

## **Characterization of phytoplankton pigments and functional community structure in the Gulf of Mannar and the Palk Bay using HPLC-CHEMTAX analysis**

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### **Abstract**

Phytoplankton marker pigments and their functional groups were identified for the first time in the Gulf of Mannar (GoM) and the Palk Bay (PB), located in the southeast coast of India using HPLC-CHEMTAX analytical techniques. The GoM generally remained more saline, productive (in terms of chlorophyll *a*) and less turbid than the PB during southwest and northeast monsoon periods. The diversity and concentration of marker pigments were high in the GoM, whereas the PB was characterized by high concentration of zeaxanthin, indicating the dominance of photosynthetic prokaryotes (cyanobacteria). The CHEMTAX analysis revealed that the phytoplankton biomass (chlorophyll *a*) in the PB was mainly derived from cyanobacterial community. However, abundance of fucoxanthin and peridinin in the GoM indicated microphytoplankton (20-200µm) as the dominant group. The CHEMTAX results showed that more than 50% of chlorophyll *a* in the GoM was contributed by microphytoplankton, in particular diatoms and dinoflagellates. The substantial increase in the photoprotective carotenoids (PPCs) and photoprotection index (PI) in the PB was indicative of its low productivity, probably caused by the warm and turbid waters.

**Key words:** Phytoplankton; HPLC; Biomarkers; Cyanobacteria; Palk Bay; Gulf of Mannar

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## 1. Introduction

Studies on phytoplankton are very useful for understanding the bio-geochemical processes and efficiency of the marine food web (Chisholm, 1992; Legendre and Rassoulzadegan, 1996). Since phytoplankton is a main source of organic production, information on their biomass, composition and community structure are so decisive for monitoring of an aquatic environment (Paerl et al., 2003). The growth and distribution of these microscopic communities are mainly controlled by a variety of abiotic and biotic factors (Chisholm, 1992). Besides chlorophyll *a*, (proxy for phytoplankton biomass), phytoplankton have evolved taxon-specific suites of various pigments (biomarkers) for performing photosynthetic activities (Wright and Jeffrey, 2006). These photopigments (e.g., fucoxanthin, peridinin, chlorophyll *b*, zeaxanthin, and alloxanthin) are diagnostic of specific phytoplankton functional groups (diatoms, dinoflagellates, chlorophytes, cyanobacteria and cryptophytes, respectively) and in a few cases can determine phytoplankton composition at the species level (Paerl et al., 2003; Barlow et al., 2008). Each of these phytoplankton functional groups (PFGs) are indicators of primary production that may change with varying environmental conditions (Barlow et al., 2007). Analysis of phytoplankton pigments using HPLC technique does not afford anything on species identification rather than the class level information (Suzuki et al., 1997; Barlow et al., 2008); however, this measurement is faster and reproducible for analysing a large set of samples compared to the time consuming and tedious microscopic identification (Jeffrey et al., 1997).

Since marker pigments can reveal a variety of taxonomic composition (Gibb et al., 2000; Barlow et al., 2007), physiological state and grazing effect (Trees et al., 2000; Veldhuis and Kraay, 2004), the pigment characterisation by HPLC is widely used to study the properties of phytoplankton. CHEMTAX is a statistical software used to estimate biomass of various PFGs by calculating the ratio of marker pigments to chlorophyll *a* (Mackey et al., 1996; Riegman and Kraay 2001). Photosynthetic pigments and their ratios (indices) are normally used for determining the phytoplankton groups (Aiken et al., 2009) under varying environmental conditions (Vidussi et al., 2001; Griffith et al., 2010). For example, the carotenoid pigment ratio is used for understanding the productivity patterns of various water bodies (Kirk, 1994; Barlow et al., 2002; Moreno et al., 2012). High concentration of photosynthetic carotenoids (PSCs) normally indicates high productivity, whereas high photoprotective carotenoids (PPCs) signify low productivity (Gibb et al., 2000; Barlow et al., 2002). These variations are generally associated with the changes in the phytoplankton community of a system as well as their cellular responses (Barlow et al., 2008; Moreno et al., 2012).

A large number of studies have been carried out in the Indian waters on phytoplankton growth and distribution (Bhattathiri et al., 1996; Madhupratap et al., 1996; Madhu et al., 2006;

Habeebrehman et al., 2008), and community composition (Sawant and Madhupratap 1996; Madhu et al., 2006; Paul et al., 2007). However, information on photosynthetic pigments from Indian waters are scanty along the west (Parab et al., 2006 & 2013; Roy et al., 2006 & 2011) and east (Naik et al., 2011) coasts of India. The GoM and the PB, located in the southeast coast of India, are poorly understood for their phytoplankton characteristics (Yapa, 2000; Rao et al., 2008; Sridhar et al., 2010). The PB and the GoM are generally less productive during the northeast monsoon due to increased turbidity and cloud cover (Bindu and Muniyandi, 2005). There are major differences in hydrological properties of these bays caused by the coastal circulation (Sridhar et al., 2008; Rao et al., 2008). Recent studies on phytoplankton using fluorescence microscopy and flow-cytometry apparently revealed that the GoM is supporting autotrophic pico-eukaryotes and nanoplankton communities, whereas the PB waters are dominated by cyanobacteria (*Synechococcus*) and heterotrophic nanoplankton during northeast monsoon (Jyothibabu et al., 2013). In this study, an attempt has been made to characterize the phytoplankton community and its seasonality in the GoM and the PB using marker pigments by HPLC technique. CHEMTAX was used to estimate the biomass of various PFGs in terms of chlorophyll *a* and explain their diversity in distribution in these two adjacent water bodies.

