support for the phylogenetic branching indicated by the optimal tree.

A total of 732 bacterial strains were characterized phenotypically from marine fishes and crustaceans. About 26 bacterial strains comprising halophilic, pigmented, starch hydrolyzing (amylase producing), cellulase producing and heat tolerance were identified using 16S rDNA sequence analysis. These include *Halomonas marina* strain DSM 4741 (GenBank Accession No. AJ306890), *H. aquamarina* (GenBank Accession No. EU440965), *Planococcus maritimus* (GenBank Accession No.EU624446), *Sporosarcina saromensis* (GenBank Accession No.AB243864), *Arthrobacter* spp. (GenBank Accession No. EU797642), *Pseudomonas aeruginosa* (GenBank Accession No. FJ665510), *Stenotrophomonas* spp. (GenBank Accession No. EU816585), *Bacillus marisflavi* (GenBank Accession No. DQ105973), *Bacillus* spp. ZH4(GenBank Accession No. EU236750), *Microbacterium esteraromaticum* (GenBank Accession No. JQ581525), *Micrococcus luteus* ATCC = 4698 (GenBank Accession No. EU236750), *Bacillus circulans* (GenBank Accession No. JQ58152), *Bacillus cereus* (GenBank Accession No. JN793477), *Pseudomonas stutzeri* (GenBank Accession No. JQ581527), *Bacillus circulans* (GenBank Accession No. JQ581528), *Brachybacterium conglomeratum* (GenBank Accession No. JQ581529), *Staphylococcus warneri* (GenBank Accession No. JQ581530), *Bacillus nealsonii* (GenBank Accession No. JN710379), *Vibrio alginolyticus* (GenBank Accession No. JN710378), *Bacillus* *atrophaeus* (GenBank Accession No. JN712298), *Pseudomonas* spp.(GenBank Accession No. JN710377), *Aeromonas hydrophila* (GenBank Accession No.JN712299), *Bacillus* spp. (GenBank Accession No. JN712300), *Bacillus subtilis* (GenBank Accession No. JN710380) and *Klebsiella oxytoca* (GenBank Accession No. JN712301).

Study on functionally diverse bacterial isolates from fishes/ crustaceans were a new concept and were carried out under the ICAR network project Application of Microorganisms in Agriculture and Allied Sectors (AMAAS). The exact role played by bacteria on fish skin, gills or viscera is unknown. The only known fact was that marine bacteria are potential source of unusual, novel bioactive compounds which can find wide application for industrial, agricultural, environmental, pharmaceutical and medical uses. Many bacterial strains of novel characteristics like extreme halophiles which can thrive and multiply in salt content ranging from 2% to 25%, fluorescent strains, pigmented strains, strains which can utilise cellulose and starch, that can produce carotenoids *etc* could be isolated and characterized during the study. By genotypic characterization, the variable portions of the 16S rDNA gene provided unique signatures that enabled the identification of selected bacteria up to species level. The strains that find application in agriculture and allied sectors were also deposited in the culture collection at National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, Uttar Pradesh for future reference by researchers.

Fish Marketing - A Market Structure Analysis of Kozhikode and Alappuzha districts

Shyam.S.Salim, Ramees Rahman. M., Pushkaran K.N., Nashad M.*, and Soma.S
ICAR-Central Marine Fisheries Research Institute, Kochi
**Nansen Environmental Research Centre (India), Kochi*

Kerala with a coastline of over 590 km, covering nine coastal districts with 222 fishing villages and a fishworker population of about 10 lakhs has 187 marine fish landing centres (Marine Fisheries Census, 2010). Contributing about 20 per cent in the national marine fish production, the fisheries sector of Kerala poses great prospects for further expansion by the balanced and fullest utilization of the resources. The districts of Kozhikode and Alappuzha are among the leading coastal districts in marine fish production as well as marine fish consumption in Kerala. The marine fisheries profiles of the two districts are given in Table 1.

Fish market structure analysis

The concept of a market structure is understood as those characteristics of a market that influence the behavior and results of the firms working in that market. The market structure analysis was based on the major dimensions namely, location (indicated by latitude – longitude), type of market, access, timing, conduct, arrival and disposal sources, infrastructural adequacy, market unions and regulation. The market structure of three major markets viz., Beypore, Puthiyappa, and the Kozhikode Central market of Kozhikode district and the Kalavur, Dara and Kakkazham markets of Alappuzha district was analyzed and the details are given in Tables 2 and 3 respectively.

The market accessibility was found to be good for the markets of Kozhikode facilitating easy transportation of fish from one place to another. Puthiyappa Fishing Harbor is the nearest major landing centre for the Puthiyappa market and the Central market, where as the Beypore Fishing Harbor is the nearest one for the Beypore market. The nearest major railway station to all the three markets

Table 1. Marine Fisheries Profile of Kozhikode and Alappuzha District

Sl	Parameter	Kozhikode	Alappuzha
1.	Total coastline (km)	71	82
2.	Number of landing centers	19	16
3.	Number of fishing villages	35	30
4.	Number of fisher families	14,157	20,278
5.	Total fisher folk population (lakhs)	0.95	0.92
6.	Number of fish markets	147	158
(i)	Number of wholesale markets	3	61
(ii)	Number of retails markets	144	97
7.	Number of export units	5	29
8.	Number of crafts	3,156	8,646
(i)	Number of mechanized fishing crafts	1,065 (33.75 %)	41 (0.47%)
(ii)	Number of motorized boats	1,831 (58.02 %)	3,313 (38.32 %)
(iii)	Number of non-motorized boats	260 (8.24 %)	5,292 (61.21 %)
9.	Marine fish production (2014) in lakh tonnes	1.02	0.81

