SPATIAL AND TEMPORAL VARIATION OF PHYTOPLANKTON COMMUNITY AND THEIR GROWTH LIMITING FACTORS IN COCHIN BACKWATER

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UNDER THE FACULTY OF MARINE SCIENCES

By

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Certificate

This is to certify that the thesis entitled ***"Spatial and Temporal Variation of Phytoplankton Community and Their Growth Limiting Factors in Cochin Backwater"*** *is an authentic record of the research work carried out by* ***Ms. C.K. Haridevi,*** *under my supervision and guidance in National Institute of Oceanography, Regional Centre, Kochi, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Marine Biology of Cochin University of Science and Technology and no part of this has been presented for the award of any other degree, diploma or associate ship in any university.*

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Declaration

I hereby declare that the thesis entitled "**Spatial and Temporal Variation of Phytoplankton Community and Their Growth Limiting Factors in Cochin Backwater**" is an authentic record of research work done by me under the supervision of Dr. C. Revichandran, National Institute of Oceanography, Regional Centre, Kochi, and no part of this has been presented for any other degree or diploma earlier.

Kochi-18
May, 2013

C.K. Haridevi

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Chapter 1
Introduction

Phytoplankton is an autotrophic organism of the plankton community. The name comes from the Greek word phyton meaning “plant” and plankton, meaning “wanderer” or “drifter” (Thurman, 2007). Phytoplankton is a highly diverse group of photoautotrophic non-vascular plant. They exists either as unicellular or multi-cellular microscopic algae in freshwater, brackish water and marine waters. They are diversified group of photosynthetic thallophytes which have a very significant role in productivity of the marine, estuarine and freshwater ecosystems that cover two-third of the earth’s surface. These microscopic organisms inhabit the upper (sunlit) layer of all aquatic bodies. They are agents for "primary production," the production of organic compounds from carbon dioxide in presence of light, a process that sustains the aquatic food web (Ghosal, 2011).

Studies on the diversity of phytoplankton in the marine environment are limited compared to that of terrestrial flora. Similar to terrestrial vegetation, marine phytoplankton diversity is a unimodal function of phytoplankton biomass, with maximum species diversity at intermediate levels and minimum diversity during massive blooms (Irigoien *et. al.,* 2004). Phytoplankton provides a major source of food not only for the zooplankton, but also for the larger aquatic organisms including fishes and Cetacean. Phytoplankton abundance and distribution are strongly dependent on factors such as ambient nutrient concentrations, physical

state of water column, and presence of grazers or herbivorous zooplankton.

The study of plankton is termed as planktonology. Planktons are primarily divided into broad functional (or trophic level) groups as producer, consumer and recycler groups, such as

- Virioplankton (viruses), of size range 0.02 to 0.2 µm that play a major role in nutrient cycling.
- Bacterioplankton (bacteria and archaea), of size range 0.2 to 2 µm that play an important role in re-mineralising organic material in water and sediments.
- Phytoplankton (2 to 200 µm), are autotrophic, prokaryotic or eukaryotic algae, mostly living in water surface where there is sufficient light to support photosynthesis. The important groups are diatoms, dinoflagellates, green algae, blue green algae and coccolithophores.
- Microzooplankton (20-200 µm), mostly composed of ciliates, flagellates, nauplius, copepodite stage of copepod etc. which feed on bacterioplankton and are, in turn, grazed by meso zooplankton to play a vital role in the microbial food web.
- Meso zooplankton (>200 µm) are small metazoans (e.g. copepods, ostracods etc.) that feed on phytoplankton. Some large zooplankton feed on small zooplankton. Some of the eggs and larvae of larger animals, such as fish, crustaceans etc. are included under this

group since they pass through the planktonic stages during the early development and is termed as meroplankton.

Phytoplankton is a critical component of the marine ecosystem as these organisms are responsible for approximately half the global net primary production (Field *et. al.,* 1998). They are indicators of climate changes resulting from global warming as well as other environmental impacts, such as ocean acidification (increase in sea water pH), eutrophication (excess of nutrients in the water column) etc. Increased emission of green house gases, in particular CO_2 (IPCC, 2000) in the latter half of 20^{th} century is being converted to organic carbon by the phytoplankton. They are responsible for transfer carbon dioxide from the atmosphere to the ocean through biological carbon pump. Worldwide, this "biological carbon pump" transfers about 10 gigatonnes of carbon from the atmosphere to the deep ocean each year (IPCC, 2000). Therefore, even a small change in the phytoplankton growth would affect the atmospheric carbon dioxide concentrations and global surface temperatures. The amount of carbon taken up for photosynthesis and released back to the atmosphere through respiration each year is about 1,000 times greater than the amount of carbon that moves through the geological cycle on an annual basis. In the oceans, phytoplanktonic organisms use carbon to make shells of calcium carbonate ($CaCO_3$). These shells settle to the bottom of the ocean when phytoplankton dies and get compressed over time as they are buried to be transformed into limestone. Organic matter buried over time, will also form deposits of hydrocarbon such as coal and oil. It is the non-calcium containing organic matter that is transformed into

fossil fuel. Both are biologically controlled processes in oceans and form long-term sinks for atmospheric CO_2.

The primary production is the basis of oceanic and fresh water food webs. Food chain/food web/ food network describes the feeding relationship between species at different trophic levels. Marine phytoplankton accounts for about half of the total primary productivity on earth. Lindeman's (1942) law states that the efficiency of energy transfer from one trophic level to the next is about 10%.

Phytoplankton is a key food item in both aquaculture and mariculture. In mariculture, the phytoplankton is naturally introduced into enclosures with the normal circulation of seawater. In aquaculture, phytoplankton is introduced into the system directly by culturing them in mass scale. The plankton can either be collected from a body of water or cultured, though the former method is seldom used. Phytoplankton is the food of rotifers (James *et. al.,*1993) which are in turn used to feed other organisms. Phytoplankton is also used to feed many varieties of aqua cultured organisms including shrimp larvae and molluscs such as pearl oysters and giant clams. The production of phytoplankton under artificial conditions itself is a form of aquaculture. Phytoplankton is cultured for a variety of purposes, including food for other aqua cultured organisms and a nutritional supplement for captive invertebrates in aquaria. Culture sizes range from small-scale laboratory cultures of less than one litre to several thousands of litres for commercial aquaculture (James *et. al.,* 1993).

Phytoplankton distribution and species composition change continuously with variations in salinity, light, nutrient availability, water movements and grazing pressure (Hsiao, 1992). Changes in species composition and diversity may produce changes in the phytoplankton growth rate and their response to irradiance or other limiting factors. It is important to understand how these changes are reflected in ecosystem functioning (Duarte *et. al.,* 2006). As phytoplankton is exposed to ever-fluctuating physico-chemical parameters they exhibit wide diversity in species and abundance.

Although several studies on plankton ecology are available from Cochin backwater (Qasim, 1970; Qasim *et. al.,*1972 a, b; Devassy and Bhattathiri 1974; Qasim *et. al.,* 1974; Madhupratap and Haridas 1975; Madhupratap 1987; Joy *et. al.,* 1990; Menon et.al., 2000; Haridevi *et. al.,* 2004; Jyothibabu *et. al.,* 2006; Madhu *et. al.,* 2007 and 2010; Martin *et. al.,* 2013), but the studies on factors (physical and biological) that limit the phytoplankton growth are lacking. The present study addresses the phytoplankton distribution in the Cochin backwater in relation to hydrographic parameters and the influence of salinity, light (physical factors) and copepod grazing (biological factor) on their growth.

The influence of salinity on phytoplankton varies widely, because different species have different salinity preferences. Like marine and aquatic species, many phytoplankton species exhibit tolerance to certain salinity, beyond which, it can inhibit their growth. Light is the most important factor that influences phytoplankton growth. In aquatic environments (lakes, sea or estuary) the light incident on the surface is

rapidly reduced exponentially with depth (Krik, 1994). In estuaries, the major factor influencing the light availability is the suspended particulate matter, which attenuates and scatters the light. The light changes with time of the day and the season, affecting the amount of light penetrating the water column. Similarly, biological factor like copepod grazing is a major factor influencing the standing crop of phytoplankton. The copepod can actively graze up to 75% of the phytoplankton biomass in a tropical estuary (Tan *et. al.,* 2004). It is in the context that the present study investigates the salinity, light (physical factors) and copepod grazing (biological factor) phytoplankton as the factors controlling phytoplankton growth and distribution.

The objectives for the present study are to:

1) Study the phytoplankton diversity in accordance to water quality parameters
2) Experimentally determine the phytoplankton growth rate at optimal condition
3) Experiments to elucidate the influence of salinity and light on the growth rate of phytoplankton
4) Study the influence of copepod grazing on phytoplankton biomass
5) Experiments to determine the prey size (phytoplankton) preferences of copepod.

Chapter 2

Spatio-Temporal Variation of Hydrographic Parameters

2.1 Introduction

Cochin backwater is the largest estuarine system along the southwest coast of India. It extends from Munambam (10º 10' N, 76º 15'E) in the north, to Thanneermukkom (09º 30'N, 76º 25'E) in the south (~80 km). The backwater is characterized by its long axis lying parallel to the coastline, with several small islands and interconnected waterways, and covers an area of about 300 km^2. The width of this estuarine system varies from 450 m to 4 km, and the depth ranges from 15 m at the Cochin inlet to 3 m near the head with an average depth of 2.5 m. The backwater is separated from the sea by barrier spits interrupted by tidal inlets at Munambam and Cochin. The openings at Cochin, known as the Cochin inlet, with a width of 450 m and Munambam, provide perennial connections to the Arabian Sea (Joseph 1996; Revichandran *et. al.,* 2012; Shivaprasad *et. al.,* 2013). The Cochin Port, situated on the Willington Island, is near the Cochin inlet, which provides the main entrance channel to this harbor. Seven important rivers viz. Periyar, Chalakudy, Muvattupuzha, Pamba, Meenachil, Manimala and Achankovil drain into the backwater, discharging large quantities of fresh water during the monsoon season. The river discharge into the Cochin backwater exhibits high seasonality with 60 to 70% of the total discharge occurring during the southwest monsoon period. The maximum annual discharge (6,795 Mm^3) was

recorded in Periyar and the minimum (1,250 Mm^3) in Achankovil with an annual river discharge into Cochin backwater of 22 x 10^3 Mm^3/year (Srinivas *et. al.,* 2003).

In general, the pre monsoon (March-May) experiences the lowest rainfall (386 mm $month^{-1}$), thus defining the peak "dry" season. In contrast, the southwest monsoon (June-September) receives the maximum rainfall (1891 mm $month^{-1}$), thus defining the peak "wet" season (Krishnakumar *et. al.,* 2009).

The anthropogenic activities in the region started since the latter half of the 19^{th} century and remain high up to the present day. During the early stages of developments in the country (1940's), industries were allowed to establish along the upper reaches of the estuary without understanding its complex hydrodynamics. Inadequate technology and the neglect of investing in waste water treatment eventually resulted in the accumulation of pollutants, especially in the northern region (Qasim 2003; Babu *et. al.,* 2006). Indiscriminate reclamation was another major intervention, which reduced the estuarine volume by 40% an estimate made nearly three decades ago (Gopalan *et. al.,* 1983). The construction of salinity barrier upstream of the estuary to support agricultural activities during the 1970's exacerbated the situation by reducing the flushing characteristics of the estuary and increasing the sedimentation. The annual maintenance dredging volume of 10 x 10^6 m^3 from the Cochin harbor region indicates the intensity of sedimentation (Rasheed 1997; CPT 2000).

The backwater provides ideal breeding grounds for many fin fishes and shellfishes and serves as a nursery for completing the early life stages of their life cycle. The tidal influence in the backwater reaches up to 60 km southwards where the range of the spring tide is about 20 cm. The configuration of the land is such that all drains and rivers flow in one direction.

Cochin backwater is one of the productive estuarine systems in India and perhaps the most intensively investigated area in the country during the last five decades. However, studies on the distribution, species diversity and abundance of phytoplankton, particularly in relation to ecological parameters are very few. The present study was carried out in order to address the role of environmental parameters on phytoplankton distribution and diversity in the Cochin backwater, both by observational and experimental approach.

2.2. Review of Literature

A number of hydrographical studies have been undertaken in the Cochin backwater during the past five decades. Some of the important works are mentioned here. Ramamritham and Jayaraman (1963) observed that during January, February and March the surface salinity was quite high and the maximum was noticed during March and April and by late May salinity started to decrease. Josanto (1971) and Joseph (1974) observed salinity values ranging from purely marine to almost limnetic condition and (Kunjukrishna Pillai *et. al.,* 1975) reported bimodal fluctuation of salinity. Kumaran and Rao (1975) observed salinity range of 1 to 34 between Narakkal and Aroor. Balakrishnan and Shynamma (1976) observed that in most cases salinity distribution followed the tidal rhythm with high values during high tide

and low values during low tide in Cochin harbor area. Ramaraju et.al., (1979) observed that during southwest monsoon, no marked variations in the salinity values of the surface layer in relation to tide was noticed in the Cochin harbor mouth area due to high influence of the fresh water influx. According to (Lakshmanan *et. al.,* 1982), surface salinity increased markedly during pre monsoon south of the inlet than on the northern part where near freshwater conditions prevailed. According to (Joseph and Kurup 1990) salinity varies diurnally in phase with the tides, showing increase during flood tide and decrease during ebb tide in the Cochin harbor area. Rasheed *et. al.,* (2000) studied the short-term impacts of dredging on salinity in Cochin backwater. Varma *et.al.,* (2002) made time series observation of daily salinity and temperature for nearly two years from a location near Panangad in Vembanad Lake and observed a bimodal variation in salinity with a range of 0 to 32 and noticed high salinity (>30) during April. Studies on tidal propagation and currents in Cochin backwater was studied by (Srinivas and Dinesh Kumar 2002; Srinivas *et.al.,* 2003; Antony et.al. 2007 and 2009) and reported that the maximum tidal height in Cochin backwater is 1 m. Balachandran *et. al.,* (2008) have developed a two dimensional hydrodynamical model for the Cochin backwater, focusing on the pollution dispersion characteristics. Revichandran *et.al.,* (2012) reported the complexity of tidal propagation in Cochin backwater and concluded that Cochin backwater is the second largest wetland ecosystem in India with its uniqueness by virtue of its dual connection to the Arabian Sea. Shivaprasad *et. al.,* (2013) studied the intratidal and spring-neap variations in stratification of water column in dry and wet seasons and also the

horizontal extent of salt intrusion and the relation between salinity and its property distributions.

The Cochin backwater being a tropical estuary, temperature variations is not as critical as salinity. Studies made by (Sankaranarayanan and Qasim 1969; Nair and Tranter 1971; Kunjukrishna Pillai *et. al.,* 1975; Balakrishnan and Shynamma 1976; Ramaraju *et. al.,* 1979; Lakshmanan *et. al.,* 1982; Varma *et. al.,* 2002) have shown reported that the decrease in surface temperature observed during monsoon was not only by the influx of freshwater into the estuarine system but also by the incursion of cold water from the Arabian Sea. Report also says that the surface temperature of Cochin to Azhikode stretch ranged between 24.5º C and 30.5º C with the annual maximum during April and minimum during July to August.

Kumaran and Rao, (1975), Kunjukrishna Pillai *et. al.,* (1975), Balakrishnan and Shynamma (1976) and Saraladevi et.al. (1979) have remarked that dissolved oxygen was higher during the monsoon and low during the pre monsoon months in the estuary near the industrial area and generally the surface values were higher. The annual range was 2 to 6 ml L^{-1}. Dissolved oxygen in the mangrove areas of Cochin backwater near Nettoor ranged between 2 and 8.75 ml L^{-1} (Sheeba *et. al.,* 1996).

Macro nutrients like (Nitrate, Phosphate and Silicate) in Cochin backwater have been studied by (Sankaranarayanan and Qasim 1969; Joseph 1974; Manikoth and Salih 1974; Balakrishnan and Shynamma 1976; Sankaranarayanan *et. al.,* 1986; Lakshmanan *et. al.,* 1987; Anirudhan and Nambisan 1990; Saraladevi *et. al.,* 1991; Sheeba *et. al.,* 1996 and Martin *et.*

al., 2008 and 2013). They observed that during pre monsoon when the system was predominantly sea water dominant, the nutrient concentration was low and high during monsoon due to the influx of freshwater. They also observed that silicate cycle was entirely dependent upon the freshwater discharge, as evidenced by the fact that the values decreased from the surface to bottom and also an inverse relationship obtained with salinity. They also reported that north of Cochin harbor was richer in nutrients than the southern parts because of the cluster of industries spread over the banks of northern estuary and also the silicate concentration in the estuary was largely dependent on the sources such as river discharge and land drainage.

Pioneering works on hydrographical parameters have also been made along the west and east coasts of India. Gunaga and Neelakanta (1987); Kusuma *et. al*., (1988); Menon and Neelakanta (1992) have studied the annual variability of environmental variables in Kali estuary in Karnataka (West coast). According to their findings the annual variation of salinity in Kali estuary was low during monsoon (1.33 and 8.02) compared to pre monsoon (27.92 to 31.12). Whereas in the case of dissolved oxygen monsoon values were relatively high (5.26 to 5.42 mg l^{-1}) than the pre monsoon values (4.67 to 4.81 mg l^{-1}).

In the Mandovi and Zuari estuaries of Goa (West coast) hydrographic studies were initiated by (Dehadrai 1970; Das *et. al.,* 1972; Murty and Das 1972; Singbal 1973; Shetye and Murty 1987; De Sousa and Sen Gupta 1988; Unnikrishnan *et. al.,* 1997; Shetye, 1999). According to them the riverine flow of fresh water was minimal during pre monsoon and maximum during

monsoon which in turn decreased the salinity during monsoon in the study region. The salinity recorded in these regions was <5 during monsoon and >30 during pre monsoon. Water temperature increased from January to May. It was followed by a decline during the monsoon months (June to September). The annual difference in the temperature was only 5-7 °C. During pre and post monsoons the oxygen concentration was found to be uniform in the Mandovi and Zuari estuaries from surface to bottom when the water was in well mixed condition. During the monsoon season, because of stratified conditions in the water column, high oxygen values were recorded at the surface and low values at the bottom. Nutrients showed significant seasonality especially in the case of nitrate and silicate with high values 20 µM and 190 µM respectively during the monsoon when the rainfall and land runoff were maximum. During the pre monsoon the nitrate (7 µM) and silicate (70 µM) values were the least.

In the Rushikulya estuary in Orissa coast, East coast (Gouda and Panigrahy 1989, 1991, 1992, 1993, 1995 a & b and Patnaik and Misra 1990) has studied the seasonal changes in salinity, temperature, transparency, dissolved oxygen and pH. Surface salinity was the lowest during August/September (0.05) and highest in April/May (34.7). Transparency in the estuary was minimum during the monsoon season (July to October) and maximum from April to June. Turbidity was largely caused by silt-laden land runoff and increased transparency by the intrusion of clear seawater. Nitrate showed the peak values during the monsoon period as in the case of silicate.

Ramana *et. al.,* (1989); Sai-Sastry and Chandramohan (1990); John *et. al.,* (1993); Reddy and Rao *et. al.,* (1994); Bandyopadhyay *et. al.,* (1994); Sarma *et. al.,* (2009 and 2010) were the pioneer workers in Godavari estuarine system (East coast). They have reported high salinity values during the pre monsoon season (March-June) and low values during the monsoon season. Turbidity of water was found to be the maximum during the monsoon months and minimum during the pre monsoon. Similarly in the case of nitrate and silicate, high values were reported during the monsoon and low values during the pre monsoon.

There are numerous studies describing the hydrography of Vellar estuary (East coast) (e.g., Rangarajan, 1958; Seshaiya, 1959; Ramamoorthi, 1971; Purushothaman and Venugopal 1972; Krishnamurthy and Sundaraj 1973; Venugopalan and Rajendran 1975; Chandran, 1985; Ramachandran and Venugopalan 1987; Maruthanayagam and Subramanian 1999; Govindasamy *et. al.,* 2000; Senthilkumar *et. al.,* 2002; Santhanam and Perumal 2003; Rajasegar, 2003). These studies have reported that salinity was high during the peak summer and low during the peak monsoon. The high values (35.7) could be attributed to the low amount of rainfall and high rate of evaporation. During the monsoon season, the rainfall and the freshwater inflow from the land reduced the salinity (14.5). Higher values of dissolved oxygen were recorded during the monsoon months. Dissolved oxygen was observed to be low during the post monsoon and summer seasons, which could be due to the gradual saline water incursion and increasing temperature. The recorded highest nitrate value (52.9 µM) during the monsoon season could be due to the high organic materials received from

the catchment area. The silicate content was higher than other nutrients and the highest value (140.5 µM) was observed during the monsoon which may be due to heavy inflow of fresh water derived from land drainage.

2.3. Materials and Methodology

2.3.1. Sampling site

Sampling was carried out from north to south of the Cochin backwater (Fort Kochi to Thaneermukkom) from June 2008 to May 2009 (Figure 2.3.1). For the study, eight stations were selected based on their features representing northern arm (Stations 1- 4) and southern arm (Stations 5 - 8) of the backwater. All the stations were 3 - 5 km apart from each other so as to cover the entire backwater region (Table.2.3.1).

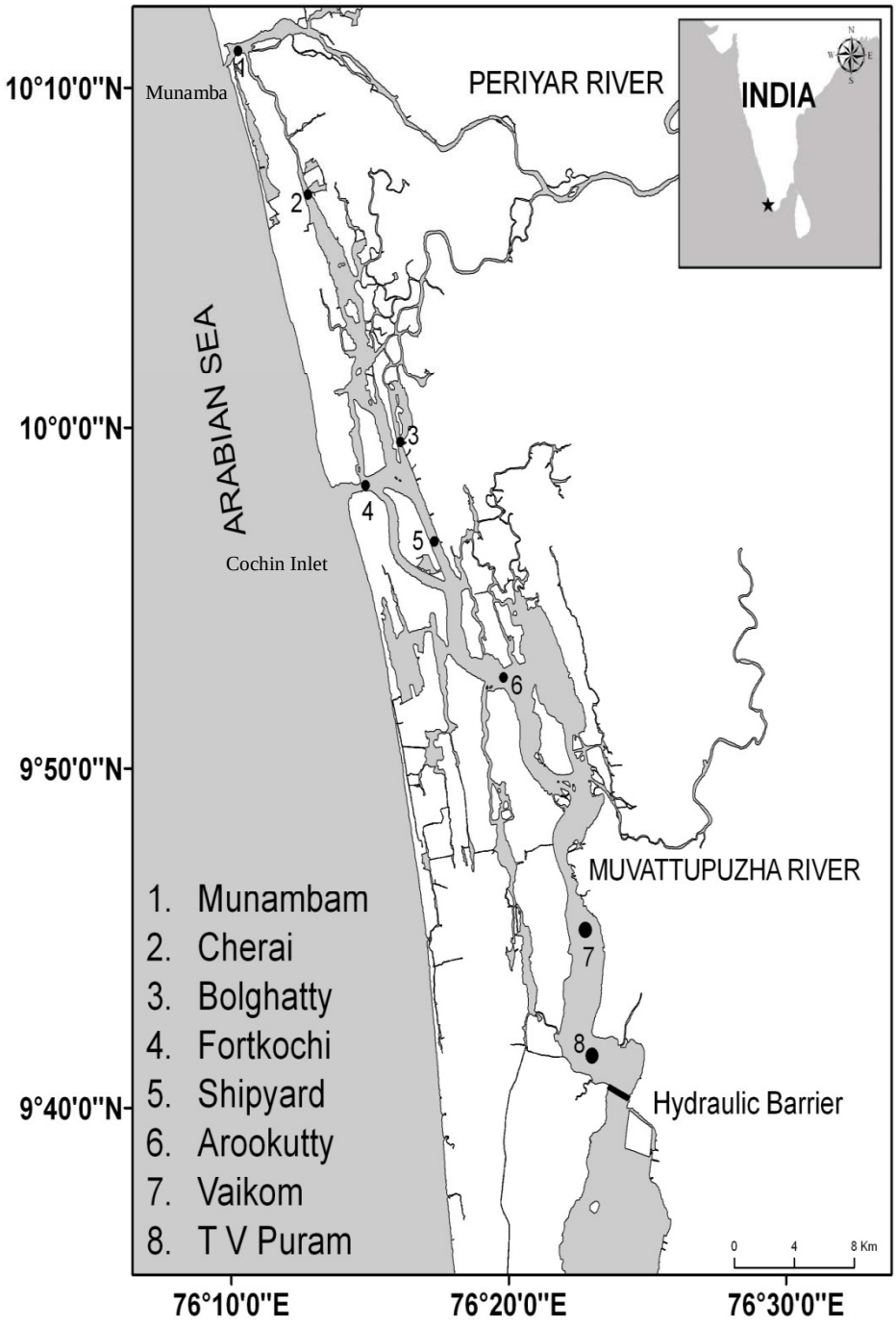

Figure 2.3.1 Sampling site

Table.2.3.1. Description of Sampling site

Station No	Station Name	Station description
Northern Arm		
1	**Munambam**	Northern inlet of Cochin back water (CBW). Marine condition, and salinity fluctuation is less. Periyar river bifurcates at Aluva and one arm joins at Munambam.
2	**Cherai**	Narrow and inner region of CBW, fishing and aquaculture activities are very high in this region
3	**Bolghatty**	Narrow and inner region of CBW, recreational activities are high, Periyar river enters the estuary, just north of this region
4	**Fort Kochi**	Major inlet of CBW (450 m wide), Ship channel, harbor area, tidal influence is high
Southern Arm		
5	**Shipyard**	Harbor area, ship building centre, oil tanker berth is situated in this region, ship channel.
6	**Arookutty**	Broader area of CBW, Muvattupuzha river enters to the south of this region. Aquaculture activities, clam shell, prawn pealing centres etc are located in this region.
7	**Vaikom**	Broad area of CBW, brackish water fishery region, clam shell deposition
8	**T V Puram**	Broad area of CBW, distillery unit, brackish water fishery, shrimp processing unit. ~ 40 km from Kochi inlet.

2.3.2. Sampling strategy

Each sampling was done during the spring tide, when the water samples were collected from euphotic zone (0.5 m) using Niskin sampler and brought to the laboratory in ice box in 3 L carbouys.

Water samples were collected during the three seasons of the study period; pre monsoon (February-May) when there is least river discharge, monsoon (June-September) with large volume of river discharge into the backwater and post monsoon (October-January) the intermediate phase. The average monthly rainfall of river basin in the study region is shown in Figure 2.3.2.

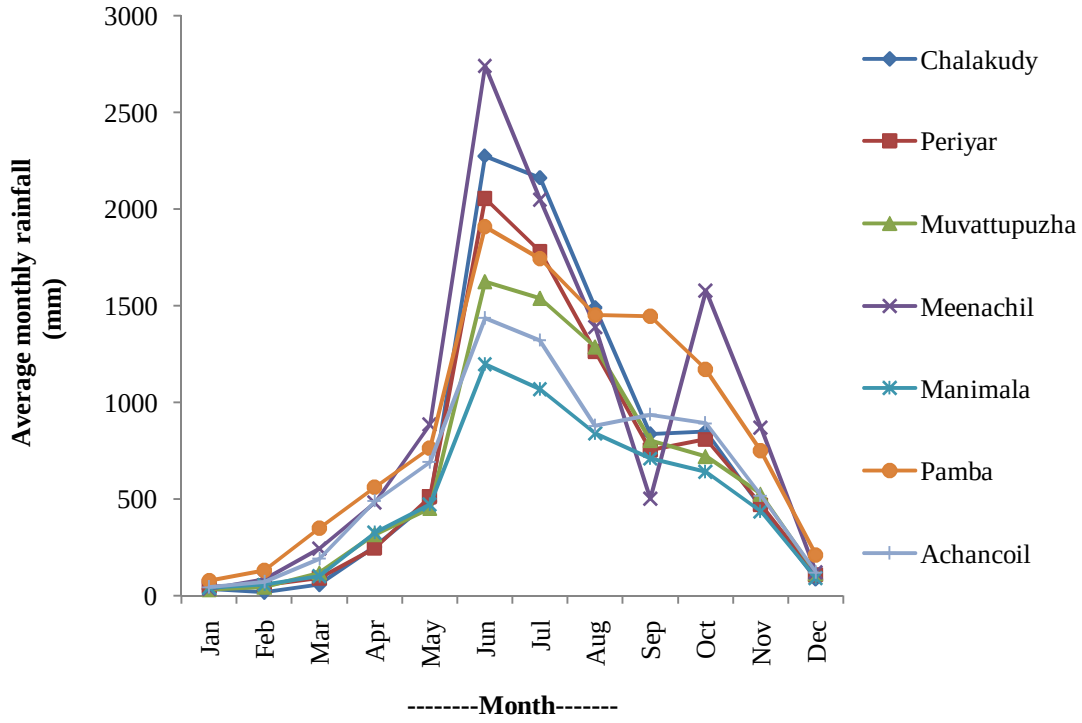

Figure 2.3.2 Average Monthly Rainfall (mm) of River Basins in this study region (Revichandran *et.al.*, 2012)

2.3.3 Salinity and Temperature

A portable Conductivity-Temperature-Depth profiler (CTD, SBE Model 911 PLUS) was operated to measure salinity and temperature at all stations.

2.3.4 Suspended Particulate Matter (SPM)

SPM was determined by filtering 250 ml of water sample on to a pre-weighed Millipore filter paper (0.45 µm pore size) and drying the residue at 80º C. The weight of the particles collected on the filter paper was gravimetrically determined following the standard procedure described in (APHA, 2005).

2.3.5 pH

A portable pH meter (Metller bench top Model No.117, accuracy, ± 0. 01) was used for measuring the pH.

2.3.6 Dissolved oxygen (DO)

Dissolved oxygen was determined by the Winkler's method, as recommended by Strickland and Parsons (1972) with standard iodimetric titration. The principle of the determination and the possible sources of systematic errors are discussed by (Grasshoff, 1983).

2.3.7 Nitrite-Nitrogen (NO_2-N)

Nitrite-N was measured by the method (Bendschneider and Robinson, 1952). In this method, nitrite in the water sample when treated with sulphanilamide in acid solution results in a diazo compound, which reacts with N-l-naphthyl ethylene diamine dihydrochloride to form an azo dye. The absorbance of this colour complex was measured at 543 nm.

2.3.8 Nitrate-Nitrogen (NO_3-N)

Nitrate-N in the water sample was quantitatively reduced to nitrite by passing through a reduction column filled with copper coated cadmium granules and measured as nitrite. During the reduction stage, ammonium chloride buffer was added to the sample to maintain a stable pH (Grasshoff, 1983). The sample after reduction was analyzed for nitrite-N as described in section 2.3.7.

2.3.9 Ammonia-Nitrogen (NH_4-N)

Ammonia-N was determined according to the indo phenol blue method of (Koroleff, 1983). In a moderately alkaline medium, ammonia reacts with hypochlorite to form mono chloramine, which in the presence of phenol, catalytic amount of nitro prusside ions and excess of hypochlorite forms indophenols blue. The formation of mono chloramine requires a pH between 8 and 11.5. At higher pH, ammonia is incompletely oxidized to nitrite. Both calcium and magnesium ions in seawater precipitate as hydroxide and carbonate at pH higher than 9.6, however their precipitation can be prevented by complexing them with citrate buffer. Great care has to be taken to ensure that samples, blanks and standards are not contaminated during the course of analysis. The samples were 'fixed' by the addition of reagents immediately after collection and the absorbance, after the colour development (about 6 hours) were measured at 630 nm. The measurement of ammonia included both free dissolved ammonia gas and the ammonium ions. This method estimates the sum of NH_4 + and NH_3 and is denoted here as NH_4-N.

2.3.10 Phosphate-Phosphorus (PO_4-P)

Phosphate-P was determined as inorganic phosphate by the formation of a reduced phospho molybdenum blue complex in an acid solution containing molybdic acid and ascorbic acid. The most popular methods relying on this reaction, which was developed by (Murphy and Riley, 1962) is that of (Strickland and Parsons, 1972). A variation of this method described by (Grasshoff, 1983) is adopted in the present work.

Instead of single solution reagent as in the Murphy and Riley procedure, two stable reagent solutions were used here. 0.5 ml of the mixed reagent containing molybdic acid and antimony tartrate followed by 0.5 ml of ascorbic acid reagent were added to 25 ml aliquots of the samples. The absorbance was measured at 882 nm within 30 minutes to reduce any possible interference from arsenite.

2.3.11 Silicate-Silica (SiO_4-Si)

The determination of dissolved silicate in seawater is based on the formation of a yellow silico molybdic acid when an acid sample is treated with a molybdate solution (Grasshoff, 1983). This is further reduced by ascorbic acid in presence of oxalic acid (to prevent interference from phosphate) to a blue coloured complex (molybdenum blue). This blue color was measured at 810 nm.

2.4. Results

Hydrographic parameters like salinity, temperature, suspended particulate matter; pH, dissolved oxygen and nutrients (nitrite, nitrate, ammonia, phosphorus and silicate) were measured in the Cochin backwater during the period June 2008 to May 2009.

2.4.1 Salinity

Spatial and temporal variation in salinity was significant in the study region. Annual variation in salinity in the northern arm ranged from 0 to 34.52 (avg. 20.51) with maximum at station 1 during April and minimum at station 2 during September. Salinity was below 10 to zero from June to September, except at station 1. Salinity gradually increased from zero and peaked to >30 during March and April except at stations 2 and 3 where the maximum salinity was only 27.6 during April (Figure 2.4.1 a).

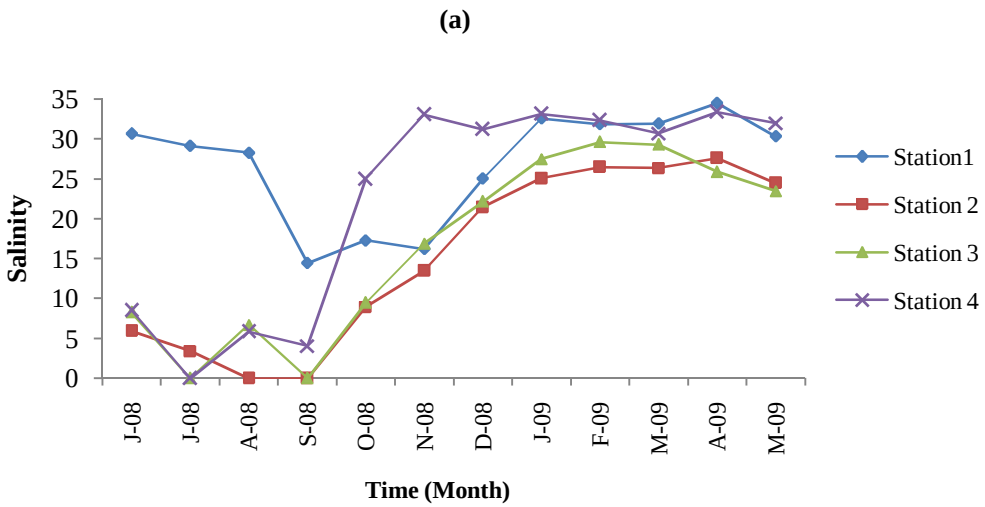

Figure 2.4.1 (a) Salinity distribution in the northern arm

Annual variation of salinity in the southern arm ranged from 0 to 33.78 (avg. 11.31). Salinity was between <5 and zero from June to October at stations 7 to 8 whereas; at stations 5 and 6 salinity started to increase from October and peaked during April. The overall annual variation in the study region showed that salinity was never >20 at stations 6, 7 and 8 whereas at station 5 the maximum salinity observed was 33.78 during May (Figure 2.4.1 b).

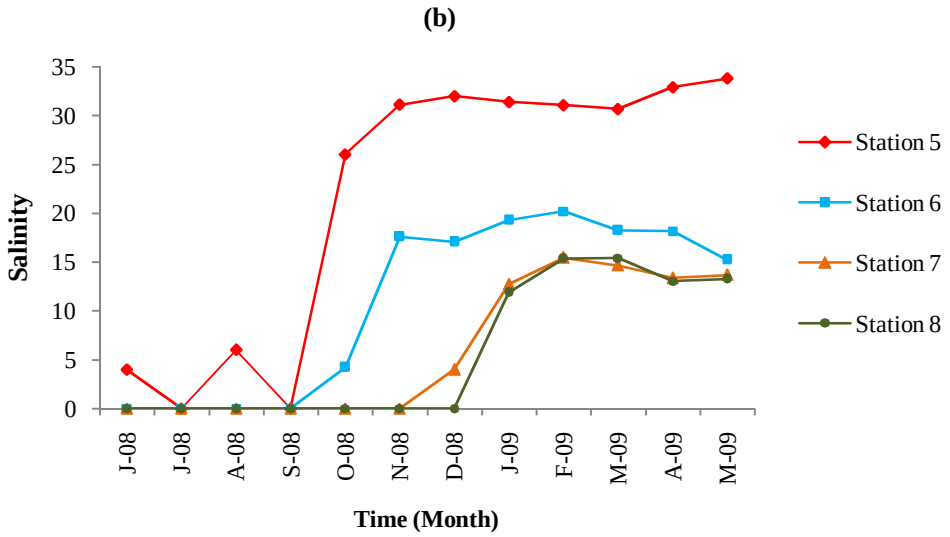

Figure 2.4.1 (b) Salinity distribution in the southern arm

Seasonal variation in salinity in the study region indicated that monsoon recorded the least salinity when compared to pre monsoon in both the arms of the Cochin backwater. Stations 4 and 5 recorded high salinity during post and pre monsoon whereas stations 6, 7 and 8 recorded least salinity during all the seasons. During monsoon only station 1 recorded high salinity (25) whereas at other stations the salinity was <5 (Figure 2.4.1 c). Seasonal distribution of salinity was distinct in the study region. The overall

seasonal salinity pattern in the study region revealed that salinity was never >20 at stations 6, 7 and 8 in all season, whereas at station 1 salinity was always >20. Monsoon recorded the least salinity (<5) at stations 2, 3, 4 and 5 and zero at stations 6, 7 and 8 but at station 1 salinity was >20 during the same period. Pre monsoon recorded the highest salinity when compared to monsoon season. Stations 1, 4 and 5 recorded the highest salinity compared to other stations.

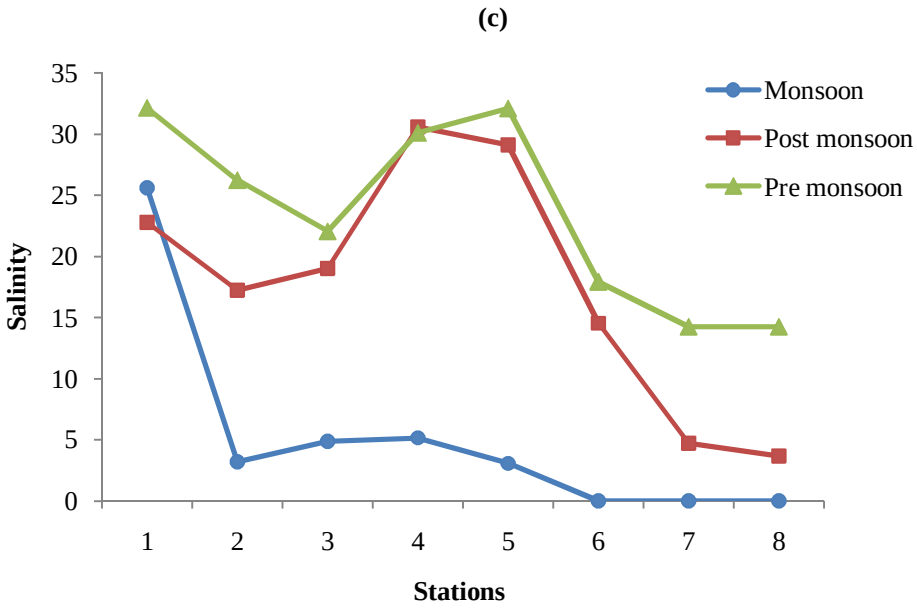

Figure 2.4.1 (c) Seasonal distribution of salinity in the study region

2.4.2 Temperature

Being a tropical estuary temperature did not show wide variations. Monthly variation in temperature was bi modal in the northern arm of the study region with two peaks; the peak seen during September to November was relatively low compared to peak observed during March to April. 3 - 4 ºC decrease in temperature was observed at stations 3 and 4 during January (Figure 2.4.2 a).

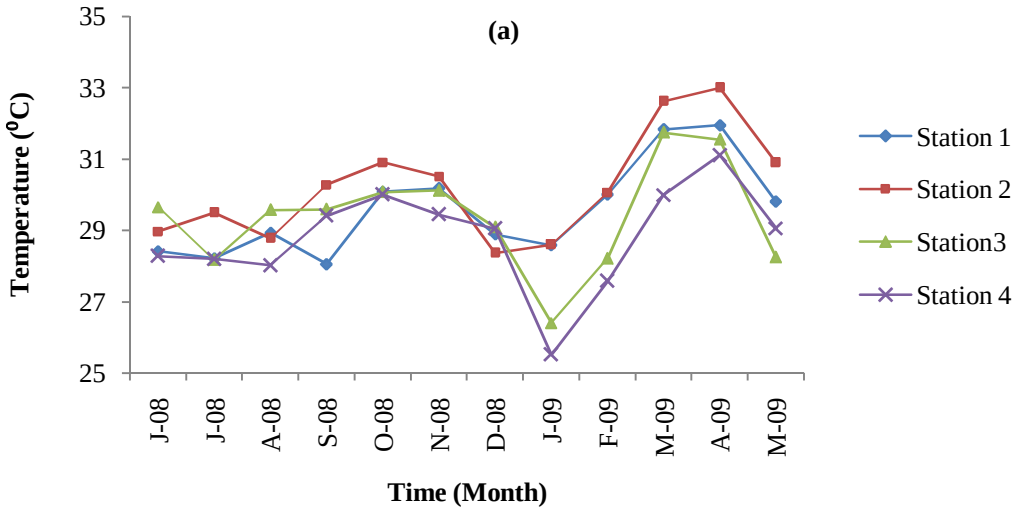

Figure 2.4.2 (a) Temperature distribution in the northern arm

Similar trend was observed in the southern arm of the study region with two peaks, a relatively low peak during September – November and a high peak from March – April. 2-3 ºC decrease was observed at stations 5 and 6 during January (Figure 2.4.2 b).

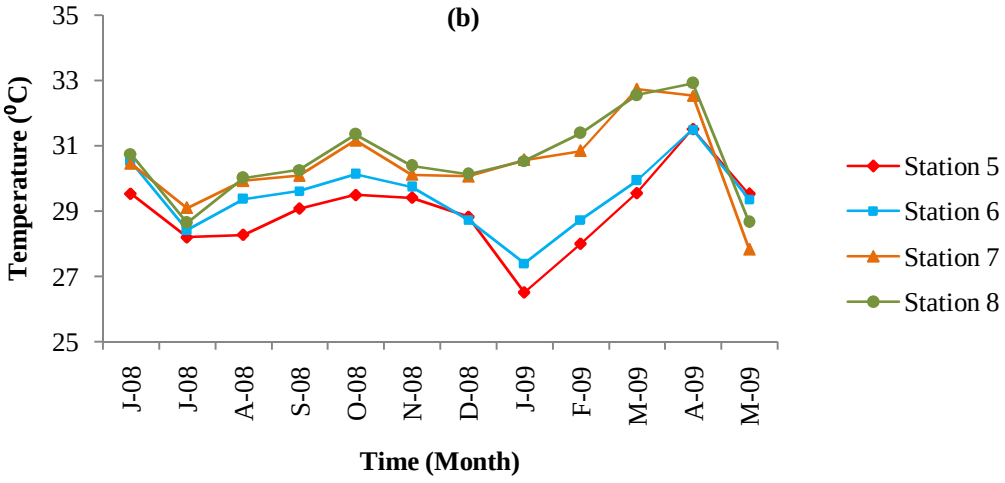

Figure 2.4.2 (b) Temperature distribution in the southern arm

Seasonality in temperature was not as significant as salinity in the study region. Pre monsoon recorded higher temperature compared to monsoon season (Figure. 2.4.2 c). During all the three seasons, stations 4 and 5 recorded relatively low temperature compared to stations 2, 6, 7 and 8.

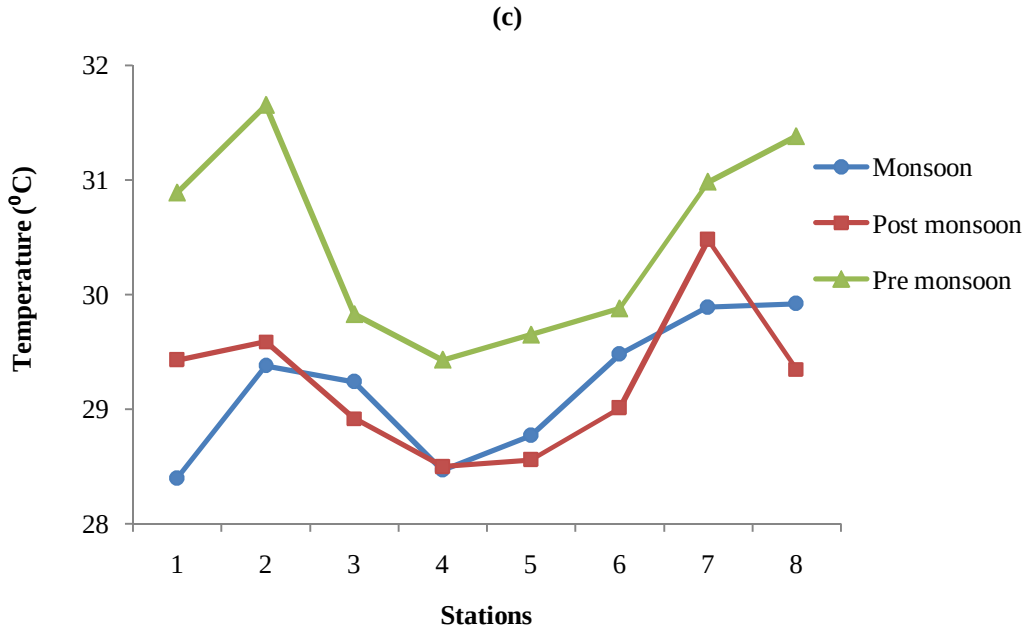

Figure 2.4.2 (c) Seasonal distribution of temperature in the study region

2.4.3 Suspended Particulate Matter (SPM)

Annual variation in SPM ranged from 22.4 to 58.0 mg L^{-1} (avg. 34.0 mg L^{-1}) in the northern arm of the study region. Marginal increase in SPM was observed from August to September at stations 1 and 2 (Figure 2.4.3 a).

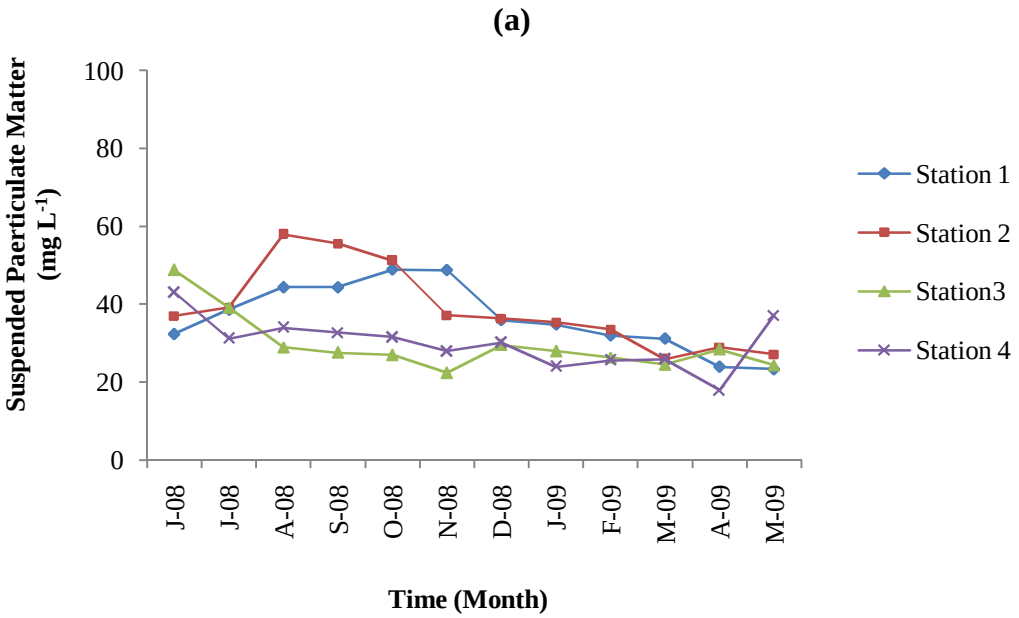

Figure 2.4.3 (a) Suspended Particulate Matter distribution in the northern arm

In the southern arm, the annual range was between 22 and 90 mg L^{-1} (avg. 44.9 mg L^{-1}). Maximum peak of SPM was observed during July at all the stations in the entire observation period (Figure 2.4.3 b).

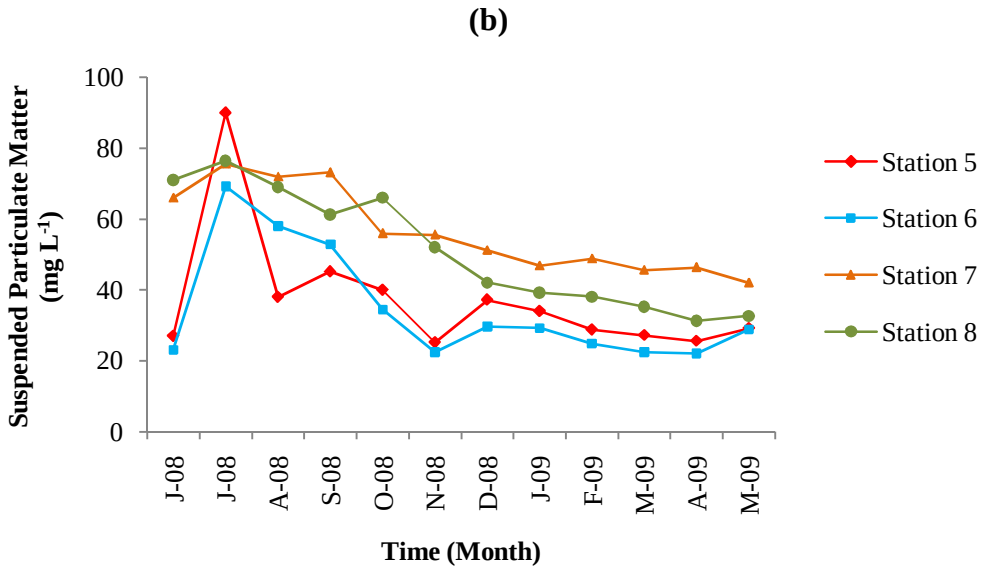

Figure 2.4.3 (b) Suspended Particulate Matter distribution in the southern arm

Seasonal distribution of SPM in the study region revealed that monsoon recorded the maximum SPM and pre monsoon the least. During monsoon SPM ranged from 40.0 to 71.7 mg L^{-1} with maximum at station 7 and minimum at station 1, whereas during pre monsoon the range was between 26.0 and 45.7 mg L^{-1} with maximum at station 8 and minimum at station 3 (Figure. 2.4.3 c). SPM was always high at stations 7 and 8 and low at station 4 during all the seasons.

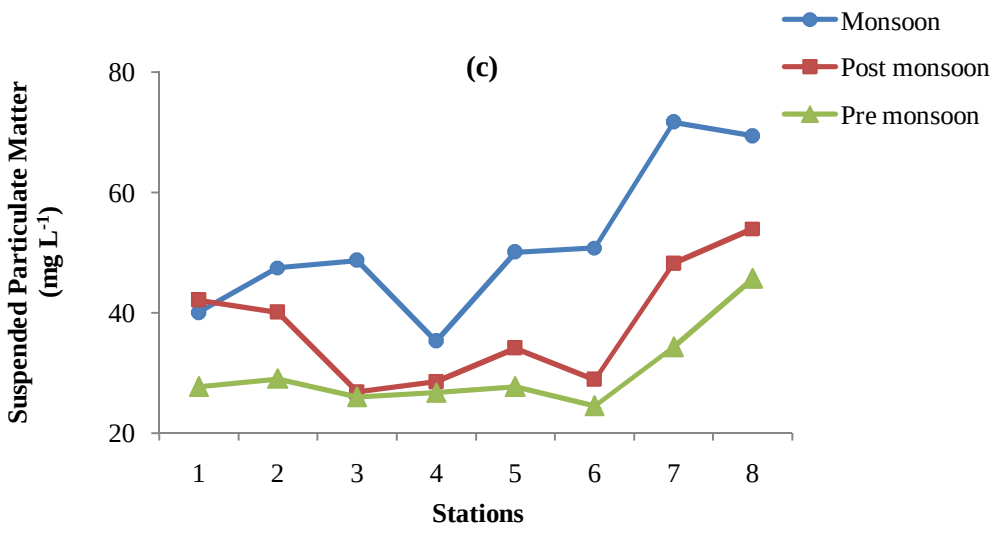

Figure 2.4.3 (c) Seasonal distribution of suspended particulate matter in the study region

2.4.4 pH

Annual range of pH in the northern arm of the backwater was 6.68 to 8.2 (avg. 7.66). It was always low at station 2 throughout the observation period and was relatively low <8 from June to October at all the stations (Figure.2.4.4 a)

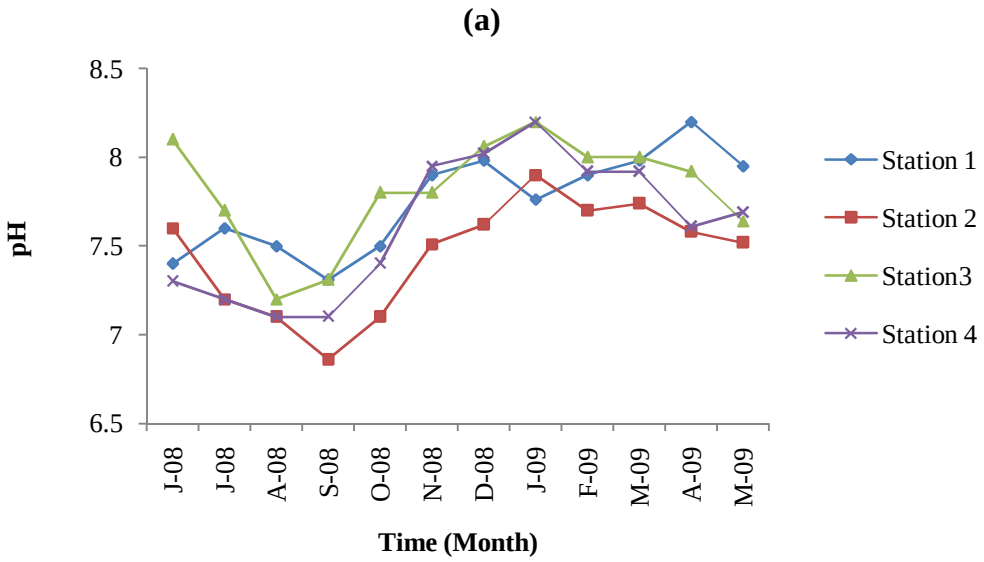

Figure 2.4.4 (a) pH distribution in the northern arm

In the southern arm of the study region pH ranged from 6.5 to 8.09 (avg.7.24). It was always found to be high at station 5 throughout the observation period except during June, July and September (Figure.2.4.4 b).

