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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 1970's 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 μM and 3.5 μM, respectively) compared to the period before (av. 2 μM 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 nutrients level in the CBW is several folds higher (NO_3 – 8 μM, PO_4 – 4 μM, SiO_4 – 10 μM and NH_4 –19 μM) than the adjacent coastal Arabian Sea (NO_3 - 0.7 μM, PO_4 - 0.5 μM, SiO_4 – 0.9 μM 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

Key words: Eutrophication, *Trichodesmium*, Nutrients, Phytoplankton, Arabian Sea, Cochin backwaters

1. 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 μM, PO_4 >3 μM, 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 *T. 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 (Carpenter 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 (Table1). 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 and (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 the important on 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 1970's 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.

2. Materials and methods

2.1. 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 which extends from Aleppey to Azhikode (between Lat. 9^{o} 30' to 10^{o} 10'N and Lon. 76^{o} 15' to 76^{o} 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).

2.2. Sampling

The typical seasonal features in the hydrography of the study area was 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 (Figure 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 (Figure 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 (Balachandran et al. 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 Figure 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 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 (Figure 2).

2.3. Methods

The surface temperature was measured using a centigrade thermometer. Salinity of the surface samples were measured using a calibrated salinometer (Digi Auto 3G). During the monthly sampling, conductivity, temperature, 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 (0.5m) and bottom using Niskin samplers. Samples for dissolved oxygen (DO) were analysed by Winkler's method. Nutrients (NO_3, PO_4, SiO_4, and NH_4) samples were filtered through Whatman No.1 filter paper (pore size 1 µm) and analysed 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. 6-8 ml of the concentrated samples (6-8 replicates of 1ml each) was scanned in a Sedgwick rafter counting chamber under an inverted epiflourescent microscope (Olympus IX 71) with 200-400X magnification. The identification of phytoplankton was carried out based on standard literature (Subrahmanyan 1959; Tomas 1997). In the 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 (Figure 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.

3. Results

3.1. Seasonal features in salinity and temperature

The seasonal variations of salinity and temperature in the study area are shown in Figure 3. During the summer monsoon, freshwater was predominant in a major part of the backwaters (Figure 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 to the coastal waters (25.3 – 31 °C).

During the pre-summer monsoon, due to increased sea water incursion, the lower reaches of the backwater behaved as an extension of the Arabian Sea with fairly high salinity (31- 33) (Figure 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 - 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 to the backwaters.

3.2. 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 (Figure 4a). By April, the lower reaches of the backwaters showed prominent marine features with salinity >32 (Figure 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 period compared to the coastal waters (Figure 4a&b). The stability (barrier layer) of the water column in the backwaters and coastal waters increased from October to April (Figure 4a) and attained a comparable level in April (Figure 4a). The attenuation coefficient of the water column in the backwaters was markedly higher (lower transparency) in the backwaters from October to February compared to the coastal waters (Figure 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.

3.3. Seasonal features of DO and nutrients

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

The DO and nutrient distribution during the pre-summer monsoon period is presented in Figure 6 f - j. DO concentration varied spatially from 4 - 7 mg L^{-1} (Figure 6f). The NO_3 and SiO_4 level during the pre-monsoon period (Figure 6 g & j) was markedly lower as compared to the summer monsoon period (Figure 6 b & e). In contrast, the concentration of NH_4 in the backwaters was higher during the pre-summer monsoon period than the summer monsoon period (Figure 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 (Figure 6d & i) compared to the summer monsoon.

3.4. Monthly variations of DO and nutrients

The monthly variations of DO and nutrients in the backwaters and coastal waters are shown in Figure 7. Except during October, DO level was consistently higher in the backwaters compared to 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 (Figure 7)

During the later part of the pre-monsoon period (March - April), the NH_4 level was also markedly higher in the backwaters compared to the coastal waters (Figure 7). Throughout the sampling period, PO_4 and SiO_4 were higher in the backwaters compared to the coastal waters (Figure 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 to the former.

3.5. Variations in chlorophyll *a* and phytoplankton

During both seasonal observations (September 2004 and March 2005), chlorophyll *a* was higher in the backwaters compared to the coastal waters (Figure 8). Except in the southern part of the coastal region, chlorophyll *a* was higher during the pre-summer monsoon period compared to 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*, *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. 64500 ± 8000 No. L^{-1}). In April, due to proliferation of *Trichodesmium*, phytoplankton abundance in the coastal waters has increased significantly (186950 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 (Figure 9).

4. Discussion

4.1. 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 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 (Figure 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 (Figure 3).

It is estimated that the backwaters is receiving 42.4 x 10^3 mol d^{-1} inorganic PO_4 and 37.6 x 10^3 mol d^{-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 x 10^3 mol d^{-1} inorganic PO_4 and 24 x 10^3 mol d^{-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 onwards, the concentrations remained high (Balachandran et al. 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.

4.2. 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 (Figure 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 (Figure 6). Located in the tropical region, Cochin backwaters receive the highest amount of solar radiation during the pre-monsoon period (626 g cal cm^{-2} d^{-1}) with 10-12 hours 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.

4.3. 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 (Figures 5; also see Saraladevi et al. 1983). However, the PO_4 levels in 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 μM; PO_4 – 0.5 μM; SiO_4 - 0.9 μM, NH_4 – 0.7 μM) were considerably lower than the backwaters (NO_3 – 8 μM; PO_4 – 4 μM; SiO_4 - 10 μM, 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).

4.4. 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 neighbouring 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 belongs to the genera *Nitzschia*, *Skeletonema* and *Thalassiosira,* having high adaptability to survive in nutrient enriched estuarine conditions (Madhu et al. 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 to 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).

