

## ***Synechococcus* as an indicator of trophic status in the Cochin backwaters, west coast of India**

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### **Abbreviations**

CB- Cochin backwaters; PP- Picophytoplankton; *SYN*- *Synechococcus*; PE- Phycoerythrin; PC- Phycocyanin; *PRO*-like- *Prochlorococcus*-like; PEUK- Picoeukaryotes; PM- Post-monsoon; PrM- Pre-monsoon; MON- Monsoon; NBW- Near bottom waters; TRIX- Trophic status index; SW-South-West; S- Station; Chl *a*- Chlorophyll *a*; IMD- Indian meteorological department; RALS- Right angle light scatter; FALS- Forward angle light scatter; DO- Dissolved oxygen; BOD- Biological oxygen demand; DIN- Dissolved inorganic nitrogen; DIP- Dissolved inorganic phosphate

### **Abstract**

Eutrophication is a major problem in coastal water bodies. Information about the trophic status of water bodies will enable proper management of coastal ecosystems. In this regard, biological organisms which are sensitive to environmental changes can serve as indicators of ecosystem trophic status. In this study, seasonal and spatial variations of picophytoplankton (PP; < 3µm size) community structure was assessed in the Cochin backwaters (CB) with respect to the prevailing environmental conditions during three seasons post-monsoon (PM-I; October 2011 and PM-II; November 2012), pre-monsoon (PrM; May 2012) and monsoon (MON; August 2012). CB, along the west coast of India, receives continuous load of nutrients throughout the year through anthropogenic wastes. Trophic status index (TRIX) scores showed that CB is highly eutrophic with a high phytoplankton biomass. *Synechococcus* was the dominant PP observed in the study area. Seasonal and spatial salinity variations influenced the PP distribution, especially *Synechococcus* where PE-rich *Synechococcus* (*SYN*-PE) were dominant in higher saline (> 30) and PC-rich *Synechococcus* (*SYN*-PC) in lower saline (< 30) waters. *SYN*-PC showed a significant positive relation with chlorophyll *a* suggesting that this group contributes substantially to the total phytoplankton biomass. TRIX scores and *SYN*-PC: *SYN*-PE abundance ratio were negatively correlated with salinity suggesting an influence of the tidal amplitude. *SYN*-PC correlated positively and *SYN*-PE negatively with TRIX scores suggesting that these groups occupy contrasting ecological niches. These findings imply that PP distribution pattern can serve as an indicator of the trophic status of coastal water bodies.

**Keywords:** Picophytoplankton, Cochin backwaters, Eutrophication, Monsoon, trophic status indicators, *Synechococcus*.

## 1. Introduction

Backwaters are interlinked bodies of waterways, rivers, inlets, lakes and natural canals. These are the largest and the most complex ecosystems in the world. These locations are highly productive and play a distinct role in the livelihood and sustenance of the local people. Physical and chemical variables are the crucial factors supporting the higher productivity. The Cochin backwaters (CB), one of the such estuarine systems along the west coast of India, is considered to be highly productive, where phytoplankton plays an important role in the food web and serves as nursery grounds for fishes and other ecologically and economically important organisms (Qasim, 2003).

Eutrophication is one of the serious problems which CB is facing presently, resulting from the increasing anthropogenic activity. This is mainly due to the location of the Cochin port in the CB, which has accelerated the industrial growth in Cochin, making it one of the fastest growing cities in India. As a consequence, eutrophication becomes a threat for trophic dynamics and functioning of the ecosystem (Madhu et al., 2007; Kaladharan et al., 2011). CB receives a lot of organic and inorganic substances from several industries like oil refineries, fertilizer plants and chemical industries. From these industries, acids, alkalis, suspended solids, fluorides, free ammonium, insecticides, dyes, trace and heavy metals and radioactive nuclei are the major contaminants (Menon et al., 2000; Martin et al., 2012; Anu et al., 2014), which create a polluted environment in CB. For its efficient functioning, such ecosystems should be in a healthy state which can be easily detected through regular monitoring of the base of the food web i.e., phytoplankton.

At the base of the food web, the smallest group of phytoplankton, i.e., picophytoplankton (PP; < 3  $\mu\text{m}$ ; Seiburth et al., 1978), which forms a major component of phytoplankton in the aquatic ecosystems, both marine and freshwater, including nutrient rich to poor ecosystems, was selected as the study organism (Stockner and Antia, 1986; Shiomoto et al., 1997). PP are significant contributors to primary productivity and total phytoplankton biomass in various ecosystems (Paerl, 1977; Platt et al., 1983). PP forms an important component of the marine microbial food web by creating a linkage with the higher trophic levels (Chiang et al., 2013). PP comprises of three groups; two of cyanobacteria i.e., *Synechococcus* (*SYN*) and *Prochlorococcus* (*PRO*) and a group of picoeukaryotes (PEUK). *SYN* is the major group of PP in well-lit coastal and estuarine waters (Jochem, 1988) with comparatively lower numbers in oligotrophic waters where *PRO* are abundant (Partensky et al., 1999). PEUK are most competitive in nutrient rich waters (Jiao et al., 2005). Although *PRO* is considered to be an oceanic group, recently researchers found *PRO*-like cells in low saline waters (Shang et al., 2007; Mitbavkar et al., 2012) and it is still speculative whether this group of cells is actually growing in these waters or is being carried from the offshore waters (Partensky et al., 1999).

*SYN* is further differentiated based on phycobilisome composition into phycoerythrin (PE) rich and phycocyanin (PC) rich in estuarine and coastal ecosystems (Murrell and Lores, 2004). Previous studies have suggested that salinity plays an important role in the spatial distribution of *SYN* where PE rich *SYN* dominates high saline waters whereas, PC rich *SYN* are abundant in lower saline waters (Murrell and Lores, 2004; Rajaneesh and Mitbavkar, 2013). Based on PE fluorescence intensity, different clades of PE rich *SYN* have been observed in the Mississippi river plume (Liu et al., 2004), Pearl River estuary (Lin et al., 2010) and the Zuari estuary (Mitbavkar et al., 2012).

CB is influenced by the South-West (SW) monsoon (MON). Generally, estuaries influenced by monsoonal rainfall are highly productive due to excess nutrient input from the landmass. Studies conducted in tropical (Qiu et al., 2010; Rajaneesh and Mitbavkar, 2013) and subtropical (Lin et al., 2010; Qiu et al., 2010; Zhang et al., 2013) regions, which come under the influence of monsoonal rainfall, have suggested that riverine runoff influences the PP growth. Physico-chemical and biological characteristics of the CB (Menon et al., 2000; Madhu et al., 2009) have suggested that this region is highly eutrophic and productive, where nanoplankton are the major component of phytoplankton (Madhu et al., 2007) and is also a perfect breeding ground for economically important fishes and other organisms (Qasim, 2003). In the monsoonal Zuari estuary along the west coast of India, rainfall intensity was found to regulate freshwater runoff, which controls the estuarine environment thereby resulting in temporal and spatial niche segregation of *SYN* groups (Rajaneesh and Mitbavkar, 2013). The present study was carried out on a seasonal basis to characterize the main environmental factors, which control the spatial distribution pattern of PP groups and consequently whether these organisms can serve as ecological indicators. Since *SYN*-PE is known to prefer clear waters and *SYN*-PC turbid waters (Stomp et al., 2007), we hypothesize that these organisms can serve as good indicators of the trophic status of the water column.

## **2. Materials and methods**

### *2.1. Study Area*

Sampling was carried out in an area within the CB, along the west coast (9° 34' 48" N, 76° 08' 24" E) of India (Fig.1). It is situated along the northern part of Kerala state, running parallel to the coastline with two permanent openings to the Arabian Sea. One opening is at the Cochin Port and another further north at Azhikode, where the estuary is flushed during ebb tide and seawater intrudes during flood tide. Periyar and Muvattupuzha rivers along with 4 others and their tributaries bring large volume of freshwater into the CB through the Vembanad Lake, which has an active influence on the prevailing salinity of the estuarine system (Jyothibabu et al., 2006). CB is a very important

estuarine system of Kerala in terms of fishing and extensive transportation of goods. It is also used for dumping industrial as well as domestic wastes. It has three dredged channels where the selected stations are located, one being the approach channel (S1, S9-S12, S21-S23) of around 10 km length and 500 m width and the two inner channels located on either side of the Willingdon Island, i.e. Ernakulam channel (S13-S20) of around 5 km length with a width of 250–500 m and Mattancherry channel (S2-S8) of 3 km length with a width of around 170–250 m (Menon et al., 2000). Tides in this region are mixed semidiurnal with a range of about 1 m (Qasim and Gopinathan, 1969). Annual air temperature range is 20°C to 35°C with maximum temperature during February to May. Annually this region experiences three seasons i.e., SW MON (June to September), post-monsoon (October to January; PM) and pre-monsoon (February to May; PrM). It has a hot and humid climate with an average annual rainfall of about 350 cm, most of which is contributed by the SW MON and rest by North-East MON. During SW MON this estuary receives large amounts of freshwater, which leads to a salt wedge condition in the CB during August to October, whereas during November to May it changes to partially mixed condition due to reduction in freshwater discharge. In June, moderately stratified to partially mixed waters are observed (Menon et al., 2000). (Fig. 1 – preferred position)

## 2.2. Sampling

All together four samplings were conducted which included two PM seasons during the consecutive years i.e., 9<sup>th</sup> to 12<sup>th</sup> October, 2011 (PM-I) and 21<sup>st</sup> to 25<sup>th</sup> November, 2012 (PM-II), one MON season, (10<sup>th</sup> to 15<sup>th</sup> August, 2012; MON) and one PrM season (26<sup>th</sup> to 29<sup>th</sup> May, 2012; PrM). Within the CB, twenty three stations were selected for sample collection including ship berths and channels (Annex Table 1). Sampling was carried out between 06:30 to 13:00 h for four days. Rainfall and tidal range data were collected from the Indian meteorological department (IMD; Annex Table 2). Tidal height was estimated from the tidal range for the respective sampling time. Temperature was determined using multiparameter Sonde DS5X (Hydrolab). Surface and near bottom water (NBW) samples were collected with a 5 L Niskin sampler. Salinity was measured with an autosal (Guildline Autosal 8400B). For chlorophyll *a* (chl *a*) estimation, seawater samples (250 ml) were filtered through Whatman GF/F filter papers. Filters were preserved with MgCO<sub>3</sub> and stored at -20°C until analysis. In the laboratory, each filter paper was placed separately in a dark vial containing 90% acetone. After extraction in the dark at 4°C for 24 h, chl *a* concentration was determined on a Turner Design 10-AU fluorometer calibrated with commercial chl *a* (Parsons et al., 1984). Nutrients such as nitrate (NO<sub>3</sub>), phosphate (PO<sub>4</sub>), nitrite (NO<sub>2</sub>), ammonium (NH<sub>4</sub>) and silicate (SiO<sub>4</sub>) were analyzed by SKALAR SAN<sup>plus</sup> ANALYSER. Dissolved oxygen (DO) and biological oxygen demand (BOD) were analyzed following standard methods (Parsons et al., 1984). For PP analysis, duplicate samples

were preserved with paraformaldehyde (0.2% final concentration) in 2 ml cryovials, flash frozen in liquid nitrogen and stored at -80°C until analysis.

