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## **Factors controlling the temporal and spatial variations in *Synechococcus* abundance in a monsoonal estuary**

Rajaneesh K. M., Smita Mitbavkar\*

CSIR-National Institute of oceanography, Dona Paula, Goa 403 004, India

\*Corresponding author. Tel.: + 91 832 2450376; fax: + 91 832 2450615.

E-mail address: mitbavkars@nio.org (Smita Mitbavkar)

### ABSTRACT

Temporal and spatial variations in *Synechococcus* abundance were investigated over an annual cycle (February'10-January'11) along a salinity gradient (0-35) in the tropical Zuari estuary, influenced by south-west monsoons. *Synechococcus* exhibited salinity preferences with phycoerythrin-rich cells at salinities  $>2$  (*Synechococcus*-PEI),  $>20$  (*Synechococcus*-PEII) and  $<1$  (*Synechococcus*-PEIII) whereas phycocyanin-rich (*Synechococcus*-PC) dominant at lower salinities. Downstream stratification during monsoon caused *Synechococcus* group segregation in the surface and near-bottom waters. During monsoon-break and non-monsoon period stabilized waters, increased salinity, temperature, solar radiation and low rainfall favored high *Synechococcus* abundance whereas unstable waters, increased turbidity and low solar radiation during active monsoon lowered abundance. *SYN*-PC positively co-related with nitrate and phosphate and *SYN*-PEI with phosphate. *Synechococcus* contribution to phytoplankton carbon biomass ranged from 9-29%. In monsoonal estuaries, rainfall intensity regulates freshwater runoff which modulates the estuarine environment, creating temporal-spatial niche segregation of *Synechococcus* groups thereby serving as indicator organisms of the estuarine hydrodynamics.

*Keywords:* Estuaries, Monsoon, Picophytoplankton, Salinity, Zuari estuary, *Synechococcus*

## 1. Introduction

Picophytoplankton (PP;  $< 3 \mu\text{m}$ ) have been recognized as significant contributors to the total phytoplankton biomass and primary production in marine (Platt et al., 1983) and freshwater ecosystems (Paerl, 1977). PP comprises two major groups of cyanobacteria, *Prochlorococcus* and *Synechococcus* (*SYN*) and small eukaryotes known as picoeukaryotes. *Prochlorococcus* is abundant in oligotrophic waters whereas picoeukaryotes are abundant in coastal waters. *SYN* proliferates in well-lit, eutrophic coastal ecosystems (Jochem, 1988) and are present in comparatively lower numbers in oligotrophic open waters at temperatures ranging from  $2^{\circ}\text{C}$  to  $30^{\circ}\text{C}$  from tropic, subtropic, temperate and Polar Regions (Partensky et al., 1999). Based on phycobilisome composition, two groups of *SYN* have been identified in estuarine ecosystems; one rich in phycoerythrin (PE) and the other in phycocyanin (PC). These studies revealed that PE rich *SYN* dominates higher saline waters whereas PC rich *SYN* are abundant in lower saline waters (Murrell and Lores, 2004). Sub-groups of PE rich *SYN* were also detected in the Mississippi river plume (Liu et al., 2004), Pearl River estuary (Lin et al., 2010) and the Zuari estuary (Mitbavkar et al., 2012) implying the importance of salinity on the distribution of the *SYN* groups. PP plays an important role in the microbial loop by forming the base of food chain and serving as food for many protists and small invertebrates species (Azam, 1983; Pomeroy, 1974). This carbon transfer through microbial food web creates the important connection between PP and higher trophic levels (Chiang et al., 2013). Studies on PP are well established in the Pacific and Atlantic Oceans whereas comparatively in the Indian Ocean only a few observations have been made (Brown et al., 1999; Campbell et al., 1998). Apart from the open ocean, the importance of PP in coastal regions is now being highlighted (Mitbavkar et al., 2012; Murrell and Lores, 2004).

Estuaries are one of the most productive natural habitats in the world which show wide variation of hydrological characteristics depending on the inputs from upstream rivers. The excess amount of nutrients available in estuarine ecosystems favor the rapid growth of phytoplankton (Madhu et al., 2009; Qiu et al., 2010). Several studies on phytoplankton and PP have been conducted in estuarine region encompassing brackish water to seawater with a wide salinity range (2 to 35). These studies suggest that salinity plays an important role in the spatial distribution of PP groups (Murrell and Lores, 2004; Ray et al., 1989) and also highlights that PP are the major component of the phytoplankton community contributing substantially to the total biomass and primary production in estuarine region of subtropical (Sin et al., 2010) and temperate estuaries (Ning et al., 2000). In tropical estuarine regions studies have mostly focused on larger phytoplankton wherein hydrology and nutrients were indicated as the major dynamic factors influencing the phytoplankton biomass

and composition (Costa et al., 2009). However, there are few studies on PP especially *SYN* distribution in tropical and subtropical estuarine and coastal environments (Lin et al., 2010; Qiu et al., 2010).

In the tropics, estuaries influenced by monsoons support very productive fisheries, which in turn are sustained via a healthy food chain supported by a strong foundation, the phytoplankton. There are few studies conducted in monsoonal estuaries. These findings showed that increase in freshwater discharge influences the PP growth (Lin et al., 2010; Qiu et al., 2010). Such areas serve as good model ecosystems for studying the dynamics of PP on temporal and spatial scales. For the first time we have studied the distribution of this organism in a monsoonal estuary where the tides and freshwater runoff regulate the hydrodynamics on an annual scale and also where short spells of breaks in monsoon are experienced during the monsoon season. The aim of the present study was to assess whether the distribution of *SYN*, the most dominant PP group, is determined by the temporal and spatial variations in environmental factors regulated by the freshwater runoff. We hypothesized that *SYN* population structure is influenced by both, the temporal and spatial variations in environmental factors, with low abundance during monsoon and dominance of different groups along the salinity gradient. As such *SYN* groups can serve as indicator organisms in estuarine regions depicting the hydrodynamics across the estuary.

## **2. Materials and Methods**

### *2.1. Study area*

Sampling was carried out in the Zuari estuary (Goa) located along the central west coast of India (Fig. 1, Table 1). It is one of the major estuaries of Goa and important for agriculture, fisheries and transportation of mines (iron and manganese ore). It originates at Hemad-Barshen in the Western Ghats and flows up to Arabian Sea with a length of 65 km. It's cross sectional area decreases from the mouth to head. This location experiences three seasons: the pre-monsoon (PrM; February to May), the southwest monsoon (MON; June to September) and the post-monsoon (PoM; October to January). During the study period, a total of 3723.5 mm rainfall was recorded which was relatively higher than that in previous years (Indian Meteorological Department). Out of that, ~92 % of precipitation occurred during the southwest MON. Consequently during MON, the main channel receives huge amounts of riverine freshwater through Kushavathi, Sanguem Rivers and small streams at many points along its length. This creates a river runoff exceeding  $400 \text{ m}^3 \text{ s}^{-1}$  whereas rest of the year freshwater runoff is  $< 10 \text{ m}^3 \text{ s}^{-1}$  (Shetye and Murty, 1987). Tides occur in this estuary up to a distance of about 50 km and the increase in the elevation of the estuarine channel prevents tides

from propagating beyond this distance (Shetye, 1999). Average depth of this estuary is ~ 5m with the catchment area of 550 km<sup>2</sup>. Tides are semidiurnal, with the highest height of 2.3 m during spring tide and ~ 1 m during neap tide (Manoj and Unnikrishnan, 2009). Cumbarjua canal, which is at about 11 km distance from the mouth of the estuary, connects the Zuari estuary to the adjacent Mandovi estuary and is also involved in the regulation of the water flow (Qasim and Sen Gupta, 1981). (Fig. 1 and Table 1 – preferred position)