4.4. Impact of environmental factors on *Trichodesmium*

High solar radiation, warm and stable waters and low nutrients level are the favourable conditions for the growth of *Trichodesmium* (Qasim 1970; Capone et al. 1998; Carpenter 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 hours of sunshine (Qasim et al. 1968). The salinity level in the backwaters and coastal waters ranged between 33 - 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 inhibits 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 μM; PO_4 – 4 μM; SiO_4 - 10 μM, NH_4- 19 μM) than the coastal waters (NO_3 – 0.7 μM; PO_4 – 0.5 μM; SiO_4 -0.9 μM, 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, favouring the proliferation of *Trichodesmium* (Devassy and Goes 1994). 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, 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 five fold increase in NO_3 and a six fold 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 haven't 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 3 to 5 fold 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 favoured with the depleted levels of nutrients.

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Figure captions

Figure 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

Figure 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 Sankaranarayanan 1972; 1970- Gopinathan 1972; 1972 - Joseph 1974; 1973 - Maniloth and Salih 1974; 1976- Lakshmanan et al. 1987; 1980 - Nair et al. 1988; 1981- Saraladevi et al. 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.

Figure 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.

Figure 4 - (a) Monthly variation in salinity, temperature, barrier layer and (b) vertical profiles of salinity and temperature at the two locations designated in Figure 1.

Figure 5- Monthly changes in the transparency in terms of attenuation coefficient at the two locations designated in Figure 1

Figure 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.

Figure 7- Monthly variations in DO (mg L^{-1}) and macronutrients (µM) at the two locations designated in Figure 1

Figure 8- Distribution of chlorophyll-*a* (mg m^{-3}) during (a) SM, (b) PSM and (c & d) monthly variation in chlorophyll *a* at the two locations designated in Figure 1

Figure 9- Monthly variations of phytoplankton abundance (No. L^{-1}) and (b) species diversity at the two locations designated in Figure 1