### 2.3. Flow cytometric analysis of picophytoplankton

In the laboratory, frozen samples were thawed and yellow green latex beads of 2 µm (polysciences co., USA) were added to 1 ml of sample as an internal standard to calibrate cell fluorescence emission and light scatter signals. Samples were analyzed with FACS Aria II flow cytometer equipped with blue (488 nm) and red (630 nm) lasers. *Prochlorococcus*-like (*PRO*-like) cells, *SYN* and *PEUK* were distinguished based on the forward and right angle light scatter (FALS and RALS, respectively which serve as proxies for cell size), red fluorescence from chlorophyll (> 650 nm) and phycocyanin (630 nm) and orange fluorescence from phycoerythrin (578 nm; Fig. 2). Two groups of *SYN* were distinguished based on their specific fluorescence characteristics; one rich in phycoerythrin (*SYN*-PE) with orange fluorescence and the other in phycocyanin (*SYN*-PC) with red fluorescence. *PEUK* and *PRO*-like cells were identified based on their larger and smaller RALS along with higher and lower red fluorescence characteristics, respectively. *SYN*-PE group comprised two sub-groups, one with a lower PE fluorescence (*SYN*-PEI) than the other (*SYN*-PEII). (Fig. 2 – preferred position)

### 2.4. Trophic status of the water column

The multivariate index of trophic state (TRIX) method was used to evaluate the trophic status of CB (Vollenweider et al., 1998; Sin et al., 2013), which was then used to assess the relationship between PP groups and trophic status of water. TRIX was calculated using the equation  $TRIX = (\log_{10} (chl\ a \times a\%O_2 \times DIN \times DIP) + k)/m$ , where chl *a* is in mg m<sup>-3</sup>, a%O<sub>2</sub> is absolute value of the percentage of DO saturation ( $abs\ |100 - \%O_2| = \%O_2$ ), DIN is dissolved inorganic nitrogen including NO<sub>3</sub>, NO<sub>2</sub>, NH<sub>4</sub> in mg m<sup>-3</sup> and DIP is dissolved inorganic PO<sub>4</sub> in mg m<sup>-3</sup>. The constants k- 3.5 and m- 0.8 are scale values obtained from Vollenweider et al. (1998) to adjust TRIX scale values (reads from 0-10) with a level of eutrophication in the CB. According to this method, TRIX scores lesser than 4 indicate high state of water quality with low eutrophication; scores between 4 to 5 indicate good state of water quality with medium eutrophication; scores between 5 to 6 indicate bad state of water quality with high eutrophication and scores greater than 6 indicate poor state of water quality with elevated levels of eutrophication.

### 2.5. Data analyses

Linear regression analysis was performed in order to understand the relationship of PP abundance (*SYN*-PEI, *SYN*-PEII, *SYN*-PC, *PEUK* and *PRO*-like; log [x+1]) with the environmental variables

(salinity, temperature, estimated tidal height, depth, DO, BOD, nutrients and chl *a*) and TRIX scores. Linear regression analysis was also performed to assess the relation of *SYN*-PC: *SYN*-PE abundance ratio with salinity. Principle Component Analysis (PCA) was applied to the ecological variables: DO, BOD, nitrate, nitrite, phosphate, ammonium and silicate ( $\log [x+1]$ ), which are indicators of anthropogenic pressure. This analysis was done using SPSS statistics software (windows 16.0) with a significance level of 0.05 in order to evaluate the ecological variables which are major indicator of anthropogenic pressure in the CB. Principle components (PC) having eigen values  $> 1$  were considered for further analysis. Subsequently, linear regression analysis was performed between the PC1 scores and cell abundance ( $\log [x+1]$ ) of individual PP groups in order to evaluate the relationship of PP groups with the indicators of anthropogenic pressure.

### 3. Results

#### 3.1. Environmental parameters

Lowest temperature was recorded during MON (24 to 29°C; Fig. 3a-b) and it varied from 27 to 31.3°C during PrM and PM in surface and NBW. Heavy precipitation was observed during MON season (total 195 mm). During PM-II, 56 mm precipitation was recorded on November 23, 2012. PrM showers were observed at the end of May 2012 (Annex Table 2). During PM-I and PM-II, tidal amplitude difference was 0.50-0.91 m. During PrM and MON, tidal amplitude difference was 0.41-0.58 m and 0.56-0.74 m, respectively (Annex Table 2). Station depths varied from ~1.71 m (S23) to ~11.69 m (S9). CB was partially mixed during PrM and PM and stratified during MON (Salinity 2 to 14 of surface waters and 4 to 34.8 of NBW) due to large amount of freshwater discharge. During PM-I, surface water salinity was higher than that during PrM at the approach channel stations (Fig. 3c-d), as PrM sampling was carried out during low tide (Annex Table 1). Higher salinity was observed across the CB during PM-II sampling which was carried out during high tide. Vertically, salinity and temperature showed differences only at those stations where the depth was  $> 5$  m. DO concentration was high in PM-I (up to 8.4 mg l<sup>-1</sup>) followed by PrM, PM-II and MON (Fig. 3e-f). DO concentrations were lower in the NBW than in the surface waters. BOD values were  $> 1$  mg l<sup>-1</sup> during all the seasons and did not show much difference between surface and NBW. On an average, BOD values were high during PM-I (3.14 mg l<sup>-1</sup>) and PM-II (1.58 mg l<sup>-1</sup>) and low during PrM and MON (Fig. 3g-h).

High NO<sub>3</sub> concentrations (up to 28.42 μM) were recorded during MON followed by PM-II, PrM and PM-I with higher values in the surface waters. NO<sub>3</sub> concentrations did not differ much between the stations except during PM-II, when inner stations (S4-S6, S14-S15) had higher NO<sub>3</sub> concentrations

(Fig. 3i-j). Higher  $\text{PO}_4$  concentrations were recorded in the surface waters during PrM followed by MON and PM (up to  $6.69 \mu\text{M}$ ; Fig. 3k-l).  $\text{NO}_2$  concentrations were lower during two successive PM seasons.  $\text{NH}_4$  concentrations were high during PrM followed by PM-I and MON ( $10$  to  $66 \mu\text{M}$ ).  $\text{SiO}_4$  concentration ranged from  $9.76$  to  $93.53 \mu\text{M}$  and were higher during PM-I in the surface waters, particularly at S15 to S19 (Fig. 3q-r). Vertically, not much difference was observed in  $\text{PO}_4$ ,  $\text{NH}_4$  and  $\text{NO}_2$  concentrations (Fig. 3k-p). Average chl *a* concentrations during the four seasons varied from  $1.4$  to  $32.46 \mu\text{g l}^{-1}$  across the CB. Compared to the mouth of the CB, chl *a* concentrations were higher at the inner stations (S2-S8 and S11-S23) with occasionally high concentrations in the surface waters during PrM ( $\sim 59.92 \mu\text{g l}^{-1}$ ; S7), PM-II ( $\sim 107.1 \mu\text{g l}^{-1}$ ; S4) and in the NBW during PM-I ( $\sim 63.23 \mu\text{g l}^{-1}$ ; S22), PrM ( $\sim 64.77 \mu\text{g l}^{-1}$ ; S7) and MON ( $\sim 84.60 \mu\text{g l}^{-1}$ ; S7; Fig. 3s-t). (Fig. 3 – preferred position)

### 3.2. Interseasonal and spatial variation of picophytoplankton

Total PP abundance ranged from  $0.1$  to  $2.29 \times 10^5$  cells  $\text{ml}^{-1}$  in surface and NBW during PM-I. During PrM, a prominent increase in PP abundance was observed across the CB (up to  $4.06 \times 10^5$  cells  $\text{ml}^{-1}$ ), which decreased during MON ( $< 1.71 \times 10^5$  cells  $\text{ml}^{-1}$ ) and increased during PM-II ( $< 2.8 \times 10^5$  cells  $\text{ml}^{-1}$ ; Fig. 4k-l). Seasonal and spatial variation of PP groups abundance is shown in the fig. 4a-l. *SYN*-PEI was the dominant group observed during PM-I (surface waters of S1, S5, S7, S9 and NBW of S1-S22) and PM-II (surface waters of S5-S8, S11, S18-S20, S22 and NBW of S1, S5-S22; Fig. 4a-b). During PrM and MON, *SYN*-PEI abundance was low ( $< 0.46 \times 10^5$  cells  $\text{ml}^{-1}$ ). During PM-I, contribution of *SYN*-PEI to total PP was higher ( $\sim 85\%$ ) in the NBW compared to the surface waters (Fig. 5a-b). Highest cell abundance was recorded in NBW of S1 ( $2.0 \times 10^5$  cells  $\text{ml}^{-1}$ ) and S7 ( $2.86 \times 10^5$  cells  $\text{ml}^{-1}$ ) compared to all other seasons. During PM-II, higher *SYN*-PEI abundance was observed at Ernakulam channel stations (surface waters and NBW) where salinity was  $> 29$  (Fig. 4a-b). Being second dominant group during PM-I, *SYN*-PC abundance was high in the Ernakulam channel stations compared to the stations near the mouth of CB (Fig. 4e-f). Throughout the season, cell abundance was higher in the surface waters than NBW, except at some stations where NBW salinity was  $< 29$ . A steep increase in *SYN*-PC abundance was observed during the PrM, with maximum cell abundance at S21 (Fig. 4e-f). This was the dominant group contributing up to  $92\%$  to the total PP abundance across the CB (Fig. 5c-d). During MON, dominance of *SYN*-PC group continued with comparatively higher cell abundance than that during PrM (Fig. 4e-f). During PM-II, *SYN*-PC dominated low saline waters ( $< 24$ ) with the highest cell abundance at S9. At S23, higher cell abundance was recorded as observed during MON (Fig. 4e-f). During PM-I, *SYN*-PEII group was absent where salinity was  $< 20$  and during PrM it was completely absent. This group was

observed in the NBW during MON with very low cell abundance. During PM-II, *SYN-PEII* was detected only at salinities > 26, with low cell abundance (Fig. 4c-d). Their contribution to total PP abundance was higher in the NBW, especially at S20 (46%) during PM-I whereas during other seasons it was < 26% (Fig. 5a-h).

*PRO*-like cells showed remarkable increase during MON with higher cell abundance in the surface waters than that in the NBW, except at Mattancherry channel stations (Fig. 4g-h). Contribution to total PP abundance was high (~ 40%) during this season and decreased (< 27%) during PM-II (Fig. 5a-h). *PEUK* abundance was high during MON exhibiting a decreasing trend from S2 to S23. In the surface waters, cell abundance was higher than in the NBW, except at Mattancherry channel stations. Highest cell abundance ( $0.49 \times 10^5$  cells ml<sup>-1</sup>) was recorded in the NBW of S7. Even though their abundance was high, their contribution to total PP was < 33%. *PEUK* abundance was reduced by an order of magnitude during PM-II. However, during PrM increased abundance was observed compared to PM-I (Fig. 4i-j). During PM-I, *PEUK* contribution to total PP abundance in the surface waters ranged up to 64% (Fig. 5a-b). Generally, their abundance was high in the surface waters compared to the NBW, except during PrM. (Fig. 4 and 5 – preferred position)

### 3.3. Intraseasonal variation of picophytoplankton

During PM-II, total PP abundance was higher compared to that during PM-I (Fig. 4k-l). *SYN-PEI* and *SYN-PEII* abundance were significantly higher during PM-II compared to that during PM-I at S22, S5-S8 and S18-S20 (Fig. 4a-d) where salinity was comparatively higher (Fig. 3c-d). *SYN-PC* showed higher cell abundance during PM-II at most of the stations compared to that during PM-I (Fig. 4e-f). The spatial distribution of *SYN-PEI* and *SYN-PC* differed during PM-II wherein the latter dominated in the surface waters of S13-S17, S1-S4, S9-S10, S12 and S21 and NBW of S11, S23 and S2-4 (Fig. 4a-f).

