# Nitrogen and carbon cycling in estuarine and marine environments

## A THESIS

*Submitted for the award of Ph. D degree of*

**MOHANLAL SUKHADIA UNIVERSITY**

*In the*

*Faculty of Science*

*By*

**Bhavya P S**

**Under the Supervision of**

**Prof. R. Ramesh**

Outstanding Scientist

Geosciences Division

Physical Research Laboratory, Ahmedabad India

DEPARTMENT OF PHYSICS

MOHANLAL SUKHADIA UNIVERSITY

UDAIPUR, INDIA

**2015**

# CERTIFICATE

I certify that the thesis entitled "Nitrogen and carbon cycling in estuarine and marine environments" by Ms. Bhavya P. S. was prepared under my guidance. She has completed the following requirements as per Ph.D. Regulations of the university,

(a) Course work as per the university rules.
(b) Residential Requirements of the university.
(c) Presented her work in the departmental committee.
(d) Published/accepted minimum of one research paper in referred research journals.

I am satisfied with the analysis of data, interpretation of results, and conclusions drawn. I recommend the submission of the thesis.

Date:

Prof. R. Ramesh

(Supervisor)

Outstanding Scientist

Physical Research Laboratory

Ahmedabad, India

Countersigned by

Head of the Department

Mohanlal Sukhadia University

# DECLARATION

I Bhavya P. S., d/o Mr. P. Sadanandan, resident of room number 120, PRL Thaltej hostel, Ahmedabad-380059, hereby declare that the research work incorporated in the present thesis entitled "Nitrogen and carbon cycling in estuarine and marine environments" is my own work and is original. This work (in part or in full) has not been submitted to any university or institute for the award of a Degree or Diploma. I have properly acknowledged the material collected from secondary sources whatever required. I solely own the responsibility for the originality of the entire content.

Date:

Bhavya P. S.

(Author)

**Dedicated to**

**my family and teachers**

# Acknowledgements

*My dreams, aims, and strength, ultimately what I am now, I owe to my parents, teachers, relatives, friends, well wishers, and Nature. I am taking this occasion of submitting my thesis as a special time to convey my heartfelt thanks to all who were with me in my ups and downs.*

*First and foremost I would like to convey my heartfelt gratitude to my PhD guide Prof. R. Ramesh for his continuous support and help during my research life in PRL. The basics of isotope geochemistry would not have been easier for me if he had not taught us during the course work. As a hardworking scientist and brilliant teacher, he is one of the highly talented people who motivated me. I am lucky to be one of his students and take this opportunity to express my gratitude to him.*

*Words fail to express my gratitude and respect towards my co-guide Dr. Sanjeev Kumar; without him I could not complete my PhD as smoothly as this. I owe my confidence and knowledge to him for developing me as a researcher and giving me courage to explore various fields of marine biogeochemistry. I am grateful to him for giving me splendid freedom in my research, ideas, writing manuscripts and often for having fun. I also appreciate him for having great patience while correcting my manuscripts and thesis which contained tons of mistakes. It was nice to work with him and I will remember him as a good teacher and perfect guide always.*

*I offer my sincere thanks to R. A. Jani and N. B. Vaghela for their continuous support during lab works. I also thank Manoj Tank, Ram*

*Mhatre, Satish, and Venkat for their support during installation and solving the technical problems of the mass spectrometer.*

*"It is the supreme art of the teacher to awaken joy in creative expression and knowledge"-Albert Einstein. I was lucky to have so many loving teachers like Jeena sister, Devayani teacher, Kalyani teacher, Kutti mash, Sarasamma teacher, Raveendran mash, Madhusoodanan mash, Yamuna teacher, T.K.G sir, and Jiji miss. I take this opportunity to express my heartfelt thanks to my teachers who inspired me.*

*I thank Prof. Anna Godhe for giving me an opportunity to be a part of mesocosm experiments conducted at the Fisheries College, Mangalore. I also thank Dr. Ann-Sofi, Prof. Lars Edler, Prof. Indrani Karunasagar, Fidel Chiriboga, and Dr. Aswin Rai for their support during the mesocosm experiments. I deeply thank Eswara uncle and his wife for making my stay at Mangalore memorable during the mesocosm experiments.*

*I am grateful to Dr. G. V. M. Gupta for his continuous help and support during estuarine and coastal sampling and preparing the manuscripts. I am also thankful to the captain and crew of FORV Sagar Sampada (299, 306, and 311), SIBER project team and ISRO-GBP for the support for scientific field trips. Thanks are also due to Dr. Mohamed Hatha and Dr. Jyothibabu for giving all freedom in using their lab during experiments in the Cochin estuary. I express my sincere thanks to my dear friends Sudharma, Dhanya K. R., Dhanya, Sudheesh, Jabir, and Maneesh for their endless support and help during my lonely field trips. I offer special thanks to my friends Sudha and Shyni for offering me a stay at Cochin during my field trip.*

*I gratefully acknowledge Prof. M. M. Sarin and Dr. J. S. Ray for their valuable suggestions and comments. I am also thankful to my teachers in*

*PRL, Prof. Ashok Singal, Dr. S. Ramachandran, Dr. Jitesh Bhatt, Dr. Varun Sheel, Dr. Dibyendru Chakraborty, Dr. Kuljeet Marhas, Dr. Buwan Joshi, and Dr. Abhijit Chakravarty. Thanks are also due to Dr. M. G. Yadava, Dr. Sunil Singh, Prof. Shyam Lal, Prof. A. K. Singhvi, Dr. Neeraj Rastogi, Dr. Ravi Bhushan, Dr. Vinay Kumar, Dr. Naveen Jual, Dr. A. D. Shukla, A. K. Sudheer, T. A. Rajesh, and Dr. Bhushit for their support at various stages.*

*I also thank people from different sections of PRL; administration, accounts, CMD, purchase, library, computer centre, dispatch, dispensary, transport, canteen and housekeeping who made my life easier in PRL. I deeply express my heartfelt thanks to late soul Abdul bhai for his countious help with a lovely smile. I also thank Alexettan who helped me during estuarine sampling.*

*"A good friend never lets you do stupid things alone". I am happy to have Lekshmy as my friend who was always with me for all stupid, crazy, and interesting explorations in PRL as well as CUSAT. I am thankful to her for being my friend and making my life interesting for the past 7 years. I express my deep gratitude to Lekshmy's father who initiated and arranged our journey to Ahmedabad; otherwise I would not have attended the PRL interview. I thank Blesson, Ranjith, Sreeja, Linto, and Neeta for saving me from homesickness by their love and care. I would also like to thank cute babies; Alan, Aron, and Vava for delighting me with their innocent smiles. I am thankful to my best friends forever; Ramya, Sudheesh, Josi, Anitha P., and Anitha C. A. for being by friends since childhood.*

*I offer my love and thanks to Midhun, Akash, Trina and my dearest juniors; Shraddha, Rupa and Niharika for filling my days with lots of fun. I am also thankful to my dear batch mates in PRL; Avodesh, Yashpal,*

*Negi, Dilip, Anjali, Damu, Priyanka, Tanmoy, Monojit, Gulab, Arko, Aathi, Reddy, Wagheesh Misra, Upendra Kumar Singh Kushwaha, Dinesh, Gaurav Sarma, Nigam, Uma, and Girish for making my PhD life enjoyable and memorable. I am so much thankful to my dear seniors; Arvind Singh, Naveen Gandhi, and Amzad Laskar for their valuable suggestions and helps during the period of my PhD.*

*Thanks are also due to all other seniors in PRL; Tanusree, Vinni bhiayya, Chinmay, Yogita, Susantha, Prasant, Srinivas, Rohit, Sunil, Arun Awasthi, Fazilul, Shashi, Naveen Chauhan, Amrendra, and Vimal. I thank my friends; Ikshu, Anirban, Sweta Srivastava, Sneha Yadav, Sneha Sawanth, Nisha, Apurv, Ashim, Devendra, Chithrabhanu, Swapna, Nita, Aswathy, Venky, Upasana, Jinia, Aslam, Abhay, Alok, and Ila Garg. I am thankful to my juniors; Girish kumar (Keju), Arun Pandey, Navpreet, Satish, Jabir, Ali, Bivin, Dipti, Wriju, Kuldeep pandey, Rukmini, Prahlad, Nijil, Vishnu, Pradeep, and Aman for making me laugh with their usual fights and leg pulling along with entertaining discussions. I express my apologies to all other PRL friends whose names are not mentioned here.*

*Words fail in expressing love, strength and motivation of my family towards me. I express my deep love and gratitude to my amma, achan, unnikuttan ammama, and achamma (late) for their endless love and support. I offer my love and thanks to my dearest cousins for filling my life with lots of nostalgia. I am thankful to Gaurav for being my best friend and his unconditional support helped to keep me cool all the time. I owe him for being with me in my ups and downs and filling my life with happiness and positivity. I also express my deepest gratitude to Gaurav's family for their love and support. I am also thankful to my relatives and neighbours for their affection and care. I specially thank Muraliettan and*

*George chettan (late) for keeping a lot of expectations on me even before I left reading "Balarama".*

*Last but not the least, I am grateful to the oceanographers worldwide who made my research life easier by putting lots of efforts in revealing the mystery of ocean biogeochemistry.*

# Abstract

Estuarine and coastal environments are highly prone to anthropogenic interferences which can potentially alter the biogeochemical cycling of these ecologically relevant systems. This thesis reports results from a set of *in situ* and simulated experiments performed to understand the influence of anthropogenic activities and climate change on the nitrogen (N) and carbon (C) cycling in the estuarine and coastal waters using N and C stable isotopic techniques. The study provides first estimates of DIN uptake and $N_2$ fixation rates in a eutrophic estuary (the Cochin estuary), from India. The results showed that N and C assimilations in this estuary are highly influenced by anthropogenic nutrient loading. Despite high DIN concentrations, the existence of diazotrophs and its ability to fix $N_2$ in the Cochin estuary was identified by the present study. The study proved that relative abundance of N and P controls DIN uptake and $N_2$ fixation rates in the Cochin estuary rather than their individual concentrations. The optimum DIN uptake rates in the estuary was observed at TN:TP close to the Redfield's ratio (16:1). The study also reports the influence of anthropogenic nutrient loading through the Cochin estuary on DIN assimilation rates at the nearshore station in the coastal Arabian Sea.

One of the major findings of this thesis is that the $^{13}C$ ($NaHCO_3$) labeling technique is not the best suited method for primary productivity measurements for aquatic environments with high $CO_{2,aq}$ concentrations. The present study also uses $\delta^{13}C$ and $\delta^{15}N$ of POM and Zooplankton along with $\delta^{13}C_{DIC}$ as tracers to understand the marine food web and DIC dynamics in the Cochin estuary and the coastal Arabian Sea. The results

indicate that DIC pool in the Cochin estuary and the coastal Arabian Sea was found to be significantly depleted in $^{13}C$.

Based on the mixing curve approximation, mineralization/respiration seems to be the major processes controlling their distribution in the both of these ecosystems. The study also confirms that the coastal Arabian Sea continues to be a major source of $CO_2$ to the atmosphere. The $\delta^{13}C$ and $\delta^{15}N$ of the zooplanktons showed an enrichment of 1.5–4 ‰ relative to POM, which is in agreement with the previous studies conducted globally. The study also constructed a first N and C isotopic flow in marine food web up to the primary consumers in the coastal Arabian Sea.

A mesocosm experiment with anticipated temperature and salinity conditions was also performed to understand the response of marine phytoplankton towards changing hydrographical conditions. The results indicate that under nutrient replete coastal conditions, salinity and temperature are important factors in modulating the assimilation rates. The lower N and C uptake rates in the decreased salinity and ambient temperature condition indicate the negative effect of osmotic stress on phytoplankton community. The results also point towards the decrease in primary productivity in future when salinity decreases with increasing precipitation.

*Key words: Coastal Arabian Sea; Cochin estuary; eutrophication; N and C cycle; $^{15}N$ and $^{13}C$ tracer techniques; diazotrophs; $\delta^{13}C_{DIC}$; marine food web; mesocosm experiments.*

# Abbreviations

| | |
|---|---|
| ‰ | Per mill (parts per thousand) |
| $\delta^{13}C$ | Isotopic composition of carbon with respect to V-PDB |
| $\delta^{15}N$ | Isotopic composition of nitrogen with respect to Air-$N_2$ |
| C | Carbon |
| CIL | Cambridge Isotope laboratories |
| Chl *a* | Chlorophyll *a* |
| CCM | $CO_2$ Concentrating Mechanism |
| CH | Cochin |
| CTD | Conductance Temperature Depth |
| DIC | Dissolved Inorganic Carbon |
| DIN | Dissolved Inorganic Nitrogen |
| DIP | Dissolved Inorganic Phosphate |
| DON | Dissolved Organic Nitrogen |
| EA | Elemental Analyzer |
| HABs | Harmful Algal Blooms |
| IPCC | Intergovernmental Panel on Climate Change |
| IAEA | International Atomic Energy Agency |
| IRMS | Isotope Ratio Mass Spectrometer |
| JGOFS | Joint Global Ocean Flux Studies |
| KSPCB | Kerala State Pollution Control Board |
| MR | Mangalore |
| N | Nitrogen |
| OM | Organic Matter |
| POC | Particulate Organic Carbon |
| POM | Particulate Organic Matter |

| | |
|---|---|
| PON | Particulate Organic Nitrogen |
| PSU | Practical Salinity Unit |
| SS | Sagar Sampada |
| SSS | Sea Surface Salinity |
| SST | Sea Surface Temperature |
| TOM | Terrestrial Organic Matter |
| TN | Total Nitrogen |
| TP | Total Phosphorous |
| V-PDB | Vienna-Pee-Dee-Belemnite |

# Contents

# List of Figures

# List of Tables

# Chapter 1

# Introduction

The nitrogen (N) cycle is perhaps the most fascinating among all biogeochemical cycles probably due to its complexity and mysterious pathways. The key paradox in the N cycle is the poor availability of reactive N despite the significant presence of $N_2$ gas (78%) in the Earth's atmosphere. Hence, as a limiting element for biological productivity, N grabs a pivotal role in aquatic as well as terrestrial biogeochemistry with a significant influence on cycling of many other elements, particularly carbon (C) and phosphorus (P). Till the industrial revolution, most of the ecosystems were N limiting due to non availability of reactive N. However, the appearance of N cycle has intensely altered since N-fertilizers and industrial revolution came into picture [*Meybeck,* 1982; *Bauer et al*., 2013; *Regnier et al*., 2013]. Elevated usage of N-fertilizers and consequent loading to the rivers and estuaries causes high dissolved inorganic and organic nitrogen concentrations in these ecosystems [*Doney et al*., 2010; *Paerl et al*., 2014]. The exact magnitudes of altered N and C fixation and loss rates are still unclear [*Cai*, 2011; *Bauer et al*., 2013; *Regnier et al*., 2013]. Also, the present-day exchange of $CO_2$ between the atmosphere and

continental shelf seas remains rudimentary, with an uncertainty as large as 50–100% [*Bauer et al.*, 2013]. Hence, it is highly important to understand the current N and C transformation rates, nutrients ($NO_3^-$, $NH_4^+$, $PO_4^{3-}$, $SiO_3$ etc.) utilization, $CO_2$ release from the estuarine-coastal coupled systems. Since C and N cycles are strongly interconnected and have a proportional alteration with ecosystem changes, it is vital to study both N and C dynamics of an ecosystem. Taking all these factors together, this thesis attempts to address the influences of anthropogenic activities and climate change on N as well as C cycles in estuarine and coastal environments in India. Below in different subsections, a brief overview of different aspects covered in thesis has been discussed.

## 1.1 Aquatic nitrogen cycle

Cycling of N in aquatic systems involves very tricky and complex transformation pathways which involve mostly biological processes [*Figure* 1.1]. The N cycle in the aquatic system starts with $N_2$ fixation by diazotrophs. The first bioavailable form of N is produced in the marine systems through $N_2$ fixation mediated by diazotrophs (e.g. *Trichodesmium*, S*ynechococcus* etc.). The $NH_4^+$ produced through $N_2$ fixation is converted subsequently to $NO_2^-$ and then to $NO_3^-$ by *nitrosomonas* and *nitrobactor*, respectively, through a process called nitrification. This $NO_3^-$ is reduced and incorporated by primary producers, the phytoplankton. This is a major step towards the formation of amino acids and consequently the organic biomass. $NH_4^+$ and urea are reduced forms of N which are released during excretion and decomposition of OM. Primary production mediated through the incorporation of $NH_4^+$ and urea is known as regenerated production. Many studies have reported that $NH_4^+$ is the most preferred substrate

among all other forms of reactive N because the oxidation state of N in $NH_4^+$ is same as that in amino acids and other nitrogenous biochemicals in phytoplankton cells [*Kirchman*, 2012].

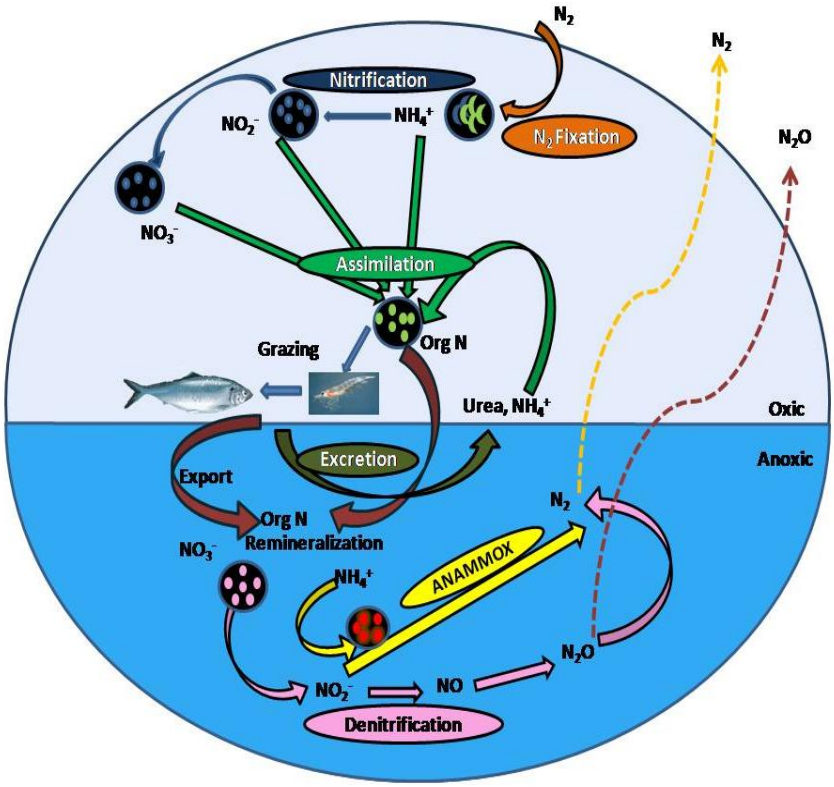


*Figure 1.1: Nitrogen cycle in the marine environment, where the light blue and dark blue shades indicate oxic and anoxic waters, respectively. Areas with dissolved oxygen concentrations ~ 5 ml/L and ~ 0ml/L are known as oxic and anoxic zones, respectively.*

The loss of N from the aquatic pool involves two processes: denitrification and anaerobic ammonium oxidation (anammox). Denitrification is a series of redox reactions which converts $NO_3^-$ to $N_2$ gas with emission of intermediate product $N_2O$ under anoxic conditions. Until 1995, it was believed that denitrification is the only process by which N escapes to the atmosphere from ocean or land. Later on the existence of anaerobic bacteria which can oxidize $NH_4^+$ with utilization of $NO_2^-$ to release $N_2$ gas was revealed [*Kuyper et al.*, 2003, *Dalsgaard et al.*, 2003].

This thesis reports the DIN ($NO_3^-$ + $NH_4^+$) uptake rates and $N_2$ fixation rates in the some estuarine and coastal environments in India. The study also covers some aspects of C cycling in these ecosystems as it requires urgent attention due to the changing hydrographic conditions. The section below briefly explains the importance of carbon cycling in the global marine biogeochemistry.

## 1.2 Aquatic carbon cycle

The increasing global atmospheric temperature with the increasing carbon dioxide ($CO_2$) concentration in the earth's atmosphere is a major concern in the anthropocene [*Hansen and Sato*, 2004; *Hansen et al*., 2007]. Terrestrial and marine photosynthesis, which converts $CO_2$ to organic matter (OM), is the foremost sink of atmospheric $CO_2$. Since ocean covers 70% of the Earth's surface, it is considered as the chief controller of atmospheric $CO_2$. The removal of atmospheric $CO_2$ by ocean involves two key processes known as the solubility pump and biological pump. The former is fundamentally dissolution and transport of $CO_2$ through cold dense ocean water from the high latitudes, where it sinks to the deep. A the tropics, where warm and less dense waters come to the surface due to upwelling, $CO_2$ is released back into the atmosphere. The net removal of $CO_2$ through primary production and consequent transport to the deeper waters is known as biological pump.

A schematic diagram of the marine C cycle is presented in *Figure* 1.2. It starts with the dissolution of atmospheric $CO_2$ in to the sea surface, which subsequently forms three different dissolved inorganic carbon (DIC) species: 90% of bicarbonate ($HCO_3^-$), 9% of carbonate ion ($CO_3^{2-}$), and 1% of $CO_2$ at the typical ocean pH of 8.17 [*Neven et al*., 2011]. The conversion of dissolved $CO_2$ into $HCO_3^-$ and $CO_3^{2-}$ helps ocean to hold

50 times as much C as in the overlying atmosphere. Inorganic carbon in the ocean is 40 times larger than that of organic carbon [*Falkowski et al.*, 2000].

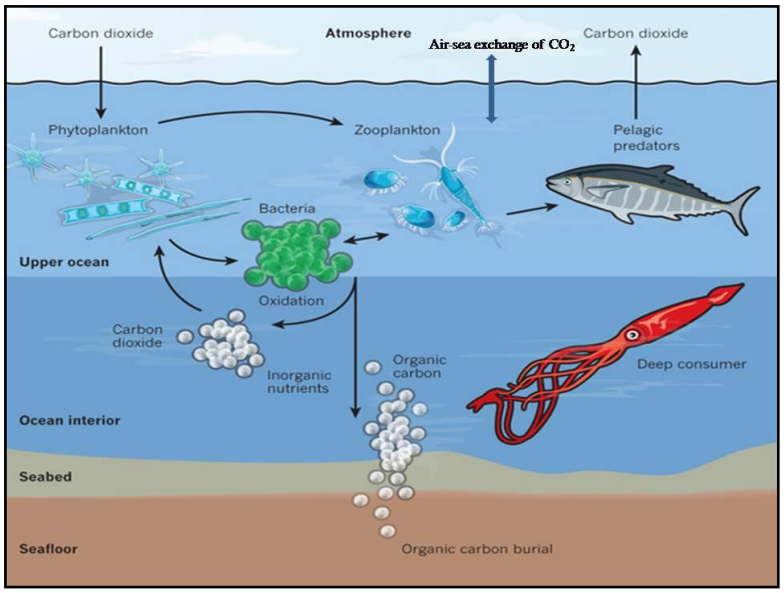


*Figure 1.2: The oceanic carbon cycle. The carbon cycle starts with the dissolution of atmospheric $CO_2$ in to the ocean. This is taken up by the marine phytoplankton during photosynthesis.*

The production of OM occurs only through the biological process, photosynthesis, the first step of the marine biological pump. With exception of $CO_3^{2-}$ species, the other two forms of DIC can be used up by phytoplankton during photosynthesis [*Riebesell et al.*, 1993; Neven et al. 2011]. A major portion of the phytoplankton colony in the surface ocean is grazed by zooplanktons, the primary consumers in the aquatic systems, which in turn are eaten up by other higher level organisms. Eventually, some part of the OM produced and consumed sinks to deeper waters, either as dead cells or fecal matter. In due course, this is used up for remineralization by bacteria present either in water column or sediments. To an extent, biological pump can control the oceanic intake of atmospheric $CO_2$ and hence, contribute to the

balancing of the $CO_2$ concentration. Conversion of $CO_2$ to the OM is controlled by a number of physical, chemical, biological and environmental factors. This process is drastically getting altered by both natural and anthropogenic influences.

The cycling of C and N in an ecosystem near human settlements, particularly estuaries and coastal waters, are highly amended by excess loading of OM and nutrients. One of the major objectives of this thesis includes the effect of anthropogenic influence on the C dynamics in estuaries and coastal systems.

## 1.3 Anthropogenic influences on nitrogen and carbon cycling in the estuaries and coastal waters: A global perspective

Being a rich stockpile of natural resources, estuaries are favorable for human settlements, which make these systems vulnerable to human induced pollution. This pollution introduces excess N which violates the balanced biogeochemical environment in these ecosystems. The colossal inputs of reactive N and terrestrial organic matter (TOM) to estuaries arise mainly from fertilizers, atmospheric deposition in drainage basins and direct sewage discharge etc. [*Howarth et al.*, 1996]. The enhanced nutrient levels, high primary productivity and high OM remineralization in such systems is known as eutrophication. Tidal activity and river runoff also cause high variability in nutrient composition and supply, leading to imbalance in N and phosphorous (P) derived nutrients [*Humborg et al.*, 1997; *Rabalais et al.*, 2009]. Phosphorus is known to be a limiting nutrient in the estuarine waters, whereas N or both N and P can be limiting in the coastal waters [*Fisher et al.*, 1999; *Neill* 2005; *Pearl et al.*, 2014]. Some studies suggest

that estuarine primary productivity is sensitive to both N and P inputs [*Ryther and Dunstan*, 1971; *Schindler*, 1975; *Rudek et al.*, 1991; *Fisher et al.*, 1999; *Boesch et al.*, 2001; *Paerl and Piehler*, 2008; *Smith and Schindler*, 2009, *Paerl et al.*, 2014].

The uptake rate measurements by *in situ* as well as culture experiments have proved that it is relative abundance of N and P that controls the metabolic rates of phytoplankton than individual nutrient concentrations [*Gilbert et al.*, 2003; *Bhavya et al.*, 2015]. Also, in the estuarine-coastal ecosystems, availability of P acts as a controlling factor for phytoplankton growth [*Karl et al.*, 1995; *Sanudo-Whilhelmy et al.*, 2001; *Mills et al.*, 2004; *Neill*, 2005].

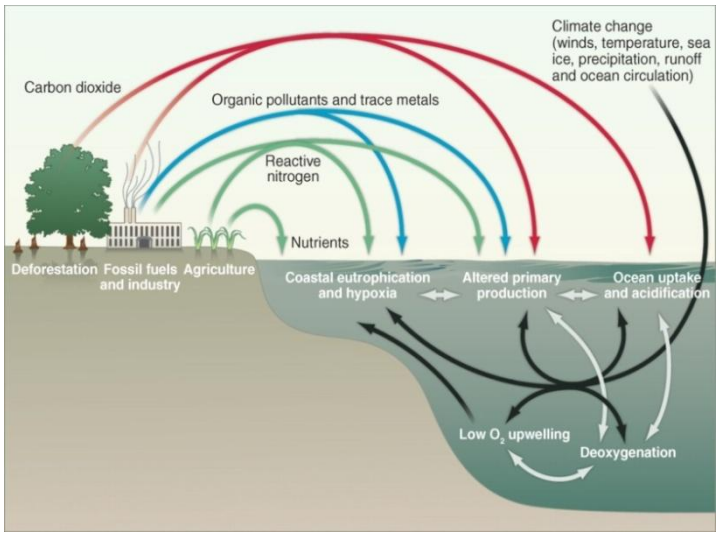


*Figure 1.3. A schematic diagram showing influence of anthropogenic activities on the coastal biogeochemistry.*

Many studies have reported that a considerable portion of terrestrial discharge gets transferred to the coastal waters adjacent to estuaries [*Nixon*, 1995; *Borum*, 1996; *Bhavya et al.*, 2015]. Hence, the biogeochemistry of coastal waters is closely linked to the terrestrial nutrient loading through rivers or estuaries. *Figure* 1.3 shows a

schematic diagram showing influence of anthropogenic activities on the coastal biogeochemistry.

However, it remains a challenge to quantify the influence of terrestrial chemical loading on various coastal ecosystems, especially tropical systems, because of perplexing physical and biogeochemical factors [*Pennock et al*. 1994; *Eyre and Balls*, 1999; *Cloern* 2001]. The influence of other nutrient sources and sinks such as the atmosphere and adjacent ocean [*Smith*, 1984; *Eyre and France*, 1997; *Smith and Hollibaugh*, 1997] also leads to complexity in the coastal ocean biogeochemistry. The reported range of primary productivity and organic matter burial of the coastal ocean are ~15% – ~30% and ~80%, respectively [*Gattuso et al*., 1998]. It also hosts most of the benthic oceanic calcium carbonate ($CaCO_3$) production, ~20% of surface pelagic oceanic $CaCO_3$ stock [*Balch et al*., 2005], and ~50% of oceanic $CaCO_3$ deposition [*Gattuso et al*., 1998]. Hence, C flow in the coastal ocean is extensively high in comparison with its surface area (~7% of total oceanic surface area). Intense air–water $CO_2$ exchange can also be expected in the coastal ocean which could be significant for $CO_2$ flux at regional [*Frankignoulle and Borges*, 2001; *Borges et al*., 2006] and global scales. It is highly important to understand the flow of N and C in these ecosystems to access the level of alteration by human influences. Further, extended studies are required at regional and global scales to construct mitigation strategies to avoid deterioration of these valuable resources.

## 1.4 Nitrogen pollution: Indian perspective

Excess OM and nutrient loading are major problems faced by Indian estuaries and coastal waters. Many of the Indian estuaries are reported with elevated TOM and nutrient concentrations, which leads

to eutrophication [*Sarma et al.*, 2001; *Mukhopadhyay et al.*, 2002; *Bouillon et al.*, 2003; *Biswas et al.*, 2004; *Gupta et al.*, 2008; *Bhavya et al.* 2015, *Bhavya et al.*, unpublished]. The N pollution in these estuaries gradually leads to heterotrophy, a state in which respiration dominates over primary productivity. Elevated nutrient levels and decrease in dissolved oxygen levels associated with heterotrophy has been reported in Indian estuaries and coastal waters [*Qasim et al.*, 1967; *Jyothibabu et al.* 2003; *Martin et al.*, 2013].

The supersaturation of $CO_2$ in Indian estuaries was reported by some recent studies [*Gupta et al.,* 2008, 2009]. There are some studies which primarily focused on the C dynamics of some Indian estuaries [*Raymond et al.*, 2000; *Sarma et al.*, 2001; *Mukhopadhyay et al.*, 2002; *Taylor et al.*, 2003; *Bouillon et al.* 2003; *Biswas et al.*, 2004; *Cai et al.*, 2004; *Borges et al.*, 2005; *Bouillon et al.*, 2007; *Gupta et al.*, 2008, 2009]. Some studies reported that a number of Indian estuaries exhibit up to 90% mineralization of TOM loads during high freshwater flow conditions [*Gupta et al.*, 2008; *Ghosh et al.*, 2013]. It has been reported that the Cochin estuary, the longest estuarine system along the west coast of India, receives approximately 1.37 kg $d^{-1}$ of inorganic phosphate and 2.69 kg $d^{-1}$ of inorganic $NO_3^-$ [*Naik*, 2000; *Balachandran et al.*, 2001]. The export to the coastal waters is around 0.91 kg $d^{-1}$ of inorganic phosphate ($PO_4^{3-}$) and 1.71 kg $d^{-1}$ of inorganic $NO_3^-$ which indicate considerable proportion of nutrients being cycled within the estuary.

In this scenario, the efficiency of these ecosystems with regards to mitigating eutrophication needs a thorough investigation. A number of studies regarding N uptake and primary productivity have been conducted in the northern Indian ocean [e.g. *Owens et al.*, 1993;

*McCarthy et al.*, 1999; *Sambrotto*, 2001; *Prakash et al.* 2008; *Kumar et al.*, 2010; *Gandhi et al.*, 2010, 2011*a*]. However, the eutrophication in estuaries and its influence on coastal environments are not studied adequately [*Bhavya et al.*, 2015]. The primary production, $N_2$ fixation, nature of OM remineralization, exchange of nutrients and TOM in the estuarine-coastal coupling systems are major focuses of this thesis.

## 1.5 Primary productivity and $CO_2$ super saturation

One of the major problems faced by eutrophic ecosystems is $CO_2$ supersaturation, which is increase in the partial pressure of $CO_2$ ($pCO_2$) in an aqueous medium relative to that of air above the water level. The prime reason for this is the dominance of respiration over primary productivity in such systems, particularly estuaries and coastal waters. The excess OM loading and heterotrophy leads such systems to sustain high level of dissolved $CO_2$ ($CO_{2,aq}$) [*Bouillon et al.*, 2001; *Gupta et al.*, 2008, 2009].

The widely used methodology for estimating primary productivity is $^{13}C$ or $^{14}C$ labeling technique [explained in *Chapter* 2]. This method assumes $HCO_3^-$ is the major substrate for primary production, which may not be the case for ecosystems with high $CO_2$ concentrations. *Burkhardt et al.* [2001] reported that $pCO_2$ accumulation may not affect the C fixation in the ocean, since $CO_{2,aq}$ and $HCO_3^-$ are equally preferred by marine autotrophs and $CO_{2,aq}$ in the ocean generally varies between 5-25 μM. However, when $CO_{2,aq}$ is higher than the half saturation constant for Rubisco (70 μM), $HCO_3^-$ uptake can be considerably suppressed. Since the oceanic $CO_2$ is only ~0.25–1.25% of the total DIC pool, an underestimation of primary productivity by the $^{13}C$-$HCO_3^-$ method is negligible. However, this may not be the case for

estuaries with $CO_2$ supersaturation and leads to a considerable underestimation of primary productivity measurements in such systems. As explained earlier, the estimation of primary productivity in eutrophic systems is highly important to understand the cycling of excess of N and C reaching these systems. However, there is lack of such studies reported on any estuarine environment with elevated rates of $CO_{2,aq}$. The present study attempts to find out the effect of high $pCO_2$ on primary productivity measurements [*Chapter* 4].

## 1.6 Application of isotopes in nitrogen and carbon dynamics

Stable isotopic composition of N and C ($\delta^{13}C$ and $\delta^{15}N$, respectively) are widely used to identify sources, infer processes, estimate metabolic rates, understand diet at different trophic levels, and validation of different biogeochemical models. Significant variation in the natural abundance of $^{15}N$ in marine organisms was first reported by *Hoering* in 1955. However, well focused studies using N and C isotopic compositions were carried out only after 1960 onwards [*Miyake and Wada*, 1967; *Wada et al*., 1975; *Wada and Hattori*, 1976; *Minagawa and Wada,* 1984].

Since molecules with different isotopes have different chemical reaction rates, the product and substrate undergoes isotopic discrimination or fractionation. This property helps in tracking the processes and sources in the ecosystems. Since isotopic differences among various materials or phases are very small, the isotopic compositions are reported relative to an internationally accepted standard. It is expressed in parts per thousand deviation from that standard. The isotopic compositions are denoted by $\delta$ which is given below,

$$\delta(‰) = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right] \times 1000$$

Where, R is the ratio of heavier isotope to lighter isotope. Isotopes are also used as tracers, where atom% is used instead of δ values, where

$$\text{Atom\%} = 100 \times \left[\frac{R_{\text{sample}}}{(1 + R_{\text{sample}})}\right]$$

The potential of stable isotopes to determine organic matter flow, inorganic nutrient dynamics, and food web structure dragged attention of many researchers [*DeNiro and Epstein*, 1978; *Fry et al.*, 1978; *Rau and Anderson*, 1981; *Peterson and Fry*, 1987; *Kumar et al.*, 2004; *Ghosh et al.*, 2011; *Bardhan et al.*, 2015]. Apart from identifying sources, $\delta^{15}N$ and $\delta^{13}C$ of phytoplankton and zooplankton can throw light on the marine food web and predator-prey relationship.