## 2.2. Sampling

Monthly sampling was carried out in the Zuari estuary from February 2010 to January 2011 (Table 1). Surface and near-bottom water (NBW) samples were collected from 10 stations with a Niskin sampler (Fig. 1). Vertical profiles of temperature and salinity were determined using portable seabird CTD (SBE 19 plus). Water transparency was measured with a secchi disk (SD). Rainfall data for the study period were acquired from the Indian Meteorological Department (IMD) (Table 2). Chlorophyll *a* (chl *a*) was measured following standard methods (Parsons et al., 1984). Nutrients (nitrate (NO<sub>3</sub>-N), phosphate (PO<sub>4</sub>-P), nitrite (NO<sub>2</sub>-N) and silicate (SiO<sub>4</sub>)) were analyzed by SKALAR SAN<sup>plus</sup> ANALYSER. For PP, seawater samples were preserved with paraformaldehyde (0.2% final concentration), quick frozen in liquid nitrogen and stored at -80°C until analysis. (Table 2 – preferred position)

## 2.3. Flow cytometric analysis of *Synechococcus*

Prior to analysis, frozen samples were thawed and then analyzed by a flow cytometer (FACS Aria II) equipped with blue (488 nm) and red (630 nm) lasers. Forward angle light scatter (FALS), right angle light scatter (RALS), red fluorescence from chlorophyll (> 650 nm) and phycocyanin (630 nm) and orange fluorescence from phycoerythrin (564-606 nm) were recorded from each particle after excitation by lasers. Data obtained was processed with the BD FACS Diva (Version 6.2) software. The different *SYN* groups present in the sample could be discriminated according to their specific fluorescence and scattering properties. Yellow green latex beads of 2 µm (polysciences co., USA) were added to the sample as internal standards to calibrate cell fluorescence emission and light scatter signals, which allowed comparison of fluorescence and cell size among different samples. Based on flow cytometric signatures, two groups of *SYN* were distinguished: one rich in phycoerythrin (*SYN*-PE) and the other in phycocyanin (*SYN*-PC) throughout the study period. RALS and FALS (proxy for cell size) signals revealed that the cell size of *SYN*-PC is bigger than *SYN*-PE whereas chlorophyll fluorescence is comparable with *SYN*-PE (Fig. 2). The *SYN*-PE group was further differentiated into 2 subgroups based on the phycoerythrin fluorescence intensity and was designated as *SYN*-PEI which had a lower fluorescence intensity and *SYN*-PEII with a comparatively

higher fluorescence intensity. Another group of *SYN*-PE whose flow cytometric signatures were similar to *SYN*-PEI but which was found only in freshwater was designated as *SYN*-PEIII. (Fig. 2 – preferred position)

#### 2.4. Carbon biomass estimation

Phytoplankton carbon biomass was derived from chl *a* using a carbon to chlorophyll ratio of 40 (Gallegos, 2001). Choice of this value was based on the fact that the dominant species of diatoms in our study area (Patil and Anil, 2011) were similar to that found in Chesapeake Bay (Marshall et al., 2009).

For calculating the *SYN* carbon biomass, initially RALS data was converted to cell diameter as explained by Worden et al. (2004). Subsequently, the cell diameter was used to estimate the biovolume using the equation,  $V = \pi/6 (d^3)$ , assuming *SYN* cells to be spherical. Factors for different *SYN* groups were derived from the biovolume to carbon conversion factor of 254 fg C  $\mu\text{m}^{-3}$  (Baudoux et al., 2007). From these estimates, the percentage contribution of *SYN* to the total phytoplankton carbon biomass was calculated.

#### 2.5. Data analysis

Three-way analysis of variance (ANOVA) was used to assess the significant temporal and spatial variations in cell abundance ( $\log(x+1)$ ) of the *SYN* groups. Principal component analysis (PCA) was performed for the environmental data to identify the key factors which influence the *SYN* groups. Principal components (PC's) having eigenvalues greater than 1 were considered for further analysis. Stepwise multiple linear regression analyses were performed between factor scores of PC's and cell abundance ( $\log(x+1)$ ) of different *SYN* groups to evaluate the possible factors that affect the *SYN* abundance. Above statistical analysis was performed using SPSS statistics 16.0 with the significance level of 0.05. Statistica 8 software was used to find the significance among environmental factors, *SYN* carbon biomass (%), phytoplankton carbon biomass and *SYN*-PC: *SYN*-PE.

### 3. Results

#### 3.1. Environmental parameters

During PrM, surface water temperature was  $\sim 27^\circ\text{C}$  in February and increased gradually to  $33^\circ\text{C}$  in May. Correspondingly, seawater intrusion towards the upstream also increased during this period (Fig. 3g and h). After onset of MON (June), a drop in surface water temperature ( $< 28^\circ\text{C}$ ) and salinity ( $< 21$ ) was observed. During MON the estuary was stratified downstream, with strong stratification in August up to S5 (Fig. 3i and j). In July, when rainfall was low, rise in temperature ( $29^\circ\text{C}$ ) and

salinity (26) was detected at S1. During PoM, temperature was low ( $< 27^{\circ}\text{C}$ ) (Fig. 3e and f). In October the estuary was stratified as a result of continuing rainfall until November (Table 2). Subsequently (December and January) salinity started increasing as a consequence of cessation in rainfall. There was not much difference in temperature and salinity between surface and NBW during PrM and PoM (except in October). The seasonal trend of chl *a* concentration (surface and NBW) was PrM  $<$  PoM  $<$  MON. During PrM, chl *a* concentration increased along the transect with highest value upstream ( $3.03 \mu\text{g l}^{-1}$ ). During MON the trend was reversed with highest value downstream ( $< 12 \mu\text{g l}^{-1}$ ). Chl *a* concentration was higher in surface waters in August as compared to NBW, whereas the reverse was observed in September (Fig. 3o and p). Chl *a* concentration peaked in January and November with slightly lower concentration in NBW (Fig. 3q and r). During MON, nitrate (22 to  $37.71 \mu\text{M}$ ) and phosphate (3 to  $7.48 \mu\text{M}$ ) concentrations were high. Nitrate concentration showed a decreasing trend from mouth to head of the estuary with higher values at surface than that in the NBW whereas phosphate showed an opposite trend (Fig. 4c, d, i and j). Nitrate and phosphate peaked in May ( $13.32 \mu\text{M}$ ) and April ( $4.78 \mu\text{M}$ ). During rest of the months, concentrations in surface and NBW were low (Fig. 4a-i). Nitrite concentration was high in April and May at S6 (Fig. 4m and n) and lowest during MON (Fig. 4o and p). Silicate concentrations showed an increasing trend from downstream to upstream of the estuary except during MON season (Fig. 4s-x). Silicate concentrations were highest in September ( $44.54$  to  $155.29 \mu\text{M}$ ). (Fig. 3 and 4– preferred position)

### 3.2. Temporal and spatial variation of *Synechococcus*

Cell abundance of all *SYN* groups showed distinct temporal variations. Three-way ANOVA indicated significant monthly ( $p < 0.001$ ) and spatial ( $p < 0.01$ ) variations in all *SYN* groups whereas vertical variation was not significant within the stations. *SYN*-PEI abundance in the surface and NBW was highest in February at the mouth of the estuary ( $0.97 \times 10^5 \text{ cells ml}^{-1}$ ) and reduced further upstream. Cell abundance reduced from March to May with a peak at S8 (Fig. 5a and b). It declined further by an order of magnitude after onset of MON (June) in surface and NBW and increased in July up to S5 (Fig. 5c and d). Subsequently, abundance decreased in August and increased in September (Fig. 5c and d). At the beginning of the PoM (October), abundance was high up to S5 with higher abundance in the NBW (Fig. 5e and f). From November to January, cell abundance was low ( $< 0.25 \times 10^5 \text{ cells ml}^{-1}$ ). *SYN*-PEII showed a decreasing trend from mouth to middle of the estuary where salinity was  $> 20$ . Monthly variation was very similar to *SYN*-PEI with lower abundance except during PoM (Fig. 5g-l). During PoM, cell abundance at the mouth of the estuary was  $\sim 0.5 \times 10^5 \text{ cells ml}^{-1}$  (surface and NBW), which was higher than *SYN*-PEI abundance. During Mon, NBW cell abundance was higher than that at the surface. *SYN*-PEIII was observed at the

upstream end (S9 and S10) during PrM (Fig. 5m and n). After the onset of MON, abundance declined in surface and NBW and it was observed from middle of the estuary where salinity was < 0.5. Low (surface and NBW; <  $0.07 \times 10^5$  cells  $\text{ml}^{-1}$ ) abundance during MON, continued in the PoM season (October) (Fig. 5o and p). From November, abundance started increasing (>  $0.13 \times 10^5$  cells  $\text{ml}^{-1}$ ). *SYN*-PC showed an increasing trend from mouth to head of the estuary in surface and NBW. Abundance was high during PrM with highest abundance recorded in the NBW of S10 ( $1 \times 10^5$  cells  $\text{ml}^{-1}$ ) in May. With the onset of MON, cell abundance declined (Fig. 5u and v) and later increased in July ( $\sim 0.34 \times 10^5$  cells  $\text{ml}^{-1}$ ) in the middle of the estuary in surface and NBW. In August, it increased ( $0.39 \times 10^5$  cells  $\text{ml}^{-1}$ ) at the estuary mouth (surface) with a decreasing trend upstream whereas in the NBW abundance was low. During PoM, distribution trend was similar to PrM with comparatively lower abundance in surface and NBW (5w and x). (Fig. 5 – preferred position)