Sources: *Marine Fisheries Census (2010) CMFRI; PANFISH Book, Kozhikode & Alappuzha District, Department of Fisheries, Kerala; NMFDC, CMFRI ; Kerala Fisheries Statistics 2014, Directorate of Fisheries, Kerala*

Table 2. Market structure analysis of fish markets of Kozhikode

Market Dimension	Central Market	Puthiyappa	Beypore
A. Location			
Year of establishment	1951	1988	1960
Type of market	Wholesale & Retail	Wholesale & Retail	Wholesale & Retail
Lat/Long Position	$11^0$25'N/$75^0$78'E	$11^0$32'N/$75^0$75'E	$11^0$18'N/$75^0$82'E
B. Market control	Calicut Corporation	Harbour Engineering Department Govt. of Kerala	Harbour Engineering Department Govt. of Kerala
C. Market access			
Nearest landing centre and distance (km)	Puthiyappa (9.0)	Puthiyappa (0.1)	Beypore (0.1)
Nearest railway station and distance (km)	Kozhikode (0.6)	Kozhikode (9.6)	Kozhikode (11.1)
Nearest bus station and distance (km)	Moffusil bus stand (2.3)	Moffusil bus stand (9.8)	Moffusil bus stand (12.5)
Nearest airport and distance (km)	Calicut International Airport (27.1)	Calicut International Airport (36.0)	Calicut International Airport (22.3)
Nearest seaport and distance (km)	Beypore port (11.8)	Beypore port (21.0)	Beypore port (1.5)
D. Market Timing (in hours)	04.30-09.00,12.00-18.00	05.00-09.00, 12.00-16.00	04.30-09.00,13.00-18.00
E. Market conduct			
Registered marketers	40	30	30

is Kozhikode Railway Station situated at an average distance of 6.7 km, whereas the nearest bus station is at an average distance of 8.2 km from the markets. Calicut International Airport is the nearest airport to the three markets.

All the three markets of Alappuzha have road connectivity and of these, Kalavur and Kakkazham are nearer to NH-47. The nearest bus station is at an average distance of 3.6 km and the Alappuzha railway station is at an average distance of 7.6 km from each market. Due to the good connectivity by roads and rail, market arrivals and disposals are done in a very short span of time. Cochin International Airport is the nearest airport to these three markets

Table 3. Market structure analysis of fish markets in Alappuzha District

Market Dimension	Kalavur	Dara market	Kakkazham
A. Location			
Year of establishment	1950	1942	1970
Type of Market	Wholesale & Retail	Wholesale & Retail	Retail
Lat/Long Position	9°34’14"N 76°19’40"E	9°29’46"N 76°20’6"E	9°23’26"N 76°21’10"E
Market control	Mannanchery Panchayath	Alappuzha Municipality	Ambalappuzha South
B. Market access			
Nearest landing centre (km)	Sasthrimukku (5)	Alappuzha Beach (3)	Valanjavazhi(2)
Nearest railway station (km)	Alappuzha (10)	Alappuzha (1)	Alappuzha (12)
Nearest bus station	Alappuzha (9)	Alappuzha (1)	Ambalappuzha (1)
Nearest airport (Km)	Cochin International Airport (73)	Cochin International Airport (82)	Cochin International Airport (95)
Nearest seaport	Cochin Port (44)	Cochin Port (53)	Cochin Port (66
C. Market Timing (in hours)	05.00-20.00 hrs	04.00-14.00 hrs	07.00-19 hrs
D. Market conduct			
Registered marketers	12	25	9
Entry fee for operation in market	₹ 5/box	₹ 5/box	₹ 5/box

located at an average distance of 83.3 km. In the trade context, Dara, Kalavur and Kakkazham markets are found out to be well structured.

Market arrivals and disposals

The study of market arrivals and disposals of the markets of Kozhikode indicate that in the Central market, the fish arrivals are mainly from Puthiyappa, Vellayil, Beypore, Koyilandy, Vadagara, Chombala, Ernakulam(Kerala)as well as from Karnataka state. The Central market often disposes fishes to various markets including Kalpetta, Sulthan Bathery, Mananthavady, Kondotty and other fish markets in Malappuram, Kannur and Ernakulam districts, as well as to the neighboring states like Karnataka, Tamil Nadu, Andhra Pradesh, Goa, and abroad also.In the case of Puthiyappa market, the major fish arrivals are from Puthiyappa, Beypore, Vellayil, Koylandi, Vadagara, Chombala landing centres or markets from Ernakulam and the states of Karnataka, Goa etc. The fish disposals from Puthiyappa market are mainly to various parts of the district as well as to other districts including Wayanad, Malappuram, Kannur, Thrissur, Ernakulam etc. Fishes are often disposed to neighboring states like Tamil Nadu, Karnataka, Andhra Pradesh, Goa, and international markets also.The fish arrivals at Beypore market are mainly from Beypore, Puthiyappa, Vellayil, Koyilandi, Vadagara, Chombala, Ernakulam, Karnataka, Goa, etc. Beypore market also disposes fishes to Puthiyappa, Wayanad, Malappuram, Kannur, Ernakulam, as well as to the neighboring states like Karnataka, Tamil Nadu, Andhra Pradesh, Goa etc.