The reported values for $\delta^{15}N$ and $\delta^{13}C$ of phytoplankton, dissolved inorganic carbon (DIC) in the Indian estuaries and coastal waters are very few [*Sonnerup and Quay*, 2000; *Bouillin et al.*, 2001; Kumar et al., 2004; *Ghosh et al.*, 2011; *Laskar et al.*, 2011; *Sarma et al.*, 2014; *Bardhan et al.*, 2015]. However, there is no well structured investigation regarding the $\delta^{15}N$ and $\delta^{13}C$ of phytoplankton and zooplankton and $\delta^{13}C$ of DIC in Indian estuaries and coastal waters. *Chapter* 5 of this thesis presents the $\delta^{13}C$ and $\delta^{15}N$ of phytoplankton, zooplankton, and DIC in the Cochin estuary and the coastal Arabian Sea.

## 1.7 Response of phytoplankton to climate change

Apart from anthropogenic chemical loading, the increase in surface temperature due to climate change and decrease in sea surface salinity (SSS) due to heavy rainfall events [*IPCC report*, 2007] is also a matter

of concern. Potential increase in sea surface temperature (SST) and decrease in coastal SSS may be close to the physiological tolerance limits for different organisms [*Walther et al.*, 2002]. Marine phytoplankton, responsible for almost half of the global primary productivity [*Field et al.*, 1998], may also get affected by such hydrographical changes, which can alter their N and C uptake potential. Studies have suggested that ocean warming has a significant impact on biological and ecological aspects, from species to community levels [*Walther et al.*, 2002], including biodiversity, population size, species community composition, geographical distribution, phenology and productivity [*Hardy*, 2003; *Edwards and Richardson*, 2004; *Behrenfeld et al.*, 2006; *Jay & Marmot*, 2009; *Boyce et al.*, 2010; *Moran et al.*, 2010]. Models also predict reduction in phytoplankton productivity due to increased SST during this century [*Bopp et al.*, 2001; *Steinacher et al.*, 2010; *Thomas et al.*, 2012].

In the context of climate change, some studies have explored temperature-related traits, such as optimal temperatures for growth and ranges of thermal tolerance for marine phytoplankton and other organisms [*Kingsolver*, 2009; *Thomas et al.*, 2012]. However, the response of the tropical phytoplankton community to the changing hydrographic conditions in terms of their production and N assimilation remains poorly constrained. The extent of such hydrographic changes on the marine food web along with their potential to contribute directly or indirectly towards initiation of harmful algal blooms (HABs) and the proliferation of marine pathogenic bacteria also remains to be explored as the frequency, intensity, and geographical distribution of HABs appear to have increased over the last few decades [*Hallegraeff et al.*, 2010]. Chapter 6

of this thesis describes a mesocosm study performed by us understand the phytoplankton response to the changing temperature and salinity.

## 1.8 Scope of the present study

The main focus of this thesis is to understand the influence of anthropogenic activities and climate change on N and C cycling in the estuarine and coastal waters. For a better understanding of of the above mentioned problems associated with the biogeochemistry of these ecosystems, stable isotopic techniques of N and C are used. The null hypotheses of this thesis are listed below,

1. Since estuarine and coastal systems receive high nutrient loading, the DIN uptake rates as well as primary productivity in these systems are higher than that of the open ocean.
2. Due to high $NH_4^+$ concentrations in the estuarine systems, $N_2$ fixation is absent.
3. The elevated $CO_{2,aq}$ concentrations in the estuary may underestimate the primary production rates using $^{13}C$-labelling technique.
4. The coastal Arabian Sea, one of the world's high productive zones, is a source of $CO_2$, because respiration dominates over primary production.
5. The isotopic enrichment in the predator relative to the prey is ≈2−3‰.
6. Hydrographic changes in future due to climate change may affect N and C uptake potential of phytoplankton.

To check the above mentioned hypotheses following studies were conducted,

1. DIN uptake rates were estimated using the $^{15}N$-labelling technique in the Cochin estuary and the coastal Arabian Sea during three seasons in 2012.
2. $N_2$ fixation rates were estimated directly using the $^{15}N_2$ tracer gas in the Cochin estuary and the coastal Arabian Sea.
3. Primary productivity measurements using $^{13}C$-labelling technique were performed in $CO_2$ supersaturated Cochin estuary and the coastal Arabian Sea.
4. Isotopic compositions of DIC in the Cochin estuary and the coastal Arabian Sea were measured during three seasons in 2012. This helped to understand the major processes governing C dynamics in this region.
5. Isotopic compositions of N and C in phytoplankton and zooplankton were analyzed during nine months of 2012. This helped to understand the food web of the Arabian Sea.
6. A mesocosm study was performed at the Fisheries College, Mangalore, to understand the response of phytoplankton community towards anticipated hydrographic changes. $^{13}C$ and $^{15}N$ labeling techniques were used to measure DIN uptake and primary production rates.

# Chapter 2

# Methodology

This thesis consists of results from three different field studies conducted in the Cochin estuary and adjacent coastal Arabian Sea along with one mesocosm experiment performed to understand the response of phytoplankton towards the anticipated hydrographic changes. The results include N and C uptake rates, $N_2$ fixation rates, $\delta^{15}N$ and $\delta^{13}C$ of particulate organic matter and zooplankton and $\delta^{13}C$ of DIC. This chapter contains different methods used for data collection and experiments during the course of this study.

## 2.1 Environmental Parameters

### 2.1.1 The Cochin estuary and coastal Arabian Sea studies

The data for temperature and salinity during the study period were collected using a Sea-Bird CTD profiler (model 911 plus for coastal [*Figure* 2.1] and model SBE 25 for the estuary). Inorganic nutrients viz. $NH_4^+$, $NO_3^-$, dissolved inorganic phosphate (DIP), and silicate (Si)

were analyzed by spectrophotometry using standard methods [*Grasshoff et al.*, 1999]. Total phosphorus (TP) and total nitrogen (TN) were estimated using persulphate oxidation [*Grasshoff et al.*, 1999]. Dissolved organic phosphorus (DOP) and nitrogen (DON) were calculated as difference between TP, TN and their respective total inorganic fractions. The analytical precision for $NH_4^+$, $NO_3^-$, DIP, Si, TN and TP were ±0.07, ±0.05, ±0.02, ±0.03, ±0.11 and ±0.06 µM, respectively. pH was measured using a potentiometric titrator (907 Titrando, Metrohm) with a precision of ±0.005 and ±0.008 for coastal and estuarine samples, respectively. Chlorophyll *a* (Chl *a*) was measured using 90% acetone extraction followed by fluorometric analysis [*Grasshoff et al.*, 1999].

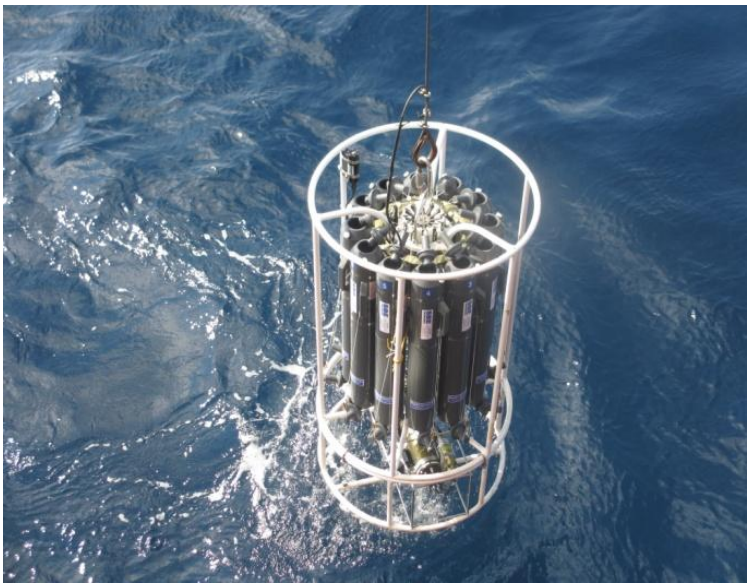

*Figure 2.1 Operation of CTD Rosette in the coastal Arabian Sea.*

### 2.1.2 Mesocosm experiment

Temperature and salinity during mesocosm experiment were measured by a thermometer and digital salinometer, respectively. Samples for $NO_3^-$, $NO_2^-$, and $NH_4^+$ were measured using autoanalyzer [*Grasshoff et*

*al*., 1999] and pH was measured using a pH meter (MKVI Systronics). On each sampling occasion, 200 ml of water in duplicate from each mesocosm tank was vacuum-filtered through GF/F filters (Whatman). Pigments were extracted overnight at 4°C, in 3 ml of 90% acetone. The absorption of the extracted pigments was measured in a spectrophotometer (Shimadzu Corporation). Chl *a* concentrations (µg $l^{-1}$) in water samples were calculated according to *Jeffrey and Humphrey* [1975]. N and C uptake rates were estimated using $^{15}N$ and $^{13}C$ isotope labelling techniques, respectively.

## 2.2 $^{13}C$ and $^{15}N$ tracer techniques

### 2.2.1 Nitrogen and carbon uptake rates

Two stable isotopes of N are $^{14}N$ and $^{15}N$ which contribute 99.636 and 0.364%, respectively, to the total N in the environment. Similarly, C has $^{12}C$ and $^{13}C$ as two stable isotopes having abundance of 98.887 and 1.113%, respectively. Lower abundance of heavier isotope is used as tool for isotope labeling technique [*Dugdale et al*., 1967; 1986]. The tracers $NaH^{13}CO_3$, $^{15}NH_4Cl$ and $Na^{15}NO_3$ are used with assumptions that:

1. Phytoplankton do not discriminate between the heavier and lighter isotopes during uptake.
2. There is no grazing by zooplankton.
3. There is no formation of nutrients during the incubation.

For estuarine-coastal and mesocosm studies, $^{13}C$ and $^{15}N$ labeling techniques were used to estimate the C and N uptake rates, respectively. For C and N uptake experiments, water samples (coastal: 1L and estuary: 500 ml) were collected and were spiked with 99 atom% enriched $NaH^{13}CO_3$, $^{15}NH_4Cl$, and $Na^{15}NO_3$ to trace C, $NH_4^+$, and $NO_3^-$

uptake rates, respectively. Tracer additions were less than 10% of the ambient concentrations. Immediately after the tracer addition, sample bottles were deployed for *in situ* incubation for 4 hrs, approximately symmetrical to the local noon.

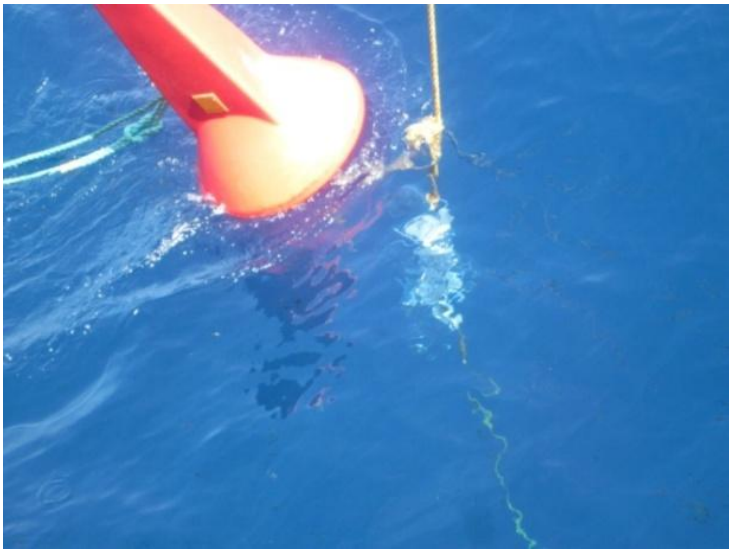

*Figure 2.2: In situ incubation of samples in the coastal Arabian Sea.*

Post incubation samples were filtered onto pre-combusted (4 hrs @ 450°C) 47 mm Whatman GF/F filters and stored for the mass spectrometric analysis after drying at 50°C overnight. The mass spectrometric analyses of post-incubation samples were performed using a continuous flow stable isotope ratio mass spectrometer (IRMS, Delta V Plus) connected to an elemental analyzer (Flash EA 2000) where, $^{15}N$ (or $^{13}C$) atom% and particulate organic N or C (PON or POC) content were measured.

The uptake rate was calculated using following equation.

Uptake rate (µmol N $l^{-1}$ $h^{-1}$) =

$$P * \Delta I_p / [T * ((I_0S_a+I_rS_t) / (S_a + S_t) - I_0)]$$

Where, P is the amount of particulate N in post incubation sample, $\Delta I_p$ is the increase in $^{15}N$ atom% in particulate N during incubation, $S_a$ and $S_t$ are ambient and added concentration, respectively, $I_r$ and $I_0$ are $^{15}N$ atom% of added tracer and natural $^{15}N$ atom%, and T is the incubation time. This equation assumes no formation of nutrient during the incubation and therefore rates presented here are representative of potential rates. The same equation can be used for estimating C uptakes rates, where, P is the amount of particulate C in post incubation sample, $\Delta I_p$ is the increase in $^{13}C$ atom% in particulate C during incubation, $S_a$ and $S_t$ are ambient and added concentration, respectively, $I_r$ and $I_0$ are $^{13}C$ atom% of added tracer and natural $^{13}C$ atom% [*Slawyak et al.*, 1977].


*Figure 2.3: Delta V plus isotope ratio mass spectrometer and FLASH EA 2000 at the Physical Research Laboratory.*

### 2.2.2 $N_2$ Fixation rates

Experiments to measure $N_2$ fixation rates during the pre-monsoon season were conducted using the $^{15}N_2$ bubble method [*Montoya et al.*, 1996] using $^{15}N_2$ (98+ atom %) tracer gas procured from Cambridge Isotope Laboratories (CIL). Water samples collected with a clean bucket (for estuary) and Niskin sampler (for coastal waters) were

transferred to four 250 ml borosilicate bottles (three light). These bottles were filled to the brim and sealed tightly with septum caps to ensure the absence of air bubbles. Five tenths ml of $^{15}N$ enriched $N_2$ gas was injected into the sample bottles using a chromatographic syringe followed by gentle mixing and 4 hrs of incubation. Post-incubated samples were filtered onto pre-combusted 25 mm Whatman GF/F filters and dried at 50°C overnight. Ambient concentrations of dissolved $N_2$ gas were estimated using the gas solubility table of *Weiss* [1970] and the $N_2$ fixation rates were calculated using *Montoya et al.* [1996]. These experiments were performed at all stations along the off Cochin and off Mangalore transects at surface, 10, and 20 m depths, except at nearshore stations, where it was performed only on surface waters. Experiments were also performed in a *Trichodesmium* bloom patch observed around MR2-0 off Mangalore [*Table* 3.1]). Similar experiments were conducted on the surface waters of estuarine stations.

The $^{15}N_2$ bubble method [*Montoya et al.*, 1996] used during the present study has some drawbacks. The main drawback is the incomplete dissolution of $^{15}N_2$ gas in the sample within the incubation time, which may underestimate the $N_2$ fixation rates [*Mohr et al.*, 2010; *Großkopf et al.*, 2012]. The release of fixed N, during the incubation, as regenerated N forms [*Capone et al.*, 1994, *Bronk et al.*, 1994, *Mulholland et al.*, 2004] may also contribute to the underestimation. A recent paper by *Dabundo et al.* [2014] reported that other N forms ($NH_4^+$, $NO_3^-$, and $N_2O$) as impurities in the $N_2$ tracer gas supplied by different distributers too can cause a misinterpretation of the $N_2$ fixation rates. However, $N_2$ gas supplied by the CIL (used during the present study) had only very low concentrations of $NH_4^+$ (0.052µmol/ 1

mol of $N_2$ gas) and non-detectable $NO_3^-$ and $N_2O$ compared to the gas supplied by Sigma-Aldrich [*Dabundo et al.*, 2014].

## 2.3 $\delta^{13}C_{DIC}$ Analysis

Samples for $\delta^{13}C_{DIC}$ were collected using a Niskin sampler and transferred to 125 ml amber glass bottles without air bubbles. 1 ml of concentrated $HgCl_2$ was added to stop the microbial activity. A gas-tight cap was placed on the bottle immediately. For mass spectrometric analysis, 12 ml septum vials containing 0.1 ml of 100 % pure orthophosphoric acid was flushed with Helium gas in a gas bench system. 1 ml of coastal samples was injected to these vials using a gas tight syringe and it was kept for 18 hours at 28°C. Isotopic analyse was carried out using continuous flow IRMS (Delta V plus) attached to a Gas Bench system [*Figure* 2.3].

$NaHCO_3$ of $\delta^{13}C = -11.4$ ‰ was used as lab standard and final values of the samples were represented with respect to international standard (V-PDB). The $\delta^{13}C$ was calculated based on the standard equation given below:

$$\delta^{13}C(‰) = \left( \frac{R_{Sample} - R_{V-PDB}}{R_{V-PDB}} \right) \times 10^3$$

The reproducibility of the isotopic measurements was better than 0.1‰.

## 2.4 $^{13}C$ and $^{15}N$ measurements in natural samples

### 2.4.1 Suspended particulate organic matter

Samples for suspended particulate organic matter (POM) were filtered through pre-combusted (450 °C for 4 h) GF/F filters (0.7 µm pore size).

The filtration volume varied from 0.5 to 2 L depending on the suspended load. The filters for $\delta^{13}C$ measurements were exposed to acid-fumes (36% HCl) to remove carbonates and dried in a clean oven at 60 ºC. A Flash EA 2000 coupled to a Thermo-Finnigan Delta V plus isotope ratio mass spectrometer [*Figure* 2.3] in continuous-flow mode was used for isotopic analyses. Isotopic ratios of C and N are expressed in the standard delta notation ($\delta^{13}C$ and $\delta^{15}N$, respectively) with respect to V-PDB and atmospheric nitrogen (Air $N_2$), respectively.

### 2.4.2 Zooplankton

Zooplankton samples were collected by trawling Bongo net with 200 µm porosity, from both the Cochin estuary and the coastal Arabian Sea [*Figure* 2.4].

*Figure 2.4 Collection of zooplankton samples using the bongo net.*

Samples dried at 60ºC and powdered fine were stored at −20°C until mass spectrometric analysis. Samples were analyzed in MAT 253 IRMS coupled to a Flash EA 2000 [*Figure* 2.5].

*Figure 2.5 MAT 253 isotope ratio mass spectrometer at Physical Research Laboratory.*

The precisions for $\delta^{13}C$ and $\delta^{15}N$ were 0.04 ‰ and 0.1 ‰, respectively. IAEA-CH-3 Cellulose and IAEA-N2-$(NH_4)_2SO_4$ were used as lab standards for $\delta^{13}C$ and $\delta^{15}N$ measurements, respectively. Final values are reported with respect to V-PDB and Air $N_2$ for C and N, respectively.

# Chapter 3

# Nitrogen dynamics in the Cochin estuary and coastal Arabian Sea

Situated at the interface of rivers, ocean, atmosphere and human settlements, estuaries are subjected to a large number of natural and anthropogenic influences, which can lead to significant ecological and biogeochemical changes within estuaries [*Cloern and Jassby*, 2012]. The countering tides and river discharge cause variations in the availability and composition of nutrients and affect major biogeochemical cycles and species diversity in estuaries and adjoining coastal waters [*Humborg et al*., 1997; *Rabalais et al*., 2009]. Although the supply of N to the estuarine-coastal ecosystems has increased significantly, P availability is the controlling factor for phytoplankton growth [*Karl et al*., 1995; *Sanudo-Whilhelmy et al*., 2001; *Mills et al*., 2004; *Neill*, 2005]. Anthropogenic inputs cause many estuarine-coastal systems to shift drastically from N limitation to an N surplus leading to eutrophication [*Bricker et al*., 1999], a major threat to most of the estuaries and coastal waters around the world [e.g., *Hoch and Kirchman,* 1995; *Guttuso et al*., 1998; *Middelburg and Nieuwenhuize*, 2000a, b; *Mulholland et al*., 2003].

Intensive autotrophic and heterotrophic activities have been observed in such ecosystems leading to an increase in anaerobic processes such as denitrification, anammox, and methanogenesis [*Fasham*, 2003]. Estuarine microbes are often sensitive to both N and P inputs [*Ryther and Dunstan*, 1971; *Schindler*, 1975; *Rudek et al.,* 1991; *Fisher et al*., 1999; *Boesch et al*., 2001; *Paerl and Piehler*, 2008; *Smith and Schindler*, 2009; *Paerl et al*., 2014]. Apart from nutrients, physical properties such as salinity [*McLusky*, 1971], pH, turbidity, and light availability [*Cloern*, 1987] also may regulate phytoplankton growth and distribution in estuaries.

At present, our knowledge of N and C cycling, particularly N uptake dynamics and its dependence on N and P distribution in tropical estuaries and the adjacent coastal waters remains rudimentary. Information about the rates of transformation of N and C in such systems is needed to develop mitigation strategies to restore and save the estuarine-coastal coupled systems from eutrophication. Some studies related to N uptake dynamics in estuaries and the adjacent coastal regions in higher latitudes provide important insights into such coupled ecosystems [e.g., *Middelburg and Nieuwenhuize*, 2000a, b; *Mulholland et al*., 2003]. In India, research on coastal and estuarine biogeochemistry has gained momentum with emphasis on C cycling [e.g., *Sarma et al*., 2001; *Bouillon et al*., 2003; *Gupta et al*., 2008, 2009; *Shoji et al*., 2008]. However, most N biogeochemical cycling processes remain understudied.

With these perspectives as guidelines, we measured N transformation rates in a tropical eutrophic estuary, the Cochin estuary and the adjacent coastal Arabian Sea. The central hypothesis of this study was that $N_2$ fixation is not significant in this estuarine system with high

DIN concentrations. Our objectives were to (1) estimate DIN uptake and $N_2$ fixation rates in the Cochin estuary and coastal waters of the Arabian Sea, (2) understand the role of N:P stoichiometry in regulating N uptake and $N_2$ fixation rates, (3) understand the effect of anthropogenic activities on N uptake rates, (4) determine the role of hydrographic parameters in modulating N uptake rates in a nutrient replete estuarine environment, and (5) investigate whether nutrient exchange between estuary and coastal waters affects N uptake rates.

## 3.1 Study area

### 3.1.1 Cochin Estuary

The Cochin estuary is a eutrophic micro-tidal system [*Figure* 3.1], with two openings to the Arabian Sea (Bar mouth and Azhikkode) exchanging saline water and nutrients [*Gupta et al.*, 2009]. Tides in the estuary are mixed, predominantly semi-diurnal type with an average tidal range of 1m [*Qasim and Gopinathan*, 1969]. Total annual freshwater input to this estuary from various sources is about $2 \times 10^{10}$ $m^3$, which makes it the largest wetland along the west coast of India [*Srinivas et al.*, 2003]. Freshwater flow into this estuary is primarily contributed by six rivers. The largest of them is the Periyar River, which feeds 30% of its discharge into the northern part of the estuary and the remaining 70% discharges directly into the Arabian Sea through the Barmouth inlet [*Sivaprasad et al.*, 2012]. Additionally, the estuary receives a large volume of unprocessed industrial ($104 \times 10^3$ $m^3$ $d^{-1}$) and domestic ($0.26 \times 10^3$ $m^3$ $d^{-1}$) sewage from the city of Cochin and the remote areas through river runoff [*KSPCB* 1982, 1986; *Balachandran et al.*, 2006]. Together, these have altered the nutrient stoichiometry of this ecosystem leading to changes in microbial activity and biogeochemistry [*Martin et al.*, 2008; *Miranda*

*et al.*, 2008; *Shoji et al.*, 2008]. Also, the eventual transfer of nutrients to the coastal Arabian Sea can potentially affect the metabolic activity of coastal phytoplankton, species diversity, and the microbial food-web.

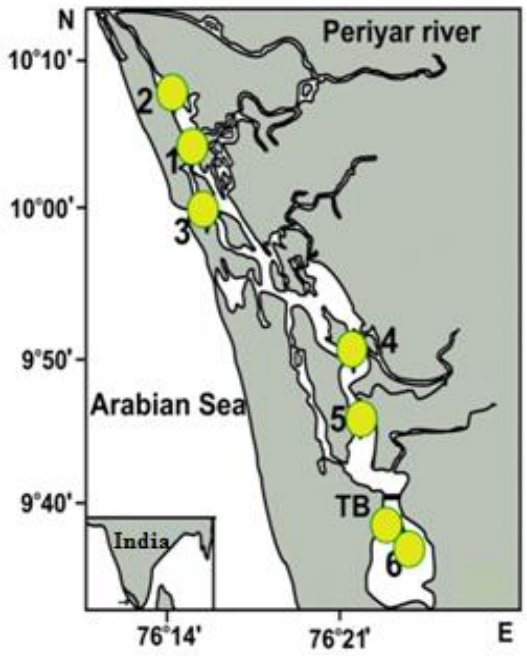


*Figure 3.1: A schematic map of Cochin estuary. The colored circles and the numbers indicate the stations at which measurements were carried out.*

In the Cochin estuary, experiments to measure $NO_3^-$ and $NH_4^+$ uptake rates were conducted (pre-monsoon: 26$^{th}$ April–1$^{st}$ May; monsoon: 16$^{th}$–20$^{th}$ September; post-monsoon: 30$^{th}$ December–2$^{nd}$ January, 2012) immediately after each coastal Arabian Sea cruise. DIN and C uptake experiments were performed at five stations (Station;1- Bolghatty Periyar, 2- Null zone, 3- Barmouth, 4- Arikkutty and 5- Vaikom) across the salinity gradient. Similar experiments were also performed at two stations (Station 6.a and 6.b; *Figure* 3.1) in the fresh water Vembanad Lake, located upstream of the estuary separated by a dam. Experiments for $N_2$ fixation were carried out in 6 stations during pre-monsoon.

### 3.1.2 Arabian Sea


*Figure 3.2: Sampling locations in the coastal Arabian Sea. Yellow stars denote the sampling stations.*

Three cruises were conducted onboard *FORV-Sagar Sampada* during pre-monsoon (April; SS 299), southwest monsoon (September; SS 306), and post-monsoon (December; SS 311) of 2012 along two transects (Off Cochin and Off Mangalore) in the Arabian [*Figure* 3.2]. *In situ* incubations for $NO_3^-$ and $NH_4^+$ uptake rates along the off Cochin transect were performed at stations with water column depth of 13 m (near shore; surface), 30 m (inner shelf; 0, 10 and 25 m depths), 50 m (mid-shelf; 0, 10, 20 and 40 m depths), and 100 m (outer shelf; 0, 10, 20, 50 and 75 m depths). The same was performed along the off Mangalore transect at 13 m (near shore; surface), 50 m (mid-shelf; 0, 10, 20 and 40 m depths), and 100 m (outer shelf; 0, 10, 20, 40, and 60 m depths) water column depth stations during the pre-monsoon [*Table* 3.1]. During southwest monsoon, the sample depths were limited up to 20 m due to the practical difficulty of mooring the samples. The same

depth stations along the off Cochin were farther from the coast compared to the off Mangalore due to the gentle slope of the continental shelf at off Cochin.

*Table 3.1: The details of sampling locations, station depth, distance from the coast in two transects in the coastal Arabian Sea.*

| Sample code | Geographical Coordinates | Station depth (m) | Distance from the coast (km) | Sampling depth (m) |
|---|---|---|---|---|
| Cochin (CH) | | | | |
| CH1-0 | 09.58º N, 76.08º E | 13 | 10.69 | 0 |
| CH2-0 | 09.58º N, 75.59º E | 30 | 25.13 | 0 |
| CH2-10 | | | | 10 |
| CH2-20 | | | | 25 |
| CH3-0 | 09.97º N,75.84º E | 50 | 44.29 | 0 |
| CH3-10 | | | | 10 |
| CH3-20 | | | | 20 |
| CH3-40 | | | | 40 |
| CH4-0 | 09.58º N,75.39º E | 100 | 65.99 | 0 |
| CH4-10 | | | | 10 |
| CH4-20 | | | | 20 |
| CH4-40 | | | | 50 |
| CH4-75 | | | | 75 |
| Mangalore (MR) | | | | |
| MR1-0 | 12.50º N, 74.50º E | 13 | 12.00 | 0 |
| MR2-0 | 12.50º N, 74.29º E | 50 | 36.59 | 0 |
| MR2-10 | | | | 10 |
| MR2-20 | | | | 20 |
| MR2-40 | | | | 40 |
| MR3-0 | 12.85º N, 74.16º E | 100 | 72.39 | 0 |
| MR3-10 | | | | 10 |
| MR3-20 | | | | 20 |
| MR3-40 | | | | 40 |
| MR3-60 | | | | 60 |

## 3.2 Environmental parameters

The Cochin estuary is an aquatic system where all the chemical biological and hydrographical parameters show large temporal and

spatial variations [*Srinivas et al.*, 2003; *Balachandran et al.*, 2006; *Martin et al.*, 2013]. Salinity in the estuary is mainly controlled by seawater intrusions and freshwater inputs, which in turn depends on the tidal cycle and season. During pre monsoon, salinity ranged from 5.8 – 15.7, possibly due to the heavy pre-monsoon rainfall and low tide condition. The salinity range during the south west monsoon season was the narrowest among the three seasons due to high fresh water inputs. Sampling period during post-monsoon was free from rainfall and was coincided with high tide phase which resulted in higher salinity range (12 – 32) among the three seasons. The estuarine pH varied from 5.73 – 8.06 throughout the sampling period, where the minimum and maximum were observed during the monsoon and post-monsoon, respectively [*Table* 3.2].

The Chl *a* concentrations in the estuary showed wide range in all three seasons (5.29 – 26.8 μg $l^{-1}$) with the highest during the post-monsoon. The narrowest range of Chl *a* was observed during monsoon (2.76 – 6.74 μg $l^{-1}$) and moderately high during the pre-monsoon (1.77 – 17.2 μg $l^{-1}$). The $NO_3^-$ and $NH_4^+$ concentrations in the estuary were higher compared to the coastal waters throughout the study period. The highest range was observed during the pre-monsoon ($NO_3^-$: 1.25 – 26.6 μM and $NH_4^+$: 2.11 – 75.2 μM) than the monsoon ($NO_3^-$: 0.73 – 16.7 μM and $NH_4^+$: 0.39 – 25.2 μM) and the post-monsoon ($NO_3^-$: 0.09 – 4.2 μM and $NH_4^+$: 1.6 – 3.2 μM) [*Table* 3.2]. Stations [station 6a & b; *Figure* 1] in the Vembanad Lake showed consistently low salinity throughout the year i.e., 0.26, 0.04, and 1.90, during the pre-monsoon, monsoon, and post monsoon, respectively [*Table* 3.2]. The Vembanad Lake showed lower pH than estuarine regions with an average of 6.13 for the study period (pre-monsoon: 6.03; monsoon: 5.61–5.59; and post-monsoon: 6.41–6.47; *Table* 3.2). The Chl *a* in the Vembanad Lake was high in all

*Table 3.2: The environmental parameters in the Cochin estuary during the pre-monsoon, monsoon, and post-monsoon seasons.*

| Sampling period | Station No: | Temperature (°C) | Salinity | pH | Chl *a* (μg $l^{-1}$) | $NO_3^-$ (μM) | $NH_4^+$ (μM) |
|---|---|---|---|---|---|---|---|
| Pre monsoon | 1 | 29.5 | 1.58 | 6.42 | 1.77 | 26.6 | 75.2 |
| | 2 | 29.9 | 15.6 | 7.26 | 17.2 | 1.25 | 5.22 |
| | 3 | 30.1 | 13.0 | 7.33 | 8.07 | 4.97 | 13.7 |
| | 4 | 30.6 | 6.27 | 6.93 | 6.44 | 8.65 | 2.11 |
| | 5 | 29.1 | 5.31 | 6.40 | 2.53 | 5.2 | 4.36 |
| | 6 | 29.4 | 0.26 | 6.03 | 12.3 | 25.4 | 5.02 |
| Monsoon | 1 | 27.2 | 0.12 | 6.10 | 3.31 | 16.7 | 25.1 |
| | 2 | 28.9 | 1.60 | 7.10 | 2.76 | 0.73 | 0.39 |
| | 3 | 28.5 | 5.36 | 7.02 | 3.05 | 12.9 | 5.51 |
| | 4 | 29.9 | 0.02 | 6.45 | 5.05 | 8.37 | 4.22 |
| | 5 | 29.7 | 0.07 | 5.73 | 6.74 | 4.71 | 4.36 |
| | 6.1 | 29.7 | 0.04 | 5.61 | 1.67 | 11.3 | 0.10 |
| | 6.2 | 29.7 | 0.04 | 5.93 | 7.76 | 5.01 | 2.84 |
| Post monsoon | 1 | 28.5 | 12.9 | 7.19 | 21.7 | 4.20 | 2.11 |
| | 2 | 28.9 | 20.4 | 7.48 | 26.8 | 0.09 | 1.59 |
| | 3 | 29.0 | 32.1 | 8.06 | 5.53 | 1.05 | 2.99 |
| | 4 | 29.6 | 18.5 | 7.69 | 22.4 | 1.13 | 2.13 |
| | 5 | 30.0 | 11.1 | 6.91 | 5.30 | 1.74 | 3.21 |
| | 6.1 | 29.8 | 1.92 | 6.47 | 13.6 | 2.05 | 2.18 |
| | 6.2 | 29.6 | 1.90 | 6.17 | 11.5 | 2.44 | 2.29 |

three seasons (pre-monsoon: 12.3, monsoon: 1.67–7.76, and post-monsoon: 11.5–13.6 μg $l^{-1}$). The excess reactive nitrogen inputs from the anthropogenic activities introduced high $NO_3^-$ (pre-monsoon: 25.4 μM, monsoon: 5.01 μM, and post-monsoon: 2.44 μM) and $NH_4^+$ (pre-monsoon: 5.02 μM, monsoon: 2.84 μM, and post-monsoon: 2.29 μM) into the Vembanad Lake.Although nutrient concentrations in the surface waters of the coastal Arabian Sea were less than that of the estuary; during the pre-monsoon and monsoon sampling periods, near shore station of the off Cochin transect showed higher DIN concentration compared to average coastal nutrient values, possibly due to inputs from the estuary.