### 3.3. Influence of environmental factors on *Synechococcus*

The total *SYN* abundance showed a significant positive correlation with temperature. (Fig. 6a). When plotted against salinity, *SYN*-PE and *SYN*-PC distribution showed a clear spatial pattern (Fig. 6b) wherein PC cells were abundant in the low saline waters whereas the PE cells were abundant in the high saline waters. The seasonal cycle of salinity also influenced *SYN* abundance in the Zuari estuary. During PrM, total *SYN* abundance showed a significant negative correlation ( $r = -0.301$ ,  $p < 0.001$ ) with salinity which was reversed during MON ( $r = 0.568$ ,  $p < 0.001$ ) and PoM ( $r = 0.256$ ,  $p < 0.05$ ). PCA displayed three factors in PrM and MON and four in PoM, which explained 68%, 64% and 87% of the variations of the environmental factors, respectively. During PrM, PC1 was highly loaded with phosphate, silicate and nitrite (Table 3). A strong load of salinity was detected in PC3. Stepwise multiple regression analysis showed that all *SYN* groups and chl *a* were significantly correlated with PC1 ( $p < 0.05$ ) and PC3 (except chl *a*;  $p < 0.01$ ), which also included chl *a* whereas only *SYN*-PC correlated with PC2 (Table 4). During MON, *SYN* groups and chl *a* were strongly associated with PC1 where phosphate and salinity were highly loaded. *SYN*-PEIII and chl *a* were negatively related to PC2. PC3 was loaded with light (0.63) and nitrite (-0.74) where *SYN*-PC was positively correlated. Like PrM, all *SYN* groups were significantly correlated to salinity which was associated with PC2 along with nitrite during PoM (Table 3 and 4). *SYN*-PC alone showed positive relation to PC1 where nitrate and phosphate were strongly loaded. None of the *SYN* groups showed correlation to PC3 which included temperature (0.75) and light (-0.90). Chl *a* indicated positive correlation to PC4 (Table 4). (Table 3 and 4; Fig. 6 – preferred position)

### 3.4. Contribution of *Synechococcus* to total phytoplankton carbon biomass

Phytoplankton carbon biomass along the estuary varied from 50 to 127  $\mu\text{g C l}^{-1}$  with higher carbon biomass (127  $\mu\text{g C l}^{-1}$ ) up to S5 at the surface (Fig. 7a and b). In the NBW, it was lower compared to that at the surface at the mouth of the estuary. *SYN* carbon contribution (%) to the total phytoplankton carbon biomass varied from 9 to 29% (surface and NBW; Fig. 7c and d). *SYN* contributed more to the total carbon at S8 and downstream end of the estuary. Compared to surface, NBW contribution was higher downstream. *SYN*-PEI (~ 13 %) and *SYN*-PEII (~ 16 %) groups contributed higher downstream. Their contribution was slightly higher in the NBW as compared to that in the surface. *SYN*-PC contributed higher (~ 17 %) upstream (surface and NBW). *SYN*-PEIII contributed (~ 4 %) only at the upstream (surface and NBW) end of the estuary. Significant negative correlation was observed between phytoplankton carbon biomass and total *SYN* carbon contribution in the estuary (Fig. 8a). Total *SYN* carbon contribution showed a significant positive correlation with salinity and phosphate (Fig. 8b and c). (Fig. 7 and 8 – preferred position)

## 4. Discussion

In the Zuari estuary, the annual variations in hydrodynamics were mainly controlled by the river runoff and tides during MON and tidal activity during non-MON periods (Qasim and Sen Gupta, 1981; Shetye and Murty, 1987). As a result, during dry season the estuary is vertically homogenous whereas during wet season it is stratified (Qasim and Sen Gupta, 1981; Shetye and Murty, 1987). Monsoonal influence can be such that the entire estuary is dominated by low saline (salinity 11) waters as was observed in August. Similar observations have been reported from the Mandovi estuary (salinity 0), which is adjacent to the Zuari estuary (Vijith et al., 2009). However, active and break phases in rainfall also brings weekly variations in water chemistry (Qasim and Sen Gupta, 1981).

### 4.1. Spatial variations of *SYN* distribution.

Variations in salinity throughout the estuary were reflected in the distribution of *SYN* groups. Transition in dominance from PC-rich to PE-rich *SYN* at salinities of ~ 20-25 found in this study is consistent with the studies carried out in subtropical estuaries such as Pensacola Bay (Murrell and Lores, 2004), lower Chesapeake Bay (Ray et al., 1989) and Pearl river estuary (Zhang et al., 2013). This illustrates that salinity plays a key role in the spatial distribution of *SYN* along this monsoon influenced tropical estuary. To support this, in the present study PC's which strongly associated with salinity showed a significant correlation to all the *SYN* groups.



Vertical variation of *SYN* abundance was not significant in the present study as was observed for the Pearl River (Lin et al., 2010) where depth was comparatively higher (< 60 m) than the Zuari estuary (Table 1). However, during MON at the estuary mouth high abundance of *SYN*-PEII in the NBW and *SYN*-PC in surface compared to that at surface and NBW respectively indicates that increased river runoff resulting in stratification of the water column influenced the distribution of these groups. Similarly, the higher abundance of *SYN*-PEI in NBW and *SYN*-PC in surface waters during early PoM (October) also shows the influence of rainfall and the resultant freshwater run off. Presence of *SYN*-PC throughout the estuary even during dry season when there is no possibility of its influx from fresh water suggests that these groups are halotolerant and they can survive at high salinities. However, their comparatively lower abundance downstream implies that their growth rates are affected at higher salinities. Waterbury et al. (1986) reported that *SYN*-PE cells have an obligate requirement for elevated concentrations of ions while marine *SYN* isolates that lack PE are halotolerant and grows equally well in the seawater or freshwater. The presence of *SYN*-PE up to the upstream end could be facilitated by the tidal entry of seawater. *SYN*-PEI and *SYN*-PEII observed at higher salinities were also previously reported from North western Arabian coast, Mississippi river plume and the Pearl River estuary (Lin et al., 2010; Liu et al., 1998; Liu et al., 2004). The high PE intensity *SYN* strain is considered as characteristic of oceanic waters (*SYN*-PEII) and other strain (*SYN*-PEI) represents the coastal waters (Campbell et al., 1998). This suggests a possibility of the *SYN*-PEII influx from offshore waters into the estuary. Increased *SYN* PEIII abundance in fresh water suggests that this could be a freshwater adapted strain. Different *SYN* strains have been reported in a variety of freshwater systems based on their pigment characteristics (Callieri, 1996).

#### 4.2. Temporal variation of *SYN* groups

Total *SYN* abundance range in the present study was comparable to previous report for the same estuary (Mitbavkar et al., 2012) and higher than that reported for the other tropical coastal waters (Agawin et al., 2003). However, it was 1 to 2 magnitudes lower than that reported for subtropical estuaries like Florida Bay (Phlips et al., 1999), Chesapeake Bay (Wang et al., 2011) and Pensacola Bay (Marshall and Nesius, 1996; Murrell and Lores, 2004). While in Chesapeake Bay *SYN* cell abundance often exceeds  $10^6$  cells ml<sup>-1</sup>, in a temperate estuary it ranged from  $10^2$  to  $10^5$  cells ml<sup>-1</sup> (Agawin et al., 1998; Ning et al., 2000). The seasonal cycle of the *SYN* population differed from that in subtropical and temperate coastal and estuarine regions which can be attributed to the influence of South West monsoon that creates a seasonal cycle of hydrodynamics that varies from other latitudinal regions.

