Project Completion Report

Harmful Algal Blooms in coastal ecosystems : implications for future aquaculture (HABAQUA)

April 2016 - May 2017

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Title: Harmful Algal Blooms in coastal ecosystems : implications for future aquaculture (HABAQUA)

1. Summary:

Project was initiated in May 2016. The aim of the project was to monitor coastal waters of India and Sri Lanka for HABs in relation to mariculture activities using in situ sampling, Remote Sensing and mathematical modelling approaches. Efforts were also made to validate the Ocean Colour data generated for the coastal waters and develop regional bio-optical algorithm for the case 2 waters in the SW coast of India.

2. Objectives:

- Time series of SPATT bag sample collection on a monthly basis at selected stations along the East and West Coasts of India and off Sri Lanka
- Concurrent auxiliary data collection at SPATT bag sample collection sites, to include water samples for phytoplankton identification and HPLC pigments, relevant environmental variables (nutrients, oxygen, temperature) and bio-optical measurements.
- Determine the relationship between toxic species cell numbers and toxin level or concentration.
- To discriminate diatoms from non-diatoms, using remote-sensing of ocean-colour Sathyendranath et al. (2004)
- Examine the water quality and circulation parameters of the sampling locations to have an understanding of the impact of aquaculture on HAB initiation.
- To predict the suitability of a location for the setting up of cage aquaculture using available data.
- Carry out outreach activities to build awareness and literacy about Marine Science to the coastal communities in Indian states and Sri Lanka.

3. Introduction

Harmful Algal Blooms (HABs) represent a major hazard for the coastal resources in India. Toxin producing HABs are recurrent phenomena leading to mass mortalities in culture cages when algal toxins are accumulated beyond regulatory levels in the fishes reared. Improved monitoring and predictive capabilities constitute the main tools to prevent or mitigate the negative impacts of HABs in coastal commodities.

Non-toxic blooms of algae can cause harm in a variety of ways. One prominent mechanism relates to the high biomass that some blooms achieve. When this biomass decays as the bloom terminates, oxygen is consumed, leading to widespread mortalities of plants and animals in the affected area. Large, prolonged blooms of non-toxic algal species can reduce light penetration to

the bottom, decreasing densities of submerged aquatic vegetation that can have dramatic impacts on coastal ecosystems, as these grass beds serve as nurseries for the food and the young of commercially important fish and shellfish. These "high biomass" blooms are sometimes linked to excessive pollution inputs, but can also occur in pristine waters.

This diversity in blooms and their impacts presents a significant challenge to those responsible for the management of coastal resources threatened by HABs. The strategies needed to protect fisheries, minimize economic and ecosystem losses, and protect public health vary considerably among locations and among HAB types.

Cage aquaculture of finfish has been initiated all along the coast of India by Central Marine Fisheries Research Institute (CMFRI). Species cultured are *Rachycentron canadum* (Cobia), *Trachinotus blochii* (Silver pompano), *Lates calcarifer* (Sea bass), *Lutjanus argentimaculatus* (Mangrove red snapper), *Epinephalus malabaricus* (Grouper). Both open sea mariculture as well as brackish water cage culture are being done along the east and west coasts of India.

4. Study area:

Fig: 1 - Cage aquaculture farms of CMFRI in India (source: Ramkumar et al., 2014)

In India, cage culture sites of CMFRI were selected for the study. Out of the locations cited in Fig. 1, cage culture site in the brackish water region of Cochin (Pizhala) in the west coast and the open sea cage culture site in the Gulf of Mannar and Palk Bay off Mandapam camp in the east coast were monitored regularly for blooms. The stations selected are regions where extensive mariculture operations are undertaken.

In addition to the cage culture sites, time series data collection was done along a horizontal transect in the coastal waters off Cochin for validation of ocean colour data. For comparison, coastal marine as well as brackish water regions were sampled once during break monsoon.

Fig 2: Sampling locations in Sri Lankan coastal waters

Off Sri Lanka:

Mirissa – 5.94N, 80.46E (south)

Tangalle – 6.02N, 80.8E (south east)

Sample collection was undertaken on a monthly basis.

5. Methodology

Biological parameters:

Phytoplankton taxonomy:

50 litres of surface water was filtered through phytoplankton net of 20µ mesh size made of bolting silk. The filtrate was preserved in 3% neutralized formaldehyde/Lugol's iodine solution. Quantitative analysis was done employing Sedgewick-Rafter counting cell. Species identification was done based on the standard keys using 'Olympus CX21i' model Trinocular Compound microscope.

The planktonic microalgae filtered from 50 L of surface water was made up to a fixed volume concentrate. One ml. of this sample was transferred to Sedgewick-Rafter counting cell (the volume of this chamber is 1ml.). The number of microalgae present in all the thousand grids was calculated. Repeated the counting for five times and took the average. The total number of planktonic algal species present in one litre of water sample was calculated using the formula,

$$N = \frac{n \times v}{V}$$

Where,

N = no. of planktonic algae per litre of water filtered

n = average no. of planktonic algae in one ml. of sample

v = volume of plankton concentrate in ml.

V = total volume of water filtered in litre.

Chlorophyll-a concentration:

Volume of water to be filtered was decided based on the turbidity of water. Chl–a was measured using a field fluorometer (model: 10-AU; Turner designs, Sunnyvale, CA, USA) following the Welschmeyer method (Welschmeyer 1992). Between 0.1 and 1.0 L of water was filtered onto 25 mm glass fibre filters (Whatman GF/F) using a vacuum pressure of less than 200 mm Hg and extracted overnight in 90% acetone. The samples were then centrifuged for 10–20 min at 2000 rpm. The raw fluorescence obtained as digital volts was converted to chl-a concentrations using calibration curves from chl-a standards (Sigma-Aldrich Company Ltd., St. Louis, MO, USA).

Primary production:

Light and dark bottle method (Gaarder and Gran, 1927) was used for the estimation of primary productivity.

A set of three ground stoppered oxygen bottles of 50 ml. capacity was used in the experiment. Out of these, one was treated as light bottle, one as control bottle (initial bottle) and the third one served as dark bottle. Water samples from the surface and sub-surface were collected using clean plastic buckets. Water was siphoned into the sampling bottles using a siphon tube; the end of the tube was fitted with nylon net/bolting silk of 200-300 µm pore size in order to remove the zooplankton, if present; which may otherwise interfere with the oxygen content in the experiment bottles. Care was taken to avoid agitation of water. All the bottles were simultaneously filled with water samples using polythene tube, which touched the bottom of the bottle while filling to avoid the formation of air bubbles. The bottles were properly stoppered without trapping air bubbles inside the bottle.

The control bottle (initial bottle) containing water sample was immediately fixed with 1.0 ml. of Manganese sulphate and 1.0 ml of alkaline Potassium Iodide (Winkler A and Winkler B). The dark bottle was wrapped with aluminum foil and kept in a black polythene bag so as to be protected completely from sunlight. The light and dark bottles were kept suspended in a transparent acrylic chamber. These bottles were incubated for a period of 3 hours in the chamber by keeping the exact light penetration and temperature as in the sampling area from which samples were collected.

After the period of incubation the bottles were taken out and were fixed in the same manner as that of the control bottle and were incubated. The oxygen content of the different bottles was

determined by Winkler's chemical titration method. The oxygen content of the light bottle relates the amount of oxygen evolved during photosynthesis minus the amount of oxygen consumed in respiration by the entrapped phytoplankton. The amount of oxygen utilized by the phytoplankton for the respiration can be estimated by using measurements of oxygen changes concurrently in control/initial and light bottles. Oxygen decreased in dark bottle (compared to that of control bottle) was only due to respiration. Hence to get the estimate of total amount of photosynthesis (gross production), negative change in O_2 content of the dark bottle has to be added with the positive change in the O_2 content of the light bottle.

The Light and Dark bottle method assumes a fixed photosynthetic quotient (PQ) of molecules of oxygen liberated during photosynthesis. Theoretically PQ is considered to be one, assuming the product of photosynthesis as starch (hexose sugars). Since it was not possible to determine the nature of photosynthetic product, a PQ value of 1.25 is invariably applied for phytoplankton.

Calculations

Gross production = O_2 content of light bottle -O_2 content of dark bottle…….. A

Net production = O_2 content of light bottle - O_2content of control bottle… B

Respiration = O_2 content of control bottle - O_2 content of dark bottle…. C

The period of incubation is 3 hours, then

Gross production (mg C/l/hr) = A x 0.375 / PQ x 3……... D

Net Production (mg C/l/hr) = B x 0.375/PQ x 3…….…. E

Gross or net production (mg C/l/day) = D or E x12 ……..... F

Gross or net production (g C/ m^3 /day) = F x 1000 x1000

Bio-optical parameters:

Techniques for operational determination of absorption coefficients for particulate and soluble matter in water samples are based on the protocols decided during the workshop at CMFRI, Mandapam. Typically, the methodology for particulate absorption consists of filtering water samples on to (25 mm 0.7 µm pore size) GF/F filters. Volume of water samples taken for filtration was determined based on the turbidity of the water. After filtration the filter pad was placed flat in the tissue capsule and stored in cryocans for further analysis in laboratory, where absorbance was measured using integrated sphere of Shimadzu® UV24500.

For measurement of absorption by soluble components, water samples from each depth were drawn directly into brown bottles (washed with Milli-q and dried). The samples were filtered onboard through 0.2 µm membrane filter into a conical flask and measured using UV-Visible dual beam spectrophotometer.

Absorption by CDOM:

CDOM samples were collected in 100 ml amber glass bottles that had been rinsed three times with sample water before filling. Water samples were filtered onboard through 0.2 µm GF/F

filters. 0.4 ml of 0.5 M $HgCl_2$ was then added to 200 ml of sample to avoid any bacterial degradation.

The sample was then preserved at low temperature until analysis in the laboratory. The sample transparency was measured using an UV/VIS spectrophotometer over the spectral range 400 to 700 nm with an interval of 1 nm against milli-Q water as blank. The spectral absorption coefficient was calculated by normalizing the wavelength to 440 nm (Kowalczuk and Kaczmark, 1996). Remote sensing studies in which the competition of CDOM with phytoplankton pigments for absorption in the blue is prominent often employ 440 nm (Carder et al., 1989; Bowers et al.2000) as the normalising wavelength and this wavelength was chosen here for the same reason.

$$a_{CDOM}(\lambda) = a_{CDOM}(440) \exp[-s(\lambda - 440)] \text{ [m-1]}$$

Where, $a_{CDOM}(440)$ is the absorption measured at 440 nm and s is the slope coefficient which was calculated as the slope of the curve resulted by plotting logarithm of a_{CDOM} against wavelength (λ). The magnitude of a_{440} gives the concentration while the spectral slope(s) indicates its composition (Stedmon and Markager, 2003). The absorption coefficients were then corrected for backscattering of small particles and colloids, which pass through filters (Green and Blough, 1994).

$$a_{CDOM}\_corr(\lambda) = a_{CDOM}(\lambda) - a_{CDOM}(700) * (\lambda/700) \text{ [m-1]}$$

Spectral Absorption Coefficient of Phytoplankton:

Seawater samples (2-5 liters) were collected and transferred, to polyethylene filtration bottles. The filtration bottles and caps were rinsed at least three times with the sample before filling. After filling, the contents were filtered under low vacuum (<25 hPa) through in-line 25 mm whatman GF/F filters. All filtration procedures are done under subdued light conditions. After the sample is filtered, each filter is transferred to a pre washed and dried filter case. The light absorption spectrum of phytoplankton can be measured by the quantitative filter technique (QFT) method (Mitchell, 1990). The absorption spectra of total particulate matter relative to a blank filter saturated with sea water were recorded in the wavelength range 350-750nm at a resolution of 1 nm with a double-beam spectrophotometer equipped with an integrating sphere. For each of the measured spectra, the optical density obtained at 750 nm was subtracted from that of all other wavelengths. Optical density of the total suspended matter was corrected for the path length amplification (ß effect) and converted into light absorption coefficients by the total particulate matter and detrital matter ($a_p(\lambda)$ and $a_d(\lambda)$ respectively) according to Cleveland and Weidemann (1993) Kyewalyanga et al. (1998) as follows.

$$ap(\lambda) = \frac{2.303\ ODs\ (\lambda)}{V/S}$$

$$ODs\ (\lambda) = 0.3780D_f\ (\lambda) + 0.523\ [OD_f\ (\lambda)]^2$$

Where OD_S (λ) is the optical density of total suspended particulate matter, v is the filtration volume (m^3) and s is the filtration area (m^2).