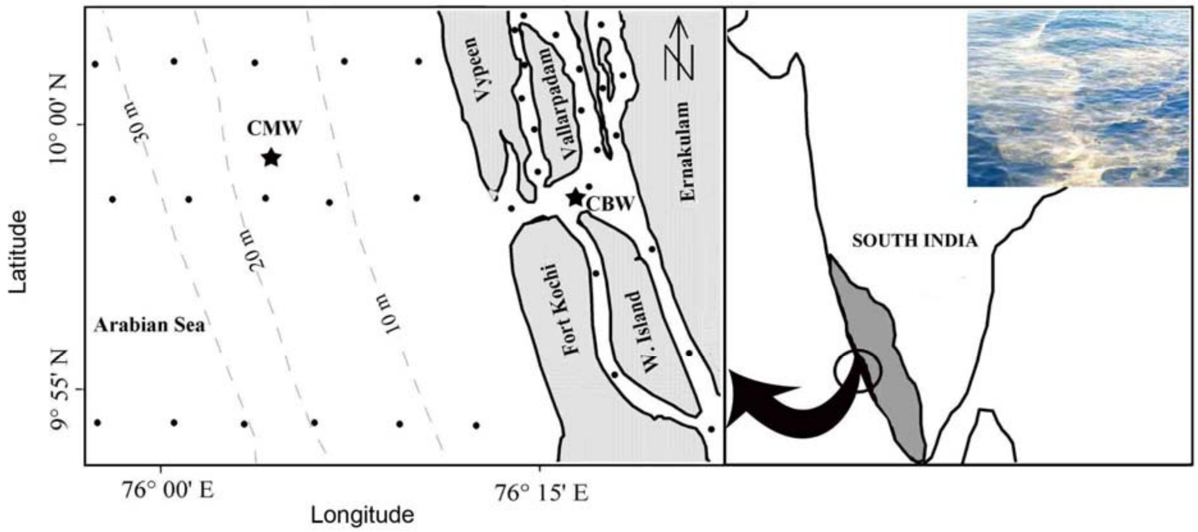


Figure 1

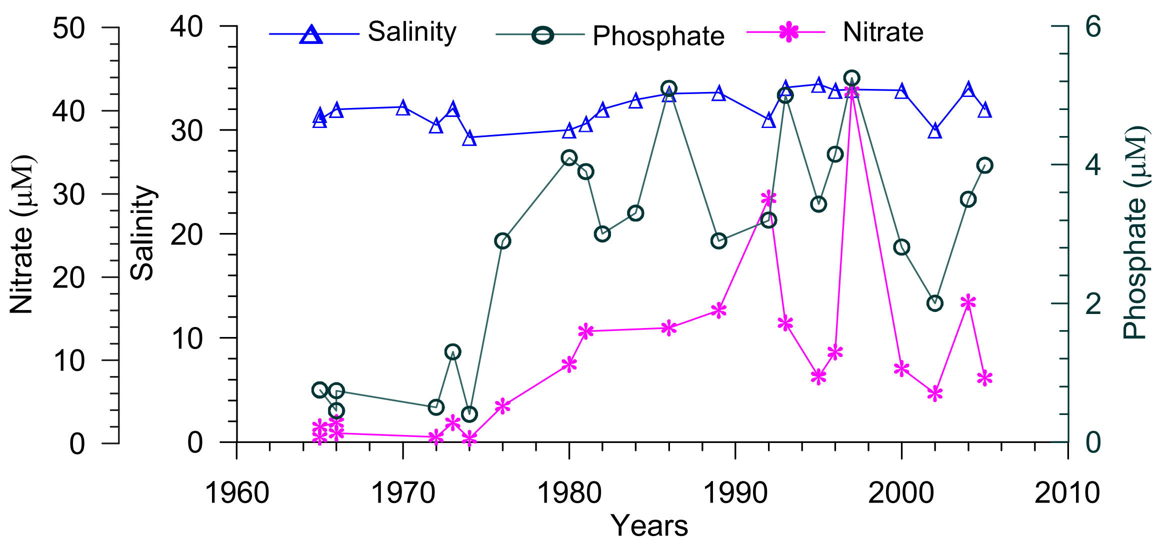


Figure 2

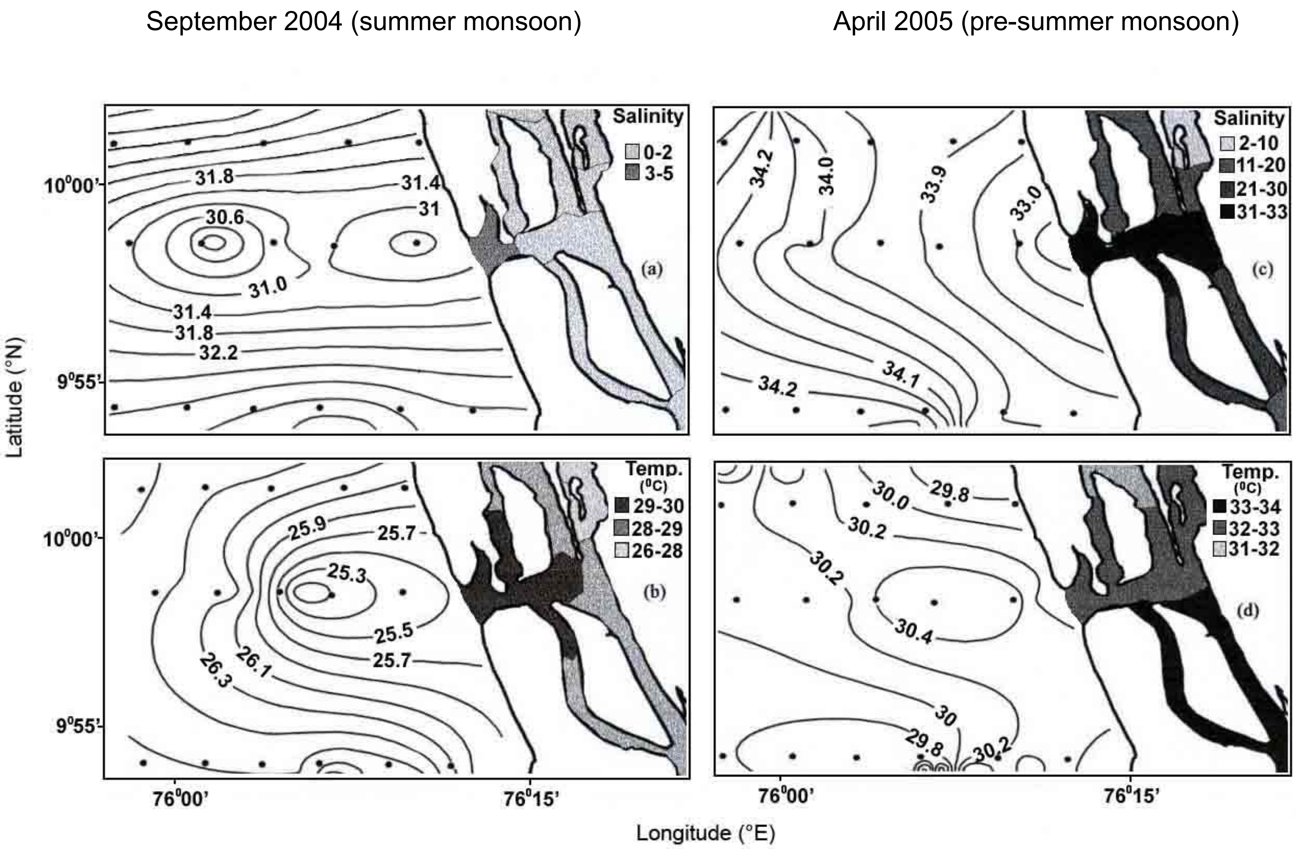
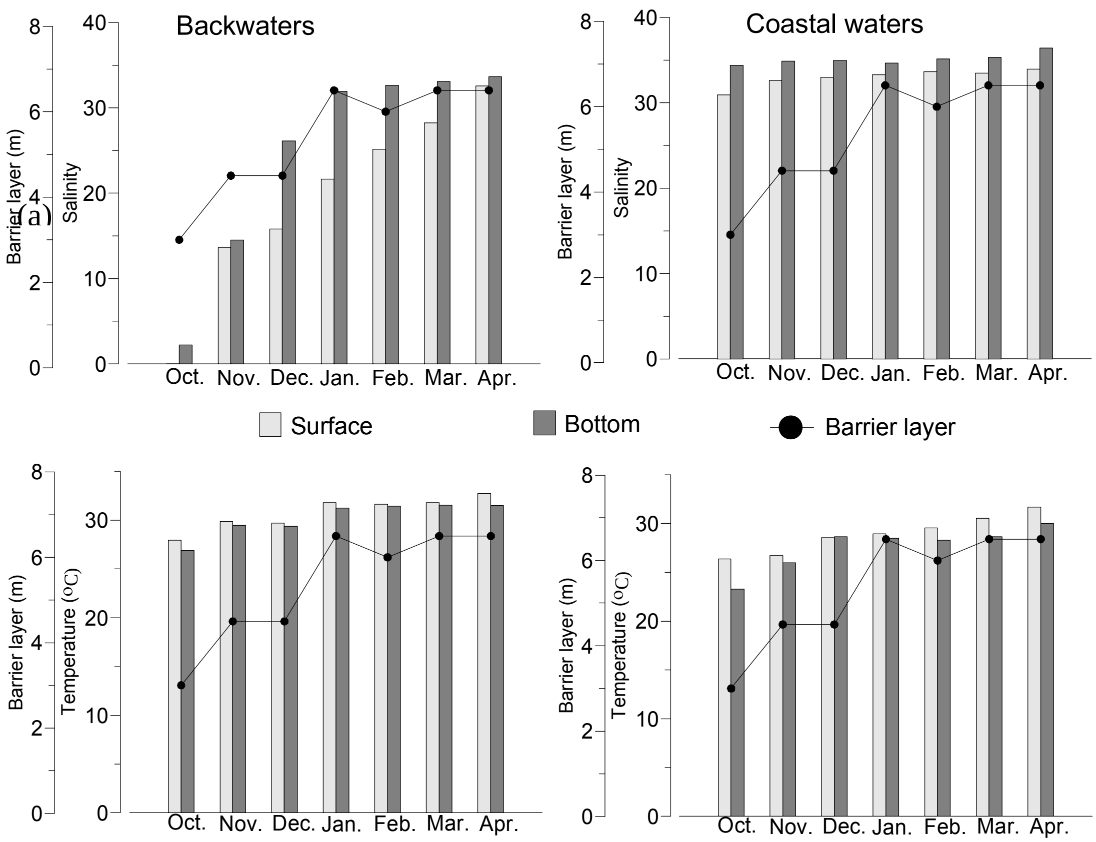