Several studies have established temperature as the key factor influencing the seasonal dynamics of *SYN* in tropics to temperate estuaries such as Chesapeake Bay (Ray et al., 1989; Wang et al., 2011), Pearl River (Qiu et al., 2010), Pensacola Bay (Murrell and Lores, 2004), Francisco Bay (Ning et al., 2000), Blanes Bay (Agawin et al., 1998), Western Pacific coast (Tsai et al., 2008) and Sagami Bay (Mitbavkar et al., 2009). These studies showed that warm period is favorable for *SYN* spp. Similarly, in the present study the total *SYN* abundance showed significant positive correlation with temperature (Fig. 6a), although a narrow temperature range exists (24 to 32°C) throughout the year as compared to that in the subtropical (10 - 30°C) and temperate estuaries (10 - 24°C). The optimum temperature range of 27 to 30°C was found to be favorable for *SYN* in the present study. Variations in the spatial distribution of salinity during the three seasons were reflected in the temporal distribution of *SYN*-PC which showed a negative relation with salinity as expected during the PrM and PoM but during MON it showed a positive relation because of the comparatively lower salinity downstream resulting in its higher abundance. *SYN*-PEI and *SYN*-PEII showed a positive and *SYN*-PEIII negative relation with salinity during the three seasons indicating that *SYN*-PEI and *SYN*-PEII preferred higher salinity and *SYN*-PEIII lower salinity.

Recent studies elucidate negative correlation between *SYN* abundance and nutrients (Qiu et al., 2010; Zhang et al., 2013). These studies suggest that *SYN* prefers reduced form of nitrate (ammonia) which was not measured in the present study. In this study, the positive correlation of *SYN*-PC to PC's which strongly associated with nitrate and phosphate during the non-MON periods and with phosphate during the MON season suggests that both these nutrients could be important for this group. A laboratory experimental study proved that PC rich *SYN* grows well in high nitrate and phosphate concentration whereas PE rich *SYN* cannot tolerate high nitrate concentration (Ernst et al., 2005). Wyman et al. (1985) reported that *SYN* can use nitrate with lower concentration efficiently and dominates the conditions. These findings corroborate the non-significant relation of *SYN*-PE subgroups with nitrate and the positive relation with phosphate of *SYN*-PEI and *SYN*-PEII during the PrM and MON season in this study (Table 4). These observations indicate that nutrients could play a vital role in the temporal variation of *SYN* groups. Since silicate is not a requirement for this PP, the positive relationship in some cases may not be a causal one.

The low cell abundance observed during active MON could be a consequence of the prevailing environmental conditions such as increased turbidity due to influx of huge quantities of fresh water and restricted light availability due to increased cloud cover (Devassy and Goes, 1988). Biological processes like grazing (Wetz et al., 2011) and viral lysis (Pan et al., 2007) are also known to play a role in controlling *SYN* abundance, which we did not account for in the present study. During MON

break increased salinity, temperature, nutrients (nitrate and phosphate), solar radiation and lower rainfall could have facilitated an increase in *SYN* abundance along with high phytoplankton biomass. Similar variations in phytoplankton biomass have been observed in this estuary with high and low biomass during MON break and during peak of MON, respectively (Patil and Anil, 2011; Pednekar et al., 2011). The increased freshwater runoff during this period was reflected in the dominance of *SYN-PC* from the mouth to head of the estuary and absence of *SYN-PEII* as well as lower cell abundance of *SYN-PEIII*. Towards the end of MON season, with the subsiding rainfall intensity and the corresponding lowered freshwater influx downstream along with the high nutrient concentrations and solar radiation, *SYN-PEI*, *SYN-PEII* and *SYN-PEIII* abundance increased in the estuary. Lowering of *SYN-PC* abundance could be a result of the gradual increase in salinity and low temperature. This period is considered to be conducive for the proliferation of phytoplankton (Patil and Anil, 2011; Pednekar et al., 2011) wherein phytoplankton biomass showed significant positive correlation with silicate. During PoM, all the *SYN* groups showed similar distribution as observed in PrM indicating return of favorable environmental conditions for their growth.

#### 4.3. Contribution of *Synechococcus* to total phytoplankton carbon biomass

The contribution of *SYN* to the total phytoplankton carbon biomass in the Zuari estuary (9 to 29 %) was lower than that reported for the Chesapeake Bay sub-estuary (34 to 52 %; salinity 19.5 to 23.7) dominated by cyanobacteria (Ray et al., 1989) and higher than that reported in a tropical coastal region (< 16 %) at the river mouth (Agawin et al., 2003). However, contribution of picoplankton biomass to total phytoplankton biomass in the Cochin estuary, along the west coast of India, influenced by the SW monsoon ranged between 6.5 % (salinity 3 to 9) and 11.2 % (salinity > 30; Madhu et al., 2009). In the present study *SYN* carbon contribution was inversely related to total phytoplankton biomass, which suggests that dominance of larger phytoplankton. Similarly a study conducted in North Carolina estuary showed inverse relation between picoplankton contribution (~ 40 %; salinity 0 to 26) and phytoplankton biomass (Gaulke et al., 2010) and concluded that variation in PP carbon contributions depends on various factors such as nutrients, light and stability of water column. These observations suggest that *SYN* contribution to total phytoplankton carbon biomass was within the range of other tropical estuaries and although lower than that reported for sub-tropical estuaries, could play a vital role in the microbial food web dynamics of estuarine regions.

In monsoonal estuaries where the fresh water runoff and tides regulate the hydrodynamics during the MON and non-MON periods respectively, the distribution pattern of these organisms both spatially (horizontal and vertical) and temporally, can serve as indicators of the source of water as

well as the water stability (mixed or stratified) at a particular location in the estuary thereby providing information about the physical factors regulating the estuarine dynamics.

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**Figure legends**

Fig. 1. Location of the study area.

Fig. 2. Flow cytometric analysis of (a-c) seawater, (d-f) brackish water, and (g-i) freshwater samples from the Zuari estuary.

Fig. 3. Temporal variations in (a-f) temperature, (g-l) salinity, (m-r) chlorophyll *a* concentrations and (s-u) secchi disk depth in the Zuari estuary.

Fig. 4. Temporal variations in nutrient concentrations. (a-f) Nitrate, (g-l) phosphate, (m-r) nitrite and (s-x) silicate concentrations.

Fig. 5. Temporal variations in distribution of *Synechococcus* groups. (a-f) *SYN*-PEI, (g-l) *SYN*-PEII, (m-r) *SYN*-PEIII and (s-x) *SYN*-PC.

Fig. 6. Relationship between (a) total *SYN* abundance with temperature and (b) ratio of PC and PE rich *SYN* with salinity in the Zuari estuary. The curve was fitted under the logarithmic equation model.

Fig. 7. (a, b) Total phytoplankton carbon biomass and (c, d) percentage contribution of *Synechococcus* to the total phytoplankton carbon biomass in the Zuari estuary.

Fig. 8. Correlation analysis of total *SYN* carbon contribution (%) with (a) total phytoplankton carbon biomass, (b) salinity, (c) phosphate. The curve was fitted under the logarithmic equation model.



Table 1. Details of sampling stations in the Zuari estuary.

Station No.	Station name	Latitude	Longitude	Distance from mouth (km)	Approximate depth (m)
1	Marmugao	15° 25' 16.9"	73° 47' 36.9"	0	16
2	Chicalim	15° 25' 8.5"	73° 47' 22.4"	5.8	5
3	Island	15° 25' 57.4"	73° 47' 57.0"	8.6	5
4	Sancoale	15° 25' 45.1"	73° 47' 30.6"	11	7.1
5	Cortalim	15° 25' 32.0"	73° 47' 50.2"	13	9.6
6	Loutulim	15° 25' 54.0"	73° 47' 24.4"	19.7	10.5
7	Borim	15° 25' 03.6"	73° 47' 58.0"	23.9	12.9
8	Shiroda	15° 25' 12.3"	73° 47' 55.5"	31.4	9.1
9	Kushavati	15° 25' 31.7"	73° 47' 28.3"	38.4	9.9
10	Sanvordem	15° 25' 01.1"	73° 47' 36.0"	42.2	4.9

Table 2. Rainfall data for the sampling days.

