ALGAL BLOOMS AND ASSOCIATED BACTERIA ALONG THE SOUTHWEST COAST OF INDIA

Thesis submitted to

Cochin University of Science and Technology

in Partial Fulfilment of the Requirements for

the Award of the Degree of

Doctor of Philosophy

in

Marine Biology

Under the Faculty of Marine Sciences

By

ANIT M. THOMAS

DEPARTMENT OF MARINE BIOLOGY, MICROBIOLOGY AND BIOCHEMISTRY
SCHOOL OF MARINE SCIENCES
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
KOCHI –682 016, INDIA

April 2014

Algal Blooms and Associated Bacteria along the Southwest Coast of India

Ph.D. Thesis under the Faculty of Marine Sciences

Author

Anit M. Thomas
Research Scholar
Department of Marine Biology, Microbiology and Biochemistry
School of Marine Sciences
Cochin University of Science and Technology
Kochi – 682 016

Supervising Guide

Dr. A.V. Saramma
Professor
Department of Marine Biology, Microbiology and Biochemistry
School of Marine Sciences
Cochin University of Science and Technology
Kochi – 682 016

Department of Marine Biology, Microbiology and Biochemistry
School of Marine Sciences
Cochin University of Science and Technology
Kochi – 682 016

April 2014

Department of Marine Biology, Microbiology and Biochemistry
School of Marine Sciences
Cochin University of Science and Technology
Kochi – 682 016

Dr. A.V. Saramma
Professor

Certificate

This is to certify that the thesis entitled **"Algal Blooms and Associated Bacteria along the Southwest Coast of India"** is a bonafide record of original research work carried out by **Mr. Anit M. Thomas** under my supervision in the Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, in partial fulfilment of the requirements for the degree of **Doctor of Philosophy in Marine Biology.** All the relevant corrections and modifications suggested by the audience during the pre-synopsis seminar and recommended by the Doctoral Committee have been incorporated in this thesis and no part thereof has been presented before for the award of any degree, diploma or associateship in any other University or Institution.

Kochi - 682 016
April 2014

Prof. (Dr.) A.V.Saramma
(Supervising Guide)

Declaration

I hereby declare that the thesis entitled **"Algal Blooms and Associated Bacteria along the Southwest Coast of India"** is an authentic record of the original research work done by me under the supervision of **Dr. A.V. Saramma,** Professor, Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, in partial fulfilment of the requirements for the degree of **Doctor of Philosophy in Marine Biology** and no part thereof has been presented before for the award of any degree, diploma or associateship or any other similar title in any other University or Institution.

Kochi - 682 016 **Anit M. Thomas**
April 2014

To my beloved parents…

Acknowledgements

This doctoral thesis has been a dream of mine, which would not have seen fruition without the help and support of numerous kind people around me.

To begin with, I would like to express my deepest gratitude to my guide Dr. A.V. Saramma, Professor, Department of Marine Biology, Microbiology and Biochemistry, CUSAT, who is captivating, honest, and the true embodiment of a mentor. Her invaluable suggestions, constant and sincere encouragement, insightful discussions, critical evaluation and excellent suggestions for improvement have been most helpful in the rough journey that has brought me here. Words fail me in expressing my thankfulness for her unfailing moral support and blessings in all ways, academically and emotionally.

I am also deeply indebted to Dr. A.A. Mohamed Hatha, Head, Department of Marine Biology, Microbiology and Biochemistry, CUSAT, for facilitating the smooth conduct of my research, providing critical and diligent interventions to overcome tricky situations, and above all for the purposeful reassurances and impassioned exhortations given during the course of my work.

This work would not have been possible without the substantial financial support provided in the form of Research Fellowship for the project entitled 'Monitoring and Surveillance of Harmful Algal Blooms' from the Centre for Marine Living Resources and Ecology (CMLRE), Ministry of Earth Sciences, Government of India, Kochi. I express my profound sense of gratitude to Dr. V.N. Sanjeevan, Former Director, CMLRE, for his benevolent support during this period. I also thank Shri. P. Madeswaran, Director, CMLRE, for his support. Technical support for the open-ocean cruises on FORV Sagar Sampada is also acknowledged. Let me take this opportunity to thank the support staff of CMLRE and FORV Sagar Sampada for all the help they have rendered.

I would like to express my sincere thanks to Prof. (Dr.) K. Sajan, Dean, Faculty of Marine Sciences, CUSAT, for his earnest support of my work.

I gratefully acknowledge the role of the members of the Doctoral Research Committee, Dr. Rosamma Philip, Prof. Aneykutty Joseph, and Dr. S. Bijoy Nandan, for their periodic review of my work, constructive criticism, wise counsel and encouragement spanning the duration of my research. I solemnly express my sincere thanks to Dr. Babu Philip, Dr. K.J. Joseph, and Dr. C.K. Radhakrishnan (Retired Professors), for their precious words of wisdom.

I owe special thanks to Prof. (Dr.) K.T. Damodaran, former Dean and Director, School of Marine Sciences, CUSAT, for his support and encouraging words.

I would also like to offer my thanks to Dr. Anilkumar Vijayan, Scientist C, CMLRE, for his active support.

This work would not have been enjoyable without the company of my ever-enterprising colleagues, Dr. M.G. Sanilkumar and Mr. K.R. Abhilash. Their cooperation and support is recognized emphatically.

The valuable help provided to me by my seniors Dr. Rejil T., Dr. Harikrishnan E., and Dr. Padmakumar K.B. is also deeply appreciated.

The tireless efforts of Ms. Vijayalakshmy K.C., Mr. Abhijith Muralidharan, Mr. Akhil P. John and Mr. Shyam Kumar S., my colleagues in the research project, have made the research a whole lot easier. I thank them also for bearing with my tantrums during testing times.

I express my gratitude towards Dr. Neil Scolastine Correya, Dr. Prabhakaran M.P., Dr. Mujeeb Rahman, Dr. Nithin Viswambaran, Dr. Johnson Zacharia, Dr. Jayesh P., Dr. Prajith K.K., Mr. Anil Kumar P.R., Mr. Harisankar H.S., Mr. Naveen Sathyan, Mr. Afsal V.V., Mr. Akhil P.S., Mr. Vishnu Prasad J., Mr. Akhilesh Vijay, Mr. Sanu V.F., Mr. Vincent P.D., Mr. Jabir T., Mr.Jayanath Gopi.,

Mr.Solly Solomon., Dr. Manjusha K., Dr. Sini P.J., Dr. Swapna P. Antony, Dr. Reema Kuriakose, Ms. Neenamol S., Ms. Lekshmi S., Ms. Jasmin K.A., Ms. Jini Jacob, Ms. Nifty John, Ms. Deepthi Augustine, Ms. Divya P.S., Ms. Neema Job, Ms. Wilsy Wilson, Ms. Deborah Alexander, Ms. Divya T. Babu, Ms. Emilda Rosmine, Ms. Chaithanya E.R., Ms. Sreedevi O.K., Ms. Varsha M.S., Ms. Bhavya K. and numerous others from my laboratory who have contributed, in their own terms, to the successful completion of my work and this thesis.

I thank Mr. Manuel P.J., Librarian, and other staff of the School of Marine Sciences Library, CUSAT, for their valuable cooperation. I am thankful to members of the administrative and supportive staff of the Department of Marine Biology, Microbiology and Biochemistry, CUSAT, for their help and cooperation. I record my gratitude to Mr. Stephen V.K., Mr. Balan V.K., Mr. Abdul Nazar M., Mr. Sajeeven T.K. and Mr. Santhosh S. for their timely help and support.

This journey would be meaningless without the two pillars of my life, my beloved parents Mr. Thomas V.M. and Mrs. Saramma Thomas. I express my deepest love and affection for them and my sister, my brother-in-law and all my family members for everything they have done for me. They have sacrificed a great deal of time, energy and material for me and have constantly stood by me, through thick and thin. Having endured my various emotions, no one understands me better than my family. I shall be eternally grateful to them for believing me and keeping me in their prayers constantly.

Above all, I thank the Almighty God for making my life a blessed one.

Anit M. Thomas

Contents

Chapter 1

General Introduction ---------- 01 - 08

Chapter 2

Materials and Methods ---------- 09 - 22

2.1 Coastal and estuarine stations along the southwest coast of India ------ 09
2.1.1 Stations ---------- 10
2.1.2 Hydrographic parameters ---------- 11
2.1.2.1 Temperature ---------- 11
2.1.2.2 Salinity ---------- 11
2.1.2.3 pH ---------- 11
2.1.2.4 Dissolved Oxygen ---------- 12
2.1.2.5 Nutrient analysis ---------- 12
2.1.2.6 Primary Productivity ---------- 12
2.1.2.7 Estimation of Pigments ---------- 14
2.1.2.7.1 Estimation of Chlorophyll ---------- 14
2.1.2.7.2 Estimation of Carotenoids ---------- 15
2.1.2.8 Phytoplankton Analysis ---------- 16
2.1.3 Sample preparation for Environmental Scanning Electron Microscopy (ESEM) ---------- 16
2.1.4 Isolation of bloom associated bacteria ---------- 17
2.1.4.1 Sampling, bacterial isolation and identification ---------- 17
2.1.4.2 Tests used for the identification of bacteria ---------- 17
2.1.4.2.1 Gram staining ---------- 17
2.1.3.4.2 Spore staining ---------- 18
2.1.4.2.3 Mannitol Motility Test ---------- 18
2.1.4.2.4 Marine Oxidation Fermentation [MOF] Test ---------- 19
2.1.4.2.5 Catalase Test ---------- 19
2.1.4.2.6 Oxidase Test ---------- 19
2.1.4.2.7 O/129 Test ---------- 20
2.1.4.2.8 Antibiotic (Kirby-Bauer) Test ---------- 20
2.1.4.3 Screening for hydrolytic enzyme production of bacteria ----- 20
2.1.4.3.1 Amylase ---------- 20
2.1.4.3.2 Lipase ---------- 21
2.1.4.3.3 Protease ---------- 21
2.1.4.3.4 Cellulase ---------- 21
2.1.4.3.5 Ligninase ---------- 21
2.1.4.3.6 Phosphatase ---------- 22
2.1.4.3.7 Alginase ---------- 22
2.2 Statistical Analysis ---------- 22

Chapter 3

Hydrography -- 23 - 38

3.1 Seasonal and spatial variation in the physico-chemical parameters in the coastal and estuarine stations along the southwest coast of India -- 23
3.1.1 Temperature-- 24
3.1.2 Salinity -- 25
3.1.3 pH-- 26
3.1.4 Nutrients-- 27
3.1.4.1 Nitrate -- 27
3.1.4.2 Nitrite-- 29
3.1.4.3 Silicate -- 30
3.1.4.4 Phosphate -- 32
3.1.5 Dissolved Oxygen-- 33
3.1.6 Primary Production -- 34
3.2 Statistical Analysis -- 36

Chapter 4

Distribution of Planktonic Microalgae along the Kerala Coast--39 - 101

4.1 Introduction-- 39
4.2 Review of Literature -- 44
4.2.1 Distribution of microalgae -- 44
4.2.2 Physico-chemical parameters -- 49
4.2.3 Primary production-- 52
4.3 Result -- 55
4.3.1 Qualitative and quantitative distribution of planktonic microalgae -----55
4.3.1.1 Station 1. Azheekode -- 55
4.3.1.2 Station 2. Balathuruth-- 58
4.3.1.3 Station 3. Kodikkal-- 61
4.3.1.4 Station 4. Mahe -- 64
4.3.1.5 Station 5. Puthiyangadi -- 67
4.3.1.6 Station 6. Thykadapuram-- 70
4.3.2 Mean annual variation in standing crop------------------------------ 73
4.3.3 Pigment composition-- 74
4.3.3.1 Chlorophyll *a*-- 74
4.3.3.2 Chlorophyll *b*-- 76
4.3.3.3 Chlorophyll *c*-- 77
4.3.3.4 Carotenoids-- 78
4.3.3.5 Mean spatial variation in pigments: 2009-2010-------------------80
4.3.3.6 Mean spatial variation in pigments: 2010-2011-------------------80
4.3.4 Spatial variation of standing crop, chlorophyll *a* and Net primary production-- 81

4.3.5 Statistical Analysis ---------- 82
4.4 Discussion ---------- 84
4.4.1 Qualitative and quantitative distribution of planktonic microalgae ---------- 84
4.4.2 Pigment composition ---------- 94
4.4.2.1 Chlorophyll *a* ---------- 94
4.4.2.2 Chlorophyll *b* ---------- 96
4.4.2.3 Chlorophyll *c* ---------- 96
4.4.2.4 Carotenoids ---------- 97
4.4.3 Mean spatial variation in standing crop, Chl *a* and Net PP ---------- 97
4.4.4 Standing crop and physical parameters ---------- 98
4.4.5 Mean spatial variation in nutrients and chlorophyll *a* ---------- 99

Chapter 5
***Algal Blooms* ---------- 103 - 145**
5.1 Introduction ---------- 103
5.2 Review of Literature ---------- 110
5.2.1 Algal Blooms: International status ---------- 110
5.2.2 Algal Bloom events along the Indian waters ---------- 112
5.2.3 Factors influencing algal blooms ---------- 115
5.2.4 Monitoring of algal blooms in Indian EEZ ---------- 116
5.3 *Prymnesium parvum* N. Carter bloom ---------- 116
5.3.1 Result ---------- 118
5.3.1.1 Standing crop and pigment composition ---------- 118
5.3.1.2 Physico-chemical parameters ---------- 118
5.3.2 Discussion ---------- 120
5.4 *Proboscia alata* (Brightwell) Sandström bloom ---------- 126
5.4.1 Result ---------- 128
5.4.1.1 Standing crop and pigment composition ---------- 128
5.4.1.2 Physico-chemical parameters ---------- 130
5.4.2 Discussion ---------- 131
5.5 *Chattonella marina* (Subrahmanyan) Hara et Chihara bloom ---------- 135
5.5.1 Result ---------- 136
5.5.1.1 Standing crop and pigment composition ---------- 136
5.5.1.2 Physico-chemical parameters ---------- 137
5.5.2 Discussion ---------- 139

Chapter 6
***Bacteria Associated with Algal Blooms and Hydrolytic Enzyme Production* ---------- 147 - 169**
6.1 Introduction ---------- 147
6.2 Review of Literature ---------- 150
6.2.1 Bacteria associated with bloom-forming microalgae ---------- 150
6.2.2 Effect of bacteria on bloom-forming microalgae ---------- 152

6.3 Result ---154
6.3.1 Bacteria associated with *Prymnesium parvum* N. Carter bloom --154
6.3.1.1 Comparative estimation of Total Heterotrophic Bacterial count----- 154
6.3.1.2 Generic composition of bloom associated bacteria ------------------- 154
6.3.1.3 Generic composition of bacteria from the non-bloom sample ------- 155
6.3.1.4 Hydrolytic enzyme production of bloom associated bacteria -------- 156
6.3.1.5 Hydrolytic enzyme production of bacteria from the non-bloom sample-- 156
6.3.2 Bacteria associated with *Proboscia alata* (Brightwell) Sandström bloom--- 157
6.3.2.1 Estimation of Total Heterotrophic Bacterial count ------------------ 157
6.3.2.2 Genera wise distribution of bloom associated bacteria ------------- 158
6.3.2.3 Genera wise distribution of bacteria from the non-bloom sample --- 159
6.3.2.4 Hydrolytic enzyme production of bloom associated bacteria ------ 160
6.3.2.5 Hydrolytic enzyme production of bacteria from the non-bloom sample --- 161
6.3.3 Bacteria associated with *Chattonella marina* (Subrahmanyan) Hara et Chihara bloom --- 162
6.3.3.1 Estimation of Total Heterotrophic Bacterial count ------------- 162
6.3.3.2 Genera wise distribution of bloom associated bacteria --------- 162
6.3.3.3 Genera wise distribution of bacteria from the non-bloom sample --- 163
6.3.3.4 Hydrolytic enzyme production of bloom associated bacteria ------- 164
6.3.3.5 Hydrolytic enzyme production of bacteria from the non-bloom sample --- 164
6.4 Discussion ---165

Chapter 7

Summary and Conclusion **----------------------------------171 - 176**

References **---177 - 235**

Appendices **--237 - 253**

Appendix – I Plates
Plate 1 Class: Bacillariophyceae (Centric Diatoms) ----------------------------238
Plate 2 Class: Bacillariophyceae (Pennate Diatoms) ---------------------------240
Plate 3 Class: Dinophyceae ---242
Plate 4 Class: Chlorophyceae ---243
Appendix – II Key used for the identification of Heterotrophic Bacteria---------- 244
Appendix– III Table 20 Media / Reagents / Stains --------------------------------245
Appendix – IV-X Tables 21-37 Data on physico-chemical variables, standing crop and pigment concentrations -------------- 247 - 253

List of Publications

List of Abbreviations

ANOVA	Analysis of Variance
ASP	Amnesic Shellfish Poisoning
AZP	Azaspiracid Poisoning
BAMPI	Ballast Water Management Programme-India
BOD	Biological Oxygen Demand
C: N: P	Ratio of Carbon, Nitrogen and Phosphorus
Carot	Carotenoids
cellsL^{-1}	Cells per litre
CFB	Cytophaga-Flavobacter-Bacteroides
CFP	Ciguatera Fish Poisoning
cfu/ml	colony forming units per millilitre
Chl *a*	Chlorophyll *a*
Chl *b*	Chlorophyll *b*
Chl *c*	Chlorophyll *c*
cm	centimetre
DB	Dark Bottle
DMS	Dimethylsulfide
DMSP	Dimethylsulfoniopropionate
DO	Dissolved Oxygen
DSP	Diarrhetic Shellfish Poisoning
E	East
EEZ	Exclusive Economic Zone
ESEM	Environmental Scanning Electron Microscope
et al.	*et alii* (Latin: 'and others')
gC/m^2/day	gram Carbon per square metre per day
gC/m^2/yr	gram Carbon per square metre per year
gC/m^3/day	gram Carbon per cubic metre per day
GEOHAB	Global Ecology and Oceanography of Harmful Algal Blooms
GF	Glass Fibre /Filter
H'	Shannon-Wiener's diversity index
H_2O_2	Hydrogen Peroxide
HAB	Harmful Algal Bloom

IB	Initial Bottle
IIOE	International Indian Ocean Expedition
INCOIS	Indian National Centre for Ocean Information Services
IOC	Intergovernmental Oceanographic Commission
IOC-FAO	Intergovernmental Oceanographic Commission - Food and ~ Agriculture Organization of the United Nations
IOC-HAB	Intergovernmental Oceanographic Commission on ~ Harmful Algal Blooms
Km	Kilometre
KORHAB	Korean Harmful Algal Bloom Research Group
L	Litre
Lat.	Latitude
LB	Light Bottle
Long.	Longitude
M	Molar
m	metre
mg	milligram
mg/m^3	milligram per cubic metre
mg/ml	milligram per millilitre
mgC/L/day	milligram Carbon per litre per day
mgC/L/hr	milligram Carbon per litre per hour
$MgCO_3$	Magnesium Carbonate
mgL^{-1}	milligram per litre
ml	millilitre
mm	millimetre
MODIS	Moderate Resolution Imaging Spectroradiometer
MOF	Marine Oxidation Fermentation
MON	Monsoon
N	North
N: P	Ratio of Nitrogen and Phosphorus
NE	Northeast
Net PP	Net Primary Production
NH_3	Ammonia
nm	nanometre

NO_2-N	Nitrite - Nitrogen
NO_3-N	Nitrate - Nitrogen
NOWPAP	Northwest Pacific Action Plan
NSP	Neurotoxic Shellfish Poisoning
O_2^-	superoxide anion radicals
OH^-	hydroxyl radicals
PBBS	Port Baseline Biological Survey
PO_4-P	Phosphate - Phosphorus
POM	Post-monsoon
PQ	Photosynthetic Quotient
PRIMER	Plymouth Routines in Multivariate Ecological Research
PRM	Pre-monsoon
PSP	Paralytic Shellfish Poisoning
psu	practical salinity unit
ROS	Reactive Oxygen Species
rpm	revolutions per minute
S	South
SD	Standard Deviation
Si: N	Ratio of Silicon and Nitrogen
SiO_4- Si	Silicate - Silicon
sp.	Species
SPSS	Statistical Package for the Social Sciences
SW	Southwest
Temp.	Temperature
THB	Total Heterotrophic Bacteria
UV-Vis Spectrophotometer	Ultraviolet-Visible Spectrophotometer
W	West
XBT	Indian Expendable Bathythermographic
μgL^{-1}	microgram per litre
$\mu molL^{-1}$	micromoles per litre
$^{\circ}C$	degree Celsius
µm	micrometre
^{14}C	Carbon-14

.....ꙮ.....

List of Publications

1) **Anit M. Thomas**, Sanilkumar M.G., Vijayalakshmy K.C., Mohamed Hatha A.A. and Saramma A.V. *Proboscia alata* (Brightwell) Sandström bloom in the coastal waters off Bekal, Southwest India.
(Accepted for publication in *Current Science*)

2) **Anit M. Thomas**, Vijayalakshmi K.C., Akhil P. John, Abhijith Muralidharan, Sanilkumar M.G., Mohamed Hatha A.A. and Saramma A.V. Occurrence of algal bloom dominated by *Fragilariopsis oceanica* from the coastal waters of Southwest India. (Communicated)

3) **Anit M. Thomas**, Sanilkumar M.G., Vijayalakshmy K.C., Sanjeevan V.N., Mohamed Hatha A.A. and Saramma A.V. Dynamic changes in bacterial population and corresponding exoenzyme activity in response to a tropical phytoplankton bloom *Chatonella marina*. (Communicated)

4) Sanilkumar M.G., **Anit M. Thomas**, Vijayalakshmy K.C., Mohamed Hatha A.A. and Saramma A.V. (2012) *Chattonella marina* bloom in the coastal Sea off Mahe, Southwest India. *Current Science* **103(6),** 624-626.

5) Sanilkumar M.G., **Anit M. Thomas**, Shyamkumar S., Rosamma Philip, Sanjeevan V.N. and Saramma A.V. (2009) First report of *Protoperidinium* bloom from Indian waters. *Harmful Algae News* **39**.

.....ꟈꟈ.....

Chapter 1

General Introduction

Earth has an ocean covering approximately 71% of the planet's surface and containing 97% of the planet's water. It influences all known life by forming part of the carbon cycle, and influencing climate and weather patterns. It is inhabited by countless species of living organisms, from tiny phytoplankton to large mammals. The biota of the oceans has huge socioeconomic value through food production, recreation, nutrient recycling, and gas regulation. A major portion of the organic carbon for Earth's petroleum reserves, especially from shallow continental shelves is provided by fossilized organic remnants of phytoplankton blooms.

Phytoplankton in the ocean play a key role as primary producers in the marine ecosystem. Also known as microalgae, more than 5000 species that have been identified form the base of marine food web especially in the shelf seas by producing organic substances. As they form the base of the food web, the evolutionary trajectories of the microalgae have also governed the evolution of organisms at higher trophic levels. Microalgal communities in the ocean comprise many different taxonomic groups; diatoms (Bacillariophyceae), dinoflagellates (Dinophyceae), blue green algae (Cyanophyceae), green microalgae (Chlorophyceae), silicoflagellates, coccolithophores and the very small

nanoplankters, which together determine primary production and various trophic level interactions. In this respect, the quantification of phytoplankton biomass and community composition is important for understanding the structure and dynamics of marine ecosystems.

The rate at which energy is stored up by microalgae determines the basic primary productivity of the ecosystem. Coastal and estuarine environments are the most productive ecological systems on earth. The stability of the oceanic photosynthetic activity by microalgae, which contributes about 80% of the atmospheric oxygen, is dependent on the physical circulation of the upper ocean. The proliferation of phytoplankton, its intensity, and spatial and temporal distribution in the ocean is determined by the depth of the upper mixed layer and the vertical fluxes of nutrients. Most of the planktonic microalgae are distributed through the euphotic zone, which can be influenced by temperature, light intensity, nutrient limitation, freshwater influence, and tidal activity.

Microalgal growth and abundance are primarily regulated by both abiotic and biotic interactions (Armstrong, 1994). Microalgae are particularly good indicators of climate change in the marine environment and any change in the physico-chemical parameters can be indicated by the distribution of microalgae because they are free floating and can respond easily to those changes. Thus, the information obtained from phytoplankton community structure can significantly contribute to assessing levels of climate change and the subsequent effect on the structure and functions of the ecosystem.

High fertility and large phytoplankton populations in estuarine environments have been frequent due to nutrient enrichment from river water,

organic pollution, and by the entrainment of coastal waters in a subsurface counter-current, which transport nutrients into the estuary. The ecological health of estuaries responds to nutrient enrichment in a positive or negative way, thereby influencing microalgal distribution.

Microalgae usually have a fairly recognizable annual cycle of growth. Sometimes this synchrony is disrupted by any drastic change in the above mentioned abiotic factors and leads to the explosive growth of some species. Under favourable conditions, this leads to the enormous concentration (millions of cells per litre) and often causing discolouration of the surface of the sea. These natural phenomena are termed as Harmful Algal Blooms (HABs). An algal bloom goes through sequential developmental phases such as initiation, growth, maintenance and termination.

The International Council for the Exploration of Seas (1984) defined algal blooms as '*those which are noticeable, particularly to the general public, directly or indirectly through their effects such as visible discolouration of the water, foam production, fish or invertebrate mortality or toxicity to humans*'. Consequences of blooms have been noticed from the positive and negative points of view. The positive aspect of algal blooms is that it may be beneficial to fisheries (D'Silva *et al*., 2012), whereas the negative impact of algal blooms includes mass mortalities of wild and farmed fish, shellfish, human illness, death of marine mammals, sea birds and alteration of marine habitats or trophic structure through shading, overgrowth or adverse effect on life-cycle of fish and other marine organisms.

Algae that can cause a variety of deleterious effects, including oxygen deficiency as mentioned above, on aquatic ecosystems are termed

"Harmful Algae". Of the several thousand planktonic algae species, only about 300 species are known to cause water discolouration. Out of these, only around 80 species are known to produce harmful toxic effects (Hallegraeff, 2003).

HAB species can be broadly classified into two groups – the high biomass producers, and the toxin producers. The high biomass producers affect fish, shellfish, and marine mammals of shallow bays or seas by inducing physical damage by virtue of gill-clogging or oxygen depletion during decay of the dead cells. The toxin producers may cause haemolytic, hepatotoxic, osmoregulatory and other unspecified toxic effects to cause mortality in animals that ingest them. Toxin accumulation in mammals and other higher levels in food chain is possible upon consumption of such contaminated shellfish whose tissues may have accumulated the toxin after digestion. The major poisoning syndromes include Paralytic Shellfish Poisoning (PSP), Ciguatera Fish Poisoning (CFP), Diarrhetic Shellfish Poisoning (DSP), Neurotoxic Shellfish Poisoning (NSP), Amnesic Shellfish Poisoning (ASP) and Azaspiracid Poisoning (AZP). Some species have characteristics of both groups.

Harmful algal toxins are secondary metabolites released in the dissolved state into sea water, that are primarily involved in allelopathy to outcompete other phytoplankton species for resources, like nutrients under limiting conditions (Graneli and Hansen, 2006). They are associated with processes such as nitrogen storage, nucleic acid biosynthesis, bioluminescence, bacterial endosymbiosis, and pheromones inducing sexuality during bloom decline as well as serving as grazer deterrents. These metabolites are only coincidentally toxic. Temperature, salinity and nutrient status influence the toxin content of

the cell, while its production is stimulated by the presence of grazers (Bates, 1998).

Non-toxic but prolonged algal blooms can have other impact on coastal ecosystem, such as reduction of light penetration to submerged aquatic vegetation which nourish and shelter fingerlings of commercially important fish and shellfish.

Despite being a natural event, human related phenomena like 'anthropogenic eutrophication' and transport of algal species by ballast water, apart from natural mechanisms of species dispersal, has led to increased reports of occurrences of harmful algal blooms over the past decades. Economic loss on account of blooms, type of resources affected, and the number of toxins and toxic species have increased dramatically throughout the world (Anderson, 1989). Anthropogenic eutrophication of the highly susceptible coastal waters occurs due to pollution on account of a dramatic increase in aquaculture activities, and ever-increasing quantities of industrial, agricultural and sewage effluents, which stimulate HABs. Consequently, more observers are involved in monitoring of HABs leading to increased awareness and advancement of monitoring systems.

The occurrence and spreading of HABs can be prevented by reducing the inflow of industrial, agricultural and sewage effluents into coastal waters. Proper monitoring for evidences of HAB events and detection of toxins in contaminated organisms can be done to mitigate its effects. Physical and chemical methods are employed to control HAB's by directly targeting the bloom cells, though they can produce unwanted side effects on the surrounding ecosystem. Biological control of HABs through application of

agents like bacteria and viruses is gaining ground. Algal blooms usually have an ordered and structured associated bacterial community rather than a random assemblage of species from the marine bacterial community. This indicates a probable role this community plays especially in bloom termination.

The interactions between algae and bacteria are an important aspect of the microbial loop. Algae represent the primary source of organic nutrients for heterotrophic microbes in the mixed layer of the ocean and the abundance of the bacteria has shown a positive correlation with algal concentrations (Kjelleberg *et al*., 1993). Their physical relationship, which may be mutually beneficial, extends from intracellular to extracellular. By providing a habitat to the intracellular bacteria, the algae benefit from the nutrients synthesized by them (Seibold *et al*., 2001). Bacteria colonizing the surface give them access to nutrients, protection against toxins, and protection against predators (Dang and Lovell, 2000).

Bacteria adhering to algal cells are potentially important players in the dynamics of HABs and this action may be of either direct or indirect mode. In accordance with the variations in the abundance and type of organic matter during an algal bloom, qualitative and quantitative changes in the associated bacteria may occur (Doucette, 1995) that ultimately reflects in the inhibitory and/or stimulatory effect on the organisms involved. Algae and bacteria may establish commensalism which, under nutrient stress, shift to competition and finally lead to killing and lysis of algae by bacteria (Mayali and Azam, 2004). The bacterial flora associated with the HABs may change at the time of bloom declination. Approximately 30% of algicidal bacteria attack their target algal

species through direct contact, and the remaining exhibit an indirect mode of attack by dissolved lytic agents.

The dissolved lytic agents mainly include biologically active compounds, exopolymers (Decho, 1990) which increase the tendency of cells to flocculate and its sticky nature may enhance the sinking rate and degradation of decaying blooms. Serine protease produced by algicidal bacteria have shown lytic activity against the diatom *Skeletonema costatum* (Lee *et al*., 2000) lending support to the theory that algicidal bacteria kill their prey using extracellular proteases.

It is essential to understand how the distribution and composition of microalgae as well as dynamics of HABs in economically important shelf seas relate to the particular physico-chemical and biological properties of the water column in which they live. In view of the importance of southwest coast of India, which is considered as one of the most biologically productive areas in the world, regular monitoring of distribution and abundance of microalgae is important. The present work is concentrated on the estuarine and coastal open sea stations along the southwest coast of India. In order to get further insights into the abiotic factors governing bloom dynamics, the physico-chemical parameters that regulated three particular bloom events during this period were studied. Bearing in mind the role of bacterial fauna associated with algal blooms as a biological factor in regulating its dynamics, isolation of bacteria associated with the algal blooms, their identification, enumeration, and ability to produce extracellular enzymes have been duly incorporated into this study.

Objectives of the study:-

- To make an in-depth study on the temporal and spatial variation in the distribution of microalgae along the southwest coast of India.
- Monitoring and surveillance of algal blooms.
- To isolate and to identify bacterial strains associated with algal blooms.
- To study the potential of associated bacteria to produce extracellular enzymes and to study their probable role in algal bloom dynamics.

The thesis comprises of seven chapters. The first chapter gives a general introduction to microalgae with special reference to algal blooms and the role of associated bacteria. In the second chapter, methodology adopted for studying planktonic microalgae, algal blooms and associated bacteria is described. Third chapter mainly comprises the analysis of physico-chemical variables, such as temperature, salinity, pH, nitrate, nitrite, silicate, phosphate, dissolved oxygen and primary productivity, which influence the distribution and abundance of microalgal species. The fluctuations of these factors for a period of two years, from pre-monsoon 2009-10 through post-monsoon 2010-11, in the estuarine and coastal monitoring stations along the southwest coast of India are described in this chapter. The distribution of microalgae is described in the fourth chapter. The fifth chapter focusses on the investigations of three algal bloom events observed along the southwest coast of India during the study period. The sixth chapter projects the determination of the bloom associated bacterial community and its ability to produce extracellular enzymes. The major findings of the study are summarised in the seventh chapter.

.....ഇൽ.....

Chapter 2

Materials and Methods

2.1 Coastal and estuarine stations along the southwest coast of India
2.2 Statistical Analysis

2.1 Coastal and estuarine stations along the southwest coast of India

Coastal and estuarine samplings on seasonal basis (pre-monsoon, monsoon and post-monsoon) from selected stations (Map 1) and continuous monitoring for algal blooms, along the southwest coast of India were carried out for a period of two years (2009-2011). The selection of stations was made on the basis of the previous occurrence of algal blooms. The details of the stations are given in Table 1. Among the stations, three are coastal (Azheekode, Kodikkal and Puthiyangadi) and the rest are estuarine (Balathuruth, Mahe and Thykadapuram). Sampling has been carried out for the study of bacteria associated with algal blooms, analysis of qualitative and quantitative distribution of microalgae and hydrographical variables. The terms PRM, MON and POM are used to represent pre-monsoon, monsoon and post-monsoon, respectively.

2.1.1 Stations

Map1
Local stations along the Kerala coast

Table 1 Coastal and estuarine station locations.

Stations	Name of the Stations	Latitude	Longitude
1	Azheekode (Thrissur)	10° 11' 02" N	76° 09' 22" E
2	Balathuruth (Kozhikode) -**E***	11° 07' 50" N	75° 49' 57" E
3	Kodikkal (Kozhikode)	11° 28' 43" N	75° 36' 10" E
4	Mahe -**E***	11° 42' 18" N	75° 32' 36" E
5	Puthiyangadi (Kannur)	12° 01' 11" N	75° 21' 89" E
6	Thykadapuram (Kasaragod) -**E***	12° 12' 39" N	75° 07' 33"E

E* stands for estuarine stations and the others are coastal stations.

Besides regular sampling from these stations, sampling has been carried out from Bekalam* (Kasaragod) (Lat. 12° 38' 02" N & Long. 75° 04' 31" E) during a bloom event from 10th to 12th October 2009.

2.1.2 Hydrographic parameters.

Hydrographic parameters such as temperature, salinity and pH were measured soon after the collection of surface water sample.

2.1.2.1 Temperature

Temperature measurement in °C (degree Celsius) was done by using a precision mercury thermometer with an accuracy ± 0.01°C.

2.1.2.2 Salinity

Salinity in psu (practical salinity unit) was measured using a hand held refractometer (Erma- Japan).

2.1.2.3 pH

pH measurement was done using a portable pH meter (Eutech eco Tester pH2) with an accuracy ± 0.01.

2.1.2.4 Dissolved Oxygen

Dissolved oxygen was determined by Winkler's (1888) method, for which water sample was collected in 50 ml ground stoppered Biological Oxygen Demand (BOD) bottle, fixed with Winkler A (Manganese Sulphate) and Winkler B (alkaline Potassium Iodide) solution.

2.1.2.5 Nutrient analysis

The quantitative estimation of major nutrients like nitrate, nitrite, silicate and phosphate were carried out in the laboratory according to the methods of Strickland and Parsons (1972) and Fischer and Zhang (2006).

2.1.2.6 Primary Productivity

Light and dark bottle method (Gaarder and Gran, 1927) was used for the estimation of primary productivity. For the analysis of primary production rate, a set of three BOD bottles of 50 ml capacity was used, in which one was taken as initial bottle or control bottle (IB), the second one as light bottle (LB) and the third one as dark bottle (DB). Surface water sample was collected by using a clean bucket and the sample was siphoned into the bottle by using a clean polythene tube. One end of the polythene tube was fitted with bolting silk, which measures about 200 µm, in order to eliminate the presence of zooplankters in the siphoned sample, which will interfere with the oxygen content in the experiment bottles. Care was taken to avoid the agitation of water. While filling the samples into the bottles, it was made sure that the polythene tube touched the bottom of the bottles and all the bottles were properly stoppered without trapping air bubbles inside the bottles. All these measures were taken to avoid the formation of air bubbles, which interfere with the oxygen content of the sample.

The initial bottle sample was immediately fixed with 0.5 ml of manganese sulphate (Winkler A) and 0.5 ml of alkaline potassium iodide (Winkler B). The dark bottle was wrapped with aluminum foil and kept in a black polythene bag in order to avoid the interference of sunlight. Both dark and light bottles were incubated in a transparent acrylic chamber for three hours. After three hour incubation the light and dark bottles were fixed with the Winkler reagents and the oxygen content of all the three bottles (LB, DB and IB) was determined by Winkler's chemical titration method.

The resultant values obtained were generalized for day/hours and the primary productivity was calculated by using the calculations described below. 1.25 was taken as the photosynthetic quotient (PQ) (Gaarder and Gran, 1927).

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_2 content of control bottle ----- B

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

The period of incubation was three hours, then

Gross production (mgC/L/hr) = A × 0.375/PQ × 3 ------------------------ D

Net Production (mgC/L/hr) = B × 0.375/PQ × 3 -------------------------- E

Gross or net production (mgC/L/day) = D or E × 12 -------------------- F

Gross or net production (gC/m^3/day) = F × 1000 ×1000

2.1.2.7 Estimation of Pigments

2.1.2.7.1 Estimation of Chlorophyll

For the chlorophyll estimation, surface water sample was collected by using a clean plastic bucket and transferred into a one litre clean black plastic can and stored in a frozen container until further analysis was carried out in the laboratory. The pigments chlorophyll *a, b, c* and carotenoids were extracted by using 90% acetone.

The surface water sample, free of zooplankters was filtered through GF/C glass filter paper having a pore size of 0.45 µm. Before filtering, 1 ml of 1% magnesium carbonate ($MgCO_3$) was added into the glass filter paper in order to avoid the development of acidity and thereby the gradual degradation of the pigment in the extract. A low suction was applied on the glass filter paper to allow the sucking out of the $MgCO_3$ as a thin layer. After the formation of a thin bed of $MgCO_3$ on the filter paper, one litre of water sample was filtered through the filtration apparatus fitted with a vacuum pump (Millipore, USA).

The filter paper was then placed in an acid free screw capped test tube containing 10 ml of 90% acetone and kept in a refrigerator for 24 hours incubation. After incubation the filter papers were taken out and ground well with a clean glass rod in order to accelerate the extraction of pigments and transferred it into a centrifuge tube and centrifuged for 10 minutes at 3500 rpm. The clear supernatant was made up to 10 ml by adding fresh 90% acetone. The absorbance of coloured acetone extract was measured by using a UV-Vis Spectrophotometer (Hitachi U-3900) against 90% acetone as blank at multiple wavelengths of 750, 665, 645, 630 and 450 nm, which are considered as the

maximum absorption wavelength of the pigments. The correction of all the extinction values for a small turbidity blank were done by subtracting the optical density of 750 nm from the 665, 645 and 630 nm absorptions (Strickland and Parsons, 1972).

The equation used to find out the chlorophyll contents are described below.

Chlorophyll *a* (C*a*) = 11.85 E665 - 1.54 E645 - 0.08 E630

Chlorophyll *b* (C*b*) = 21.03 E645 - 5.43 E665 - 2.66 E630

Chlorophyll *c* (C*c*) = 24.52 E630 - 1.67 E665 – 7.60 E645

where, 'E' is the absorbance at different wavelengths in the respective wavelengths.

$$\text{Chlorophyll } a \ (\mu g/L) = \frac{Ca \times v}{V \times I}$$

$$\text{Chlorophyll } b \ (\mu g/L) = \frac{Cb \times v}{V \times I}$$

$$\text{Chlorophyll } c \ (\mu g/L) = \frac{Cc \times v}{V \times I}$$

2.1.2.7.2 Estimation of Carotenoids

For the analysis of carotenoids the above procedure was followed and the spectrophotometric measurement was done at wavelengths of 510 and 480 nm.

Carotenoids (C*p*) µg/ L = 7.6 [(E480-E750) – (1.49 E510-E750)]

$$= \frac{Cp \times v}{V \times I}$$

where, 'v' is volume of acetone (ml), 'V' is volume of water (L) filtered for extraction and 'I' is the path length (cm) of cuvette used in spectrophotometer.

2.1.2.8 Phytoplankton Analysis

For the qualitative and quantitative analysis, microalgae were filtered from 50 litre of surface water by using 20 μm bolting silk and the filtered sample was preserved in 2-3% neutralised formaldehyde/Lugol's iodine. The quantitative estimation was carried out by using Sedgewick-Rafter counting cell.

The microalgal identification was done based on the standard keys (Allen and Cupp, 1935; Venkataraman, 1939; Cupp, 1943; Subrahmanyan, 1946; Hustedt, 1955; Desikachary, 1959; Hendey, 1964; Simonsen, 1974; Gopinathan, 1984; Jin Dexiang *et al.*, 1985; Desikachary and Sreelatha, 1989; Hallegraeff *et al.*, 1995; Tomas, 1997; Karlson *et al.*, 2010).

2.1.3 Sample preparation for Environmental Scanning Electron Microscopy (ESEM)

The algal cells were treated with 0.5% glutarldehyde prepared in distilled water and kept for 30 minutes to clean the cells thoroughly. The cells were washed with distilled water by centrifuging gently (500 rpm for 2 minutes) for three times to remove the glutarldehyde as well as the salt content completely. The sample was then air dried over a clean glass slide. Care was taken to avoid breakage of the weakly silicified algal cells. The images of the prepared sample were taken from the Environmental Scanning Electron Microscope (Carl Zeiss EVO-18) at National Institute for Interdisciplinary Science and Technology (NIIST), Trivandrum.

2.1.4 Isolation of bloom associated bacteria

2.1.4.1 Sampling, bacterial isolation and identification

Isolation of bloom associated bacteria was done as described by Croci *et al.* (2006) with some modifications. The bloom sample was filtered through 1 µm GF/C filter paper by using a sterile vacuum filtration unit to retain the phytoplankton cells. Algal cell mass with attached bacterial cells remained in the filter paper and the cells were repeatedly washed with sterile sea water in order to separate out the freely associated bacteria completely. Centrifugation (5000 rpm for 2 minutes) was carried out to isolate the loosely attached bacteria in the algal cells. The centrifuged algal cell pellets were sonicated (70 amplitude for 1 minute) by using an Ultrasonicator (Sonics and Materials Inc., USA) in order to isolate the firmly associated bacteria. The treated algal sample was serially diluted in sterile sea water and plated on to ZoBell 2216e marine agar and nutrient agar (HiMedia, India) media by standard plate count method and incubated at 28 ± 2°C for 48-72 hours to estimate the Total Heterotrophic Bacteria (THB) present in the sample. The individual bacterial colonies that developed were isolated and purified. The purified isolates were identified up to generic level based on cell morphology and biochemical reactions as per Bergey's Manual of Determinative Bacteriology (2000) (Appendix II). THB associated with reference sample was also estimated by standard plate count method.

2.1.4.2 Tests used for the identification of bacteria

2.1.4.2.1 Gram staining

For staining, smears were prepared on clean glass slides using 12-18 hours old bacterial cultures. The primary stain, ammonium oxalate crystal violet was

added to the fixed smear and allowed to stand for one minute. Then the slides were rinsed gently in running water. Then Gram's iodine solution was added as a mordant and allowed to stand for one minute. The slides were washed and treated with acetone (decolourizer) for 30 seconds. After washing the smear, the counter stain safranin-O was added and allowed to stand for one minute. The smear was rinsed, allowed to air dry and observed under oil immersion objective lens of a microscope. Gram positive bacteria appeared in violet or purple color, whereas gram negatives were pink in colour.

2.1.3.4.2 Spore staining

Gram positive strains were subjected to spore staining. Smears were prepared using 60-72 hours old cultures. The slides were flooded with malachite green and allowed to react at room temperature for one minute. Then the slides were periodically heated by using a Bunsen burner until steam arose from the stain on the slide. The slides were steamed for about 3 minutes, replacing the malachite green as it evaporated from the slides, and were then allowed to cool for about 5 minutes before rinsing with water. Then the counter stain safranin-O was added and allowed to stand for one minute. The slides were then washed and allowed to air dry, and observed under oil immersion objective lens of a microscope. Spores appeared in green colour and the vegetative cells were pink in colour.

2.1.4.2.3 Mannitol Motility Test

Mannitol motility medium was prepared and about 3-4 ml was distributed in test tubes. The tubes were sterilized in an autoclave and left for setting in a vertical position. After cooling, the inoculum from the culture was

stabbed straight to the bottom. The tubes were then incubated at room temperature for 48-72 hours. Change of color from pink to yellow in the medium showed the utilization of mannitol. Motile bacteria moved away from the line of inoculation and exhibited diffused growth.

2.1.4.2.4 Marine Oxidation Fermentation [MOF] Test

MOF medium was prepared and about 5 ml was distributed in test tubes and sterilized in an autoclave. After sterilization, the tubes were kept in a slanting position. The inoculum was stabbed and streaked on the agar slant and incubated for 48 hours. Oxidative forms showed a change in colour from pink to yellow from slope to bottom. Gas production was observed by the presence of cracks and bubbles in the hard agar of the butt area. Alkaline reactions were noticed by a deep pink color in the slope region.

2.1.4.2.5 Catalase Test

On a clean glass slide, a smear of fresh bacterial culture was prepared. A drop of concentrated hydrogen peroxide (H_2O_2) solution was added on the smear. Effervescence or bubbling was noticed in the case of cultures producing catalase enzyme.

2.1.4.2.6 Oxidase Test

Small pieces of filter paper (Whatman paper No.1) were soaked in 1% aqueous solution of N,N,N',N'-tetramethyl-p-phenylene diamine dihydrochloride and the papers were dried. A small portion of the culture was placed on the test paper with a clean platinum loop. Oxidase positive cultures showed a blue color within 10-30 seconds.

2.1.4.2.7 O/129 Test

This test was used to distinguish between *Vibrio* sp. and *Aeromonas* sp. Discs were prepared using 2, 4-diamino-6, 7-di-isopropylpteridine phosphate. The discs were placed on swabbed nutrient agar plates and incubated overnight at 30°C. Sensitive cultures showed a zone of inhibition around the disc.

2.1.4.2.8 Antibiotic (Kirby-Bauer) Test

Penicillin G sensitivity was tested to distinguish between *Pseudomonas* sp. and *Moraxella* sp. Muller Hinton agar plates were swabbed with 24 hour old bacterial broth and the antibiotic discs were placed and the plates were incubated overnight at 37°C. Clearing zone around the disc indicated sensitivity to the antibiotic.

2.1.4.3 Screening for hydrolytic enzyme production of bacteria

For the purpose of hydrolytic enzyme screening, the cultures were spot inoculated on nutrient agar medium supplemented with specific substrates viz. starch, tributyrin, gelatin, carboxy methyl cellulose, tannic acid, phenolphthalein diphosphate and sodium alginate and examined for enzyme production.

2.1.4.3.1 Amylase

The plate assay for amylase production was carried out using the method of Mac Faddin (1980). The bacterial strains were spot inoculated on to nutrient agar medium supplemented with 0.5% soluble starch and were incubated till the colonies were clearly visible. The plates were flooded with Gram's iodine solution. The amylase producing colonies exhibited a clear zone against a blue-black surrounding.

2.1.4.3.2 Lipase

The plate assay for lipase production was carried out using the method of Sierra (1957). The strains were spot inoculated on to nutrient agar medium supplemented with 1% tributyrin and were incubated for 48 hours. Clearing zone around the colonies indicated a positive reaction.

2.1.4.3.3 Protease

Protease production was studied by using gelatin as the substrate (Smibert and Kreig, 1994). Nutrient agar medium supplemented with 2% gelatin powder was inoculated and incubated for 48 hours. The proteolytic activity was observed as a clearing zone around the colonies when flooded with 1% mercuric chloride solution.

2.1.4.3.4 Cellulase

Cellulase production was screened according to the method of Hankin and Anagnostakis (1977). The strains were spotted on to nutrient agar medium supplemented with 0.5% carboxy methyl cellulose. After incubation for 3-4 days the plates were flooded with congo red dye (1 mg/ml) solution. It was further incubated for a period of 15 minutes at room temperature. The plates were washed several times using 1 M sodium chloride to remove the unbound excess dye. A clearance zone against a bright red background indicated the production of cellulase.

2.1.4.3.5 Ligninase

The bacterial strains were spot inoculated on to nutrient agar medium supplemented with 0.5% tannic acid. After 4-7 days incubation the appearance

of a clearance zone around the colony was taken as an indication of ligninase production.

2.1.4.3.6 Phosphatase

The screening for phosphatase production was carried out by the method of Baird - Parker (1966). Basal nutrient agar plates containing 1 ml of 1% solution of phenolphthalein diphosphate were spot inoculated with the bacterial culture and incubated till sufficient growth was observed. These plates were then exposed to ammonia (NH_3) vapours by inverting it over a petridish containing NH_3 solution. Pink colouration of cultures indicated the presence of phosphatase enzyme.

2.1.4.3.7 Alginase

The screening for alginase production was carried out by the method of Gacesa and Wusteman (1990). Nutrient agar medium supplemented with 1.5% sodium alginate were spot inoculated with bacterial strains. After incubation for 2-3 days the plates were flooded with 10% cetyl pyridinium chloride solution. The positive strains showed a clearance zone against an opaque white background after incubation for 10-30 minutes at room temperature.

2.2 Statistical Analysis

Statistical interpretation of data was carried out by using softwares like MS Excel, PRIMER 6, SPSS 13 and Origin Pro 7.0.

.....ഇൽ.....

Chapter 3

Hydrography

3.1 Coastal and estuarine stations along the southwest coast of India
3.2 Statistical Analysis

3.1 Seasonal and spatial variation in the physico-chemical parameters in the coastal and estuarine stations along the southwest coast of India

Phytoplankton community structure and its distribution are austerely important for balancing the marine ecosystem, because it acts as a vital contributor of energy in the marine environment, by playing a key role in the productivity of the marine ecosystem (Maric *et al*., 2012). The life-cycle and the distribution of microalgae are mostly influenced individually or collectively by the hydrographical variables (Wang *et al*., 2006a; Houliez *et al*., 2013). The interaction of physico-chemical variables with microalgae (phytoplankton) can trigger the occurrences of algal blooms in the marine ecosystem. Seasonal and spatial variation in the physico-chemical variables such as temperature, salinity, pH, inorganic nutrients (nitrate, nitrite, silicate and phosphate), dissolved oxygen and primary productivity were determined in the study stations from pre-monsoon 2009-10 to post-monsoon 2010-11.

3.1.1 Temperature

Both spatial and seasonal variation in temperature was studied from six stations during 2009-2010 and 2010-2011. In 2009-10, the highest temperature (32°C) was recorded from station 6, Thykadapuram, in the pre-monsoon and the lowest (26°C) was recorded from stations 2, Balathuruth, and 5, Puthiyangadi, in the monsoon period (Fig.1).

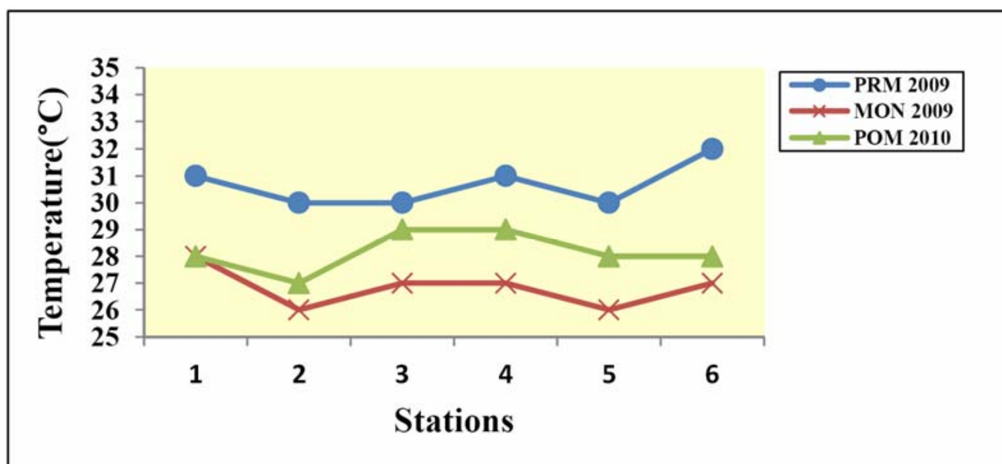


Fig.1 Seasonal and spatial variation in temperature: 2009-2010

In 2010-2011, the highest temperature of 31°C was recorded at station 6, Thykadapuram, in the post-monsoon period, whereas the lowest (26°C) was in station 1 (Azheekode), station 2 (Balathuruth) and station 4 (Mahe) in the monsoon period (Fig.2). Two-way ANOVA showed that there is significant difference in temperature with seasons ($p<0.001$) but not with stations at 0.05 and 0.001 level.

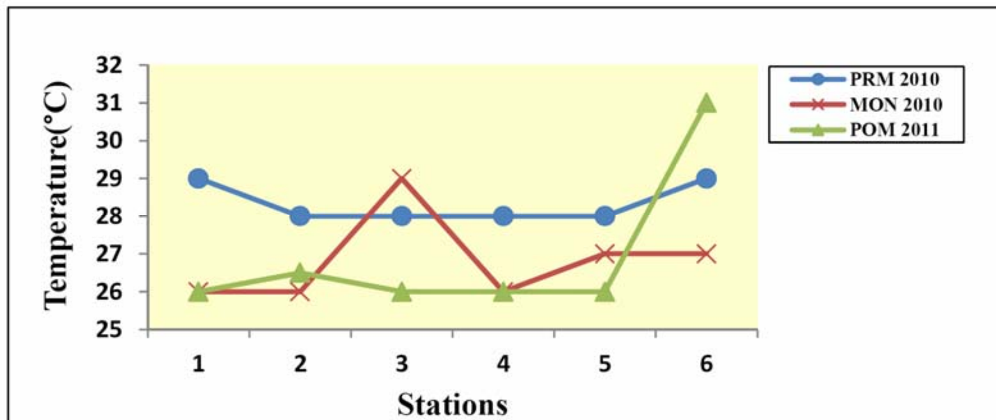


Fig.2 Seasonal and spatial variation in temperature: 2010-2011

3.1.2 Salinity

In 2009-2010, the highest salinity of 36 psu was recorded from station 2, Balathuruth, in the pre-monsoon period, whereas the lowest salinity of 2 psu was also observed at station 2 in the monsoon. Similarly, in station 6, Thykadapuram, a lower salinity of 4 psu was noted during monsoon. This might be due to the influx of freshwater from river Kadalundy and river Tejaswini to station 2 and station 6, respectively (Fig.3).

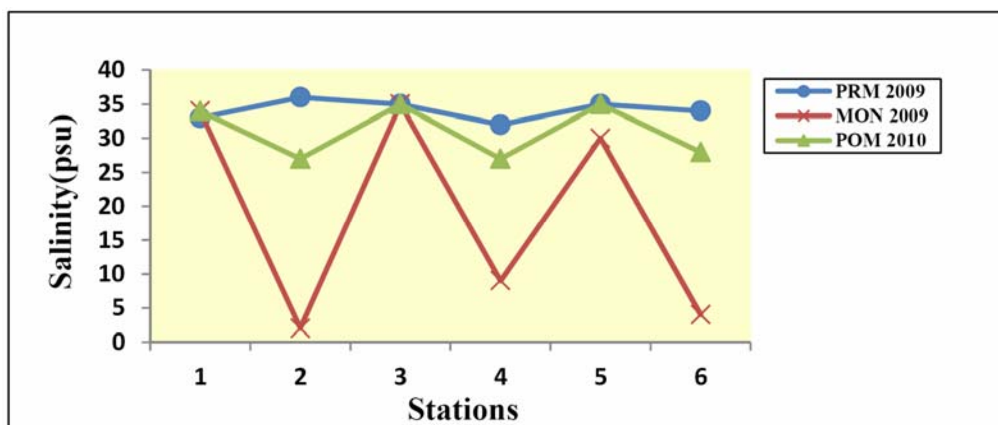


Fig.3 Seasonal and spatial variation in salinity: 2009-2010

In 2010-2011, the highest salinity of 35 psu was recorded from stations 1 (Azheekode), 3 (Kodikkal), and 5 (Puthiyangadi) in the pre-monsoon period. Station 2, Balathuruth, has shown the lowest salinity of 5 psu in the monsoon period (Fig.4). Statistically, a significant variation was found between salinity and seasons ($p<0.001$) and also with stations ($p<0.05$).

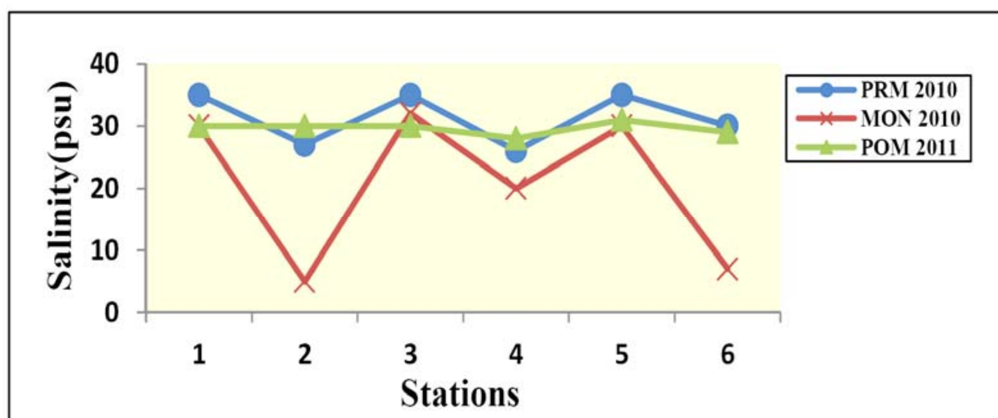


Fig.4 Seasonal and spatial variation in salinity: 2010-2011

3.1.3 pH

The pH of the stations were generally alkaline. In 2009-2010 highest pH was recorded at station 2, Balathuruth, in the monsoon and lowest was at station 4, Mahe, in the post-monsoon period (Fig.5).

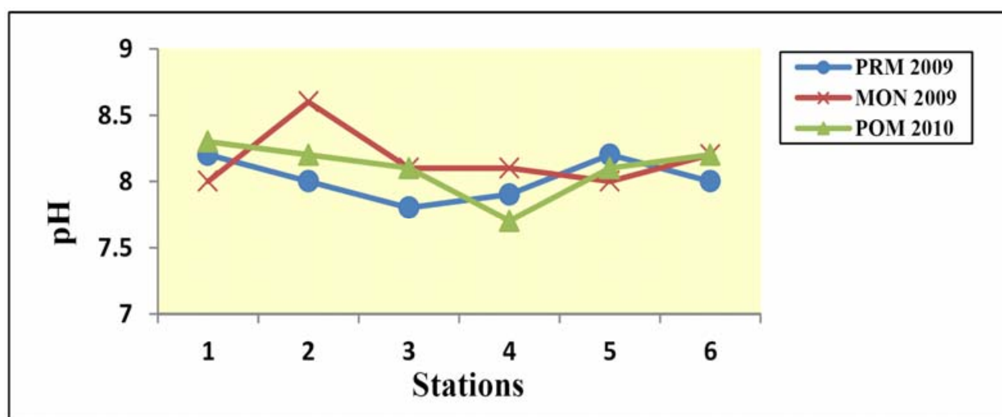


Fig.5 Seasonal and spatial variation in pH: 2009-2010

In 2010-2011, highest pH was found at stations 5 (Puthiyangadi) and 6 (Thykadapuram) in the monsoon, whereas the lowest value was found in station 6 in the post-monsoon. Alkaline condition was found in all stations throughout the investigation period (Fig.6). Two-way ANOVA showed no significant difference in pH between the stations at 0.05 and 0.001 level but had a significant difference with seasons ($p<0.05$).

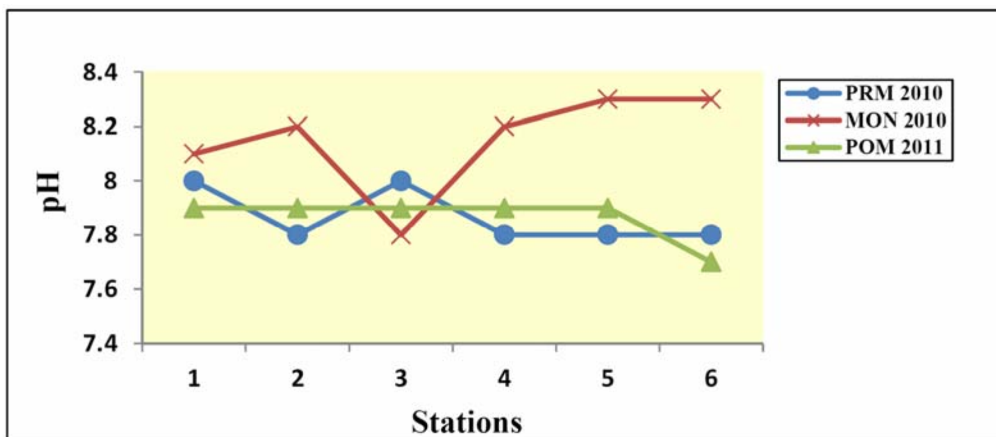


Fig.6 Seasonal and spatial variation in pH: 2010-2011

3.1.4 Nutrients

3.1.4.1 Nitrate

In 2009-2010, the nitrate concentration was in the range of 1.45 µmolL^{-1} to 11.90 µmolL^{-1}. During the pre-monsoon, the highest concentration of nitrate, 11.90 µmolL^{-1}, was recorded in station 3, Kodikkal, whereas the lowest concentration (3.05 µmolL^{-1}) was observed in station 6 (Thykadapuram). In the monsoon season, station 2 (Balathuruth) recorded the highest concentration of nitrate (8.90 µmolL^{-1}) but lowest (2.30 µmolL^{-1}) was noted in station 3, Kodikkal. The highest concentration of nitrate (3.98 µmolL^{-1}) was

observed in station 3 and the lowest (1.45 µmolL^{-1}) was in station 1 (Azheekode) during the post-monsoon period (Fig.7).

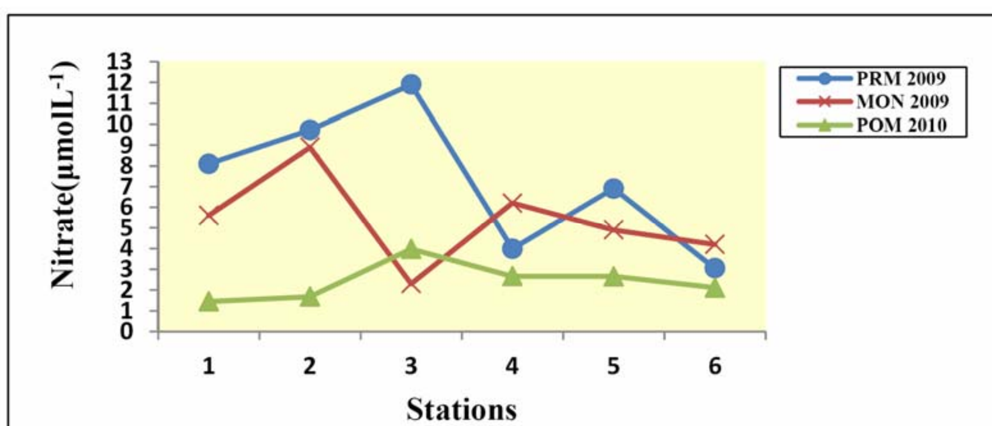


Fig.7 Seasonal and spatial variation in nitrate: 2009-2010

During 2010-2011, nitrate concentration from the surface water sample ranged from 1.18 µmolL^{-1} to 14.36 µmolL^{-1}. In the pre-monsoon period, 4.59 µmolL^{-1} and 1.24 µmolL^{-1} were the highest and lowest nitrate concentration, recorded correspondingly from stations 2 (Balathuruth) and 3 (Kodikkal). Comparatively the monsoon season recorded highest value of nitrate in the study period. The highest recorded value was from station 1, Azheekode (14.36 µmolL^{-1}) and lowest (2.99 µmolL^{-1}) from station 5, Puthiyangadi. During the post-monsoon season, the highest concentration of nitrate was noted from station 4, Mahe (4.51 µmolL^{-1}) and the lowest (1.18 µmolL^{-1}) from station 6, Thykadapuram (Fig.8). Two-way ANOVA showed significant difference in nitrate concentration with seasons ($p<0.05$) but not with stations.