Following the measurement of the total particulate absorption spectrum, the filters were extracted in 100% methanol following the procedure of Kishino et al. (1985) and then saturated with filtered seawater. Following this extraction, the absorption of the filters relative to blank filters also treated with methanol and re-saturated with filtered seawater was determined in the spectrophotometer. These spectra represent absorption by non-methanol extractable detrital matter.

$$ad(\lambda) = \frac{2.303\ ODs\ (\lambda)}{V/S}$$

Where ODs (λ) is calculated using the same equation as above. The term v and s stands for the filtration volume (m^3) and filtration area (m^2) respectively.

An estimate of phytoplankton component of the total particulate absorption [$a_{ph}(\lambda)$] was then determined by subtracting $a_d(\lambda)$ from $a_p(\lambda)$ (Kishino et al., 1985).

$$a_{ph}(\lambda) = a_p(\lambda) - a_d(\lambda)$$

The chlorophyll-specific light absorption coefficients of phytoplankton [$a^*_{ph}(\lambda)$] were obtained by dividing [a_{ph} (λ)] by the Chl-a concentration.

Quantitative estimation of TSM (detritus):

Seawater samples are collected, in clean polyethylene bottles from undisturbed or temporarily disrupted area. A known amount of water was filtered through a 0.45 µm millipore filter paper pre-washed and pre-dried at 103-105^{o}C. It was then rinsed, dried and reweighed with at least 7 digits of precision to calculate the correct TSM in mg/L.

TSM was then calculated by using the equation given below as per Strickland and Parsons, 1972.

$$TSM(mg/L) = [(A-B)\ X\ 1000]/C$$

where,

A = Final dried weight of the filter (in milligrams = mg)

B = Initial weight of the filter (in milligrams = mg)

C = Volume of water filtered (in Litres)

Physico-chemical parameters:

Temperature, salinity, pH, DO were estimated using standard procedures (Strickland and Parsons, 1972).

Nutrients

The inorganic nutrients (nitrite, nitrate, phosphate and silicate) were analyzed using the method outlined by Strickland and Parsons (1972) and measured on U-2001 spectrophotometer. A standard graph was prepared for each nutrient factor using known concentrations of standards. The nutrient values were expressed in the unit of microgram atom/litre (µg at/l).

a) **Nitrite:** Nitrite-Nitrogen present in seawater was estimated by the method described by Strickland and Parsons (1972). Fifty ml seawater samples were measured out in conical flask. One ml of sulphanilamide solution was added to the sample. After 2 minutes but not later than 8 minutes 1 ml of N.N.E.D. (N- Naphthyl Ethylene diamene Dihydrochloride) solution was added and mixed thoroughly. The absorbance was measured at 530 nm.

b) **Nitrate:** The nitrite in the water sample was reduced to nitrate and then measured in the same way as described for nitrite. To each sample a buffer reagent and reducing agent ($CuS0_4$ and hydrazine sulphate) were added and kept in dark for 20 hrs. This reduced solution was treated with sulphanilamide and intensity of colour developed was measured. 50 ml of the sample was taken in a volumetric flask and 2 ml buffer reagent (Phenol and Sodium hydroxide) was added followed by 1 ml reducing agent (Copper sulphate and Hydrazine sulphate) on gentle mixing. The sample was then kept in the dark for 20 hours. 2 ml acetone solution was added and after 2 minutes, 1 ml of sulphanilamide solution. After not less than 2 minutes and not longer than 8 minutes 1 ml of N.N.E.D. (N-Naphthyl Ethylene diamene Dihydrochloride) solution was added to the sample. The absorbance of the sample was determined after 10 minutes at 530 nm.

c) **Phosphate:** Phosphorus present in seawater in the form of dissolved orthophosphate was determined quantitatively by the ascorbic acid (Strickland and Parsons, 1972). For the determination of orthophosphate ions by the formation of a reduced phosphomolybdenum blue complex in an acid containing molybdic acid, ascorbic acid and trivalent antimony, 8 ml of mixed reagent is added to 50ml of the sample. After 5 minutes and preferably within the first 30 minutes, the absorbance was measured at 660nm.

d) **Silicate:** Silicon present in seawater in the dissolved form was estimated by the method described by Strickland and Parsons (1972). The determination of dissolved silicon compound was based on the formation of a yellow silicomolybdic acid, when a more or less acidic sample was treated with molybdate reagent. Since this acid is weak, the same was reduced by ascorbic acid to intensely coloured blue complexes. The absorption of the sample was measured against distilled water at a wavelength of 660nm. 20ml of the sample pipetted out into 50ml-graduated flask containing 3ml of the acid molybdate reagent and mixed thoroughly. After 10 minutes, 15ml of reducing agent was made up to 50ml with distilled water. The solution was allowed to stand for 3hrs and measured absorbance at 660nm.Nutrients(Nitrite, phosphate and silicate) were estimated spectrophotometrically as per standard methods (Grasshoff, 1983).

6. Results:

6.1 Work done in the aquaculture sites of Mandapam, East coast of India

Sampling was done in the vicinity of the culture cages in Gulf of Mannar (GoM) as and when transportation and other facilities for sampling were available. 4 samplings were conducted in the GoM waters - July 2016, September 2016, March 2017 and April 2017. Samples were collected from the vicinity of 4 cages deployed in the GoM. Cages deployed in the Palk Bay (PB) were also sampled, in July 2016 and March 2017. The findings were as follows:

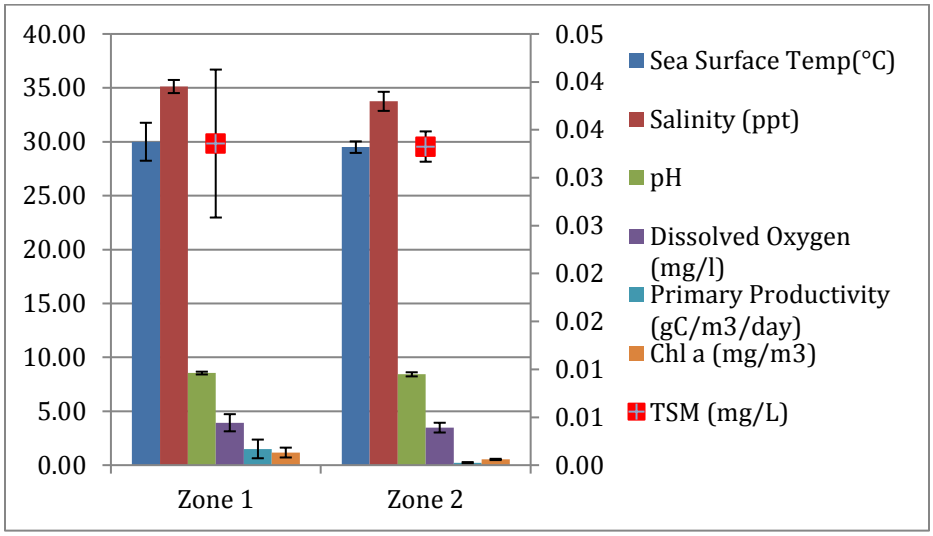


Fig. 3: Hydrography of two different cage culture zones in Mandapam (Zone 1 - GoM, Zone 2 - PB)

Hydrographic data shows that the environmental conditions were similar in both GoM and PB. Chlorophyll and primary productivity were lesser in PB when compared to GoM.

Table 1: Phytoplankton composition (no. of cells / litre) in the GoM (each month data is average from 4 cages) and PB (each month data is average from 2 cages) during 2016 - 2017

	Gulf of Mannar				Palk Bay	
Genus / species	July 2016	Sept. 2016	Mar. 2017	Apr. 2017	July 2016	Mar. 2017
Asterionella sp.	2	0	10	1	0	0
Bacillaria sp.	20267	28276	1462	146	3625	213
Bacteriastrum sp.	27	4	28	0	2	4
Bellerochea sp.	32	5	2	0	2	4
Biddulphia sp.	6	2	0	0	0	0
Chlorella sp.	2080	1722	8301	3372	482	2305
Chaetoceros sp.	297	975	2885	3007	576	526

Corethron sp.	0	6	0	0	0	0
Coscinodiscus sp.	693	1815	3125	3393	752	776
Dictyocha sp.	1	0	0	0	0	0
Ditylum sp.	3	0	4	0	2	0
Eucampia sp.	0	0	0	0	4	0
Fragilaria sp.	5	0	0	0	1	0
Guinardia sp.	0	0	0	0	62	0
Leptocylindrica sp.	47	19	305	608	32	1
Melosira sp.	261	137	17	79	8	0
Navicula sp.	1577	760	8389	3261	884	3472
Nitzschia sp.	422	395	2255	707	306	992
Planktoniella sp.	0	1	0	0	2	0
Pleurosigma sp.	10299	560	1494	806	884	487
Rhizosolenia sp.	24665	5000	9156	11397	3016	2865
Skeletonema sp.	1543	22	213	334	63	11
Thalassionema sp.	0	3	0	0	0	0
Ceratium sp.	1337	1650	1452	1696	70	518
Procentrum sp.	231	150	506	1664	0	149
Gymnodinium sp.	23	10	0	0	0	0
Peridinium sp.	2	10	30	1	0	0
Dinophysis sp.	11	27	0	0	0	0

Phytoplankton of GoM and PB were dominated by diatoms, of which *Rhizosolenia* was the most dominant one. Even though species diversity was high in GoM, species evenness was more in PB. Dinoflagellates formed very low percentage of the total phytoplankton. Interesting feature noted was that not even a single HAB was encountered during the sampling period.

Nutrients:

It was found that PB stations in the vicinity of the aquaculture cages had comparatively high amount of nutrients except silicate whereas in the stations in the GoM cages, silicate levels were high than PB. The low content of nitrite and nitrate in GoM would have acted as a triggering factor in initiating the *Trichodesmium* blooms in the GoM.

The high silicate content in GoM justifies the dominance and abundance of diatoms in GoM than in PB.