Sr No.	Sampling dates	Rainfall (mm)
1	29-Jan-10	0
2	03-Mar-10	0
3	01-Apr-10	0
4	29-Apr-10	0
5	27-Jun-10	57.8
6	13-Jul-10	0.2
7	11-Aug-10	51.7
8	17-Sep-10	69.8
9	23-Oct-10	55.4
10	06-Nov-10	3
11	06-Dec-10	0
12	10-Jan-11	0

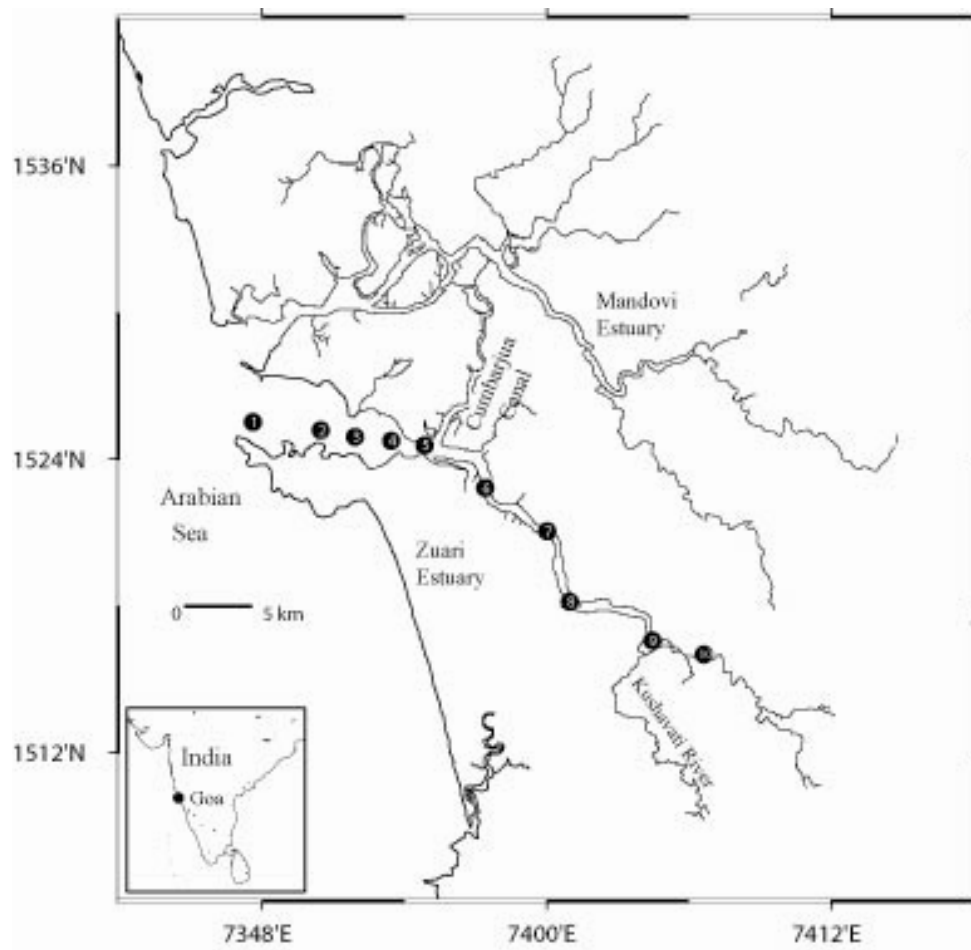
Table 3. Rotated component matrix (RCM) with varifactors (principal components, PCs) extracted in different seasons.

Parameter	Pre-monsoon (PrM)			Monsoon (MON)			Post-monsoon (PoM)			
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3	PC4
Salinity	-0.02	-0.08	<b>0.94</b>	<b>0.70</b>	0.02	-0.10	-0.24	<b>0.76</b>	0.04	-0.49
Temperature	-0.06	<b>0.72</b>	-0.05	0.33	<b>0.47</b>	0.36	0.33	0.24	<b>0.75</b>	-0.05
Secchi disk depth	-0.13	<b>-0.51</b>	0.15	-0.45	0.13	<b>0.63</b>	-0.02	0.15	<b>-0.90</b>	-0.02
Nitrate	-0.34	<b>0.63</b>	0.12	-0.47	<b>0.69</b>	-0.21	<b>0.90</b>	-0.30	0.08	-0.14
Phosphate	<b>0.92</b>	-0.16	0.11	<b>0.87</b>	-0.07	0.24	<b>0.89</b>	0.24	0.24	0.14
Nitrite	<b>0.60</b>	0.56	0.32	-0.20	0.09	<b>-0.74</b>	0.08	<b>0.89</b>	0.00	0.14
Silicate	<b>0.82</b>	0.01	-0.45	-0.02	<b>-0.87</b>	-0.02	-0.04	-0.01	-0.01	<b>0.98</b>
Eigenvalues	2.00	1.52	1.24	1.82	1.49	1.19	1.77	1.60	1.44	1.25
% of Variance	28.61	21.76	17.68	26.05	21.27	16.96	25.21	22.91	20.56	17.89
Cumulative %	28.61	50.38	68.06	26.05	47.32	64.28	25.21	48.12	68.67	86.57

Table 4. Regression analysis of the factor scores (as independent factors) for the abundance of *SYN* groups in different seasons.

Dependent factor	$R^2$	$F$	$\beta$			
			PC1	PC2	PC3	PC4
Pre-monsoon						
<i>SYN</i> -PC	0.40	51.21**	0.184*	0.250**	-0.630**	-
<i>SYN</i> -PEI	0.55	94.27**	0.200*	0.004	0.740**	-
<i>SYN</i> -PEII	0.67	163.55**	-0.197*	-0.148	0.823**	-
<i>SYN</i> -PEIII	0.39	50.11**	-0.192*	-0.560	-0.625**	-
Chlorophyll <i>a</i>	0.16	14.56**	0.060	0.139	-0.397**	-
Monsoon						
<i>SYN</i> -PC	0.41	13.81**	0.555**	-0.740	0.327**	-
<i>SYN</i> -PEI	0.29	30.69**	0.534**	0.038	0.058	-
<i>SYN</i> -PEII	0.10	8.95**	0.323**	0.206*	0.055	-
<i>SYN</i> -PEIII	0.16	15.47**	-0.410**	-0.226*	0.032	-
Chlorophyll <i>a</i>	0.05	4.21*	0.213*	-0.228*	0.054	-
Post-monsoon						
<i>SYN</i> -PC	0.43	51.12**	0.257*	-0.655**	-0.004	0.516**
<i>SYN</i> -PEI	0.51	70.58**	-0.011	0.714**	0.000	-0.162*
<i>SYN</i> -PEII	0.84	84.22**	-0.079	0.736**	0.001	-0.538**
<i>SYN</i> -PEIII	0.50	16.01**	0.060	-0.691**	0.004	0.125
Chlorophyll <i>a</i>	0.30	4.16*	-0.508	0.030	-1.980	0.208*

$R^2$ - regression coefficient;  $F$ - value of the full model;  $\beta$ - standardized coefficient. \*  $p < 0.05$ , \*\*  $p < 0.01$