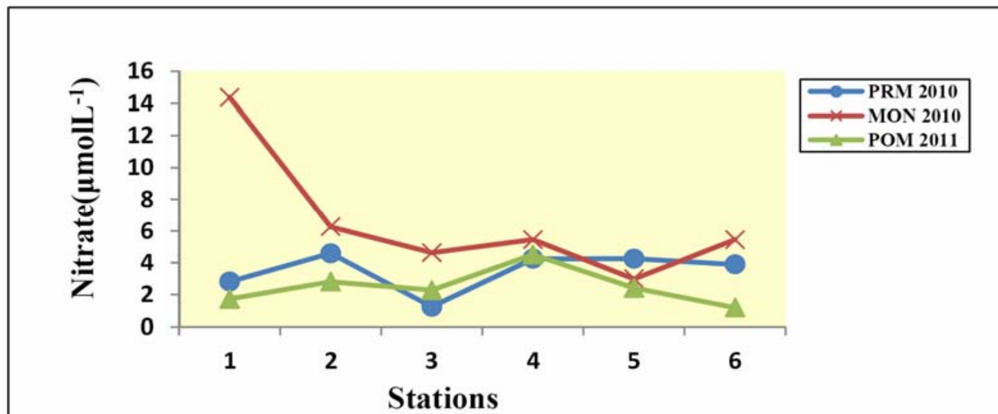


Fig.8 Seasonal and spatial variation in nitrate: 2010-2011

3.1.4.2 Nitrite

Nitrite concentration was found to be higher during the monsoon period as compared with the pre-monsoon and the post-monsoon seasons in all the stations during 2009-2010. The highest concentration, 1.40 $\mu molL^{-1}$, was observed in station 2 (Balathuruth) during the monsoon, whereas the lowest concentration of 0.19 $\mu molL^{-1}$ was recorded from station 2 in the pre-monsoon season (Fig.9).

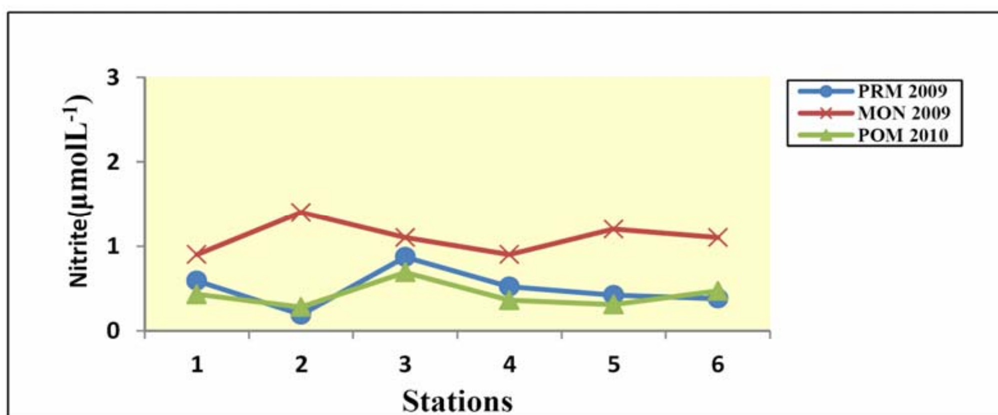


Fig. 9 Seasonal and spatial variation in nitrite: 2009-2010

In 2010-2011, the highest concentration of nitrite, 1.33µmolL^{-1}, was recorded at stations 1 (Azheekode) and 6 (Thykadapuram) in the post-monsoon period and the lowest concentrations, 0.03 µmolL^{-1} and 0.06 µmolL^{-1}, were recorded at stations 1 and 2 (Balathuruth) respectively, in the pre-monsoon season (Fig.10). Two-way ANOVA showed significant difference with seasons ($p<0.05$) and not with stations at 0.05 and 0.001 level.

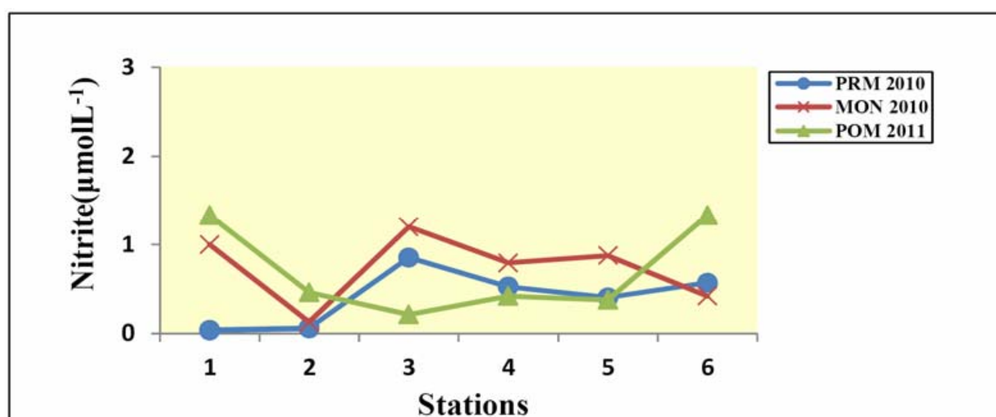


Fig. 10 Seasonal and spatial variation in nitrite: 2010-2011

3.1.4.3 Silicate

In 2009-2010, during the pre-monsoon, highest concentration of silicate (34.70 µmolL^{-1}) was recorded at station 4, Mahe, and the lowest concentration (3.23 µmolL^{-1}) at station 1, Azheekode. During the monsoon period, highest concentration of silicate (144.90 µmolL^{-1}) was observed in station 2, Balathuruth, while station 3, Kodikkal, recorded the lowest concentration of 19.70 µmolL^{-1}. The silicate concentration ranged from 2.98 µmolL^{-1} to 35.04 µmolL^{-1} during the post-monsoon period and the highest (35.04 µmolL^{-1}) was in station 4 and the lowest (2.98 µmolL^{-1}) in station 1 (Fig.11).

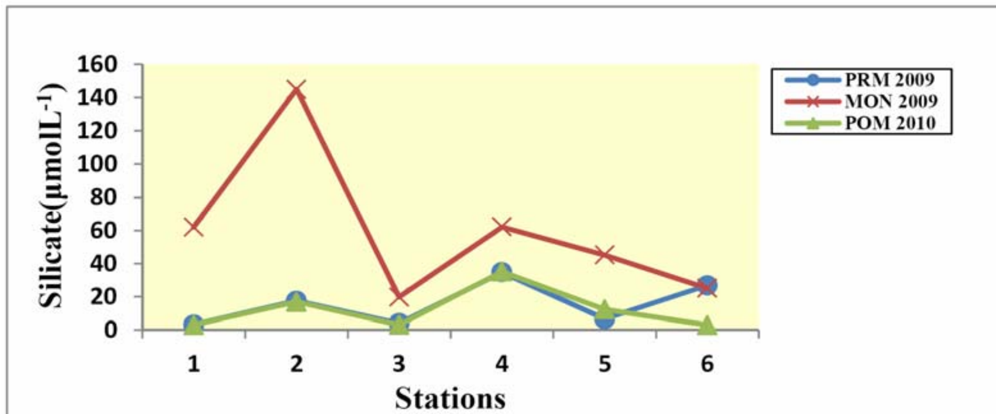


Fig. 11 Seasonal and spatial variation in silicate: 2009-2010

In 2010-2011, during the pre-monsoon, 55.04 µmolL^{-1} was the highest concentration of silicate recorded (station 5, Puthiyangadi) and station 3, Kodikkal, recorded the lowest concentration (15.16 µmolL^{-1}). During the monsoon, stations 4 (Mahe) and 1 (Azheekode) recorded the highest (55.41 µmolL^{-1}) and lowest (10.59 µmolL^{-1}) concentrations of silicate, respectively. In the post-monsoon, 50.91 µmolL^{-1} was the highest concentration of silicate recorded (station 4) and lowest (12.83 µmolL^{-1}) was at station 3 (Fig.12). Two-way ANOVA showed significant difference with seasons ($p<0.05$) but not with stations.

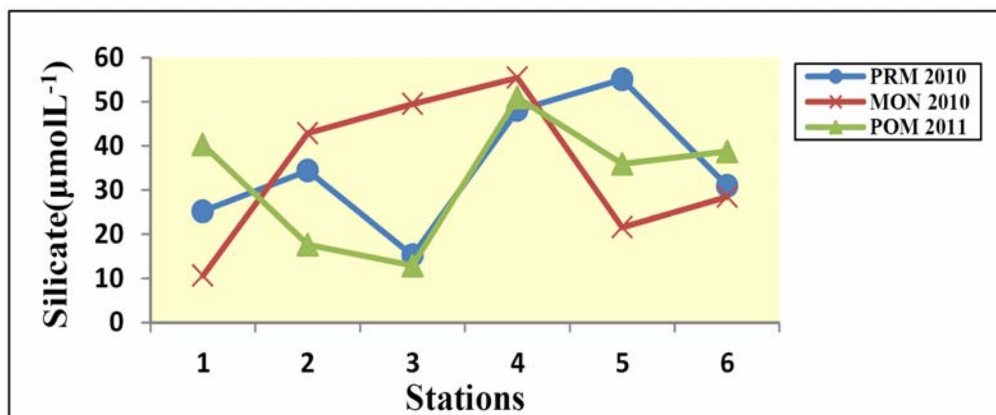


Fig. 12 Seasonal and spatial variation in silicate: 2010-2011

3.1.4.4 Phosphate

In 2009-2010, the highest concentration of phosphate (1.15 µmolL^{-1}) was recorded in station 4, Mahe, and the lowest concentration was at station 2, Balathuruth (0.55 µmolL^{-1}), in the pre-monsoon season. Comparatively high range of phosphate concentration was observed in the monsoon season, the highest (1.90 µmolL^{-1}) at station 1, Azheekode and the lowest (1.30 µmolL^{-1}) in station 4. During the post-monsoon, station 2 recorded the highest concentration (0.81 µmolL^{-1}) and negligible values were recorded from stations 3, Kodikkal and 6, Thykadapuram (Fig.13).

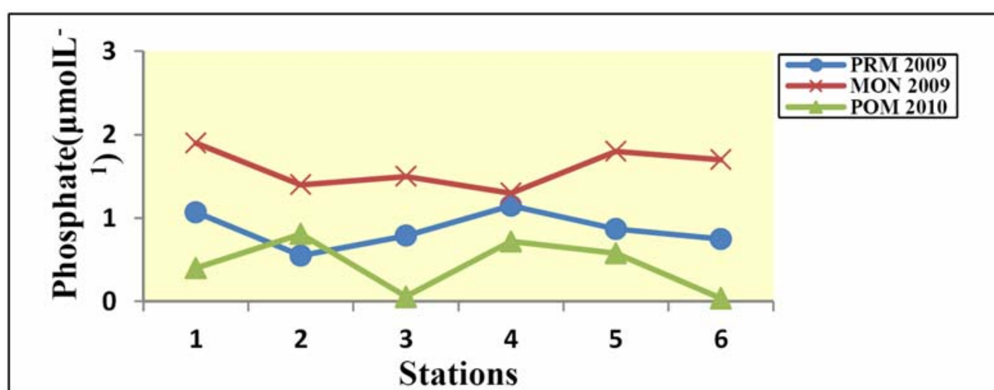


Fig. 13 Seasonal and spatial variation in phosphate: 2009-2010

In 2010-2011, the pre-monsoon season recorded relatively lower phosphate concentration in all the stations except station 1, Azheekode. During the monsoon season, the highest concentration was recorded in the station 2, Balathuruth (2.62 µmolL^{-1}) while station 6, Thykadapuram had the lowest concentration (0.77 µmolL^{-1}). In the post-monsoon, station 4, Mahe recorded the highest concentration (2.54 µmolL^{-1}), whereas the lowest (0.79 µmolL^{-1}) was observed in station 5, Puthiyangadi (Fig.14). Two-way ANOVA showed significant difference with seasons ($p<0.05$) but not with stations.

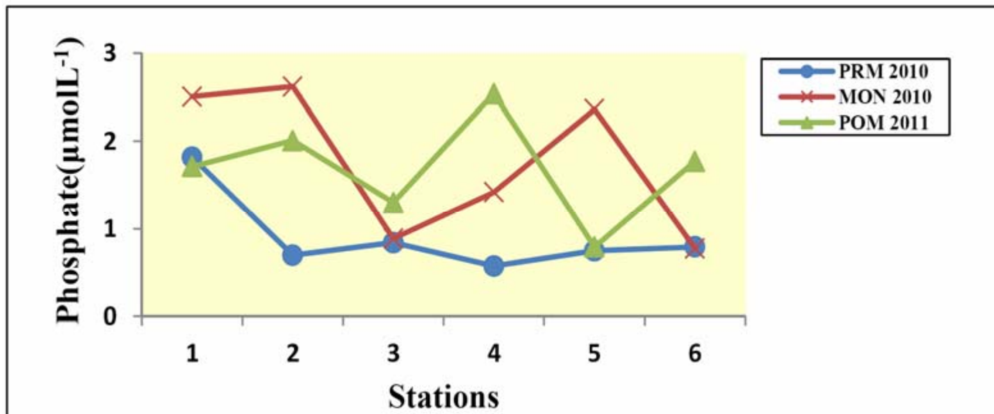


Fig. 14 Seasonal and spatial variation in phosphate: 2010-2011

3.1.5 Dissolved Oxygen

In 2009-2010, in the pre-monsoon period, the highest value of dissolved oxygen (6.53 mgL^{-1}) was recorded in station 6, Thykadapuram, whereas the lowest was (3.84 mgL^{-1}) in station 2, Balathuruth. During the monsoon season, the highest value was recorded in station 2 (6.54 mgL^{-1}) and the lowest from station 1, Azheekode (3.38 mgL^{-1}). The highest value of DO was recorded in station 3, Kodikkal (6.24 mgL^{-1}) and the lowest (5.31 mgL^{-1}) in station 6 during the post-monsoon period (Fig.15).

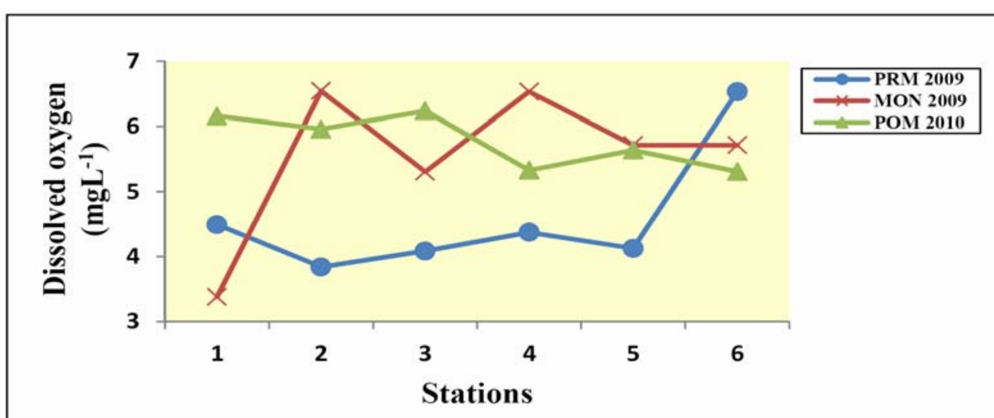


Fig. 15 Seasonal and spatial variation in dissolved oxygen: 2009-2010

In 2010-2011, considerably higher level of dissolved oxygen was recorded in the pre-monsoon period and the highest dissolved oxygen value was at station 5, Puthiyangadi (9.55 mgL^{-1}) and lowest in station 2, Balathuruth (7.14 mgL^{-1}). In the monsoon, 8.04 mgL^{-1} was the highest and 5.90 mgL^{-1} was the lowest values of dissolved oxygen recorded from stations 4 (Mahe) and 3 (Kodikkal), respectively. During the post-monsoon, relatively lower values were recorded and the highest was from station 6, Thykadapuram (6.53 mgL^{-1}) and the lowest (4.49 mgL^{-1}) in station 1, Azheekode (Fig.16). Two-way ANOVA showed no significant difference in DO values with seasons and with stations.

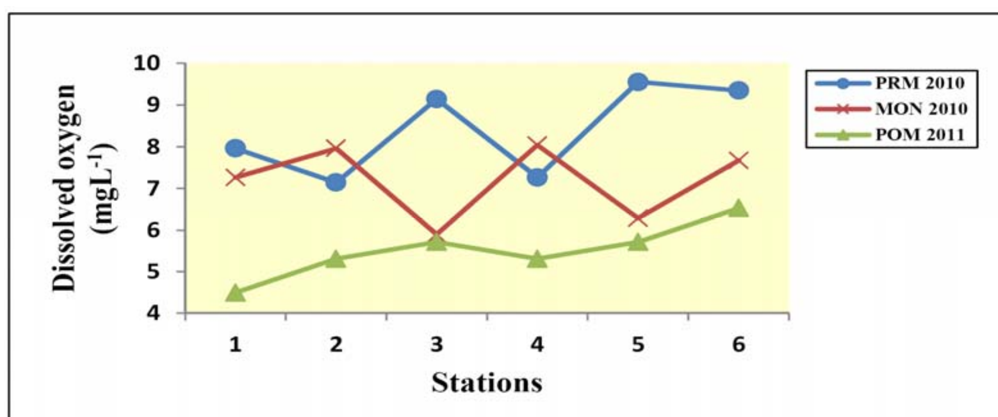


Fig.16 Seasonal and spatial variation in dissolved oxygen: 2010-2011

3.1.6 Primary Production

In 2009-2010, in the pre-monsoon, the net primary production ranged from 4.24 $gC/m^3/day$ (station 4, Mahe) to 16.96 $gC/m^3/day$ (station 6, Thykadapuram). During the monsoon, the net primary production was found to be highest at station 1, Azheekode (18.16 $gC/m^3/day$) and lowest recorded was 4.24 $gC/m^3/day$ (station 5, Puthiyangadi). In the post-monsoon period, 16.92 $gC/m^3/day$ was the highest net primary production recorded (station 5) and the station 6 showed the lowest value of 1.04 $gC/m^3/day$ (Fig.17).

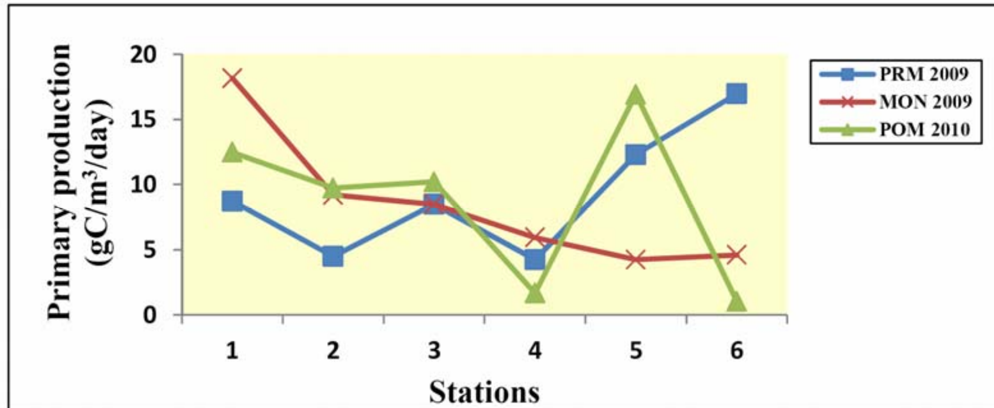


Fig. 17 Seasonal and spatial variation in primary production: 2009-2010

During 2010-2011, in the pre-monsoon, the highest value of net primary production was recorded in station 5, Puthiyangadi (9.75 gC/m^3/day) and 1.70 gC/m^3/day was the lowest value (station 1, Azheekode). In the monsoon season, the primary production ranged from 1.91 gC/m^3/day (station 2, Balathuruth) to the highest of 9.65 gC/m^3/day (station 4, Mahe). During the post-monsoon, the highest value, 28.83 gC/m^3/day was recorded in station 5 and lowest, 1.09 gC/m^3/day in station 1 (Fig.18). Two-way ANOVA showed no significant difference in primary production values with seasons and with stations.

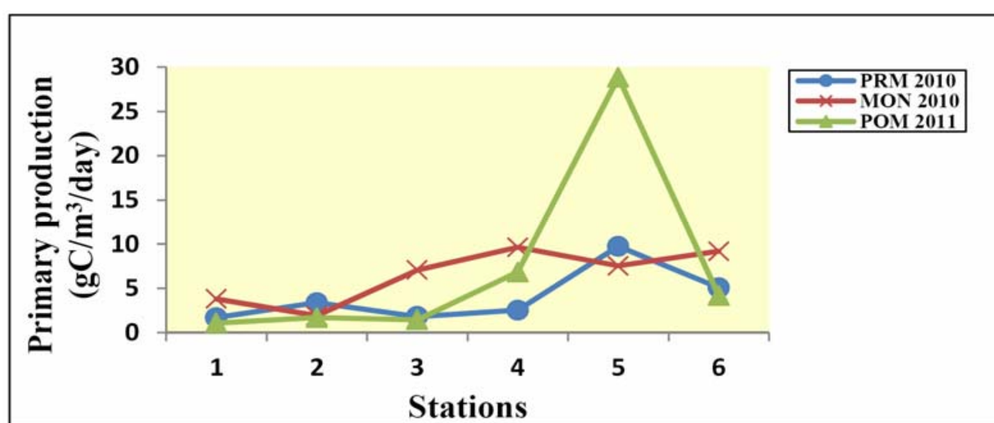


Fig.18 Seasonal and spatial variation in primary production: 2010-2011

3.2 Statistical Analysis

Two-way ANOVA (Analysis of Variance) of physico-chemical variables between seasons and stations for the period from pre-monsoon 2009 to post-monsoon 2011 is shown in Table 2. The Pearson Correlation analysis of the hydrographic parameters is shown in Table 3.

Table 2 Analysis of Variance (ANOVA) of physico-chemical variables

Temperature					
Source of Variation	SS	DF	MS	F	P
Season	48.097	2	24.049	13.948	<0.001
Station	12.701	5	2.540	1.473	0.230
Error	48.278	28	1.724		
Total	109.076	35			
Salinity					
Source of Variation	SS	DF	MS	F	P
Season	1131.722	2	565.861	14.166	<0.001
Station	1058.139	5	211.628	5.298	0.002
Error	1118.444	28	39.944		
Total	3308.306	35			
pH					
Source of Variation	SS	DF	MS	F	P
Season	0.317	2	0.159	4.744	0.017
Station	0.159	5	0.032	0.951	0.464
Error	0.936	28	0.033		
Total	1.412	35			
Nitrate					
Source of Variation	SS	DF	MS	F	P
Season	84.317	2	42.158	5.892	0.007
Station	25.976	5	5.195	0.726	0.610
Error	200.356	28	7.156		
Total	310.649	35			

Nitrite					
Source of Variation	SS	DF	MS	F	P
Season	1.438	2	0.719	6.359	0.005
Station	0.583	5	0.117	1.030	0.419
Error	3.167	28	0.113		
Total	5.188	35			
Silicate					
Source of Variation	SS	DF	MS	F	P
Season	4444.978	2	2222.489	4.068	0.028
Station	4556.816	5	911.363	1.668	0.175
Error	15297.192	28	546.328		
Total	24298.986	35			
Phosphate					
Source of Variation	SS	DF	MS	F	P
Season	4.166	2	2.083	5.649	0.009
Station	1.847	5	0.369	1.002	0.435
Error	10.325	28	0.369		
Total	16.338	35			
Dissolved Oxygen					
Source of Variation	SS	DF	MS	F	P
Season	5.122	2	2.561	1.008	0.378
Station	4.491	5	0.898	0.353	0.876
Error	71.141	28	2.541		
Total	80.754	35			
Primary Production					
Source of Variation	SS	DF	MS	F	P
Season	11.817	2	5.909	0.178	0.838
Station	279.466	5	55.893	1.680	0.172
Error	931.665	28	33.274		
Total	1222.948	35			

Table 3 Pearson Correlation analysis of physico-chemical variables

		Temp	Salinity	pH	NO_3-N	NO_2-N	SiO_4-Si	PO_4-P	DO	Net PP
Temp	**Pearson Correlation**	1.000								
	Sig. (2-tailed)									
	N	36								
Salinity	**Pearson Correlation**	0.473**	1.000							
	Sig. (2-tailed)	0.004								
	N	36	36							
pH	**Pearson Correlation**	-0.305	-0.472**	1.000						
	Sig. (2-tailed)	0.071	0.004							
	N	36	36	36						
NO_3-N	**Pearson Correlation**	0.020	-0.168	0.190	1.000					
	Sig. (2-tailed)	0.909	0.328	0.268						
	N	36	36	36	36					
NO_2-N	**Pearson Correlation**	-0.122	-0.177	0.114	0.142	1.000				
	Sig. (2-tailed)	0.477	0.302	0.510	0.407					
	N	36	36	36	36	36				
SiO_4-Si	**Pearson Correlation**	-0.310	-0.536**	0.145	0.123	0.380*	1.000			
	Sig. (2-tailed)	0.066	0.001	0.398	0.474	0.022				
	N	36	36	36	36	36	36			
PO_4-P	**Pearson Correlation**	-0.463**	-0.265	0.082	0.183	0.285	0.261	1.000		
	Sig. (2-tailed)	0.004	0.118	0.634	0.285	0.092	0.124			
	N	36	36	36	36	36	36	36		
DO	**Pearson Correlation**	-0.213	-0.240	-0.010	-0.149	-0.121	0.180	0.002	1.000	
	Sig. (2-tailed)	0.212	0.159	0.952	0.384	0.482	0.294	0.990		
	N	36	36	36	36	36	36	36	36	
Net PP	**Pearson Correlation**	0.082	0.168	0.183	-0.016	-0.065	0.057	-0.230	-0.174	1.000
	Sig. (2-tailed)	0.634	0.328	0.286	0.928	0.708	0.741	0.178	0.309	
	N	36	36	36	36	36	36	36	36	36

*** Correlation is significant at 0.05 level.**
**** Correlation is significant at 0.01 level.**

.....ꙮ.....

Chapter 4

Distribution of Planktonic Microalgae along the Kerala Coast

4.1 Introduction
4.2 Review of Literature
4.3 Result
4.4 Discussion

4.1 Introduction

The term phytoplankton literally comprises two Greek words, meaning "plant" (phyto) and "wanderer" (plankton). Phytoplankton comprises a diverse, polyphyletic group of single-celled and colonial aquatic photosynthetic organisms that drift with the currents (Falkowski and Raven, 1997). Phytoplankton can be classified based on their size as picophytoplankton (0.2-2μm), nanophytoplankton (2-20μm), microphytoplankton (20-200μm), mesophytoplankton (200μm-2mm) and macrophytoplankton (>2mm) (Sieburth, 1978).

Despite constituting less than one percent of Earth's photosynthetic biomass, the marine phytoplankton are responsible for more than forty-five percent of its net primary production (Field *et al*., 1998) underpinning the marine food web especially in the shelf seas (Fehling *et al*., 2012). These tiny organisms are the most important producers of organic substances and the rate at which energy is stored up by them determines the basic primary productivity

of the ecosystem. They comprise many different taxonomic groups which, together with their distribution, determine primary production and various trophic level interactions (Roy *et al*., 2006) providing a good indication of energy turnover in aquatic environments (Forsberg, 1982; Devassy and Goss, 1988). Photosynthetic pigments like chlorophyll *b* (chlorophytes), fucoxanthin (diatoms), 190-hexanoyloxyfucoxanthin (haptophyte), 190-butanoyloxyfucoxanthin (pelagophytes and haptophyte), peridinin (dinophytes), alloxanthin (cryptophytes) and zeaxanthin (cyanobacteria) are the taxon-specific indicators of major taxonomic groups used for studying the composition and physiological status of phytoplankton (Jeffrey and Vesk, 1997). Determination of microalgal population is essential for understanding the dynamics of the primary production because the species composition can play a dominant role in the variations of production, in well-mixed interface areas, especially in estuaries (Shaw and Purdie, 2001; Behrenfeld *et al*., 2004). Within these environments phytoplankton are located at the base level and are represented as a major source of organic carbon (Trivedy and Goel, 1984; Gaikwad *et al*., 2004). They are important contributors to global carbon fluxes (Falkowski *et al*., 1998) as marine primary production is responsible for at least 30% of total global carbon fixation (Peterson *et al*., 1986).

The factors that control the composition, diversity and distribution of oceanic phytoplankton are complicated and are mainly bounded to the physico-chemical factors like light, temperature, salinity, availability of nutrients, strong seasonal variations in accordance with climate conditions, upwelling, turbulence and biotic factors which include the grazing rate of herbivorous zooplankton (Boyce *et al*., 2010; Jang *et al*., 2013).

Continental shelf and adjacent slope waters represent only 10 - 20% of surface area of global oceans, but these ocean margins are highly productive, and contribute 25 - 50% of total oceanic primary production (Walsh, 1988). The coastal and estuarine environment, as compared to the open sea, has high fertility and large phytoplankton population basically due to the increased nutrient input from river water and land run off (Ketchum, 1967; Wang *et al*., 2006a).

Estuaries are economically important ecosystems for fisheries in tropical regions (Kawabata *et al*., 1993). Biomass and productivity of phytoplankton of different size ranges are important factors regulating the productivity of higher trophic-level organisms. The pelagic algal communities make important contributions to the smooth functioning of estuarine ecosystem (Kawabata *et al*., 1993). Phytoplankton species distribution shows wide spatio-temporal variations due to the differential effect of hydrographical factors on individual species and they serve as good indicators of water quality, including pollution (Sun *et al*., 2001; Liu *et al*., 2004).

Estuaries act as a transitional zone between land and sea and act as a filter that traps both natural and anthropogenic materials transferring from the land to the open sea, and thus are mainly susceptible to anthropogenic impact and their integrity is currently under risk worldwide (Flindt *et al*., 1999; Kiddon *et al*., 2003). Many studies (Cederwall and Elmgren 1990; Kahru *et al*., 1994; Escaravage *et al*., 1996; Schollhorn and Graneli, 1996) have reported that anthropogenic activities increase the nutrient level of estuarine and coastal areas which may result in the alteration of phytoplankton population and biomass production and thereby affect the ecosystem structure and function (FAO, 1992). Nitrogen and phosphorus contained in agricultural effluents and

industrial discharges are the main cause of eutrophication (Karthikeyan *et al*., 2010). Phytoplankton follow a fairly recognizable annual cycle of growth, but sometimes the synchrony in their normal annual cycle is disrupted by explosive growth of some species (Vaulot, 2001), termed algal blooms, which can be counted as a negative influence of the above factors leading to adverse consequences on the ecosystem (Gianesella *et al*., 2000).

The availability of nutrients in the euphotic zone and its subsequent biochemical response is the basis of biological productivity of phytoplankton community structure (Malone, 1980; Platt *et al*., 1983; Fisher *et al*., 1999). The productivity, phytoplankton biomass and its distribution is largely associated with nutrient availability at large spatial scales; at medium and small scales with biophysical processes such as the light environment, water column stratification /turbulence, temperature and grazing (Smith, 2006). In highly turbid coastal waters where nutrients are present at moderate to high concentrations, light availability is usually the limiting factor for primary production (Underwood and Kromkamp, 1999). Hence, the distribution of phytoplankton biomass can be expected to reflect horizontal gradients in those variables that modulate light availability (Pennock, 1985). Phytoplankton productivity is also directly dependent on the seasonally changing wind system leading to strong seasonality in export production with peaks during the SW- and NE-monsoons in the Arabian Sea (Nair *et al*., 1989; Haake *et al*., 1993).

The environmental conditions of the ecosystem, reflected by the availability of nutrients play a significant role in phytoplankton species composition, succession and community composition (Rhyther and Dunstan, 1971; Smayda, 1980; Riegman *et al*., 1990). If the supply of nutrients is less than the uptake by

phytoplankton, nutrient concentrations decrease and limit additional growth of phytoplankton (Tilman *et al*., 1982). The limiting nutrient concentrations vary with season, location and phytoplankton community structure (Fisher *et al*., 1992). Under conditions of increasing eutrophication, initial changes in the aquatic communities begin with the succession in the species composition and abundance of phytoplankton (Aktan *et al*., 2005). Additionally, variation in nutrient ratio due to disproportionate inputs of nutrients has also been shown to profoundly affect the phytoplankton species composition and production, and the ecosystem structure and function (Smith *et al*., 1999). Species diversity responds to changes in environmental gradients and may characterize many interactions that can establish the intricate pattern of community structure. Any slight alteration in the environmental status can change diversity until there is no adaptation or gene flow from non-adaptive sources. Hence a high diversity count suggests a healthy ecosystem, the reverse of this indicates a degraded environment and in the latter situation only a few organisms can thrive and flourish, which represents the "paradox of enrichment" effect (Ghosh *et al*., 2012).

Nutrient limitation in natural phytoplankton communities is primarily identified from bioassays in which its response to nitrogen or phosphorus is measured by additions of one or both nutrients in micro/mesocosm or, inferred from elemental ratios which could enable the prediction of phytoplankton abundance and assemblages. The ability of phytoplankton cells to synthesize new biomass depends on their ability to assimilate sufficient carbon, nitrogen and phosphorus, as well as minor nutrients, to ensure replication. As a consequence of depletion of nutrients in the surrounding waters due to growth of phytoplankton assemblages, future generations must adapt to decreasing

nutrient concentrations. Therefore, the environment in which phytoplankton grow is significantly modified by their growth, and over time-periods of only a few days, assemblages may move from a situation of nutrient excess to nutrient depletion (Robin *et al.*, 2010). This may limit total biomass of phytoplankton assemblage, but need not necessarily reduce the growth rate of those cells which are adapted to the assimilation of nutrient at very low concentration (Rees *et al.*, 1999). Life in the plankton involves a balance between behaviour and the organisms' environment (McManus and Woodson, 2012).

Even though the governing factors of phytoplankton distribution are mainly location specific, it is important to determine how the distribution and composition of phytoplankton populations in economically important shelf seas relate to the particular chemical and physical properties of the water column in which they live (Fehling *et al.*, 2012). With this view, the present study was an attempt to investigate the correlation between the respective physico-chemical properties with the spatio-temporal distribution and abundance of planktonic microalgae along southwest coast of India, with special emphasis on those species with a known history of bloom formation.

4.2 Review of Literature

4.2.1 Distribution of microalgae

A plant microorganism was first seen by Anton van Leeuwenhoek through his invented microscope in 1674 from Dutch waters. Joseph Banks in 1768-71, observed the "sea sawdust", *Oscillatoria* (*Trichodesmium*) spp. while accompanying James Cook on his voyage on '*Endeavour*' to the South Seas. Muller collected plankton for the first time in 1845 by towing fine meshed plankton net. Hooker (1847) first recognized the wide distribution, abundance

and general ecological significance of phytoplankton as a result of his observations during an Antarctic cruise. Though the name 'plankton' was derived from Greek, it was coined by a German scientist Hensen (1887). Haeckel (1890) more precisely defined the word plankton.

A number of species of diatoms were recorded from Java Sea, West Indian Archipelago, Baffin Bay and coastal Sweden by Cleve (1873, 1878, 1894, 1896). The scientific results of the voyage of H.M.S. *Challenger* during the years 1873-76, especially from the family Diatomaceae was reported by Castracane (1886). Cupp (1937) prepared a monograph on '*Marine plankton diatoms of the West coast of North America*' based on the seasonal distribution and occurrence of marine diatoms and dinoflagellates from Alaska and it has been used as a key textbook on diatom taxonomy worldwide.

Hendey (1937) made a series of pioneer studies on phytoplankton. In 1954, he published a compiled check-list on British marine diatoms, which contained 771 species from 104 genera. In 1964, a monograph titled "*An introductory account of the smaller algae of the coastal waters. Part V. Bacillariophyceae (Diatoms)"* was published. Later on, in 1974 he published a revised check-list of the British marine diatoms. During 1933-34, 'John Murray Expedition' was conducted which explored the Arabian Sea. Gilson (1937) estimated the organic production in the Arabian Sea as part of the John Murray expedition and expressed in wet weight of algae.

Subrahmanyan and co-workers (1946, 1958a and b, 1959, 1960, 1965) described over 500 species of phytoplankton forms of all groups together representing over 150 genera from both coasts of India. In 1946, he recorded 171 forms of marine planktonic diatoms at Madras, representing 15 families,

64 genera, 134 species, 17 varieties and several other forms. Subrahmanyan and Sharma (1960) listed a total of 291 species of phytoplankton from Calicut. The IIOE expedition (1962-65) focused on the plankton with a view to assessing organic production.

Reynolds (1973, 1984, 2006), the most reputed scientist in contemporary phycology, focused on the ecology of phytoplankton and published two pioneer monographs on phytoplankton ecology in 1984 and 2006. Seasonal and spatial distribution of phytoplankton in Cochin backwaters was studied by Joseph and Pillai (1975). Joseph and Nair (1975) made an analysis on the growth constants, mean generation time and chlorophyll in relation to cell numbers and ^{14}C uptake in a few unialgal cultures of selected phytoplankters isolated from Cochin estuary and found that the highest growth constant and lowest mean generation time were during the exponential growth phase. The diversity of phytoplankton species, pigments and succession with a note on primary production at a tidal zone in the Vellar estuary, east coast of India was studied by Vijayalakshmi and Venugopalan (1975). Gopinathan (1975a and b) described the diatoms present in various estuarine systems in India, their seasonal fluctuations, occurrence, and distribution, particularly from Cochin backwaters and also from the Indian Seas.

Krammer and Lange-Bertalot (1985, 1986) did astonishing work on the diatoms, especially on the family Naviculaceae, and prepared a monograph, *Bibliotheca Diatomologica*. Atlases of Diatoms contributed by Desikachary and his colleagues (1986, 1987a, b and c, 1988, 1989) are considered as the most valuable reference books worldwide.

During the 9th Indian Antarctic Expedition (1989-1990), the distribution of chlorophyll *a*, phaeopigments, zooplankton and physico-chemical parameters in the surface waters from Southern Indian Ocean were studied out by Jiyalalram and Goswami (1993). Sarno *et al*. (1993) investigated the phytoplankton population at Fusaro lagoon (Mediterranean Sea) from 1989 to 1990 and calculated species composition, temporal succession and standing stock of the different species.

An investigation on the phytoplankton community structure in the Arabian Sea during and after the SW monsoon during 1994 was carried out by Tarran *et al*. (1999) and they reported that during the SW monsoon period Prochlorophytes were restricted to oligotrophic stratified waters, but these algae were found at all stations along the transect during the inter-monsoon as a dominating phytoplankton standing stocks in the oligotrophic region. Shalapyonok *et al.* (2001) observed that phytoplankton community composition in the Arabian Sea is highly variable, both spatially and temporally.

Sarojini and Nittala (2001) investigated the vertical distribution in phytoplankton in the upper 200m water column at five stations around the Andaman Islands. Gowda *et al.* (2001) examined the variations in abundance and distribution of phytoplankton in the Nethravathy estuary; diatoms were the dominant components of phytoplankton followed by green algae, dinoflagellates, blue green algae and silicoflagellates. Nedumaran *et al.* (2001) studied the ecology of phytoplankton of Vellar estuary and adjoining brackish water systems along the east coast of India and found that density of phytoplankton often changes and each species appears to have its own peak period in different estuaries.

Huang *et al*. (2004) examined the taxonomic composition, abundance, and spatial distribution of phytoplankton in the Pearl River estuary during cruises of July 1999 and January 2001 and found that there was no major difference in the phytoplankton species composition between monsoon seasons, and inter monsoon. Cermeno *et al*. (2006) noted that large sized phytoplankton have greater potential to export organic matter through a short, classical food chain, whereas the small-sized phytoplankton are utilized by complex microbial food webs that favour the recycling of organic matter.

Abundance and seasonal variations of phytoplankton in the creek waters of western mangrove of Kachchh-Gujarat was studied in 2008 by Saravanakumar *et al*. Vengadesh *et al*. (2009) studied the seasonal variations of plankton diversity in the Kaduviyar estuary, southeast coast of India. Madhu *et al*. (2010a) studied the short term variability of water quality and its implications on phytoplankton production in the Cochin estuary and pointed out that the transient variations in the water quality play a significant role on phytoplankton behaviour. Madhu *et al*. (2010b) also studied the monsoon-induced changes in the size-fractionated phytoplankton biomass and production rate in the estuarine and coastal waters of southwest coast of India and pointed out that the heavy cloud cover and increased water column turbidity not only limit the growth of large-sized phytoplankton in the estuary and coastal waters but also supported the proliferation of nanoplankton community during the monsoon season.

Alkawri and Ramaiah (2011) studied the distribution of diatom *Pseudo-nitzschia* and dinoflagellates of *Dinophysis* spp. along the coast off Goa and noted that out of 179 species of phytoplankton observed 11 of them are

potentially toxic. Fehling *et al.* (2012) studied the relationship between phytoplankton distribution and water column characteristics in northwest European shelf sea waters and observed that temperature, salinity, water density and inorganic nutrient concentrations have a significant relation with phytoplankton community composition. Sivaprasad *et al.* (2013) studied the seasonal stratification and property distributions of Cochin estuary, southwest coast of India, which established a strong connection with the distribution of chemical and biological parameters.

4.2.2 Physico-chemical parameters

Dyer (1973) classified the estuaries into three types based on their longitudinal salinity distribution: partially mixed, vertically homogeneous or well-mixed, and highly stratified or salt wedge type. Tilman *et al.* (1982) pointed out that if the supply of nutrients is less than the uptake by phytoplankton, nutrient concentrations decrease and limit additional growth of phytoplankton. Smith (1990) noted that phosphorus would be limiting in marine coastal waters only when large nutrient loads with high N: P ratios reach coastal waters.

Oviatt *et al.* (1986) documented that conditions like high pH, high phytoplankton production, and low oxygen are the characteristic of nutrient enriched systems and are often found in coastal waters. De-Pauw and Naessens (1991) studied the nutrient induced competition between species of marine diatoms and pointed out that elemental ratio of nitrate, phosphate and silicate from water samples can sometimes be used as an indicator of the status of nutrient loading or to predict productivity.

Fisher *et al.* (1992) reported that the limiting nutrient concentrations on abundance and the distribution of phytoplankton vary with season, location and phytoplankton community structure. Oviatt *et al.* (1995) reported that for most marine coastal waters, nitrogen is the most limiting nutrient for phytoplankton production. Different diatom species have different demands for Si compared to P (Si: N = 96:1 to 1:1) (Hecky and Kilham, 1988). Diatoms with a low Si: P demand (around 1) would need very small amounts of Si compared to N even if N: P demand is 16:l, the Redfield ratio (Graneli *et al.*, 1999).

Smith *et al.* (1999) pointed out that in coastal waters, increasing nutrient enrichment accompanied by a variation in nutrient ratio due to disproportionate inputs of nutrients would profoundly affect the phytoplankton species composition and production. Falkowski (2000) observed that oceanic cycles of life's essential elements carbon, nitrogen and phosphorus are closely coupled through the metabolic requirements of phytoplankton, in the average proportions of C: N: P = 106:16:1, the Redfield ratio.

Turner *et al.* (2003) carried out an important study on the fluctuating Si: N ratios and coastal plankton food webs especially on the diatom growth in the coastal ecosystem and found that marine diatoms require dissolved silicate to form an external shell, and their growth becomes Si-limited when the atomic ratio of silicate to dissolved inorganic nitrogen (Si: N) approaches 1:1. Yin (2002) studied the monsoonal influence on seasonal variations in the nutrients and phytoplankton biomass in the coastal waters of Hong Kong in the vicinity of Pearl River estuary and observed that the low phosphorus concentrations play an important role in controlling phytoplankton biomass production during spring and summer.

Klausmeser *et al*. (2004) reported that the canonical Redfield N: P ratio of 16 is not a universal biochemical optimum and optimal N: P ratios will vary from 8.2 to 45.0, depending on the ecological conditions. Aktan *et al.* (2005) emphasised that based on the study of the qualitative and quantitative characteristics of phytoplankton community structure and the environmental factors that affect its distribution and the changes in Izmit Bay, a eutrophic bay of Marmara Sea, the primary production was mainly influenced by nutrients like nitrogen and silicate by acting as a limiting factor.

Jouenne *et al*. (2005) pointed out that change in taxonomic composition according to tidal forcing led to variations in primary production levels based on the study of biological and physico-chemical factors controlling short-term variability in phytoplankton primary production and photosynthetic parameters in eastern English Channel. The distributions of phytoplankton taxa and biomass was recorded by Calliari *et al.* (2005), to assess their association to environmental variables in the Rio de la Plata, a shallow and highly turbid estuary. Sanilkumar (2009) in his pioneer work on microalgae along the southwest coast of India observed that the planktonic microalgal standing crop and chlorophyll *a* values are well supported by the higher Si: N ratios along the coastal and estuarine stations.

Joseph and Ouseph (2010) reported that the run off components like municipal waste discharge and riverine water carrying industrial and agricultural wastes are responsible for nutrient enrichment influencing the estuarine water quality. Boyce *et al*. (2010) studied the trend on global phytoplankton abundance and stated that global phytoplankton concentration has declined over the past century and the fluctuations of phytoplankton abundance were strongly correlated

with climate change, particularly increasing sea surface temperature. Successional shifts in microalgal community structure are mainly influenced by changes in environmental variables such as nutrients and other physico-chemical variables which mainly influence the distribution and abundance of plankton communities in estuaries (Madhu *et al.,* 2007). Sankar and Padmavati (2012) reported the higher phytoplankton densities and low diversity in harbour areas as compared to coastal waters based on the study of species composition, abundance and distribution of phytoplankton in the harbour areas and coastal waters of Port Blair, South Andaman. George *et al.* (2012) studied the influence of hydro-chemical parameters on phytoplankton distribution along Tapi estuarine area of Gulf of Khambhat, India and pointed out that physico-chemical properties play a major role in determining the density, diversity and occurrence of phytoplankton in an estuarine ecosystem.

4.2.3 Primary production

A pioneer attempt to understand primary production in tropical marine areas was made by Steemann (1959) who observed that the estimation of oceanic production is easy by seeing the colour of the sea in which the deep blue sea is the most unproductive water. Ryther *et al.* (1966) proved that the Western Indian Ocean is one of the most productive regions in the world, after an extensive measurement of primary production on board the *Anton Bruun*.

Qasim and Reddy (1967) studied the plant pigments of Cochin backwaters during the monsoon season and pointed out the inconsistency in their relationship, because the extract contained high amount of dead chlorophylls and their derivatives coming from the detritus and stirred up

sediments. Qasim *et al.* (1968) studied solar radiation and its penetration in tropical estuaries and observed that the zone of optimum illumination for maximum photosynthesis was 0 and 1.5 m depth.

Nair *et al.* (1968) pointed out that the potential production of phytoplankton in terms of carbon for the west coast as 46×10^6 tons and for east coast of India as 15×10^6 tons. The nutrient influx of the Cochin estuary in relation to environmental characteristics was studied by Sankaranarayanan and Qasim (1969). Qasim *et al.* (1969) estimated the organic production of tropical estuaries and found that annual gross production fell within the range of 273 to 293 gC/m^2/yr and the net production ranged from 184 to 202 gC/m^2/yr.

Nair *et al.* (1975) made an investigation on primary production in the Vembanad Lake and observed that the maximum production was during pre- and post-monsoon period, unlike the adjacent marine environment. The annual gross production ranged from 150-650 gC/m^2/day. The estimated total organic production in the Vembanad Lake was 10,00,000 tons of carbon.

The plankton production in relation to the environmental parameters in Vembanad Lake was studied by Pillai *et al.* (1975) and they noted the relative influence of environmental factors such as temperature, salinity, dissolved oxygen and nutrients on the plankton production. Seasonal fluctuations in the abundance of phytoplankton in the Cochin backwaters were studied for a period of one year by Kumaran and Rao (1975) who reported that phytoplankton production was high and moderately stable during the pre-monsoon. *Skeletonema costatum* was the dominant diatom with the highest

percentage of the total cell density. They also noticed a sudden brief shoot in the plankton abundance soon after or following a break in monsoon.

Nair and Gopinathan (1981) investigated the primary productivity of the exclusive economic zone (EEZ) of India and the factors governing phytoplankton production. Phytoplankton pigments in relation to primary production and nutrients in the inshore waters of Tuticorin, southeast coast of India were studied by Gopinathan *et al.*, in 1994. Menon *et al.* (2000) studied the primary production in Cochin estuary and identified it as one of the most productive estuarine systems along the west coast of India.

Redekar and Wagh (2000) pointed out on the basis of study on growth of fouling diatoms from the Zuari estuary, west coast of India, that different hydrographical parameters like pH, temperature, salinity and solar radiation determine the diversity, distribution, succession and abundance of phytoplankton. Distribution of chlorophyll *a* and *b* in the 40 to 200 m depth zone of Varaval coastal waters in the pre-monsoon was documented by Gopinathan *et al.* (2001).

Alkershi *et al.* (2003) studied the contribution of size fractions of planktonic algae to the primary organic productivity in the coastal waters off Cochin. Chlorophyll *a* was found to be the most abundant of all pigments, followed by fucoxanthin, along the southwest coast of India towards the end of upwelling season, on the basis of the study conducted on the spatial variation of phytoplankton pigments by Roy *et al.* (2006). Madhu *et al.* (2006) investigated the western Bay of Bengal during summer, winter, and spring inter-monsoon periods and found that there was a lack of pronounced seasonal variation in the phytoplankton standing stock (chlorophyll *a*) and

primary production. Phytoplankton community structure and primary production in small intertidal estuarine-bay ecosystem (eastern English Channel, France) was studied by Jouenne *et al*. (2007). Shevchenko *et al.* (2013) studied the phytoplankton of the Amur River Estuary (Sea of Okhotsk) during the summer periods of 2005–2007 and noted that the increased nutrient input via the river run off caused the high cell density and biomass of phytoplankton.

4.3 Result

4.3.1 Qualitative and quantitative distribution of planktonic microalgae

4.3.1.1 Station 1. Azheekode

During 2009-2010, the highest standing crop was found in the monsoon season (8×10^7 cellsL^{-1}) which was due to a monospecific bloom of *Prymnesium parvum* N. Carter and the lowest cell abundance was in the post-monsoon season, with 372 cellsL^{-1}. In the year 2010-2011, the cell density was found to be highest in the post-monsoon season (3034 cellsL^{-1}), whereas lowest cell density of 764 cellsL^{-1} was found in the pre-monsoon season. *Thalassionema* sp. was found to be dominant among diatoms, whereas *Biceratium furca* was found to be dominant among dinoflagellates (Table 4). The Shannon Weiner diversity index (H') was found to be high during post-monsoon 2011 (Table 5) and the Bray Curtis similarity Dendrogram of planktonic microalgal abundance (Fig.19) showed 35% similarity between pre-monsoon 2009 and monsoon 2010. The monsoon 2009 behaved entirely different from all other seasons due to the bloom of *Prymnesium parvum* N. Carter which showed 100% monospecific standing crop.

Table 4 Qualitative and quantitative distribution of planktonic microalgae at station 1 (Azheekode) during 2009-2011

Sl No	Class	2009-2010			2010-2011		
		PRM	MON	POM	PRM	MON	POM
Prymnesiophyceae							
1	*Prymnesium parvum*		8×10^7				
	Total		**8×10^7**				
Bacillariophyceae							
1	*Asterionellopsis glacialis*				48		167
2	*Biddulphia rhombus*				48		
3	*Chaetoceros curvisetus*						107
4	*Chaetoceros danicus*					90	
5	*Chaetoceros decipiens*	80				150	134
6	*Chaetoceros didymus*						80
7	*Coscinodiscus asteromphalus*	80			32		80
8	*Coscinodiscus radiatus*				28		
9	*Cylindrotheca closterium*				32		167
10	*Cylindrotheca gracilis*			16			333
11	*Entomoneis alata*						80
12	*Gyrosigma acuminatum*						27
13	*Leptocylindrus danicus*				32		
14	*Nitzschia sigma*						60
15	*Odontella aurita*				24		100
16	*Paralia sulcata*				164		
17	*Petroneis marina*			16			
18	*Planktoniella sol*				12		
19	*Pleurosigma aestuarii*				24		147
20	*Pleurosigma angulatum*			44			
21	*Proboscia alata*					72	
22	*Skeletonema costatum*				132		
23	*Surirella fluminensis*			64			
24	*Surirella striatula*						53
25	*Thalassionema frauenfeldii*						500
26	*Thalassionema nitzschioides*						400
27	*Thalassiosira coramandeliana*			13			
28	*Thalassiosira subtilis*			10	32		100
29	*Trieres mobiliensis*	64			20	60	
	Total	**224**		**163**	**628**	**372**	**2535**

Dinophyceae							
1	*Alexandrium monilatum*	40					
2	*Biceratium furca*	1040		83	12		266
3	*Dinophysis caudata*	220					
4	*Diplopsalis lenticula*			19			
5	*Prorocentrum gracile*	60		43	24	240	
6	*Prorocentrum lima*				28	336	
7	*Prorocentrum micans*	12					
8	*Protoperidinium conicum*						20
9	*Protoperidinium oblongum*			38			
10	*Protoperidinium oceanicum*			26	72	66	66
11	*Pyrophacus horologium*	28				48	
12	*Pyrophacus steinii*						80
	Total	**1400**	**0**	**209**	**136**	**690**	**432**
Dictyochophyceae							
1	*Dictyocha fibula*						67
	Total						**67**
	***Grand total**	**1624**	**8×10^7**	**372**	**764**	**1062**	**3034**
***CellsL^{-1}**							

Table 5 Diversity indices of planktonic microalgae at station 1 (Azheekode) during 2009-2011

Diversity indices of planktonic microalgae at station 1						
Seasons	**S**	**N**	**d**	**J'**	**H'(log2)**	**1-Lambda'**
PRM2009	9	96	1.754	0.8958	2.84	0.8317
MON2009	1	8944	0	****	0	0
POM2010	11	61	2.436	0.9774	3.381	0.9141
PRM2010	17	106	3.431	0.9756	3.988	0.9408
MON2010	8	87	1.568	0.9715	2.915	0.8692
POM2011	21	234	3.667	0.9742	4.279	0.9485

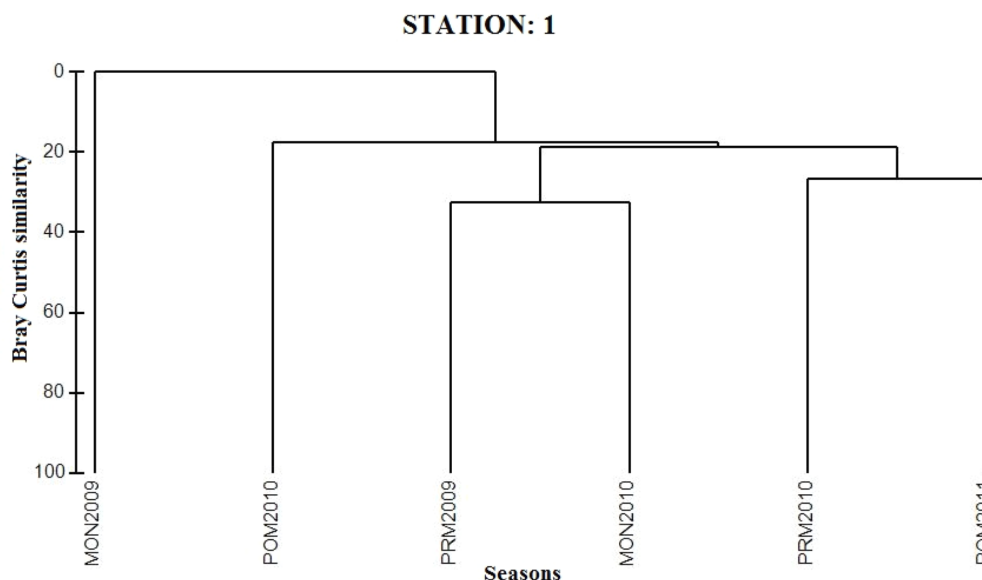


Fig.19 Bray Curtis similarity Dendrogram of planktonic microalgal composition at station 1 (Azheekode) during 2009-2011

4.3.1.2 Station 2. Balathuruth

The highest cell abundance of 2369 cellsL^{-1} was recorded in the pre-monsoon season, whereas lowest cell abundance of 394 cellsL^{-1} was in the monsoon season during 2009-10. During 2010-11, the monsoon season recorded the highest cell abundance of 939 cellsL^{-1} and the lowest of 536 cellsL^{-1} was recorded in the post-monsoon season. *Biceratium furca* was the dominant species among dinoflagellates. Among diatoms, *Asterionellopsis glacialis* was found to be predominant in both the years. Chlorophycean members were represented by ten genera (Table 6). Highest diversity index (H') was found during the pre-monsoon 2010 period (Table 7) where the diatom species showed the predominance. Bray Curtis similarity Dendrogram showed 35% similarity of planktonic microalgal abundance between pre-monsoon and post-monsoon 2010 (Fig.20).

Table 6 Qualitative and quantitative distribution of planktonic microalgae at station 2 (Balathuruth) during 2009-2011

Sl No	Class	2009-2010			2010-2011		
		PRM	MON	POM	PRM	MON	POM
Chlorophyceae							
1	*Cosmarium quadrilaterum*		12				
2	*Micrasterias foliacea*		48				
3	*Neochlorosarcina minor*		16				
4	*Oedogonium* sp		12				
5	*Pediastrum duplex*		16				
6	*Spirogyra* sp		12				
7	*Staurastrum asteroideum*		24				
	Total		**140**				
Bacillariophyceae							
1	*Halamphora coffeaeformis*			10			
2	*Asterionellopsis glacialis*			115	150		
3	*Biddulphia sinensis*			26			
4	*Campylodiscus ecclesianus*	43		48	54		
5	*Chaetoceros affinis*			38			
6	*Chaetoceros danicus*			58	21		
7	*Coscinodiscus asteromphalus*		20	10			23
8	*Coscinodiscus centralis*		96			70	
9	*Coscinodiscus marginatus*				24		
10	*Coscinodiscus radiatus*	54		42			
11	*Cyclotella meneghiniana*				30	63	
12	*Cylindrotheca closterium*					19	168
13	*Cylindrotheca gracilis*				12		92
14	*Diploneis crabro*						92
15	*Ditylum brightwelli*				48		
16	*Entomoneis alata*				33		
17	*Gyrosigma acuminatum*				66	89	
18	*Gyrosigma fasciola*		8				
19	*Gyrosigma tenuissimum*			22			
20	*Licmophora flabellata*				15		
21	*Licmophora juergensii*		24				
22	*Lyrella lyra*						46
23	*Navicula hasta*					32	

24	*Nitzschia palea*				24		23
25	*Nitzschia sigma*				18	127	
26	*Odontella aurita*				24		
27	*Odontella longicruris*	40			18		
28	*Paralia sulcata*	29					
29	*Planothidium hauckianum*		5				
30	*Pleurosigma aestuarii*		17			127	
31	*Pleurosigma angulatum*	54					
32	*Pleurosigma falx*					114	
33	*Pleurosigma normanii*	79					
34	*Rhizosolenia hebetata*			27			
35	*Surirella fastuosa*	162			165	127	
36	*Surirella fluminensis*			54			
37	*Surirella striatula*		24		126		
38	*Thalassionema frauenfeldii*						92
39	*Thalassionema nitzschioides*		20	51	12		
40	*Thalassiosira coramandeliana*			10	15		
41	*Thalassiosira subtilis*		26				
42	*Trieres mobiliensis*		14		39	95	
43	*Trieres regia*	72					
	Total	**533**	**254**	**511**	**894**	**863**	**536**
Dinophyceae							
1	*Biceratium furca*	1566			9		
2	*Ceratium fusus*	36					
3	*Dinophysis caudata*	54					
4	*Diplopsalis lenticula*			10			
5	*Prorocentrum compressum*	72					
6	*Protoperidinium oceanicum*	54					
7	*Protoperidinium steinii*	40					
8	*Pyrophacus steinii*				24		
	Total	**1822**		**10**	**33**		
Dictyochophyceae							
1	*Dictyocha crux*	14				76	
	Total	**14**				**76**	
	***Grand total**	**2369**	**394**	**521**	**927**	**939**	**536**
$*CellsL^{-1}$							

Table 7 Diversity indices of planktonic microalgae at station 2 (Balathuruth) during 2009-2011

Diversity indices of planktonic microalgae at station 2						
Seasons	**S**	**N**	**d**	**J'**	**H'(log2)**	**1-Lambda'**
PRM2009	15	142	2.825	0.9119	3.563	0.8886
MON2009	17	77	3.686	0.979	4.002	0.9455
POM2010	14	80	2.966	0.9741	3.709	0.9303
PRM2010	21	127	4.132	0.9687	4.255	0.9496
MON2010	11	99	2.178	0.9862	3.412	0.9129
POM2011	7	58	1.477	0.9708	2.725	0.856

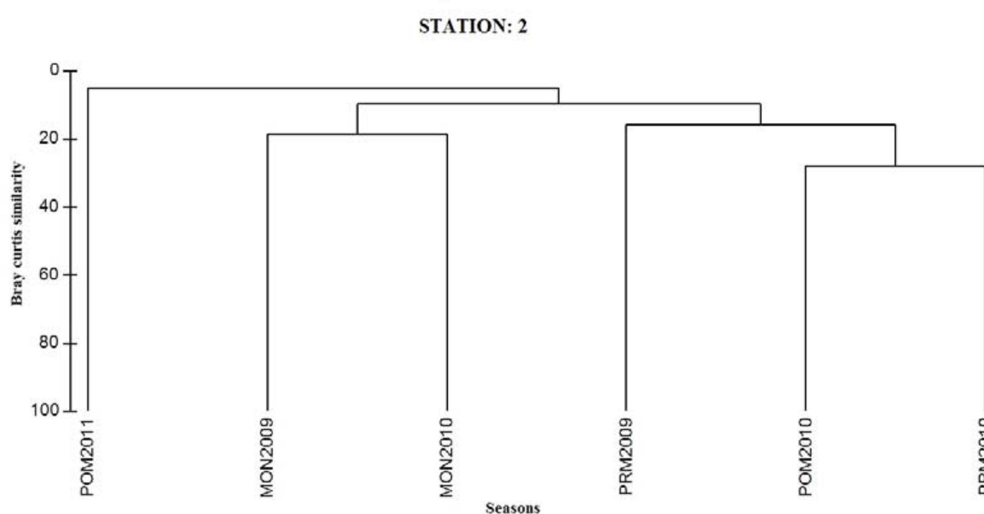


Fig.20 Bray Curtis similarity Dendrogram of planktonic microalgal composition at station 2 (Balathuruth) during 2009-2011

4.3.1.3 Station 3. Kodikkal

In 2009-10, highest cell density (7608 cellsL^{-1}) was recorded in the pre-monsoon while the lowest was (332 cellsL^{-1}) in the monsoon season, whereas in 2010-11 highest standing crop of 1239 cellsL^{-1} was observed during the post-monsoon and the lowest of 319 cellsL^{-1} during the pre-monsoon. Among dinoflagellates *Biceratium furca* was found in all the seasons in the entire

period whereas, among diatoms the predominant species was *Coscinodiscus asteromphalus* (Table 8). Pre-monsoon 2010 showed highest diversity index (H') than other seasons (Table 9). Bray Curtis similarity Dendrogram of planktonic microalgal abundance (Fig.21) indicated 35% similarity between monsoon 2009 and post-monsoon 2010, and 33% similarity between pre-monsoon 2010 and post-monsoon 2011. Pre-monsoon 2009 showed least resemblance to other seasons due to the high abundance of dinoflagellate *Biceratium furca*.