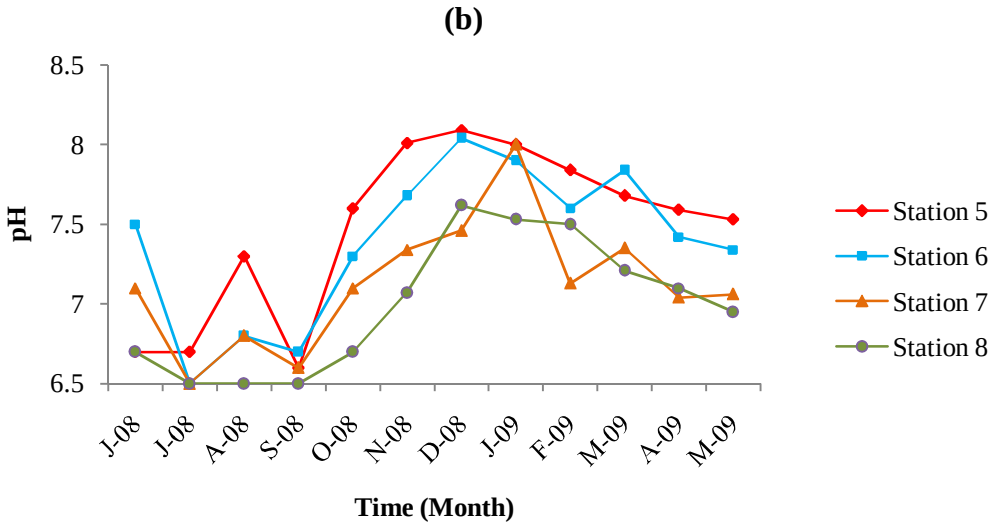

Figure 2.4.4 (b) pH distribution in the southern arm

Seasonal variation was observed with relatively high pH during post and pre monsoon and comparatively low during monsoon in both the arms of the study region. pH was always >7.5 at stations 7 and 8 during three season (Figure. 2.4.4 c).

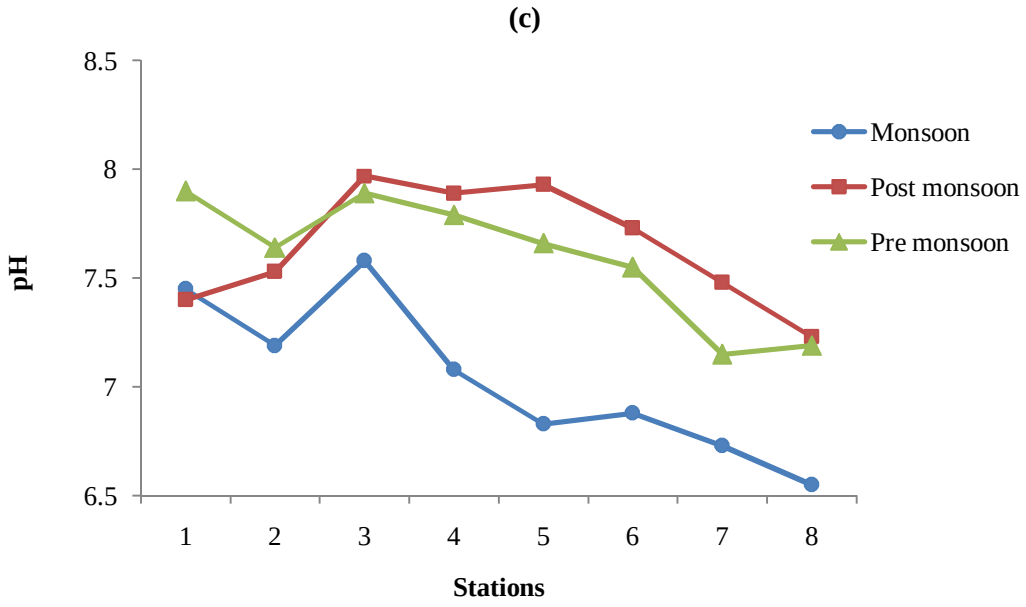

Figure 2.4.4 (c) Seasonal distribution of pH in the study region

2.4.5 Dissolved Oxygen (DO)

Annual variation of DO in the northern arm of the study region ranged from 2.6 to 8.7 mg L^{-1} (avg. 5.9 mg L^{-1}). The maximum DO was observed during April at station 3 and minimum at station 2 during April. Station 3 recorded relatively high DO throughout the observation period except during June, November and May (Figure 2.4.5 a).

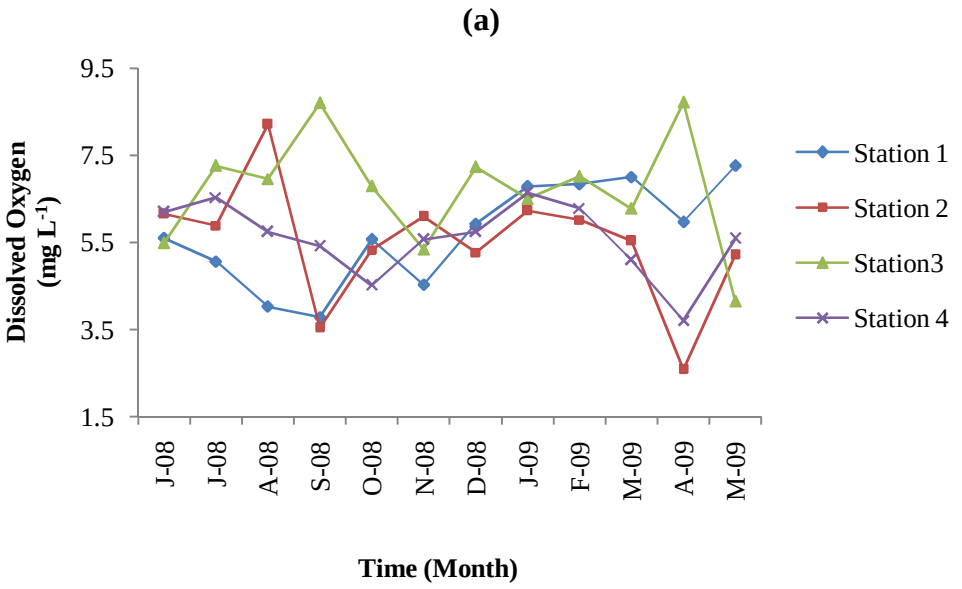

Figure 2.4.5 (a) Dissolved Oxygen distribution in the northern arm

In the southern arm, DO was relatively low at station 5 throughout the observation period (Figure 2.4.5 b). DO ranged from 3.2 to 8.7 mg L^{-1} (avg. 6.6 mg L^{-1}). The high DO values were recorded at stations 8 and the low values at station 4 during September and April.

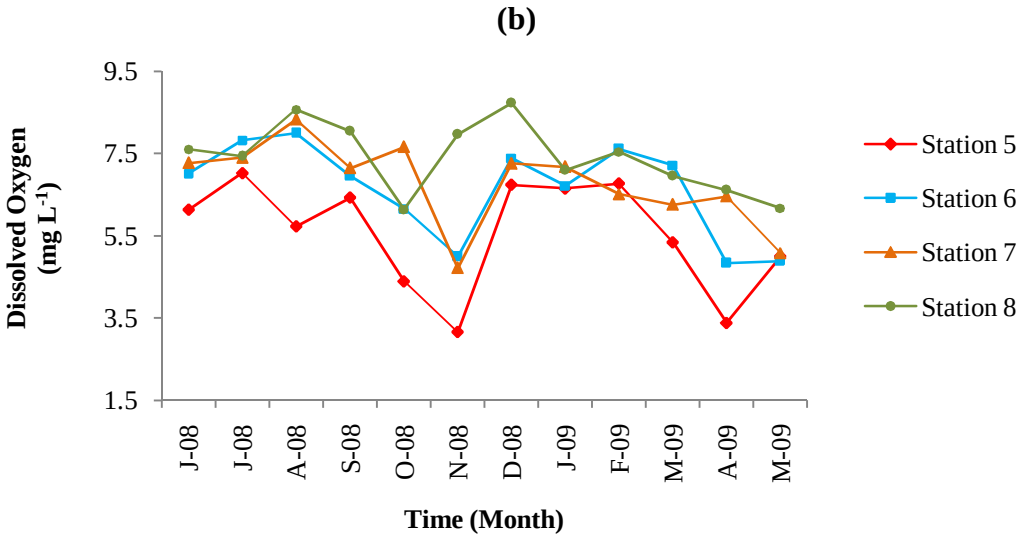

Figure 2.4.5 (b) Dissolved Oxygen distribution in the southern arm

Seasonal variation in DO in the study region revealed high DO (>5 mg L^{-1}) during monsoon at all the stations except at station 1. Whereas during pre monsoon season, stations 1 and 3 recorded the high DO compared to other stations. DO values showed an increasing trend from stations 6 to 8 during all the seasons (Figure 2.4.5 c).

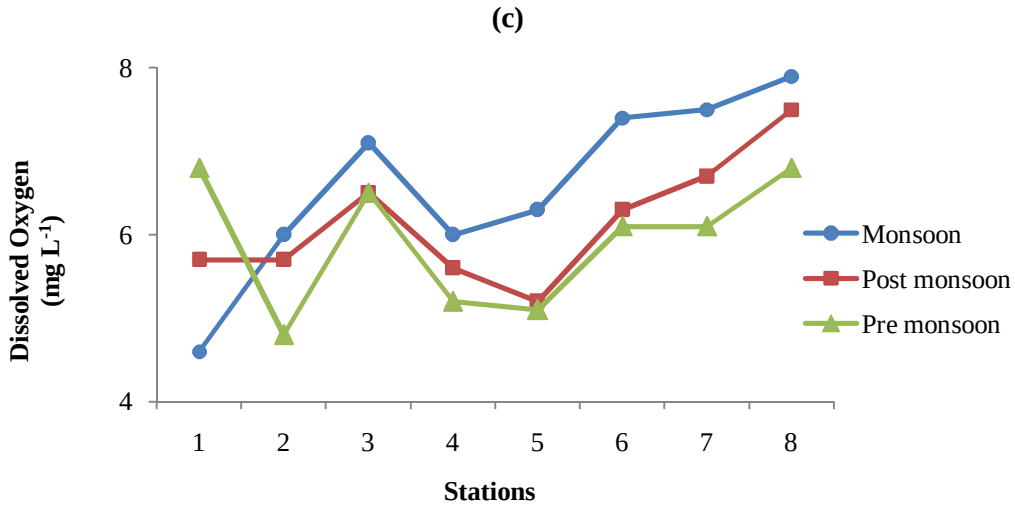

Figure 2.4.5 (c) Seasonal distribution of dissolved oxygen in the study region

2.4.6 Nitrite (NO_2-N)

Nitrite in northern arm ranged between 0.15 and 0.74 µM (av. 0.37 µM). Spatial and temporal distribution of nitrite in the study region was more or less same at all the stations except during September (0.74 µM) and October (0.73 µM) at station 4 with relatively high values (Figure 2.4.6 a).

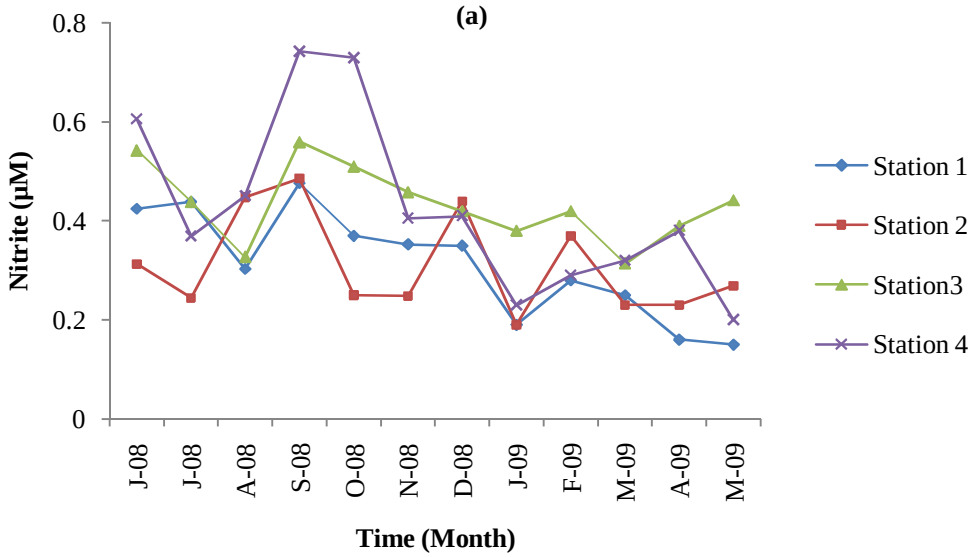

Figure 2.4.6 (a) Nitrite distribution in the northern arm

Nitrite in the southern arm of the estuary ranged from 0.1 to 0.74 µM (avg. 0.36 µM). Relatively high values were observed during October (0.74 µM) and January (0.62 µM) at station 5 (Figure. 2.4.6 b).

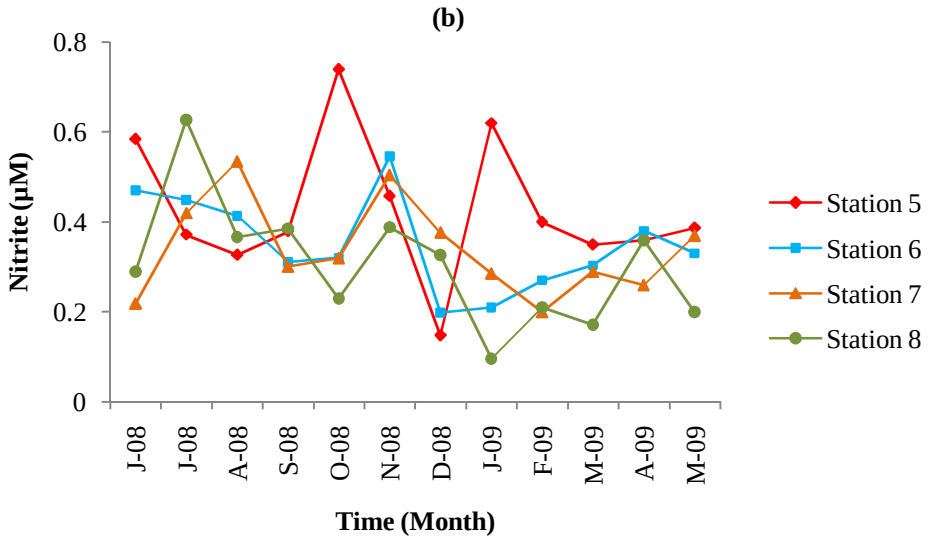

Figure 2.4.5 (b) Nitrite distribution in the southern arm

Seasonal distribution of nitrite showed that comparatively low during pre monsoon when compared to monsoon throughout the backwater. The range during the monsoon was 0.37 to 0.54 µM with maximum at station 4 and minimum at stations 2 and 7 (Figure 2.4.6 c).

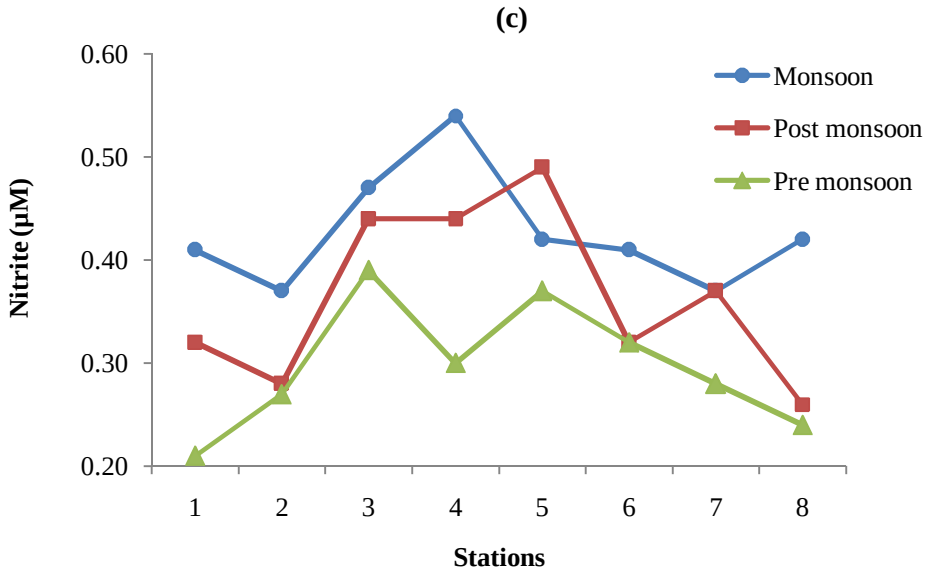

Figure 2.4.6 (c) Seasonal distribution of Nitrite in the study region

2.4.7 Nitrate (NO_3-N)

Nitrate showed tri modal trend in the northern arm of the backwater with a relatively high peak during July - September and two minor peaks during December to January and March to April (Figure. 2.4.7 a), with an overall range of 2.28 - 33.9 µM (av. 13.31 µM). Maximum was recorded at station 3 during September and very low values were observed at station 2 (2.28 µM), 1 (2.4 µM) and 3 (2.76 µM), respectively.

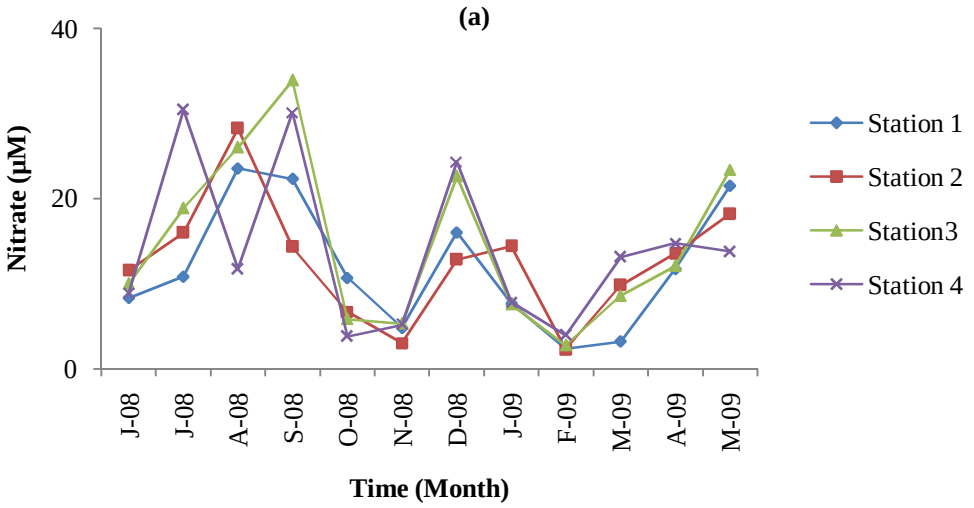

Figure 2.4.7 (a) Nitrate distribution in the northern arm

Similarly in the southern arm also the nitrate content showed tri modal trend with major peak during July-September and minor peaks during December- January and March-April (Figure 2.4.7 b). The annual range was between 1.34 and 32.19 µM (avg. 14.48 µM). Maximum nitrate was recorded at station 5 during July and minimum at station 8 during October.

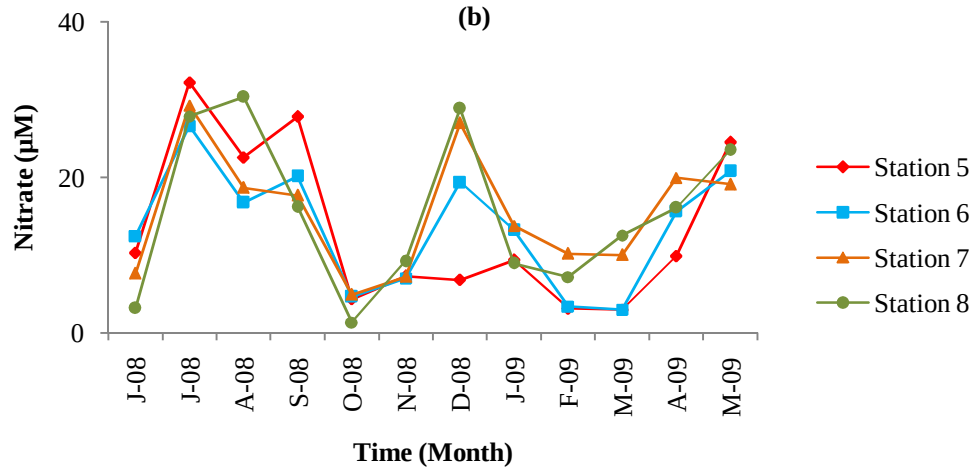

Figure 2.4.7 (b) Nitrate distribution in the southern arm

Seasonal distribution of nitrate was significant with high values during monsoon and low values during post and pre monsoon (Figure. 2.4.7 c). The monsoon range was 16.26 to 26.9 µM with minimum at station 1 and maximum at station 8. The post monsoon range was between 6.96 to 16.08 µM with minimum at station 2 and maximum station 5.

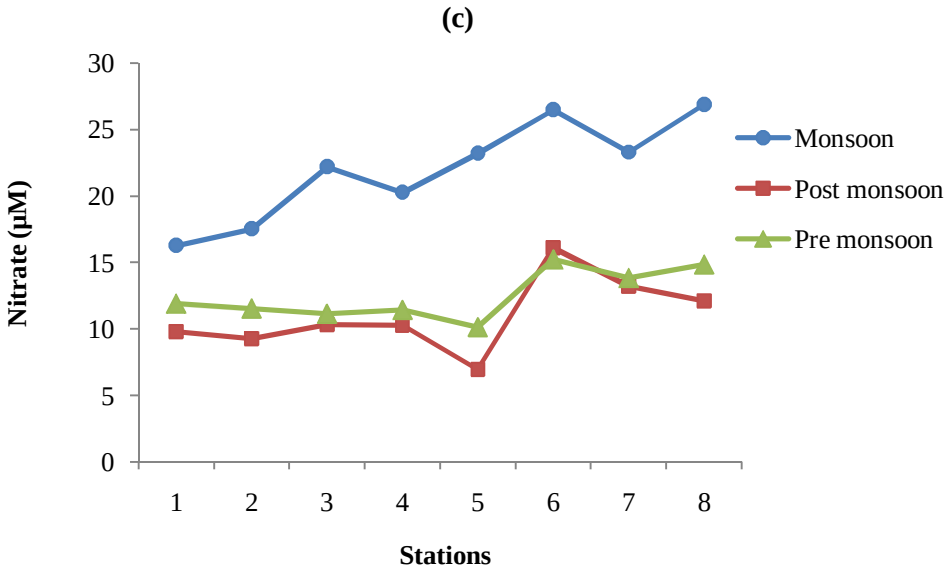

Figure 2.4.7 (c) Seasonal distribution of Nitrate in the study region

2.4.8 Ammonia (NH_4-N)

Spatial and temporal variation in ammonia was observed in the northern arm of the study region ranged from 1.13 to 77.2 µM (avg.17.6 µM). Ammonia level in the study region was found to be high (Figure. 2.4.8 a) during September (46.36 µM), October (55.25 µM) and December (62.40 µM) at station 4 and during December at station 2 (77.20 µM) and also during November at station 1 (50.23 µM).

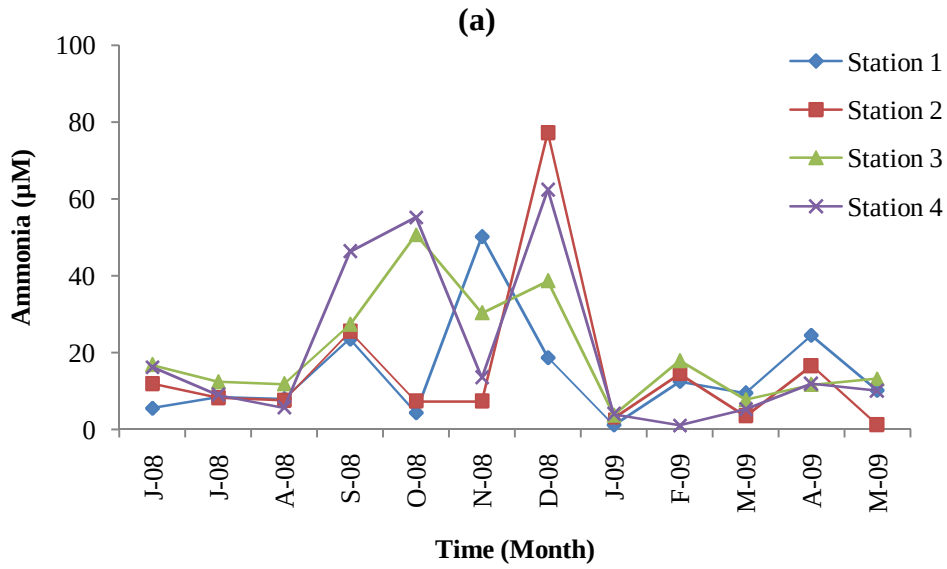

Figure 2.4.8 (a) Ammonia distribution in the northern arm

Ammonia in the southern arm of the backwater ranged from 1.0 to 95.2 µM (avg. 18.67 µM). Ammonia level in the study region was found to be > 40 µM during September and November at all the stations except during November at station 8 (Figure 2.4.8 b). From December to May the ammonia level was below 10 µM except at station 5.

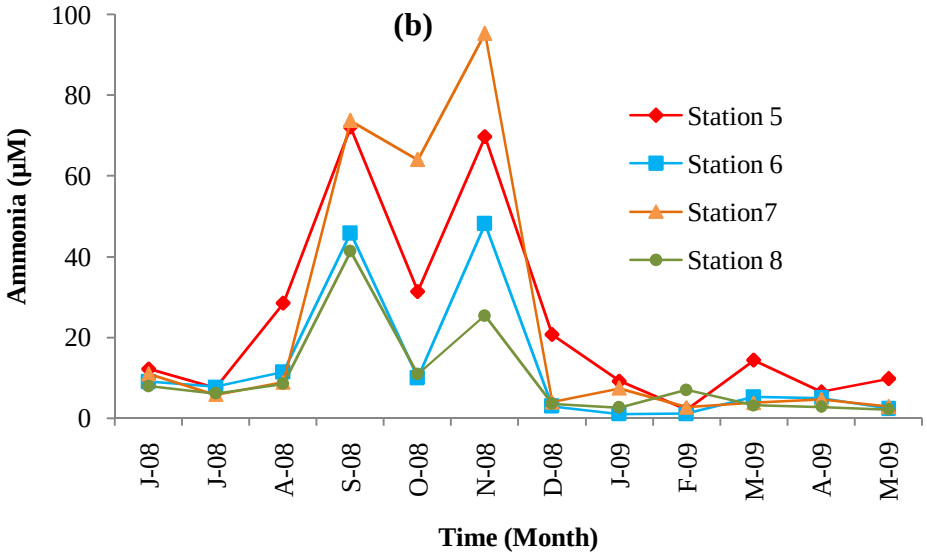

Figure 2.4.8 (b) Ammonia distribution in the southern arm

Seasonal variation in ammonia was significant with maximum during post monsoon from station 1 to 5 (Figure.2.4.8). During monsoon ammonia ranged from 11.4 to 30.1 µM with maximum at station 5 and minimum at station 1. During post monsoon the range was between 10.6 and 42.7 µM with maximum at station 7 and minimum at station 8 and during pre monsoon it was 3.4 to 14.2 µM with maximum at station 1and minimum at station 6.

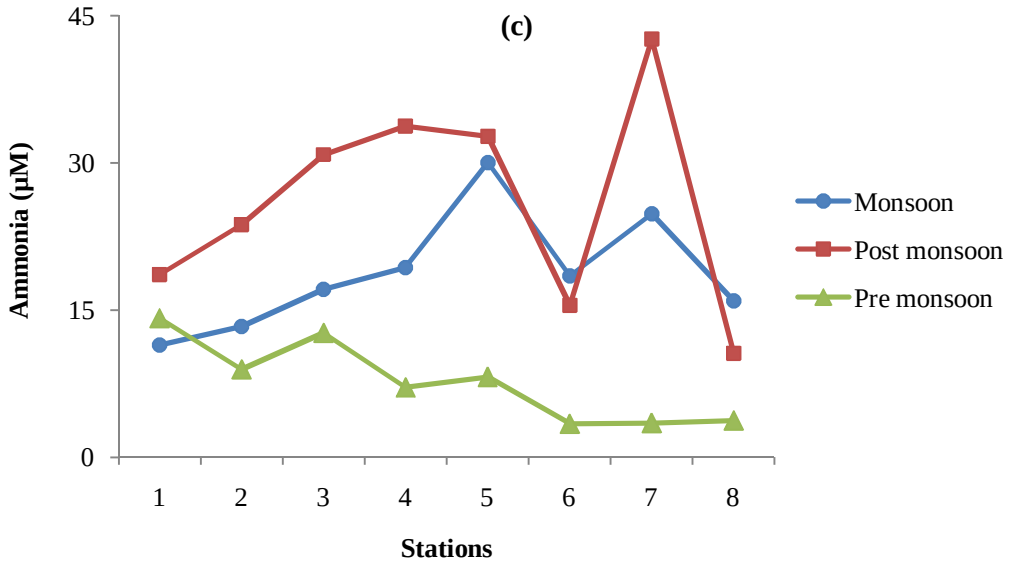

Figure 2.4.8 (c) Seasonal distribution of Ammonia in the study region

2.4.9 Phosphate (PO_4-P)

Spatial and temporal variation in phosphate showed only marginal variation. Phosphate distribution in the northern arm of the backwater ranged from 1.1 to 5.4 µM (avg. 2.02 µM). At station 1, the concentration was uniformly low during the study period. The maximum phosphate was observed during August at station 3 (Figure 2.4.9 a).

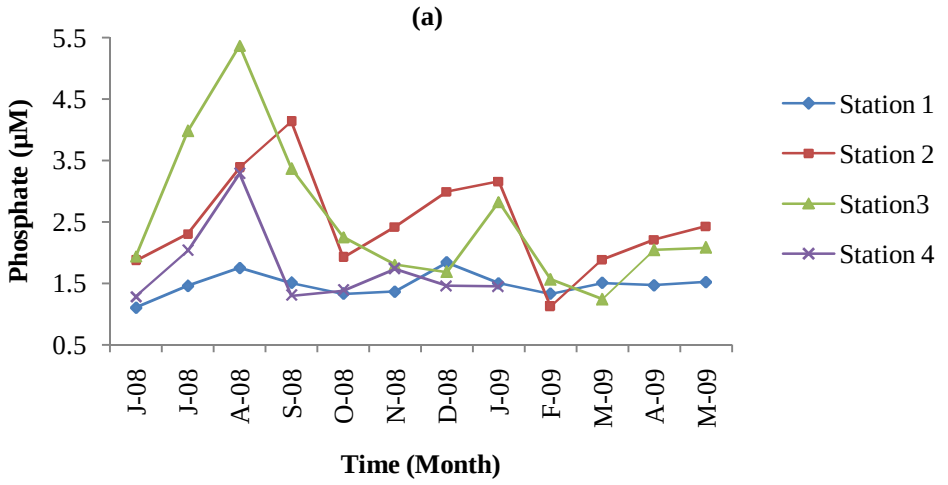

Figure 2.4.9 (a) Phosphate distribution in the northern arm

Phosphate in the southern arm of the study region did not show significant spatial and temporal variation and the range was between 1.07 and 2.0 µM (avg. 1.5 µM) with maximum at station 8 during September and minimum at station 5 during March (Figure 2.4.9 b). Phosphate value was never above 2 µM in the southern arm of the study region.

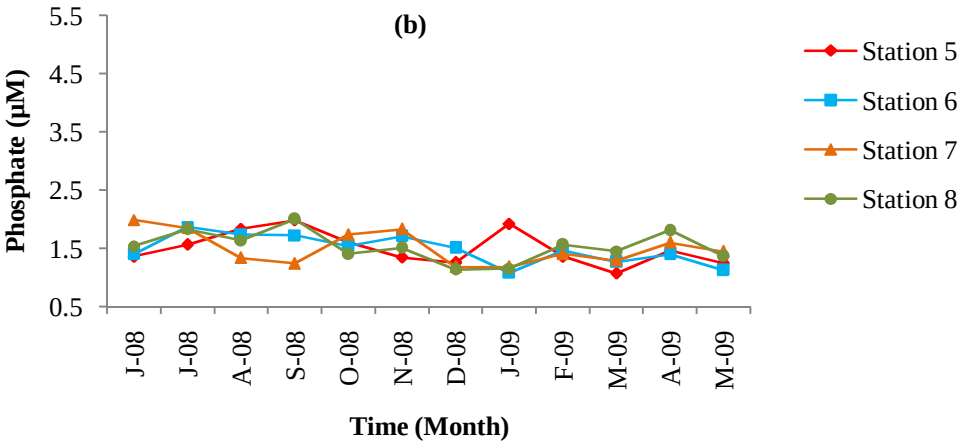

Figure 2.4.9 (b) Phosphate distribution in the southern arm

Seasonal variation in phosphate was marginal in the study region (Figure. 2.4.9 c) except at stations 2 and 3. During monsoon it ranged from 1.4 to 3.7 µM with maximum at station 3 and minimum at station 1, during post monsoon the range was from 1.3 to 2.6 µM with maximum at station 2 and minimum at station 8 and during pre monsoon it ranged from 1.3 to 2.0 µM, with maximum at station 2 and minimum at station 5. Phosphate content was high in the northern arm compared to in the southern arm. During all the three season phosphate values were relatively high at stations 2 and 3 compared to other stations.

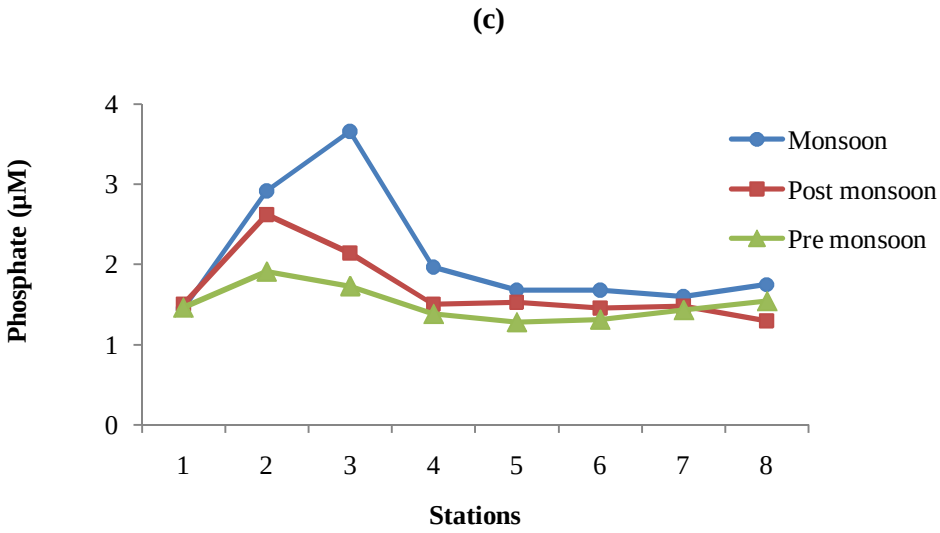

Figure 2.4.9 (c) Seasonal distribution of Phosphate in the study region

2.4.10 Silicate (SiO_4-Si)

Monthly variation of silicate showed noticeable differences. It was found that from July to September silicate was high at all the four stations along the northern arm of the study region (Figure. 2.4.10 a). Silicate range in the northern arm of the estuary was 5.3 to 95.3 µM (avg. 41.8 µM).

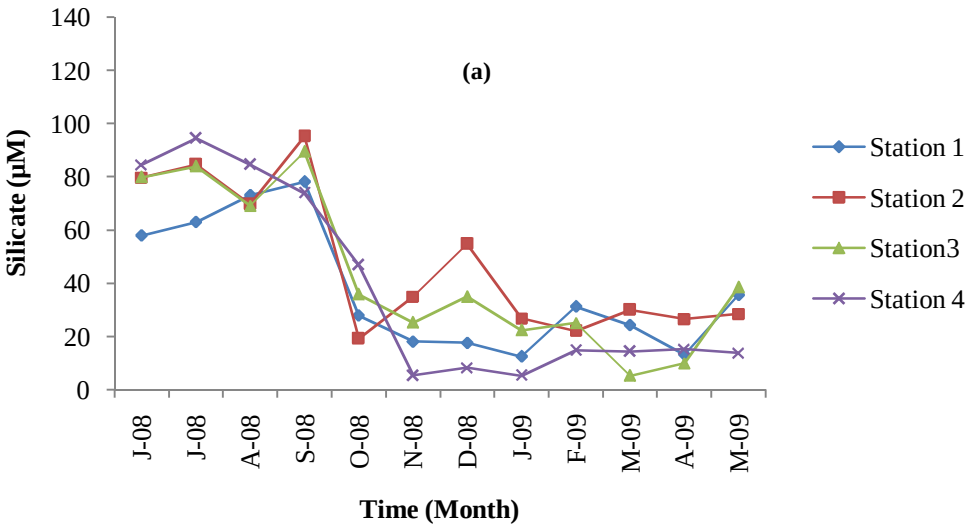

Figure 2.4.10 (a) Silicate distribution in the northern arm

Monthly variation of silicate in southern arm of the estuary ranged from 7.2 to 124.6 µM (avg. 63.0 µM) and high values were observed from June to September (> 80 µM) at all the station in the south (Figure. 2.4.10 b).

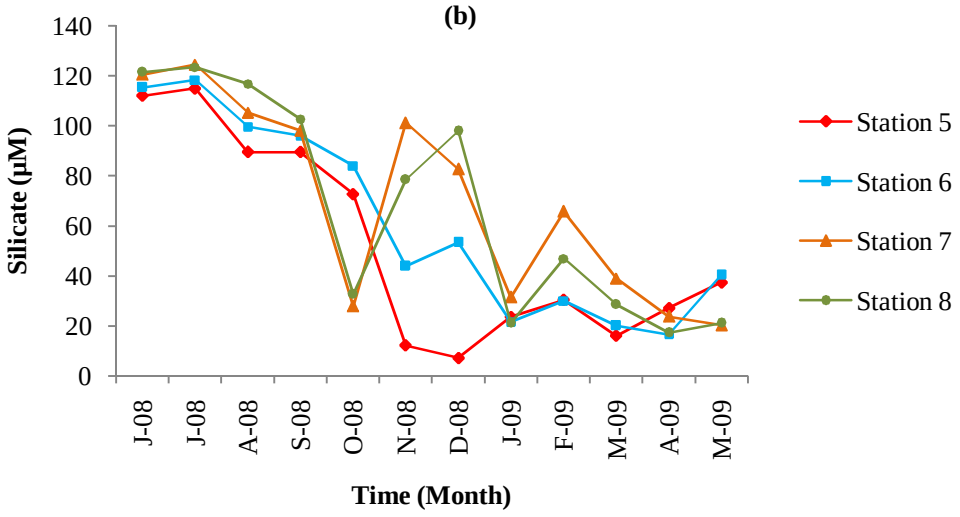

Figure 2.4.10 (b) Silicate distribution in the southern arm

Silicate content in the study region showed noticeable seasonality with high peaks during monsoon and low peak during pre monsoon (Figure 2.4. 10 c). During monsoon, the silicate concentration ranged from 68.0 to 116.1 µM with maximum at station 8 and minimum at station 1. During post monsoon the range was between 16.5 and 60.7 µM with maximum at station 7 and minimum at station 4 and during pre monsoon it was 14.6 to 37.2 µM with maximum at station 7 and minimum at station 4. Silicate content in the southern arm of the backwater was higher than that of the northern arm.

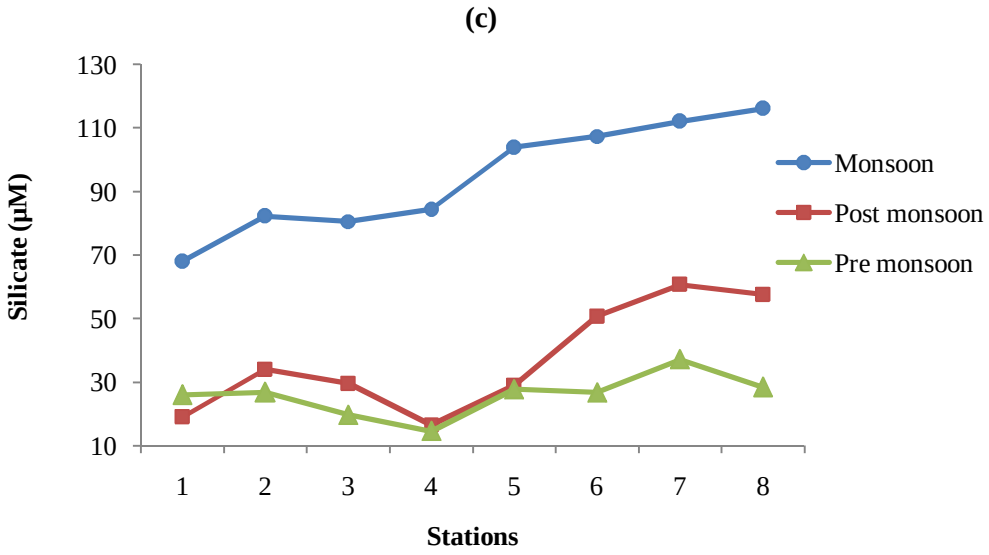

Figure 2.4.10 (c) Seasonal distribution of Silicate in the study region

2.5 Statistical analysis

2.5.1 Principal Component Analysis (PCA)

In the bi plot, the relative directions of the different physico-chemical variables denote the linear correlations among these variables. The location of the sampling stations inside the plot helps to understand which of the

physico-chemical characteristics are important in that location. Based on this, stations 6, 7 and 8 were found to be characterized by high temperature, DO, SPM, NO_3, NH_4 and SiO_4 and relatively low salinity, pH, NO_2 and PO_4 during monsoon season (Figure 2.5.1 a).

During the post monsoon season, NO_3, SiO_4 and DO were relatively high at stations 6 and 7. Parameters like NH_4, NO_2 and pH were relatively high at station 3, 4 and 5 (Figure 2.5.1 b).

During pre monsoon, stations 7 and 8 were characterized by relatively high DO, SPM, SiO_4 and NO_3 whereas salinity, pH and NH_4 were high at stations 4 and 5 (Figure 2.5.1 c).

From the PCA bi-plot it is clear that the northern estuary was more saline with relatively high pH, PO_4 and low SPM, DO and SiO_4. In the southern arm, the trends were just the opposite. This region remained low saline area with relatively low pH, PO_4 and high SPM, DO and SiO_4. PCA analysis thus indicated that these two regions are different with respect to the physico-chemical conditions.

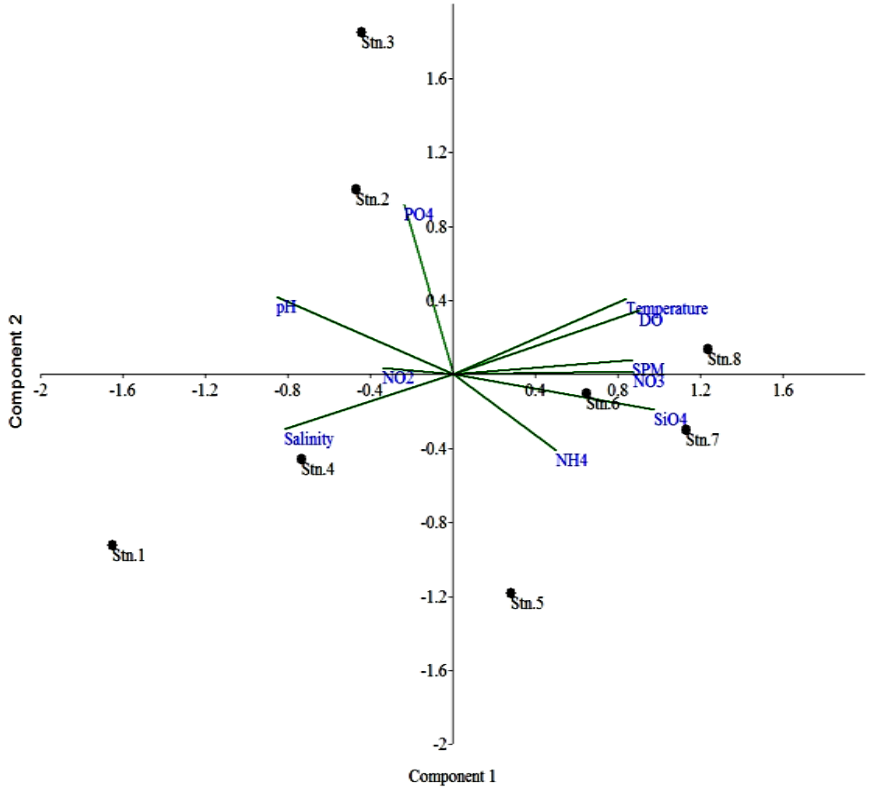

Figure 2.5.1 (a) PCA analysis on physical parameters during monsoon

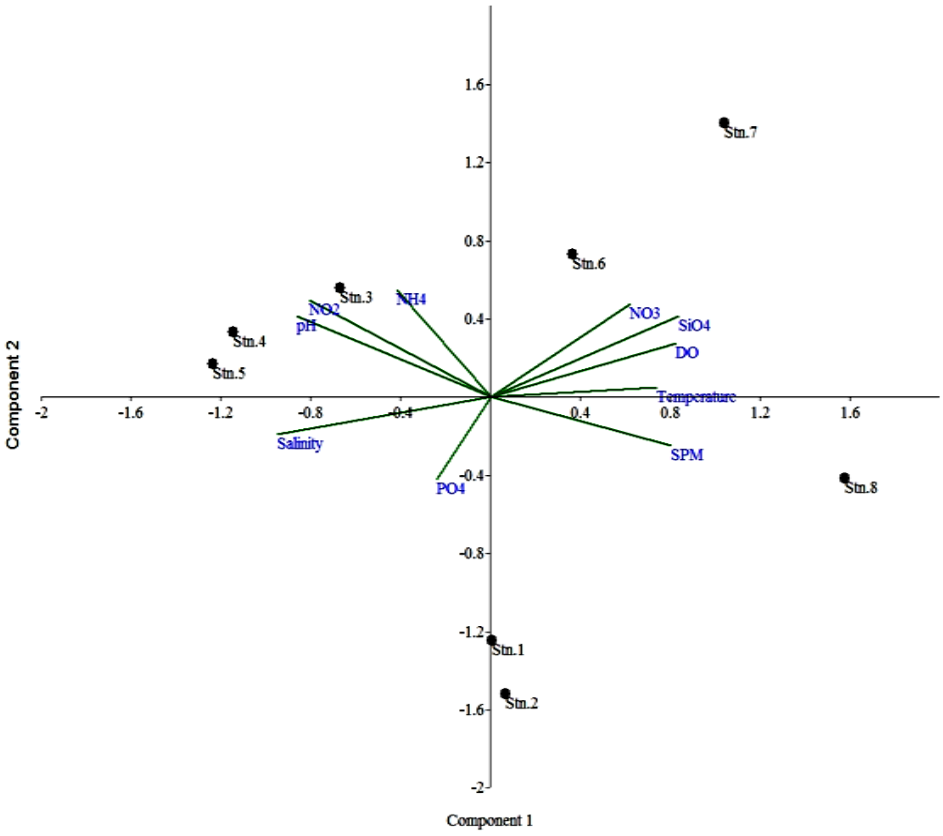

Figure 2.5.1 (b) PCA analysis on physical parameters during post monsoon

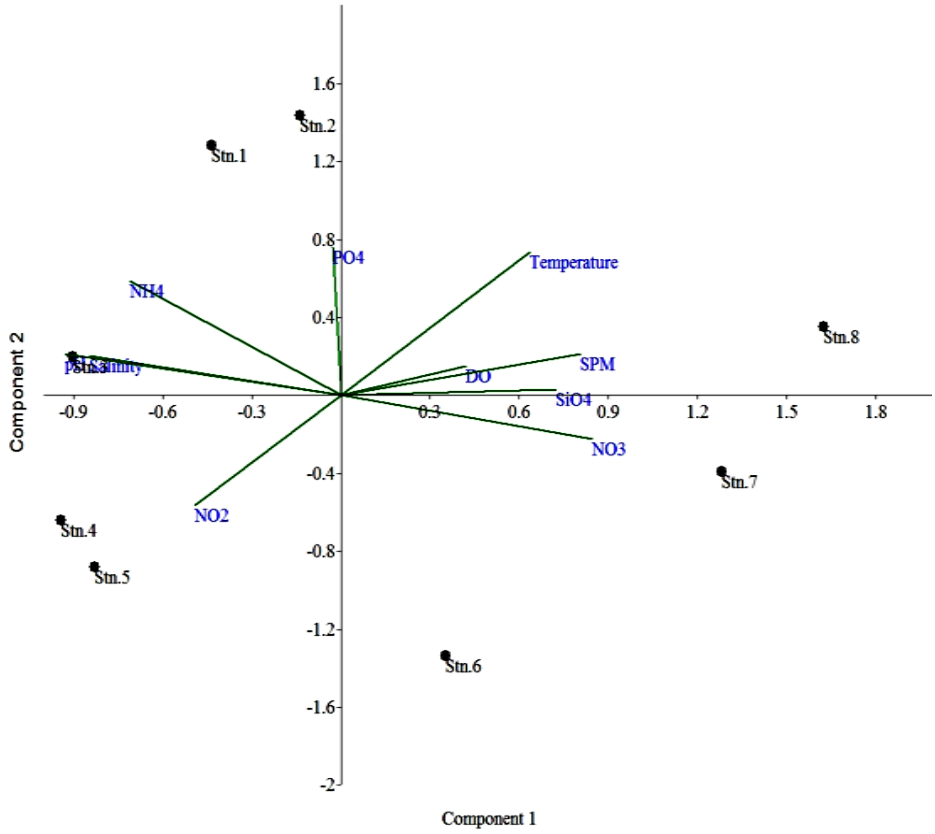

Figure 2.5.1 (c) PCA analysis on physical parameters during pre monsoon

2.5.2. Mann-Whitney U Test

Mann-Whitney U test was performed between the physico-chemical parameters of the northern and southern arms of the estuary for all the seasons. It was observed that there was significant difference between the two arms of the backwater with respect to physico-chemical parameters like salinity, SPM, pH, DO, PO_4 and SiO_4. Salinity, pH and PO_4 were relatively high in the northern arm, whereas SPM, DO and SiO_4 were high in southern arm (Table. 2.5.3).

Thus, the results from the PCA and Mann-Whitney U test showed that the northern and southern arms of the study region are environmentally different.

Table. 2.5.2 Mann-Whitney U test table

Mann Whitney Test	Salinity	SPM	pH	DO	PO_4	SiO_4
Column A vs Column B	Column A vs Column B					
P value	0.0175	0.047	0.0283	0.0302	0.0248	0.0391
Are medians significant different? (P<0.05)	Yes					
Exact or approximate P value?	Gaussian Approximation					
One or two tailed	One					
Sum of rank in Column A,B	187, 113	120.5, 179.5	183.5, 116.5	117, 183	184.5, 115.5	119, 181
Mann Whitney	35	42.5	38.5	39	37.5	41

2.6 Discussion

Salinity distribution in the study region showed well defined seasonal pattern with maximum salinity during pre monsoon and minimum during monsoon season. Northern and southern arm of the study region remained fresh water dominant during monsoon. During pre monsoon, they behaved differently as the northern arm high saline while southern arm remained as brackish (Figure 2.4.1 c). When fresh water discharge was minimum, the salinity increased to maximum values to create homogenous condition which remained undisturbed till the onset of south-west monsoon. During monsoon season, on the other hand, the backwater was subjected to considerable dilution by enormous volumes of fresh water discharged into it

by the seven rivers. Earlier studies made by (Ramamritham and Jayaraman 1963; Joseph *et. al.,* 1975; Udayavarma *et. al.,* 1981; Joseph and Kurup 1990; Balachandran *et. al.,* 2008; Martin *et. al.,* 2008; Revichandran *et. al.,* 2012 and Shivaprasad *et. al.,* 2013) have revealed that Cochin backwater experienced a well defined seasonal pattern in salinity. Salinity varied from time to time, depending on the fresh water influx and tidal activity in the backwater. Whereas during monsoon period the backwater experienced a considerable dilution of enormous fresh water discharges.