*Table 3.3: The nutrients and Chl a concentrations in the Cochin and Mangalore transects in the coastal Arabian Sea during pre-monsoon, monsoon, and post monsoon seasons.*

| | Sample code | Chl *a* (µg $l^{-1}$) | $NO_3^-$ (µM) | $NH_4^+$ (µM) | $PO_4^{3-}$ (µM) | Chl *a* (µg $l^{-1}$) | $NO_3^-$ (µM) | $NH_4^+$ (µM) | $PO_4^{3-}$ (µM) | Chl *a* (µg $l^{-1}$) | $NO_3^-$ (µM) | $NH_4^+$ (µM) | $PO_4^{3-}$ (µM) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pre-monsoon | | | | Monsoon | | | | Post-monsoon | | | |
| Cochin transect | CH1-0 | 2.60 | 0.89 | 1.94 | 0.24 | 9.5 | 1.84 | 4.00 | 0.43 | 1.91 | 0.57 | 1.46 | 0.17 |
| | CH2-0 | 0.11 | 0.05 | 0.22 | 0.50 | | 0.41 | 0.27 | 0.10 | 0.19 | 0.14 | 0.08 | 0.38 |
| | CH2-10 | 0.22 | 0.09 | 0.24 | 0.18 | | 11.2 | 0.53 | 1.13 | 0.25 | 0.25 | BDL | 0.31 |
| | CH2-20 | 0.45 | 0.09 | 0.22 | 0.55 | | 13.3 | 0.03 | 1.18 | 0.43 | 0.31 | 0.26 | 0.67 |
| | CH3-0 | 0.24 | 0.13 | 1.73 | 0.22 | 1.39 | 0.35 | 2.81 | 0.36 | 0.15 | 0.33 | BDL | 0.11 |
| | CH3-10 | 0.21 | 0.07 | 1.51 | 0.23 | 3.15 | 2.81 | 1.08 | 0.46 | 0.14 | 0.36 | BDL | 0.11 |
| | CH3-20 | 0.31 | 0.20 | 1.47 | 0.23 | 4.33 | 19.7 | 0.73 | 0.95 | 0.18 | 0.42 | BDL | 0.13 |
| | CH3-40 | 2.05 | 5.77 | 1.67 | 0.47 | 0.13 | 22.6 | 1.21 | 1.71 | 0.21 | 1.17 | BDL | 0.15 |
| | CH4-0 | 0.11 | 0.06 | 0.41 | 0.11 | 0.40 | 0.15 | 0.35 | 0.27 | 0.13 | 0.23 | 0.48 | 0.13 |
| | CH4-10 | 0.10 | 0.06 | 0.87 | 0.17 | 0.38 | 0.25 | 0.54 | 0.27 | 0.13 | 0.09 | 0.73 | 0.40 |
| | CH4-20 | 0.13 | 0.15 | 0.76 | 0.08 | 0.73 | 11.2 | 0.41 | 0.91 | 0.15 | 0.19 | 0.45 | 0.22 |
| | CH4-40 | | | | | | | | | 0.16 | 0.21 | 0.62 | 0.18 |
| | CH4-50 | 0.28 | 3.98 | 1.28 | 0.53 | | | | | | | | |
| | CH4-75 | 0.16 | 13.7 | 0.68 | 1.23 | | | | | | | | |
| Mangalore transect | MR1-0 | 2.31 | 0.24 | 0.92 | 0.40 | 4.79 | 0.09 | 2.97 | 0.40 | 7.10 | 1.71 | 0.89 | 0.63 |
| | CH2-0 | | | | | 1.26 | 0.29 | 2.01 | 0.21 | 0.37 | 0.59 | 0.37 | 0.18 |
| | CH2-10 | | | | | 20.3 | 12.6 | 0.06 | 0.21 | 0.32 | 0.43 | 0.28 | 0.20 |
| | CH2-20 | | | | | 2.49 | 0.49 | 4.37 | 0.24 | 0.68 | 1.91 | 0.94 | 0.53 |
| | MR2-0 | 0.08 | 0.04 | 1.69 | 0.05 | 0.62 | 0.27 | 0.62 | 0.05 | 0.27 | 0.17 | 0.09 | 0.11 |
| | MR2-10 | 0.11 | 0.08 | 0.57 | 0.06 | 11.17 | 16.2 | 0.75 | 0.06 | 0.26 | 0.01 | BDL | 0.11 |
| | MR2-20 | 0.14 | 0.03 | 0.26 | 0.05 | 0.82 | 20.4 | 0.83 | 0.05 | 0.43 | 0.08 | BDL | 0.13 |
| | MR2-40 | 0.14 | 0.05 | 4.89 | 0.09 | | | | | | 2.82 | 0.08 | 0.47 |
| | MR3-0 | 0.15 | 0.11 | 1.53 | 0.03 | 0.67 | 0.97 | 0.64 | 0.03 | 0.14 | 0.06 | 0.05 | 0.11 |
| | MR3-10 | 0.11 | 0.11 | 1.19 | 0.05 | 0.40 | 3.48 | 1.20 | 0.05 | 0.17 | 0.04 | 0.14 | 0.15 |
| | MR3-20 | 0.11 | 0.07 | 0.75 | 0.09 | 0.35 | 20.1 | 1.05 | 0.09 | 0.18 | 0.01 | 0.09 | 0.22 |
| | MR3-40 | 0.06 | 0.14 | 1.06 | 0.12 | | | | | 0.24 | 0.63 | | 0.42 |
| | MR3-60 | | 3.30 | 0.56 | 0.49 | | | | | 0.18 | | | |

During the pre-monsoon, at the near shore surface waters, both $NO_3^-$ and $NH_4^+$ concentrations were higher for the off Cochin (0.89 μM and 1.94 μM, respectively) compared to that of the off Mangalore transect (0.24 μM and 0.92 μM, respectively) [*Table* 3.3]. Near shore station in the off Cochin transect (1.84 μM and 4.00 μM, respectively) had higher $NO_3^-$ and $NH_4^+$ concentrations than off Mangalore transect (0.09 μM and 2.97 μM, respectively) during monsoon as well. In contrast, during the post-monsoon, the near shore station at the off Mangalore transect (1.71 μM) showed higher $NO_3^-$ concentration compared to that of the off Cochin transect (0.57 μM). However, $NH_4^+$ concentration was still higher in the off Cochin transect (1.46 μM) than that of the off Mangalore transect (0.89 μM) at the nearshore station [*Table* 3.3].

## 3.3 Nitrogen sources to the Cochin estuary

One of the prime reasons for nutrient replete conditions in the Cochin estuary is inorganic and organic N loadings into the estuary from several anthropogenic sources [*Gupta et al.*, 2008; *Balachandran et al.*, 2006]. The shallow photic depth (~1 m, equivalent to 10-30% of entire water column) of the estuary suggests that mineralization of OM in the dark bottom waters could be another source of DIN. The leaching/remineralization of resuspended sediments due to navigational activities in the lower estuary are important sources of N [*Gopalan et al.*, 1983, *Martin et al.*, 2010]. The mesohaline regions in the estuary receive 7–11 times high organic matter as lateral input as compared to the inputs through rivers [*Shoji et al.*, 2008; *Gupta et al.*, 2009]. Another possible source of N to the estuarine and coastal waters is atmospheric deposition [*Paerl,* 1993, 1995]. A recent study reported atmospheric $NO_3^-$ and $NH_4^+$ inputs to the Arabian Sea to be around 6.5 nmol $m^{-3}$ and 0.4 nmol $m^{-3}$, respectively [*Singh et al.*, 2012; *Srinivas*

*and Sarin*, 2013], which is too low to make a significant contribution to the DIN pool of the estuary or the coastal waters.

## 3.4 Nitrogen uptake rates in the Cochin estuary

### 3.4.1 Total DIN uptake rates

The high nutrient and chlorophyll concentrations resulted in high uptake rates for $NO_3^-$ (pre-monsoon: 0.01–0.38; monsoon: 0.016–0.15; post monsoon: 0.03–0.85 µmol N $h^{-1}$) and $NH_4^+$ (pre-monsoon: 0.32–0.95; monsoon: 0.06–0.63; post monsoon: 0.26–0.52 µmol N $h^{-1}$) [*Figure* 3.3].

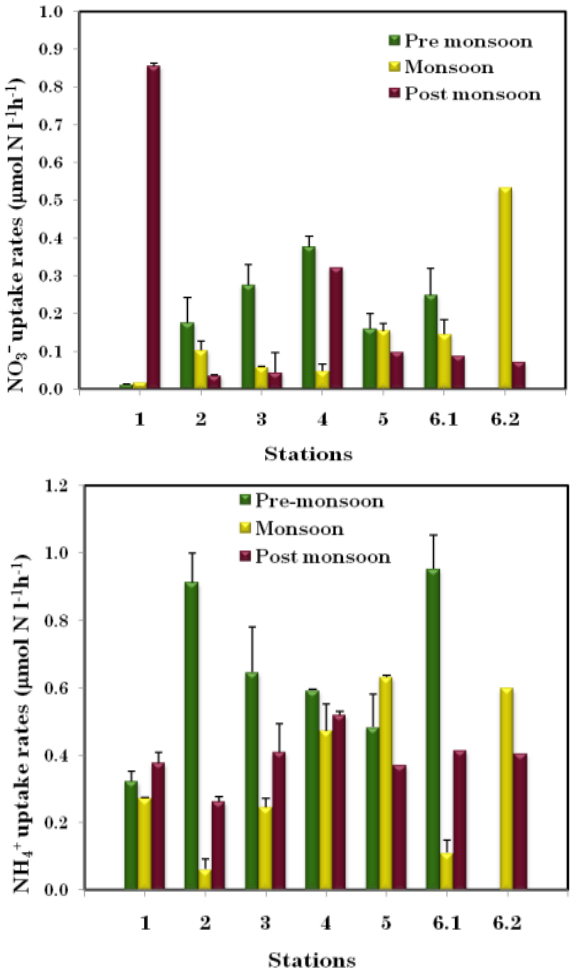


*Figure 3.3: The $NO_3^-$ and $NH_4^+$ uptake rates in the Cochin estuary.*

In general, the lowest range for $NH_4^+$ uptake was observed during monsoon and $NO_3^-$ was relatively lower during the post-monsoon. However, $NO_3^-$ uptake rate (0.85 μmol N $h^{-1}$) at station 1 during post-monsoon was observed to be the highest in the Cochin estuary during the study period. In general, $NH_4^+$ uptake dominated during all seasons.

### 3.4.2 Nanoplankton uptake rates

It is well known that small phytoplankton and the heterotrophic bacteria play a significant role in N uptake [*Laws et al.*, 1985; *Kirchman*, 1994; *Middelburg and Nieuwenhuize*, 2000b]. Among them nanophytoplankton whose size ranges from 2 to 20 μm in diameter contributes nearly 90% of the world's marine biomass. Although we could not estimate the bacterial removal of N forms we attempted to account nanoplankton size effects in the Cochin estuary. *Figure* 3.4 shows the values of $NO_3^-$ and $NH_4^+$ uptake rates by nanaoplankton in the Cochin estuary during the study period.

The results show nanoplankton contributions to the total DIN uptake rates during monsoon and post monsoon varied between 75-95% and 75-98%, respectively. Similarly, the fraction of DIN uptake rates by nanoplankton (monsoon: 77-98% and post monsoon: 79-96%) were also in agreement with higher influence of nanoplankton in N removal in this eutrophic system. Previous studies reported that nanoplankton (2-20 μm) contribute about 80% of primary production in the Cochin estuary and coastal waters followed by microplankton (>20 μm) and picoplankton (0.2-2.0 μm) during summer monsoon [*Madhu et al.*, 2010]. However, ours is the first study to account the contribution of nanoplankton activity directly using the $^{15}N$ labelling technique in a tropical eutrophic estuary, particularly in India.

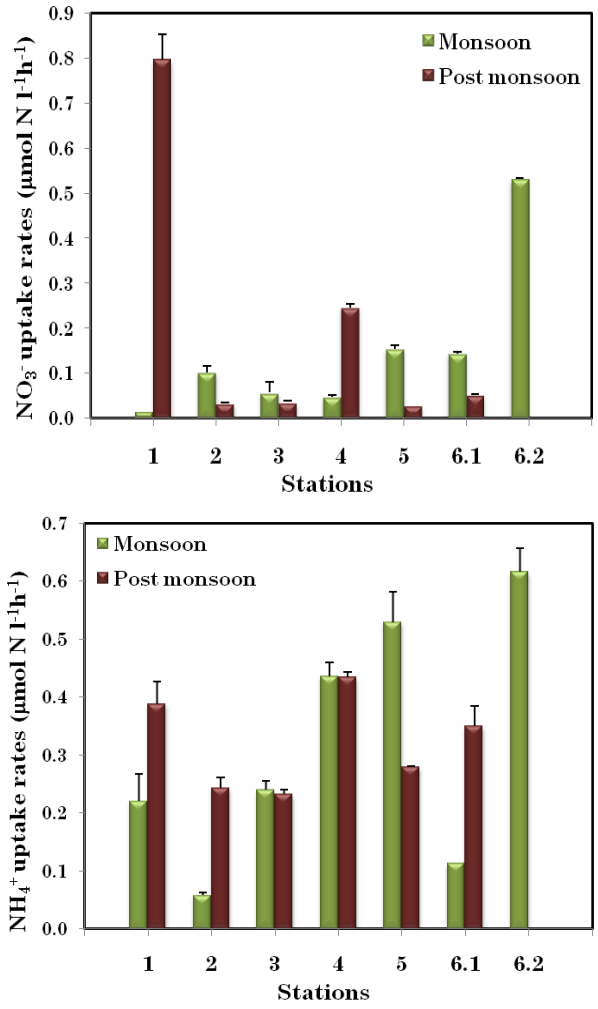


*Figure 3.4: $NO_3^-$ and $NH_4^+$ uptake rates by nanaoplankton in the Cochin estuary during the pre monsoon and monsoon seasons in 2012.*

## 3.5 Nitrogen assimilation in the coastal Arabian Sea

The $NO_3^-$ uptake rates in the two transects in the coastal Arabian Sea (off Cochin; pre-monsoon: 0.003–0.176 µmol N $h^{-1}$, monsoon: 0.011–0.34 µmol N $h^{-1}$, post-monsoon: 0.001–0.01 µmol N $h^{-1}$ and off Mangalore; pre-monsoon: 0.003–0.03 µmol N $h^{-1}$, monsoon: 0.008–0.052 µmol N $h^{-1}$, post-monsoon: 0.0005–0.26 µmol N $h^{-1}$) were observed to be lower compared to the Cochin estuary.

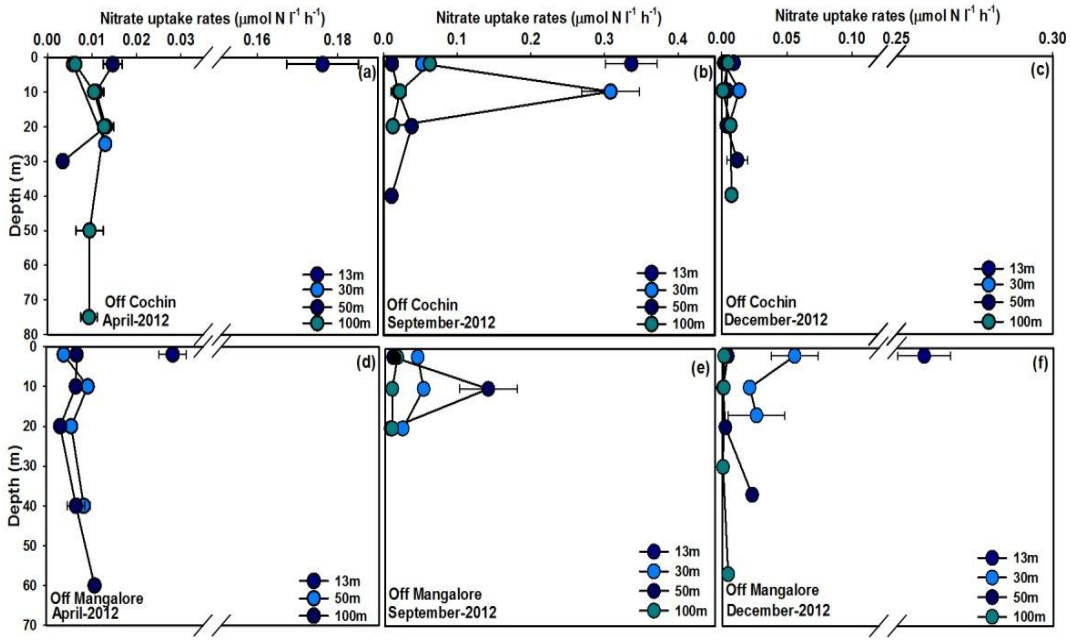


*Figure 3.5: (a), (b), and (c) show the depth profiles of $NO_3^-$ uptake rates in the off Cochin transect during pre-monsoon, monsoon and post-monsoon, respectively and (d), (e), and (f) shows the corresponding profiles in the off Mangalore transect.*

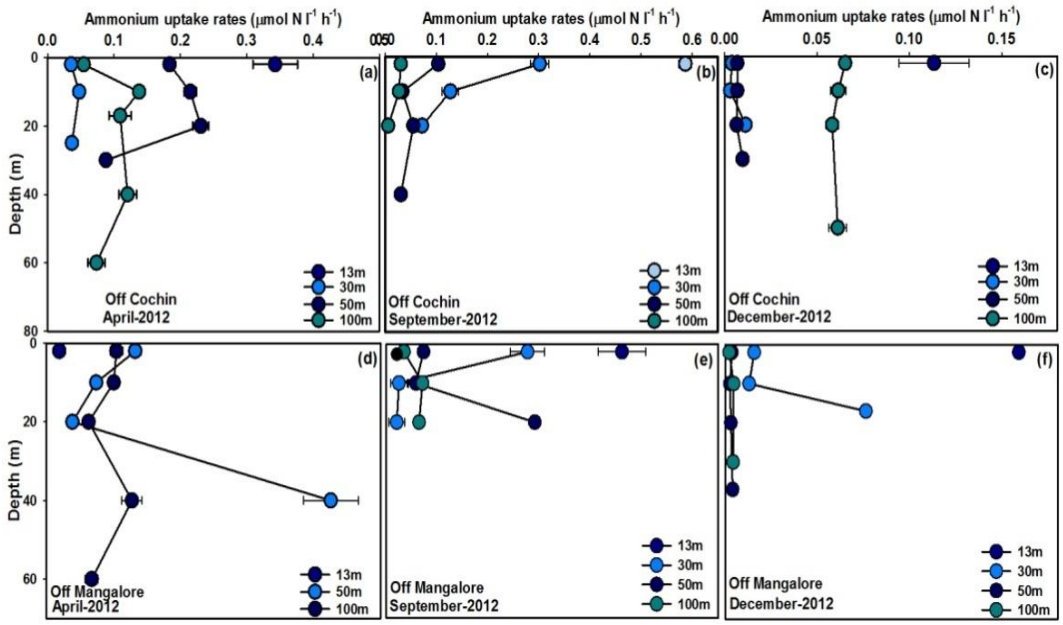


*Figure 3.6: (a), (b), and (c) show the depth profiles of $NH_4^+$ uptake rates in the off Cochin transect during pre-monsoon, monsoon and post-monsoon, respectively and (d), (e), and (f) shows the corresponding profiles in the off Mangalore transect.*

In general, $NH_4^+$ uptake rates (off Cochin; pre-monsoon: 0.035–0.34 µmol N $h^{-1}$, monsoon: 0.006–0.59 µmol N $h^{-1}$, post-monsoon: 0.003–0.11 µmol N $h^{-1}$ µmol N $h^{-1}$ and off Mangalore; pre-monsoon: 0.019–0.43 µmol N $h^{-1}$, monsoon: 0.02–0.46 µmol N $h^{-1}$, post-monsoon: 0.002–0.16 µmol N $h^{-1}$) were higher than $NO_3^-$ uptake rates [*Figure* 3.5 and 3.6]. These rates were significantly higher than that of oligotrophic waters [*Gandhi et al.*, 2011a; *Kumar et al.*, 2010]. The coastal Arabian is known for its sensitivity towards the seasonal wind reversal and following upwelling systems which alter the biogeochemical cycling [*Prakash et al.*, 2008; *Gandhi et al.*, 2011]. Apart from unique geographical features, anthropogenic inputs through the coastal waters also affect the biogeochemical dynamics of the Arabian Sea.

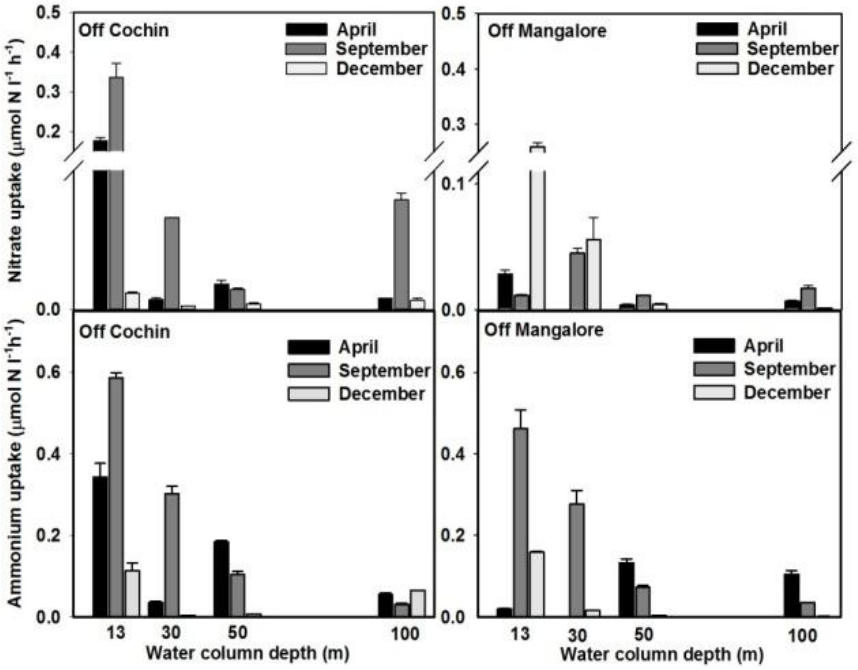


*Figure 3.7: $NO_3^-$ and $NH_4^+$ uptake rates in the surface waters in the two coastal transects (Cochin and Mangalore) with distance from the coast. The x-axis denotes the water column depth at sampling locations.*

In the coastal Arabian Sea nutrients and light conditions were observed to be the major factors controlling the DIN uptake rates. The

DIN uptake rates (pre-monsoon: $NO_3^-$ ~ 0.18 and $NH_4^+$ ~ 0.34; monsoon: $NO_3^-$ ~ 0.33 and $NH_4^+$ ~ 0.58 μmolN $L^{-1}$ $h^{-1}$) in the nearshore station off Cochin were observed to be higher than that of off Mangalore (pre-monsoon: $NO_3^-$ ~ 0.03 and $NH_4^+$ ~ 0.02; monsoon: $NO_3^-$ ~ 0.01 and $NH_4^+$ ~ 0.46 μmolN $L^{-1}$ $h^{-1}$) [*Figure* 3.7].

The direct interaction with the Cochin estuary nourishes the offshore Cochin transect and this coupled system exchanges lots of organic and inorganic matters [*Gopalan et al.*, 1983; *Gupta et al.*, 2009] whereas, off Mangalore is relatively away from nutrient loading, apart from its weak connection a minor estuarine system, the Nethravathi estuary. The higher N uptake rates in the off Cochin region and Cochin estuary has been reported recently [*Bhavya et al.*, 2015].

## 3.6 $N_2$ Fixation in the estuary and coastal waters

$N_2$ fixation rates were measured to ensure the relevance of diazotrophic activity in the eutrophic Cochin estuary and the coastal Arabian Sea during the pre-monsoon season. *Trichodesmium*, one of the important diazotrophs, fixes atmospheric N and contributes to the bio-available N pool in aquatic systems [*Subramaniam and Carpenter*, 1994; *Capone et al.*, 1997; *Mulholland and Capone*, 2009]. A gradual decline in *Trichodesmium* abundance from 1970–2005 along with an increase in nutrient loading has been observed in the Cochin estuary [*Qasim et al.*, 1969; *Thomson*, 2002; *Martin et al.*, 2013]. Recent observations indicate the presence of diazotrophs, particularly *Synechocystis* and *Synechococcus*, in the Cochin estuary [*Jabir et al.*, personal communication]. $N_2$ fixation rates in are generally expected to be low in estuaries because high $NH_4^+$ concentrations (>1 μM) can suppress the nitrogenase activity [*Mulholland and Capone*, 2001].

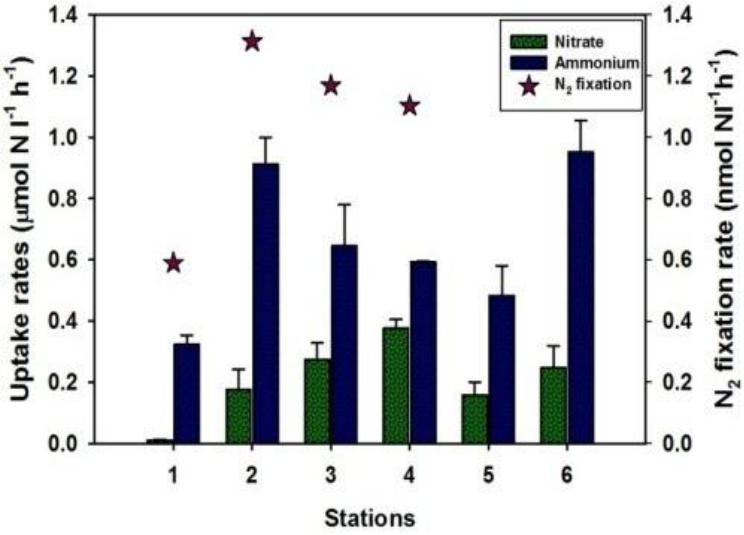


*Figure 3.8: DIN uptake and $N_2$ fixation rates during pre-monsoon season in the Cochin estuary.*

However, despite high DIN concentrations in the estuary, considerable $N_2$ fixation rates were observed (0.59–1.3 nmol N $l^{-1}$ $h^{-1}$) [*Figur*e 3.8]. Even though these rates are significantly lower than the DIN uptake rates, they provide evidence of diazotrophic activity in this eutrophic estuary.

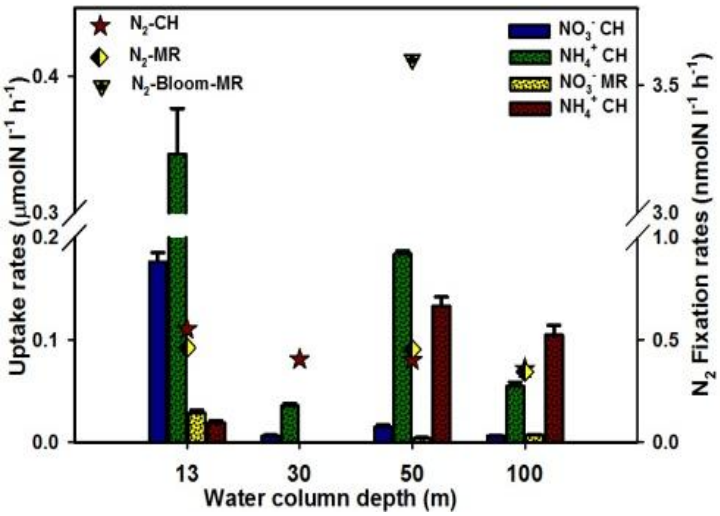


*Figure 3.9: DIN uptake rates and $N_2$ fixation rates in the coastal Arabian Sea. The horizontal axis represents the water column depth of each station in the two transects of the coastal Arabian Sea.*

In general, $N_2$ fixation in the coastal ocean at our regular stations ranged from 0.33–0.55 nmol N $l^{-1}$ $h^{-1}$. The nearshore surface waters off Cochin (CH1-0: 0.55 nmol N $l^{-1}$ $h^{-1}$) and Mangalore (MR1-0: 0.46 nmol N $l^{-1}$ $h^{-1}$) showed higher $N_2$ fixation rates among the coastal non-bloom stations. High concentrations of dissolved iron and P in inner shelf waters of Cochin [*Balachandran*, 2001] enhance $N_2$ fixation in this region. Based on the estimations from *Mulholland et al* [2006], $N_2$ fixation was examined as a new N source here. Assuming that a maximum of 50% of the fixed N is released in the form $NH_4^+$, the contribution of $N_2$ fixation to the $NH_4^+$ pool in the Cochin estuary was less than 0.05% (0.04 ± 0.042 %). This result was mainly due to higher ambient $NH_4^+$ concentration in the estuary. Similar calculations for coastal stations revealed a contribution of less than 0.5% (average ~ 0.17 ± 0.14 %) with the highest at the *Trichodesmium* bloom patch (0.42%). $N_2$ fixation was not a significant source of new N to the Cochin estuary and the coastal Arabian Sea during our study period. Previous studies in the open Arabian Sea, however, indicate that the *Trichodesmium* bloom patches are a significant source of new N in the surface waters [*Bange et al.*, 2000; *Gandhi et al.*, 2011].

## 3.7 Environmental parameters and N uptake rates

The possible controls of N uptake dynamics in this estuary has been examined thoroughly in this study by checking correlations of N uptake rates with nutrient concentrations ($NO_3^-$, $NH_4^+$, DIP, and Si), salinity, temperature, Chl *a* and TN: TP. There was no significant correlation observed between nutrients and N uptake rates. Overall, $NH_4^+$ was observed to be the preferred substrate throughout the sampling except some stations where $NO_3^-$ concentrations are significantly higher than $NH_4^+$ concentrations. Since the intra annual

temperature variation is not significant in this tropical estuary, the temperature control on uptake rates was not observed.

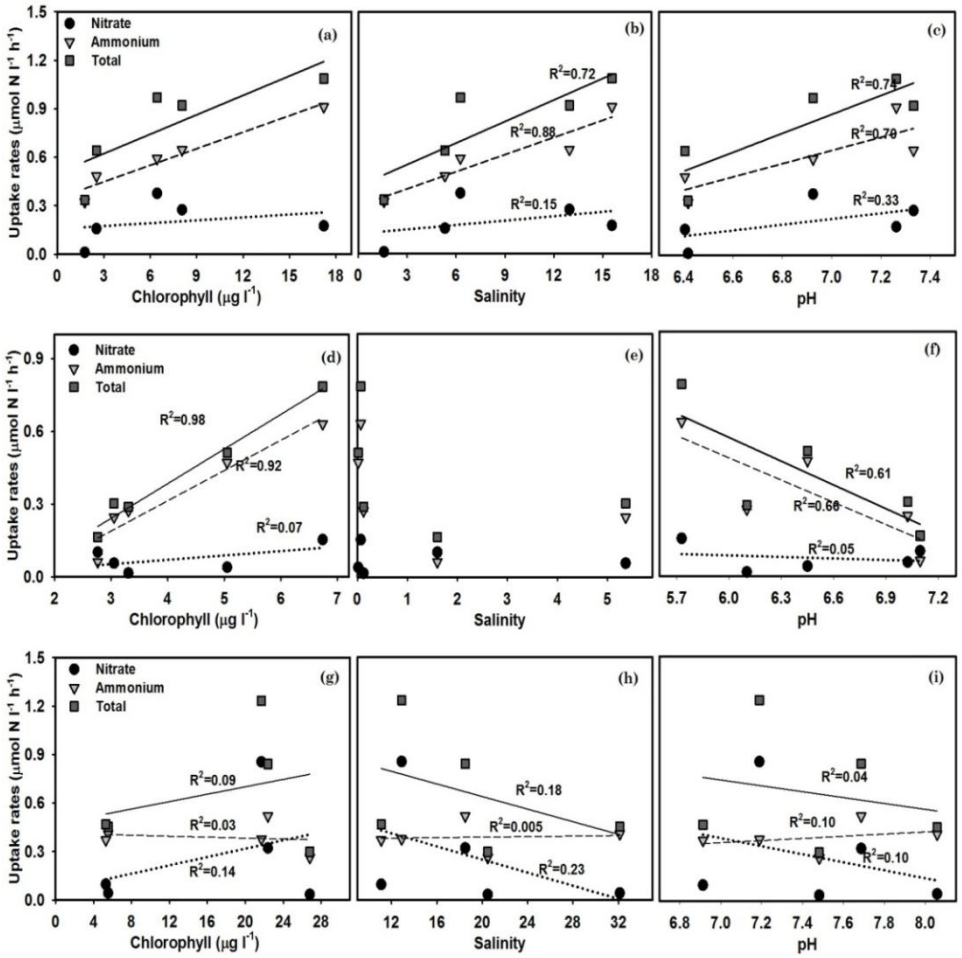


*Figure 3.10: The relationship of N uptake rates with Chlorophyll (a, d, and g), Salinity (b, e, and h), and pH (c, f, and i) in the Cochin estuary during per-monsoon, monsoon and post-monsoon, respectively.*

During the pre-monsoon, $NH_4^+$ uptake rates were in strong positive correlation with salinity, Chl *a*, and pH. However, this dependency of $NH_4^+$ uptake rates with salinity and pH was not sustained during monsoon and post-monsoon. Chl *a* concentration in the estuary was higher throughout with maximum during the post-monsoon. The $NH_4^+$ uptake rates were also showed strong positive correlation with Chl *a* during the pre-monsoon ($R^2$ = 0.94) and monsoon ($R^2$ = 0.92). In

contrast, $NH_4^+$ and $NO_3^-$ uptakes during the post-monsoon did not show any correlation with Chl *a* concentrations during post monsoon [*Figure* 3.10]. Interestingly, the DIN concentrations in the estuary were considerably lower than the DON concentrations, which indicate the possibility of DON uptake along with DIN uptake as a N source to the OM production. This may also be a reason for the poor relationship of DIN uptake rates with Chl *a*. Strong positive relationship with Chl *a* and PON concentrations during the three seasons is in agreement with higher primary productivity and hence N uptake. However, during the present study the DON uptake rates in this estuary were not accounted. The higher productivity followed by fast nutrient consumption might have led to the high chlorophyll concentration and low DIN concentrations.

## 3.8 TN:TP as a major controlling factor

One of the major problems related to high nutrient loading is the imbalance in the nutrient stoichiometry, which is required to maintain potential assimilation rates [*Bhavya et al.*, 2015]. Although estuaries, in general, sustain high nutrient concentrations, phytoplankton growth may ultimately be limited by the nutrient which is in lower proportion relative to another. It is reported that the major regulator of autotrophic assimilation is TN:TP than individual nutrient concentrations [*Gilbert et al.*, 2003; *Bhavya et al.*, 2015]. Although P concentrations are higher than those in the open ocean, it is often the limiting nutrient in freshwater and estuarine systems due to relatively higher proportion of N-nutrients [*Schindler*, 1977; *Hecky & Kilham*, 1988; *Yin et al.*, 2001; *Bhavya et al.*, 2015], where N:P plays an important role in regulating N metabolism.

During the present study, pre-monsoon $NH_4^+$ uptake rates showed a strong negative relationship with TN:TP which varied from 6.5:1 to 32:1. The effect of co-limitation of P on the total N uptake implies that more than higher nutrient concentration, co-availability matters more on the potential assimilation. Also, the higher N:P ratio at the low saline region indicate the freshwater supplied to the estuary by the rivers contains relatively higher proportion of N than P.