The market study of Alappuzha indicates that in Kalavur market fish arrivals are from Sasthrimukku, Thaickal, Punnapra, Alappuzha, Cochin Fisheries Harbour, Mangalore and Tamil Nadu. The Kalavur market often sends fishes to different local markets such as Mannancherry, Kattoor, Kanjikkuzhi and Marrarikkulam. All these three markets charges ₹ 5 as an entry fee for operating in the markets. In Dara market, major fish arrivals are from Neendakara, Punnapra, Munambam, Thottappally, Mangalore, Karnataka and Tamil Nadu and the market trades marine fishes to the entire Kuttanad regions i.e., Muhamma, Pallathuruthi, Cherthala, Aryadu, Aravukadu, Punnamada, Nedumudi,Thumboli, Zakkariyabazar and Thanneermukkom. Mostly the fish arrivals of retail market at Kakkazham are from Thottappally, Punnapra, Neendakara and Tamil Nadu. The major fish disposals are done to nearest localities like Valanjavazhi, Ambalappuzha, Purakkad, Vandanam and Neerkunnam.

Species Traded

Nearly 25 to 30 marine fish species are marketed in all the three markets of Kozhikode where as around 20 to 25 marine and inland species are marketed in the markets of Alappuzha. The details regarding the market price and the quantity of the major species traded in the markets of Kozhikode and Alappuzha markets are explained in Tables 4 and 5.

The trade union activities in the markets of both Kozhikode and Alappuzha are restricted to loading, unloading, transporting and allied activities. While analyzing the quantum of fish trade in the markets of Kozhikode, the average quantity of fish traded in the Central market is almost 18 tonnes (t) whereas around 20 t is traded in Puthiyappa and 15 tonnes in Beypore. The average value of transaction across the

Table 4. Major species traded - Quantum and average price in the selected markets of Kozhikode (March-April, 2015)

Species	Central Market		Puthiyappa		Beypore	
	Qty (Kg)	Price(₹/Kg)	Qty (Kg)	Price(₹/Kg)	Qty (Kg)	Price(₹/Kg)
Anchovies	1000	80	1000	80	1000	80
Barracuda	800	200	850	200	-	-
Black Spot Snapper	800	200	900	200	500	250
Cat fish	800	150	750	130	-	-
Catla	500	120	-	-	500	120
False Trevally	800	120	800	100	800	100
Mackerel	1000	120	1000	90	800	100
Malabar Sole	900	120	1000	100	750	100
Milk fish	1000	150	1200	100	900	130
Mussels	500	150	200	100	500	150
Pomfret	500	300	700	250	500	250
Prawns	1500	175	2000	150	1500	200
Rays	900	300	1000	250	1000	300
Red Snapper	800	250	1000	250	800	200
Sardine	1500	120	1500	120	800	100
Seer fish	1000	400	1200	350	1000	300
Sharks	800	250	1200	200	1200	200
Silver Moony	400	80	350	60	-	-
Sword fish	800	200	1000	150	1000	150
Threadfin Bream	900	160	1000	150	1000	150
Tuna	1000	270	1500	250	1000	250
Total	18200		20150		15350	

Table 5. Major species traded - Quantum and average price in the selected markets of Alappuzha (January – February 2015)

Species	Dara Market		Kalavur		Kakkazham	
	Qty (kg)	Average Price (in ₹)	Qty (kg)	Average Price (in ₹)	Qty (kg)	Average Price (in ₹)
Anchovies	1,000	100	-	-	200	120
Barracuda	750	230	500	250	150	260
Big jawed jumper	250	150	-	-	-	-
Chanos chanos	150	350	-	-	-	-
Clam	500	80	-	—	-	—
Coryphaena	1,500	225	300	250	-	-

Crab	500	120	-	-	150	140
Croakers	500	75	-	-	-	-
Cuttlefish	750	225	-	-	-	-
Grouper	500	120	-	—	-	—
Half and full beaks	750	180	150	200		
Horse mackerel	500	150				
Mackerel	3,500	180	1000	210	750	220
Mullets	500	250	-	-	-	-
Catfish	250	250	-	-	-	-
Pearl spot	1,250	350	-	—	-	—
Pomfrets	500	300	-	-	150	325
Prawn	1000	160	250	175	100	200
Priacanthus	500	160	200	180	-	-
Ribbon fish	250	150	-	-	-	-
Sardine	7500	100	2500	120	1500	130
Scad	1000	210	450	230	150	240
Sea bass	500	200			150	220
Seer fish	1750	350	500	400	150	400
Shark	1500	260	250	300	150	320
Silver bellies	250	100	-	-	100	120
Snake head	250	280	50	300	-	-
Snapper	500	180	-	-	200	200
Sole	750	75	500	80	250	100
Squid	500	170	150	200	150	210
Tilapia	500	115	250	125	-	-
Threadfin breams	750	125	-	-	200	140
Thrysa	250	40	-	-	-	-
Tuna	2500	250	600	280	250	300
White sardine	250	100	-	-	-	-
Wolf herring	250	175	-	-	-	-
Miscellaneous	600	65	350	75	250	80
Total	35000		8000		5000	

three markets amounts to around ₹ 34 lakhs each across Central market and Puthiyappa and ₹ 27 lakh in Beypore market.

The analysis of daily market turnover of the different markets of Alappuzha indicated that the Dara market trades around 35 t of fish valued at ₹ 62.41 lakhs followed by Kalavur, trading eight t valued at ₹15.20 lakhs. Kakkazham market trade around five t of fish daily valued over ₹ 9.20 lakhs on an average.