Reports from Kali, Mandovi and Zuari estuaries in the west coast, Godavari and Vellar estuaries in the east coast revealed more or less similar kind of salinity distribution pattern and observed low salinity during monsoon and high during pre monsoon. The contrast in salinity pattern in both the season was attributed to the riverine flow of fresh water which was minimal during pre monsoon and maximal during monsoon in Kali estuary (Menon and Neelakanta 1992); in Mandovi and Zuari estuaries (Shetye, 1999); in Godavari estuary (Sarma *et. al.,* 2009 and 2010); and in Vellar estuary (Santhanam and Perumal 2003; Rajasegar, 2003).

Temperature generally showed an annual fluctuation in the study region (Figure 2.4.2 a & b). A decrease of temperature 3 - 4º C was observed at stations 3 and 4 and 2 - 3º C decrease at stations 5 and 6 during January. The entire backwater was relatively warm during March and April. Ramamritham and Jayaraman (1963) reported maximum surface temperature during April (32°C) and minimum during July (29° C). Seasonal distribution of temperature revealed that pre monsoon was warmer compared to monsoon

and post monsoon. But there was no noticeable difference in temperature between post and pre monsoon (Figure 2.4.2. c). According to (Sankaranarayanan and Qasim 1969), the decrease in surface temperature during monsoon was caused not only by the influx of cold and fresh water into the backwater, but also by the incursion of cold water from the Arabian sea. Nair and Tranter (1971) have reported that the temperature does not vary much between pre and post monsoon seasons even though there are diurnal variations. Menon *et. al.,* (1971), reported that with the onset of monsoon, there was a decrease in surface temperature and a certain amount of uniformity was maintained in temperature till the end of the monsoon. Since the Cochin backwater is geographically located in the tropical region, there are only minor seasonal variations in temperature (Madhupratap, 1987).

The major sources of SPM in the open waters are clay and silt (erosion), re-suspension, organic detritus, dead plants and animal materials. Dredging operations have also increased the suspended load in water column. SPM was found to be high during monsoon (July to September) and low during pre monsoon (February to May). SPM was comparatively high in the southern arm of the estuary than in northern arm (Figure 2.4.3 a, b & c). During the monsoon period water was turbid due to suspended particulate matter (Joseph, 1974). Saraladevi *et. al.,* (1989) also reported that during monsoon, the backwater was heavily loaded with suspended particulate matter and least during pre monsoon. SPM was also found to be maximum during monsoon months and minimum during pre monsoon in Godavari estuary (Sarma *et. al.,* 2009 and 2010).

The pH range expected for normal seawater is 8 - 8.3 and that for coastal water 7.9 - 8.2. It varies widely in backwater due to influx of fresh water and intrusion of sea water. In the present study, pH was relatively low (avg. 7.24) in the southern arm of the study region when compared to the northern arm (Figure 2.4.4 a & b). This may be due to fresh water influx from five rivers (Pamba, Meenachil, Achankovil, Manimala and Muvattupuzha). Martin *et. al.,* (2008) reported that during monsoon the river discharge lowers the pH to below 6 and during pre monsoon when the river discharge is the least, pH is recovered.

Concentration of dissolved oxygen (DO) was more or less uniform in both arms of the backwater (Figure 2.4.5 a & b). Ramamritham *et. al.,* (1986) observed uniformity in DO values in the Cochin backwater. DO was marginally high during monsoon than during pre monsoon in the study region. Marginal differences were found between post monsoon and monsoon in DO (Kumaran and Rao, 1975). Madhu *et. al.,* (2007) also observed relatively low DO (avg. 5.8 ± 0.9 ml L^{-1}) during pre monsoon season. Saraladevi *et.al.,* (1991) found that DO was low during pre monsoon months and the range was between 2 to 6 ml L^{-1}. Similarly in Vellar estuary also dissolved oxygen was lower during monsoon and post monsoon values and higher during summer seasons due to the gradual saline water incursion and increasing temperature (Santhanam and Perumal 2003; and Rajesegar, 2003).

Nutrients, also known as the bio stimulants, are usually represented by nitrate, phosphate and silicate, which are utilized by photosynthetic organisms to form organic matter. The availability of the nutrients determines the growth

of phytoplankton. The available sources of inorganic nitrogen, in an aquatic environment are nitrate, nitrite and ammonia. These are formed as part of the nitrogen cycle, either by oxidation of ammonia or by reduction of nitrate to nitrite and ammonia. The latter process has been referred to as denitrification and has been found to occur both in the water column and sediment, at low oxygen concentrations (Vaccaro, 1965).

Nitrite did not show any seasonality, whereas nitrate showed significant seasonality with peak during monsoon (Figure 2.4.7 c). The seasonal variability of nitrate in the backwater was in accordance with the fresh water discharge. High values were associated with the early monsoon when the fresh water influx was maximum but it was also found that further increase of freshwater discharge did not increase nitrate concentration (Sankaranarayanan and Qasim 1969; Saraladevi *et. al.,* 1991and Martin *et.al.,* 2008). The high values of nitrate during monsoon season may be due to increased fresh water influx into the backwater (Saraladevi *et. al.,* 1991; Martin *et. al.,* 2008). Nitrate showed high values (20 µM) in the Mandovi and Zuari estuaries during monsoon when the rainfall and runoff were maximum and showed minimum values (7 µM) during pre monsoon when the rain and runoff were minimal (Unnikrishnan *et. al.,* 1997 and Shetye, 1999). Similarly in Rushikulya, Godavari and Vellar estuaries also high values of nitrate were reported during monsoon and low values during pre monsoon (Gouda and Panigrahy 1995; Bandyopadhyay *et. al.,* 1994; Sarma *et. al.,* 2009 and 2010; Senthilkumar *et.al.,* 2002; Santhanam and Perumal 2003).

The seasonal variability of ammonia in the study region revealed that during post monsoon and monsoon seasons the ammonium content was high compared to pre monsoon (Figure 2.4.8 c). Madhu *et. al.,* (2007) reported that ammonia showed a sharp increase during monsoon (avg. 47.8 ± 26.5 µM) and post monsoon (avg. 55.2 ± 51.8 µM). Martin *et. al.,* (2008) have reported that greater values of ammonia were always associated with freshwater flow.

Seasonal cycle of the inorganic phosphorus (Figure 2.4.9 c), showed that during the period when homogenous conditions prevailed, i.e from January to April, phosphate values were low. Towards May - June, when the pre monsoon and monsoon showers set in, the phosphate values attained the first peak. This probably indicates that the phosphate contribution to the estuary is largely dependent upon external sources such as land drainage and fresh water runoff. Sankaranarayan and Qasim (1969) observed very high phosphate values during June and July and very low phosphate content during pre monsoon period. They indicated that in addition to the enrichment of phosphate by run-off and land drainage, there may be some other mechanisms for its enrichment in this estuary. Phosphate values were always high from stations 1 to 4 (northern arm) regardless of the season (Figure 2.4. 9 c). This is probably because the stations in the northern arm are in and around the industrial region. This result was in concurrence with the report of (Qasim, 2003) that high phosphate content was from the sources of industrial discharges from various factories situated along the banks of Periyar River which empty into the northern arm of the Cochin backwater.

The silicate values remained low from December to April, but increased during the monsoon (June to September) months (Figure 2.4.10 c). The silicate-Si cycle is entirely dependent upon the freshwater discharge. Maximum value was recorded during July – August, which indicates that the silicate is associated with the heavy silt load of the estuary. Joseph (1974) observed decrease in silicate values in the Cochin backwater during pre monsoon which can be attributed to the reduction in silt present in the river matter. A two fold increase in silicate (>90 µM) during monsoon and post monsoon compared to pre monsoon (avg. 44 µM) was observed by (Madhu *et. al.,* 2007). Silicate was higher than that of the other macro nutrients and the higher concentration (140.5 µM) was during monsoon may be due to heavy inflow of fresh water (Gouda and Panigrahy 1995; Bandyopadhyay *et. al.,* 1994; Sarma *et. al.,* 2009 and 2010; Senthilkumar *et.al.,* 2002; Santhanam and Perumal 2003).

Results of the PCA bi- plot and Mann Whitney U test indicated that the northern and southern arms of the study region behaved differently. The northern arm of the study region was more saline with relatively high pH and PO_4 values and with low SPM, DO and SiO_4. This was probably due to the marine condition that prevailed during most of the time except during the monsoon season. High values of PO_4 observed in the northern arm were mainly due to non-point (local) sources such as industrial effluents and domestic sewages. The industrial development and intensification of agricultural practices have considerably accelerated the eutrophication (Balachandran 2001; Martin *et. al.,* 2008). In the case of SPM, DO and SiO_4,

low values indicate the absence of point sources like shoreline erosion, river runoff and re-suspended bottom sediments were minimal in northern region.

But in the southern arm the trend was just the opposite with low salinity low pH and PO_4 with high SPM, DO and SiO_4. Low salinity and pH in the southern arm was observed because this region is brackish water during most of the period due to high river discharge and low salinity incursion. PO_4 was found to be low because there were no non-point sources in this region, whereas SPM and SiO_4 were high in this region due to silt discharge from the five rivers.

In the present study the hydrographic features of the northern and southern arms of the backwater have been compared and it was observed that the two arms of the backwater are characteristically different. This variation will have a major role to play on the phytoplankton distribution, community structure and growth.

Chapter 3

Variation in the Phytoplankton Biomass, Abundance and Composition

3.1. Introduction

Phytoplankton are photosynthesizing microscopic organisms found in sunlit layer of almost all oceans and bodies of fresh water. They are agents of "organic production," from carbon dioxide in presence of sunlight, a process that sustains the aquatic food web (Ghosal *et. al.,* 2011). Phytoplankton obtain energy through the process of photosynthesis in the well-lit surface layer (termed the “euphotic zone”) of an ocean, sea, lake, or other bodies of water. Phytoplankton accounts for half of all photosynthetic activity on Earth (NASA, 2009) and are responsible in balancing the oxygen content in the Earth's atmosphere (NASA, 2005). Marine phytoplankton constitutes less than 1% of Earth’s photosynthetic biomass, yet they are responsible for more than 45% of our planet’s annual net primary production (Field *et. al.,* 1998). Their evolutionary trajectories have shaped trophic dynamics and are capable of altering the global biogeochemical cycles (Katz *et. al*., 2004). The high efficiency in transferring solar energy into tertiary trophic level via photosynthesis indicates there in the overall structure and function of marine pelagic ecosystems (Nixon, 1988). The primary producers have always been at the base of the food web.

Phytoplankton biomass, distribution and species composition do undergo variations with change in the light, temperature, tide, waves, grazing pressure, and even with time of day (Hsiao, 1992). Therefore, the phytoplankton dynamics can reveal the functioning of a particular ecosystem and how they respond to environmental changes (Patil and Anil 2011).

Size structure of phytoplankton is very important as it changes with the environmental conditions, affecting food chain (Legendre and Le Fevre 1988). The scaling system and nomenclature of (Sieburth *et. al.,* 1978) has been widely adopted in phytoplankton ecology to distinguish functional separations within the phytoplankton. The classification of phytoplankton according (Sieburth *et. al.,* 1978) is as follows:

Plankton	Size (µm)
Microplankton	**20-200**
	50-500
	60-500
Nanoplankton	**2-20**
	5-50
	5-60
	<45
	<100
	<64
	15-64
Ultra plankton	<5
	0.5-5
	1-10
	1-15
	<15
Pico plankton	**0.2-2**
Femto plankton	0.02-0.2

Phytoplankters are the primary producers of the pelagic marine ecosystems capable of indicating long-term ecological changes in the environment. Endogenous rhythms also affect the diel distribution patterns of phytoplankton (Sournia, 1974; Nelson and Brand 1979; Kamykowski, 1981; Cullen and Horrigan 1981; Kana *et. al.,* 1985; Demers *et. al.,* 1986 and Hsiao,1992).

Although the oceans cover 70% of the Earth's surface, our knowledge of diversity patterns in marine phytoplankton are very limited compared to that of plants in the terrestrial world. Similar to terrestrial vegetation, marine phytoplankton diversity is a unimodal function of phytoplankton biomass, with maximum diversity at intermediate levels and minimum diversity during massive blooms. Several studies have shown that biodiversity and production decrease due anthropogenic activities (Lehman and Smith 1991: Turner and Rabalais 1994: Lehman, 2000: and Huang et. al. 2004). The dominant phytoplankton classes in the present study region are Bacillariophyceae (Diatoms), Dinophyceae (Dinoflagellates), Chlorophyceae (green algae) and Cyanophyceae (blue green algae).

Bacillariophyceae (Diatoms)

Diatoms form a major group of algae, with a cell size ranging from 2 to 200 µm and are covered by siliceous frustules (Horner, 2002). This siliceous wall can be highly patterned with a variety of pores, ribs, minute spines, marginal ridges and elevation; all of which can be utilized to delineate the genus and species. The external morphology of diatoms is

based on the solid silica shell or frustules in common. It is this shell that is used in species identification and comparison. All diatom skeletons are made of silica and consist of two parts or frustules that fit inside each other like a petri dish: the epitheca and the hypotheca (Alexopoulos, 1967). The hypotheca is smaller and fits inside the larger epitheca. The shapes of the frustules are the defining feature that is used to break the diatoms into two distinct classes: the centric or Centro bacillariophyceae and the pennate or Pennati bacillariophyceae. The centric diatoms are usually radially symmetrical while the pennate diatoms are generally bilaterally symmetrical (Alexopoulos, 1967). These two classes can be found in both marine and freshwater habitats, but centric diatoms are more likely found in the oceans while the pennate diatoms are predominately found in freshwater (Round, 1990).

Tiffany (1968) writes that marine diatoms are considered "grass of the sea". Another important use of diatoms in the biological realm is in water quality testing. Research by (Dixit *et. al.,* 1999) showed that diatoms can not only be used for determining the present water quality but also to determine the former water quality and trends over the years. The sediments of lakes and rivers hold chemical and biological clues to the environment and water quality of the past and present. Diatoms are one of these clues. Because diatoms are ecologically diverse in almost every freshwater habitat, the dead and living diatoms can be found in the substrate. Diatoms in the first centimeter represent the current condition of the water, while the diatoms found in deeper sediment are representative of past water quality. The high reproductive rate of diatoms makes them

respond quickly to environmental changes and many diatom species, as well, have specific tolerances for water quality. An important result of this research is that diatoms can be used to determine former water quality. This means that pre-colonial water quality can be estimated and used as a baseline to work from in determining anthropogenic effects on water quality. Diatoms help biologists see trends from past to present based on the sheer number, diversity and tolerance of diatoms in the sediment (Dixit *et. al.,* 1999).

Dinophyceae (Dinoflagellates)

Dinoflagellates are a large group of flagellate protists. Dinoflagellates are unicellular forms with one to three flagellae. Usually, they possess two flagellae: one which extends towards the posterior, called the longitudinal flagellum, and the other forming a lateral circle, called the transverse flagellum. In many forms, these are set into grooves, called the sulcus and cingulum. The transverse flagellum is ribbon-like and coiled, provides most of the force propelling the cell, and often imparts to it a distinctive whirling motion, which is what gives them their name. The longitudinal flagellum acts mainly as a rudder, but provides a small amount of propulsive force, as well. Many reviews (Fenson *et. al.,* 1993; Spector, 1984; Taylor, 1987 and Edward, 1993) have been written on dinoflagellates that are mostly marine plankton, but are also common in freshwater habitats, as well. Many dinoflagellates are known to be photosynthetic, but a large fraction of these are in fact mixotrophic, combining photosynthesis with ingestion of prey (Stoecker, 1999).

Dinoflagellates are the largest group of marine eukaryotes aside from the diatoms. Being primary producers makes them an important part of the aquatic food chain. Some species, called zooxanthellae, are endosymbionts of marine animals and play an important part in the biology of coral reefs. Other dinoflagellates are colourless predators on other protozoa, and a few forms are parasitic. Dinoflagellates produce resting stages, called dinoflagellate cysts or dinocysts, as part of their life cycles. Dinoflagellates are considered to be protists, with their own division, Dinoflagellata. About 1,555 species of free-living marine dinoflagellates are currently described (Gomez, 2005). Another estimate suggests ca. 2000 living species, of which more than 1700 are marine and about 220 are from freshwater (Taylor *et. al.,* 2008). The latest estimates suggest a total of 2,294 living dinoflagellate species, which includes marine, freshwater and parasitic dinoflagellates (Gomez, 2012). An algal bloom of dinoflagellates can result in a visible coloration of the water colloquially known as red tide. Red tides can have harmful effects and certain species of dinoflagellates produce potent toxins. These toxins are carried up in the food chain, ultimately to humans and can, sometimes result in permanent neurological damage or even death (Fukuyo, 1981).

Dinoflagellates are protists which have been classified using both the International Code of Botanical Nomenclature (ICBN) and the International Code of Zoological Nomenclature (ICZN). Approximately half of living dinoflagellate species are autotrophs possessing chloroplasts and half are non-photosynthesising heterotrophs. It is now widely accepted that the ICBN should be used for their classification.

Dinoflagellates include 130 genera with about 2000 living and 2000 fossil species (Van den Hoek *et. al.,* 1995). Their cell covering structure known as thecca, differentiates them from other algal groups. Cells are either armoured or unarmoured, and the former have thecae divided into plates composed of cellulose or polysaccharides. The theca may be smooth and simple or laced with spines, pores and/or grooves and may be variously ornamented. Dinoflagellates share features common to both plants and animals: they can swim, many have cell walls, and both photosynthetic and heterotrophic species are known.

Chlorophyceae (Green Algae)

Chlorophyceae (from the Greek word chloros, meaning “green”) make up an extremely large and important class of **green algae**. Members may be unicellular, colonial, or filamentous. Cells of unicellular and colonial chlorophyceans may have two or more flagella.

There are about 2,650 living species of chlorophyceans. These include about 500 genera and approximately 8000 species (Van Den Hoek, *et. al.,* 1995). Most of them are prone to freshwater habitat and many are reported to thrive well in marine and terrestrial environments. Chlorophyceae are one of the classes of green algae, distinguished mainly on the basis of ultra structural morphology. The chloroplast may be discoid, plate-like, reticulate, cup-shaped, spiral or ribbon shaped in different species. Most of the members have one or more storage bodies called Pyrenoids located in the chloroplast. Pyrenoids contain protein besides starch. Some algae may store food in the form of oil droplets.

Green algae usually have a rigid cell wall made up of an inner layer of cellulose and outer layer of pectose. Green algae are the major primary producers of the freshwater ecosystems. In estuarine systems, they are frequently distributed during the monsoon periods and provide high biomass and productivity.

Cyanophyceae (Blue Green Algae)

Cyanobacteria are prokaryotic unicellular and colonial species. Colonies may form filaments, sheets or even hollow balls. Cyanobacteria are arguably the most successful group of microorganisms on earth. They are the most genetically diverse; they occupy a broad range of habitats across all latitudes, widespread in freshwater, marine and terrestrial ecosystems, and they are found in the most extreme niches such as hot springs, salt works, and hypersaline bays. Cyanophyceae, contains about 150 genera and 2000 species, found in most diverse habitats, in freshwater and in the sea; on damp soil, glaciers, deserts and can grow over a wide range of temperatures such as hot springs. The species can also occur as symbionts of protozoa, diatoms and lichen-forming fungi, and vascular plants. Considerable proportion of marine phytoplankton consists of blue-green algae, particularly in picoplankton (0.2 to 2µm). Coccoid blue-green algae appear to be everywhere in temperate and tropical parts of the ocean and even be the main contributors to photosynthetic primary production (Fogg, 1987). They are most abundant in nutrient rich coastal and estuarine waters and occur together with diatoms and dinoflagellates. They are also found in oligotrohic parts of

tropical and subtropical seas. *Trichodesmium* sp. can often form extensive blooms in tropical and sub-tropical oceans and are visible as orange-brown wind rows on the surface of water. This species is capable of fixing atmospheric nitrogen and probably the most important biological fixer of nitrogen in the open ocean. Cyanophytes have a capacity to change their colour in relation to the wavelength of the incident light. Very often characteristic blue or red colouration is imparted to the marine environment when the bloom of blue green algae appear consequent to eutrophication. Several species of blue green algae produce toxins, which may be either neurotoxic or hepatotoxic.

3.2. Review of Literature

Venkataraman (1939) made the systematic account on both fresh and estuarine diatoms in and around Madras coast, with taxonomic description. Subrahmanyan *et. al.,* (1946, 1958, 1959, 1960 & 1965) were the pioneers in the phytoplankton taxonomy, and made outstanding contributions from the Indian inshore seas. Subrahmanyan *et. al.,* (1946) described over 500 species of the phytoplankton forms of all groups together representing over 150 genera from both coasts of India. In 1946, he described 171 forms of marine planktonic diatoms from Madras coast, representing 15 families, 64 genera and 134 species. Iyengar and Venkataraman (1951) studied the ecology and seasonal succession of the diatom flora of the estuarine waters of India. They studied the estuarine parts of the Cooum river near Madras coast.

Desikachary (1959) published a monograph on Cyanophyta. Nair (1959) studied the marine planktonic diatoms of Trivandrum coast quite extensively. Gopinathan (1972) made the qualitative and quantitative estimation of phytoplankton of the Cochin backwater and described 120 species. Devassy and Bhattathiri (1974) studied the phytoplankton ecology in the Cochin estuary. Vijayalakshmi and Venugopalan (1975) studied the diversity of phytoplankton species, pigments and succession with a note on primary production at tidal zone in the Velar estuary, East coast of India. Joseph and Pillai (1975) studied the seasonal and spatial distribution of phytoplankton in Cochin backwater.

Kumaran and Rao (1975) studied the seasonal fluctuations in the abundance of phytoplankton in Cochin backwaters. Gopinathan (1975 a) described an account of the estuarine diatoms present in various estuarine systems in India, their occurrence, seasonal fluctuations and distribution, particularly from Cochin backwater. Gopinathan (1975 b), described some new distributional records of planktonic diatoms from the Indian seas.

Thresiamma and Nair (1980) investigated the qualitative and quantitative analysis of planktonic diatoms of Vizhinjam, the southwest coast of India. Pant *et. al.,* (1980) studied the contribution of phytoplankton photosynthesis in a mangrove ecosystem. Desikachary and his colleagues, (1986, 1987 a, b and c, 1988, 1989) made massive contribution to the field of algology in India. Their Atlases of diatoms are considered as the most valuable reference books worldwide. Jayalakshmi *et. al.,* (1986) studied the seasonal distribution of phytoplankton in Cochin

backwaters. Santra *et. al.,* (1989) studied the seasonal fluctuations of phytoplankton in Bhagirathi-Hoogly estuary, in West Bengal. Rasheed *et. al.,* (2000) estimated the photosynthetic pigments at the dredged and non-dredged sites in the Cochin harbour and observed that the higher concentration of chlorophyll *a* in the bottom is indicative of the detachment of benthic flora from the sediments due to dredging. Seasonal variation of phytoplankton abundance and productivity were studied in the surf zone of the sea at Cochin backwaters with reference to cell counts, chlorophyll *a*, photosynthesis and hydrographic parameters by (Selvaraj *et. al.,* 2003). Sheeba and Ramanujan (2003) identified 38 genera of phytoplankton together with physicochemical parameters of water at monthly intervals in the estuarine environment of Ithikkara River, southern Kerala. Geetha and Kondalarao (2004) carried out the qualitative and quantitative distribution of phytoplankton with regional and seasonal variations in the coastal waters of east coast of India. The study revealed that the dinoflagellates were the dominant taxa with 131 species, followed by 111 species of diatoms and 7 species of cyanophytes. Renjith *et. al.,* (2004) studied the primary production at selected stations in Cochin Estuary and showed a tri modal annual variation with peaks during April, July and November. Maximum production was in November and minimum in June.

Joy *et. al.,* (1990) studied the effect of industrial discharges on the ecology of phytoplankton production in the river Periyar. Madhu *et. al.,* (2007 and 2010) studied on phytoplankton abundance and seasonal variations in Cochin estuary and concluded that salinity is the major abiotic factor that controls the distribution and diversity in Cochin estuary. Martin

et. al., (2012) studied the impact of eutrophication on the occurrences of *Trichodesmium* in the Cochin backwater.

Malone (1980), Raimbault *et. al*., (1988), Legendre and Le Fevre (1989), Amstrong (1994), Chisholm (1992), Riegman *et. al.,* (1993), Tang (1995), Shiomoto (1997), Cermeno *et. al.,* (2006) and Jyothibabu *et. al.,* (2006) have explained that the size structure of phytoplankton is very important as it changes with the environmental conditions reflecting the pathways of carbon cycling in the pelagic food chain. They also reported that large sized phytoplankton has greater potential to export organic matter through a short, classical food chain, whereas the small-sized phytoplankton is utilized by complex microbial food webs that favour the recycling of organic matter. Earlier studies have also confirmed that small sized phytoplankton is an integral component of the plankton community, although, their relative contribution to the total community varies with the abundance of large sized phytoplankton. Generally nutrient enrichment favors the growth of large phytoplankton while the production of small phytoplankton (nano) is mainly controlled by microzooplankton (cilites and flagellates) grazing. It is known that those nanoplanktons are more efficient in using low light intensities and ambient nutrient concentrations for photosynthesis than larger counterparts. Earlier studies made (Qasim *et. al.,* 1974; Madhu *et. al.,* 2007 and 2010) have shown that nanoplankton contribute greatly to the total Chlorophyll *a*.

Similar kind of studies made by (Ragathaman and Rao 1977) reported that in Adayar estuary in east coast the predominant species

found are *S.costatum, C. meneghiniana, A. coefeaeformis, N. halophila, N. closterium* and *A. japonica*. Gouda and Panigrahy (1989) reported salinity and dissolved oxygen remained high during the bloom period. Senthilkumar and Sivakumar (2008) studied the phytoplankton diversity in response to abiotic factors in Veeranam Lake Tamil Nadu. Perumal *et. al.,* (2009) studied the seasonal variations of phytoplankton diversity in the Kaduviyar estuary, Nagapattinam, southwest coast of India.

Bhargava and Diwedi (1974 and 1976) studied the seasonal distribution of phytoplankton pigments in the estuarine system of Goa. Devassy and Goes (1989) reported the seasonal pattern of phytoplankton biomass and productivity in Mandovi-Zuari estuaries. Krishnakumari and John (2003) studied the biomass and quantitative indices of phytoplankton in Mandovi-Zuari estuaries. Mitbavkar *et. al.,* (2007) reported the picophytoplankton community in Goa waters. Madondkar *et. al.,* (2007) studied the phytoplankton diversity, biomass and production. D'Silva *et. al.,* (2008) tracked the history of dinoflagellate distribution in Goa coast. Patil and Anil (2011) studied the variations in phytoplankton community in Mandovi and Zuari estuaries.

3.3. Material and Methodology

3.3.1. Estimation of phytoplankton biomass (chlorophyll *a*):

For the estimation of chlorophyll a,250 ml of surface water was filtered through GF/F filters (pore size 0.7 mm), extracted with 90% acetone for 24 h in the dark and the extinction was measured in a spectrophotometer (Model -UV 1650 PC, Shimadzu) before and after

acidification. For size fractionationed phytoplankton biomass (chlorophyll *a*) was measured by sequential filtering of 500 ml water, initially through 20 µm nylon sieve and subsequently through 2 µm and 0.2 µm polycarbonate filters. The cells retained by the 20 µm sieve are the microplankton, whereas those retained by 2 and 0.2 µm filters constitute the nano- and pico fractions, respectively. After filtration, pigments were extracted in 90% acetone for 24 h in the dark at 4°C and the pigment concentration was determined spectrophotometrically (UV-VIS Spectrophotometer, Model – 1650 PC, Shimadzu) according to standard protocol (Parsons, 1984).

3.3.2. Estimation of phytoplankton abundance using Sedgewick-Rafter Counting Cell

One litre of sea water sample was fixed in Lugol's iodine and kept for sedimentation for 48 h. The samples were then concentrated to 10 ml and 1 ml of each subsample was counted under inverted microscope (Olympus IX 51) and identified up to species level (Subramanian 1946; Desikachary 1959; Gopinathan 1984; Tomas *et. al.,* 1997). The number of phytoplankton present in all the thousand grids was counted. Repeated the counting for three times and took the average. The total number of planktonic algal species present in one litre of water sample was calculated using the formula (Subramanian 1946).

N= n * v/V

Where,

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

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

v = volume of plankton concentrate in ml.

V = total volume of water filtered in litre

3.3.3 Statistical Analysis

3.3.3.1 Pearson correlation

Pearson correlation was calculated using SPSS version 10. The most common measure of predictability is Person's Coefficient, which can have a value anywhere between -1 and 1. Pearson correlation was performed to analyze the relation between various measured variables (two-tailed significance assumed at P<0.05 and P<0.01).The equation used in the software for calculation was:

$$r = \frac{\sum_i (x_i - \overline{x})(y_i - \overline{y})}{\sqrt{\sum_i (x_i - \overline{x})^2} \sqrt{\sum_i (y_i - \overline{y})^2}}$$

3.3.3.2 Principal Component Analysis

Principal component analysis (PCA) was carried out using the statistical programme PAST version 2.02, to understand the interrelationships between the abiotic variables during different seasons. Analysis was also done between biotic and abiotic parameters.

3.3.3.3 Mann-Whitney test

For each biotic and abiotic variables, the Mann-Whitney U test was performed for northern and southern estuary during all seasons to understand the difference between these two regions. The statistical software Graph Pad Prism (version 5.01) was used for this purpose.

3.3.3.4 Species Diversity Index

Species diversity was computed as Shannon diversity index (H') using PRIMER version 5.2.8 (Clark and Warwick, 1994). The Shannon diversity index is commonly used to characterize species diversity of a community.

$$H' = -\sum_{i=1}^{R} p_i \log p_i$$

Species evenness was computed as Pielou's evenness index (J') using PRIMER version 5.2.8 (Clark and Warwick, 1994). Species evenness refers to closeness in numbers each species in an environment. **J'** is constrained between 0 and 1. The less variation between the species in the community, the **J'** is higher. The evenness of a community can be represented by Pielou's evenness index:

$$J' = \frac{H'}{H'_{\max}}$$

3.4. Result

3.4.1. Phytoplankton biomass (Chlorophyll *a*)

3.4.1.1 Total Phytoplankton Biomass

Annual variation of total phytoplankton biomass (chlorophyll *a*) in the northern arm of the study region ranged from 2.1 to 66.2 mg m^{-3} (av. 12.1 mg m^{-3}) with maximum biomass at station 3 (66.2 mg m^{-3}) during April followed by station 1 (50.2 mg m^{-3}) during August, station 3 (34.2 mg m^{-3}) during October, station 2 (33.1 mg m^{-3}) during March and station 3 (29.9 mg m^{-3}) during May. Rest of the period the phytoplankton biomass was < 15 mg m^{-3} at all the stations (Figure 3.4.1.1 a).

(a)

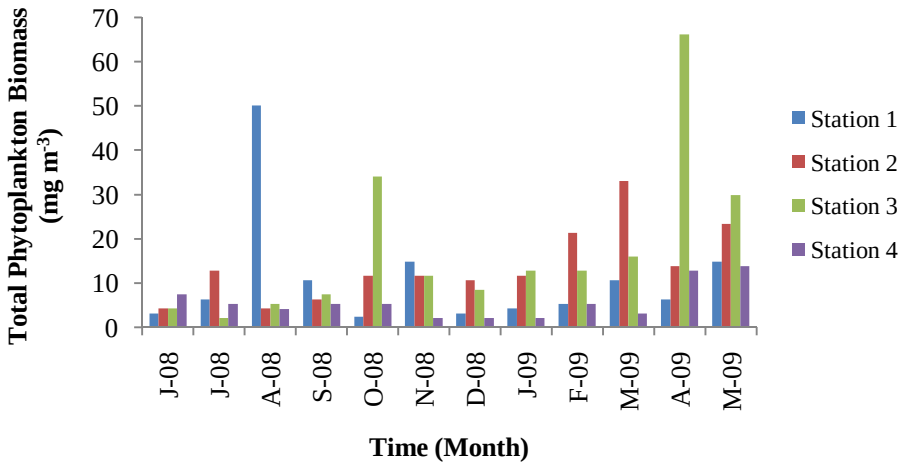

Figure 3.4.1.1 (a) Distribution of total phytoplankton biomass in the northern arm

Annual variability in total phytoplankton biomass (chlorophyll *a*) distribution in the southern region ranged from 2.1 to 19.2 mg m^{-3} (av. 7.3 mg m^{-3}) with maximum at station 7 during May and minimum at station 5 during November, December and January (Figure 3.4.1.1 b).

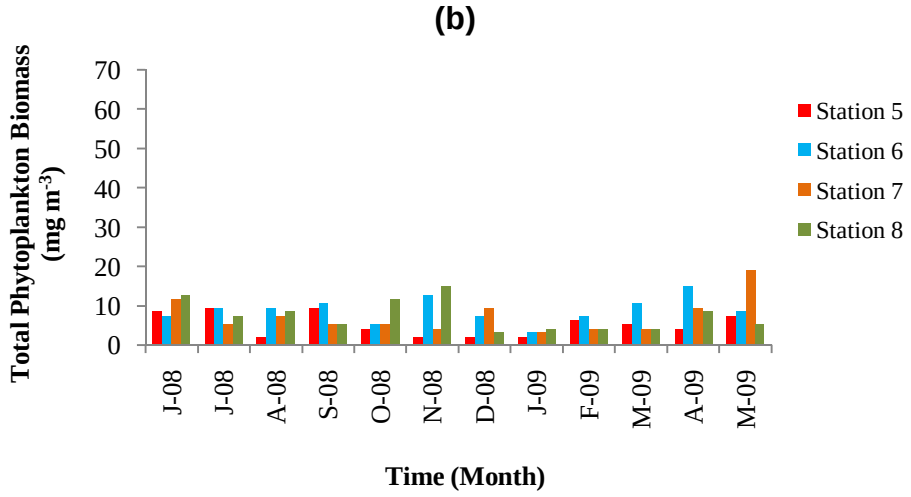

Figure 3.4.1.1 (b) Distribution of total phytoplankton biomass in the southern arm

Seasonal distribution of total phytoplankton biomass (chlorophyll *a*) revealed that during pre monsoon and post monsoon total phytoplankton biomass was high at stations 2 and 3. Station 4 onwards the total phytoplankton biomass was more or less same regardless of season. During monsoon a relatively high peak in total phytoplankton biomass was observed at station 1 and in rest of the stations the total phytoplankton was more or less same (Figure 3.4.1.1 c).

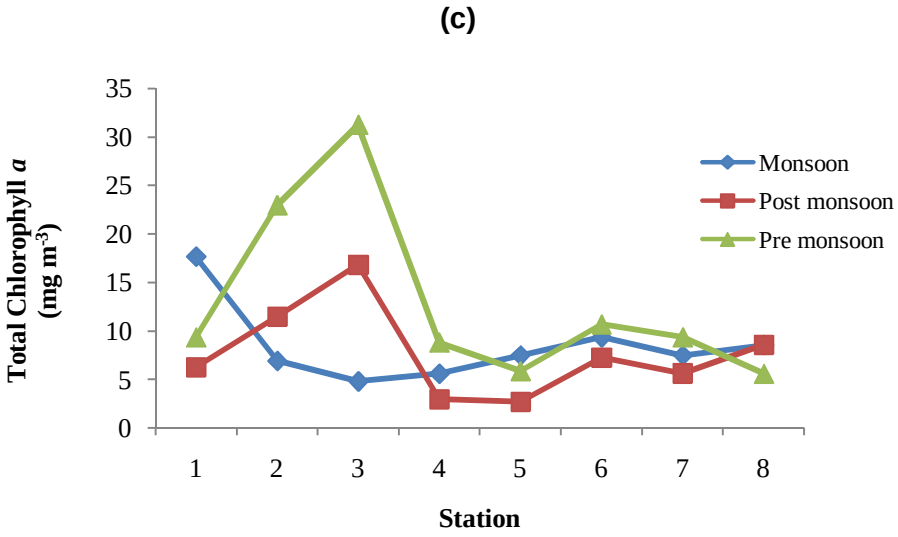

Figure 3.4.1.1 (c) Seasonal distribution of total phytoplankton biomass in the study region

3.4.1.2 Size Fractionated Phytoplankton Biomass

Size fractionated biomass comprised of three fractions, viz. pico (<2 µm), nano (2-20 µm) and micro (>20 µm). This is to quantify which fraction has contributed more to the total phytoplankton biomass.

Annual range of pico fraction in northern arm ranged from 0.1 to 1.6 mg m^{-3} (av. 0.4 mg m^{-3}) with maximum at station 1 (1.6 mg m^{-3}) during August and September and at station 2 (1.6 mg m^{-3}) during July (Figure 3.4.1.2 a).

(a)

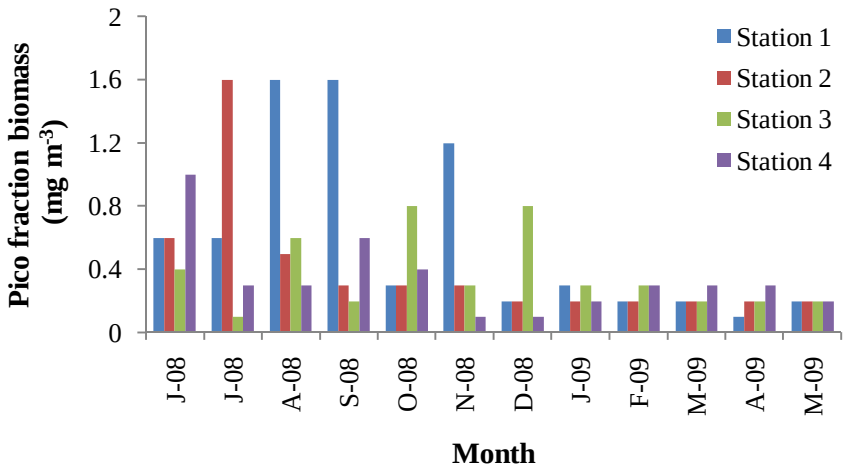

Figure 3.4.1.2 (a) Distribution of pico fraction in the northern arm

In southern arm the range was between 0.1 to 1.2 (av. 0.5 mg m^{-3}) maximum was observed during July at station 6 (1.2 mg m^{-3}) and during June at station 8. The second highest peak was at station 5 (1.1 mg m^{-3}) during July and September and at station 6 during June and August (Figure 3.4.1.2 b).

(b)

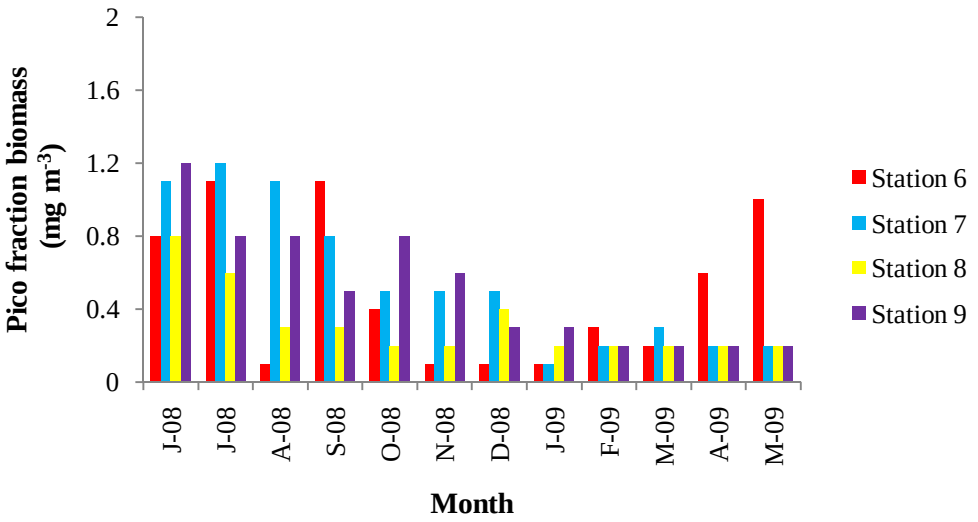

Figure 3.4.1.2 (b) Distribution of pico fraction in the southern arm

In general pico fraction got reduced gradually from June to May at all the stations in both the arm of the study region.

Annual range of nano fraction in the northern arm (Figure 3.4.1.2 c) of the study region ranged from 1 to 36.3 mg m^{-3} (av. 6.5 mg m^{-3}) with maximum at station 3 during April. The next highest peak was at station 1 during August (26.3 mg m^{-3}). Minor peaks were also observed at station 3 during October (18.8 mg m^{-3}) and May (15.6 mg m^{-3}) and at station 2 during February (12.2 mg m^{-3}) and March (18.8 mg m^{-3}) and May (12.3 mg m^{-3}).

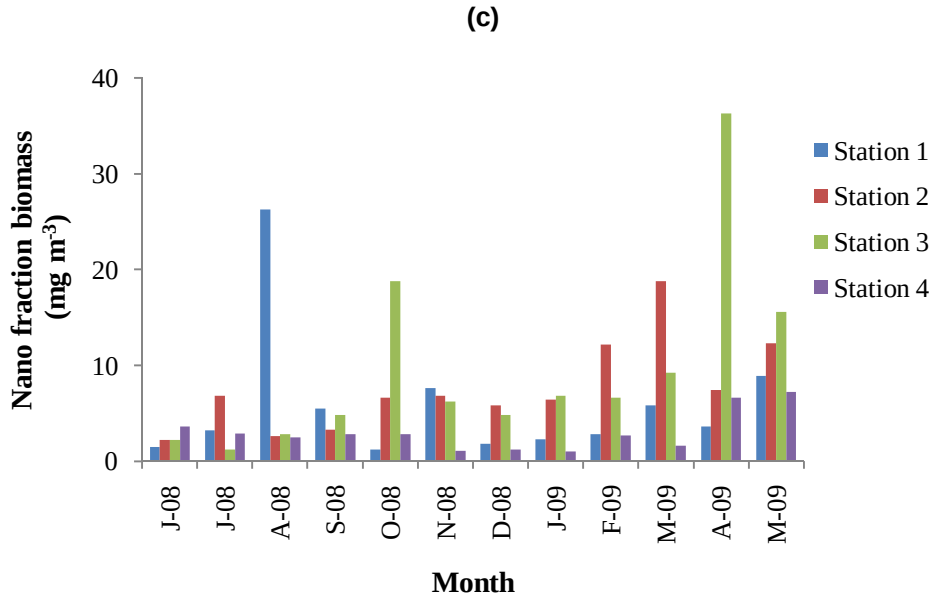

Figure 3.4.1.2 (c) Distribution of nano fraction in the northern arm

Annual range of nano fraction in the southern arm of the study region ranged from 1.1 to 10.6 mg m^{-3} (av.4.0 mg m^{-3}) with maximum at station 7 during May (Figure 3.4.1.2 d). Throughout the observation period the nano fraction biomass was <10 mg m^{-3} at all the stations except at station 7 (10.6 mg m^{-3}) during May.

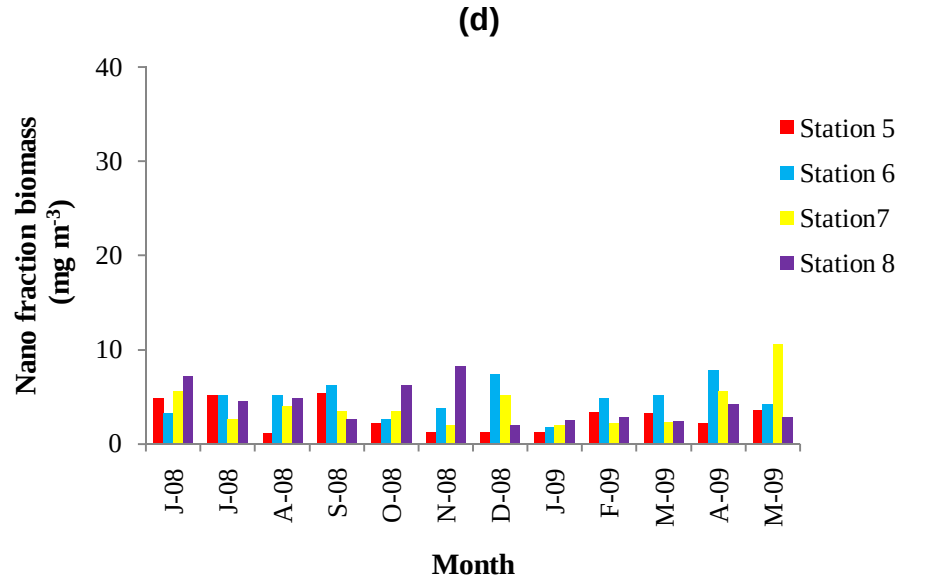

Figure 3.4.1.2 (d) Distribution of nano fraction in the southern arm

In general nano fraction biomass was comparatively low in southern arm compared to northern arm.

Annual range of micro fraction in the northern arm of the study region ranged from 0.8 to 28.6 mg m^{-3} (av. 4.6 mg m^{-3}) with maximum at station 3 during April. The second highest peak was observed at station 1 (20.6 mg m^{-3}) during August (Figure 3.4.1.2 e). Minor peaks were also observed at stations 3 during October (18.8 mg m^{-3}) and May (15.6 mg m^{-3}) and at station 2 during February (12.2 mg m^{-3}), March (18.8 mg m^{-3}) and May (12.3 mg m^{-3}). Rest of the period the micro fraction biomass was < 7 mg m^{-3} at all the stations.

(e)

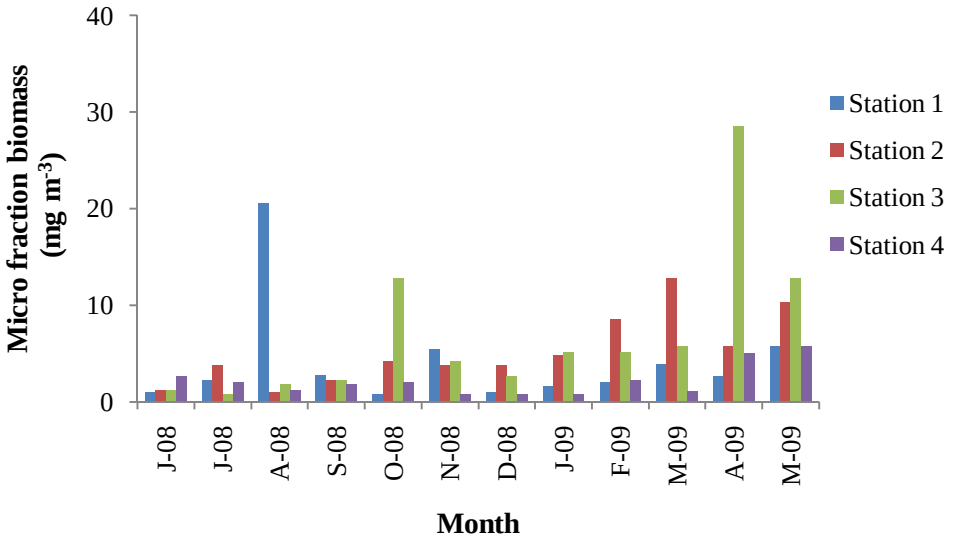

Figure 3.4.1.2 (e) Distribution of micro fraction in the northern arm

Annual range of micro fraction in the southern arm of the study region ranged from 0.8 to 7.6 mg m^{-3} (av. 2.6 mg m^{-3}) with maximum during April at station 7 (Figure 3.4.1.2 f). The micro fraction biomass was never >7 mg m^{-3} at any station in the southern arm, except at station 7 (7.6 mg m^{-3}) during April.

(f)

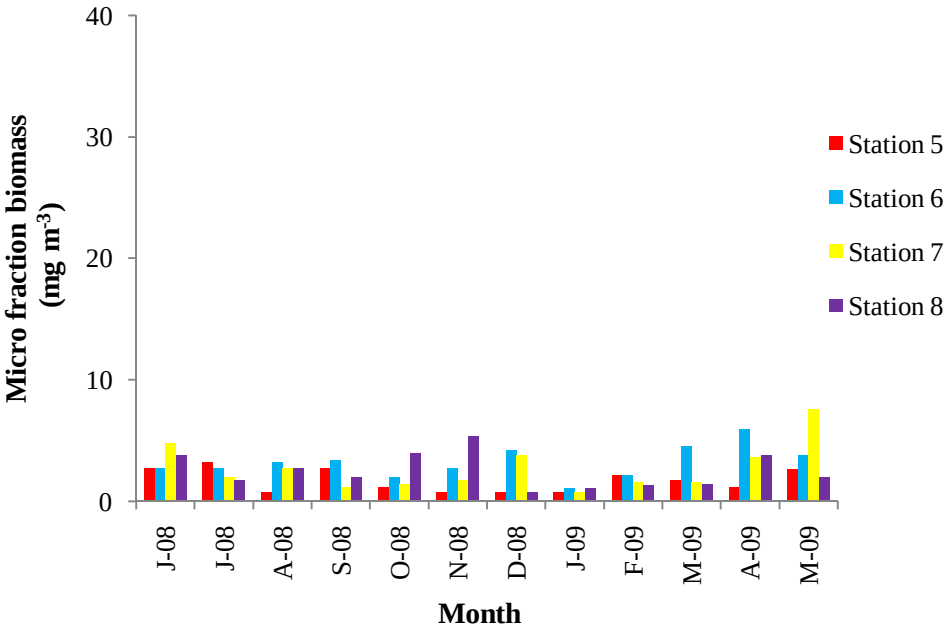

Figure 3.4.1.2 (f) Distribution of micro fraction in the southern arm

In general micro fraction biomass was comparatively low in the southern arm compared to the northern arm.

3.4.1.3 Percentage contribution of size fractionated phytoplankton biomass

Percentage contribution of fractionated phytoplankton biomass revealed that pico fraction ranged from 1 to 11%, nano fraction from 51 to 61% and micro fraction from 32 to 46% over the season in the northern arm of the study region. The overall results revealed that there was 3- 10% decrease in pico fraction and 3-10% increase in micro fraction over the season. Percentage contribution of nano fraction did not show significant variations over the season (Figure 3.4.1.3 g).

In the southern arm of the study region pico fraction biomass contributed 2 to 12%, nano 41 to 61% and micro 29 to 52%. The overall results revealed that there 3- 10% decrease in pico fraction and 3-10% increase in micro fraction over the season. Nano fraction showed only marginal variation over the season (Figure 3.4.1.3 h).

The overall results revealed that pico fraction trend was just the opposite of micro fraction. The percentage contribution of pico fraction gradually decreased from monsoon to pre monsoon whereas micro fraction gradually increased from monsoon to pre monsoon.