Table 8 Qualitative and quantitative distribution of planktonic microalgae at station 3 (Kodikkal) during 2009-2011

Sl No.	Class	2009-2010			2010-2011		
		PRM	MON	POM	PRM	MON	POM
Cyanophyceae							
1	*Trichodesmium erythraeum*	36					
	Total	**36**					
Bacillariophyceae							
1	*Achnanthes brevipes*	48					
2	*Amphiprora gigantea*				20		
3	*Asterionellopsis glacialis*			31			
4	*Campylodiscus ecclesianus*			10			73
5	*Chaetoceros affinis*			28		142	
6	*Chaetoceros decipiens*					198	
7	*Chaetoceros lorenzianus*					227	
8	*Coscinodiscus asteromphalus*				18	312	40
9	*Coscinodiscus centralis*		55	10			
10	*Coscinodiscus radiatus*		11		8		
11	*Cyclotella meneghiniana*		15				47
12	*Cylindrotheca gracilis*			19	18		113
13	*Ditylum brightwelli*				8		
14	*Ditylum sol*				45		
15	*Grammatophora marina*						40
16	*Gyrosigma acuminatum*				9		40
17	*Gyrosigma tenuissimum*			12			
18	*Nitzschia sigma*				12		

19	*Odontella aurita*				17		
20	*Odontella longicruris*		11				
21	*Planktoniella sol*				5		
22	*Pleurosigma aestuarii*						80
23	*Pleurosigma falx*		13	12		113	
24	*Proboscia alata*					34	
25	*Rhizosolenia hebetata*				15		20
26	*Skeletonema costatum*					91	
27	*Surirella fastuosa*						113
27	*Surirella fluminensis*			48			
29	*Surirella striatula*	36					
30	*Thalassionema nitzschioides*				95		240
31	*Thalassiosira coramandeliana*					40	
32	*Trieres mobiliensis*						80
	Total	**84**	**105**	**170**	**270**	**1157**	**886**
Dinophyceae							
1	*Biceratium furca*	7200	11	180	12	153	313
2	*Ceratium fusus*		88	36		*227*	
3	*Ceratium gibberum*		11				
4	*Ceratium trichoceros*		7				
5	*Ceratium vultur*		18			57	
6	*Dinophysis acuminata*		13				
7	*Dinophysis caudata*	80	55	17		85	
8	*Diplopsalis lenticula*	32		14			
9	*Prorocentrum gracile*	84					
10	*Prorocentrum lima*	60					
11	*Prorocentrum micans*					56	
12	*Protoperidinium compressum*			26			
13	*Protoperidinium oblongum*						
14	*Protoperidinium oceanicum*			7	20		
15	*Protoperidinium pellucidum*			24			
16	*Pyrophacus steinii*		24	48		85	
	Total	**7456**	**227**	**352**	**32**	**663**	**313**
Dictyochophyceae							
1	*Dictyocha crux*	32			6		40
2	*Dictyocha fibula*				11		
	Total	**32**			**17**		**40**
	***Grand total**	**7608**	**332**	**522**	**319**	**1820**	**1239**

***CellsL^{-1}**

Table 9 Diversity indices of planktonic microalgae at station 3 (Kodikkal) during 2009-2011

Diversity indices of planktonic microalgae at station 3						
Seasons	**S**	**N**	**d**	**J'**	**H'(log2)**	**1-Lambda'**
PRM2009	9	141	1.617	0.6793	2.153	0.6215
MON2009	13	60	2.927	0.968	3.582	0.9242
POM2010	16	82	3.402	0.9655	3.862	0.9342
PRM2010	16	65	3.587	0.9712	3.885	0.9399
MON2010	14	152	2.588	0.9804	3.733	0.9273
POM2011	13	118	2.518	0.9704	3.591	0.9181

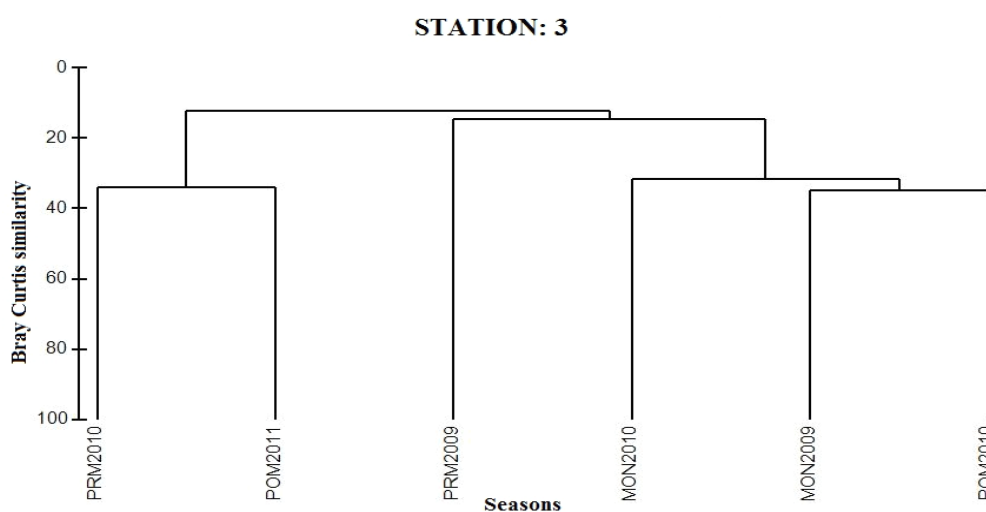


Fig.21 Bray Curtis similarity Dendrogram of planktonic microalgal composition at station 3 (Kodikkal) during 2009-2011

4.3.1.4 Station 4. Mahe

In 2009-10, highest cell abundance (516 cellsL^{-1}) was recorded during the post-monsoon season, whereas the lowest (82 cellsL^{-1}) was recorded in the monsoon season. In the second year, the highest cell density was recorded in the post-monsoon with 1628 cellsL^{-1} and the lowest of 648 cellsL^{-1} was recorded in the monsoon season. Among diatoms, *Surirella fastuosa* and

Cylindrotheca gracilis were found to be dominant. *Biceratium furca* was found to be predominant among dinoflagellates (Table 10). Highest diversity index (H') was found in the pre-monsoon 2010 where the diatom species showed the predominance (Table 11). Bray Curtis similarity Dendrogram of planktonic microalgal abundance (Fig.22) showed 25% similarity between pre-monsoon 2009 and post-monsoon 2010 and 23% similarity between monsoon 2010 and post-monsoon 2011.

Table 10 Qualitative and quantitative distribution of planktonic microalgae at station 4 (Mahe) during 2009-2011

Sl No.	Class	2009-2010			2010-2011		
		PRM	MON	POM	PRM	MON	POM
Bacillariophyceae							
1	*Aneumastus maculosus*	22					
2	*Asterionellopsis glacialis*				117		
3	*Biddulphia rhombus*			17	95		
4	*Biddulphia sinensis*			12			
5	*Chaetoceros decipiens*					186	205
6	*Chaetoceros danicus*				35		
7	*Coscinodiscus asteromphalus*		29	19		96	220
8	*Coscinodiscus marginatus*	90					
9	*Coscinodiscus radiatus*				48	96	
10	*Cylindrotheca closterium*			17			
11	*Cylindrotheca gracilis*						477
12	*Detonula pumila*				65		
13	*Ditylum brightwelli*				52		
14	*Entomoneis alata*					60	
15	*Licmophora flabellata*						29
16	*Mastogloia exigua*						51
17	*Melosira numuloides*				69		
18	*Navicula distans*	58					
19	*Nitzschia palea*		6		13		
20	*Nitzschia sigma*						51

21	*Odontella aurita*					90	
22	*Odontella longicruris*				43		
23	*Pleurosigma aestuarii*	44					125
24	*Pleurosigma angulatum*		19				
25	*Proboscia alata*				48		
26	*Psammodictyon panduriforme*	72					
27	*Rhizosolenia hebetata*				43		
28	*Skeletonema costatum*				74		
29	*Surirella fastuosa*	90		384	65		147
30	*Surirella striatula*					60	
31	*Thalassionema frauenfeldii*						74
32	*Thalassionema nitzschioides*	44		12	9		
33	*Thalassiosira subtilis*						51
34	*Trieres mobiliensis*				56		
	Total	**420**	**54**	**461**	**832**	**588**	**1430**
Dinophyceae							
1	*Biceratium furca*			12	65		51
2	*Dinophysis caudata*		28				
3	*Prorocentrum gracile*						59
4	*Prorocentrum micans*			7	52		
5	*Protoperidinium oceanicum*			26			
6	*Pyrophacus steinii*					60	
	Total		**28**	**45**	**117**	**60**	**110**
Dictyochophyceae							
1	*Dictyocha crux*			10			
2	*Dictyocha fibula*						88
	Total			**10**			**88**
	***Grand total**	**420**	**82**	**516**	**949**	**648**	**1628**
***CellsL^{-1}**							

Table 11 Diversity indices of planktonic microalgae at station 4 (Mahe) during 2009-2011

Diversity indices of planktonic microalgae at Station 4						
Seasons	**S**	**N**	**d**	**J'**	**H'(log2)**	**1-Lambda'**
PRM2009	7	53	1.511	0.9878	2.773	0.867
MON2009	4	17	1.048	0.971	1.942	0.7762
POM2010	10	54	2.261	0.8855	2.941	0.8353
PRM2010	17	123	3.325	0.9873	4.036	0.945
MON2010	7	66	1.432	0.9897	2.779	0.8642
POM2011	13	134	2.45	0.9686	3.584	0.9161

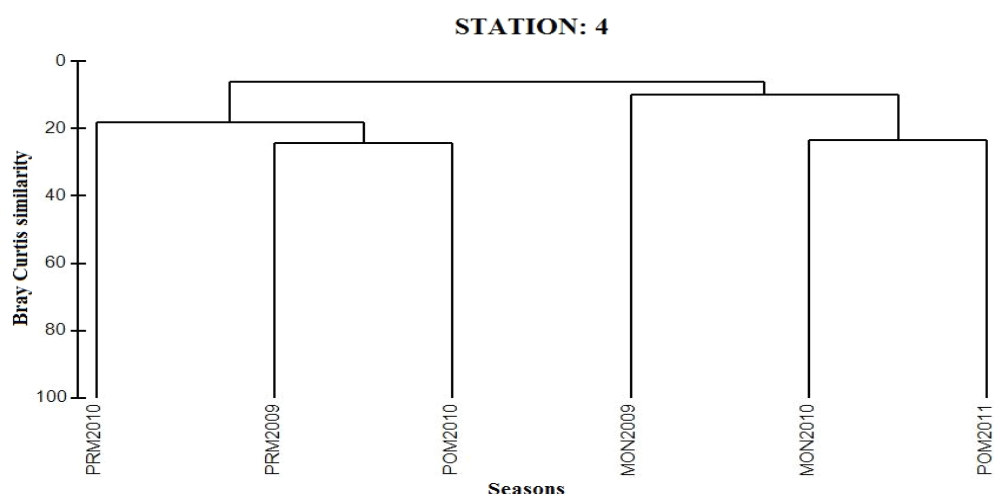


Fig.22 Bray Curtis similarity Dendrogram of planktonic microalgal composition at station 4 (Mahe) during 2009-2011

4.3.1.5 Station 5. Puthiyangadi

In 2009-10, highest standing crop was observed in the pre-monsoon period (2277 cellsL^{-1}) and the lowest of 326 cellsL^{-1} in the monsoon season. However, in 2010-11, the highest recorded cell abundance was 2198 cellsL^{-1} in the pre-monsoon season and the lowest of 354 cellsL^{-1} in the monsoon season. *Biceratium furca* was found to be predominant among dinoflagellates, whereas among diatoms, *Asterionellopsis* sp., *Biddulphia* sp., *Trieres* sp., *Coscinodiscus* sp.

and *Proboscia* sp. were predominant (Table 12). Shannon Weiner diversity index (H') was found to be high during the pre-monsoon 2010 season (Table 13) and the Bray Curtis similarity Dendrogram of planktonic microalgal abundance (Fig.23) showed 35% similarity among pre-monsoon 2009 and 2010.

Table 12 Qualitative and quantitative distribution of planktonic microalgae at station 5 (Puthiyangadi) during 2009-2011

Sl No.	Class	2009-2010			2010-2011		
		PRM	MON	POM	PRM	MON	POM
Bacillariophyceae							
1	*Asterionellopsis glacialis*				107	107	
2	*Biddulphia rhombus*				400		
3	*Cerataulina bicornis*						112
4	*Chaetoceros affinis*			72	59		
5	*Chaetoceros decipiens*					14	150
6	*Coscinodiscus asteromphalus*		30		32	40	
7	*Coscinodiscus marginatus*		14		69		
8	*Coscinodiscus nitidus*	34					
9	*Coscinodiscus radiatus*				80	32	
10	*Cyclotella meneghiniana*						67
11	*Cyclotella striata*					40	
12	*Cylindrotheca closterium*						172
13	*Cylindrotheca gracilis*		22				
14	*Ditylum brightwelli*				59		
15	*Entomoneis alata*				5		
16	*Gyrosigma acuminatum*		20		37		
17	*Halamphora tumida*			20			
18	*Lyrella hennedyi*			48			
19	*Lyrella lyra*		10	40			
20	*Navicula hasta*			32			
21	*Navicula marina*	51					
22	*Nitzschia palea*			44			
23	*Nitzschia sigma*		16				
24	*Odontella aurita*				43		
25	*Paralia sulcata*		12				
26	*Planktoniella sol*				59		

27	*Pleurosigma falx*	24	34				188
28	*Proboscia alata*	27			165		
29	*Rhizosolenia hebetata*				80	21	
30	*Skeletonema costatum*						32
31	*Surirella fastuosa*						112
32	*Surirella fluminensis*			60			
33	*Thalassionema frauenfeldii*						112
34	*Thalassionema nitzschioides*				32		
35	*Thalassiosira coramandeliana*		40				
36	*Thalassiosira subtilis*			32	37		
37	*Trieres mobiliensis*	102			187	35	
38	*Trieres regia*		12				
39	*Tropidoneis longa*						30
	Total	**238**	**210**	**348**	**1446**	**289**	**975**
Dinophyceae							
1	*Biceratium furca*	1496		84	400	27	
2	*Ceratium fusus*		24				
3	*Ceratium gibberum*	119					
4	*Ceratium symmetricum*	58					
5	*Ceratium trichoceros*	34					
6	*Ceratium tripos*					27	
7	*Dinophysis acuminata*		22				
8	*Dinophysis caudata*	27	50		59	11	
9	*Dinophysis sp*			12			
10	*Diplopsalis lenticula*		20	32			
11	*Prorocentrum gracile*	68			64		
12	*Prorocentrum lima*			100			
13	*Prorocentrum micans*			104			
14	*Protoperidinium oblongum*				69		
15	*Protoperidinium oceanicum*	136		120	53		
16	*Pyrophacus horologium*	85			107		
	Total	**2023**	**116**	**452**	**752**	**65**	
Dictyochophyceae							
1	*Dictyocha fibula*	16					82
	Total	**16**					**82**
	***Grand total**	**2277**	**326**	**800**	**2198**	**354**	**1057**
***CellsL^{-1}**							

Table 13 Diversity indices of planktonic microalgae at station 5 (Puthiyangadi) during 2009-2011

Diversity indices of planktonic microalgae at station 5						
Seasons	**S**	**N**	**d**	**J'**	**H'(log2)**	**1-Lambda'**
PRM2009	14	135	2.652	0.9058	3.449	0.8807
MON2009	14	66	3.106	0.9898	3.768	0.9389
POM2010	14	101	2.815	0.9825	3.741	0.9313
PRM2010	22	200	3.964	0.9691	4.322	0.9497
MON2010	10	57	2.23	0.9791	3.253	0.9055
POM2011	10	99	1.958	0.9829	3.265	0.9017

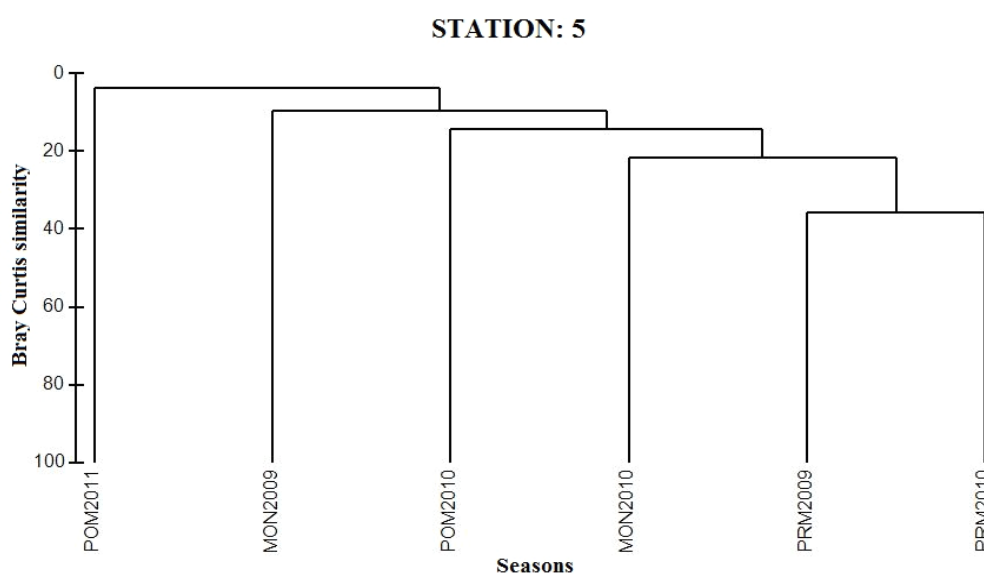


Fig.23 Bray Curtis similarity Dendrogram of planktonic microalgal composition at station 5 (Puthiyangadi) during 2009-2011

4.3.1.6 Station 6. Thykadapuram

In 2009-10, highest cell density of 664 cellsL^{-1} was in the pre-monsoon season, whereas the lowest of 149 cellsL^{-1} was in the post-monsoon period. In 2010-11, highest cell density (1493 cellsL^{-1}) was observed in post-monsoon

and the lowest cell density of 776 cellsL^{-1} was recorded in the pre-monsoon. Among dinoflagellates, *Biceratium furca* and *Dinophysis caudata* were found to be predominant. *Coscinodiscus radiatus* and *Pleurosigma falx* were found to be predominant among diatoms (Table 14). Pre-monsoon 2010 (Table 15) showed highest diversity index (H') and Bray Curtis similarity Dendrogram of planktonic microalgal abundance (Fig.24) showed 27% similarity between monsoon 2009 and post-monsoon 2010 and 20% similarity between pre-monsoon 2009 and monsoon 2010.

Table14 Qualitative and quantitative distribution of planktonic microalgae at station 6 (Thykadapuram) during 2009-2011

Sl No.	Class	2009-2010			2010-2011		
		PRM	MON	POM	PRM	MON	POM
Chlorophyceae							
1	*Pleurotaenium* sp.		10				
2	*Scenedesmus quadricauda*		8				
3	*Staurodesmus convergens*		7				
	Total		**25**				
Bacillariophyceae							
1	*Amphiprora gigantea*				26		
2	*Biddulphia rhombus*						101
3	*Biddulphia sinensis*				19		
4	*Campylodiscus ecclesianus*			11			
5	*Cerataulina bicornis*						44
6	*Chaetoceros decipiens*					60	
7	*Coscinodiscus asteromphalus*				32		101
8	*Coscinodiscus centralis*	30					
9	*Coscinodiscus marginatus*						44
10	*Coscinodiscus radiatus*	150	38		13	228	
11	*Cyclotella meneghiniana*				54		
12	*Cylindrotheca closterium*				26		
13	*Cylindrotheca gracilis*						133
14	*Diploneis elliptica*	120					
15	*Ditylum brightwelli*						19
16	*Gyrosigma acuminatum*	40					
17	*Gyrosigma fasciola*						19

18	*Lyrella hennedyi*		19				101
19	*Lyrella lyra*						70
20	*Navicula directa*		17				
21	*Navicula hasta*				56		
22	*Nitzschia longissima*		26				
23	*Nitzschia sigma*					60	
24	*Odontella longicruris*	30					
25	*Petroneis marina*						76
26	*Pleurosigma aestuarii*		14	29			133
27	*Pleurosigma falx*		36	43	32	120	127
28	*Podosira montagnei*		10			72	
29	*Proboscia alata*						95
30	*Psammodictyon panduriforme*	20					
31	*Rhaphoneis amphiceros*			18			
32	*Surirella fastuosa*	40					
33	*Surirella striatula*				40		82
34	*Thalassionema frauenfeldii*	8					
35	*Thalassionema nitzschioides*						114
36	*Thalassiosira coramandeliana*				43		
	Total	**438**	**160**	**101**	**341**	**540**	**1259**
Dinophyceae							
1	*Biceratium furca*	120		26	40		190
2	*Ceratium fusus*				69		
3	*Ceratium tripos*	30					
4	*Dinophysis acuminata*	30					
5	*Dinophysis caudata*					492	
6	*Dinophysis miles*				40		
7	*Prorocentrum gracile*	14					
8	*Prorocentrum micans*				43		
9	*Protoperidinium conicum*						44
10	*Protoperidinium oceanicum*			22	56		
11	*Pyrophacus horologium*					60	
12	*Pyrophacus steinii*	32			107	102	
	Total	**226**		**48**	**355**	**654**	**234**
Dictyochophyceae							
1	*Dictyocha crux*				11		
2	*Dictyocha fibula*				69		
	Total				**80**		
	***Grand total**	**664**	**185**	**149**	**776**	**1194**	**1493**
$*CellsL^{-1}$							

Table 15 Diversity indices of planktonic microalgae at station 6 (Thykadapuram) during 2009-2011

Diversity indices of planktonic microalgae at station 6						
Seasons	**S**	**N**	**d**	**J'**	**H'(log2)**	**1-Lambda'**
PRM2009	13	85	2.698	0.9667	3.577	0.9198
MON2009	10	41	2.419	0.9819	3.262	0.9136
POM2010	6	29	1.48	0.9882	2.554	0.8555
PRM2010	18	114	3.588	0.9874	4.117	0.9488
MON2010	8	90	1.555	0.9626	2.888	0.8624
POM2011	17	153	3.179	0.9853	4.027	0.9427

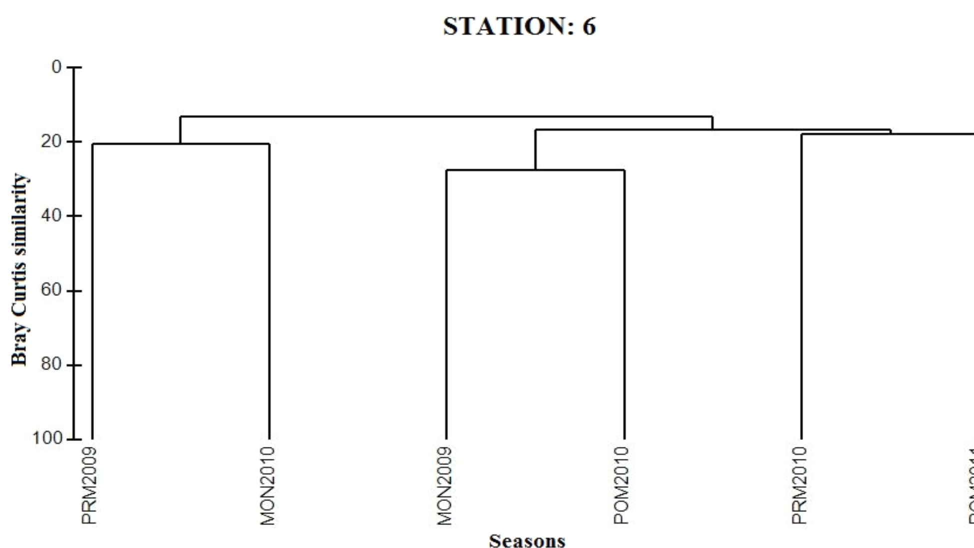


Fig.24 Bray Curtis similarity Dendrogram of planktonic microalgal composition at station 6 (Thykadapuram) during 2009-2011

4.3.2 Mean annual variation in standing crop

During 2009-10, the mean annual variation of standing crop was highest in station 1 (Azheekode) and the lowest was at station 6 with 333 (SD $\pm$288) cellsL^{-1}. The mean annual variation of the standing crop during

2010-11 was also highest at station 1 having 1620 (SD ±1234) cellsL^{-1}, whereas the lowest was at station 2 with 801(SD ±229) cellsL^{-1} (Fig.25).

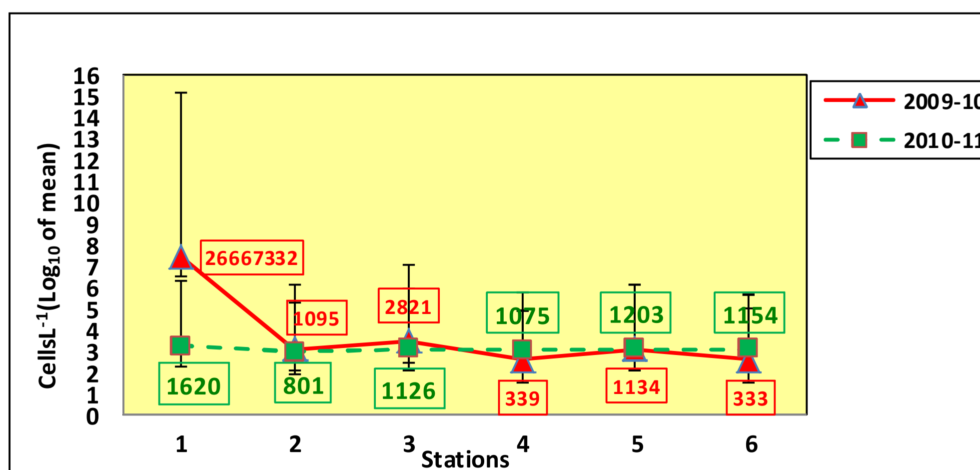


Fig. 25 Mean annual variation in standing crop: 2009-2011

4.3.3 Pigment composition

4.3.3.1 Chlorophyll *a*

In 2009-10, during pre-monsoon, chlorophyll *a* was found to be highest at station 3 (6.01 µgL^{-1}), whereas station 6 recorded the lowest (2.14 µgL^{-1}). In the monsoon season, the highest chlorophyll *a* value (13.54 µgL^{-1}) was at station 1 where a blooming of *Prymnesium parvum* was observed and lowest was at station 4 (1.10 µgL^{-1}). During the post-monsoon season, station 5 recorded the highest chlorophyll *a* value (4.32 µgL^{-1}) and station 6 showed the lowest of 1.68 µgL^{-1} (Fig.26).

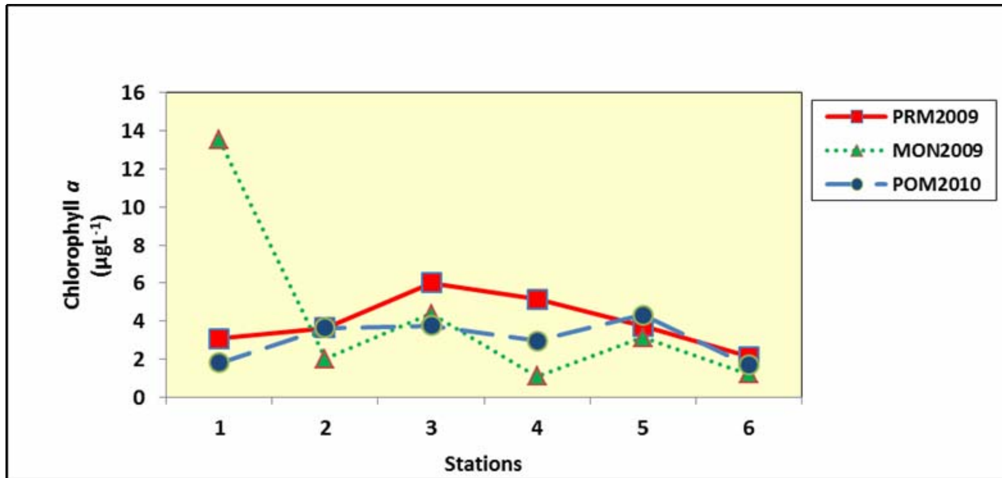


Fig. 26 Seasonal and spatial variation in chlorophyll *a:* 2009-2010

In the second year, the concentration of chlorophyll *a* was found to be highest at station 5 (10.46 μgL^{-1}), whereas lowest was recorded at station 3 (3.72 μgL^{-1}) in the pre-monsoon season. During the monsoon season, station 1 showed the highest chlorophyll *a* value (8.61 μgL^{-1}) and the lowest at station 5(1.95 μgL^{-1}). In the post-monsoon season, the highest chlorophyll *a* value recorded was 9.17 μgL^{-1} (station 1) and the lowest 2.82 μgL^{-1} at station 2 (Fig.27).

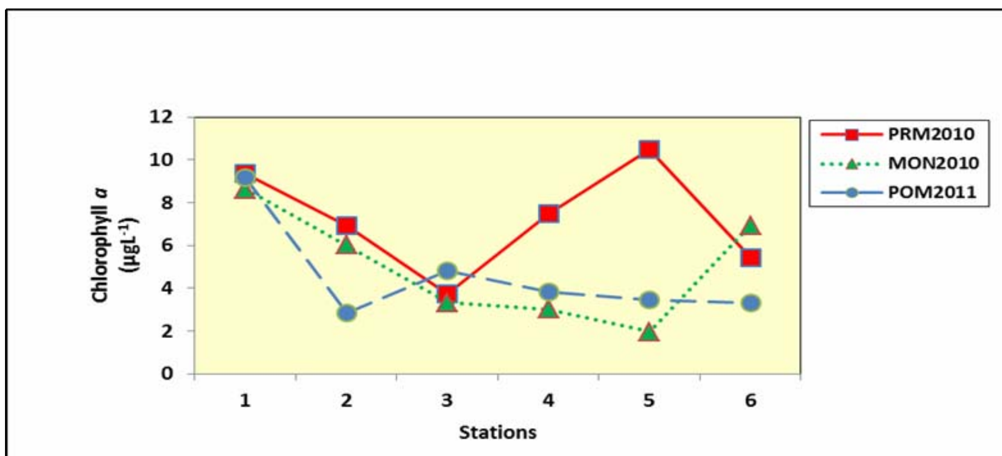


Fig. 27 Seasonal and spatial variation in chlorophyll *a*: 2010-2011

4.3.3.2 Chlorophyll *b*

During the first year, in the pre-monsoon season, the highest chlorophyll *b* value was recorded at station 5 (0.41 µgL-1), whereas station 6 recorded the lowest value (0.03 μgL^{-1}). In the monsoon season, the chlorophyll *b* value ranged from the lowest of 0.01 μgL^{-1} at station 1 to the highest of 1.58 μgL^{-1} at station 2. In the post-monsoon season, the highest chlorophyll *b* value was recorded at station 6 (0.29 μgL^{-1}) and the lowest value (0.04 μgL^{-1}) at station 5 (Fig.28).

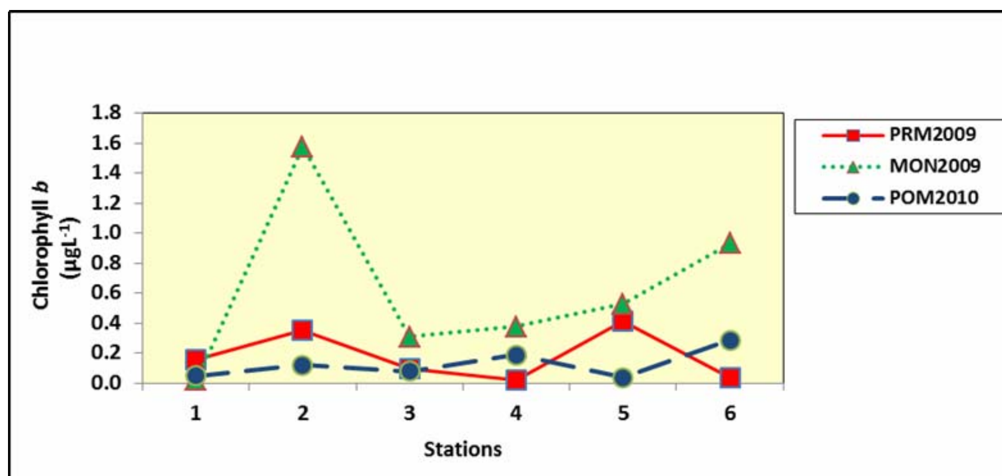


Fig. 28 Seasonal and spatial variation in chlorophyll *b*: 2009-2010

In 2010-11, the highest chlorophyll *b* value was at station 3 (1.18 μgL^{-1}) and the lowest at station 1 (0.24 μgL^{-1}) during the pre-monsoon season. In the monsoon season, station 6 recorded the highest chlorophyll *b* value (1.14 μgL^{-1}) and the lowest at station 5 (0.01 μgL^{-1}). During the post-monsoon season, station 5 had the highest value of chlorophyll *b* (0.94 μgL^{-1}) and the lowest of 0.01 μgL^{-1} at station 6 (Fig.29).

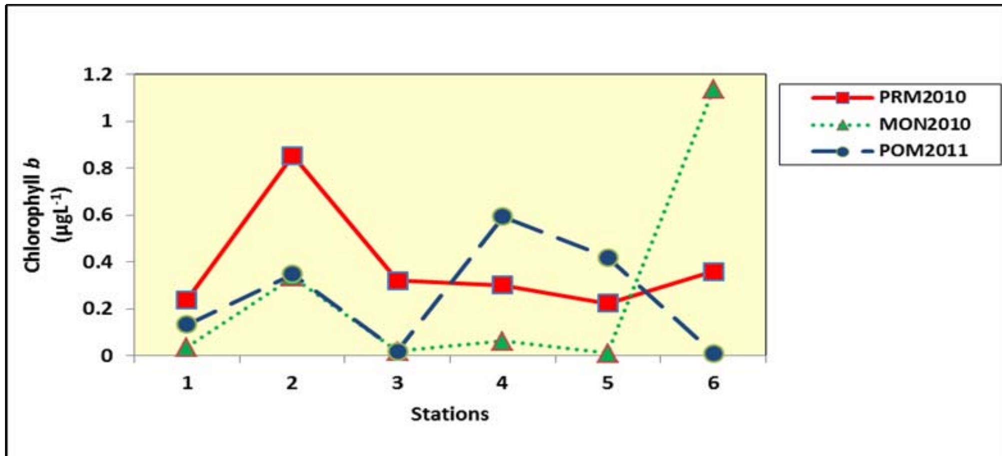


Fig. 29 Seasonal and spatial variation in chlorophyll *b*: 2010-2011

4.3.3.3 Chlorophyll *c*

In the first year, during the pre-monsoon season, the highest value of chlorophyll *c* was recorded at station 6 (1.56 µgL-1) and lowest at station 3 (0.56 µgL^{-1}). In the monsoon season, station 1 recorded the highest chlorophyll *c* value (3.44 µgL^{-1}), whereas the lowest value of 0.48 µgL^{-1} was recorded at station 4.

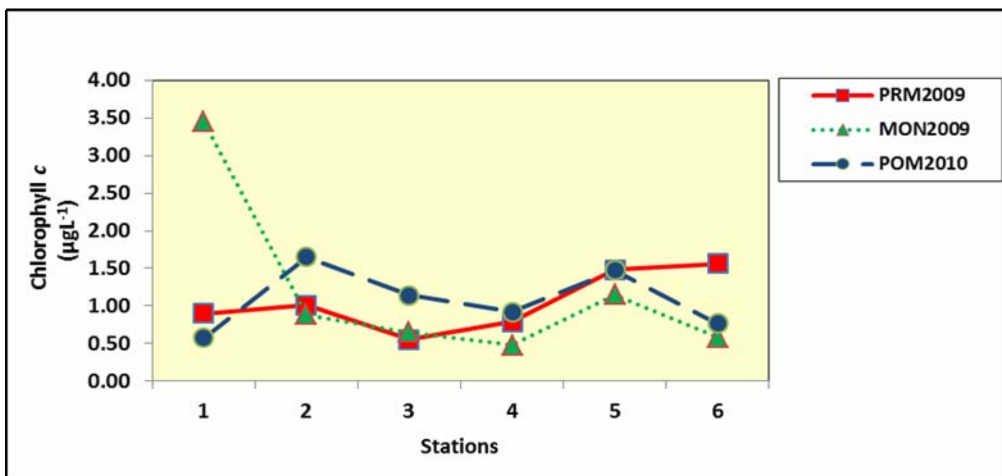


Fig. 30 Seasonal and spatial variation in chlorophyll *c*: 2009-2010

During post-monsoon, the chlorophyll *c* value ranged from the lowest of 0.58 µgL^{-1} at station 1 to the highest of 1.65 µgL^{-1} at station 2 (Fig.30).

During 2010-11, in the pre-monsoon season, the chlorophyll *c* value was highest at station 1 (2.46 µgL^{-1}) and lowest at station 6 (0.66 µgL^{-1}). During the monsoon period, the highest chlorophyll *c* value (2.95 µgL^{-1}) was recorded at station 3, whereas the lowest (0.56 µgL^{-1}) was at station 6. In the post-monsoon season, the chlorophyll *c* value ranged from the lowest (0.61 µgL^{-1}) at station 2 to the highest (3.06 µgL^{-1}) at station 1 (Fig31).

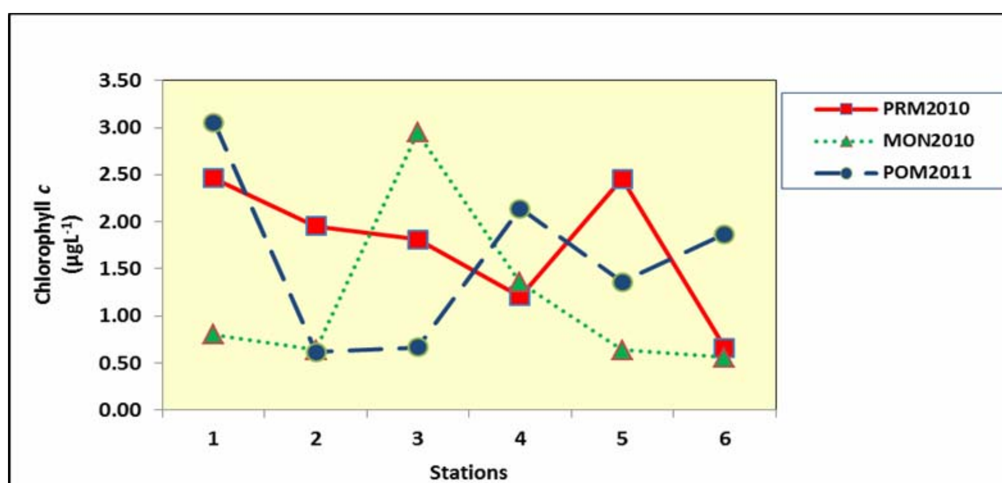


Fig. 31 Seasonal and spatial variation in chlorophyll *c:* 2010-2011

4.3.3.4 Carotenoids

In 2009-10, the carotenoid pigment showed the maximum concentration at station 2 (1.81 µgL^{-1}) and the lowest at station 3 (0.49 µgL^{-1}) in the pre-monsoon season. During the monsoon season, station 1 recorded the highest carotenoid value (1.91 µgL^{-1}), whereas station 4 showed the lowest value (0.02 µgL^{-1}). In the post-monsoon season, the highest value was at station 4 (1.11 µgL^{-1}) and the lowest was at station 6 with 0.60 µgL^{-1} (Fig.32).

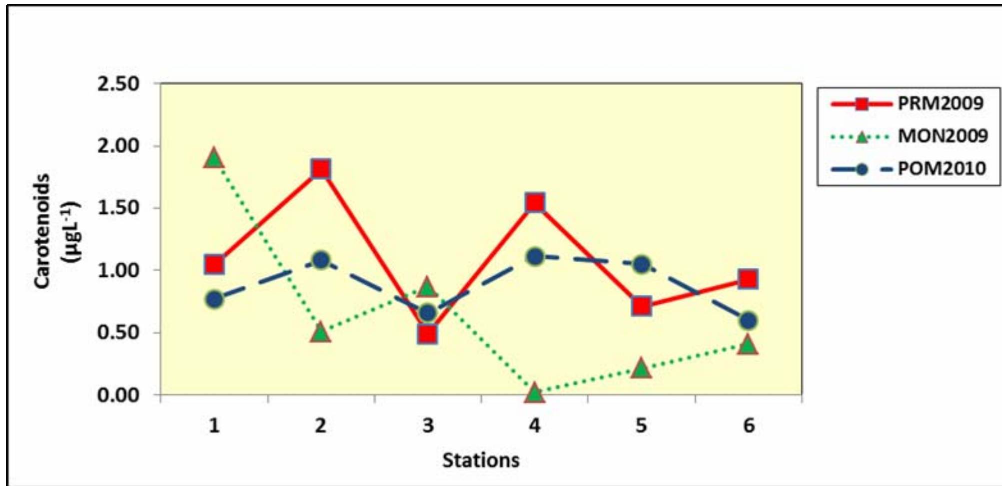


Fig. 32 Seasonal and spatial variation in carotenoids: 2009-2010

In the pre-monsoon season of 2010-2011, the highest value of carotenoids was 3.95 μgL^{-1} at station 1 and the lowest being 1.17 μgL^{-1} at station 3. In the monsoon season, it varied from the lowest of 0.44 μgL^{-1} at station 2 to the highest of 1.88 μgL^{-1} at station 6. In the post-monsoon period, the carotenoid values varied between 0.81 μgL^{-1} at station 2 to 2.93 μgL^{-1} at station 1 (Fig.33).

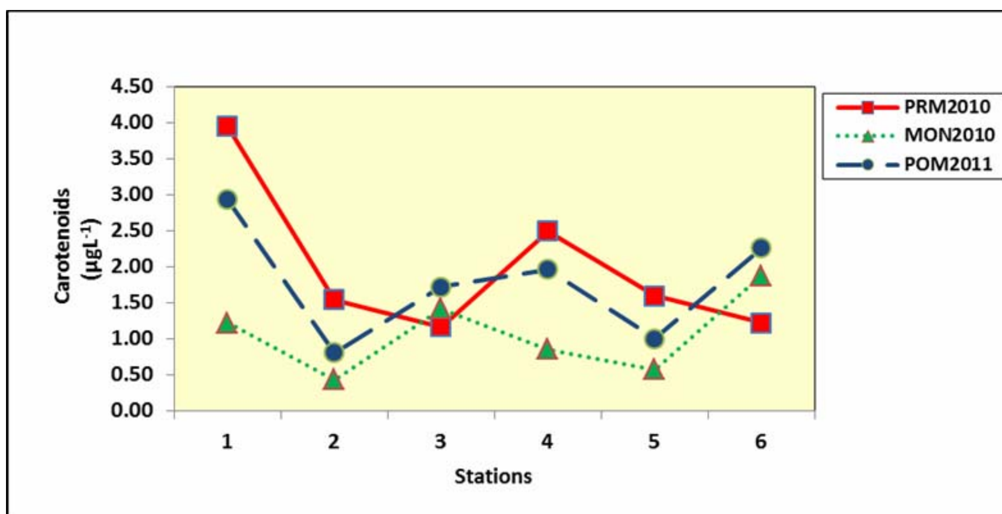


Fig. 33 Seasonal and spatial variation in carotenoids: 2010-2011

4.3.3.5 Mean spatial variation in pigments: 2009-2010

Chlorophyll *a* showed the highest value at station 1 (6.14 ±6.44 μgL^{-1}) and the lowest value (1.68 ±0.474 μgL^{-1}) at station 6. Chlorophyll *b* was highest at station 2 with 0.68 ±0.78 μgL^{-1}, whereas it was lowest at station 1 (0.08 ±0.07 μgL^{-1}). The highest value of chlorophyll *c* was found at station 1 (1.64 ±1.57 μgL^{-1}) and the lowest of 0.73 ±0.23 μgL^{-1} at station 4. Carotenoid pigments showed the highest value at station 1 with 1.24 ±0.59 μgL^{-1} and the lowest of 0.65 ±0.26 μgL^{-1} at station 6 (Fig.34).

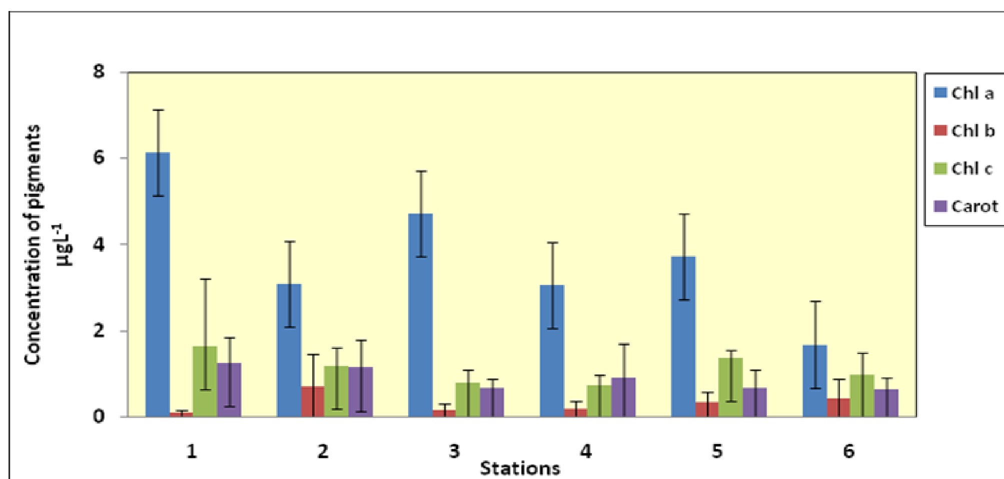


Fig. 34 Mean spatial variation in pigments: 2009-2010

4.3.3.6 Mean spatial variation in pigments: 2010-2011

The highest mean annual variation of chlorophyll *a* was found at station 1 (9.05 ±0.39 μgL^{-1}) and the lowest of 3.95 ±0.76 μgL^{-1} at station 3. Chlorophyll *b* was recorded as highest in station 5, with 0.65 ±0.21 μgL^{-1}, whereas it was lowest at station 1, having 0.14 ±0.10 μgL^{-1}. The highest value of chlorophyll *c* was found at station 1, with 2.11 ±1.17 μgL^{-1} while the lowest value was at station 6 with 1.03 ±0.73 μgL^{-1}. Highest carotenoid value was recorded in

station 1 (2.70 ±1.38 µgL^{-1}), whereas the lowest value of 0.93 ±0.56 µgL^{-1} was at station 2 (Fig.35).

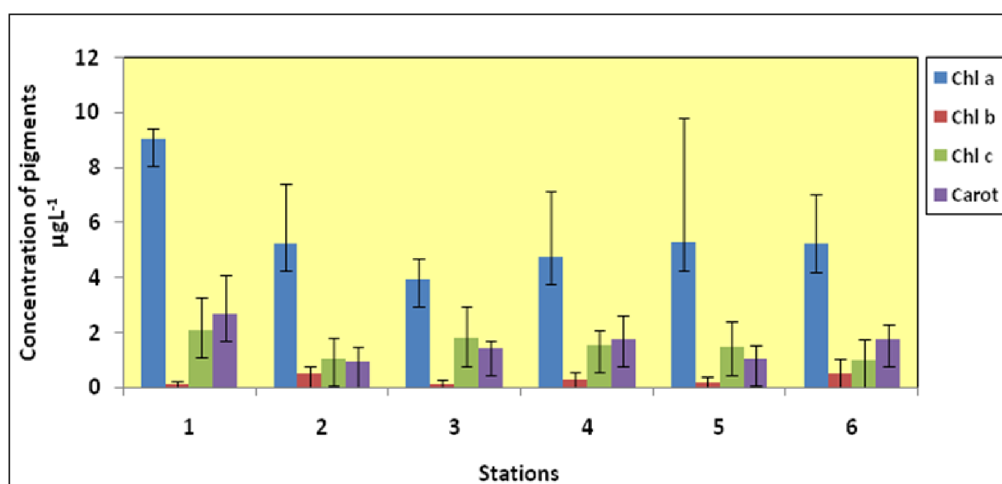


Fig. 35 Mean spatial variation in pigments: 2010-2011

4.3.4 Spatial variation of standing crop, chlorophyll *a* and Net primary production

During 2009-10, the mean annual variation of chlorophyll *a* and standing crop was found to be directly proportional. The cell abundance was found to be highest in station 1 (Azheekode) along with chlorophyll *a* at 6.14 (SD ±6.44) mg/m^3. The primary productivity was also found to be highest in station 1 with 1.05 (SD ±0.35) gC/m^3/day (Fig.36).

The mean annual chlorophyll *a* and the standing crop were found to be directly proportional in the second year also except in station 1. The highest chlorophyll *a* and standing crop were recorded at station 1 with, 9.05 ±0.39 mg/m^3 and 4860 ±1234 cellsL^{-1} respectively, whereas primary production was found to be highest in station 5 with, 1.28 ±0.98 gC/m^3/day (Fig.37).

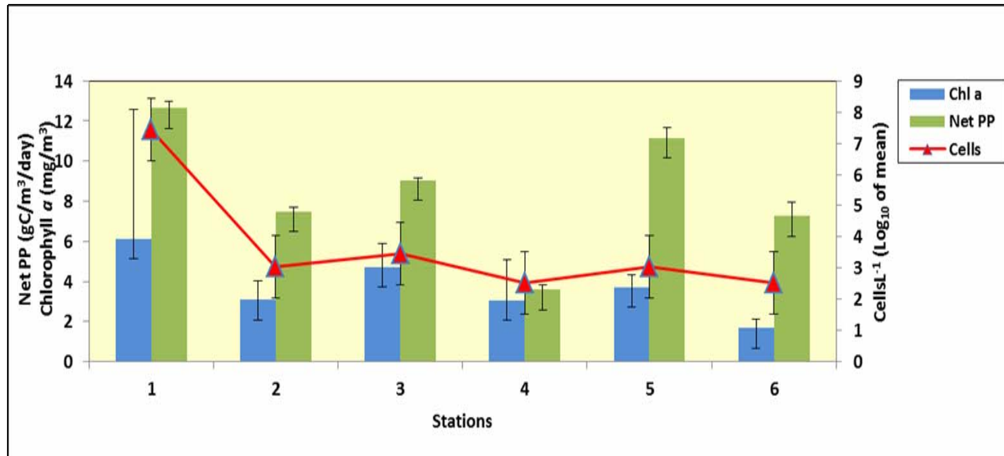


Fig. 36 Mean spatial variation in Standing crop, Chl *a* and Net PP: 2009-2010

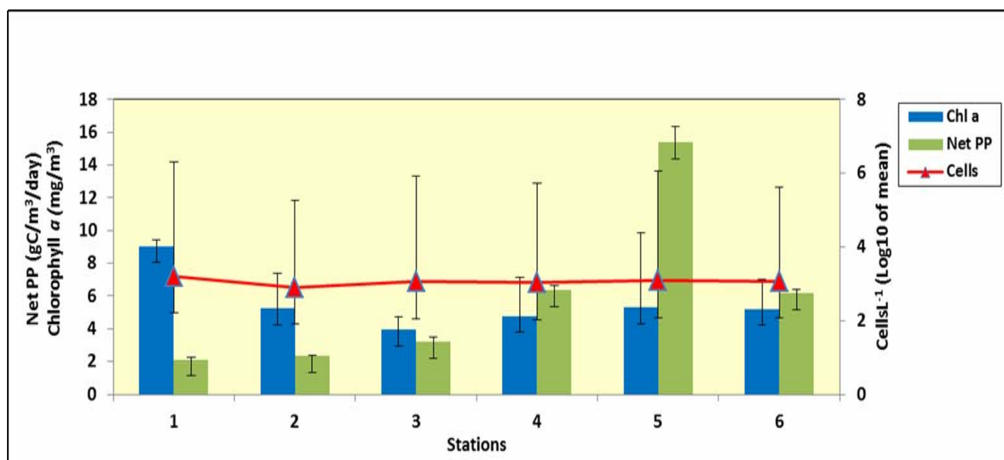


Fig. 37 Mean spatial variation in standing crop, Chl *a* and Net PP: 2010-2011

4.3.5 Statistical Analysis

The two-way ANOVA of pigments with stations and seasons is shown in Table 16. No significant relationship could be observed between these parameters.

Table 16 Analysis of Variance (ANOVA) of pigments with stations and seasons

Chlorophyll *a*					
Source of Variation	SS	DF	MS	F	P
Season	19.375	2	9.687	1.395	0.265
Station	65.947	5	13.189	1.899	0.126
Error	194.472	28	6.945		
Total	279.794	35			
Chlorophyll *b*					
Source of Variation	SS	DF	MS	F	P
Season	0.253	2	0.127	0.847	0.439
Station	0.912	5	0.182	1.220	0.326
Error	4.186	28	0.149		
Total	5.351	35			
Chlorophyll *c*					
Source of Variation	SS	DF	MS	F	P
Season	0.334	2	0.167	0.252	0.779
Station	2.924	5	0.585	0.882	0.506
Error	18.566	28	0.663		
Total	21.824	35			
Carotenoids					
Source of Variation	SS	DF	MS	F	P
Season	2.920	2	1.460	2.726	0.083
Station	4.600	5	0.920	1.718	0.163
Error	14.993	28	0.535		
Total	22.514	35			

Pearson correlation of pigments is shown in Table 17, in which chlorophyll *c* has shown 0.001 level of significance with chlorophyll *a*, and carotenoids at 0.001 level with both chlorophyll *a* and *c*.

Table 17 Pearson Correlation analysis of pigments

		Chl *a*	Chl *b*	Chl *c*	Carotenoids
Chl *a*	Pearson Correlation	1			
	Sig. (2-tailed)				
	N	36			
Chl *b*	Pearson Correlation	-0.021	1		
	Sig. (2-tailed)	0.903			
	N	36	36		
Chl *c*	Pearson Correlation	0.558**	-0.037	1	
	Sig. (2-tailed)	0	0.829		
	N	36	36	36	
Carotenoids	Pearson Correlation	0.623**	-0.085	0.593**	1
	Sig. (2-tailed)	0	0.622	0	
	N	36	36	36	36

* Correlation is significant at 0.05 level
** Correlation is significant at 0.01 level.

4.4 Discussion

4.4.1 Qualitative and quantitative distribution of planktonic microalgae

Distribution of planktonic microalgae varies widely from station to station and from season to season. In the first year of observation 2009-2010, at station 1 off Azheekode, both diatoms and dinoflagellates were recorded with 9 species each. An extensive monospecific haptophycean bloom of *Prymnesium parvum* N. Carter was observed in the monsoon season. None of the diatom species were found to be common in all the seasons. Harmful dinoflagellate *Biceratium furca* was found to be dominant among dinoflagellates. This observation was in accordance with the study of Taylor *et al*. (2008) who observed that *Ceratium* spp. were more abundant in the tropical waters. Potent DSP producing *Dinophysis caudata* was recorded in pre-monsoon season with a cell density of

220 cellsL^{-1}. This species is widely distributed in tropical and temperate waters and can appear abundantly in coastal waters (Nishitani *et al*., 2008). The red tide of *Biceratium furca* associated with mass mortalities of fish and the DSP intoxication in green mussels were reported (Okaichi, 1967; Holmes *et al*., 1999). *Dinophysis caudata* was reported to have mixotrophic nutrition (Nishitani *et al*., 2008) which also helps them to survive under adverse condition. Potentially toxic *Alexandrium* sp. was recorded in the pre-monsoon season with low cell density. The relative high temperature (31°C) in the pre-monsoon season favoured the increased abundance of dinoflagellates (Lehman, 2000).

During 2010-11, 33 species of microalgae were recorded from station 1 of which 25 species were diatoms, 7 species were dinoflagellates and the remaining species were grouped under Dictyochophyceae. Among these *Thalassionema frauenfeldii* was the dominant one. *Protoperidinium oceanicum* was the only species reported from all the consecutive seasons. Previous studies also had shown the abundance of *Protoperidinium* spp. in Indian waters (Subrahmanyan, 1971; Gopinathan, 1972; Sanilkumar *et al*., 2009). Harmful dinoflagellates *Biceratium furca* and *Prorocentrum lima* were also observed in the present study. *Prorocentrum lima* is a widespread and common dinoflagellate species in both tropical and temperate benthic environments (Richardson, 1997; Taylor *et al*., 2008) and its occurrence as planktonic microalgae is probably due to the mixing of sediments, because station 1 is located near the bar-mouth. The standing crop was found to be high in the post-monsoon season. Bloom-forming diatom *Leptocylindrus danicus* was found in low cell density in the post-monsoon.

During the study period, a total of 43 species of microalgae were recorded from station 1 of which 29 species were diatoms, 12 species were dinoflagellates and one species each belonged to Haptophyceae and Dictyochophyceae. A gradual increase in the diversity index was observed from the first year to the following year and it was due to the increased abundance of diatoms in the post-monsoon. Bray Curtis similarity Dendrogram of planktonic microalgal abundance (Fig.19) showed 35% similarity between pre-monsoon 2009 and monsoon 2010 due to the similarity in species composition among diatoms. Distribution of microalgae in monsoon 2009 was entirely different from other seasons due to the bloom of *Prymnesium parvum* which showed 100% monospecific standing crop. Highest standing crop of diatom was observed during the post-monsoon 2011 which might be due to the increased Si: N ratio (13:1) as reported by earlier workers (Turner *et al*., 2003; Piehler *et al*., 2004; Aktan *et al*., 2005; Sanilkumar *et al*., 2009).

Distribution of microalgae at station 2 (Balathuruth), an estuarine station, was also found to be varying considerably depending on the season. During 2009-10, 42 species of microalgae were documented of which 27 species belonged to diatoms and seven species each of Chlorophyceae and Dinophyceae. Dictyochophyceae was represented by one species. None of the species were found to be common in all the seasons. During the pre-monsoon, the abundance of harmful dinoflagellate *Biceratium furca* was observed. In a similar study, high diversity and dominance of dinoflagellates, particularly long horned members of *Ceratium* sp. in the tropical regions were reported by Taylor (2004). In estuarine conditions during the monsoon, the floral composition will change in accordance with the lowering of salinity (Gopinathan, 1972; Joseph and Pillai, 1975; Gao and Song, 2005). Low

salinity of 2 psu in station 2 favoured the distribution of chlorophycean species during monsoon. Abundance of diatom species was found to be low when compared with other seasons and none of the dinophycean members were found. The presence of *Gyrosigma tenuissimum,* a typical marine diatom species (Jin-Dexiang *et al.*, 1985) in the post-monsoon where the salinity was 27 psu indicated its wide salinity tolerance. The same species was reported from this station in the monsoon as well as in the post-monsoon seasons as the new distributional records in India (Sanilkumar, 2009).

In the year 2010-11, a total of 31 species were recorded from this station of which 29 species were diatoms and remaining two were dinoflagellates. None of the species were found to be common in all the seasons. The high Si: N ratio favoured the increased abundance of diatoms (Piehler *et al.*, 2004; Aktan *et al.*, 2005) in the monsoon and post-monsoon seasons.

Bray Curtis similarity Dendrogram of planktonic microalgal abundance (Fig.20) showed 35% similarity between pre-monsoon and post-monsoon 2010. This was due to the similarity in species composition among diatoms. Altogether, sixty species of microalgae were observed in this station of which predominant group was diatoms (44 species) followed by dinoflagellates (8 species), Chlorophyceae (7 species) and one species of Dictyochophyceae.

At station 3, off Kodikkal in 2009-10, dinoflagellates were found to be predominant with 14 species followed by diatoms (13 species) and one species each of Cyanophyceae and Dictyochophyceae. Harmful bloom-forming *Biceratium furca* was found to be present in all the seasons and it was the dominant one (7200 cellsL^{-1}) in the pre-monsoon season. *Ceratium* sp. is a

ubiquitous thecate dinoflagellate, characteristically found during all seasons, contributing substantially to annual primary production in the world ocean (Graham, 1941; Nordli, 1953; Elbrachter, 1973; Weiler, 1980; Dodge and Marshall, 1994). *Biceratium furca* and *Ceratium gibberum* can cause chocking of gills of fish by its epithecal and hypothecal horns. Baek *et al.* (2008) also reported *Biceratium furca* and *Ceratium fusus* as the most common bloom-forming species in the coastal and estuarine waters of the world. Moderately high temperature (30°C) and optimum N: P ratio (16:1) (Redfield *et al.*, 1963) in the pre-monsoon season might have caused the increased abundance of dinoflagellates. Higher temperature influences the abundance and the distribution of flagellates (Lehman, 2000). Toxic dinoflagellate *Dinophysis caudata* was also recorded in low numbers in all the seasons. *Trichodesmium erythraeum,* an important carbon and nitrogen fixing microalgae (Capone and Carpenter 1982; Zehr *et al.*, 2001; Lugomela *et al.*, 2002; Capone *et al.*, 2005) in the oceanic water, which mainly grow in nitrate limiting condition (Parab and Matokandar, 2012) was also observed in low cell density (36 cellsL^{-1}) in the pre-monsoon season. *Trichodesmium* sp. can often form extensive blooms in tropical and sub-tropical oceans (Capone *et al.*, 2005).

In 2010-11, of the total of 34 species that were recorded in station 3, 25 species belonged to diatoms, while dinoflagellates comprised 7 species and one species belonged to Dictyochophyceae. The bloom-forming centric diatom *Coscinodiscus asteromphalus* was recorded as predominant among diatoms, whereas among dinoflagallates *Ceratium fusus* was the predominat species. *Biceratium furca* was found in all the seasons. Dominance of diatoms over dinoflagellates was noticed which might be due to the high Si: N ratio (Piehler *et al.*, 2004; Aktan *et al.*, 2005) in all the seasons.

Bray Curtis similarity Dendrogram of planktonic microalgal abundance (Fig.21) showed 35% similarity between monsoon 2009 and post-monsoon 2010, due to the similarity in species composition among Dinophyceae. Pre-monsoon 2010 and post-monsoon 2011 showed 33% similarity, due to the similarity in species composition among diatoms. Due to the high abundance of *Biceratium furca*, pre-monsoon 2009 showed least resemblance to other seasons. A total of 57 species of microalgae were recorded from this station during the entire investigation period; diatoms were the dominant group (32 species) followed by dinoflagellates (16 species), Dictyochophyceae (2 species) and one species of Cyanophyceae. The harmful bloom-forming dinoflagellate *Biceratium furca* was found in all the seasons during the entire study period.

At station 4, Mahe, in the year 2009-10 the overall microalgal distribution was 14 species of diatoms, 4 species of dinoflagellates and one species of Dictyochophyceae. Diatom species were found to be dominant in all seasons, favoured by a high Si: N ratio (10:1) (Piehler *et al.*, 2004). *Surirella fastuosa* was recorded as the dominant alga (384 cellsL^{-1}) in the post-monsoon season. Potential DSP producing *Dinophysis caudata* was recorded in low cell density in the monsoon season.

In 2010-11, among 32 species of microalgae recorded, 27 species were diatoms, 4 species were dinoflagellates and the remaining one was Dictyochophyceae. None of the species were recorded throughout the season. Diatom *Cylindrotheca gracilis* was found to be dominant (477 cellsL^{-1}) in the post-monsoon season. Harmful dinoflagellate *Biceratium furca* was recorded in both pre- and post-monsoon seasons in low cell densities. *Dictyocha fibula*

an oceanic species (Tomas, 1997) was also observed in this estuary in low cell count (88 cells L^{-1}).

Bray Curtis similarity Dendrogram of planktonic microalgal abundance (Fig.22) showed that pre-monsoon 2009 and post-monsoon 2010 had 25% similarity due to the similarity in species composition among diatoms. 23% similarity between monsoon 2010 and post-monsoon 2011 was due to the increased abundance of bloom-forming diatom *Chaetoceros decipiens* and *Coscinodiscus asteromphalus.* Altogether 43 algal species were recorded; 35 species were diatoms followed by dinoflagellates (6 species) and Dictyochophyceae (2 species). Diatoms were found to be predominant in all the seasons. Relatively high silicate concentration (Kristiansen and Hoell, 2002; Piehler *et al.*, 2004; Aktan *et al.*, 2005) and high pH (Round *et al.*, 1990; Kenneth, 2002) might have favoured the dominance of diatoms.