September 2004 (summer monsoon)
April 2005 (pre-summer monsoon)
Salinity
0-2
3-5
31.8
31.4
31
30.6
31.0
31.4
31.8
32.2
(a)
Salinity
2-10
11-20
21-30
31-33
34.2
34.0
33.9
33.0
34.1
34.2
(c)
Temp. (°C)
29-30
28-29
26-28
25.9
25.7
25.3
25.5
25.7
26.1
26.3
(b)
Temp. (°C)
33-34
32-33
31-32
30.0
29.8
30.2
30.2
30.4
30
29.8
30.2
(d)
10°00'
9°55'
Latitude (°N)
76°00'
76°15'
Longitude (°E)

Figure 3

(a)


Backwaters
Coastal waters
Barrier layer (m)
Salinity
Temperature (°C)
Oct. Nov. Dec. Jan. Feb. Mar. Apr.
Surface
Bottom
Barrier layer

(b)

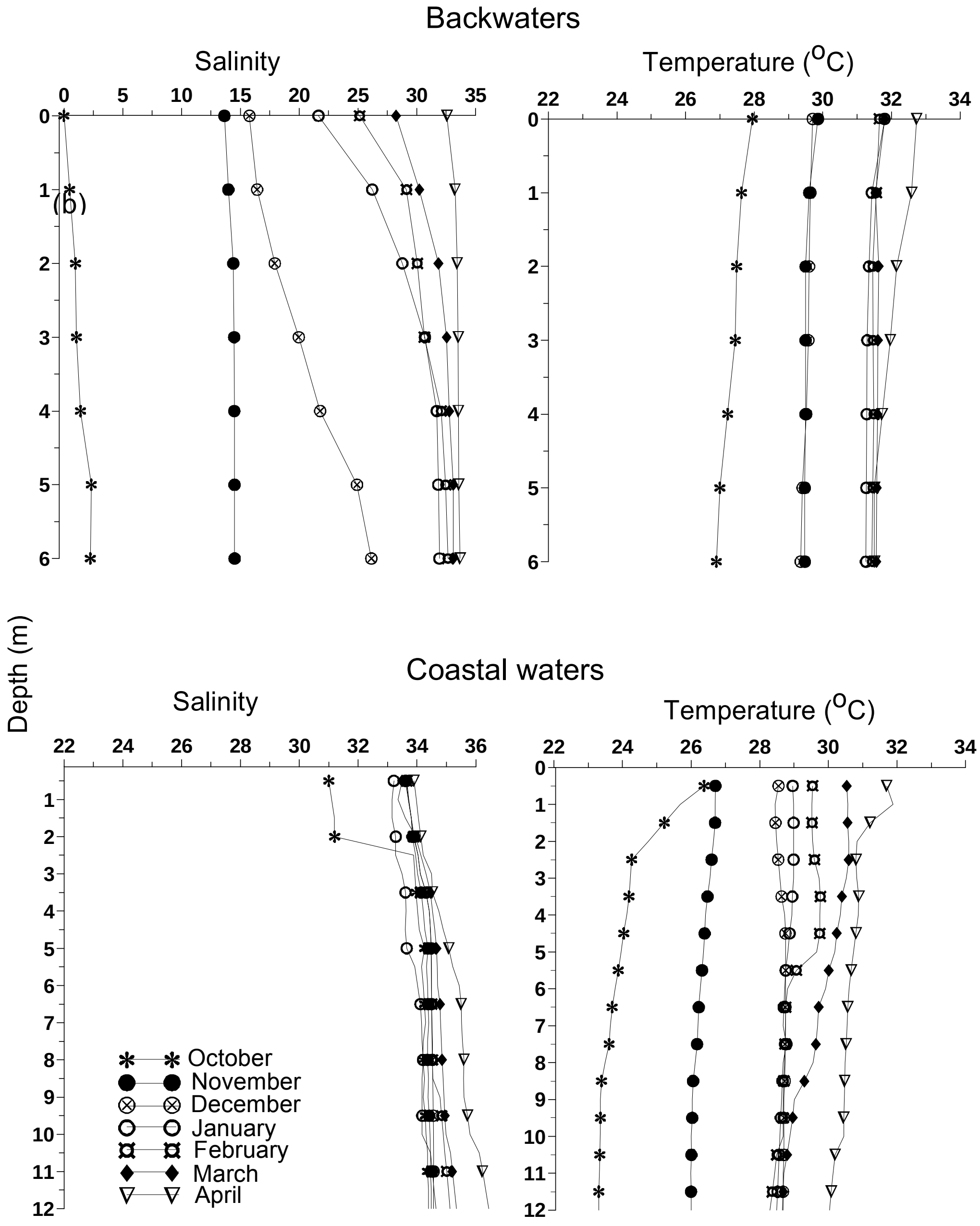

Backwaters
Salinity
Temperature (°C)
Coastal waters
Salinity
Temperature (°C)
Depth (m)
(b)
October
November
December
January
February
March
April

Figure 4

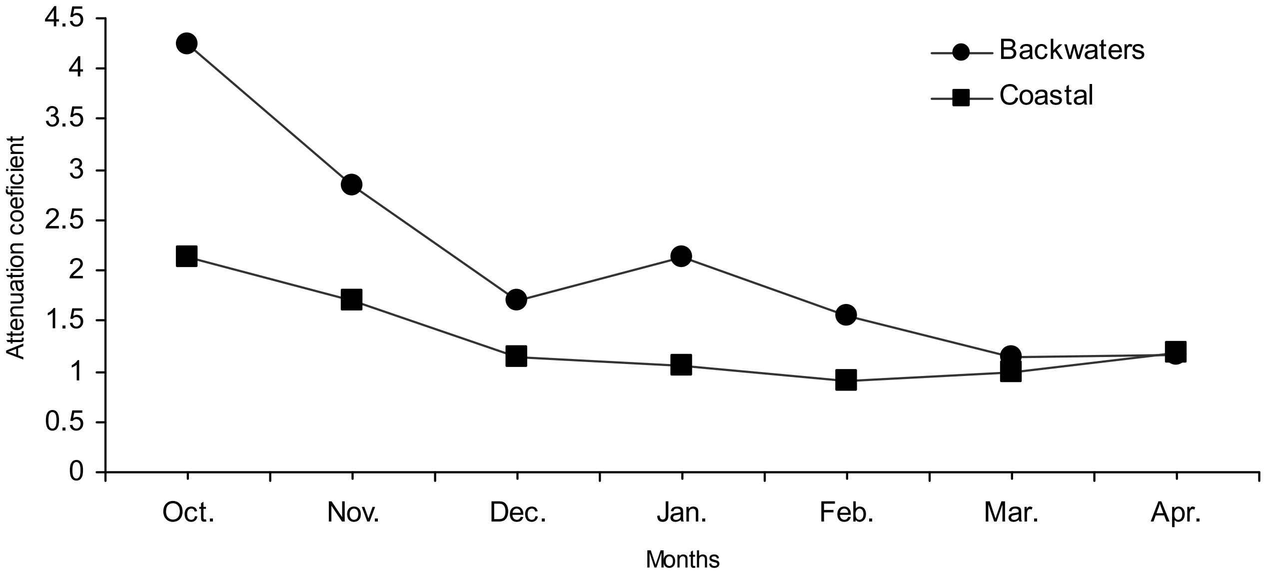
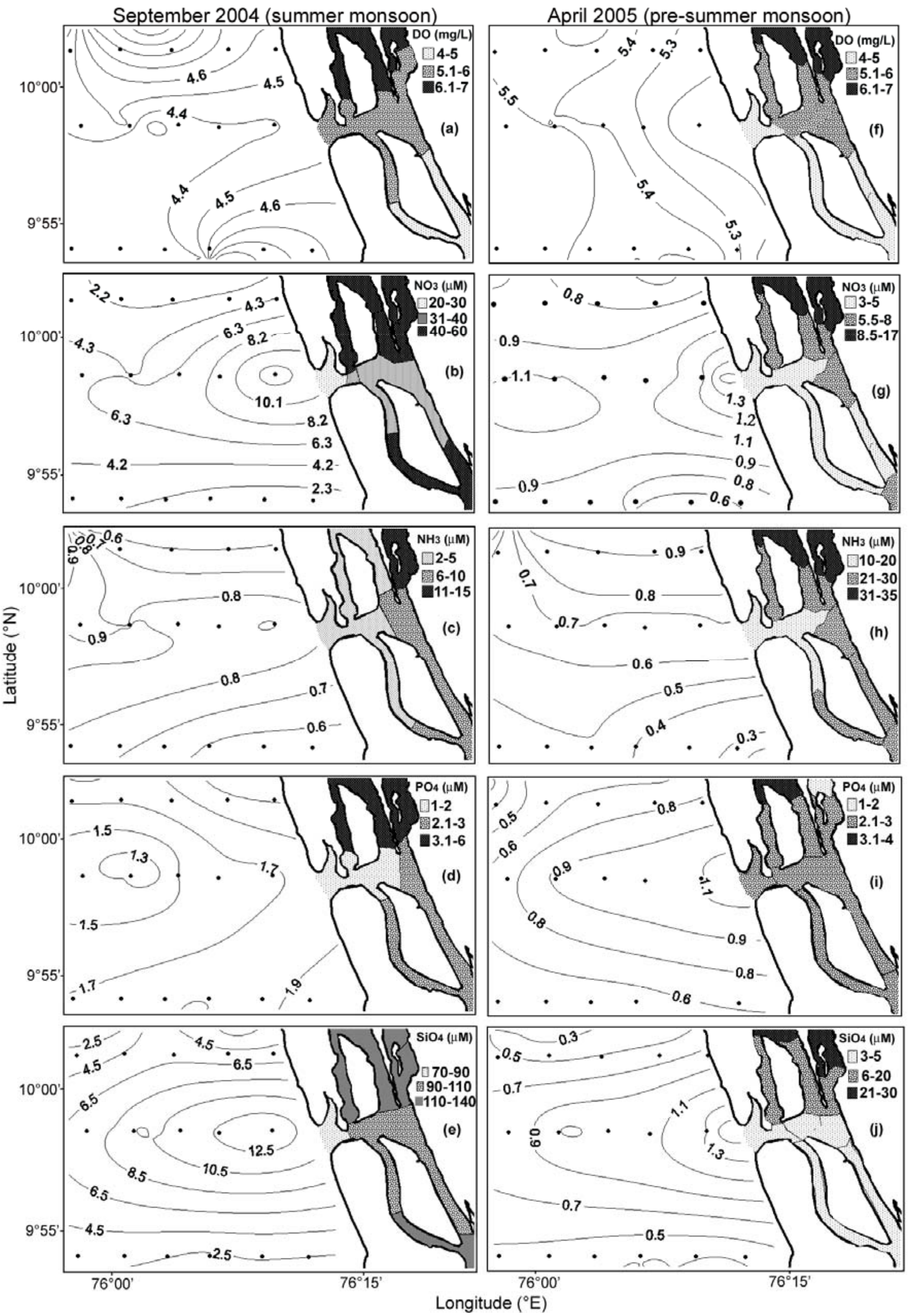