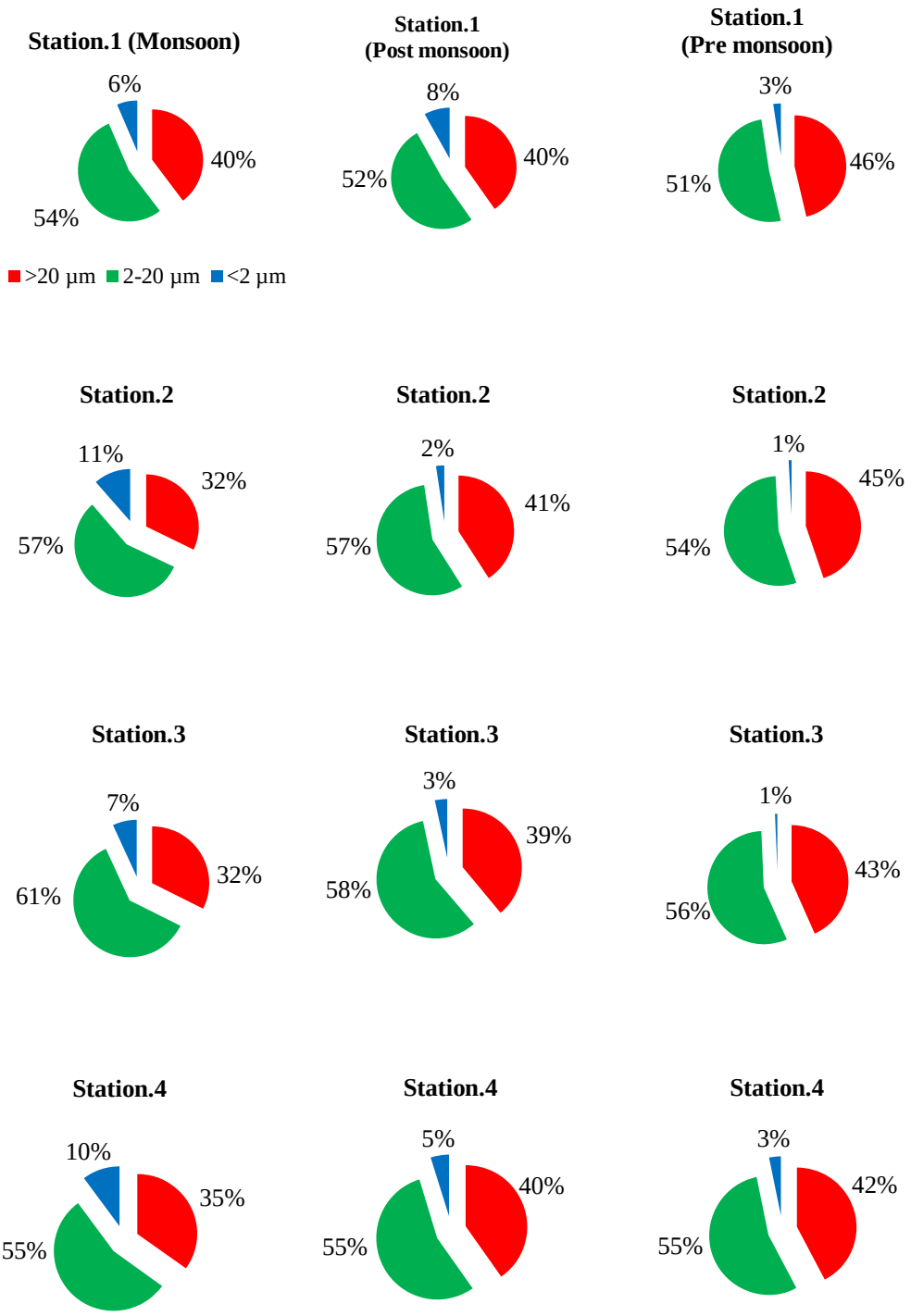

Figure 3.4.1.3 (g) Percentage contribution of fractionated chlorophyll *a* in northern arm

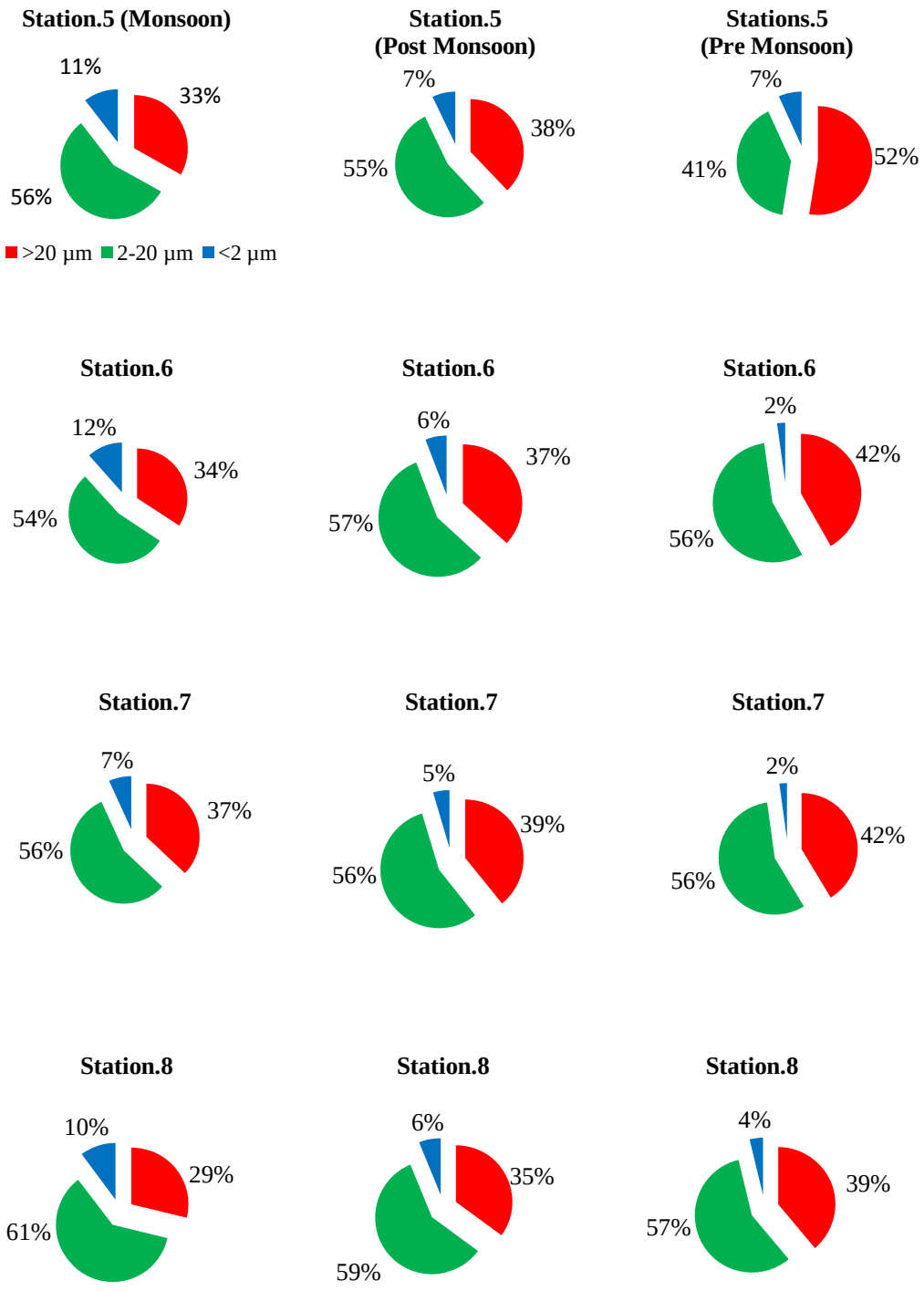

Figure 3.4.1.3 (h) Percentage contribution of fractionated chlorophyll *a* in southern arm

3.4.2. Total Phytoplankton Density (Abundance)

Annual range of phytoplankton abundance in the northern arm of the study region ranged from 0.2 to 49.5 x 10^5 Cells L^{-1} (av. 4.7 x 10^5 Cells L^{-1}). Maximum abundance was observed at station 3 in April (49.5 x 10^5 Cells L^{-1}) followed by station 1 in August (38.6 x 10^5 Cells L^{-1}), station 3 in May (15.9 x 10^5 Cells L^{-1}) and rest of the period, the abundance was < 10 x 10^5 Cells L^{-1} (Figure 3.4.2 a).

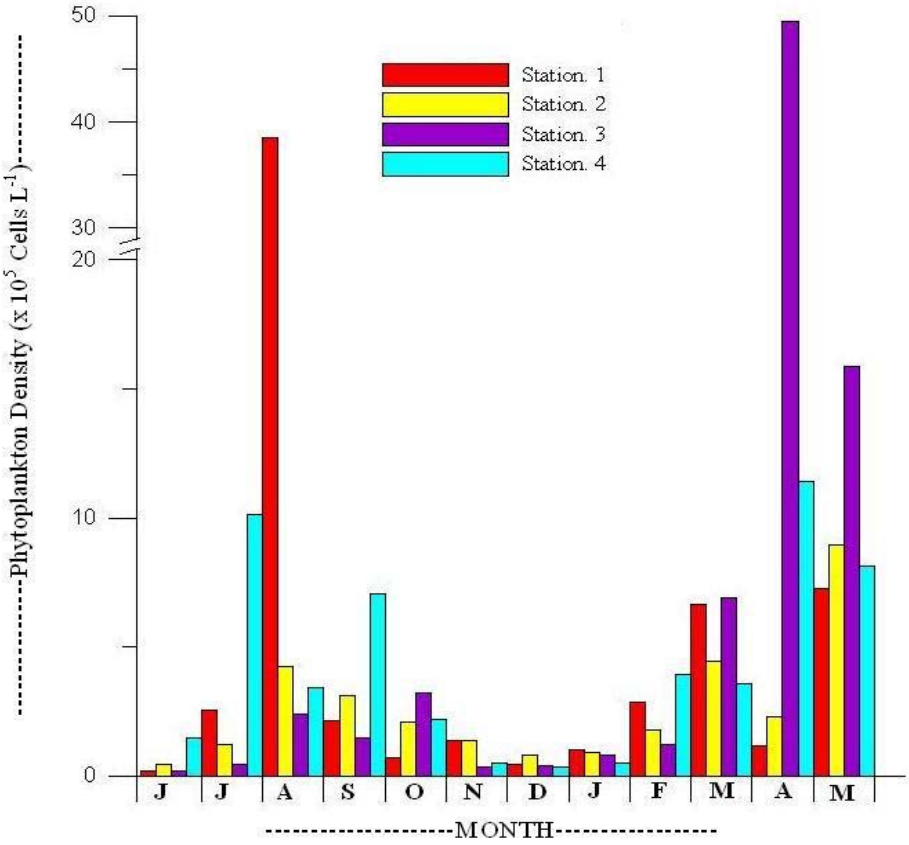

Figure 3.4.2 (a) Distribution of total phytoplankton abundance in the northern arm

Annual range of phytoplankton abundance in the southern arm of the study region (Figure 3.4.2 b) ranged from 0.1 to 17.0 x 10^5 Cells L^{-1}

(av. 2.6 x 10^5 Cells L^{-1}) and was noticeably low compared to stations in the northern arm (Figure 3.5.2 b). The highest abundance was observed at station 6 (17.0 x 10^5 Cells L^{-1}) during November, and was <10 x 10^5 Cells L^{-1} at all the stations throughout the observation period.

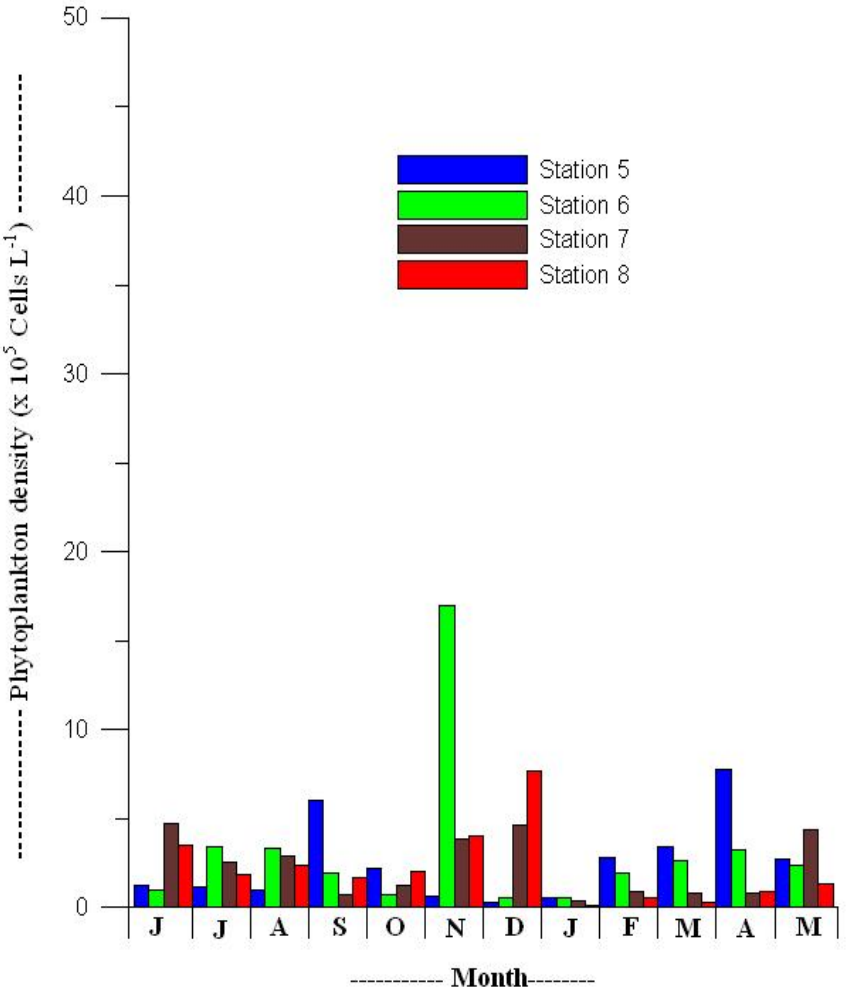

Figure 3.4.2 (b) Distribution of total phytoplankton abundance in the southern arm

Seasonal distribution of total phytoplankton abundance in the study region (Figure 3.4.2 c) showed that during monsoon only at station 1 (10.9 x 10^5 Cells L^{-1}) and station 4 (5.5 x 10^5 Cells L^{-1}) the abundance was relatively high compared to other stations. During post monsoon season the abundance was relatively high only at station 6 (4.7 x 10^5 Cells L^{-1}) and

during pre monsoon season the maximum abundance (18.4 x 10^5 Cells L^{-1}) was recorded at station 3.

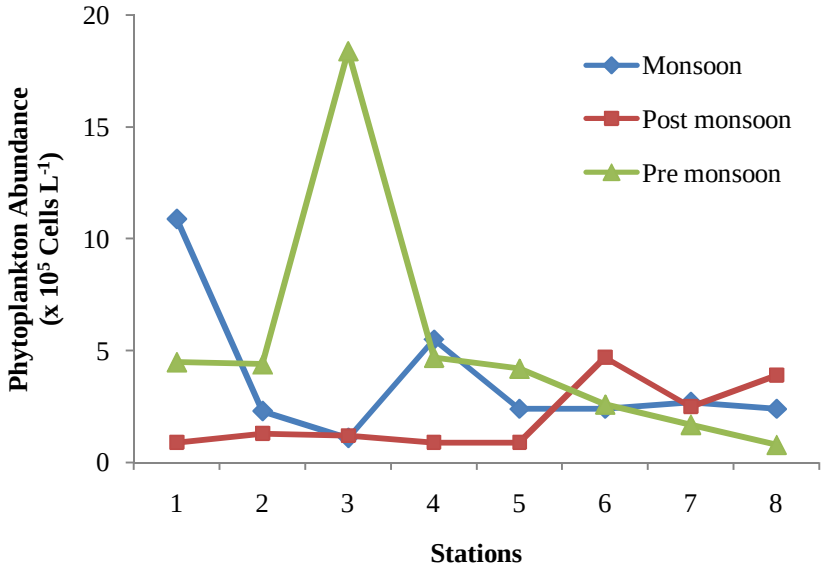

Figure 3.4.2 (c) Seasonal distribution of total phytoplankton abundance in the study region

3.4.3. Phytoplankton Community structure

Phytoplankton community was composed of four main classes: Bacillariophycea (diatoms), Dinophycea (dinoflagellates), Chlorophyceae (green algae) and Cyanophycea (blue green algae). However, diatoms dominated (> 80%) followed by dinoflagellates (> 5%), green algae (< 5%) and blue green algae (< 3%).

Percentage distribution of phytoplankton community in the northern arm of the study region revealed that diatom community was more or less same throughout the season, whereas dinoflagellates gradually increased and maximum was observed during pre monsoon except at station 1 where the maximum was observed during monsoon (16%). But in the case

of green algae the percentage contribution decreased from monsoon to pre monsoon at all the stations along the northern arm whereas green algae did not show any significant variation over the season (Figure 3.4.3 a).

Similar pattern was observed in southern arm of the study region with diatoms as the dominant community followed by dinoflagellates, green algae and blue green algae. The percentage contribution of green algae and blue green algae marginally varied over the season (Figure 3.4.3 b).

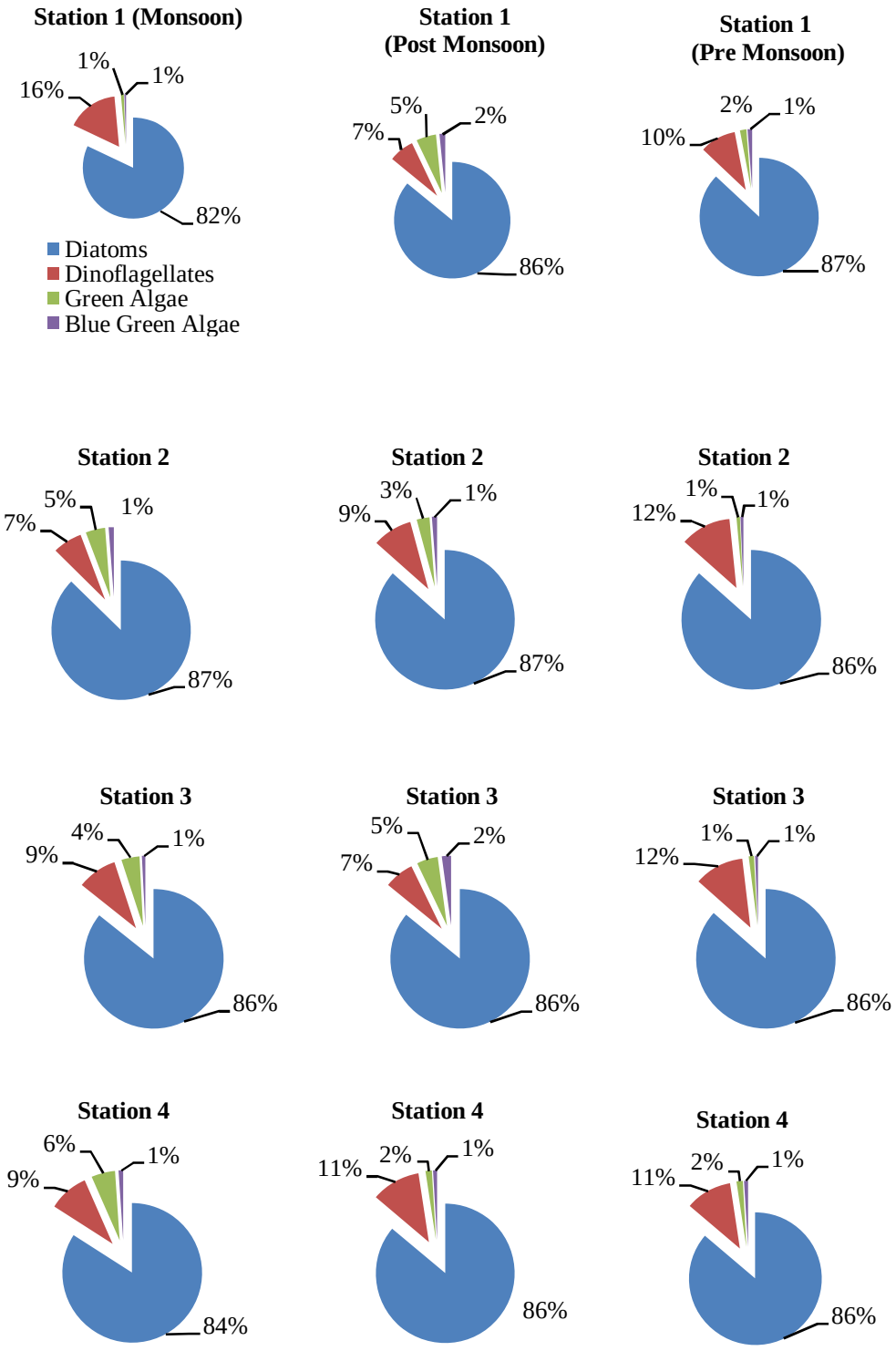

Figure 3.4.3 (a) Seasonal distribution of phytoplankton community in northern arm

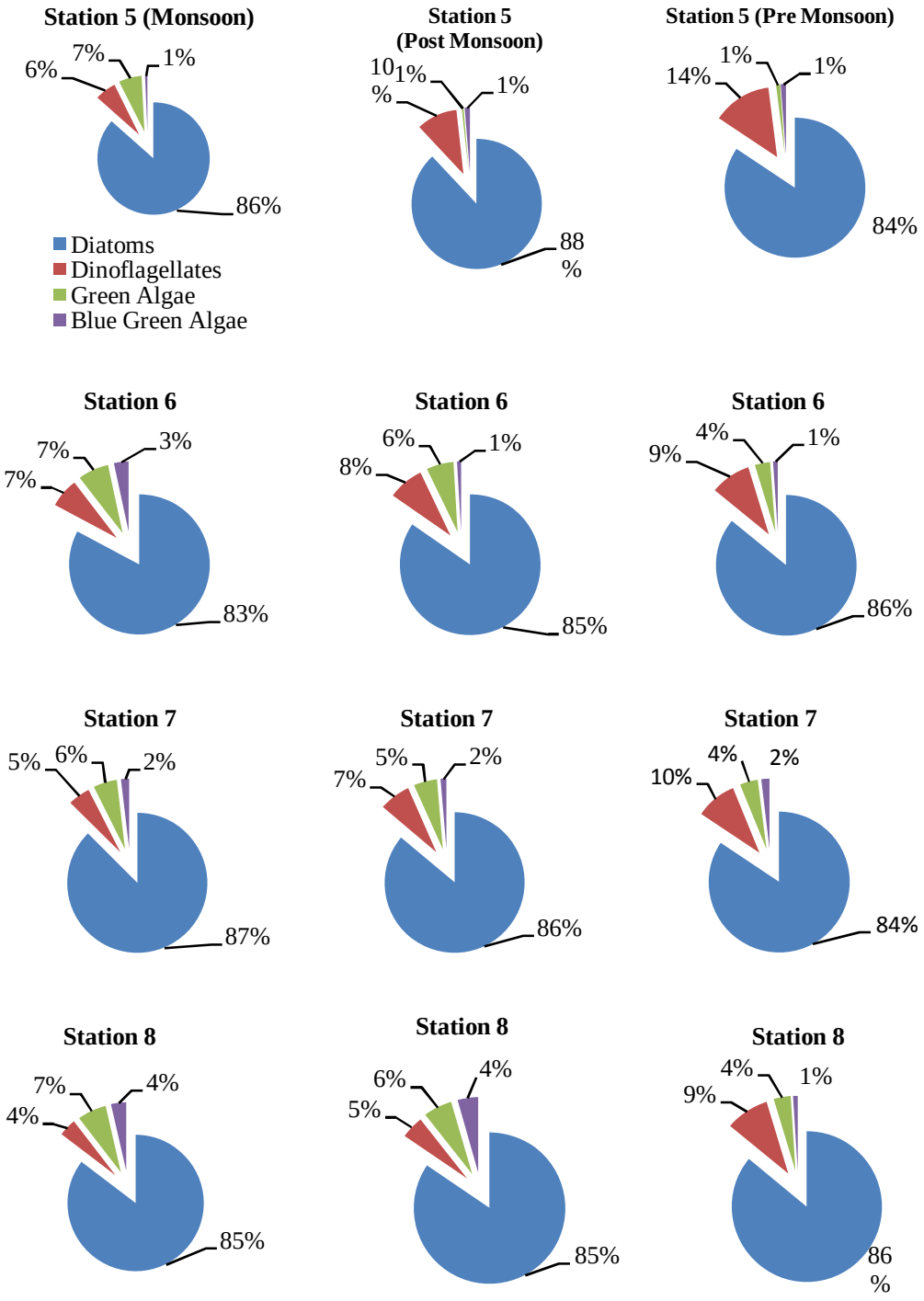

Figure 3.4.3 (b) Seasonal distribution of phytoplankton community in southern arm

3.4.4 Phytoplankton species density

The average number of phytoplankton species density in the study region was computed (Figure 3.4.4 a- h). It was observed that the average phytoplankton species density was maximum at Station 4 (93) followed by station 1 (84) and station 5 (82). Station 3 recorded the lowest species density (58).

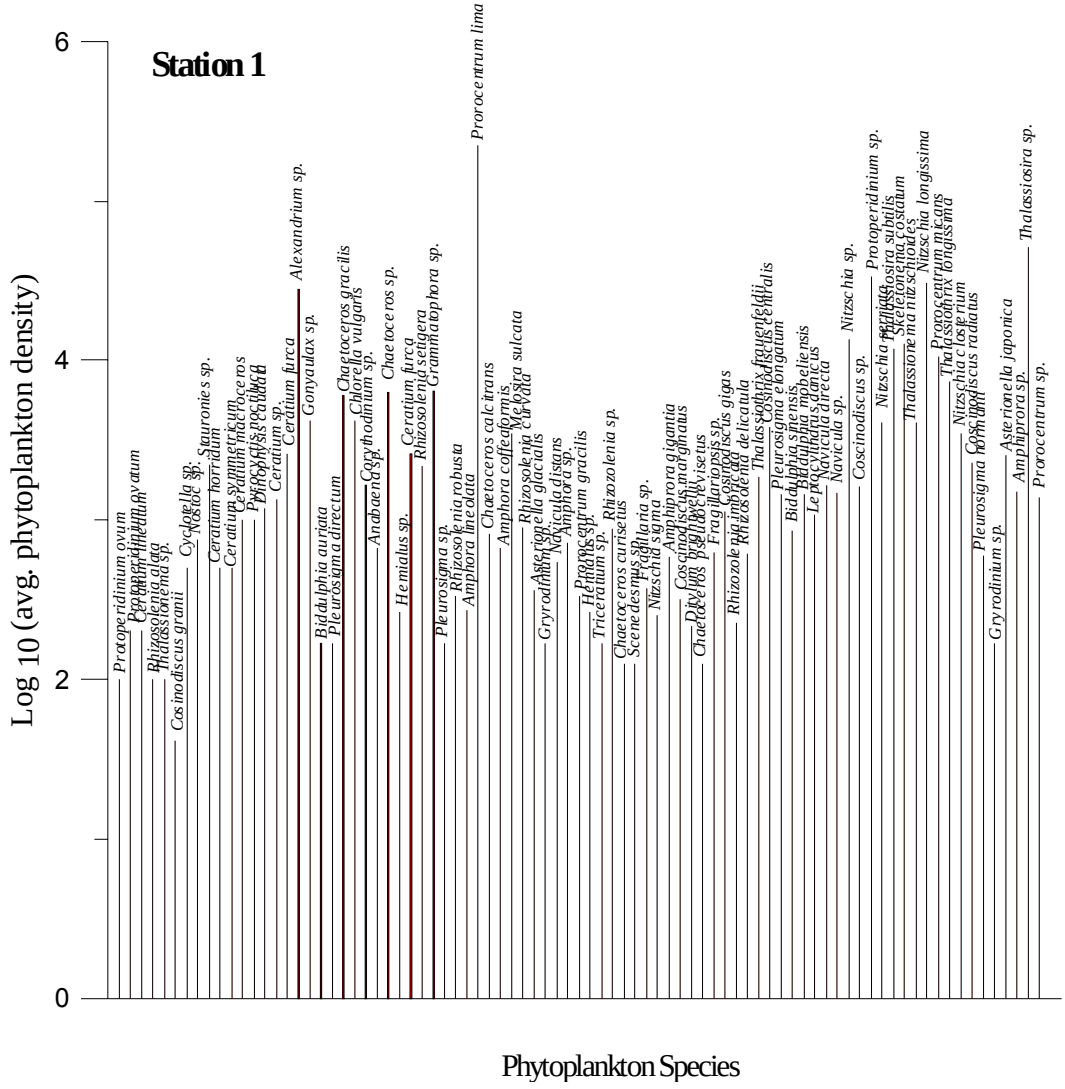

Figure 3.4.4 (a) Average phytoplankton species density

The average density of individual species (log $_{10}$) ranged from 1.6 to 5.3. The highest density was contributed by *P. lima*, *Alexandrium* sp.,

Protoperidinium sp., *Thalassiosira* sp., and the lowest by *Scendesmus* sp., *Gyrodinium* sp., *Pleurosigma* sp., *C. gigas*. Although some species of dinoflagellates were present in higher density at this station, diatoms were represented by more number of species.

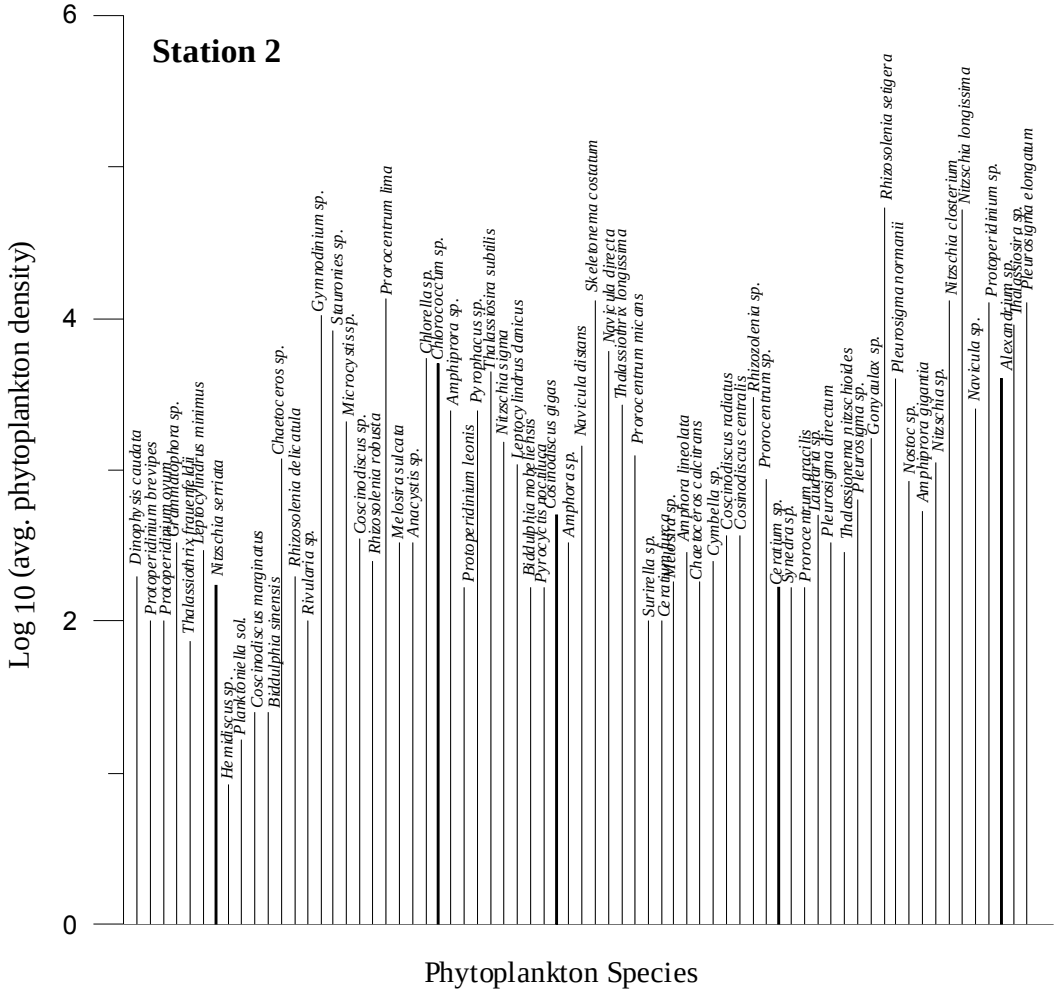

Figure 3.4.4 (b) Average phytoplankton species density

The average individual species density (log $_{10}$) at this station ranged from 1.8 to 5.3. The maximum density was contributed by *S. costatum, N. closterium, T. subtilis, N. longissima, R. setigera* and minimum by *P. simplex, Melosira* sp, and *Synedra* sp.

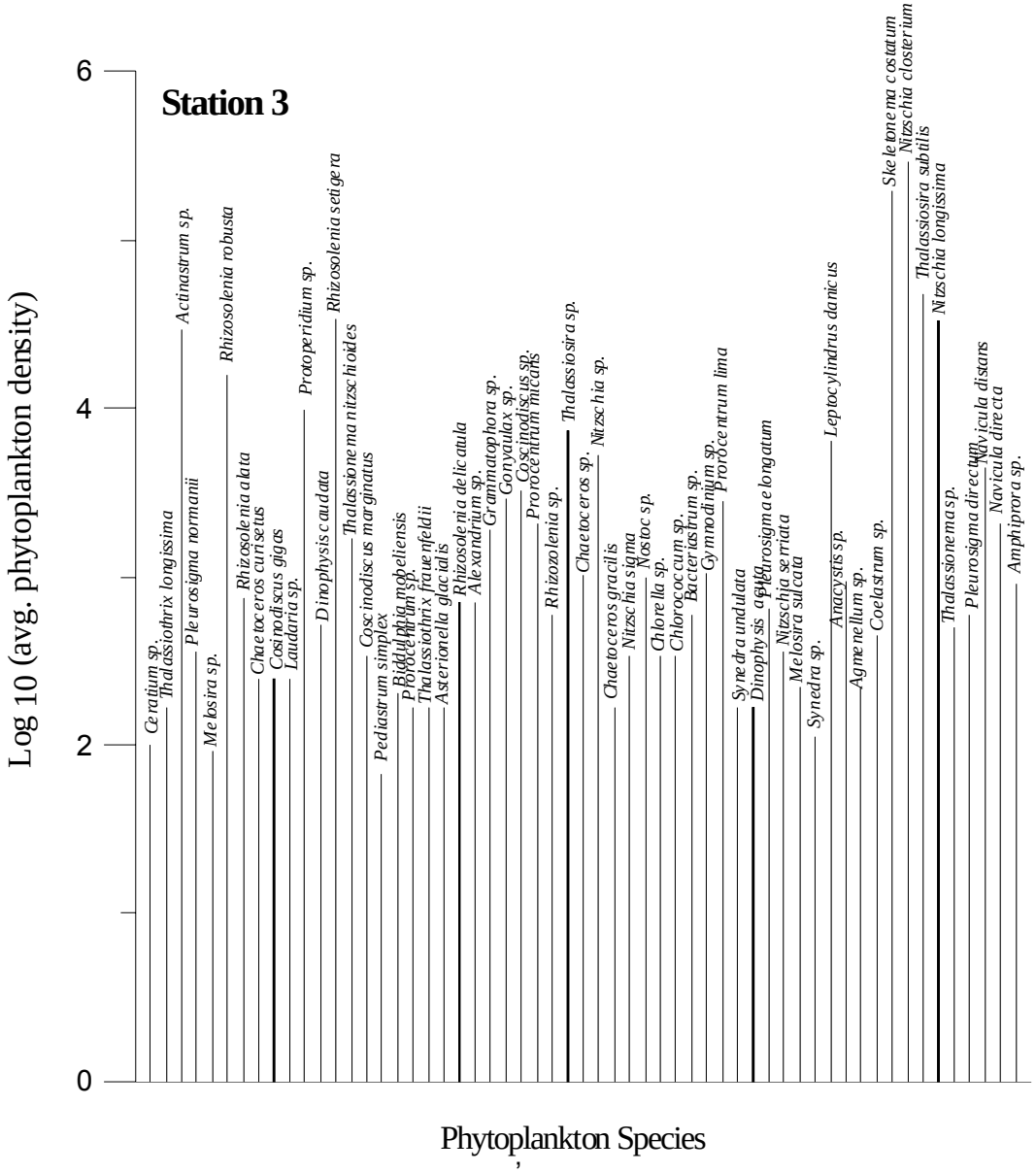

Figure 3.4.4 (c) Average phytoplankton species density

The average density of species (log $_{10}$) ranged from 0.9 to 4.7. *S. costatum, N. closterium, T. subtilis, R. setigera* were the dominant species while and minimum density was contributed by *Hemidiscus* sp.

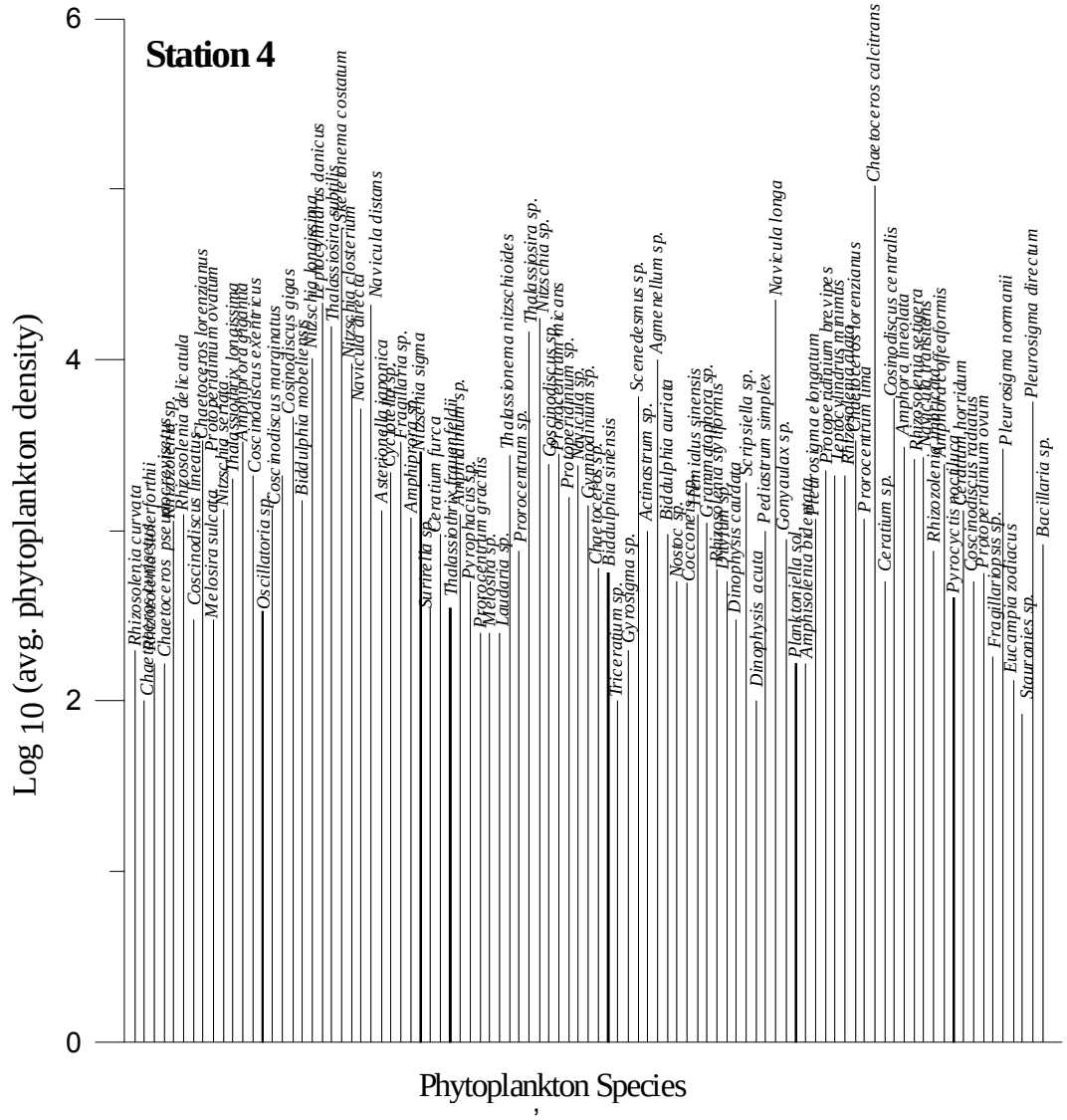

Figure 3.4.4 (d) Average phytoplankton species density

At this station, the average density of individual species (log $_{10}$) ranged from 1.9 to 5.0. The maximum density was exhibited by *C. calcitrans, S. costatum, L. danicus, N. closterium* and the minimum by *Stauronies* sp., *Triceratium* sp., *D. acuta*.

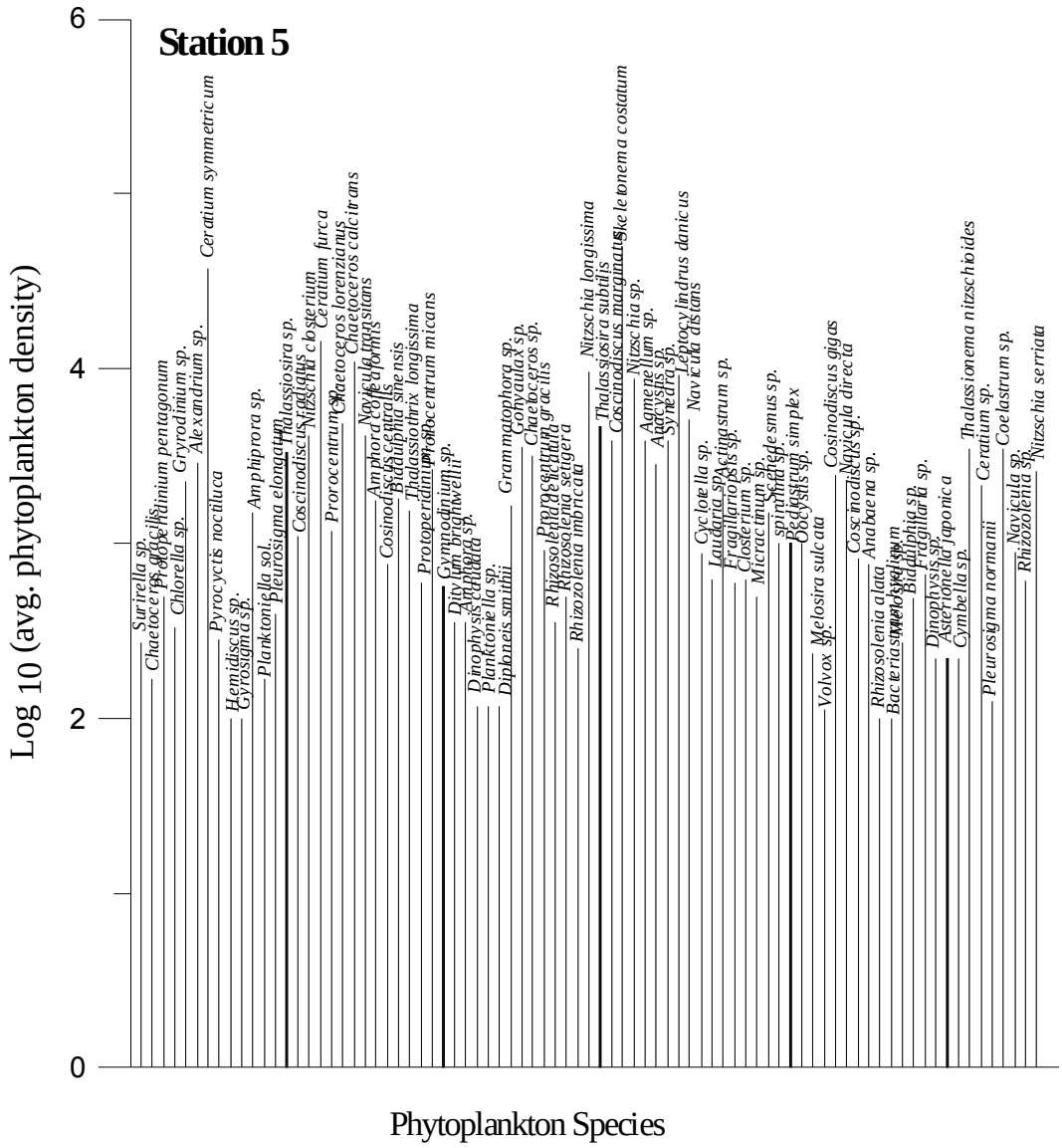

Figure 3.4.4 (e) Average phytoplankton species density

At this station the average individual species density (log $_{10}$) ranged from 2.0 to 4.7. *S. costatum, C. symmetricum, C. calcitrans, N. longisimma* occurred in high densities while *Hemidiscus* sp, *Gyrosigma* sp., *Volvox* sp., *R. alata*, *Bacteriastrum* sp., *P.* normanii were present in low densities.

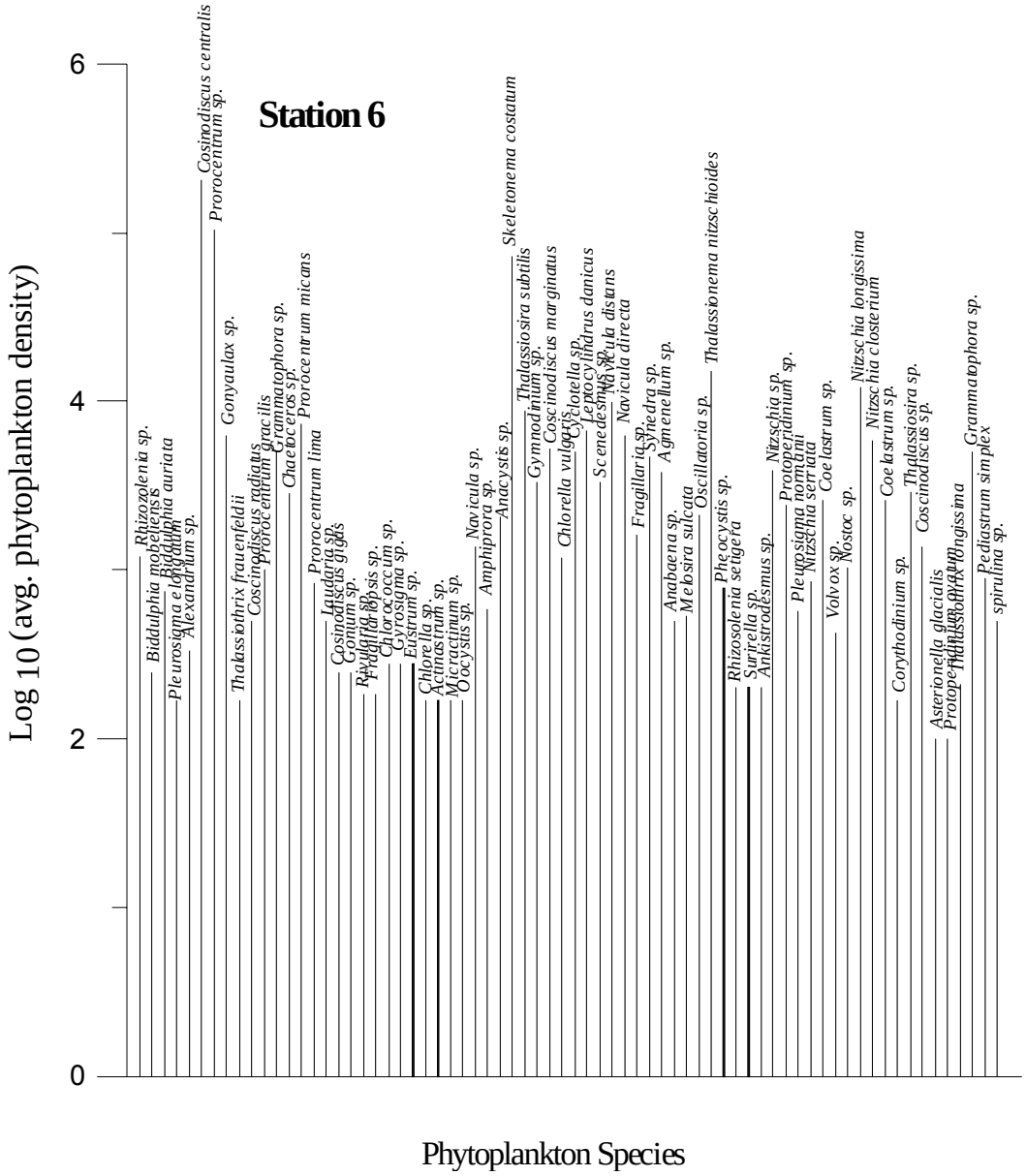

Figure 3.4.4 (f) Average phytoplankton species density

The range in average density of individual species (log $_{10}$) was 2.0 - 5.3. The maximum density was contributed by *C. centralis, Procentrum sp,. S. costatum, T. nitzschioides and* the minimum *by A. glacialis* and *P. ovatum*.

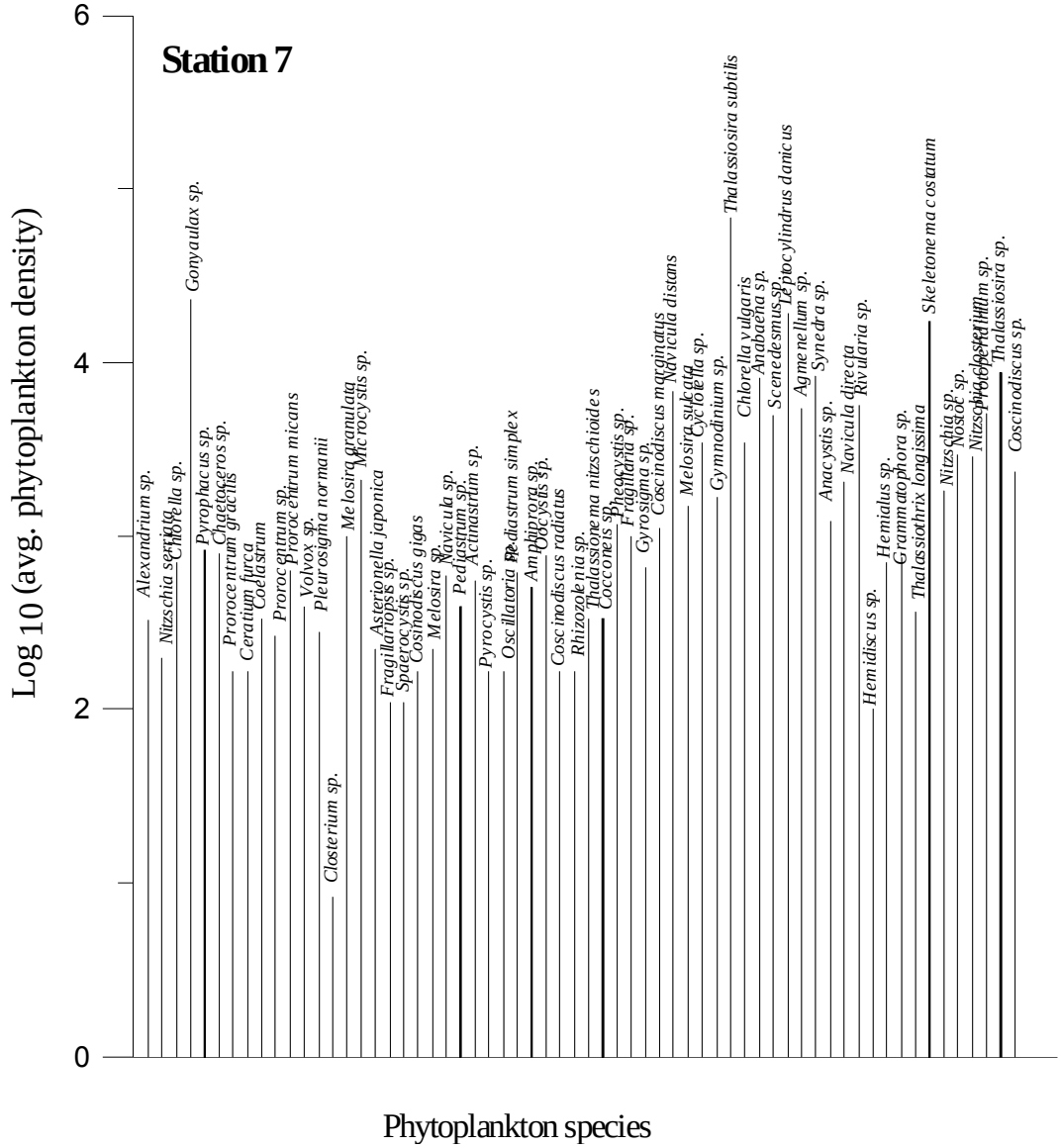

Figure 3.4.4 (g) Average phytoplankton species density

The average density of species (log $_{10}$) ranged from 0.9 to 4.8. The maximum contribution was by *T. subtilis, S. costatum, L. danicus, Gonyaulax* sp. and the minimum by *Ceratium* sp. *Hemidiscus* sp.

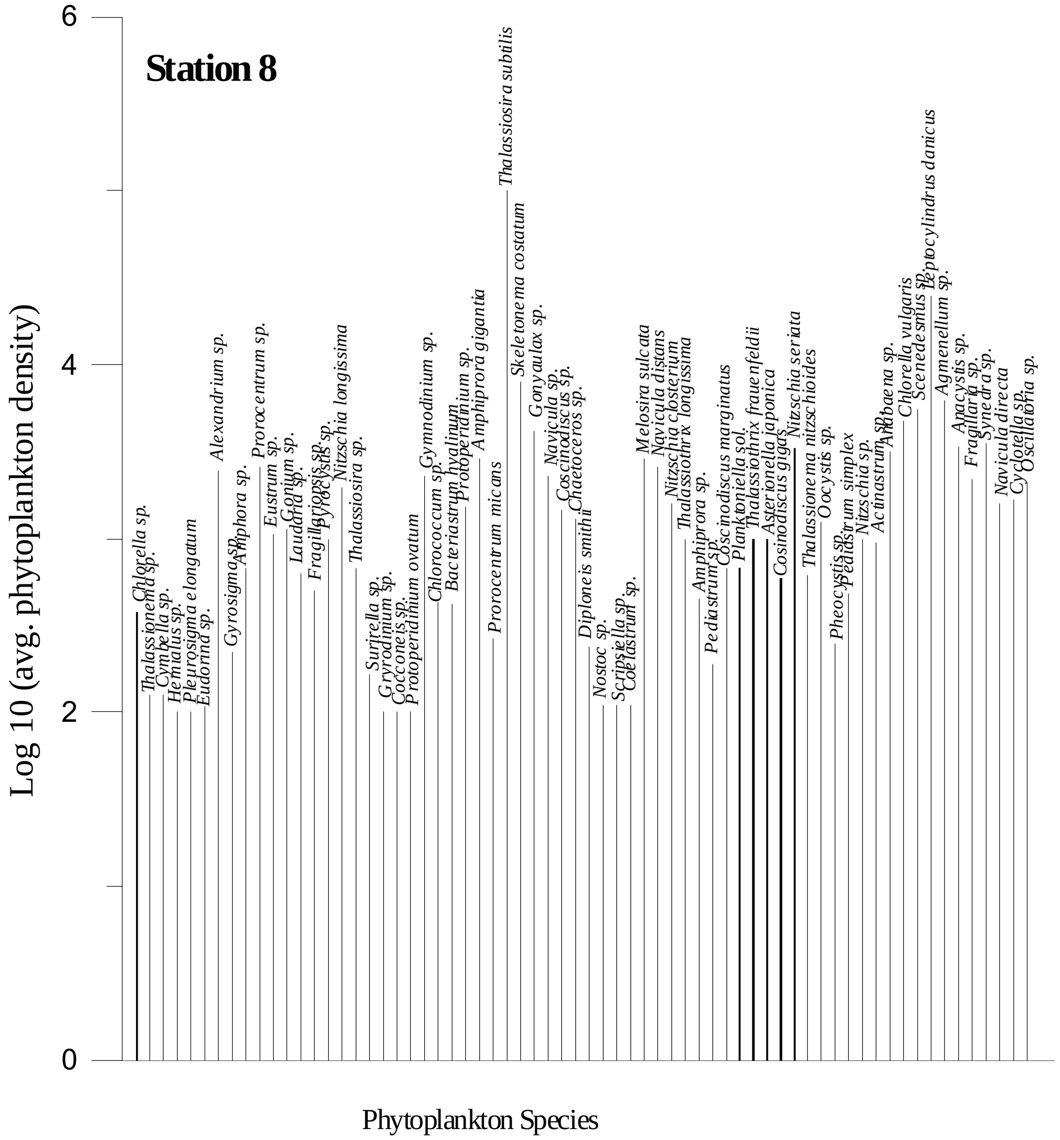

Figure 3.4.4 (h) Average phytoplankton species density

The average individual species density (log $_{10}$) ranged from 2.0 to 5.0. The maximum was contributed by *T. subtilis, L. danucus, S. costatum, C. vulgaris* and the minimum by *Nostoc* sp, *Scripsiella* sp., *Ceratium* sp., *Pheocyctis* sp.