Bio-optical properties

Chlorophyll specific absorption Coefficient of Phytoplankton [a^*_{ph} (m^2/mg)]

a^*_{ph} values increased with decreasing chlorophyll, as most attenuation occurs in the blue part of the spectrum. As the relative contribution of detrital matter to total absorption tends to increase with decreasing chlorophyll (Bricaud and Stramski 1990), imperfectly eliminated

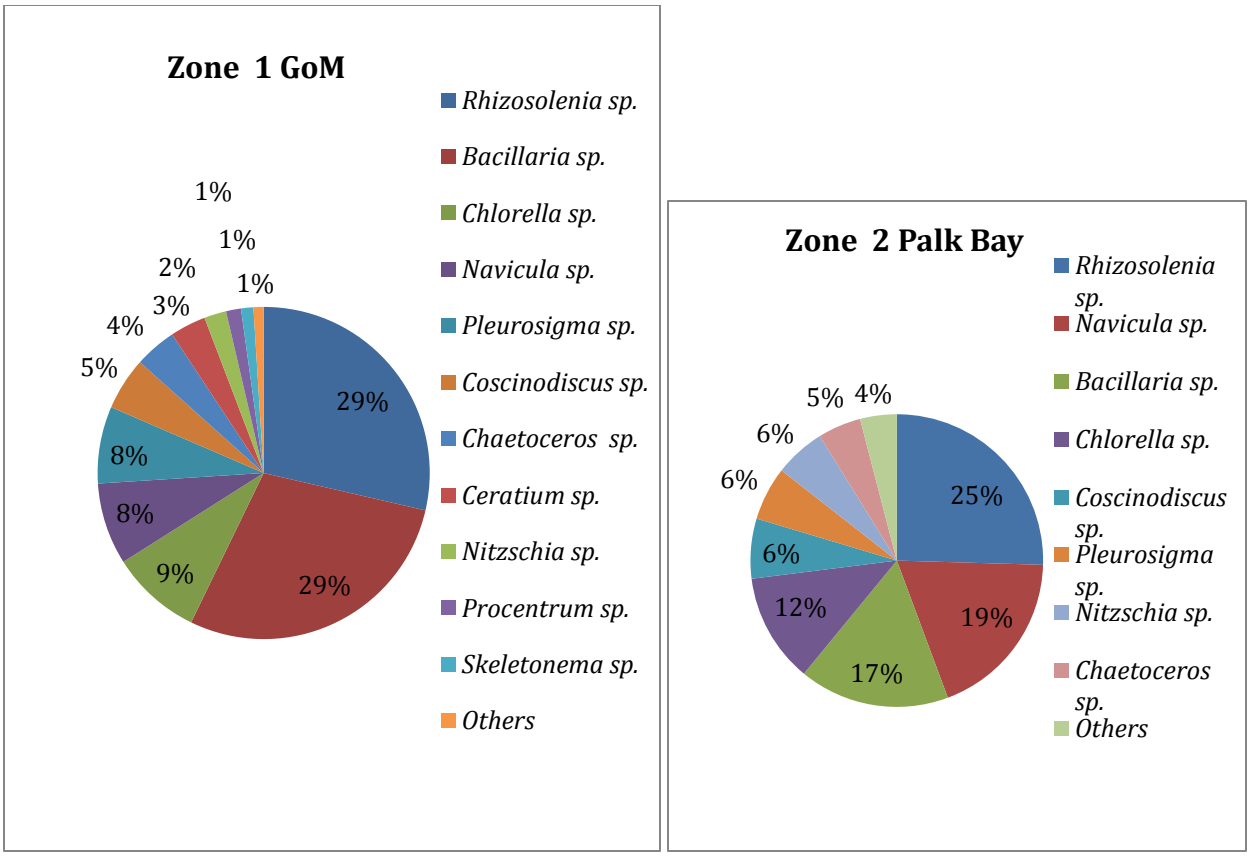


Fig. 4: Percentage contribution of different genera of phytoplankton in the cage culture zones of Gulf of Mannar and Palk Bay

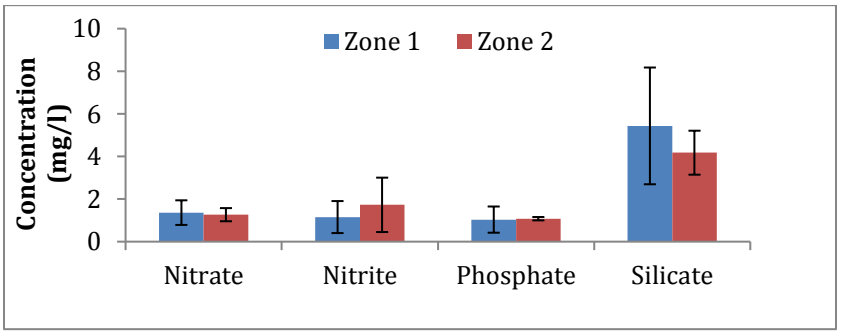


Fig. 5: Nutrient concentration in the cage culture zones of Gulf of Mannar (Zone 1) and Palk Bay (Zone 2)

detrital absorption could lead to an artificial increase of a^*_{ph} at low chlorophyll. The dominant pigments in phytoplankton include chlorophyll and phycoerythrin, which absorb the sunlight energy that drives photosynthesis (Siddiqui et al., 1991). However, during none of the samplings, formation of peak at wavelength 489nm - 495nm, characteristic of phycoerythrin, the dominant pigment of *Trichodesmium* was found.

Phytoplankton light absorption is a major factor contributing to the variation in optical properties of oceanic and coastal waters. The properties of phytoplankton spectral absorption form an integral part of a variety of bio-optical algorithms to estimate phytoplankton biomass and other constituents. Phytoplankton spectral absorption can vary as a consequence of composition and concentration of pigments as well as of pigment packaging. Absorption by phytoplankton **(**a_{ph}) value varied in the blue and red wavelengths of the spectrum in accordance with the phytoplankton diversity. When diversity was high, a_{ph} was maximum whereas when the diversity was less, a_{ph} was minimum and the peak shifted to 430 from 440.

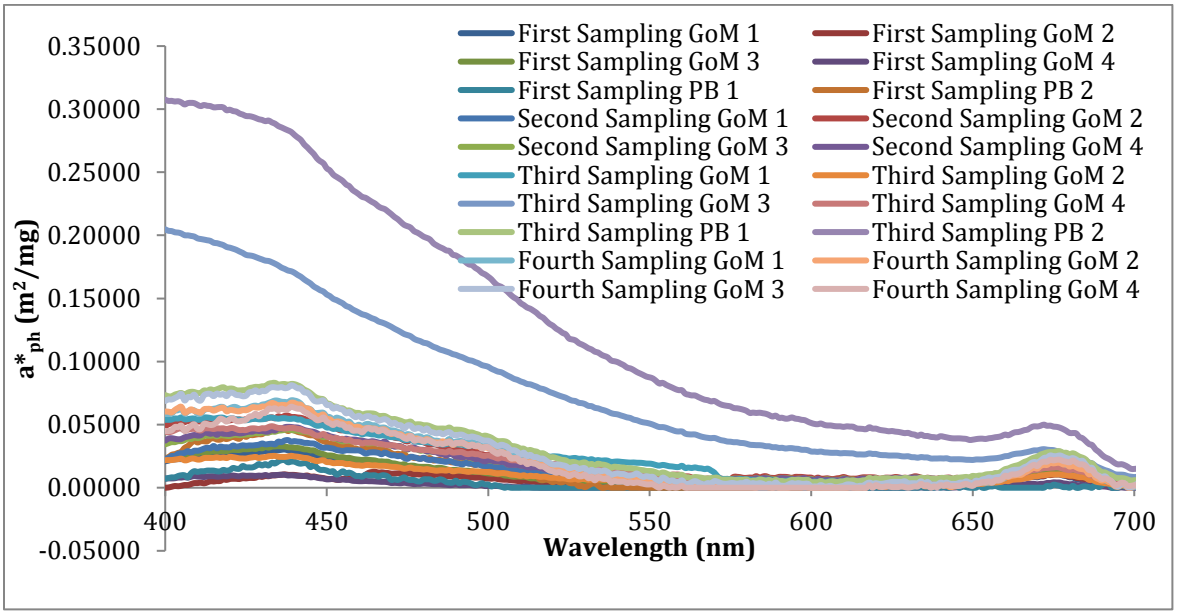


Fig. 6: Chlorophyll specific absorption in the GoM and PB waters during each sampling

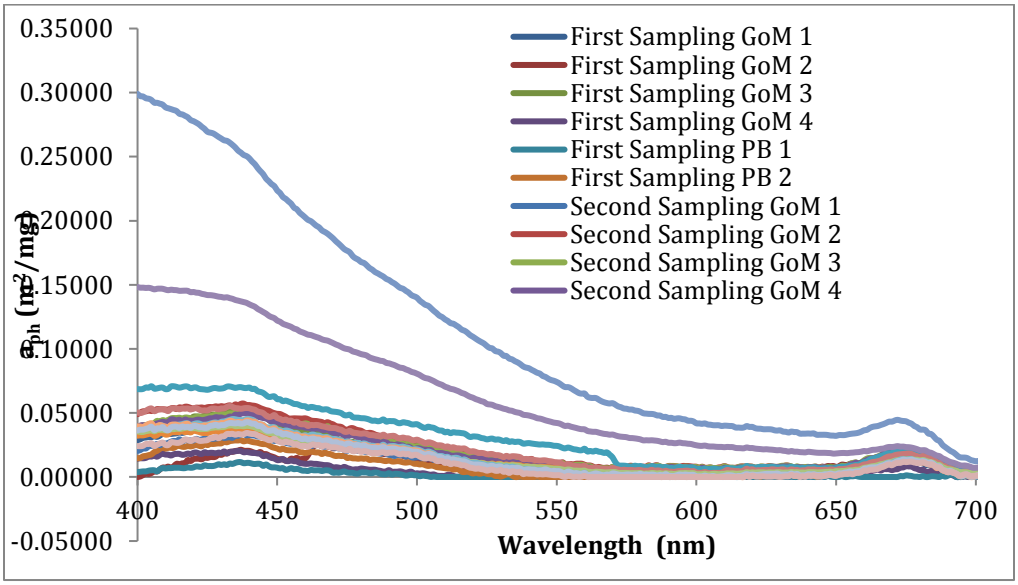


Fig. 7: Absorption by phytoplankton [a_{ph} (m^2/mg)] in the GoM and PB waters during each sampling

The a_d value is a direct indication of the presence of total suspended matter in the water column. This has direct relevance to the optical properties as the TSM interferes with the light absorption by chlorophyll. When the phytoplankton abundance was high, the $a_d(440)$ showed a high value of 0.19 m^{-1}.

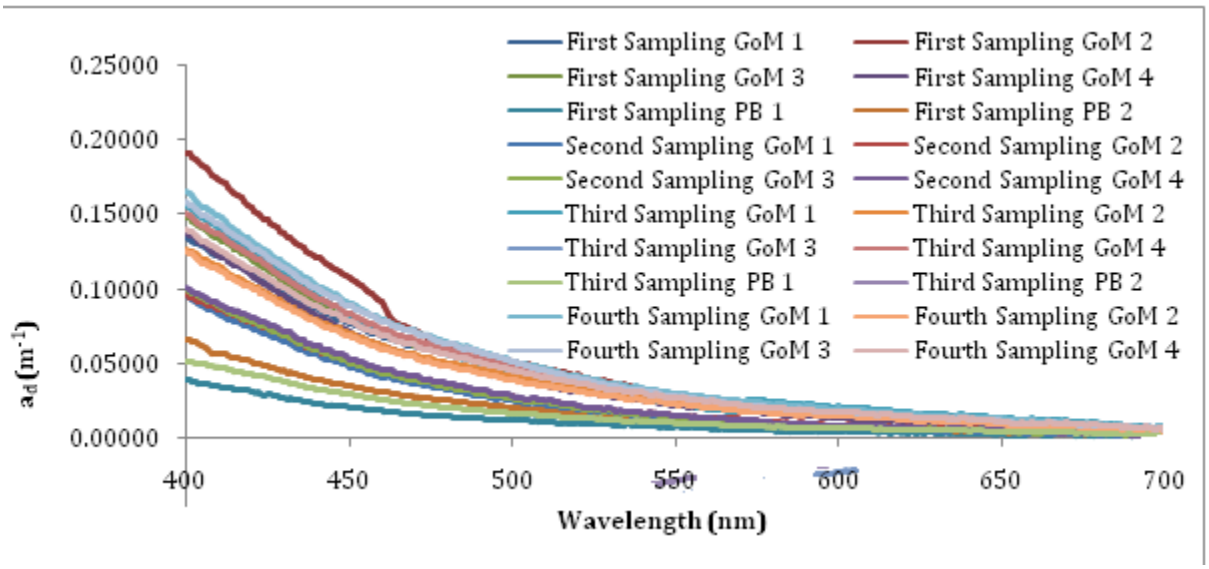


Fig. 8: Detrital absorption [a_d (m-1)] in the GoM and PB waters during each sampling

All the above results show that there was neither phytoplankton blooms nor presence of *Trichodesmium* in the GoM or PB during the time of sampling. However, in the months July to December, *Trichodesmium* blooms were a regular feature in the GoM waters. With the help of scientists of Mandapam Regional centre of CMFRI, most of the HAB incidents were covered and water quality parameters were measured.