### 3.4. *TRIX* scores for Cochin backwaters

The average values of *TRIX* (5.15) for the study period revealed that the CB is highly eutrophic with a bad state of water quality. *TRIX* scores for the study region ranged from 1.64 to 7.37 during all the seasons (Fig. 6a-b). During PrM and MON, most of the stations showed elevated conditions of eutrophication. Only during PM-I medium level of eutrophication with good state of water quality was observed for the surface waters. (Fig. 6 – preferred position)

### 3.5. Relationship between environmental parameters and picophytoplankton groups

Linear regression analysis showed that *TRIX* scores correlated negatively with salinity and estimated tidal height (Table 1). *SYN-PEI* and *SYN-PEII* correlated positively with salinity, estimated

tidal height and station depth, whereas *SYN-PC* and *PEUK* correlated negatively. *PRO*-like cells correlated negatively with salinity. *SYN-PC* correlated positively with temperature whereas *SYN-PEII* and *PRO*-like cells correlated negatively (Table 1). DO correlated negatively with station depth. *SYN-PC* and *PEUK* correlated positively with nutrients whereas, *SYN-PEI* and *SYN-PEII* correlated negatively (Table 1). *SYN-PC* and *PRO*-like cells correlated positively with total chl *a*. *SYN-PC* correlated positively with *TRIX* scores, whereas *SYN-PE* correlated negatively (Fig. 7a-b; Table 1). *SYN-PC*: *SYN-PE* abundance ratio correlated negatively with salinity (Fig. 8). (Fig. 7, Fig. 8 and Table 1 preferred position)

PCA exhibited 2 PC's which explained 65% variation of the ecological variables (DO, BOD, nitrate, nitrite, phosphate, ammonium and silicate) which are indicators of anthropogenic pressure. The first factor, PC1 accounted for 37% of the variance with a positive load of PO<sub>4</sub>, NH<sub>4</sub>, SiO<sub>4</sub>, NO<sub>2</sub>, and NO<sub>3</sub>. NH<sub>4</sub> and PO<sub>4</sub> were the most significant variables. Weak negative loading of the BOD was observed in PC1 (Table 2). Positive load of the DO and BOD was observed at PC2 which explained 27% of the variance. Linear regression analysis showed that *SYN-PEI* and *SYN-PEII* correlated negatively with PC1 scores, whereas *SYN-PC*, *PEUK* and chl *a* correlated positively (Fig. 9a-f). (Table 2 and Fig. 9 preferred position)

## 4. Discussion

### 4.1. Hydrography of Cochin backwaters

Over the past few years, investigators have revealed that CB is contaminated by anthropogenic activities (Balachandran et al., 2005; Martin et al., 2012; Anu et al., 2014) such as industrialization (10<sup>4</sup> million liters of partially treated and untreated industrial effluents are discharged everyday by a large number of industries), agriculture, transportation and domestic sewage effluent discharge (Unnithan et al., 1975; Vijayan et al., 1976; Menon et al., 2000; Qasim, 2003). As a consequence, concentration of toxic metals in the surficial sediments has been reported in moderate to heavily polluted condition (Martin et al., 2012). In the present study, *TRIX* scores showed that CB is highly eutrophic.

Hydrography of the CB reflected typical tropical estuarine conditions where temperature gradually increased from PM to PrM season and subsequently decreased during MON. During MON, stratification developed due to increased freshwater influx and formed a decreasing salinity gradient from the mouth towards upstream of the CB. During non-MON period, as freshwater influx reduced, water column was partially mixed as is observed in estuaries influenced by monsoonal rainfall (Joseph and Kurup, 1989; Shetye, 1999). Recently, Jacob et al. (2013) reported that during non-

MON high tide, saltwater intrudes up to 40 km in CB. Thus, in the present study, stations located in all three channels were influenced by the incoming high saline waters during the high tide.

An unique feature of CB is the surplus amount of nutrient load that it receives throughout the year *via* land drainage, agricultural activities and river discharge during MON (Devi et al., 1991; Madhu et al., 2007), when annual discharge of freshwater is  $20000 \times 10^6 \text{ m}^3$  (Srinivas et al., 2003). High nutrient supply during MON is a profound characteristic of estuaries influenced by monsoonal rainfall (Qasim and Sen Gupta, 1981). High  $\text{PO}_4$  concentrations were observed during the PrM as reported in earlier studies (Sankaranarayanan and Qasim, 1969). Martin et al. (2012) has reported a steady increase in  $\text{PO}_4$  concentration from December to April. This is believed to be the result of high salinity/pH combined with tidal activity during the PrM, which causes desorption of phosphate from the suspended particles (Reddy and Sankaranarayanan, 1972; Martin et al., 2008). In this study, PCA suggested that  $\text{PO}_4$  and  $\text{NH}_4$  are the major indicators of the eutrophicated environment in the CB.

Comparatively lower concentrations of DO in the NBW than that in the surface waters indicated higher utilization of oxygen than production. This observation was consistent with previous reports (Madhu et al., 2009; Martin et al., 2011) and was substantiated by the BOD values of  $>1.5 \text{ mg l}^{-1}$  at most of the stations suggesting that respiration by aquatic animals, decomposition by bacteria and various chemical reactions were active in the CB.

#### *4.2. Interseasonal variation of picophytoplankton in Cochin backwaters*

Several studies have been conducted on the biological aspects in the CB (Madhu et al., 2007; Martin et al., 2008; Madhu et al., 2009). These studies emphasize that irrespective of the season, CB facilitates luxurious growth of phytoplankton due to excess level of nutrient availability (Balachandran et al., 2005; Madhu et al., 2007). In the study region, maximum phytoplankton biomass recorded was higher (average  $17.05 \mu\text{g l}^{-1}$ ; ranged up to  $107 \mu\text{g l}^{-1}$ ) than that reported in previous studies in CB ( $49 \mu\text{g l}^{-1}$ ; Madhu et al., 2007; Madhu et al., 2009; Martin et al., 2011) and also in the Mandovi and Zuari estuaries located along the west coast of India (Pednekar et al., 2011; Patil and Anil, 2011). This could be due to the nutrient enrichment by anthropogenic activities, which triggers the massive growth of nanoplankton ( $< 20 \mu\text{m}$ ; Madhu et al., 2007; Martin et al., 2011) and phytoplankton blooms which are common in the CB when the intermediate salinity condition exists (Devassy, 1974; Madhu et al., 2010; Martin et al., 2013). Another reason could be the weeds and water hyacinths, which proliferate in the upstream waters that severely restrict the natural flushing (Shivaprasad et al., 2012) and enter the CB during MON due to influx of low saline

waters. In the PrM and PM-II seasons the higher chl *a* concentration in the surface (64.77  $\mu\text{g l}^{-1}$ ; S7) and NBW (59.99  $\mu\text{g l}^{-1}$ ; S8), could be the result of showers that occurred during the sampling period (Annex Table 2), which probably drive the weeds and water hyacinths into the CB or from stirred up sediments. In addition, during PM-II, the highest chl *a* concentration recorded at S4 (104  $\mu\text{g l}^{-1}$ ) one day after heavy showers confirms that the incoming freshwater is a source of the high chl biomass.

Consistently high PP abundance ( $10^5$  cells  $\text{ml}^{-1}$ ) during all the seasons indicates that PP could be an important component of the phytoplankton community in CB. During MON the prevailing environmental factors influenced the distribution of PP groups, especially *SYN-PE* and *SYN-PC*, wherein the former is known to be abundant in high saline waters and latter in low saline waters (Murrell and Lores, 2004). During non-MON seasons, tide controls the salinity distribution in CB (George and Kartha, 1963). Salinity variation due to tidal impact clearly influenced *SYN-PC* and *SYN-PE* distribution in the CB, both horizontally and vertically. PM-II sampling was carried out during high tide. This could be the reason for high salinity in Ernakulam channel where *SYN-PEI* was the dominant group in the surface and NBW. PrM sampling was carried out during low tide which resulted in low surface salinity across the estuary where *SYN-PC* was dominant. These observations suggest that tide is also an influential factor for *SYN* distribution in the CB wherein *SYN-PE* enters the CB from the coastal waters during high tide and *SYN-PC* during the low tide from the upstream end. This is substantiated by observations from the monsoonal Zuari estuary (Rajaneesh and Mitbavkar, 2013), wherein during the non-MON period due to high tidal activity, *SYN-PE* showed higher abundance upstream whereas, during MON, *SYN-PC* abundance was higher downstream due to strong freshwater runoff. The significant positive relation of *SYN-PE* abundance and negative relation of *SYN-PC* and *PEUK* abundance with salinity and estimated tidal height (Annex Table 1) indicates that tide is a prominent controller of PP community structure in CB. Significant relationship of PP groups with salinity is consistent with studies carried out in subtropical and temperate regions (Ray et al., 1989; Murrell and Lores, 2004), especially for *SYN*. Transition in dominance from *SYN-PC* to *SYN-PE* at salinities of ~20 - 25 found in this study (Fig. 8), was previously observed in subtropical estuaries (Ray et al., 1989; Murrell and Lores, 2004; Zhang et al., 2013) and tropical estuaries (Rajaneesh and Mitbavkar, 2013). In addition, negative relation of *SYN-PC* and positive relation of *SYN-PE* with the station depths indicate that higher salinity in the NBW favors *SYN-PE* groups. This was well reflected in MON when the high saline waters harboring *SYN-PE* were capped by the low saline waters harboring *SYN-PC* in the approach channel stations (Fig 5c and d). These findings suggest that *SYN* distribution pattern can serve as an indicator of the seasonal water column hydrography (stratified or mixed) influenced by physical forces such as tides and

freshwater runoff. The presence of an additional group of *SYN*-PE with high PE intensity at higher salinities (*SYN*-PEII) in the estuarine waters indicates that this group could have been introduced from the offshore waters during high tide. This observation is consistent with previous reports from the Zuari estuary, North western Arabian coast, Mississippi river plume and the Pearl River estuary (Campbell et al., 1998; Liu et al., 1998; Liu et al., 2004; Lin et al., 2010; Rajaneesh and Mitbavkar, 2013). *PRO*-like cells detected in the low saline waters of the CB were also observed recently in the Zuari estuary along the South West coast of India (Mitbavkar et al., 2012). Shang et al. (2007) reported *PRO*-like cells in brackish and in freshwater. Similarly in the present study, *PRO*-like cells were higher in the low saline waters as seen from the negative correlation with salinity. However, work on *PRO*-like cells is very limited and effect of environmental condition on these cells is still not clear. Recently, Liu et al. (2013) identified a previously suggested group of *PRO*-like cells as *SYN*-PC based on laboratory experiments. However, to confirm the strain in the CB, molecular approaches are required.

Variation in the spectral light quality is one of the factors altering the PP composition in oceanic, coastal and estuarine waters (Wood, 1985; Scanlan, 2003). In coastal and estuarine waters, generally, *SYN*-PE group abundance is high in clear waters where the green light predominates due to the low concentrations of suspended particles and dissolved organic matter concentrations (Li et al., 1983; Wood, 1985; Stomp et al., 2007). *SYN*-PC is higher in turbid waters loaded with dissolved particulate organic matter or rich in chlorophyll where the spectral light quality is altered from green to red (Stomp et al., 2007). In CB, water is highly turbid throughout the year and comparatively higher during the MON (Qasim and Reddy, 1967). Hence, this could be one of the factors responsible for the predominance of *SYN*-PC in the CB throughout the year, due to its better ability to utilize the red wavelength along with its ability to proliferate at lower salinities. In addition, the positive correlation of *SYN*-PC with chl *a* suggests that this group is not only dominant, but also significantly contributes to total phytoplankton biomass in the CB. Increasing temperature, irradiance, salinity and comparatively lower turbidity could be responsible for the higher abundance of *SYN*-PE groups during PM.

The negative relationship of TRIX with the estimated tidal height and salinity suggests that low tide causes a higher trophic index due to more influence of freshwater rich in anthropogenic contaminants from the upstream whereas, high tide brings offshore waters in to the CB, which leads to dilution of eutrophic waters (Table 1). The positive and negative relation of *SYN*-PC and *SYN*-PE, respectively, with TRIX scores suggests that these groups occupy contrasting ecological niches (Fig. 7a-b). In the hypertrophic waters of French Mediterranean lagoon, which was described as an anthropogenically

influenced eutrophicated area, abundance of *SYN-PC* was higher than that of *SYN-PE* (Bec et al., 2011). Munawar and Weisse (1989) reported that autotrophic picoplankton avoided contaminated environments but were high in contaminated eutrophicated areas. They attributed this to availability of excess nutrients, which may have complexing effects resulting in the detoxification of contaminants. Aneeshkumar and Sujatha (2012) reported that zeaxanthin pigment indicative of cyanobacteria was found more in the sediments of sites influenced by anthropogenic activities (near to S23) in the CB. Since zeaxanthin is a marker pigment of both *SYN-PE* and *SYN-PC* and we found *SYN-PC* > *SYN-PE* throughout the study period at S23, which is a sewage discharge point, we assume that *SYN-PC* was the dominant group found in this area by Aneeshkumar and Sujatha (2012). These observations corroborate our findings and suggest that *SYN* could serve as a potential indicator of the trophic status of water bodies.