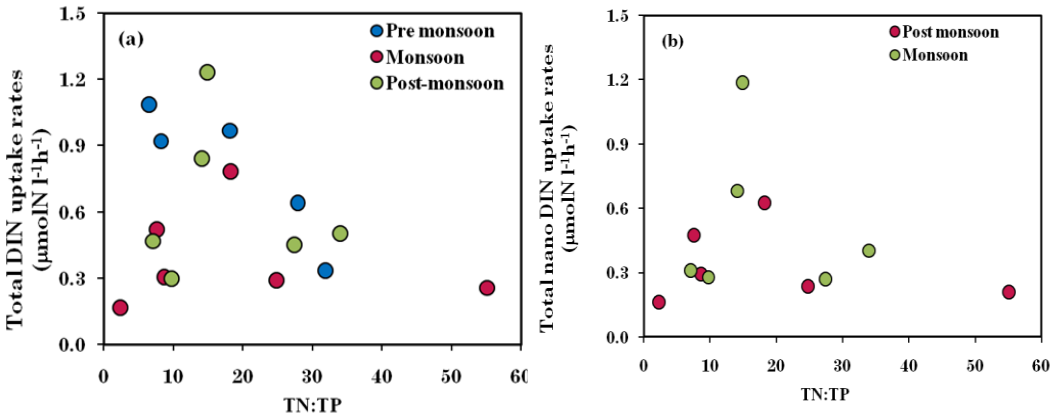


*Figure 3.11: The variability of DIN uptake rates with TN:TP in the cochin estuary during the pre-monsoon, monsoon, and post-monsoon.*

This relationship is not sustained during the monsoon and post monsoon. Interestingly, the stations with TN:TP close to the Redfield ratio were observed with higher DIN uptake rates. This indicate the potential N assimilation is favored with optimum TN:TP and co-limited by either N or P at stations having TN:TP deviating from the Redfield ratio [*Figure* 3.11]. It is worth noticing that the highest TN (118 µM) and TP (3.6 mM) during the whole study were observed at station 1, a location where the river Periyar meets the estuary, but it showed comparative limitation of P (TN:TP ~31.9:1). $N_2$ fixation rates in DIN enriched waters are generally expected to be low because high $NH_4^+$ concentrations (>1 µM) can suppress the nitrogenase activity [*Mulholland and Capone*, 2001].

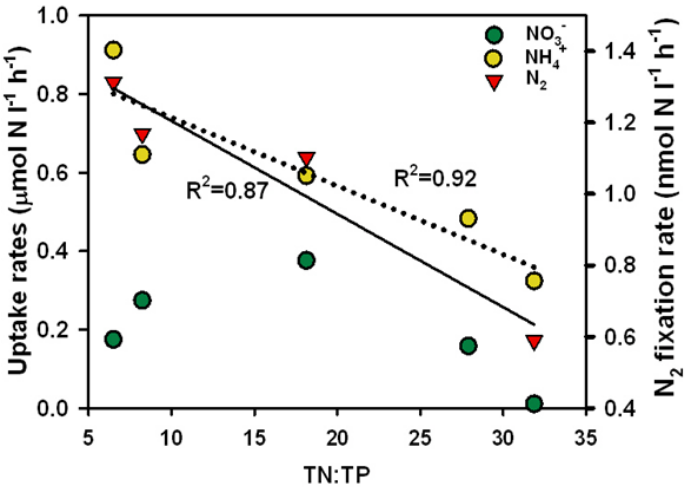


*Figure 3.12: The relationship of TN:TP on estuarine DIN uptake rates and $N_2$ fixation rates during the pre-monsoon season.*

A significant negative correlation ($R^2 = 0.89$) of $N_2$ fixation rates with $NH_4^+$ concentrations was observed in the Cochin estuary as well [*Figure* not shown). Interestingly, in contrast to results in coastal waters, positive correlations of estuarine $N_2$ fixation rates with $NH_4^+$ uptake ($R^2 = 0.89$), $NO_3^-$ uptake ($R^2 = 0.45$), and total DIN uptake ($R^2 = 0.97$) rates were also observed (*Figure* not shown). The occurrence of diazotrophic activity in nutrient replete waters of the estuary and its relationship with DIN rates is intriguing but it may stem from the fact, as discussed earlier, that the N assimilation rates in the estuary depend more on N:P ratios than on DIN concentrations alone. DIN uptake as well as $N_2$ fixation rates increase with the relative availability of P.

Importantly, the negative relationship observed during the present study between TN:TP and $NH_4^+$ uptake rates (as well as $N_2$ fixation; *Figure* 3.12) suggests that the N assimilation in the Cochin estuary, in general, is co-limited by P. Higher DIN uptake and $N_2$ fixation rates were observed at locations where TN:TP was low. Station 1, despite having very high TN and TP (also the highest DIN; *Figure* 3.12)

showed the lowest DIN uptake and $N_2$ fixation rates; whereas station 2, with lower DIN concentrations, showed the highest uptake rates.

## 3.9 Annual budget for nitrogen in the Cochin estuary and the coastal Arabian Sea

An attempt has been made to construct an annual N budget for the Cochin estuary and the coastal Arabian Sea between the off Cochin and off Mangalore transects. The data for various pathways of N cycle has been taken from the present study and previous studies [*Figure* 3.13].

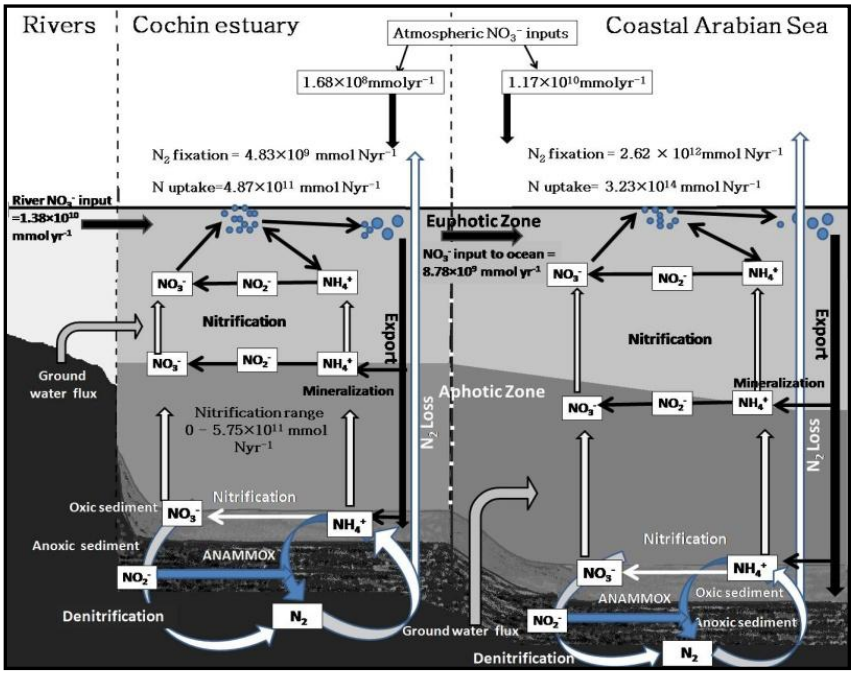


*Figure 3.13: Schematic diagram of rates of different N pathways in the Cochin estuary and the adjacent coastal Arabian Sea.*

For the Cochin estuary the euphotic depth is assumed to be 1m. However, the euphotic depth at coastal transects was calculated by multiplying Secchi depth with 2.8 [*French et al.*, 1982]. Surface area of Cochin estuary from Thannermukkam barrier to Azhikkode is 132 × $10^6$ $m^2$ and that of Vembanad lake is 99× $10^6$ $m^2$ (SOI toposheet, 2002).

The total $NO_3^-$ input to the estuary is 1.38 × $10^{10}$ mmol/year [*Balachandran*, 2006]. The present study estimated annual $N_2$ fixation rate in the Cochin estuary to be nearly 4.83 × $10^9$ mmol/year. This indicates that despite diazotrophic activity being present in this estuary, $N_2$ fixers are not a significant contributor to the DIN pool in comparison with N loading from the rivers. Nitrification in the Cochin estuary ranges from 0-5.75 × $10^{11}$ mmol/year [*Miranda et al.*, 2008]. The total DIN uptake as per the present study in this eutrophic estuary is 4.87 × $10^{11}$ mmol/year. Similarly, potential N uptake for the ocean euphotic zone between off Cochin and off Mangalore is 3.24 × $10^{14}$ mmolN/ year per 7.88 × $10^{11}$ $m^3$ volume of the water. Overall, most of the N transformation rates required for constructing a biogeochemical model of this region are lacking in this estuary due to limited number studies.

## 3.10 Conclusions

Significantly higher DIN uptake rates in the Cochin estuary and at a nearshore location in the adjacent Arabian Sea compared to other coastal locations provide a clear evidence of anthropogenic effects on N uptake dynamics in the region. These results suggest that nutrients delivered via rivers from anthropogenic activities upstream have the potential ability to modulate the estuarine-coastal biogeochemistry. Measurable $N_2$ fixation rates despite very high DIN concentrations were observed in the estuary, which disproved our central hypothesis. Significant relationship of $NH_4^+$ uptake and $N_2$ fixation rates with TN:TP suggest that the N assimilation in the estuary depends more on the relative balance of N and P than on just N or P concentrations alone. The potential DIN assimilation in this estuary is found to be peaked at TN:TP close to the Redfield ratio.

# Chapter 4

# Primary productivity in the Cochin estuary and coastal Arabian Sea

Estuarine and coastal systems are highly susceptible to anthropogenic modulations. One of the direct aftermaths of anthropogenic activities is freshwater effluents comprised of excess nutrients and terrestrial organic matter (TOM). These nutrients enter the estuarine and coastal system in the form of organic and inorganic N and P through rivers and atmosphere, jeopardizing the ecosystem [*Galloway et al.,* 2004]. Consequently, these ecosystems sustain high primary productivity and elevated heterotrophy as discussed in many previous works [e.g., *Middelburg and Nieuwenhuize* 2000; *Mulholland et al.,* 2003].

Apart from physico-chemical parameters such as light intensity, temperature, and salinity, the potential primary productivity substantially depends upon the availability of nutrients [*Bernhardt and Mulholland*, 2005; *Thomas et al.,* 2013]. Recent studies have reported that the imbalances in the relative availability of N and P due to variations in the nutrient loading can also be a major factor in determining the potential primary productivity [*Glibert*; 2003;

*Arrigo et al.,* 2005; *Bhavya et al.,* 2015]. Ambient N:P ratios also have the potential to tune the autotrophic diversity and HABs developments [*Hodgkiss and Ho*, 1997]. Primary production along the continental margins is tightly linked to its estuarine effluents and biogeochemical processes such as ocean–atmosphere $CO_2$ exchange, upwelling, and ground water discharge. Therefore, primary production in the coastal waters adjacent to estuarine systems is subjected to consequences of anthropogenic loading.

Another major concern about the estuarine-coastal coupled systems is the gradual change from autotrophic to heterotrophic nature due to eutrophication [*Hoch and Kirchman,* 1995; *Guttuso et al.,* 1998; *Bricker et al.,* 1999; *Middelburg &Nieuwenhuize*, 2000a, b; *Mulholland et al.,* 2003; *Cloern et al.,* 2014]. Intensive autotrophic as well as heterotrophic activities have been observed in such ecosystems, which ultimately promote microbial respiration leading to increase in $CO_{2,aq}$ levels [*Fasham*, 2003; *Gupta et al.,* 2009]. Some studies through culture experiments have pointed out that increase in $CO_2$ levels would not affect the total oceanic primary productivity [e.g, *Burkhardt et al.,* 2001]. However, the possible influence of high $pCO_2$ on primary productivity in $CO_2$ supersaturated estuaries has not been investigated in detail.

The isotope labeling experiment using $NaHCO_3$ with 99 atom% enriched $^{13}C$ or $^{14}C$ has been widely used for the measurement of primary productivity in both ocean and estuaries [*Nielsen*, 1952; *Slawyk et al.,* 1977; *Gandhi et al.,* 2011]. However, this method was not tested adequately under high $CO_{2,aq}$ concentrations. One of the assumptions during this measurement is that the major substrate for photosynthesis is $HCO_3^-$. Since $HCO_3^-$ is the most abundant species in the marine realm, carbon fixation was believed to be not limited by

DIC substrate. This conviction was challenged by a study [*Riebesell et al.,* 1993] combining model and laboratory data that phytoplankton can be subjected to C limitation, when its DIC acquisition is based on the passive uptake of $CO_{2,aq}$ [*Neven et al.,* 2011]. However, this may not be true for all environments, especially for the ecosystems with high concentrations of $CO_{2,aq}$.

A sufficient supply of $CO_{2,aq}$ is crucial for activation of the main carbon-fixing enzyme in photosynthesis:ribulose-1,5-bisphosphate-carboxylase/oxygenase (Rubisco). But, oceanic DIC is comprised of <1% of $CO_{2,aq}$, about 90% of $HCO_3^-$ and about 10% of $CO_3^{2-}$ [*Zeebe et al.*, 2001], and hence, the $CO_{2,aq}$ concentrations (10–25 μM) in the ocean cannot meet the requirement of Rubisco for its half saturation carboxylation constant (i.e., the $CO_{2,aq}$ concentration at which Rubisco catalyses chemical reactions at 50% of its maximum rate) which is typically at 20–70 μM [*Neven et al.,* 2011]. It is $CO_2$ concentration mechanism (CCM) which helps autotrophs to initiate photosynthesis by extracellular catalytic conversion of $HCO_3^-$ to $CO_{2,aq}$ in the presence of carbonic anhydrase [*Badger et al.,* 1998; *Kaplan et al.,* 1998; *Su¨ltemeyer et al.,* 1998]. However, it may be difficult to estimate the contribution of $CO_{2,aq}$ and $HCO_3^-$ individually to the total organic matter production and the extent to which C uptake is affected by changes in $CO_{2,aq}$ levels [*Burkhardt et al.,* 2001]. In an aquatic system with high $CO_{2,aq}$ concentrations, the measurements of primary productivity using only by $^{13}C$-$HCO_3^-$ tracer technique may not necessarily provide the correct estimate of primary productivity.

Taking these factors together, a study was conducted in the Cochin estuary and in the adjacent coastal Arabian Sea to understand the issues associated with the methodology and nutrient imbalance vis-à-vis primary productivity measurements. On methodological front, the

central hypothesis of the present study was that under a high $CO_{2,aq}$ environment, primary productivity may be underestimated when measured using the $^{13}C\text{-}HCO_3^-$ tracer technique. The main objectives of this study were to (1) estimate primary productivity in tropical estuarine-coastal coupled system, (2) understand the influence of TN:TP ratio on primary productivity, (3) check the influence of estuarine effluences on the coastal primary productivity, and (4) understand the effect of high $CO_{2,aq}$ levels on the primary productivity measurements using the $^{13}C\text{-}HCO_3^-$ labeling technique.

## 4.1 Carbon uptake rates in the Cochin estuary

In general, Cochin estuary showed high rates of carbon fixation with significant seasonal variations (pre-monsoon: 2.78–9.99 μmolC $l^{-1}h^{-1}$; monsoon: 2.01–8.07 μmolC $l^{-1}h^{-1}$; and post-monsoon: 4.02–16.36 μmolC $l^{-1}h^{-1}$; *Figure* 4.1).

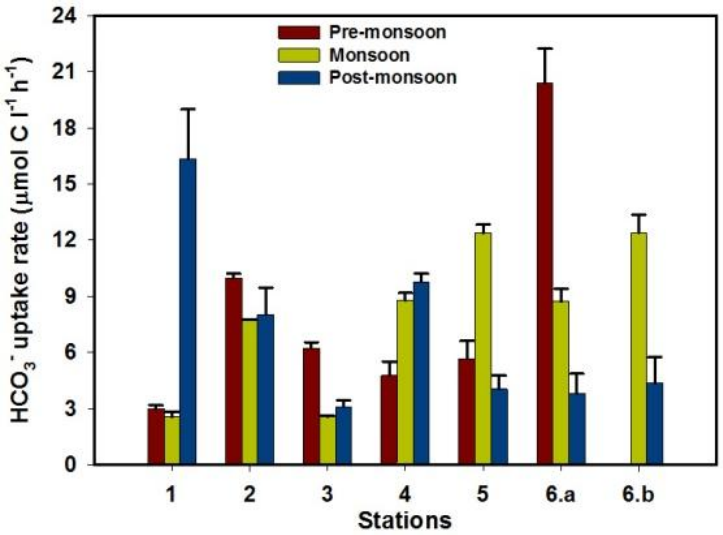


*Figure 4.1: $HCO_3^-$ uptake rates in the Cochin estuary during three seasons (pre-monsoon, monsoon and post-monsoon).*

The rates observed during the present study are comparable with previous estimates using direct and indirect methods in this and other

estuaries around the world [e.g., *Gupta et al.,* 2009; *Cloern et al.,* 2014]. Like Cochin estuary, majority of the estuaries affected by anthropogenic influences tend to exhibit high primary productivity, heterotrophy and high $CO_{2,aq}$ concentrations [*Cloern et al.,* 2014]. One aspect of the primary productivity measurements in such estuaries that needs to be explored is the viability of $^{13}C$ tracer method to measure primary productivity. We explore below this aspect in the light of the results obtained during the present study.

## 4.2 $^{13}C$-tracer technique and $CO_2$ supersaturation

Simultaneous uptake of $CO_2$ and $HCO_3^-$ in seawater by marine eukaryotic microalgae has been reported [*Riebesell et al.,* 1993, *Colman and Rotatore*, 1995, *Rotatore et al.,* 1995, *Korb et al.,* 1997, *Tortell et al.,* 1997, *Elzenga et al.,* 2000, *Zeebe et al.,* 2001, *Hopkinson et al.,* 2005, *Neven et al.,* 2011]. However, these studies have focused on the effect of increasing $CO_{2,aq}$ on primary productivity and did not address the possibility of underestimation of primary productivity while using $HCO_3^-$ based $^{13}C$ or $^{14}C$ tracers. Our results show that the composition of DIC ($CO_{2,aq}$ and $HCO_3^-$) has significant influence on the primary productivity estimations. During the present study, $CO_{2,aq}$ concentrations in the estuary varied from 14.33–1352 μM with an average of 317 ± 391 μM, ~1–82% of the total DIC pool in the estuary [*Table* 4.1]. This can enhance $CO_{2,aq}$ uptake by suppressing $HCO_3^-$ uptake [*Kaplan et al.,* 1998, *Su¨ltemeyer et al.,* 1998, *Burkhardt et al.,* 2001, *Zeebe et al.,* 2001, *Neven et al.,* 2011], leading to an underestimation of primary productivity using $H^{13}CO_3^-$ method. Unfortunately, we could not measure the carbon fixation by $CO_{2,aq}$ independently due to the difficulty in procuring $^{13}CO_2$ tracer.

*Table 4.1: The hydrobiological parameters in the Cochin estuary during the sampling. The units of TP, TN, and DIC are in μM; Chl a is in $\mu g l^{-1}$, and C uptake rates are in $\mu mol\ C\ l^{-1}h^{-1}$.*

| Season | Stn. | T (°C) | Salinity | Chl *a* | TP | TN | TN/TP | pH | DIC | $\%CO_2$ | C uptake rate |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pre-monsoon | 1 | 29.5 | 1.58 | 1.77 | 3.70 | 118 | 31.9 | 6.418 | 822 | 44.6 | 3.00 |
| | 2 | 29.9 | 15.6 | 17.2 | 3.15 | 20.4 | 6.50 | 7.255 | 1156 | 6.09 | 9.98 |
| | 3 | 30.1 | 13.0 | 8.06 | 2.80 | 23.1 | 8.24 | 7.325 | 916 | 5.50 | 6.22 |
| | 4 | 30.6 | 6.27 | 6.44 | 0.78 | 14.2 | 18.1 | 6.920 | 584 | 15.5 | 4.76 |
| | 5 | 29.1 | 5.31 | 2.53 | 0.38 | 10.7 | 27.9 | 6.402 | 357 | 39.1 | 5.67 |
| | 6.a | 29.4 | 0.26 | 12.2 | 0.80 | 37.3 | 46.4 | 6.030 | 1004 | 71.2 | 20.4 |
| | | | | | | | | | | | |
| Monsoon | 1 | 27.2 | 0.12 | 3.31 | 1.86 | 46.0 | 24.8 | 6.278 | 1191 | 60.0 | 2.55 |
| | 2 | 28.9 | 1.60 | 2.76 | 3.83 | 8.83 | 2.30 | 7.275 | 855 | 10.1 | 7.73 |
| | 3 | 28.5 | 5.36 | 3.05 | 2.86 | 24.7 | 8.64 | 7.192 | 624 | 9.45 | 2.57 |
| | 4 | 29.9 | 0.02 | 5.05 | 2.83 | 21.5 | 7.58 | 6.629 | 647 | 41.3 | 8.77 |
| | 5 | 29.7 | 0.07 | 6.74 | 0.81 | 14.8 | 18.2 | 5.906 | 1301 | 78.1 | 12.4 |
| | 6.a | 29.7 | 0.04 | 1.67 | 0.33 | 18.2 | 55.1 | 5.782 | 1633 | 82.9 | 8.71 |
| | 6.b | 29.7 | 0.04 | 7.76 | 1.28 | 22.2 | 17.3 | 6.112 | 1216 | 69.4 | 12.4 |
| | | | | | | | | | | | |
| Post-monsoon | 1 | 28.5 | 12.9 | 21.7 | 1.36 | 20.3 | 14.9 | 7.547 | 916 | 5.85 | 16.3 |
| | 2 | 28.9 | 20.5 | 26.8 | 2.58 | 25.3 | 9.80 | 7.635 | 1154 | 4.07 | 8.03 |
| | 3 | 29.0 | 32.1 | 5.53 | 0.78 | 21.5 | 27.5 | 8.058 | 1567 | 0.91 | 3.10 |
| | 4 | 29.6 | 18.5 | 22.4 | 1.93 | 27.3 | 14.1 | 7.527 | 959 | 2.26 | 9.77 |
| | 5 | 30.0 | 11.1 | 5.30 | 2.44 | 17.3 | 7.08 | 7.364 | 511 | 14.0 | 4.04 |
| | 6.a | 29.8 | 1.92 | 13.6 | 0.59 | 20.0 | 34.0 | 6.466 | 403 | 41.0 | 3.81 |
| | 6.b | 29.6 | 1.89 | 11.5 | 0.60 | 27.1 | 45.4 | 6.414 | 473 | 44.0 | 4.34 |

Therefore, the primary productivity measured during the estuarine study will be referred to as $HCO_3^-$ uptake. To analyze the effect of $CO_{2,aq}$ on the measured $HCO_3^-$ uptake rates on an annual scale, the estuarine stations were divided into two groups: $CO_{2,aq}$<100 µM and $CO_{2,aq}$>100 µM. The stations with $CO_{2,aq}$>100 µM showed a significant positive correlation ($R^2$=0.96) with DIC, indicating that $CO_{2,aq}$ is the major component of the DIC [*Figure* 4.2a].

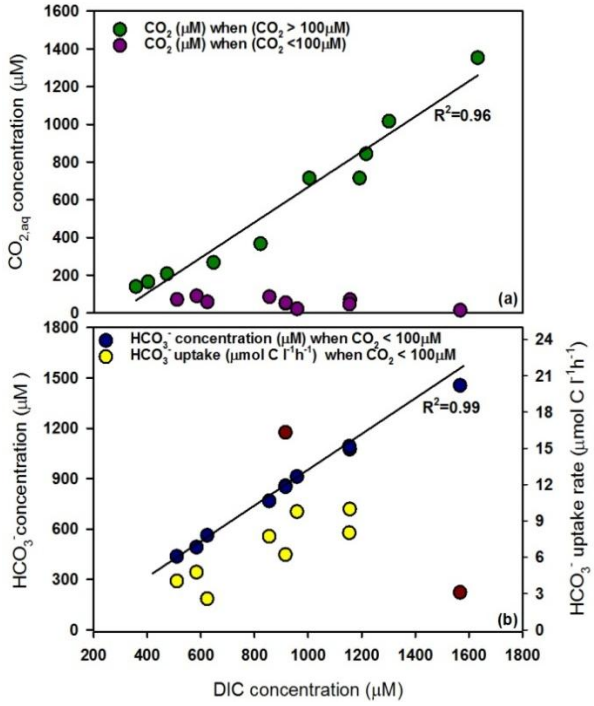


*Figure 4.2: Stations have been classified into two groups; $CO_{2,aq}$>100 µM and $CO_{2,aq}$< 100 µM, where (a) shows variation of $CO_{2,aq}$ concentrations with DIC concentrationsfor stationsunder both groups (b) variation of $HCO_3^-$ concentration and $HCO_3^-$ uptake rates with DIC concentration when $CO_{2,aq}$ concentration<100 µM. The maroon coloured circles which did not follow the increasing trend represent station 3 (near oceanic inlet;uptake rate ~ 3.10 µmolC $l^{-1}h^{-1}$) and station 1 (near river inlet; uptake rate ~16. 33 µmolC $l^{-1}h^{-1}$).*

However, stations with $CO_{2,aq}$<100 µM did not exhibit any relation with DIC [*Figure* 4.2a], but showed a significant correlation ($R^2$=0.99) with $HCO_3^-$, indicating $HCO_3^-$ as a major DIC component [*Figure* 4.2b].

Stations with $CO_{2,aq}$<100 µM showed a significant positive trend between measured $HCO_3^-$ uptake rates and DIC pool [*Figure* 4.2b], which was absent for stations with $CO_{2,aq}$>100 µM (data not shown). There was also a decreasing trend for measured $HCO_3^-$ uptake with increasing % $CO_{2,aq}$ in DIC [*Figure* 4.3]. Taken together, it appears that C uptake rates are possibly underestimated at stations where the contribution of $CO_2$ is high. Therefore, it may be erroneous to attribute $HCO_3^-$ uptake as primary productivity or 'total' C uptake in high $CO_{2,aq}$ environments.

Since the Cochin estuary is a tropical estuary; the temperature control on primary productivity is negligible due to narrow spread of the annual surface temperature (28–30°C). The salinity in the Cochin estuary was highly dynamic due to interplay of freshwaters and tidal intrusion of sea waters [*Table* 4.1]. Even though microbes in the estuary are adapted to fluctuating salinity range, there are direct and indirect influences of freshwater inputs, such as nutrient stoichiometry imbalance, increased $pCO_2$ levels, fluctuating pH etc. [*Gupta et al.*, 2009].

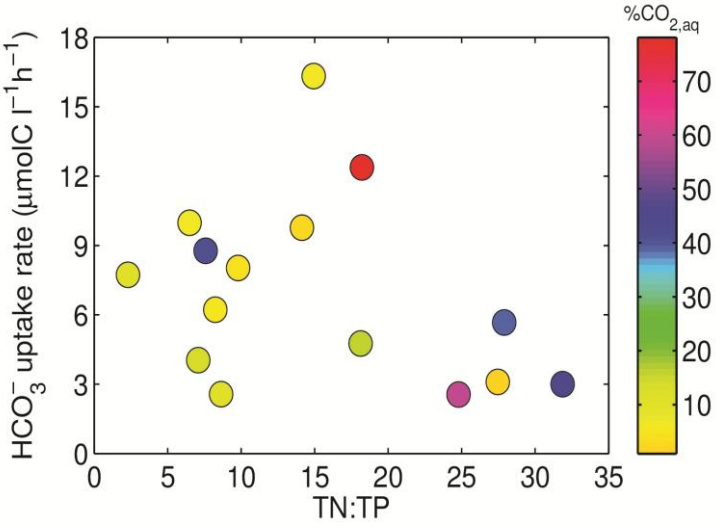


*Figure 4.3: Variation in $HCO_3^-$ uptake with TN: TP and % $CO_{2,aq}$. The colour bar indicates % $CO_{2,aq}$ to the total DIC pool.*

It has been observed that the relative abundance of N and P is the major factor which controls the DIN uptake mechanism in this estuary, rather than the individual nutrient concentrations [*Bhavya et al.*, 2015]. *Figure* 4.3 explains the relative influence of TN:TP and percentage contribution of $CO_{2,aq}$ to the total DIC (% $CO_{2,aq}$) on $HCO_3^-$ uptake rates. With the exception of one station, at stations where $\%CO_2$ is low (<15% ~ $CO_{2,aq}$ concentration <100 μM), $HCO_3^-$ uptake rates appear to increase with increase in N:P ratio to peak near the Redfield ratio. The exceptional station lies near the inlet point of the Arabian Sea to the estuary (salinity ~ 32) and was observed with the lowest $CO_{2,aq}$ (0.91 % of DIC) and highest $HCO_3^-$ (1567 μM) concentrations.

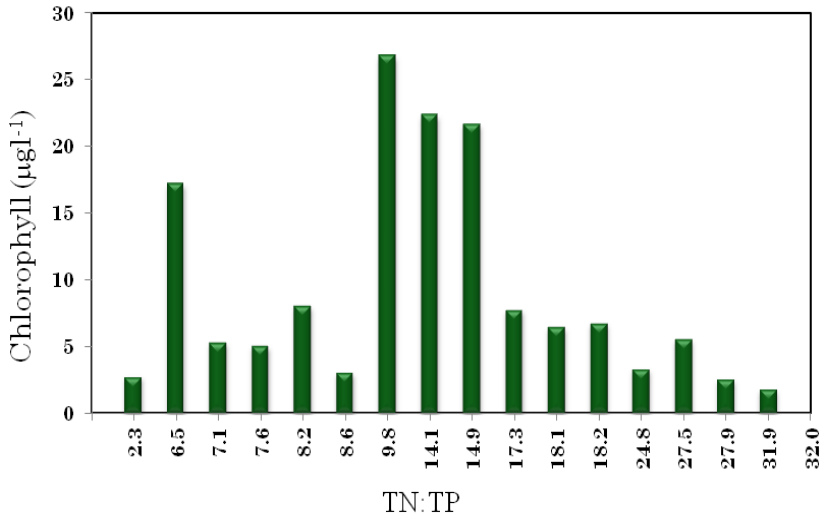


*Figure 4.4: The variation in Chl a with the TN: TP ratio in the Cochin estuary during three seasons.*

Similarly, with the exception of one station (Red) the $HCO_3^-$ uptake rates drop for stations with higher $CO_{2,aq}$ percentage ( > 30 %). The higher $HCO_3^-$ uptake at this station (Red) may be due to optimum N:P ratio. Over all, it appears that in a tropical eutrophic estuary, the TN:TP ratio and percentage contribution of $CO_{2,aq}$ to the total DIC pool affects the C uptake rates measured using $HCO_3^-$ tracer technique.

Therefore, this methodology is best suited for the environment where DIC pool is dominated by $HCO_3^-$ and N:P is close to the Redfield ratio. In addition to that, N assimilation rates in the culture experiments on *Prorocentrum donghaiense* showed maximum values near Redfield ratio and lower values otherwise [*Glibert et al.*, 2011]. Similarly, during the present study, the peak Chl *a* concentrations were observed at N:P ratio close to the Redfield ratio [*Figure* 4.4]. In general, autotrophs prefer a balanced N:P (16:1), where in the Cochin estuary, TN:TP varied between 2.30–31.9.

Another methodological consideration is the measurement of carbon uptake rates in the environment with varying pH. The principle behind $^{13}C$ or $^{14}C$ labeling experiments is that $NaHCO_3$ splits into $Na^+$ and $HCO_3^-$ thereby making $HCO_3^-$ as a major substrate for the carbon uptake [*Slawyk et al.,* 1977], which is not the case when $CO_{2,aq}$ is high. $HCO_3^-$ will be converted to $CO_{2,aq}$ under low pH and vice versa. Therefore, during incubation experiments, there is a chance that a fraction of the tracer added may get converted to $CO_{2,aq}$ at stations with low pH and diffuse to the headspace of the experimental bottle and get isotopically exchanged. However, it is cumbersome to calculate the percentage of $H^{13}CO_3^-$ converted to $^{13}CO_2$ through this process and its overall effect on measurements of carbon uptake rates. The Cochin estuary showed spatial and temporal fluctuations in pH (pre-monsoon: 6.022–7.325, monsoon: 5.782–7.275, post-monsoon: 6.414–8.058), therefore, a certain percentage of added $HCO_3^-$ tracer might have converted to $CO_{2,aq}$ and got assimilated by autotrophs.

## 4.3 Primary productivity in the coastal waters

*Burkhardt et al.* [2001] reported that $pCO_2$ accumulation may not affect the C fixation in the ocean, since $CO_{2,aq}$ and $HCO_3^-$ are equally

preferred by marine autotrophs. However, when $CO_{2,aq}$ is higher than half saturation constant for Rubisco, $HCO_3^-$ uptake can be considerably suppressed. Since the oceanic $CO_2$ shares only ~ 0.25–1.25% of the total DIC pool, an underestimation of primary productivity by $^{13}C$-$HCO_3^-$ method would be negligible. Unlike the estuary, the influence of TN:TP on primary productivity was not significantly evident in oceanic waters. The exchange between the estuary and ocean was clearly reflected in many of the hydrological parameters [*Table* 4.1 & *Table* 3.3].

The nutrient concentrations in the nearshore stations were significantly higher than at stations farther from the coast. During the pre-monsoon, $NO_3^-$ and $NH_4^+$ concentrations at the nearshore station off Cochin (0.89 μM and 1.94 μM, respectively) were higher compared to that of off Mangalore transect (0.24 μM and 0.92 μM, respectively). Concentrations were higher at off Cochin (1.84 μM and 4.00 μM, respectively) than at off Mangalore (0.09 μM and 2.97 μM, respectively) during monsoon as well. The export fluxes from the Cochin estuary might have played a significant role in elevating the nutrient levels.

However, the nearshore waters of off Mangalore (1.71 μM) showed higher $NO_3^-$ concentration during post-monsoon compared to off Cochin (0.57 μM), though $NH_4^+$ continued to be high at Cochin (1.46 μM) than at off Mangalore (0.89 μM). The C fixation rates in the off Cochin transect (pre-monsoon: 0.004–1.08, monsoon: 0.009–4.91 μmol C $l^{-1}h^{-1}$) were significantly higher than that of off Mangalore transect (pre-monsoon: 0.004–0.38, monsoon: 0.011–1.61 μmol C $l^{-1}h^{-1}$), while it was opposite during post-monsoon (off Cochin: 0.007–0.11, off Mangalore: 0.01–3.4 μmol C $l^{-1}h^{-1}$). The observed C fixation rates at the farther stations in the coastal Arabian Sea are comparable with the previously

reported values from this region [*Gandhi et al.*, 2011], except at nearshore stations in the off Cochin transect during the pre-monsoon and monsoon [*Figure* 4.5].