Constraint analysis

The constraint analysis of markets was done on the basis of the opinion of different market functionaries. The major constraints perceived by 10 marketers from each market were analyzed using Garrett ranking and the ranks with score are presented in Tables 6 and 7 respectively for Kozhikode and Alappuzha.The Garrett's ranking technique is usually used to rank the preference indicated by the respondents on different factors. The ranks assigned by the respondents for different factors are converted into scores.

$$\text{Percent position} = \frac{100\,(R_{ij} - 0.5)}{N_j}$$

Table 6. Problems/ Constraints faced at markets of Kozhikode

Items	Central Market		Puthiyappa		Beypore	
	Rank	Score	Rank	Score	Rank	Score
High marketing cost	II	75.00	I	91.67	I	91.67
Cut throat competition among traders	III	58.33	II	75.00	II	75.00
Lack of infrastructure & amenities	I	91.67	VI	8.33	IV	41.67
Price discrimination	VI	8.33	III	58.33	V	25.00
High transportation cost	IV	41.67	IV	41.67	VI	8.33
Lack of access facilities	V	25.00	V	25.00	III	58.33

Table 7. Problems/ Constraints faced at markets of Alappuzha

Items	Dara Market		Kalavur		Kakkazham	
	Rank	Score	Rank	Score	Rank	Score
High marketing cost	IV	50.00	IV	50.00	III	64.29
Lack of infrastructure and amenities	I	92.86	I	92.86	I	92.86
Price discrimination	V	35.71	III	64.29	IV	50.00
High transportation cost	VI	21.43	VI	21.43	V	35.71
Lack of access facilities	III	64.29	VII	7.14	VII	7.14
Cut-throat competition among traders	II	78.57	V	35.71	VI	21.43
Inadequate supply of fish	VII	7.14	II	78.57	II	78.57

Where, Rij = Rank given for the i^{th} variable by j^{th} respondents

Nj = Number of variable ranked by j^{th} respondents

The factors with highest mean value are considered to be the most important factor.

Accordingly the major constraint faced by the marketers of Kozhikode was the high marketing cost and price discrimination. Lack of adequate infrastructure facilities like parking area, waste management, drinking water, freezers etc. were also cited as constraints. The infrastructure amenities at the Puthiyappa market and the Beypore market are commendable. The markets possessed good drainage, drinking water facilities and adequate parking facilities. In the case of the Central market, the infrastructure facilities were less. The market was less hygienic with a poor drainage system. The space constraint and absence of drinking water facility added to these problems. The traders expressed that lack of infrastructure and allied amenities in markets including waste management facilities, parking areas, freezers, drinking water etc. were the major problems besides the inadequate supply of fish across the fish markets.

Conclusion

The analysis of the three major fish markets of Kozhikode district suggests the need for improved marketing infrastructure. The major problems and constraints which affects the proper working of the markets highlighted indicates the need for governmental interventions in providing infrastructure to improve fish trade in the major fish markets of Kozhikode. The market structure analysis of major fish markets of Alappuzha indicate that there is significant fish trade which caters to the demand for fish in the nearby towns and cities. However lack of appropriate infrastructure and inadequate amenities are limiting factors in fish trade in these markets.

Oil sardine from Oman enter fish markets of Kerala

Prakasan, D., Jinesh, P.T., Beni, N., Rema, K.V. and Ganga.U
ICAR-Central Marine Fisheries Research Institute, Kochi

The Oil sardine *Sardinella longiceps* is a major fishery resource in India and catches in 2014 were an estimated 5.44 lakh tonnes (t). Of this, Kerala accounted for 1.55 lakh t forming 28% of the All India Oil sardine landings. In the domestic retail markets of Kerala when Oil sardine prices were ranging between ₹ 80 - 140 per kilogram, large sized sardines with average weights > 120 g each were also appearing and being sold at prices 40 - 60% higher than the locally caught sardines. Although market sources informed that these large sized Oil sardine were coming from Oman, the claim could not be traced. However, during a weekly visit to Kalamukku landing centre, Kochi for sampling fish on 23.6.2015, the unloading of packed cartons containing large sized Oil sardine was observed and these were traced to being sourced from Oman. Identity of the sardine as the Oil sardine *Sardinella longiceps* was later confirmed using morphometric and DNA barcoding tools (Fig. 1).

The whole frozen oil sardine packed in 10 kg cardboard crates bearing labels mentioning contents as a Product of Oman dated 12.5.2015 and a shelf life of one year was brought in trucks. The cartons were opened by local dealers, the sardines unpacked and washed in fresh water, immediately re-iced and despatched to local markets (Figs. 2 & 3). These repacked sardines were priced at ₹ 200 and above per kg which was much higher than the prices of ₹ 100–140 normally got for locally caught Oil sardine. The total length of individual fish ranged from 22-24 cm and weighed on an average 160 g, with 6-8 numbers adding up to a kilogram. During the same period, due to heavy rains and windy weather effects caused by the monsoon, ring seine and gillnet fishing activities were temporarily suspended by the local fishermen leading to low availability of fish in the markets. The supply - demand gap being high, the generally big - sized sardines from Oman fetched a very good price. In the subsequent days also similar market arrivals of Oil sardine from Oman were recorded. A few specimens analysed to gather information on their biology indicated females had developing gonads containing oocytes in early yolk development stage of 0.1 - 0.2 mm diameter size. Guts contained very little food and copepods (zooplankton), *Thalassiosira* and *Peridinium* (phytoplankton) were noted. The sardine fishery in