SPATT bags were regularly deployed in the cage region and stored in -70^{o}C deep freezer for analysis of toxins accumulated.

Next section explains the detailed study conducted during and after the incidence of *Trichodesmium* bloom and the resultant mass mortality occurred in the cages.

Fig. 9: (Clockwise from top left) - Culture cages in GoM, deployment of SPATT bags near the cage, procurement of water samples from the cage

6.1.1. Report on the mass mortality of cage farmed Cobia at Mandapam Regional Centre of CMFRI

A total of 10 cages were installed in the sea cage farm of the Centre. The details on the species of fishes, their average weights, total biomass of each cage and the stock position before and after the mass mortality are summarized in Table 1.

Table 2: Details of the mass mortality and stock position of fishes in sea cages as 22.07.2016

Cage No.	Species	Kind of fishes	Stock position (nos.) as on 15.07.2016	Average weight of each fish (kg)	Total Biomass (kg)	No. of fishes died as on 22.07.2016
1	Cobia	Brooder-1	22	30	660	16
2	Cobia	Brooder-2	26	28	728	20
3	Cobia	Brooder-3	34	20	680	26
4	Cobia	Brooder-4	36	15	540	36
5	Cobia	Grow outs-1	680	1.3	884	620
6	Cobia	Grow outs-2	1000	0.4	400	450

7	Silver Pompano	Brooders	35	2.5	87.50	2
8	Silver Pompano	Grow outs	450	0.18	80	10
9	Snappers	Sub-adults	45	4.0	180	Nil
10	Sea bass	Sub-adults	30	4.0	120	Nil

On 13th July 2016 at 3 pm, a harmful algal bloom (HAB) of *Trichodesmium* sp was observed (although the scum formation was not observed) just near the seashore (within 50 m from the shore) adjoining the sea cage farm of the Centre. The sea cages were inspected immediately. However, there was no visible bloom near the cages. Water samples from the shore as well as the cage sites were collected to confirm the bloom species and to estimate the water quality parameters. The microscopic examination of the water samples confirmed the algal species to be *Trichodesmium sp.* (Figure 10).

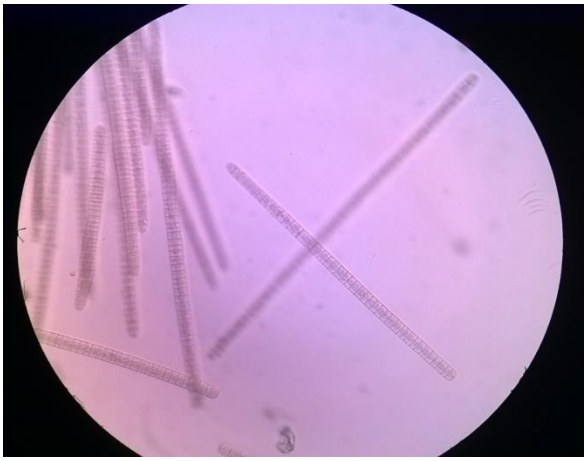


Fig. 10: *Trichodesmium sp.* (the bloom species) under microscope

Fig. 11: Patchy scum formation of the bloom only very near to the shore

To monitor the conspicuousness of the bloom, the water near the sea shore and the cages were observed on the morning (when the water was calm) of the next day (i.e. 14th July 2016). At that time the bloom was conspicuous and discontinuous patches of surface scum were observed only near the shore but not at the cage site (Fig. 11). Water samples were again taken for analysis from the shore as well as from the cage sites. The bloom rapidly vanished within one hour when the sea became rough. Since then bloom (especially the visible scum) was not observed despite regular observation. The water samples were analyzed for their planktonic composition and the results were presented in Table 3. It was found that *Trichodesmium* was the major species of the bloom and the mean *Trichodesmium* density (trichomes mL^{-1} of water) was insignificant at the cage sites during any days of the bloom. However, the trichome density was 176 mL^{-1} near shore during 13th July which increased to 679 mL^{-1} during 14th July which was followed by non-

significant densities during the subsequent days. Preliminary investigation on the dissolved oxygen, water temperature, salinity and pH of the water samples collected on the different dates are presented in Table 4.

The sea cages were also monitored for any possible mortality of the fish in the cages and no unusual mortality was found during 13th and 14th July 2016. Nevertheless, the cages were moved preemptively little far into the sea to avoid mortalities. However, after the decay of the bloom, high mortalities of the fishes in the cages were noticed on 18th, 19th, 20th, 21st and 22nd July. The details on the mortality of fishes in the sea cages are given in Table 2. The temporal relationship in the occurrence of the bloom and the fish mortality is depicted in the following Figure 12.

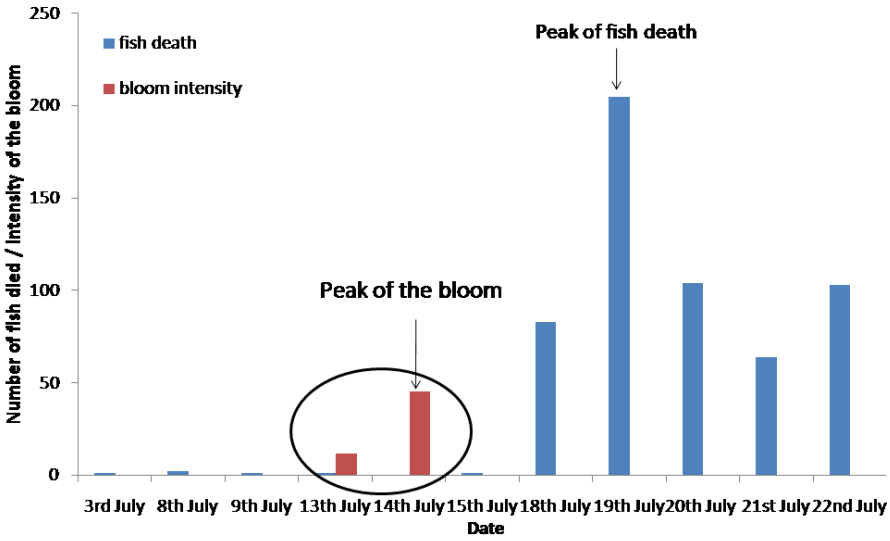
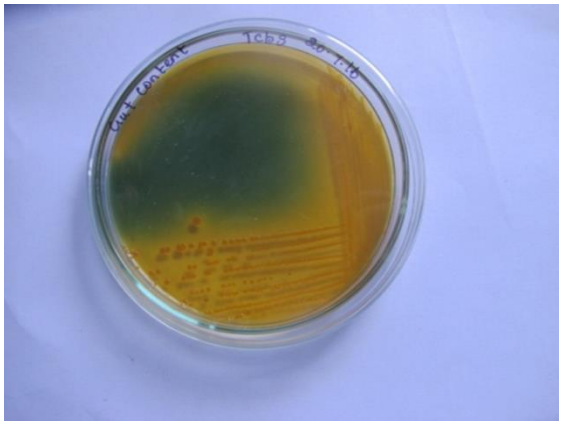


Fig. 12: Temporal relationship of the bloom and the fish mortality

Following this unusual death of fish, they were examined *post mortem* and tissue samples were taken for further examination for finding the possible causes of the death. During the postmortem, no external lesion was found on the fishes. However, hemorrhages on the stomach epithelium were observed. The gut was found almost empty. The small amount of gut content was analyzed under microscope in which no whole or remnants of planktonic/conspicuous organisms were observed.

Fig. 13: Dead brooders of cobia

The samples were analyzed for the possible microbial as well as any chemical (bloom algal toxins, pesticides) causes of the disease. Microbiological plating of gut content on the TCBS agar showed growth of yellow and green colored mixed colonies. Based on the colony morphology, the isolated bacterium was suspected as *Vibrio sp.* Similarly, blood samples were processed by peptone enrichment and subsequently streaked on Zobell marine agar and other selective medias such as TCBS agar (*Vibrio* sp.), MacConkey agar (Gram negative bacteria), Mannitol salt agar (*Staphylococcus sp*.) and Cetrimide agar (*Pseudomonas sp.)*. The species isolated through the selective media were *Vibrio sp*., *Vibrio parahaemolyticus*, *Bacillus sp*., and *Pseudomonas aeruginosa*. The growth, colony morphology and the biochemical characteristics of the isolated bacteria are given in the Table 5. The colony morphology is shown in the following figures (Figures 14 to 16).



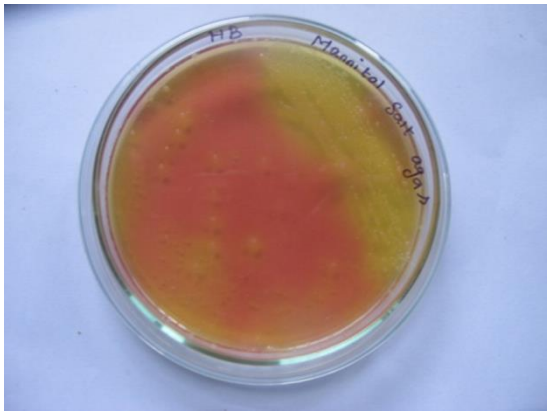


Fig 14: Colony on TCBS agar - gut content Fig. 15: Colony on Mannitol salt agar- blood sample

The *Trichodesmium* species, as such do not cause direct toxicity in fishes. But *Trichodesmium* blooms function as a habitat for diverse range of organisms, including *Vibrio* spp., other cyanobacteria, eukaryotic microalgae, protozoa, fungi, hydrozoans and copepods (Capone *et al.*, 1997). When the *Trichodesmium* bloom crashes and settle at the bottom, it would lead to sudden reduction of dissolved oxygen in the water and causes flaring up of associated

bacteria, eventually leading to stress, shock, septicemia and death in fishes. The stress caused by the low dissolved oxygen concentration accompanied by the septicemia might have caused the mass mortality in the fishes. The mortality had occurred only in cobia not in other species of fishes which might be due to the fact that cobia, being the fast growing fish with very high metabolic rate, has very high oxygen demand and is highly sensitive to low dissolved oxygen. The Silver Pompano is highly resilient and survived the occurrence. Therefore, low dissolved oxygen concentration at the peak of the bloom and during the mortality observed in the present case, might be a possible factor for the mass mortality.