4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Attenuation coeficient
Backwaters
Coastal
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
Months

Figure 5



September 2004 (summer monsoon)
April 2005 (pre-summer monsoon)
DO (mg/L)
4-5
5.1-6
6.1-7
NO3 (µM)
20-30
31-40
40-60
3-5
5.5-8
8.5-17
NH3 (µM)
2-5
6-10
11-15
10-20
21-30
31-35
PO4 (µM)
1-2
2.1-3
3.1-6
3.1-4
SiO4 (µM)
70-90
90-110
110-140
6-20
21-30
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
Latitude (°N)
10°00'
9°55'
Longitude (°E)
76°00'
76°15'

Figure 6

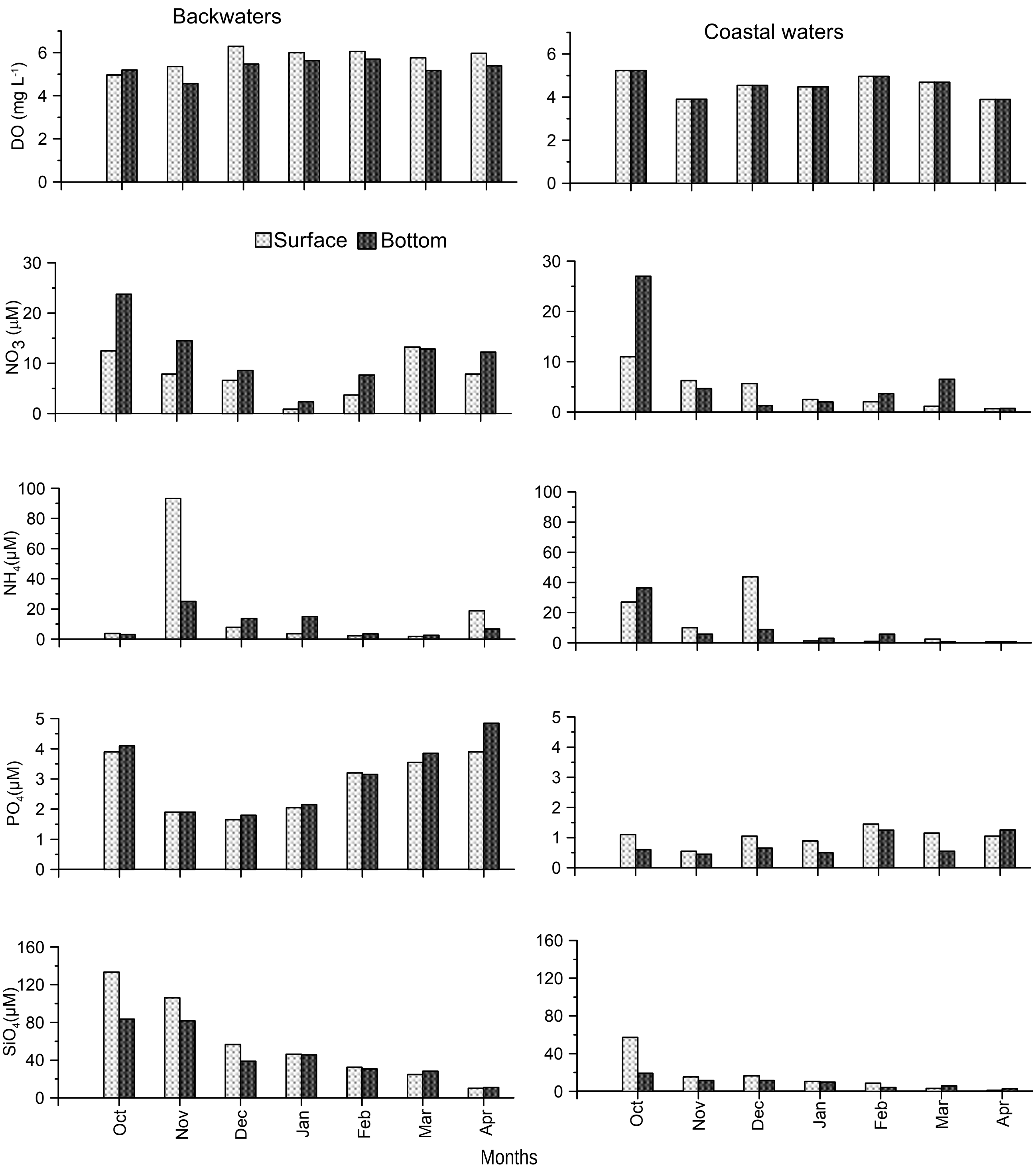

Backwaters
Coastal waters
DO (mg L-1)
Surface
Bottom
NO3 (µM)
NH4(µM)
PO4(µM)
SiO4(µM)
Oct
Nov
Dec
Jan
Feb
Mar
Apr
Months