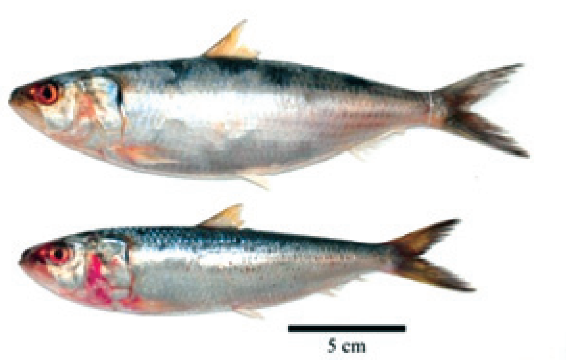


Fig. 1. *Sardinella longiceps* from Oman (top) compared to oil sardine caught locally off Kochi

Fig. 2. Cardboard crates containing frozen oil sardine and bearing label with product details

Fig. 3. Oil sardine being repacked for local markets

Oman is well established and average annual catches reported during 2002-09 period was about 35,000 t.They are harvested using beach seines and gill nets operated close to the shore. They are consumed fresh as well as in dried form for using directly as fodder for cattle and goats when green fodder availability is low and at times also exported to other Gulf countries like the UAE. Sardine abundance in Oman Seas is reported during September to April months with peak during December- January when temperatures are lower (Jufaili and Al-Jahwari, 2011, Sultan Quaboos University, Oman). In comparison, along the Kerala coast the Oil sardine fishery peaks during June to September with annual catches recorded in 2014 being 1.5 lakh against a record landing of 3.99 lakh t in 2012. Catch trends indicate steady decline since 2012. During June, 2015 landings were conspicuously low along the Central Kerala coast although catches were reported from other parts of Karala and neighbouring states like Karnataka and Tamil Nadu.

Large scale exploitation of the Unicorn leatherjacket by multiday trawlers

Lingappa Chaniyappa M., Appaya Naik, R., Rajesh, K.M. and Prathibha Rohit
Mangalore Research Centre of ICAR-Central Marine Fisheries Research Institute, Mangaluru

Aluterus monoceros commonly known *as* the Unicorn leatherjacket or filefishes were exploited by multiday trawlers in Karnataka, but catch volumes were always low. However since mid-April 2015, large quantities were landed both at Mangalore and Malpe Fishing Harbours. The fish has limited preference in local markets but fetches a very good price in other domestic markets and the fishers were happy with the unusual catches.

While small unicorn leatherjacket prefer estuaries and coastal habitas with plenty of weed and reef cover, the larger ones occur in offshore waters. In Karnataka, the trawlers operating at depths ranging from 70 - 100 m were exploiting the larger sized *A. monoceros.* Locally known ‘batmeenu’ soon after landing is skinned and packed in boxes. The fish which originally has a pale grey to brown thick leathery skin then looks smooth with a pinkish white colouration and is sold to local restaurants under the trade name “China pomfret”. The landing price ranged from ₹150 - 200 per kg depending on the size and quantity landed. During the second half of April, around 53 units landed at Mangalore and Malpe Harbours daily with the catch per unit ranging from 0.25 to 4.5 t and the trend continued during May also.

Skinned Unicorn leatherjacket proceeding for sale

Revival of *Rampani* fishing for mackerel in Maharashtra

Punam A. Khandagale, Nilesh A. Pawar, Vaibhav D. Mhatre, Ramkumar S., Anulekshmi Chellappan and V. V. Singh
Mumbai RC of ICAR-Central Marine Fisheries Research Institute, Mumbai

A characteristic shore seine fishery is practised along southern Maharashtra, Goa and northern Karnataka region mainly for catching Indian mackerel *Rastrelliger kanagurta*. The mackerel shoals occurring close to shores during post monsoon season from November to January are spotted by the experts among the fishing community and caught with the shore seine popularly known as *Rampani*. The net is operated from the beach by means of a boat laying the net in semicircular manner, consequent to which both ends of the hauling ropes are pulled simultaneously on to the beach. About 50-80 people are needed for operation of the net so that the process of shooting and hauling is carried out as fast as possible. The net can be operated only in shallow, calm waters where sea bottom is smooth.

The estimated total landings of mackerel in Maharashtra during 2005 to 2014 shows an increasing trend up to year 2011 and thereafter a decrease. The catch of mackerel landed by purse seine a recorded spectacular increase from 1593 t in 2005 to 21575 t in 2011and in gill nets from 1592 t in 2005 to 5835 t in 2010. *Rampani* net operations were very limited until 2005 when only around 12 t of mackerel was landed. In 2011, *Rampani* operations in Sindhudurg district landed 1364 t and thereafter in 2014, highest landing of 1523 t was recorded. During the same period landings of mackerel by trawl net remained steady at around 1600 t. Due to the unprecedented abundance of mackerel, many trawlers were converted into purse seiners by fitting drum winch and other accessories in addition to the net during this time.