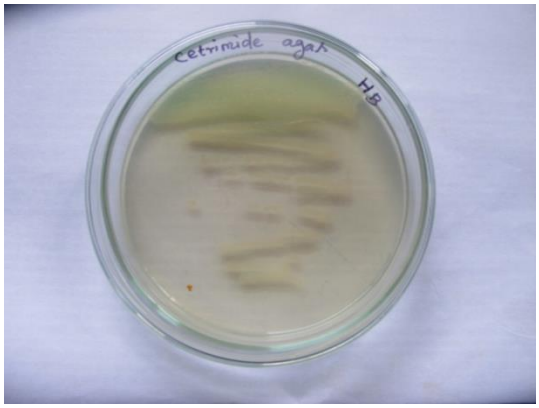


Fig. 16: Colony on Cetrimide agar- blood sample

Table 3: Phytoplankton composition of the water samples from sea shore (SS) and cage sites (CS)

Phytoplankton	13/7/16 Nos/ml		14/7/16 Nos/ml		15/7/16 Nos/ml		16/7/16 Nos/ml		17/7/16 Nos/ml	
	SS	CS	SS	CS	SS	CS	SS	CS	SS	CS
Diatom								2		
Coscinodiscus sp.	1			3		1				
Thallassiosira sp.	3	3	3	3						
Biddulphia sp.	3			2	8				10	
Pleurosigma sp.	6			2	2	1			1	
Navicula sp.	5	6			5	7		1	10	
Rhizosolenia sp.		1	1	2		1		1	4	
Cyanobacteria										
Trichodesmium sp. (Filaments)	233		871	5			1	3	3	
Trichodesmium sp. (Bundles of Trichomes)	23	13	70	10			1	1		

Table 4: Water Quality Parameters

Date	Seashore	Cage 1	Cage 2	Cage 3
13-7-2016	DO- 5.207; Temperature- 31 ˚C; Salinity 35 ppt	DO- 4.381; Temperature- 32 ˚C; Salinity 35 ppt	DO- 4.150; Temperature- 32 ˚C; Salinity 35 ppt	-----
14-7-2016	DO-3.503; Temperature- 30 ˚C; Salinity 36 ppt; pH 7.54	DO- 3.770; Temperature- 30 ˚C; Salinity 36 ppt; pH 7.72	DO- 3.617; Temperature- 30 ˚C; Salinity 34 ppt; pH 7.74	DO- 3.579; Temperature- 30.5 ˚C; Salinity 36 ppt; pH 7.65
20-7-2016	DO- 3.503; Temperature- 31.5 ˚C; Salinity 35 ppt; pH 7.94	------	------	------
21-7-2016	DO-4.15, salinity 36, Temperature 30 ˚C	DO-3.198, salinity 36, Temperature 32.0 ˚C	DO-3.27, salinity 37, Temperature 31.5 ˚C	DO-3.274, salinity 37, Temperature 31.5 ˚C

Table 5: Growth, colony morphology and biochemical characteristics of the bacterial isolates

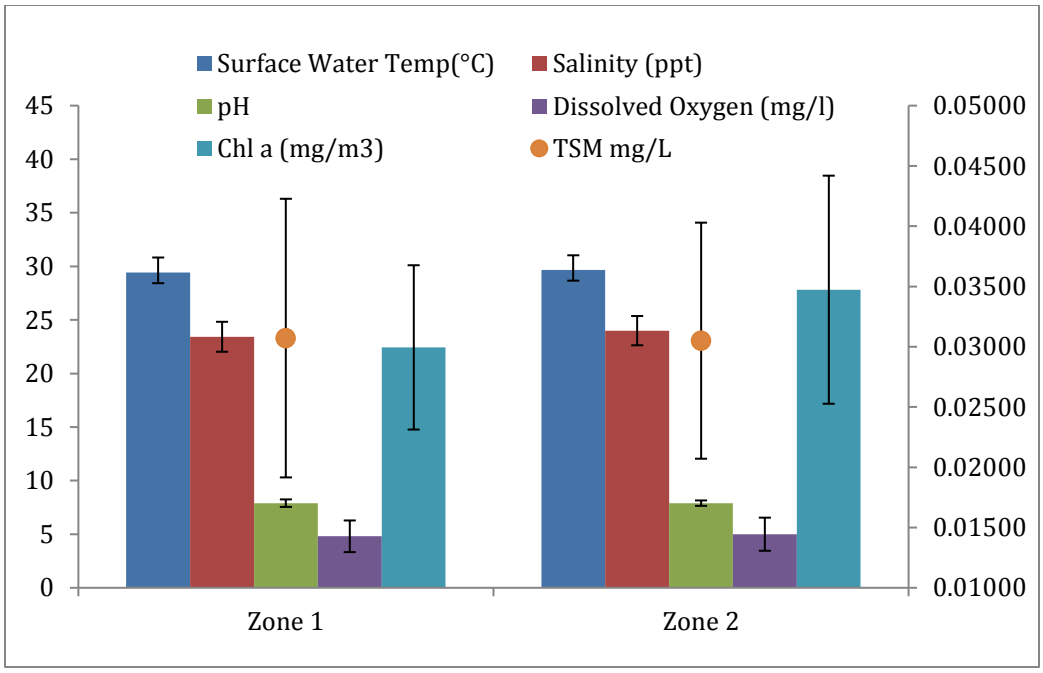
Growth & colony morphology	TCBS		Mannitol	MAC	Cetrimide
	c-1	c-2			
Shape	Circular	Circular	Circular	Circular	Circular
Margine	Entire	Entire	Entire	Entire	Entire
Elevation	Convex	Umbonate	Convex	Convex	Flat
Size	Moderate	Small	Moderate	Small	Small
Texture	Smooth	Smooth	Smooth	Smooth	Smooth
Appearance	Shiny	Dull	Shiny	Dull	Dull
Pigmentation	Yellow	Green	Yellow	Pale pink	Blue-green
Optical property	Opaque	Translucent	Opaque	Opaque	Opaque
Gram stain	G-ve Rod	G - ve Rod	G+ve Rod	G-ve Rod	G- ve Rod
Catalase test	+	+	+	+	+
Sucrose fermentation	+	-	+	-	-
Lactose fermentation	+	-	-	+	- ve
Isolate	***Vibrio spp***	***Vibrio parahaemolyticus***	***Bacillus spp***	***Pseudomonas aeroginosa***	***Pseudomonas aeroginosa***

6.2 Work done at Pizhala, Kochi

Similar to the monitoring of the culture cages in GoM and PB, routine monitoring was undertaken in the aquaculture area in Pizhala, a location in the brackish water lake in Kochi, south west coast of India. The environmental conditions in Pizhala are very much different from that of GoM and PB.

Water quality parameters clearly show that Pizhala is a brackish water region with lesser salinity than GoM. Salinity was in the range of 20-25 psu. Waters were well oxygenated during all the sampling months. Chl-a was also very high corroborating the high diversity and abundance of phytoplankton in these waters. TSM was low in the waters, indicating good quality and clarity of the water.

Nutrient concentration was high in the waters which could be due to the influence of riverine run off adding large amount of silicates into the water. This helps in maintaining a diverse and densely populated phytoplankton in the region.



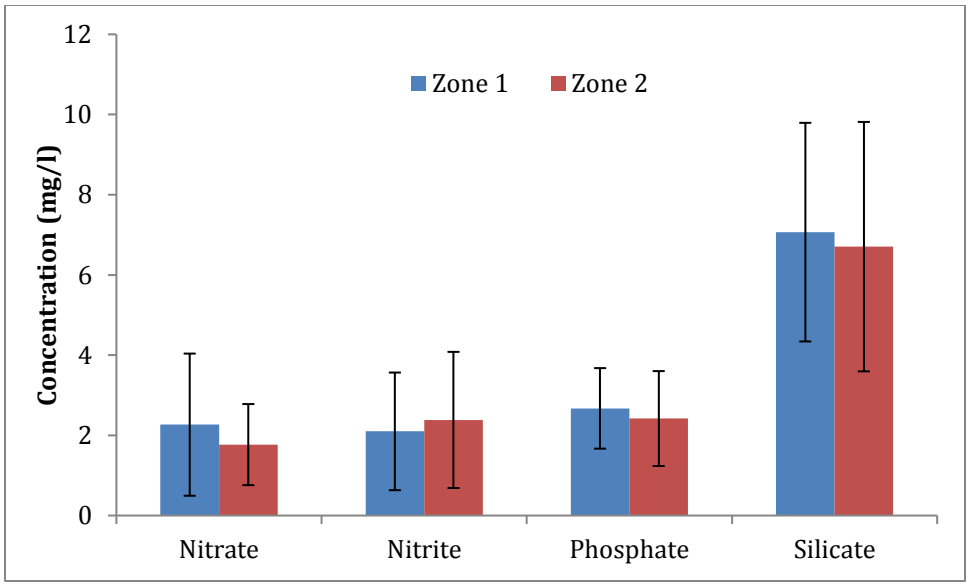


Fig. 18: Nutrient concentration of the culture cage environment in two different zones in Pizhala, Kochi

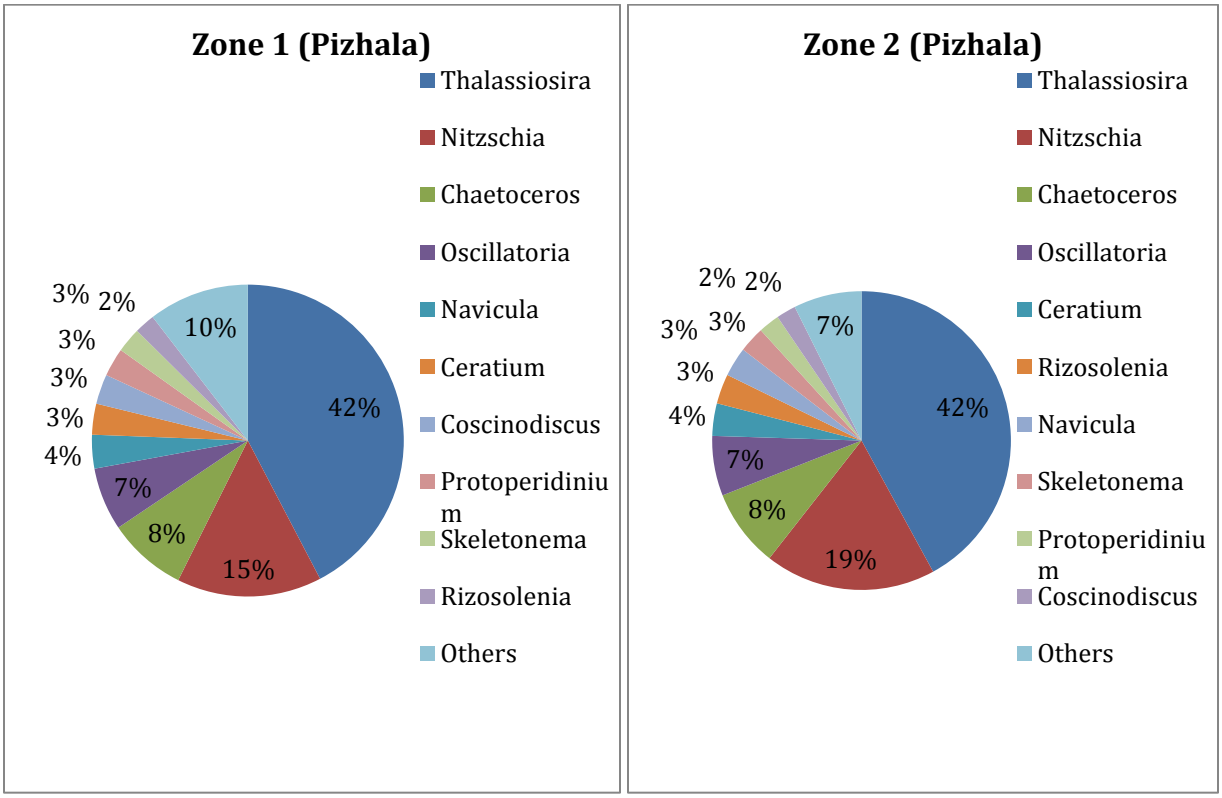


Fig. 19: Percentage contribution of different groups of phytoplankton in the two zones of cage culture sites in Pizhala