**Fig. 1**

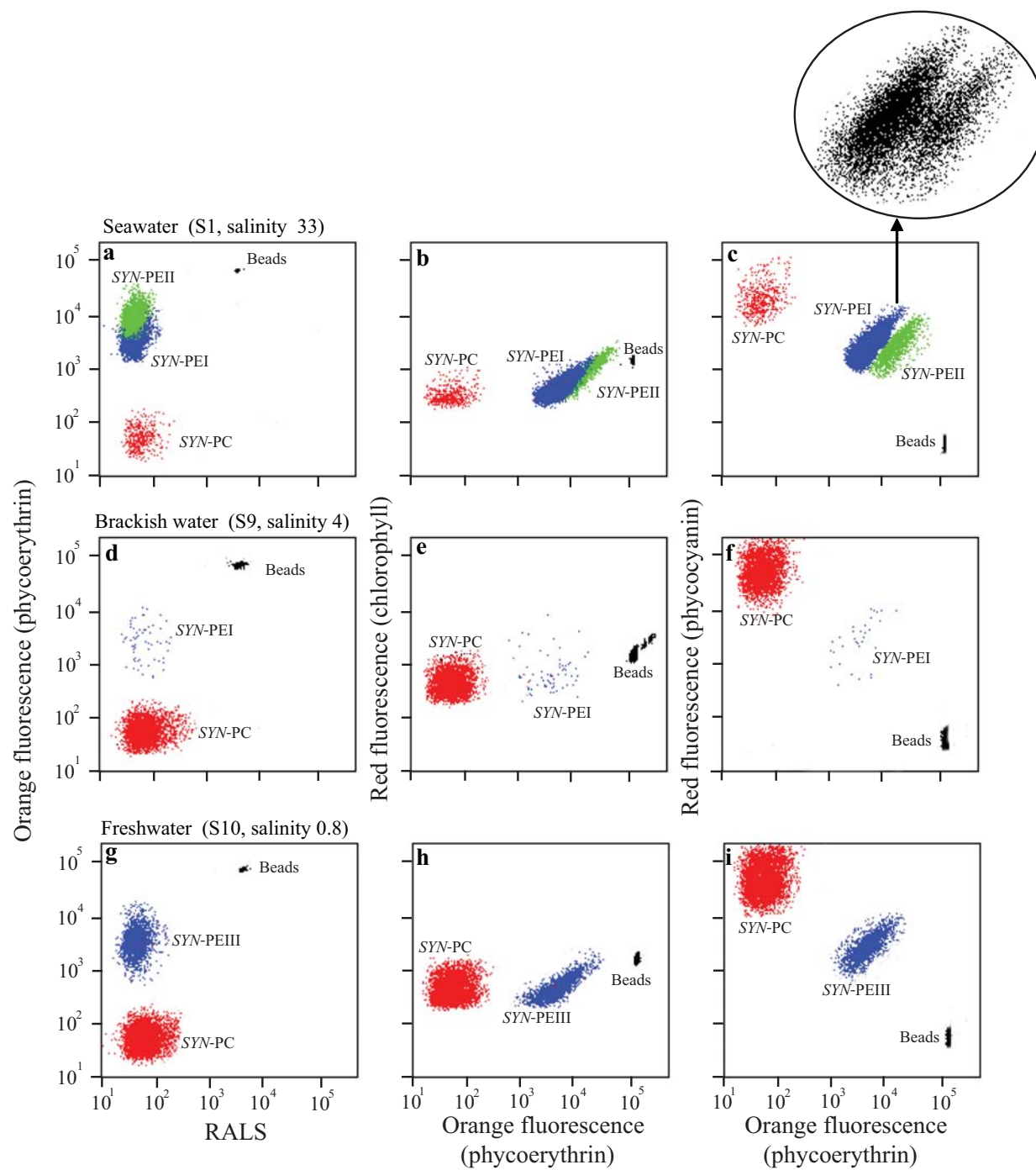


Fig. 2

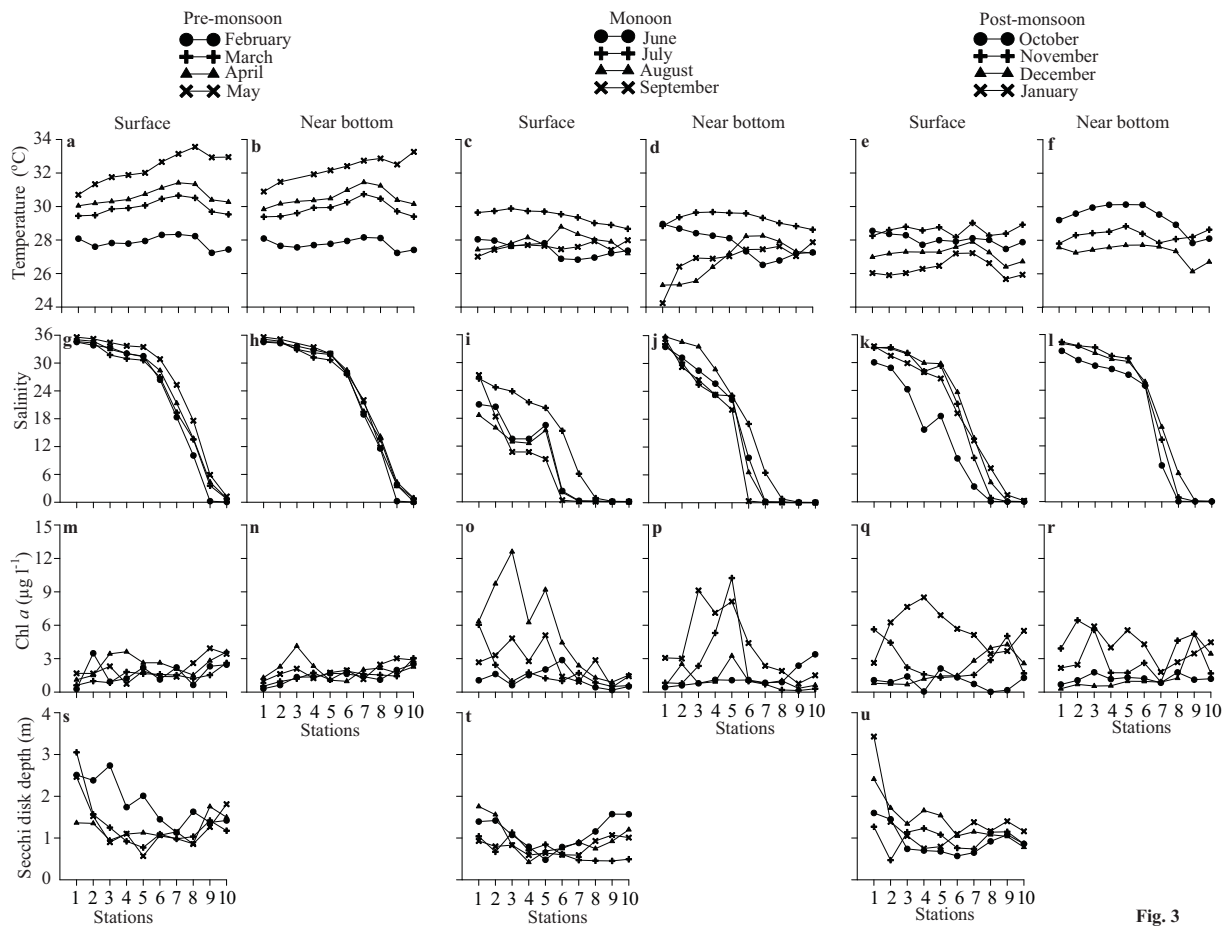