Temperature in the tropical regions does not show much annual variation (8°C in the present study) as in temperate regions (14°C). As a consequence, PP abundance was high throughout the year in the tropics as compared to the temperate regions where abundance peaks are observed only during summer (Agawin et al., 1998; Chiang et al., 2002; Murrell and Lores, 2004). This suggests that seasonal temperature exercises a latitudinal variation on the PP distribution. Significant positive relation of total PP abundance with temperature is a profound characteristic of tropical, subtropical and temperate estuaries (Ray et al., 1989; Agawin et al., 1998; Qiu et al., 2010; Wang et al., 2011). However, in the present study, only *SYN-PC* showed a positive correlation ( $p < 0.05$ ) with temperature. Some studies conducted in tropical and subtropical estuaries showed that PP negatively correlated with nutrients (Qiu et al., 2010; Zhang et al., 2013). However, these studies considered total *SYN* (*SYN-PC* and *SYN-PE*) abundance in order to evaluate the relationship with nutrients. In the present study, although the positive relation of *SYN-PC*, PEUK and negative relation of *SYN-PE* groups with NO<sub>3</sub>, PO<sub>4</sub> and NH<sub>4</sub> may not be cause and effect relationships, it indicates that nutrient concentrations could influence the seasonal variations of these groups. Similar results were obtained from the Zuari estuary, India (Rajaneesh and Mitbavkar, 2013). In the Uchiumi Bay, Japan, PO<sub>4</sub> addition showed seasonal variations on the growth rates of *SYN* and PEUK (Katano et al., 2005). They presumed that the *in situ* nutrient concentrations or difference in species could be responsible for such a seasonal response. Though PEUK are the most competitive among PP groups (Pan et al., 2007), it was not the dominant group in CB, even in high nutrient concentrations. Previous studies in tropical and subtropical regions have reported that this group dominated the nutrient rich conditions (Jiao et al., 2005; Qiu et al., 2010). Probably, this group abundance was controlled by the high microzooplankton grazing rates which we did not consider in the present study (Wetz et al., 2011).

*SYN-PE* groups attained higher abundance during PM-I and PM-II seasons indicating its preference for increased temperature after MON. Along with temperature, irradiance is also known to influence the seasonal distribution of *SYN* abundance and biomass (Agawin et al., 1998; Tsai et al., 2008). It is believed that there are multifactors, which are controlling the *SYN-PE* growth in coastal and estuarine ecosystems (Chang et al., 2003; Zhang et al., 2013). Increasing temperature during PrM and decreasing temperature and salinity during MON could be the reason for low abundance during these periods. *SYN-PC* was the dominant group present in the CB with highest abundance in PrM and their contribution was substantial to total PP. Similarly, studies carried out in the subtropical and temperate estuaries have shown that abundance of *SYN-PC* was greatest during warm periods (Ray et al., 1989; Murrell and Lores, 2004). Temperature was the limiting factor for PEUK in the previous studies where temperature varied between 20 to 27°C (Pan et al., 2007). However, in the present study area, where the temperature range was 24 to 32°C, PEUK were not significantly affected by temperature.

#### *4.3. Intraseasonal variation of picophytoplankton in Cochin backwaters*

As the PM-I sampling was carried out in October soon after the MON, the freshwater influence was still felt in the CB as compared to that during PM-II sampling which was carried out in November. As a result, salinity and temperature were comparatively lower during PM-I. This was reflected in the PP distribution wherein higher PP abundance was observed during the PM-II. The dominance of *SYN-PC* over *SYN-PEI* during PM-II in some of the stations was mainly due to salinity < 25 as a result of rainfall that occurred on the sampling day (Annex Table 2). Higher salinity in the NBW during PM-II favored higher *SYN-PEI* and *SYN-PEII* abundance in the CB.

### **5. Conclusion**

Hydrography of the CB reflected typical tropical estuarine conditions with stratification during MON and partially mixed condition during non-MON seasons. Irrespective of the season, high concentrations of nutrients were recorded in the estuary. TRIX scores showed that this estuary is highly eutrophic. Consistently high PP abundance ( $10^5$  cells ml<sup>-1</sup>) during all the seasons indicates that PP is an important component of the phytoplankton community in the CB. During non-MON, tide was an influential factor for *SYN* distribution wherein *SYN-PE* was found to be high during high tide and *SYN-PC* during the low tide suggesting their influx from the coastal waters and upstream, respectively. During MON, due to stratification by freshwater influx, surface waters dominated by *SYN-PC* capped the *SYN-PE* dominated NBW of inner stations. Along with low salinity, high turbidity that affects the light penetration with predominance of red light could be another factor

favoring the *SYN-PC* abundance in the low saline waters. *SYN-PC* showed a significant positive relation with chl *a* suggesting its significant contribution to total biomass. Although the relation of *SYN-PC* and *SYN-PE* groups with nutrients may not be cause and effect relationships, it indicates that nutrient concentrations could influence the seasonal variations of these groups. *SYN-PE* groups attained higher abundance during PM-I and PM-II seasons indicating its preference for higher temperature. *SYN-PC* and PEUK showed positive and *SYN-PE* showed negative relation with TRIX scores which suggests that these groups occupy contrasting ecological niches. These findings suggest that PP distribution pattern can serve as an indicator of the trophic status of coastal water bodies.

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### **References**

- Agawin, N. S., Duarte, C. M., Agusti, S., 1998. Growth and abundance of *Synechococcus* sp. in a Mediterranean Bay: seasonality and relationship with temperature. *Mar. Ecol. Prog. Ser.* 170, 45-53.
- Aneeshkumar, N., Sujatha, C., 2012. Biomarker pigment signatures in Cochin backwater system—A tropical estuary south west coast of India. *Estuar. Coast. Shelf. Sci.* 99, 182-190.
- Anu, P., Jayachandran, P., Sreekumar, P., Nandan, S.B., 2014. A review on Heavy Metal Pollution in Cochin Backwaters, Southwest Coast of India. *Int. J. Mar. Sci.* 4, 92-98.
- Balachandran, K., Raj, L., Nair, M., Joseph, T., Sheeba, P., Venugopal, P., 2005. Heavy metal accumulation in a flow restricted, tropical estuary. *Estuar. Coast. Shelf. Sci.* 65, 361-370.
- Bec, B., Collos, Y., Souchu, P., Vaquer, A., Lautier, J., Fiandrino, A., Benau, L., Orsoni, V., Laugier, T., 2011. Distribution of picophytoplankton and nanophytoplankton along an anthropogenic eutrophication gradient in French Mediterranean coastal lagoons. *Aquat. Microb. Ecol.* 63, 29-45.
- Campbell, L., Landry, M., Constantinou, J., Nolla, H., Brown, S., Liu, H., Caron, D., 1998. Response of microbial community structure to environmental forcing in the Arabian Sea. *Deep-Sea Res. Pt II* 45, 2301-2325.
- Chang, J., Lin, K. H., Chen, K. M., Gong, G. C., Chiang, K. P., 2003. *Synechococcus* growth and mortality rates in the East China Sea: range of variations and correlation with environmental factors. *Deep-Sea Res. Pt II* 50, 1265-1278.

- Chiang, K. P., Kuo, M. C., Chang, J., Wang, R. H., Gong, G. C., 2002. Spatial and temporal variation of the *Synechococcus* population in the East China Sea and its contribution to phytoplankton biomass. *Cont. Shelf. Res.* 22, 3-13.
- Chiang, K. P., Tsai, A.Y., Tsai, P. J., Gong, G. C., Tsai, S. F., 2013. Coupling of the spatial dynamic of picoplankton and nanoflagellate grazing pressure and carbon flow of the microbial food web in the subtropical pelagic continental shelf ecosystem. *Biogeosci. Discuss.* 10, 233-263.
- Devi, K. S., Sankaranarayanan, V., Venugopal, P., 1991. Distribution of nutrients in the Periyar river estuary. *Indian J. Mar. Sci.* 20, 49-54.
- Devassy, V., 1974. Observations on the bloom of a diatom *Fragilaria Oceanica* Cleve. *Mahasagar* 7, 101-105.
- George, M., Kartha, K., 1963. Surface salinity of Cochin backwater with reference to tide. *J. Mar. Biol. Ass. India* 5, 178-184.
- Jacob, B., Revichandran, C., NaveenKumar, K., 2013. Salt intrusion study in Cochin estuary-using empirical models. *Indian J. Geo. Mar. Sci.* 42, 304-313.
- Jiao, N., Yang, Y., Hong, N., Ma, Y., Harada, S., Koshikawa, H., Watanabe, M., 2005. Dynamics of autotrophic picoplankton and heterotrophic bacteria in the East China Sea. *Cont. Shelf. Res.* 25, 1265-1279.
- Jochem, F., 1988. On the distribution and importance of picocyanobacteria in a boreal inshore area (Kiel Bight, Western Baltic). *J. Plank. Res.* 10, 1009-1022.
- Joseph, J., Kurup, P., 1989. Volume transport and estuarine features at Cochin inlet. *Mahasagar* 22, 165-172.
- Jyothibabu, R., Madhu, N., Jayalakshmi, K., Balachandran, K., Shiyas, C., Martin, G., Nair, K., 2006. Impact of freshwater influx on microzooplankton mediated food web in a tropical estuary (Cochin backwaters–India). *Estuar. Coast. Shelf. Sci.* 69, 505-518.
- Kaladharan, P., Krishnakumar, P., Prema, D., Nandakumar, A., Khambadkar, L., Valsala, K., 2011. Assimilative capacity of Cochin inshore waters with reference to contaminants received from the backwaters and the upstream areas. *Ind. J. Fish.* 58, 75-83.
- Katano, T., Nakano, S. I., Ueno, H., Mitamura, O., Anbutsu, K., Kihira, M., Satoh, Y., Drucker, V., Sugiyama, M., 2005. Abundance, growth and grazing loss rates of picophytoplankton in Barguzin Bay, Lake Baikal. *Aquat. Ecol.* 39, 431-438.
- Li, W., Rao, S., Harrison, W., Smith, J., Cullen, J., Irwin, B., Platt, T., 1983. Autotrophic picoplankton in the tropical ocean. *Science(Washington)* 219, 292-295.
- Lin, D., Zhu, A., Xu, Z., Huang, L., Fang, H., 2010. Dynamics of photosynthetic picoplankton in a subtropical estuary and adjacent shelf waters. *J. Mar. Biol. Assoc. U.K.* 90, 1319-1329.
- Liu, H., Campbell, L., Landry, M., Nolla, H., Brown, S., Constantinou, J., 1998. *Prochlorococcus* and *Synechococcus* growth rates and contributions to production in the Arabian Sea during the 1995 Southwest and Northeast Monsoons. *Deep-Sea Res. Part II* 45, 2327-2352.
- Liu, H., Dagg, M., Campbell, L., Urban-Rich, J., 2004. Picophytoplankton and bacterioplankton in the Mississippi River plume and its adjacent waters. *Estuar. Coast.* 27, 147-156.