In station 5, off Puthiyangadi, during 2009-10, 21 species of diatoms, 14 species of dinoflagellates and 1 species of Dictyochophyceae were recorded (total 36 species). None of these species were recorded during all the seasons. The increased abundance of the harmful dinoflagellate *Ceratium* spp. in the present study was significant as their distribution was reported more in tropical waters (Taylor *et al.*, 2008). This might be due to the high N: P ratio (8:1), as it was found that the growth rate of the *Ceratium* species increased readily in high N: P nutrient conditions (Baek *et al.,* 2008) as well as at relatively increased temperature (Lehman, 2000). The DSP producing *Dinophysis* spp. were present in low cell densities and *Dinophysis caudata* was present in both monsoon and pre-monsoon seasons, while *D. acuminata* (22 cellsL^{-1}) in the monsoon season. *D. acuminata* can cause intoxication, if the cell count

reaches above 200 $cellsL^{-1}$ (Yasumoto *et al*., 1978). The mixotrophic nature of *Dinophysis* spp. helps them to survive in all the adverse conditions, especially in the low irradiance climate. The occurrence of *Dinophysis* spp. were totally independent of changes in salinity and temperature because in the pre-monsoon season, temperature and salinity were 30°C and 35 psu, respectively, whereas in the monsoon season it shifted to 26°C and 30 psu. Yasumoto *et al.* (1978) reported that *Dinophysis* spp. were adapted to a wide range of salinity and temperature. Harmful dinoflagellate *Prorocentrum lima*, adapted to benthic environments (Richardson, 1997; Taylor *et al*., 2008), was also found in the post-monsoon among the planktonic algae, which can be attributed to the influence of bar-mouth in the present station. *Prorocentrum micans*, a harmful bloom-forming dinoflagellate was recorded in the post-monsoon.

In the following year, among the total of 34 recorded microalgal species, 26 species belonged to diatoms, 7 species to dinoflagellates and 1 species to Dictyochophyceae. The significant increase in diatom diversity and abundance was substantiated by high concentration of Si: N ratio (10:1) (Piehler *et al*., 2004: Aktan *et al*., 2005). Toxic bloom-forming *D. caudata* and harmful *Biceratium furca* were recorded in low cell densities in the pre-monsoon and post-monsoon seasons. Diatom species like *Asterionellopsis, Trieres, Coscinodiscus,* and *Proboscia*, which can proliferate into blooms were recorded in both pre-monsoon and monsoon seasons.

Bray Curtis similarity Dendrogram of planktonic microalgal abundance (Fig.23) showed 35% similarity between pre-monsoon 2009 and 2010. This was mainly due to the abundance of the bloom-forming species of *Biceratium*, *Trieres* and *Proboscia*. Altogether 57 species of microalgae were recorded in

this station, of which diatoms were predominant (39species) followed by dinoflagellates (17 species) and Dictyochophyceae (1 species).

At station 6, Thykadapuram, in the year 2009-10, out of 25 species of microalgae recorded, 16 species belonged to diatoms, 6 species belonged to dinoflagellates and 3 species belonged to Chlorophyceae. Chlorophycean species were present in the monsoon season when the salinity was 4 psu. The low abundance of Chlorophyceae might be due to the low proportion of nitrogen and phosphorus (3:1), whereas the optimum levels reported was 27:1 (Arrigo, 2005). Harmful dinoflagellate *Biceratium* sp. and diatom *Coscinodiscus* sp. were found to be the predominant species.

In the successive year, 36 species of microalgae were recorded at this station. Diatoms were the predominant group (25 species) followed by dinoflagellates (9 species) and Dictyochophyceae (1 species). Pennate diatom *Pleurosigma falx* was recorded in all the seasons. Pennate diatoms have generally higher surface to volume ratio and thus are able to assimilate nutrients even when their concentrations are limited or very low. Si: N ratio of 15:1 favoured the diatoms in the post-monsoon season. Toxic dinoflagellate *D. caudata* was recorded in the monsoon season where the salinity and temperature were 7 psu and 27°C respectively, which shows that *Dinophysis* spp. are adapted to a wide range of salinity and temperature (Yasumoto *et al.*, 1978; Nishitani *et al.*, 2008).

Bray Curtis similarity Dendrogram of planktonic microalgal abundance (Fig.24) showed 27% similarity between monsoon 2009 and post-monsoon 2010, due to the abundance of *Pleurosigma* sp., whereas 20% similarity was observed between pre-monsoon 2009 and monsoon 2010 due to the abundance

of *Coscinodiscus* sp. A total of 53 species of microalgae were recorded in which the majority were diatoms (68%) followed by dinoflagellates (22%), Chlorophyceae (6%) and Dictyochophyceae (4%).

Altogether, one hundred and fourteen species of microalgae classified into sixty four genera were recorded during the entire study period from 2009- 2011. These genera come under 6 classes Bacillariophyceae (43 genera) Dinophyceae (8 genera), Chlorophyceae (10 genera), Dictyochophyceae (1 genus), Cyanophyceae (1 genus), and Prymnesiophyceae (1 genus). Class Bacillariophyceae dominated with 75 species (66%) followed by Dinophyceae (22%), Chlorophyceae (9%), Dictyochophyceae (1%), Cyanophyceae (1%) and Prymnesiophyceae (1%).

Among class Bacillariophyceae, *Chaetoceros* was found to be the dominant genus with 6 species followed by *Coscinodiscus* (5 species), *Pleurosigma* and *Navicula* (4 species each), *Gyrosigma, Nitzschia* and *Surirella* (3 species each). Members of diatoms are the predominant groups occurring in the coastal waters (Hendey, 1964; Simonsen, 1974; Van den Hoek *et al*., 1995; Tomas, 1997). In a similar study, abundance of *Navicula* sp., *Nitzschia* sp., *Chaetoceros* sp. and *Coscinodiscus* sp. in the southwest coastal waters of India was reported by Sanilkumar (2009). Among dinoflagellates, harmful bloom-forming *Ceratium* and *Protoperidinium* (6 species each) were dominant followed by potentially toxic *Dinophysis* (4 species) and *Prorocentrum* (4 species). *Ceratium* sp. and *Protoperidinium* sp. were abundant in the tropical waters (Balkis, 2003; Baek *et al*., 2008). The occurrence of one species of nitrogen fixing Cyanophyceae, *Trichodesmium* sp. was also observed in the coastal waters. Low salinity in the monsoon season in the estuarine waters

favoured the abundance of chlorophycean members. In the present investigation, at stations 2 and 6 low salinity favoured the occurrence of members of Chlorophyceae (10 species), although in low abundance. Two species of the genus *Dictyocha* were reported from Dictyochophyceae. Prymnesiophyceae was represented by potent bloom-forming *Prymnesium parvum.*

4.4.2 Pigment composition

4.4.2.1 Chlorophyll *a*

During 2009-10, the mean annual concentration of chlorophyll *a* was found to be highest in the monsoon season (4.23 $\pm$4.72 μgL^{-1}). Peak chlorophyll *a* concentration was found in station 1 in the monsoon and station 3 in the pre-monsoon period. This high chlorophyll *a* value was substantiated by the high standing crop due to the blooming of *Prymnesium parvum* at station 1 (monsoon) and by dinoflagellates especially *Biceratium furca* (7200 cells L^{-1}) at station 3 (pre-monsoon). The chlorophyll *a* values were slightly higher in the pre-monsoon season than the post-monsoon. In the following year 2010-11, chlorophyll *a* value showed a wide fluctuation and the peak was shown in station 5 and 1 in the pre-monsoon. Station 1 also showed a higher level of chlorophyll *a* in the monsoon as well as in the post-monsoon when compared with other stations due to the increased microalgal biomass in this station. The mean annual concentration of chlorophyll *a* was found to be highest in the pre-monsoon season (7.23 $\pm$2.48 μgL^{-1}). Two-way ANOVA showed no significant difference in chlorophyll *a* value between stations and seasons. The mean annual chlorophyll *a* showed a slight deviation from the earlier reports. Sarupria and Bhargava (1998) have reported the annual average of chlorophyll *a* for the entire euphotic zone of Indian EEZ for the period from 1962 to 1988 as 13.4 μgL^{-1}, with a seasonal average of 18 μgL^{-1}, 7.9 μgL^{-1} and 8.3 μgL^{-1} in

the pre-monsoon, monsoon and post-monsoon periods respectively. Sanilkumar (2009) reported mean annual average of chlorophyll *a* along the southwest coast of India as 28.57 μgL^{-1} and in the successive seasons, 7.29 μgL^{-1}, 49.6 μgL^{-1} and 28.7 μgL^{-1} from 2007-2009. However, in the present study the mean annual average of chlorophyll *a* value recorded was 4.66 μgL^{-1} with the seasonal average of 5.59 μgL^{-1}, 4.60 μgL^{-1} and 3.79 μgL^{-1} in the pre-monsoon, monsoon and post-monsoon seasons respectively. Usually, the surface chlorophyll *a* concentration is generally low during inter-monsoon periods (March-May and October-December) which ranged from 0.03 to 0.05 μgL^{-1}, while during the NE and SW monsoons the chlorophyll *a* concentrations went up to 11 μgL^{-1} in the western Arabian Sea (Brock and McClain, 1992). The surface chlorophyll *a* concentration varied from 0.21 μgL^{-1} to 30.82 μgL^{-1} off Mangalore, west coast of India (Lingadhal *et al.*,2003), whereas Harnstrom *et al.* (2009) reported chlorophyll *a* concentration from 1.67 μgL^{-1} to 4.87 μgL^{-1} in the post-monsoon season from off Mangalore and also noted that individual microalgal taxonomic group can influence the chlorophyll *a* concentration. Usually, the monsoon period showed high chlorophyll *a* value due to the nutrient rich land run-off in the coastal waters (Hopkinson and Vallino, 1995).The peak in the chlorophyll *a* value in the pre-monsoon season in the present study was substantiated by high standing crop of dinoflagellate *Biceratium furca* in all the stations. In the present study there was no relative variation of pigments in accordance with the seasons because the individual planktonic taxon might influence the microalgal biomass irrespective of seasons. Harnstrom *et al.* (2009) reported a positive correlation between chlorophyll *a* and dinoflagellates, along off Mangalore, coastal Arabian Sea. A high chlorophyll *a* concentration was reported from Cochin

backwaters in the pre-monsoon period rather than monsoon period (Nair *et al.*, 1975). The Pearson correlation analysis showed a positive correlation between standing crop and chlorophyll *a*.

4.4.2.2 Chlorophyll *b*

In the present investigation, the mean chlorophyll *b* value was found to be highest in the monsoon season (0.66 $\pm$0.55 μgL^{-1}) in 2009-10. This is because of the abundance of Chlorophyceae species at stations 2 and 6. The highest chlorophyll *b* value recorded was 1.58 μgL^{-1} (station 2). However, Sanilkumar (2009) recorded the highest chlorophyll *b* value as 20.41 μgL^{-1} along the southwest coast in the monsoon, which is much higher than the present value. In 2010-11, the peak in chlorophyll *b* was recorded at station 6 in the monsoon season, whereas the mean seasonal average was highest in the pre-monsoon (0.38 $\pm$0.24 μgL^{-1}). No chlorophycean species were recorded throughout the year and the hike in chlorophyll *b* especially in the estuarine stations might be due to the presence of nanoplanktonic microalgae (2–20 µm) (Van den Hoek *et al.*, 1995) which could not be counted by Sedgewick–Rafter counting cell. Mean average of chlorophyll *b* in the entire study period was high in the monsoon season (0.45 $\pm$0.25 μgL^{-1}); similar observations were made by Menon *et al.* (2000). Two-way ANOVA showed no significant difference in chlorophyll *b* value with stations and with seasons.

4.4.2.3 Chlorophyll *c*

The mean average chlorophyll *c* concentration was found to be high in the monsoon period (1.20 $\pm$1.13 μgL^{-1}) of 2009-10. Bloom of *Prymnesium parvum* accounted for the increased chlorophyll *c*, since it is one of its major accessory pigments (Jeffrey *et al.*, 1997; Gibb *et al.*, 2001; Roy *et al.*, 2006). In the

successive year 2010-11, diatom abundance and chlorophyll *c* value was directly proportional since it is the major accessory pigment in diatoms. (Hendey, 1964; Van den Hoek *et al.*, 1995) Two-way ANOVA showed no significant difference in chlorophyll *c* with stations and with seasons.

4.4.2.4 Carotenoids

Station 1 in the monsoon of 2009-10 showed a relatively high carotenoid value, due to the monospecific blooming of *Prymnesium parvum*. The major pigment in carotenoids was fucoxanthin, the key pigment for diatom and prymnesiophytes (Jeffrey *et al.*, 1997; Gibb *et al.*, 2001; Roy *et al.*, 2006). In 2010-11, both post- and pre-monsoon seasons showed a comparatively high carotenoid value, probably due to the increased abundance of diatom species. Two-way ANOVA showed no significant difference in carotenoid value with stations and with seasons. Pearson correlation analysis (Table 17) revealed that chlorophyll *a* had a positive correlation with chlorophyll *c* and carotenoids. Significant correlation was also found between chlorophyll *c* and carotenoids at 0.01 level. Among these, the strongest correlation was found between chlorophyll *a* and carotenoids (0.623).

4.4.3 Mean spatial variation in standing crop, Chl *a* and Net PP

The mean annual variation of standing crop and chlorophyll *a* were found to be directly proportional in the entire study period but primary production did not show any relationship with chlorophyll *a* and standing crop. Similar observation was made from the studies on the southwest coast of India by Sanilkumar (2009). The highest primary productivity value of 28.83 $gC/m^3/day$ was recorded in station 5 in the post-monsoon season of 2010-11. In the first year, the highest average mean value was 12.64 $gC/m^3/day$ at station 1,

whereas in the successive year it was 15.38 gC/m^3/day at station 5. From the observations made from 2006-2008 along the southwest coast of India by Sanilkumar (2009), the highest average productivity was recorded as 28.28 gC/m^3/day, and it ranged from 2.44 gC/m^3/day to 28.28 gC/m^3/day. In the present study the average productivity ranged from 2.12 gC/m^3/day to 15.38 gC/m^3/day which is higher than the average production of Vembanad Lake, 1.2 gC/m^3/day as reported by Nair *et al*. (1975).

4.4.4 Standing crop and physical parameters

In the present study, variation in temperature was comparatively very low and hence did not influence the plankton distribution much. Gopinathan (1975a) observed that temperature would never be a limiting factor for diatoms in the tropical marine ecosystem. But high temperature favoured the dinoflagellates (Lehman, 2000). Salinity influenced the floral composition especially in the monsoon season. The occurrence of chlorophycean species in the estuarine stations 2 and 6 revealed the influence of salinity. In coastal ecosystems salinity is a thriving force in the distribution of microalgae (Joseph and Pillai, 1975; Nair *et al*., 1975; Pillai *et al*., 1975; Huang *et al*., 2004). Despite the inactivation and replacement of marine species with freshwater species in the estuary on account of freshwater influx during the monsoon season (Joseph and Pillai, 1975), an optimum nutrient level was also essential (Arrigo, 2005) for the better growth of freshwater species. This might be the reason for the scarcity of chlorophycean members in the present study even though the salinity was too low. The shift from monsoon to post-monsoon alters the phytoplankton community (Joseph and Pillai, 1975; Nair *et al*., 1975). Statistically there was no correlation between hydrographical parameters with

chlorophyll *a*, standing crop and primary productivity in the present study. Similar observations were made by many workers (Pillai *et al.*, 1975; Sanilkumar, 2009).

4.4.5 Mean spatial variation in nutrients and chlorophyll *a*

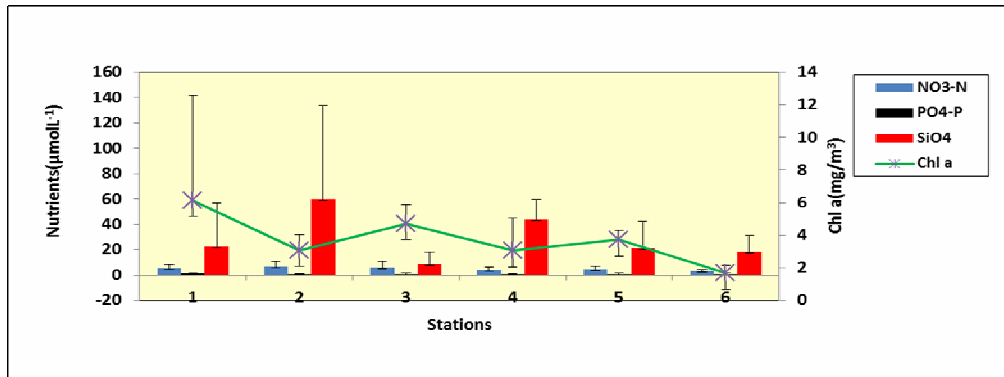


Fig.38 Mean spatial variation in nutrients and chlorophyll *a:* 2009-2010

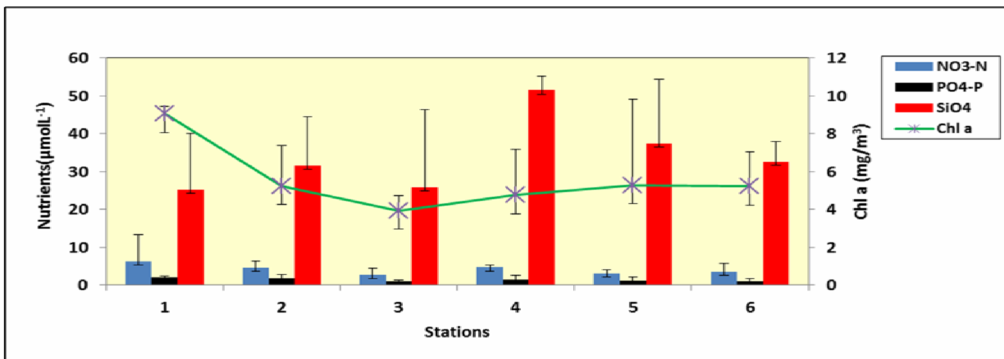


Fig.39 Mean spatial variation in nutrients and chlorophyll *a:* 2010-2011

In the present study, no direct relationship could be observed between nutrients (nitrate, silicate and phosphate) and chlorophyll *a* during the first year of observation. Highest annual mean value of nitrate (6.77 ±4.22 µmolL^{-1}) and silicate (59.77 ±73.72 µmolL^{-1}) were found in station 2 but here the chlorophyll *a* concentration was comparatively less. The highest annual mean

of chlorophyll *a* value (6.14 $\pm$6.44 mg/m^3) was found in station 1 where the mean value of silicate (22.74 $\pm$34.0 µmolL^{-1}) and nitrate (5.05 $\pm$3.36 µmolL^{-1}) were low. The silicate and nitrate concentrations were comparatively low in station 3 where the chlorophyll *a* value was in a higher range as compared with other stations. Likewise in the following year, station 1, where the highest mean annual chlorophyll *a* value (9.05 $\pm$0.39 mg/m^3) was recorded, showed lower mean concentration of nitrate (6.30 $\pm$7.0 µmolL^{-1}) and silicate (25.31 $\pm$14.82 µmolL^{-1}). From these observations it is clear that, apart from the nutrient concentration, many other factors like magnitude and position of the turbidity maximum, tidal amplitude and freshwater discharge volume, water column stratification and grazing rates of zooplankton influence phytoplankton distribution. (Platt, 1972; Therriault *et al.*, 1978; Joint and Pomeroy, 1981; Harrison *et al.*, 1990, 1991; Pennock and Sharp, 1994; Gao and Song, 2005). The demand for nutrients by phytoplankton is species-specific (Mochizuki *et al.*, 2002). Graneli *et al.* (1999) suggested that the demand for silica by diatoms appears to differ with different species. Gopinathan (1975a) inferred that nutrient requirement of diatoms may vary and high concentrations of nitrate and phosphate alone may not support the substantial increase in the production of diatom. The widely accepted fact, that multiple resources control or limit the microalgal growth in the world ocean (Arigo, 2005), has now replaced the importance of individual nutrients on microalgal distribution and biomass (Calliari *et al.*, 2005). In the present study, it was imperative to state that the spatial and temporal distribution and the abundance of microalgae were seldom dependant on individual factors; rather they depended on multiple resources.

In all, 114 species of microalgae coming under 64 genera, among 6 different classes such as Bacillariophyceae, Dinophyceae, Prymnesiophyceae,

Cyanophyceae, Chlorophyceae and Dictyochophyceae were recorded in the present study. The predominant groups were diatoms and dinoflagellates. Chlorophycean members were found in the monsoon seasons. Phytoplankton standing crop/abundance and chlorophyll *a* were positively correlated. Diatoms were the dominant group during the entire study period except in the pre-monsoon of 2010-11, when a high cell abundance of harmful bloom-forming dinoflagellate *Biceratium furca* was observed. Potentially toxic dinoflagellates *Dinophysis* sp. and *Alexandrium* sp., harmful bloom-forming *Prorocentrum lima* and *Prorocentrum micans* and *Ceratium* spp. were also observed. Besides, bloom-forming diatoms, *Coscinodiscus* sp., *Odontella* sp., *Trieres* sp., *Asterionellopsis*, sp., *Chaetoceros* sp. *Thalassiosira* sp., *Pleurosigma* sp. and *Proboscia* sp. were present along the Kerala coast during the entire study period suggesting the possibility of blooming of these species provided the conditions are optimal in the environment.

.....൬൫.....

Chapter **5**

Algal Blooms

5.1 Introduction
5.2 Review of Literature
5.3 *Prymnesium parvum* N. Carter bloom
5.4 *Proboscia alata* (Brightwell) Sandström bloom
5.5 *Chattonella marina* (Subrahmanyan) Hara et Chihara bloom

5.1 Introduction

The enormous proliferation of planktonic microalgae into millions of cells per litre when sufficient light and nutrients are present is termed as algal blooms. Algal blooms are usually natural phenomena and most of the blooms are beneficial to the marine ecosystem.

In some situations algal blooms can have a negative effect. As per the International Council for the Exploration of Seas (1984), algal blooms are defined as 'those which are noticeable, particularly to the general public, directly or indirectly through their effects such as visible discolouration of the water, foam production, fish or invertebrate mortality or toxicity to humans'.

The negative impacts of algal bloom events appear to have increased in frequency, intensity and geographic distribution in the past two decades

(Daranas *et al*., 2001). Harmful Algal Blooms (HABs) are becoming a potent threat all over the world by affecting human health, natural and cultured resources, tourism and ecosystems, and the economy. Whereas a normal algal bloom preferably supports the fishery resources, the fishes avoid the area having harmful algal blooms due to the increased proliferation or the presence of toxic substance which are harmful to it.

Usually blooms have very high population density but its potential harmful effect is influenced by seasonal, regional and species-specific characteristics. Thus, a low and high biomass of the bloom can cause harmful effect (Smayda, 1997). However, it is very difficult to define the cell count that cause potential harmful effect, as some species are so toxic that their presence even in relatively low numbers may impart high level of lethal effect (IOC, 2001).

There are two primary factors that are attributed to algal blooms: natural processes such as circulation, upwelling relaxation, and river flow; and anthropogenic loadings leading to eutrophication. The latter is generally assumed to be the primary cause of all algal blooms (Anderson *et al*., 2002). The occurrences of algal bloom are increasing throughout the world's oceans. The reasons for this obvious increase remain unclear, which include not only eutrophication but increased observation efforts in coastal zones of the world.

Of around 5000 known species of microalgae around the world, only about 300 species are known to have the harmful algal bloom-forming effect, particularly of water discolouration, of which only about 80 species produce potent toxins (Hallegraeff, 2003).

Based on the problems caused by the algal blooms, they can be classified into four major groups, the species which cause water discolouration, species non-toxic to humans but harmful to filter feeding invertebrates and fishes, species which produce toxins causing illness to humans through aerosols from bloom area to the coast, and species which produce potent toxins that can affect humans through seafood (Hallegraeff, 1995).

Basically, the species which cause water discolouration and those which are non-toxic to human but cause harmful effects in the fauna comprised non-toxic species. But under some conditions, due to huge growth that generates anoxic conditions, these blooms also may lead to the death of the fishes and invertebrates.

'Toxins' produced by the microalgae are commonly secondary metabolites and are primarily involved in bioluminescence, nitrogen storage, nucleic acid biosynthesis, bacterial endosymbiosis and pheromones.

Important human illnesses caused by toxic algae are Paralytic Shellfish Poisoning (PSP), Ciguatera Fish Poisoning (CFP), Diarrhetic Shellfish Poisoning (DSP), Neurotoxic Shellfish Poisoning (NSP), Amnesic Shellfish Poisoning (ASP) and Azaspiracid Poisoning (AZP).

Generally the toxin producing algal blooms may cause haemolytic, hepatotoxic and osmoregulatory and many other toxic effect on fauna that ingest these species. The toxicity and other negative effects caused by harmful algae are not limited to a particular genera but are distributed among several taxonomic groups, and the high taxonomical diversity of the harmful algae result in the variety of toxins (Anderson, 1997).

Among the HAB producing microalgae, species belonging to the group dinoflagellates are potent toxin producers. About one hundred and eighty five species are harmful in nature of which sixty species are able to produce toxins. *Amphidinium, Alexandrium, Ceratium, Cochlodinium, Dinophysis, Gymnodinium, Gyrodinium, Heterocapsa, Peridinium, Pfiesteria, Prorocentrum, Protoperidinium,* and *Pyrodinium* are the major harmful genera. Among these, many species can produce potent toxins that cause severe illnesses in humans. *Alexandrium* sp., *Gymnodinium catenatum*, and *Pyrodinium bahamense* are the causative microalgae of Paralytic Shellfish Poisoning (PSP). PSP induces muscular paralysis and in severe cases can lead to the death through paralysis of respiratory system. PSP is caused by 'PSP toxins', which is a combination of eighteen different toxins mainly saxitoxins, neosaxitoxins and gonyautoxins.

Gambierdiscus toxicus, Prorocentrum lima, Ostreopsis siamensis, Coolia monitis, Thecadinium sp. and *Amphidinium carterae* are the causative organisms of Ciguatera Fish Poisoning (CFP) in which the major toxins are Ciguatoxins and Maitotoxin. Ciguatoxins are very potent neurotoxins. Ciguatera fish poisoning (CFP) generate gastrointestinal, neurological and cardiovascular disorders in humans.

Dinophysis sp., *Prorocentrum lima, Protoceratium maculosum, Protoceratium reticulatum* and *Coolia* sp. cause Diarrhetic Shellfish Poisoning (DSP), by producing the toxins Okadaic acid, Dinophysis toxins, Yessotoxins and Pectenotoxins. Major symptoms of DSP are stomach pain, nausea, vomiting and diarrhoea.

Karenia brevis (formerly known as *Gymnodinium breve*) is the causative organism of Neurotoxic Shellfish Poisoning (NSP), which produces a potent

toxin called brevetoxin. NSP produces intoxication leading to gastrointestinal and neurological problems. Garthwaite (2000) reported the burning of the eyes and nasal passages, leading to cough and asthma like symptoms.

Major harmful algal genera under the class Prymnesiophyceae are *Chrysochromulina, Prymnesium* and *Phaeocystis*. The toxins produced by these have a wide range of biological effects, including icthyotoxicity, neurotoxicity, cytotoxicity, hepatotoxicity, haemolytic, allelopathic and antibacterial activity.

Chatonella and *Heterosigma* are the major harmful algae under the class Raphidophyceae, which produces neurotoxins and free fatty acids, whose reactive oxygen species are involved in tissue injury and mucus production.

Usually diatoms cause harmful effects either by physical stress or by oxygen depletion. However, some diatoms like *Pseudo-nitzschia pungens* f. *multiseries, P. australis, P. pseudodelicatissima, P. seriata, Nitzschia actydrophila* and *Amphora coffeaeformis* are able to cause Amnesic Shellfish Poisoning (ASP) in humans by producing a potent neurotoxin called domoic acid. Symptoms mainly include gastroenteritis, but in severe cases neurological symptoms along with dizziness, headache, disorientation, short term memory loss, respiratory difficulty and coma have also been observed (Perl *et al*., 1990).

Among cyanobacteria, major harmful genera comprises *Anabaena, Aphanizomenon, Calothrix, Cylindrospermopsis, Lyngbya, Microcystis, Nodularia, Nostoc, Oscillatoria, Planktothrix, Scytonema* and *Trichodesmium*, of which the species of *Anabaena, Aphanizomenon, Cylindrospermopsis, Microcystis,*

Nodularia, Nostoc and *Oscillatoria* have toxic strains which are responsible for cyanophycean toxins.

In recent decades, the frequency, intensity and spreading of toxic and non-toxic planktonic algae and the HAB events have increased worldwide. (Hallegraeff *et al*., 1995; Lewitus *et al*., 2012). There are five possible reasons for increase in frequency and geographical extent of HAB events (Hallegraeff, 1993) (1) improved methods for detection and monitoring methods of blooms that would previously have gone unreported, (2) species dispersal through currents, storms or other natural mechanisms, (3) introduction of new algal species into inshore areas through ship ballast water exchange or aquaculture, (4) long-term climatic changes and (5) cultural eutrophication. These reasons may vary from one bio-region to the other with regional environmental settings. Bio-invasion is considered as one of the vectors for global expansion of HABs in other parts of the world (Blackburn *et al*., 2001; Marangoni *et al*., 2001).

Arising from growing concerns of such an increase in the occurrence of HABs, a number of national, regional and international programmes viz. the Intergovernmental Oceanographic Commission on Harmful Algal Blooms (IOC-HAB), Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB), the Northwest Pacific Action Plan (NOWPAP), the Korean Harmful Algal Bloom Research Group (KORHAB), have been implemented to understand the features and mechanisms underlying the population dynamics of HABs and to improve and develop management and amelioration strategies.

India, being one of the major maritime countries, is endowed with a coastline of approximately 7,500 km which is embraced by two important

seas, the Arabian Sea on the west coast and the Bay of Bengal on the east coast. Studies on algal blooms in Indian waters indicated that the west coast is a more bloom prone area compared to the east, since it is one of the most biologically productive areas among the World Oceans. Most blooms occurring in Indian waters are naturally driven due to physical forcing such as monsoonal influence, riverine discharge and seasonal upwelling. Besides these factors, variations in temperature, salinity, irradiance, water stability and nutrient enriched waters are important conditions that influence bloom formation. Some species-specific blooms of diatoms, raphidophytes and cyanophytes which followed a seasonal pattern are common in Indian waters, whereas the non-seasonal dinoflagellates blooms respond to short-term events such as sunny, calm weather (D'Silva *et al*., 2012). However, the algal bloom outbreaks are sporadic and unpredictable. Regular monitoring of bloom-prone areas will provide significant insights into bloom dynamics and its impact on the ecosystem and human community.

A national coordinated multi-institutional research programme for monitoring of ''HABs in the Indian EEZ'' and monitoring of phytoplankton under the Indian Expendable Bathythermographic (XBT) programme has been initiated by the Ministry of Earth Sciences, Government of India. Ballast Water Management Programme-India (BAMPI) and Port Baseline Biological Survey (PBBS) by the Council of Scientific and Industrial Research and the Ministry of Shipping, Government of India, and Moderate Resolution Imaging Spectroradiometer (MODIS)/Aqua data of remote sensing by INCOIS are the other programmes actively involved in the surveillance of HABs along the Indian coast.

In view of this, as part of the Ministry of Earth Sciences, Government of India sponsored project on ‘Monitoring and Surveillance of Algal Blooms’, funded by Centre for Marine Living Resources and Ecology, Ministry of Earth Sciences, Government of India, regular monitoring and surveillance of planktonic algal blooms along the southwest coast of India from the coastal/estuarine stations has been carried out. During the present investigation, occurrences of three blooms *Prymnesium parvum* N. Carter, *Proboscia alata* (Brightwell) Sandström and *Chattonella marina* (Subrahmanyan) Hara et Chihara have been observed from the northern Kerala coast.

5.2 Review of Literature

5.2.1 Algal Blooms: International status

The first written reference regarding the harmful algal bloom was (back in 1000 years B.C.) in the Bible,“... all the waters that were in the river were turned to blood and the fish that was in the river died; and the river stank, and the Egyptians could not drink of the water of the river” (Exodus 7: 20-21). One of the first recorded fatal cases of human poisoning after eating shellfish contaminated with dinoflagellate toxins was in 1793. Captain George Vancouver and his crew, when landed in British Columbia, noticed that the local Indian tribes were intoxicated with contaminated shellfish when sea water became phosphorescent due to dinoflagellate blooms (Dale and Yentsch, 1978). However, there is fossil evidence that HABs have occurred long before this. The main constrain in the study of algal blooms is the lack of historical data and the restricted number of good long-term data series.

With the emergence of HAB studies, a series of conferences were held in Boston, Massachusetts, in November 1974 (LoCicero, 1974), at Miami, Florida,

in 1978 and at St. Andrews, Canada, in 1985. The First International Symposium on Red Tides was held in 1987 (Hallegraeff *et al.*, 1995). The concept for the 'Manual on Harmful Marine Microalgae' was discussed in the first Session of the IOC-FAO Intergovernmental Panel on Harmful Algal Blooms in 1992, which was published in 1995. Hallegraeff (1993) made a review on harmful algal blooms. 'A Review On Seafood Toxins: Present Approaches and Future Options' was made by Wright in 1995.

Smayda (1997) reported that in order to define algal blooms, subjective difference and arbitrary criteria are essential. Zingone and Enevoldsen (2000) reported that HABs show high diversity with regard to causative organisms, bloom dynamics and type of impact. Bio-invasion is considered as one of the vectors for global expansion of HABs (Marangoni *et al.*, 2001). The intoxication of shellfish, fish fauna and avian community (Shumway *et al.*, 2003) and ultimately of humans due to toxic bloom events have increased worldwide (Okolodkov, 2005). Paralytic Shellfish Poisoning (PSP) (Dale and Yentsch, 1978; Usup *et al.*, 2002; Nguyen-Ngoc, 2004; Vilaa *et al.*, 2005), Diarrhetic Shellfish Poisoning (DSP) (Kat, 1979, 1985; Kumagai *et al.*, 1986; Lassus *et al.*, 1985; Dahl and Yndestad, 1985; Cembella, 1989; Marasigan *et al.*, 2001, Madigan *et al.*, 2005), Amnesic Shellfish Poisoning (ASP) (Jeffery *et al.*, 2004), Neurotoxic Shellfish Poisoning (NSP) (Magana *et al.*, 2003; Kirkpatrick *et al.*, 2004) and the outbreak with the toxicity of *Prymnesium* sp. (Holdway *et al.*, 1978; Kaartvedt *et al.*, 1991; Guo *et al.*, 1996; Edvardsen and Paasche, 1998; Amsinck *et al.*, 2005; Lundholm and Moestrup, 2006; Graneli *et al.*, 2008; Baker *et al.*, 2007, 2009) have been reported.

5.2.2 Algal Bloom events along the Indian waters

In the Indian EEZ, occurrence of algal blooms is more prevalent along the west coast than on the east coast. Hornell (1908) made the first observations on algal blooms that caused massive fish mortality while cruising along the Malabar Coast to the Laccadive Islands.

Diatom blooms have been reported from the west coast of India and the causative organisms were *Ditylum* sp. and *Thalassiosira* sp. (Hornell and Nayudu, 1923), *Fragilariopsis oceanica* (Devassy, 1974), *Nitzschia sigma* (Devassy and Bhattathiri, 1974), *Skeletonema costatum* (Devassy and Bhattathiri, 1974; Tiwari and Nair, 1998) and *Coscinodiscus asteromphalus* (Padmakumar *et al*., 2007). Diatom blooms by *Fragilariopsis oceanica* and *Skeletonema costatum* have been reported as a recurring annual feature (Gopinathan, 1974; Devassy, 1983; Devassy and Goes, 1988; Tiwari and Nair, 1998; Mitbavkar and Anil, 2002; Patil and Anil, 2008).

Dinoflagellate blooms along the west coast were mainly caused by *Glenodinium* sp. (Hornell and Nayudu, 1923), *Gymnodinium* spp. (Hornell and Nayudu, 1923; Bhimachar and George, 1950; Karunasagar, 1993), *Prorocentrum* spp. (Hornell and Nayudu, 1923), *Cochlodinium* spp. (Hornell and Nayudu, 1923; O'Herald, 2001), *Noctiluca* spp. (Bhimachar and George, 1950; Venugopal *et al*., 1979; Devassy *et al*., 1979; Devassy and Nair, 1987; Katti *et al*., 1988; Nayak and Karunasagar, 2000; Naqvi *et al*., 1998; Sahayak *et al*., 2005; Padmakumar *et al*., 2008; Sanilkumar *et al*., 2009; Padmakumar *et al*., 2010), *Dinophysis* sp. (Bhimachar and George, 1950), *Gonyaulax* sp. (Prakash and Sarma, 1964), *Karenia* sp. (Iyer *et al*., 2008; Madhu *et al*., 2011)

and *Protoperidinium* sp. (Sanilkumar *et al*., 2009). Among these, blooms of *Noctiluca* spp. were observed to be predominant.

Cyanobacterial blooms especially of *Trichodesmium* sp. are predominant in the Indian waters (Prabhu *et al*., 1965; Nagabhushanam, 1967; Qasim, 1970; Ramamurthy *et al*., 1972; Devassy *et al*., 1978; Verlancar, 1978; Sarangi *et al*., 2004; Anoop *et al*., 2007). Blooms of raphidophycean *Chatonella marina (*formerly *Hornellia marina)* was reported by various workers (Subrahmanyan, 1954; Jugnu and Kripa, 2009; Sanilkumar *et al*., 2012).

Diatom blooms in the east coast were mainly caused by *Rhizosolenia* sp. (Raghu Prasad, 1956), *Asterionellopsis* spp. (Subba Rao, 1969; Mani *et al*., 1986; Choudhury and Panigrahy, 1989; Panigrahy and Gouda, 1990; Mishra and Panigrahy, 1995; Satpathy and Nair, 1996; Sasamal *et al*., 2005), *Thalassiothrix* sp. and *Coscinodiscus* spp. (Mishra and Panigrahy, 1995).

Dinoflagellate blooms were mainly caused by *Noctiluca miliaris* (Aiyar, 1936; Raghu Prasad, 1953, 1958; Santha, 1975) and *Noctiluca scintillans* (Silas *et al*., 1982; Sargunam and Rao, 1989; Eashwar *et al*., 2001; Dharani *et al*., 2004; Mohanty *et al*., 2007; Gopakumar *et al*., 2009).

Trichodesmium erythraeum was the predominant bloom forming cyanobacteria on the east coast (Chacko, 1942; Chidambaram and Unny, 1944; Ramamurthy, 1968, 1970a and b, 1973; Chellam and Alagarswami, 1978; Jyothibabu *et al*., 2003; Satpathy *et al*., 2007; Anantharaman *et al*., 2010). *Microcystis aeruginosa* bloom was reported by Santhosh *et al*. (2010).

Phytoplankton blooms which occurred along the Indian coast during the period from 1982 to 1987 have been documented by Mathew *et al*. (1988). Algal blooms, particularly HAB occurrences, along the Indian coast have been reviewed by Karunasagar and Karunasagar (1990). D'Silva *et al*. (2012) made the pioneer review regarding the occurrence of algal blooms from 1908 to 2009, and showed that there has been an exponential increase in algal bloom events along the Indian coasts, which have direct or indirect effects on coastal waters, fisheries, other marine organisms and humans.

However, the intoxication of humans by algal bloom is comparatively less in Indian waters (D'Silva *et al*., 2012). Paralytic Shellfish Poisoning by an unidentified toxic species, due to the consumption of bloom infected *Meretrix casta* resulted in casualties in Tamil Nadu in 1981 and in Mangalore (Karnataka) in 1983 (Bhat, 1981; Silas *et al*., 1982; Karunasagar *et al*., 1984; Devassy and Bhat, 1991). Low levels of PSP were recorded in shellfish from surrounding estuaries near Mangalore on two occasions during 1985 and 1986 (Segar *et al*., 1989). Planktonic and cyst forms of *Gymnodinium catenatum*, a PSP-producing dinoflagellate were recorded from Mangalore (Godhe *et al*., 1996). In 1997 at Vizhinjam (Kerala), shell fish poisoning by *Gymnodinium* resulted in causalities and hospitalisation of people inhabiting the coastal belt (Karunasagar *et al*., 1998). In 2004, a bloom event that occurred along the coasts of Kollam and Trivandrum due to species belonging to the genera *Gonyaulax*, *Cochlodinium* and *Karenia* caused massive fish kill and hospitalization of people, especially children, due to the consumption of intoxicated sea food (Sahayak *et al*., 2005, Iyer *et al*., 2008). Subsequent to this event, an unidentified holococcolithophore (Ramaiah *et al*., 2005) was reported from southern Malabar Coast.

5.2.3 Factors influencing algal blooms

The formation of algal blooms is influenced by physical processes such as upwelling, cyclones and eddies (Vinayachandran and Mathew, 2003; GEOHAB, 2005; McGillicuddy *et al.*, 2007), chemical processes such as increased nutrient conditions (Anderson *et al.*, 2002; Smayda, 2005), biological processes like competition, grazing and allelopathy (Smayda, 1998; Graneli and Johansson, 2003) in combination with local meteorological conditions of the geographical region (Naik *et al.*, 2011).

Algal blooms along the west coast of India, one of the highly productive regions of the world's oceans (Smith *et al.*, 1991; Banse, 1994) were mainly influenced by upwelling during the monsoon period (Venugopal *et al.*, 1979; Mathew *et al.*, 1988; Banse *et al.*, 1996; Madhupratap *et al.*, 2001) which leads to high nutrient conditions triggering high primary production and bloom events (De Sousa *et al.*, 1996; Raghukumar and Anil, 2003; Patil and Anil, 2008; D'Silva *et al.*, 2012).

The break in monsoon can cause sudden changes of salinity and water temperatures, and might induce the blooming of certain species that prefer a particular range of salinity and temperature in the presence of sufficient nutrients in the coastal waters (Subrahmanyan, 1959; Gopinathan, 1974; Patil, 2003; Patil and Anil, 2008; Wang *et al.*, 2011). Nutrient availability together with light and temperature are primary determinants of phytoplankton growth and biomass accumulation (Kooistra *et al.*, 2007; Patil and Anil, 2008) which in turn is linked to anthropogenic eutrophication (Glibert *et al.*, 2005).

The most important nutrients that limit the microalgal growth in the coastal waters are silicate, an important nutrient for diatoms (Tilstone *et al*., 1994; Kristiansen and Hoell, 2002; Kudela, 2008) and nitrogen (Wilkerson and Dugdale, 2008).

5.2.4 Monitoring of algal blooms in Indian EEZ

Since, Indian fisheries economy depends heavily upon the coastal zone for marine products, keen attention has been taken regarding the occurrence of toxic microalgae and its proliferation even on a low scale, because such harmful or toxic blooms cause substantial impacts on the growth, recruitment and mortality of fish population. This causes direct and severe damage to coastal fishing industries, and thus emphasizes the need for efficient monitoring systems to minimize damage to fisheries and to reduce public health risks (D'Silva *et al.,* 2012).

5.3 *Prymnesium parvum* N. Carter bloom

Prymnesium parvum N. Carter, commonly referred as "golden alga", is a microscopic haptophyte. Blooming of *P. parvum* is common in brackish and coastal waters, especially in nutrient rich condition (Edverdsen and Paasche, 1998). This species can produce exotoxins called prymnesins under certain conditions and have wide range of biological activities, including icthyotoxic, neurotoxic, cytotoxic, hepatotoxic and haemolytic activity towards a wide range of marine organisms including fish and shellfish (Yariv and Hestrin, 1961; Manning and LaClaire, 2010). Blooms of *Prymnesium* sp. may cause serious economic losses (Moestrup, 1994).

A monospecific bloom of *Prymnesium parvum* was observed off Azheekode (Lat. 10° 11’ 02” N & Long. 76° 09’ 22” E) in the southwest coast of India, during the monsoon 2009 (Fig.40). The surface water colour was turned into pale brownish, which extended up to 8-10 nautical miles from the coast. However, there was no foam production and fish mortality during the bloom event. The bloom lasted only for one day as heavy rain dissipated the cells. This is the first report of *Prymnesium parvum* bloom from Indian waters.

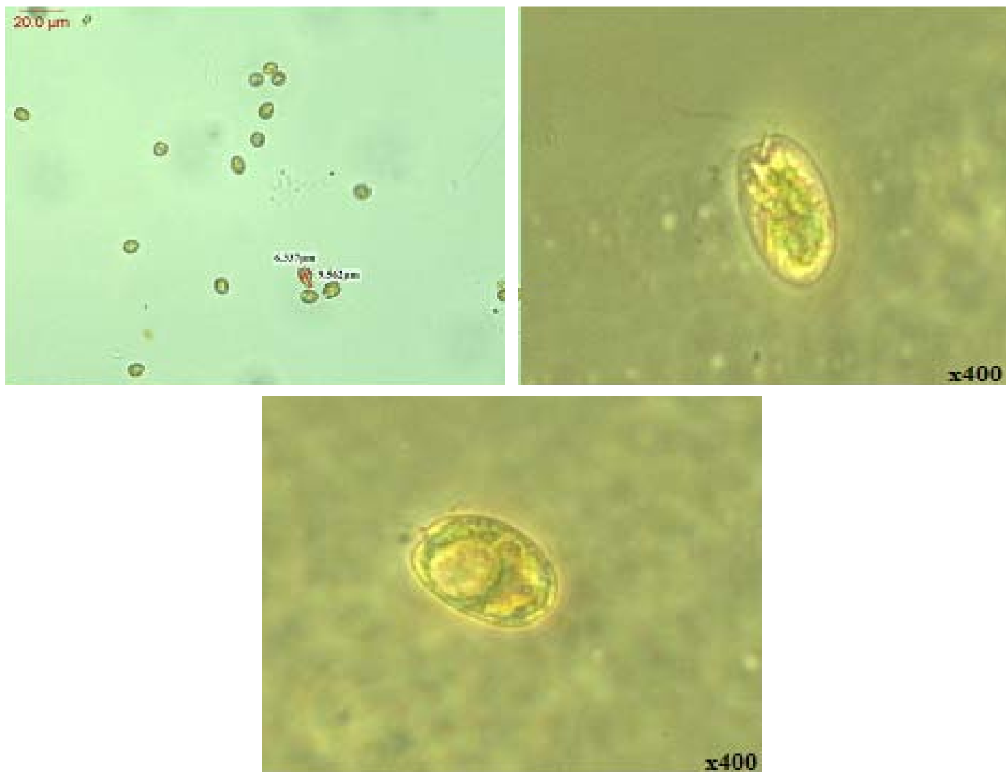


Fig. 40 Phase contrast images of *Prymnesium parvum*

5.3.1 Result

5.3.1.1 Standing crop and pigment composition

At the time of bloom observation, a monospecific standing crop of the *P. parvum* was observed with the cell density of 8×10^{7} cellsL^{-1}. Chlorophyll *a* concentration was higher, 13.54 µgL^{-1}, whereas on the previous and successive years of the same season at the same station chlorophyll *a* was found to be only of 3.82 µgL^{-1} and 2.61 µgL^{-1}, respectively. Chlorophyll *c* and carotenoid values were 3.44 µgL^{-1} and 1.91 µgL^{-1} at the time of bloom event, whereas comparatively lower concentration of chlorophyll *c* and carotenoid was observed during the previous and successive years (Fig.41).

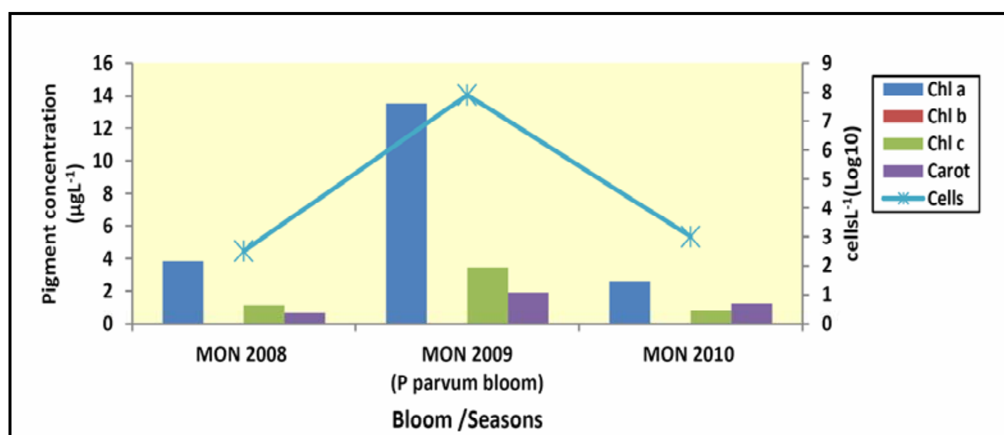


Fig.41 Comparison of standing crop and pigment concentrations of *P. parvum* bloom (MON 2009) with those during MON 2008 and 2010 off Azheekode.

5.3.1.2 Physico-chemical parameters

At the time of boom event, the sea surface temperature was 28°C, salinity 34 psu and pH 8. When compared with the previous and successive years of the same season at the same station (temperature 26°C, salinity 35 psu, pH 7.8 and temperature 26°C, salinity 30 psu, pH 8.1, respectively) no drastic change in physical parameters was noticed (Fig.42).

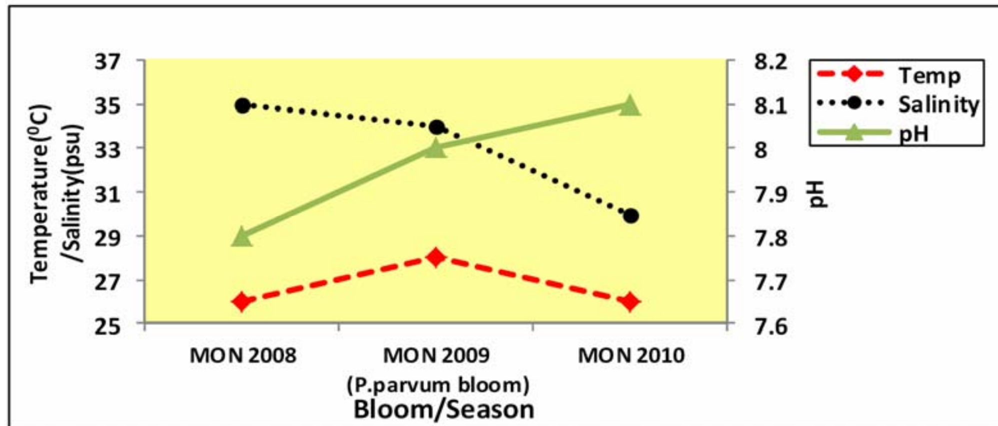


Fig.42 Comparison of physical variables at the time of *P. parvum* bloom (MON 2009) with those during MON 2008 and 2010 off Azheekode.

During the *P. parvum* bloom event, nitrate concentration was 5.6 µmolL^{-1}, phosphate was 1.9 µmolL^{-1} and silicate was 62 µmolL^{-1}. However, in the previous and successive years of the same season, higher level of nitrate concentration was recorded, whereas phosphate concentration was lowest in the 2008 monsoon season (0.36 µmolL^{-1}) but highest at 2010 monsoon season.

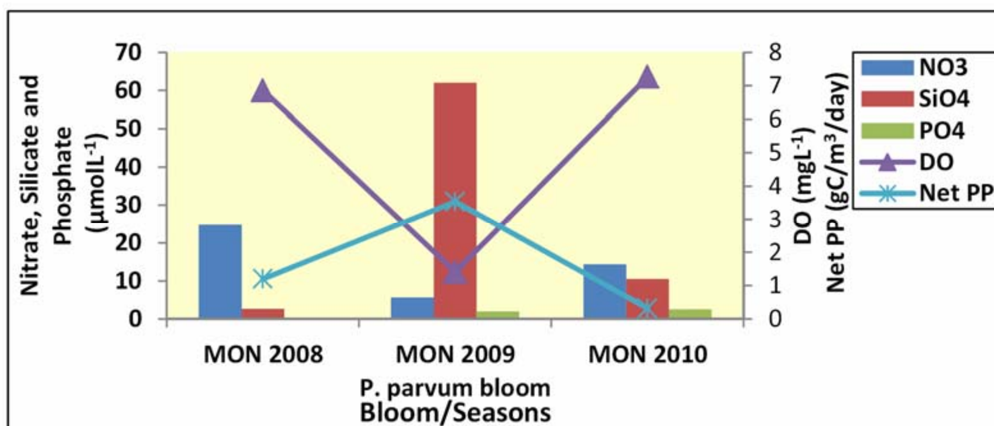


Fig.43 Comparison of nutrient concentrations, DO and Net PP of *P. parvum* bloom (MON2009) with those during MON 2008 and 2010 off Azheekode.

In the bloom event, dissolved oxygen was found to be lower (1.41 mgL^{-1}), whereas net primary production was higher (3.5 $gC/m^3/day$) when compared with the previous and successive years of the same season at Azheekode (Fig.43).

5.3.2 Discussion

5.3.2.1 Standing crop and pigment composition

Prymnesium parvum is a common member of the marine phytoplankton (Lee, 1980; Bold and Wynne, 1983; Larsen, 1999). It is a uninucleate, unicellular flagellate with an ellipsoid or narrowly oval cell shape (Prescott, 1968; Lee, 1980). *P. parvum* cell has two equal flagella and a well-developed haptonema. The flagella are used for motility and the haptonema may be involved in attachment and/or phagotrophy (McLaughlin, 1958; Prescott, 1968). Bold and Wynne (1983) described *P. parvum* as photosynthetic with possible heterotrophic growth (phagotrophy) when cells sink below the euphotic zone which enable them to sustain under nutrient deficient condition. It is a euryhaline and eurythermal organism tolerating a broad range of salinities and temperatures.

P. parvum was first identified as the culprit of mass fish mortalities in the brackish waters of Denmark and Holland (Shilo and Aschner, 1953; McLaughlin, 1958) in the Ketting Nor off the coast of Jutland and again in 1939 in the Selso So located on a peninsula of Sjalland Island (Reichenbach-Klinke, 1973). Shilo and Shilo (1953) reported *P. parvum* bloom occurrence in 1947 with large fish mortality in Israel waters. Bales *et al.* (1993) noted multiple fish mortalities associated with *P. parvum* in England starting in 1969 and becoming less severe until 1975, this bloom event was supposed to be stimulated by gull-guano from the large number

of black-headed gulls nesting in the area. In Norway, from 1989-1996, mixed blooms of *P. parvum* have occurred every summer in the Sandsfjord system (Larsen and Bryant, 1998). Hallegraeff (1992) also noted that since the 1970's, *P. parvum* blooms have been related to recurrent fish kills in Vasse-Wonnerup estuary of Australia. Harmful blooms of *P. parvum* associated with fish kills have been reported from China (Guo *et al*., 1996), Europe and Australia (Edvardsen and Paasche, 1998; Lindholm *et al*., 1999), Morocco (Sabour *et al*., 2000), Israel (Gordon and Colorni, 2008) and North America (Roelke *et al*., 2007). Usually, blooming of *P. parvum* with faunal mortalities is quite common in temporal waters when compared with tropical waters. Even a cell density of 5×10^{7} cellsL^{-1} could bring about faunal mortality (Lindholm and Virtanen, 1992). However, in the present bloom event, even though the cell density was 8×10^{7} cellsL^{-1} no faunal mortality was observed. The toxic effect of different strains will vary depending up-on the strain and the physico-chemical factors. The same species which cause blooming and faunal mortality in one area were not supposed to have the same effect on everywhere because both physico-chemical variables and the biogeography of the area play a significant role in the metabolic activity of algal strains. This was quite true in the case of blooming of *P. parvum.* The factors that are responsible for the formation of toxic *P. parvum* blooms have yet to be determined. Apart from these, the environmental conditions conducive to blooms and the factors that lead to the formation and termination of harmful algal blooms in general are complex (Paerl, 1988; Roelke and Buyukates, 2001).

However, the factors that are likely to contribute to *P. parvum* bloom formation include the production of chemicals toxic to grazers (Graneli and Johansson, 2003; Tillmann, 2003; Barreiro *et al*., 2005; Michaloudi *et al*., 2009; Brooks *et al*., 2010), use of alternative energy and nutrient sources through mixotrophy and saprophytic nourishment (Nygaard and Tobiesen, 1993; Skovgaard and Hansen, 2003; Lindehoff *et al*., 2009), suppression of competitors through allelopathy (Fistarol *et al*., 2003, 2005; Graneli and Johansson, 2003; Roelke *et al*., 2007; Errera *et al*., 2008) and resistance to the allelopathic effects of other algae (Suikkanen *et al*., 2004; Tillmann *et al*., 2007). In the present bloom event, no other microalgae were enumerated other than *P. parvum.* The monospecific nature of the present bloom event could be substantiated by the alleopathic effect of the *P. parvum* because, by producing allelopathic chemicals *P. parvum* can immobilize plankton and suppress competitors, thereby fuelling bloom development and persistence (Fistarol *et al*., 2003, 2005; Graneli and Johansson, 2003; Uronen *et al*., 2005; Roelke *et al*., 2010).

Chlorophyll *a, c* and carotenoid concentrations during the bloom were much higher than the values obtained during the previous and subsequent years. The chlorophyll *a* was found to be 13.54 μgL^{-1}, with a standing crop of 8×10^7 $cellsL^{-1}$. Lindholm and Virtanen (1992) reported a bloom of *P. parvum* with toxicity and fish mortality in Finland waters during June 1990, where chlorophyll *a* was below 10 $\mu gL^{-1}$and standing crop was 5×10^7 $cellsL^{-1}$. A significant hike in the chlorophyll *c* and carotenoids were present in the bloom event. This is because chlorophyll *c* and carotenoid (fucoxanthin) are the major accessory pigment of the haptophycean members. *P. parvum* cells have large amounts of fucoxanthin, a carotenoid pigment that gives its characteristic golden colour (Moestrup and Thomsen, 2003).

5.3.2.2 Physico-chemical parameters

Shilo and Aschner (1953) observed that temperatures greater than 30°C were inhibitory to the growth of *P. parvum*, and 35°C resulted in cell lysis, however, the cells could survive at 2°C for many days. McLaughlin (1958) noted erratic growth of *P. parvum* above 32°C with death occurring at 34°C. Larsen *et al*. (1993) found that *P. parvum* has a growth temperature optimum of 26°C and growth was found to be severely limited at 10°C. However, different strains of *P. parvum* tested by Larsen and Bryant (1998) exhibited maximum growth rate at 15°C and tolerated wide temperature range of 5°C to 30°C. An outbreak of *P. parvum* occurred in Morocco waters where the temperature was between 15°C - 23.5°C (Sabour *et al.*, 2000). All these investigations suggest that *P. parvum* is a eurythermal organism. In the present *P. parvum* bloom event also the sea surface temperature was 28°C, which is well within the optimal range of growth temperature of *P. parvum*.

Salinity may also play an important role in the blooming of *P. parvum* and its toxicity. Optimum salinity of bloom formation depends on the strains. Larsen *et al.* (1993) reported growth of *P. parvum* in the salinity range of 8-25 psu. Larsen and Bryant (1998) noted that different strains of *P. parvum* survived in salinities ranging from 3 to 30 psu. Sabour *et al*. (2000) reported that *P. parvum* bloom associated with the fish kill in Morocco was characterized by a salinity of 8.6 to 12.4 psu. In the present bloom observation, the salinity was 34 psu. A rapid change in physical variables especially of salinity and temperature in the bloom event as a result of heavy rain could be one reason that caused the *P. parvum* bloom deterioration on the very next day. Roelke

et al. (2011) observed that an increase in anthropogenic activity and change in the climate can influence the frequency and magnitude of *P. parvum* bloom. Usually an alkaline pH favoured the blooming of *P. parvum* (Lindholm *et al.*, 1999; Prosser *et al.*, 2012). Sabour *et al.* (2000) reported that the *P. parvum* outbreak in Morocco occurred in water with a pH of 7.67-9.04. In the present study, the pH was found to be 8 during the bloom which was favourable for the growth of this alga.

Usually, high nutrient supply can promote algal blooms by supporting rapid reproductive growth. After a population has grown, its ultimate abundance is often controlled by the supply of a critical nutrients, such as nitrogen or phosphorus, because conversion of nutrients to algal biomass occurs through the process of consumption and growth. Since *P. parvum* bloom occurs usually in the eutrophic waters, nitrogen and phosphorus can play a key role in the bloom dynamics and toxicity. In the present bloom event, nitrate concentration was 5.6 $\mu molL^{-1}$, nitrite was 0.8 $\mu molL^{-1}$ and phosphate was 1.9 $\mu molL^{-1}$. The low nitrate to phosphate ratio might be one reason for the blooming. Similarly, Michaloudi *et al.* (2009) reported that low nitrogen concentrations favoured the initiation of the *P. parvum* bloom in northern Greece. The present bloom station, off Azheekode was located near the northern end of the Cochin estuary, which carry large influx of rain water. Since the bloom event occurred in the monsoon season, it might have played a significant role in the bloom dynamics. However, the important point to be noted in connection with the planktonic microalgal blooms is not why they occur but rather what mechanisms control the species which occur at a given time and place (Richardson, 1997). The marine environment provides different niches that can be exploited by different microalgal

species and each species has its own specific combination of necessities such as light, micro and macro nutrients. The triggering of a particular microalga to its bloom stage is not only dependent on a particular factor but also on a combination of all the favourable factors like physico-chemical, geographical, biological as well as the occurence of the target species at that time.

The toxicity of *P. parvum* increased markedly under nitrogen or phosphorus deficient conditions compared to nutrient sufficient conditions (Johansson and Graneli, 1999) suggesting that the production of toxins is a chemical response to low nutrient levels. However, the presence of blooms of *P. parvum* did not necessarily mean that the algae will produce and secrete toxins into the water, and in fact studies suggested that bloom density and toxicity are not strongly correlated (Shilo, 1981) which was quite evident in the present bloom event also. Even though *P. parvum* cell density was high, there was no faunal mortality or foam production; this might be due to the sufficiency in nitrogen and phosphorus concentration at the present bloom event. So, it could be clear that apart from the geographical variation, which provide a species-specific privilege, nutrients played a significant role in the harmless effect at the time of the present *P. parvum* bloom. A low concentration of dissolved oxygen was recorded at the time of bloom observation, since the bloom was in the decline stage. Ramaiah *et al.* (2005) noted a very low level of dissolved oxygen concentration as a consequence of excessive organic loading due to crash of the bloom in southern coast of Kerala. So, it could be inferred that, rather as a specific factor, the multiple resources like temperature, salinity, pH, nutrients, other environmental factors and geographical adaptations, in a favourable range for a particular species,

commonly referred to as ‘species-specific’, played a significant role in the present bloom dynamics.

5.4 *Proboscia alata* (Brightwell) Sandström bloom

Proboscia alata (Brightwell) Sandström is widespread in boreal, tropical and subtropical realms of the World Ocean and the seas of the middle latitudes. It substantially contributes to the abundance and biomass of the total phytoplankton and carbon fluxes in pelagic ecosystems (Jordan *et al*., 1991; Takahashi *et al*., 1994). *Proboscia* sp. are quite important in the aspect of diatom–diazotrophic cyanobacterial association and its episodic, monospecific bloom formation, since it can contribute high rate of carbon and nitrogen fixation in the marine ecosystem. *Proboscia alata* can dominate phytoplankton biomass in highly productive areas (Garate-Lizarraga *et al*., 2003).

The coastal sea off Bekal (Lat. 12° 38' 02" N & Long. 75° 04' 31" E), experienced a bloom of centric diatom *Proboscia alata* (Brightwell) Sandström; (formerly *Rhizosolenia alata)* during 10th to 12th October 2009, in the early post-monsoon (Fig.44). During the bloom, there was a pale brown discolouration of sea surface water, which extended around 3 nautical miles along the coastal area. There was no fish mortality and foam production. For a comparative analysis, sampling was also done from two reference stations, Off Thykadapuram (St.1) (Lat. 12° 22' 84" N & Long. 75° 10' 94" E) and Off Puthur (St.2) (Lat. 12° 55' 18" N & Long. 74° 95' 18" E), one before and one after the bloom station in the same latitude.