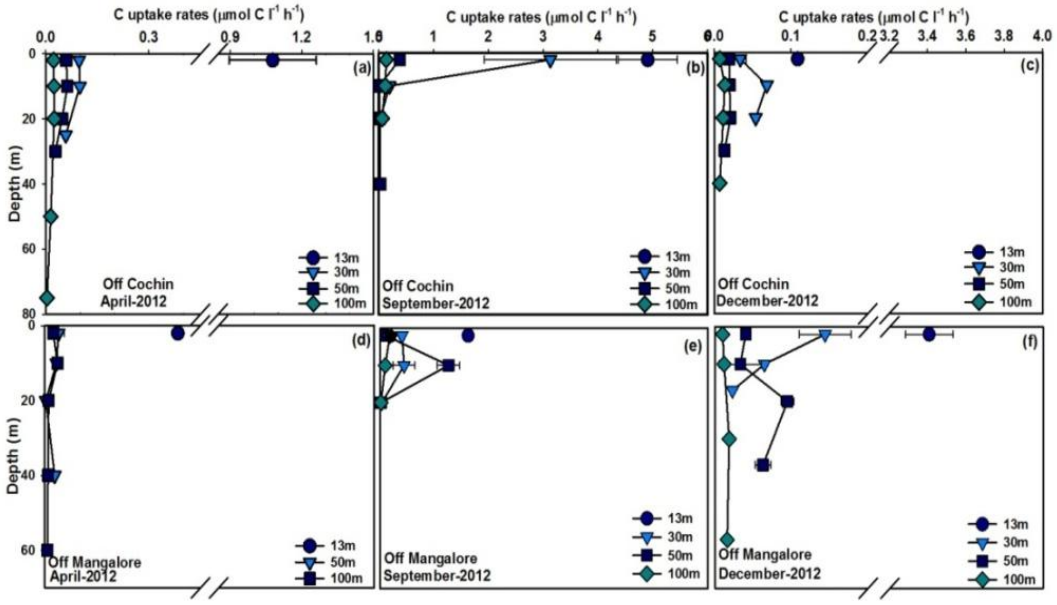


*Figure 4.5: The depth profile of coastal primary productivity at the off Cochin (a, b, c) and off Mangalore (d, e, f) during pre-monsoon, monsoon and post-monsoon, respectively.*

The seasonality of winds and hence the ocean currents import a dynamic nature to the mixed layer in the Arabian Sea [*Burkill et al.*, 1993]. Various studies have reported the influence of physical processes such as upwelling and winter cooling in the Arabian Sea on enhancement of its primary productivity [*Madhupratap et al.*, 1996; *Kumar et al.*, 2010]. The present study reports the depth wise variations in primary productivity over different seasons in the coastal Arabian Sea. During the monsoon, primary production is known to be the strongest contributor to the standing C stock in the coastal Arabian Sea [*Barber et al.*, 2001]. Significantly high primary productivity was observed in the Cochin transect during the monsoon compared to the pre- and post-monsoon.

The distribution of C uptake rates showed seasonality in primary productivity and influence of upwelling [Figure 4.5]. The upwelling in the Indian Ocean is generally initiated as early as in March near the equator and progresses towards the north, while it strengthens during May-July. Towards the beginning of post-monsoon season, the upwelling features progress much northwards to off Mangalore, while it remains subdued at off Cochin. The temperature and nutrient concentrations are also in agreement with this observation [*Gupta et al*., unpublished]. Overall, it appears that primary productivity in the nearshore waters are influenced by estuarine discharge whereas farther stations carry the signature of upwelling.

## 4.4 Conclusions

Experiments to estimate primary productivity using the $^{13}C$ tracer technique conducted in the Cochin estuary, a tropical $CO_2$ supersaturated estuary, pointed towards the evidence of underestimation in the primary productivity. Findings of this study suggests that primary productivity measurements using $^{13}C$ technique is not the best suited for aquatic environments with high $CO_{2,aq}$ concentrations. Further, the primary production in the Cochin estuary was found to be optimum, when the TN:TP ratio was closer to the Redfield ratio. Nearshore stations are affected by estuarine discharge, pointing towards the capability of anthropogenic inputs to modulate the coastal biogeochemistry.

# Chapter 5

# Stable isotopes of carbon and nitrogen in marine environments

## 5.1 Carbon dynamics and $\delta^{13}C_{DIC}$

In aquatic ecosystems, the C isotopic composition of DIC ($\delta^{13}C_{DIC}$) can provide information about its sources, and in turn, the role of anthropogenic and natural processes in our ecosystems [*Degens et al.*, 1969]. $\delta^{13}C_{DIC}$ is influenced by several major processes that alter the input and output of the DIC in an ecosystem [*Oana and Deevey*, 1960]. DIC inputs to an ecosystem can be through diffusion of atmospheric $CO_2$, organic respiration, surface runoff and groundwater inflow, calcite dissolution, and methane oxidation. The outputs of DIC include evasion of $CO_2$, calcite precipitation, photosynthetic uptake, and water outflow. In general, the preference for $^{12}C$ during the microbial assimilation makes the aqueous C pool enriched in $^{13}C$ with respect to resultant compounds. During remineralisation, however, the $CO_2$ derived from this OM or biota is depleted in $^{13}C$, thereby introducing a C pool depleted in $^{13}C$ into the aqueous medium. Therefore, higher primary productivity results in higher $\delta^{13}C_{DIC}$, whereas higher rates of decomposition of biota lower the $\delta^{13}C_{DIC}$ [*Mook and Tan*, 1991]. In addition to the atmospheric influence, the exchange between the

terrestrial and marine carbon pools also play an important role in controlling the oceanic $\delta^{13}C_{DIC}$ [*Broecker and Peng*, 1993; *Keir et al.*, 1998].

The determination of long term changes in $\delta^{13}C_{DIC}$ is difficult due to the paucity of data [*Quay et al.*, 1992]. In general, $\delta^{13}C_{DIC}$ of surface ocean water ranges from 0 to 2 ‰ whereas values in deeper waters below the mixed layer are around +0.5±0.5 ‰ [*Mook and Tan,* 1991]. DIC in the surface waters is expected to be enriched in $^{13}C$ due to removal of isotopically light carbon during primary productivity [*Mook*, 1970]. In the coastal ocean, sea surface $\delta^{13}C_{DIC}$ distribution is controlled by air–sea $CO_2$ exchange, biological productivity, vertical mixing, and sea surface temperature. However, the bottom waters along the continental shelf are mostly influenced by community respiration and organic matter degradation. They are also influenced by estuarine and ground water effluents. The relative dominance of these processes varies depending on the region and season [*Recapé et al.*, 2010].

Very few studies have been conducted in the tropical regions, especially in India, to understand the C dynamics of these ecologically important estuary-coastal coupled systems on these lines [e.g., *Ghosh et al.*, 2012; *Boullin et al.*, 2006]. The present study attempted to decipher sources and pathways of DIC in one such coupled system from India, i.e., the Cochin estuary, a eutrophic tropical estuary in the southwest coast of India, and the adjacent coastal Arabian Sea during three different seasons (April: pre-monsoon, September: southwest monsoon and December: post monsoon) of 2012. The major objectives of the study were to (1) understand the factors influencing $\delta^{13}C_{DIC}$ of the Cochin estuary and coastal Arabian Sea, (2) characterize the seasonal

variations in $\delta^{13}C_{DIC}$ of the estuarine-coastal coupled systems (3) determine whether the coastal Arabian Sea is a source or sink of $CO_2$, and (4) test for the presence or absence of signatures of oceanic Suess effect in the coastal Arabian Sea.

For the present study, surface samples were collected for $\delta^{13}C_{DIC}$ at five stations across the salinity gradient and two stations in the Vembanad fresh water Lake, located upstream of the estuary but separated by a dam (*Figure* 3.1 of *Chapter* 3). During post-monsoon, sixteen samples from the estuary and four from the Vembanad Lake were collected. Coastal samples were collected along the two transects at locations representing (13, 20, 30, 40, 50, and 100 m water column depths) with an interval of 10m (*Table* 3.1 of *Chapter* 3).

### 5.1.1 Environmental parameters

Estuarine environmental parameters of the seven regular stations during the study period are shown in *Table* 5.1 (the data of other 16 stations during post monsoon is not included in the table; however explained in this section). Vembanad Lake showed consistently low salinity throughout the study period with average values of 0.26, 0.04, and 1.90. The estuarine pH was higher during the post-monsoon (6.994–8.058; all 22 stations) compared to the pre-monsoon (6.402–7.325) and the monsoon (5.906–7.275).

pH of the Vembanad lake showed consistently lower values than estuarine stations (pre monsoon: 6.030–6.523; monsoon: 5.782–6.112; post monsoon: 6.414–6.466).The average DIC concentrations in the estuary were lower during the pre-monsoon (752 ± 310 μmol $kg^{-1}$) compared to the monsoon (905 ± 296 μmol $kg^{-1}$) and the post-monsoon (1061 ± 411 μmol $kg^{-1}$) with lower values generally at upstream and higher values at downstream. Partial pressure of $CO_2$ ($pCO_2$) in the

estuary ranged from 1803 to 12230 µatm (pre-monsoon), 1955 to 33796 µatm (monsoon) and 439 to 2536 µatm (post-monsoon). The salinity patterns clearly show the impacts of tidal movements and the monsoon driven freshwater inflow on the coastal waters. The surface data of the above mentioned parameters in the coastal transect are given in *Table* 5.2 & 5.3 (The details of station location and depths are given in *Table* 3. 1 of *Chapter* 3). The salinity ranged in the off Cochin transect from 33.9 to 35.5 (pre-monsoon), 31.7 to 35.6 (monsoon) and 33.8 to 36.7 (post-monsoon); whereas in the Mangalore transect, it varied from 34.6 to 35.8 (pre-monsoon), 27.1 to 35.3 (monsoon) and 34.6 to 35.9 (post-monsoon).

Average surface pH in the off Cochin transect (pre-monsoon: 8.131 and monsoon: 8.101) was less than that of the off Mangalore transect (pre-monsoon: 8.139 and monsoon: 8.144), whereas the opposite was observed during the post-monsoon (off Cochin: 8.165 and off Mangalore: 8.124). The values of $pCO_2$ during the pre-monsoon (off Cochin: 417–720 µatm; off Mangalore: 404–844 µatm) and the monsoon (off Cochin: 253–1052 µatm; off Mangalore: 39 –1220 µatm) were in a higher range than the post-monsoon (off Cochin: 395–598 µatm; off Mangalore: 390–855 µatm) with exceptions at some depths.

### 5.1. 2 $\delta^{13}C_{DIC}$ in the Cochin estuary

The prevailing eutrophication and augmented heterotrophy in the Cochin estuary persuaded researchers to focus on the carbon dynamics of this ecosystem [*Gupta et al.*, 2009]. The conclusions from each study divulged the amplified primary productivity, decreased oxygen level, increased bacterial activities and OM degradation, species shift, and supersaturation of $CO_2$ in the estuarine water column [*Gupta et al.*, 009; *Shoji et al.*, 2009; *Martin et al.*, 2013; *Bhavya et al.*, unpublished].

*Table 5.1: The environmental parameters in the Cochin estuary during the three sampling periods. The units of Si, $PO_4^{3-}$, DIC, Talk, and $CO_3^{2-}$ are given in μmol $l^{-1}$ whereas the unit of $pCO_2$ is in μatm.*

| Season | Stn. | T (°C) | Salinity | Si | $PO_4^{3-}$ | pH | DIC | TAlk | $pCO_2$ | $CO_3^{2-}$ | $\delta^{13}C_{DIC}$ (‰) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pre-monsoon | 1 | 29.5 | 1.58 | 47.60 | 3.64 | 6.418 | 822 | 456 | 12230 | 0.14 | -12.3 |
| | 2 | 29.9 | 15.6 | 42.14 | 2.95 | 7.255 | 1156 | 1104 | 2540 | 10.2 | -3.26 |
| | 3 | 30.1 | 13.0 | 41.10 | 2.68 | 7.325 | 916 | 882 | 1803 | 8.59 | -4.21 |
| | 4 | 30.6 | 6.27 | 48.50 | 0.51 | 6.920 | 584 | 496 | 3165 | 1.2 | -5.95 |
| | 5 | 29.1 | 5.31 | 70.90 | 0.3 | 6.402 | 357 | 218 | 4699 | 0.14 | -8.26 |
| | 6.a | 29.4 | 0.26 | 112.6 | 0.54 | 6.030 | 1004 | 289 | 23601 | 0.02 | -9.75 |
| | 6.b | 29.4 | 0.26 | 90.24 | 0.57 | 6.523 | 777 | 308 | 15531 | 0.04 | -8.08 |
| Monsoon | 1 | 27.2 | 0.12 | 57.96 | 1.11 | 6.278 | 1191 | 477 | 22316 | 0.04 | -13.6 |
| | 2 | 28.9 | 1.6 | 144.5 | 3.13 | 7.275 | 855 | 774 | 2832 | 1.74 | -8.27 |
| | 3 | 28.5 | 5.36 | 109.6 | 2.11 | 7.192 | 624 | 570 | 1955 | 2.19 | -5.69 |
| | 4 | 29.9 | 0.02 | 101.6 | 2.5 | 6.629 | 647 | 380 | 8932 | 0.07 | -9.28 |
| | 5 | 29.7 | 0.07 | 40.29 | 0.33 | 5.906 | 1301 | 284 | 33796 | 0.01 | -9.25 |
| | 6.a | 29.7 | 0.04 | 80.68 | 0.26 | 5.782 | 1633 | 278 | 45015 | 0.01 | -11.7 |
| | 6.b | 29.7 | 0.04 | 83.30 | 0.61 | 6.112 | 1216 | 372 | 28067 | 0.02 | -11.8 |
| Post-monsoon | 1 | 28.5 | 12.9 | 47.39 | 0.727 | 7.547 | 916 | 885 | 1145 | 10.4 | -3.81 |
| | 2 | 28.9 | 20.5 | 42.43 | 1.268 | 7.635 | 1154 | 1130 | 1043 | 14.7 | -1.67 |
| | 3 | 29.0 | 32.1 | 37.22 | 0.485 | 8.058 | 1567 | 1693 | 439 | 98.4 | 0.79 |
| | 4 | 29.6 | 18.5 | 29.65 | 1.286 | 7.527 | 959 | 980 | 1094 | 25.1 | -0.38 |
| | 5 | 30.0 | 11.1 | 36.56 | 1.789 | 7.364 | 511 | 443 | 786 | 1.49 | -5.35 |
| | 6.a | 29.8 | 1.92 | 76.35 | 0.438 | 6.466 | 403 | 238 | 5552 | 0.09 | -4.93 |
| | 6.b | 29.6 | 1.89 | 79.57 | 0.336 | 6.414 | 473 | 265 | 6966 | 0.09 | -11.6 |

*Table 5.2: The environmental parameters of the surface waters of the off Cochin transect during the three sampling periods. The units of $PO_4^{3-}$, Si, DIC, and DIC are given in μmol $l^{-1}$, where unit of $pCO_2$ is in μatm.*

| Season | Name | Temp (°C) | Salinity | $PO_4^{3-}$ | Si | pH | DIC | $pCO_2$ | $\delta^{13}C_{DIC}$ (‰) | $DIC_{mix}$ | $\delta^{13}C_{mix}$ (‰) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pre-monsoon 2012 | C1-0 | 30.2 | 33.9 | 0.52 | 1.59 | 8.103 | 2008 | 514 | 0.93 | 1911 | 0.72 |
| | C2-0 | 30.1 | 34.0 | 0.33 | 1.26 | 8.133 | 1874 | 444 | 0.72 | 1916 | 0.75 |
| | C3-0 | 30.4 | 34.5 | 0.50 | 0.68 | 8.130 | 1927 | 459 | 0.87 | 1931 | 0.83 |
| | C 4-0 | 30.7 | 34.8 | 0.21 | 0.50 | 8.133 | 1944 | 459 | 0.90 | 1940 | 0.87 |
| | C 5-0 | 30.2 | 34.8 | 0.22 | 0.85 | 8.140 | 1982 | 458 | 1.00 | 1940 | 0.87 |
| | C 6-0 | 30.1 | 35.0 | 0.11 | 0.94 | 8.150 | 1958 | 442 | 1.07 | 1949 | 0.91 |
| Monsoon 2012 | C 1-0 | 25.8 | 31.7 | 0.43 | 8.03 | 7.981 | 2024 | 690 | 0.04 | 1866 | -0.19 |
| | C 2-0 | 26.2 | 31.7 | 0.28 | 3.86 | 8.176 | 1786 | 377 | 0.89 | 1867 | -0.18 |
| | C 3-0 | 26.3 | 31.9 | 0.19 | 3.19 | 8.322 | 1749 | 254 | 2.02 | 1872 | -0.11 |
| | C 4-0 | 25.9 | 34.1 | 1.02 | 6.01 | 7.865 | 2115 | 936 | -0.20 | 1919 | 0.46 |
| | C 5-0 | 26.7 | 33.1 | 0.36 | 1.45 | 8.126 | 1953 | 464 | 0.98 | 1897 | 0.20 |
| | C 6-0 | 27.4 | 33.7 | 0.27 | 1.42 | 8.139 | 2003 | 459 | 1.10 | 1909 | 0.34 |
| Post monsoon 2012 | C1-0 | 29.9 | 35.0 | 0.17 | 10.8 | 8.156 | 1916 | 436 | 0.32 | 1908 | 0.82 |
| | C2-0 | 29.6 | 34.6 | 0.08 | 8.71 | 8.178 | 1862 | 395 | 0.95 | 1891 | 0.79 |
| | C3-0 | 29.5 | 33.8 | 0.09 | 6.39 | 8.168 | 1882 | 407 | 0.99 | 1860 | 0.75 |
| | C 4-0 | 29.5 | 34.0 | 0.07 | 4.18 | 8.165 | 1945 | 423 | 0.72 | 1867 | 0.76 |
| | C 5-0 | 30.2 | 33.9 | 0.11 | 2.04 | 8.156 | 1921 | 429 | 1.15 | 1866 | 0.76 |
| | C 6-0 | 29.7 | 34.7 | 0.13 | 3.13 | 8.165 | 1924 | 417 | 0.27 | 1895 | 0.80 |

*Table 5.3: The environmental parameters of the surface waters of the off Mangalore transect during three seasons. The units of $PO_4^{3-}$, Si, DIC, and DIC are given in μmol $l^{-1}$, whereas unit of $pCO_2$ is in μatm.*

| Season | Name | Temp (°C) | Salinity | $PO_4^{3-}$ | Si | pH | DIC | $pCO_2$ | $\delta^{13}C_{DIC}$ (‰) |
|---|---|---|---|---|---|---|---|---|---|
| Pre monsoon 2012 | M1-0 | 31.4 | 35.2 | 0.40 | 5.29 | 8.125 | 1832 | 442 | 1.00 |
| | M2-0 | 31.0 | 35.0 | 0.44 | 3.76 | 8.121 | 1827 | 446 | 0.81 |
| | M3-0 | 31.0 | 34.9 | 0.21 | 3.46 | 8.145 | 1960 | 450 | 0.96 |
| | M 4-0 | 30.5 | 34.7 | 0.57 | 3.96 | 8.144 | 1816 | 417 | 1.01 |
| | M 5-0 | 30.1 | 34.6 | 0.05 | 1.94 | 8.150 | 1917 | 433 | 1.03 |
| | M 6-0 | 30.1 | 35.0 | 0.03 | 1.70 | 8.151 | 1924 | 431 | 1.13 |
| | | | | | | | | | |
| Monsoon, 2012 | M1-0 | 26.9 | 27.1 | 0.40 | 3.03 | 8.146 | 1697 | 404 | 0.76 |
| | M2-0 | 27.5 | 29.8 | 0.40 | 6.68 | 8.109 | 1804 | 462 | 0.95 |
| | M3-0 | 27.0 | 30.7 | 0.21 | 2.55 | 8.143 | 1840 | 428 | 0.91 |
| | M 4-0 | 27.2 | 31.5 | 0.12 | 1.30 | 8.159 | 1803 | 400 | 0.89 |
| | M 5-0 | 26.8 | 32.2 | 0.05 | 1.68 | 8.136 | 2018 | 471 | 1.19 |
| | M 6-0 | 27.2 | 33.1 | 0.03 | 0.82 | 8.175 | 1867 | 393 | 1.02 |
| | | | | | | | | | |
| Post monsoon 2012 | M1-0 | 29.3 | 34.6 | 0.63 | 2.58 | 8.001 | 1920 | 626 | 0.04 |
| | M2-0 | 29.3 | 34.9 | 0.28 | 1.78 | 8.087 | 1919 | 504 | 0.88 |
| | M3-0 | 29.3 | 35.0 | 0.18 | 1.06 | 8.148 | 1880 | 424 | 0.55 |
| | M 4-0 | 29.8 | 35.1 | 0.13 | 2.32 | 8.170 | 1880 | 401 | 0.48 |
| | M 5-0 | 29.3 | 35.0 | 0.11 | 2.75 | 8.169 | 1912 | 408 | 1.08 |
| | M 6-0 | 29.4 | 35.2 | 0.11 | 1.30 | 8.171 | 1931 | 409 | 0.54 |

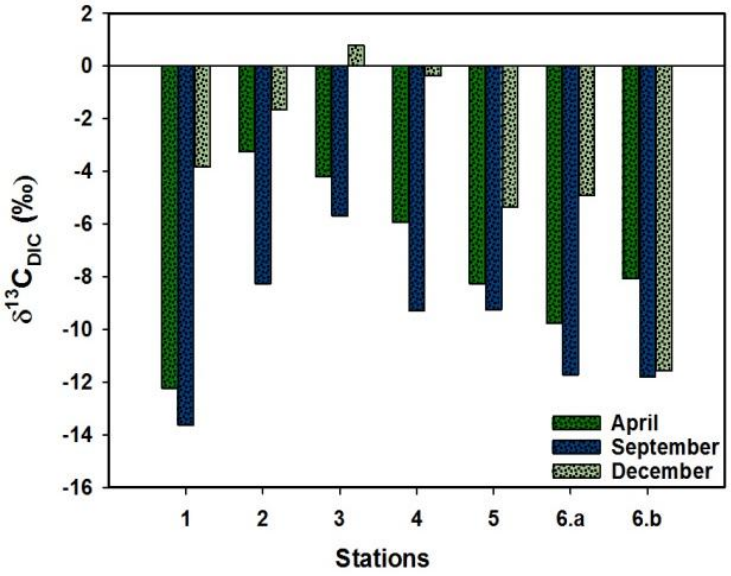


*Figure 5.1.1: $\delta^{13}C_{DIC}$ variation in the Cochin estuary during the three seasons (pre-monsoon, monsoon and post monsoon).*

The present study attempted to understand the major processes which affected the $\delta^{13}C_{DIC}$ and DIC concentrations in the estuary using the concept of mixing curve approximation [*Alling et al.* 2012]. $\delta^{13}C_{DIC}$ in the estuarine region during the pre-monsoon varied from −12.24 – −3.26 ‰ with an average of −6.78 ± 3.59 ‰ and a comparable range (−13.64 – −5.68 ‰) was observed during the monsoon with slightly depleted average of −9.23 ± 2.87‰. $\delta^{13}C_{DIC}$ was observed to be slightly enriched (−5.35 – +0.78‰) during the post-monsoon with the highest at station 3, the meeting point of the Arabian Sea with the estuary [*Figure* 5.1.1].

### 5.1.3 Conservative mixing of DIC and $\delta^{13}C_{DIC}$ in the Cochin estuary

Under conservative mixing, the values of DIC and $\delta^{13}C_{DIC}$ at a particular point are assumed to be independent of the local biogeochemical processes, but are dependent on the fraction of each

end members at that place. In this study, the meeting point of the Periyar River and average surface values of the coastal Arabian Sea (30-100 m depth stations) have been taken as fresh water and seawater end members, respectively, for both $\delta^{13}C_{DIC}$ and DIC. The major factor which controls the DIC and $\delta^{13}C_{DIC}$ is the mixing of waters of two different salinities; i.e., freshwater and sea water. To create a hypothetical mixing curve for DIC and $\delta^{13}C_{DIC}$, we assumed that the salinity at particular location is dominantly dependent on the fraction of freshwater mixed with seawater ($F_{fw}$). To calculate the mixing curve approximation and signatures of biogeochemical processes as discussed below, we followed *Alling et al.*, [2012]. $F_{fw}$ can be calculated using the mass balance equation for salinity [*Equation* 1].

$$S_{mix} = S_{fw}F_{fw} + S_{sw}\,[1 - F_{fw}] \quad \text{.....[1]}$$

Where, $S_{mix}$ is the salinity measured at each station, $S_{fw}$ and $S_{sw}$ represent the salinities of freshwater and seawater end members. Using $F_{fw}$, we calculated the conservative mixing values of DIC concentrations and $\delta^{13}C_{DIC}$ [*Equation* 2 and 3, respectively].

$$[DIC]_{fw}F_{fw} + [DIC]_{sw}\left(1\text{-}F_{fw}\right) = [DIC]_{mix} \quad \text{.....[2]}$$

$$\delta^{13}C_{mix} = \frac{[DIC]_{fw}\delta^{13}C_{fw}F_{fw} + [DIC]_{sw}\delta^{13}C_{sw}[1\text{-}F_{fw}]}{[DIC]_{fw}F_{fw} + [DIC]_{sw}[1\text{-}F_{fw}]} \quad \text{.....[3]}$$

Where [DIC] is the concentration of DIC, subscripts *sw* and *fw* denotes the seawater and freshwater, respectively, [*Equation* 2]. The subscript *mix* represents the estuarine water from the conservative mixing of the freshwater and seawater end members. The $\delta^{13}C_{mix}$ is a theoretical value of $\delta^{13}C_{DIC}$, where there are no sources or sinks of DIC and is purely a function of salinity and [DIC] [*Equation* 3]. *Figure* 5.1.2a and

5.1.2b represent the variation of actual and conservative mixing values of $\delta^{13}C_{DIC}$ and [DIC], respectively, with salinity in the estuary for all the three seasons.

It appears that, the [DIC] and $\delta^{13}C_{DIC}$ are highly controlled by dynamic estuarine nature; majorly primary productivity and OM degradation. *Erlenkeuser et al.,* [2003] have reported that the deviations of [DIC] and $\delta^{13}C_{DIC}$ from the conservative mixing values can be used as proxy to understand the process in the estuary. Equation 4 and 5 below has been used to calculate these deviations, i.e., $\Delta\delta^{13}C_{DIC}$ and $\Delta$[DIC].

$$\Delta\delta^{13}C_{DIC} = \delta^{13}C_{sample} - \delta^{13}C_{mix} \quad \text{.....[4]}$$

$$\Delta[DIC] = \frac{[DIC]_{sample} - [DIC]_{mix}}{[DIC]_{mix}} \quad \text{.....[5]}$$

*Figure* 5.1.3 shows the $\Delta\delta^{13}C_{DIC}$ of the samples plotted against the corresponding $\Delta$[DIC] values. For the end members, no data is shown and they lie at the origin; while all other points are spread in the four quadrants with reference to the major processes controlling them. In general, locations where $[DIC]_{sample} > [DIC]_{mix}$, indicate the addition of DIC to the existing pool due to OM decomposition, whereas the opposite is true for primary productivity.

The samples which lie in the upper left quadrant indicate the $^{13}C$ enrichment along with decrease in the DIC concentration. The possible reasons could be out gassing of $CO_2$ and primary productivity. Both these processes selectively remove $^{12}C$ from the DIC pool and reduce its concentration. During the present study, it appears that the estuarine $\delta^{13}C_{DIC}$ and [DIC] during the pre-monsoon and the monsoon seasons were mainly controlled by primary productivity and degassing of $CO_2$. The high $pCO_2$ observed in the Cochin estuary is also in agreement

with this. The consistently high microbial respiration in the Cochin estuary enhances the turnover rates of surplus OM [*Shoji et al.*, 2008], which in turn becomes the reason for ample $pCO_2$ levels in this estuary [*Gupta et al.*, 2009].

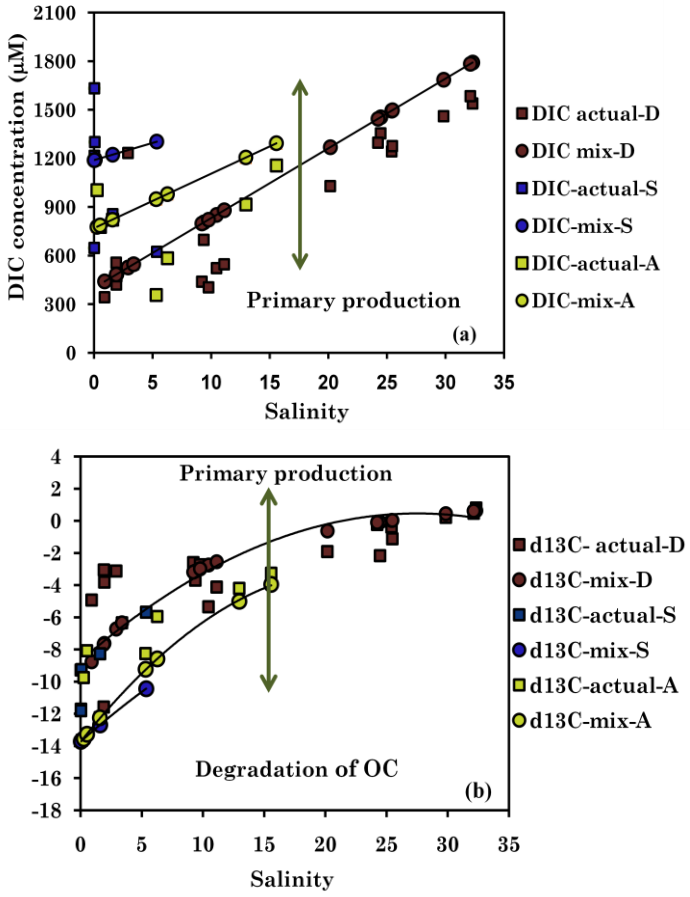


*Figure 5.1.2: (a) DIC concentrations plotted against salinity and [b] $\delta^{13}C_{DIC}$ in the Cochin estuary plotted against salinity. Circles in both plots denote the conservative mixing values and the rectangles define the actual values. The arrows indicate the influence of primary productivity (PP) and OM degradation on the isotopic composition and concentrations of DIC. The A, S, and D in the legend indicate the three seasons; the pre-monsoon (April), monsoon (September), and post-monsoon (December), respectively.*

In addition, the $pCO_2$ concentration in the estuary is exceptionally high due to elevated OM remineralization and may cause high rates of $CO_2$ emission to the atmosphere. $CO_2$ produced due to degradation of OM

diffuses into the atmosphere immediately. Hence, the nonexistence of data in the lower right quadrant does not mean the absence of OM degradation. Studies show that Indian estuaries mineralize up to 90% of terrestrial organic loads during high-flow conditions [*Gupta et al.,* 2008]. This might be a possible reason for higher $pCO_2$ during the pre-monsoon and the monsoon when the estuary received heavy fresh water runoff with plenty of lateral inputs of OM.

Station 1 situated at the inlet point of the Periyar River and reception point of excess OM from the city of Cochin exhibited the highest $pCO_2$. Eventhough smaller in comparison, Muvattupuzha River also appears to have brought enough OM to Station 4. The lower left quadrant represents the carbonate precipitation, where both [DIC] and $\delta^{13}C_{DIC}$ decrease [*Alling et al.,* 2012]. During post-monsoon, both lack of freshwater supply as well as high tide phase, facilitates high pH and salinity range in the estuarine zone. The majority of the stations falling in the lower left corner belongs to the post-monsoon and indicates the dominance of slow rate of calcite precipitation.

However, % contribution of calcite precipitation and OM mineralization in $CO_2$ emission from this estuary cannot be determined due to the close coupling between calcite precipitation and aerobic respiration [*Frankignoulle et al.,* 1994]. The upper right quadrant represents the stations with calcite dissolution as the major process. $\Delta\delta^{13}C_{DIC}$ and $\Delta$[DIC] >0 at these stations indicate the increase in [DIC] as well as enrichment of $^{13}C$. These are stations with relatively low pH and higher $pCO_2$ levels (5, 6.a, & 6.b). Incidentally, the macro benthos of this region is heavily dominated with bivalves [~50%, *Martin et al.*, 2010], so calcite dissolution is regarded as an important $CO_2$ source here at low pH conditions.

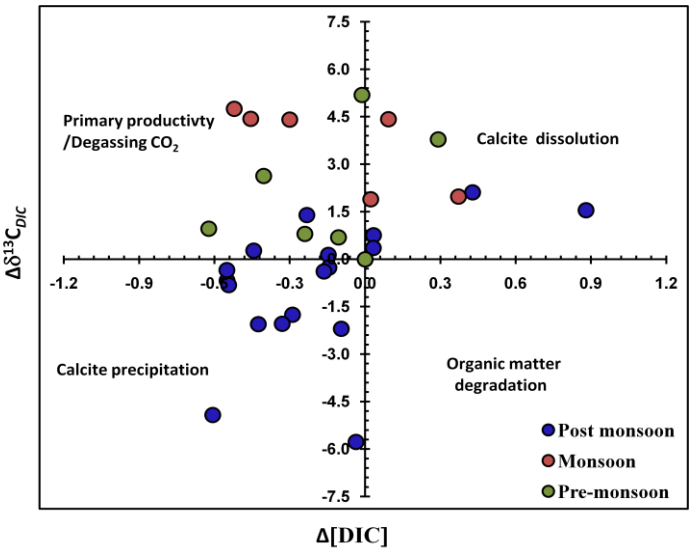


*Figure 5.1.3: The change in $\delta^{13}C_{DIC}$ ($\Delta\ \delta^{13}C_{DIC}$) vs change in DIC concentrations (Δ[DIC]) from the corresponding conservative mixing values in the Cochin estuary. The four quadrants explain the processes which are the most likely to affect the $\delta^{13}C_{DIC}$ in the estuary.*

Taken together, based on this mixing curve approximation, the major estuarine processes that are controlling estuarine C cycling are primary productivity, OM degradation and $CO_2$ outgassing during the pre-monsoon and monsoon. Calcium carbonate precipitation is a dominant process during post-monsoon. However, these findings can be highly influenced by the selection of freshwater inputs. Since the Cochin estuary receives OM loading from other small rivers and tributaries, the C dynamics in this estuary can be significantly influenced by them. But we have assumed supply only from the Periyar River and have taken it as the freshwater end member. So these results serve as a guide only to a preliminary identification of processes in this eutrophic estuary.

### 5.1.4 $\delta^{13}C_{DIC}$ in the coastal Arabian Sea

Along with the seasonal variations, the biogeochemistry of the coastal Arabian Sea is significantly influenced by the effluents from the Cochin

estuary [*Bhavya et al.,* 2015; *Gupta et al.,* unpublished]. The nutrient loading from the estuary enhances the uptake of $CO_2$ through primary productivity. However, the OM accumulated through high export production as well as OM discharged from the Cochin estuary cause high OM degradation and hence the coastal Arabian Sea acts as a source of $CO_2$ as reported by some previous studies [*Sarma*, 2003; *Takahashi et al*., 2009; *Valsala and Maksyutov*, 2010; *Sarma et al*., 2012]. Therefore, the low values of $\delta^{13}C_{DIC}$ observed during the present study in the region are not likely to be due to the oceanic Suess effect.