- Liu, H., Jing, H., Wong, T. H., Chen, B., 2013. Co-occurrence of phycoerythrin-and phycoerythrin-rich *Synechococcus* in subtropical estuarine and coastal waters of Hong Kong. *Env. Microbiol. Rep.* 6, 90-99.
- Madhu, N., Jyothibabu, R., Balachandran, K., Honey, U., Martin, G., Vijay, J., Shiyas, C., Gupta, G., Achuthankutty, C., 2007. Monsoonal impact on planktonic standing stock and abundance in a tropical estuary (Cochin backwaters–India). *Estuar. Coast. Shelf. Sci.* 73, 54-64.
- Madhu, N. V., Jyothibabu, R., Balachandran, K. K., 2009. Monsoon-induced changes in the size-fractionated phytoplankton biomass and production rate in the estuarine and coastal waters of southwest coast of India. *Environ. Monit. Assess.* 166, 521-528.
- Madhu, N., Balachandran, K., Martin, G., Jyothibabu, R., Thottathil, S. D., Nair, M., Joseph, T., Kusum, K., 2010. Short-term variability of water quality and its implications on phytoplankton production in a tropical estuary (Cochin backwaters—India). *Environ. Monit. Assess.* 170, 287-300.
- Martin, G., Vijay, J., Laluraj, C., Madhu, N., Joseph, T., Nair, M., Gupta, G., Balachandran, K., 2008. Fresh water influence on nutrient stoichiometry in a tropical estuary, South West coast of India. *Appl. Ecol. Env. Res.* 6, 57-64.
- Martin, G., Nisha, P., Balachandran, K., Madhu, N., Nair, M., Shaiju, P., Joseph, T., Srinivas, K., Gupta, G., 2011. Eutrophication induced changes in benthic community structure of a flow-restricted tropical estuary (Cochin backwaters), India. *Environ. Monit. Assess.* 176, 427-438.
- Martin, G., George, R., Shaiju, P., Muraleedharan, K., Nair, S., Chandramohanakumar, N., 2012. Toxic metals enrichment in the surficial sediments of a eutrophic tropical estuary (Cochin Backwaters, Southwest coast of India). *Sci. World J.* 2012.
- Martin, G., Jyothibabu, R., Madhu, N., Balachandran, K., Nair, M., Muraleedharan, K., Arun, P., Haridevi, C., Revichandran, C., 2013. Impact of eutrophication on the occurrence of *Trichodesmium* in the Cochin backwaters, the largest estuary along the west coast of India. *Environ. Monit. Assess.* 185, 1237-1253.
- Menon, N., Balchand, A., Menon, N., 2000. Hydrobiology of the Cochin backwater system—a review. *Hydrobiol.* 430, 149-183.
- Mitbavkar, S., Rajaneesh, K., Anil, A., Sundar, D., 2012. Picophytoplankton community in a tropical estuary: Detection of *Prochlorococcus*-like populations. *Estuar. Coast. Shelf. Sci.* 107, 159-164.
- Munawar, M., Weisse, T., 1989. Is the ‘microbial loop’ an early warning indicator of anthropogenic stress? *Hydrobiol.* 188, 163-174.
- Murrell, M. C., Lores, E. M., 2004. Phytoplankton and zooplankton seasonal dynamics in a subtropical estuary: importance of cyanobacteria. *J. Plank. Res.* 26, 371-382.
- Paerl, H. W., 1977. Ultraphytoplankton biomass and production in some New Zealand lakes. *New Zeal. J. Mar. Fresh.* 11, 297-305.
- Pan, L., Zhang, J., Zhang, L., 2007. Picophytoplankton, nanophytoplankton, heterotrophic bacteria and viruses in the Changjiang Estuary and adjacent coastal waters. *J. Plank. Res.* 29, 187-197.
- Parsons, T., Maita, Y., Lalli, C., 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford.

- Partensky, F., Hess, W., Vaulot, D., 1999. *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. *Microbiol. Mol. Biol. R.* 63, 106-127.
- Patil, J. S., Anil, A.C., 2011. Variations in phytoplankton community in a monsoon-influenced tropical estuary. *Environ. Monit. Assess.* 182, 291-300.
- Pednekar, S. M., Matondkar, S. G. P., Gomes, H. D. R., Goes, J. I., Parab, S., Kerkar, V., 2011. Fine-scale responses of phytoplankton to freshwater influx in a tropical monsoonal estuary following the onset of southwest monsoon. *J. Earth. Syst. Sci.* 120, 545-556.
- Platt, T., Rao, D. S., Irwin, B., 1983. Photosynthesis of picoplankton in the oligotrophic ocean. *Nature* 301, 702-704.
- Qasim, S., Gopinathan, C., 1969. Tidal cycle and the environmental features of Cochin Backwater (a tropical estuary), *Proceedings of the Indian Academy of Sciences*, 336-353.
- Qasim, S., Reddy, C., 1967. The estimation of plant pigments of Cochin Backwater during the monsoon months. *Bull. Mar. Sci.* 17, 95-110.
- Qasim, S., Sen Gupta, R., 1981. Environmental characteristics of the Mandovi-Zuari estuarine system in Goa. *Estuar. Coast. Shelf. Sci.* 13, 557-578.
- Qasim, S.Z., 2003. *Indian estuaries*. Allied publishers.
- Qiu, D., Huang, L., Zhang, J., Lin, S., 2010. Phytoplankton dynamics in and near the highly eutrophic Pearl River Estuary, South China Sea. *Cont. Shelf. Res.* 30, 177-186.
- Rajaneesh, K., Mitbavkar, S., 2013. Factors controlling the temporal and spatial variations in *Synechococcus* abundance in a monsoonal estuary. *Mar. Environ. Res.* 92, 133-143.
- Ray, R. T., Haas, L. W., Sieracki, M. E., 1989. Autotrophic picoplankton dynamics in a Chesapeake Bay sub-estuary. *Mar. Ecol. Prog. Ser.* 52, 273-285.
- Reddy, C., Sankaranarayanan, V., 1972. Phosphate regenerative activity in the muds of a tropical estuary. *Indian J. Mar. Sci.* 1, 57-60.
- Sankaranarayanan, V., Qasim, S., 1969. Nutrients of the Cochin Backwater in relation to environmental characteristics. *Mar. Biol.* 2, 236-247.
- Sieburth, J.M., Smetacek, V., Lenz, J., 1978. Pelagic ecosystem structure, heterotrophic compartments of the plankton and their relationship to plankton size fractions. *Limnol. Oceanogr.* 23, 1256-63.
- Sin, Y., Hyun, B., Jeong, B., Soh, H. Y., 2013. Impacts of eutrophic freshwater inputs on water quality and phytoplankton size structure in a temperate estuary altered by a sea dike. *Mar. Environ. Res.* 85, 54-63.
- Scanlan, D. J., 2003. Physiological diversity and niche adaptation in marine *Synechococcus*. *Adv. Microb. Physiol.* 47, 1-64.
- Shang, X., Zhang, L., Zhang, J., 2007. *Prochlorococcus*-like populations detected by flow cytometry in the fresh and brackish waters of the Changjiang Estuary. *J. Mar. Biol. Assoc. U.K.* 87, 643-648.
- Shetye, S. R., 1999. Propagation of tides in the Mandovi and Zuari estuaries. *Sadhana (Academy Proceedings in Engineering Sciences)*. Ind. Acad. Sci. 5-16.

- Shiomoto, A., Tadokoro, K., Monaka, K., Nanba, M., 1997. Productivity of picoplankton compared with that of larger phytoplankton in the subarctic region. *J. Plank. Res.* 19, 907-916.
- Shivaprasad, A., Vinita, J., Revichandran, C., Manoj, N., Srinivas, K., Reny, P., Ashwini, R., Muraleedharan, K., 2012. Influence of Saltwater Barrage on Tides, Salinity, and Chlorophyll *a* in Cochin Estuary, India. *J. Coast. Res.* 29, 1382-1390.
- Srinivas, K., Revichandran, C., Maheswaran, P., Asharaf, T. M., Murukesh, N., 2003. Propagation of tides in the Cochin estuarine system, southwest coast of India. *J. Mar. Sci.* 32, 14-24.
- Stockner, J. G., Antia, N. J., 1986. Algal picoplankton from marine and freshwater ecosystems: a multidisciplinary perspective. *Can. J. Fish. Aquat. Sci.* 43, 2472-2503.
- Stomp, M., Huisman, J., Voros, L., Pick, F.R., Laamanen, M., Haverkamp, T., Stal, L. J., 2007. Colourful coexistence of red and green picocyanobacteria in lakes and seas. *Ecol. Lett.* 10, 290-298.
- Tsai, A. Y., Chiang, K. P., Chang, J., Gong, G. C., 2008. Seasonal variations in trophic dynamics of nanoflagellates and picoplankton in coastal waters of the western subtropical Pacific Ocean. *Aquat. Microb. Ecol.* 51, 263-274.
- Unnithan, R., Vijayan, M., Remani, K., 1975. Organic pollution in Cochin backwaters. *Indian J. Mar. Sci.* 4, 39-42.
- Vijayan, M., Ramani, K., Unnithan, R., 1976. Effects of organic pollution on some hydrographic features of Cochin backwaters. *J. Mar. Sci.* 5, 196-200.
- Vollenweider, R., Giovanardi, F., Montanari, G., Rinaldi, A., 1998. Characterization of the trophic conditions of marine coastal waters, with special reference to the NW Adriatic Sea: proposal for a trophic scale, turbidity and generalized water quality index. *Environmetrics* 9, 329-357.
- Wang, K., Wommack, K. E., Chen, F., 2011. Abundance and distribution of *Synechococcus* spp. and cyanophages in the Chesapeake Bay. *Appl. Environ. Microb.* 77, 7459-7468.
- Wetz, M. S., Paerl, H. W., Taylor, J. C., Leonard, J. A., 2011. Environmental controls upon picophytoplankton growth and biomass in a eutrophic estuary. *Aquat. Microb. Ecol.* 63, 133.
- Wood, A. M., 1985. Adaptation of photosynthetic apparatus of marine ultraphytoplankton to natural light fields. *Nature* 316, 253-255.
- Zhang, X., Shi, Z., Ye, F., Zeng, Y., Huang, X., 2013. Picophytoplankton abundance and distribution in three contrasting periods in the Pearl River Estuary, South China. *Mar. Freshwater Res.* 64, 692-705.

## Figure legends

Fig. 1. Sampling stations located in the Cochin backwaters, west coast of India. 1. Custom buoy, 2. Fishery harbour, 3. Dry dock, 4. South coal berth, 5. Quay-1, 6. Quay-2, 7. North coal berth, 8. Boat train pier, 9. Container terminal, 10. DC jetty, 11. Quay-6, 12. Quay-8, 13. Quay-10, 14. Ro-Ro jetty, 15. Naval jetty, 16. Cochin shipyard, 17. Bunker oil jetty, 18. Integrated fisheries project jetty, 19. South tanker berth, 20. North tanker berth, 21. Ernakulam ferry jetty, 22. Cochin oil terminal, 23. Ernakulam creek mouth.

Fig. 2. Flow cytometric analysis of picophytoplankton community from (a-c) low saline and (d-f) high saline water samples.

Fig. 3. Seasonal and spatial variations in (a-b) temperature, (c-d) salinity, (e-f) dissolved oxygen, (g-h) biological oxygen demand, (i-j) nitrate, (k-l) phosphate, (m-n) nitrite, (o-p) ammonium, (q-r) silicate and (s-t) chlorophyll *a* in the Cochin backwaters.

Fig. 4. Seasonal and spatial variations in the picophytoplankton community structure in the Cochin backwaters. (a-b) *SYN*-PEI, (c-d) *SYN*-PEII, (e-f) *SYN*-PC, (g-h) *PRO*- like cells, (i-j) PEUK and (k-l) total picophytoplankton cell abundance.

Fig. 5. Contribution (%) of individual picophytoplankton groups to the total picophytoplankton abundance during (a-b) post-monsoon I, (c-d) pre-monsoon, (e-f) monsoon and (g-h) post-monsoon II seasons.

Fig. 6. TRIX scores during different seasons for Cochin backwaters. (a) surface and (b) Near bottom waters.

Fig. 7. Linear regression analysis of (a) *SYN*-PEI and (b) *SYN*-PC abundance with TRIX scores.

Fig. 8. Linear regression analysis between *SYN*-PC:*SYN*-PE abundance ratio and salinity in the Cochin backwaters. The curve was fitted under the logarithmic equation model.

Fig. 9. Linear regression analysis of (a) *SYN*-PEI, (b) *SYN*-PEII, (c) *SYN*-PC, (d) PEUK, (e) *PRO*-like cells and (f) chl *a* with principle component scores (PC1) for the ecological variables (Dissolved oxygen, biological oxygen demand, nitrate, phosphate, nitrite, ammonium and silicate).

Table 1. Results of linear regression analysis for the environmental variables, TRIX scores and picophytoplankton abundance.