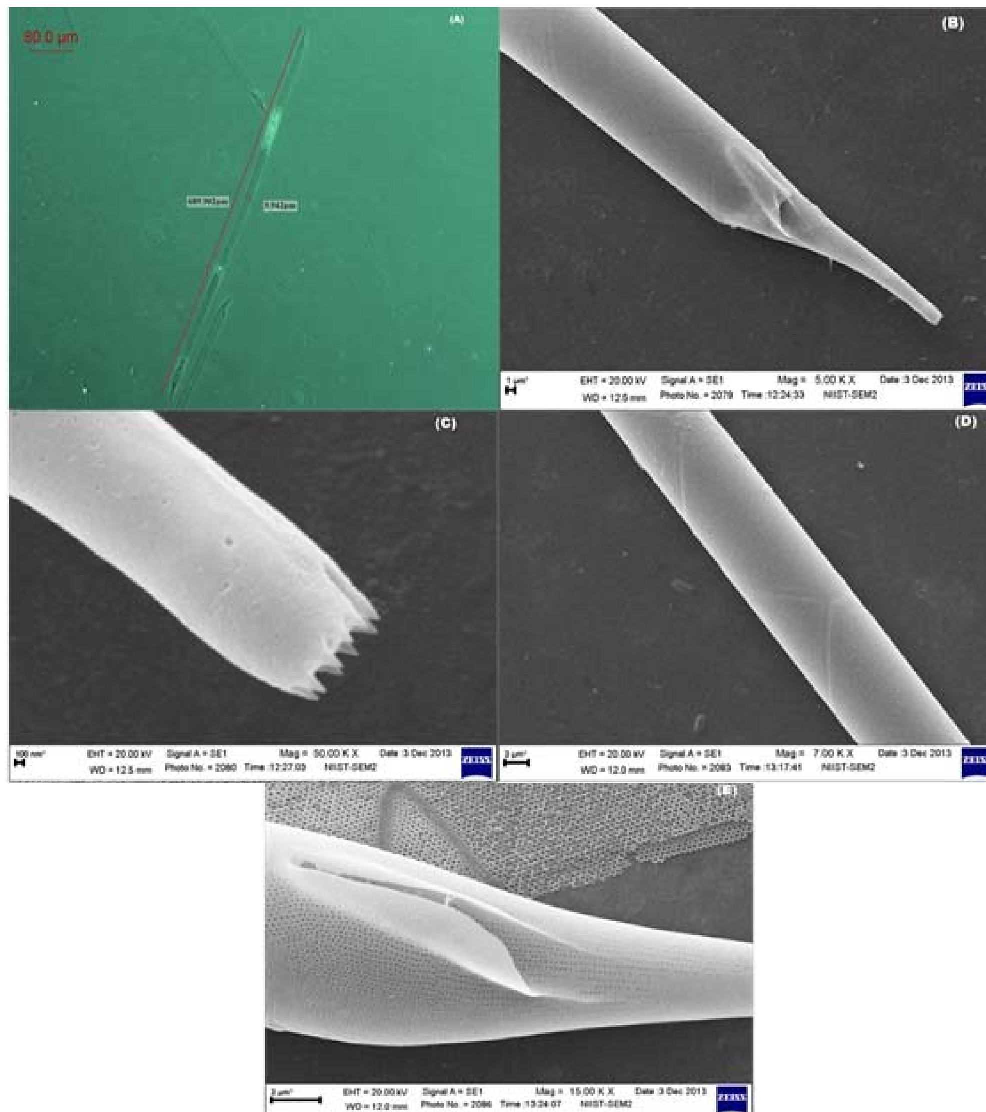


Fig. 44 *Proboscia alata*. (A) A complete cell, (Phase contrast microscopy). (B) Apical part of valve, (ESEM). (C) Proboscis structure, varied spinules (ESEM). (D) Girdle segments, (ESEM). (E) Details of clasper and contiguous area, (ESEM).

5.4.1 Result

5.4.1.1 Standing crop and pigment composition

Table 18 Abundance of standing crop during *Proboscia alata* bloom

Sl. No.	Class	Off Bekal		Off Thykadapuram	Off Puthur
		10-10-2009	12-10-2009	Reference st.1	Reference st.2
Bacillariophyceae					
1	*Asterionellopsis glacialis*				18
2	*Chaetoceros constrictus*			96	24
3	*Chaetoceros curvesetus*			54	14
4	*Chaetoceros decipiens*		115		
5	*Coscinodiscus asteromphalus*		79		6
6	*Coscinodiscus centralis*			48	
7	*Cylindrotheca closterium*		29		
8	*Entomoneis alata*				12
9	*Lyrella lyra*			4	28
10	*Navicula hasta*				36
11	*Nitzschia sigma*		18	86	
12	*Pleurosigma falx*			70	86
13	*Proboscia alata*	80000	28000	59	66
	Total	**80000**	**28241**	**417**	**290**
Dinophyceae					
1	*Biceratium furca*			48	23
2	*Ceratium fusus*		119		
3	*Ceratium trichoceros*		72		
4	*Dinophysis acuminata*			38	
5	*Dinophysis caudata*			80	17
6	*Pyrophacus steinii*		360		34
	Total		**551**	**166**	**74**
	***Grand total**	**80000**	**28792**	**583**	**364**

***cellsL^{-1}**

The abundance of standing crop during the bloom is presented in Table 18. On 10th October, the total standing crop comprised only *P. alata*, with 8×10^4 cellsL^{-1}. On 12th October, the abundance of *P. alata* decreased to 2.8×10^3 cellsL^{-1} and a few species of other diatoms and dinoflagellates were also observed. Both the reference stations showed the predominance of diatoms.

On 10th October, the chlorophyll *a* concentration was found to be highest, 10.8 µgL^{-1}, whereas it receded to 6.48 µgL^{-1} on 12th October with an average of 8.64 µgL^{-1}. Lower concentration of chlorophyll *b* was detected from the bloom event. Chlorophyll *c* concentration was highest on the first day with 4.62 µgL^{-1}, and on 12th October, it was 3.97 µgL^{-1} with an average of 4.29 µgL^{-1}. On 10th October, carotenoid concentration was 2.39 µgL^{-1}, while on 12th October it receded to 2.01 µgL^{-1} with an average value of 2.20 µgL^{-1}. In the reference stations, the average chlorophyll *a* concentration (4.34 µgL^{-1}) was lower than the bloom event. Both chlorophyll *c* and carotenoid average values (2.39 µgL^{-1} and 1.13 µgL^{-1} respectively) were also found to be in a lower range than the *P. alata* bloom event (Fig.45).

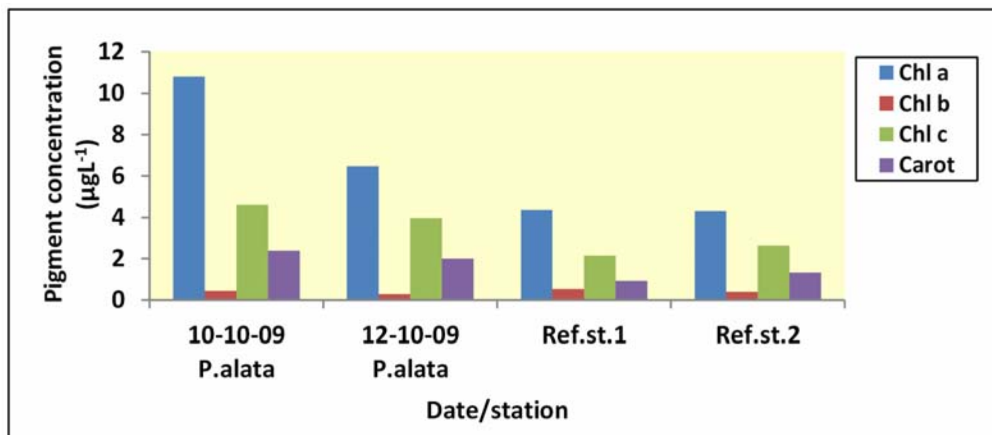


Fig.45 Concentration of pigments during *Proboscia alata* bloom

5.4.1.2 Physico-chemical parameters

In the bloom event, the sea surface temperature was 27°C on the first day while on 12th October it was 28°C. Salinity was stable at 35 psu in the entire bloom event, whereas the pH showed a gradual decrease from 8.4 on the first day to 8.2 on 12th October, when the bloom almost crashed. The pH observed in the reference stations were comparatively lower (7.6 and 7.8 in Ref. stations 1 and 2 respectively) (Fig.46).

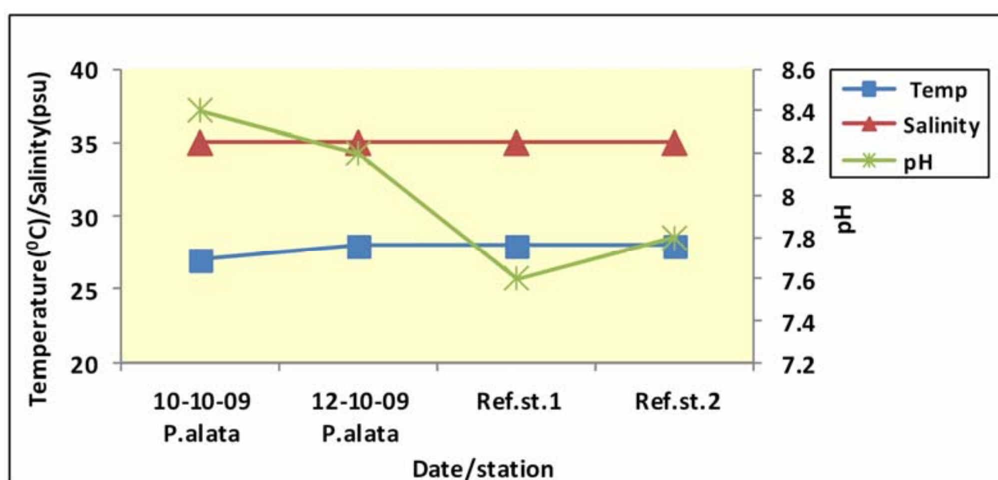


Fig.46 Variation in temperature, salinity and pH during *Proboscia alata* bloom

During the bloom event, on the first day, concentration of silicate was high (38.31 µmolL-1) which receded to 14.2 µmolL-1 on the last day. Concentration of nitrate ranged from 2.11 µmolL^{-1} to 1.4 µmolL^{-1}, whereas nitrite was below the detectable range. Phosphate concentration was found to be ranged from 1.40 µmolL^{-1} to 1.20 µmolL^{-1} during the bloom event. Dissolved oxygen concentration was 5.42 mgL^{-1} on the first day, whereas at the end of bloom event, it was 4.09 mgL^{-1}. Primary production was found to be highest (1.87 gC/m^3/day) on first day of bloom event, whereas the lowest

(1.05 gC/m^3/day) was recorded on the last day of the bloom. In the reference stations (st.1 and st.2), concentration of silicate was 1.86 µmolL^{-1} and 2.46 µmolL^{-1}, and nitrate was 3.03 µmolL^{-1}and 2.65 µmolL^{-1}, respectively. Phosphate concentration was found to be very low. Dissolved oxygen value was 6.25 mgL^{-1} and 5.24 mgL^{-1} and net primary production was 0.76 gC/m^3/day and 1.41 gC/m^3/day, respectively in reference stations 1 and 2 (Fig.47).

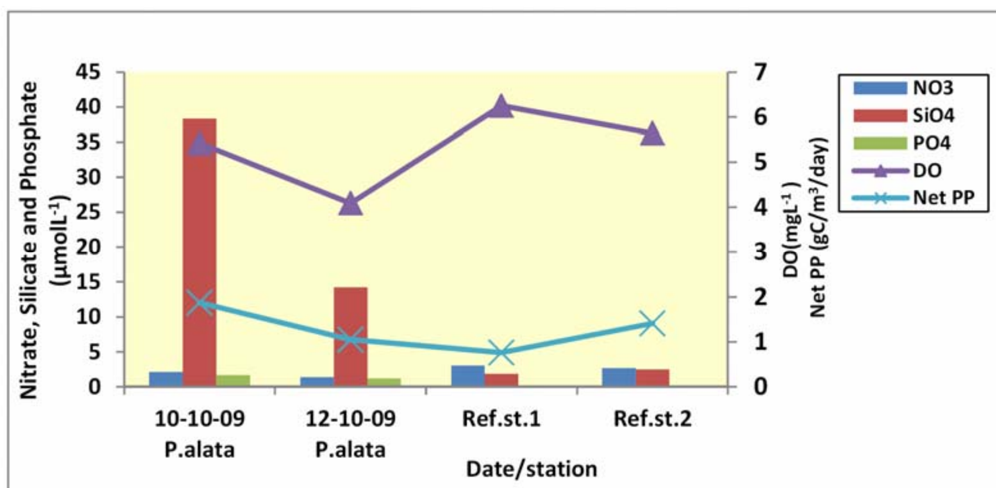


Fig.47 Chemical variables during *Proboscia alata* bloom

5.4.2 Discussion

The west coast of India is one of the most biologically productive areas of the World Oceans (Smith *et al*., 1991; Banse, 1994) mainly influenced by the seasonally reversing monsoon systems, southwest monsoon (June- September) and northeast monsoon (November-February), leading to upwelling. The microalgal blooms triggered by upwelling influenced eutrophication is common in this area. Hence the occurrence of the present *P. alata* bloom soon after the monsoon can be coupled with upwelling which leads to high nutrient conditions, triggering high primary production (De Sousa *et al*., 1996).

5.4.2.1 Standing crop and pigment composition

On the first day of the bloom event, *Proboscia alata* was seemingly in a monospecific condition with a cell count of 8×10^4 cellsL^{-1}. There was no significant difference in the cell abundance on the 2nd day of the bloom event. However, on the 3rd day (12th October 2009), the surface water discolouration nearly faded and the cell abundance reduced to 2.8×10^3 cellsL^{-1}. Along with *P. alata* a number of other diatom and dinoflagellate species like *Chaetoceros decipiens* Cleve; *Coscinodiscus asteromphalus* Ehrenberg; *Cylindrotheca closterium* (Ehrenberg) Reimann & J.C. Lewin; *Ceratium fusus* (Ehrenberg) Dujardin; *Ceratium trichoceros* (Ehrenberg) Kofoid and *Pyrophacus steinii* (Schiller) Wall & Dale, were enumerated from the sample on the third day, which apparently indicated that the bloom was in a stage of decline. In the reference stations also diatoms were found to be the predominant flora with 11 species, but the cell numbers were considerably less. The dinoflagellates (4species) which were prominent in the reference stations were also found in the bloom station when the bloom was at the decline stage. *Proboscia alata* was found in lower cell density of 59 cellsL^{-1} and 66 cellsL^{-1} respectively, in the reference stations. So it could be inferred that during the algal bloom as the number of blooming species increases the diversity of microalgae decreases. At the end of the bloom event, the diversity further increased and the abundance of *P. alata* decreased.

The magnitude and the composition of the algal bloom were indicated by the concentration of the pigments, especially of chlorophyll *a, c* and carotenoid. On the first day of the bloom event, the chlorophyll *a* was found to be high, which gradually receded in the last day of the bloom event. A hike in

concentration of chlorophyll *c* and carotenoids were also found on the first day which substantiated the diatom bloom event, since these are the major accessory pigments of diatoms, and showed a gradual decrease on the last day of the bloom. The concentration of pigments particularly of chlorophyll *a, c* and carotenoids in the reference stations were comparatively much lower. The occurrence of chlorophyll *b* especially in the initial stages of the bloom sample might be due to the presence of prochlorophytes / euglenophytes / chlorophytes, which could not be enumerated by Sedgewick-Rafter counting cell.

5.4.2.2 Physico-chemical parameters

In the present bloom event, the pH was found to be high (8.4) on the first day, when the bloom was monospecific and a gradual decrease in pH was observed by the third day when the bloom was in the decline stage. The hydrogen ion concentration in the coastal environment is probably altered through nutrient enrichment. Upon the availability of more nutrients the phytoplankton proliferates into bloom condition which may progressively drive the pH higher (Kenneth, 2002). However, in the reference stations the hydrogen ion concentration did not show much variation. Both the salinity and temperature did not change drastically during the bloom event, which were found to be almost similar to the reference stations.

During the bloom event, the concentration of silicate was much higher compared to the reference stations and its concentration reduced gradually in the second and third day. Since silicate is utilised for the formation of siliceous frustules of the diatoms, it is considered as the most important nutrient regulating the growth and proliferation and ultimately the blooming of diatoms (Kristiansen and Hoell, 2002). *Proboscia* species are weakly silicified and

they can adjust their buoyancy and migrate to deeper levels below the euphotic zone to obtain nutrients. In seasonal upwelling regions, this migration often enables them to reach nutrient rich water layers before the mixing of photic zone, resulting in a high contribution to the primary production by these diatoms. Therefore, remnants of *Proboscia* species may serve as biomarkers for upwelling conditions (Tilstone *et al*., 1994). Southwest coast of India is a known area of upwelling during southwest monsoon period and since *Proboscia* is a common species, comparatively high silicate value probably due to the coastal upwelling might have influenced the formation of this bloom. Usually, the diatom growth in marine waters is likely to be limited by dissolved silica when Si: N ratios are less than 1 (Piehler *et al*., 2004). On the first day of the bloom event, the Si: N ratio was 17:1, whereas on the third day, when the bloom almost crashed, it receded to 9:1. This depletion in Si: N ratio was found to be positively correlated with variations of chlorophyll *a* and standing crop in the bloom event. However, no such remarkable variation in the individual concentration of nutrients especially of silicate and Si: N ratio was observed in the reference stations. Hence, it could be inferred that high Si: N ratio played a significant role in the formation of the *P. alata* bloom. Dissolved oxygen and primary production were high in the initial stages of the bloom, whereas it gradually decreased as the bloom crashed. As a consequence of excessive organic loading, during the crash of the bloom, the dissolved oxygen concentration might decrease (Ramaiah *et al*., 2005). So it may be inferred that, an increased Si: N ratio with alkaline pH favoured the *P. alata* bloom and lowering of Si: N ratio with decreasing pH might have caused the bloom declination.

5.5 *Chattonella marina* (Subrahmanyan) Hara et Chihara bloom

Marine raphidophycean algae, especially *Chattonella* sp. have been implicated in major fish kills in various parts of the world (Tiffany *et al*., 2001). Blooms of *Chattonella marina* have been linked to mass mortality of marine life along the southwest coast of India [reported as *Hornellia marina* by Subrahmanyan (1954); Jugnu and Kripa, 2009; Padmakumar *et al*., 2011; Sanilkumar *et al*., 2012).

A conspicuous brown discolouration of surface water was observed in the coastal sea off Mahe (Lat. 11° 42' 18" N & Long. 75° 32' 36" E), along the northern part of Kerala from October 27th to November 1st 2011. This was due to the massive bloom of marine raphidophyte, *Chattonella marina* (Subrahmanyan) Hara et Chihara (Fig.48) which extended up to about two kilometres inside the Mahe (Mayyazhi) estuary during high tide. The spreading of visible water discolouration (about 1 km in width) extended from the bar mouth to both the northern and southern sides. The bloom event lasted for a period of one week.

Fig.48 Phase contrast photo micrograph of *C. marina*

Fig.49 Gills of Mullet fish being choked by *C. marina* cells

5.5.1 Result

5.5.1.1 Standing crop and pigment composition

Table 19 Abundance of standing crop during *Chattonella marina* bloom

Sl. No.	Class	Mahe		
		27-10-2011	29-10-2011	01-11-2011
Raphidophyceae				
1	*Chattonella marina*	45000000	40000000	3800000
	Total	**45000000**	**40000000**	**3800000**
Bacillariophyceae				
1	*Campylodiscus ecclesianus*			8
2	*Chaetoceros decipiens*			12
3	*Coscinodiscus asteromphalus*		11	48
4	*Coscinodiscus radiatus*		7	18
5	*Cylindrotheca closterium*			10
6	*Cylindrotheca gracilis*			26
7	*Hantzschia marina*			8
8	*Odontella aurita*		6	12
9	*Pleurosigma aestuarii*			18
	Total		**24**	**160**
Dinophyceae				
1	*Biceratium furca*			4
2	*Prorocentrum gracile*			7
	Total			**11**
	***Grand total**	**45000000**	**40000024**	**3800171**
***cellsL^{-1}**				

The abundance of standing crop during the bloom is presented in Table 19. The bloom was in a monospecific condition on the first day of investigation (27th October) with the cell abundance of 4.5×10^6 cellsL^{-1}. There was a gradual

decrease in the cell numbers on the 29th of October, with 4×10^6 cellsL^{-1}. On the 3rd day of observation, 1st November, a steady decrease in cell abundance was observed with 3.8×10^5 cellsL^{-1}.

On the first day of observation the chlorophyll *a* concentration was 10.89 µgL^{-1}, which receded gradually, and on the last day of observation it was 6.69 µgL^{-1}. The highest concentration of chlorophyll *b* was 0.69 µgL^{-1} on the first day and the lowest being 0.49 µgL^{-1} on the final day. Concentration of chlorophyll *c* varied from 2.44 µgL^{-1} to 1.75 µgL^{-1}, while concentration of carotenoid pigment varied from 3.19 µgL^{-1} to 2.29 µgL^{-1} by the last day of bloom event (Fig.50).

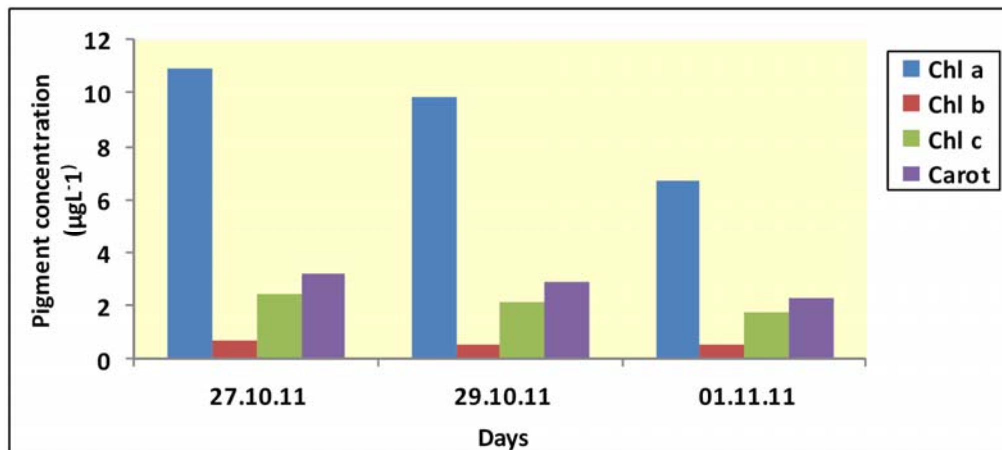


Fig.50 Concentration of pigments during *Chattonella marina* bloom

5.5.1.2 Physico-chemical parameters

During the bloom event temperature varied from 25°C to 27°C. Salinity was 30 psu on the first day of bloom, whereas it receded to 28 psu on the last day of bloom event. pH was 7.9 on the first day of investigation and on the last day it was 7.7 (Fig.51).

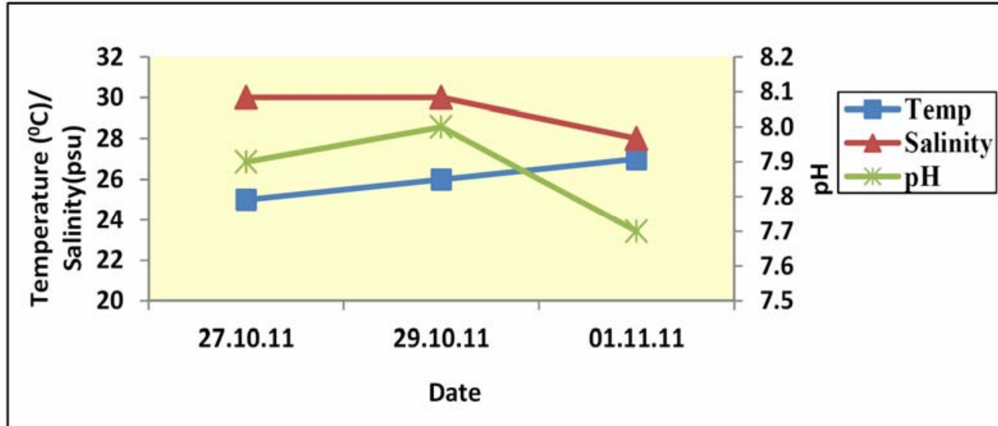


Fig.51 Variation of temperature, salinity and pH during *Chattonella marina* bloom

The concentration of nitrate ranged from 12.54 µmolL^{-1} on 27th October, to 6.99 µmolL^{-1} on 1st November. Silicate concentration varied from 3.47 µmolL^{-1} to 2.38 µmolL^{-1}, whereas the phosphate concentration varied from 0.56 µmolL^{-1} to 0.49 µmolL^{-1}. The concentration of nitrite was below detectable range. Dissolved oxygen concentration varied from 5.2 mgL^{-1} to 4.4 mgL^{-1}, whereas net primary production ranged from 4.2 gC/m^3/day to 1.2 gC/m^3/day in the bloom event (Fig.52).

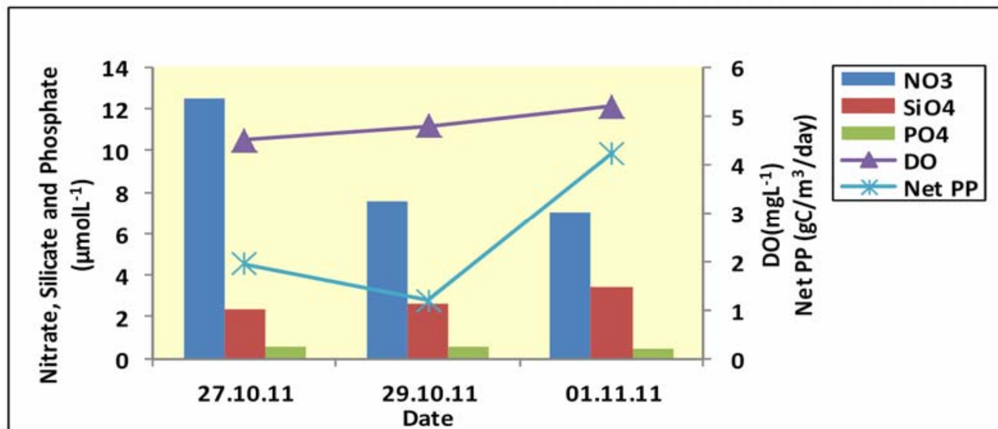


Fig.52 Chemical variables during *Chattonella marina* bloom

5.5.2 Discussion

Blooms of marine raphidophyte *Chattonella marina* (Subrahmanyan) Hara et Chihara are known to have deleterious effects on the marine fauna by having potent ability to produce haemolytic, haemoagglutinating compounds and reactive oxygen species (ROS) including superoxide anion radicals (O_2^-), hydrogen peroxide and hydroxyl radicals (OH^-) (Onoue and Nozawa, 1989; Oda *et al*., 1994). The toxic effects of *Chattonella marina* have been attributed to the production of brevetoxins and potent neurotoxins similar to those of dinoflagellate, *Gymnodinium breve* (Ahmed *et al*., 1995; Khan *et al*., 1995). Even in a nanomolecular concentration, these polyether compounds can act as an icthyotoxin and the gills become highly susceptible absorptive area for these brevetoxins from the water column. Hence, exposure of fish to *C. marina* causes gill epithelium to become swollen with massive mucous production (Endo *et al*., 1985, 1992) and fish appear to smother even in well-oxygenated waters. The bloom of the same species had been reported as to have appeared in different colours like green (Subrahmanyan, 1954; Jugnu and Kripa, 2009) and brownish-red (Padmakumar *et al*., 2011). Usually, *C. marina* had numerous bright green, disc-shaped chromatophores, uniformly distributed all over the body around its peripheral region (Subrahmanyan, 1954). Here, the chromatophores appeared in golden brown colour instead of the normal green, which might be the reason for the brown discolouration of the surface water during the bloom.

5.5.2.1 Standing crop and pigment composition

In the present study, on the first day of investigation, *Chattonella marina* bloom was found to be monospecific with a standing crop of 4.5×10^6 cellsL^{-1}.

On the second day, the standing crop of *Chattonella marina* decreased to 4×10^{6} cellsL^{-1} and a few species of diatoms like *Coscinodiscus asteromphalus* Ehrenberg, *Coscinodiscus radiatus* H.L.Smith *and Odontella aurita* (Lyngbye) C.Agardh were also observed. On the last day of observation the standing crop of *Chattonella marina* receded to 3.8×10^{5} cellsL^{-1}. The abundance of diatoms (9 species) and dinoflagellates (2 species) increased profoundly. *Chattonella marina* bloom had been reported in Kerala coast earlier. A bloom of the same species occurred during September 2002 and 2003 with a standing crop of $28x10^{7}$ and $135x10^{5}$ cellsL^{-1} respectively, along the Calicut coast (Jugnu and Kripa, 2009) and it was associated with huge fish mortality. In the case of this bloom event also the bloom was monospecific initially; however, the species diversity increased gradually during the crash of the bloom. A species succession could be noted during algal bloom and its crash.

The concentrations of pigments like chlorophyll *a, b, c* and carotenoids were significantly related with the bloom event, since a gradual decrease in the pigment concentrations were observed from the first day to the last day of the bloom event. The pigment concentration changed with different phases of the bloom. On the first day, the pigment concentration and the standing crop revealed that the bloom was in an exponential phase. On the second day of observation, slight decrease in the standing crop and pigment concentration were noted which pointed out the transition of exponential phase to the stationary phase. On the last day of the bloom event, the decreased standing crop and the pigment concentration indicated the late stationary phase, which led to decline phase of the bloom. Significant hike in chlorophyll *c* and carotenoids were admissible to *Chattonella marina* bloom since Chl c_1+c_2 and fucoxanthin dominated carotenoid were the major accessory pigments of

marine/brackish water golden brown raphidophytes. The mean chlorophyll *a* concentration in the bloom event (9.14 ±2.18 μgL^{-1}) was found to be higher when compared with the five year mean value of the same at Mahe for the same season (3.48 ±3.10 μgL^{-1}). Similarly, the mean concentrations of chlorophyll *c* and carotenoid at the time of present bloom event (2.11 ±0.35 μgL^{-1} and 2.79 ±0.46 μgL^{-1} respectively) were also higher when compared with the five year mean concentrations at Mahe (1.57 ±0.71μgL^{-1} and 1.63 ±2.13 μgL^{-1} respectively). The chlorophyll *b* value indicated the presence of chlorophytes / prochlorophytes / euglenophytes which are very small and could not be counted by Sedgewick-Rafter counting cell.

5.5.2.2 Physico-chemical parameters

The surface water temperature showed a gradual increase from 25°C to 27°C from the first day to third day of observation. The optimum growth of *C. marina* in laboratory conditions was shown to be at 25°C (Marshall and Hallegraeff, 1999). The bloom condition observed here was found to be optimum for the maximum growth of *C. marina*. The sea surface temperature range was 15°C to 35°C in the Salton Sea during the bloom of the same species (Tiffany *et al*., 2001). However, *C. marina* preferred an optimal temperature of 25°C and 18°C as the minimum for its survival (Wang *et al*., 2011). Here on 27th October, the temperature was exactly 25°C and then increased to 27°C. Even though the variation was very minor, intense bloom appeared when temperature was at 25°C and the bloom receded with increasing surface water temperature during the following days. The temperature optima of *C.marina* from different geographical areas differ with seasons. *C. marina* blooms mostly occur in summer along the Japanese coast,

when water temperature was within a range of 20–25°C. The raphidophycean blooms commonly occur when the temperature varies from 20–32°C during spring and autumn in the southern Chinese coastal waters (Wang *et al.*, 2006 b). On the first two days of bloom event, when the cell abundance was high, the salinity was 30 psu, which was optimum for the maximum growth of *C. marina* (Marshall and Hallegraeff, 1999). In accordance with the decrease in salinity (28 psu on the last day) the cell abundance of *C. marina* was also found to be decreased.

The nutrient concentrations did not show any marked variation during the bloom days except nitrate (12.54, 7.54 and 6.99 $\mu molL^{-1}$). The mean nitrate concentration during the bloom period was 9.02 $\pm$3.06 $\mu molL^{-1}$ against the 2006-2010 mean of 6.59 $\pm$1.90 $\mu molL^{-1}$ (Fig.49). *C. marina* prefer nitrogen rich environment for their better growth and survival. Studies from the Chinese coasts also have shown that *Chattonella* blooms are nitrogen dependent. The increase of nitrogen, particularly nitrate, was thought to be the important cause of the bloom of *C. marina* in the Daya Bay, South China Sea (Wang *et al.*, 2006b; Wang *et al.*, 2011). Here also the increased nitrate concentrations, when compared to the five year mean value, might have influenced the development of bloom.

The mean phosphate level during the bloom event was 0.52 $\pm$0.04 $\mu molL^{-1}$, which was comparatively lesser than that of the five year mean concentration (1.92 $\pm$1.90 $\mu molL^{-1}$). *C. marina* could efficiently utilize all organic phosphate compounds as noted for several other harmful algal bloom species (Yamaguchi *et al.*, 2008). It could maintain growth and survive in phosphate free conditions, which suggest that this species has the ability to store

phosphorus in an internal pool for sustaining population numbers (Yamaguchi *et al*., 2008; Wang *et al*., 2011).

The levels of silicate are usually higher during the early post-monsoon season at Mahe and the five year mean (2006-10) value was 12.48 $\pm$10.73 µmolL^{-1}. Diatoms were the dominant group found here and they have an absolute requirement for silicon as they are the most important silicifying algal group (Kristiansen and Hoell, 2002). During the bloom event, the silicate concentration was comparatively very low, (2.38 to 3.47 µmolL^{-1}) hence it has limited the proliferation of diatoms. The diatom growth in marine waters is likely to be limited by dissolved silica when Si: N ratios are less than one (Piehler *et al.*, 2004). In this bloom event, the Si: N ratios were less than one against the five year mean of 3:1. Since there was no competition from diatoms, *C. marina* could proliferate well and produced the bloom.

The N: P ratios showed highest value on 27th October with 26:1, while it decreased to 12:1 on 1st November. Five year mean of 4:1 ratio prevailed in the location since 2006.The high nitrate concentration might have played a major role in this bloom. Similar observations were made during the *C.marina* bloom along Chinese coast by Shen *et al*. (2006).

5.5.2.3 Fish mortality during *Chattonella marina* bloom

During the bloom event, mortality of a few fishes like Pearl Spot (*Etroplus suratensis*) and Mullets (*Mugil cephalus*) was observed on 27th October, in the region of one kilometre inside the estuary (Fig.49). It was found that the gills were fully choked with algal cells and the death might have been due to suffocation. No other faunal mortalities were observed. The

avoidance of fishes from the bloom areas along this coast was very much evident. Commercially important shoaling fishes shift from bloom waters to other favourable grounds during the *C. marina* bloom incidents (Subrahmanyan, 1954; Jugnu and Kripa, 2009). There are several reports on the production of icthyotoxin during the bloom of *C. marina* (Endo *et al*., 1992; Jugnu and Kripa, 2009). Contrary to this, the fish killing activity of *C. marina* is attributed to the ROS (Reactive Oxygen Species) produced by these algae (Kim and Oda, 2010). However, the impact of harmful algal blooms depends on the concentration of the harmful species; even the most toxic species must have a minimum cell concentration to exert the harmful effect (Smayda, 1997). The present harmful algal bloom incident was not at all a prolonged one and no other faunal mortalities were observed. Hence, it is imperative to comment that even though mortality of a few fishes were observed during the bloom, it may not have been associated with the effect of toxins formed during the present *Chattonella marina* bloom event.

It could be inferred from the present bloom events that, most of the bloom formations are naturally driven by physical forcing such as monsoonal influence, riverine discharge and seasonal upwelling, which result in variations in temperature, salinity, irradiance, water stability, nutrient enrichment (eutrophication) etc. The optimum conditions for blooming will vary from species to species. Occurrence of *Chattonella marina* bloom, which is considered as a ‘species-specific bloom’, following a seasonal pattern (D’Silva *et al*., 2012) is mainly attributed to the presence of higher concentration of nitrate, since N: P ratio was found to be high during the initial stage of the bloom. Optimum temperature for this bloom was found to be 25°C.

The occurrence of *Proboscia alata* bloom soon after the monsoon was coupled with upwelling, which led to high nutrient conditions (eutrophication) especially of silicate which impart high Si: N ratio that played a significant role in the bloom initiation. Even though there was no specific factor that could be attributed to *Prymnesium parvum* bloom, the combination of eutrophic condition with the species-specific optimum favourable conditions of physical variables could have favoured the bloom event. The bloom was non-toxic in nature which might be due to the availability of sufficient nutrients, nitrogen and phosphorus in the medium. It can be concluded that algal blooming is not triggered by a single factor. A combination of species-specific, optimum favourable physico-chemical, geographical and biological factors can play a key role in the microalgal bloom dynamics.

.....ꕥ.....

Chapter 6

Bacteria Associated with Algal Blooms and Hydrolytic Enzyme Production

6.1 Introduction
6.2 Review of Literature
6.3 Result
6.4 Discussion

6.1 Introduction

The outbreaks and termination of algal blooms in the marine environments are influenced by physico-chemical and biological factors. Of the biological factors that regulate bloom dynamics, algal-bacterial interactions are of great importance as they are potential regulators of both, enhancing and decreasing algal blooming (Doucette, 1995; Doucette *et al.*, 1998). Algal blooms have an ordered and structured bacterial community associated with it rather than a random assemblage of species from the marine bacterial community. The algal-bacterial association is very specific, with this specificity transcending a wide range of taxonomical groups, from algal class (Imai *et al.*, 1995) to specific species (Fukami *et al.*, 1992). The lethal effects of bacteria are influential factors in controlling phytoplankton bloom dynamics (Fukami *et al.*, 1996; Kim *et al.*, 1998; Yoshinaga *et al.*, 1998; Imai *et al.*, 2001).

Algal–bacterial interactions can be classified into 4 types; (1) symbiotic, where both partners benefit from each other's presence; (2) parasitic, where

bacteria can lyse algae and algal antibiosis can inhibit bacterial growth; (3) commensalistic, where the bacteria have no actual negative effects on the host; and (4) bacteria are competitors for limiting nutrients like phosphate by being loosely associated with algae (Grossart, 1999).

Phycosphere is a mutually beneficial region around the algal cell (Bell and Mitchell, 1972) where growth promoting nutrients are exchanged between phytoplankton and bacteria, the latter are especially found to feed on dissolved organic material released by the algal cells (Riquelme *et al.*, 1988). The presence of the bacterial community and of specific populations have distinct effects on the growth and organic matter release of marine diatoms and other algae (Grossart and Simon, 2007). Apart from influencing algal growth, the secondary metabolites produced by the associated bacteria can also inhibit settlement of potential competitors and antagonise other bacteria (Holmstrom and Kjellebeg, 1994).

Reduced sulphur in the form of the osmolyte dimethylsulfoniopropionate (DMSP) is abundantly produced by marine phytoplankton (especially dinoflagellates and coccolithophores) and is rapidly converted to the gas dimethylsulfide (DMS) by bacteria, thereby aiding cloud formation over the oceans, which is important for atmospheric cooling (Charlson *et al.*, 1987). *Roseobacter* found primarily in association with phytoplankton, are most proficient at converting DMSP to DMS (Gonzalez *et al.*, 1999). Thus, these associated bacteria may also contribute immensely in the microbial loop of the marine biogeochemical cycle.

Apart from these, the bioactive compounds produced by the epiphytic bacteria regulate the ontogenesis of marine organisms which either enable them

to survive under adverse conditions or strictly regulate its dynamics. As a control measure against algal blooms, this is important because the physical and chemical methods like yellow loess (Na *et al.*, 1996; Choi *et al.*, 1998) and clay (Sun *et al.*, 2004) employed to mitigate the algal blooms have secondary effects upon the feeding habits and ecology of the bottom-dwelling organisms (Rhoads and Young, 1970; Bricelj and Malouf, 1984). Chemical agents such as copper sulfate, hydrogen peroxide, and triosyn are effective in controlling blooms within a short period after application (Steidinger, 1983; Ryu *et al.*, 1998) but their use can have wide spectrum effects upon even beneficial organisms (Jeong *et al.*, 2000). But, the biological factors especially of bacteria (Imai *et al.*, 1995) have least adverse effect (Kim *et al.*, 2007). The mode of action of these bacteria may be either through direct contact with the algal cell or indirectly through the release of dissolved lytic agents (Lovejoy *et al.*, 1998; Wang *et al.*, 2005). Extracellular protease produced by algicidal bacteria exert their effects on algae as dissolved lytic agent. The algal cells which were treated with algicidal strains probably secreting algicidal proteins showed a change in morphological characteristics, with loss of cell wall integrity, discolouration and disruption of cells, ultimately the cellular substances were decomposed and released (Wang *et al.*, 2012).

Usually, algicidal bacteria have repeatedly been observed in coastal environments where harmful algal blooms often occur (Imai *et al.*, 1993; Lovejoy *et al.*, 1998). Bacteria having algicidal effects are vivaciously involved in the termination and decomposition of algal blooms (Liu *et al.*, 2008). In the present study, the bloom associated cultivable bacteria were isolated, identified and their ability to produce extracellular enzymes such as amylase, lipase, protease, cellulase, ligninase, phosphatase and alginase were ascertained.

6.2 Review of Literature

Interactions between algae and bacteria are commonly observed in both freshwater and marine ecosystems with bacteria increasingly cited as responsible for the regulation of growth and dynamics of phytoplankton blooms (Doucette *et al.*, 1998; Hold *et al.*, 2001; Mayali and Azam, 2004). Bacteria are inherent part of the physical environment of microalgae both in the laboratory and natural environments (Gallacher and Smith, 1999). The physiological and ecological relevance of the algal-bacterial interactions in stimulating and inhibiting each other's growth and in the biogeochemical cycling has been reported earlier (Fukami *et al.*, 1996; Lovejoy *et al.*, 1998; Imai *et al.*, 2001). By means of direct or indirect modes of action (Mayali and Azam, 2004) associated bacteria can influence toxin production in algae (Bates *et al.*, 1995). Kim *et al.* (1998) observed that the population dynamics of algicidal bacteria has a close relationship to the blooms of the phytoplankton and that in the marine ecosystems, algicidal bacteria targeting specific phytoplankton may be one of the agents which regulate the change of species structure of phytoplankton communities.

6.2.1 Bacteria associated with bloom-forming microalgae

Simidu *et al.* (1971) observed that free living and algal associated bacteria are very different from each other and the latter are usually gram negatives, with *Vibrio* sp. and *Aeromonas* sp. constituting 70% of the bacterial flora. As part of the association, the microalgae benefits from bacterial products, mainly remineralized nutrients (Golterman, 1972) while the bacteria benefits from phytoplankton products, such as exudates (Bell *et al.*, 1974; Cole, 1982). The association of the bacteria with the physical environment of the microalgae may be either loose or close (Caldwell, 1977; Alavi *et al.*, 2001) and can also be intracellular (Cole, 1982; Franca *et al.*, 1995).

The bacteria mainly associated with algal bloom were dominated by alpha-proteobacteria, beta-proteobacteria, gamma-proteobacteria and Cytophaga-Flavobacter-Bacteroides (CFB) (De Long *et al.*, 1993; Gonzalez and Moran, 1997; Meusnier *et al.*, 2001). *Pseudomonas* and *Moraxella* were found to be associated with *Amphidinium carterae* while proteobacteria and Cytophaga group were found in association with *Alexandrium catenella* (Nayak *et al.*, 1997). *Marinobacter hydrocarbonoclasticus* associated with *Alexandrium fundyense* was able to metabolise complex unusual hydrocarbon molecules associated with the algae (Rontani *et al.*, 1997).

Liu *et al.* (2000) reported extracellular and intracellular bacteria associated with *Alexandrium minutum*. Maki and Imai (2001) reported the presence of bacteria in the cytoplasm and the food vacuoles of *Heterocapsa circularisquama*. Dinoflagellate *Pfiesteria* showed the association of as many as thirty bacterial genera including *Pseudomonas, Vibrio, Nocardia, Moraxella, Cytophaga, Acinetobacter* and *Roseobacter* (Alavi *et al.*, 2001).

Among alpha-proteobacteria, the most frequently associated member is the *Roseobacter* clade (Hold *et al.*, 2001) in microalgal culture (Alavi *et al.*, 2001) and field bloom (Fandino *et al.*, 2001). Within gamma-proteobacteria, *Marinobacter* spp. and *Alteromonas* spp. appear to have an association with dinoflagellates (Hold *et al.*, 2001; Ferrier *et al.*, 2002) and algal cultures (Mayali and Azam, 2004). The bacterial group most often associated with dinoflagellates and diatoms are alpha-proteobacteria and gamma-proteobacteria (Green *et al.*, 2004). Beta-proteobacteria, recorded to be rare in the marine system was found to be dominant as an intracellular bacterial flora of the dinoflagellate *Gymnodinium instriatum* (Alverca *et al.*, 2002).

Rooney-Verga *et al*. (2005) observed the occurrence of CFB group in association with algal blooms. Grossart *et al.* (2005) reported the association of Flavobacteria–Sphingobacteria group in diatoms. Alpha and beta-proteobacteria were found in association with *Pseudo-nitzschia multiseries* (Kaczmarska *et al*., 2005; Sapp *et al*., 2006). During *Lingulodinium polyedrum* bloom event, 11 associated bacterial taxa were detected, which mainly belonged to the proteobacteria and CFB groups (Mayali *et al*., 2011). An increased presence of gamma-proteobacteria populations during the bloom of *Akashiwo sanguinea* and its gradual decrease in the post-bloom from Chinese waters was recently reported by Yang *et al*. (2012).

6.2.2 Effect of bacteria on bloom-forming microalgae

Usually bacteria have significant impacts on aquatic biogeochemical processes such as carbon flux and nutrient regeneration (Azam, 1998; Doucette *et al.*, 1998; Copley, 2002) and may influence the initiation, growth, maintenance, and/or termination of bloom populations (Imai *et al*., 1998, 2001; Kodama *et al*., 2006). Specifically, a bacterial assemblage can have symbiotic (Silva, 1962), inhibitory (Doucette *et al*., 1999) or stimulatory effects (Fukami *et al.*, 1991) including algal toxin production during a bloom event (Riquelme *et al.*, 1988; Tamplin, 1990; Simon *et al.*, 2002).

Kodama *et al*. (1988) reported a toxin producing bacteria associated with dinoflagellate. Yoshinaga *et al*. (1998) isolated 96 bacterial strains from Hiroshima Bay which have lethal effect on *Heterosigma carterae.* Lovejoy *et al*. (1998) reported the wide range algicidal effect of *Pseudoalteromonas,* an associated bacterial strain on *Chatonella, Heterosigma* and *Gymnodinium* blooms

and thereby plays an important role in regulating the onset and development of harmful algal blooms.

An algicidal extracellular protease by *Pseudoalteromonas* sp. against *Skeletonema costatum* was reported by Lee *et al.* in 2000. Skerrat *et al.* (2002) reported five algicidal bacterial strains, *Pseudoalteromonas, Bacillus cereus, Zobellia* sp*., Cellulophaga lytica* and *Planomicrobium* sp., from Huon estuary, Australia that were effective against *Gymnodinium catenatum.* Mayali and Doucette (2002) studied the effect of *Cytophaga*, an algicidal bacterium, on *Karenia brevis* and found that there should be threshold concentration to trigger an algicidal response.

Ferrier *et al*. (2002) reported stimulatory effect of *Alteromonas* sp. on *Alexandrium fundyense.* Amaro *et al.* (2005) found that *in situ* environmental condition modulates the algicidal expression in bacteria associated with *Alexandrium catenella*. A bacterial strain *Shewanella* sp. had inhibitory effect (Hare *et al.*, 2005; Pokrzywinski *et al*., 2012) on dinoflagellate *Pfiesteria piscicida* but stimulatory effect on diatoms and raphidophytes.

Su *et al*. (2007) isolated *Pseudoalteromonas* which produced a heat tolerant, acid unstable algicidal compound against toxic dinoflagellate *Alexandrium tamarense*. Liu *et al*. (2008) noted the presence of algicidal effect of bacteria on raphidophyte blooms *Chatonella subsalsa, Heterosigma akashiwo* and *Fibrocapsa japonica* and found variability in the taxonomic specificity of the algicidal bacterial effect and raphidophyte susceptibility. Kang *et al.* (2008) isolated algicidal bacteria *Variovorax paradoxus, Acidovorax delafieldii, Hydrogenophaga palleronii* and *Pseudomonas plecoglossicida* that have algicidal effects on diatom *Stephanodiscus hantzschii* and dinoflagellate *Peridinium bipes*. *Idiomarina* sp. isolated from the east sea areas of China has algicidal effect on

the toxic dinoflagellate *Alexandrium tamarense* (Su *et al.*, 2011). *Aquimarina* sp. under the family Favobacteriaceae (Chen *et al.*, 2011) and *Ochrobactrum* sp. (Mu *et al.*, 2012) were reported to possess algicidal activity against toxic cyanobacterium *Microcystis aeruginosa*.

6.3 Result

6.3.1 Bacteria associated with *Prymnesium parvum* N. Carter bloom

6.3.1.1 Comparative estimation of Total Heterotrophic Bacterial count

During the *Prymnesium parvum* bloom, the total heterotrophic load of associated bacteria was 19.2×10^4 cfu/ml, whereas in the non-bloom sample, the THB was 6.1×10^4 cfu/ml (Fig.53). The variation in total heterotrophic bacterial load between bloom and non-bloom sample was significant ($P<0.001$, Student-Newman-Keuls-Multiple Comparisons Test).

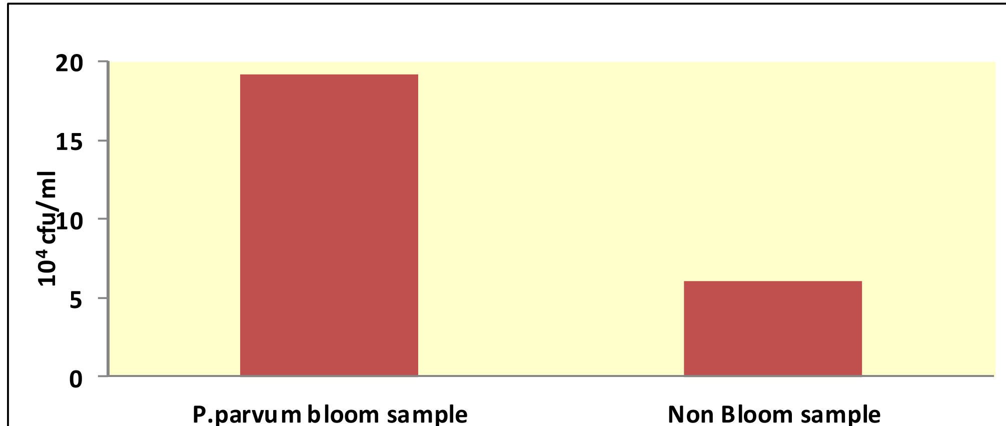


Fig.53 Total Heterotrophic Bacterial (THB) count in *Prymnesium parvum* bloom and non-bloom sample

6.3.1.2 Generic composition of bloom associated bacteria

Flavobacterium (38%) was found to be the predominant bacterial genera associated with *P. parvum* bloom, which was followed by *Pseudomonas* (19%),

Vibrio (13%), 6% each *of Bacillus, Moraxella, Micrococcus, Acinetobacter* and *Corynebacterium* (Fig.54).

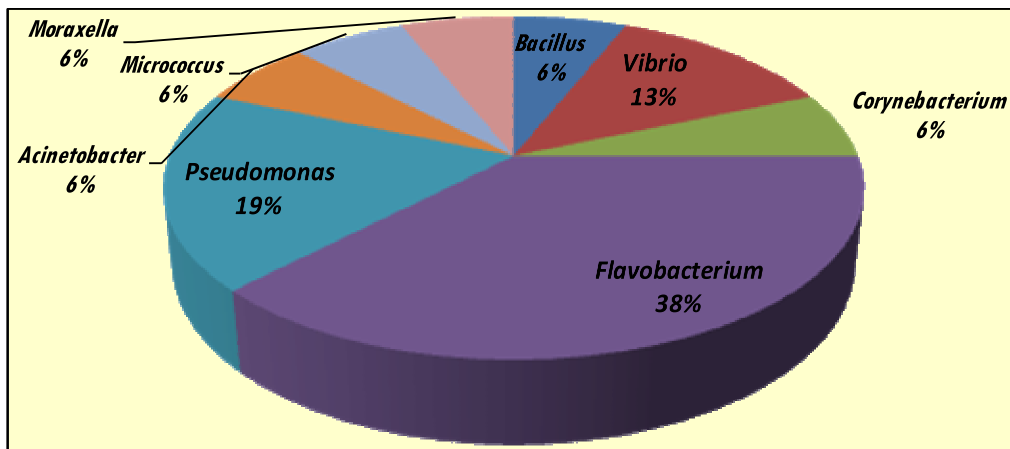


Fig.54 Generic composition of bacteria associated with *Prymnesium parvum* bloom

6.3.1.3 Generic composition of bacteria from the non-bloom sample

In the non-bloom sample, the major bacterial genera were *Acinetobacter* and *Alcaligenes* (25% each) followed by *Micrococcus* (17%), *Staphylococcus* (9%), *Corynebacterium*, *Bacillus* and *Flavobacterium* (8% each) (Fig.55).

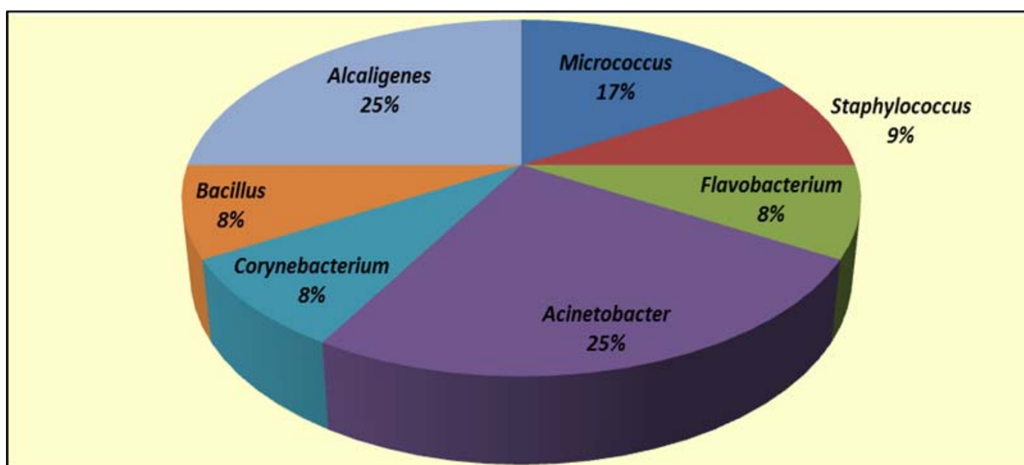


Fig.55 Generic composition of heterotrophic bacteria in the non-bloom sample

6.3.1.4 Hydrolytic enzyme production of bloom associated bacteria

The hydrolytic enzyme production of the heterotrophic bacteria associated with the *P. parvum* bloom showed a high percentage of proteolytic bacteria (31%). The percentage of other hydrolytic enzyme producers were lipases (16%), ligninase and phosphatases (15% each), amylase and cellulase (10% each) and alginase (3%) (Fig.56).

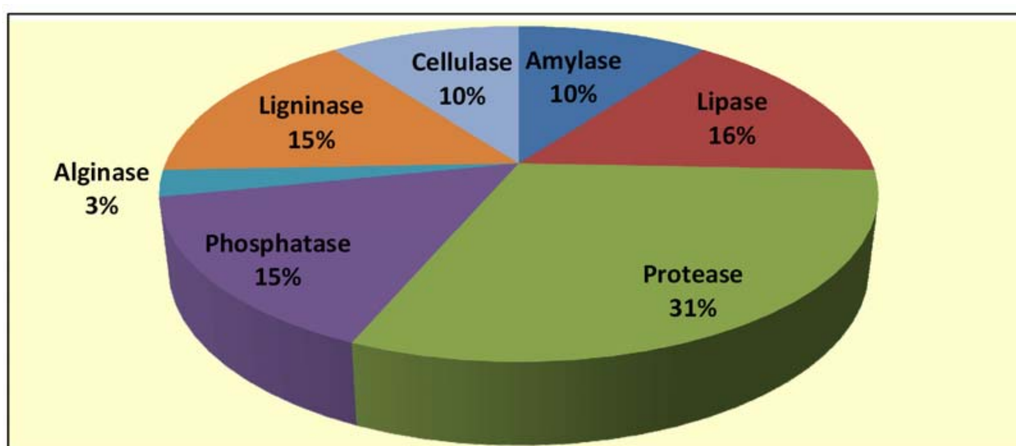


Fig.56 Hydrolytic enzyme production of bacteria associated with *Prymnesium parvum* bloom

6.3.1.5 Hydrolytic enzyme production of bacteria from the non-bloom sample

Hydrolytic enzyme producers from the non-bloom samples showed the predominance of lipase producers (23%) followed by amylase and phosphatase producers (20% each). 14% of isolates could produce ligninase, whereas only 11% could produce protease. 7% of isolates were able to produce cellulose, whereas alginase producers were only 5% (Fig.57).

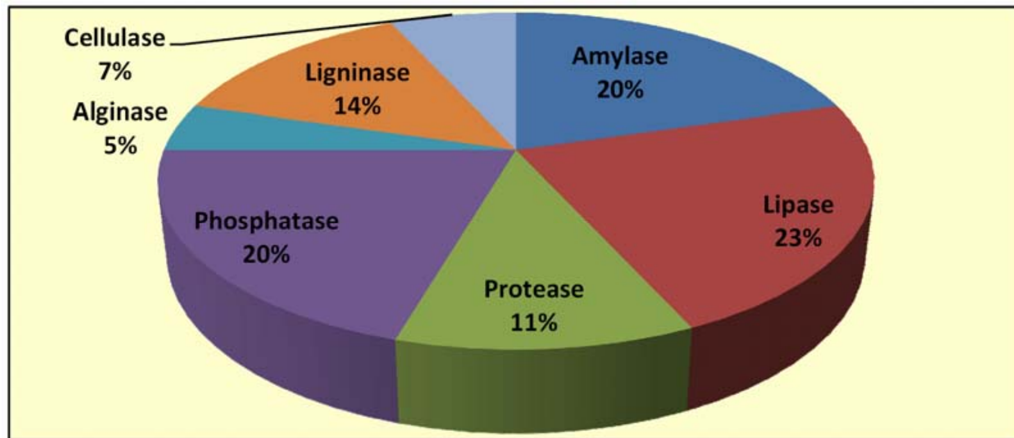


Fig.57 Hydrolytic enzyme production of heterotrophic bacteria from the non-bloom sample

6.3.2 Bacteria associated with *Proboscia alata* (Brightwell) Sandström bloom

6.3.2.1 Estimation of Total Heterotrophic Bacterial count

During the *Proboscia alata* bloom, the total heterotrophic load of associated bacteria on the first day was 30×10^4 cfu/ml, whereas it increased to 40×10^4 cfu/ml on the last day of observation. However, in the non-bloom sample, the THB was only 12×10^4 cfu/ml. (Fig.58).

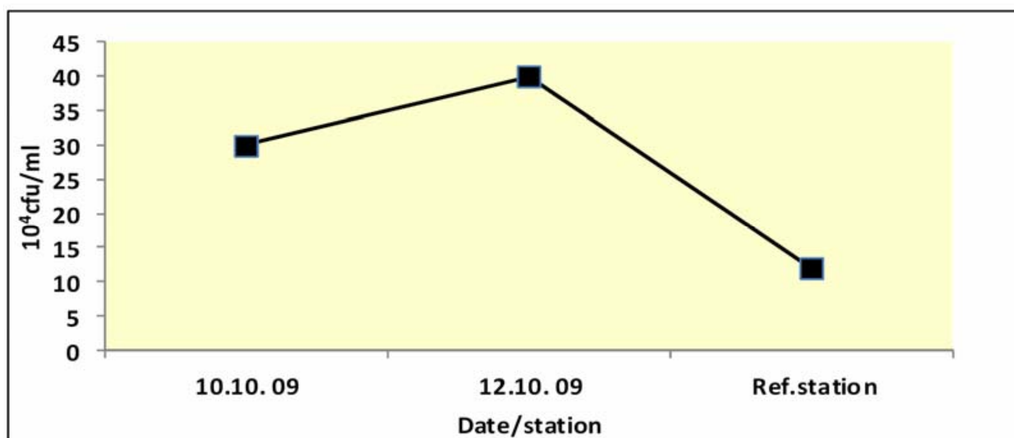


Fig.58 Total Heterotrophic Bacterial (THB) count in *Proboscia alata* bloom and non-bloom sample

The variation in total heterotrophic bacterial load between bloom and non-bloom sample was significant (P<0.001, Student-Newman-Keuls-Multiple Comparisons Test).

6.3.2.2 Genera wise distribution of bloom associated bacteria

On the first day of the bloom event, the generic composition of the bloom associated bacteria showed the predominance of *Flavobacterium* (25%) followed by *Corynebacterium*, *Acinetobacter*, *Micrococcus* (13% each) and 12% each of *Bacillus, Pseudomonas* and *Alcaligenes* (Fig.59).

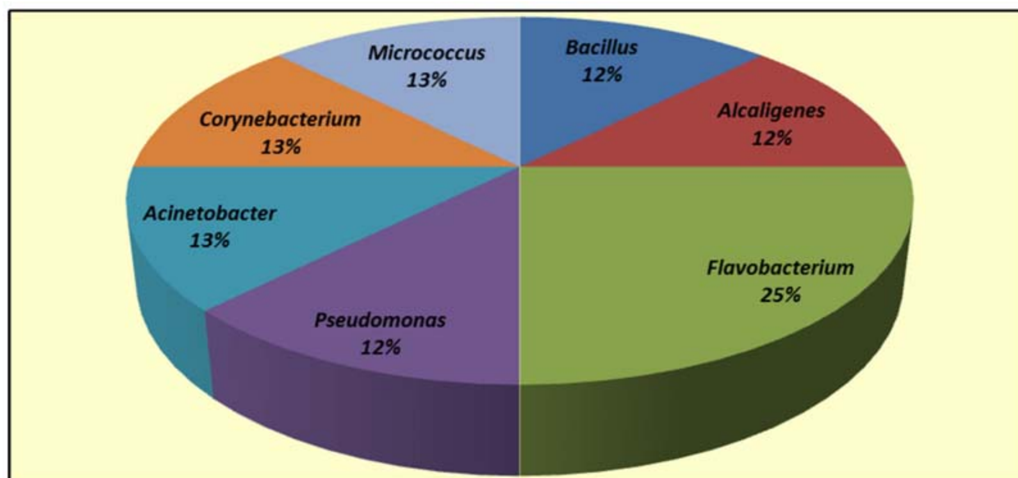


Fig.59 Generic composition of bacteria associated with *Proboscia alata* bloom on the first day of observation

But on the last day of the bloom event, the generic composition of the bloom associated bacteria showed the predominance of *Flavobacterium* (40%), and *Pseudomonas* (20%), followed by 10% each of *Moraxella, Acinetobacter, Bacillus* and *Vibrio* (Fig.60).

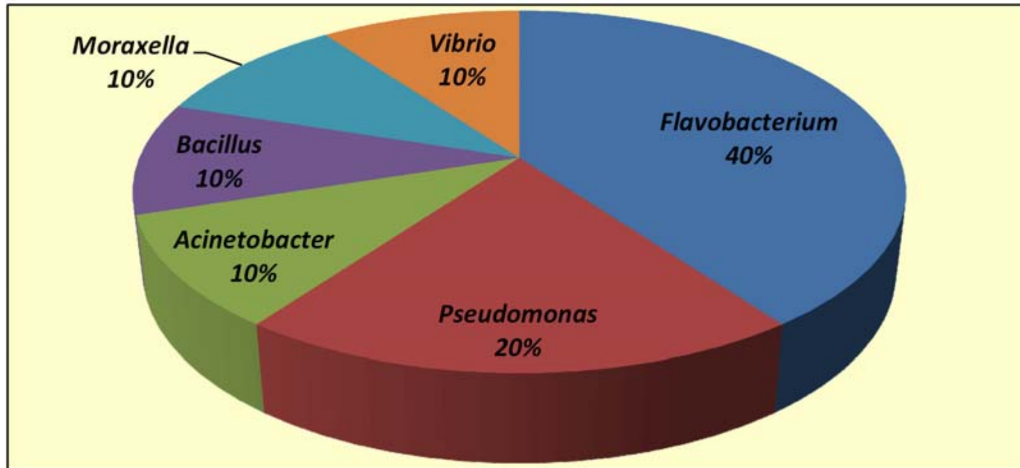


Fig.60 Generic composition of bacteria associated with *Proboscia alata* bloom on the last day of observation

6.3.2.3 Genera wise distribution of bacteria from the non-bloom sample

In the reference sample, the predominant forms were *Micrococcus* and *Moraxella* (25% each) followed by 13% each of *Bacillus* and *Alcaligenes*, and 12% each of *Pseudomonas* and *Acinetobacter* (Fig.61).