Figure 7

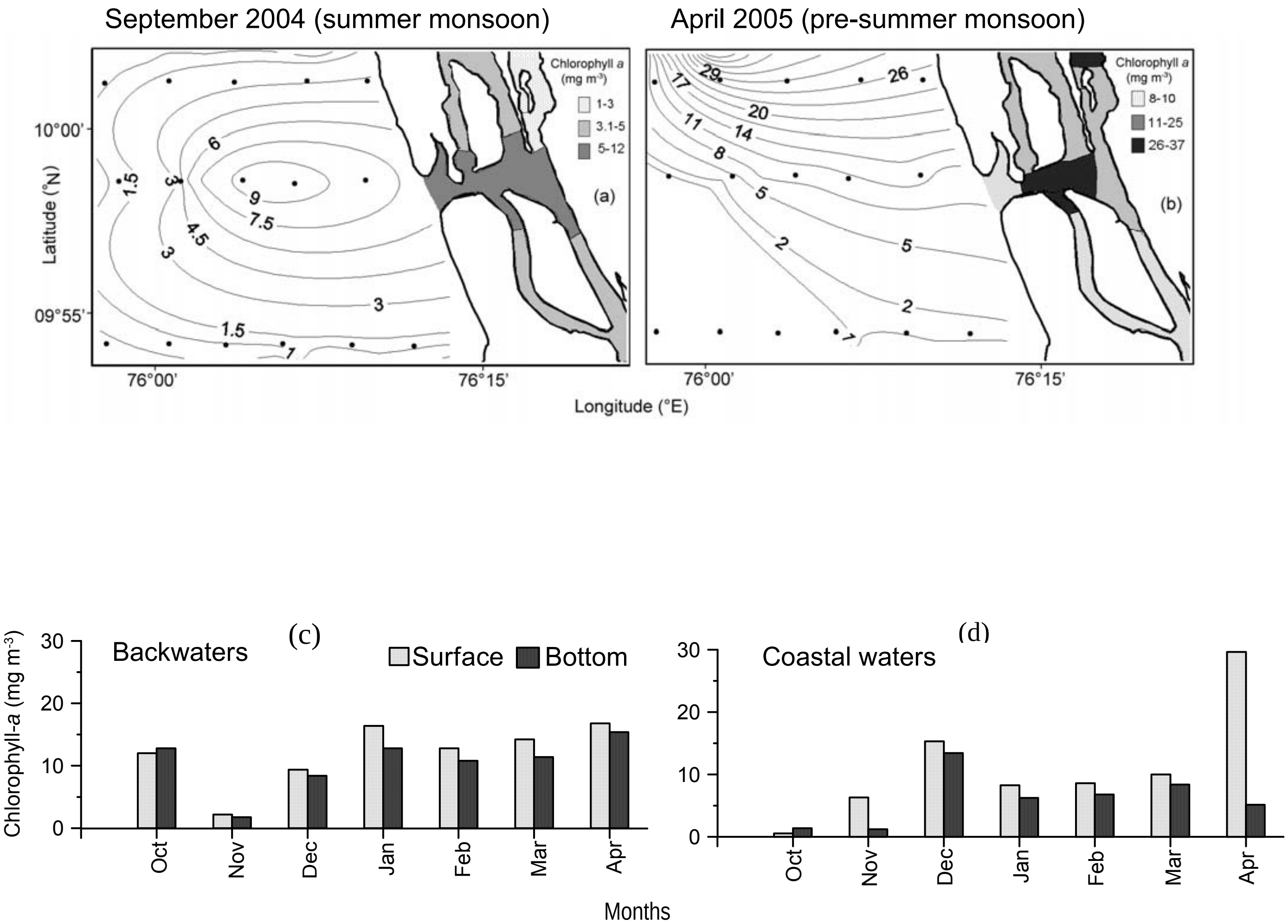

September 2004 (summer monsoon)
April 2005 (pre-summer monsoon)
Chlorophyll a (mg m-3)
1-3
3.1-5
5-12
(a)
Chlorophyll a (mg m-3)
8-10
11-25
26-37
(b)
10°00'
09°55'
Latitude (°N)
76°00'
76°15'
Longitude (°E)
(c)
Backwaters
Surface
Bottom
Chlorophyll-a (mg m-3)
Oct
Nov
Dec
Jan
Feb
Mar
Apr
(d)
Coastal waters
Months