Landings of Mackerel in *Rampani* net

During the post monsoon period, abundance of mackerel off Ratnagiri in Sindhudurg district of Maharashtra was very high and around 70-80 *Rampani* net operators were busy fishing for mackerel. Mackerel landings ranged from 2 to 6 t at Devbag and Wairi landing centres. The mackerel catch from *Rampani* fishing in Sindhudurg district was 3.5 t during November 2014 to January 2015. Price of mackerel at landing centres was low at ₹ 10 -15 per kg while the same in retail market was up to ₹ 80 per kg. Such activity among Rampankars or mackerel fishery along the southern coast of Maharashtra was seen after a gap of many years. For the past few years, purse seiners from Goa were reported to be operating in coastal waters of southern Maharashtra and therefore *Rampani* fishery was defunct. From 2010 onwards, strict implementation of Monsoon Fishing Ban from June to August was observed in Maharashtra. The revival of the *Rampani* fishery was attributed by the fishermen to this ban. However, the extension of mackerel even to the northern districts of Maharashtra and further towards Gujarat coast could also be due to the high cyclic abundance of the species, as observed in the past.

Multiday fishing during southwest monsoon along the Kerala coast

Jinesh P. T., Prakasan D., Retheesh T. B. and Ganga U.
ICAR-Central Marine Fisheries Research Institute, Kochi

In 2015, the uniform Monsoon Fishing Ban along the west wast was promulgated for a period of 61 days by the Government of India, but was observed only for 47 days (June 15th - July 31st) in the state of Kerala. The traditional fishermen operating artisanal crafts as well as motorized fishing crafts with outboard motors (OBM) were permitted to operate in the territorial waters during this period. The major gears that operated during this period in the past years were ring seines. During the monsoon, the sea is very rough making it difficult for even experienced fishermen to go fishing. However, a gillnet and hook and line fishery have emerged recently and is gaining popularity by exploiting large pelagic fishes such as tunas, dolphin fishes, cobia etc. during these lean fishery months. It is mainly carried out using motorized fishing vessels of 30-37 feet OAL and 9.9 HP dual engine. The engine with a fuel capacity of 32.5 litres can run for about 4.5 hours with an average speed of 5 knots per hour. There are usually 5-7 people onboard these vessels which mainly operates up to a distance of 25-35 nautical miles (nmi) away from the shore. Sometimes it takes 4-7 days for each fishing trip. These fishers are highly adventurous going, fishing in the rough sea conditions during monsoon with just a polythene sheet as roof on their crafts. The gillnet operations are mainly carried out during night time, while hook and line fishing is carried out during the day hours. It takes about 5-7 hours for operating the gear. The fish hold has the capacity to store about 2 - 4 tonnes (t) of fish catch. This mainly comprises large fishes like tunas, seer fish, barracudas, dolphin fish, cobia, sail fishes, carangids, pelagic sharks and rays. The majority of the fishermen engaged in this fishery are from Poziyoor and Poovar villages of southern Kerala. According to the fishermen although it is risky to fish in the rough seas during monsoon, it provides them a decent livelihood during the lean season.

Fig 1. Traditional OBM crafts using gillnets and hooks and line set sail during monsoon

Landings and size range recorded as Total length (TL) from a boat landed at Munambam Fishing Harbour on 17.07.2015 were Cobia (*Rachycentron canadum,* 69-81cm), Seer fish (*Scomberomorus commerson,* 60-80 cm), Dolphin fish (*Coryphaena hippurus,* 98-132 cm), Marlin (*Makaira mazara,* 100-128 cm), Sail fish (*Istiophorus platypterus,* 89-110 cm), Yellowfin tuna (*Thunnus albacares,* 69-81cm), Little tunny (*Euthynnus affinis,* 36-59 cm) and carangid (*Caranx ignobilis,* 85-110 cm) caught by in hook and line. Species like (*Mene maculata* 18-25 cm), Bullet tuna (*Auxis rochei,* 20-25 cm), Big eye scad (*Selar crumenophthalmus,* 20-25 cm), Rainbow runner (*Elagatis bipinnulata,* 40 - 64 cm) were observed in the gillnet catches.

Record sized Kiddi prawn caught off Mumbai

Sawant, A. D., Johny R. Dias, Vaibhav, D. M., Amey R. J., Swapnil S. Tandel and Ratheesh Kumar, R.
Mumbai Research Centre of ICAR-Central Marine Fisheries Research Institute, Mumbai

Parapenaeopsis stylifera is a penaeid prawn commercially called as *Kiddi* prawn and locally known as 'Jinga'. A large sized *P. stylifera* was collected from New Ferry Wharf on 29.04.2014 during regular field sampling. The mechanized shrimp trawler which had landed the specimen was fishing in 40 - 60 m depths off Gujarat coast. It landed about 50 - 60 kg *P. stylifera* along with the catches of other penaeid prawns such as *Metapenaeus affinis*, *M. monoceros* and *Solenocera crassicornis*. The particular specimen was a female measuring 158 mm in total length and weighing 27.6 grams. It appears to be the maximum recorded size for the species as the earlier maximum sizes recorded by different researchers range between 113-145 mm in total length (Table 1). The specimen was deposited with Accession number (ED. 1.3.4.6.1) in the Designated National Repository of Central Marine Fisheries Research Institute, Kochi.