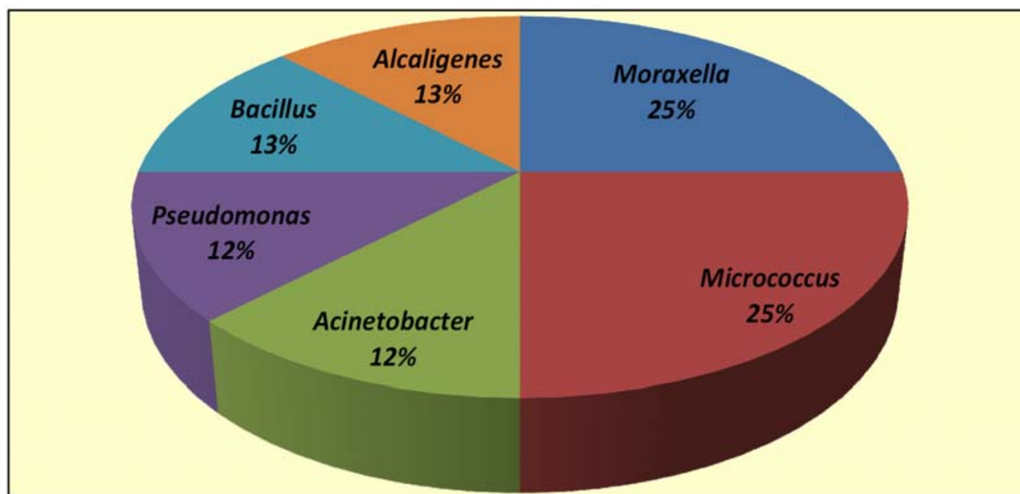


Fig.61 Generic composition of heterotrophic bacteria in the non-bloom sample

6.3.2.4 Hydrolytic enzyme production of bloom associated bacteria

The hydrolytic enzyme production of the heterotrophic bacteria associated with the bloom on the first day showed a high percentage of proteolytic bacteria (36%). Other hydrolytic enzyme producers were amylase and phosphatase (14% each), lipase, alginase, ligninase and cellulase (9% each) (Fig.62).

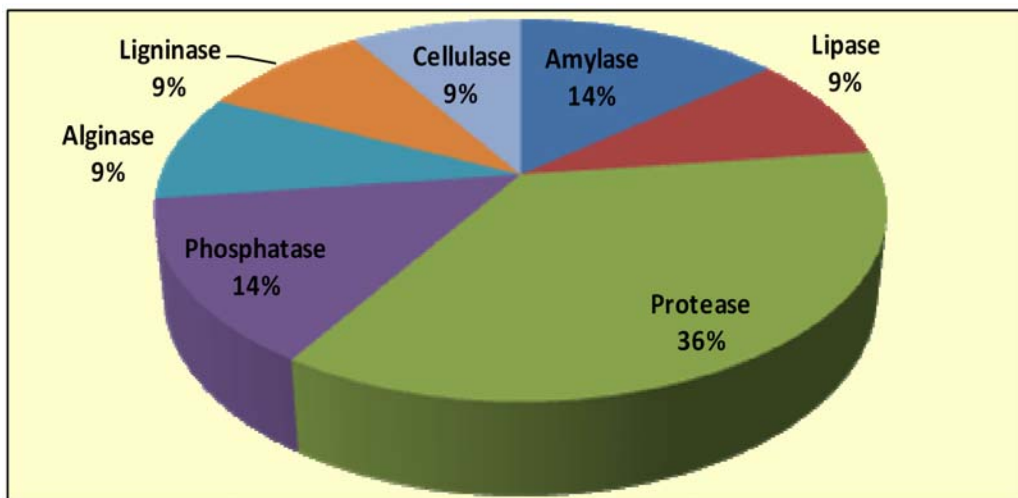


Fig.62 Hydrolytic enzyme production of bacteria associated with *Proboscia alata* bloom on the first day of observation

On the last day of the bloom event, the presence of proteolytic bacteria increased to 38%. Percentage of other hydrolytic enzyme producers were cellulase and ligninase (12% each), lipase and phosphatase (11% each), amylase and alginase (8% each) (Fig.63).

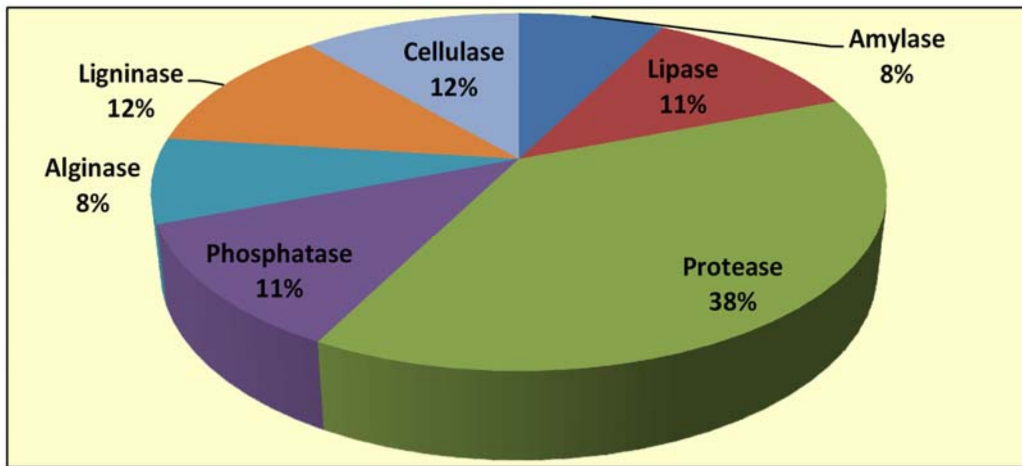


Fig.63 Hydrolytic enzyme production of bacteria associated with *Proboscia alata* bloom on the last day of observation

6.3.2.5 Hydrolytic enzyme production of bacteria from the non-bloom sample

Hydrolytic enzyme producers in the non-bloom samples showed the predominance of amylase and lipase producers (20% each) followed by phosphatase (17%), protease and cellulase (13% each), ligninase (10%) and alginase (7%) (Fig.64).

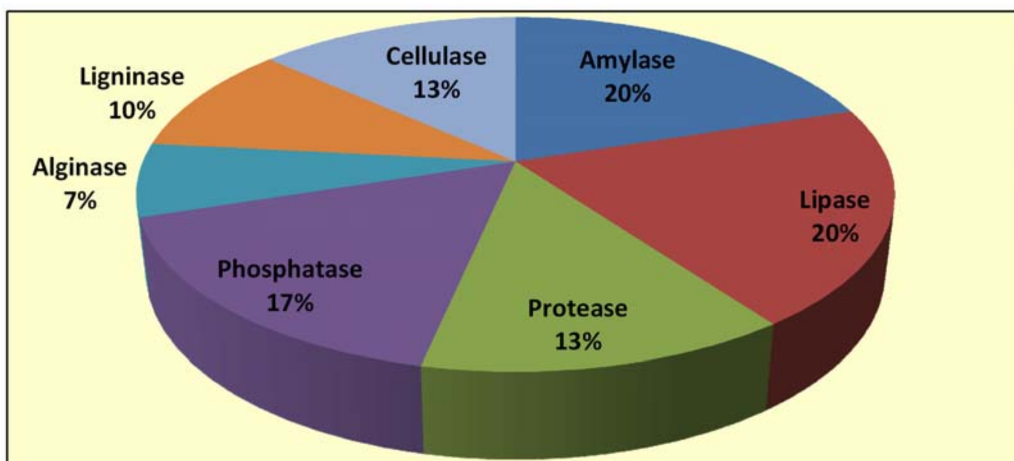


Fig.64 Hydrolytic enzyme production of heterotrophic bacteria in the non-bloom sample

6.3.3 Bacteria associated with *Chattonella marina* (Subrahmanyan) Hara et Chihara bloom

6.3.3.1 Estimation of Total Heterotrophic Bacterial count

During the *Chattonella marina* bloom event, the total heterotrophic load of associated bacteria ranged from 10.9×10^4 cfu/ml on the first day to 175×10^4 cfu/ml on the last day. But in the non-bloom sample, the THB was in the range of 1.2×10^4 cfu/ml to 4.9×10^4 cfu/ml (Fig.65). The variation in total heterotrophic bacterial load between bloom and non-bloom was significant (P<0.001, Student-Newman-Keuls-Multiple Comparisons Test).

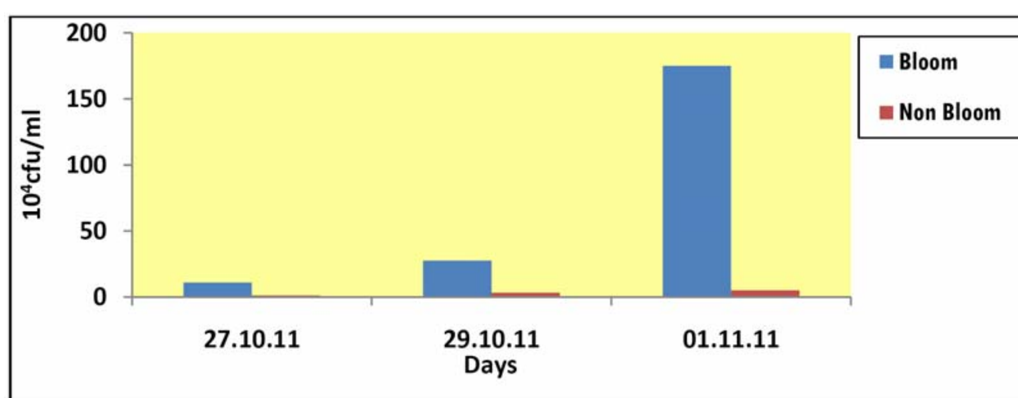


Fig. 65 Total Heterotrophic Bacterial (THB) count in *Chattonella marina* bloom sample and non-bloom sample

6.3.3.2 Genera wise distribution of bloom associated bacteria

The generic composition of the bloom associated bacteria showed the predominance of *Bacillus, Micrococcus, Vibrio* and *Flavobacterium* on the first day. In the second sampling, species of *Bacillus, Micrococcus, Staphylococcus, Moraxella, Vibrio, Streptococcus, Flavobacterium* and *Pseudomonas* were recorded, whereas on the last day of the bloom event, the predominant genera were *Flavobacterium, Pseudomonas, Vibrio, Corynebacterium, Staphylococcus, Listeria, Micrococcus* and *Bacillus* (Fig.66).

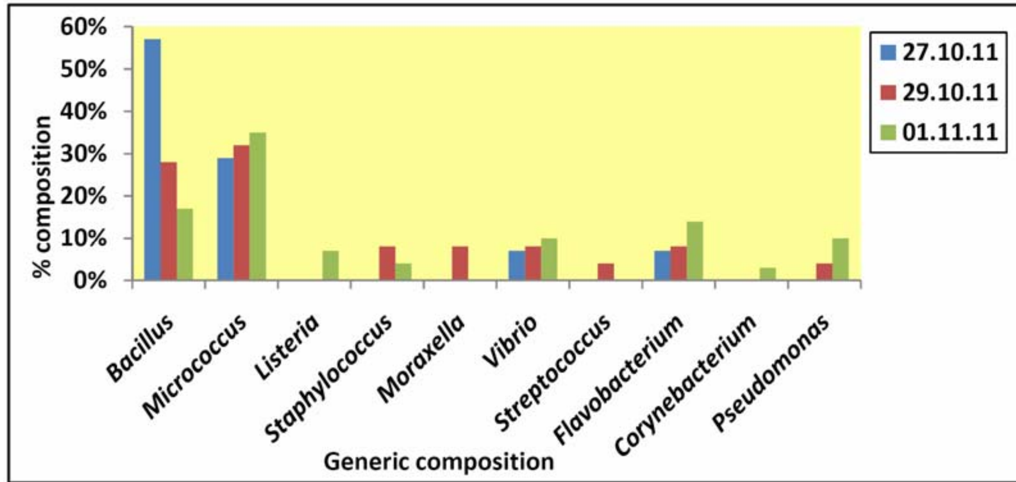


Fig.66 Percentage composition of bacteria associated with *Chattonella marina* bloom

6.3.3.3 Genera wise distribution of bacteria from the non-bloom sample

The bacteria in the non-bloom sample comprised mainly of *Bacillus, Moraxella, Listeria, Streptococcus, Corynebacterium* and *Staphylococcus* (Fig.67).

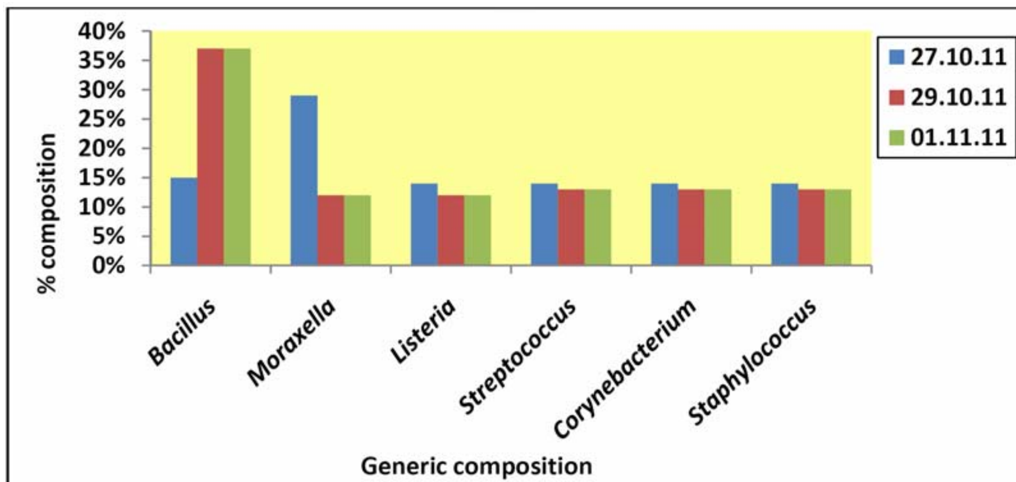


Fig.67 Generic composition of heterotrophic bacteria in the non-bloom samples

6.3.3.4 Hydrolytic enzyme production of bloom associated bacteria

The hydrolytic enzyme production of the heterotrophic bacteria associated with the bloom on the successive days of sampling showed a high percentage of proteolytic bacteria, with a gradual increase from 43% on the first day, 45% on the second day to 48% on the last day (mean 45%). Other hydrolytic enzyme producers were amylase (13%), lipase (11%), phosphatase (9%), alginase (8%), ligninase (5%) and cellulase (8%) (Fig.68).

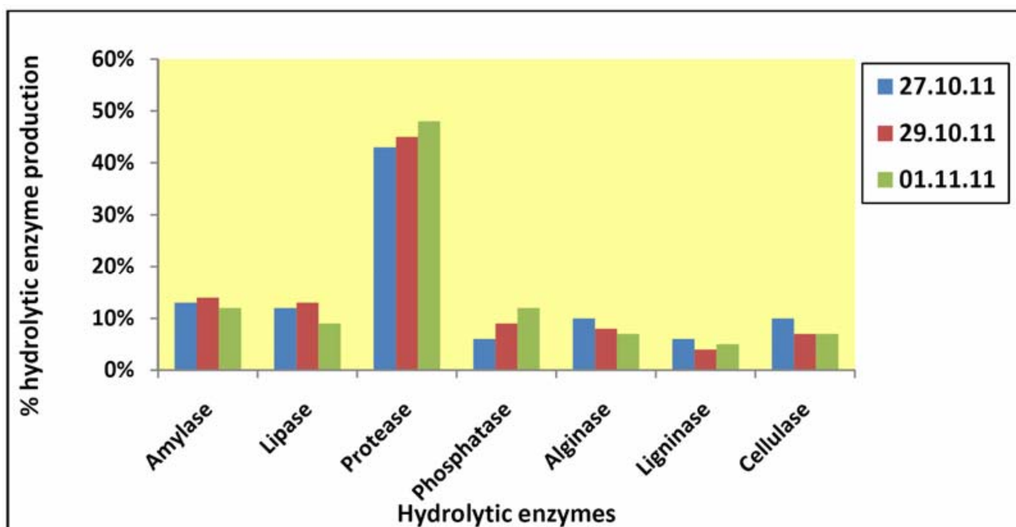


Fig.68 Hydrolytic enzyme production of bacteria associated with *Chattonella marina* bloom

6.3.3.5 Hydrolytic enzyme production of bacteria from the non-bloom sample

Hydrolytic enzyme producers in the non-bloom samples were amylase (21%), protease (20%), cellulase (17%), phosphatase (16%), lipase and alginase (13% each) while none of the strains were able to produce ligninase (Fig.69).

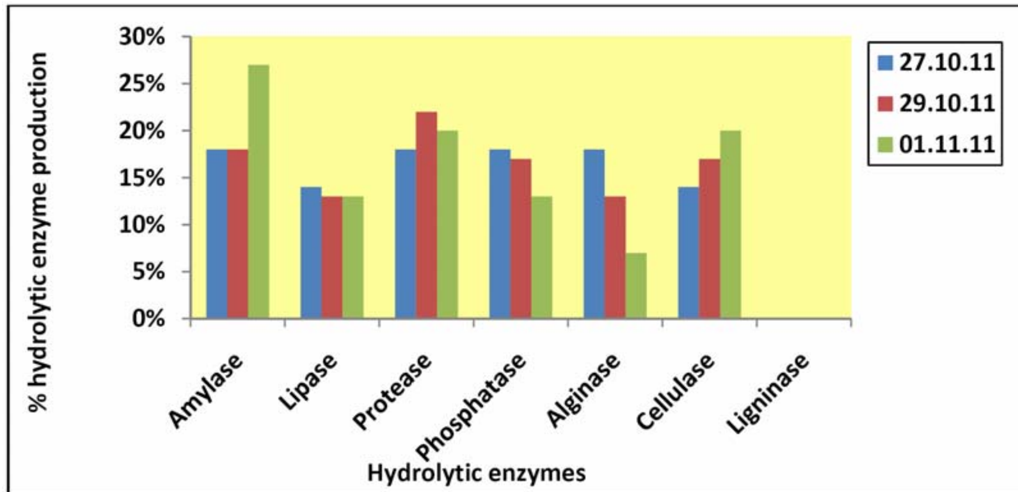


Fig.69 Hydrolytic enzyme production of heterotrophic bacteria in the non-bloom samples

6.4 Discussion

Interactions between bacteria and harmful algal bloom have been considered as an important factor regulating the population of microalgae (Doucette *et al.*, 1998). In the present study, a considerable increase in the culturable bacterial community has been observed in the bloom sample. In all the three bloom events observed, total heterotrophic bacterial count was found to be higher in the bloom samples than in the reference samples. There was a gradual increase in heterotrophic bacterial population from the first day to the last day in the bloom events of *P. alata* and *C. marina*.

THB was found to be maximum during the decline stage of the bloom indicating the probable role of bacteria in bloom termination. Hence it can be assumed that during the algal bloom event, there is a considerable change in associated bacteria, the number slowly increases from the initial stage and reaches its maximum during the decline stage of the bloom. These associated

bacteria must have played a crucial role in bloom dynamics, some acting as beneficial ones supporting the bloom and another group playing a detrimental role in bloom decline. Previous studies have also shown that bacterial community change quantitatively during an algal bloom and this may either play a beneficial or detrimental role in controlling algal growth (Doucette, 1995; Doucette *et al*., 1999).

The bacterial-algal bloom relationships involve more than just trophic interactions and ultimately reflect a balance between processes inhibitory and/or stimulatory to the organisms involved. Gamma-proteobacteria, beta-proteobacteria and Cytophaga-Flavobacterium-Bacteroides (CFB) are the major bacterial groups reported to have close association with algal blooms (Rooney-Verga *et al*., 2005). Gamma-proteobacteria frequently encountered in the algal bloom fields act as a potentially significant factor in the bloom decline (Skerratt *et al*., 2002). CFB group of bacteria are also reported to have algicidal activity (Doucette *et al*., 1999) and like gamma-proteobacteria, this group also could be important in the bloom termination. Based on several reviews, about 50% of the algicidal strains belong to the CFB group, while about 45% are members of gamma-proteobacteria; the remaining strains represent the gram-positive genera *Micrococcus, Bacillus,* and *Planomicrobium* (Fandino *et al*., 2001; Fukuyo *et al*., 2002; Mayali and Azam, 2004; Hare *et al*., 2005; Jasti *et al*., 2005).

During *C. marina* bloom, it was clearly noticed that there was a gradual increase in *Flavobacterium*, gram-negative gamma-proteobacteria such as *Pseudomonas* and *Vibrio,* and gram-positive *Micrococcus* from initial stage to the decline stage of the bloom. The bacterial genera which was abundant especially at the decline stage of bloom was absent or present in very few

numbers in the non-bloom samples. Likewise, it was also noticed that during the *P. alata* bloom event, as the bloom reached its decline stage, the predominance of associated bacteria mainly shifted to *Flavobacterium,* with other members of the gamma-proteobacteria like *Pseudomonas, Vibrio* and *Moraxella* having an increased percentage composition as compared with the first day of the bloom event.

During *P. parvum* bloom also the predominant bacterial genera were *Flavobacterium, Pseudomonas* and *Vibrio*. So, in all the bloom events studied, the predominant associated bacteria comprised of members of gamma-proteobacteria and CFB groups and its abundance was also found to be increased in the successive stages of the bloom event with a peak in the decline stage of bloom. The relative difference in the composition and abundance of the associated bacteria in the bloom samples with non-bloom samples indicated its specific association behaviour. These findings were positively correlated with the recorded reports that in early stages of algal blooms bacteria often are virtually absent or less in abundance (Azam and Ammerman, 1984; Lancelot and Rousseau, 1994), whereas late bloom stages coincide with increased colonization by associated bacteria (Lancelot and Rousseau, 1994; Smith *et al.*, 1995). Recently, Yang *et al.* (2012) also noticed the abundance of gamma-proteobacteria populations significantly during the decline phase of *Akashiwo sanguinea* bloom.

The association of bacteria on algae was complex and diverse in the sense of many effects on algae (Imai *et al.*, 1995; Lovejoy *et al.*, 1998; Lee *et al.*, 2000). The algicidal effect of the associated bacteria could play a vital role in the algal bloom dynamics especially of the bloom termination (Liu *et al.*, 2008).

In a natural system the algae lytic bacteria closely respond to the algal bloom dynamics; as bloom proceeds through their initation, maintenance and decline stage, the relative algicidal bacterial abundance also increases (Doucette *et al.*, 1999), ultimately reaching the threshold concentration, where algicidal activity becomes detectable and leads to the rapid destruction of algal cells (Fukami *et al.*, 1992; Mitsutani *et al.*, 1992; Imai *et al.*, 1993). During the decline stage, the abundance of the algicidal bacteria become high in number and maintain a top-down control mechanism (Liu *et al.*, 2008).

The algicidal effect of *Flavobacterium* sp. (Yoshinaga *et al.*, 1997), *Pseudomonas* sp. (Baker and Herson, 1978; Yoshinaga *et al.*, 1997; Lee and Park, 1998; Kitaguchi *et al.*, 2001), *Vibrio* sp. (Ishio *et al.*, 1989; Yoshinaga *et al.*, 1997; Wang *et al.*, 2010) and *Micrococcus* sp. (Park *et al.*, 1998) against different microalgae have already been documented.

Usually the mode of action of algicidal bacteria are of two types, those which attack algal cells through direct contact and others which attack through the release of a dissolved lytic agent (Wang *et al.*, 2005). Approximately 30% of the phylogenetically classified algicidal bacteria attack their target algal species through direct contact, and approximately 70% of algicidal bacteria exhibit an indirect mode of attack where dissolved lytic agents, especially extracellular metabolites, are released into the surroundings that effectively antagonises algal cells without the need of physical contact (Hare *et al.*, 2005).

Many algicidal bacteria exert their effects on algae through extracellular protease (Lee *et al.*, 2000; Wang *et al.*, 2012). So, the hydrolytic enzyme production potential of the bloom associated bacteria was assessed. The percentage of protease producers were very high in the bloom sample. During

C. marina bloom, the percentage of protease producers was significantly higher when compared with the other hydrolytic enzyme producers and a gradual increase in protease producers was noticed from the initial stage to the decline stage of the bloom event. But in the non-bloom sample, such an increase in protease producers was not observed. Similarly, the hydrolytic enzyme production potential of the associated bacteria of *P. alata* on the successive days showed a significantly higher number of protease producers when compared with other enzyme producers and a gradual increase in protease producers was found in the decline stage of the bloom. Similar phenomenon was also observed during the *P. parvum* bloom event. Among the various genera associated with HAB, *Flavobacterium, Pseudomonas* and *Vibrio* were found to be more potent protease producers in all the bloom events. The occurrence of higher number of proteolytic bacteria was found to be unique in all the bloom events. The proteases produced by the bacteria were found to have algicidal activity. Lee *et al*. (2000) reported the algicidal activity of the extracellular protease produced by the strains of *Pseudoalteromonas* against the diatom *Skeletonema costatum.* Recently, Wang *et al*. (2012) also documented the algicidal effect of the protein produced by two algicidal bacteria, *Vibrio* and *Pseudoalteromonas,* against the toxic dinoflagellate *Alexandrium tamarense*. The unique nature of the associated bacterial composition, abundance, high proteolytic activity and its gradual increase at the time of bloom declination in all the present bloom events pointed out the specific nature of the bloom associated bacteria and its significant role in the bloom dynamics especially of the bloom termination.

.....ꕤ.....

Chapter 7

Summary and Conclusion

Microalgae play a key role as primary producers in the marine ecosystem, forming the base of marine food web. The biomass, distribution and species composition of microalgae change continuously with variations in physico-chemical variables such as temperature, light, nutrient availability, grazing pressure, sediment characteristics, tide and water movements, seasons, and even with time of the day.

Direct microscopic observation, accurate identification and enumeration are essential for documenting species composition and biomass. Generally, microalgae have a fairly recognizable annual cycle of growth; sometimes the synchrony in their normal annual cycle is disrupted by explosive growth of some species leading to algal blooms.

Algal blooms can cause severe economic loss to aquaculture, fisheries and tourism, major environmental disturbances and significantly affect human health. The outbreak and termination of algal blooms in marine environments are mainly controlled by physical, chemical, and biological factors. With respect to the biological factor, the associated bacterial community acts as potential regulators by enhancing or decreasing algal blooms through direct or indirect modes of action. Thus, it is significant to understand how the

distribution and composition of phytoplankton populations in economically important shelf seas relate to the particular chemical and physical properties of the water column in which they live.

The results of the present study over a period of two years revealed the distribution and composition of microalgae at three estuarine and three coastal stations along the southwest coast of India, with special emphasis on bloom-forming microalgae, algal bloom dynamics, and the role of bloom associated bacterial community on algal bloom dynamics.

Physico-chemical variables that were investigated in this study were temperature, salinity, pH, nitrate, nitrite, silicate, phosphate, dissolved oxygen and primary productivity. The fluctuations of these factors over a period of two years from pre-monsoon 2009-10 to post-monsoon 2010-11 at the stations are described.

In all, one hundred and fourteen species of microalgae were enumerated during the study and are distributed among sixty four genera under six classes, Bacillariophyceae (43 genera), Dinophyceae (8 genera), Chlorophyceae (10 genera), Dictyochophyceae (1 genus), Cyanophyceae (1 genus) and Prymnesiophyceae (1 genus).

Class Bacillariophyceae was found to be the predominant group with 75 species (66%) followed by Dinophyceae (25 species, 22%), Chlorophyceae (10 species, 9%), Dictyochophyceae (1%), Cyanophyceae (1%) and Prymnesiophyceae (1%). From the class Bacillariophyceae, *Chaetoceros* was found to be predominant with 6 species followed by *Coscinodiscus* (5 species), *Pleurosigma* and *Navicula* (4 species each), *Gyrosigma, Nitzschia*

and *Surirella* (3 species each). From the class Dinophyceae, harmful bloom-forming *Ceratium* and *Protoperidinium* (6 species each) were dominant followed by potentially toxic *Dinophysis* (4 species) and *Prorocentrum* (4 species). High cell abundance of harmful bloom-forming dinoflagellate *Biceratium furca* was recorded during the entire study period.

Significant correlation was found between standing crop and chlorophyll *a*. Salinity influenced the floral composition during the monsoon season. No direct relationship could be observed between nutrients and chlorophyll *a* during the entire study period. The spatial and temporal distribution and the abundance of microalgae were dependent on cumulative multiple factors rather than on individual factors.

Several known bloom-forming microalgal species such as *Halamphora coffeaeformis* (C. Agardh) Levkov; *Asterionellopsis glacialis* (Castracane) Round; *Proboscia alata* (Brightwell) Sandström; *Biceratium furca* (Ehrenberg) Vanhoeffen; *Dinophysis acuminata* Claparède and Lachmann; *Dinophysis caudata* Saville-Kent; *Dinophysis miles* Cleve; *Alexandrium monilatum* (J.F. Howell) Balech; *Prorocentrum lima (*Ehrenberg) F. Stein; *Prymnesium parvum* N. Carter and *Chattonella marina* (Subrahmanyan) Hara et Chihara, were recorded during the study, indicating the possibility of potential threat of harmful blooms in the coastal environment of southwest India.

During the present study period three algal bloom events were recorded. A monospecific bloom of *Prymnesium parvum* N. Carter was observed off Azheekode (Lat. 10° 11' 02" N & Long. 76° 09' 22" E) during the monsoon season of 2009. This is the first report of *Prymnesium parvum* N. Carter bloom from Indian waters. A multitude of factors which included low N: P ratio,

alkaline pH, species-specific optimum salinity and temperature, and other environmental factors and geographical adaptations in a favourable range for the particular species might have favoured the blooming of *Prymnesium parvum* and influenced its dynamics.

The coastal sea off Bekal (Lat. 12° 38' 02" N & Long. 75° 04' 31" E) experienced a bloom of centric diatom *Proboscia alata* (Brightwell) Sandström from 10th to 12th October 2009 in the early post-monsoon season. Upwelling leading to high nutrient conditions especially of silicate, which imparted high Si: N ratio, along with an alkaline pH, significantly influenced the initiation of the bloom. Low Si: N ratio with a decreasing pH might have caused the bloom to decline.

A massive bloom of the marine raphidophyte, *Chattonella marina* (Subrahmanyan) Hara et Chihara; was observed in the coastal sea off Mahe (Lat. 11° 42' 18" N & Long. 75° 32' 36" E) from October 27th through November 1st 2011. High nitrate concentration which caused high N: P ratio, along with optimum temperature (25°C) and salinity (30 psu) might have favoured the blooming. However, it was observed that though the algal cells choked and caused suffocation in the fish due to mechanical rupturing of gills, there wasn't any significant fish mortality.

The studies on the bloom associated cultivable bacterial community isolated from all the bloom events (*Prymnesium parvum* N. Carter, *Proboscia alata* (Brightwell) Sandström and *Chattonella marina* (Subrahmanyan) Hara et Chihara) revealed a considerable increase in the number of bacteria from the first day to the last. When compared to the reference stations, THB was much higher in the bloom samples.

The important bacterial flora associated with the bloom-forming algae were *Flavobacterium* sp., *Pseudomonas* sp., *Vibrio* sp., *Bacillus* sp., *Moraxella* sp., *Micrococcus* sp., *Acinetobacter* sp., *Corynebacterium* sp., *Alcaligenes* sp., *Staphylococcus* sp., *Listeria* sp. and *Streptococcus* sp. These bacterial species were also able to produce extracellular enzymes such as protease, amylase, lipase, cellulase, ligninase, phosphatase and alginase.

An increase in the abundance of members of gamma-proteobacteria (*Pseudomonas* sp. and *Vibrio* sp.) and CFB groups (*Flavobacterium* sp.) were found at the bloom decline stage. A gradual increase in proteolytic bacterial population, which is attributed to have algicidal activity, was found during the transition of the bloom events from the initial stage to the decline stage suggesting the probable role of extracellular protease in algal bloom control.

The unique nature of the associated bacterial composition, abundance, high proteolytic activity and its gradual increase at the time of bloom declination in all the bloom events revealed the specific nature of the bloom associated bacteria and its probable role in the bloom dynamics especially in bloom termination.

Thus, an inference can be drawn that algal blooming is a consequence of the combination of multiple, species-specific, optimum favourable physico-chemical, biological and geographical factors rather than of a single factor. All these also play a key role in the microalgal distribution and abundance.

The study also indicates that bacterial strains are potential agents for the control of algal blooms. However, further investigations are essential for establishing the role of associated bacteria, especially of proteolytic bacteria, in termination of algal blooms.

.....ℵℵ.....

References

Ahmed, M. S., Arakawa, O. and Onoue, Y. (1995) Toxicity of cultured *Chattonella marina*. In: *Harmful Marine Algal Blooms* (eds.) Lassus, P., Arzul, G., Gentien, P. and Marcaillou, C. pp.499-504. Lavoisier Inc.

Aiyar, R. G. (1936) Mortality of fish of the Madras coast in June 1935. *Curr. Sci.* **4**, 488-489.

Aktan, Y., Tufekci, V., Tufekci, H. and Aykulu, H. G. (2005) Distribution patterns, biomass estimates and diversity of phytoplankton in Izmit Bay (Turkey). *Estuar. Coast. Shelf Sci.* **64,** 372-384.

Alavi, M., Miller, T., Erlandson, K., Schnieder, R. and Belas, R. (2001) Bacterial community associated with *Pfiesteria*-like dinoflagellate cultures. *Environ. Microbiol.* **3(6)**, 380-396.

Alkawri, A. A. S. and Ramaiah, N. (2011) Distribution of diatom *Pseudo-nitzschia* and dinoflagellates of *Dinophysis* spp. along coast off Goa. *J. Environ. Biol.* **32,** 65-70.

Alkershi, A., Menon, N. R. and Joseph, K. J. (2003) Contribution of size fractions of planktonic algae to primary organic productivity in the coastal waters of Cochin. *J. Mar. Biol. Ass. India* **45,** 1-8.

Allen, W. E. and Cupp, E. E. (1935) Plankton diatoms of the Java Sea. *Ann. Jard. Bot. Buitenzorg* **44(2),** 101-174, 127 figs.

Alverca, E., Biegala, I. C., Kennaway, G. M., Lewis, J. and Franca, S. (2002) In situ identification and localization of bacteria associated with *Gyrodinium instriatum* (Gymnodiniales, Dinophyceae) by electron and confocal microscopy. *Eur. J. Phycol.* **37,** 523-530.

Amaro, A. M., Fuentes, M. S., Ogalde, S. R., Venegas, J. A. and Suárez-Isla, B. A. (2005) Identification and characterization of potentially algal-lytic marine bacteria strongly associated with the toxic dinoflagellate *Alexandrium catenella*. *J. Eukaryot. Microbiol.* **52(3)**, 191-200.

Amsinck, S. L., Jeppesen, E. and Landkildehus, F. (2005) Inference of past changes in zooplankton community structure and planktivorous fish abundance from sedimentary sub fossils—a study of coastal lake subjected to major fish kill incidents during the past century. *Arch. Hydobiol.* **162,** 363-382.

Anantharaman, P., Thirumaran, G., Arumugam, R., Kannan, R. R. R., Hemalatha, A., Kannathasan, A., Sampathkumar, P., Balasubramanian, T. (2010) Monitoring of *Noctiluca* bloom in Mandapam and Keelakarai coastal waters; South-East coast of India. *Recent Res. Sci. Tech.* **2(10),** 51-58.

Anderson, D. M. (1989) Toxic algal blooms and red tides: a global prospective. In: *Red tides: Biology, Environmental Science and Toxicology* (eds.) Okaichi, T., Anderson, D. M. and Nemoto, T. pp.11-16. NewYork: Elsevier Science Publishing.

Anderson, D. M. (1997) Bloom dynamics of toxic *Alexandrium* species in the north western US. *Limnol. Oceanogr.* **42,** 1009-1022.

Anderson, D. M., Gilbert, P. M. and Burkholder, J. M. (2002) Harmful algal blooms and eutrophication: nutrient sources, composition and consequences. *Estuaries* **25,** 704-726.

Anoop, A. K., Krishnakumar, P. K. and Rajagopalan, M. (2007) *Trichodesmium erythraeum* (Ehrenberg) bloom along the southwest coast of India (Arabian Sea) and its impact on trace metal concentrations in seawater. *Estuar. Coast. Shelf Sci.* **71,** 641-646.

Armstrong, R. A. (1994) Grazing limitation and nutrient limitation in marine ecosystems: Steady state solutions of an ecosystem model with multiple food chains. *Limnol. Oceanogr.* **39(3),** 597-608.

Arrigo, K. R. (2005) Marine microorganisms and global nutrient cycles. *Nature* **437,** 349-355.

Azam, F. (1998) Microbial control of oceanic carbon flux: the plot thickens. *Science* **280**, 594-696.

Azam, F. and Ammerman, J. W. (1984) Cycling of organic matter by bacterioplankton in pelagic marine ecosystems, micro environmental considerations. In: *Flows of energy and materials in marine ecosystems* (ed.) Fasham, M. J. R. pp.345-360. New York: Plenum Press.

Baek, S. H., Shimode, S., Han, M. S. and Kikuchi, T. (2008) Reproductive Ecology of the dominant dinoflagellate, *Ceratium fusus*, in Coastal Area of Sagami Bay, Japan. *J. Oceanogr.* **63,** 35- 45.

Baird-Parker, A. C. (1966) Methods for classifying staphylococci and micrococci. In: *Identification methods from microbiologists (*eds.) Gibbs, B. M. and Skinner, F. A. pp.59-64. London: Academic Press.

Baker, J.W., Grover, J. P., Bryan, W. B., Fabiola, U. B., Daniel, L. R., Reagan, E. and Richard, L. K. (2007) Growth and toxicity of *Prymnesium parvum* (Haptophyta) as a function of salinity, light and temperature. *J. Phycol.* **43**, 219-227.

Baker, J. W., Grover, J. P., Ratheesh, R., Black, C., Valenti, T. W., Brooks, B. W. and Daniel, L. R. (2009) Growth at the edge of the niche: an experimental study of the harmful alga *Prymnesium parvum. Limnol. Oceanogr.* **54**, 1679-1687.

Baker, K. H. and Herson, D. S. (1978) Interaction between the diatom *Thcillnsiosiru pseudonaniio* and an associated pseudomonad in a mariculture system. *Appl. Environ. Microbiol.* **35,** 791-796.

Bales, M., Moss, B., Phillips, G., Irvine, K. and Stansfield, J. (1993) The changing ecosystem of a shallow, brackish lake, Hickling Broad, Norfolk, U. K. II. Long-term trends in water chemistry and ecology and their implications for restoration of the lake. *Freshwater Biol.* **29 (1),** 141-165.

Balkis, N. (2003) Seasonal variations in the phytoplankton and nutrient dynamics in the neritic water of Buyukcekmece Bay, Sea of Marmara. *J. Plank. Res.* **25,** 703-717.

Banse, K. (1994) On the coupling of hydrography, phytoplankton, zooplankton and settling of organic particles offshore in the Arabian Sea. *P. Indian Acad. Sci. - Earth* **103(83),** 125-161.

Banse, K., Vijayaraghavan, S. and Madhupratap, M. (1996) On the possible causes of the seasonal phytoplankton blooms along the west coast of India. *Indian J. Mar. Sci.* **25,** 283-289.

Barreiro, A., Guisande, C., Maneiro, I., Lien, T. P., Legrand, C., Tamminen, T., Lehtinen, S., Uronen, P. and Graneli, E. (2005) Relative importance of the different negative effects of the toxic haptophyte *Prymnesium parvum* on *Rhodomonas salina* and *Brachionus plicatilis*. *Aquat. Microb. Ecol.* **38,** 259-267.

Bates, S. S. (1998) Ecophysiology and metabolism of ASP toxin production. In: *Physiological Ecology of Harmful Algal Blooms* (eds.) Anderson, D. M., Cembella, A. D. and Hallegraeff, G. M. pp.405-426. Heidelberg: Springer-Verlag.

Bates, S. S., Douglas, D. J., Doucette, G. J. and Léger, C. C. (1995) Enhancement of domoic acid production by reintroducing bacteria to axenic cultures of the diatom *Pseudo-nitzschia multiseries. Nat. Toxins* **3**, 428-435.

Behrenfeld, M. J., Prasil, O., Babin, M. and Bruyant, F. (2004) In search of a physiological basis for variations in light-limited and light saturated photosynthesis. *J. Phycol.* **40,** 4-25.

Bell, W. H. and Mitchell, R. (1972) Chemotactic growth responses of marine bacteria to algal extracellular products. *Biol. Bull.* **143**, 265-277.

Bell, W. H., Lang, J. M. and Mitchell, R. (1974) Selective stimulation of marine bacteria by algal extracellular products. *Limnol. Oceanogr.* **19**, 833-839.

Bergey, D. H. and Holt, J. G. (2000) *Bergey's manual of determinative bacteriology*. 9th edition. p.1246. Baltimore: Williams and Wilkins Co.

Bhat, R. V. (1981) A report on an outbreak of mussel poisoning in coastal Tamil Nadu, India. p.9. Hyderabad: National Institute of Nutrition.

Bhimachar, B. S. and George, P. C. (1950) Abrupt set-backs in the fisheries of the Malabar and Kanara coasts and ‘‘red water’’ phenomenon as their probable cause. *P. Indian Acad. Sci. B* **31,** 339-350.

Blackburn, S. I., Bolch, C. J. S., Haskard, K. A. and Hallegraeff, G. M. (2001) Reproductive compatibility among four global populations of the toxic dinoflagellate *Gymnodinium catenatum* (Dinophyceae). *Phycologia* **40**, 78-87.

Bold, H. D. and Wynne, M. J. (1983) *Introduction to the algae.* 2nd edition. pp.417-428. New Jersey: Prentice-Hall, Inc.

Boyce, D. G., Lewis, M. R. and Worm, B. (2010) Global phytoplankton decline over the past century. *Nature* **466,** 591-596.

Bricelj, V. M. and Malouf, R. E. (1984) Influence of algal and suspended sediment concentrations on the feeding physiology of the hard clam *Mercenaria mercenaria*. *J. Mar. Biol.* **84,** 155-165.

Brock, J. C. and McClain, C. R. (1992) Inter annual variability in phytoplankton blooms observe in the north western Arabian Sea during the south western monsoon. *J. Mar. Res.* **97(C1),** 733-750.

Brooks, B. W., James, S. V., Valenti Jr., T. W., Urena-Boeck, F., Serrano, C., Berninger, J. P., Schwierzke, L., Mydlarz, L. D., Grover, J. P. and Roelke, D. L. (2010) Comparative toxicity of *Prymnesium parvum* in inland waters. *J. Am. Water Resour. As.* **46,** 45-62.

Caldwell, D. E. (1977) The planktonic micro flora of lakes. *Crit. Rev. Microbiol.* **5,** 305-370.

Calliari, D., Gomez, M. and Gomez, N. (2005) Biomass and composition of the phytoplankton in the Rıo de la Plata: large-scale distribution and relationship with environmental variables during a spring cruise. *Cont. Shelf Res.* **25,** 197-210.

Capone, D. G. and Carpenter, E. J. (1982) Nitrogen fixation in the marine environment. *Science* **217,** 1140-1142.

Capone, D. G., Burns, J. S., Michaels, A. F., Montoya, J. P., Subramaniam, A. and Carpenter, E. J. (2005) Nitrogen fixation by *Trichodesmium* spp. An important source of new nitrogen to the tropical and subtropical North Atlantic Ocean. *Global Biogeochem. Cy.* **19,** 1-17.

Castracane, A. F. (1886) Report on the Diatomaceae collected by H.M.S. Challenger during the years 1873-76. Vol. 4. p.178.

Cederwall, H. and Elmgren, R. (1990) Biological effects of eutrophication in the Baltic Sea, particularly the coastal zone. *Ambio* **19,** 109-112.

Cembella, A. D. (1989) Occurrence of okadaic acid, a major diarrheic shellfish toxin, in natural populations of *Dinophysis* spp. from the eastern coast of North America. *J. Appl. Phycol.* **1**, 307-310.

Cermeno, P., Maranon, E., Perez, V., Serret, P., Fernandez, E. and Castro, C. G. (2006) Phytoplankton size structure and primary production in highly dynamic coastal ecosystem (Ria de Vigo, NW-Spain): Seasonal and short time scale variability. *Estuar.Coast. Shelf Sci.* **67,** 251-266.

Chacko, P. I. (1942) An unusual incidence of mortality of marine fauna. *Curr. Sci.* **11,** 404

Charlson, R. J., Lovelock, J. E., Andreae, M. O. and Warren, S. G. (1987) Oceanic phytoplankton, atmospheric sulfur, cloud albedo and climate. *Nature* **326,** 655-661.

Chellam, A. and Alagarswami, K. (1978) Blooms of *Trichodesmium thiebautii* and their effect on experimental pearl culture at Veppalodai. *Indian J. Fish.* **25**, 237-239.

Chen, W. M., Sheu, F. S. and Sheu, S. (2011) Novel L-amino acid oxidase with algicidal activity against toxic cyanobacterium *Microcystis aeruginosa* synthesized by a bacterium *Aquimarina* sp. *Enzym. Microb. Tech.* **49,** 372-379.

Chidambaram, K. and Unny, M. M. (1944) Note on the swarming of the planktonic algae *Trichodesmium erythraeum* in the Pamban area and its effect on the fauna. *Curr. Sci.* **13,** 263.

Choi, H. G., Kim, P. J., Lee, W. C., Yun, S. J., Kim, H. G. and Lee, H. J. (1998) Removal efficiency of *Cochlodinium polykrikoides* by yellow loess. *J. Kor. Fish. Soc.* **31,** 109-113.

Choudhury, S. B. and Panigrahy, R. C. (1989) Occurrence of bloom of diatom *Asterionella glacialis* in near-shore waters of Gopalpur, Bay of Bengal. *Indian J. Mar. Sci.* **18,** 204-206.

Cleve, P. T. (1873) Examination of the diatoms found on the surface of the sea of Java. *Bih. Svensk Vetensk Akad. Handl.* **1(11),** 1-13.

Cleve, P. T. (1878) Diatoms from the West Indian Archepelago. *Bih. Svensk Vetensk Akad. Handl.* **5,** 1-22.

Cleve, P. T. (1894) Synopsis of the naviculoid diatoms. Part I. *Bih. Svensk Vetensk Akad. Handl.* **27,** 1-219.

Cleve, P. T. (1896) Diatoms from the Baffin Bay and Davis Strait. *Bih. Svensk Vetensk Akad. Handl.* **22,** 1-76.

Cole, J. J. (1982) Interactions between bacteria and algae in aquatic ecosystems. *Ann. Rev. Ecol. Syst.* **13,** 291-314.

Copley, J. (2002) All at sea. *Nature* **415,** 572-574.

Croci, L., Cozzi, L., Suffredini, E., Ciccaglioni, G., Toti, L., Milandri, A., Ceredi, A., Benzi, M. and Poletti, R. (2006) Characterization of microalgae and associated bacteria collected from shellfish harvesting areas. *Harmful Algae* **5,** 266-274.

Cupp, E. E. (1937) Seasonal distribution and occurrence of marine diatoms and dinoflagellates at Scotch Cap, Alaska. *Bull. Scripps Inst. Oceanogr.* **4,** 71- 100.

Cupp, E. E. (1943) Marine plankton diatoms of the west coast of North America. In: *Bulletin of the Scripps Institution of Oceanography of the University of California La Jolla, California* (eds.) Sverdrup, H. U., Fleming, R. H., Miller, L. H. and Zobell, C. E. **5(1),** p.238. London: Cambridge University Press.

D'Silva, M. S., Anil, A. C., Naik, R. K. and D'Costa, P. M. (2012) Algal blooms: a perspective from the coasts of India. *Nat. Hazards* **63,** 1225 - 1253.

Dahl, E. and Yndestad, M. (1985) Diarrhetic shellfish poisoning (DSP) in Norway in the autumn 1984 related to the occurrence of *Dinophysis* spp. In: *Toxic Dinoflagellates* (eds.) Anderson, D. M., White, A. W. and Baden, D. G. pp.495-501. Amsterdam: Elsevier.

Dale, B. and Yentsch, C. M. (1978) Red tide and paralytic shellfish poisoning. *Oceanus* **21,** 41-49.

Dang, H. and Lovell, C. R. (2000) Bacterial primary colonization and early succession on surfaces in marine waters as determined by amplified rRNA gene restriction analysis and sequence analysis of 16S rRNA genes. *Appl. Environ. Microbiol.* **66(2),** 467-475.

Daranas, A. H., Norte, M. and Fernandez, J. J. (2001) Toxic marine microalgae. *Toxicon* **39,** 1101-1132.

De Sousa, S. N., Dileepkumar, M., Sardessai, S., Sarma, V. V. S. S. and Shirodkar, P. V. (1996) Seasonal variability in oxygen and nutrients in the central and eastern Arabian Sea. *Curr. Sci.* **71,** 847-851.

Decho, A. W. (1990) Microbial exopolymer secretions in ocean environment: their role(s) in food webs and marine processes. *Oceanogr. Mar. Biol. Ann. Rev.* **28,** 73-153.

De Long, E. F., Franks, D. G. and Alldredge, A. L. (1993) Phylogenetic diversity of aggregateattached vs. free-living marine bacterial assemblages. *Limnol. Oceanogr.* **38(5),** 924-934.

De-Pauw, N. and Naessens, F. E. (1991) Nutrient induced competition between species of marine diatoms. *Hydrobiol. Bull.* **25,** 23-28.

Desikachary, T. V. (1959) *Cyanophyta*. p.686. New Delhi: Indian Council of Agricultural Research.

Desikachary, T. V. (1988) Marine diatoms of the Indian Ocean region. In: *Atlas of Diatoms, Fascicle V* (ed.) Desikachary, T. V. pp.1-13. Madras: Madras Science Foundation.

Desikachary, T. V. and Prema, P. (1987a) Diatoms from the Bay of Bengal. In: *Atlas of Diatoms, Fascicle III* (ed.) Desikachary, T. V. pp.1-10. Madras: Madras Science Foundation.

Desikachary, T. V. and Renjitha Devi, K. A. (1986) Marine fossil diatoms from India and Indian Ocean. In: *Atlas of Diatoms, Fascicle I* (ed.) Desikachary, T. V. pls.1-77. Madras: Madras Science Foundation.

Desikachary, T. V. and Sreelatha, P. M. (1989) *Oamaru Diatoms*. pp.1-330. Berlin: J. Cramer.

Desikachary, T. V., Gowthaman, S. and Latha, Y. (1987b) Diatom flora of some sediments from the Indian Ocean region. In: *Atlas of Diatoms, Fascicle II* (ed.) Desikachary, T. V. pls.78-221. Madras: Madras Science Foundation.

Desikachary, T. V., Prasad, A. K. S. K., Hema, P., Sreelatha, P. M., Sreedharan, V. T. and Subrahmanyan, R. (1987c) Marine diatoms from the Arabian Sea and Indian Ocean. In: *Atlas of Diatoms, Fascicle IV* (ed.) Desikachary, T. V. pp.1-7, pls. 332-400A. Madras: Madras Science Foundation.

Devassy, V. P. (1974) Observations on the bloom of a diatom *Fragilaria oceanica* Cleve. *Mahasagar* **7,** 101-105.

Devassy, V. P. (1983) Plankton ecology of some estuarine and marine regions of the west coast of India. *PhD thesis*, University of Kerala.

Devassy, V. P. and Bhat, S. R. (1991) The Killer tides. *Scientific Reporter* 16-19.

Devassy, V. P. and Bhattathiri, P. M. A. (1974) Phytoplankton ecology of the Cochin backwater. *Indian J. Mar. Sci.* **3**, 46-50.

Devassy, V. P. and Goes, J. I. (1988) Phytoplankton community structure and succession in a tropical estuarine complex central west coast of India. *Estuar. Coast. Shelf Sci.* **27,** 671-685.

Devassy, V. P. and Nair, S. R. S. (1987) Discolouration of water and its effect on fisheries along the Goa coast. *Mahasagar* **20,** 121-128.

Devassy, V. P., Bhattathiri, P. M. A. and Qasim, S. Z. (1978) *Trichodesmium* phenomenon. *Indian J. Mar. Sci.* **7,** 168-186.

Devassy, V. P., Bhattathiri, P. M. A. and Qasim, S. Z. (1979) Succession of organisms following *Trichodesmium* phenomenon. *Indian J. Mar. Sci.* **8**, 89-93.

Dharani, G. K., Abdul Nazar, A. K., Kanagu, L., Venkateshwaran, P., Kumar, T. S., Ratnam, S., Venkatesan, R. and Ravindran, M. (2004) On the recurrence of *Noctiluca scintillans* bloom in Minnie Bay, Port Blair: impact on water quality and bioactivity of extracts. *Curr. Sci.* **87,** 990-994.

Dodge, J. D. and Marshall, H. G. (1994) Biogeographic analysis of the armoured Planktonic dinoflagellate *Ceratium* in the North Atlantic and adjacent seas. *J. Phycol.* **30,** 905- 922.

Doucette, G. (1995) Interactions between bacteria and Harmful Algae: A Review. *Nat. Toxins* **3,** 65-74.

Doucette, G., Kodania, M., Franca, S. and Gallacher, S. (1998) Bacterial interactions with harmful algal bloom species: bloom ecology, toxigenesis, and cytology. In: *Physiological Ecology of Harmful Algal Blooms* (eds.) Anderson, D. M., Cembella, A. D. and Hallegraeff, G. M. pp.619-648. Heidelberg: Springer-Verlag.

Doucette, G. J., McGovern, E. R. and Babinchak, J. A. (1999) Algicidal bacteria active against *Gymnodinium breve* (Dinophyceae). I. Bacterial isolation and characterization of killing activity. *J. Phycol.* **35,** 1447-1457.

Dyer, K. R. (1973) Estuaries: A Physical introduction. 1st edition. p.140. London: John Wiley and Sons.

Eashwar, M., Nallathambi, T., Kuberaraj, K. and Govindarajan, G. (2001) *Noctiluca* blooms in Port Blair, Andaman. *Curr. Sci.* **81,** 203-206

Edvardsen, B. and Paasche, E. (1998) Bloom dynamics and physiology of *Prymnesium* and *Chrysochromulina*. In: *The physiological ecology of harmful algal blooms* (eds.) Anderson, D. M., Cembella, A. D. and Hallegraeff, G. M. pp.193-208. Springer.

Elbrachter, M. (1973) Population dynamics of *Ceratium* in coastal waters of the Kiel Bay. *Oikos* **15,** 43-48.

Endo, M., Onoue, Y. and Kuroki, A. (1992) Neurotoxin-induced cardiac disorder and its role in the death of fish exposed to *Chattonella marina*. *Mar. Biol.* **112,** 371-376.

Endo, M., Sakai, T. and Kuroki, A. (1985) Histological and histochemical changes in the gills of yellowtail *Seriola quinqueradiata* exposed to the raphidophycean flagellate *Chattonella marina. Mar. Biol.* **87,** 193-197.

Errera, R. M., Roelke, D. L., Kiesling, R., Brooks, B. W., Grover, J. P., Schwierzke, L., Urena-Boeck, F., Baker, J. W. and Pinckney, J. L. (2008) The effect of imbalanced nutrients and immigration on *Prymnesium parvum* community dominance and toxicity: results from in-lake microcosm experiments, Texas, US. *Aquat. Microb. Ecol.* **52,** 33-44.

Escaravage, V., Prins, T. C., Smaal, A. C. and Peeters, J. C. H. (1996) The response of phytoplankton communities to phosphorus input reduction in mesocosm experiments. *J. Exp. Mar. Biol. Ecol.* **198,** 55-79.

Falkowski, P., Barber, R. and Smetacek, V. (1998) Biogeochemical controls and feedbacks on ocean primary production. *Science* **281,** 200-206.

Falkowski, P. G. (2000) Rationalizing elemental ratios in unicellular algae. *J. Phycol.* **36,** 3-6.

Falkowski, P. G. and Raven, J. A. (1997) *Aquatic Photosynthesis.* p.375. Massachusetts: Blackwell Science, Malden.

Fandino, L. B., Riemann, L., Steward, G. F., Long, R. A. and Azam, F. (2001) Variations in bacterial community structure during a dinoflagellate bloom analysed by DGGE and 16S rDNA sequencing. *Aquat. Microb. Ecol.* **23(2),** 119-130.

Fehling, J., Davidson, K., Bolch, C. J. S., Brand, T. D and Narayanaswamy, B.E. (2012) The relationship between phytoplankton distribution and water column characteristics in North West European shelf sea waters. *PLOS ONE* **7(3),** 1-16.

Ferrier, M., Martin, J. L. and Rooney-Verga, J. N. (2002) Stimulation of *Alexandrium fundyense* growth by bacterial assemblages from the Bay of Fundy. *J. Appl. Microbiol.* **92(4),** 706-716.

Field, C., Behrenfeld, M., Randerson, J. and Falkowski, P. (1998) Primary production of the biosphere: integrating terrestrial and oceanic components. *Science* **281,** 237-240.

Fischer, C. J. and Zhang, J. Z. (2006) A simplified resorcinol method for direct spectrophotometric determination of nitrate in sea water. *Mar. Chem.* **99**, 220- 226.

Fisher, T. R., Gustafson, A. B., Sellner, K., Lacourte, R., Hass, L. W., Wetzel, R. L., Magnien, R., Eweritt, D., Michaels, B. and Karrh, R. (1999) Spatial and temporal variation of resource limitation in Chesapeake Bay. *Mar. Biol.* **133,** 763-778.

Fisher, T. R., Peele, E. R., Ammerman, J. W. and Harding, L. W. (1992) Nutrient limitation of phytoplankton in Chesapeake Bay. *Mar. Ecol. Prog. Ser.* **82,** 51-63.

Fistarol, G. O., Legrand, C. and Graneli, E. (2003) Allelopathic effect of *Prymnesium parvum* on a natural plankton community. *Mar. Ecol. Prog. Ser.* **255,** 115-125.

Fistarol, G. O., Legrand, C. and Graneli, E. (2005) Allelopathic effect on a nutrient-limited phytoplankton species. *Aquat. Microb. Ecol.* **41,** 153-161.

Flindt, M. R., Pardal, M. A., Lillebo, A. I., Martins, I. and Marques, J. C. (1999) Nutrient cycling and plant dynamics in estuaries: a brief review. *Acta Oceanol. Sin.* **20,** 237-248.

Forsberg, C. (1982) Limnological research can improve and reduce the cost of monitoring and control of water quality. *Hydrobiologia* **86,** 143-146.

Franca, S., Veigas, S., Mascarenhas, V., Pinto, L. and Doucette, G. J. (1995) Prokaryotes in association with a toxic *Alexandrium lusitanicum* in culture. In: *Harmful Marine Algal Blooms* (eds.) Lassus, P., Arzul, G., Erard, E., Gentien, P. and Marcaillou, C. pp.45-51. Paris: Lavoisier.

Fukami, K., Nishijima, T., Murata, H., Doi, S. and Hata, Y. (1991) Distribution of bacteria influential on the development and the decay of *Gyrnriodiniurn itcignsnkiense* red tide and their effect on algal growth. *Nippon Suisniz Gukkuishi* **57,** 2321-2326.

Fukami, K., Sakaguchi, K., Kanou, M. and Nishijima, T. (1996) Effects of bacterial assemblages on the succession of blooming phytoplankton from *Skeletonema costatum* to *Heterosigma akashiwo*. In: *Harmful and toxic algal blooms: Intergovernmental Oceanographic Commission of UNESCO* (eds.) Yasumoto, T., Oshima, Y. and Fukuyo, Y. pp.35-338. Paris.

Fukami, K., Yuzawa, A., Nishijima, T. and Hata, Y. (1992) Isolation and properties of bacterium inhibiting the growth of *Gymnodinium nagasakiense*. *Nippon Suisan Gakkaishi* **58,** 1073-1077.

Fukuyo, Y., Imai, I., Kodama, M. and Tamai, K. (2002) Red tides and other harmful algal blooms in Japan. In: *Harmful Algal Blooms in the PICES Region of the North Pacific* (eds.) Taylor, F. J. R. M. and Trainer, V. L. pp.7-20. North Pacific Marine Science Organization.

Gaarder, T. and Gran, H. H. (1927) Investigations of the production of plankton in the Oslo Fjord. *Rapp. Cons. Explor. Mer.* **42,** 1-48.

Gacesa, P. and Wusteman, F.S. (1990) Plate assay for simultaneous detection of alginate lyases and determination of substrate specificity. *Appl. Environ. Microbiol.* **56(7)**, 2265-2267.

Gaikwad, S. R., Tarot, S. R. and Chavan, T. P. (2004) Diversity of Phytoplankton and Zooplankton with respect to pollution status of river Tapi in North Maharashtra region. *Curr. Sci.* **5,** 749-754.

Gallacher, S. and Smith, E. A. (1999) Bacteria and paralytic shellfish toxins. *Protist* **150,** 245-255.

Gao, X. and Song, J. (2005) Phytoplankton distributions and their relationship with the environment in the Changjiang Estuary, China. *Mar. Pollut. Bull.* **50,** 327-335.

Garate-Lizarraga, I., Siquieros Betrones D.A. and Maldonado-Lopez, V. (2003) First record of a *Rhizosolenia debyana* bloom in the Gulf of California, Mexico. *Pac. Sci.* **57,** 141-145.

Garthwaite, I. (2000) Keeping shellfish safe to eat: a brief review of shell fish toxins and methods for their detection. *Trends Food Sci. Tech.* **11,** 235-244.

GEOHAB, (2005) Global Ecology and Oceanography of Harmful Algal Blooms, GEOHAB core research project: HABs in upwelling systems (eds.) Pitcher, G., Moita, T., Trainer, V., Kudela, R., Figueiras, P. and Probyn, T. p.82. IOC and SCOR, Paris and Baltimore.

George, B., Nirmal Kumar, J. I. and Kumar, R. N. (2012) Study on the influence of hydro-chemical parameters on phytoplankton distribution along Tapi estuarine area of Gulf of Khambhat, India. *Egyptian J. Aquat. Res.* **38,** 157-170.

Ghosh, S., Barinova, S. and Keshri, J. P. (2012) Diversity and seasonal variation of phytoplankton community in the Santragachi Lake, West Bengal, India. *QScience Connect* **3,** 1-19.

Gianesella, S. M. F., Saldanha-Correa, F. M. P. and Teixeira, C. (2000) Tidal effects on nutrients and phytoplankton distribution in Bertioga Channel, Sao Paulo, Brazil. *Aquat. Ecosyst. Health* **3,** 533-544.

Gibb, S. W., Cummings, D. G., Irigoien, X., Barlow, R. G., Fauzi, R. and Mantoura, C. (2001) Phytoplankton pigment chemotaxonomy of north eastern Atlantic. *Deep-Sea Res. II* **48,** 795-823.

Gilson, H. C. (1937) The Nitrogen cycle. Scientific Reports. John Murray Expedition 1933-34. **2,** 21-81.

Glibert, P. M., Anderson, D. M., Gentien, P., Graneli, E. and Sellner, K. G. (2005) The global complex phenomenon of harmful algal blooms. *Oceanography* **18,** 136-147.

Godhe, A., Karunasagar, I. and Karunasagar, I. (1996) *Gymnodinium catenatum* on west coast of India. *Harmful Algae News* **15,** 1.

Golterman, H. L. (1972) The role of phytoplankton in detritus formation. In: *Detritus and its role in aquatic ecosystems*. Proceedings of the IBP-UNESCO Symposium, pp.89-103. Pallanza, Italy.

González, J. M. and Moran, M. A. (1997) Numerical dominance of a group of marine bacteria in the alpha subclass of the class Proteobacteria in coastal seawaters. *Appl. Environ. Microbiol.* **63(11),** 4237-4242.

Gonzalez, J. M., Kiene, R. P. and Moran, M. A. (1999) Transformation of sulfur compounds by an abundant lineage of marine bacteria in the alpha subclass of the class Proteobacteria. *Appl. Environ. Microbiol.* **65,** 3810-3819.

Gopakumar, G., Sulochanan, B. and Venkatesan, V. (2009) Bloom of *Noctiluca scintillans* (McCartney) in Gulf of Mannar, southeast coast of India. *J. Mar. Biol. Ass. India* **55(1),** 75-80.

Gopinathan, C. P. (1972) Seasonal abundance of phytoplankton in the Cochin Backwaters. *J. Mar. Biol. Ass. India* **14,** 568-577.