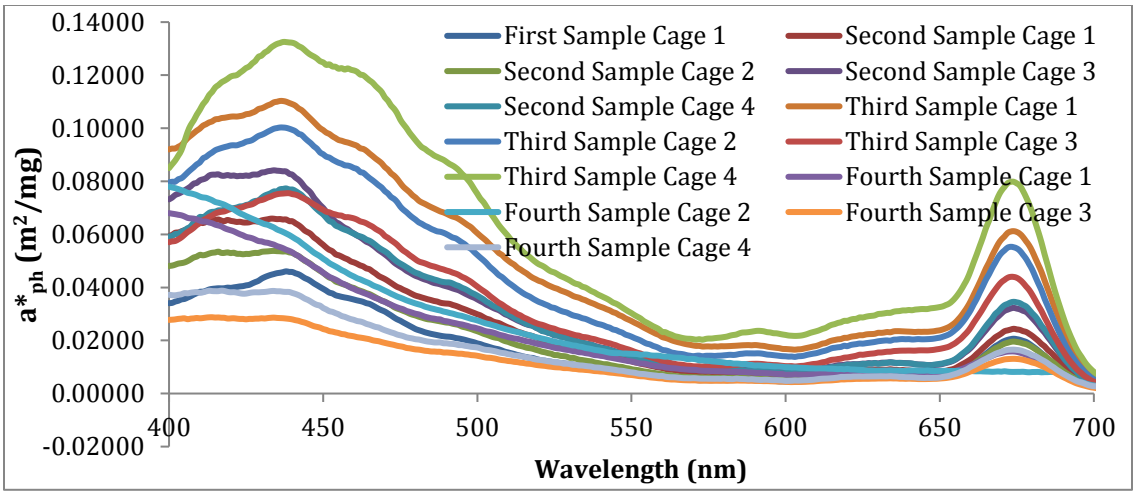


Fig. 20: Chlorophyll specific absorption [a^*_{ph} (m^2/mg)] of the waters in the culture cages in Pizhala, Kochi

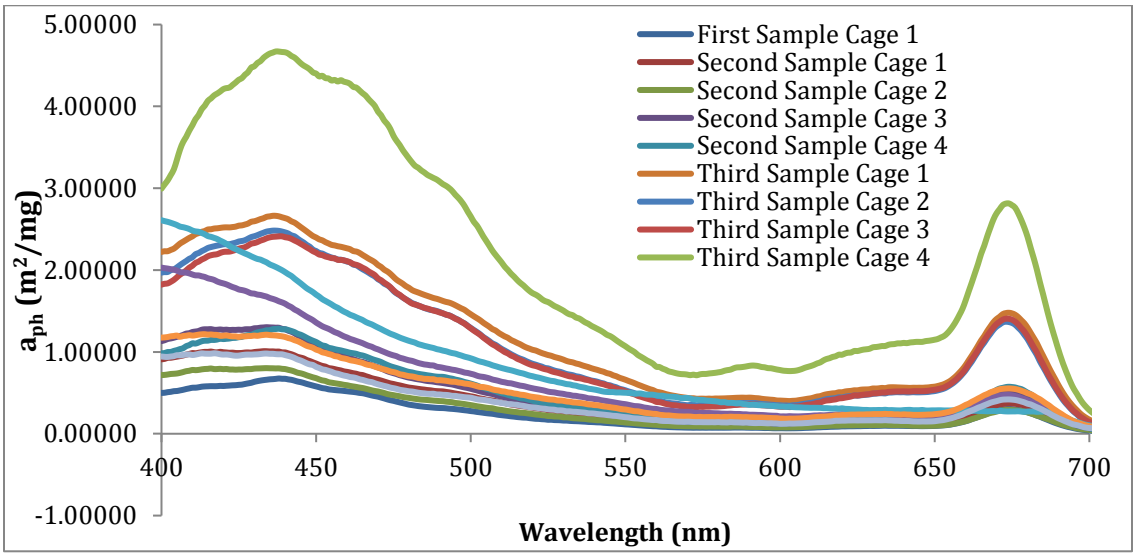


Fig. 21: Absorption by phytoplankton [a_{ph} (m^2/mg)] of the waters in the culture cages in Pizhala, Kochi

The dominant pigments in phytoplankton collected from Pizhala include chlorophyll and phycoerythrin, which absorb the sunlight energy that drives photosynthesis (Siddiqui et al., 1991). In the case of a_{ph}, some of the spectra show flattened peaks whereas some others show a distinct peak. This variation is due to the difference in cell size, with small cells having distinct and sharp peaks while large cells have flattened peaks, even though both belong to the class diatoms, which show a characteristic peak around 450 nm. Presence of the cyanobacteria *Oscillatoria* in large numbers resulted in the formation of a second distinct peak at wavelength 489nm - 495nm.

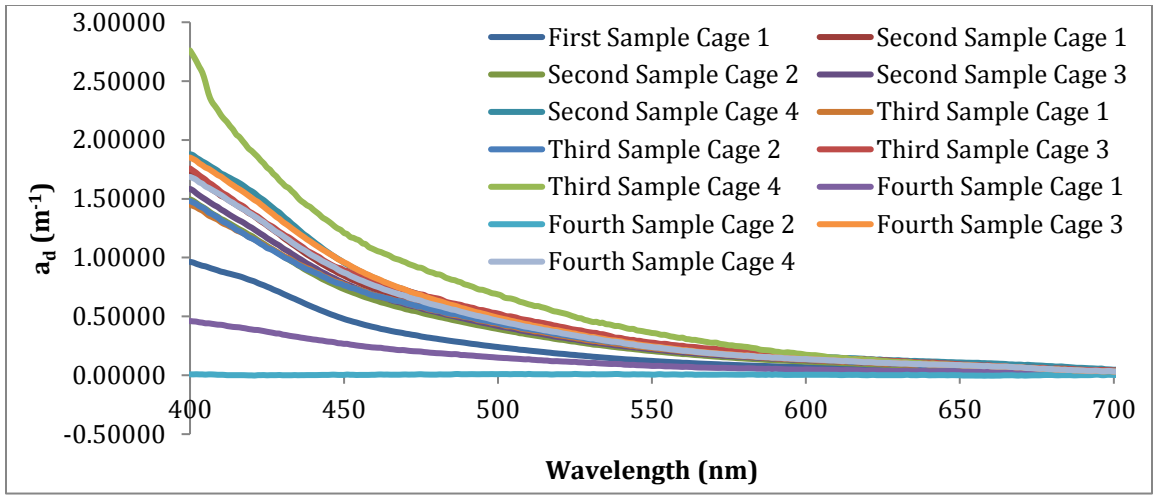


Fig. 22: Absorption by detritus [a_d (m^{-1})] of the waters in the culture cages in Pizhala, Kochi

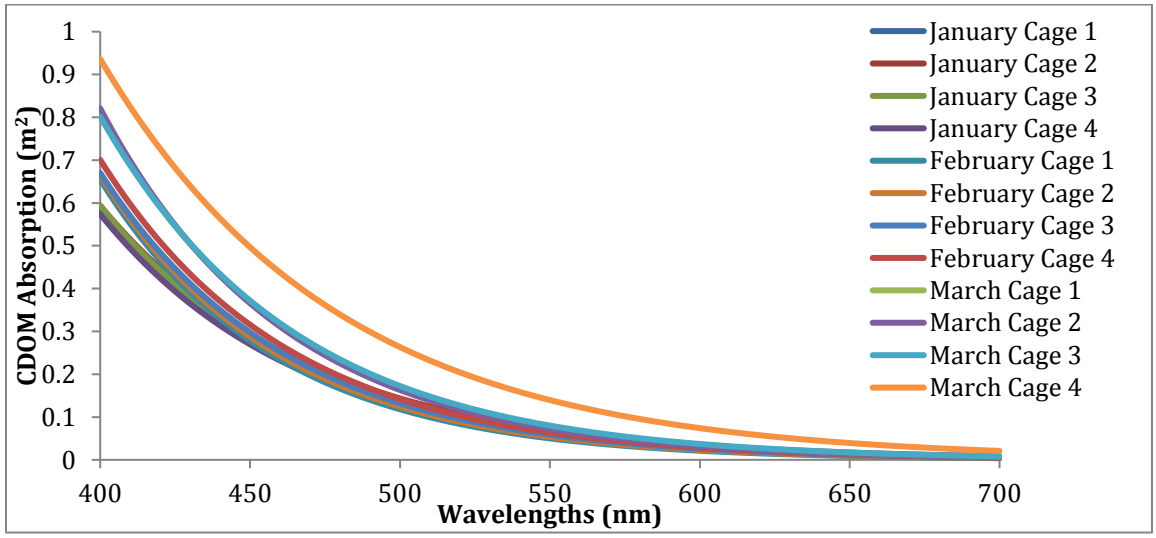


Fig. 23: Absorption spectra of Coloured Dissolved Organic Matter (CDOM) for different cage culture stations in Pizhala, Kerala

At all stations, the magnitude of CDOM absorption was high, which implies its relation to salinity and river run off.

Table 6: Phytoplankton composition in the vicinity of the culture cages in the two zones of Pizhala

Genus name	Zone 1	Zone 2
Amphora sp.	998	860
Ankistrodesmus	674	274
Asterionellopsis	0	0

Bacteriastrum	24	14
Biddulphia	1064	1258
Cerataulina sp.	4	0
Ceratium	2020	2346
Chaetoceros	5184	5642
Chlamydomonas	342	354
Corethron	72	44
Coscinodiscus	1952	1416
Cyclotella sp.	0	0
Dinophysis	152	128
Ditylum	0	0
Gonyaulax	24	0
Gossleriella	0	0
Guinardia	722	692
Gymnodinium	12	0
Gyrosigma	298	0
Gyrodinium	0	0
Hemidiscus	0	0
Leptocylindrus	66	0
Melosera	16	0
Minidiscus	0	0
Navicula	2202	2124
Nitzschia	9480	12372
Notiluca	0	0
Odontella	574	602
Ornithocerus	0	0
Oscillatoria	4120	4326
Pharacroma	0	0
Pleurosigma	508	508
Procentrum	46	0
Peridinium	778	154
Protoperidinium	1860	1522
Pseudo- nitzschia	0	38
Rizosolenia	1342	2152
Scenedesmus	216	0
Skeletonema	1624	1870
Sphaerocystis	0	0
Stephanopyxis	0	0

Striatella	0	0
Surirella sp.	0	0
Thalassionema	0	0
Thalassiosira	26702	28072

There was no incidence of HABs in spite of the high nutrient content. Dinoflagellate contribution was less than diatoms and the major dinoflagellates noticed were *Ceratium, Prorocentrum, Protoperidinium*. Interestingly toxic species like *Gonyaulax* were noticed in the phytoplankton community. *Oscillatoria, Thalassiosira, Nitzschia* were also dominant representatives in the phytoplankton community.

Fig. 23 : Sampling location at Pizhala, Kochi Rithin with water samples collected

Highlights at a glance:

- In estuaries, cage culture is the most viable technique to rear fin fishes. Stationary cages fixed to the poles at their corners are used for culture in Pizhala.
- Even though the nutrient concentration was high due to the confluence of terrestrial and riverine influence, the waters never reached the stage of eutrophication.
- No massive blooms of phytoplankton were observed during the sampling period. However, presence of toxic dinoflagellates like *Gonyaulaux* was noted in the phytoplankton community.
- Salinity fluctuations were found to be less when compared to other stretches of the estuary. Cobia, highly adaptable to salinity fluctuations were reared in these cages.
- Waters had very less amount of TSM indicating less rate of siltation in culture beds and further deterioration of water quality.