Fig 1. Record sized specimen of *P. stylifera*

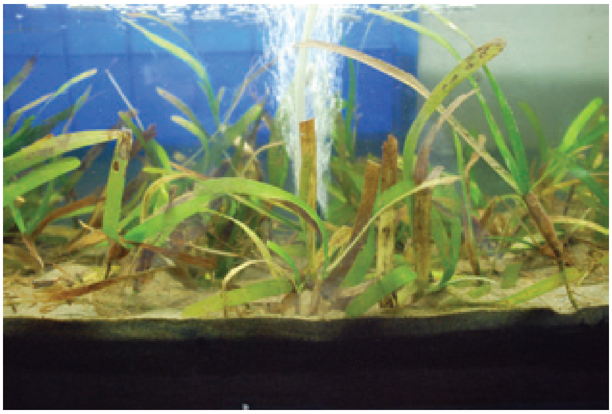
Table 1. Records of large sized *P. stylifera*

Earlier recorded size (mm)	Recorded area	Reference
126-130	Malabar coast	Menon M. K., 1953
145	Mumbai, Maharashtra	Shaikhmahmud and Tembe, 1960
140	Mumbai, Maharashtra	Mohamed, K. H., 1967
123	Ambalapuzha, Kerala	Kurup and Rao, 1974
113	Mangalore, Karnataka	Ramamurthy S., 1980
115	Cochin, Kerala	Suseelan and Rajan,1989
135	Needakara, Kerala	Geetha and Nair, 1992
125	Cochin, Kerala	Suseelan *et. al.*, 1993
115	North-West Coast of India	Ramamurthy S., 1994
135	Mangalore, Karnataka	Anantha *et al.*, 1998
129	Karachi, Pakistan	Ayub, Z. 1998
143	Mumbai, Maharashtra	Deshmukh, V. D., 2001
130	Calicut, Kerala	Sarada, P. T., 2002
140	Saurashtra, Gujarat	Dineshbabu, A. P., 2005
125	Valanjavazhi, Kerala	Nandakumar *et al.*, 2005
158	Mumbai, Maharashtra	Present observation, 2014

Seagrass transplantation protocol for marine aquarium

Kaladharan, P. and Jishnu Mohan
ICAR- Central Marine Fisheries Research Institute, Kochi

Maintaining a marine aquarium though a bit cost and effort intensive, offers pleasure to many people irrespective of age, gender and status. Whle ornamental plants for freshwater aquaria are common, in marine aquariums phytal components are rare. If a marine aquarium is set with seaweeds or



Marine aquarium with seagrass

seagrasses, the aesthetic value will be more and water quality inside the aquarium also will improve. A simple protocol for transplanting seagrass to set up a marine aquarium is described here. Seagrass *Cymodocea serrulata* along with rhizomes and roots collected from Tuticorin coast were acclimatized in the laboratory providing light and aeration for four days. Shoot containing rhizome and lateral roots were tied to thin wooden reapers (85 x 0.5 cm) at every 10 cm interval. Five such reapers were attached together longitudinally with 7 cm interval between and placed inside a glass aquarium tank of 90 x 36 x 38 cm. A narrow 50 cm long siphon was also attached at one of the corners. The tank was filled with 100 litres filtered seawater of 33 ppt. Beach sand collected at the lowest low tide level was slowly added to the tank to fill 10 cm height uniformly ensuring the wooden raft is well below the sand. The turbid water along with floating debris if any were siphoned out. Freshly filtered seawater of 33 ppt was slowly added to the tank without disturbing the bed so that the water level was maintained about 35 cm. Aeration was provided and the tank was illuminated for 10 hours through two fluorescent lamps set from the top of the tank with a regime of 10:14 h light /dark period. Nutrient solutions (Sol. A & B) of Walne's medium at a concentration of 0.5 ml/L each were administered fortnightly through the siphon using a hypodermic syringe so that the nutrient solutions diffused directly to the sediment. The aquarium tank settled within a week and the seawater became clear allowing fish to be introduced. During the 70 days observation period, though the old leaves were shed and fresh leaves appeared regularly, no fresh shoot emerged from the nodes of the rhizomes. The above protocol for transplanting seagrasses in marine aquarium will also be useful for acclimatization studies, nursery rearing of seagrass through vegetative propagation and for carbon sequestration studies under different levels of dissolved CO_2 and temperatures.

Field observation of asexual reproduction by fission in sea cucumber *Holothuria atra*

Asha P. S. and Diwakar, K.

Tuticorin Research Centre of ICAR-Central Marine Fisheries Research Institute,Tuticorin

Asexual reproduction by fission and regeneration are two primitive characters exhibited by echinoderms. Generally they reproduce asexually fragmentation (fission), budding, parthenogenesis, and polyembryony. Fission (autotomy) a common method of reproduction usually involves the division of the body into two or more parts and regeneration of the missing body parts. This phenomenon can occur in populations that are reproducing sexually also. Around ten species of sea cucumbers have been reported to reproduce asexually including *Holothuria atra, H.parvula, H.edulis, H. leucospilota, Actinopyga mauritiana* and *Stichopus chloronotus*. *H. atra* commonly called as Black sea cucumber and locally as "kuchi attai" is the most common holothurian in Gulf of Mannar and Palk Bay.

During field surveys off Tuticorin, the natural fission process was recorded in *H. atra*. It occurred in an enclosed break water system inside the Tuticorin port where adults and fission pieces (both anterior and posterior resulted from autotomy) of *H. atra* were abundantly distributed. The animal followed a twisting pattern of movement in opposite direction, leading to the formation of constriction in the mid body part and throwing out of the internal organs especially the gonad and eventually separating the body into two halves (Fig.1). The entire process lasted for half an hour and the resultant pieces were not having any profound external injuries, unlike those resulting from laboratory experimental induction techniques. The fission pieces were noticed in locations where *H.atra* was densely populated and hardly noticed in locations with sparse populatios. This confirms that natural autotomy is a population density dependent phenomenon among sea cucumbers. It was also observed that the fission pieces dominated during the cooler months, hence environmental factors especially sea water temperature also may have a role in triggering the process of autotomy. A disadvantage of this type of reproduction is the lack of genetic variation, and being genetically identical the progeny cannot withstand big changes in environment. Research on the triggering factors both environmental and hormonal inducing asexual reproduction in *H. atra* and the genetic variation among the populations in Indian waters is needed to throw more light on this aspect.