Variables	Temperature	Salinity	Est. tidal height	Station depth	TRIX	DO	BOD	NO <sub>3</sub>	NO <sub>2</sub>	NH <sub>4</sub>	PO <sub>4</sub>	SiO <sub>4</sub>	Chlorophyll <i>a</i>	
Salinity	0.11													
Est. tidal height	-0.14	<b>0.20**</b>												
Station depth	<b>-0.25**</b>	<b>0.43**</b>	0.03											
TRIX	<b>-0.21**</b>	<b>-0.18*</b>	<b>-0.20**</b>	0.06										
DO	<b>0.46**</b>	<b>-0.19**</b>	-0.07	<b>-0.50**</b>	-									
Chlorophyll <i>a</i>	-0.08	0.02	-0.13	0.05	<b>0.54**</b>	-	<b>0.43**</b>	<b>0.40**</b>	0.11	<b>0.58**</b>	0.12	0.14	-0.01	
SYN-PEI	0.06	<b>0.26**</b>	<b>0.36**</b>	<b>0.33**</b>	<b>0.24**</b>	-0.10	<b>0.18*</b>	-	<b>0.40**</b>	<b>0.30**</b>	<b>0.28**</b>	<b>0.25**</b>	<b>0.31**</b>	-0.06
SYN-PEII	<b>-0.24**</b>	<b>0.20**</b>	<b>0.33**</b>	<b>0.28**</b>	-0.16	-0.13	<b>0.15*</b>	-	<b>0.29**</b>	<b>0.23**</b>	<b>0.34**</b>	<b>-0.17*</b>	<b>0.24**</b>	-0.01
SYN-PC	<b>0.19*</b>	<b>-0.35**</b>	<b>-0.20**</b>	<b>-0.43**</b>	<b>0.34**</b>	0.06	-	<b>0.25**</b>	<b>0.36**</b>	<b>0.36**</b>	<b>0.16*</b>	<b>0.22**</b>	<b>0.38**</b>	<b>0.34**</b>
PEUK	-0.11	<b>-0.32**</b>	<b>-0.32**</b>	<b>-0.20**</b>	0.14	0.13	-0.06	<b>0.20**</b>	<b>0.29**</b>	<b>0.27**</b>	<b>0.25**</b>	<b>0.27**</b>	<b>0.27**</b>	-0.09
PRO-like	<b>-0.27**</b>	<b>-0.19**</b>	-0.07	-0.01	0.02	-	<b>0.30**</b>	<b>0.25**</b>	-0.09	<b>0.33**</b>	-0.10	-0.11	-0.08	<b>0.31**</b>

\*  $p < 0.05$ , \*\*  $p < 0.01$ ,

Table 2. Principle component analysis with varifactors (PC's) extracted for the ecological variables. Bold text denotes significant loading.

Variables	PC1	PC2
DO	-0.239	<b>0.868</b>
BOD	<b>-0.460</b>	<b>0.739</b>
NO <sub>3</sub>	<b>0.590</b>	0.299
NO <sub>2</sub>	<b>0.634</b>	<b>-0.440</b>
NH <sub>4</sub>	<b>0.739</b>	0.206
PO <sub>4</sub>	<b>0.781</b>	0.209
SiO <sub>4</sub>	<b>0.639</b>	0.524
Eigenvalues	2.58	1.94
% of variance	36.9	27.8
Cumulative %	36.9	64.7

Annex table 1. Sampling details for each sampled station in the Cochin backwaters along the west coast of India.

Stn. No.	Station name	Latitude (N)	Longitude (E)	Average depth (m)	Post-monsoon I			Pre-monsoon			Monsoon			Post-monsoon II		
					Date (d-m-y)	Time (h)	Est. tidal height (m)	Date (d-m-y)	Time (h)	Est. tidal height (m)	Date (d-m-y)	Time (h)	Est. tidal height (m)	Date (d-m-y)	Time (h)	Est. tidal height (m)
1	Custom buoy	9° 58' 05''	76° 15' 11''	4.88	11-10-2011	07:30	0.56	27-05-2012	12:08	0.54	12-08-2012	08:07	0.64	24-11-2012	09:19	0.78
2	Fishery harbour	9° 56' 24''	76° 15' 47''	5.6	11-10-2011	12:45	0.72	28-05-2012	11:12	0.46	13-08-2012	07:20	0.56	24-11-2012	07:06	0.55
3	Dry dock	9° 56' 42''	76° 16' 01''	5.05	11-10-2011	11:30	0.78	26-05-2012	10:44	0.44	13-08-2012	08:18	0.62	24-11-2012	07:28	0.52
4	South coal berth	9° 57' 11''	76° 16' 01''	6.91	11-10-2011	10:45	0.80	26-05-2012	12:17	0.58	13-08-2012	09:30	0.69	24-11-2012	07:51	0.50
5	Quay-1	9° 57' 14''	76° 16' 01''	7.01	11-10-2011	09:45	0.74	26-05-2012	11:31	0.51	13-08-2012	10:51	0.73	21-11-2012	08:32	0.76
6	Quay-4	9° 57' 29''	76° 15' 54''	8.33	11-10-2011	09:00	0.68	26-05-2012	11:01	0.46	13-08-2012	11:42	0.71	21-11-2012	10:25	0.63
7	North coal berth	9° 57' 50''	76° 15' 40''	11.69	11-10-2011	08:15	0.62	26-05-2012	10:10	0.38	12-08-2012	09:57	0.69	21-11-2012	10:59	0.59
8	Boat train pier	9° 57' 54''	76° 15' 36''	6.85	12-10-2011	07:00	0.54	28-05-2012	08:40	0.44	12-08-2012	09:16	0.70	21-11-2012	09:41	0.68
9	Container terminal	9° 58' 30''	76° 15' 07''	11.25	12-10-2011	07:45	0.59	28-05-2012	09:15	0.41	12-08-2012	07:36	0.61	24-11-2012	09:56	0.73
10	DC jetty	9° 58' 08''	76° 15' 14''	9.27	12-10-2011	08:30	0.65	28-05-2012	07:52	0.48	12-08-2012	06:49	0.57	23-11-2012	10:19	0.69
11	Quay-6	9° 58' 01''	76° 16' 01''	9.25	12-10-2011	09:30	0.71	28-05-2012	07:29	0.47	11-08-2012	09:33	0.65	23-11-2012	09:52	0.72
12	Quay-8	9° 57' 58''	76° 16' 12''	11.44	10-10-2011	07:30	0.60	27-05-2012	06:55	0.45	11-08-2012	08:56	0.66	23-11-2012	09:20	0.75
13	Quay-10	9° 57' 50''	76° 16' 30''	9.19	10-10-2011	08:00	0.65	27-05-2012	11:03	0.45	11-08-2012	08:07	0.67	23-11-2012	09:02	0.77
14	Ferry jetty	9° 57' 36''	76° 16' 41''	8.88	10-10-2011	08:45	0.71	27-05-2012	10:37	0.41	11-08-2012	07:22	0.66	23-11-2012	06:59	0.86
15	Naval jetty	9° 57' 25''	76° 16' 52''	2.69	10-10-2011	09:30	0.77	29-05-2012	09:56	0.49	11-08-2012	06:57	0.64	23-11-2012	06:35	0.84
16	Cochin shipyard	9° 57' 18''	76° 17' 10''	7.22	10-10-2011	10:15	0.81	29-05-2012	10:03	0.49	10-08-2012	10:03	0.58	22-11-2012	06:43	0.91
17	Bunker oil jetty	9° 57' 29''	76° 17' 06''	8.57	10-10-2011	11:15	0.74	27-05-2012	10:34	0.42	10-08-2012	10:30	0.57	22-11-2012	07:20	0.86
18	IFP jetty	9° 57' 36''	76° 17' 02''	3.75	09-10-2011	12:30	0.66	29-05-2012	08:45	0.53	10-08-2012	09:18	0.60	22-11-2012	07:54	0.82
19	South tanker berth	9° 57' 43''	76° 16' 48''	10.69	09-10-2011	11:30	0.70	29-05-2012	11:38	0.51	10-08-2012	08:42	0.62	22-11-2012	08:45	0.77
20	North tanker berth	9° 57' 50''	76° 16' 44''	10.37	09-10-2011	10:45	0.77	27-05-2012	10:57	0.44	10-08-2012	07:50	0.64	22-11-2012	09:27	0.72
21	Ernakulam ferry jetty	9° 58' 16''	76° 16' 44''	2.28	09-10-2011	10:00	0.79	29-05-2012	07:58	0.56	15-08-2012	10:43	0.74	25-11-2012	06:40	0.78
22	Cochin oil terminal	9° 58' 12''	76° 16' 12''	11.11	12-10-2011	11:00	0.77	27-05-2012	12:27	0.57	11-08-2012	10:12	0.64	22-11-2012	10:46	0.63
23	Ernakulam creek mouth	9° 58' 41''	76° 16' 30''	1.71	09-10-2011	09:00	0.73	27-05-2012	07:07	0.43	10-08-2012	07:04	0.66	22-11-2012	10:12	0.66

Stn- Stations; Est- Estimated

Annex table 2. Rainfall data for the sampling periods.

Post-monsoon I		Pre-monsoon		Monsoon		Post-monsoon II	
Date	Rainfall	Date	Rainfall	Date	Rainfall	Date	Rainfall
(d-m-y)	(mm)	(d-m-y)	(mm)	(d-m-y)	(mm)	(d-m-y)	(mm)
06-10-2011	0.0	24-05-2012	0.0	08-08-2012	15.5	19-11-2012	0.0
07-10-2011	0.0	25-05-2012	0.0	09-08-2012	46.4	20-11-2012	0.0
08-10-2011	0.0	26-05-2012	0.6	10-08-2012	31.9	21-11-2012	0.0
09-10-2011	0.0	27-05-2012	5.4	11-08-2012	29.0	22-11-2012	0.0
10-10-2011	0.0	28-05-2012	10.6	12-08-2012	6.1	23-11-2012	56.0
11-10-2011	0.0	29-05-2012	0.0	13-08-2012	7.2	24-11-2012	1.0
12-10-2011	0.0	30-05-2012	0.1	14-08-2012	0.2	25-11-2012	0.0
13-10-2011	0.0	31-05-2012	0.8	15-08-2012	28.2	26-11-2012	0.0
14-10-2011	0.0	01-06-2012	0.1	16-08-2012	31.0	27-11-2012	0.0

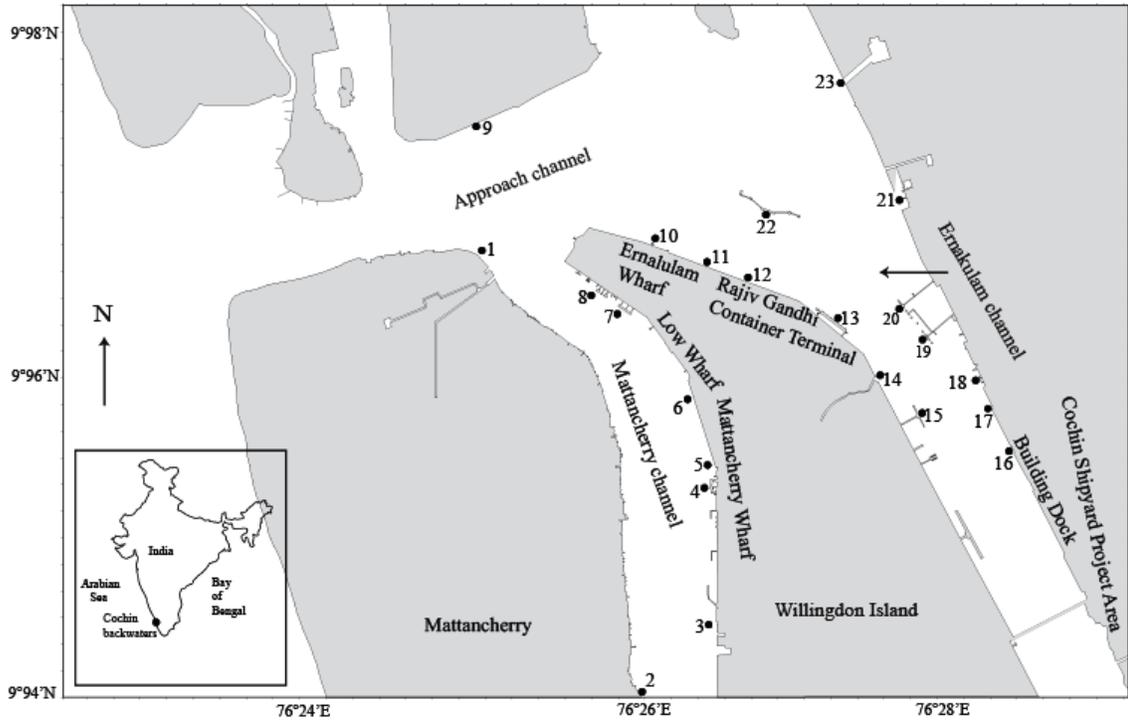


Fig.1.