Figure 8

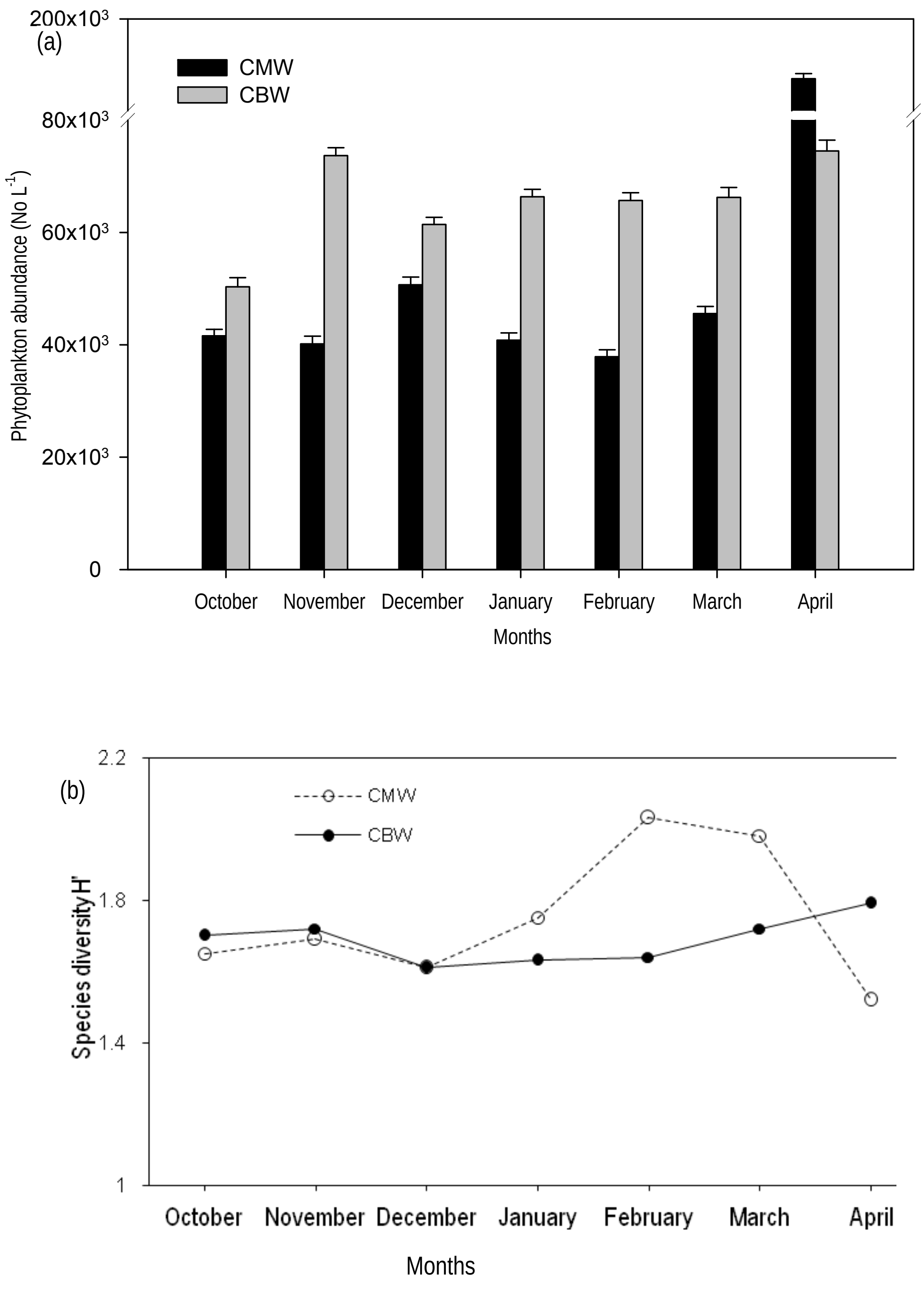

(a)
200x10³
80x10³
60x10³
40x10³
20x10³
0
CMW
CBW
Phytoplankton abundance (No L-1)
October
November
December
January
February
March
April
Months
(b)
2.2
1.8
1.4
1
CMW
CBW
Species diversity H'
October
November
December
January
February
March
April
Months

Figure 9

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

Table 1 – Major genera of phytoplankton reported from the Cochin backwaters since 1970
(SM- Summer monsoon; PM- Post-summer monsoon; PSM- Pre-summer monsoon; – Not available; * study exclusively on cyanobacteria)

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	11300	17520	19400	15600	17900	9600	16400	2600	14500	4560	16500	6560	23000	10900
Coscinodiscus sp.	130	340	150	60	420	410	250	50	210	600	180	60	1100	260
C. lineatus	440	200	200	-	50	-	-	220	-	220	50	50	240	180
Leptocylindrus danicus	5200	450	4100	210	2500	100	1240	130	1950	530	360	260	2600	1300
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	1600	200	800	1600	720	1300	2400	1350	1900	3500	2900	900	3000	1200
Thalassiosira sp.	4200	4500	15600	6500	18400	8000	7500	5560	11500	7560	5300	6400	12600	35560
Pleurosigma sp.	160	200	420	160	-	100	2600	120	240	200	190	200	320	320
Pleurosigma normani	-	100	-	-	-	50	-	200	-	220	-	180	-	-
Navicula sp.	6420	4500	8300	4600	4500	13200	2400	12900	2600	11500	11300	11900	6400	12400
Nitzschia sigma	100	100	420	-	200	-	-	-	240	160	-	-	230	230
N. closterium	16000	12000	21300	9400	15400	16700	32000	14000	29000	5000	24000	11000	21820	7820
Pyrrophyceae														
Peridinium sp.	200	60	420	120	300	300	1200	1500	1080	-	1300	230	1300	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														
Trichodesmium erythraeum	-	-	-	-	-	-	-	300	-	420	-	2100	-	112000
Others	4200	1100	2100	1300	720	600	2400	1000	1600	2400	3200	4200	1100	3600

Table 2- Monthly variation of dominant phytoplankton species (ind.L^{-1}) in the CBW and CMW; (-) indicate absence. *Trichodesmium* filaments are counted during the study.

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

Table 3- Correlation between chlorophyll *a* and macronutrients