Fig.1. *Holothuria atra* in the process of fission

A note on the Slug *Oxynoe viridis* inhabiting seaweeds

Veena, S.[1] and Kaladharan, P.[2]
[1]*Mangalore Research Centre of ICAR-CMFRI, Mangaluru*
[2]*ICAR-Central Marine Fisheries Research Institute, Kochi*

Sacoglossa (Mollusca, Opisthobranchia) consists of mostly herbivorous, marine and estuarine sea slugs with nearly 400 described species. This note reports the observation of *Oxynoe viridis* (Pease, 1861) and its association with seaweed *Caulerpa racemosa*. While on a hydrographic data collection trip to Thotlakonda , Visakhapatnam, seaweeds were collected by the first author from the shallow waters of less than 2 m for laboratory observations on 23rd August 2009. The identification of the associated sea slug among the seaweeds was carried out according to Rudman (1999).

SYSTEMATICS
Order SACOGLOSSAvon Ihering, 1876
Superfamily OXYNOOIDEA Adams, H. & A., 1854
Family OXYNOIDAE Stoliczka, 1868
Genus: *Oxynoe* Rafinesque, 1814
Oxynoe viridis (Pease, 1861)

Fig. 1. *Oxynoe viridis*

The specimen was 15 mm in length and had a fragile bubble-shaped shell partially enclosed by parapodial flaps. Spots colouring bluish white were seen on the margins of the parapodia (Fig. 1). A sticky white secretion oozed out when the animal was disturbed and caught by hand during shifting. In our study, the specimen of *Oxynoe viridis* was observed grazing upon *Caulerpa racemosa* and not on *Ulva lactuca* that was also being cultured in the same tank. As the growth of the seaweeds deteriorated after 2 days, the slug also could not survive. Species of *Oxynoe* are reported to feed on a variety of species of *Caulerpa* where they are well camouflaged because of their colour.

A note on a stranded Humpback dolphin

Thirumalaiselvan, S.[1], Anasukoya, A[1]. and Jeyabaskaran, R[2]
[1]*Calicut Research Centre of ICAR-Central Marine Fisheries Research Institute, Kozhikode*
[2]*ICAR-Central Marine Fisheries Research Institute, Kochi*

On 17th September, 2015, a dead dolphin was found at Beypore beach, Kozhikode. On enquiry with local fishermen, it was reported to be washed ashore eight days before. It was identified as Indo-Pacific Humpback dolphin *Sousa chinensis* (Osbeck, 1765) with the help of external characters especially the number of teeth. It had a stocky, robust body with a well-defined beak set off from the rounded melon. The specimen was a male measuring 107 cm in length and weighing about 18 kg. It had deep cut in the ventral region. The morphometric measurements of the Humpback dolphin are given below.

Table 1. Morphometrics of *Sousa chinensis*

Morphometrics	**cm**
Diameter of eye	2
Tip of snout to notch of caudal fluke	107
Tip of snout to origin of dorsal fin	46
Tip of snout to centre of eye	14.5
Tip of snout to blow hole	15.6
Tip of snout to origin of flipper	26.4
Tip of snout to centre of anus	68
Length of flipper along outer margin	17
Length of flipper along inner margin	12
Length of dorsal fin along outer margin	25.4
Length of dorsal fin along inner margin	12
Depth at origin of dorsal fin	24.5
Length of upper jaw	18
Length of lower jaw	17

Sighting of the Short-finned pilot whale

Sajikumar. K.K., Jestin Joy and Gishnu Mohan
ICAR-Central Marine Fisheries Research Institute, Kochi

A small pod of Short-finned pilot whale *Globicephala macrorhynchus* (Gray, 1846) was observed during a fishing cruise of *FV Silver Pompano* on 19th April, 2015 for exploration of the oceanic squid *Sthenoteuthis oualaniensis* in the Southern Arabian Sea. The pod was observed in the

Pilot whale spouting

Pilot whale pods exhibiting resting behaviour

Southeast side of Pitty Islands (10° 38′ 05′′ N; 72° 46′ 02′′ E) and contained nine members of which four were large and five medium sized. The average length of the whales was estimated as 5 metres. They were travelling in a Northwest direction at an approximate speed of 2 knots per hour. The surface water temperature at the location of sighting was 28^0C and salinity was 34 ppt. The IUCN lists this species as data deficient in the Red List of threatened species (IUCN 2012). In India all the cetaceans are listed under Schedule-1 of Wild Life (Protection) Act, 1972.

Reports of marine mammal strandings

K. G. Baby
ICAR-Central Marine Fisheries Research Institute, Kochi

A Spinner dolphin, *Stenella longirostris* was washed ashore at Puthankadapuram landing centre in Thrissur district on 10th March 2015. The animal was in decayed condition and had an injury near caudal base. The total length of animal (snout to notch of caudal flukets) was 242 cm. The length of the upper jaw and lower jaw were 38 cm each. The total number of teeth on each side of the upper jaw was 87.

On 29.05.2014, a Baleen whale measuring 16.5 metres (m) in total length was washed ashore at Blangad Landing Centre, Thrissur district, Kerala. The whale was in a highly putrified condition and Panchayat authorities buried it.

Baleen whale stranded at Blangad


MFIS