3.4.5 Phytoplankton Species Composition

Phytoplankton species composition in the study region revealed the occurrence of total 148 species of which 87 species belonged to diatoms, 32 to dinoflagellates, 17 to green algae and 12 to blue green algae. Their seasonal distribution is given in Table 3.4.5 a, b and c.

Table 3.4.5 (a) Phytoplankton Species composition during monsoon in the study region
(-) absent, (+) 10-1000, (++) 1000-10,000 and (+++) >10,000 Cells L^{-1}

Phytoplankton Species/Stations	1	2	3	4	5	6	7	8
Diatoms (Centrales)								
Melosira sp.	+	+						
Melosira sulcata		+	+	+	+	+	+	++
Melosira granulata								+
Skeletonema costatum	++	++	++	+++	++	++	+	+
Thalassiosira sp.	++	+		++	+	++	++	++
Thalassiosira subtilis	++	++	+	++	+	++	++	++
Coscinodiscus sp.	++	+	++	+	+			
Coscinodiscus centralis	+	+		+			+	+
Coscinodiscus marginatus	+	+	+	+	+		+	+
Coscinodiscus excentricus				+				
Coscinodiscus radiates	++	+		++	+		+	
Coscinodiscus gigas	++	++		++	+	+		+
Coscinodiscus granii	+							
Coscinodiscus lineatus								
Hemidiscus sp.		+						
Planktoniella Sol.		+			+			
Cyclotella sp.					+	++	+	+
Laudaria sp.					+	+		+
Leptocylindrus sp.								
Leptrocylindrus danicus	+	++	++	++		++	++	++
Leptocylindrus minimus		+			++			
Rhizosolenia sp.	+		+	+	+	+	+	
Rhizosolenia delicatula	++		++					
Rhizosolenia setigera								
Rhizosolenia robusta		++						
Rhizosolenia imbricate								
Rhizosolenia alata								
Rhizosolenia curvata								
Rhizosolenia styliformis								
Rhizosolenia stolerforthii								
Bacteriastrum sp.			+					
Bacteriastrum hyalinum								
Chaetoceros sp.	+							
Chaetoceros gracilis	+	+		+				

Chaetoceros calcitrans								
Chaetoceros lorenzianus								
Chaetoceros pseudocrevisetus	+							
Biddulphia sp								
Biddulphia sinensis	+	+						
Biddulphia mobeliensis	+	+		+				
Biddulphia auriata								
Ditylum sp.								
Ditylum brightwellii	+				+			
Hemialus sp.								
Hemialus sinensis								
Eucampia sp.								
Eucampia zodiacus								
Diatoms (Pennales)								
Fragillaria sp.	+			+		+	+	+
Fragillariopsis sp	+			+	+	+	+	+
Grammatophora sp.			+			+	+	+
Synedra sp.								
Synedra undulata			+				++	
Thalassionema sp								
Thalassionema nitzschiodes	++	+		+	+	+	+	+
Thalassiothrix sp.								
Thalassiothrix longisimma	+	++		+	+	+	+	+
Thalassiothrix frauendfeldii	++	+						
Asterionella japonica	++			++			+	+
Asterionella glacialis								
Cocconeis sp.								
Navicula sp.	+			+	+	+	+	+
Navicula directa	+	++	++	++	+	++	+	+
Navicula distans	+	++	++	++	++	++	++	+
Navicula transitans								
Navicula longa								
Gyrosigma sp.						+	+	
Pleurosigma sp.								
Pleurosigma elongatum	+	++	+					
Pleurosigma normanii	+	+	+	+	+	+	+	+
Pleurosigma directum			+					
Pleurosigma aesturii			++	+				
Amphiprora sp.	+		++	+	+	+	+	+
Amphiprora gigantia	+			+				
Amphora sp.	+	+			+			
Amphora lineolata								
Amphora coffeaformis	+			+				
Nitzschia sp.	++	+				+	+	+
Nitzschia closterium	++	++	+	++	++	++	+	+
Nitzschia longisimma	++	+++	+	+	+	+		+
Nitzschia sigma	+	++		+	+			
Nitzschia seriata	++	+	+	+	+	+		+
Stauronies sp.	+	+++						
Suirella sp.								

Surirella ovalis								
Diploneis sp.								
Diploneis smithii					+			+
Dinoflagellates								
Prorocentrum sp.	+	+						
Prorocentrum micans	++	+	+		+			
Prorocentrum lima	+++	+++	+++					
Prorocentrum gracilis								
Protoperidinium sp.	+++	+	++			+	+	+
Protoperidinium ovum								
Protoperidnium ovatum								
Protoperidinium brevipes								
Protoperidinium leonis								
Ceratium sp.	+							
Ceratium furca	+							
Ceratium horridum	+							
Ceratium fusus	+							
Ceratium lineatum								
Ceratium symmetricum	+							
Ceratium macroceros	++	+						
Ceratium tripos								
Alexandrium sp.	+++							
Pyrocystis sp.							+	
Pyrocycstis noctiluca	++	+						
Gymnodinium sp.	+++	+++	++	+	+	+	+	+
Gyrodinium sp.								
Amphidinium sp.								
Corythodium sp.								
Gonyaulax sp.	+++					+		+
Scripsiella sp.								
Pyrophacus sp.								
Dinophysis sp.								
Dinophysis caudata	+		+		+			
Dinophysis acuta		++	+					
Amphisolenia bidendata								
Ornithocercus sp.								
Pheodactylum triconatum								
Green Algae								
Chlorella sp.						+	+	+
Chlorella salina							+	+
Chlorella vulgaris						+	++	++
Eudoria sp.								
Volvox sp.					+		+	+
Pediastrum sp.							+	+
Pediastrum simplex			+	+	++	+	+	+
Actinastrum sp.			+	+	+	+	+	+
Euastrum sp.								
Coelastrum sp.			+		++			+
Agmenellum sp.			+	++	++	++	+	+
Scenedesmus sp.	+				+	+	+	+
Micractinum sp.					+	+		
Closterium sp.					+	+		
Gomphosphaeria sp.								

Ankistrodesmus sp.								
Chlorococcum sp.						+		+
Blue Green Algae								
Anabaena sp.						+	+	+
Oscillatoria sp.				+		+	+	+
Lyngbya sp.								
Oocystis sp.					+	+	+	+
Microcystis sp.							+	
Sphaerocystis sp.							+	
Phaeocystis sp.						+	+	+
Anacystis sp.					+		+	+
Gonium sp.						+		
Nostoc sp.			+			+		
Rivularia sp.			+			+	++	
Spirulina sp.					+	+		

During monsoon (Table. 3.4.5 a) it was observed that 20 species belonged to class diatom were recorded in all the eight stations in varying density (*M. sulcata, Skeletonema costatum, Thalassiosira sp., T. subtilis, Coscinodiscus sp., C. marginatus, C. radiates, C. gigas, Leptocylindrus danicus, Rhizosolenia sp., Fragillariopsis sp., Thalassionema nitzschiodes, Thalassiothrix longissima, Navicula directa, N.distans, Plueorosigma normanii, Amphiprora sp., Nitzschia closterium, N.longissima and N.seriata*), 2 belonged to class dinoflagellates (*Protoperidinium* sp. and *Gymnodinium* sp.), It was observed that species belonging to class green algae and blue green algae were found from stations 5 to 8 (south of the study region) of that the most common belonged to class green algae were (*Chlorella vulgaris, Pediastrum simplex*, *Actinastrum* sp. and *Agmenellum* sp.) and blue green algae (*Anabaena* sp., *Oscillatoria* sp., *Oocystis* sp., and *Phaeocystis* sp.).

Table 3.4.5 (b) Phytoplankton Species composition during post monsoon in the study region (-) absent, (+) 10-1000, (++) 1000-10,000 and (+++) >10,000 Cells L^{-1}

Phytoplankton Species/Stations	1	2	3	4	5	6	7	8
Diatoms (Centrales)								
Melosira sp.								
Melosira sulcata	++							
Skeletonema costatum	++	+	+++	+++	+++	+++	+++	++
Thalassiosira sp.	+	+	+	++	+	++	++	
Thalassiosira subtilis	+		+	++		+	+++	+++
Coscinodiscus sp.	+	+	+	++	+	+	+	+
Coscinodiscus centralis								
Coscinodiscus marginatus					+			
Coscinodiscus exentricus								
Coscinodiscus radiates					+			
Coscinodiscus gigas								
Coscinodiscus granii								
Coscinodiscus lineatus				+				
Hemidiscus sp.								
Planktoniella Sol.								
Cyclotella sp.						+		+
Laudaria sp.								
Leptocylindrus sp.								
Leptrocylindrus danicus	+			+		+	+	++
Leptocylindrus minimus				+				
Rhizosolenia sp.	+	+	+	+	++	++		
Rhizosolenia delicatula		+	+	+	+			
Rhizosolenia setigera	+		+					
Rhizosolenia robusta								
Rhizosolenia imbricata	+							
Rhizosolenia alata	+				+			
Rhizosolenia curvata	++			+				
Rhizosolenia styliformis								
Rhizosolenia stolerforthii				+				
Bacteriastrum sp.								
Bacteriastrum hyalinum					+			++
Chaetoceros sp.	++	+	+	+	++	++		+
Chaetoceros gracilis								
Chaetoceros calcitrans								
Chaetoceros lorenzianus								
Chaetoceros crevisetus				+				
Chaetoceros pseudocrevisetus				+				
Biddulphia sp.					+			
Biddulphia sinensis				+				
Biddulphia mobeliensis			+			++		
Biddulphia auriata								
Ditylum sp.								
Ditylum brightwellii								
Hemialus sp.	+							
Hemialus sinensis								
Eucampia sp.								
Eucampia zodiacus								

Diatoms (Pennales)								
Fragillaria sp.					++			+
Fragillariopsis sp.								
Grammatophora sp.	+	+	+		+	+		
Synedra sp.								+
Synedra undulata								
Thalassionema sp.	+							
Thalassionema nitzschiodes	+		+	++	++			
Thalassiothrix sp.								
Thalassiothrix longisimma		+	+	+		+	+	
Thalassiothrix frauendfeldii	+		+	+				
Asterionella japonica				+	+			
Asterionella glacialis	+		+			+		
Cocconeis sp.								
Navicula sp.	+	+		+	+			+
Navicula directa					+		+	
Navicula distans		+						
Navicula transitans								
Navicula longa								
Gyrosigma sp.								+
Pleurosigma sp.								
Pleurosigma elongatum	+	+				+		
Pleurosigma normanii	+	+				+		
Pleurosigma directum								
Pleurosigma aesturii								
Amphiprora sp.								
Amphiprora gigantia		+		+				++
Amphora sp.								++
Amphora lineolata								
Amphora coffeaformis								
Cymbella sp.					+			
Nitzschia sp.	+	+	+	+	+	+	++	++
Nitzschia closterium	+	+	+++	++			++	
Nitzschia longisimma	+	++	+++	++				
Nitzschia sigma		+						
Nitzschia seriata	+			++	+		+	++
Stauronies sp.								
Suirella sp.								
Surirella ovalis								
Diploneis sp.								
Diploneis smithii								
Dinoflagellates								
Prorocentrum sp.	+	+	+	++	+	+++	+	++
Prorocentrum micans	+	+		+	+	++	+	+
Prorocentrum lima						++		
Prorocentrum gracilis					+			
Protoperidinium sp.	+	++	+	+		+	++	+
Protoperidinium ovum		+						
Protoperidnium ovatum				+		+		+
Protoperidinium brevipes								
Protoperidinium leonis								

Ceratium sp.			+		++			
Ceratium furca	+				+			
Ceratium horridum								
Ceratium symmetricum								
Ceratium macroceros								
Ceratium tripos								
Gonyaulax sp.	+		+				+	+
Pyrocystis sp.								
Pyrocycstis noctiluca				+				
Gymnodinium sp.	+		+	+	+	++		+
Gyrodinium sp.	+							+
Amphidinium sp.								
Corythodium sp.								
Alexandrium sp.	+	+	+		+	++	+	++
Pyrophacus sp.								
Scripsiella sp.								
Dinophysis sp.					+			
Dinophysis caudata				+	+			
Dinophysis acuta								
Amphisolenia bidendata								
Ornithocercus sp.								
Pheodactylum triconatum								
Green Algae								
Chlorella sp.							++	
Chlorella salina								
Chlorella vulgaris							++	++
Eudoria sp.							+	
Volvox sp.								
Pediastrum sp.								
Pediastrum simplex						+		
Actinastrum sp.			+++					+
Euastrum sp.								
Coelastrum sp.								
Agmenellum sp.					+		++	++
Scenedesmus sp.							++	++
Micractinum sp.								
Closterium sp.								
Gomphosphaeria sp.								
Ankistrodesmus sp.								
Chlorococcum sp.								
Blue Green Algae								
Anabaena sp.					+		+	+
Oscillatoria sp.								
Lyngbya sp.								
Oocystis sp.								
Microcystis sp.								
Sphaerocystis sp.								
Phaeocystis sp.								
Anacystis sp.								++
Gonium sp.								+
Nostoc sp.		+					+	
Rivularia sp.		+					+	
Spirulina sp.								

During post monsoon (Table.3.4.5 b) the species occupied in the study region were (*S.costatum*, *Thalassiosira* sp., *T. subtilis*, *Rhizosolenia* sp., *Chaetoceros* sp., *Nitzschia* sp., *N. closterium*) which belonged to class diatoms, among dinoflagellates 5 species were (*Prorocentrum* sp., *P. micans*, *Protoperidinium* sp., *Gymnodinium* sp., *Alexandrium* sp.) commonly found in varying density in all the stations (stations 1-8). Green algae and blue green algae were found more concentrated towards the south of the study region but when compared to distribution pattern during monsoon the density distribution was low.

Table 3.4.5 (c) Phytoplankton Species composition during pre monsoon in the study region (-) absent, (+) 10-1000, (++) 1000-10,000 and (+++) >10,000 Cells L^{-1}

Phytoplankton Species/Stations	1	2	3	4	5	6	7	8
Diatoms (Centrales)								
Melosira sp.			+					
Melosira sulcata								
Skeletonema costatum	++	+++	+++	+++	+++	+++	++	++
Thalassiosira sp.	+++	+++	++	+++	++	+++	+++	+
Thalassiosira subtilis	++		+++	+++	++	++	+++	++
Coscinodiscus sp.	+		++	++		+	+	+
Coscinodiscus centralis	+++	+		+++	++			
Coscinodiscus marginatus				++	++	+++		
Coscinodiscus exentricus								
Coscinodiscus radiates		+		++	++	+		
Coscinodiscus gigas	+			+	++			
Coscinodiscus granii								
Coscinodiscus lineatus								
Hemidiscus sp.	+				+		+	
Planktoniella Sol.				+	+			
Cyclotella sp.	+			++	+	+	+	+
Laudaria sp.			+	+				+
Leptocylindrus sp.								
Leptrocylindrus danicus	+		++	+				+
Leptocylindrus minimus				++				
Rhizosolenia sp.				+		+		
Rhizosolenia delicatula					+			
Rhizosolenia setigera	+	+++	+++	++	+			
Rhizosolenia robusta	+		++			+		
Rhizosolenia imbricate				++	+			
Rhizosolenia alata			+					

Rhizosolenia curvata								
Rhizosolenia styliformis				++				
Rhizosolenia stolerforthii								
Bacteriastrum sp.								
Bacteriastrum hyalinum								
Chaetoceros sp.	+		+	+	+	++	+	+
Chaetoceros gracilis	++		+		+			
Chaetoceros calcitrans	+	+	+	+++	+++			
Chaetoceros lorenzianus				++				
Chaetoceros pseudocrevisetus								
Biddulphia sp								
Biddulphia sinensis	+			+	++			
Biddulphia mobeliensis	+			+		+		
Biddulphia auriata	+			+		++		
Ditylum sp.								
Ditylum brightwellii								
Hemialus sp.	+						+	+
Hemialus sinensis				+				
Eucampia sp.								
Eucampia zodiacus				+				
Diatoms (Pennales)								
Fragillaria sp.				+				
Fragillariopsis sp	++		++	+	++	++	+	
Grammatophora sp.								
Synedra sp.								
Synedra undulate								
Thalassionema sp								
Thalassionema nitzschiodes	+		++	+	++	+++		
Thalassiothrix sp.								
Thalassiothrix longisimma					+		+	
Thalassiothrix frauendfeldii	+++			+		+		
Asterionella japonica	+			+				
Asterionella glacialis								
Cocconeis sp.								
Navicula sp.	+			+		+	+	+
Navicula directa	+	++		+	+			
Navicula distans	+		++	++	+			
Navicula transitans	+			+	+++			
Navicula longa	+			+				
Gyrosigma sp.				+	+			
Pleurosigma sp.	+							
Pleurosigma elongatum	+	++	++	++	++			+
Pleurosigma normanii	+	+	++	+		+	+	
Pleurosigma directum	+		++	+++				
Pleurosigma aesturii								
Amphiprora sp.	+			+				
Amphiprora gigantia	+				+			+
Amphora sp.	+	+						
Amphora lineolata	+		+	+				

Amphora coffeaformis	+		+	+				
Nitzschia sp.	++		++	+++	+	++	++	
Nitzschia closterium	+	+++	+++	+++	+++	+++		++
Nitzschia longisimma	+++	+++	+++	+++	+++	+++		+++
Nitzschia sigma			++	+				
Nitzschia seriata	+		+		+	++		
Stauronies sp.				+				
Suirella sp.				+	+	+		
Surirella ovalis								
Diploneis sp.								
Diploneis smithii								+
Dinoflagellates								
Prorocentrum sp.				+	+	+	+	
Prorocentrum micans	+		++	+	+	++	++	+
Prorocentrum lima	+		+					
Prorocentrum gracilis	+			+	+	++	++	+
Protoperidinium sp.	+	++	++	+	+	++	++	+
Protoperidinium ovum				+				
Protoperidnium ovatum				+				
Protoperidinium brevipes				+				
Protoperidinium leonis		+		+				
Protoperidinium pentagonum					+			
Ceratium sp.	+			+				
Ceratium furca	+			+	++		+	
Ceratium horridum	+			+				
Ceratium symmetricum					+++			
Ceratium macroceros								
Ceratium tripos								
Alexandrium sp	+	+			+		+	
Pyrocystis sp.								
Pyrocycstis noctiluca								
Gymnodinium sp.	+			++			+	
Gyrodinium sp.					+			
Amphidinium sp.				+				
Corythodium sp.	+					+		
Gonyaulax sp	+		++	+	+	++	+++	++
Pyrophacus sp.	+			+			+	
Scripsiella sp.				+				
Dinophysis sp.								
Dinophysis caudata	++		+	+				
Dinophysis acuta				+				
Amphisolenia bidendata				+				
Ornithocercus sp.								
Pheodactylum triconatum								
Green Algae								
Chlorella sp.		++	+		+			+
Chlorella salina								
Chlorella vulgaris	++					++	+	+
Eudoria sp.								+
Volvox sp.						+		
Pediastrum sp.								
Pediastrum simplex			+	+		+	+	

Actinastrum sp.				+				
Euastrum sp.								
Coelastrum sp.			+			+	+	
Agmenellum sp.			+	+++				
Scenedesmus sp.				++		+		+
Micractinum sp.								
Closterium sp.								
Gomphosphaeria sp.								
Ankistrodesmus sp.								
Chlorococcum sp.		+++	+					
Blue Green Algae								
Anabaena sp.	+				+		+	+
Oscillatoria sp.				++				
Lyngbya sp.								
Oocystis sp.								
Microcystis sp.								
Sphaerocystis sp.								
Phaeocystis sp.								
Anacystis sp.		+	+					
Gonium sp.						+		+
Nostoc sp.	+	+	+	++			+	
Rivularia sp.								
Spirulina sp.						+		

During pre monsoon (Table.3.4.5 c) frequently observed phytoplankton species 16 belonged to diatoms (*S. costatum*, *Thalassiosira* sp., *T. subtilis*, *Coscinodiscus* sp., *Cyclotella* sp., *Rhizosolenia setigera*, *Chaetoceros sp*., *Chaetoceros calcitrans*, Fragillatiopsis sp., *Pleurosigma elongatum, P.normanii,* Nitzschia sp., *N. closterium, N. longisimma*), 3 belonged to dinoflagellates (*Prorocentrum micans*, Protoperidinium sp., *Gonyaulax* sp.). Species which belonged to green algae and blue green algae were sparsely distributed in the study region during pre monsoon. Among green algae species like *Chlorella vulgaris, Pediastrum simplex*, *Scenedesmus* sp. and among blue green algae Anabaena sp., *Nostoc* sp. were the common.

3.4.6 Phytoplankton Genera

A comparison of phytoplankton genera (Table.3.4.6) was made before Thanneermukkam bund was built (1972-1975), after (1975-2007) and present study (2008-2009). It was observed that out of 43 genera reported under the class diatom 8 were not present after Thanneermukkam bund was commissioned (*Hyalodiscus* sp., *Stephanopyxis* sp., *Schroderella* sp., *Climacodium* sp., *Steptotheca*., *Lithodesmium* sp., and *Denticula* sp) similarly out of 23 genera reported under class green algae 8 were not observed (*Cosmarium* sp., *Dismobryan* sp., *Holopedium* sp., *Hydrodictyon* sp., *Kirchneriella* sp., *Selenastrum* sp., *Xanthidium* sp., and *Mougoetia* sp.) during the present study. In the case of blue green algae, *Katagnymene* sp. was not observed.

It was also observed that few genera of diatoms, dinoflagellates, green algae and blue green algae which were non existant before the commission of the bund, has appeared after the bund was built. They were (*Asteromphalus* sp., *Actinoptycus* sp. and *Stauronies* sp.) representing diatoms, (*Pyrocystis* sp., *Prophacus* sp., *Ornithocerus* sp., *Amphisolenia* sp., *Pheodactylum* sp., and *Podolampus* sp.) dinoflagellates, (*Eudoria* sp., *Agmenellum* sp., *Gophosphaeria* sp., *Ankistrodesmus* sp. and *Pandoria* sp.) green algae and (*Sphaerocystis* sp. *Phaeocystis* sp.) blue green algae.

Table. 3.4.6 Major phytoplankton genera reported from the Cochin backwater (+) present and (-) absent

Sl. No.	Phytoplankton genera	1972-1975 (Before Thanneermukkam bund built)	1975-2007 (After Thanneermukkam bund built)	2008-2009 (present study)
	DIATOM			
1	*Melosira* sp.	+	+	+
2	*Skeletonema* sp.	+	+	+
3	*Thalassiosira* sp.	+	+	+
4	*Coscinodiscus* sp.	+	+	+
5	*Hemidiscus* sp.	+	+	+
6	*Asteromphalus* sp.	-	+	+
7	*Actinoptycus* sp.	-	+	+
8	*Planktoniella* sp.	+	+	+
9	*Cyclotella* sp.	+	+	+
10	*Laudaria* sp.	+	+	+
11	*Leptocylindrus* sp.	+	+	+
12	*Rhizosolenia* sp.	+	+	+
13	*Bacteriastrum* sp.	+	+	+
14	*Chaetoceros* sp.	+	+	+
15	*Biddulphia* sp.	+	+	+
16	*Ditylum* sp.	+	+	+
17	*Hemialus* sp.	+	+	+
18	*Eucampia* sp.	+	+	+
19	*Fragillaria* sp.	+	+	+
20	*Fragillariopsis* sp.	+	+	+
21	*Grammatophora* sp.	+	+	+
22	*Synedra* sp.	+	+	+
23	*Thalassionema* sp.	+	+	+
24	*Thalassiothrix* sp.	+	+	+
25	*Asterionella* sp.	+	+	+
26	*Cocconies* sp.	+	+	+
27	*Navicula* sp.	+	+	+
28	*Gyrosigma* sp.	+	+	+
29	*Pleurosigma* sp.	+	+	+
30	*Amphiprora* sp.	+	+	+

31	*Amphora* sp.	+	+	+
32	*Nitzschia* sp.	+	+	+
33	*Stauronies* sp.	-	+	+
34	*Surirella* sp.	+	+	+
35	*Diploneis* sp.	+	+	+
36	*Hyalodiscus* sp.	+	-	-
37	*Stephanopyxis* sp.	+	-	-
38	*Schroderella* sp.	+	-	-
39	*Climacodium* sp.	+	-	-
40	*Steptotheca* sp.	+	-	-
41	*Bellarochea* sp.	+	-	-
42	*Lithodesmium* sp.	+	-	-
43	*Denticula* sp.	+	-	-
DINOFLAGELLATE				
1	*Prorocentrum* sp.	+	+	+
2	*Protoperidinium* sp.	+	+	+
3	*Ceratium* sp.	+	+	+
4	*Alexandrium* sp.	+	+	+
5	*Pyrocystis* sp.	-	+	+
6	*Gymnodinium* sp.	+	+	+
7	*Gyrodinium* sp.	+	+	+
8	*Amphidinium* sp.	+	+	+
9	*Corythodium* sp.	-	+	+
11	*Gonyaulax* sp.	+	+	+
12	*Prophacus* sp.	-	+	+
13	*Scripsiella* sp.	+	+	+
14	*Dinophysis* sp.	+	+	+
15	*Amphisolenia* sp.	-	+	+
16	*Ornithocerus* sp.	-	+	+
17	*Pheodactylum* sp.	-	+	+
18	*Podolampus* sp.	-	+	+
19	*Phalacroma* sp.	+	+	+
20	*Cladopyxis* sp.	+	-	-
GREEN ALGAE				
1	*Chlorella* sp.	+	+	+
2	*Eudoria* sp.	-	+	+

3	*Volvox* sp.	+	+	+
4	*Pediastrum* sp.	+	+	+
5	*Actinastrum* sp.	+	+	+
6	*Euastrum* sp.	+	+	+
7	*Coelastrum* sp.	+	+	+
8	*Agmenellum* sp.	-	+	+
9	*Scenedesmus* sp.	+	+	+
10	*Micractinum* sp.	+	+	+
11	*Closterium* sp.	+	+	+
12	*Gomphosphaeria* sp.	-	+	+
13	*Ankistrodesmus* sp.	-	+	+
14	*Chlorococcum* sp.	+	+	+
15	*Pandorina* sp.	-	+	+
16	*Cosmarium* sp.	+	-	-
17	*Dismobryan* sp.	+	-	-
18	*Holopedium* sp.	+	-	-
19	*Hydrodictyon* sp.	+	-	-
20	*Kirchneriella* sp.	+	-	-
21	*Selenastrum* sp.	+	-	-
22	*Xanthidium* sp.	+	-	-
23	*Mougoetia* sp.	+	-	-
BLUE GREEN ALGAE				
1	*Anabaena* sp.	+	+	+
2	*Oscillatoria* sp.	+	+	+
3	*Lyngbya* sp.	+	+	+
4	*Oocystis* sp.	+	+	+
5	*Microcystis* sp.	+	+	+
6	*Sphaerocystis* sp.	-	+	+
7	*Phaeocystis* sp.	-	+	+
8	*Anacystis* sp.	+	+	+
9	*Gonium* sp.	+	+	+
10	*Nostoc* sp.	+	+	+
11	*Rivularia* sp.	+	+	+
12	*Spirulina* sp.	+	+	+
13	*Katagnymene* sp.	+	-	-

3.5. Statistical analysis

3.5.1 Pearson Correlation

Pearson correlation was carried out to find the influence of physico-chemical parameters (salinity, temperature, suspended particulate matter (SPM), pH, dissolved oxygen (DO), nitrite (NO_2), nitrate (NO_3), ammonia (NH_4), phosphate (PO_4) and silicate (SiO_4)) on biological parameters (phytoplankton density (TPD), total phytoplankton biomass (TPB), micro plankton biomass (MPB), nano plankton biomass (NPB) and pico plankton biomass (PPB)).

Table. 3.5.1 (a-h) Show the correlation between the physico-chemical parameters and biological parameters

(a) Station 1

	Salinity	Temperature	SPM	pH	DO	NO_2	NO_3	NH_4	PO_4	SiO_4
TPD	0.706*									
TPB										
MPB										
NPB										
PPB	-0.576*		0.667*	-0.628*	-0.896**					0.706*

**P*=0.576, significant correlations at the 0.05 level (two-tailed); ***P*=0.708, significant correlations at the 0.01 level (two-tailed)

In station 1 (Table. 3.5.1 a) TPD showed significant positive correlation (at $P \leq 0.05$) with salinity (r=0.706). Whereas PPB showed significant negative correlation ($P \leq 0.05$) with salinity (r= -0.576) and pH (r= -0.628) and positive correlation ($P \leq 0.05$) with SPM (r= 0.667). It was also

observed that PPB showed significant negative correlation ($P \leq 0.01$) with DO ($r^2 = -0.896$).

(b) Station 2

	Salinity	Temperature	SPM	pH	DO	NO_2	NO_3	NH_4	PO_4	SiO_4
TPD										
TPB	0.702*	0.610*	-0.705*							-0.615*
MPB	0.759**	0.614*	-0.727**							-0.663*
NPB	0.694*	0.605*	-0.685*							-0.614*
PPB	-0.579*									0.633*

*P=0.576, significant correlations at the 0.05 level (two-tailed); **P=0.708, significant correlations at the 0.01 level (two-tailed)

TPB and NPB at station 2 (Table. 3.5.1 b) showed significant correlation ($P \leq 0.05$) with salinity (r=0.702) and (r=0.694) and with temperature (r=0.610) and (r=605) whereas MPB showed significant positive correlation ($P \leq 0.01$) with salinity (r=0.759) and negative correlation ($P \leq 0.01$) with SPM (r= -0.727). TPB, MPB and NPB showed significant negative correlation ($P \leq 0.05$) with SiO_4 (r= -0.615, r= -0.663, r= -0.614) respectively whereas PPB showed positive correlation ($P \leq 0.05$) with SiO_4 (r=0.633).

(c) Station 3

	Salinity	Temperature	SPM	PH	DO	NO_2	NO_3	NH_4	PO_4	SiO_4
TPD								0.694*		
TPB										
MPB										
NPB										
PPB										

**P*=0.576, significant correlations at the 0.05 level (two-tailed); ***P*=0.708, significant correlations at the 0.01 level (two-tailed)

In station 3 (Table. 3.5.1 c) none of the physical or chemical parameters had any significant influence on phytoplankton parameters like TPD, TPB, MPB, NPB and PPB except TPD against NH_4 with a significant positive correlation (r=0.694) at ($P \leq 0.05$) significant level.

(d) Station 4

	Salinity	Temperature	SPM	PH	DO	NO_2	NO_3	NH_4	PO_4	SiO_4
TPD	0.586*						0.579*			
TPB										
MPB										
NPB										
PPB	-0.588*		0.581*	-0.581*		0.621*				0.639*

**P*=0.576, significant correlations at the 0.05 level (two-tailed); ***P*=0.708, significant correlations at the 0.01 level (two-tailed)

Pearson correlation at station 4 (Table. 3.5.1 d) showed that TPD had positive correlation ($P \leq 0.05$) with salinity (r = 0.586) and NO_3 (r =0.579) and PPB negative correlation ($P \leq 0.05$) with salinity (r= -0.588), pH (r = -0.581) and positive correlation with SPM (r= 0.581), NO_2 (r= 0.621) and SiO_4 (r= 0.639).

(e) Station 5

	Salinity	Temperature	SPM	PH	DO	NO_2	NO_3	NH_4	PO_4	SiO_4
TPD	-0.667*	0.635*								
TPB				-0.811**						0.632*
MPB				-0.763**						0.599*
NPB	-0.598*			-0.829**						0.634*
PPB				-0.801**			0.712**			0.627*

**P*=0.576, significant correlations at the 0.05 level (two-tailed); ***P*=0.708, significant correlations at the 0.01 level (two-tailed)

TPD and NPB showed significant negative correlation (*P*≤0.05) with salinity (*r*= -0.667) and (*r*= -0.598) at station 5 (Table. 3.5.1 e). TPB, MPB, NPB and PPB showed significant negative correlation (*P*≤0.01) with pH. With SiO_4, TPB, MPB, NPB and PPB had a significant positive correlation (*P*≤0.05) significant level.

(f) Station 6

	Salinity	Temperature	SPM	PH	DO	NO_2	NO_3	NH_4	PO_4	SiO_4
TPD	-0.621*					0.682*		0.676*		
TPB		0.638*								
MPB		0.641*								
NPB										
PPB	-0.885**		0.708**	-0.684*					0.728*	0.942**

**P*=0.576, significant correlations at the 0.05 level (two-tailed); ***P*=0.708, significant correlations at the 0.01 level (two-tailed)

TPD and PPB showed significant negative correlation (*P*≤0.05) with salinity (*r*= -0.621) and (*r*= -0.885) and TPB and MPB showed positive correlation (*P*≤0.05) with temperature (*r*=0.638) and (*r*=0.641) (Table. 3.5.1 f).

(g) Station 7

	Salinity	Temperature	SPM	PH	DO	NO_2	NO_3	NH_4	PO_4	SiO_4
TPD	-0.674*									
TPB										
MPB										
NPB										
PPB	-0.588*		0.642*							0.747**

**P*=0.576, significant correlations at the 0.05 level (two-tailed); ***P*=0.708, significant correlations at the 0.01 level (two-tailed)

TPD and PPB showed significant negative correlation (*P*≤0.05) with salinity (*r*= -0.674) and (*r*= -0.588) and PPB showed positive correlation with SPM (*r*= 0.642) and PPB showed significant positive correlation (*P*≤0.01) with SiO_4 (*r*=0.747) (Table. 3.5.1 g).

(h) Station 8

	Salinity	Temperature	SPM	PH	DO	NO_2	NO_3	NH_4	PO_4	SiO_4
TPD	-0.583*				0.611*					0.549*
TPB	-0.559									
MPB										
NPB	-0.572*		0.549*							
PPB	-0.802**		0.917**	-0.692*						0.695*

**P*=0.576, significant correlations at the 0.05 level (two-tailed); ***P*=0.708, significant correlations at the 0.01 level (two-tailed)

In station 8 (Table. 3.5.1 h) TPD, TPB and NPB showed negative correlation (*P*≤0.05) with salinity (*r*= -0.583), (*r*= -0.559) and (*r*= -0.572) and NPB showed positive correlation with SPM (*r*= 0.549). PPB showed positive correlation (*P*≤0.01) with salinity (*r*= 0.802) and SPM (*r*= 0.917).

The overall results from (Table. 3.5.1, a-h) showed that salinity and SPM has a major role in the distribution of phytoplankton biomass and density in the study region. Nutrients (NO_2, NO_3, NH_4 and PO_4) did not have any influence on phytoplankton abundance and biomass whereas SiO_4 showed positive and negative correlation with the phytoplankton biomass and density. This indicates that the system was dominated by diatoms because for their cell growth SiO_4 is an essential major nutrient.

3.5.2 Principle component analysis (PCA)

PCA was carried out between the physico-chemical variables and biological parameters to understand the influence of environmental conditions on the distribution of phytoplankton. During monsoon, the PC1 and PC2 axes explained 52% of the relationship, when all the biotic variables showed a close association with salinity and it was most prominent with the total phytoplankton density (TPD) (Figure 3.5.2 a).

During post monsoon, the first two axes could explain, 42.5 % of the variability, all biotic components (except PPB) were positively related to PO_4, especially at station 2 (Figure 3.5.2 b.). Though of the fractionated phytoplankton was relatively high at station 1 during monsoon, it was lowest during the post monsoon. The phytoplankton density was positively correlated to SiO_4, NO_3, SPM and temperature, especially in the southern part (Stations 6, 7 and 8) of the study region.

During pre monsoon, the first two PCAs explained 43% of the variability (Figure.3.5.2c). Total phytoplankton biomass (TPB) and fractionated phytoplankton biomass (NPB, MPB) was closely related whereas, PPB, a component of TPB showed distant relationship with other

biotic variables (TPB, NPB and MPB) especially at stations 4 and 5. During this period, except PPB all other biotic variables were closely related with PO_4 at stations 2 and 3.

The overall PCA bi plot revealed that the biotic parameters are positively related to salinity. PPB was significantly related with other biotic parameters (TPB, NPB and MPB). It was also found that the biotic parameters have positive relationship with PO_4. The total phytoplankton density (TPD) was also significantly related to SiO_4, NO_3, SPM and temperature in the southern part (stations 6, 7 and 8).

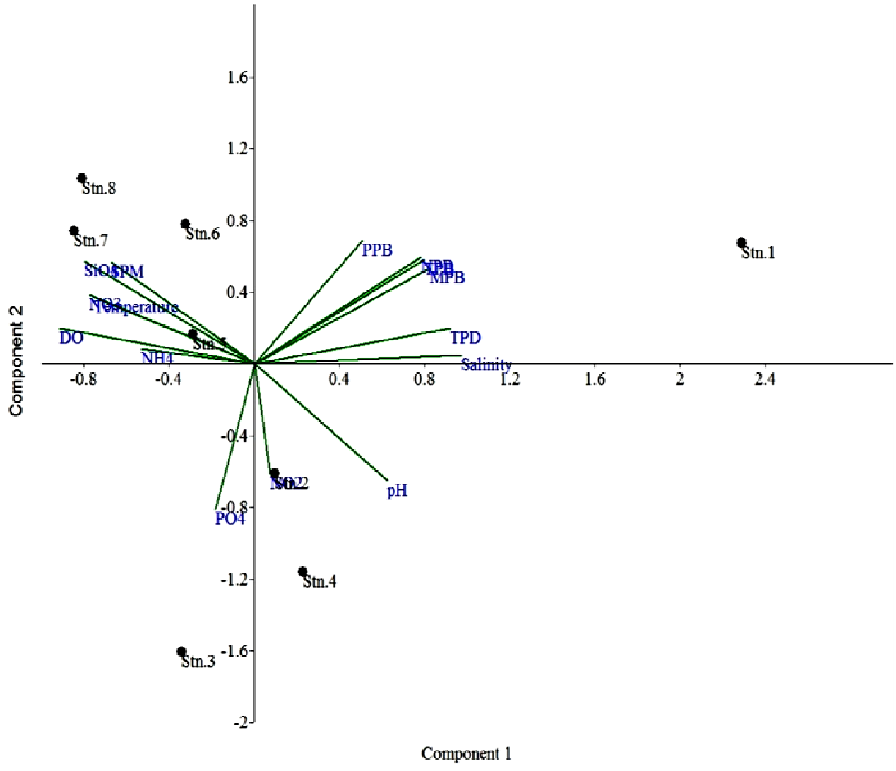

Figure. 3.5.2 (a) PCA analysis on physical, chemical and biological parameters during monsoon

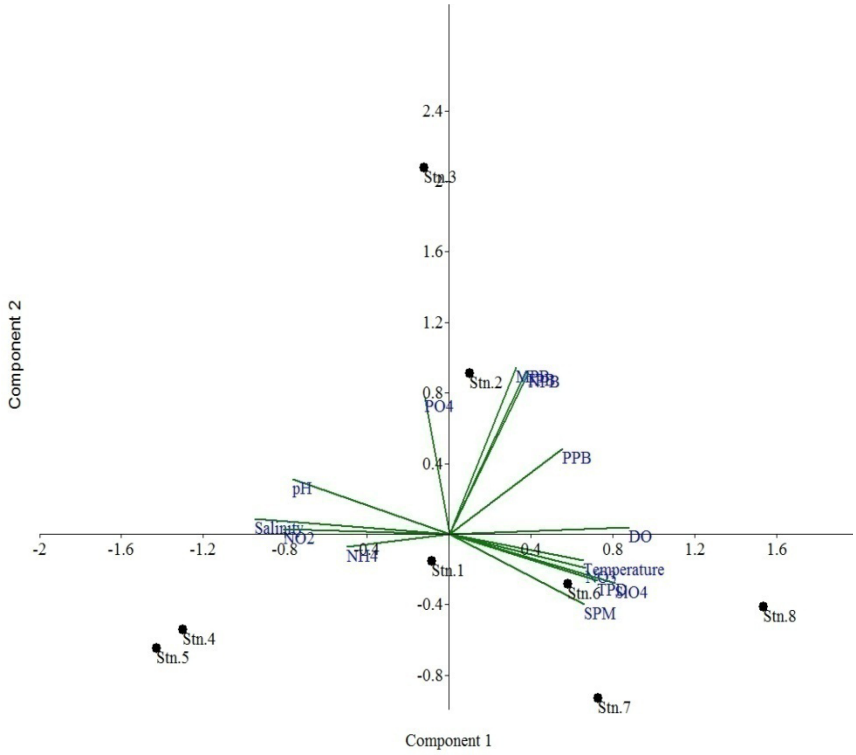

Figure. 3.5.2 (b) PCA analysis on physical, chemical and biological parameters during post monsoon

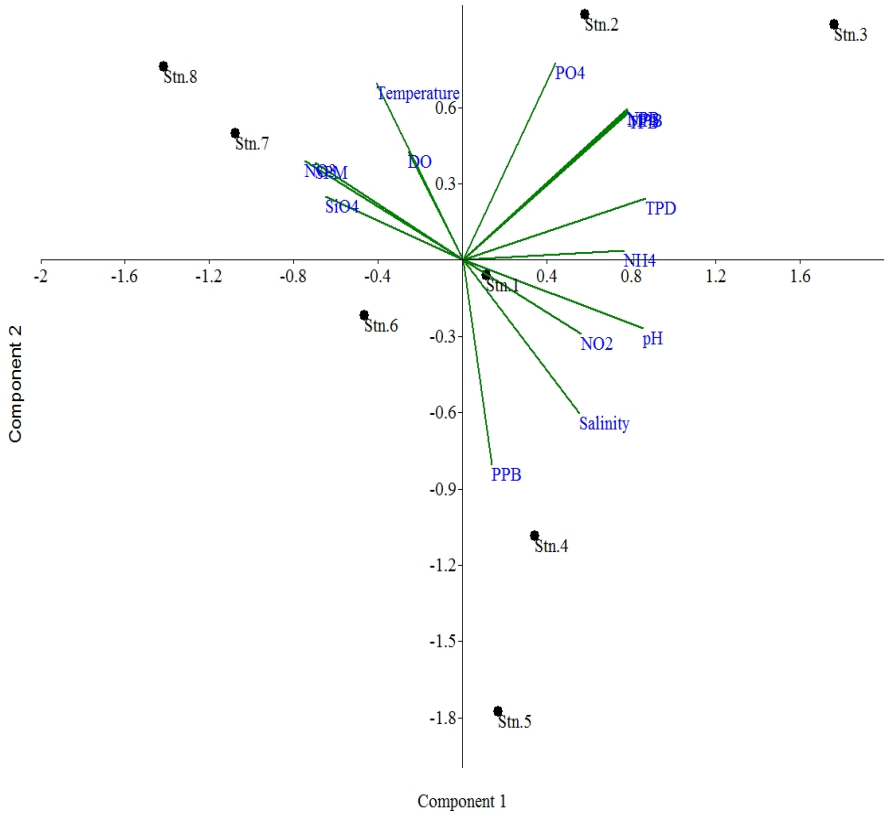

Figure. 3.5.2 (c) PCA analysis on physical, chemical and biological parameters during pre monsoon

3.5.3 Mann-Whitney U test

To distinguish the distribution pattern of phytoplankton in the two arms of the backwater, Mann-Whitney U test was performed. It was observed that no such significant differences between the two arms of the backwater with respect to biological parameters. This indicated that the phytoplankton species in the study region were dominated by euryhaline phytoplankton which is tolerant to any salinity range (Table. 3.6.3).

Table. 3.5.3 Mann-Whitney U test table

Mann Whitney Test	TPB	PPB	NPB	MPB	TPD
Column A (Northern arm) vs Column B (Southern arm)	Column A vs Column B				
P value	0.1631	0.431	0.1704	0.1427	0.3325
Are medians significant different? (P<0.05)	NS				
Exact or approximate P value?	Gaussian Approximation				
One or two tailed	One				
Sum of rank in Column A,B	167.5, 132.5	146.5, 153.5	167, 133	169, 131	158, 142
Mann Whitney	54.5	68.5	55	53	64

* NS= not significant

3.5.4. Species Diversity Index

3.5.4.1 (a) Shannon diversity index (H')

Species diversity index was computed using Shannon diversity index (H') using PRIMER version 5.2.8 (Clark and Warwick, 1994). The Shannon diversity index is commonly used to characterize species diversity in a community.

$$H' = -\sum_{i=1}^{R} p_i \log p_i$$

Species diversity index in the northern arm of the study region ranged from 0.8 to 3.0 (av. 1.9) with maximum at station 4 during March and minimum at station 2 during October. It was observed that station 1 showed comparatively high species diversity except during August (1.2)

and March (1.1). Species diversity at stations 2 and 3 was relatively low during October (0.8) and April (0.9) (Figure 3.5.4.1 a).

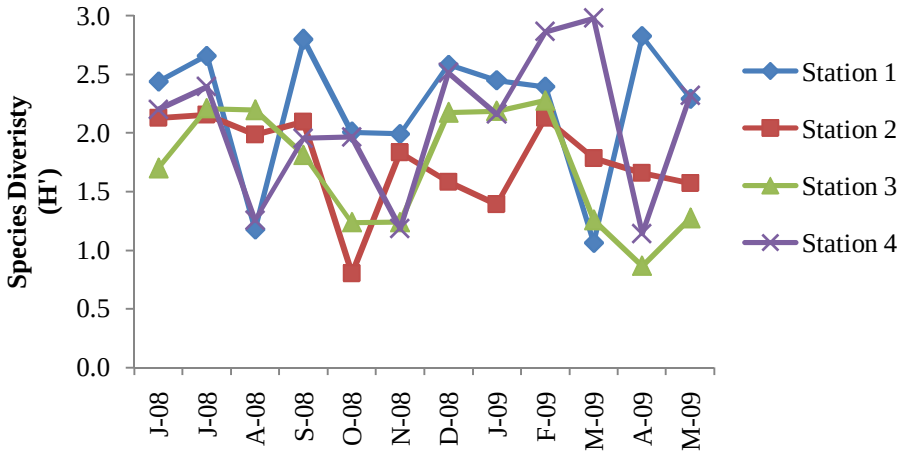

Figure 3.5.4.1 (a) Species diversity index in the northern arm

In the southern arm, the species diversity between 0.2 and 2.8 (av. 1.9) was relatively high during July to September with maximum at station 5 during February and minimum at station 8 during December (Figure 3.5.4.1 b)

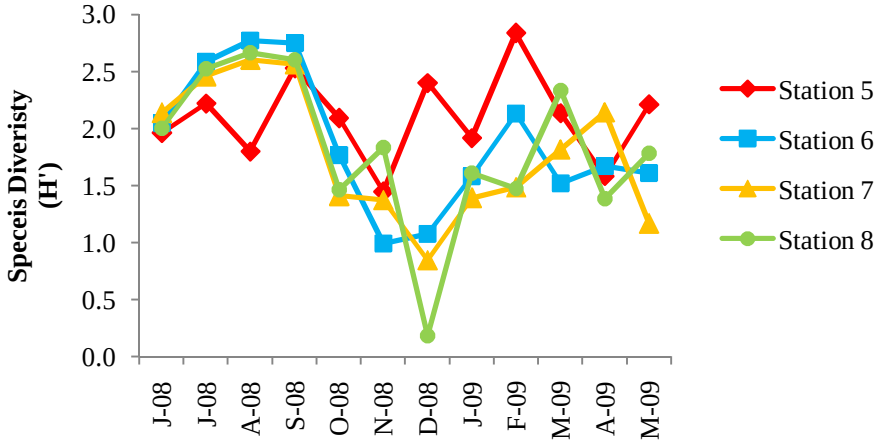

Figure 3.5.4.1 (b) Species diversity index in the southern arm

Seasonal distribution revealed that the species diversity was high at station 1 and low at station 3 during all the three seasons. It was relatively high during monsoon at stations 1, 6, 7 and 8. During post monsoon and pre monsoon high diversity index was observed only at stations 1, 4 and 5 (Figure 3.5.4.1 c). Diversity pattern was more or less similar at stations 4 and 5 during all the seasons.

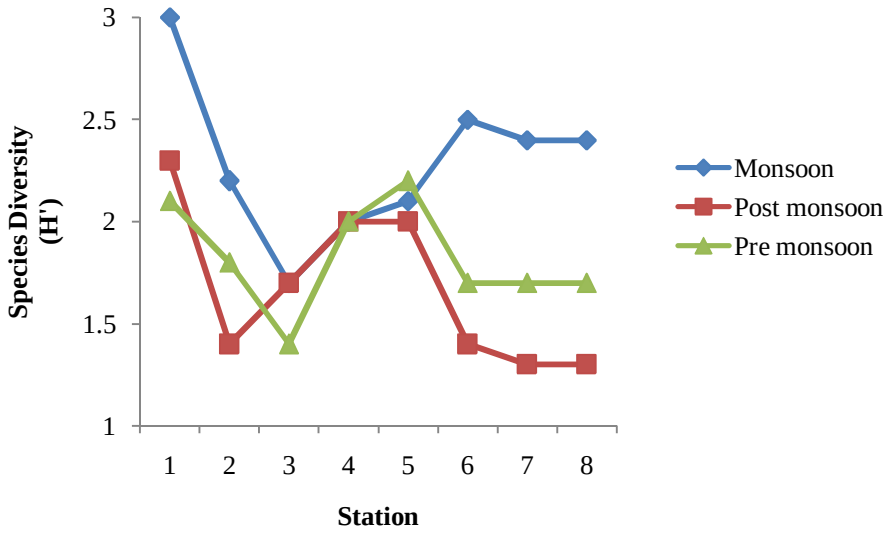

Figure 3.5.4.1 (c) Seasonal pattern of species diversity index in the study region

3.5.4.2 (a) Pielou's evenness index (J')

Species evenness index was computed by Pielou's evenness index (J') using PRIMER version 5.2.8 (Clark and Warwick, 1994). Species evenness refers to how close in numbers each species in an environment. **J'** is constrained between 0 and 1. The less variation between the species in the community, the **J'** is higher. The evenness of a community can be represented by Pielou's evenness index:

$$J' = \frac{H'}{H'_{max}}$$

Species evenness index ranged from 0.33 to 0.93 (av.0.69) in the northern arm with high values at station 1 during September, December and April, at station 3 during July, December and February and at station 4 during December. Species evenness index was low at station 1 during August and March and at station 2 during October (Figure 3.5.4.2 a). The lowest value (0.33) for the study period was observed at station 4 during April.

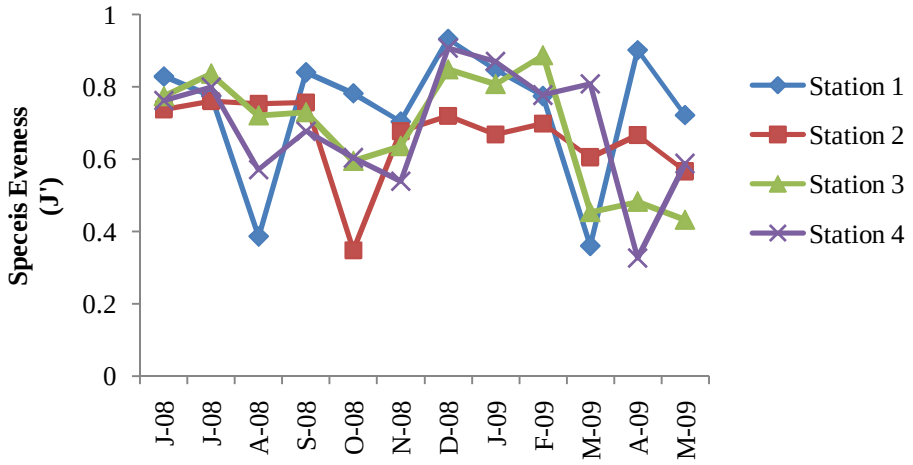

Figure 3.5.4.2 (a) Species evenness index in the northern arm

In the southern arm the species evenness index ranged between 0.11 and 1.00 (av. 0.7) with higher values at station 8 during September (0.96), January (1.0) and March (0.95) and at station 5 during December to February (0.88-0.92). The highest value (0.89) at stations 6 and 7 was observed during September. The lowest J' value (0.11) was observed at station 8 during December (Figure 3.5.4.2 b).

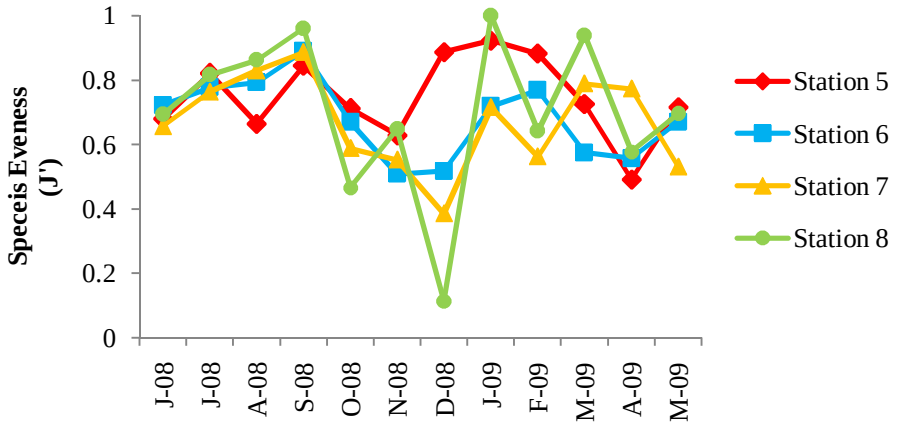

Figure 3.5.4.2 (b) Species evenness index in the southern arm

Species evenness index was generally high during the monsoon season as compared to pre and post monsoon season (Figure 3.5.4.2 c). It ranged from 0.7 (Station 4) to 0.83 (Station 8). The highest (0.82) value for the post monsoon season was observed at station 1 and the lowest (0.56) at station 8. During the pre monsoon season, the species evenness index was relatively low at all the stations compared to other two seasons with the highest value (0.73) at station 8 and the lowest value (0.56) at station 3.