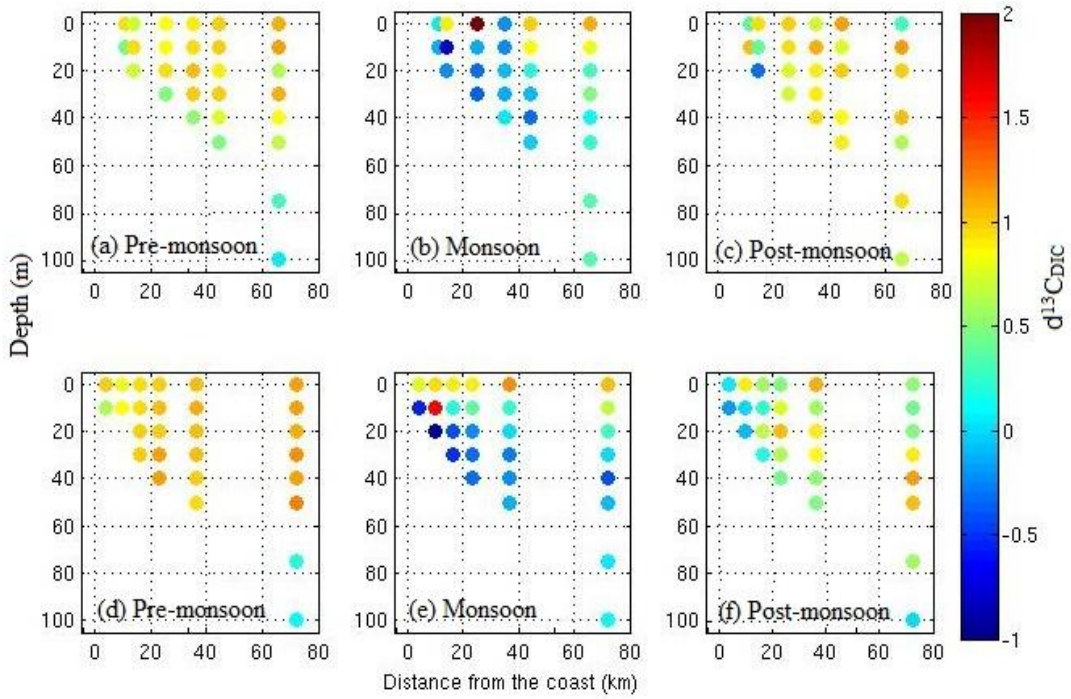


*Figure 5.1.4: Depth profile of the $\delta^{13}C_{DIC}$ in the coastal Arabian Sea; a, b and c are the depth profiles in the Cochin transects during pre-monsoon, monsoon, and the post-monsoon respectively and d, e and f are the same for Mangalore transect.*

The influence of biogeochemical parameters along with the tidal forcing is reflected by the $\delta^{13}C_{DIC}$ during all the three seasons. In general, the surface $\delta^{13}C_{DIC}$ during the pre-monsoon was almost similar

in both the off Cochin (0.86–1.07‰) and off Mangalore (0.81– 1.13‰) transects. During the monsoon, the off Cochin transect surface $\delta^{13}C_{DIC}$ (−0.20–2‰) showed a broader range than the off Mangalore transect (0.75–1.19‰). During the post-monsoon, surface $\delta^{13}C_{DIC}$ in the off Cochin transect were relatively higher (0.31 – 1.14‰) than in the off Mangalore transect (0.04 – 1.07‰; *Figure* 5.1.4). The observed surface $\delta^{13}C_{DIC}$ during the pre-monsoon was nearly similar at both the off Cochin (0.86–1.07‰) and the off Mangalore (0.81–1.13 ‰) transects, whereas during the monsoon, the off Cochin transect (−0.20 – 1.10‰) was depleted compared to the off Mangalore transect (0.75 – 1.19 ‰) except for the high value observed at surface of 30 m deep station (2.0‰).

The seasonal depth wise variations of $\delta^{13}C_{DIC}$ indicate the effects of upwelling. During the post-monsoon, the scenario was different with comparatively higher values of surface $\delta^{13}C_{DIC}$ in the off Cochin transect (0.31–1.14‰) and lower in the off Mangalore transect (0.04–1.07‰) [*Figure* 5.1.4]. While focusing on the depth profile of $\delta^{13}C_{DIC}$, the pre-monsoon was observed with positive $\delta^{13}C_{DIC}$ values which shifted to negative during the monsoon. *Figure* 5.1.4 clearly shows highly depleted surface waters at the off Cochin transect, at least up to a distance of 35 km from the coast, compared to corresponding waters at the Mangalore transect during the monsoon which evidently showed the influence of discharge from the Cochin estuary.

In contrast, the subsurface waters and outer shelf waters at the off Mangalore transect are more depleted in $^{13}C$ with lower $\delta^{13}C_{DIC}$ than at the off Cochin transect. The off Mangalore shelf experiences a high intrusion of relatively highly mineralized and oxygen depleted upwelled waters from the offshore oxygen minimum zone compared to

the Cochin region [*Gupta et al.*, communicated], and the further oxygen demand turns the subsurface shelf waters to anoxic and anaerobic up to about 60 km from the coast [*Sudheesh et al.,* in preparation]. The highest $^{13}C$ depletion in DIC at the near shore bottom waters of off Mangalore during the monsoon [*Figure* 5.1.3e] is due to the strong sulfate reduction in these highly anoxic waters/sediments [*Naqvi et al.,* 2009; *Sudheesh et al.,* in preparation].

### 5.1.5 Mixing curve approximation in the coastal Arabian Sea

The surface average $\delta^{13}C_{DIC}$ values observed during three seasons (pre-monsoon: CH=0.78; MR=0.85, monsoon: CH=0.69, MR=0.82 and post-monsoon: CH=0.63, MR=0.51‰] are lower than the model predicted open ocean values [*Figure* 5.1.4]. Previous studies have reported that the Arabian Sea is a source of $CO_2$ to the atmosphere rather than a sink [*Sarma*, 2003; *Takahashi et al.*, 2009; *Valsala and Maksyutov*, 2010; *Sarma et al.*, 2012] hence; the oceanic Suess effect can be neglected. Therefore, $\delta^{13}C_{DIC}$ in the coastal Arabian Sea is most likely dependent on physical, biological and thermo dynamical processes in the ocean as well as its surroundings rather than atmospheric $CO_2$ concentration.

To confirm this hypothesis we have used a similar mixing curve approximation for this region as well. Like in the estuary, the Periyar River and average surface sea water values has been taken as freshwater and seawater end members, respectively. *Figure* 5.1.5 (a, b, c) shows the $\delta^{13}C_{DIC}$ conservative mixing line plotted against salinity during the pre-monsoon, the monsoon and the post-monsoon and *Figure* 5.1.5 (d, e, f) shows that of DIC concentrations in the off Cochin transect. Since the freshwater end member of off Mangalore is not

dominant, samples were not collected from the corresponding river end member and hence, we could not apply the mixing curve approximation for the off Mangalore transect.

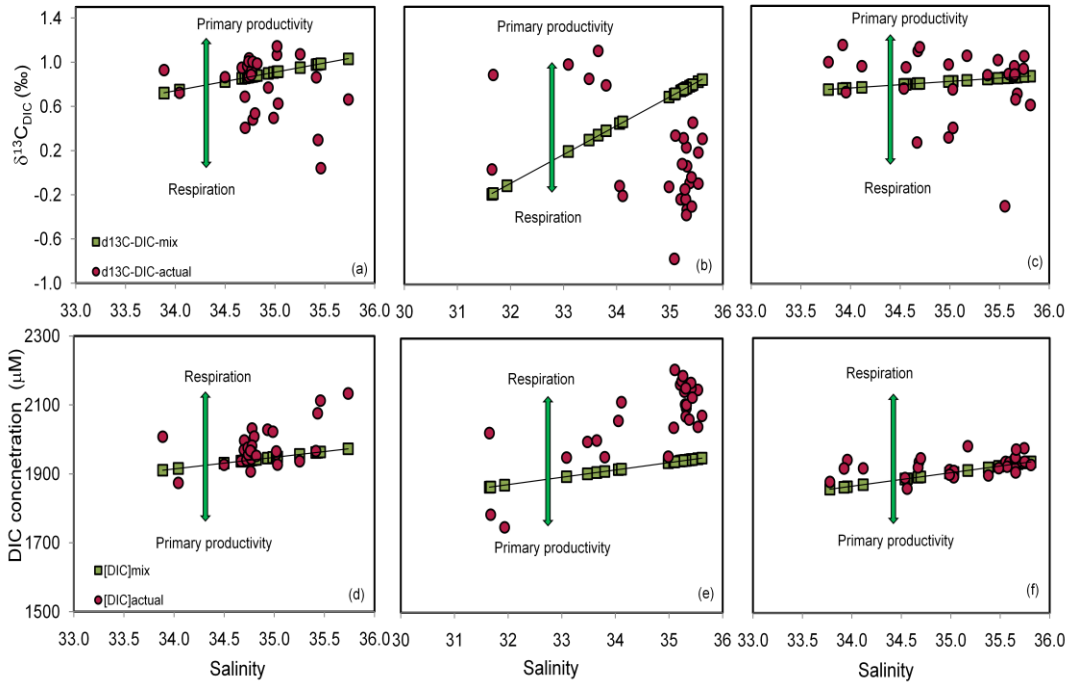


*Figure 5.1.5: (a), (b) and (c) represent the values of $\delta^{13}C_{DIC}$ conservative (green squares) and actual (red circles) plotted against salinity in the off Cochin transect during the pre-monsoon, monsoon and post-monsoon, respectively. Figure (d), (e), and (f) indicate the corresponding plots with DIC. The arrows indicate the influence of primary productivity (PP) and respiration on the $\delta^{13}C$ and concentration of DIC.*

The deviations from the conservative mixing values in the off Cochin transect during the pre-monsoon and monsoon seasons of both DIC and $\delta^{13}C_{DIC}$ show the dominance of respiration/OM degradation over primary productivity. Although the summer monsoon coincides with the peak of the Arabian Sea primary production, the results show the dominance of respiration/OM degradation to drive the Arabian Sea as a source of $CO_2$ to the atmosphere. During the post-monsoon, the actual values of DIC and $\delta^{13}C_{DIC}$ were found to be close to conservative mixing

lines with some exceptions. However, the values at either side of the conservative mixing lines are considered to be altered by *in situ* physical, chemical and biological processes. Physical processes include ground water influx (weathered $CO_2$), upwelling, and $CO_2$ emission from vents. The diffusion or emission of atmospheric $CO_2$ and calcite dissolution can be considered as a chemical processes, whereas biological processes include primary production, decomposition, respiration, and calcite precipitation.

## 5.2 Aquatic food web and stable isotopes

As $\delta^{13}C$ of DIC is used to identify the sources of dissolved inorganic carbon and processes in ecosystems, $\delta^{15}N$ and $\delta^{13}C$ of POM and zooplankton are used for tracing the flow of N and C in the marine food web. The measurement of $\delta^{15}N$ and $\delta^{13}C$ plays a major role in resolving the contribution of isotopically distinct N and C sources to the corresponding elemental budget of a system. The distinct isotopic composition of different sources of N to the estuaries and coastal waters consequently propagate to the primary producers and then to higher trophic level organisms through the marine food web [*Rau et al.,* 1981; *Spice et al.,* 1989; *McClelland and Valiela,* 1998; *Tucker et al.*, 1999]. Each stage involves a proportionate isotopic fractionation mainly due to excretion of isotopically depleted $NH_4^+$ [*Checkley and Miller*, 1989]. The isotopic enrichment of resulting zooplankton body can be used as an efficiency index of N transfer within the marine food web.

A number of studies have been conducted in estuaries and coastal systems to investigate the isotopic flow of N and C in the marine food web [*Rau et al.,* 1981; *Goering et al.*, 1990; *Montoya et al.*, 1991; *Tucker et al.*, 1999]. Limited number of studies has reported $\delta^{15}N$ and $\delta^{13}C$ of

the suspended POM in northern Indian Ocean and estuarine systems [e.g., *Kumar et al.*, 2004; *Sarma et al.* 2014; *Bardhan et al.*, 2015]. However, isotopic measurement of zooplankton in the Indian region is lacking. The present study attempted to generate a marine food web for the coastal Arabian Sea using N and C stable isotopic ratio.

Samples from the coastal Arabian Sea were collected during 9 months (January, March, April, May, June, July, September, October, November, and December) in 2012 to understand the inter-annual variability of the prey to predator isotopic flow. The values of $\delta^{15}$N-POM are reported only for four months (April, October, November and December) in this chapter. Sample collection and analysis are described in *Chapter* 2.

### 5.2.1 $\delta^{15}$N and $\delta^{13}$C of POM in the coastal Arabian Sea

Natural oceanic phytoplankton community has a wide range of $\delta^{13}$C values as obtained by many studies conducted [*Degens et al.,* 1968; *Rau et al.,* 1983; *Gearing et al.,* 1984; *Kumar et al.*, 2004, 2005]. The major factors that control the $\delta^{13}$C-POM are $\delta^{13}$C-DIC, the morphology of species, the type of carboxylating enzyme involved, and ultimately the growth rate of plankton [*Burkhardt et al.*, 1999]. However, in the case of ocean $\delta^{13}$C-DIC may not be a major factor regulating $\delta^{13}$C-POM, since it is highly abundant and its variability in the spatial and temporal frames is too small [*Bhavya et al.*, unpublished]. The results from the present study show that phytoplankton growth is a major process which controls the $\delta^{13}$C-POM.

*Figure* 5.2.1 shows the dependency of $\delta^{13}$C-POM on POM concentration, which is a measure of phytoplankton growth. The high POM concentrations and $\delta^{13}$C-POM during May-October indicate the

influence of mixing of bottom waters from the onset to withdrawal of southwest the monsoon on marine primary productivity.

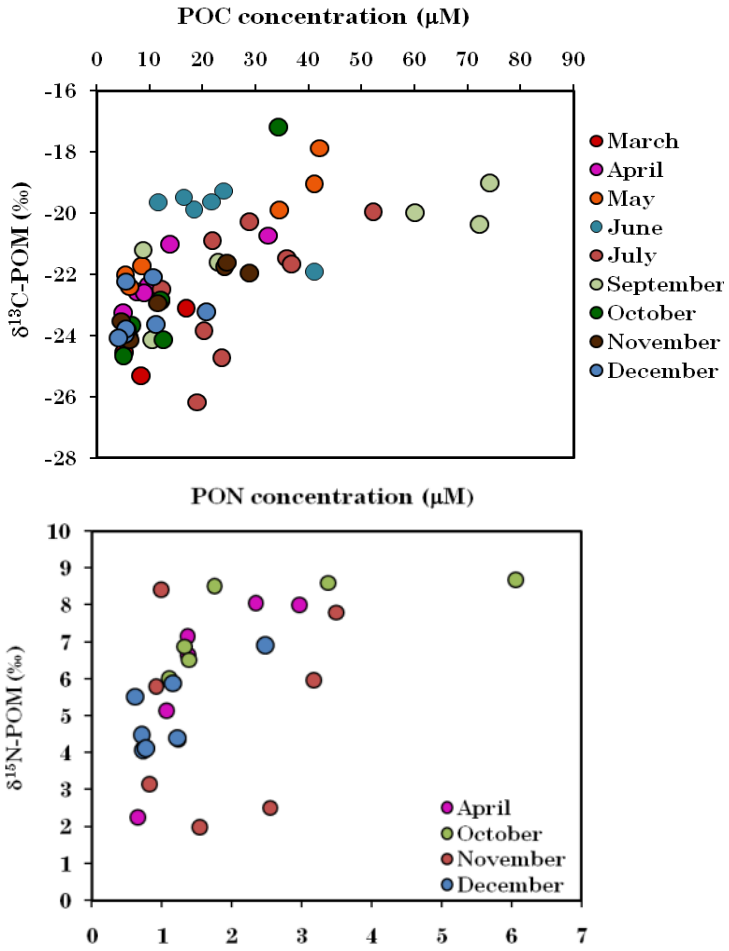


*Figure 5.2.1: Relationship between $\delta^{13}$C-POM and $\delta^{15}$N-POM with POC and PON, respectively, in the surface waters of the coastal Arabian Sea in 2012.*

Similarly, the $\delta^{15}$N-POM also showed a strong positive relationship with PON concentration [*Figure* 5.2.1]. The above explanation is applicable in the case of fractionation of N isotopes during primary production. However, unlike the DIC pool, the DIN pool cannot sustain an invariable isotopic composition. The relatively lower concentration, over consumption, and complex metabolic pathways make $\delta^{15}$N of the substrate pool highly variable.

### 5.2.2 The predator and prey relationship

The isotopic composition of zooplankton depends up on its diet, which is highly variable around the globe. The summary of earlier research works indicates that in general, the C and N isotopic composition of predator is enriched by ≈3 ‰ relative to its prey in different trophic levels [*Rau et al.,* 1981; *Spice et al.,* 1989; *McClelland and Valiela,* 1998]. The results from our study show that the $\delta^{13}C$ and $\delta^{15}N$ of zooplankton are enriched by ≈1.5–4 ‰ than those of POM, which is in agreement with the global observations.

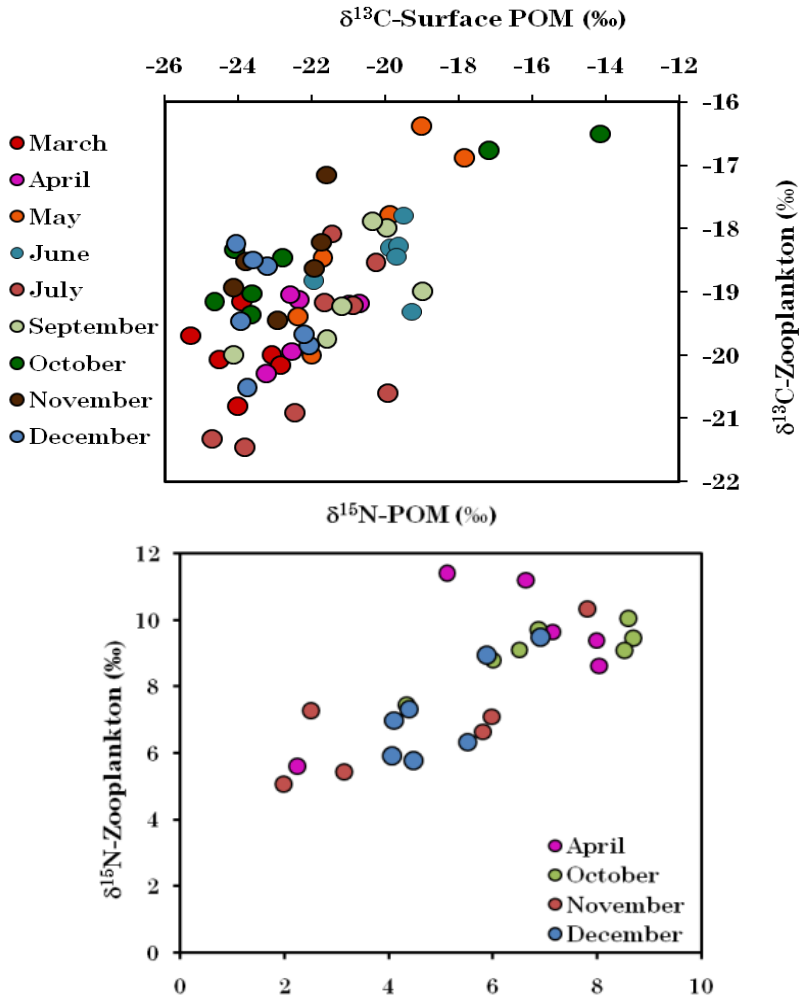


*Figure 5.2.2: The relationship of $\delta^{13}C$ and $\delta^{15}N$ of zooplankton with its corresponding POM.*

The $\delta^{13}C$ of zooplankton near the inlet point of the Cochin estuary was slightly depleted than corresponding values of POM during October. The possible reason for this depletion is the consumption of OM brought by estuary, which is highly depleted (−24 to −28 ‰). In general, the predator-prey isotopic relationship indicates the proportionate flow of OM from the lower trophic level to the higher. However, there is no data available for the isotopic composition of consumers in the high trophic levels in the Arabian Sea food chain so far. Based on our data we attempted to construct a schematic diagram which picturizes the isotopic enrichment in the zooplankton with respect to its diet [*Figure* 5.2.3].

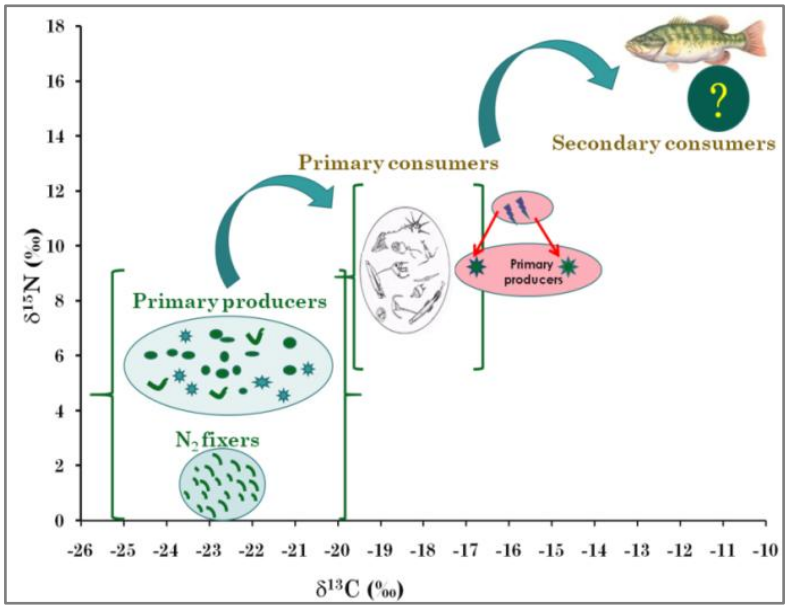


*Figure 5.2.3: The schematic diagram of isotopic enrichment in the marine zooplankton with respect to its diet in the coastal Arabian Sea.*

The picture contains ranges of $\delta^{13}C$ and $\delta^{15}N$ of primary producers and primary consumer (zooplankton) based on the present study. $\delta^{13}C$ and $\delta^{15}N$ of POM varied between −26.17 and −14.16‰ and from 1.98 and 9.02‰, respectively. The values of $\delta^{13}C$ and $\delta^{15}N$ of the zooplankton

exhibited a range from −23.38 to −16.38‰ and from 5.07 to 11.43‰, respectively. Exceptionally enriched $\delta^{15}N$ and $\delta^{13}C$ values observed at two stations during October are represented by different symbols in *Figure* 5.2.3. Some species of diatoms do exhibit $\delta^{13}C$ enrichment up to −14‰ [*Fry and Wainright*, 1991]. It is well known that diatoms growth is proliferated by high Si abundance. The peak of Si concentration in the coastal Arabian Sea is reported to be observed during withdrawal of southwest monsoon (October). Hence, high POC and the lowest $\delta^{13}C$-POM are in agreement with the enhanced diatom growth and large OM production during October.

## 5.3 Conclusions

The DIC pool in the Cochin estuary and the coastal Arabian Sea was found to be depleted in $^{13}C$. Based on the mixing behavior, mineralization and respiration seem to be the major processes controlling their distribution in the Cochin estuary. The study also confirms that the coastal Arabian Sea continues to be a major source of $CO_2$ to the atmosphere. The $\delta^{13}C$ and $\delta^{15}N$ of the POM in the coastal Arabian Sea indicate the seasonal variability of primary production. The $\delta^{13}C$ and $\delta^{15}N$ of the zooplanktons show an enrichment of 1.5–4 ‰ relative to POM. The study also constructed a first isotopic flow in marine food web up to the primary consumers in the coastal Arabian Sea.

# Chapter 6

# Mesocosm experiment

Observations of increase in the global average air and ocean temperatures along with widespread melting of ice and rising sea level provide unequivocal evidence of global warming [*IPCC*, 2007]. Due to continued greenhouse gas (GHG) emissions at or above the present rates, a further warming in global climate system during 21st century has been predicted by climate models. Several associated parameters of climate warming, such as increasing sea surface temperature (SST), ocean acidification, changes in vertical mixing, upwelling, precipitation and evaporation patterns, may in turn impact the resilience of many ecosystems [*Moore et al.*, 2008].

Increase in SST, one of the most important components of climate, is a major indicator of global warming. According to the *IPCC* [2007] report, an average increase of ~1°C in SST across the global ocean is evidently seen during the last century and is expected to increase further during the 21st century. An integrated study conducted from different platforms (satellite, ships and buoys) has also shown upward trends in SST at all latitudes, with rates of change being greater than 2°C per century in many tropical seas [*Hoegh-Guldberg*, 1999].

Sea surface salinity (SSS), another important characteristic of seawater, is one of the determining factors for water density, column stability and circulation patterns in the ocean. Unlike changes in SST, which follows relatively general global pattern, changes in sea surface salinity (SSS), particularly in coastal areas of the world, are largely determined by local meteorological processes, such as precipitation, evaporation and mixing [*Henderson-Sellers & McGuffie*, 2012]. Precipitation data, in general, show considerable spatial and temporal variability. In the last century, precipitation has mostly increased over land, particularly in the high northern latitudes, while decreases have dominated the tropics since the 1970s [*IPCC*, 2007]. The frequency of extreme precipitation, however, has increased over most areas [*Bates et al.*, 2008]. Different climate models agree with a consistent increase in precipitation at higher latitudes and parts of the tropics during the 21$^{st}$ century [*Bates et al.*, 2008].

The Arabian Sea constitutes the north-western part of the Indian Ocean. Its uniqueness is the influence of seasonally reversing monsoonal wind forcings on the biogeochemical cycling, a characteristic which has attracted scientists worldwide to carry out biogeochemical studies. However, the primary focus during major scientific programs such as Joint Global Ocean Flux Studies (JGOFS) has been the open ocean with limited attention to the coastal ecosystems [e.g., *Madhupratap et al.*, 1996; *McCarthy et al.*, 1999; *Watts and Owens*, 1999; *Sambrotto*, 2001; *Kumar et al.*, 2010; *Gandhi et al.*, 2010]. Like many other oceanic basins, hydrographic settings of the coastal Arabian Sea may also display increase of SST, higher freshwater runoff and decreased surface salinity, stronger stratification and changed ratios of macronutrients.

Observations suggest a statistically significant increasing trend of positive SST anomaly over the tropical Indian Ocean along with significant increases in frequency and intensity of extreme monsoon rain events in central India, a source of freshwater to the coastal Arabian Sea and Bay of Bengal [*Goswami et al.*, 2006].

Given the importance of the Arabian Sea as a biogeochemical system and its potential to undergo changes due to warming, it is prudent to examine the N and C uptake capabilities of its pelagic community under different temperature-salinity To explore the above-mentioned aspects, a mesocosm study simulating different temperature and salinity conditions was conducted on natural pelagic microbial community from the coastal waters of the Arabian Sea, a tropical basin. This work formed a part of this study with the main objective to understand potential changes in N uptake capabilities of phytoplankton community of the coastal Arabian Sea, when subjected to different temperature-salinity conditions; we have attempted to mimic the temperature and salinity range predicted by different climate models.

## 6.1 Study area and experimental set up

Mesocosms are large tanks in which culture experiments can be performed in large volumes of sample which would help to mimic the environment to a better extent. *Figure* 6.1 shows the mesocosm set up used for the present study. For the whole study, sixteen mesocosms, each containing 1000 L of seawater, were used to simulate four different conditions with four replicates for each condition. The conditions simulated were (i) ambient temperature - ambient salinity (28°C-35), (ii) ambient temperature-decreased salinity (28°C-31), (iii) increased temperature-ambient salinity (31°C-35), and (iv) increased temperature-decreased salinity (31°C-31). Henceforth, for the purpose

of discussion, these conditions will be referred as mesocosms 1 to 4, respectively [*Figure* 6.2].

*Figure 6.1: Mesocosm tanks in the laboratory for stimulation experiments.*

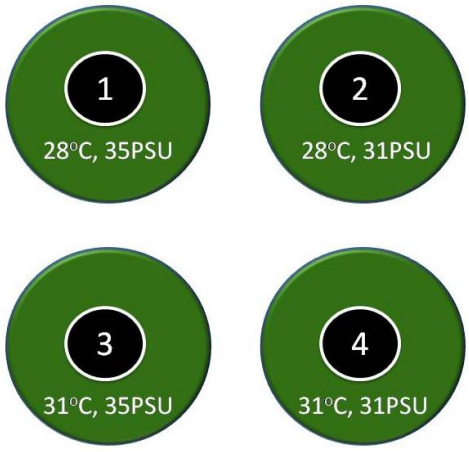


*Figure 6.2: Temperature and salinity conditions set during the experiment in mesocosm tanks. Salinity is given in practical salinity unit (PSU)*

The experiment was performed near Mangalore, central west coast of India [*Figure* 6.3]. Seawater collected from the adjoining beach (12°47′024″ N, 74°51′192″ E) was transported to the mesocosm facility and transferred in a common reservoir (20,000 L). Before releasing it into each mesocosm tank through a network of interconnected pipes,

seawater was filtered through 0.5 µm sand filters and simultaneously ozonated to eliminate living microorganisms. After filling with 1000 L of filtered and ozonated seawater, all tanks were inoculated with equivalent volumes of phytoplankton inoculum collected from 1 nautical mile away from the shore.

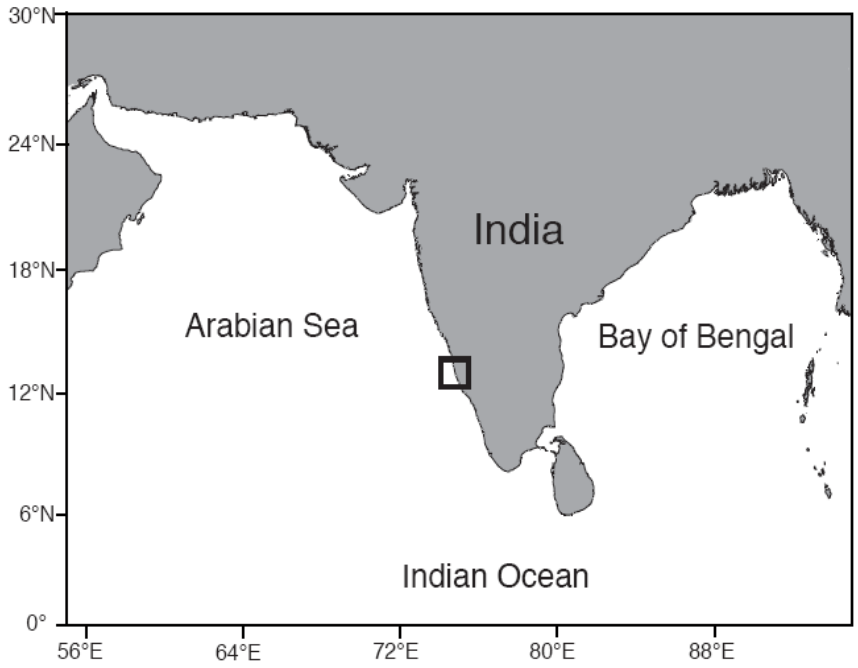


*Figure 6.3: The location in the coastal Arabian Sea from where sea water was collected for the Mesocosm study*

The inoculum was collected using plankton net of 10 µm mesh size during a single episode in the first week of December 2011. Inoculation was followed by two day period of gradual increase in temperature and decrease of salinity to avoid any sudden physical and metabolic shock on inoculated microorganisms. The manipulation was done step-wise by increasing the temperature and decreasing the salinity, respectively, every 6$^{th}$ hours during acclimatization. In mesocosm set up for increased temperature (31°C), a set of 4 underwater heaters were placed to attain the same. Mesocosm with ambient temperature were also provided with a set of heaters to ensure a constant water temperature (28°C). The lowering of salinity, from 35 to 31, was done

by slowly extracting previously calculated volumes of seawater from the mesocosms through submerged plankton net, not to lose or damage any cells, and thereafter adding equivalent volume of sterilized fresh water. In the end, all mesocosms had the final volume of 1000 L and the equal biomass of phytoplankton cells. Water temperature and salinity were identical among the four replicates within each mesocosm, initiating the subsequent sampling period.

In each mesocosm, surface light intensity was maintained at 50 µmol photon $s^{-1}m^{-2}$ using four plant-growth fluorescent light lamps approximately 30 cm above water surface. The diurnal light cycle was set to 12 hours. To maintain equal surface light conditions and to prevent unwanted particles from falling in, reflective blankets were put up above each tank with slits to facilitate air circulation. The mesocosms were mixed by continuous aeration, and gently stirred by a wooden stick (one for each mesocosm in order to avoid contamination among the different treatments) 30 minutes before sampling on alternate days. Nutrient concentrations ($NO_3^-$ and $NH_4^+$) in the mesocosm water were measured prior to the start of the experiment to ensure nutrient replete conditions for algal and bacterial growth.

## 6.2 Nitrogen and carbon uptake experiments

Four out of sixteen mesocosms, each belonging to different conditions, were selected for N uptake experiments. These experiments were performed on four days [($8^{th}$-Dec-2011)$2^{rd}$, ($11^{th}$-Dec-2011)$5^{th}$, ($13^{th}$-Dec-2011)$7^{th}$ and ($16^{th}$-Dec-2011)$10^{th}$ day] of the whole experiment period, representing initial, peak and declining phase of phytoplankton growth. For the convenience in discussion, these four days when uptake experiments were conducted will be considered as first to fourth day of experiment, with first and last day as representatives of the

beginning and the declining phases, and second and third days as the middle phase.

On each experiment day, water samples from each mesocosm were collected into 1 L acid-washed polycarbonate Nalgene bottles in duplicate for $NH_4^+$ and $NO_3^-$ uptake. Samples were spiked with 98+ atom% enriched $Na^{15}NO_3$ and $^{15}NH_4Cl$ to trace $NO_3^-$ and $NH_4^+$ uptake, respectively. Subsequent to tracer additions, bottles were incubated inside respective mesocosm tanks for four hours. Post-incubation samples were filtered onto precombusted GF/F filters (400˚C at 4hrs) and subsequently oven dried for mass spectrometric analysis where $^{15}N$ atom% and particulate organic N (PON) content were measured using continuous flow stable isotope mass spectrometer (Delta V Plus) connected to an elemental analyzer (Flash EA 2000). The coefficient of variation in $^{15}N$ atom% measurement was < 5% for $NO_3^-$ and $NH_4^+$ uptake samples whereas the average difference in PON measurement of duplicate samples was < 8%. The uptake rate was calculated using the equation of *Dugdale & Wilkerson*, [1986] (see *Chapter* 2).

## 6.3 Response of phytoplankton towards temperature and salinity

In the beginning of the experiment, average $NO_3^-$ and $NH_4^+$ concentrations in the mesocosms were 8.4 ± 1 μM and 2.0 ± 0.5 μM, respectively [*Figure* 6.4]. The slight difference in initial nutrient concentration is due to the nutrient consumption by plankton during acclimatization. Chl *a* values were low during the initial phase of the experiment, peaked in the middle and reduced subsequently [*Figure* 6.5]. A day after inoculation, Chl *a* concentrations in all four mesocosms were less than 5 μg $l^{-1}$, which increased to higher than 30 μg $l^{-1}$ during the peak days, except for mesocosm 2 (28°C-31). Diatoms

were dominating the phytoplankton community in all mesocosms (data not shown).