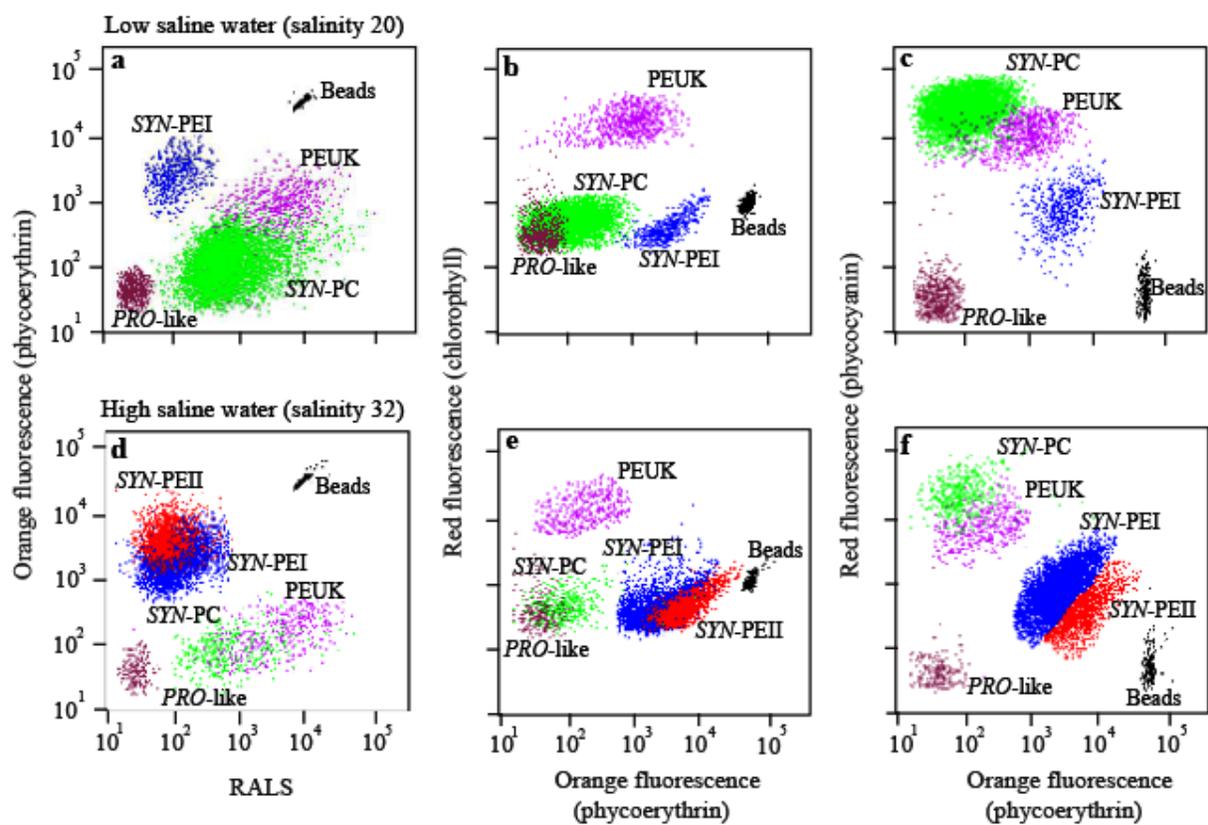


Fig. 2

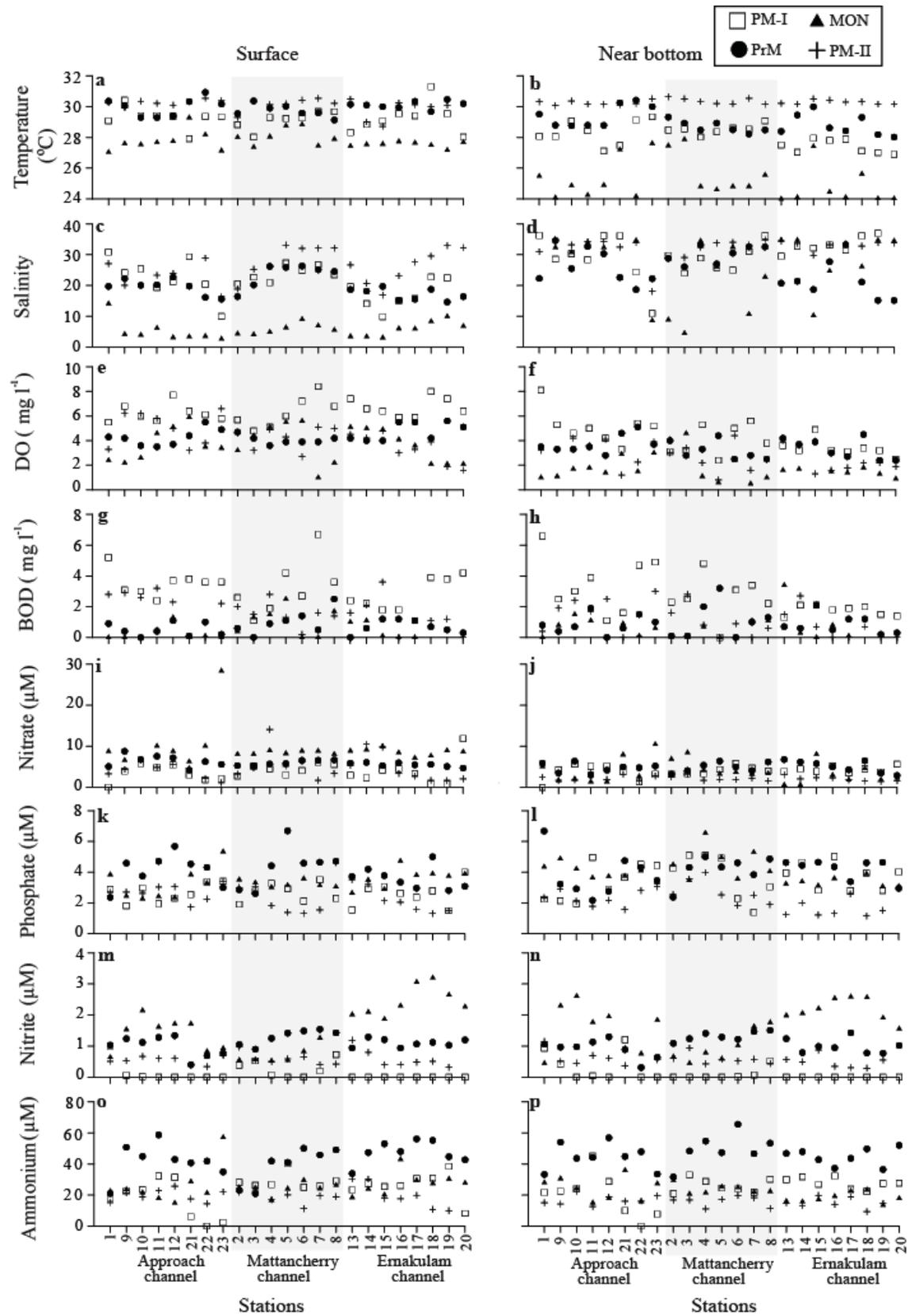


Fig. 3

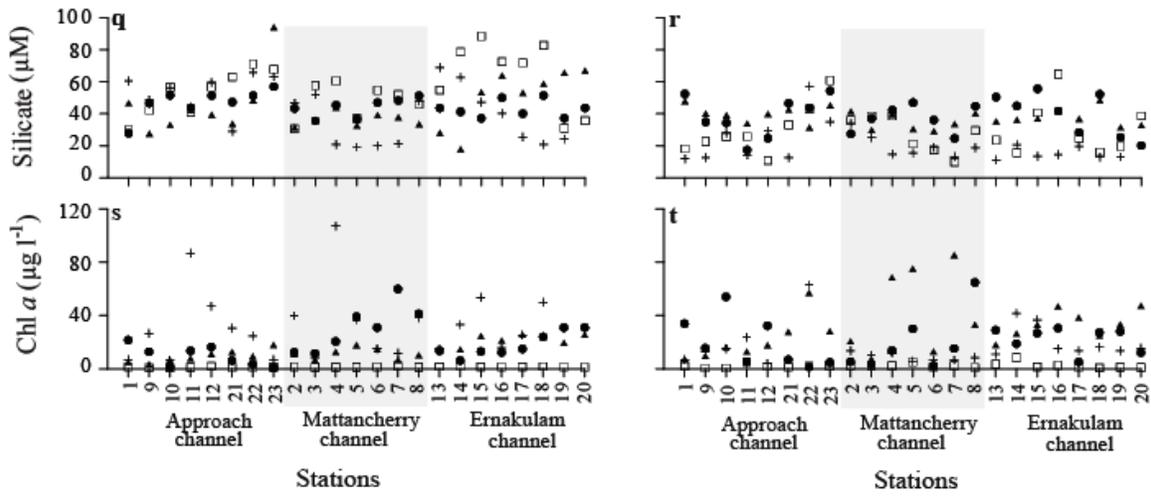


Fig. 3 continued

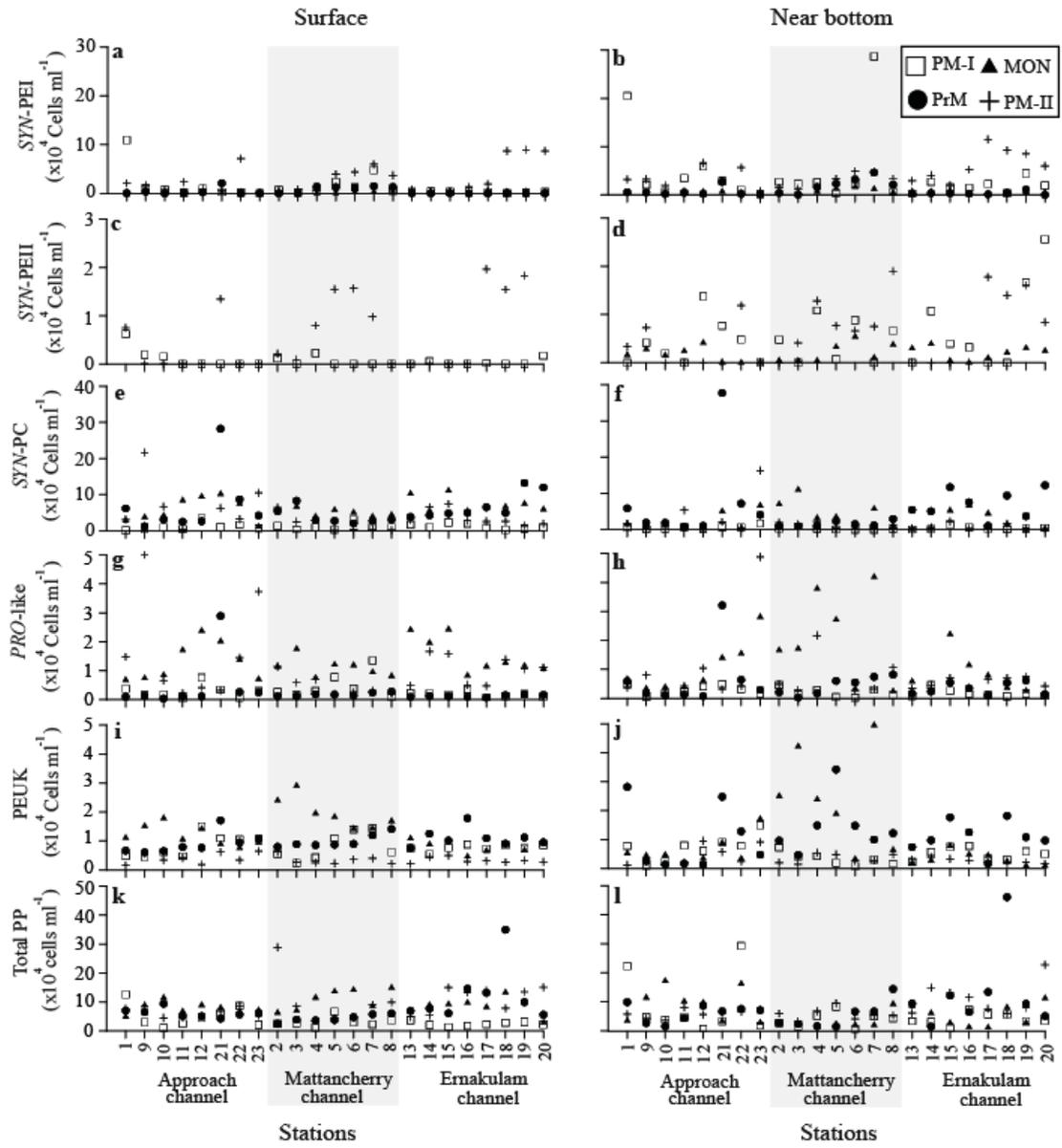


Fig. 4