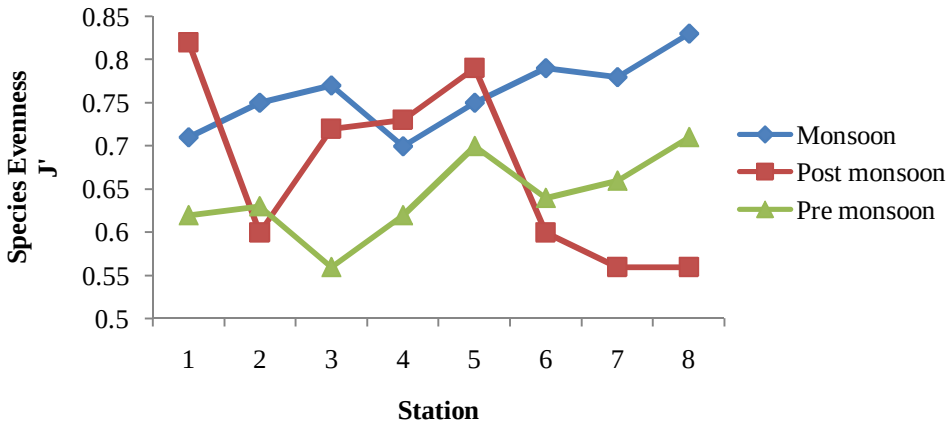

Figure 3.5.4.2 (c) Seasonal pattern of species evenness index in the study region

3.6 Discussion

Total phytoplankton biomass exhibited seasonal variation with high values in the northern and low values the southern sector of the study region. A fourfold decrease in biomass was observed in the southern region compared to north of the study region mainly due to the type of phytoplankton prevailing during the period. In the southern region only oligohaline species occurred, whereas in the northern arm the total phytoplankton biomass was contributed by euryhaline and marine species. Madhu *et. al.,* (2010) reported that high values of phytoplankton biomass were observed in the region where seawater incursion was high and low biomass in the riverine influx regions; thus substantiating the present observation.

The percentage contribution of size fractionated phytoplankton biomass (Figure 3.4.1.3 g & h) revealed that the pico fraction (<2 µm) was reduced from 11 % to 1% when the backwater was transformed from freshwater (monsoon) to marine (pre monsoon) condition. The reduction in the pico fraction during pre monsoon may be due to the presence of major planktonic grazers such as micro zooplankton (Protozoans) which is capable of grazing the pico plankton biomass considerably (Reigman *et.al.,* 1993). Micro zooplankton constitutes a considerable portion of zooplankton biomass in marine and estuarine environment (Pierce and Turner, 1992). Jyothibabu *et. al.,* (2006) reported high grazing rate of phytoplankton by micro zooplankton in the Cochin backwater during high saline period (pre monsoon season). In the case of nano plankton biomass (2-20 µm), no such significant change in biomass was seen throughout the

season. Therefore, it can be concluded that the major contribution to the total phytoplankton biomass is by nano plankton biomass. Qasim *et. al.,* (1974) and Madhu *et. al.,* (2007) have reported that the Cochin backwater is mainly constituted by nano plankton community and are more or less evenly distributed in the study region. These reports are in conformity with the current results. Nano phytoplankton has faster growth rate particularly in turbid waters, (Qasim 1974; Shiomoto 1997). In the case of micro fraction (>20 µm) there was a gradual increase in biomass from monsoon to pre monsoon as the larger cells need high light intensity for their proliferation (Glover *et. al.,* 1985; Cermeno *et. al.,* 2005). In the present study region the solar radiation during monsoon was less than 350 ly day^{-1} due to the cloud cover and rainfall (avg. 400 mm day^{-1}; Qasim 2003). In addition, during this season, the suspended particulate matter due to the terrestrial and riverine inputs may also be responsible for the reduction in intensity of light (Saraladevi *et. al.,* 1989).

The phytoplankton density was generally similar to the pattern of total phytoplankton biomass, with higher abundance in the northern region compared to the southern part of the study region (Figure 3.4.2 a & b). The abundance was high in April at station 3 (49.5 x 10^5 Cells L^{-1}) and August at Station 1 (38.6 x 10^5 Cells L^{-1}). These high values were contributed by *S. costatum, T. subtilis, N. closterium* and *R. setigera* at station 3 and *P. lima*, *Protoperidinium* sp., *Gymnodinium* sp., *Alexandrium* sp. at station 1 (Figure. 3.4.2 a). In the southern arm, the high abundance (19.2 x 10^5 Cells L^{-1}) at station 6 during November was contributed by *S. costatum, N. closterium, C.centralis and Prorocentrum* sp. (Figure 3.4.2 b). The

phytoplankton distribution in this study is in agreement with those reported by (Gopinathan, 1972; Gopinathan *et.al.,* 1974; Devassy and Bhattatiri 1974; Gopinathan *et. al.,* 1975; Joseph and Pillai 1975; Joy *et. al.,* 1990; Menon *et. al.,* 2000; Madhu *et. al.,* 2007 and 2010) from the Cochin backwater.

The seasonal phytoplankton abundance (Figure 3.4.2 c) at station 3 in the northern arm during pre monsoon showed higher abundance when compared to other stations. This may probably be due to the eutrophic condition that is prevailing in this region as a result of industrial discharge (Joy *et. al.,* 1990) and also due to sea water intrusion during this period which brings marine species into the backwater (Menon *et. al.,* 2000; Madhu *et. al.,* 2007 and 2010).

Phytoplankton community was composed of Diatoms, Dinoflagellates, Green algae and Blue green algae. The percentage contribution of each class to the total abundance was similar in both the arms of the study region. Diatom dominated (> 80%) the community followed by dinoflagellates (> 5%), green algae (< 5%) and blue green algae (< 3%) in the northern and southern arm (Figure 3.4.3 a & b). There was not much variation in the overall percentage contribution of phytoplankton community with the earlier findings of (Gopinathan 1972; Gopinathan *et. al.,* 1974; Gopinathan *et. al.,* 1975; Joseph and Pillai 1975; Devassy and Bhattatiri 1981; Joy *et.al.,* 1990; Menon *et. al.,* 2000; Madhu *et. al.,* 2007 and 2010). It was observed that diatoms in the study region did not vary much, but dinoflagellates, increased during post monsoon and peaked during pre monsoon. The green and blue green algae showed a

reducing trend over the seasons. The dominance of diatoms in the study region throughout the year may be due to their euryhaline and eurythermal nature which allows them to grow quickly under eutrophic conditions (Huang *et. al.,* 2004). On the other hand, dinoflagellates mostly prefer oligotrophic waters and hence fail to survive in eutrophic waters by competing with diatoms (Menzel *et. al.,* 1963; Cushing, 1989). During pre monsoon season, dinoflagellate density showed an increasing trend when the backwater nutrient level was getting depleted and salinity level was increasing as compared to monsoon season. In short, the system was turning to slightly oligotrophic condition during pre monsoon season (Joseph and Pillai 1975). In the case of green algae and blue green algae their density was found to be high during monsoon season, owing to their affinity towards low salinity. In the earlier works (Gopinathan *et.al.,* 1975; Joseph and Pillai 1975 and Madhu *et. al.,* 2010) it has been reported that green algae and blue green algae are fresh water forms.

A qualitative study on phytoplankton species composition revealed that *C. marginatus, C. centralis, C. lorenzianus, C. calcitrans, A. lineolata, A. coffeaformis, P. lima, Gymnodinium* sp*., Peridinium* sp*.,* occurred in higher density only during pre monsoon when the estuary was sea water dominant suggesting that these are stenohaline species. On the other hand, species like *S. costatum, T. subtilis, P. elongatum, P. directum, N. directa, N. distans, N. transitans, N. closterium* are euryhaline species as these were present in the study region during all seasons, tolerating change in salinity. However, species like *T. nitzschioides, C. salina, C. vulgaris, Scenedesmus* sp*., Closterium sp.* and *Anabaena* sp*.,* are

freshwater to brackish water forms as these species occurred in freshwater to low saline waters. The present phytoplankton species are in partial agreement with the earlier works of (Joy *et. al.,* 1990 and Madhu *et. al.,* 2010).

A comparison made on the distribution of phytoplankton genera (Table.3.4.6) before Thanneermukkam bund was built (1972-1975), after (1975-2010) and present study (2008-2009), it was observed that out of 43 genera reported under the class diatom 8 species have disappeared after Thanneermukkam bund was commissioned. Similarly out of 23 genera reported under class green algae 8 were not observed (*Cosmarium* sp., *Dismobryan* sp., *Holopedium* sp., *Hydrodictyon* sp., *Kirchneriella* sp., *Selenastrum* sp., *Xanthidium* sp., and *Mougoetia* sp.) whereas, in the case of blue green algae, *Katagnymene* sp. was not observed. It was also observed that few genera under the class diatoms, dinoflagellates, green algae and blue green algae which did not exist before the commission of the bund have newly appeared after the bund was built. This may be due to the construction of Thaneermukkam bund in 1975, as it has stopped free movement of fresh and brackish water phytoplankton geneses resulting in the elimination/reduction. It can also be due to effect of eutrophication which causes exponential growth of some selected species of phytoplankton causing the loss of biodiversity (Verlekar *et. al.,* 2006).

Among the dinoflagellates, *C. fusus, P. lima, Gymnodinium* sp*., Peridinium* sp*.* and *D. caudata* were found only during pre monsoon when the estuary was sea water dominant. The present observation is in accordance with the earlier studies of (Joy *et. al.,* 1990 and Madhu *et. al.,*,

(2010). Dinoflagellates can also survive at lower nutrient concentrations than diatoms (Qasim *et.al.,* 1972; Joseph and Pillai 1975 ; and Madhu *et.al.,* 2007 and 2010), which explains their appearance during the pre monsoon months.

The PCA analysis (Figure. 3.5.2 a-c) revealed that the biotic parameters like total phytoplankton density (TPD), total phytoplankton biomass (TPB) and size fractionated phytoplankton biomass (MPB, NPB and PPB) were influenced by salinity in most of the stations but was more prominent at stations 1, 4 and 5 as these stations were sea water dominant during pre monsoon. This indicates that salinity is an important factor influencing the phytoplankton community. The flow pattern affects their distribution through physical flushing as well as by controlling the salinity and nutrient gradients to which the cells are exposed (Patil and Anil 2011).

Mann-Whitney U test (Table. 3.6.3) confirmed that there was no significant difference in the phytoplankton species distribution between northern and southern arms even though fresh water and marine species existed. From the U test, it can be concluded that the system was dominated by euryhaline species. They are the major players in the backwater and density was not affected by any physico-chemical parameters, they were present in the backwater throughout period. Phytoplankton in the Cochin backwater are broadly divided into two (a) flora which are well adapted to the fluctuating estuarine conditions and (b) those which are not adapted to the fluctuating estuarine condition. The former comprise of typical estuarine forms which are euryhaline and may

be permanent resident and the latter represents either freshwater or marine forms which migrate to the estuary and inhabit only for short periods (Joseph and Pillai 1975). The study region is chiefly represented by the euryhaline forms, but at the inlet regions marine forms are found in large numbers during pre monsoon and in the upstream the fresh water and brackish water forms during monsoon and post monsoon. This shows the community shifts of phytoplankton due to the changes in salinity that commonly occurred in the Cochin backwater (Devassy and Bhattathiri 1974).

The result show a relatively high species diversity index H' during monsoon compared to post monsoon and pre monsoon season (Figure 3.4.4.1 c). This was probably caused by the co-existence of euryhaline (*S. costatum, T. subtilis, P. elongatum, P. directum, N. distans, N. transitans, N. closterium*), fresh water (*Volvox* sp., *Rivularia* sp. *Lyngbya* sp. *Gonium* sp., *Spirulina* sp.) and brackish water species (*T. nitzschioides*, *C. salina*, *C. vulgaris*, *Scenedesmus sp*., *Closterium sp*. *Anabaena sp*.). During post monsoon and pre monsoon seasons, only the euryhaline and marine species were present because the increased salinity. The salinity during the present study was maximum during the pre monsoon season and minimum during the monsoon season. Hence, salinity could be a major factor controlling the phytoplankton population in the estuary. Devassy and Bhattathiri (1974) have reported that the species diversity index in the Cochin estuary ranged from 1.58 to 4.5 with maximum during monsoon (> 4) and minimum during pre monsoon season. In the Veeranam lake, species diversity ranged from 0.49 to 4.2 (Senthilkumar and Sivakumar

2008), and the in Mandovi and Zuari estuaries the phytoplankton species diversity index was 0.49 to 4.2 and 1.1 to 4.1 respectively. It appears that the low species diversity (3.0) observed in the present study could be due to the effect of the salinity barrier at Thaneermukkam that stopped free movement of fresh water into the estuary, resulting in elimination/reduction of some species. Although information on phytoplankton species diversity index is not available from this region, a reduction in the species diversity compared to that of 1974 could be due to the construction of the bund and other modifications in the backwater. Studies have indicated that the construction of Thanneermukkom bund in the southern estuary has not only restricted the entry of saline water into the paddy fields, but also prevented the estuarine migration of prawns and fishes (Qasim and Madhupratap 1979). Secondly, a large area of the backwater around Vallarpadom and Ramanthuruth (lower estuary) has been modified as an oil tanker jetty and crude oil Terminal. This has resulted in altering the circulation pattern causing severe depletion of oyster beds and spat fall (Purushan, 1978). Moreover, heavy siltation resulting from dumping of dredged material has led to depletion of fauna and flora of the Cochin backwater (Gopinathan and Qasim 1971).

Chapter 4

Factors Influencing the Growth of Phytoplankton: An Experimental Approach

4.1. Introduction

In-situ observations on environmental variables and phytoplankton growth provide only a broad relationship. Experimental studies on the influence of environmental factors on phytoplankton cultures is perhaps the only reliable approach for determining the extent of limitation imposed by different factors. The significance of such experiments lies on the prediction of ecological processes through mathematical models. Thus, the purpose of this study was to conduct laboratory studies undertaken to measure phytoplankton growth under simulated environmental conditions. The factors considered for this study are: (a) salinity (b) light (c) copepod grazing and (d) prey (phytoplankton) size selectivity by copepod.

The conclusion derived in Chapter 3 was that salinity and light are the major physical forces and grazing of phytoplankton by copepod is the major biological factor that limits the growth of phytoplankton in the study region. The present experiment was therefore, designed to measure the

extent to which salinity, light intensity and grazing by copepods control the distribution and growth of phytoplankton in the Cochin backwater.

In estuaries, the mixing creates the well-known estuarine salinity gradient, with seawater near the mouth of the estuary to freshwater near the head of the estuary (Admiraal, 1977; Miller and Kamykowski 1986; Rijstenbil 1989; Kirst 1990; Flameling and Kromkamp 1994; Bisson and Kirst 1995). The salinity gradients influence the phytoplankton growth, because different species have different salinity preferences. Some phytoplankton are freshwater species, others are of marine origin, whereas some others prefer environments that are more saline than others. Phytoplankton generally exhibit a tolerance to a range in the salinity beyond which, they inhibit the growth.

In aquatic environments (sea, estuaries or lakes), the amount of light incident on the surface is rapidly reduced with depth by an exponential function (i.e. not linear). In general, light intensity declines exponentially with depth as described by the Beer-Lambert Equation. The depth of the euphotic zone suitable for photosynthesis is the depth where light energy is reduced to 1% of the intensity (Krik and Oliver 1995). A major interference to the light availability in estuaries is suspended particulate matter (SPM), brought through land runoff which leads to turbidity in the water column. This in, turn attenuates and scatters the light. As the amount of SPM increases, the photic depth decreases (i.e. photic depth above 1% of surface incident light). Light availability in the photic depth influences phytoplankton growth, pigment content and

photosynthetic rate (Yentsch and Ryther 1957). Phytoplankton growth is linearly related to the amount of light intensity or irradiance falling on an individual cell up to a point when no further increase occurs, i.e. saturation (Falkowski and Raven 1997). Photosynthesis vs light can be represented by a P-I curve (Webb *et. al.,* 1974), which is now widely accepted as a useful relationship for examining the photophysiology of phytoplankton (Henley, 1993).

Copepods, the dominant species of mesozooplankton in any aquatic system, (Calbet *et. al.,* 2000; Froneman 2000 and Lo *et. al.,* 2004) play a pivotal role in transferring energy from the primary trophic level to higher trophic levels. (Raymont 1980; Humes 1994). Hence, quantifying the rates of phytoplankton grazing by copepods is essential for understanding the mechanism that regulate phytoplankton populations in any aquatic ecosystems (Morales et. al. 1990; Landry *et. al*., 1995 a, b; Froneman *et. al.,* 1997; Sautour *et. al.,* 2000).

4.2. Review of Literature

Salinity in an estuary is a dynamic entity regulated by the river discharge, rainfall and tide. Phytoplankton communities are adapted to a certain range of salinity and show complex pattern of distribution along the salinity gradient.

Qasim *et. al.,* (1972 a) reported that tropical phytoplankton species show wide adaptability to changes in salinity.

Desikachary and Rao (1972) studied the salinity preferences of cultured diatoms grouped into euryhaline (tolerate wide range of salinity) and stenohaline (tolerance to very narrow salinity range) species. Any change in salinity is sensitive enough to affect stenohaline phytoplankton species and could alter the phytoplankton community structure into new stable community.

Qasim *et. al.,* (1968) reported that Cochin backwater receives maximum solar radiation (500-580 g cal cm^{-2} d^{-1}) from January to April and minimum (250-300 g cal cm^{-2} d^{-1}) during July and August. The high turbidity prevailing in the backwater greatly reduces light penetration and hence, production of phytoplankton. Qasim *et. al.,* (1972 b) studied the effect of solar illumination on phytoplankton using ^{14}C technique and reported that light is never a limiting factor for phytoplankton growth in tropics, but the turbidity due to suspended particulate matter do limit the phytoplankton growth.

Similar kind of studies made elsewhere (Alpine and Cloern, 1988; Lusia and Cantera, 1993; Macedo *et. al.,* 2001) have shown that phytoplankton growth is largely controlled by light availability. According to these studies the phytoplankton cells reside in a turbulent medium of an upper photic zone sustains photosynthesis, but the lower aphotic zone does not. Cloern (1987) reported that the photic depth is characteristically shallow in estuaries because of high suspended particulate matter. Hence the mean light exposure of phytoplankton cells and their growth rates are relatively low.

Guillard and Rhyther (1962) studied the growth rate of marine phytoplankton and reported that salinity changes can result in osmotic stress and affect the cellular ionic ratio in phytoplankton. Underwood and Provot (2000) studied the preferences of estuarine diatoms across a range of salinity. Hayatti (2007) studied the effect of salinity on growth and distribution of freshwater diatoms.

Menon *et. al.,* (1971) studied the biomass and faunal composition of the zooplankton in the Cochin backwater. Zooplankton distribution along salinity gradient in Cochin backwater was reported by Nair and Tranter (1971). Haridas *et. al.,* (1973) have studied the salinity, temperature, dissolved oxygen and zooplankton biomass of the backwater from Cochin to Alleppy. Rao *et. al.,* (1975) studied the distribution of zooplankton in space and time in the Cochin backwater. Madhupratap and Haridas (1976) have explained the composition and variations in the abundance of zooplankton of backwater from Cochin to Alleppy. Madhupratap (1978 and 1980) also studied the distribution, community structure and species succession of copepods in the Cochin backwater. Annual variation in zooplankton from a polluted coastal environment was reported by (Haridas *et. al.,* 1980; Madhu *et.al.,* 2007) studied the monsoonal impact on the standing stock and distribution of plankton. Despite these numerous work on copepod ecology, the feeding behavior of copepods on phytoplankton community is not yet studied in the Indian waters. The only study on copepod feeding from the Indian waters is that of (Goes *et. al.,* 1999) where in they have studied the inter-relationship between phytoplankton and copepods. Achuthankutty *et. al.,* (2000), have studied the influence of

salinity on feeding, survival rate, growth and neonate production of cladocera which is a mesozooplankton, but coming under different class.

The effect of phytoplankton size on copepod feeding have been of major concern in a number of recent studies (Hansen *et al.,* 1997; Romam and Gauzens 1997; Gowen *et. al.,* 1999; Head *et. al.,* 1999) made outside the Indian waters.

4.3. Materials and Methodology

4.3.1. Isolation and culture of phytoplankton

Phytoplankton was isolated from the lower estuary Fort Kochi (Stn. 4 described in Chapters 2 & 3) by collecting 3 L of sea water during the spring tide and as brought to the laboratory in ice box. In the laboratory, seawater was filtered through 200 µm bolting silk to remove larger grazers; the filtered seawater was allowed to settle for a minimum five hours. After sedimentation of sea water sample was concentrated to 100 ml and from this 5 ml was added to ten sets of F/2 media prepared in autoclaved seawater. The whole experimental setup was then kept in algal rack provided with ambient light (12 L: 12 D) for a period of 14 days until colour developed inside the flask.

Phytoplankton was isolated from the mixed culture following (a) serial dilution and (b) agar plating method.

(a) Dilution culture method

Serial dilution was followed by the procedure of (Michael *et. al.,* 2004). Dispensed 9 ml sterilized f/2 medium in 5 glass vials of capacity 15 ml, added 1 ml of sample taken from the mixed stock culture into first glass vial and made the dilution 10^{-1}. From this dispensed 1 ml sample to the second vial and made the dilution 10^{-2} and continued the dilution until 10^{-5}.

(b) Agar plating method

1.5 g of bacterio agar (HiMedia) was added to 1 L of filtered (0.22 µm) estuarine water. The solution was then sterilized in an autoclave for 15 minutes under 150 lb pressure and 120º C temperature. After cooling, the medium was poured into sterilized petri plates and kept for 24 hr. From the concentrated phytoplankton sample, 1 ml of sub sample was streaked on to the agar plate and kept for incubation. These agar plates were then incubated in an algal chamber for 7-8 days providing light and dark period (12 L-12 D hr). Phytoplankton colonies developed on the agar plates were isolated species wise (individual cells) using micro blades under an inverted epifluorescence microscope (Olympus CK IX 51) and transferred to culture tubes and grown as mass mono culture (Michael *et. al.,*, 2004).

4.3.2. Phytoplankton growth rate (optimal and varying condition)

Common and major phytoplankton species were isolated using the above techniques (a & b) and were mono cultured. To study the optimum growth rate of phytoplankton, ambient conditions were provided and incubated for 7 to 8 days and growth rate were measured once in a days.

Growth rate studies on varying salinities were carried out by selecting the salinity ranges in the order 0, 5, 10, 15, 20, 25, 30 and 35 which is the salinity range encountered in the study region. These salinities were achieved by diluting the sea water with distilled water and artificial nutrient medium was provided (F/2 medium, HiMedia) to maintain the nutrients level in the cultures till the end of the experiment (Robertson, 2005). The growth rate was calculated

$\mu\ (d^{-1}) = (\ln N_t - N_0)/t_2\text{-}t_1$

Where,

N_t = Final density

N_o = Initial density

t_2 = Final incubation

t_1 = Initial incubation

F/2 Media Preparation Chart

f/2 medium* (Guillard and Ryther 1963)
Updated April 2007

FOUR STOCK SOLUTIONS (1-4)
OBS! For all solutions, use sterilized distilled deionized water!

		For 1L	For 0.5L
1. $NaNO_3$ stock solution	$NaNO_3$	75.0 g	37.5 g
2. NaH_2PO_4 stock solution	NaH_2PO_4	5.0 g	2.5 g

3. Trace Metals stock solution
To distilled water add the following:

	For 1L	For 0.5L
Na_2EDTA	4.36 g	2.18 g
$FeCl_3 \cdot 6H_2O$ (Ferric Chloride)	3.15 g	1.575 g
Primary Metals Stocks (below)	1ml of each of the five	0.5ml of each of five

Primary Trace Metals stock solutions (make up five separate stocks)
To the chosen volume of sterile distilled deionized water add the following:

	100ml	50ml	10ml
$CuSO_4 \cdot 5H_2O$	*1.0 g*	0.50 g	0.10 g
$ZnSO_4 \cdot 7H_2O$	*2.2 g*	1.10 g	0.22 g
$CoCl_2 \cdot 6H_2O$	*1.0 g*	0.50 g	0.10 g
$MnCl_2 \cdot 4H_2O$	*1.8 g*	0.90 g	0.18 g
$NaMoO_4 \cdot 2H_2O$	*0.63 g*	0.315g	0.063 g

4. Vitamin Stock solution
Light sensitive – keep covered in foil!

	For 1.0 L	For 0.5L
Biotin	10.0 mL of 0.1 $mg \cdot mL^{-1}$ solution (1mg in 10ml)	5.0 mL
Vitamin B_{12}	1.0 mL of 1.0 $mg \cdot mL^{-1}$ solution (1mg in 1ml)	0.5 mL
Thiamine HCl	0.2g	0.1 g

Lastly: Making Final Medium
To 950 mL of 0.22 µM filtered seawater (FSW) add:

		To make 100 tubes:
$NaNO_3$ Stock solution	1.0 mL	100 ml
NaH_2PO_4 Stock Solution	1.0 mL	100 ml
Trace Metals Stock Solution	1.0 mL	100 ml
Vitamin Stock Solution	0.5 mL	50 ml

Filter sterilize at 0.22 µM before use and store at 4° C. * Si has been removed from this recipe to reduce the growth of contaminating diatoms.
Tip: Make up a larger batch, just multiply each stock by how many tubes you want to set up. For example, for 100 tubes of 3.5ml total of f/2 final medium stock, add 100ml of each of the first three stocks and 50 ml of the Vitamin stock. That gives a total of 350 ml, which gives 100 15ml falcon tubes of 3.5 ml each of f/2 final medium stock, each of which is ready to make up one each of 1.0L working f/2 media (one tube of 3.5 ml plus 950ml of filtered seawater.

4.3.3 Photosynthesis-Irradiance (P/I) experiment

Water samples were filtered through 200 µm nylon meshes and dispensed into culture bottles (60 ml) and each culture bottle was spiked

with approximately 1 ml of 5 µ Ci of $NaH^{14}CO_3$. The culture bottles were then incubated in light gradient incubator with an external light provided by 1500 W tungsten halogen lamps. Irradiance (ambient condition) was measured using LICOR meter (Bio Spherical Instruments, USA). Attenuation was achieved by neutral density filters and incubated for 2 hr in ambient temperature. Heat produced by the lamp was dissipated using a cold water flow system. Following incubation, samples were filtered in low vacuum (≤250 mm Hg) onto GF/F filter paper (0.7 µm pore size, 25 mm dia) and each filter paper was placed in separate scintillation vial, after fumigating with concentrated HCl. The photosynthetic rate was normalized by dividing with chlorophyll *a* and expressed in (mg C (mg Chl *a*) $^{-1}$ h^{-1}). P/I curves were plotted based on the equation [Pm^B (1-exp (-αI/Pm^B))] (Platt *et. al.,* 1980), using the software ROPE (R Ocean Production Extensions, Version 1.1, Canada 2007).

4.3.4. Estimation of copepod grazing (Gut pigment content)

4.3.4. (a) Copepod Grazing on phytoplankton biomass

Mesozooplankton samples for this study were collected from Cochin backwater (Stn. 4, Fort Kochi, Chapter 2 & 3) during monsoon (2008) and pre monsoon (2009) period using WP net (working plankton net) of mesh size 200 µm and brought to laboratory in ice box. The frozen zooplankton samples were thawed and washed with filtered seawater to remove adhering algae and debris. Copepods (Calanoid) which were the dominant (comprising > 70% of the total mesozooplankton) were carefully sorted and extracted in 5 ml of 90% aqueous acetone maintained at 4º C

in the dark without homogenization in a refrigerator (Atkinson 1996, Hwang et al. 1998, Wong et al. 1998). After extraction overnight, the solution was centrifuged, and the upper clear layer was measured using a Turner Design Model 7200 fluorometer in the laboratory. The extract was then acidified with 0.1ml of 10% HCl and measured again. Due to pheopigment loss during the experiment, all pheopigment values were multiplied by a factor of 1.51 according to (Dagg and Wyman 1983). Gut pigment content was expressed as ng chlorophyll *a* per individual copepod obtained from the addition of Chl *a* and pheopigment (pheophorbide expressed as Chl *a* equivalent) concentrations in the gut. Gut pigment content was calculated using the formula:

$$\textbf{ng chlorophyll/copepod} = K(F_0 - F_a)/n$$

$$\textbf{ng pheophytin/copepod} = K(RF_0 - F_a)/n$$

Where,

K= machine calibration constant

F_0= before acidification

F_a= after acidification

R= acidification ratio

n= number of copepods

4.3.4. (b) Prey-size selectivity of Copepod

From the copepod samples *Acartia tropica* and *Pseudodiaptomous annandalei* were sorted out from the zooplankton sample which was dominant species during monsoon and pre monsoon. They were carefully sorted out and transferred to filtered seawater and starved overnight. Three sets of grazing experiments were conducted in which the increase in total gut pigment (Chl *a*) was used as a measure of ingestion rates. Experiments were performed with phytoplankton like *Chlorella vulgaris* (6 µm), *Skeletonema costatum* (10 µm), *Nitzschia closterium* (45 µm) and *Coscinodiscus centralis* (105 µm) as prey with different size fractions for studying the size selectivity of copepods.

4.4. Result

4.4.1. Phytoplankton growth at optimal and at varying salinity condition

Growth rate (per day division of the cell) study is one important way of expressing the relative ecological succession of phytoplankton species or strains in adapting to its natural environment or the experimental environment imposed upon it. In an experimental condition there are four main phases of growth (lag, exponential, stationary and death phase). In Plate 4.4.1 only three phases are demonstrated because increase in growth rate occurs only during these three phases. Lag phase is the acclimatization period of phytoplankton to new environment. Once adapted to the conditions, the rate of cell division accelerates and increase in phytoplankton cell number in the culture, this

period is called exponential phase. Cell division rate then slows as light penetration through the culture is limited and also the nutrient. The culture then enters the stationary phase.

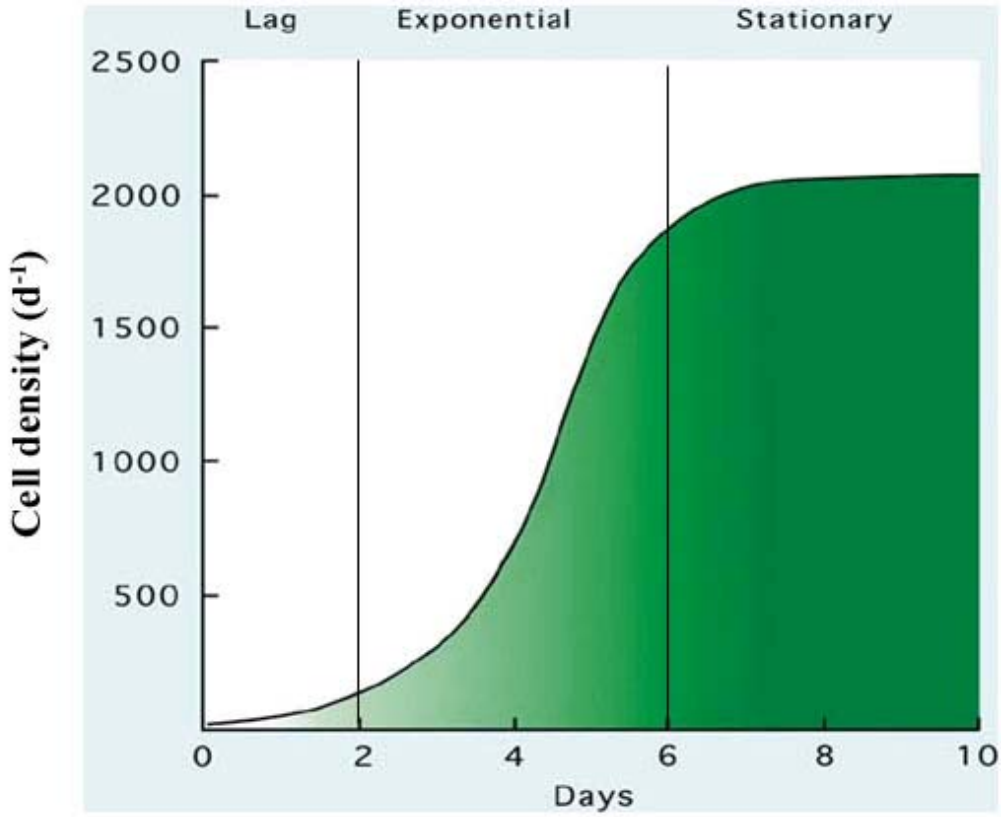

Plate. 4.4.1 Growth phases of phytoplankton in an experimental condition

To study the growth rate (optimal condition) of phytoplankton in the study region, ten phytoplankton species commonly found in Cochin backwater were isolated and mono cultured following the procedure (4.3.1 a & b). Among them eight were diatoms (*S. costatum, C. calcitrans, C. centralis, B. sinensis, N. closterium, N. distans, P. elongatum and A. coeffeaeformis*), and the other two belonged to green algae (*C. vulgaris*) and blue green algae (*Anabaena* sp.). The species belonged to nano (2-20 µm) and micro (20-200 µm) planktonic sizes (Figure.4.4.1 a; Table.

4.4.1 a). The growth rate of nano and micro phytoplankton studied are the first attempt for the Cochin backwater. The nano phytoplankton growth rate ranged from 0.92 to 2.12 d^{-1} and micro phytoplankton growth rate range was between 0.56 to 0.85 d^{-1}. Nano phytoplankton showed a faster growth than micro phytoplankton. Nano phytoplankton was contributed by diatoms (*S. costatum, C. calcitrans, N. closterium, N. distans, P. elongatum and A. coeffeaeformis*), green algae (*C. vulgaris*) and blue green algae (*Anabaena* sp.), whereas the micro phytoplankton was mainly the diatoms (*C. centralis* and *B. sinensis*).

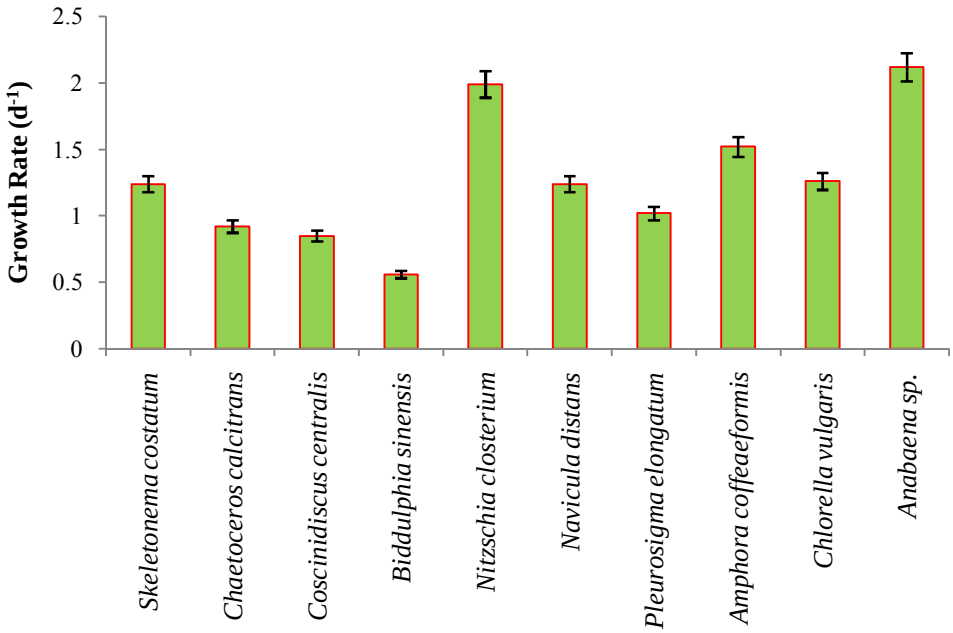

Figure. 4.4.1 (a) Growth rate of common phytoplankton isolated from the study region

Table. 4.4.1 (a) Phytoplankton growth rate (optimal condition) and their size

Species	Size (µm)	Growth rate (d^{-1})
Skeletonema costatum	15	1.24 (± .002)
Chaetoceros calcitrans	17	0.92 (±0.12)
Coscinodiscus centralis	105	0.85 (±0.01)
Biddulphia sinensis	92	0.56 (±0.01)
Nitzschia closterium	16	1.24 (±0.02)
Navicula distans	12	1.02 (±0.02)
Pleurosigma elongatum	17	1.02 (±0.1)
Amphora coffeaeformis	26	1.52 (±0.02)
Chlorella vulgaris	6	1.26 (±0.03)
Anabaena sp.	6	2.12 (±0.05)

The salinity preferences of the various phytoplankton species revealed that the optimum salinity required for maximum growth for each phytoplankton species varied in the range 15 - 25 (Figure 4.4.1 b). It was found that half of the species *S. costatum, N. closterium, N. distans, P. elongatum and A. coffeaeformis* studied were euryhaline, whereas except *C. calcitrans*, *C. centralis, C. vulgaris* and *Anabaena* sp. were stenohaline in nature. The optimum salinity prefered by *C. calcitrans,* and *C. centralis* were in a narrow range of 30 – 35, while for *C. vulgaris* and *Anabaena* sp., it was 10 - 20 (Table 4.4.1 b).

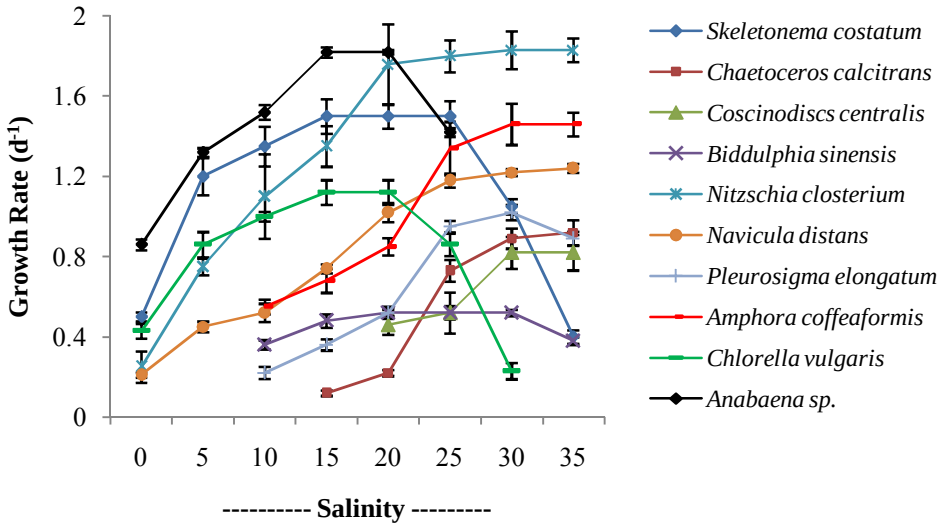

Figure. 4.4.1 (b) Salinity preference of various phytoplankton species in Cochin backwater

Table.4.4.1 (b) Range in salinity showing maximum growth for different unialgal species

Phytoplankton species	Optimum Salinity range for maximum growth	Nature
Skeletonema costatum	15-25	Euryhaline (Estuarine)
Chaetoceros calcitrans	30-35	Stenohaline (Marine)
Coscinodiscus centralis	30-35	Stenohaline (Marine)
Biddulphia sinensis	15-30	Euryhaline (Estuarine)
Nitzschia closterium	20-35	Euryhaline (Estuarine)
Navicula distans	25-35	Euryhaline (Estuarine)
Pleurosigma elongatum	25-30	Euryhaline (Estuarine)
Amphora coffeaeformis	25-35	Euryhaline (Estuarine)
Chlorella vulgaris	10-20	Stenohaline (Brackish)
Anabaena sp.	10-20	Stenohaline (Brackish)

4.4.2. Influence of light on photosynthetic uptake of phytoplankton

The process of photosynthesis involves the conversion of inorganic carbon into organic carbon with light as the energy source. In plant physiology, the rate of this conversion is called the photosynthetic rate or uptake. Light becomes a limiting factor for photosynthetic uptake of phytoplankton in Cochin backwater during monsoon due to high suspended particulate matter (SPM). Therefore a comparative study was made on photosynthetic uptake of phytoplankton during monsoon (high SPM) and pre monsoon (low SPM). Sampling was done at a single station (Stn. 4, Fort Kochi, Chapter 2 & 3) in both the season at euphotic depth (0.5 m) during spring tide.

The functional response of phytoplankton to available light can be studied through use of the photosynthesis-irradiance (P-I) experiment. Different components of P-I like photosynthetic uptake (P^B), saturated photosynthetic uptake (P^Bm), photoadaptation (I_K) and photosynthetic efficiency (α^B) explain about the photophysiology of phytoplankton and their relationship to environmental variables (Plate. 4.4.2). P^B and P^Bm are functions of phytoplankton biomass and species composition. I_K and α^B are functions of specific characteristics of dominant phytoplankton species with respect to their light capturing capacity and environmental variables. Among the environmental variables, salinity and SPM varied considerably during monsoon and pre-monsoon seasons.

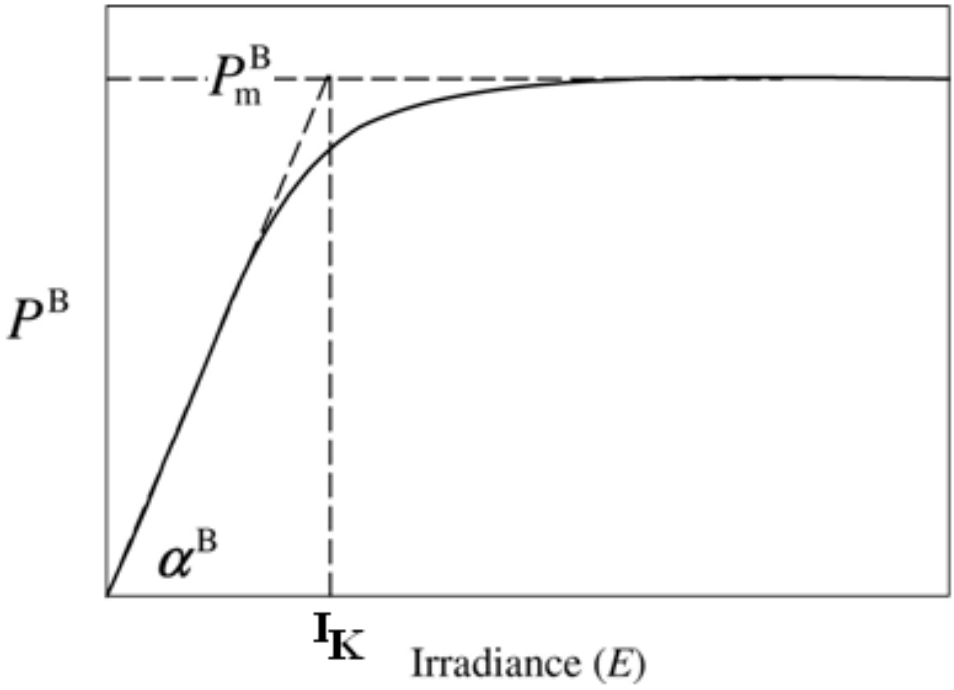

Plate. 4.4.2. Schematic diagram of Photosynthesis-irradiance experiment

Photosynthetic uptake (P^B) during monsoon ranged from 2.8 to 6.7 mg C mg Chl^{-1} h^{-1} (av. 4.5 mg C mg Chl^{-1} h^{-1}) with maximum during June and minimum during July and August (Figure. 4.4.2 a; Table 4.4.2). High P^B during June can be attributed to the high biomass and relatively low SPM during the period. Low P^B during July and August was due to comparatively high SPM during July (50.0 mg L^{-1}) and August (45.3 mg L^{-1}) which blemished the quality of light. During pre monsoon P^B ranged from 5.7 to 7.0 mg C mg Chl^{-1} h^{-1} (av. 6.5 mg C mg Chl^{-1} h^{-1}). Maximum P^B was recorded during March and minimum February (Figure. 4.4.2 b; Table 4.4.2). The variability in P^B was mainly due to dominant phytoplankton species present. It was observed that maximum diversity was observed during March when compared to April and May even though biomass was

high during April (12.8 mg m^{-3}) and May (13.9 mg m^{-3}) compared to March (7.2 mg m^{-3}).

$P^B m$ is the saturated photosynthetic uptake which followed the trend of P^B with maximum range was during pre monsoon (5.5- 6.8 mg C mg Chl^{-1} h^{-1}) and minimum during monsoon (2.7-6.2 mg C mg Chl^{-1} h^{-1}).

The photo adaptation (I_K) showed wide variation during monsoon and pre monsoon, the differences in I_K was mainly due to phytoplankton species diversity (Table. 4.4.2; Figure 4.4.2 d). During monsoon maximum photoadaptation of phytoplankton was during August (111.3 µE m^{-2} s^{-1}) and minimum during July (66.7 µE m^{-2} s^{-1}) the variations in the I_K can be attributed to the differences in phytoplankton species. The relative abundance of *L. danicus* was high during July but was completely absent in August similarly *T. subtilis* which was abundant during August was completely absent during July. During pre monsoon I_K was maximum during March (340 µE m^{-2} s^{-1}) and minimum during May (131.4 µE m^{-2} s^{-1}) this may be probably due to the phytoplankton taxonomical composition differences. Photosynthetic efficiency (α^B) during monsoon and pre monsoon did not show noticeable differences except during June. This indicates that efficiency of fixing carbon by phytoplankton in the study region is more or less similar.

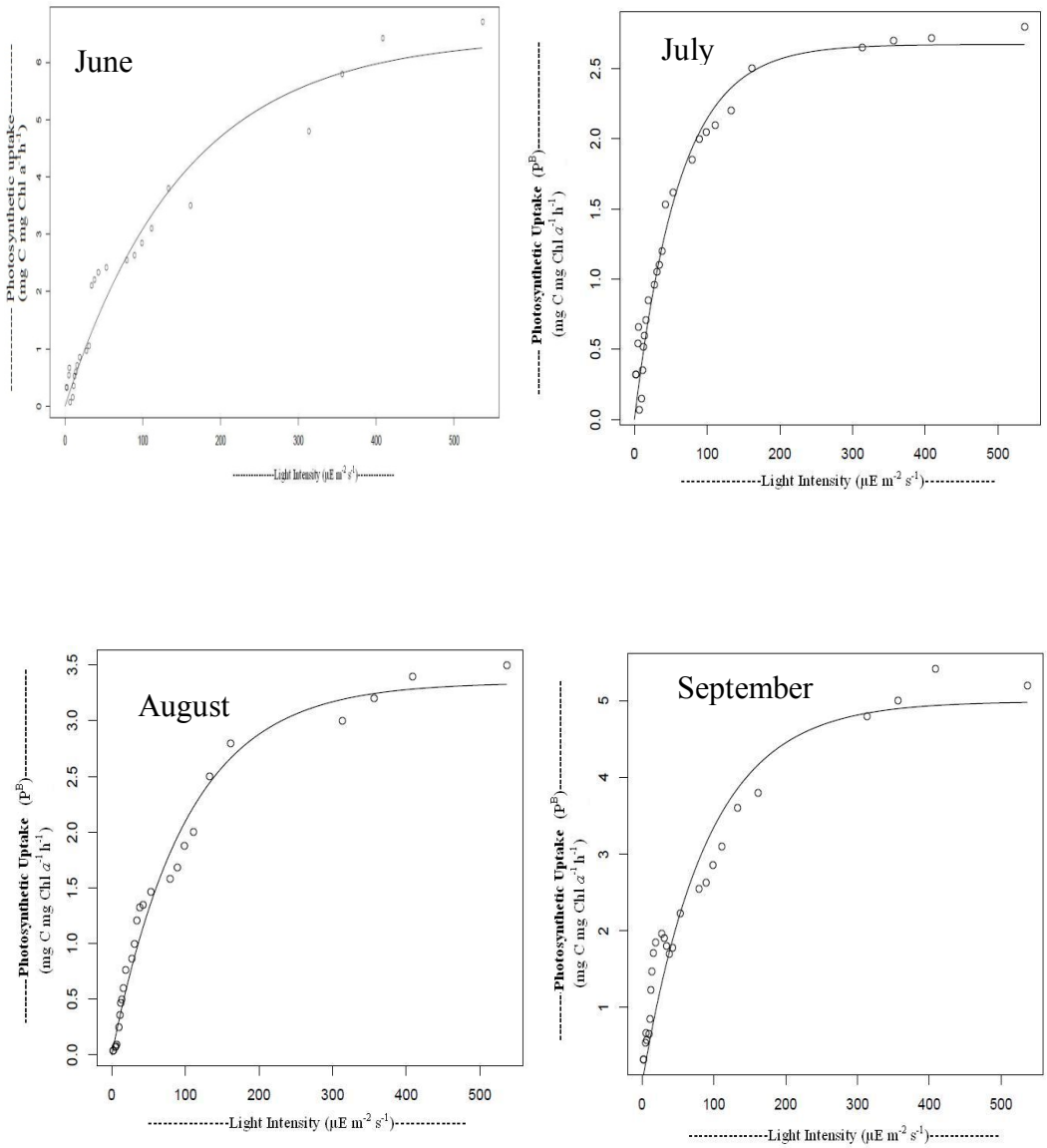

Figure 4.4.2 (a) Photosynthetic uptake of phytoplankton during monsoon season in the study region

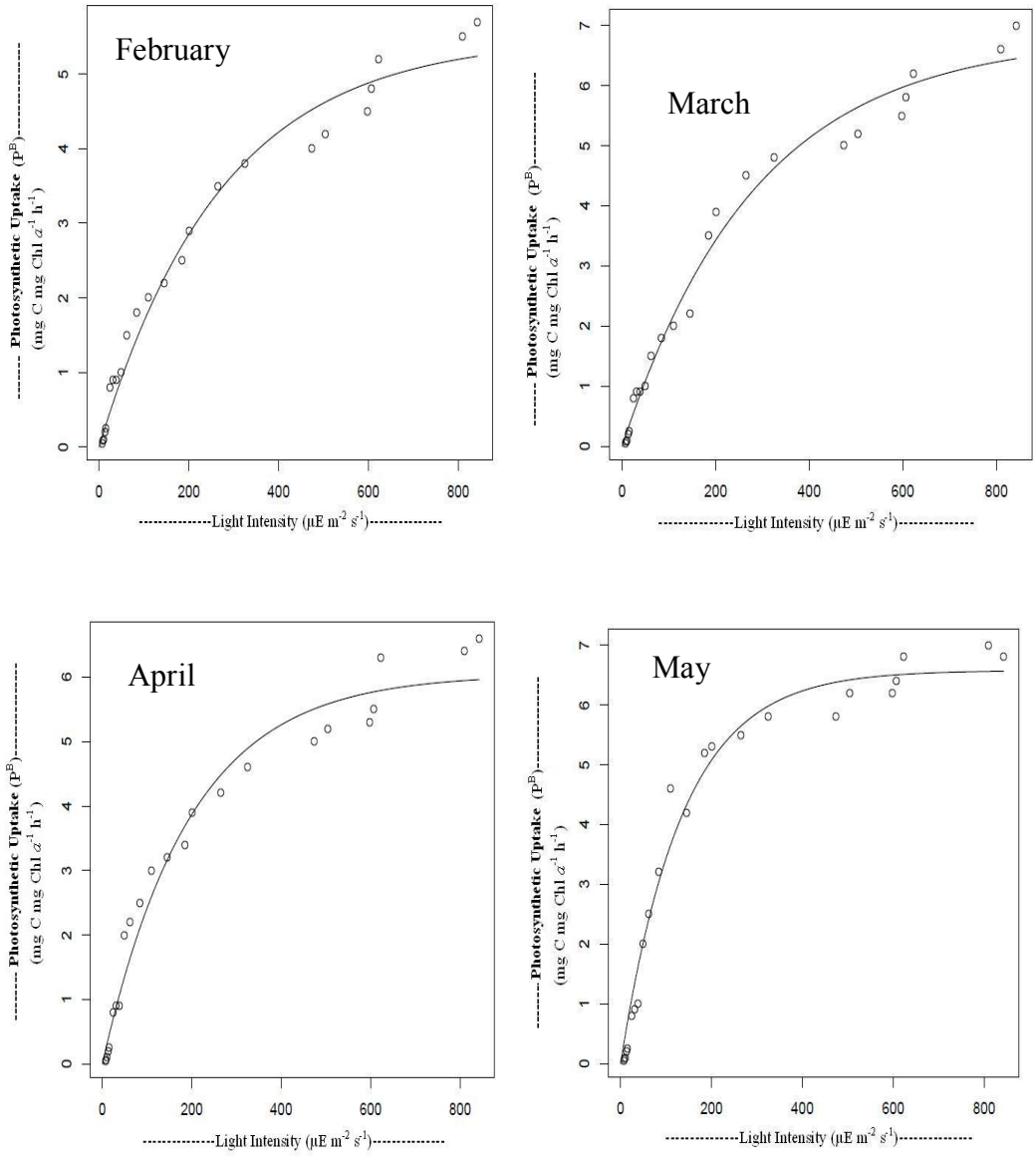

Figure 4.4.2 (b) Photosynthetic uptake of phytoplankton during pre-monsoon season in the study region

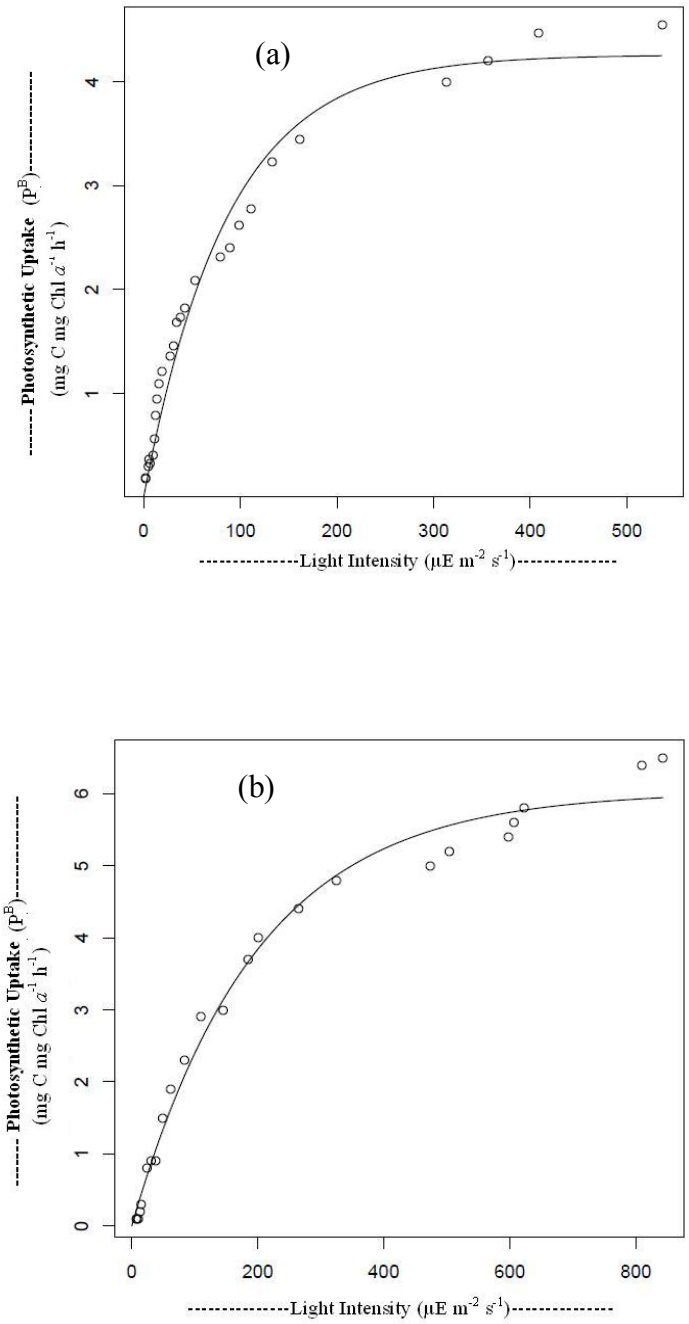

Figure 4.4.2 (c) Photosynthetic uptake (average) of phytoplankton during (a) monsoon (b) pre monsoon in the study region

Table.4.4.2 Photosynthetic uptake (P/I) components and related physical and biological parameters in the study region

	Monsoon 2008					Pre monsoon 2009				
Parameters	**June**	**July**	**Aug**	**Sept**	**Avg.**	**Feb**	**March**	**April**	**May**	**Avg.**
P^B	6.7	2.8	3.5	5.2	**4.5**	5.7	7.0	6.6	6.8	**6.5**
P^Bm	6.2	2.7	3.3	5.0	**4.3**	5.5	6.8	6.4	6.6	**6.0**
I_K	77.0	66.7	111.3	99.8	**85**	274.5	340	201.3	131.4	**201**
α^B	0.07	0.04	0.03	0.05	**0.05**	0.02	0.02	0.03	0.05	**0.03**
Chl. *a*	7.5	5.3	4.2	5.5	**5.6**	5.4	7.2	12.8	13.9	**9.8**
Light	852	277	347	568	**511**	706	988	793	774	**815**
Salinity	8.5	0.0	5.8	4.1	**5.2**	32.3	30.7	23.4	24.0	**27.6**
SPM	27.6	50.8	45.3	33.6	**39.3**	25.6	26.0	28.8	27.2	**26.9**

Units

PB and P^Bm = mg C mg Chl a^{-1} h^{-1}

IK= µE m^{-2} s^{-1}

αB= mg C mg Chl a^{-1} h^{-1} µE m^{-2} s^{-1}

Light= µE m^{-2} s^{-1}

Biomass= mg m^{-3}

Suspended particulate matter (SPM) = mg L^{-1}

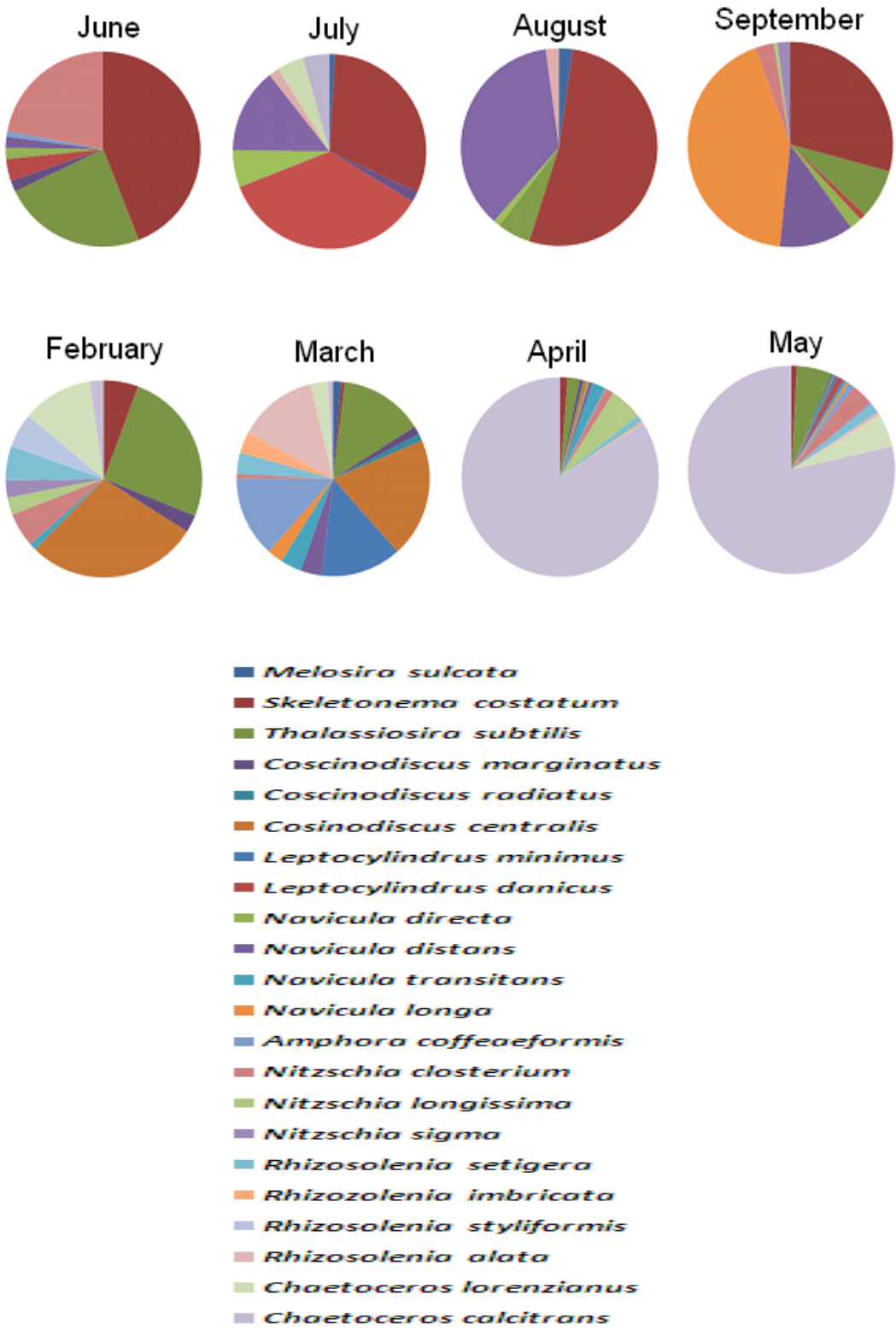

Figure. 4.4.2 (d) Relative abundance (%) of the main phytoplankton species

Photosynthetic uptake is also a function of the dominant phytoplankton species (Figure. 4.4.2 d). Phytoplankton species distribution during the study period showed differences in their relative abundance. During monsoon the dominant species were *S. costatum, T. subtilis, N. distans, N.longa, N. directa* and *N. closterium* whereas during pre monsoon the dominant species were *T. subtilis, C. lorenzianus, C. centralis, N. directa, A. coffeaeformis, R. styliformis during* February and March whereas during April and May more than 80% of the abundance was contributed by *Chaetoceros calcitrans*. The differences in the floral composition played a major role in the in the photosynthetic uptake and light utilization of phytoplankton.