Fig. 3

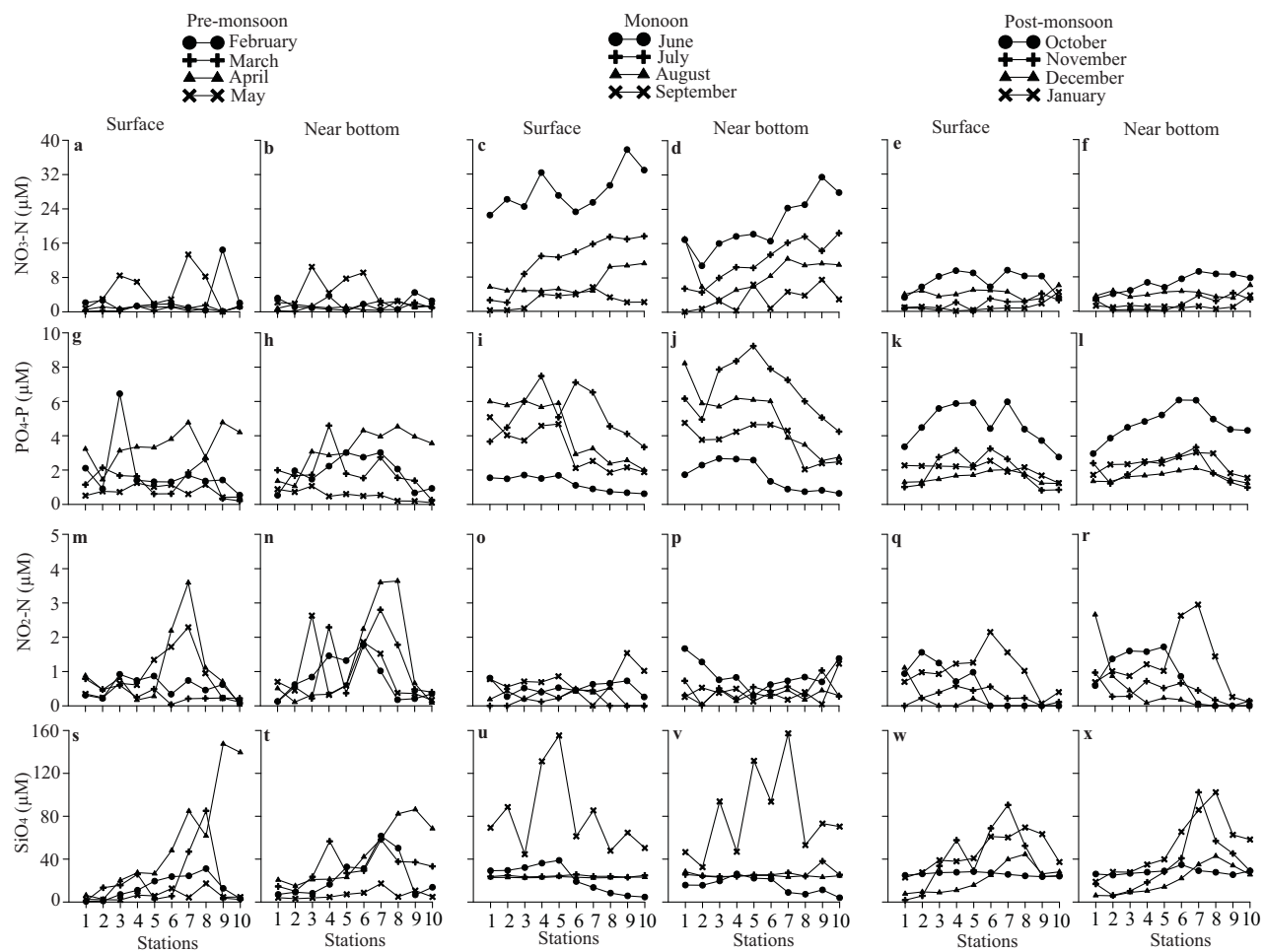
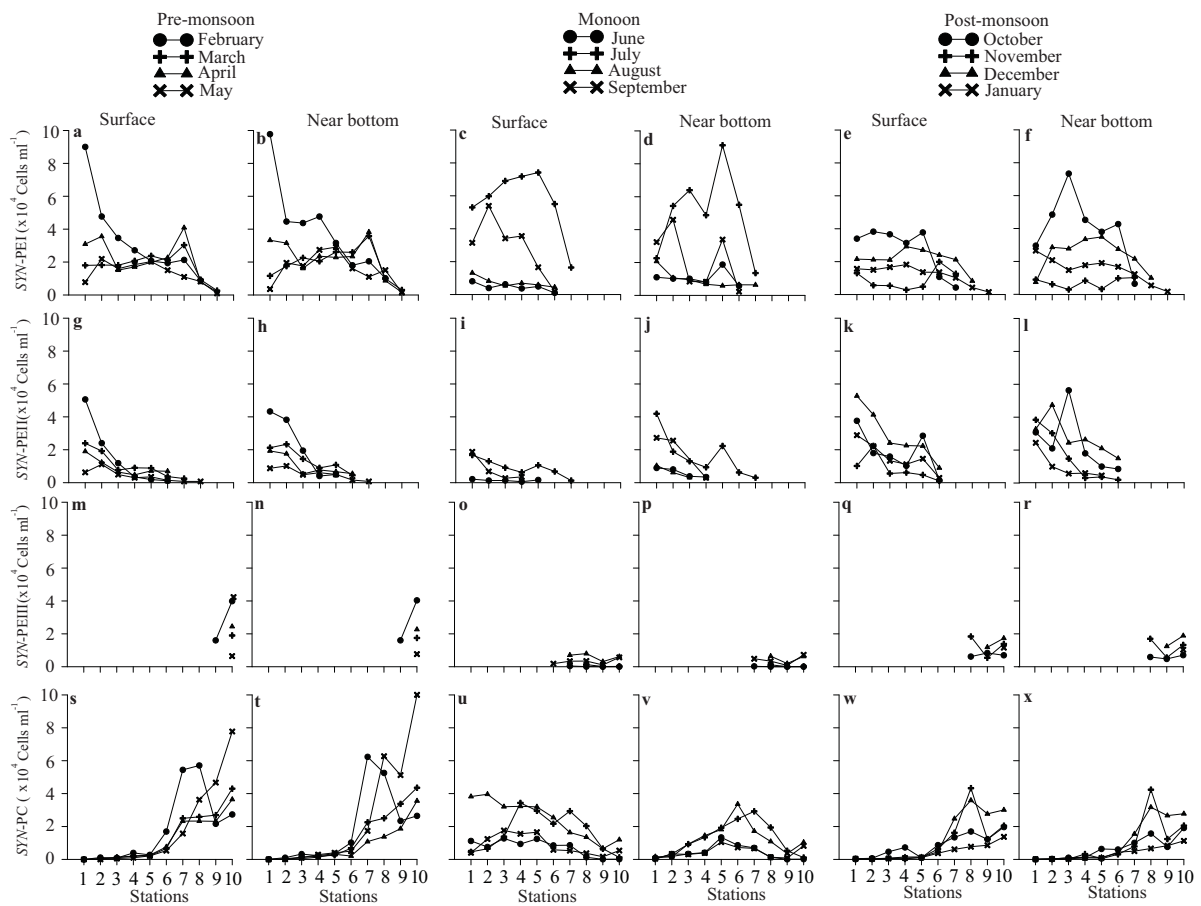