6.3 Calibration exercise to compare the water quality of cage cultures with normal estuarine and coastal waters

The sampling area consisted of 8 stations marked as A1 – A4 and B1 – B4. Based on the location and circulatory parameters, these stations were demarcated as two broad regions - wave breaking zone of barmouth region (estuary) and nearshore coastal waters of Kochi.

Field sampling for estimation of water quality of the above mentioned area was conducted on 4th August 2016. Various physico-chemical and biological parameters were measured to assess the water quality.

It was found that the surface temperature was uniform throughout all 8 stations – 26-27ºC. At the same time, salinity showed clear cut variation in that the coastal waters had lower salinity (30 psu) than the estuarine stations near barmouth (33-35 psu). This difference may be due to the reason that the sampling was done during high tide time. Joseph et al. (2009) have found that in Kochi backwaters, fortnightly spring–neap variability in temperature–salinity is observed, with with lower temperature and higher salinity during spring tide. High salinity could be also due to the reduction in the amount of rainfall received in August 2016, in spite of the fact that its part of monsoon season.

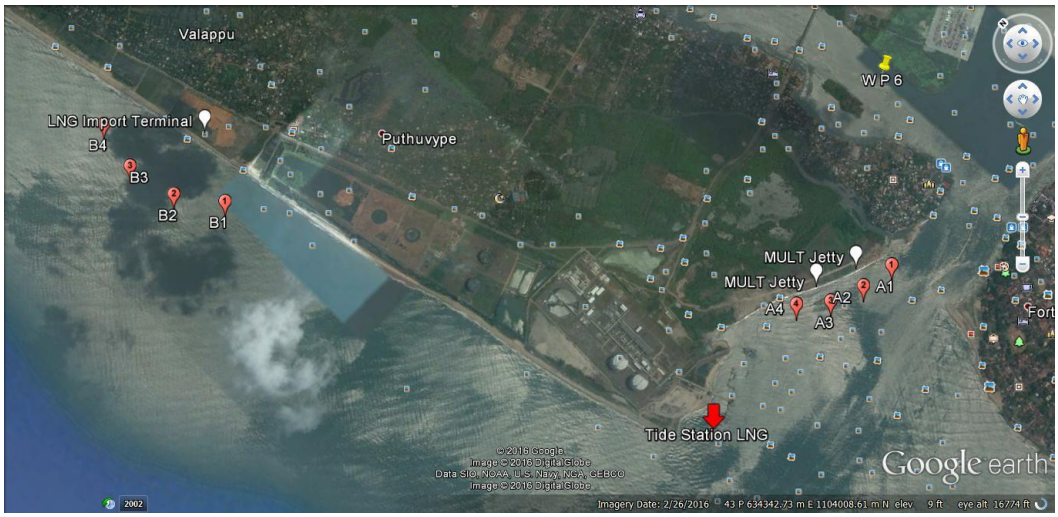


Fig. 24: Sampling stations in the Cochin estuary (A1-A4) as well as nearshore coastal waters of Kochi (B1-B4) used for calibration exercise

pH of the water retained the characteristic value of seawater of 8.4 – 8.7 at all stations.

Transparency of the water column as measured by secchi disc reading was higher in the coastal waters when compared to the backwater stations. Barmouth region which is part of the backwaters is characterized by high amount of suspended sediments probably brought in by the tidal influence. Otherwise with the reduction in amount of rainfall, barmouth region should also have shown high transparency with very less suspended solid load. Coastal waters as usual showed higher transparency than the estuarine waters.

Table 7: Variation in physical, chemical and biological parameters at the 8 sampling stations in the estuarine and coastal waters of Kochi during August 2016

Stations	A1	A2	A3	A4	B1	B2	B3	B4
Sea Surface Temp(°C)	27	26	27	26	26	26	27	27
Salinity (ppt)	33	31	32	35	30	30	30	30
pH	8.5	8.4	8.5	8.4	8.7	8.6	8.6	8.7
Transparency (cm)	33	37	25	28	93	130	103	87
Chl a (mg/m3)	14.04	19.17	11.1	12.12	27.75	16.77	24.66	15.27
Dissolved Oxygen (mg/l)	2.8	2.0	1.6	1.6	2.4	2.4	2.4	2.4
Primary Productivity (gC/m3/day)	1.44	1.44	1.44	2.88	2.88	2.88	4.32	4.32

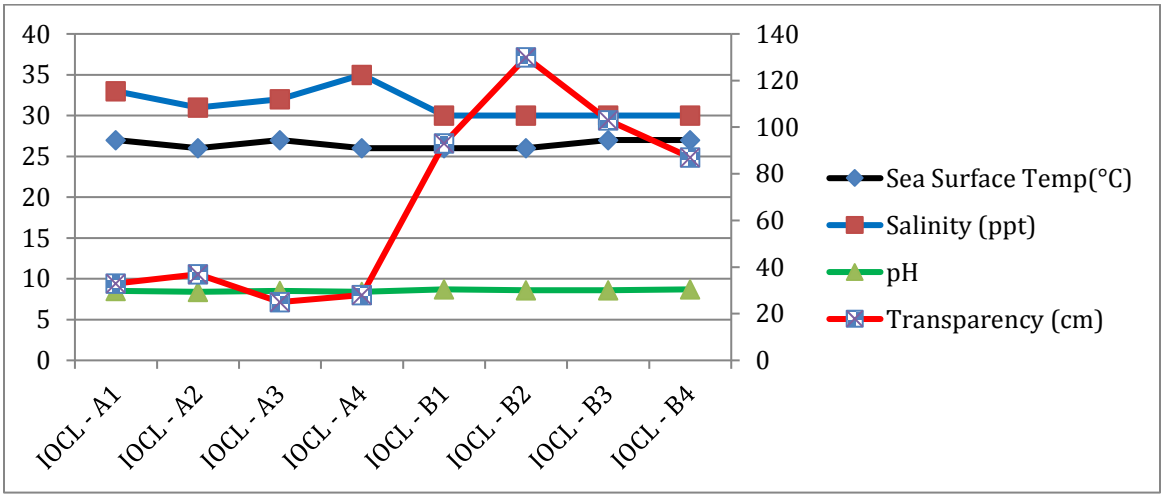


Fig. 25: Variation in physical and chemical parameters at the 8 sampling stations in the estuarine and coastal waters of Kochi during August 2016

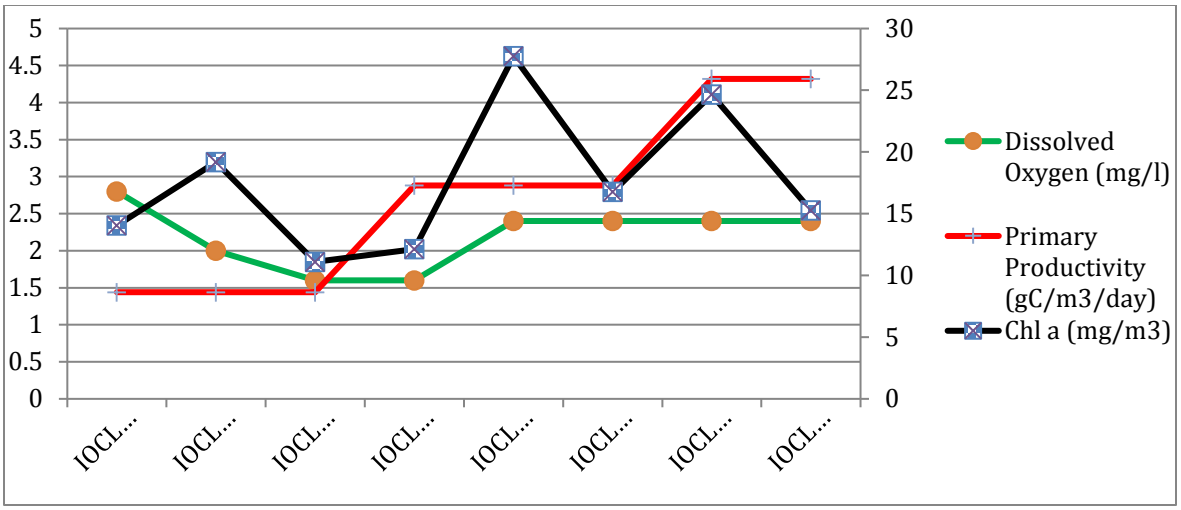


Fig. 26: Variation in biological parameters at the 8 sampling stations in and around coastal waters of Kochi during August 2016

In the case of chlorophyll and primary production (PP), contrary to the usual scenario where backwater stations exhibit high chlorophyll and PP, here the coastal stations had more chlorophyll and primary production. It was found that chlorophyll values did not closely follow the primary production pattern in the study area. Reason for the less chlorophyll content and PP in estuary could be due to the high turbidity preventing penetration of solar radiation for photosynthesis. Madhu et al. (2007) have also shown that the increase in the concentration of suspended particulate matter (SPM) due to the terrestrial and riverine run off also lead the reduction of sufficient light for photosynthesis by enhancing water column turbidity.

DO values were maximum in the station A1, probably being the station located far from the bar mouth and having maximum riverine influence among the backwater stations. In general, DO was high in coastal waters than backwater stations.

Table 8: Phytoplankton diversity and abundance in the 4 inshore stations in the bar mouth region of Kochi during August 2016

Genus / Species name	**A1** (cells /L)	**A2** (cells /L)	**A3** (cells /L)	**A4** (cells /L)
Bacillariophyceae				
Actinoptychus	34	40	0	
Amphipleura	0	40	0	
Amphora	34	0	0	0
Biddulphia	102	160	160	0
Chaetoceros	34	40	0	0
Coscinodiscus	612	880	320	1020

Cyclotella	0	40	0	0
Cymbella	34			
Diploneis	0	0	0	150
Gyrosigma				270
Leptocylindrus	714	560	440	2510
Melosira	612	960	1120	60
Navicula	0	40	40	300
Nitzchia	68	0	40	60
Pinnularia			80	30
Planktoniella				120
Pleurosigma	0	40	40	150
Podosira		40		
Pseudonitzchia	0	0	40	120
Roperia	0	0	0	30
Skeletonema	34			
Surirella	68	40	80	60
Thalassionema	0	40	80	150
Thalassiosira	34	0	0	60
Trachyneis	34			
Triceratium				180
Cynophyceae				
Anabaena	34	0	0	0
Aphanocapsa	34	0	0	
Oscillatoria	34	40	40	0
Chlorophyceae				
Botryococcus	34	0	0	0
Scenedesmus	34	0	0	150
Treubaria	34	40		
Euglenophyceae				
Euglenamorpha	0	40	0	
Phacus	340			
Dinophyceae				
Ceratium	306	160	560	240
Dinophysis	34			
Prorocentrum	68	120	200	0
Protoperidinium		160	40	120
Dictyochophyceae				

Dictyocha	0	0	40	

Fig. 27: Water sample analysis on board by Rithin Raj during the calibration exercise

Table 9: Phytoplankton diversity and abundance in the 4 coastal stations of Kochi during August 2016

Genus / Species name	**B1** (cells /L)	**B2** (cells /L)	**B3** (cells /L)	**B4** (cells /L)
Bacillariophyceae				
Amphora sp.	0	7	0	4
Asterionella sp.	0	13	11	23
Biddulphia spp.	262	335	243	416
Chaetoceros spp.	782	1715	3075	3122
Corethron sp.	0	2	0	0
Coscinodiscus sp.	1664	2272	2229	2718
Cyclotella sp.	0	0	5	1
Ditylum sp.	1	9	7	4

Eucampia sp.	0	13	11	14
Fragilaria sp.	0	21	9	12
Guinardia sp.	0	0	7	0
Gyrosigma sp.	0	0	0	4
Hemiaulus sp.	1	0	1	0
Lauderia sp.	25	0	4	0
Leptocylindrus sp.	7	14	12	7
Melosira sp.	28	31	11	16
Navicula sp.	0	14	1	15
Nitzschia sp.	0	7	0	0
Odontella spp.	249	463	263	374
Peudo-nitzschia	0	9	0	0
Planktionella	0	0	0	0
Pleurosigma sp.	0	0	12	0
Rhizosolenia sp.	2	7	26	9
Skeletonema sp.	30	30	102	45
Thalassionema sp.	0	15	2	0
Thalassiosira sp.	8	9	9	56
Triceratium sp.	0	7	0	0
Dinophyceae				
Ceratium sp.	14	0	77	0
Neoceratium	0	0	0	0
Prorocentrum sp.	9	16	19	18
Preperidinium sp.	0	0	0	0
Pyrophacus sp.	0	0	0	0
Dinophysis caudata	0	2	5	2
Gonyaulax sp.	0	0	0	0
Protoperidinum sp.	0	4	0	7
Chlorophyceae				
Pediastrum sp.	0	0	0	5

Highlights at a glance

- The comparison exercise showed that both the estuarine and coastal areas used for cage culture in Pizhala and Mandapam respectively are not experiencing any deterioration in water quality.
- The physico-chemical and biological data show that the parameters are within the range that does not disrupt the existence of communities in the waters selected for cage culture.