4.4.3. Influence of copepod grazing on phytoplankton biomass and their prey size selectivity

Copepods are the major group in a mesozooplankton sample; more than 80% of the total mesozooplankton is contributed by them. They are efficient grazers of phytoplankton and play an important role as intermediaries for nutrient/energy transformation from primary to tertiary trophic level. So it is essential to study their grazing effect on phytoplankton biomass. Copepod grazing was measured using Chlorophyll *a* (Phytoplankton biomass) as a proxy. Copepod grazing controls phytoplankton biomass and distribution.

Copepod grazing is a major biological factor that was responsible for controlling the phytoplankton biomass in the study region. Time series observations (24 hr) made during monsoon (2008) and pre-monsoon

(2009) seasons revealed that during both periods 90% of the meso zooplankton biomass was contributed by copepods (Figure 4.4.3 a & b). During monsoon, the meso zooplankton biomass ranged from 0.03 ml m^{-3} (±0.02) to 0.175 ml m^{-3} (± 0.04) and copepod biomass ranged from 0.025 (± 0.01) to 0.14 ml m^{-3} (± 0.03). During pre-monsoon, the range in meso zooplankton biomass was between 0.06 (± 0.01) and 0.52 ml m^{-3} (± 0.15) and that of copepod was between 0.03 (± 0.04) and 0.39 ml m^{-3}(± 0.18). During both the seasons the meso zooplankton and copepod biomass were found to be high in night hours (9 pm) and early morning (1 am & 5 am). However, the values for the respective time were 2-3 fold higher during pre monsoon as compared to the monsoon.

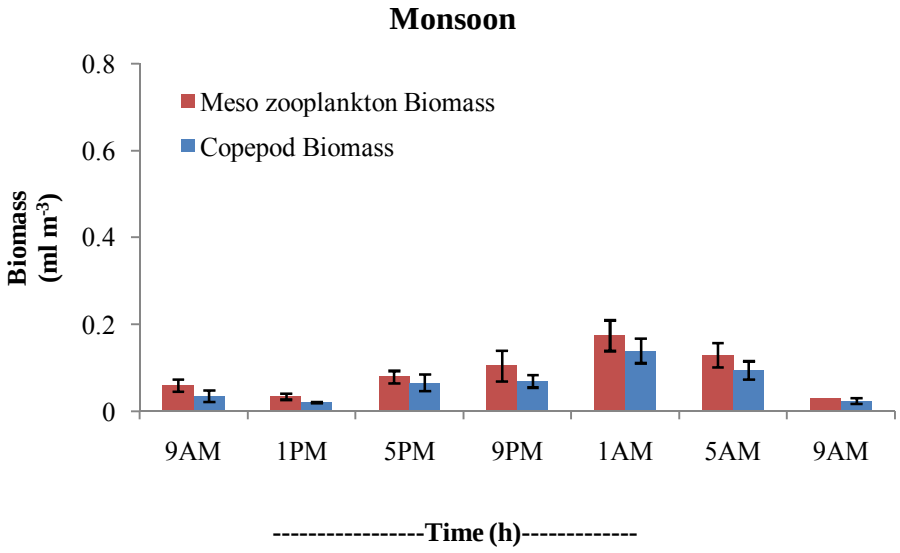

Figure 4.4.3 (a) Time series (24 hr) observation of meso zooplankton and copepod biomass during monsoon in the study region

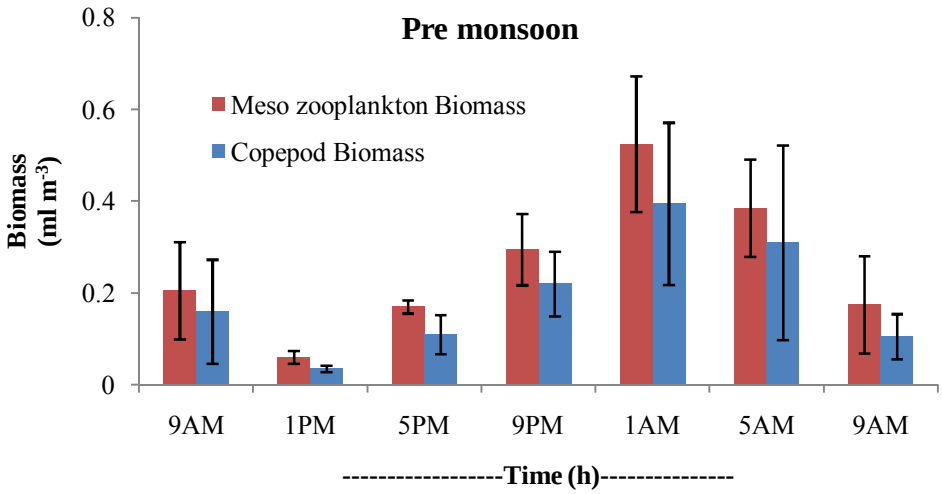

Figure 4.4.3 (b) Time series (24 hr) observation of meso zooplankton and copepod biomass during pre monsoon in the study region

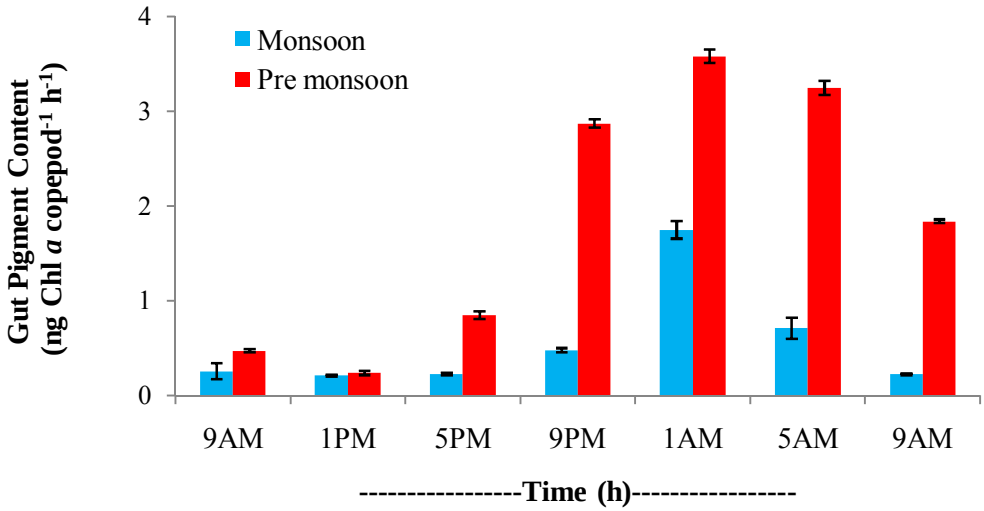

Figure 4.4.3 (c) Time series (24 hr) observation of copepod grazing during monsoon and pre monsoon in the study region

Copepod grazing during monsoon and pre monsoon revealed that grazing was relatively high during pre monsoon compared to monsoon. During monsoon the grazing rate ranged from 0.21 to 1.75 ng Chl *a* copepod^{-1} h^{-1} and during pre monsoon the range was between 0.24 and

3.58 ng Chl *a* copepod^{-1} h^{-1} (Figure 4.4.3 c). Grazing was high during 9 pm to 5 am and the peak grazing time was 1am during both monsoon and pre monsoon. Two fold increases in grazing of copepod was observed during night and early morning hours in pre monsoon.

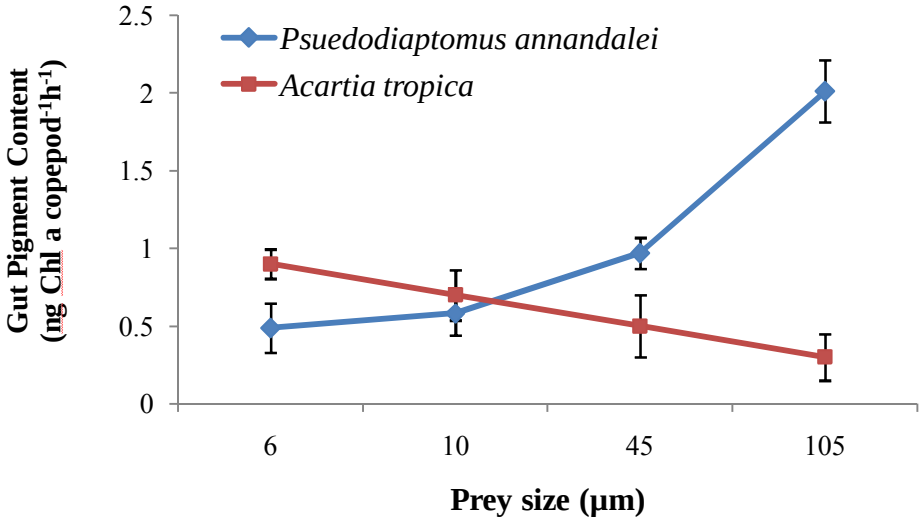

Figure 4.4.3 (d) Prey-size selectivity of *Acartia tropica* and *Psuedodiaptomus annadalie*

Grazing also indicates the size selectivity of the prey (phytoplankton) by copepods. It was found that *Acartia tropica* dominated during monsoon and contributed more than 70% of copepod density. The grazing rate of this species decreased as the size of phytoplankton increased. In the case of *Psuedodiaptomus annandalei* which contributed more than 80% of the copepod density during pre monsoon, preferred larger cell size (Fig. 4.4.3 d). Grazing rate of *A. tropica* ranged from 0.32 to 0.92 ng Chl *a* copepod^{-1} h^{-1} and the size preference ranged from 6 to 10 µm whereas for *P. annandalei* the grazing rate ranged from 0.49 to 1.0 ng Chl *a* copepod^{-1} h^{-1} and the size preference ranged from 45 to 105 µm.

The result indicates that *A. tropica* prefers only nano planktonic size while *P. annandalei* can take both nano and micro planktons. It is also observed that *A. tropica* and *P. annandalei* can graze nano planktonic size (10 µm) with more or less same efficiency.

4.5 Discussion

4.5.1 Phytoplankton growth at optimal and varying salinity condition

Phytoplankton growth rate study was a first time approach in the Cochin backwater. There are only a few reports available in the Indian waters (Phatarpekar *et. al.,* 2000 and Gireesh *et. al.,* 2008). Similar kind of studies from elsewhere is that of (Raven, 1986; Tang, 1995; Raven and Kubler 2002 and Geraldine *et. al.,* 2005). They obtained maximum growth rates ranging from 0.2 to 3.3 d^{-1} with an average of 1.5 ± 0.8 d^{-1} under conditions of saturating light and nutrient sufficiency. In the present observation the phytoplankton growth rate ranged from 0.56 to 2.12 d^{-1} which is within the range of above reported values.

It was also found that smaller cells exhibited faster growth rate. *N. closterium* and *Anabaena* sp. showed faster growth rate than *C. centralis* and *B. sinensis*. *N. closterium* (16 µm) and *Anabaena* sp. (6 µm) belong to nano planktonic size whereas *C. centralis* (105 µm) and *B. sinensis* (92 µm) are micro planktonic size. The latter two species registered were slower growth rate with a cell division rate of 0.5 and 0.8 d^{-1} while the former species showed cell division rate of 1.99 and 2.12 d^{-1}. The

reduction in the growth rate with increasing cell size implies that small cells have a distinct advantage over large ones (Raven 1986). Faster growth rate can be related to cell size, which has long been recognized as an important cause of interspecific variability (Kagami and Urabe 2001).

Salinity preference of different phytoplankton species revealed that maximum growth rate was attained in the salinity range 15 to 25 and also most of these species were euryhaline in nature. Observational studies made by (Qasim 1974; Devassy and Bhattathiri 1974; Gopinathan 1975; and Madhu *et. al.,* 2007 and 2010) have reported that maximum biomass and density were observed during low saline period (15-25). Similar kind of observations made in the Mandovi estuary by (Matondkar *et. al.,* 2007) revealed that phytoplankton bloom in the estuary coincides with low saline period (20-25). Patil and Anil (2011) have reported that salinity stratification (17 to 18) favors phytoplankton bloom in the Zuari estuary which incidentally opens to the Arabian Sea where the Mandovi estuary also opens. All these studies points to the fact that salinity is the key controlling factor for phytoplankton growth.

Phytoplankton in the present study region was mostly euryhaline in nature. Of the ten phytoplankton species studied, six were euryhaline (*S. costatum, N. closterium, N. distans, P. elongatum, B. sinensis and A. coeffeaeformis*) and four (*C. calcitrans*, *C. centralis, C. vulgaris* and *Anabaena* sp.) were stenohaline species. *C. calcitrans,* and *C. centralis* were marine which could not tolerate salinity less than <25 and *C. vulgaris* and *Anabaena* sp. were brackish water in nature and could not survive in

salinity >20. A few observational studies made by Devassy and Bhattathiri (1974) and Menon *et. al.,* (2000) have reported that *C. vulgaris* and *Anabaena* sp are fresh/brackish water forms and occur in high density during monsoon period. In the present experiment also, the results were similar in that *C. vulgaris* and *Anabaena* sp showed optimal growth in low salinity. Phytoplankton classified based on their salinity preferences in the Cochin backwater showed that *C. centralis* and some *Chaetoceros* species are purely marine and are stenohaline in nature. These reports substantiate the above results. The observational work made by (Menon *et. al.*, 2000) *B. sinensis* was classified under marine forms which are stenohaline in nature but in the present study it has been experimentally proved that it is a euryhaline form and hence, estuarine in nature.

4.5.2 Effect of light on photosynthetic uptake of phytoplankton

Light is an important abiotic factor for photosynthesis that limits phytoplankton growth. The variability in photosynthetic uptake (P^B) and saturated photosynthetic uptake (P^Bm) was mainly due to the optical property of dominant phytoplankton present during the period. P^B, P^Bm and I_K are functions of biomass (Chlorophyll *a*), specific characteristics of the locally dominant phytoplankton species and also changes in environmental conditions. In the present study the environmental conditions during monsoon and pre monsoon were entirely different especially in the case of salinity and SPM. Qasim (1973) while studying biological productivity of the Indian Ocean has reported the assimilation rate (photosynthetic uptake) ranged from 0.6 to 14.0 mg C mg $Chl^{-1}h^{-1}$ with

maximum during pre-monsoon and minimum during monsoon. Similar types of observations have been made from other regions (Falkowski, 1981; Cote and Platt, 1983; Falkowski and Raven, 1997) and it has been reported that assimilation rate (P^B and P^B max) was observed during high light intensity. I_K which is frequently used to describe the physiological adjustments of phytoplankton to changing environmental conditions also showed significant variation in the study region. It is also known that seasonal changes in I_K may occur in response to changing photoperiod and species composition (Cote and Platt 1983). In the present study I_K showed wide variation during monsoon and pre monsoon which can be attributed to the characteristics of locally dominant phytoplankton and their optical properties (Macedo *et. al.,* 2001).

4.5.3 Effect of copepod grazing on phytoplankton and their prey size selectivity

The grazing of copepod (Calanoid) revealed that meso zooplankton and copepod biomass was high during pre-monsoon. Biomass was high in the night and early morning during both the seasons. Similar kinds of results have been reported by (Madhupratap and Haridas 1975; Rao *et. al.,* 1975; Madhupratap 1979 and 1987; Haridevi *et. al.,* 2004; Madhu *et. al.,* 2007 and Molly and Krishnan 2009). The abundance of copepod is associated with salinity, because the Cochin backwater becomes an arm of the adjoining sea during pre monsoon season, supporting the entry of marine zooplankton.

Quantitative study on field caught copepod (Calanoid) revealed that grazing (gut pigment content) was high during pre monsoon period as compared to monsoon period (Figure 4.4.3 c). Grazing rate during pre monsoon ranged from 0.24 to 3.58 ng Chl *a* copepod^{-1} h^{-1}, whereas during monsoon, it was 0.21 to 1.75 ng Chl *a* copepod^{-1} h^{-1}. Earlier studies cited above form the Cochin backwater have not quantified the gut pigment content in copepod. These studies only explained the facts drawn out from filed observations and reported that during pre monsoon season there could be active grazing of copepod on phytoplankton independently or in combination with microzooplankton because the herbivorous copepods are capable of grazing up to 75% of the phytoplankton in a tropical estuary. The present work is a first time attempt to quantify phytoplankton biomass consumed by copepod in the Cochin backwater. Differences of phytoplankton biomass (Chl. *a*) available in the system and the biomass consumed by the copepod were computed. Phytoplankton biomass in the system ranged from 1.8 to 4.3 mg m^{-3} during monsoon and 2.1 to 6.6 mg m^{-3} and the remaining biomass after the grazing of copepod ranged from 1.2 to 4.2 mg m^{-3} during monsoon and 0.9 to 6.5 mg m^{-3} during pre monsoon. From the present study it was observed that there was always surplus of food available in the Cochin backwater regardless of season. This is because; the grazing by copepod was much lower than the growth of phytoplankton, as revealed from the growth and grazing experiment. Qasim (1970) estimated the amount of food available in Cochin backwater in terms of carbon. According to him the gross production in the backwater ranged from 270-295 g C/m^2/y (av. 280 g C/m^2/y) while net production, for

days only, is 180-200 g C/m^2/y (av. 195 g C/m^2/y). The estimated annual consumption by the zooplankton herbivores is only about 30 g C/m^2. This indicates there is large surplus of basic food in the backwater. The lacking of (Qasim, 1970) work was that they took gross or net production as proxies to measure the grazing pressure of zooplankton on phytoplankton. But these proxies are not the direct measurement of phytoplankton biomass grazed by copepods. Primary productivity is controlled by factors like light and the efficiency of phytoplankton to fix the carbon. But present study overrules these factors since phytoplankton biomass is taken as the proxy to measure the food availability in the system.

The flows of organic matter in pelagic food webs are determined by the food selectivity of the pelagic grazers. Several criteria may be involved in food selection, including prey size, motility, surface characteristics, biochemical composition, electrostatic forces etc. (Poulet and Marsot 1978). Among these criteria, prey size is generally believed to play a major role (Sheldon *et.al.,* 1977; Conover and Huntley, 1980). The present work on prey size selectivity of two copepods (Calanoid) in the Cochin backwater revealed that their grazing rate was more or less similar, but the prey size selectivity was different. The result indicated that *A. tropica* prefers only nano plankton size while *P. annandalei* can graze both nano and micro planktonic prey. Grazing rate of *A. tropica* ranged from 0.32 to 0.92 ng Chl *a* copepod^{-1} h^{-1} whereas for *P. annandalei,* it ranged from 0.49 to 1.0 ng Chl *a* copepod^{-1} h^{-1}. Goes *et. al.,,* (1999) have obtained the grazing rate to be 1.21 ng Chl copepod^{-1} h^{-1} from Indian waters.

Dominance of *A. tropica* has been reported during monsoon season and *P. annendalie* during pre-monsoon season in the Cochin backwater (Madhupratap and Haridas, 1975; Rao *et. al.,* 1975; Madhupratap, 1979). Therefore, the prey (phytoplankton) size selectivity of copepod will be according to the prevailing size of the phytoplankton. According to Støttrup and Jensen (1990) *Acartia* sp. selectively graze on phytoplankton with a size <10 µm. Therefore, copepods can shift their feeding as ominivores when the phytoplankton size becomes too small (mainly pico) for their consumption. On the other hand, copepods prefer to be herbivores when phytoplankton available is of suitable size (mainly micro) (Stoecker and Capuzzo, 1990; Gifford and Dagg, 1991; Foreman, 2002). The prey size selectivity of copepod in the Cochin backwater made during the present study is new information for the Cochin backwater.

Plate. 4.4.3 (a) Laboratory set up for experimental work

Plate. 4.4.3 (a) Copepod grazing experiment set up

Chapter 5

Summary and Conclusion

The Cochin backwater is one of the productive estuaries in India and perhaps the most intensively investigated systems during the last five decades. However, a precise understanding on the distribution, species diversity and abundance of the phytoplankton community, in relation to the ecological parameters is severely lacking in the backwater. The present study was carried out to address the role of environmental parameters on phytoplankton distribution and diversity in the Cochin backwater, both by observational and experimental approach.

The present study shows that variability in total phytoplankton biomass is mainly due to the type of phytoplankton prevailing in the region. A four- fold decrease in total phytoplankton biomass was observed in the southern region compared to north of the study region because, the southern region remained oligohaline, whereas in the northern arm was euryhaline supporting high abundance of the marine species.

Size fractionated phytoplankton biomass study revealed that the major contributor to the total phytoplankton biomass was the nano fraction (2-20 µm) due to their faster growth rate and efficient utilization of light and nutrients, particularly in turbid waters where the euphotic zone is shallow and nutrients rich. In the case of pico fraction (<2 µm), there was a

reduction of biomass from 11 % to 1% when the backwater transformed from freshwater (monsoon) to marine (pre monsoon) condition. The reduction in the pico fraction during pre monsoon was because of the active grazing by micro zooplankton (Protozoans) which are capable of grazing the pico plankton biomass. In the case of micro fraction (>20 µm), there was a gradual increase in biomass from monsoon to pre monsoon. This is because the larger cells need high light intensity for their proliferation. In the present study region the total radiation during monsoon is less than 350 ly day^{-1} due to heavy cloud cover. In addition, the high suspended particulate matter also lead to reduction of light intensity during monsoon.

Diatoms dominated the study region throughout the year contributing 80% to the total phytoplankton community. The dominancy of diatoms in the entire study region throughout the year was due to their euryhaline nature which allows them to proliferate under eutrophic conditions. On the other hand, dinoflagellates mostly prefer oligotrophic waters, and hence, fail to their maximum to the total in eutrophic waters by competing with diatoms. Dinoflagellate density showed an increasing trend when the backwater nutrient levels were low as compared to the monsoon season. In short, the system was turning to slightly oligotrophic condition during pre monsoon season. In the case of green algae and blue green algae their density was found to be high during the monsoon season, owing to their affinity towards low salinity.

The qualitative study on phytoplankton species revealed that in the present study region there exist two kinds of phytoplankton species, (a)

those well adapted to the fluctuating estuarine conditions and (b) those which are not adapted to the fluctuating estuarine condition. The former comprise typical estuarine forms, which are euryhaline and the latter stenohaline that represent either freshwater or marine forms which migrate to the estuary and are seen only for short periods.

A comparison made on the distribution of phytoplankton genera from early reported work and the current study, it was observed a reduction/elimination of phytoplankton genera was observed which could be due to the effect of eutrophication which causes exponential growth of some selected species causing the loss of biodiversity.

Species diversity index (H') was also low (3.0) probably due to the effect of the salinity barrier at Thaneermukkam that considerably reduced the tidal expanse of the backwater. A reduction in the species diversity compared to previous reports could reflect the impact to the construction of the bund and other modifications in the backwater.

Estimation of some of the species especially from the upstream section of the backwater could be related to the consequence of the climate change. It is established that the backwater has been transformed to eutrophic state and there has been considerable deterioration in the water quality following the construction of salinity barrier in the south of backwater (Thanneermukkam).

Phytoplankton growth rate experiment revealed that smaller cells exhibited faster growth over the larger one. The reduction in the growth rate with increasing cell size implies that smaller cells have a distinct advantage over large ones. Similarly, faster growth rate can be related to

cell size, which has long been recognized. Salinity preferences of different phytoplankton species in the study region were mostly euryhaline forms.

Light, which is an important abiotic factor for photosynthesis, was also found to be a limiting factor for the growth of phytoplankton in the Cochin backwater. Photosynthetic rate (P^B and P^Bm) are functions of biomass (Chlorophyll *a*), species specific and changes in the water environment. The environmental conditions in the present study region during monsoon and pre monsoon were entirely different especially in the case of salinity and SPM. The variability in photosynthetic rate was therefore, due to the variations in optical properties of the dominant phytoplankton species.

Grazing of phytoplankton by copepod is a factor regulating the phytoplankton growth. Copepods are the major secondary producers that transfer energy between phytoplankton and the nektons. Quantitative study of field caught copepod (Calanoid) revealed that grazing (gut pigment content) was high during pre monsoon period as compared to monsoon period. Earlier studies lacked in quantifying the biomass reduction by copepod grazing. The grazing pressure of copepod was much lower than the growth of phytoplankton. Hence, there seems to be surplus food available in Cochin backwater, regardless of season.

The pathways for flow of organic matter in pelagic food webs are to a large extent determined by the food selectivity of the pelagic grazers. The present work on prey size selectivity of two major copepods (Calanoid) found in Cochin backwater during monsoon and pre monsoon season revealed that *A. tropica* (dominant during monsoon) prefers only

nano plankton while *P. annandalei* (dominant during pre monsoon) can prey on nano and micro plankton. Quantification of copepod grazing on phytoplankton biomass and prey-size (phytoplankton) selectivity of copepod is first time information for the Cochin backwater.

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Growth and proximate composition of the *Chaetoceros calcitrans* f. *pumilus* under different temperature, salinity and carbon dioxide levels

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Abstract

The marine diatom *Chaetoceros calcitrans* f. *pumilus* has been examined for its potential source as live feed in aquaculture. The present study investigated effects of temperature (20, 25 and 30 °C), salinity (25 and 35) and carbon dioxide addition (air+CO_2) on the growth and proximate composition of *C. calcitrans* under laboratory conditions. The growth and biomass of *C. calcitrans* were primarily affected by carbon dioxide addition, and to a lesser extent by temperature and salinity. In general, lipid and carbohydrate contents were higher at lower temperatures (20 and 25 °C), while the protein content was unaffected. Carbon dioxide addition increased protein, while lowering carbohydrates, but had no effect on lipid content. Carbohydrates were increased while lipids and protein decreased at the highest salinity (35 ± 0.9). These results should be taken into consideration when evaluating the dietary value of this micro alga for aquaculture.

Keywords: *Chaetoceros calcitrans*, growth, composition, temperature, salinity, carbon dioxide, live feed

Introduction

Micro algae are the major food source for many aquatic organisms and the main live feed component in marine hatchery operations because they serve as a natural resource for polyunsaturated fatty acids. The diatom *Chaetoceros calcitrans* is considered as the most popular strain used in hatcheries, especially for shrimp larvae. This species gives vital energy and organic nutrients for the growth and development of larvae and juveniles (Jeffrey, Brown & Garland 1994). Its culture is an important activity, which influences the nutritional value of aquatic herbivores (Whyte, Bourne & Hodgson 1989) as well as the economic aspects of their culture (Coutteau & Sorgeloos 1992). Very few investigations on the optimal condition for the growth of *C. calcitrans* are available. Liang (1985) reported the effect of silicate and its optimum level on growth of this diatom. The operation of a cultivation column in airlift mode was proven to be successful and a high growth rate could be achieved even with a lower light intensity than the optimal. The mass production of *C. calcitrans* through a bioreactor and high growth rate have been reported (Sontaya, Worapannee, Sorawit & Prasert 2005). Many specific characteristics are thought to influence the nutritional value of micro algae, such as cell wall digestibility (Epifanio, Valenti & Turk 1981), cell size and biochemical composition (Fernandez-Reiriz, Perez-Camacho, Ferreiro, Blanco, Planas, Campos & Labarta 1989). However, no information is available on the environmental conditions for the growth of the diatom *C. calcitrans* and its effects in proximate compositions. The biochemical composition of micro algae depends on their environmental conditions, growth rates or the micro algal life cycle (Richmond 1986). The important factors used to evaluate the nutritional value of a species are growth rates, in terms of cell numbers or biomass and biochemical composition, which should be optimized in terms of vital nutrients. The

main factors controlling micro algal growth and composition are light, nutrients, temperature and pH (Tzovenis, De Pauw & Sorgeloos 1997; Zhu, Lee & Chao 1997), but other factors such as salinity can also be important for a few species (Chu, Phang & Goh 1996). The aim of the present work was to determine the effect of temperature, salinity and carbon dioxide addition on the biochemical composition and growth of the marine diatom *C. calcitrans* at different levels in a tropical commercial hatchery condition. The work mainly focuses on the total proximate composition.

Materials and methods

Micro alga culture was obtained from the algal stock collection of Central Marine Fisheries Research Institute, Kochi, India, and maintained under laboratory conditions. Experiments were performed to test the effects of temperature, salinity and carbon dioxide concentration on the growth and gross biochemical constituents of this species individually. Temperature was tested at three levels (20, 25 and 30 °C), while two salinities (25 and 35) and two CO_2 conditions [with (+) and without (−) CO_2] were used for other experiments. Carbon dioxide concentration was monitored by the free carbon dioxide method, which is based on the titration of dissolved carbon dioxide with NaOH (0.03 N), with the end-point reaction at pH 7.9 (Baumgarten, Rocha & Nienchesky 1996). Cultures were maintained using Walne medium (Walne 1974) in a Hauffkine culture flask (4 L) in a temperature-controlled algal chamber. Before each experiment, cultures remained at the determined experimental conditions for an adaptation period of approximately five generations. The cultures were started by inoculating a volume of 5–10% the total volume. Cell counts were performed daily to determine the maximum cell density and specific growth rate (K_2), which was calculated by linear regression of the $\log_2$ cell concentration on time, at the exponential growth phase (Guillard 1973). Each experiment was conducted in triplicate. Chlorophyll *a* concentration was determined at the initial and final phases of the experiment, by filtering a known culture volume on GF/F filters and extracting the pigment in 90% acetone solution for 24 h at − 20 °C. Fluorescence was then determined in the extract with a Turner Designs TD 700 fluorometer, according to Welschmeyer (1994). Light intensity was kept at 500 μmol m^{-2} s^{-1} under a photoperiod of 12 h L:12 h D. Experiments were conducted in batch cultures, which were grown until either late exponential phase or early stationary phase. Algal biomass was obtained by concentrating the three replicates from each treatment on GF/F Whatman filters, using a Millipore peristaltic pump. The samples retained on the filters were dried at 60 °C until constant weight. The filters with algae samples were stored at − 20 °C until chemical analysis. The biochemical composition of *C. calcitrans* was determined in terms of total protein, total lipids and total carbohydrates. Total lipids were extracted according to Bligh and Dyer (1959), as modified by Whyte (1987). In the lipid extract residue (polymeric fraction), total protein was determined by the Kjeldahl technique (Whyte 1987). Samples retained in GF/F filters were hydrolyzed in 10 mL 80% sulphuric acid (Myklestad & Haug 1972), and carbohydrates were determined according to Dubois, Gilles, Hamilton, Rebers and Smith (1956). Statistical analysis included one-way analysis of variance (ANOVA) and Tukey test. Total protein, total carbohydrate and total lipid percentages were transformed using arcsine before statistical analysis.

Results

The effect of temperature, salinity and carbon dioxide addition on the growth of *C. calcitrans*

Temperature had a significant effect ($P < 0.05$) on the growth rate of *C. calcitrans* (Fig. 1a) under a salinity of 25 but not under high salinity (35). Higher growth rates of *C. calcitrans* occurred when carbon dioxide was added to the cultures (Fig. 1a). The highest temperature (30 °C) caused the growth rate to be lower at salinity 25, with no addition of carbon dioxide. Temperature showed no effect ($P > 0.05$) on the maximum cell concentration (Fig. 1b), although a visible trend of high values at 25 °C was observed when carbon dioxide was added. Carbon showed lower values at 30 °C (Fig. 1c), while chlorophyll per cell (Fig. 1d) was not affected by temperature or any other factors tested. Salinity (25–35) had no significant effect ($P > 0.05$) on *C. calcitrans* growth, maximum cell density, biomass and chlorophyll per cell (Fig. 1a–d). However, an affinity of higher growth and biomass as well as low cell concentration was observed at the lower salinity.

The effect of temperature, salinity and carbon dioxide addition on the biochemical composition of *C. calcitrans*

Temperature seems to influence the biochemical composition. At temperatures 20 and 25 °C, lipids

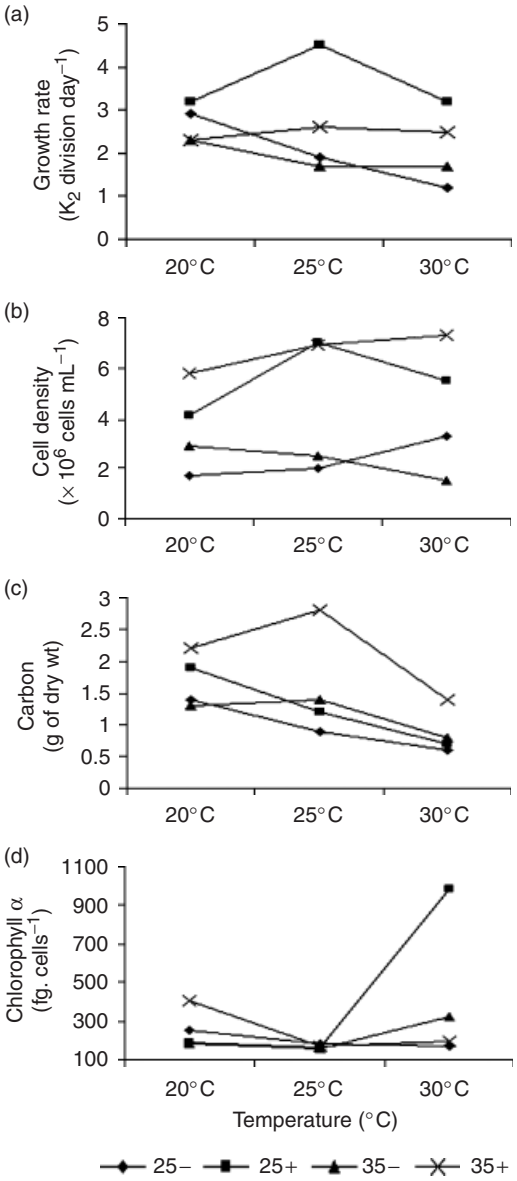

Figure 1 (a) Growth rate, (b) cell density, (c) carbon and (d) chlorophyll *a* under different temperatures, salinities (25 and 35) and with (+) and without (–) addition of CO_2.

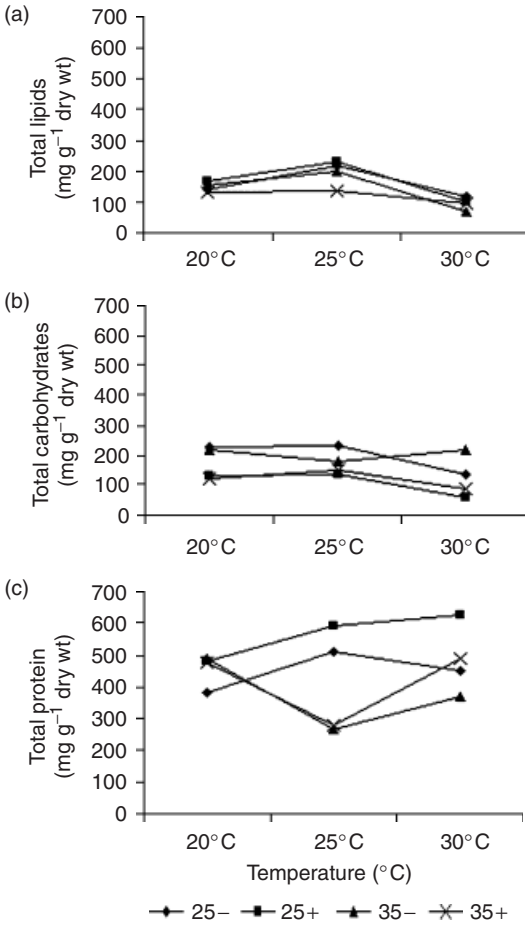

Figure 2 (a) Total lipids, (b) carbohydrates and (c) protein under different temperatures, salinities (25 and 35) and with (+) and without (–) addition of CO_2.

and carbohydrates were higher than at 30 °C under salinity 25 (Fig. 2a and b). Protein was not significantly affected by temperature, but an affinity for lower values was observed at 25 °C under salinity 35 (Fig. 2c). The effect of carbon dioxide on the biochemical composition of *C. calcitrans* is shown in Fig. 2a–c. An increase in protein content (Fig. 2c) and a decrease in carbohydrates (Fig. 2b) were noted.

Discussion

The effect of temperature on the growth rate of micro algae has been observed in other species. Significant increases in growth rates of *Chaetoceros pseudocurvisetus*, *Skeletonema hantzschii*, *Skeletonema costatum* with a maximum at 25 °C were observed by Yoshihiro and Takahashi (1995). Renaud, Thinh, Lambridis and Parry (2002) attributed the higher growth rate of *Chaetoceros* sp. to an increase in temperature from 25 to 30 °C. Fogg and Thake (1987) stated that a lower micro algae growth rate could be a result of the increase in respiration due to rise in temperature above the species' optimum level. It is possible that all these effects are related to the results observed in growth rate, and therefore in cell density and biomass in this work. As the results suggest, the adequate

temperature for *C. calcitrans* is between 20 and 25 °C, under the conditions used in these experiments.

Salinity (25–35) had no significant effect on growth, maximum cell density, biomass and chlorophyll per cell, although a tendency of higher growth, biomass and lower cell density was observed at the lower salinity. The observed contrast between higher growth rate and lower maximum cell density could be explained by a limitation in some nutritional factor not determined at the lowest salinity (25), as the medium of this salinity was obtained by dilution of the seawater. Further tests are needed to determine the cause of such results.

Higher growth rates occurred when carbon dioxide was added to the cultures, indicating that although other factors may be sufficient, the nutrient can limit the algal growth. Increases in growth rate have been observed in other micro algae species with carbon dioxide addition (Olaizola, Duerr & Freeman 1991). It was also reported that addition of carbon dioxide to algal culture extends the exponential phase, which is important in the hatchery system, as it provides maximum nutritional value for aquatic animals (Fabregas, Otero, Dominguez & Patino 2001).

In the present study, lipids and carbohydrates were high at low temperatures, whereas the protein value was low at 25 °C. According to Renaud, Zhou, Parry, Thinh and Woo (1995), the maximum lipid content coincides with the optimal range in growth temperature in many species and varies at temperatures below and above this range. Another investigation showed a higher lipid content at 25 °C for *Chaetoceros* sp., while *Rhodomonas* sp., *Cryptomonas* sp. and *Isochrysis* sp. showed higher concentrations between 27 and 30 °C (Renaud *et al.* 2002). All the species studied showed a significantly lower protein content at temperatures above 27 °C. Carbohydrates in *Chaetoceros* sp. were significantly higher between 25 and 30 °C and became lower at higher temperatures. In general, the results of biochemical composition of *C. calcitrans* are in accordance with other similar works. Lipids and carbohydrates are considered as stored energy products (Thompson, Guo & Harrison 1992) and their decrease can negatively affect the growth and metabolic activities of cells.

The present study suggests that temperatures between 20 and 25 °C could be used to optimize the nutritional value of *C. calcitrans* due to the higher lipid and carbohydrate and adequate protein content under these conditions. Higher levels of carbohydrates are reported to produce higher growth of *Ostrea edulis* juveniles (Enright, Newkirk, Craigie & Castell 1986) and larvae of *Patinopecten yessoensis* (Whyte *et al.* 1989).

The effects of salinity on proximate composition of algae are shown in Fig. 2a–c. Protein content was low at a salinity of 35, while lipids and carbohydrate increased slightly by mineral fraction. Although many species of micro algae are tolerant to great variations in salinity, their chemical composition can be affected (Brown, Jeffrey & Garland 1989; Roessler 1990). Protein, lipids and carbohydrates seem slightly affected by a wide range of salinity for most micro algae species (Richmond 1986). However, in some species, increases in ash and lipid content were observed at higher salinity (Ben-Amotz, Fishler & Schneller 1987). Fabregas, Herrero, Abalde and Cabezas (1985) reported a decrease in the protein content with an increase in salinity. The result shows that a salinity of 25 is optimum for *C. calcitrans* in terms of growth and chemical composition.

The effect of carbon dioxide on the biochemical composition of *C. calcitrans* is shown in Fig. 2a–c. A decrease in carbohydrate (Fig. 2b) and an increase in protein content (Fig. 2c) were noted when carbon dioxide was added to the culture. Brown, Jeffrey, Volkman and Dunstan (1997) noticed an increase (100%) in protein content when cultures were enriched with 1% carbon dioxide in many species in different groups of micro algae. Lipids and carbohydrates, on the other hand, were not affected. In *Phaeodactylum tricornutum*, the protein content was increased with carbon dioxide addition (Chrismadha & Borowitzka, 1994). Chu *et al.* (1996) observed increases in lipids and carbohydrates at protein expenses in the diatom *Nitzschia inconspicua*, when the culture was enriched with 5% (v/v) of carbon dioxide. In the present work, *C. calcitrans* apparently directed the extra-assimilated carbon mainly to protein synthesis, indicating a positive effect on cell physiology. Probably, the cells were investing the excess of carbon assimilated much more in protein synthesis and growth rather than lipids and carbohydrates as reserve substances in micro algae.

According to the results, a salinity of 25, temperature between 20 and 25 °C and addition of carbon dioxide seems more adequate for enhanced growth of *C. calcitrans* and high biochemical composition in terms of protein, lipids and carbohydrates. The system could be useful for the high algal production and successful operation of a hatchery system.

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Impact of eutrophication on the occurrence of *Trichodesmium* in the Cochin backwaters, the largest estuary along the west coast of India

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Abstract Phytoplankton studies in early 1970s have shown the annual dominance of diatoms and a seasonal abundance of *Trichodesmium* in the lower reaches of the Cochin backwaters (CBW) and adjacent coastal Arabian Sea during the pre-summer monsoon period (February to May). Surprisingly, more recent literature shows a complete absence of *Trichodesmium* in the CBW after 1975 even though their seasonal occurrence in the adjacent coastal Arabian Sea continued without much change. In order to understand this important ecological feature, we analyzed the long-term water quality data (1965–2005) from the lower reaches of the CBW. The analyses have shown that salinity did not undergo any major change in the lower reaches over the years and values remained >30 throughout the period. In contrast, a tremendous increase was well marked in levels of nitrate (NO_3) and phosphate (PO_4) in the CBW after 1975 (av. 15 and 3.5 μM, respectively) compared with the period before (av. 2 and 0.9 μM, respectively). Monthly time series data collected in 2004–2005 period from the lower reaches of the CBW and coastal Arabian Sea have clearly shown that the physical characteristics like salinity, temperature, water column stability, and transparency in both regions are very similar during the pre-summer monsoon period. In contrast, the nutrient level in the CBW is several folds higher (NO_3, 8; PO_4, 4; SiO_4, 10; and NH_4, 19 μM) than the adjacent coastal Arabian Sea (NO_3, 0.7; PO_4, 0.5; SiO_4, 0.9; and NH_4, 0.6 μM). The historic and fresh time series data evidences a close coupling between enriched levels of nutrients and the absence of *Trichodesmium* in the Cochin backwaters

Keywords Eutrophication · *Trichodesmium* · Nutrients · Phytoplankton · Arabian Sea · Cochin backwaters

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Introduction

One of the well-documented consequences of human alterations of environment is the eutrophication of estuaries and coastal seas. The term 'eutrophication' refers primarily to the increase in compounds of nitrogen and phosphorus in aquatic ecosystems. Eutrophication may cause exponential growth of a few species of phytoplankton causing the loss of biodiversity (Verlekar et al. 2006). High growth of a few species of phytoplankton may disrupt the balance of the ecosystem by depletion of oxygen near the bottom, production of toxins, etc., thereby negatively influencing the associated

organisms. Normally, eutrophication is a very gradual natural process, but large-scale human activities can greatly accelerate the rate at which nutrients enter into aquatic ecosystems (Anderson et al. 2002).

The deterioration of water quality of many Indian estuaries in recent times has been reported (Balachandran et al. 2005; Mukhopadhyay et al. 2006; Ramaiah et al. 2006; Martin et al. 2011). The need for protecting these estuaries from further eutrophication is an important concern due to mounting human settlements and developmental activities. In this direction, Cochin backwaters, situated along the southwest coast of India, is a good example to examine. It is the largest estuarine system along the southwest coast of India with high levels of nutrients throughout the year (NO_3>8, PO_4>3, SiO_4> 5 μM). The nutrients in backwater reach exceptionally high levels (NO_3>50 μM, PO_4>50 μM, SiO_4>125 μM) during the summer monsoon (Saraladevi et al. 1983, 1991; Sankaranarayanan et al. 1986; Jyothibabu et al. 2006; Martin et al. 2008, 2010). Runoff from agriculture, industrial discharge, aquaculture, and domestic wastes are the major sources of nutrients in the backwater (Saraladevi et al. 1991; Vijayan et al. 1976; Madhu et al. 2007). The coastal region of the southeastern Arabian Sea (also known as Laccadive Sea), which receive the influx of backwater, shows marked decrease in nutrient and phytoplankton pigment levels due to mixing with marine waters (Sankaranarayanan and Qasim 1969; Saraladevi et al. 1991; Lierheimer and Banse 2002).

Trichodesmium is a gaseous nitrogen-fixing cyanobacterium common in the tropical and temperate waters (Capone et al. 1998; Devassy et al. 1978; Jyothibabu et al. 2003; Krishnan et al. 2007; Hegde et al. 2008). Among several species of *Trichodesmium* potent to form red tides, two species *Trichodesmium erythraeum* and *Trichodesmium thiebautii* are common in Indian waters (Devassy et al. 1978; Nair et al. 1992; Krishnan et al. 2007; Jyothibabu et al. 2008). The blooming of *Trichodesmium* in tropical waters is believed as a response to surface layer stratification and nitrogen limitation (Carpentor et al. 1999; Krishnan et al. 2007; Hegde et al. 2008; Jyothibabu et al. 2008). Along the Indian coasts, *Trichodesmium* blooms are common during the pre-summer monsoon period (Devassy et al. 1978; Jyothibabu et al. 2003, 2008; Hegde et al. 2008), and its formation was reported in a wide range of salinity (28–34) (Devassy et al. 1978; Nair et al. 1992; Rao and Sarojini 1992; Devassy and Goes 1988; Jyothibabu et al. 2003, 2008; Krishnan et al. 2007).