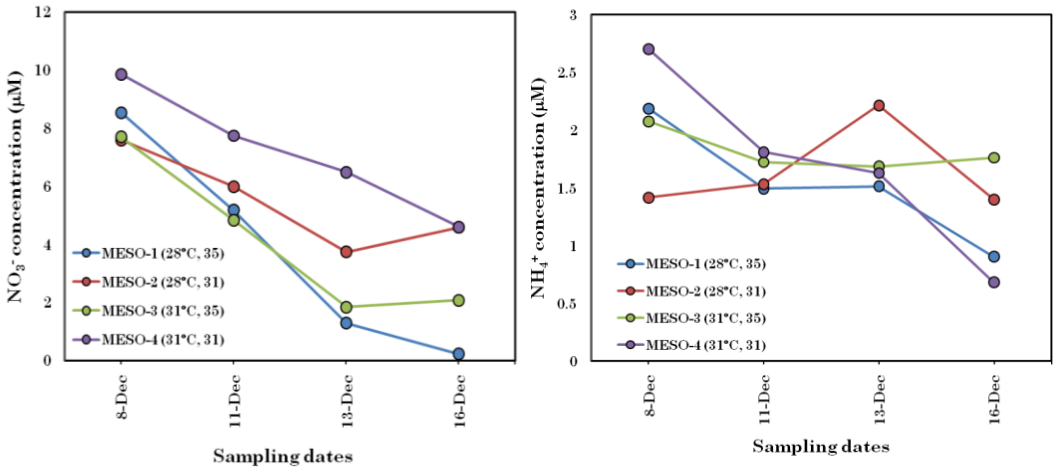


*Figure 6.4: The variation of $NH_4^+$ and $NO_3^-$ concentrations in the mesocosm tanks during the sampling period.*

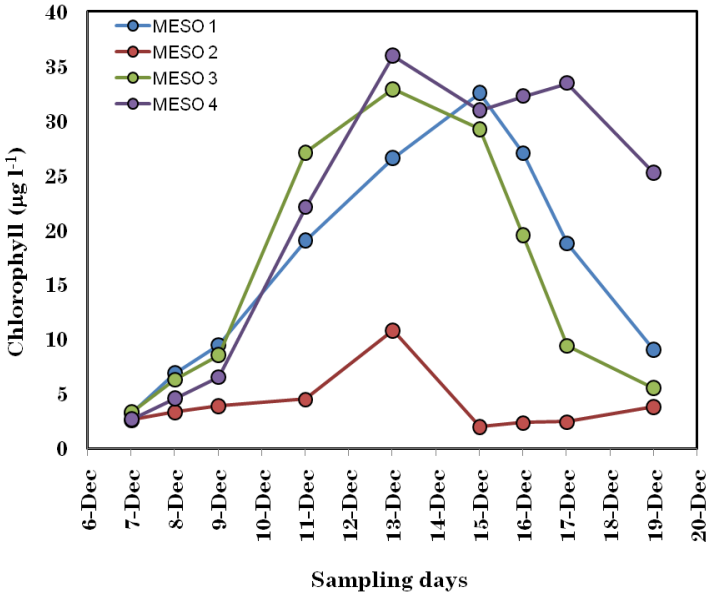


*Figure 6.5: Variation of Chlorophyll concentration in the four mesocosms throughout the duration of the experiment.*

## 6.4 Nitrogen and carbon uptake rates

For all mesocosms, consistent with the Chl *a*, the total DIN and C uptake rates also showed similar patterns, i.e., relatively lower values during the beginning and the end of the experiment with peaks in the

middle. In general, the highest total DIN uptake rate on each experimental day was observed for mesocosm 1, maintained in ambient conditions (28°C-35), whereas mesocosm 2 with ambient temperature and reduced salinity (28°C-31) showed significantly lower values compared to the other treatments. The highest total DIN uptake for mesocosm 2 was observed on the third day of the experiment (0.13 µmol N $l^{-1}$ $h^{-1}$), which was lower than the minimum (0.16 µmol N $l^{-1}$ $h^{-1}$) observed for mesocosm 1. Increase in DIN uptake in the mesocosm 2 on third day might be due to addition of nutrients due to remineralization which was shown in *Figure* 6.6. In mesocosm 4, with increased temperature and reduced salinity (31°C-31), total N uptake rates, in general, were higher than the mesocosm 2 (28°C-31).

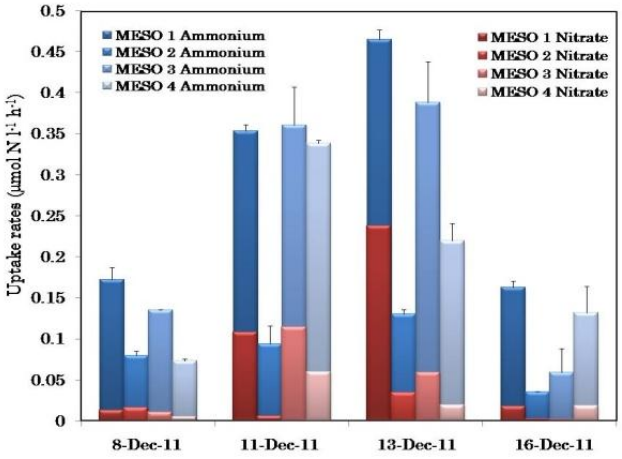


*Figure 6.6: Variations in the $NO_3^-$ and $NH_4^+$ uptake rates in the four different mesocosm tanks.*

Between the two mesocosms where salinity remained the ambient (35) but temperature was increased from 28 to 31°C (mesocosms 1 and 3), total N uptake rates did not differ significantly, particularly in the middle of the experimental period. Individually, $NH_4^+$ uptake rates followed a similar pattern as the total DIN uptake which increased with time to peak in the middle and decreased again at end of the experiment [*Figure* 6.6]. The lowest $NH_4^+$ uptake rate (0.03 µmolN $l^{-1}$ $h^{-}$

$^{1}$) was observed for mesocosm 2 (28°C-31). $NH_4^+$ uptake rates in this mesocosm were significantly lower than those observed for other mesocosms. The highest $NH_4^+$ uptake (0.32 µmol N $l^{-1}$ $h^{-1}$) was observed for mesocosm 3 with increased temperature and ambient salinity (31°C-35) on the third day of the uptake experiment.

The $NO_3^-$ uptake rates also followed a similar trend as the $NH_4^+$ and the total uptake [*Figure* 6.6]. At the start of the experiment, the $NO_3^-$ uptake rates were similar for all mesocosms and increased on the second day except for the mesocosm 2 (28°C-31), where it showed a decrease. The highest $NO_3^-$ uptake (0.23 µmol N $l^{-1}$ $h^{-1}$) was observed for mesocosm 1 with ambient conditions (28°C-35) on the third day of the experiment, when $NO_3^-$ uptake rates for all other conditions were significantly lower. Ammonium was the preferred substrate for phytoplankton under all mesocosms. In the beginning, $NO_3^-$ uptake contribution to the total uptake was less than 10% for all mesocosms, except mesocosm 2 (28°C-31), where it was 20%. However, the contribution of $NO_3^-$ uptake increased in the middle of the study period, i.e., on the second and third day, to reduce again in the end. Overall, $NO_3^-$ uptake was less than 32 % of the total uptake, except for mesocosm 1 (28°C-35), in which it was around 50 % on the third day.

Carbon uptake rates in mesocosm 2 did not show such an increase on third day, rather it gradually decayed as the experiment progressed. All other conditions except mesocosm 2 were observed to be favorable for phytoplankton growth with high C uptake rates [*Figure* 6.7]. However, the species diversity in the mesocosm reduced except in the mesocosm with natural conditions [*Kahlon et al.* unpublished], with the survival of opportunistic species *Skeletonema grevilleii*. Over the study period, total N uptake was significantly different among mesocosms ($p = 0.01$). This indicates that ambient temperature and salinity

conditions are the best suited for phytoplankton in the tropics, which is already at its optimum environmental conditions. Any further change in SST or SSS may alter the species abundance, diversity and potential assimilation rates.

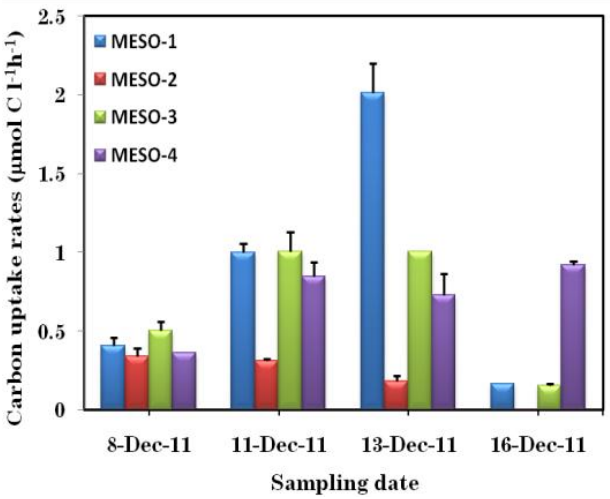


*Figure 6.7: The variation of carbon uptake rates in the mesocosm tanks.*

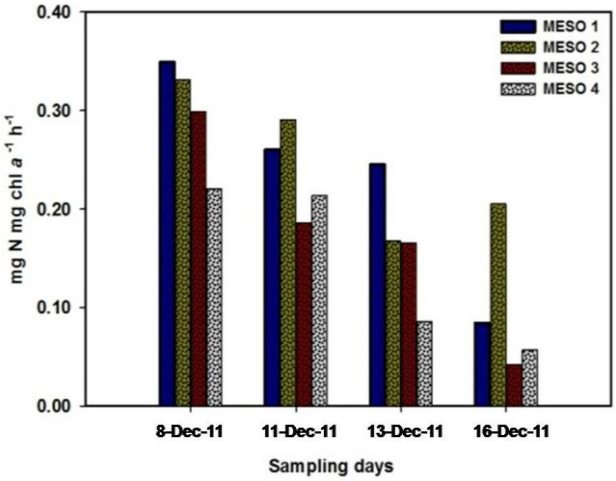


*Figure 6.8: The quantum yield of nitrogen for unit chlorophyll.*

For all mesocosms, calculations for total N uptake per unit Chl *a* showed an interesting pattern, with a gradual decline over time [*Figure* 6.8]. High total N uptake per unit Chl *a* (µgN (µgChl *a*)$^{-1}$ h$^{-1}$) was observed at the start of the experiment with the highest value

(0.35) for mesocosm 1 (28°C-35). Interestingly, contrary to the general trend observed for other mesocosms, this value showed a slight increase from the third (0.17) to the fourth (0.20) day for mesocosm 2, the only increase during this study [*Figure* 6.8].

## 6.5 Controlling factors of nitrogen and carbon uptake rates

In all mesocosms, the DIN uptake rates did not show any significant relationship with substrate concentrations [*Figure* not shown], where the average initial $NO_3^-$ (8.4 ± 1 μM) and $NH_4^+$ (2.0 ± 0.5 μM) concentrations were high. This indicates that the variations observed during the present study are due to the modulating role played by simulated temperature and salinity conditions on the DIN uptake rates. Generally, the mechanism of nutrient supply and its kinetics of utilization determines the productivity and size structure of phytoplankton [*Harrison et al.*, 1996], which follows concentration-dependent uptake of dissolved inorganic DIN according to Michaelis-Menten kinetics for enzymes [*Menten and Michaelis*, 1913].

But it appears that under nutrient replete tropical conditions, factors such as temperature and salinity become important. It has been reported earlier that kinetic relationships, such as nutrient uptake, exhibit wide range of variability even for a given substrate and species. These relationships can be a function of physiological state of the phytoplankton cells, time of exposure to nutrients and environmental factors [*Glibert et al.,* 2012]. The present experimental results probably favours to argue that under changing hydrographical conditions the DIN uptake potential of coastal tropical phytoplankton may tend to behave independently of ambient substrate concentrations.

Significantly lower total DIN uptake rates for mesocosm 2 with reduced salinity and ambient temperature entail important consequences for potential DIN uptake in the coastal Arabian Sea under increased precipitation. Total DIN uptake rates displayed an increase when temperature was increased from 28 to 31°C, while maintaining reduced salinity (31) condition (mesocosm 4). This implies that elevated temperature probably favours increase in N uptake. However, ambient condition shows higher N uptake, which probably is due to adaptation and affinity to local environmental conditions in which the phytoplankton lived. Overall, N uptake potential in tropical coastal ecosystem such as during the present study, may be an interplay between salinity and temperature conditions. While N uptake rates tend to decrease with the reduction in salinity, slight increase in temperature may contribute to its increase. However, as mentioned earlier, it has also been predicted that increase in temperature may be adverse for the growth of phytoplankton in tropical regions [*Thomas et al.*, 2012]. In such a case, the thermal niche width, the temperature range over which growth rate is positive, remains to be carefully explored. *Thomas et al.* [2012] showed a wide range (approximately 22°C to 32°C) optimum temperatures for the growth of phytoplankton strains from the same geographical region where the inoculum was collected for the present experimental study.

A few studies show the effect of salinity and temperature stress on DIN uptake rates. A limited number of studies from estuarine ecosystem have observed the relationship between uptake rates and salinity [e.g., *Middelburg & Nieuwenhuize*, 2000; *Mulholland et al.*, 2003]. In a completely different salinity range (0.03-10.5), higher DIN uptake has been observed for higher salinity waters in a sub-estuary of the Chesapeake Bay despite lower $NO_3^-$ and $NH_4^+$ concentrations compared

to fresher waters [*Mulholland et al.*, 2003]. A similar trend has been observed in the Cochin estuary, a tropical ecosystem on the southwest coast of India, during the pre-monsoon [*Bhavya et al.*, 2015]. This appears to show that salinity has a role to play in modulating DIN uptake rates, however the exact biological mechanisms for the same are presently not understood.

Independent of mesocosm conditions, $NH_4^+$ was assimilated in preference to $NO_3^-$ and the primary production appears to be mainly supported by ammonium based regenerated N. It is important to note that the ambient $NO_3^-$ concentration during the present study was more than that of $NH_4^+$. This observation is consistent with other N uptake studies from the open Arabian Sea [*McCarthy et al.*, 1999; *Sambrotto*, 2001; *Kumar et al.*, 2010]. The preference for reduced or recycled forms of N such as $NH_4^+$ is well known in aquatic environments [e.g., *Dugdale and Goering*, 1967; *McCarthy and Eppley*, 1972; *Glibert et al.*, 1982] due to the higher energy requirement for the assimilation of $NO_3^-$ [*McCarthy and Carpenter*, 1983].

Although contribution of $NO_3^-$ to the total uptake increased during the middle phase of the experiment compared to the beginning and the end, it remained lower than the $NH_4^+$ uptake throughout, except on the third day for mesocosm 1 [*Figure* 6.6]. It appears that some physical or biological controls were limiting $NO_3^-$ utilization despite the high primary productivity, which has been observed in the open Arabian Sea as well [*McCarthy et al.*, 1999]. Inhibition of $NO_3^-$ uptake in the presence of $NH_4^+$ could be one of the reasons for lower $NO_3^-$ uptake, a commonly observed feature in the world oceans [e.g., *Glibert et al.*, 1982; *Harrison et al.*, 1987; *McCarthy et al.*, 1999].

Although the extent of inhibition is much more variable, the inhibition is potentially greater under nutrient replete conditions and the least

when phytoplankton are N-starved [*Conway*, 1977; *Dortch*, 1990]. During the present study, ambient conditions were nutrient replete and this might have contributed to relatively lower $NO_3^-$ uptake. Yet another reason could be higher than required addition of $^{15}NH_4^+$ tracer during the experiment, which could also trigger artificial stimulation of $NH_4^+$ uptake [*Watts and Owens*, 1999]. However, during the present study, the addition of $NH_4^+$ was not large enough to stimulate the artificial uptake, evident by very low correlation ($r^2$~0.006) between the percentages of $NH_4^+$ addition relative to ambient and $NH_4^+$ uptake rates for all mesocosms. One more reason for $NO_3^-$ underutilization could be iron and silicon limitation [*Landry et al.*, 1997] but the ranges of iron [*Measures and Vink*, 1999] and silica [*Morrison et al.*, 1998] concentrations in the Arabian Sea, especially in the coastal area, are greater than the known limiting values.

## 6.6 Conclusions

Our results indicate that under nutrient replete coastal conditions salinity and temperature are important in modulating the assimilation rates of the pelagic microbial community and thereby affecting the pool of inorganic nutrients. The lower N and C uptake rates in the decreased salinity and ambient temperature condition indicate the negative effect of osmotic stress on plankton community. This points towards the likely decreasing primary productivity in future if salinity decreases with higher precipitation.

# Chapter 7

# Summary and scope for future work

The finding of the present study provides important insights to the biogeochemical research of the Cochin estuary and the coastal Arabian Sea. The study reveals the major consequences of anthropogenic activities and climate change on the N and C cycling in these ecosystems. The highlights of the present study are summarized below.

## 7.1 Nitrogen dynamics in the Cochin estuary and the coastal Arabian Sea

- DIN uptake rates in the Cochin estuary is highly influenced by anthropogenic nutrient loading.

- The existence of diazotrophs and their ability to fix $N_2$ in nutrient replete conditions has been identified in the Cochin estuary.
- The results show that the relative abundances of N and P controls DIN uptake and $N_2$ fixation rates in the Cochin estuary rather than their individual concentrations.

- The optimum DIN uptake rates in the estuary is observed at a TN:TP close to the Redfield's ratio (16:1).

- The highest value of $N_2$ fixation is observed at the station with a low TN:TP ratio, where P was not a limiting factor.

- DIN assimilation at the nearshore station in the coastal Arabian Sea was found to be influenced by the estuary.

## 7.2 Carbon dynamics in the Cochin estuary and the coastal Arabian Sea

- The $^{13}C$ ($NaHCO_3$) labeling technique is not the best suited method for primary productivity measurements for aquatic environments with high $CO_{2,aq}$ concentrations
- Primary production in the Cochin estuary was found to be optimum, when the TN:TP ratio was closer to the Redfield ratio.

## 7.3 Nitrogen and carbon isotopes as tracers of carbon dynamics and the marine food web

- Based on the mixing curve approximation, respiration seems to dominate in the Cochin estuary and coastal Arabian Sea.
- The study also confirms that the coastal Arabian Sea continues to be a major source of $CO_2$ to the atmosphere.
- The $\delta^{13}C$ and $\delta^{15}N$ of the zooplankton show an enrichment of 1.5–4 ‰ relative to POM.
- The first C and N isotopic flow in marine food chart up to primary consumers in the coastal Arabian Sea has been constructed.

## 7.4 Response of marine phytoplankton to the future temperature and salinity conditions

- Under nutrient replete coastal conditions, salinity and temperature modulate N and C assimilation rates.
- Lower N and C uptake rates in decreased salinity and ambient temperature conditions indicate the negative effect of osmotic stress on phytoplankton.

- The results also point towards the decrease in primary productivity in future when salinity decreases with increasing precipitation.

## 7.5 Scope for future work

- The role of relative abundances of N and P in the nutrient replete Cochin estuary is the major highlight of the present study. A detailed culture experiment with varying N and P concentrations and salinity is recommended for the better understanding C and N assimilation processes in the nutrient rich environments.

- The presence of diazotrophs and their ability to fix $N_2$ was identified in the present study using $^{15}N_2$ bubble method. Comparison of bubble method and newly developed dissolution method should be performed in the estuarine and coastal environments, to improve the N budget of these environments.

- Individual analysis of isolated $N_2$ fixing species under varying nutrient and salinity conditions could be performed to understand species wise response to the changing nutrient and hydrographic conditions.

- The rates of many N pathways in the Cochin estuary and the coastal Arabian Sea are not investigated so far, particularly the anaerobic processes such as denitrification and anammox. Experiments using $^{15}N$ tracers could be performed to understand the rates of N loss from these ecosystems.

- Our study reported the underestimation of primary productivity while using $NaH^{13}CO_3$ labelling technique in the high $pCO_2$

environments. It is highly recommended to use both $^{13}CO_2$ and $NaH^{13}CO_3$ to estimate the potential primary productivity in such $CO_2$ supersaturated environments.

- During the present study, $\delta^{13}C_{DIC}$ is used as a proxy to understand the sources of DIC and processes in the estuarine and coastal environment. A detailed study in multiple stations is required to reveal the complexity of estuarine C dynamics.

- The present study attempted to construct the flow of C and N isotopes in the marine food web up to primary consumers. More studies are needed on the isotopic composition and its enrichment in higher trophic levels in the Cochin estuary and the coastal Arabian Sea. A detailed monthly measurement of $\delta^{15}N$ and $\delta^{13}C$ in various trophic levels could be performed to understand the marine food web thoroughly, which would help in marine conservation and management.

- Smaller size phytoplankton and heterotrophic bacteria are known to play a significant role in N uptake. Hence, the response of bacteria and picoplankton under such simulated conditions as during the present study warrants attention in future. Also, the extent of dissolved organic N as an alternative source of N was not considered in this study and could be a component of N uptake in future studies.

# References


Alling, V., Porcelli, D., Mörth, C. M., Anderson, L. G., Sanchez-Garcia, L., Gustafsson, Ö., Anderson, P. S., and Humborg, C. (2012). Degradation of terrestrial organic carbon, primary production and out-gassing of $CO_2$ in the Laptev and East Siberian Seas as inferred from $\delta^{13}C$ values of DIC. *Geochimica Cosmochimica Acta*, *95*, 143-159.

Arrigo, K. R. (2005). Marine microorganisms and global nutrient cycles. *Nature*, *437*(7057), 349-355.

Badger, M. R., Andrews, T.J., Whitney, S.M., Ludwig, M., Yellowlees, D.C., Leggat, W., Price, G.D. (1998). The diversity and co-evolution of Rubisco, plastids, pyrenoids, and chloroplast-based $CO_2$-concentrating mechanisms in algae. *Canadian Journal of Botony*, 76, 1052– 1071.

Balachandran, K. K., Laluraj, C. M., Martin, G. D., Srinivas, K., & Venugopal, P. (2006). Environmental analysis of heavy metal deposition in a flow-restricted tropical estuary and its adjacent shelf. *Environmental Forensics*, *7*(4), 345-351.

Balachandran, K. K., Reddy, G. S., Revichandran, C., Srinivas, K., Vijayan, P. R., & Thottam, T. J. (2008). Modelling of tidal hydrodynamics for a tropical ecosystem with implications for pollutant dispersion (Cohin Estuary, Southwest India). *Ocean Dynamics*, *58*(3-4), 259-273.

Balachandran, K.K. 2001. Chemical oceanographic studies of the coastal waters of Cochin. *Ph.D Thesis,* Cochin University of Science and Technology, 1-264pp.

Balch, W. M., Gordon, H. R., Bowler, B. C., Drapeau, D. T., & Booth, E. S. (2005). Calcium carbonate measurements in the surface global ocean based on Moderate-Resolution Imaging Spectroradiometer data. *Journal of Geophysical Research: Oceans (1978–2012)*, *110*(C7).

Bange, H. W., Rixen, T., Johansen, A. M., Siefert, R. L., Ramesh, R., Ittekkot, V., ... & Andreae, M. O. (2000). A revised nitrogen budget for the Arabian Sea. *Global Biogeochemical Cycles*, *14*(4), 1283-1297.

Barber, R. T., Marra, J., Bidigare, R. C., Codispoti, L. A., Halpern, D., Johnson, Z., ... & Smith, S. L. (2001). Primary productivity and its regulation in the Arabian Sea during 1995. *Deep Sea Research Part II: Topical Studies in Oceanography*, *48*(6), 1127-1172.

Bardhan, P., Karapurkar, S. G., Shenoy, D. M., Kurian, S., Sarkar, A., Maya, M. V., ... & Naqvi, S. W. A. (2015). Carbon and nitrogen isotopic composition of suspended particulate organic matter in Zuari Estuary, west coast of India. *Journal of Marine Systems*, *141*, 90-97.

Bates, B., Kundzewicz, Z. W., Wu, S., & Palutikof, J. (2008). *climate change and Water: technical Paper vi*. Intergovernmental Panel on Climate Change (IPCC).

Behrenfeld, M. J., O'Malley, R. T., Siegel, D. A., McClain, C. R., Sarmiento, J. L., Feldman, G. C., ... & Boss, E. S. (2006). Climate-driven trends in contemporary ocean productivity. *Nature*, *444*(7120), 752-755.

Bhavya, P. S., Kumar, S., Gupta, G. V. M., Sudheesh, V., Sudharma, K. V., Varrier, D. S., ... & Saravanane, N. (2015). Nitrogen uptake dynamics in a tropical eutrophic estuary (Cochin, India) and adjacent coastal waters. *Estuaries and Coasts*, 1-14.

Biswas, H., Mukhopadhyay, S. K., De, T. K., Sen, S., & Jana, T. K. (2004). Biogenic controls on the air-water carbon dioxide exchange in the Sundarban mangrove environment, northeast coast of Bay of Bengal, India. *Limnology and Oceanography*, *49*(1), 95-101.

Boesch, D., Burreson, E., Dennison, W., Houde, E., Kemp, M., Kennedy, V., ... & Kidwell, S. (2001). Factors in the decline of coastal ecosystems. *Science*, *293*(5535), 1589-1591.

Bopp, L., Monfray, P., Aumont, O., Dufresne, J. L., Le Trent, H., Madec, G., ... & Orr, J. C. (2001). Potential impact of climate change on marine export. *Global Biogeochemical Cycles*, *15*(1), 81-99.

Borges, A. V., Schiettecatte, L. S., Abril, G., Delille, B., & Gazeau, F. (2006). Carbon dioxide in European coastal waters. *Estuarine, Coastal and Shelf Science*, *70*(3), 375-387.

Borum, J. (1996). Shallow waters and land/sea boundaries. *Eutrophication in coastal marine ecosystems*, 179-203.

Bouillon S, Dahdouh-Guebas F, Rao AVVS, Koedam N, Dehairs F (2003) Sources of organic carbon in mangrove sediments: variability and some possible implications forecosystem functioning. Hydrobiologia 495:33–39.

Bouillon, S., Dahdouh-Guebas, F., Rao, A. V. V. S., Koedam, N., & Dehairs, F. (2003). Sources of organic carbon in mangrove sediments: variability and possible ecological implications. *Hydrobiologia*, *495*(1-3), 33-39.

Bouillon, S., Frankignoulle, M., Dehairs, F., Velimirov, B., Eiler, A., Abril, G., ... & Borges, A. V. (2003). Inorganic and organic carbon biogeochemistry in the Gautami Godavari estuary (Andhra Pradesh, India) during pre-monsoon: The local impact of extensive mangrove forests. *Global biogeochemical cycles*, *17*(4).

Boyce, D. G., Lewis, M. R., & Worm, B. (2010). Global phytoplankton decline over the past century. *Nature*, *466*(7306), 591-596.

Bricker, S. B., C. G. Clement, D. E. Pirhalla, S. P. Orlando, D. R. G. Farrow. 1999. National Estuarine Eutrophication Assessment: Effects of Nutrient Enrichment in the Nation's Estuaries. *NOAA, National Ocean Service, Special Projects office and the National Centres for Coastal Ocean Science. Silver Spring, MD*.

Bronk, D. A., P. M. Glibert, and B. B. Ward. 1994. Nitrogen uptake, dissolved organic nitrogen release and new production, *Science*, 265, 1843–1846.

Burkhardt, S., Amoroso, G., Riebesell, U., & Sültemeyer, D. (2001). $CO_2$ and $HCO_3^-$ uptake in marine diatoms acclimated to different $CO_2$ concentrations. *Limnology and Oceanography*, *46*(6), 1378-1391.

Burkhardt, S., Riebesell, U., & Zondervan, I. (1999). Effects of growth rate, $CO_2$ concentration, and cell size on the stable carbon isotope fractionation in marine phytoplankton. *Geochimica et Cosmochimica Acta*, *63*(22), 3729-3741.

Burkill, P. H., Mantoura, R. F. C., & Owens, N. J. (1993). Biogeochemical cycling in the northwestern Indian Ocean: a

brief overview. *Deep Sea Research Part II: Topical Studies in Oceanography*, *40*(3), 643-649.

Cai, W. J., Pomeroy, L. R., Moran, M. A., & Wang, Y. (1999). Oxygen and carbon dioxide mass ba Capone, D. G., J. Zehr, H. Paerl, B. Bergrnan, E. J. Carpenter. 1997. *Trichodesmium*: A globally significant marine cyanobacterium. *Science* 276: 1221-1229.

Capone, D. G., Ferrier, M. D., & Carpenter, E. J. (1994). Amino acid cycling in colonies of the planktonic marine cyanobacterium Trichodesmium thiebautii. *Applied and Environmental Microbiology*, *60*(11), 3989-3995.

Cloern, J. E. (1987). Turbidity as a control on phytoplankton biomass and productivity in estuaries. *Continental Shelf Research* 7: 1367-1381.

Cloern, J. E. (2001). Our evolving conceptual model of the coastal eutrophication problem. *Marine ecology progress series*, *210*(2001), 223-253.

Cloern, J. E., & Jassby, A. D. (2012). Drivers of change in estuarine-coastal ecosystems: Discoveries from four decades of study in San Francisco Bay. *Reviews of Geophysics*, *50*(4).

Cloern, J. E., Foster, S. Q., & Kleckner, A. E. (2014). Phytoplankton primary production in the world's estuarine-coastal ecosystems. *Biogeosciences*, *11*(9), 2477-2501.

Colman, B., & Rotatore, C. (1995). Photosynthetic inorganic carbon uptake and accumulation in two marine diatoms. *Plant, Cell & Environment*, *18*(8), 919-924.

Conway, H. L. (1977). Interactions of inorganic nitrogen in the uptake and assimilation by marine phytoplankton. *Marine Biology*, *39*(3), 221-232.

Dabundo, R., Lehmann, M. F., Treibergs, L., Tobias, C. R., Altabet, M. A., Moisander, P. H., & Granger, J. (2014). The contamination of commercial $^{15}N_2$ gas stocks with $^{15}N$–labeled nitrate and ammonium and consequences for nitrogen fixation measurements.

Dalsgaard, T., Canfield, D. E., Petersen, J., Thamdrup, B., & Acuña-González, J. (2003). $N_2$ production by the anammox reaction in

the anoxic water column of Golfo Dulce, Costa Rica. *Nature*, *422*(6932), 606-608.

DeNiro, M. J., & Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et cosmochimica acta*, *42*(5), 495-506.

Dickson, A. G., and Goyet C., (Eds.) (1998), Hand book of methods for the analysis of the various parameters of the carbon dioxide system in seawater, version 2, Rep. ORNL/CDIAC-74, Dep. of Energy, Oak Ridge, Tenn.

Doney, S. C., Ruckelshaus, M., Duffy, J. E., Barry, J. P., Chan, F., English, C. A., ... & Talley, L. D. (2012). Climate change impacts on marine ecosystems. *Marine Science*, *4*.

Dortch, Q. (1990). The interaction between ammonium and nitrate uptake in phytoplankton. *Marine Ecology progress Series* 61. 183-201.

Dugdale, R. C., & Goering, J. J. (1967). Uptake of new and regenerated forms of nitrogen in primary productivity. *Limnology and oceanography*, *12*(2), 196-206.

Dugdale, R. C., & Wilkerson, F. P. (1986). The use of $^{15}N$ to measure nitrogen uptake in eutrophic oceans; experimental considerations. *Limnol. Oceanogr*, *31*(4), 673-689.

Edwards, M., & Richardson, A. J. (2004). Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature*, *430*(7002), 881-884.

Elzenga, J. T. M., Prins, H., & Stefels, J. (2000). The role of extracellular carbonic anhydrase activity in inorganic carbon utilization of Phaeocystis globosa (Prymnesiophyceae): A comparison with other marine algae using the isotopic disequilibrium technique. *Limnology and Oceanography*, *45*(2), 372-380.

Erlenkeuser, H., Cordt, H. H., Simstich, J., Bauch, D., & Spielhagen, R. (2003). DIC stable carbon isotope pattern in the surface waters of the southern Kara Sea, Sep. 2000. In *Siberian River Run-off in the Kara Sea: Characterisation, Quantification, Variability and Environmental Significance, Proc. Mar. Sci* (Vol. 6, pp. 91-110).

Evans, J., McGregor, J. L., McGuffie, K., Henderson-Sellers, A., & McGuffie, K. (2012). Future regional climates. *Future of the World's climate. Elsevier*, 223-252.

Eyre, B., & Balls, P. (1999). A comparative study of nutrient behavior along the salinity gradient of tropical and temperate estuaries. *Estuaries*, *22*(2), 313-326.

Eyre, B., & France, L. (1997). Importance of marine inputs to the sediment and nutrient load of coastal-plain estuaries: a case study of Pumicestone Passage, south-eastern Queensland, Australia. *Marine and freshwater research*, *48*(4), 277-286.

Falkowski, P., Scholes, R. J., Boyle, E. E. A., Canadell, J., Canfield, D., Elser, J., ... & Steffen, W. (2000). The global carbon cycle: a test of our knowledge of earth as a system. *Science*, *290*(5490), 291-296.

Fasham, M. J. (2003). Ocean Biogeochemistry: The Role of the Ocean Carbon Cycle in the Global Change. *Springer Science & Business Media*.

Field, C. B., Behrenfeld, M. J., Randerson, J. T., & Falkowski, P. (1998). Primary production of the biosphere: integrating terrestrial and oceanic components. *Science*, *281*(5374), 237-240.

Fisher, T. R., Gustafson, A. B., Sellner, K., Lacouture, R., Haas, L. W., Wetzel, R. L., ... & Karrh, R. (1999). Spatial and temporal variation of resource limitation in Chesapeake Bay. *Marine Biology*, *133*(4), 763-778.

Frankignoulle, M., & Borges, A. V. (2001). Direct and indirect pCO2 measurements in a wide range of $pCO_2$ and salinity values (the Scheldt estuary). *Aquatic Geochemistry*, *7*(4), 267-273.

French, R. H., Cooper, J. J., & Vigg, S. (1982). Secchi Disc Relationships. *Water resources bulletin*, *18 (1),* 121-123.

Fry, B., Woei-Lih, J., Scalan, R. S., Parker, P. L., & Baccus, J. (1978). $\delta^{13}C$ food web analysis of a Texas sand dune community. *Geochimica et Cosmochimica Acta*, *42*(8), 1299-1302.

Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P., ... & Vöosmarty, C. J. (2004). Nitrogen cycles: past, present, and future. *Biogeochemistry*, *70*(2), 153-226.

Gandhi, N., Prakash, S., Ramesh, R., and Kumar, S. (2010). Nitrogen uptake rates and new production in the northern Indian Ocean. *Indian Journal of Marine Sciences 39(2)*, 1–7.

Gandhi, N., Singh, A., Prakash, S., Ramesh, R., Raman, M., Sheshshayee, M. S., & Shetye, S. (2011a). First direct measurements of $N_2$ fixation during a Trichodesmium bloom in the eastern Arabian Sea. *Global Biogeochemical Cycles*, *25*(4).

Gandhi, N., Kumar, S., Prakash, S., Ramesh, R., & Sheshshayee, M. S. (2011b). Measurement of marine productivity using 15N and $^{13}C$ tracers: Some methodological aspects. *Journal of earth system science*, *120*(1), 99-111.

Gardner, W. S., Lavrentyev, P. J., Cavaletto, J. F., McCarthy, M. J., Eadie, B. J., Johengen, T. H., & Cotner, J. B. (2004). Distribution and dynamics of nitrogen and microbial plankton in southern Lake Michigan during spring transition 1999–2000. *Journal of Geophysical Research: Oceans (1978–2012)*, *109*(C3).

Gattuso, J. P., Frankignoulle, M., & Wollast, R. (1998). Carbon and carbonate metabolism in coastal aquatic ecosystems. *Annual Review of Ecology and Systematics*, 405-434.

Ghosh, P., Chakrabarti, R., & Bhattacharya, S. K. (2013). Short-and long-term temporal variations in salinity and the oxygen, carbon and hydrogen isotopic compositions of the Hooghly Estuary water, India. *Chemical Geology*, *335*, 118-127.

Gilbert, J. A., Steele, J. A., Caporaso, J. G., Steinbrück, L., Reeder, J., Temperton, B., ... & Field, D. (2012). Defining seasonal marine microbial community dynamics. *The ISME journal*, *6*(2), 298-308.

Glibert, P. M., & Alexander, J. A. (2011). Effects of ambient DIN: DIP ratio on the nitrogen uptake of harmful dinoflagellate Prorocentrum minimum and Prorocentrum donghaiense in turbidistat. *Chinese Journal of Oceanology and Limnology*, *29*(4), 746-761.