- Proper selection of location that permits regular flushing of the water in and out of the cages and the circulation and wind features prevent the build up of wastes and other nutrients in the vicinity of the cages.
- The occurrence of *Trichodesmium* blooms in the GoM waters were not due to the adverse effects of the culture. The bloom started far away from the area of the cages and drifted towards the cage area causing mass mortality of fishes.
- Modelling and remote sensing tools can be of immense use in the management of the culture cages. Grinson George and team are exploring this possibility.

6.4 Work done at SriLanka

Time series of SPATT bag sample collection in southern coast of Sri Lanka was carried out in 2016 at the two locations Tangalle (6.02N, 80.8E) and Mirissa (5.94N, 80.46E).

The following table indicates dates of collection of SPATT samples in 2016 at the two sites.

Tangalle	**Mirissa**
Feb 02, 2016	Feb. 02, 2016
Mar 22, 2016	Jun 29, 2016
May 24, 2016	Jul 28, 2016
Jun 28, 2016	
Jul 30, 2016	
Aug 09, 2016	
Aug 30, 2016	

All samples have been shipped in April, 2017 under frozen conditions to Dr. Rajdeep Roy, Goa, India for analysis.

7. Outreach activities

Grinson George

Fig. 28: Inauguration of Zoology Association of St. Xavier's college, Aluva, Kerala and delivering lecture on 'Climate change issues in the marine ecosystem'

Fig. 29: Invited lecture entitled "Application of remote sensing in marine fisheries" and interaction with audience at the International seminar on Coastal Biodiversity Assessment (COBIA 2017) at St. Gregarios college, Kottarakkara, Kerala

Ravidas Naik

Fig. 30: Lecture on Phytoplankton with reference to HAB and later having interaction with the students from Regina Mundi School, Goa

Fig. 31: Interaction with a) inspire school students, b) undergraduate students of Chowgule college and c) undergraduate students of Dempe College, Goa on HAB

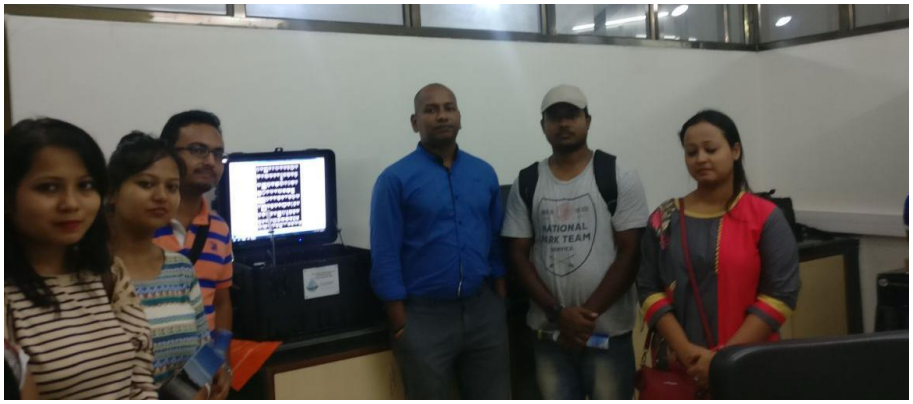


Fig. 32: Interaction with M.Tech students from Gauhati University of ASSAM

Fig. 33: Lecture, appreciation and prize distribution to students all at one platform of a socio-cultural group of coastal community.

Science from outreach activities

Small gaps arising in the project work were filled up by assigning dissertations to post graduate students of Goa University. The topics were as follows:

No.	Student	M.Sc.	Dissertation topic
1	Ms. Pooja Bisht	Marine Microbiology	Comparative studies on the protein content of diatom monocultures
2	Ms. Prisma D'Costa	Marine Microbiology	Seasonal variation in coliform abundance in an intertidal sandflat
3	Ms. Mary Fernandes	Marine Microbiology	Studies on lipid content of selected diatom monocultures
4	Ms. Minette Fernandes	Marine Microbiology	Carbohydrate concentration in active growth and decomposition stages of selected diatom monocultures
5	Ms. Sonali Narvekar	Marine Microbiology	Studies on the bacteria associated with diatom monocultures
6	Ms. Dakshata Phadte	Marine Microbiology	Preliminary studies on metal tolerance of bacteria isolated from intertidal sandflats

Nandini Menon

Fig. 34: Invited lecture entitled "Major threats to our oceans" at the International seminar on Coastal Biodiversity Assessment (COBIA 2017) at St. Gregarios college, Kottarakkara, Kerala

Fig. 35: Invited lecture entitled "Ocean colour for climate change studies" and interaction with audience at the International seminar on Coastal Biodiversity Assessment (COBIA 2017) at St. Gregarios college, Kottarakkara, Kerala

Science from outreach activities

The practice of assigning work components to M. Sc students for their dissertation was continued in 2016-17 also. The phytoplankton samples collected from coastal waters of Kochi were given to M. Sc students of St, Teresa's College, Kochi, Kerala to conduct taxonomic studies under the supervision of Dr. Nandini Menon.

Table 10: Dissertation topics given to M. Sc. students at Kochi

No.	Student	Master of Science	Dissertation topic
1	Merin Verghese	Zoology	Studies on bacteria associated with selected phytoplankton of Vembanad Lake

2	Nicy Augustine	Zoology	Phytoplankton ecology in the coastal waters off Cochin, south west coast of India

Rithin Raj

Fig. 36: Lecture on "Ocean resources and services" to Higher secondary students of Central Institute of Science, Technology and Management, Pazhayanoor, Kerala.

8. Collaboration or Networking activities undertaken in HABAQUA project

a. Fluorecence of Chlorophyll *a* samples were analysed at CIFT, Cochin using Turner 10 AU Fluorometer. (Dr. Muhamed Ashraf, Sr. Scientist - NANO member).
b. Central Marine and Fisheries Research Institute (CMFRI), Mandapam: facilities provided for the conduct of training programme, field sampling & analysis of samples (Dr. Amir Kumar Sanal, Dr. Abdul Nasar - NANO friends).
c. Central Marine and Fisheries Research Institute (CMFRI), Kochi: Support provided for sampling in the culture cages at Pizhala, Cochin (Dr. Imelda Joseph _ NANO friend)
d. Dept. of Marine Biology, Microbiology and Biochemistry, Cochin University of Science and Technology (CUSAT): for providing facilities for storage and analysis of samples and sharing the hired boat for field sampling (Dr. A. A. Mohamed Hatha - NANO friend).

9. Publications produced out of HABAQUA project

Alienor C. C. Vaz, Velitta Silveira, Priya M. D'Costa, Protein estimation using Folin assay from whole cell, culture filtrate and cell extracts of diatom cultures, at National Seminar on 'Biopolymers and Biosensors: What's New, What's Next?' organized by the Department of Microbiology, P.E.S's R.S.N. College of Arts and Science, Farmagudi, Ponda – Goa on 25th February 2017.

Alienor C. C. Vaz, Venicia Fernandes, Priya M. D'Costa, Protein content in selected phytoplankton species: comparison between active and decomposition phases of growth, at National Seminar on Advances in Sustainable Biotechnology, organized by Department of Biotechnology, St. Xavier's College, Mapusa – Goa during 27-28 January, 2017.

Grinson George, Muhammad Shafeeque, Nandini Menon, Shubha Sathyendranath and Trevor Platt. 2017. Inter-annual variability in Sardinella longiceps in response to ENSO event in the coastal waters of India. Presented at the International Symposium on Drivers of Dynamics of Small Pelagic Fish Resources, Victoria, BC, Canada from 6-11, March 2017

Jobin Clement, Priya M. D'Costa, Diversity of phytoplankton in a rocky, intertidal environment along the west coast of India, at National Seminar on 'Biopolymers and Biosensors: What's New, What's Next?' organized by the Department of Microbiology, P.E.S's R.S.N. College of Arts and Science, Farmagudi, Ponda – Goa on 25th February 2017.

Nandini Menon N, Syam Sankar, Smitha A, Grinson George, Shubha Sathyendranath and Trevor Platt. 2017. Application of phytoplankton biomass as an aid in management of marine resources of the southeastern Arabian Sea. Presented at the International Symposium on Drivers of Dynamics of Small Pelagic Fish Resources at Victoria, BC, Canada from 6-11, March 2017

Nashad M, N. Nandini Menon, C. Ajith Joseph, Lasse. H. Pettersson and N. R. Menon 2017. First report of *Leptocylindrus* sp. bloom in the coastal waters of Kerala (southeast Arabian Sea). J. Mar. Biol. Ass. India, 59 (1), 1-6. doi: 10.6024/jmbai.2017.59.1.1937-00

Rukma S. S. Kunkolienkar, Arti G. Naik, Priya M. D'Costa, Studies on bacteria associated with the toxic dinoflagellate - *Prorocentrum rhathymum*, at National Seminar on Advances in Sustainable Biotechnology, organized by Department of Biotechnology, St. Xavier's College, Mapusa – Goa during 27-28 January, 2017.

Sonvi D. Naik, Dipaswi H. Naik and Priya M. D'Costa, Exploring the sensitivity of raphidophytes to metals and organic pollutants, at National Seminar on Advances in Sustainable Biotechnology, organized by Department of Biotechnology, St. Xavier's College, Mapusa – Goa during 27-28 January, 2017.

Sweta Naik, Rima Desai, Priya M. D'Costa, Studies on the culturable bacteria associated with selected diatom monocultures, at National Seminar on Advances in Sustainable Biotechnology, organized by Department of Biotechnology, St. Xavier's College, Mapusa – Goa during 27-28 January, 2017.

Venicia Fernandes, Alienor Vaz, Priya M. D'Costa, Preliminary studies on the lipid content of selected marine phytoplankton isolates, at National Seminar on Advances in Sustainable Biotechnology, organized by Department of Biotechnology, St. Xavier's College, Mapusa – Goa during 27-28 January, 2017.

Venicia Fernandes, Priya M. D'Costa, Analysis of carbohydrates in dinoflagellate and raphidophyte strains isolated from the Eastern Arabian Sea, at National Seminar on 'Biopolymers and

Biosensors: What's New, What's Next?' organized by the Department of Microbiology, P.E.S's R.S.N. College of Arts and Science, Farmagudi, Ponda – Goa on 25th February 2017.

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