Several records in the early 1970s have shown the occurrence of *Trichodesmium* in the lower reaches of the Cochin backwaters during the pre-summer monsoon period (Gopinathan 1972; Gopinathan et al. 1974; also see the review by Verma and Agarwal 2000). However, *Trichodesmium* has not been encountered in the Cochin backwaters in any of the phytoplankton studies conducted in the last three decades (Table 1). At the same time, the incidence of *Trichodesmium* bloom had frequently been reported from the adjacent coastal waters during the pre-summer monsoon period (Nair et al. 1992; Krishnan et al. 2007; Ashadevi et al. 2010). Therefore, the absence of *Trichodesmium* in the Cochin backwaters in recent decades is intriguing given that the physical characteristics of the lower reaches of the backwaters and the coastal Arabian Sea are very similar during the pre-summer monsoon period (Madhupratap 1987; Menon et al. 2000).

In order to study the possible reasons for the intriguing absence of *Trichodesmium* in the backwaters in recent decades, we analyzed three sets of data (a) long-term data of water quality in the lower reaches of CBW, (b) the seasonal hydrographic conditions in the Cochin backwaters and coastal marine waters, and (c) monthly time series water quality data from the lower reaches of the CBW and coastal Arabian Sea spanning from summer monsoon to spring intermonsoon (till the bloom occurred in the coastal Arabian Sea). This examination is primarily because major physical factors like temperature, salinity, and transparency are important in the occurrence and blooming of *Trichodesmium* (Capone et al. 1998; Hood et al. 2001). The major objectives of the present study can be stated as (a) to understand the major environmental change that occurred in the Cochin backwaters in 1970s and to understand whether this change has any link to the absence of *Trichodesmium* in the backwaters and (b) to delineate the reason behind the current absence of *Trichodesmium* in the lower reaches of the backwaters and its presence in the adjacent coastal Arabian Sea.

Materials and methods

Study area

The estuarine system located around the city of Cochin (renamed as Kochi) is known as Cochin backwaters. It consists of the northern part of the backwaters of Kerala

Table 1 Major genera of phytoplankton reported from the Cochin backwaters since 1970

SL. no.	Phytoplankton genera	Hydrography				Year (no. of sampling locations in brackets)	Source
		Salinity	Temp. (°C)	NO_3 (μM)	PO_4 (μM)		
1	(SM) *Triceratium,Fragellaria, Coscinodiscus, Planktoniella*	1.0	27.7	–	–	1970	Gopinathan 1972
	(PM) *Fragellaria, Coscinodiscus, Pleurosigma, Skeletonema*	14.6	28.2	–	–	(2)	
	(PSM) *Skeletonema, Biddulphia, Coscinodiscus, Trichodesmium*	32.3	31.5	–	–		
2	(SM) *Spirogyra, Euastrum,Cosmarium, Closterium*	2.0	29.0	3.0	2.0	1972	Gopinathan et al. 1974
	(PSM) *Skeletonema, Prorocentrum, Ceratium, Trichodesmium*	30.5	30.5	0.75	0.5	(4)	
3	(SM) *Skeletonema, Nitzschia, Coscinodiscus, Asterionella*	1.0	27.5	–	–	1972	Kumaran and Rao 1975
	(PM) *Skeletonema, Nitzschia, Coscinodiscus, Suriella*	6.0	29.5	–	–	(3)	
	(PSM) *Skeletonema, Nitzschia, Coscinodiscus, Pleurosigma*	30.5	30.0	–	–		
4	(SM) *Spirogyra, Euastrum,Cosmarium*	0.5	28.0	1.6	0.3	1974	Gopinathan 1981
	(PSM) *Skeletonema, Prorocentrum, Ceratium, Dictyocha*	30.2	29.3	0.6	0.4	(6)	
5	(SM) *Fragellaria, Eucampia, Nitzschia, Coscinodiscus*	1.7	–	19.0	2.5	1981	Gopalakrishnan et al. 1988
	(PM) *Eucampia, Coscinodiscus, Thalassiosira, Fragellaria*	3.4	–	5.8	2.9	(9)	
	(PSM) *Oscillatoria, Skeletonema, Coscinodiscus, Microcystis*	30.8	–	3.2	3.4		
6	(PSM) *Peridinium, Oscillatoria, Pleurosigma, Navicula*	30.0	31.5	–	2.8	1992 (2)	Balasubramanian et al. 1995
7	(SM) *Asterionella, Thalassiosira, Skeletonema, Nitzschia*	34	26.0	5.89	2.7	2000	Alkershi 2002
	(PM) *Thalassisothrix, Asterionella, Chaetoceros, Skeletonema*	31	30.0	1.44	0.9	(1)	
	(PSM) *Thalassiosira, Chaetoceros, Rhizosolenia, Asterionella*	33	31.0	2.92	1.1		
8[a]	(SM) *Aphanothece, Chroococcus, Dactylococcopsis, Gloeocapsa*	5	28.6	12.0	5.0	2002	Joseph 2005
	(PM) *Chroococcus, Coelosphaerium, Coelosphaerium, Gloecapsa*	27	29.0	7.0	1.5	(8)	
	(PSM) *Aphanothece, Chroococcus, Gloeocapsa, Synechococcus*	30	31.2	6.0	2.0		
9	(SM) *Nitzschia, Skeletonema, Synedera, Cocconeis*	0.8	281	6.5	3.4	2003	Madhu et al. 2007
	(PM) *Skeletonema, Coscinodiscus, Leptocylindrus, Nitzschia*	9.2	29.1	4.9	1.2	(2)	
	(PSM) *Nitzschia, Skeletonema, Synedera, Thalassiosira*	29.2	31.5	10.5	1.2		
10	(SM) *Nitzschia, Skeletonema, Navicula, Leptocylindrus*	0	27.9	12.5	3.9	2005	Present study
	(PM) *Thalassiosira, Skeletonema, Nitzschia, Navicula*	17.0	29.7	6.6	1.7	(1)	
	(PSM) *Nitzschia, Skeletonema, Navicula, Thalassiosira*	30.1	32.7	7.9	3.9		

(*SM* summer monsoon, *PM* post-summer monsoon, *PSM* pre-summer monsoon; *en dash* not available

[a] Study exclusively on cyanobacteria)

which extends from Aleppey to Azhikode (between Lat. 9° 30′ to 10° 10′N and Lon. 76° 15′ to 76° 25′E). The backwaters is a complex, shallow estuarine network running parallel to the coastline of Kerala with two permanent opening to the Arabian Sea—one at Cochin and the other at Azhikode. Six rivers (Pamba,

Achancovil, Manimala, Meenachil, Periyar, and Muvattupuzha) with their tributaries and several canals bring large volumes of freshwater into the backwaters. Among these rivers, Periyar and Muvattupuzha discharge into the northern part of the backwaters and hence have an active influence on the prevailing salinity in the Cochin backwaters.

Based on the climatology of the study area, seasons have traditionally been classified into monsoon/summer monsoon/southwest monsoon (June to September), post-monsoon (October to January), and pre-summer monsoon (February to May—see Menon et al. 2000). Among these seasons, summer monsoon period accounts for 60–65 % of the total annual rainfall in the study area (Menon et al. 2000). As a result of heavy rainfall during the peak monsoon period, salinity over a large extent of the backwaters reaches near zero values. During the post-summer monsoon period, river discharge into the backwaters diminishes and salinity gradually increases. As pre-summer monsoon begins, fresh water input into the backwaters considerably decreases due to low rainfall over the region. Hence, a gradient of salinity develops from the mouth to the head of the backwaters, and thus the lower reaches behave as an extension of the Arabian Sea (Madhupratap 1987). Since the backwaters is geographically located in the tropical region, there is only minor seasonal variation of temperature (Madhupratap 1987).

In the backwaters, phytoplankton biomass and production remains largely constant throughout the year, although marked salinity variations arise seasonally as a result of heavy freshwater influx (Menon et al. 2000). High river influx seems to have only minor effect on the overall phytoplankton production in the backwaters (Qasim 2003). However, a qualitative shift in phytoplankton composition has been reported in the backwaters during extremely low saline conditions (Menon et al. 2000). Among various size classes of phytoplankton in the backwaters, nano-size fraction contributes majority of the primary standing stock and production all through the year (Menon et al. 2000; Qasim 2003).

Sampling

The typical seasonal features in the hydrography of the study area were generated based on observations from 20 stations in the backwaters and 16 in the coastal waters during the summer monsoon (September 2004) and pre-summer monsoon (April 2005). The idea behind the sampling was to differentiate the seasonal hydrographic features in the backwaters and coastal waters (Fig. 1). The sampling time was selected based on the understanding that the pre-monsoonal and summer monsoonal hydrographical features would be fairly reflected in observations during April and September, respectively (Menon et al. 2000). In addition to the seasonal measurements described above, from October 2004 to April 2005, monthly sampling was carried out at two locations in the lower reaches of backwaters and coastal waters. This sampling was to present the gradual environmental changes that occur in the backwaters and coastal waters from the summer monsoon to pre-summer monsoon conditions (Fig. 1).

The six rivers that empty into the backwater are responsible for the exceptionally high concentration of SiO_4 (Sankaranarayanan and Qasim 1969). In contrast, non-point (local) sources have a major role in causing high nitrate levels in the backwaters (Saraladevi et al. 1991). The industrial developments and intensification of agriculture practices in the early 1970s have considerably accelerated the eutrophication in the backwaters

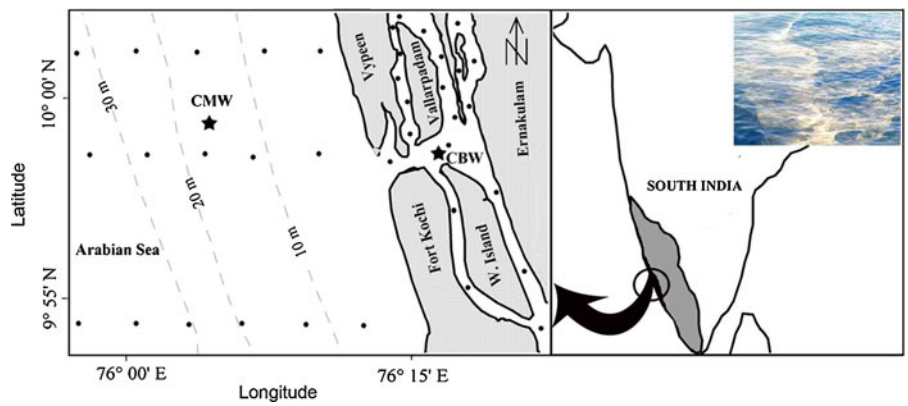

Fig. 1 Station locations in the cochin backwaters and coastal waters; '*stars*' designate locations of monthly sampling; *Trichodesmium* bloom observed in the coastal waters during April 2005 in the *inset*

(Balachandran 2001; Martin et al. 2008). The resultant increase in NO_3 and PO_4 concentration in the backwaters is well reflected in the long-term data of NO_3 and PO_4 levels from the lower reaches of the Cochin backwaters presented in Fig. 2. The marked increase in NO_3 and PO_4 concentration began in the early 1970s and attained elevated levels since 1980. It is important to see that the high nutrient levels that prevailed in the backwaters over several years have not caused any massive phytoplankton bloom or oxygen depletion within the estuary so far, possibly due to adequate renewal of estuarine waters by the combined action of river discharge and tidal exchange. Contrasting to NO_3 and PO_4, the salinity in the lower reaches of the backwaters do not show any appreciable change over the years (Fig. 2).

Methods

The surface temperature was measured using a centigrade thermometer. Salinity of the surface samples were measured using a calibrated salinometer (Digi Auto 3 G). During the monthly sampling, conductivity, temperature, and depth (CTD) profiler recorded the vertical variation of temperature and salinity. From the CTD data, the stratification of water column was decided in terms of 'barrier layer' which is the difference between isothermal and isopycnal depths. In coastal areas where fresh water influx governs the stability of the water column, the barrier layer thickness is a direct representation of the strength of the surface stratification (Balachandran et al. 2008a). In order to understand the transparency of the water column, a Secchi disc was operated in the coastal and backwater locations during the monthly sampling. Attenuation coefficient of the water column during different months was calculated from the Secchi disc data based on Pickard and Emery (1982).

Water samples were collected from surface (0.5 m) and bottom using Niskin samplers. Samples for dissolved oxygen (DO) were analyzed by Winkler's method. Nutrient (NO_3, PO_4, SiO_4, and NH_4) samples were filtered through Whatman no.1 filter paper (pore size 1 μm) and analyzed using a spectrophotometer (Shimazdu, Japan) following standard procedures (Grasshoff et al. 1983). Water samples (500 ml) were filtered through Whatman GF/F filter papers (pore size 0.7 μm), and the chlorophyll *a* was extracted using 90 % acetone. The measurements were carried out using a spectrophotometer following the procedure of Strickland and Parsons (1972). Water samples (500 ml) were also collected for qualitative and quantitative analysis of phytoplankton and preserved in 4 % acid Lugol's iodine. Water samples were concentrated to 10 ml following the settling and siphoning procedure. The 6–8 ml of the concentrated samples (six to eight replicates of 1 ml each) was scanned in a Sedgewick rafter counting chamber under an inverted epiflourescent microscope (Olympus IX 71) with ×200–400 magnification. The identification of phytoplankton was carried out based on standard literature (Subrahmanyan 1959; Tomas 1997). In the

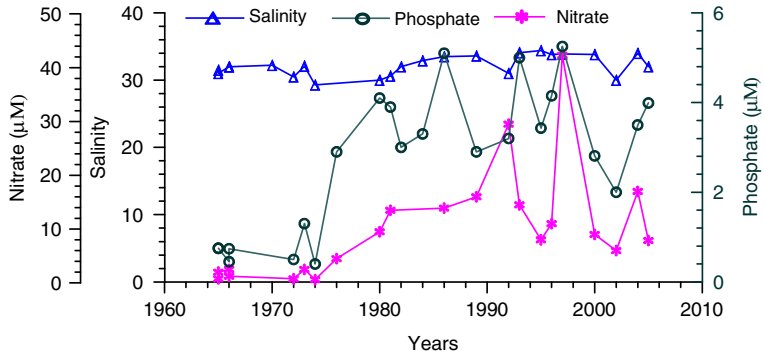

Fig. 2 Long-term variation in salinity, nitrate (NO_3), and phosphate (PO_4) in the lower reaches of CBW during the PSM period. Source: 1965—Sankaranarayanan and Qasim 1969; 1966—Qasim and Gopinathan 1969; 1968—Reddy and Sankaranarayan 1972; 1970—Gopinathan 1972; 1972—Joseph 1974; 1973—Maniloth and Salih 1974; 1976—Lakshmana et al. 1987; 1980—Nair et al. 1988; 1981—Saraladevi 1986; 1982—Sankaranarayanan et al. 1986; 1984—NIO data unpublished; 1986—Anirudhan 1988; 1989, 1992, 1993, 1995, 1996—NIO data unpublished; 1997—Sheeba 2000; 2000—Balachandran 2001; 2004—Martin et al. 2008; 2005—Present study

case of *Trichodesmium,* which formed a bloom in the coastal waters in April with an areal extension of about 5 km, individual filaments were counted during the phytoplankton analyses (Fig. 1). In order to make a measure of the phytoplankton diversity in the backwaters and coastal waters, Shannon-Weaver index (Shannon and Weaver 1963) was calculated using the species abundance data of the monthly sampling.

Results

Seasonal features in salinity and temperature

The seasonal variations of salinity and temperature in the study area are shown in Fig. 3. During the summer monsoon, freshwater was predominant in a major part of the backwaters (Fig. 3), and as a result, the salinity in the barmouth area was also very low (3–5). In contrast, high salinity with less variability between locations (av. 32±1) was found in the coastal waters. During the summer monsoon period, the surface water was warmer in the backwaters (26–34 °C) compared with the coastal waters (25.3–31 °C).

During the pre-summer monsoon, due to increased seawater incursion, the lower reaches of the backwater behaved as an extension of the Arabian Sea with fairly high salinity (31–33) (Fig. 3). The low freshwater influx was the main causative factor for the high salinity level (>31) in the backwater during the pre-summer monsoon period. As usual, salinity was low (<1) at the upstream north of the backwater. The surface temperature in the backwaters during the pre-summer monsoon period varied from 31 °C to 33.5 °C with relatively high values in the upstream region. As observed during the summer monsoon period, the surface temperature was lesser in the coastal waters (29.8–30.5 °C) compared with the backwaters.

Monthly variations of salinity, temperature, and transparency

The surface salinity in the backwaters gradually increased from near zero in October to 33 in April whereas, in the coastal waters, it increased from 31 in October to 33.7 in April (Fig. 4a). By April, the lower reaches of the backwaters showed prominent marine features with salinity >32 (Fig. 4a, b). The warming of surface waters from October to April was evident both in the backwaters and coastal waters. The backwater was warmer throughout the study

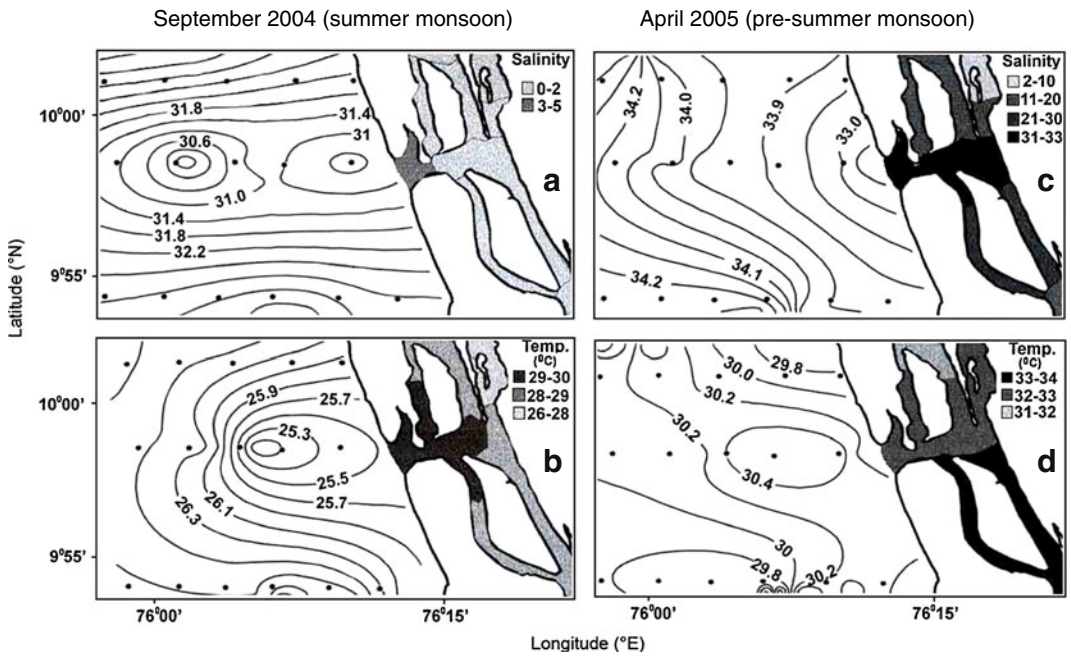

Fig. 3 Distribution of salinity and temperature during (**a**, **b**) September 2004 and (**c**, **d**) April 2005. The distribution plots are prepared similar to Balachandran et al. 2005, pages 363–364

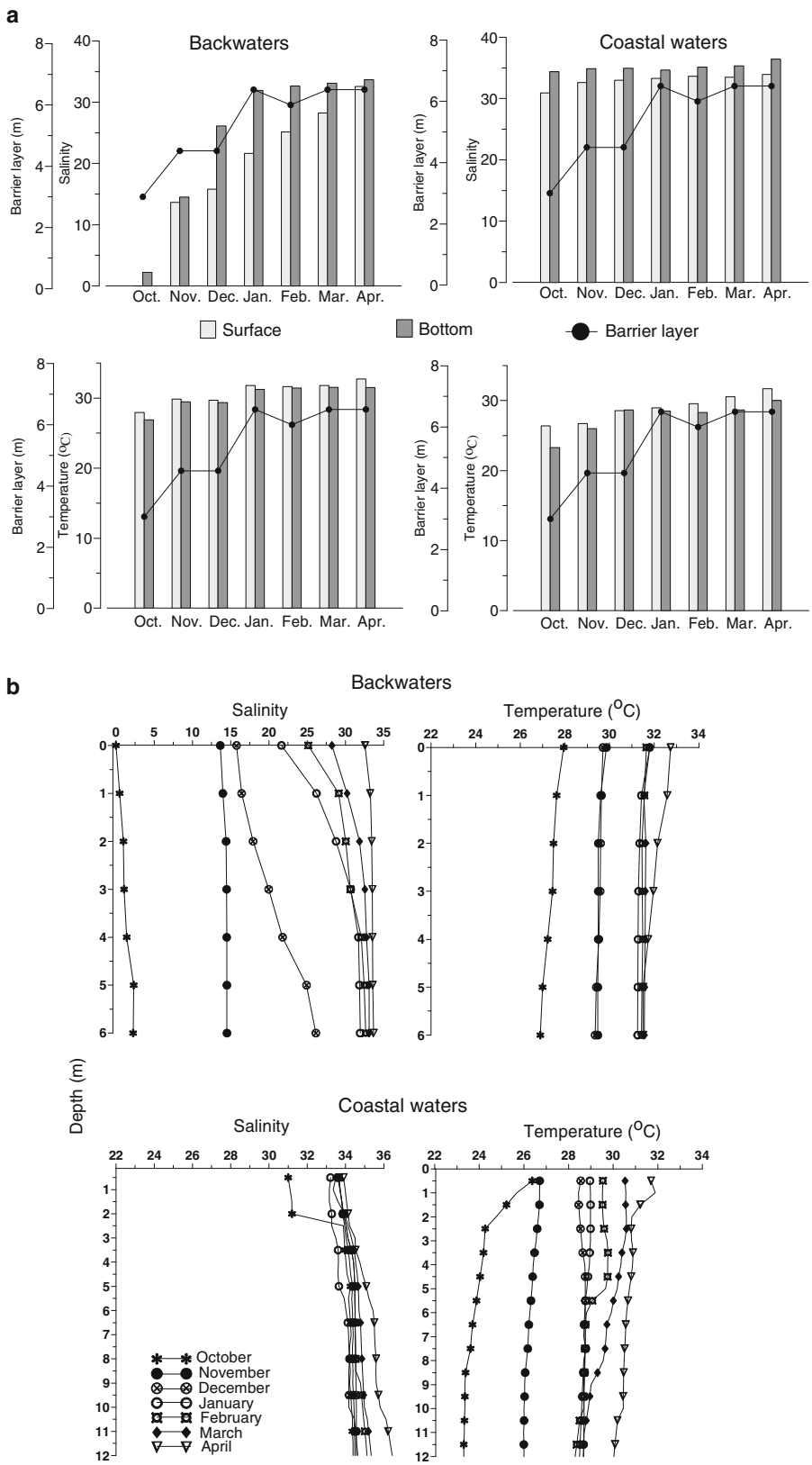

Fig. 4 **a** Monthly variation in salinity, temperature, barrier layer, and **b** vertical profiles of salinity and temperature at the two locations designated in Fig. 1

period compared with the coastal waters (Fig. 4a, b). The stability (barrier layer) of the water column in the backwaters and coastal waters increased from October to April (Fig. 4a) and attained a comparable level in April (Fig. 4a). The attenuation coefficient of the water column in the backwaters was markedly higher (lower transparency) in the backwaters from October to February compared with the coastal waters (Fig. 5). By March, the water column transparency in the backwaters and coastal waters reached comparable magnitude, and in April, both regions attained almost same amount of solar light availability in the subsurface waters.

Seasonal features of DO and nutrients

During the summer monsoon period, DO concentration varied from 4.1 to 7 mg L^{-1} with higher concentration in the backwaters compared with the coastal waters (Fig. 6a). During the period, all major nutrients were found to be high (NO_3>2; NH_4>0.5, PO_4> 1 μM, and SiO_4>2.5 μM) both in the backwaters as well as in the coastal waters (Fig. 6a–e). Among the sampling regions, the backwaters showed higher nutrient concentration as compared with the coastal waters (Fig. 6b–e). Very high concentration of NO_3 and silicate (>40 and >90 μM, respectively) was found in the upper reaches of the backwater during the period (Fig. 6b, e).

The DO and nutrient distribution during the pre-summer monsoon period is presented in Fig. 6f–j. DO concentration varied spatially from 4 to 7 mg L^{-1} (Fig. 6f). The NO_3 and SiO_4 level during the pre-monsoon period (Fig. 6g, j) was markedly lower as compared with the summer monsoon period (Fig. 6b, e). In contrast, the concentration of NH_4 in the backwaters was higher during the pre-summer monsoon period than the summer monsoon period (Fig. 6c, h), with relatively high values in the upper estuary (21–35 μM). Similarly, the PO_4 concentration in the backwaters was also higher during the pre-summer monsoon period (Fig. 6d, i) compared with the summer monsoon.

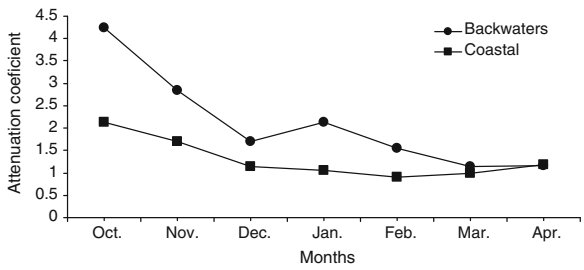

Fig. 5 Monthly changes in the transparency in terms of attenuation coefficient at the two locations designated in Fig. 1

Monthly variations of DO and nutrients

The monthly variations of DO and nutrients in the backwaters and coastal waters are shown in Fig. 7. Except during October, DO level was consistently higher in the backwaters compared with the coastal waters. The NO_3 levels in the backwaters decreased initially from October to January and then increased towards April (18 μM at the surface and 14 μM at the bottom). In the coastal waters, the concentration of NO_3 decreased considerably from October (11 μM at the surface and 28 μM at the bottom) to April (0.4 μM at the surface and 0.5 μM at the bottom). During most of the observations, especially during the pre-summer monsoon period, NO_3 level in the backwaters was higher than that of the coastal waters (Fig. 7).

During the later part of the pre-monsoon period (March–April), the NH_4 level was also markedly higher in the backwaters compared with the coastal waters (Fig. 7). Throughout the sampling period, PO_4 and SiO_4 were higher in the backwaters compared with the coastal waters (Fig. 7). SiO_4 level in the backwaters and coastal waters showed a gradual decrease from October to April with consistently lower values in the latter region compared with the former.

Variations in chlorophyll a and phytoplankton

During both seasonal observations (September 2004 and March 2005), chlorophyll a was higher in the backwaters compared with the coastal waters (Fig. 8). Except in the southern part of the coastal region, chlorophyll *a* was higher during the pre-summer monsoon period compared with summer monsoon. The concentration of chlorophyll a was very high (>10 mg m^{-3}) in the backwaters during most of the monthly observations whereas it was relatively low (<8 mg m^{-3}) in the coastal waters throughout the observations.

The phytoplankton community in the lower reaches of the backwaters and coastal waters were more or less similar in composition (Table 2) and *Nitzschia*,

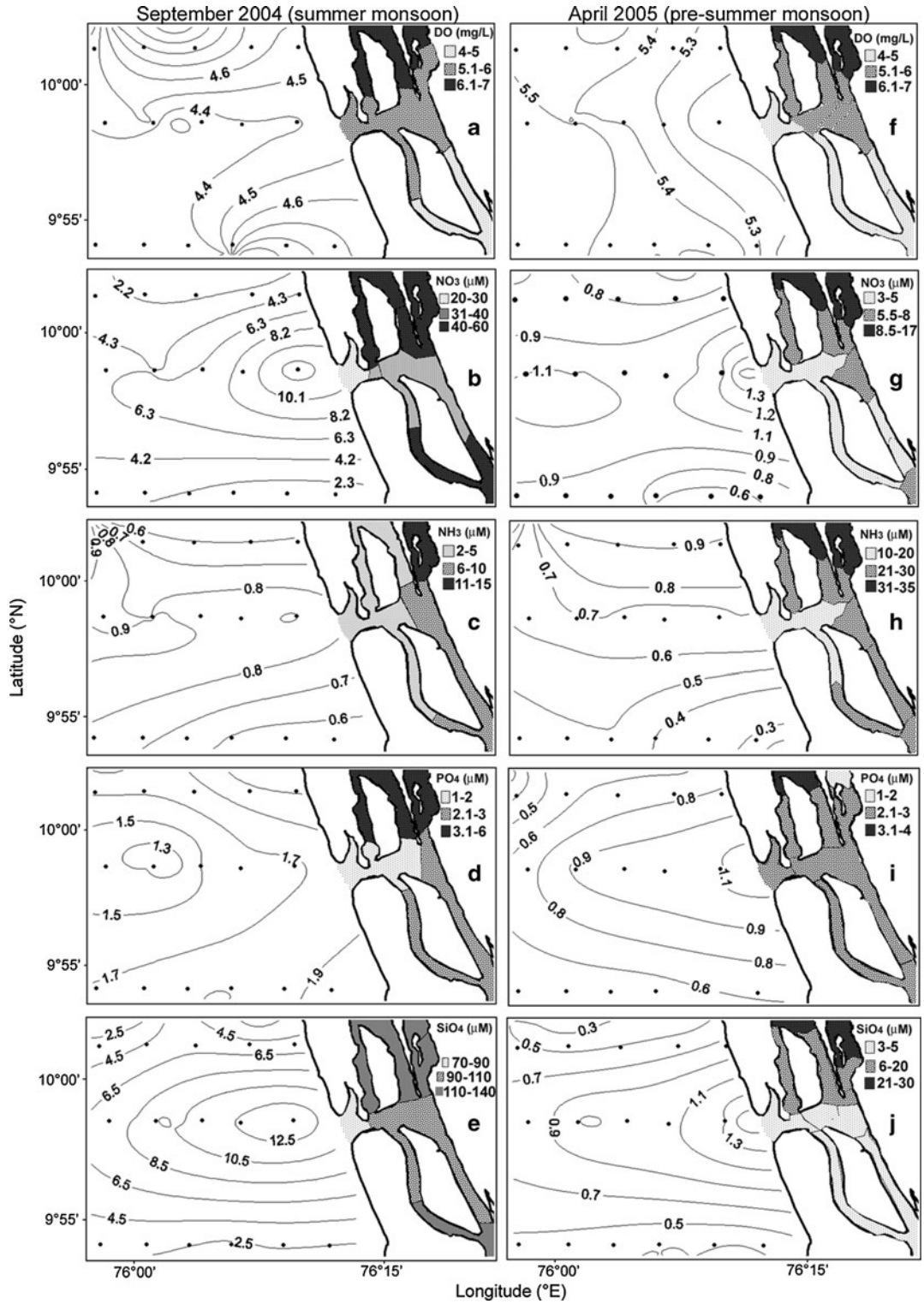

Fig. 6 Distribution of DO and macronutrients during (**a**, **b**, **c**, **d**, **e**) September 2004 and (**f**, **g**, **h**, **i**, **j**) April 2005. The distribution *plots* are prepared similar to Balachandran et al. 2005, pages 363–364. The concentration in the backwaters is shown as ranges whereas contouring by Surfer software is used for coastal waters

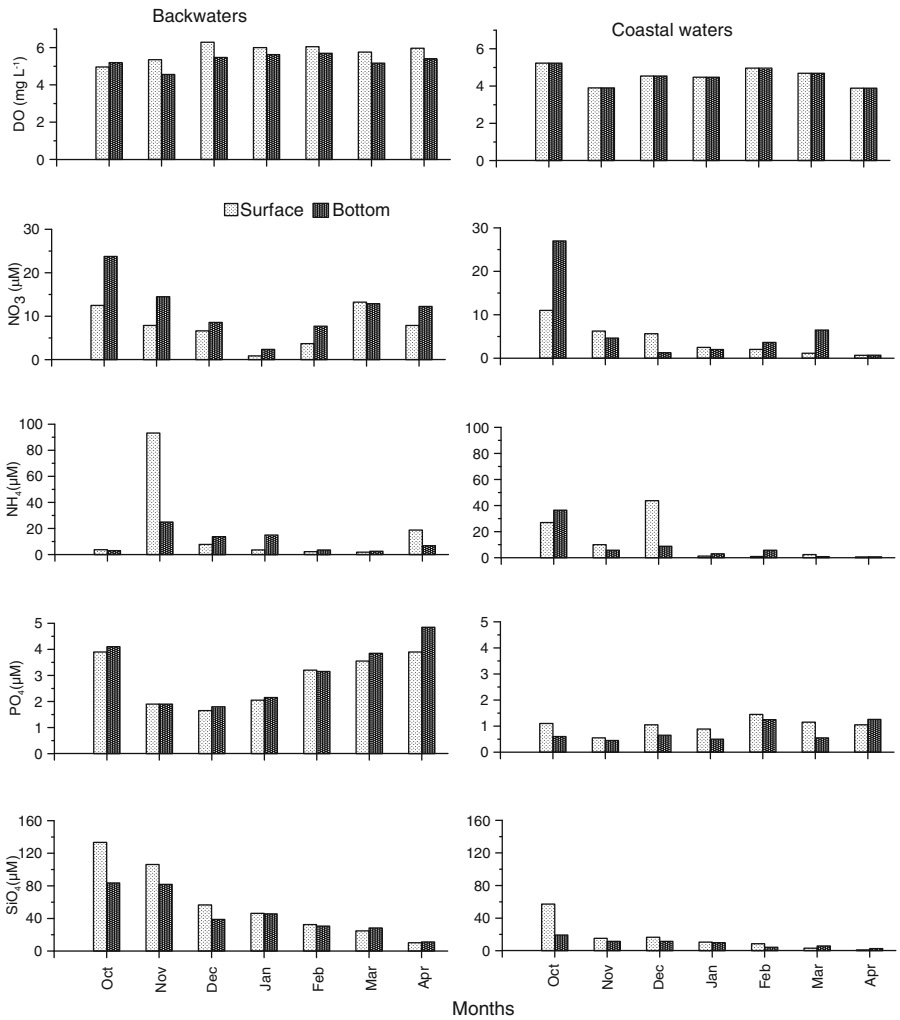

Fig. 7 Monthly variations in DO (milligrams per liter) and macronutrients (micromolars) at the two locations designated in Fig. 1

Skeletonema, Thalassiosira, and *Thalassionema* were the dominant genera of diatoms in both regions. *Trichodesmium* was not recorded in the backwaters during the study, whereas it was present in the coastal waters during January to April period. From October to March, phytoplankton abundance was high in the backwaters (av. 64,500±8,000 no. L^{-1}). In April, due to proliferation of *Trichodesmium*, phytoplankton abundance in the coastal waters has increased significantly (186,950 no. L^{-1}). The species diversity of phytoplankton was high in the backwaters in October, November, and April (1.70, 1.72, and 1.79, respectively) whereas it was high in the coastal waters in January, February, and March (Fig. 9).

Discussion

Anthropogenic influence and eutrophication

A significant change in the estuarine ecology due to human interference of the environment was reported from the Hooghly estuary, at the head of the Bay of Bengal (Sinha et al. 1996; De et al. 1994). The above study reported a considerable shift in phytoplankton composition including an elimination of *Trichodesmium* sp. in recent decades. This was attributed primarily to the construction of Farakka Barrage on the River Ganga in April 1975. This barrage has brought about significant increase in freshwater discharge into

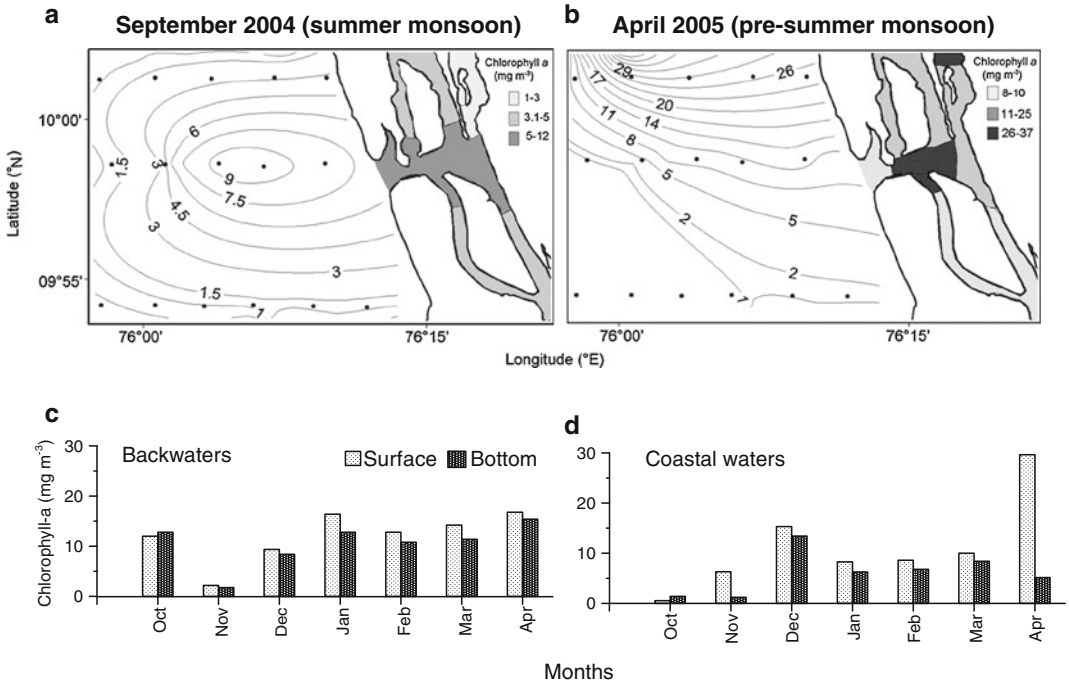

Fig. 8 Distribution of chlorophyll a (milligrams per cubic meter) during **a** SM, **b** PSM, and **c**, **d** monthly variation in chlorophyll a at the two locations designated in Fig. 1

the Hooghly estuary, causing a major qualitative shift in the biological components (Sinha et al. 1996). However, such major decrease in salinity has not been observed in backwater over the years (Fig. 2). During the pre-summer monsoon, the lower reaches of the estuary continues to have marine features and behave as an extension of the Arabian Sea (Fig. 3).

It is estimated that the backwaters receive 42.4×10^3 inorganic PO_4 and 37.6×10^3 mol day^{-1} of inorganic nitrogen through River Periyar, the major river associated with the backwaters (Naik 2000). Out of these nutrient inputs, there is an export 28.2×10^3 mol day^{-1} inorganic PO_4 and 24×10^3 mol day^{-1} inorganic nitrogen into the coastal waters which indicate the amount of the surplus inorganic nutrients available in the backwaters (Naik 2000). The long-term data shows that NO_3 and PO_4 were in low levels up to early 1970s, and since then, it increased due to augmented industrial and agriculture activities. During 1965, the surface PO_4 and NO_3 were 0.75 and 2.0 μM, which increased to 2.9 and 6 μM, respectively, by 2000. The overall trend shows a prominent increase of NO_3 and PO_4 after 1975, and from 1980 onward, the concentrations remained high (Balachandran 2001). It is important to note that this comparison is based on available data from the lower reaches of the backwaters as several researchers have sampled this region since 1965.

Seasonal changes in physical features in the backwaters and coastal waters

Normally, the surface layer stratification in marine waters is largely governed by solar heating (Pickard and Emery 1982). However, in areas of high freshwater influx, the water column stability is governed primarily by the upper layer of freshwater (Pickard and Emery 1982). This was found true during the present study also, since the stability of the water column in terms of barrier layer thickness was high in the coastal waters (Fig. 4). Water column in the backwaters attained stability comparable to that of the coastal waters during April, when the surface salinity in the former region was more or less same as that of the latter (Fig. 6). Located in the tropical region, Cochin backwaters receive the highest amount of solar radiation during the pre-monsoon

Table 2 Monthly variation of dominant phytoplankton species (individual per liter) in the CBW and CMW

Phytoplankton	October		November		December		January		February		March		April	
	CBW	CMW	CBW	CMW	CBW	CMW	CBW	CMW	CBW	CMW	CBW	CMW	CBW	CMW
Bacillariophyceae														
Skeletonema costatum	11,300	17,520	19,400	15,600	17,900	96,00	16,400	26,00	14,500	45,60	16,500	65,60	23,000	10,900
Coscinodiscus sp.	130	340	150	60	420	410	250	50	210	600	180	60	1,100	260
C. lineatus	440	200	200	–	50	–	–	220	–	220	50	50	240	180
Leptocylindrus danicus	5,200	4,50	4,100	2,10	2,500	100	1,240	130	1,950	530	360	260	2,600	1,300
Rhizosolenia sp.	120	40	60	300	40	120	240	30	60	150	120	120	60	130
R. alata	–	–	–	–	–	–	–	200	–	120	–	160	–	–
R. imbricata	–	–	–	–	–	–	–	400	–	60	–	120	–	160
Biddulphia sinensis	130	–	40	–	–	–	–	50	240	150	60	250	–	–
Thalassionema nitzschioides	1,600	200	800	1600	720	1,300	2,400	1,350	1,900	3,500	2,900	900	3,000	1,200
Thalassiosira sp.	4,200	4,500	15,600	6,500	18,400	8,000	7,500	5,560	11,500	7,560	5,300	6,400	12,600	35,560
Pleurosigma sp.	160	200	420	160	–	100	2,600	120	240	200	190	200	320	320
Pleurosigma normani	–	100	–	–	–	50	–	200	–	220	–	180	–	–
Navicula sp.	6,420	4,500	8,300	4,600	4,500	13,200	2,400	12,900	2,600	11,500	11,300	11,900	6,400	12,400
Nitzschia sigma	100	100	420	–	200	–	–	–	240	160	–	–	230	230
N. closterium	16,000	12,000	21,300	9,400	15,400	16,700	32,000	14,000	29,000	5,000	24,000	11,000	21,820	7,820
Pyrrophyceae														
Peridinium sp.	200	60	420	120	300	300	1,200	1,500	1,080	–	1,300	230	1,300	260
Gonyaulax sp	40	50	240	40	160	100	360	130	450	230	600	430	160	–
Gymnodinium sp	–	100	–	–	–	40	–	–	–	120	–	100	–	–
Ornithocercus sp.	40	60	20	120	60	–	100	20	220	–	–	–-	–	20
Ceratium furca	20	70	100	100	40	50	–	60	180	60	240	120	620	400
C. lineatum	–	–	–	50	–	–	–	–	–	120	–	100	–	–
Cyanophyceae														
T. erythraeum	–	–	–	–	–	–	–	300	–	420	–	2,100	–	11,2000
Others	4,200	1,100	2,100	1,300	720	600	2,400	1,000	1,600	2,400	3,200	4,200	1,100	3,600

Trichodesmium filaments are counted during the study

En dash indicates absence

period (626 g cal cm^{-2} day^{-1}) with 10–12 h of sunshine (Qasim et al. 1968). However, monsoon associated heavy river runoff bring high amount of suspended sediments into the backwaters which considerably reduce the transparency of the water column having implications on the phytoplankton composition and physiology (Qasim et al. 1968). This seasonal feature in solar radiation availability in the subsurface waters was well reflected in the Secchi disc data collected during the present study showing higher attenuation coefficient, more prominent in the coastal waters, during the monsoon period. As river runoff decreases by pre-monsoon period, the water column in the lower reaches of the backwaters and coastal waters attains similar transparency level.

Seasonal changes in chemical parameters

Land drainage and river discharge during the summer monsoon brings in nutrient-enriched waters into the backwaters (Saraladevi et al. 1983, 1986, 1991). As the rain and river flow decreases from October to April, the nutrient input also decreases (Fig. 5; also see Saraladevi et al. 1983). However, the PO_4 levels in

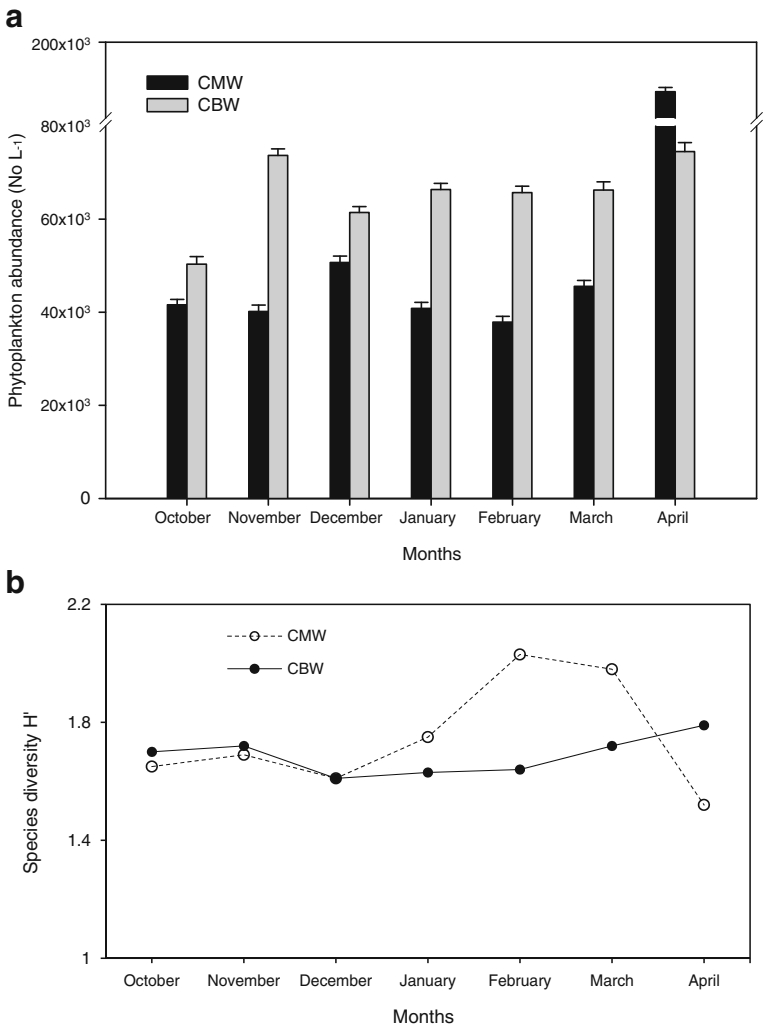

Fig. 9 Monthly variations of phytoplankton abundance (number per liter) and **b** species diversity at the two locations designated in Fig. 1

the backwaters showed a steady increase from December to April, but such changes were not very obvious in the coastal waters. The observed increase in PO_4 levels is believed to be the result of high salinity/pH combined with tidal activity during the pre-summer monsoon which causes desorption of phosphate from the suspended particles (Reddy and Sankaranarayanan 1972; Martin et al. 2008). It is important to note that concentrations of all nutrients in the coastal waters (NO_3, 0.7; PO_4, 0.5; SiO_4, 0.9, NH_4, 0.7 μM) were considerably lower than the backwaters (NO_3, 8; PO_4, 4; SiO_4, 10; NH_4, 19 μM) during the pre-summer monsoon. The high concentration of nitrogen compounds in the backwaters was due to the discharge of industrial, domestic, and agricultural wastes (Vijayan et al. 1976; Saraladevi et al. 1991; Qasim 2003).

Phytoplankton composition and nutrient levels

It is usual that higher amount of phytoplankton stock occurs in the estuaries along the southwest coast of India than the neighboring coastal waters. This was primarily due to the surplus levels of nutrients available in the backwaters throughout the year (Madhu et al. 2007; Balachandran et al. 2008b). This feature is found true during the present study also, since none of the correlations between major nutrients and chlorophyll a showed significant positive relationship (Table 3).

The most common diatoms in the backwaters belong to the genera *Nitzschia*, *Skeletonema*, and *Thalassiosira,* having high adaptability to survive in nutrient-enriched estuarine conditions (Madhu et al.

Table 3 Correlation between chlorophyll a and macronutrients

Parameters		R^2	N	Significance
Chlorophyll *a*	Nitrate (NO_3)	0.02	7	$P>0.05$
Chlorophyll *a*	Phosphate (PO_4)	0.29	7	$P>0.05$
Chlorophyll *a*	Silicate (SiO_4)	0.39	7	$P>0.05$
Chlorophyll *a*	Ammonia (NH_4)	−0.5	7	$P>0.05$

2007). The high abundance of *Thalassiosira* can also be considered as an indication of the deteriorated water quality (Ramaiah et al. 1998; Raman and Prakash 1989). Similarly, *Skeletonema* dominate in areas where organic waste inputs are high (Ramaiah et al. 1998). Prominent decrease in phytoplankton diversity observed in the backwaters during January to March can be related with enriched levels of nutrients, which favors the proliferation of a few species of diatoms (Ramaiah et al. 1998).

The diversity of phytoplankton in the backwaters and coastal waters were more or less comparable during October to December, when nutrient concentrations were high in both regions. During January to March, the phytoplankton diversity in the coastal waters has increased compared with the backwaters which may be linked to the marked decrease in NO_3 and SiO_4 levels. The low NO_3 and SiO_4 levels in the coastal waters might have decreased the ecological advantage of diatoms *Skeletonema costatum* and *Nitzschia closterium*, favoring the co-occurrence of other diatom species in the environment. The environmental condition of high transparency and low nutrients has also favored the proliferation of *Trichodesmium* in the coastal waters in April which in turn decreased phytoplankton species diversity. In contrast, *Trichodesmium* was not encountered in the backwaters as was the observation in the earlier studies (Alkershi 2002; Joseph 2005).

Impact of environmental factors on *Trichodesmium*

High solar radiation, warm and stable waters, and low nutrients level are the favorable conditions for the growth of *Trichodesmium* (Qasim 1970; Capone et al. 1998; Carpentor et al. 1999). Recent modeling studies have suggested that *Trichodesmium* distribution in marine waters is defined by high light intensity, stratified waters, and low concentrations of dissolved inorganic nitrogen (Hood et al. 2001). The physiological response of *Trichodesmium* to environmental features is difficult to measure, but efforts are progressing with laboratory cultures elsewhere (Ohki et al. 1986; Lin et al. 1998; Stihl et al. 2001; Bell et al. 2005). Some of such studies showed that *Trichodesmium* grows actively on a wide range of irradiances with optimal growth at 7 W m^{-2} (Ohki et al. 1986). Similarly, *Trichodesmium* grows actively over a wide range of salinity (22–37), with optimum growth in the range 30–37 (Bell et al. 2005; Hegde et al. 2008). Field studies from the coastal waters of Bay of Bengal and Arabian Sea have also shown that the local species of *Trichodesmium* could form massive blooms with salinity range of 29–31 (Jyothibabu et al. 2003).

During the pre-summer monsoon, solar radiation in the Cochin backwaters and coastal waters is at its seasonal highest with 10–12 h of sunshine (Qasim et al. 1968). The salinity level in the backwaters and coastal waters ranged between 33 and 33.5 which is well within the optimal salinity range (30–37) suggested for the proliferation of *Trichodesmium* (Bell et al. 2005). The warm waters (>30 °C) present in the study area was also conducive for *Trichodesmium* growth (Capone et al. 1998; Hegde et al. 2008). Therefore, it is evident that salinity, solar radiation, and temperature present in the backwaters during the pre-summer monsoon period were conducive for the growth of local species of *Trichodesmium*, and therefore these environmental factors do not act as limiting factors in the study area.

Recent studies have shown that *Trichodesmium* can assimilate compounds of nitrogen (NO_3, NH_4, amino acids, and dissolved organic nitrogen) from solutions. However, the normal growth and physiology of *Trichodesmium* are inhibited by nutrients when present in high concentrations; presence of NO_3 as low as 0.5 μM is found to inhibit the growth of *Trichodesmium*, and large initial concentration of NO_3 (>10 μM) completely stops the N_2-fixation (Holl and Montoya 2005). Similarly, addition of NH_4 to *Trichodesmium* cultures is found to inhibit growth and nitrogen fixation (Lin et al. 1998). Some other studies showed that high PO_4 concentration also has a strong inhibitory effect on the *Trichodesmium* growth (Ohki et al. 1986; Stihl et al. 2001). It is important here to recall the fact that, during the pre-summer monsoon period, Cochin backwaters have shown the presence of exceptionally high levels of nutrients (NO_3, 8; PO_4, 4; SiO_4, 10; NH_4, 19 μM) than the coastal waters

(NO_3, 0.7; PO_4, 0.5; SiO_4, 0.9, NH_4, 0.7 μM). It is also to be noted that the disappearance of *Trichodesmium* in the Cochin backwater coincides ever since (from mid-1970s) pronounced eutrophication has been noticed. Therefore, we propose the exceptionally high levels of nutrients in the backwaters as the primary cause for the absence of *Trichodesmium* in recent times. During the pre-summer monsoon, depleted nutrients level in the coastal waters decrease the ecological advantage of a few species of diatoms over other phytoplankton, favoring the proliferation of *Trichodesmium* (Devassy and Goes 1988). Certainly, more studies would be needed to explore the extent of physiological impact of eutrophication on *Trichodesmium* in the backwater. It is also important to verify the limiting effect of eutrophication on the occurrence of *Trichodesmium* proposed in this paper and in other similar estuarine systems along the Indian coast.

Conclusions

The environmental quality in the CBW and coastal Arabian Sea and its role on the differential occurrence of *Trichodesmium* in respective regions during the pre-monsoon were analyzed. Long-term data from the lower reaches of the backwaters evidenced a fivefold increase in NO_3 and a sixfold increase in PO_4 levels after 1975. Earlier studies on phytoplankton (before 1975) have shown the seasonal occurrence of *Trichodesmium* in the lower reaches of backwater and coastal waters during the pre-summer monsoon. However, studies after 1975 have not encountered *Trichodesmium* in the backwaters, whereas this species has frequently been reported from the neighboring coastal waters during the pre-summer monsoon. While the physical features (salinity, temperature, water column stability, and transparency) in the backwaters and coastal waters were comparable, the nutrient levels in the former region were three- to fivefold higher than the latter. Based on the current understanding, it is proposed that high ambient level of nutrients in the Cochin backwaters is responsible for the absence of *Trichodesmium* in recent times. High level of NO_3 and PO_4 in the backwaters possibly inhibit the normal growth of *Trichodesmium* as observed in earlier experimental studies, whereas its occurrence in the coastal Arabian Sea was favored with the depleted levels of nutrients.

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