Gopinathan, C. P. (1974) Studied on the phytoplankton of the Cochin backwater, a tropical estuary. *Indian J. Fish.* **21(2),** 50-513.

Gopinathan, C. P. (1975a) Studies on the estuarine diatoms of India. *Bull. Dept. Mar. Sci. Cochin Univ.* **7(4),** 995-1004.

Gopinathan, C. P. (1975b) On new distributional records of plankton diatoms from the Indian Seas. *J. Mar. Biol. Ass. India* **17,** 223-240.

Gopinathan, C. P. (1984) A systematic account of the littoral diatoms of the southwest coast of India. *J. Mar. Biol. Ass. India* **26,** 1-31.

Gopinathan, C. P., Gireesh, R. and Smitha, K. S. (2001) Distribution of chlorophyll *a* and *b* in the eastern Arabian Sea (West coast of India) in relation to nutrient during pre-monsoon season. *J. Mar. Biol. Ass. India* **43,** 21-30.

Gopinathan, C. P., Rodigro, J. X., Qasim, M. H. and Rajagopalan, M. S. (1994) Phytoplankton pigments in relation to primary production and nutrients in the inshore waters of Tuticorin, South West coast of India. *Indian J. Mar. Sci.* **23,** 204-212.

Gordon, N. and Colorni, A. (2008) *Prymnesium parvum*, an ichthyotoxic alga in an ornamental fish farm in southern Israel. *Israeli J. Aquacult.* **60,** 5-8.

Gowda, G., Gupta, T. R. C., Rajesh, K. M., Gowda, H., Lingadhal, C. and Ramesh, A. M. (2001) Seasonal distribution of phytoplankton in Nethravathi estuary. *J. Mar. Biol. Ass. India* **43,** 31-40.

Graham, H. W. (1941) An oceanographic consideration of the dinoflagellate genus *Ceratium*. *Ecol. Monogr.* **11,** 99-116.

Graneli, E. and Hansen, P. J. (2006) Alleopathy in harmful algae: A mechanism to compete for resources? In: *Ecology of Harmful Algae.* Vol.189 (eds.) Graneli, E. and Turner, J. T. p.415. Berlin-Heidelberg: Springer-Verlag.

Graneli, E. and Johansson, N. (2003) Effects of the toxic haptophyte *Prymnesium parvum* on the survival and feeding of a ciliate: the influence of different nutrient conditions. *Mar. Ecol. Prog. Ser.* **254,** 49-56.

Graneli, E., Carlsseon, P., Turner, J. T., Ptester, B. and Dawson, R. (1999) Effect of N:P:Si ratios and zooplankton grazing on phytoplankton communities in the Northern Adriatic Sea. I. Nutrients, phytoplankton biomass and polysaccharide production. *Aquat. Microb. Ecol.* **18,** 37-54.

Graneli, E., Weberg, M. and Salomon, P. S. (2008) Harmful algal blooms of allelopathic microalgal species: the role of eutrophication. *Harmful Algae* **8,** 94-102.

Green, D. H., Llewellyn, L. E., Negri, A. P., Blackburn, S. I. and Bolch, C. J. S. (2004) Phylogenetic and functional diversity of the cultivable bacterial community associated with the paralytic shellfish poisoning dinoflagellate *Gymnodinium catenatum*. *FEMS Microbiol. Ecol.* **47,** 345-357.

Grossart, H. P. (1999) Interactions between marine bacteria and axenic diatoms (*Cylindrotheca fusiformis*, *Nitzschia laevis*, and *Thalassiosira weissflogii*) incubated under various conditions in the lab. *Aquat. Microb. Ecol.* **19,** 1-11.

Grossart, H. P. and Simon, M. (2007) Interactions of planktonic algae and bacteria: effects on algal growth and organic matter dynamics. *Aquat. Microb. Ecol.* **47,** 163-176.

Grossart, H. P., Levold, F., Allgaier, M., Simon, M. and Brinkhoff, T. (2005) Marine diatom species harbour distinct bacterial communities. *Environ. Microbiol.* **7,** 860-873.

Guo, M., Harrison, P. J. and Taylor, F. J. R. (1996) Fish kills related to *Prymnesium parvum* N. Carter (Haptophyta) in the People's Republic of China. *J. Appl. Phycol.* **8(2),** 111-117.

Haake, B., Ittekkot, V., Rixen, T., Ramaswamy. V., Nair, R. R. and Curry, W. B. (1993) Seasonality and inter annual variability of particle fluxes to the deep Arabian Sea. *Deep-Sea Res.* **40(7),** 1323-1344.

Haeckel, E. (1890) Plankton-Studien. Jena. p.124. (English translation in Rep. U.S. Fish. Comm., 1893. For 1889-1891).

Hallegraeff, G. M. (1992) Harmful algal blooms in the Australian region. *Mar. Pollut. Bull.* **25(5-8),** 186-190.

Hallegraeff, G. M. (1993) A review of harmful algal blooms and their apparent global increase. *Phycologia* **32,** 79-99.

Hallegraeff, G. M. (1995) Harmful algal blooms: a global overview. In: *Manual on Harmful Marine Microalgae* (eds.) Hallegraeff, G. M., Anderson, D. M. and Cembella, A. D. Vol 33. pp.1-22. Paris: IOC-UNESCO.

Hallegraeff, G. M. (2003) Harmful Algal Blooms: a global review. In: *Manual of Marine Microalgae* (eds.) Hallegraeff, G. M., Anderson, D. M. and Cembella, A. D. Vol 11. 2nd edition. pp.25-49. Paris: IOC-UNESCO.

Hallegraeff, G. M., Anderson, D. M. and Cembella, A. D. (1995) *Manual on Harmful Marine Microalgae*. IOC Manuals and Guides No. 33. p.551. Paris: IOC- UNESCO.

Hankin, L. and Anagnostakis, S. L. (1977) Solid media containing Carboxymethylcellulose to detect Cx cellulase activity of micro-organisms. *J. Gen. Microbiol.* **98,** 109-115.

Hare, C. E., Demir, E., Coyne, K. J., Cary, S. C., Kirchman, D. L. and Hutchins, D. A. (2005) A bacterium that inhibits the growth of *Pfiesteria piscicida* and other dinoflagellates. *Harmful Algae* **4,** 221-234.

Harnstrom, K., Karunasagar, I. and Godhe, A. (2009) Phytoplankton species assemblages and their relationship to hydrographic factors-a study at the old port in Mangalore, coastal Arabian Sea. *Indian J. Mar. Sci.* **8(2),** 24-234.

Harrison, P. J., Clifford, P. J., Cochlan, W. P., Yin, K., St. John, M.A., Thompson, P. A., Sibbald, M. J. and Albright, L. J. (1991) Nutrient and phytoplankton dynamics in the Fraser River plume, Strait of Georgia, British Columbia. *Mar. Ecol. Prog. Ser.* **70,** 291-304.

Harrison, P. J., Hu, M. H., Yang, Y. P. and Lu, X. (1990) Phosphate limitation in estuarine and coastal waters of China. *J. Exp. Mar. Biol. Ecol.* **140,** 79-87.

Hecky, R. E. and Kilham, P. (1988) Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent evidence on the effects of enrichment. *Limnol. Oceanogr.* **33,** 796-822

Hendey, N. I. (1937) The plankton diatoms of the southern seas. *'Discovery' Rep.* **16,** 151-364.

Hendey, N. I. (1964) *An introductory account of the smaller algae of the British coastal waters. Part V. Bacillariophyceae (Diatoms).* p.317. Koenigstein: Otto Koeltz Science Publishers.

Hensen, V. (1887) Über die Bestimmung des Plankton's oder des im Meere treibenden Materials an Pflanzen und Tieren. *Fünfter Ber. Komm. Unters. Dtsch. Meere. Kiel* (1882-1886), **1,** 12-16.

Hold, G. L., Smith, E. A., Rappé, M. S., Maas, E. W., Moore, E. R. B., Stephen, J. R., Prosser, J. I., Birkbeck, T. H. and Gallacher, S. (2001) Characterisation of bacterial communities associated with toxic and non-toxic dinoflagellates: *Alexandrium* spp. and *Scrippsiella trochoidea. FEMS Microbiol. Ecol.* **37,** 161-173.

Holdway, P. A., Watson, R. A. and Moss, B. (1978) Aspects of the ecology of *Prymnesium parvum* (Haptophyta) and water chemistry in the Norfolk Broads, England. *Freshwater Biol.* **8(4),** 295-311.

Holmes, M. J., Teo, S. L. M., Lee, F. C. and Khoo, H. W. (1999) Persistent low concentrations of diarrhetic shellfish toxins in green mussels *Perna viridis* from the Johor Strait, Singapore: First record of diarrhetic shellfish toxins from South-East Asia. *Mar. Ecol. Prog. Ser.* **181,** 257-268.

Holmstrom, C. and Kjelleberg, S. (1994) The effect of external biological factors on settlement of marine invertebrate larvae and new antifouling technology. *Biofouling* **8,** 147-160.

Hooker, J. D. (1847) *The Botany of the Antarctic Voyage of H.M. Discovery Ships Erebus and Terror in the Years 1839-1843*. p.684. London: Reeve Bros.

Hopkinson, C. S. and Vallino, J. J. (1995) The relationships among man's activities in watersheds and estuaries: A model of runoff effects on patterns of estuarine community metabolism. *Estuaries* **18,** 598-621.

Hornell, J. (1908) Report of the results of a fishery cruise along the Malabar coast to the Laccadive Islands in 1908. *Madras Fish. Bull.* **4**, 71-126.

Hornell, J. and Nayudu, M. R. (1923) A contribution to the life history of the Indian sardine with note, on the plankton of the Malabar coast. *Madras Fish. Bull.* **17,** 129.

Houliez, E., Lizon, F., Artigas, L. F., Lefebvre, S. and Schmitt, F. G. (2013) Spatio-temporal variability of phytoplankton photosynthetic activity in a macro tidal ecosystem (the Strait of Dover, eastern English Channel). *Estuar. Coast. Shelf Sci.* **129,** 37-48.

Huang, L., Jian, W., Song, X., Huang, X., Liu, S., Qian, P., Yin, K. and Wu, M. (2004) Species diversity and distribution for phytoplankton of the Pearl River estuary during rainy and dry seasons. *Mar. Pollut. Bull.* **49,** 588-596.

Hustedt, F. (1955) Marine littoral diatoms of Beaufort, North Carolina. *Bulletin of the Duke University Marine Station* **6,** 1-67.

Imai, I., Ishida, Y. and Hata, Y. (1993) Killing of marine phytoplankton by a gliding bacterium *Cytophaga* sp., isolated from the coastal sea of Japan. *Mar. Biol.* **116,** 527-532.

Imai, I., Ishida, Y., Sakaguchi, K., and Hata, Y. (1995) Algicidal marine bacteria isolated from northern Hiroshima Bay, Japan. *Fish. Sci.* **61,** 628-636.

Imai, I., Kim, M. C., Nagasaki, K., Itakura, S. and Ishida, Y. (1998) Relationship between dynamics of red tide-causing raphidophycean flagellates and algicidal micro-organisms in the coastal sea of Japan. *Phycol. Res.* **46,** 139-146.

Imai, I., Sunahara, T., Nishikawa, T., Hori, Y., Kondo, R. and Hiroishi, S. (2001) Fluctuation of the red-tide flagellates *Chattonella* spp. (Raphidophyceae) and the algicidal bacterium *Cytophaga* sp. in the Seto Inland Sea, Japan. *Mar. Biol.* **138,** 1043-1049.

Intergovernmental Oceanographic Commission, www.ioc-unesco.org

International Council for the Exploration of Seas 1984, www.ices.dk

Ishio, S., Mangindaan, R. E., Kuwahara, M. and Nakagawa, H. (1989) A bacterium hostile to flagellates: identification of species and characters. In: *Red Tides: Biology, Environmental Science, and Toxicology* (eds.) Okaichi, T., Anderson, D. and Nemoto, T. pp.205-208. Elsevier Science Publishing Co., Inc.

Iyer, C. S. P., Robin, R. S., Sreekala, M. S. and Kumar, S. S. (2008) *Karenia mikimotoi* bloom in Arabian Sea. *Harmful Algae News* **37,** 9-10.

Jang, P. G., Shin, H. H., Baek, S. H., Jang, M. C., Lee, T. S. and Shin, K. (2013) Nutrient distribution and effects on phytoplankton assemblages in the western Korea/Tsushima Strait. *New Zeal. J. Mar. Fresh.* **47,** 21-36.

Jasti, S., Michael, E. S., Nicole, J. P., Michael, W. G. and Juliette, N. R. V. (2005) Phylogenetic diversity and specificity of bacteria closely associated with *Alexandrium* spp. and other phytoplankton. *Appl. Environ. Microbiol.* **71(7),** 3483-3494.

Jeffery, B., Barlow, T., Moizer, K., Paul, S. and Boyle, C. (2004) Amnesic shellfish poison. *Food Chem. Toxicol.* **42,** 545-557.

Jeffrey, S. W. and Vesk, M. (1997) Introduction to marine phytoplankton and their pigment signatures, In: *Phytoplankton pigments in oceanography: guidelines to modern methods. Monographs on Oceanographic Methodology* (eds.) Jeffrey, S. W., Mantoura, R. F. C. and Wright, S. W. Vol.10. pp.37-84. Paris: UNESCO Publishing.

Jeong, J. H., Jin, H. J., Sohn, C. H., Suh, K. H. and Hong, Y. K. (2000) Algicidal activity of the seaweed *Corallina pilulifera* against red tide microalgae. *J. Appl. Phycol.* **12,** 37-43.

Jin, D., Zhaodi, C., Jumin, L. and Liu, S. (1985) *Marine benthic diatoms in China*. Vol.1. p.331. Beijing: China Ocean Press.

Jiyalalram, M. and Goswami, S. C. (1993) Observations on phytoplankton pigments, zooplankton and physico-chemical parameters in surface waters from Southern Indian Ocean and Antarctic region. *Mahasagar* **26,** 123-131.

Johansson, N. and Graneli, E. (1999) Influence of different nutrient conditions on cell density, chemical composition and toxicity of *Prymnesium parvum* (Haptophyta) in semi-continuous cultures. *J. Exp. Mar. Biol. Ecol.* **239 (2),** 243-258.

Joint, I. R. and Pomeroy, A. J. (1981) Primary production in a turbid estuary. *Estuar. Coast. Shelf Sci.* **13,** 303-316.

Jordan, T. E., Correll, D. L., Miklas, J. and Weller, D. E. (1991) Long-term trends in estuarine nutrients and chlorophyll, and short-term effects of variation in watershed discharge. *Mar. Ecol. Prog. Ser.* **75,** 121-132.

Joseph, K. J. and Nair, P. V. R. (1975) Growth characteristics of certain estuarine phytoplankters. *Bull. Dept. Mar. Sci. Cochin Univ.* **7,** 151-159.

Joseph, K. J. and Pillai, V. K. (1975) Seasonal and spatial distribution of phytoplankters in Cochin Backwaters. *Bull. Dept. Mar. Sci. Cochin Univ.* **7,** 171-180.

Joseph, S. and Ouseph, P. P. (2010) Assessment of nutrients using multivariate statistical techniques in estuarine systems and its management implications: A case study from Cochin Estuary. *Water Environ. J*. **24,** 126-132.

Jouenne, F., Lefebvre, S., Veron, B. and Lagadeuc, Y. (2005) Biological and physicochemical factors controlling short-term variability in phytoplankton primary production and photosynthetic parameters in a macrotidal ecosystem (eastern English Channel). *Estuar. Coast. Shelf Sci.* **65**, 421-439.

Jouenne, F., Lefebvre, S., Veron, B. and Lagadeuc, Y. (2007) Phytoplankton community structure and primary production in small intertidal estuarine-bay ecosystem (eastern English Channel, France). *Mar. Biol.* **151**, 805-825.

Jugnu, R. and Kripa, V. (2009) Effect of *Chattonella marina* [(Subrahmanyan) Hara et Chihara 1982] blooms in the coastal fishery resources along Kerala coast, India. *Indian J. Mar. Sci.* **38**, 77-88.

Jyothibabu, R., Madhu, N. V., Murukesh, N., Haridas, P., Nair, K. K. C. and Venugopal, P. (2003) Intense blooms of *Trichodesmium erythraeum* (Cyanophyta) in the open waters along east coast of India. *Indian J. Mar. Sci.* **32,** 165-167.

Kaartvedt, S., Johnsen, T. M., Aksnes, D. L., Lie, U. and Svendsen, H. (1991) Occurrence of the toxic phytoflagellate *Prymnesium parvum* and associated fish mortality in a Norwegian Fjord system. *Can. J. Fish. Aquat. Sci.* **48(12),** 2316-2323.

Kaczmarska, I., Ehrman, J. M., Bates, S. S., Green, D. H., Leger, C. and Harris, J. (2005) Diversity and distribution of epibiotic bacteria on *Pseudo-nitzchia muliseries* (Bacillariophyceae) in culture and comparison with those of diatoms in native sea water. *Harmful Algae* **4,** 725-741.

Kahru, M., Horstmann, U. and Rud, O. (1994) Increased cyanobacterial blooming in the Baltic Sea detected by satellites: Natural fluctuation or ecosystem change? *Ambio* **23**, 469-472.

Kang, Y. K., Cho, S. Y., Kang, Y. H., Katano, T., Jin, E. S., Kong, D. S. and Han, M. S. (2008) Isolation, identification and characterization of algicidal bacteria against *Stephanodiscus hantzschii* and *Peridinium bipes* for the control of freshwater winter algal blooms. *J. Appl. Phycol.* **20,** 375-386.

Karlson, B., Cusack, C. and Bresnan, E. (2010) *Microscopic and molecular methods for quantitative phytoplankton analysis*. IOC Manuals and Guides No. 55. p.120. Paris: UNESCO.

Karthikeyan, P., Jayasudha, S., Sampathkumar, P., Manimaran, K., Santhoshkumar, C., Ashokkumar, S. and Ashokprabu, V. (2010) Effect of industrial effluent on marine diatom *Chaetoceros simplex* (Ostenfeld, 1901). *J. Appl. Sci. Environ. Manage.* **14**(**4**), 35-37.

Karunasagar, I. (1993) *Gymnodinium* kills farm fish in India. *Harmful Algae News* **5,** 3.

Karunasagar, I., Gowda, H. S. V., Subhurah, M., Venugopal, M. N. and Karunasagar, I. (1984) Outbreak of paralytic shellfish poisoning in Mangalore, West coast of India. *Curr. Sci.* **53**, 247.

Karunasagar, I., Joseph, B., Philipose, K. K. and Karunasagar, I. (1998) Another outbreak of PSP in India. *Harmful Algae News* **17,** 1.

Karunasagar, I. and Karunasagar, I. (1990) Effects of eutrophication and harmful algal blooms on coastal aquaculture and fisheries in India. In: *Advances in Aquatic biology and Fisheries*. **14**, 162-172.

Kat, M. (1979) The occurrence of *Prorocentrum* species and coincidental gastrointestinal illness of mussel consumers. In: *Toxic Dinoflagellate Blooms* (eds.) Taylor, D. and Seliger, H. H. pp.215-220. Amsterdam: Elsevier.

Kat, M. (1985) *Dinophysis acuminata* blooms, the distinct cause of Dutch mussel poisoning. In: *Toxic Dinoflagellates* (eds.) Anderson, D. M., White, A. W. and Baden, D. G. pp.73-77. Amsterdam: Elsevier.

Katti, R. J., Chandrashekhara, T. R. and Shetty, H. P. C. (1988) On the occurrence of 'green tide' in the Arabian Sea off Mangalore. *Curr. Sci.* **57(7)**, 380-381.

Kawabata, Z., Magendran, A., Palanichamy, S., Venugopalan, V. K. and Tatsukawa, R. (1993) Phytoplankton biomass and productivity of different size fractions in the Vellar estuarine system, southeast coast of India. *Indian J. Mar. Sci.* **22,** 294-296.

Kenneth, R. H. (2002) Effects of pH on coastal marine phytoplankton. *Mar. Ecol. Prog. Ser.* **238,** 281-300.

Ketchum, B. H. (1967) Phytoplankton nutrients in estuaries. In: *Estuaries* (ed.) Lauff, G. H. pp.329-335. Washington, DC: American Association for the Advancement of Science, Publ. 83.

Khan, S., Ahmed, M. S., Arakawa, O. and Onoue, Y. (1995) Properties of neurotoxins separated from a harmful red tide organism *Chattonella marina*. *Israeli J. Aquacult.* **47,** 137-141.

Kiddon, J. A., Paul, J. F., Buffum, H. W., Strobel, C. S., Hale, S. S., Cobb, D. and Brown, B. S. (2003) Ecological condition of US Mid-Atlantic estuaries, 1997-1998. *Mar. Pollut. Bull.* **46**, 1224-1244.

Kim, D. and Oda, T. (2010) Possible factors responsible for the fish-killing mechanisms of the red tide phytoplankton, *Chattonella marina* and *Cochlodinium polykrikoides*. In: *Coastal Environmental and Ecosystem Issues of the East China Sea* (eds.) Ishimatsu, A. and Lie, H. J. pp.245-268. Nagasaki University.

Kim, J. D., Kim, B. and Lee, C. G. (2007) Alga-lytic of *Pseudomonas fluorescens* against the red tide causing marine alga *Heterosigma akshiwo* (Raphidophyceae). *Biol. Control* **41,** 296-303.

Kim, M. C., Yoshinaga, I., Imai, I., Nagasaki, K., Itakura, S. and Ishida, Y. (1998) A close relationship between algicidal bacteria and termination of *Heterosigma akashiwo* (Raphidophyceae) blooms in Hiroshima Bay, Japan. *Mar. Ecol. Prog. Ser.* **170,** 25-32.

Kirkpatrick, B., Fleming, L. E., Squicciarini, D., Backer, L. C., Clark, R., Abraham, W., Benson, J., Cheng, Y. S., Johnson, D., Pierce, R., Zaias, J., Bossart, J. D. and Baden, D. G. (2004) Literature review of Florida red tide: implications for human health effects. *Harmful Algae* **3**, 99-115.

Kitaguchi, H., Hiragushi, N., Mitsutani, A., Yamaguchi, M. and Ishida, Y. (2001) Isolation of an algicidal bacterium with activity against the harmful dinoflagellate *Heterocapsa circularisquama* (Dinophyceae). *Phycologia* **40**, 275-279.

Kjelleberg, S., Flardh, K. B. G., Nystrom, T. and Moriarty, D. J. W. (1993) Growth limitation and starvation of bacteria. In: *Aquatic microbiology: An Ecological Approach* (ed.) Ford, T. E. pp.289-320. Oxford: Blackwell Scientific Publications.

Klausmeler, C. A., Litchman, E., Daufresne, T. and Levin, S. A. (2004) Optimal nitrogen to phosphorus stoichiometry of phytoplankton. *Nature* **429**, 171-174.

Kodama, M., Doucette, G. J. and Green, D. H. (2006) Relationships between bacteria and harmful algae. In: *Ecology of Harmful Algae* (eds.) Graneli, E. and Turner, J. T. Vol.189. pp.243-255. Berlin-Heidelberg: Springer-Verlag.

Kodama, M., Ogata, T. and Sato, S. (1998) Bacterial production of saxitoxin. *Agr. Biol. Chem.* **52,** 1075-1077.

Kooistra, W. H. C. F., Gersonde, R., Medlin, L. K. and Mann, D. G. (2007) The origin and evolution of the diatoms: their adaptation to a planktonic existence. In: *Evolution of primary producers in the sea* (eds.) Falkowski, P. G. and Knoll, A. H. pp 207-249. Amsterdam: Elsevier, Academic Press.

Krammer, K. and Lange-Bertalot, H. (1985) Naviculaceae. Bibliotheca Diatomologica. Vol. 9. p.230. Berlin-Stuttgart: J Cramer.

Krammer, K. and Lange-Bertalot, H. (1986) Bacillariophyceae. 1. Teil: Naviculaceae. In: *Süsswasser flora von Mitteleuropa, B and 2/1* (eds.) Ettl, H., Gerloff, J., Heynig, H. and Mollenhauer, D. p.876. Stuttgart-New York: Gustav Fischer Verlag.

Kristiansen, S. and Hoell, E. E. (2002) The importance of silicon for marine production. *Hydrobiologia* **484**, 21-31.

Kudela, R. M. (2008) Silicon: Nitrogen interactions in the marine environment. In: *Nitrogen in the Marine Environment* (eds.) Capone, D., Bronk, D., Mulholland, M. and Carpenter, E. pp.1589-1626. New York: Elsevier.

Kumagai, M., Yamagi, T., Murata, M., Yasumoto, T., Kat, M., Lassus, P. and Rodoriguez-Vazquez, J. A. (1986) Okadaic acid as the causative toxin of diarrhetic shellfish poisoning in Europe. *Agr. Biol. Chem.* **50,** 2853-2867.

Kumaran, S. and Rao, T. S. S. (1975) Phytoplankton distribution and abundance in the Cochin Backwaters during 1971-72. *Bull. Dept. Mar. Sci. Cochin Univ.* **7**, 791-799.

Lancelot, C. and Rousseau, V. (1994). Ecology of *Phaeocystis*: the key role of colony forms. In: *The Haptophyte Algae* (eds.) Green, J. C. and Leadbeater, B. S. C. pp.229-245. Oxford: Clarendon Press.

Larsen, A. (1999) *Prymnesium parvum* and *P. patelliferum* (Haptophyta) - one species. *Phycologia* **38(6),** 541-543.

Larsen, A. and Bryant, S. (1998) Growth rate and toxicity of *Prymnesium parvum* and *Prymnesium patelliferum* (Haptophyta) in response to changes in salinity, light and temperature. *Sarsia* **83(5),** 409-418.

Larsen, A., Eikrem, W. and Paasche, E. (1993) Growth and toxicity in *Prymnesium patelliferum* (Prymnesiophycea) isolated from Norwegian waters. *Can. J. Bot.* **71,** 1357-1362.

Lassus, P., Bardouil, M., Truquet, I., Truquet, P., Baut, L. and Pierre, M. J. (1985) *Dinophysis acuminata* distribution and toxicity along the Southern Brittany coast (France): Correlation with hydrological parameters. In: *Toxic Dinoflagellates* (eds.) Anderson, D. M., White, A. W. and Baden, D. G. pp.159-164. Amsterdam: Elsevier.

Lee, R. E. (1980) *Phycology*. Cambridge: Cambridge University Press, UK, p.473.

Lee, S. O., Kato, J., Takiguchi, N., Kuroda, A., Ikeda, T., Mitsutani, A. and Ohtake, H. (2000) Involvement of an extracellular protease in algicidal activity of the marine bacterium *Pseudoalteromonas* sp. strain A28. *Appl. Environ. Microbiol.* **66,** 4334-4339.

Lee, W. J. and Park, Y. T. (1998) Isolation of marine bacteria killing red tide microalgae II. Isolation and algicidal properties of *Pseudomonas* sp. LG-2 possessing killing activity for dinoflagellate, *Prorocentrum micans. J. Korean Fish. Soc.* **31,** 852-858.

Lehman, P. W. (2000) The influence of climate on phytoplankton community biomass in San Francisco Bay estuary. *Limnol. Oceanogr.* **45,** 580-590.

Lewitus, A. J., Horner, R. A., Caron, D. A., Garcia-Mendoza, E., Hickey, B. M., Hunter, M. and Huppert, D. D. (2012) Harmful algal blooms along the North American west coast region : History, trends, causes, and impacts. *Harmful Algae* **19,** 133-159.

Lindehoff, E., Graneli, E. and Graneli, W. (2009) Effect of tertiary sewage effluent additions on *Prymnesium parvum* cell toxicity and stable isotope ratios. *Harmful Algae* **8,** 247-253.

Lindholm, T. and Virtanen, T. (1992) A bloom of *Prymnesium parvum* Carter in a small coastal inlet in Dragsfjärd, south-western Finland. *Environ. Toxic. Water Qual.* **7,** 165-170.

Lindholm, T., Ohman, P., Kurki-Helasmo, K., Kincaid, B. and Meriluoto, J. (1999) Toxic algae and fish mortality in a brackish-water lake in Aland, SW Finland. *Hydrobiologia* **397,** 109-120.

Lingadhal, C., Gupta, T. R. C. and Ramesh, A. M. (2003) Distribution of chlorophyll pigments in the Arabian Sea off Mangalore (west coast of India) in relations to nutrients. *J. Mar. Biol. Ass. India* **46**, 126-132.

Liu, D., Sun, J. and Liu, Z. (2004) The effects of spring-neap tide on the phytoplankton community development in the Jiaozhou Bay, China. *Acta Oceanol. Sin.* **23**, 687-697.

Liu, J., Lewitus, A., Jason, W. K. and Susan, B. W. (2008) The association of algicidal bacteria and raphidophyte blooms in South Carolina brackish detention ponds. *Harmful Algae* **7,** 184-193.

Liu, Y., Chai, T. and Hwang, D. (2000) Isolation of bacteria from toxic dinoflagellate *Alexandrium minitum* and their effects on algal toxicity. *J. Nat. Toxins* **9,** 409-417.

LoCicero, V. R. (ed.) (1974) Proceedings First International Conference Toxic Dinoflagellate Blooms. Massachusetts Science and Technology Foundation, p.541.

Lovejoy, C., Bowman, J. P. and Hallegraeff, G. M. (1998) Algicidal effects of a novel marine *Pseudoalteromonas* isolate (class Proteobacteria, gamma subdivision) on harmful algal bloom species of the genera *Chattonella, Gymnodinium,* and *Heterosigma*. *Appl. Environ. Microbiol.* **64(8),** 2806-2813.

Lugomela, C., Lyimo, T. J., Bryceson, I., Semesi, A. K. and Bergman, B. (2002) *Trichodesmium* in coastal waters of Tanzania: diversity, seasonality, nitrogen and carbon fixation. *Hydrobiologia* **477,** 1-13.

Lundholm, N. and Moestrup, Ø. (2006) The biogeography of harmful algae. In: *Ecology of Harmful Algae* (eds.) Graneli, E. and Turner, J. T. pp.23-35. Berlin, Germany: Springer.

Macfaddin, J. F. (1980) *Biochemical tests for identification of medical bacteria*. 2nd edition. The Baltimore: Williams and Wilkins Co.

Madhu, N. V., Balachandran, K. K., Martin, G. D., Jyothibabu, R., Thottathil, S. D., Maheswari, N., Joseph, T. and Kusum, K. K. (2010a) A short-term variability in water quality and its implications for phytoplankton production in a tropical estuary (Cochin backwaters - India). *Environ. Monit. Assess.* **170,** 287-300.

Madhu, N. V., Jyothibabu, R. and Balachandran, K. K. (2010b) Monsoon-induced changes in the size-fractionated phytoplankton biomass and production rate in the estuarine and coastal waters of southwest coast of India. *Environ. Monit. Assess.* **166(1-4),** 521-528.

Madhu, N. V., Jyothibabu, R., Balachandran, K. K., Honey, U. K., Martin, G. D., Vijay, J. G., Shiyas, C. A., Gupta, G. V. M. and Achuthankutty, C. T. (2007) Monsoonal impact on planktonic standing stock and abundance in a tropical estuary (Cochin Backwaters - India). *Estuar. Coast. Shelf Sci.* **73,** 54-64.

Madhu, N. V., Jyothibabu, R., Maheswaran, P. A., Gerson, V. J., Gopalakrishnan, T. C. and Nair, K. K. C (2006) Lack of seasonality in phytoplankton standing stock (chl.*a*) and production in the western Bay of Bengal. *Cont. Shelf Res.* **26,** 1868-1883.

Madhu, N. V., Reny, P. D., Paul, M., Ulhas, N. and Resmi, P. (2011) Occurrence of red tide caused by *Karenia mikimotoi* (toxic dinoflagellate) in the south-west coast of India. *Indian J. Geo. Mar. Sci.* **40(6),** 821-825.

Madhupratap, M., Nair, K. N. V., Gopalakrishnan, T. C., Haridas, P., Nair, K. K. C., Venugopal P. and Gauns, M. (2001) Arabian Sea oceanography and fisheries of the west coast of India. *Curr. Sci.* **81(4),** 355-361.

Madigan, T. L., Lee, K. G., Padula, D. J., McNabb, P. and Pointon, A. M. (2005) Diarrhetic shellfish poisoning (DSP) toxins in South Australian shellfish. *Harmful Algae* **5,** 119-123.

Magana, H. A., Contreras, C. and Villareal, T. A. (2003) A historical assessment of *Karenia brevis* in the western Gulf of Mexico. *Harmful Algae* **2,** 163-171.

Maki, T. and Imai, I. (2001) Relationship between intracellular bacteria and the bivalve killer dinoflagellate *Heterocapsa circularsquama* (Dinophyceae). *Fish. Sci.* **67,** 794-803.

Malone, T. C. (1980) Algal size. In: *The Physiological Ecology of Phytoplankton* (ed.) Morris, I. pp.433-463. Oxford: Blackwell Scientific Publications.

Mani, P., Krishnamurty, K. and Palaniappan, R. (1986) Ecology of phytoplankton blooms in Vellar estuary, East Coast of India. *Indian J. Mar. Sci.* **15,** 24-28.

Manning, S. R. and LaClaire, J. W. (2010) Prymnesins: toxic metabolites of the golden alga, *Prymnesium parvum* Carter (Haptophyta). *Marine Drugs* **8(3),** 678-704.

Marangoni, C., Pienaar, R. N. and Sym, S. D. (2001) Possible introduction of alien phytoplankton via shipping ballast water: a South African perspective. *S. Afr. J. Bot.* **67,** 465-474.

Marasigan, A. N., Sato, S., Fukuyo, Y. and Kodama, M. (2001) Accumulation of a high level of diarrhetic shellfish toxins in the green mussel *Perna viridis* during a bloom of *Dinophysis caudata* and *Dinophysis miles* in Sapian Bay, Panay Island, the Philippines. *Fish. Sci.* **67,** 994-996.

Maric, D., Kraus, R., Godrijan, J., Supic, N., Djakovac, T. and Precali, R. (2012) Phytoplankton response to climatic and anthropogenic influences in the north-eastern Adriatic during the last four decades. *Estuar. Coast. Shelf Sci.* **115,** 98-112.

Marshall, J. A. and Hallegraeff, G. M. (1999) Comparative eco-physiology of the harmful alga *Chattonella marina* (Raphidophyceae) from South Australian and Japanese waters. *J. Plankton Res.* **21,** 1809-1822.

Mathew, K. J., Thomas, P. A., George, R. M., Girijavallabhan, K. G., Siraimeetan, P., Naomi, T. S., Nair, K. R., Anthony, G., Bhat, G. S. and Selvaraj, M. (1988) Plankton blooms along the Indian coasts, some highlights. *Mar. Fish. Inf. Serv. Tech. Ext. Ser.* **84,** 11-13.

Mayali, X. and Azam, F. (2004) Algicidal Bacteria in the Sea and their Impact on Algal Blooms. *J. Eukuryot. Microbiol.* **51(2),** 139-144.

Mayali, X. and Doucette, G. J. (2002) Microbial community interactions and population dynamics of an algicidal bacterium active against *Karenia brevis* (Dinophyceae). *Harmful Algae* **1,** 277-293.

Mayali, X., Peter, J. S. F. and Ronald, S. B. (2011) Temporal attachment dynamics by distinct bacterial taxa during a dinoflagellate bloom. *Aquat. Microb. Ecol.* **63,** 111-122.

McGillicuddy, D., Anderson, L. A., Bates, N. R., Bibby, T., Buesseler, K. O., Carlson, C., Davis, C. S., Ewart, C., Falkowski, P. G., Goldthwait, S. A., Hansel, D. A., Jenkins, W. J., Johnson, R., Kosnyrev, V. K., Ledwell, J. R., Li, Q. P., Siegel, D. A. and Steinberg, D. K. (2007) Eddy-wind interactions stimulate extraordinary mid-ocean plankton blooms. *Science* **316,** 1021.

McLaughlin, J. J. A. (1958) Euryhaline chrysomonads: nutrition and toxigenesis in *Prymnesium parvum*, with notes on *Isochrysis galbana* and *Monochrysis lutheri*. *J. Protozool.* **5(1),** 75-81.

McManus, M. A. and Woodson, C. B. (2012) Plankton distributions and Ocean Dispersal. *J. Exp. Biol.* **215,** 1008-1016.

Menon, N. N., Balchand, A. N. and Menon, N. R. (2000) Hydrobiology of the Cochin backwater system. *Hydrobiologia* **430,** 149-183.

Meusnier, J. L., Olsen, W. T., Stam, C., Destombe, C. and Valero, M. (2001) Phylogenetic analysis of *Caulerpa taxifolia* (Chlorophyta) and of its associated bacterial micro flora provides clues to the origin of the Mediterranean introduction. *Mol. Ecol.* **10,** 931-946.

Michaloudi, E., Moustaka-Gouni, M., Gkelis, S. and Pantelidakis, K. (2009) Plankton community structure during an ecosystem disruptive algal bloom of *Prymnesium parvum*. *J. Plankton Res.* **31,** 301-309.

Mishra, S. and Panigrahy, R. C. (1995) Occurrence of diatom blooms in Bahuda estuary, East Coast of India. *Indian J. Mar. Sci.* **24,** 99-101.

Mitbavkar, S. and Anil, A. C. (2002) Diatoms of the microphytobenthic community: population structure in tropical intertidal sand flat. *Mar. Biol.* **140,** 41-57.

Mitsutani, A., Takesue, K., Kirita, M. and Ishida, Y. (1992) Lysis of *Skeletonema costamm* by *Cytophaga* sp. isolated from the coastal water of the Ariake Sea. *Nippon Suisan Gakkaishi* **58,** 2159-2169.

Mochizuki, M., Shiga, N., Saito, M. and Imai, K. (2002) Seasonal changes in nutrients, chlorophyll *a* and phytoplankton assemblage of the western subarctic gyre in Pacific Ocean. *Deep Sea Res. II* **49,** 5421-5439.

Moestrup, Ø. (1994) Economic aspects, blooms, nuisance species, and toxins. In: *The Haptophyte Algae* (eds.) Green, J. C. and Leadbeater, B. S. C. Vol. 51. pp.265-285. Oxford: Systematics Association. Clarendon Press.

Moestrup, Ø. and Thomsen, H. A. (2003) Taxonomy of toxic haptophytes (prymnesiophytes). In: *Manual on harmful marine microalgae* (eds.) Hallegraeff, G. M., Anderson, D. M. and Cembella, A. D. pp.433-463. Paris: UNESCO Publishing.

Mohanty, A. K., Satpathy, K. K., Sahu, G., Sasmal, S. K., Sahu, B. K. and Panigrahy, R. C. (2007) Red tide of *Noctiluca scintillans* and its impact on the coastal water quality of the near-shore waters, off the Rushikulya River, Bay of Bengal. *Curr. Sci.* **93,** 616-618.

Mu, R., Ma, G., Sun, C., and Jia, J. (2012) Inhibition of *Microcystis aeruginosa* and microcystin-LR with one algicidal bacterium isolated from a eutrophic lake. *J. Biotechnol.* **11(2),** 460-465.

Na, G. H., Choi, W. J. and Chun, Y. Y. (1996) A study on red tide control with loess suspension. *Kor. J. Aquacult.* **9,** 239-245.

Nagabhushanam, A. K. (1967) On an unusually dense phytoplankton 'bloom' around Minicoy Island (Arabian Sea) and its effect on the local tuna fisheries. *Curr. Sci.* **36,** 611-612.

Naik, R. K., Hegde, S. and Anil, A. C. (2011) Dinoflagellate community structure from the stratified environment of the Bay of Bengal, with special emphasis on harmful algal bloom species. *Environ. Monit. Assess.* **182,** 15-30.

Nair, P. V. R. and Gopinathan, C. P. (1981) Productivity of the Exclusive Economic Zone of India. *J. Mar. Biol. Ass. India* **23,** 48-54.

Nair, P. V. R., Joseph, K. J., Balachandran, V. K. and Pillai, V. K. (1975) A Study of the primary production in the Vembanad Lake. *Bull. Dept. Mar. Sci. Cochin Univ.* **7,** 161-170.

Nair, P. V. R., Samuel, S., Joseph, K. J. and Balachandran, V. K. (1968) Primary production and potential fishery resources in the seas around India. *Proceed. Symp. Living Res. Seas around India, CMFRI Special Publication*, 184-198.

Nair, R. R., Ittekkot, V., Manganini, S. J., Ramaswamy, V., Haake, B., Degens, E. T., Desai, B. N. and Honjo, S. (1989) Increased particle flux to the deep ocean related to monsoons. *Nature* **338,** 749-751.

Naqvi, S. W. A., George, M. D., Narvekar, P. V., Jayakumar, D. A., Shailaja, M. S., Sardesai, S., Sarma, V. V. S. S., Shenoy, D. M., Naik, H., Maheswaran, P. A., Krishnakumari, K., Rajesh, G., Sudhir, A. K. and Binu, M. S. (1998) Severe fish mortality associated with 'red tide' observed in the sea off Cochin. *Curr. Sci.* **75,** 543-544.

Nayak, B. and Karunasagar, I. (1997) Influence of bacteria on the growth and haemolysin production by the marine dinoflagellate *Amphidinium carterae*. *Mar. Biol.* **130,** 35-39.

Nayak, B. B. and Karunasagar, I. (2000) Bacteriological and physico-chemical factors associated with *Noctiluca milicans* bloom along Mangalore, Southwest coast of Indian. *Indian J. Mar. Sci.* **29,** 139-143.

Nedumaran, T., Ashok, P. V. and Perumal, P. (2001) Ecology of phytoplankton of Vellar estuary, southeast coast of India. *Seaweed Res. Util.* 23 **(1 and 2),** 157-162

Nguyen-Ngoc, L. (2004) An autecological study of the potentially toxic dinoflagellate *Alexandrium affine* isolated from Vietnamese waters. *Harmful Algae* **3,** 117-129.

Nishitani, G., Nagai, S., Sakiyama, S. and Kamiyama, T. (2008) Successful cultivation of the toxic dinoflagellate *Dinophysis caudata* (Dinophyceae). *Plankton Benthos Res.* **3(2),** 78-85.

Nordli, E. (1953) Salinity and temperature as controlling factors for distribution and mass occurrence of *Ceratia. Blyttia* **2,** 16-18.

Nygaard, K. and Tobiesen, A. (1993) Bacterivory in algae—a survival strategy during nutrient limitation. *Limnol. Oceanogr.* **39,** 273-279.

O'Herald. (2001) NIO discovers toxic algae off Goa. O'Herald Newspaper Goa, 29th October 2001.

Oda, T., Ishimatsu, A., Takeshita, S. and Muramatsu, T. (1994) Hydrogen peroxide production by the red tide flagellate *Chattonella marina*. *Biosci. Biotech. Bioch.* **58,** 957-958.

Okaichi, T. (1967) Red tides found in and around the Seto Inland Sea in 1965. *Tech. Bull. Fac. Agri. Kagawa Univ.* **18,** 181-185.

Okolodkov, Y. B. (2005) The global distributional patterns of toxic, bloom dinoflagellates recorded from the Eurasian Arctic. *Harmful Algae* **4,** 351-369.

Onoue, Y. and Nozawa, K. (1989) Separation of toxin from harmful red tides occurring along the coast of Kagoshima Prefecture. In: *Red tides - Biology, Environmental Science and Toxicology* (eds.) Okaichi, T., Anderson, D. M. and Nemeto, T. pp.371-374. New York: Elsevier.

Oviatt, C., Doering, P., Nowicki, B., Recd, L., Cole, J. and Frithsen, S. (1995) An ecosystem level experiment on nutrient limitation in temperate coastal marine environments. *Mar. Ecol. Prog. Ser.* **116,** 171-179.

Oviatt, C. A., Keller, A. A., Samoou, P. A. and Beatty, L. L. (1986) Patterns of productivity during eutrophication: A mesocosm experiment. *Mar. Ecol. Prog. Ser.* **28,** 69-80.

Padmakumar, K. B., Sanilkumar, M. G. and Saramma, A. V. (2007) A 'red tide' caused by the diatom *Coscinodiscus* on the South-West coast of India. *Harmful Algae News* **35,** 14.

Padmakumar, K. B., Sanilkumar, M. G., Saramma, A. V., Sanjeevan, V. N. and Menon, N. R. (2008) ''Green tide'' of *Noctiluca miliaris* in the Northern Arabian Sea. *Harmful Algae News* **36,** 12.

Padmakumar, K. B., Sreerenjima, G., Fanimol, C. L., Menon, N. R. and Sanjeevan, V. N. (2010) Preponderance of heterotrophic *Noctiluca scintillans* during a multi-species diatom bloom along the South-West coast of India. *Int. J. Oceans Oceanogr.* **4,** 5-63.

Padmakumar, K. B., Thomas, L. C., Salini, T. C., John, E., Menon, N. R. and Sanjeevan, V. N. (2011) Monospecific bloom of noxious raphidophyte *Chattonella marina* in the coastal waters of South-West coast of India. *Int. J. Biosci.* **1(1)**, 57-69.

Paerl, H. W. (1988) Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. *Limnol. Oceanogr.* **33,** 823-847.

Panigrahy, R. C. and Gouda, R. (1990) Occurrence of bloom of the diatom *Asterionella glacialis* (Castracane) in the Rushikulya estuary, east coast of India. *Mahasagar* **23,** 179-182.

Parab, S. G. and Matondkar, S. G. P. (2012) Primary Productivity and Nitrogen fixation by *Trichodesmium* spp. in the Arabian Sea. *J. Mar. Syst.* **105(108),** 82-95.

Park, Y. T., Park, J. B., Chung, S. Y., Song, B. C., Lee, W. J., Lim, W. A. and Kim, C. (1998) Isolation of marine bacteria killing red tide microalgae: 1. Isolation and algicidal properties of *Micrococcus* spp. LG-1 possessing killing activity for harmful dinoflagellate *Cochlodinium polykrikoides*. *J. Korea Fish. Soc.* **31,** 767-773.

Patil, J. S. (2003) Diatoms in benthic and pelagic environment. In *Studies on ecology of diatoms*. *PhD Thesis*, University of Goa, pp.47-50.

Patil, J. S. and Anil, A. C. (2008) Temporal variation of diatom benthic propagules in a monsoon influenced tropical estuary. *Cont. Shelf Res.* **28,** 2404-2416.

Pennock, J. R. (1985) Chlorophyll distribution in the Delaware estuary: regulation by light limitation. *Estuar. Coast. Shelf Sci.* **21,** 711-725.

Pennock, J. R. and Sharp, J. H. (1994) Temporal alternation between light and nutrient limitation of phytoplankton production in a coastal plain estuary. *Mar. Ecol. Prog. Ser.* **111,** 275-288.

Perl, T. M., Dedard, L., Kotsatsky, T., Hockin, J. C., Todd, E. C. D. and Remis, M. D. (1990) An outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid. *New Engl. J. Med.* **322**, 1775-1780.

Peterson, B. J., Hobbie, J. E. and Corliss, T. L. (1986) Carbon flow in a tundra stream ecosystem. *Can. J. Fish. Aquat. Sci.* **43,** 1259-1270.

Piehler, M. F., Twomey, L. J., Hall, N. S. and Paerl, H. W. (2004) Impacts of inorganic nutrient enrichment on phytoplankton community structure and function in Pamlico Sound, NC, USA. *Estuar. Coast. Shelf Sci.* **61(2),** 197-209.

Pillai, V. K., Joseph, K. J. and Nair, A. K. K. (1975) The plankton production in the Vembanad lake and adjacent waters in relation to the environmental parameters. *Bull. Dept. Mar. Sci. Cochin Univ.* **1,** 137-150.

Platt, T. (1972) Local phytoplankton abundance and turbulence. *Deep-Sea Res.* **19,** 183-187.

Platt, T., Subba, R. D. V. and Irwin, B. (1983) Photosynthesis of picoplankton in the oligotrophic ocean. *Nature* **300,** 702-704.

Pokrzywinski, K. L., Place, A. R., Warner, M. E. and Coyne, K. J. (2012) Investigation of the algicidal exudate produced by *Shewanella* sp. IRI-160 and its effect on dinoflagellates. *Harmful Algae* **19,** 23-29.

Prabhu, M. S., Ramamurthy, S., Kuthalingam, M. D. K. and Dhulkhed, M. H. (1965) On an unusual swarming of the planktonic blue-green algae *Trichodesmium* spp., off Mangalore. *Curr. Sci.* **34,** 95.

Prakash, A. and Sarma, V. (1964) On the occurrence of 'red water' phenomenon on the west coast of India. *Curr. Sci.* **33,** 168-170.

Prescott, G. W. (1968) The algae: A review. Houghton Mifflin Co., Boston. p.436.

Prosser, K. N., Valenti Jr., T. W., Hayden, N. J., Neisch, M. T., Hewitt, N. C., Umphres, G. D., Gable, G. M., Grover, J. P., Roelke, D. L. and Brooks, B. W. (2012) Low pH pre-empts bloom development of a toxic haptophyte, *Harmful Algae* **20,** 156-164.

Qasim, S. Z. (1970) Some characteristics of a *Trichodesmium* bloom in the Laccadives. *Deep-Sea Res.* **17**, 655-660.

Qasim, S. Z. and Reddy, C. V. G. (1967) The estimation of plant pigments of Cochin backwater during the monsoon months. *Bull. Mar. Sci.* **17(1),** 95-110.

Qasim, S. Z., Bhattathiri, P. M. A. and Abidi, S. A. H. (1968) Solar radiation and its penetration in a tropical estuary. *J. Exp. Mar. Biol. Ecol.* **2,** 87-103.

Qasim, S. Z., Wallershaus, S., Bhattathiri, P. M. A. and Abidi, S. A. H. (1969) Organic production in a tropical estuary. *P. Indian Acad. Sci. B* **59,** 51-94.

Raghu Prasad, R. (1953) Swarming of *Noctiluca* in the Palk Bay and its effect on the 'Choodai' fishery with a note on the possible use of *Noctiluca* as an indicator species. *P. Indian Acad. Sci. B* **38,** 40-47.

Raghu Prasad, R. (1956) Further studies on the plankton of the inshore waters off Mandapam. *Indian J. Fish.* **1**, 4231.

Raghu Prasad, R. (1958) A note on the occurrence and feeding habits of *Noctiluca* and their effects on the plankton community and fisheries. *P. Indian Acad. Sci. B* **331**, 33-76.

Raghukumar, S. and Anil, A. C. (2003) Marine biodiversity and ecosystem functioning: a perspective. *Curr. Sci.* **84,** 884-892.

Ramaiah, N., Paul, J. T., Fernandes, V., Raveendran, T., Raveendran, O., Sundar, D., Revichandran, C., Shenoy, D. M., Mangesh, G., Kurian, S., Gerson, V. J., Shoji, D. T., Madhu, N. V., Sreekumar, S., Lokabharathi, P. A. and Shetye, S. R. (2005) The September 2004 stench off the southern Malabar coast-A consequence of holococcolithophore bloom. *Curr. Sci.* **88,** 551-554.

Ramamurthy, V. D. (1968) Studies on phytoplankton in Porto Novo waters with special reference to *Trichodesmium erythraeum*. *PhD Thesis*, Annamalai University.

Ramamurthy, V. D. (1970a) Antibacterial activity traceable to marine blue green alga *Trichodesmium erythraeum* in the gastrointestinal contents of two pelagic fishes. *Hydrobiologia* **159,** 163-361

Ramamurthy, V. D. (1970b) Studies on red water phenomenon in Porto Novo waters (11° 29' N-79° 49' E, South India) caused by *Trichodesmium erythraeum* (Marine blue green algae). *P. Joint Oceanogr. Assembly* (Abstract) (ed.) UdaTokyo, M. p.562.

Ramamurthy, V. D. (1973) Infra-red spectral analysis of antibacterial substance isolated from *Trichodesmium erythraeum* (Marine blue green algae). *Hydrobiologia* **247,** 250-412.

Ramamurthy, V. D., Selvakumar, R. A. and Bhargava, R. M. S. (1972) Studies on the blooms of *Trichodesmium erythraeum* (Ehr.) in the waters of the central west coast of India. *Curr. Sci.* **41,** 803-804.

Redekar, P. D. and Wagh, A. B. (2000) Growth of fouling diatoms from the Zuari estuary, Goa (West coast of India) under different salinities in the laboratory. *Seaweed Res. Util.* **22 (1 and 2),** 121-124.

Redfield, A. C., Ketchum, B. H. and Richards, F. A. (1963) The influence of organisms on the composition of sea-water. In: *The Sea* (ed.) Hill, M. N. Vol.2. pp.26-77. New York: Interscience Publishers.

Rees, A., Woodward, M. and Joint, I. (1999) Measurement of nitrate and ammonium uptake in oligotrophic waters of the northeast Atlantic. *Mar. Ecol. Prog. Ser.* **187,** 295-300.

Reichenbach-Klinke, H. (1973) *Fish Pathology*. p.512. New Jersey: T.F.H. Publications.

Reynolds, C. S. (1973) The seasonal periodicity of planktonic diatoms in a shallow, eutrophic lake. *Freshwater Biol.* **3,** 89-110.

Reynolds, C. S. (1984) *The ecology of freshwater phytoplankton*. p.384. Cambridge: Cambridge University Press.

Reynolds, C. S. (2006) *The ecology of phytoplankton*. p.552. Cambridge: Cambridge University Press.

Rhoads, D. C. and Young, D. K. (1970) The influence of deposit feeding organisms on sediment stability and community trophic structure. *J. Mar. Res.* **28,** 150-178.

Richardson, K. (1997) Harmful or exceptional phytoplankton blooms in the marine ecosystems. *Adv. Mar. Biol.* **36,** 301-385.

Riegman, R., Colijn, F., Malschaert, J. F. P., Kloosterhuis, H. T. and Cadee, G. C. (1990) Assessment of growth rate limiting nutrients in the North Sea by the use of nutrient-uptake kinetics. *Neth. J. Sea. Res.* **26,** 53-60.

Riquelme, C. E. (1988) Interaction between microalgae and bacteria in coastal sea water. *PhD Thesis*, Kyoto University.

Robin, R. S., Vishnu Vardhan, K., Muduli, P. R., Rajkumar, J. S. I. and Swaminathan, P. (2010) Vertical distribution of biological characteristics and phytoplankton community structure in the shelf waters off southwest coast of India. *Int. J. Curr. Res.* **8,** 16-34.

Roelke, D. L. and Buyukates, Y. (2001) The diversity of harmful algal bloom-triggering mechanisms and the complexity of bloom initiation. *Hum. Ecol. Risk Assess.* **7,** 1347-1362.

Roelke, D. L., Errera, R., Kiesling, R., Brooks, B. W., Grover, J. P., Schwierzke, L., Urena-Boeck, F., Baker, J. and Pinckney, J. L. (2007) Effects of nutrient enrichment on *Prymnesium parvum* population dynamics and toxicity: results from field experiments, Lake Possum Kingdom, US. *Aquat. Microb. Ecol.* **46,** 125-140.

Roelke, D. L., Grover, J. P., Brooks, B. W., Glass, J., Buzan, D., Southard, G. M., Fries, L., Gable, G. M., Schwierzke-Wade, L., Byrd, M. and Nelson, J. (2011) A decade of fish killing *Prymnesium parvum* blooms in Texas: roles of inflow and salinity. *J. Plankton Res.* **33,** 243-253.

Roelke, D. L., Schwierzke, L., Brooks, B. W., Grover, J. P., Errera, R. M., Valenti Jr., T. W. and Pinckney, J. L. (2010) Factors influencing *Prymnesium parvum* population dynamics during bloom initiation: results from in-lake mesocosm experiments. *J. Am. Water Resour. As.* **46,** 76-91.

Rontani, J. F., Gilewicz, M. J., Michotey, V. D., Zheng, T. L., Bonin, P. C. and Bertrand, J. C. (1997) Aerobic and anaerobic metabolism of 6,10,14-trimethylpentadecan-2-one by the denitrifying bacterium isolated from marine sediments. *Appl. Environ. Microbiol.* **63,** 636-643.

Rooney-Verga, J. N., Giewat, M. W., Savin, M. C., Sood, S., LeGresley, M. and Martin, J. L. (2005) Links between phytoplankton and bacterial communities dynamics in a coastal marine environment. *Microb. Ecol.* **49(1),** 163-175.

Round, F. E., Crawford, R. M. and Mann, D. G. (1990) *The Diatoms: Biology and Morphology of the Genera.* p.747. Cambridge: Cambridge University Press.

Roy, R., Pratihary, A., Gauns, M. and Naqvi, S. W. A. (2006) Spatial variation of phytoplankton pigments along the southwest coast of India. *Estuar. Coast. Shelf Sci.* **69,** 189-195.

Ryther, J. H. and Dunstan, W. M. (1971) Nitrogen, phosphorus, and eutrophication in the coastal marine environment. *Science* **171,** 1008-1013.

Ryther, J. H., Hall, J. R., Pease, A. K., Bakeem, A. and Jones, M. M. (1966) Primary organic production in relation to the chemistry and hydrography of the western Indian Ocean. *Limnol. Oceanogr.* **11,** 371-380.

Ryu, H. Y., Shim, J. M., Bang, J. D. and Lee, C. (1998) Experimental chemical treatments for the control of dinoflagellate, *Cochlodinium polykrikoides* in the land-based culture of olive flounder *Paralichthys olivaceus*. *Kor. J. Aquacult.* **11,** 285-294.

Sabour, B., Loudiki, L. M., Oudra, B., Oubraim, S., Fawzi, B., Fadlaoui, S., Chlaida, M. and Vasconcelos, V. (2000) Blooms of *Prymnesium parvum* associated with fish mortalities in a hypereutrophic brackish lake in Morocco. *Harmful Algae News* **21,** 8-9.

Sahayak, S., Jyothibabu, R., Jayalakshmi, K. J., Habeebrehman, H., Sabu, P., Prabhakaran, M. P., Jasmine, P., Shaiju, P., Rejomon, G., Thresiamma, J. and Nair, K. K. C. (2005) Red tide of *Noctiluca miliaris* off south of Thiruvananthapuram subsequent to the 'stench event' at the southern Kerala coast. *Curr. Sci.* **89,** 1472-1473.

Sanilkumar, M. G. (2009) Microalgae in the southwest coast of India. *PhD Thesis*, Cochin University of Science and Technology, p.285.

Sanilkumar, M. G., Anit, M. T., Philip, R., Hatha, A. A. M., Sanjeevan, V. N. and Saramma, A. V. (2009) First report of *Protoperidinium* bloom from Indian waters. *Harmful Algae News* **39,** 15.

Sanilkumar, M. G., Anit, M. T., Vijayalakshmi, K. C., Hatha, A. A. M., and Saramma, A. V. (2012) *Chattonella marina* bloom in the coastal Sea off Mahe, southwest India. *Curr. Sci.* **103(6)**, 624-626.

Sanilkumar, M. G., Padmakumar, K. B., Menon, N. R., Joseph, K. J., Sanjeevan, V. N. and Saramma, A. V. (2009) Algal blooms along the coastal waters of southwest India during 2005-08. *J. Mar. Biol. Ass. India* **51(1),** 69-74.

Sankar, S. R. and Padmavati, G. (2012) Species Composition, abundance and distribution of phytoplankton in the harbour areas and coastal waters of Port Blair, South Andaman. *Int. J. Oceanogr. Mar. Ecol. Syst.* **1,** 76-83.

Sankaranarayanan, V. N. and Qasim, S. Z. (1969) Nutrients in the Cochin Backwaters in relation to environmental characteristics. *Mar. Biol.* **2,** 236-247.

Santha, J. P. (1975) Seasonal distribution of phytoplankton in the Vellar estuary, east coast of India. *Indian J. Mar. Sci.* **4,** 98-200.

Santhosh, K. C., Prabu, V. A., Sampathkumar, P. and Anantharaman, P. (2010) Occurrence of algal bloom *Microcystis aeruginosa* in the Vellar estuary, South-East coast of India. *Int. J. Curr. Res.* **5**, 52-55.

Sapp, M., Schwaderer, A. S., Wiltshire, K. H., Hoppe, H. G., Gerdts, G. and Wichles, A. (2006) Species specific bacterial communities in the phycosphere of microalgae. *Microb. Ecol.* **53(4),** 683-699.

Sarangi, R. K., Chauhan, P. and Nayak, S. R. (2004) Detection and monitoring of *Trichodesmium* blooms in the coastal waters off Saurashtra coast, India using IRS-P4 OCM data. *Curr. Sci.* **86(12),** 1636-1641.

Saravanakumar, A., Rajkumar, M., Serebiah, S. J. and Thivakaran, G. A. (2008) Seasonal variations in physico- chemical characteristics of water, sediment and soil texture in arid zone mangroves of Kachchh - Gujarat. *J. Environ. Biol.* **29(5),** 725-732.

Sargunam, C. A. and Rao, V. N. R. (1989) Occurrence of *Noctiluca* bloom in Kalpakkam coastal waters, east coast of India. *Indian J. Mar. Sci*. **18,** 289-290.

Sarno, D., Zingone, A., Saggiomo, V. and Carrada, G. C. (1993) Phytoplankton biomass and species composition in a Mediterranean coastal lagoon. *Hydrobiologia* **271,** 27- 40.

Sarojini, Y. and Nittala, S. S. (2001) Vertical distribution of phytoplankton around Andaman and Nicobar Islands, Bay of Bengal. *Indian J. Mar. Sci.* **30,** 65-69.

Sarupria, J. S. and Bhargava, R. M. S. (1998) Seasonal distribution of chlorophyll *a* in the exclusive economic zone (EEZ) of India. *Indian J. Mar. Sci.* **27,** 292-297.

Sasamal, S. K., Panigrahy, R. C. and Misra, S. (2005) *Asterionella* blooms in the north-western Bay of Bengal during 2004. *Int. J. Remote Sens.* **26,** 3853-3858.

Satpathy, K. K. and Nair, K. K. V. (1996) Occurrence of phytoplankton bloom and its effect on coastal water quality. *Indian. J. Mar. Sci.* **25,** 145-147

Satpathy, K. K., Mohanty, A. K., Sahu, G., Prasad, M. V. R., Venkatesan, R., Natesan, U. and Rajan, M. (2007) On the occurrence of *Trichodesmium erythraeum* (Ehr.) bloom in the coastal waters of Kalpakkam, east coast of India. *Indian J. Sci. Tech.* **1(2),** 1-9.

Schollhorn, E. and Graneli, E. (1996) Influence of different nitrogen to silica ratios and artificial mixing on the structure of a summer phytoplankton community from the Swedish west coast (Gullmar fjord). *J. Sea Res.* **35,** 159-167.

Segar, K., Karunasagar, I. and Karunasagar, I. (1989) Dinoflagellate toxins in shellfishes along the coast of Karnataka. In: *The first Indian fisheries forum proceedings* (ed.) Joseph, M. M. pp.389-390. Mangalore: Asian Fisheries Society, Indian Branch.

Seibold, A., Wichels, A. and Schutt, C. (2001) Diversity of endocytic bacteria in the dinoflagellate *Noctiluca scintillans. Aquat. Microb. Ecol.* **25,** 229-235.

Shalapyonok, A., Olson, R. J. and Shalapyonok, L. S. (2001) Arabian Sea phytoplankton during Southwest and North- east Monsoons 1995: composition, size structure and biomass from individual cell properties measured by flow cytometry. *Deep-Sea Res. II* **48,** 1231-1262.

Shaw, P. J. and Purdie, D. A. (2001) Phytoplankton photosynthesis-irradiance parameters in the near-shore UK coastal waters of the North Sea: temporal variation and environmental control. *Mar. Ecol. Prog. Ser.* **216,** 83-94.

Shen, Z., Liu, Q., Wu, Y. and Yao, Y. (2006) Nutrient structure of seawater and ecological responses in Jiaozhou Bay, China. *Estuar. Coast. Shelf Sci.* **69,** 299-307.

Shevchenko, O. G., Selina, M. S., Orlova, T. Y., Morozova, T. V., Stonik, I. V., Zvalinsky, V. I. and Tishchenko, P. Y. (2013) Phytoplankton of the Amur River Estuary (Sea of Okhotsk) during the Summer Periods of 2005-2007. *Russ. J. Mar. Biol.* **39(2),** 92-106.

Shilo, M. (1981) The toxic principles of *Prymnesium parvum*. In: *The Water Environment. Algal Toxins and Health* (ed.) Carmichael, W. W. pp.37-47. New York: Plenum Press.