Fig. 4





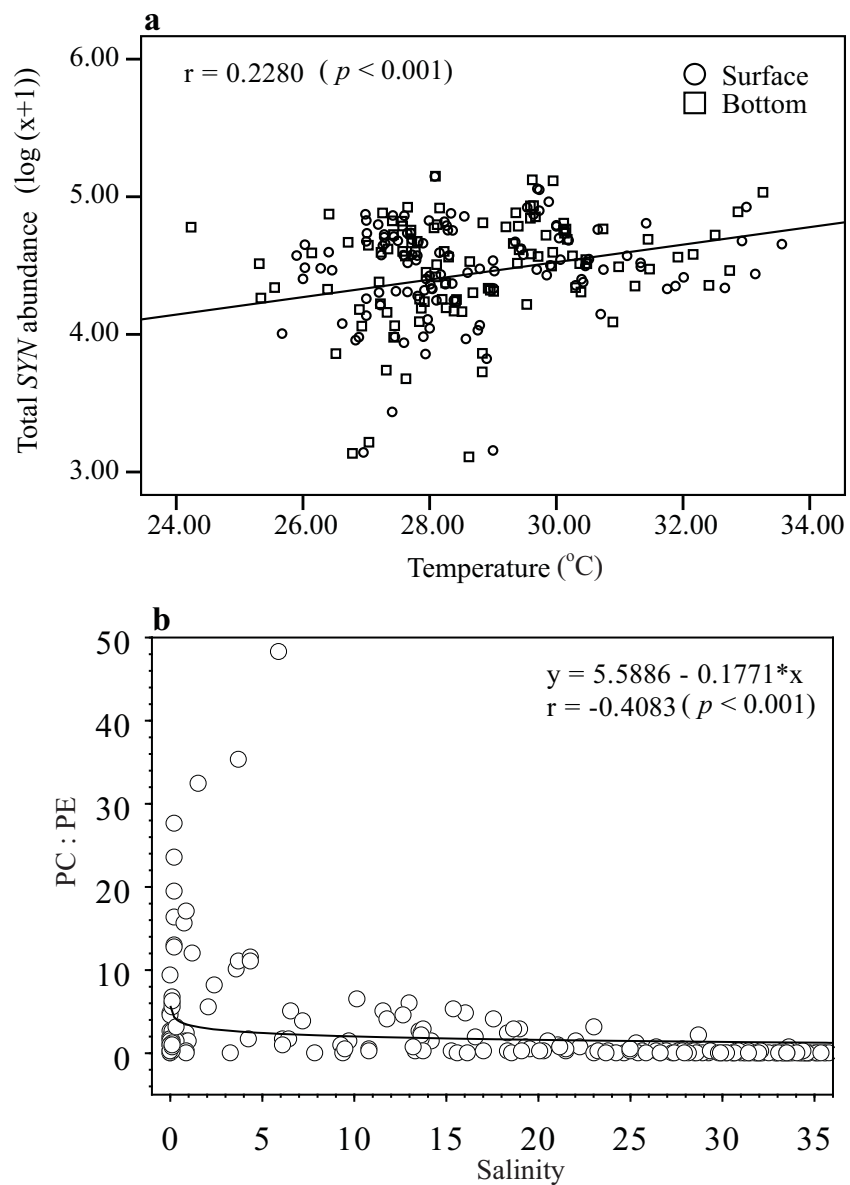


Fig. 6

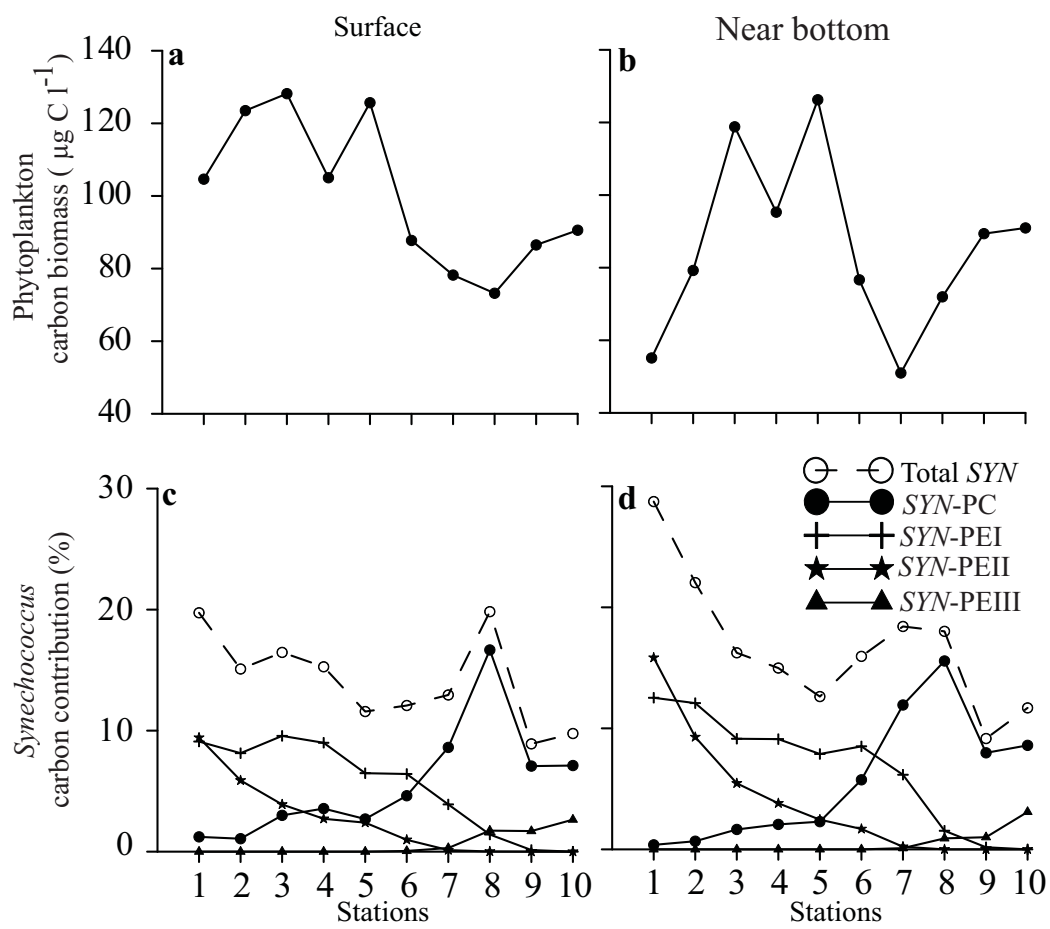


Fig. 7

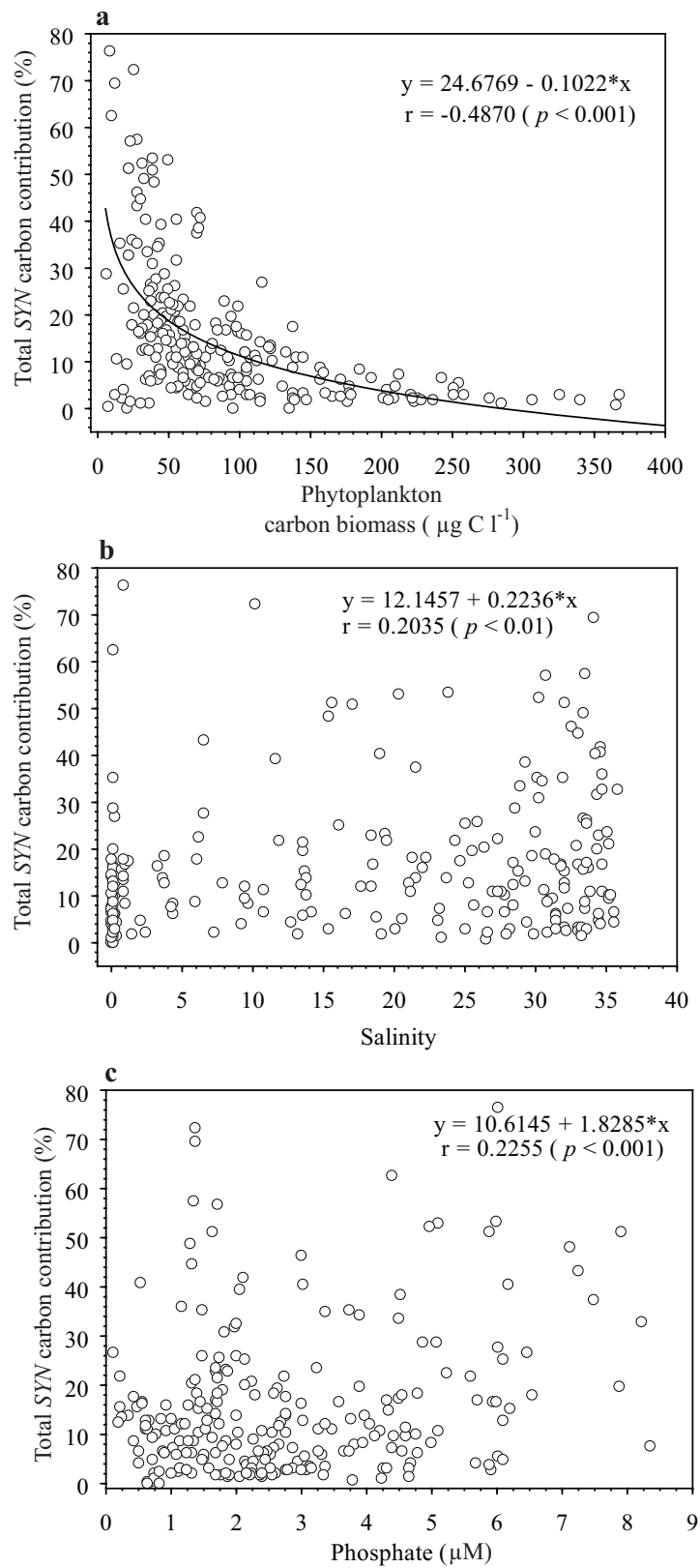


Fig. 8