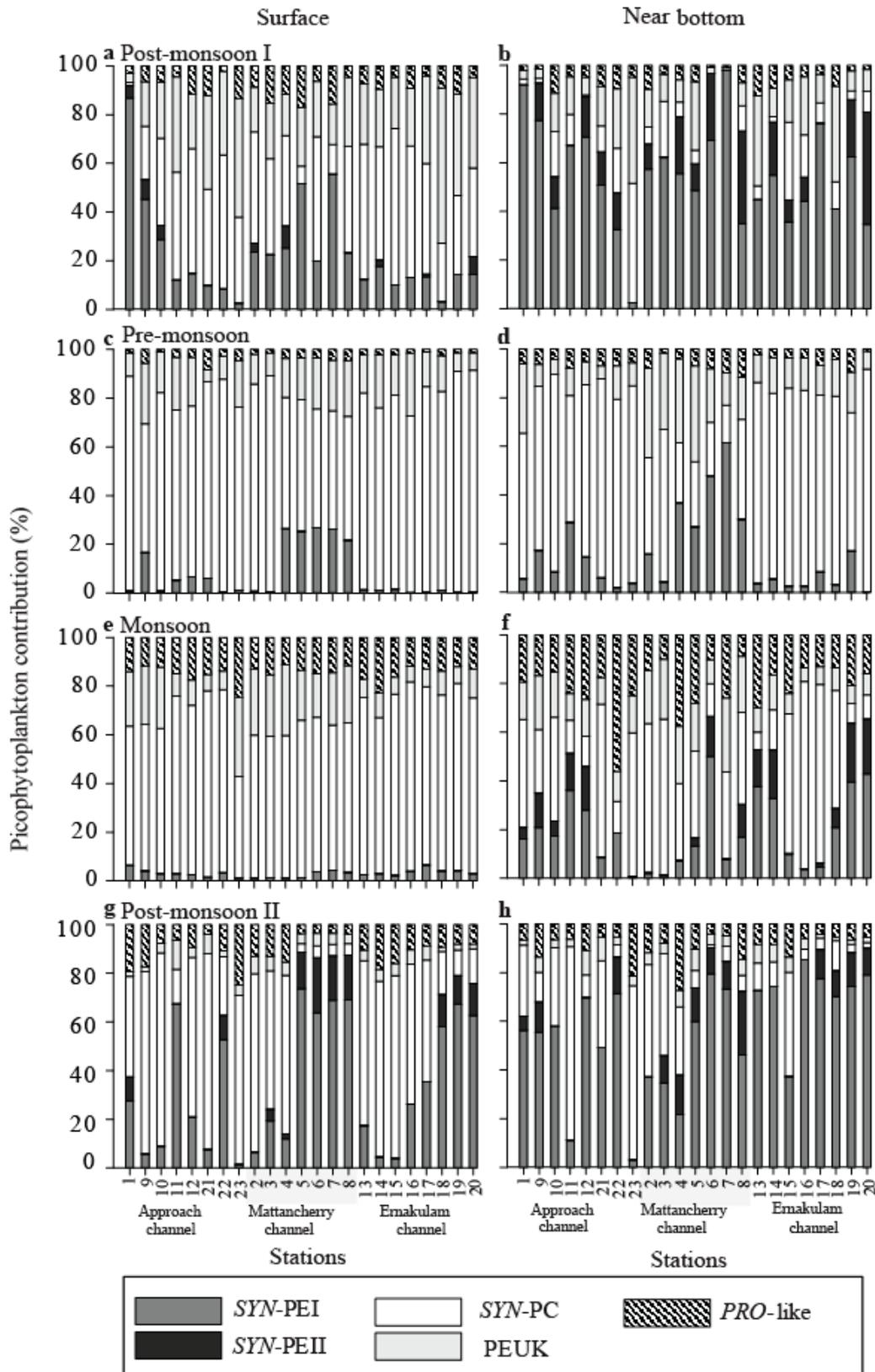


Fig. 5

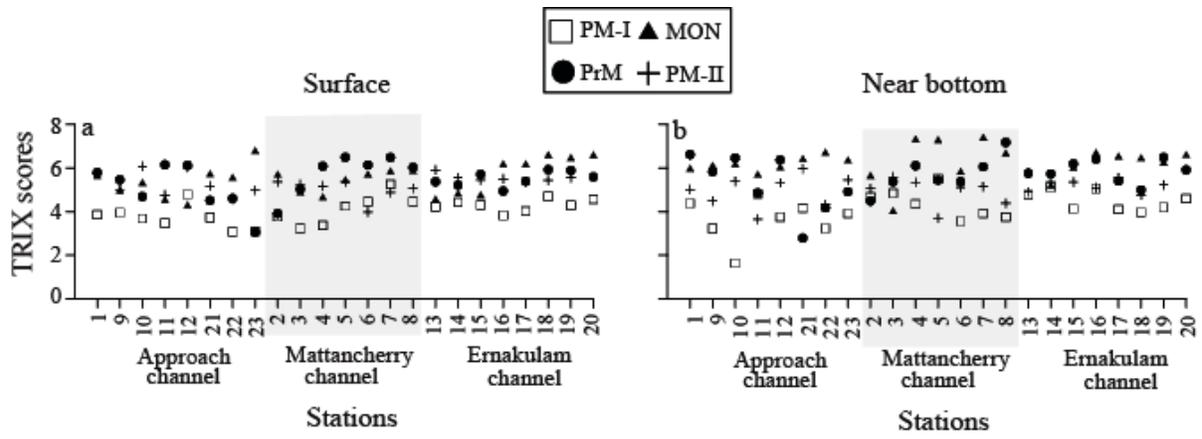


Fig. 6

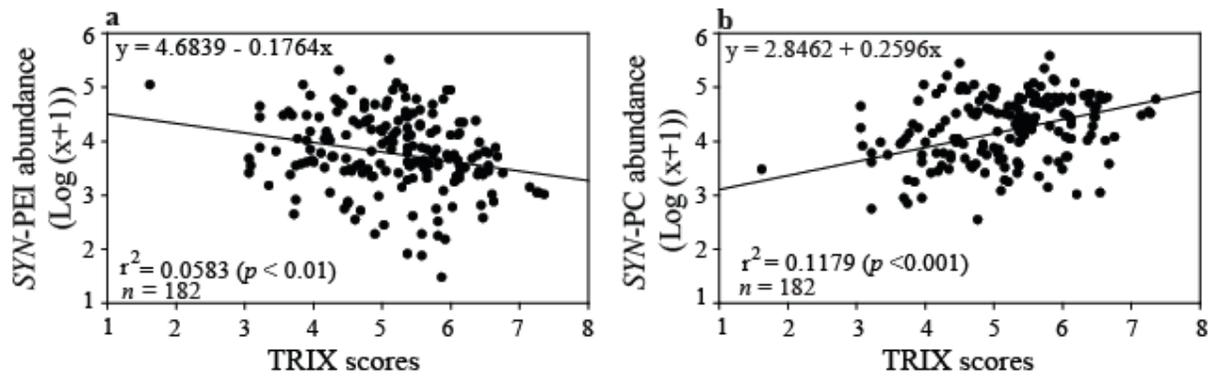


Fig. 7

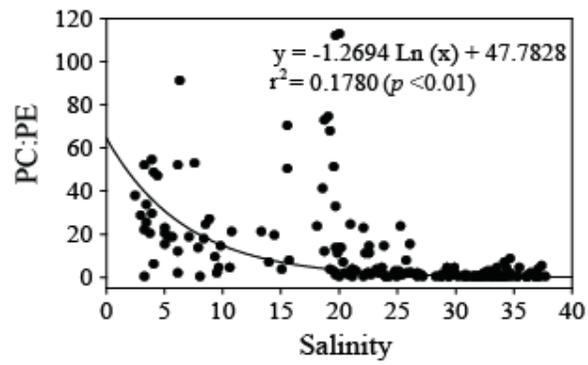


Fig. 8

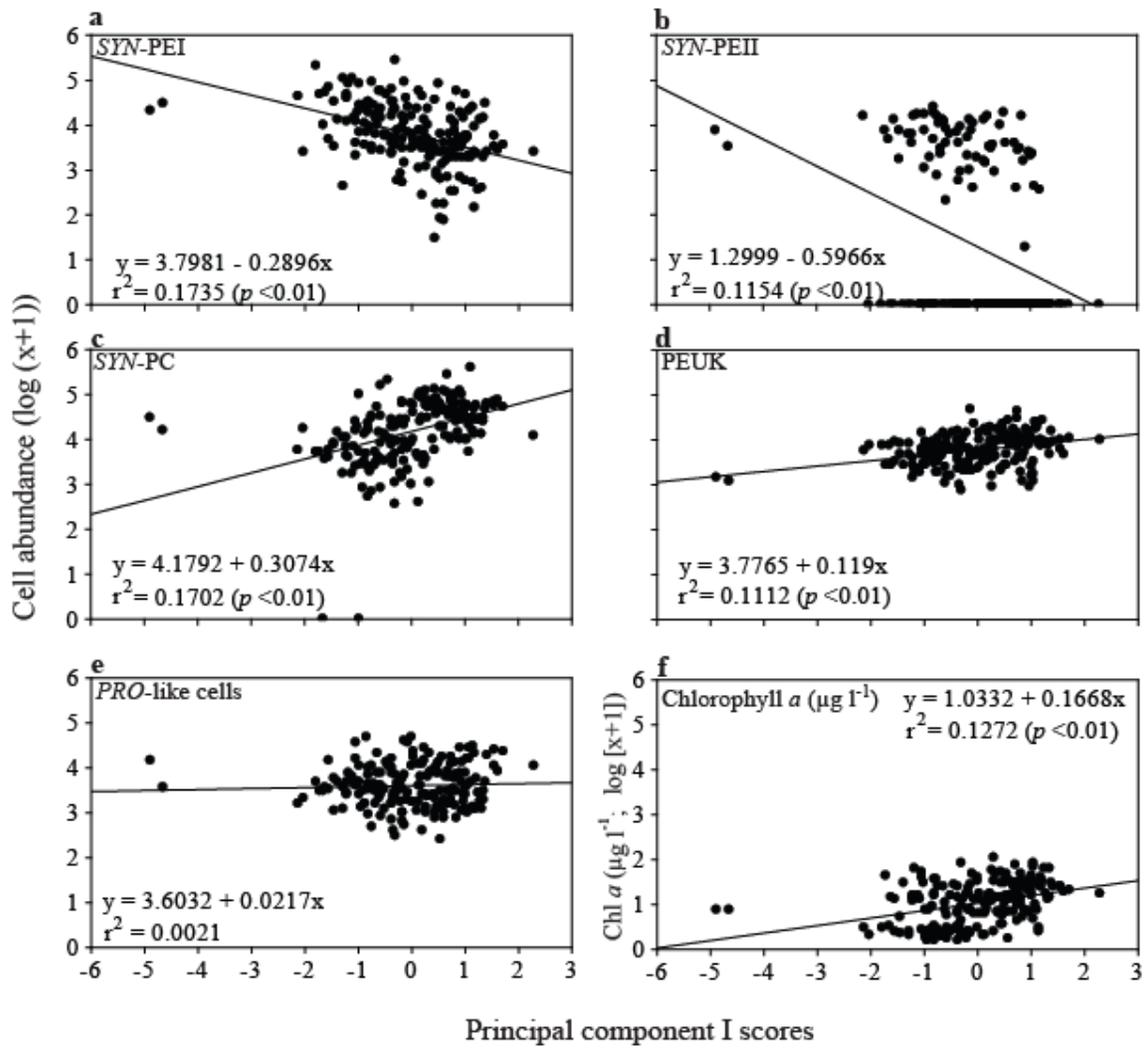


Fig. 9