Shilo, M. and Aschner, M. (1953) Factors governing the toxicity of cultures containing phytoflagellate *Prymnesium parvum* Carter. *J. Gen. Microbiol.* **8**, 333-343.

Shilo, M. and Shilo, M. (1953) Conditions which determine the efficiency of ammonium sulphate in the control of *Prymnesium parvum* in fish breeding ponds. *Appl. Microbiol.* **1**, 330-333.

Shivaprasad, A., Vinita, J., Revichandran, C. P. D., Reny, M. P., Deepak, K., Muraleedharan, R. and Naveen Kumar, K. R. (2013) Seasonal stratification and property distributions in a tropical estuary (Cochin estuary, west coast, India). *Hydrol. Earth Syst. Sci.* **17,** 187- 199.

Shumway, S. E., Allen, S. M. and Dee Boersma, P. (2003) Marine birds and harmful algal blooms: sporadic victims or under-reported events? *Harmful Algae* **2,** 1-17.

Sieburth, J. M. (1978) Bacterioplankton: Nature, biomass, activity and relationships to the protist plankton. *Arch. Mikrobiol.* **100,** 419-436.

Sierra, G. A. (1957) A simple method for the detection of lipolytic activity of microorganisms and observation of the influence of the contact between cells and fatty substrates. *Antonie Van Leeuwenhoek* **23,** 15-22.

Silas, E. G., Alagarswami, K., Narasimham, K. A., Appukuttan, K. K. and Muthiah, P. (1982) Country Report—India. In: *Bivalve culture in Asia and the Pacific. Proceedings of Workshop held in Singapore, 16-19 Feb 1982* (eds.) Davy, F. B. and Graham, M. pp.34-431. Ottawa: International Development Centre.

Silva, M. (1962) Some observations on the dinoflagellates cultures. III *Gonyaulax spinifera* (Clap and Lach.) Dies., *Gonyaulax tamarensis* Leb., and *Peridinium trochoideum* (Stein) Lemm. *Notas. Estud. Inst. Biol. Mar.* **26,** 1-21.

Simidu, U., Austrio, K. and Kaneka, E. (1971) Bacterial flora of phyto and zooplanktonin inshore water of Japan. *Can. J. Microbio.* **17,** 1157-1160.

Simon, N., Biegala, I. C., Smith, E. A. and Vaulot, D. (2002) Kinetics of attachment of potentially toxic bacteria to *Alexandrium tamarense*. *Aquat. Microb. Ecol.* **28,** 249-256.

Simonsen, R. (1974) *The diatom plankton of the Indian Ocean Expedition of RV"Meteor" 1964-1965*. p.66. Stuttgart: Gebruder Borntraeger.

Skerratt, J. H., Bowman, J. P., Hallegraeff, G. M., James, S. and Nichols, P. D. (2002) Algicidal bacteria associated with blooms of a toxic dinoflagellate in a temperate Australian estuary. *Mar. Ecol. Prog. Ser.* **244,** 1-15.

Skovgaard, A. and Hansen, P. J. (2003) Food uptake in the harmful alga *Prymnesium parvum* mediated by excreted toxins. *Limnol. Oceanogr.* **48,** 1161-1166.

Smayda, T. J. (1980) Phytoplankton species succession. In: *The Physiological Ecology of Phytoplankton* (ed.) Morris, I. pp.493-570. Oxford: Blackwell Scientific Publications.

Smayda, T. J. (1997) What is a bloom? A commentary. *Limnol. Oceanogr.* **42,** 1132-1136.

Smayda, T. J. (1998) Ecophysiology and bloom dynamics of *Heterosigma akashiwo* (Raphidophyceae). In: *Physiological ecology of harmful algal blooms NATO ASI Series Vol. G 41* (eds.) Anderson, C. M., Cembella, A. D. and Hallegraeff, G. M. pp 113-131. Berlin: Springer.

Smayda, T. J. (2005) Eutrophication and phytoplankton. In: *Integrated approaches to drainage basin nutrient inputs and coastal eutrophication. An Integrated approach* (eds.) Wassmann, P. and Olli, K. pp.89-98. Norway: University of Tromso.

Smibert, R. M. and Krieg, N. R. (1994) Phenotypic characterisation. In: *Methods for General and Molecular Bacteriology* (eds.) Gerhardt, P., Murray, R. G. E., Wood, W. A. and Krieg, N. R. pp.607-654. Washington DC: American Society for Microbiology.

Smith, D. C., Steward, G. F., Long, R. A. and Azam, F. (1995) Bacterial mediation of carbon fluxes during a diatom bloom in a mesocosm. *Deep-Sea Res. II* **42,** 75-97.

Smith, S. L., Banse, K., Cochran, J. K., Codispoti, L. A., Ducklow, H. W., Luther, M. E., Olson, D. B., Peterson, W. T., Prell, W. L., Surgi, N., Swallow, J. C. and Wishner, K. (1991) U.S. JGOFS: Arabian Sea Process Study, U.S. JGOFS Planning Report Number 13. p.164. Woods Hole: Woods Hole Oceanographic Institution.

Smith, V. H. (1990) Nitrogen, phosphorus and nitrogen fixation in lacustrine and estuarine ecosystems. *Limnol. Oceanogr.* **35,** 1852-1859.

Smith, V. H. (2006) Responses of estuarine and coastal marine phytoplankton to nitrogen and phosphorus enrichment. *Limnol. Oceanogr.* **51,** 377-384.

Smith, V. H., Tilman, G. D. and Nekola, J. C. (1999) Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environ. Pollut.* **100,** 179-196.

Steemann, N. E. (1959) Primary production in tropical marine areas. *J. Mar. Biol. Ass. India* **1(1),** 7-12.

Steidinger, K. A. (1983) A re-evaluation of toxic dinoflagellate biology and ecology. In: *Progress in Phycological Research* (eds.) Round, F. E. and Chapman, D. J. pp.147-188. New York: Elsevier Science Publishing Company.

Strickland, J. D. H. and Parsons, T. R. (1972) *A Practical Handbook of Seawater Analysis*. 2nd edition Bulletin 167. p.310. Ottawa: Fisheries Research Board of Canada.

Su, J., Yang, X., Zhou, Y. and Zheng, T. (2011) Marine bacteria antagonistic to the harmful algal bloom species *Alexandrium tamarense* (Dinophyceae). *Biol. Control* **56,** 132-138.

Su, J. Q., Yang, X. R., Zheng, T. L., Yun, T., Nian, Z. J., Cai, L. Z. and Hua, S. H. (2007) Isolation and characterization of a marine algicidal bacterium against the toxic dinoflagellate *Alexandrium tamarense. Harmful Algae* **6,** 799-810.

Subba Rao, D. V. (1969) *Asterionella japonica* bloom and discolouration off Waltair, Bay of Bengal. *Limnol. Oceanogr.* **14,** 632-634.

Subrahmanyan, R. (1946) A systematic account of the marine plankton diatoms of the Madras coast. *P. Indian Acad. Sci. B* **24**, 85-197.

Subrahmanyan, R. (1954) On the life history and ecology of *Hornellia marina* Gen. Ersp. Nov, (Chloromonodinaeae) causing green discolouration of the sea and mortality among marine organisms off the Malabar Coast. *P. Indian. Acad. Sci. B* **39,** 182-203.

Subrahmanyan, R. (1958a) Ecological studies on the marine phytoplankton on the west coast of India. *J. Indian Bot. Soc.* **1,** 145-151.

Subrahmanyan, R. (1958b) Plankton organisms of the Arabian Sea of the west coast of India. *J. Indian Bot. Soc.* **37(4),** 435-441.

Subrahmanyan, R. (1959) Studies on the phytoplankton of the west coast of India-part II. Physical and chemical factors influencing the production of phytoplankton with remarks on the cycle of nutrients and on the relationship of the phosphate content to fish landings. *P. Indian Acad. Sci. B* **50,** 113-252

Subrahmanyan, R. (1971) The Dinophyceae of Indian Seas. Part 2. Family Peridiniaceae Shutt emend Lindemann, Mem. II. *J. Mar. Biol. Ass. India*. p.334.

Subrahmanyan, R. and Sharma, V. A. H. (1960) Studies on the plankton of the west coast of India: Part III. *Indian J. Fish.* **7(2),** 307-336.

Subrahmanyan, R. and Sharma, V. A. H. (1965) Studies on the phytoplankton of the west coast of India: Part IV. *Magnitude of the standing crop for 1955-1962, with observations on nanoplankton and its significance to fisheries. J. Mar. Biol. Ass. India* **7(2),** 406-419.

Suikkanen, S., Fistarol, G. O. and Graneli, E. (2004) Allelopathic effects of the Baltic cyanobacteria *Nodularia spumigena*, *Aphanizomenon flosaquae* and *Anabaena lemmermannii* on algal monocultures. *J. Exp. Mar. Biol. Ecol.* **308,** 85-101.

Sun J., Liu, D. and Qian, S. (2001) Preliminary study on the seasonal succession and development pathway of phytoplankton community in the Bohai Sea. *Acta Oceanol. Sin.* **20,** 251-260.

Sun, X. X., Choi, J. K. and Kim, E. K. (2004) A preliminary study on the mechanism of harmful algal bloom mitigation by use of sophorolipid treatment. *J. Exp. Mar. Biol. Ecol.* **304,** 35-49.

Takahashi, K., Jordan, R. and Priddle, J. (1994) The diatom genus *Proboscia* in Subarctic waters. *Diatom Res.* **9(2),** 411-428.

Tamplin, M. L. (1990) A bacterial source of tetrodotoxins and saxitoxins. In: *Marine Toxins: Origins, Structure and Molecular Pharmacology* (eds.) Hall, S. and Strichartz, G. R. pp.78-85. Washington DC: American Chemical Society.

Tarran, G. A., Burkill, P. H., Edwards, E. S., Malcolm, E. and Woodward, S. (1999) Phytoplankton community structure in the Arabian Sea during and after the SW monsoon, 1994. *Deep-Sea Res.II* **4,** 655-676.

Taylor, F. J. R. (2004) Harmful dinoflagellate species in space and time and the value of morphospecies. In: *Harmful Algae* 2002. (eds.) Steidinger, K. A., Landsberg, J. H., Tomas, C. R. and Vargo, G. A. pp.555-559. St. Petersburg: IOC-UNESCO.

Taylor, F. J. R., Hoppenrath, M. and Saldarriaga, J. F. (2008) Dinoflagellate diversity and distribution. *Biodiv. Cons.* **17,** 407-418.

Therriault, J. C., Lawrence, D. L. and Platt, T. (1978) Spatial variability of phytoplankton turn over in relation to physical processes in a coastal environment. *Limnol. Oceanogr.* **2,** 900-911.

Tiffany, M. A., Barlow, S. B., Matey, V. E. and Hurlbert, S. H. (2001) *Chattonella marina* (Raphidophyceae), a potentially toxic alga in the Salton Sea, California. *Hydrobiologia* **66,** 187-194.

Tillmann, U. (2003) Kill and eat your predator: a winning strategy of the planktonic flagellate *Prymnesium parvum. Aquat. Microb. Ecol.* **32,** 73-84.

Tillmann, U., John, U. and Cembella, A. D. (2007) On the allelochemical potency of the marine dinoflagellate *Alexandrium ostenfeldii* against heterotrophic and autotrophic protists. *J. Plankton Res.* **29,** 527-543.

Tilman, D., Kilham, S. S. and Kilham, P. (1982) Phytoplankton community ecology: The role of limiting nutrients. *Annu. Rev. Ecol. Syst.* **13,** 349-372.

Tilstone, G. H., Figueiras, F. G. and Fraga, F. (1994) Upwelling-downwelling sequences in the generation of red tides in a coastal upwelling system. *Mar. Ecol. Prog. Ser.* **112,** 241-253.

Tiwari, L. R. and Nair, V. R. (1998) Ecology of phytoplankton from Dharamtar creek, west coast of India. *Indian J. Mar. Sci.* **27,** 302-309.

Tomas, C. R. (ed.) (1997) *Identifying Marine diatoms and dinoflagellates*. p.858. New York: Academic press.

Trivedi, M. K. and Goel, P. G. (1984) *Chemical and biological methods for pollution studies*. p.215. Karad: Environmental Publications.

Turner, R. E., Rabalais, N. N., Justic, D. and Dortch, Q. (2003) Future aquatic nutrient limitations. *Mar. Pollut. Bull.* **46(8),** 1032-1034.

Underwood, G. J. C. and Kromkamp, J. (1999) Primary production by phytoplankton and microphytobenthos in estuaries. *Adv. Ecol. Res.* **29,** 93-153.

Uronen, P., Lehtinen, S., Legrand, C., Kuuppo, P. and Tamminen, T. (2005) Haemolytic activity and allelopathy of the haptophyte *Prymnesium parvum* in nutrient limited and balanced growth conditions. *Mar. Ecol. Prog. Ser.* **299,** 137-148.

Usup, G., Pin, L. C., Ahmad, A. and Teen, L. P. (2002) *Alexandrium* (Dinophyceae) species in Malaysian waters. *Harmful Algae* **1,** 265-275

Van den Hoek, C., Mann, D. G. and Jans, H. M. (1995) *Algae. An Introduction to phycology*. p.623. Cambridge: Cambridge University Press.

Vaulot, D. (2001). *Phytoplankton*. Centre Nationale de la Recherche Scientific et Universite Pierre et Marie Curie, Roscoff, France.

Vengadesh, P. N., Rajkumar, M., Perumal, P. and Thillai, R. K. (2009) Seasonal variations of plankton diversity in Kaduviyar estuary, Nagapattinam, Southeast coast of India. *J. Environ. Biol.* **30(6),** 1035-1046.

Venkataraman, G. (1939) A systematic account of some south Indian diatoms. *P. Indian Acad. Sci. B* **10,** 293-368.

Venugopal, R., Haridas, P., Madhupratap, M. and Rao, T. S. S. (1979) Incidence of red water along South Kerala coast. *Indian J. Mar. Sci.* **94,** 9782.

Verlancar, X. N. (1978) Some observations on the *Trichodesmium* bloom along the south-west coast of India. *Mahasagar* **11,** 221-224.

Vijayalakshmi, G. S. and Venugopalan, V. K. (1975) Studies on diversity of phytoplankton species, pigments and succession with a note on primary production at a tidal zone in the Vellar estuary. *Rec. Res. Estuar. Biol.* 237-246.

Vilaa, M., Giacobbeb, M. G., Masoa, M., Gangemib, E., Pennac, A., Sampedroa, N., Azzarob, F., Campa, J. and Gallu, L. (2005) A comparative study on recurrent blooms of *Alexandrium minutum* in two Mediterranean coastal areas. *Harmful Algae* **4,** 673-695.

Vinayachandran, P. N. and Mathew, S. (2003) Phytoplankton bloom in the Bay of Bengal during the northeast monsoon and its intensification by cyclones. *Geophys. Res. Lett.* **30,** 1572.

Walsh, J. J. (1988) *On the Nature of Continental Shelves*. p.520. New York: Academic Press.

Wang, B., Xiao, R. Y., Jinglin, L., Yanyan, Z., Jianqiang, S., Yun, T., Jun, Z., Wang, G. and Tianling, Z. (2012) A marine bacterium producing protein with algicidal activity against *Alexandrium tamarense*. *Harmful Algae* **13,** 83-88.

Wang, B. X., Zhou, Y. Y., Bai, S. J., Su, J. Q., Tian, Y., Zheng, T. L. and Yang, X. R. (2010) A novel marine bacterium algicidal to the toxic dinoflagellate *Alexandrium tamarense*. *Lett. Appl. Microbiol.* **51(5),** 552-557.

Wang, X. L., Gong, L. Y., Liang, S. K., Han, X. R., Zhu, C. J. and Li, Y. B. (2005) Algicidal activity of rhamnolipid biosurfactants produced by *Pseudomonas aeruginosa. Harmful Algae* **4,** 433-443.

Wang, Z., Meiling, Y., Yu, L. and Songhui, L. (2011) Effects of temperature and organic and inorganic nutrients on the growth of *Chattonella marina* (Raphidophyceae) from the Daya Bay, South China Sea. *Acta Oceanol. Sin.* **30**, 124-131.

Wang, Z., Qi, Y., Chen, J., Xu, N. and Yang, Y. (2006a) Phytoplankton abundance, community structure and nutrients in cultural areas of Daya Bay, South China Sea. *J. Mar. Syst.* **62,** 85-94.

Wang, Z., Yuzao, Q. and Jufang, C. (2006b) Population dynamics of *Chattonella* in spring in Daya Bay, the South China Sea and the cause of its blooms. *Acta Hydrobio. Sin.* (in Chinese), **30(4)**, 394-398.

Weiler, C. S. (1980) Population structure and in situ division rates of *Ceratium* in oligitrophic water of the North Pacific central gyre. *Limnol. Oceanogr.* **25,** 610-619.

Wilkerson, F. and Dugdale, R. (2008) Coastal upwelling. In: *Nitrogen in the Marine Environment* (eds.) Capone, D., Bronk, D., Mulholland, M. and Carpenter, E. 2nd edition. pp.771-807. New York: Elsevier.

Winkler, L. W. (1888) Die Bestimmung des im Wasser gelosten Sauerstoffes. *Chem. Ber.* **21**, 2843-2855.

Wright, J. L. C. (1995) Dealing with seafood toxins: present approaches and future options. *Food Res. Int.* **28,** 341-358.

Yamaguchi, H., Sakamoto, S. and Yamaguchi, M. (2008) Nutrition and growth kinetics in nitrogen- and phosphorus-limited cultures of the novel red tide flagellate *Chattonella ovata* (Raphidophyceae). *Harmful Algae* **7,** 26-32.

Yang, C., Yanyan, Z. Y. L., Zheng, W., Yun, T. and Zheng, T. (2012) Bacterial community dynamics during a bloom caused by *Akashiwo sanguinea* in the Xiamen sea area, China. *Harmful Algae* **20,** 132-141.

Yariv, J. and Hestrin, S. (1961) Toxicity of the extracellular phase of *Prymnesium parvum* cultures. *J. Gen. Microbiol.* **24,** 165.

Yasumoto, T., Oshima, Y. and Sugaware, W. (1978) Identification of *Dinophysis fortii* as the causative organisms of diarrhetic shelfish poisoning. *Bull. Jap. Soc. Sci. Fish.* **46,** 1405-1411.

Yin, K. (2002) Monsoonal influence on seasonal variations in nutrients and phytoplankton biomass in the coastal waters of Hong Kong in the vicinity of Pearl River estuary. *Mar. Ecol. Prog. Ser.* **245,** 111-122.

Yoshinaga, I., Kawai, T. and Ishida, Y. (1997) Analysis of algicidal ranges of the bacteria killing the marine dinoflagellate *Gymnodinium mikimotoi* isolated from Tanabe Bay (Wakayama Prefecture, Japan) *Fish. Sci.* **63,** 94-98.

Yoshinaga, I., Kim, M. C., Katanozaka, N., Imai, I., Uchida, A. and Ishida, Y. (1998) Population structure of algicidal marine bacteria targeting the red tide-forming alga *Heterosigma akushiwo* (Raphidophyceae), determined by restriction fragment length polymorphism analysis of the bacterial 16s ribosomal RNA genes. *Mar. Ecol. Prog. Ser.* **170,** 3344.

Zehr, J. P., Waterbury, J. B., Turner, P. J., Montoya, J. P., Omoregie, E., Steward, G. F., Hansen, A. and Karl, D. M. (2001) Unicellular cyanobacteria fix N_2 in the subtropical North Pacific Ocean. *Nature* **412,** 635-638.

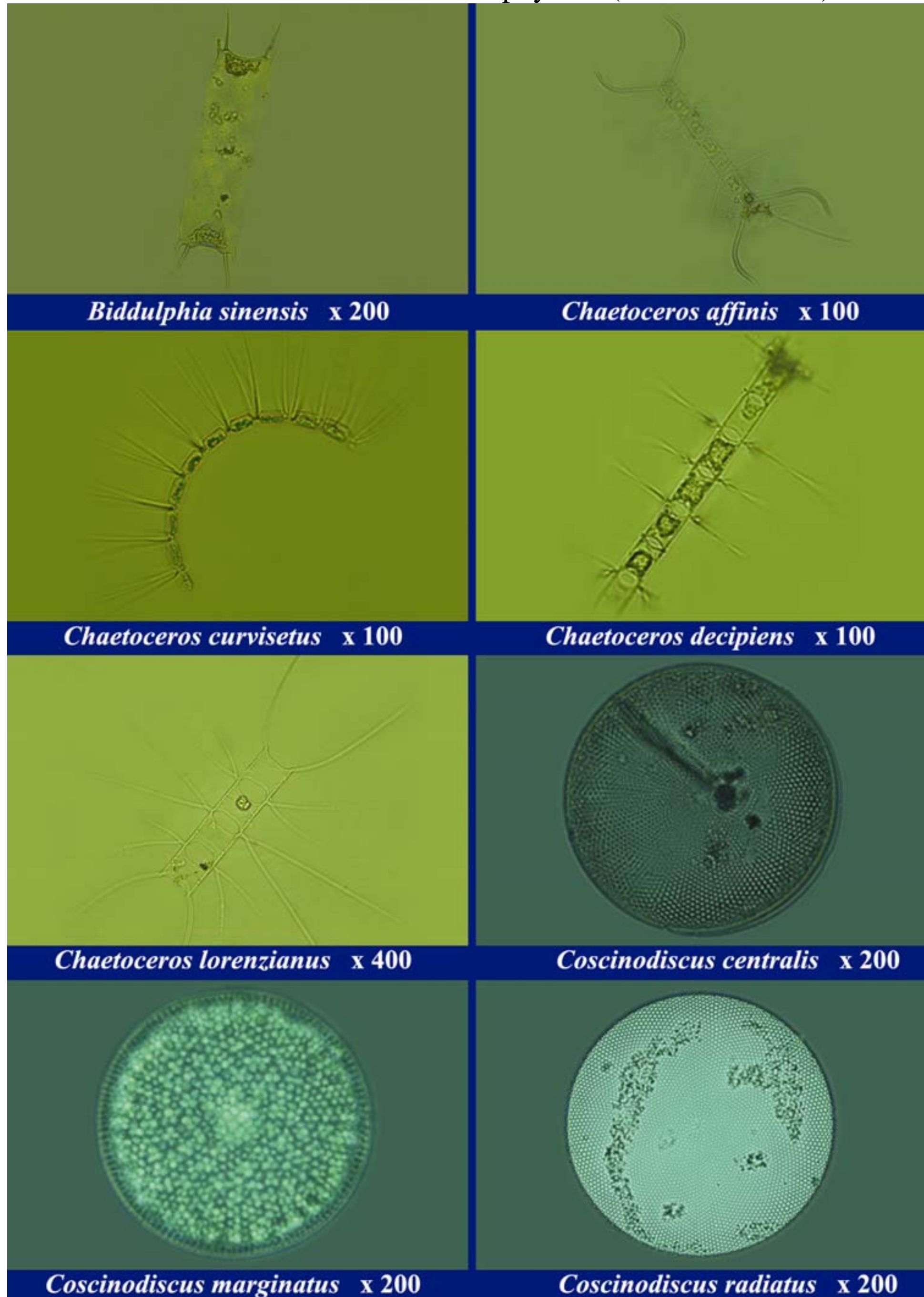
Zingone, A. and Enevoldsen, H. O. (2000) The diversity of harmful algal blooms: a challenge for science and management. *Ocean Coast. Manage.* **43,** 725-748.

.....ഇൽ.....

APPENDICES

Appendix – I

Plate 1 Class: Bacillariophyceae (Centric Diatoms)



Conti….

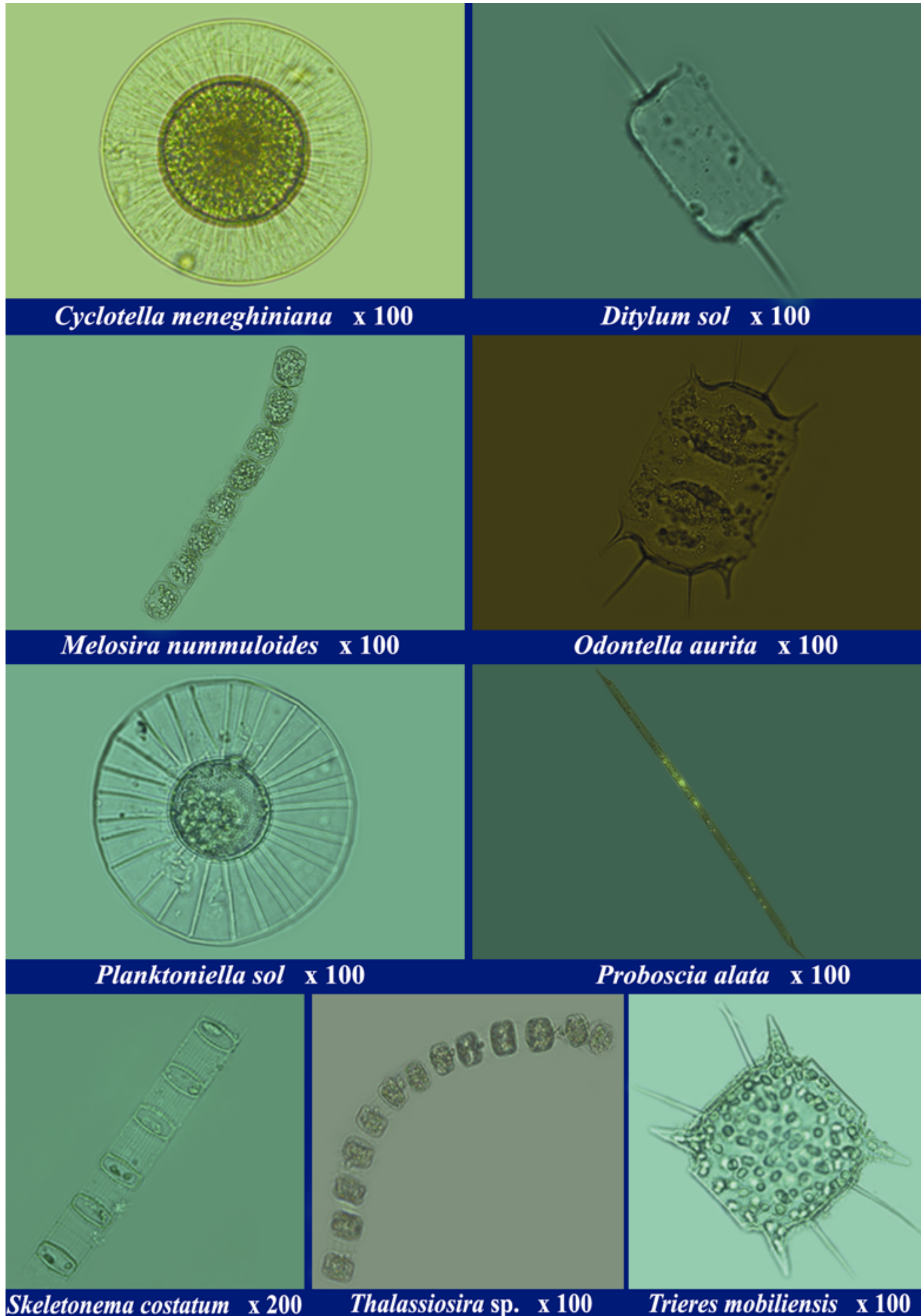
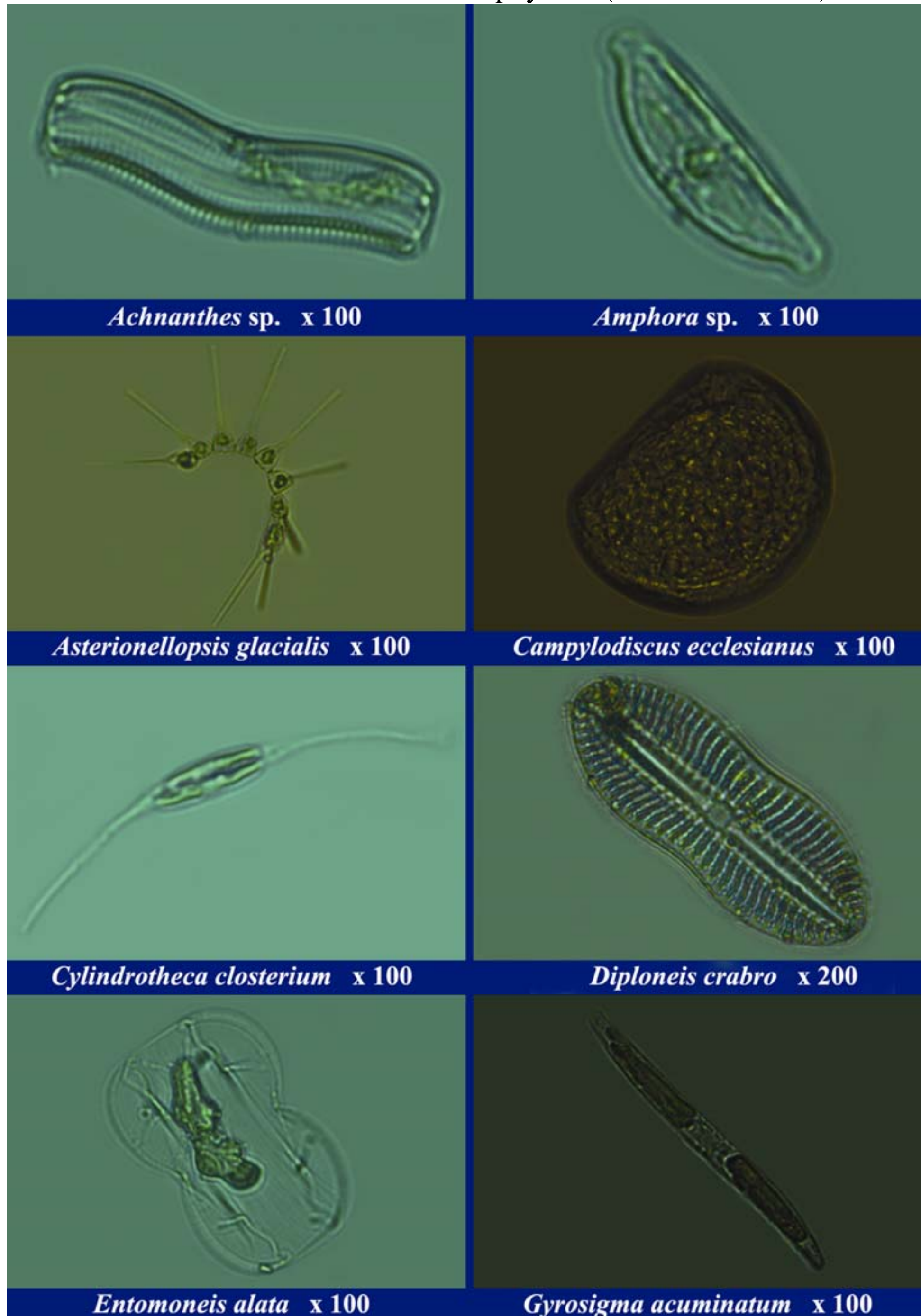

Cyclotella meneghiniana x 100
Ditylum sol x 100
Melosira nummuloides x 100
Odontella aurita x 100
Planktoniella sol x 100
Proboscia alata x 100
Skeletonema costatum x 200
Thalassiosira sp. x 100
Trieres mobiliensis x 100

Plate 2 Class: Bacillariophyceae (Pennate Diatoms)



Achnanthes sp. x 100

Amphora sp. x 100

Asterionellopsis glacialis x 100

Campylodiscus ecclesianus x 100

Cylindrotheca closterium x 100

Diploneis crabro x 200

Entomoneis alata x 100

Gyrosigma acuminatum x 100

Conti….

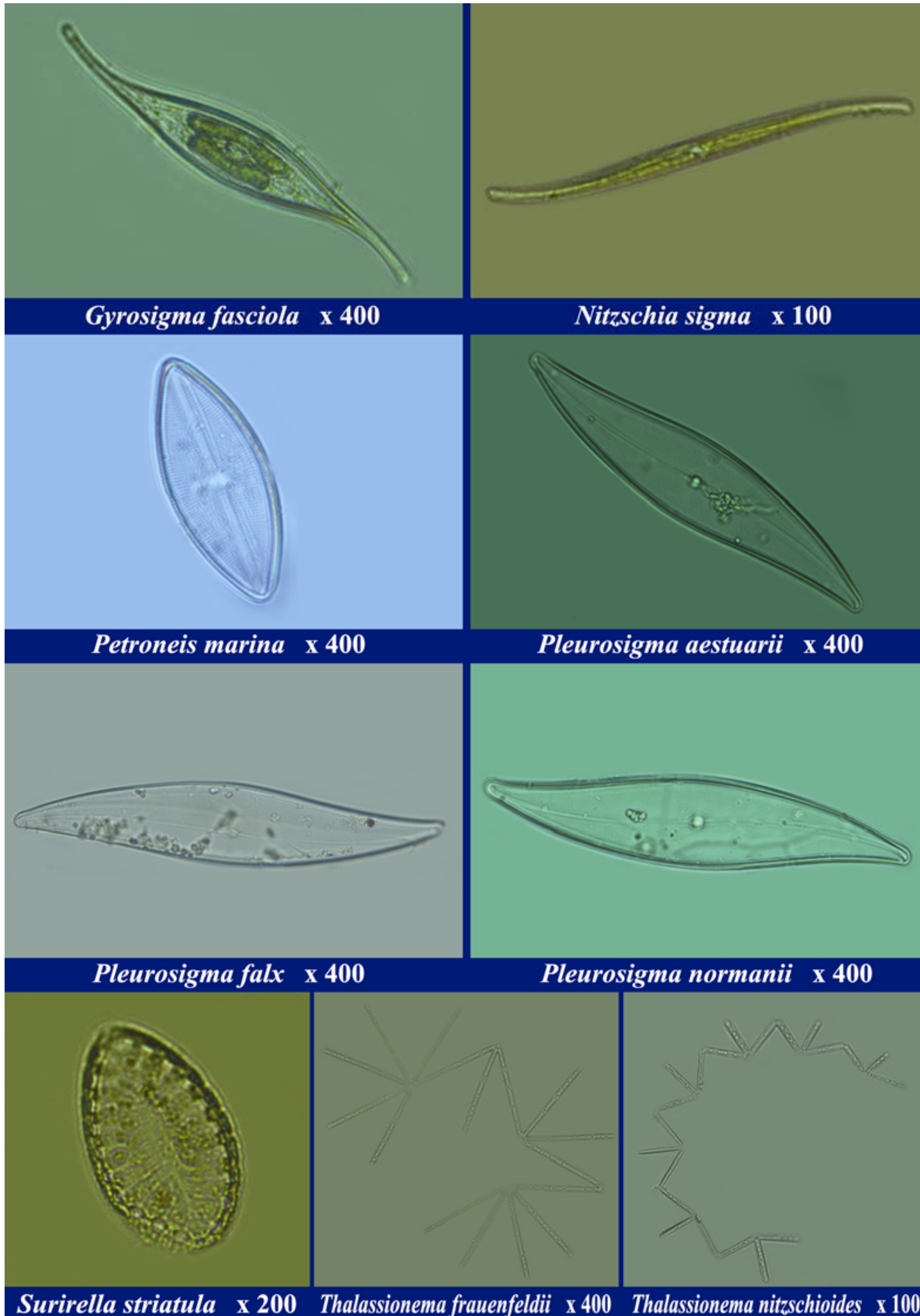

Gyrosigma fasciola x 400
Nitzschia sigma x 100
Petroneis marina x 400
Pleurosigma aestuarii x 400
Pleurosigma falx x 400
Pleurosigma normanii x 400
Surirella striatula x 200
Thalassionema frauenfeldii x 400
Thalassionema nitzschioides x 100

Plate 3 Class: Dinophyceae

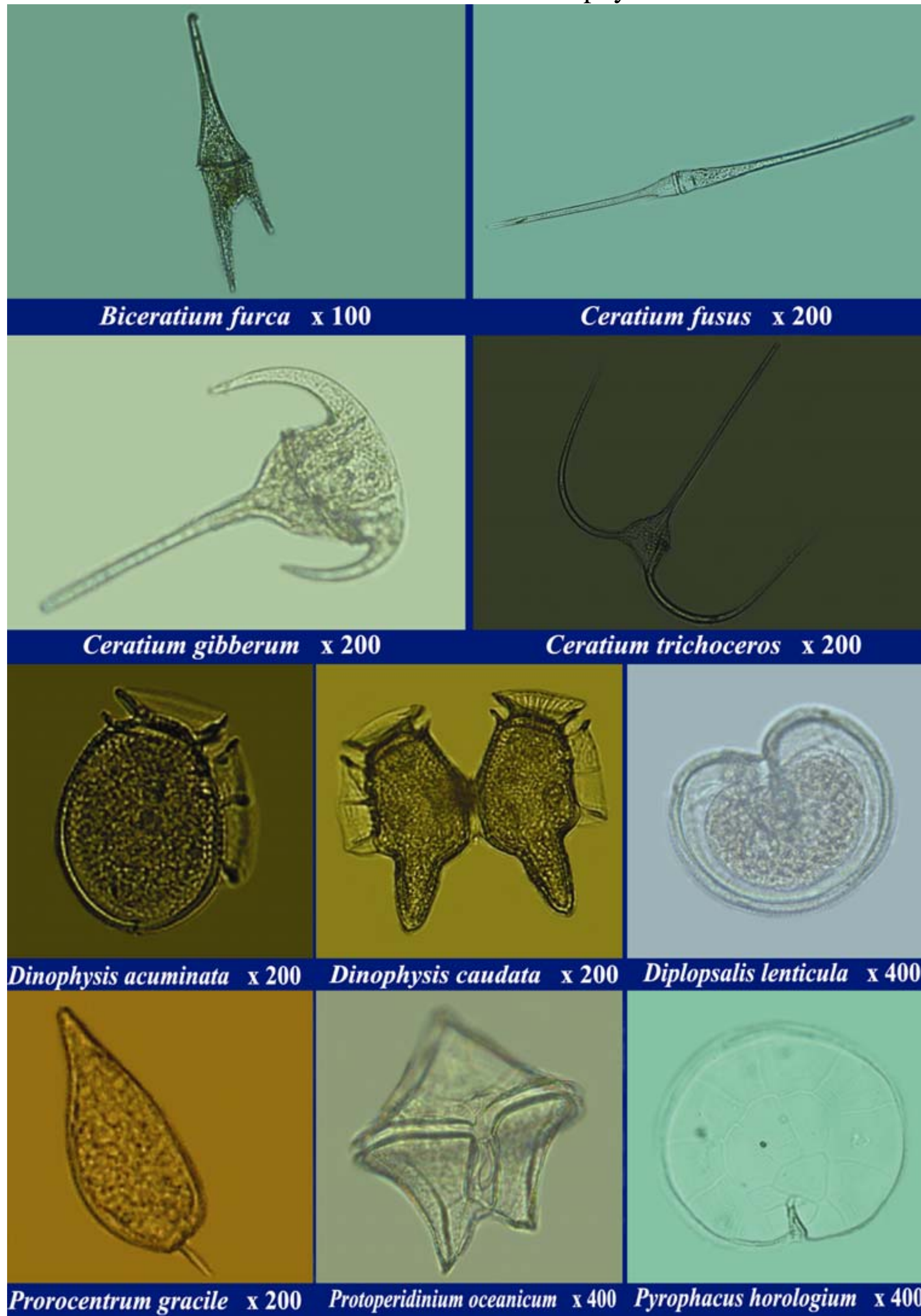
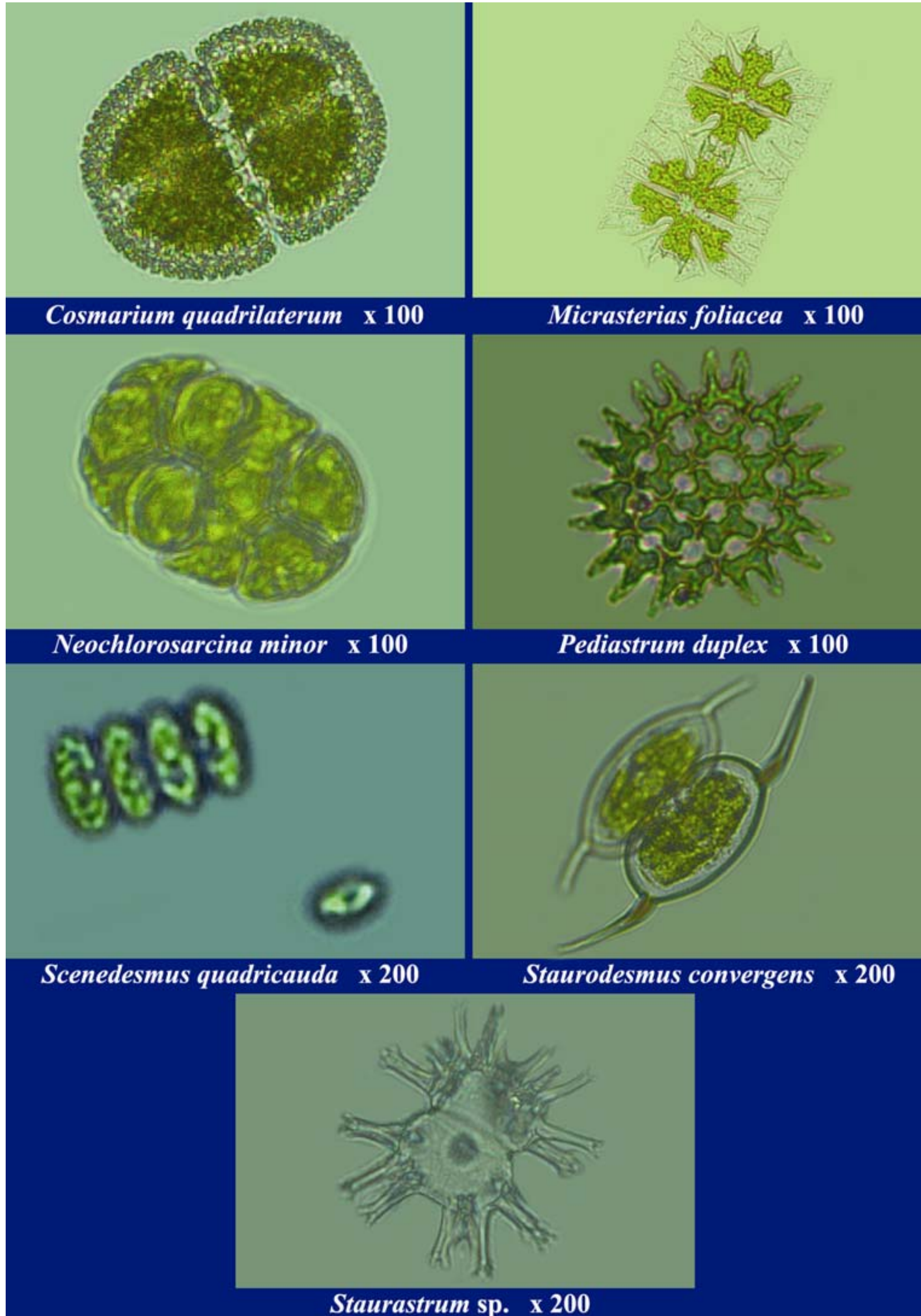


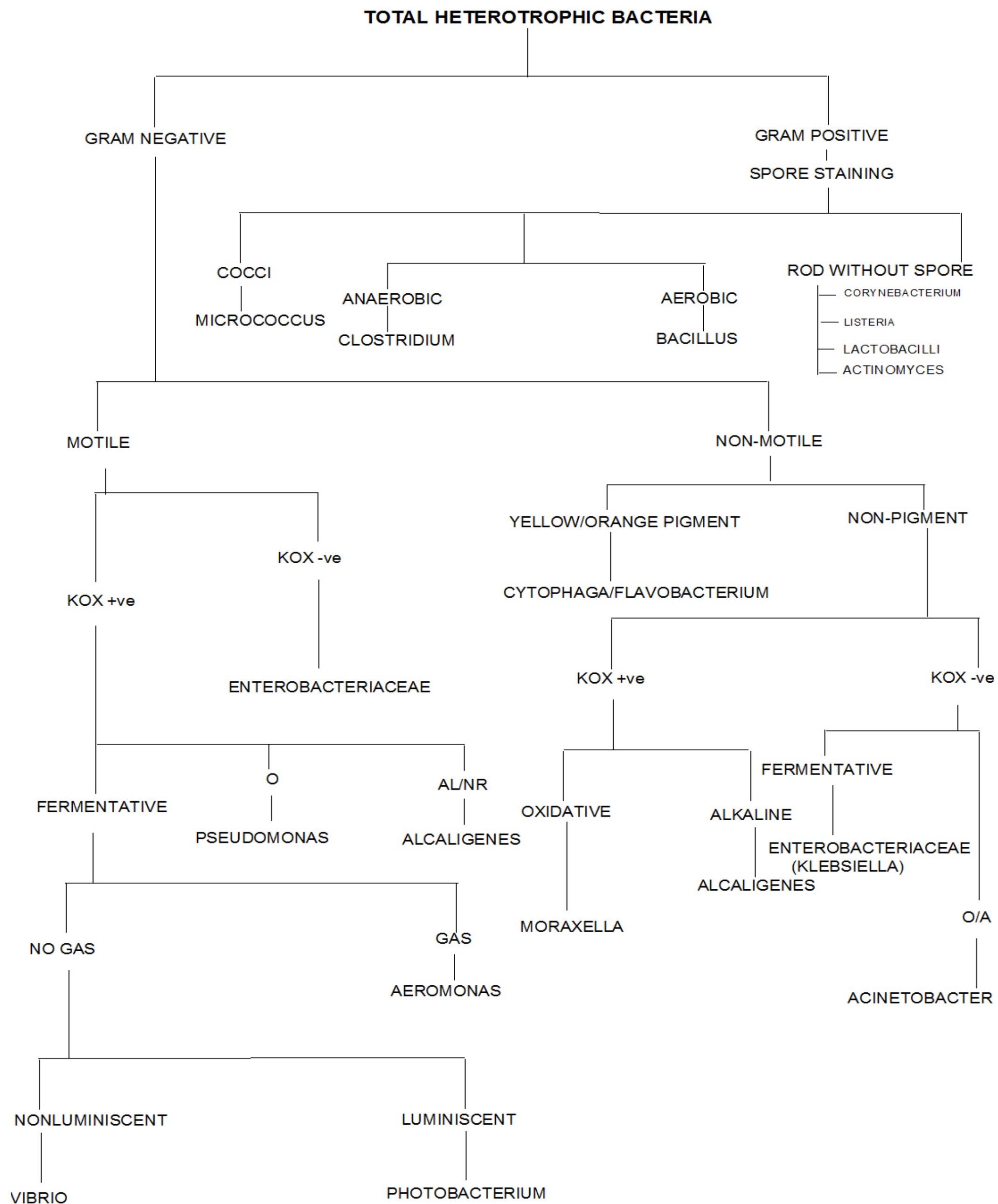
Plate 4 Class: Chlorophyceae



Appendix - II

KEY FOR THE IDENTIFICATION OF HETEROTROPHIC BACTERIA

(Bergey's Manual of Determinative Bacteriology, 2000)



Appendix - III

Table 20 Media / Reagents / Stains

1. ZOBELL'S MARINE AGAR 2216e	**2. NUTRIENT AGAR MEDIUM**
Peptone : 5 gm Yeast extract : 1 gm Ferric phosphate : 0.02 gm Agar : 20.00 gm 50% sea water : 1000 ml pH : 7.2	Peptone : 5 gm Beef extract : 3 gm Agar : 20 gm 50% sea water : 1000 ml pH : 7.2
3. MANNITOL MOTILITY AGAR MEDIUM Mannitol : 26gm . NaCl : 15gm Agar : 4 gm Distilled Water : 1000ml pH : 7.2	**4. MARINE OXIDATION PERMENTATION MEDIUM (MOF)** MOF : 22 gm Dextrose : 10gm NaCl : 15gm Agar : 1.5gm Distilled Water : 1000ml pH : 7.2
5. STARCH AGAR MEDIUM Peptone : 5 gm Beef extract : 3 gm Soluble starch : 5 gm Agar : 20 gm 50% sea water : 1000 ml pH : 7.2	**6. TRIBUTYRIN AGAR MEDIUM** Peptone : 5 gm Beef extract : 3 gm Tributyrin : 10 ml Agar : 20 gm 50% sea water : 1000 ml pH : 7.2
7. GELATIN AGAR MEDIUM Peptone : 5 gm Beef extract : 3 gm Gelatin : 20 gm Agar : 20 gm 50% sea water : 1000 ml pH : 7.2	**8. CELLULOSE AGAR MEDIUM** Peptone : 5 gm Beef extract : 3 gm CMC : 5 gm Agar : 20 gm 50% sea water : 1000 ml pH : 7.2
9. LIGNIN AGAR MEDIUM Peptone : 5 gm Beef extract : 3 gm Agar : 20 gm 50% Sea water : 1000ml Tannic acid : 5 gm pH : 7.2	**10. PHENOLPHTHALEIN DI PHOSPHATE AGAR MEDIUM** Peptone : 5 gm Beef extract : 3 gm Agar : 20 gm 50% Sea water : 1000ml Phenolphthalein di phosphate : 10 ml of 1% solution pH : 7.2

11. ALGINATE AGAR MEDIUM

Peptone	: 5 gm
Beef extract	: 3 gm
Agar	: 20 gm
50% Sea water	: 1000ml
Sodium Alginate	: 15 gm
pH	: 7.2

Gram's Iodine		Safranin-'O'	
Iodine Potassium iodide Distilled Water	1gm 2gm 300ml	Safranin-0 Distilled water Ethyl alcohol (95%)	0.25gm 90ml 10ml
Gram's stain			
Solution-A		Solution-B	
Crystal Violet Ethyl alcohol (95%)	2gm 20ml	Ammonium oxalate Distilled Water Solution A and B mixed	0.8gm 80ml
Spore stain			
Malachite green Distilled water			5gm 100ml

Appendix - IV

Table 21 Physical variables during 2009-2010

Stations, No.	2009-2010								
	Temperature (°C)			Salinity (psu)			pH		
	PRM 2009	MON 2009	POM 2010	PRM 2009	MON 2009	POM 2010	PRM 2009	MON 2009	POM 2010
Azheekode. 1	31	28	28	33.0	34.0	34.0	8.2	8.0	8.3
Balathuruth. 2	30	26	27	36.0	2.0	27.0	8.0	8.6	8.2
Kodikkal. 3	30	27	29	35.0	35.0	35.0	7.8	8.1	8.1
Mahe. 4	31	27	29	32.0	9.0	27.0	7.9	8.1	7.7
Puthiyangadi. 5	30	26	28	35.0	30.0	35.0	8.2	8.0	8.1
Thykadapuram. 6	32	27	28	34.0	4.0	28.0	8.0	8.2	8.2

Table 22 Physical variables during 2010-2011

Stations, No.	2010-2011								
	Temperature (°C)			Salinity (psu)			pH		
	PRM 2010	MON 2010	POM 2011	PRM 2010	MON 2010	POM 2011	PRM 2010	MON 2010	POM 2011
Azheekode. 1	29.0	26.0	26.0	35	30	30	8.0	8.1	7.9
Balathuruth. 2	28.0	26.0	26.5	27	5	30	7.8	8.2	7.9
Kodikkal. 3	28.0	29.0	26.0	35	32	30	8.0	7.8	7.9
Mahe. 4	28.0	26.0	26.0	26	20	28	7.8	8.2	7.9
Puthiyangadi. 5	28.0	27.0	26.0	35	30	31	7.8	8.3	7.9
Thykadapuram. 6	29.0	27.0	31.0	30	7	29	7.8	8.3	7.7

Appendix - V

Table 23 Nutrient parameters during 2009-2010

Stations, No.	2009-2010											
	NO_3-N ($\mu molL^{-1}$)			NO_2-N ($\mu molL^{-1}$)			SiO_4-Si ($\mu molL^{-1}$)			PO_4-P ($\mu molL^{-1}$)		
	PRM 2009	**MON 2009**	**POM 2010**	**PRM 2009**	**MON 2009**	**POM 2010**	**PRM 2009**	**MON 2009**	**POM 2010**	**PRM 2009**	**MON 2009**	**POM 2010**
Azheekode. 1	8.10	5.60	1.45	0.59	0.90	0.43	3.23	62.00	2.98	1.07	1.90	0.40
Balathuruth. 2	9.72	8.90	1.68	0.19	1.40	0.28	17.50	144.90	16.92	0.55	1.40	0.81
Kodikkal. 3	11.90	2.30	3.98	0.87	1.10	0.69	4.00	19.70	3.05	0.79	1.50	0.06
Mahe. 4	3.99	6.20	2.66	0.52	0.90	0.36	34.70	62.10	35.04	1.15	1.30	0.72
Puthiyangadi. 5	6.90	4.90	2.65	0.42	1.20	0.31	6.60	45.10	12.46	0.87	1.80	0.58
Thykadapuram. 6	3.05	4.20	2.11	0.38	1.10	0.47	26.80	25.00	3.01	0.75	1.70	0.04

Table 24 Nutrient parameters during 2010-2011

Stations, No.	2010-2011											
	NO_3-N ($\mu molL^{-1}$)			NO_2-N ($\mu molL^{-1}$)			SiO_4-Si ($\mu molL^{-1}$)			PO_4-P ($\mu molL^{-1}$)		
	PRM 2010	**MON 2010**	**POM 2011**	**PRM 2010**	**MON 2010**	**POM 2011**	**PRM 2010**	**MON 2010**	**POM 2011**	**PRM 2010**	**MON 2010**	**POM 2011**
Azheekode. 1	2.82	14.36	1.73	0.03	1.00	1.33	25.10	10.59	40.24	1.82	2.50	1.71
Balathuruth. 2	4.59	6.27	2.81	0.06	0.12	0.46	34.32	42.81	17.56	0.70	2.62	2.01
Kodikkal. 3	1.24	4.63	2.27	0.85	1.20	0.21	15.16	49.49	12.83	0.84	0.89	1.30
Mahe. 4	4.25	5.45	4.51	0.52	0.79	0.42	48.04	55.41	50.91	0.57	1.42	2.54
Puthiyangadi. 5	4.26	2.99	2.41	0.40	0.87	0.37	55.04	21.50	35.88	0.75	2.36	0.79
Thykadapuram.6	3.90	5.45	1.18	0.57	0.42	1.33	30.87	28.37	38.67	0.79	0.77	1.77

Appendix – VI

Table 25 Dissolved Oxygen and Net Primary Production during 2009-2010

2009-2010						
Stations, No.	**DO (mgL^{-1})**			**Net PP ($gC/m^3/day$)**		
	PRM 2009	**MON 2009**	**POM 2010**	**PRM 2009**	**MON 2009**	**POM 2010**
Azheekode. 1	4.49	3.38	6.16	8.72	18.16	12.48
Balathuruth. 2	3.84	6.54	5.96	4.48	9.20	9.72
Kodikkal. 3	4.08	5.31	6.24	8.48	8.48	10.20
Mahe. 4	4.49	6.53	5.33	4.24	5.94	1.68
Puthiyangadi. 5	4.12	5.72	5.64	12.30	4.24	16.92
Thykadapuram. 6	6.53	5.72	5.31	16.96	4.60	1.04

Table 26 Dissolved Oxygen and Net Primary Production during 2010-2011

2010-2011						
Stations, No.	**DO (mgL^{-1})**			**Net PP ($gC/m^3/day$)**		
	PRM 2010	**MON 2010**	**POM 2011**	**PRM 2010**	**MON 2010**	**POM 2011**
Azheekode. 1	7.96	7.27	4.49	1.70	3.81	1.09
Balathuruth. 2	7.14	7.96	5.31	3.39	1.91	1.70
Kodikkal. 3	9.14	5.90	5.72	1.82	7.08	1.45
Mahe. 4	7.27	8.04	5.31	2.54	9.65	6.84
Puthiyangadi. 5	9.55	6.29	5.72	9.75	7.54	28.83
Thykadapuram. 6	9.35	7.67	6.53	5.09	9.21	4.20

Appendix - VII

Table 27 Pigment composition during 2009-2010

Stations, No.	2009-2010											
	Chlorophyll *a* (μgL^{-1})			Chlorophyll *b* (μgL^{-1})			Chlorophyll *c* (μgL^{-1})			Carotenoids (μgL^{-1})		
	PRM 2009	MON 2009	POM 2010	PRM 2009	MON 2009	POM 2010	PRM 2009	MON 2009	POM 2010	PRM 2009	MON 2009	POM 2010
Azheekode. 1	3.07	13.54	1.82	0.16	0.01	0.05	0.90	3.44	0.58	1.05	1.91	0.77
Balathuruth. 2	3.63	2.02	3.63	0.35	1.58	0.12	1.01	0.88	1.65	1.81	0.51	1.08
Kodikkal. 3	6.01	4.38	3.75	0.10	0.31	0.08	0.56	0.65	1.14	0.49	0.87	0.66
Mahe. 4	5.14	1.10	2.95	0.02	0.38	0.19	0.79	0.48	0.92	1.54	0.02	1.11
Puthiyangadi. 5	3.72	3.14	4.32	0.41	0.53	0.04	1.48	1.15	1.47	0.71	0.22	1.05
Thykadapuram. 6	2.14	1.21	1.68	0.03	0.53	0.29	1.56	0.59	0.76	0.93	0.41	0.60

Table 28 Pigment composition during 2010-2011

Stations, No.	2010-2011											
	Chlorophyll *a* (μgL^{-1})			Chlorophyll *b* (μgL^{-1})			Chlorophyll *c* (μgL^{-1})			Carotenoids (μgL^{-1})		
	PRM 2010	MON 2010	POM 2011	PRM 2010	MON 2010	POM 2011	PRM 2010	MON 2010	POM 2011	PRM 2010	MON 2010	POM 2011
Azheekode. 1	9.35	8.61	9.17	0.24	0.04	0.13	2.46	0.80	3.06	3.95	1.23	2.93
Balathuruth. 2	6.90	6.02	2.82	0.86	0.34	0.35	1.95	0.64	0.61	1.55	0.44	0.81
Kodikkal. 3	3.72	3.32	4.80	1.18	0.02	0.01	1.81	2.95	0.67	1.17	1.42	1.72
Mahe. 4	7.48	3.02	3.82	0.30	0.06	0.60	1.21	1.36	2.14	2.50	0.86	1.97
Puthiyangadi. 5	10.46	1.95	3.45	0.92	0.01	0.94	2.45	0.64	1.36	1.60	0.58	1.01
Thykadapuram. 6	5.43	6.93	3.31	0.36	1.14	0.01	0.66	0.56	1.87	1.23	1.88	2.27

Appendix - VIII

Table 29 Standing crop and pigment concentrations during MON 2008, at the time of *P. parvum* bloom, and during MON 2010 off Azheekode.

Bloom/Season	Chlorophyll *a* (μgL^{-1})	Chlorophyll *b* (μgL^{-1})	Chlorophyll *c* (μgL^{-1})	Carotenoids (μgL^{-1})	Cells L^{-1} (Log10)
MON 2008	3.82	0.05	1.14	0.66	2.5
MON 2009 (*P. parvum* bloom)	13.54	0.02	3.44	1.91	7.9
MON 2010	2.61	0.04	0.80	1.23	3

Table 30 Physical variables during MON 2008, at the time of *P. parvum* bloom, and during MON 2010 off Azheekode.

Bloom/Season	Temperature (°C)	Salinity (psu)	pH
MON 2008	26	35	7.8
MON 2009 (*P. parvum* bloom)	28	34	8.0
MON 2010	26	30	8.1

Table 31 Nutrient concentrations, DO, and Net PP during MON 2008, at the time of *P. parvum* bloom, and during MON 2010 off Azheekode.

Bloom/Season	NO_3-N ($\mu molL^{-1}$)	SiO_4-Si ($\mu molL^{-1}$)	PO_4-P ($\mu molL^{-1}$)	DO (mgL^{-1})	Net PP ($gC/m^3/day$)
MON 2008	24.75	2.60	0.26	6.86	1.20
MON 2009 (*P. parvum* bloom)	5.60	62.00	1.90	1.41	3.53
MON 2010	14.36	10.50	2.50	7.27	0.32

Appendix - IX

Table 32 Concentration of pigments during *Proboscia alata* bloom

Date/station	Chlorophyll *a* (μgL^{-1})	Chlorophyll *b* (μgL^{-1})	Chlorophyll *c* (μgL^{-1})	Carotenoids (μgL^{-1})
10-10-09 *P. alata* bloom	10.8	0.44	4.62	2.39
12-10-09 *P. alata* bloom	6.48	0.28	3.97	2.01
Ref.st.1	4.36	0.53	2.14	0.93
Ref.st.2	4.32	0.39	2.64	1.33

Table 33 Temperature, salinity and pH during *Proboscia alata* bloom

Date/station	Temperature (°C)	Salinity (psu)	pH
10-10-09 *P. alata* bloom	27	35	8.4
12-10-09 *P. alata* bloom	28	35	8.2
Ref.st.1	28	35	7.6
Ref.st.2	28	35	7.8

Table 34 Chemical variables during *Proboscia alata* bloom

	10-10-2009 *P. alata* bloom	12-10-2009 *P. alata* bloom	Ref.st.1	Ref.st.2
NO_3-N ($\mu molL^{-1}$)	2.11	1.40	3.03	2.65
SiO_4-Si ($\mu molL^{-1}$)	38.31	14.20	1.86	2.46
PO_4-P ($\mu molL^{-1}$)	1.70	1.20	0.13	0.08
DO (mgL^{-1})	5.42	4.09	6.25	5.64
Net PP ($gC/m^3/day$)	1.87	1.05	0.76	1.41

Appendix - X

Table 35 Concentration of pigments during *Chattonella marina* bloom

Date	Chlorophyll *a* (μgL^{-1})	Chlorophyll *b* (μgL^{-1})	Chlorophyll *c* (μgL^{-1})	Carotenoids (μgL^{-1})
27.10.11	10.89	0.67	2.44	3.19
29.10.11	9.83	0.53	2.14	2.89
01.11.11	6.69	0.49	1.75	2.29

Table 36 Temperature, salinity and pH during *Chattonella marina* bloom

Date	Temperature ($^{\circ}C$)	Salinity (psu)	pH
27.10.11	25	30	7.9
29.10.11	26	30	8.0
01.11.11	27	28	7.7

Table 37 Chemical variables during *Chattonella marina* bloom

Date	NO_3-N ($\mu molL^{-1}$)	SiO_4-Si ($\mu molL^{-1}$)	PO_4-P ($\mu molL^{-1}$)	DO (mgL^{-1})	Net PP ($gC/m^3/day$)
27.10.11	12.54	2.38	0.51	4.50	1.94
29.10.11	7.54	2.58	0.56	4.80	1.20
01.11.11	6.99	3.47	0.49	5.20	4.24

.....ꙮꙮ.....

List of Publications

1) **Anit M. Thomas**, Sanilkumar M.G., Vijayalakshmy K.C., Mohamed Hatha A.A. and Saramma A.V. *Proboscia alata* (Brightwell) Sandström bloom in the coastal waters off Bekal, Southwest India.
(Accepted for publication in *Current Science*)

2) **Anit M. Thomas**, Vijayalakshmi K.C., Akhil P. John, Abhijith Muralidharan, Sanilkumar M.G., Mohamed Hatha A.A. and Saramma A.V. Occurrence of algal bloom dominated by *Fragilariopsis oceanica* from the coastal waters of Southwest India. (Communicated)

3) **Anit M. Thomas**, Sanilkumar M.G., Vijayalakshmy K.C., Sanjeevan V.N., Mohamed Hatha A.A. and Saramma A.V. Dynamic changes in bacterial population and corresponding exoenzyme activity in response to a tropical phytoplankton bloom *Chatonella marina*. (Communicated)

4) Sanilkumar M.G., **Anit M. Thomas**, Vijayalakshmy K.C., Mohamed Hatha A.A. and Saramma A.V. (2012) *Chattonella marina* bloom in the coastal Sea off Mahe, Southwest India. *Current Science* **103(6),** 624-626.

5) Sanilkumar M.G., **Anit M. Thomas**, Shyamkumar S., Rosamma Philip, Sanjeevan V.N. and Saramma A.V. (2009) First report of *Protoperidinium* bloom from Indian waters. *Harmful Algae News* **39**.

.....