Glibert, P. M., Biggs, D. C., & McCarthy, J. J. (1982). Utilization of ammonium and nitrate during austral summer in the Scotia Sea. *Deep Sea Research Part A. Oceanographic Research Papers*, *29*(7), 837-850.

Goering, J., Alexander, V., & Haubenstock, N. (1990). Seasonal variability of stable carbon and nitrogen isotope ratios of organisms in a North Pacific bay. *Estuarine, Coastal and Shelf Science*, *30*(3), 239-260.

Gopalan, U. K., Vengayil, D. T., Udaya Varma, V. P., and Krishnankutty, M. (1983). The shrinking backwaters of Kerala. *Journal of Marine Biological Assessment of India* 25: 131–141.

Goswami, B. N., Venugopal, V., Sengupta, D., Madhusoodanan, M. S., & Xavier, P. K. (2006). Increasing trend of extreme rain events over India in a warming environment. *Science*, *314*(5804), 1442-1445.

Grasshoff, K., Kremling, K., & Ehrhardt, M. (Eds.). (2009). *Methods of seawater analysis*. John Wiley & Sons.

Großkopf, T., Mohr, W., Baustian, T., Schunck, H., Gill, D., Kuypers, M. M., ... & LaRoche, J. (2012). Doubling of marine dinitrogen-fixation rates based on direct measurements. *Nature*, *488*(7411), 361-364.

Gupta, G. V. M., Sarma, V. V. S. S., Robin, R. S., Raman, A. V., Kumar, M. J., Rakesh, M., & Subramanian, B. R. (2008). Influence of net ecosystem metabolism in transferring riverine organic carbon to atmospheric $CO_2$ in a tropical coastal lagoon (Chilka Lake, India). *Biogeochemistry*, *87*(3), 265-285.

Gupta, G. V. M., Thottathil, S. D., Balachandran, K. K., Madhu, N. V., Madeswaran, P., & Nair, S. (2009a). CO2 supersaturation and net heterotrophy in a tropical estuary (Cochin, India): influence of anthropogenic effect. *Ecosystems*, *12*(7), 1145-1157.

Hallegraeff, G. M. (2010). Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. *Journal of Phycology* 46(2), 220-235.

Hansen, J., & Sato, M. (2004). Greenhouse gas growth rates. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(46), 16109-16114.

Hansen, J., Sato, M., Ruedy, R., Kharecha, P., Lacis, A., Miller, R., ... & Zhang, S. (2007). Climate simulations for 1880–2003 with GISS modelE. *Climate Dynamics*, *29*(7-8), 661-696.

Hardy, J. T. (2003). Climate change: causes, effects, and solutions. *John Wiley & Sons.*

Harrison, W. G., Harris, L. R., & Irwin, B. D. (1996). The kinetics of nitrogen utilization in the oceanic mixed layer: Nitrate and ammonium interactions at nanomolar concentrations. *Limnology and Oceanography*, *41*(1), 16-32.

Harrison, W. G., L. Harris, and B. Irwin. 1996. The kinetics of nitrogen utilization in the oceanic mixed layer: Nitrate and ammonium interactions at nanomolar concentrations. *Limnology and Oceanography* 41: 16–32.

Hecky, R. E., & Kilham, P. (1988). Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment1. *Limnology and Oceanography*, *33*(4part2), 796-822.

Hoch, M. P., & Kirchman, D. L. (1995). Ammonium uptake by heterotrophic bacteria in the Delaware estuary and adjacent coastal waters. *Limnology and Oceanography*, *40*(5), 886-897.

Hodgkiss, I. J., & Ho, K. C. (1997, January). Are changes in N: P ratios in coastal waters the key to increased red tide blooms?. In *Asia-Pacific Conference on Science and Management of Coastal Environment* (pp. 141-147). Springer Netherlands.

Hoegh-Guldberg, O. (1999). Climate change, coral bleaching and the future of the world's coral reefs. *Marine and freshwater research*, *50*(8), 839-866.

Hoering, T. (1955). Variations of nitrogen-15 abundance in naturally occurring substances. *Science*, *122*(3182), 1233-1234.

Hooper, D. U., Adair, E. C., Cardinale, B. J., Byrnes, J. E., Hungate, B. A., Matulich, K. L., ... & O'Connor, M. I. (2012). A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature*, *486*(7401), 105-108.

Hopkinson, C. S., & Smith, E. M. (2005). Estuarine respiration: an overview of benthic, pelagic, and whole system respiration. *Respiration in aquatic ecosystems*, 122-146.

Howarth, R. W., Billen, G., Swaney, D., Townsend, A., Jaworski, N., Lajtha, K., ... & Zhao-Liang, Z. (1996). Regional nitrogen budgets and riverine N & P fluxes for the drainages to the North Atlantic Ocean: Natural and human influences. In *Nitrogen cycling in the North Atlantic Ocean and its watersheds* (pp. 75-139). Springer Netherlands.

Humborg, C., Ittekkot, V., Cociasu, A., & Bodungen, B. V. (1997). Effect of Danube River dam on Black Sea biogeochemistry and ecosystem structure. *Nature*, *386*(6623), 385-388.

Jabir, T., Dhanya, V., Jesmi, Y., Prabhakaran, M. P., Saravanane, N., Gupta, G. V. M., & Hatha, A. A. M. (2013). Occurrence and Distribution of a Diatom-Diazotrophic Cyanobacteria Association during a Trichodesmium Bloom in the Southeastern Arabian Sea. *International Journal of Oceanography*, *2013*.

Jay, M., & Marmot, M. G. (2009). Health and climate change. *BMJ*, *339*.

Jeffrey, S. T., & Humphrey, G. F. (1975). New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochem Physiol Pflanz BPP*.

Jyothibabu, R., Madhu, N. V., Jayalakshmi, K. V., Balachandran, K. K., Shiyas, C. A., Martin, G. D., & Nair, K. K. C. (2006). Impact of freshwater influx on microzooplankton mediated food web in a tropical estuary (Cochin backwaters–India). *Estuarine, Coastal and Shelf Science*, *69*(3), 505-518.

Kanda, J., Itoh, T., Ishikawa, D., & Watanabe, Y. (2003). Environmental control of nitrate uptake in the East China Sea. *Deep Sea Research Part II: Topical Studies in Oceanography*, *50*(2), 403-422.

Kanda, J., Ziemann, D. A., Conquest, L. D., & Bienfang, P. K. (1989). Light-dependency of nitrate uptake by phytoplankton over the spring bloom in Auke Bay, Alaska. *Marine Biology*, *103*(4), 563-569.

Kaplan, A., Ronen-Tarazi, M., Zer, H., Schwarz, R., Tchernov, D., Bonfil, D. J., ... & Reinhold, L. (1998). The inorganic carbon-concentrating mechanism in cyanobacteria: induction and ecological significance. *Canadian journal of botany*, *76*(6), 917-924.

Karl, D. M., Letelier, R., Hebel, D., Tupas, L., Dore, J., Christian, J., & Winn, C. (1995). Ecosystem changes in the North Pacific subtropical gyre attributed to the 1991–92 El Nino.

Kerala State Pollution Control Board (KSPCB). (1982). Environmental Status Report on Greater Cochin. Kerala (with special reference to water pollution). *KSPCB*, Trivandrum, Kerala 32 pp.

Kerala State Pollution Control Board (KSPCB). (1986). Water Quality Studies-Kuttanad Water Balance Study Project-inception Report. *KSPCB*, Trivandrum, Kerala, 56 pp.

Kingsolver, J. G. (2009). The Well-Temperatured Biologist. *The American Naturalist*, *174*(6), 755-768.

Kirchman, D. L. (1994). The uptake of inorganic nutrients by heterotrophic bacteria. *Microbial Ecology*, *28*(2), 255-271.

Kirchman, D. L. (2012). *Processes in microbial ecology*. Oxford University Press.

Korb, R. E., Raven, J. A., & Johnston, A. M. (1998). Relationship between aqueous CO~ 2 concentrations and stable carbon isotope discrimination in the diatoms Chaetoceros calcitrans and Ditylum brightwellii. *Marine Ecology-Progress Series*, *171*, 303-305.

Kumar, S., Ramesh, R., Bhosle, N. B., Sardesai, S., & Sheshshayee, M. S. (2004). Natural isotopic composition of nitrogen in suspended particulate matter in the Bay of Bengal. *Biogeosciences*, *1*(1), 63-70.

Kumar, S., Ramesh, R., Dwivedi, R. M., Raman, M., Sheshshayee, M. S., & D'Souza, W. (2010). Nitrogen uptake in the northeastern Arabian Sea during winter cooling. *International Journal of Oceanography*, *2010*.

Kumar, S., Ramesh, R., Sheshshayee, M. S., Sardessai, S., & Patel, P. P. (2005). Signature of terrestrial influence on nitrogen isotopic composition of suspended particulate. *Current Science*, *88*(6), 770-774pp.

Kuypers, M. M., Sliekers, A. O., Lavik, G., Schmid, M., Jørgensen, B. B., Kuenen, J. G., ... & Jetten, M. S. (2003). Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. *Nature*, *422*(6932), 608-611.

Laskar, A. H., Gandhi, N., Thirumalai, K., Yadava, M. G., Ramesh, R., Mahajan, R. R., & Kumar, D. (2014). Stable carbon isotopes in dissolved inorganic carbon: extraction and implications for quantifying the contributions from silicate and carbonate weathering in the Krishna River system during peak discharge. *Isotopes in environmental and health studies*, *50*(2), 156-168.

Laws, E. A., Harrison, W. G., & DiTullio, G. R. (1985). A comparison of nitrogen assimilation rates based on 15 N uptake and autotrophic protein synthesis. *Deep Sea Research Part A. Oceanographic Research Papers*, *32*(1), 85-95.

Lundry, M. R., Barber, R. T., Bidare, R. R., Chai, F., Coale, K. H., Dam, H. G., ... & White, J. R. (1997). Iron and grazing constraints on primary production in the central equatorial Pacific: an EqPac synthesis. *Limnology and Oceanography*, *42*(3), 405-418.

Madhu, N. V., Jyothibabu, R., & Balachandran, K. K. (2010). Monsoon-induced changes in the size-fractionated phytoplankton biomass and production rate in the estuarine and coastal waters of southwest coast of India. *Environmental monitoring and assessment*, *166*(1-4), 521-528.

Madhupratap, M., S. Prasanna Kumar, P.M.A. Bhattathiri, M. Dileep Kumar, S. Raghukumar, K.K.C. Nair, and N. Ramaiah (1996), Mechanism of the biological response to winter cooling in the northeastern Arabian Sea, *Nature*, *384,* 549-552.

Martin, G. D., Jyothibabu, R., Madhu, N. V., Balachandran, K. K., Nair, M., Muraleedharan, K. R., ... & Revichandran, C. (2013). Impact of eutrophication on the occurrence of Trichodesmium in the Cochin backwaters, the largest estuary along the west coast of India. *Environmental monitoring and assessment*, *185*(2), 1237-1253.

Martin, G. D., Vijay, J. G., Laluraj, C. M., Madhu, N. V., Joseph, T., Nair, M., ... & Balachandran, K. K. (2008). Fresh water influence on nutrient stoichiometry in a tropical estuary, southwest coast of India. *Applied ecology and environmental research*, *6*(1), 57-64.

McCarthy, J. J., & Carpenter, E. J. (1983). Nitrogen cycling in near-surface waters of the open ocean. *Nitrogen in the marine environment*, 487-512.

McCarthy, J. J., & Eppley, R. W. (1972). A comparison of chemical, isotopic, and enzymatic methods for measuring nitrogen assimilation of marine phytoplankton. *Limnology and Oceanography*, *17*(3), 371-382.

McCarthy, J. J., Garside, C., & Nevins, J. L. (1999). Nitrogen dynamics during the Arabian Sea northeast monsoon. *Deep Sea Research Part II: Topical Studies in Oceanography*, *46*(8), 1623-1664.

McClelland, J. W., & Valiela, I. (1998). Linking nitrogen in estuarine producers to land-derived sources. *Limnology and Oceanography*, *43*(4), 577-585.

McLusky, D. S. (1971). Ecology of estuaries. Heinemann Educational, London.

Measures, C. I., & Vink, S. (1999). Seasonal variations in the distribution of Fe and Al in the surface waters of the Arabian Sea. *Deep Sea Research Part II: Topical Studies in Oceanography*, *46*(8), 1597-1622.

Menon, N. N., Balchand, A. N., & Menon, N. R. (2000). Hydrobiology of the Cochin backwater system–a review. *Hydrobiologia*, *430*(1-3), 149-183.

Meybeck, M. (1982). Carbon, nitrogen, and phosphorus transport by world rivers. *Am. J. Sci*, *282*(4), 401-450.

Michaelis, L., & Menten, M. L. (1913). Die kinetik der invertinwirkung. *Biochem. z*, *49*(333-369), 352.

Middelburg, J. J., and Nieuwenhuize, J. (2000a). Uptake of dissolved inorganic N in turbid, tidal estuaries. *Marine Ecology Progress Series* 192: 79–88.

Middelburg, J. J., and Nieuwenhuize, J. (2000b). N uptake by heterotrophic bacteria and phytoplankton in the nitrate rich Thames estuary. *Marine Ecology Progress Series* 203:13–21.

Mills, M. M., Ridame, C., Davey, M., La Roche, J., & Geider, R. J. (2004). Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. *Nature*, *429*(6989), 292-294.

Minagawa, M., & Wada, E. (1984). Stepwise enrichment of 15 N along food chains: further evidence and the relation between δ 15 N and animal age. *Geochimica et Cosmochimica Acta*, *48*(5), 1135-1140.

Miranda, J., Balachandran, K. K., Ramesh, R., & Wafar, M. (2008). Nitrification in Kochi backwaters. *Estuarine, Coastal and Shelf Science*, *78*(2), 291-300.

Miyake, Y., & Wada, E. (1967). The abundance ratio of 15N/14N in marine environments. *Records of Oceanographic Works in Japan*, *9*(1), 37-53.

Mohr, W., Grosskopf, T., Wallace, D. W., & LaRoche, J. (2010). Methodological underestimation of oceanic nitrogen fixation rates. *PLOS one*, *5*(9), e12583.

Montoya, J. P., Korrigan, S. G., & McCarthy, J. J. (1991). Rapid, storm-induced changes in the natural abundance of 15 N in a planktonic ecosystem, Chesapeake Bay, USA. *Geochimica et Cosmochimica Acta*, *55*(12), 3627-3638.

Montoya, J. P., Voss, M., Kahler, P., & Capone, D. G. (1996). A Simple, High-Precision, High-Sensitivity Tracer Assay for N (inf2) Fixation. *Applied and environmental microbiology*, *62*(3), 986-993.

Mook, W. G., & Tan, F. C. (1991). Stable carbon isotopes in rivers and estuaries. *Biogeochemistry of major world rivers*, *42*, 245-264.

Moore, S. K., Trainer, V. L., Mantua, N. J., Parker, M. S., Laws, E. A., Backer, L. C., & Fleming, L. E. (2008). Impacts of climate variability and future climate change on harmful algal blooms and human health. *Environmental Health*, *7*(2), S4.

Moran, X. A. G., Lopez-Urrutia, A., Calvo-Diaz, A., Li, W. K. W., (2010). Increasing importance of small phytoplankton in a warmer ocean. *Global Change Biology*, *16*(3), 1137-1144.

Morrison, J. M., Codispoti, L. A., Gaurin, S., Jones, B., Manghnani, V., & Zheng, Z. (1998). Seasonal variation of hydrographic and nutrient fields during the US JGOFS Arabian Sea Process Study. *Deep Sea Research Part II: Topical Studies in Oceanography*, *45*(10), 2053-2101.

Mukhopadhyay, S. K., Biswas, H., De, T. K., Sen, S., & Jana, T. K. (2002). Seasonal effects on the air–water carbon dioxide exchange in the Hooghly estuary, NE coast of Bay of Bengal, India. *Journal of Environmental Monitoring*, *4*(4), 549-552.

Mulholland, M. R., & Bernhardt, P. W. (2005). The effect of growth rate, phosphorus concentration, and temperature on N2 fixation, carbon fixation, and nitrogen release in continuous cultures of Trichodesmium IMS101. *Limnology and oceanography*, *50*(3), 839-849.

Mulholland, M. R., & Capone, D. G. (2001). Stoichiometry of nitrogen and carbon utilization in cultured populations of Trichodesmium IMS101: Implications for growth. *Limnology and Oceanography*, *46*(2), 436-443.

Mulholland, M. R., & Capone, D. G. (2009). Dinitrogen fixation in the Indian Ocean. *Indian Ocean Biogeochemical Processes and Ecological Variability*, 167-186.

Mulholland, M. R., Bernhardt, P. W., Heil, C. A., Bronk, D. A., & O'Neil, J. M. (2006). Nitrogen fixation and release of fixed nitrogen by Trichodesmium spp. in the Gulf of Mexico. *Limnology and Oceanography*, *51*(4), 1762-1776.

Mulholland, M. R., Bronk, D. A., & Capone, D. G. (2004). Dinitrogen fixation and release of ammonium and dissolved organic nitrogen by Trichodesmium IMS101. *Aquatic Microbial Ecology*, *37*(1), 85-94.

Mulholland, M. R., Lee, C., & Glibert, P. M. (2003). Extracellular enzyme activity and uptake of carbon and nitrogen along an estuarine salinity and nutrient gradient. *Marine ecology. Progress series*, *258*, 3-17.

Naqvi, S. W. A., Moffett, J. W., Gauns, M. U., Narvekar, P. V., Pratihary, A. K., Naik, H., ... & Ahmed, S. I. (2010). The Arabian Sea as a high-nutrient, low-chlorophyll region during the late Southwest Monsoon.

Neven, I. A., Stefels, J., van Heuven, S. M., de Baar, H. J., & Elzenga, J. T. M. (2011). High plasticity in inorganic carbon uptake by Southern Ocean phytoplankton in response to ambient CO 2. *Deep Sea Research Part II: Topical Studies in Oceanography*, *58*(25), 2636-2646.

Nixon, S. W. (1995). Coastal marine eutrophication: a definition, social causes, and future concerns. *Ophelia*, *41*(1), 199-219.

O'Neill, L. P., Benitez-Nelson, C. R., Styles, R. M., Tappa, E., & Thunell, R. C. (2005). Diagenetic effects on particulate phosphorus samples collected using formalin-poisoned sediment traps. *Limnology and Oceanography: Methods*, *3*(7), 308-317.

Owens, N. J. P., Burkill, P. H., Mantoura, R. F. C., Woodward, E. M. S., Bellan, I. E., Aiken, J., ... & Llewellyn, C. A. (1993). Size-fractionated primary production and nitrogen assimilation in the northwestern Indian Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography*, *40*(3), 697-709.

Owens, N. J., & Rees, A. P. (1989). Determination of nitrogen-15 at sub-microgram levels of nitrogen using automated continuous-flow isotope ratio mass spectrometry. *Analyst*, *114*(12), 1655-1657.

Paerl, H. W. (1993). Emerging role of atmospheric nitrogen deposition in coastal eutrophication: biogeochemical and trophic perspectives. *Canadian Journal of Fisheries and Aquatic Sciences*, *50*(10), 2254-2269.

Paerl, H. W. (1995). Coastal eutrophication in relation to atmospheric nitrogen deposition: current perspectives. *Ophelia*, *41*(1), 237-259.

Paerl, H. W., & Piehler, M. F. (2008). Nitrogen and marine eutrophication. *Nitrogen in the marine environment*, *2*, 529-567.

Paerl, H. W., Hall, N. S., Peierls, B. L., & Rossignol, K. L. (2014). Evolving paradigms and challenges in estuarine and coastal

eutrophication dynamics in a culturally and climatically stressed world. *Estuaries and coasts*, *37*(2), 243-258.

Pennock, J. R., Sharp, J. H., & Schroeder, W. W. (1994). What controls the expression of estuarine eutrophication? Case studies of nutrient enrichment in the Delaware Bay and Mobile Bay Estuaries, USA Jonathan R. Pennock1, Jonathan H. Sharp2 & William W. Schroeder1 1. University of Alabama, Marine Environmental Sciences Consortium. *Changes in fluxes in estuaries: implications from science to management*, 139.

Peterson, B. J., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual review of ecology and systematics*, 293-320.

Prakash, S., & Ramesh, R. (2007). Is the Arabian Sea getting more productive?. *Current Science*, *92*(5), 667-670.

Qasim, S. Z., & Gopinathan, C. K. (1969). Tidal cycle and the environmental features of Cochin Backwater (a tropical estuary). In *Proceedings of the Indian Academy of Sciences, Section B* (Vol. 69, No. 6, pp. 336-353). Indian Academy of Sciences.

Qasim, S. Z., Wellershaus, S., Bhattathiri, P. M. A., & Abidi, S. A. H. (1969, February). Organic production in a tropical estuary. In *Proceedings of the Indian Academy of Sciences-Section B* (Vol. 69, No. 2, pp. 51-94). Springer India.

Quay, P., Sonnerup, R., Stutsman, J., Maurer, J., Körtzinger, A., Padin, X. A., & Robinson, C. (2007). Anthropogenic $CO_2$ accumulation rates in the North Atlantic Ocean from changes in the $^{13}C/^{12}C$ of dissolved inorganic carbon. *Global biogeochemical cycles*, *21*(1).

Rabalais, N. N., Turner, R. E., Díaz, R. J., & Justić, D. (2009). Global change and eutrophication of coastal waters. *ICES Journal of Marine Science: Journal du Conseil*, *66*(7), 1528-1537.

Rau, G. H., & Anderson, N. H. (1981). Use of $^{13}C/^{12}C$ to trace dissolved and particulate organic matter utilization by populations of an aquatic invertebrate. *Oecologia*, *48*(1), 19-21.

Rees, A. P., N. Owens, J. P., and Woodward, E. M. S. (1995). Phytoplankton nitrogen assimilation at low nutrient concentrations in the NW-Mediterranean Sea (July 1993). In:

Martin JM, Barth H (eds) *Water Pollution Reports. No. 32. European Commission*, Brussels p 141-148.

Regnier, P., Friedlingstein, P., Ciais, P., Mackenzie, F. T., Gruber, N., Janssens, I. A., ... & Thullner, M. (2013). Anthropogenic perturbation of the carbon fluxes from land to ocean. *Nature Geoscience*, *6*(8), 597-607.

Riebesell, U., Wolf-Gladrow, D. A., & Smetacek, V. (1993). Carbon dioxide limitation of marine phytoplankton growth rates.

Rotatore, C., Colman, B., & Kuzma, M. (1995). The active uptake of carbon dioxide by the marine diatoms Phaeodactylum ticornutum and Cyclotella sp. *Plant, Cell & Environment*, *18*(8), 913-918.

Rudek, J., Paerl, H. W., Mallin, M. A., & Bates, P. W. (1991). Seasonal and hydrological control of phytoplankton nutrient limitation in the lower Neuse River Estuary, North Carolina. *Marine ecology progress series. Oldendorf*, *75*(2), 133-142.

Ryther, J. H., & Dunstan, W. M. (1971). Nitrogen, phosphorus, and eutrophication in the coastal marine environment. *Science*, *171*(3975), 1008-1013.

Sambrotto, R. N. (2001). Nitrogen production in the northern Arabian Sea during the Spring Intermonsoon and Southwest Monsoon seasons. *Deep Sea Research Part II: Topical Studies in Oceanography*, *48*(6), 1173-1198.

Sañudo-Wilhelmy, S. A., Kustka, A. B., Gobler, C. J., Hutchins, D. A., Yang, M., Lwiza, K., ... & Carpenter, E. J. (2001). Phosphorus limitation of nitrogen fixation by Trichodesmium in the central Atlantic Ocean. *Nature*, *411*(6833), 66-69.

Sarma, V. V. S. S. (2003). Monthly variability in surface pCO2 and net air-sea CO2 flux in the Arabian Sea. *Journal of Geophysical Research: Oceans (1978–2012)*, *108*(C8).

Sarma, V. V. S. S., Krishna, M. S., Prasad, V. R., Kumar, B. S. K., Naidu, S. A., Rao, G. D., ... & Reddy, N. P. C. (2014). Distribution and sources of particulate organic matter in the Indian monsoonal estuaries during monsoon. *Journal of Geophysical Research: Biogeosciences*, *119*(11), 2095-2111.

Sarma, V. V. S. S., Viswanadham, R., Rao, G. D., Prasad, V. R., Kumar, B. S. K., Naidu, S. A., ... & Murty, T. V. R. (2012). Carbon dioxide emissions from Indian monsoonal estuaries. *Geophysical Research Letters*, *39*(3).

Sarma, V. V., Kumar, M. D., & Manerikar, M. (2001). Emission of carbon dioxide from a tropical estuarine system, Goa, India. *Geophysical Research Letters*, *28*(7), 1239-1242.

Schindler, D. W. (1975). Whole-lake eutrophication experiments with phosphorus, nitrogen and carbon. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie* 19: 3221-3231.

Schindler, D. W. (1977). Evolution of phosphorus limitation in lakes. *Science*, *195*(4275), 260-262.

Shivaprasad, A., Vinita, J., Revichandran, C., Manoj, N. T., Srinivas, K., Reny, P. D., & Muraleedharan, K. R. (2012). Influence of saltwater barrage on tides, salinity, and Chlorophyll a in Cochin Estuary, India. *Journal of Coastal Research*, *29*(6), 1382-1390.

Singh, A., Gandhi, N., & Ramesh, R. (2012). Contribution of atmospheric nitrogen deposition to new production in the nitrogen limited photic zone of the northern Indian Ocean. *Journal of Geophysical Research: Oceans (1978–2012)*, *117*(C6).

Slawyk, G., Collos, Y., & Auclair, J. C. (1977). The use of the $^{13}C$ and $^{15}N$ isotopes for the simultaneous measurement of carbon and nitrogen turnover rates in marine phytoplankton1. *Limnology and Oceanography*, *22*(5), 925-932.

Smith, S. V. (1984). Phosphorus versus nitrogen limitation in the marine environment1. *Limnology and oceanography*, *29*(6), 1149-1160.

Smith, S. V., & Hollibaugh, J. T. (1997). Annual cycle and interannual variability of ecosystem metabolism in a temperate climate embayment. *Ecological Monographs*, *67*(4), 509-533.

Smith, V. H., & Schindler, D. W. (2009). Eutrophication science: where do we go from here?. *Trends in Ecology & Evolution*, *24*(4), 201-207.

Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., ... & Miller, H. L. (2007). IPCC, Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change.

Sonnerup, R. E., Quay, P. D., & McNichol, A. P. (2000). The Indian Ocean 13C Suess effect. *Global biogeochemical cycles*, *14*(3), 903-916.

Srinivas, B., & Sarin, M. M. (2013). Atmospheric deposition of N, P and Fe to the Northern Indian Ocean: implications to C-and N-fixation. *Science of the total environment*, *456*, 104-114.

Srinivas, K., Revichandran, C., Maheswaran, P. A., Asharaf, T. M., & Murukesh, N. (2003). Propagation of tides in the Cochin estuarine system, southwest coast of India. *Indian Journal of Marine Sciences*, *32*(1), 14-24p.

Steeman-Nielsen, E. (1952). The use of radioactive carbon (C14) for measuring organic production in the sea. *J. Conseil, Conseil Perm. Intern. Exploration Mer.* 18: 117-140.. 1965.

Steinacher, M., Joos, F., Frolicher, T. L., Bopp, L., Cadule, P., Cocco, V., ... & Segschneider, J. (2010). Projected 21st century decrease in marine productivity: a multi-model analysis. *Biogeosciences*, *7*(3).

Subramaniam, A., & Carpenter, E. J. (1994). An empirically derived protocol for the detection of blooms of the marine cyanobacterium Trichodesmium using CZCS imagery. *International Journal of Remote Sensing*, *15*(8), 1559-1569.

Sültemeyer, D. (1998). Carbonic anhydrase in eukaryotic algae: characterization, regulation, and possible function during photosynthesis. *Canadian Journal of Botany*, *76*(6), 962-972.

Thomas, M. K., Kremer, C. T., Klausmeier, C. A., & Litchman, E. (2012). A global pattern of thermal adaptation in marine phytoplankton. *Science*, *338*(6110), 1085-1088.

Thomson, K. T. (2002). Economic and social issues of biodiversity loss in Cochin backwaters. In *Technical report* (pp. 51-82). Cochin University of Science and Technology Cochin, India.

Thottathil, S. D., Balachandran, K. K., Gupta, G. V. M., Madhu, N. V., & Nair, S. (2008). Influence of allochthonous input on autotrophic–heterotrophic switch-over in shallow waters of a tropical estuary (Cochin Estuary), India. *Estuarine, Coastal and Shelf Science*, *78*(3), 551-562.

Tortell, P. D., DiTullio, G. R., Sigman, D. M., & Morel, F. M. (2002). $CO_2$ effects on taxonomic composition and nutrient utilization in an Equatorial Pacific phytoplankton assemblage. *Marine Ecology-Progress Series*, *236*, 37-43.

Tucker, J., Sheats, N., Giblin, A. E., Hopkinson, C. S., & Montoya, J. P. (1999). Using stable isotopes to trace sewage-derived material through Boston Harbor and Massachusetts Bay. *Marine Environmental Research*, *48*(4), 353-375.

Wada, E., & Hattori, A. (1976). Natural abundance of 15 N in particulate organic matter in the North Pacific Ocean. *Geochimica et Cosmochimica Acta*, *40*(2), 249-251.

Wada, E., Kadonaga, T., & Matsuo, S. (1975). 15N aboundance in nitrogen of naturally occurring substances and global assessment of denitrification from isotopic viewpoint. *Geochemical Journal*, *9*(3), 139-148.

Walther, G. R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J., ... & Bairlein, F. (2002). Ecological responses to recent climate change. *Nature*, *416*(6879), 389-395.

Watts, L. J., & Owens, N. J. P. (1999). Nitrogen assimilation and the f-ratio in the northwestern Indian Ocean during an intermonsoon period. *Deep Sea Research Part II: Topical Studies in Oceanography*, *46*(3), 725-743.

Weiss, R. F. (1970). The solubility of nitrogen, oxygen, and argon in water and seawater. *Deep Sea Research and Oceanographic Abstracts* 17(4), 721–735.

Yin, C., & Shan, B. (2001). Multipond systems: a sustainable way to control diffuse phosphorus pollution. *AMBIO: A Journal of the Human Environment*, *30*(6), 369-375.

Zeebe, R. E., & Wolf-Gladrow, D. A. (2001). CO2 in seawater: equilibrium, kinetics, isotopes (Vol. 65). *Gulf Professional Publishing*.

## Research publications

1. "Primary production in Red Sea" *Journal of Marine Systems*.132 (2014) 75–82. doi:10.1016/j.jmarsys.2014.01.006.

   Qurban, A., C. Balala, Sanjeev Kumar, **P. S. Bhavya**, M. Wafar.

2. **"**Nitrogen uptake dynamics in a tropical eutrophic estuary (Cochin estuary) and adjacent coastal regions during pre-monsoon" (2015). *Estuaries and coasts*. 10.1007/s12237-015-9982-y.

   **P. S. Bhavya,** Sanjeev Kumar, G.V.M. Gupta, V. Sudheesh, K.V. Sudharma, D.S. Varrier, K.R. Dhanya, N. Saravanane.

3. "Tracing of sources of dissolved inorganic carbon in a tropical estuary (Cochin estuary, India) using $\delta^{13}C_{DIC}$", 28th ISMAS conference proceedings: ISBN 978-81-904442-6-2: Page-271-273.
   **Bhavya, P. S.,** Sanjeev Kumar, G.V.M., Gupta, V., Sudheesh, D.S., Varrier, K.V., Sudharma.

4. "Primary productivity measurements in $CO_2$ supersaturated tropical estuary and adjacent coastal waters using $^{13}C$-labelling technique". (*under review: Journal of marine systems*).

   **P. S. Bhavya**, Sanjeev Kumar, and G. V. M. Gupta.

5. "Carbon isotopic composition of suspended particulate matter and dissolved inorganic carbon in the Cochin estuary during post-monsoon". (*Under review: Current science*).

   **P. S. Bhavya,** Sanjeev Kumar, G.V.M. Gupta, V. Sudheesh, K.V. Sudharma.

6. "Nitrogen uptake potential under different temperature-salinity conditions: Implications for nitrogen cycling under climate change scenarios" (*To be resubmitted to: JGR Biogeosciences*).

Sanjeev Kumar, **P. S. Bhavya**, R. Ramesh, Fidel Chiriboga, Arvind Singh, G.V.M. Gupta, Indrani Karunasagar, Ashwin Rai, Ann-Sofi Rehnstam-Holm, Lars Edler and Anna Godhe.

7. "Response of a tropical pelagic microbial community to changed salinity and temperature: Implications for structure and production in coastal water under climate change scenarios". (*Under review: Aquatic microbial ecology*).

   Gurpreet Kaur-Kahlon, Sanjeev Kumar, Ann-Sofi Rehnstam-Holm, Ashwin Rai, **P. S. Bhavya**, Lars Edler, Arvind Singh, Björn Andersson, Indrani Karunasagar, Rengaswamy Ramesh, Anna Godhe.

## Articles under preparation

1. "Nitrogen cycling in the estuarine-coastal coupling systems: role of N:P ratio on transformation rates"

   **P. S. Bhavya**, Sanjeev Kumar, G.V.M. Gupta, Sudheesh. V, Sudharma.

2. Natural abundance of $^{13}C$ and $^{15}N$ in marine particulate organic matter and zooplankton in the Cochin estuary and the coastal Arabian Sea.

   **P. S. Bhavya,** Sanjeev Kumar, G.V.M. Gupta, V., Sudheesh, K.V. Sudharma, K. R. Dhanya, and Dhanya varrier.

3. "Dynamics of dissolved inorganic carbon in a eutrophic estuary and adjacent southeastern Arabian Sea".

   **P. S. Bhavya,** Sanjeev Kumar, G. V. M. Gupta, V. Sudheesh, K. V. Sudharma, and K. R. Dhanya.

## Abstracts presented in conferences

1. **Bhavya, P. S.,** Sanjeev Kumar, G.V.M. Gupta, V., Sudheesh, D.S., Varrier, K.V., Sudharma, K.R., Dhanya, N., Saravanane, *The nitrogen uptake rates in a tropical eutrophic estuary and adjacent coastal Arabian Sea*, in IMBER IMBIZO III workshop at NIO-Goa, 28th-31st -Jan-2013.

2. **Bhavya, P. S**, Sanjeev Kumar, G. V. M. Gupta, V. Sudheesh, K. V. Sudharma, D. S. Varrier, K. R. Dhanya, *The nitrogen uptake rates in a tropical eutrophic estuary and adjacent coastal Arabian Sea*, in EUR-OCEANS-"hot topic conference-A changing ocean", at PLOCAN, Las Palmas, Gran Canaria, Spain, 6th-8th-Nov-2013.

3. **Bhavya, P. S.,** Sanjeev Kumar, G.V.M., Gupta, V., Sudheesh, D.S., Varrier, K.V., Sudharma. *Tracing of sources of dissolved inorganic carbon in a tropical estuary (Cochin estuary, India) using* $\delta^{13}C_{DIC}$, 28th ISMAS conference-9th-14th-Mar-2014.