## **2. Materials and Methods**

### **2.1. Study area**

The Gulf of Mannar and the Palk Bay, located between Sri Lanka and south-eastern margin of Indian subcontinent, are two adjacent bays with a diverse flora and fauna (Rao et al., 2008; Kannan et al., 1998). The GoM is generally deeper and wider compared to the PB. Because of the rich flora and fauna (coral reefs, seaweeds and sea grass beds), the GoM is considered as one of the marine bioresources of India (Rao et al., 2008). Compared to the GoM, the PB is an enclosed bay that remains turbid due to shallowness and suspended sediments brought from the Bay of Bengal (Chandramohan et al., 2001; Sanilkumar et al., 2002; Sridhar et al., 2008). These basins are separated by a narrow strip of land mass (Rameswaram island and the submerged Adam's bridge) and show significant differences in their hydrographical (Murty and Varma, 1964; Bindu and Muniyandi, 2005) and biological properties (Rao et al., 2008; Jyothibabu et al., 2013; Anjusha et al., 2013). The seasonal reversal of surface currents along the Indian coast enable the entry of Arabian Sea (AS) waters into the GoM and Bay of Bengal (BoB) waters into the PB (Jagadeesan et al., 2013). Thus, during southwest monsoon (SWM, June-September), the West India Coastal Current (WICC) brings AS (high saline) waters into the GoM, whereas during the northeast monsoon (NEM, December-February), the East India Coastal Current (EICC) brings low saline BoB waters into the

PB (Rao et al., 2011). It has been shown that despite being separated, there is a water exchange between the PB and the GoM (Jagadeesan et al., 2013; Anjusha et al., 2013).

## 2.2. Station locations and water sampling

The study area encompasses the western margin of the GoM and the PB between depths 5 and 30m (Fig. 1). Two hydrographic surveys were carried out during the southwest monsoon (15<sup>th</sup> September - 5<sup>th</sup> October 2009) and northeast monsoon (2<sup>nd</sup>-25<sup>th</sup> January 2010) periods. Sampling locations were selected in such a manner that there are 11 transects with 3 stations in each transect, at depths of 5, 10 and >20m. Thus, 15 stations were fixed in the GoM and 18 stations in the PB (Fig. 1). Water samples were collected from the near surface (0.5m) using 5L Niskin samplers for estimating the water quality parameters such as suspended particulate matter (SPM), dissolved inorganic nutrients and phytoplankton pigments. Sea surface temperature (SST) and sea surface salinity (SSS) were recorded using a portable Seabird conductivity-temperature-depth profiler (SBE, Seabird Electronics, USA with temperature accuracy  $\pm 0.01^\circ\text{C}$  and salinity accuracy  $\pm 0.001$ ). SPM was estimated by filtering the seawater (2 L) gravimetrically through Millipore membrane filters (0.45  $\mu\text{m}$ ) and weighing the filters after drying at  $70^\circ\text{C}$  for 6 to 8 hours. Major inorganic nutrients such as nitrate ( $\text{NO}_3^-$ ) and phosphate ( $\text{PO}_4^{3-}$ ) were determined following Grasshoff et al (1983). Phytoplankton (>5 $\mu\text{m}$ ) identification and counting were carried out using a binocular stereoscope inverted microscope (Olympus CK 30) following the standard protocols (Utermohl 1958; Tomas 1997).

## 2.3. Phytoplankton photosynthetic pigments

Two to four litres of near surface water was filtered onto Whatman GF/F filters (nominal pore size 0.7 $\mu\text{m}$ ; 25mm diameter) under a vacuum of <500mb and the filters were kept in deep freezer ( $-20^\circ\text{C}$ ). The frozen filters were disrupted for 30 seconds in 4ml of 90% acetone using a sonicator (Model 100, Fischer Scientific) at  $4^\circ\text{C}$  and extracted by passing through 0.2 $\mu\text{m}$  membrane filters (25mm diameter). The pigment extracts (400 $\mu\text{L}$ ) were injected into the HPLC (Agilent 1100) using a reverse phase C8 column (150 x 4.6mm, 3.5 $\mu\text{m}$  particle size) with three phase solvent gradient at a flow rate 1.1 $\mu\text{L}/\text{minutes}$  (van Heukelem, 2002). The absorption spectra of the pigments were measured at two wave lengths of 450 and 665 nm using a Diode Array Detector (DAD). From the absorption spectra, these pigments were identified and quantified based on their retention time against authentic standards (Table 1). The HPLC system was calibrated with standard pigments obtained commercially (DHI - Institute for Water and Environment, Denmark).

## 2.4. CHEMTAX analysis and pigment indices

The CHEMTAX is a mathematical programme used to calculate the ratio of marker pigments to chlorophyll *a*, and an estimate of individual phytoplankton class biomass in terms of chlorophyll *a*. It is based on matrix factorization explained by Mackey et al (1996). The initial pigment ratios of major phytoplankton classes (diatoms, dinoflagellates, cyanobacteria and cryptophytes) were obtained from literature (Schluter et al., 2011) and the major pigments detected by the HPLC were also loaded to CHEMTAX (see Table 2). The optimized pigment ratio of the matrix derived by CHEMTAX is presented in Table 3. This programme is currently used worldwide to classify the phytoplankton community with respect to their chlorophyll *a* content (Llewellyn et al., 2005; Eker-Develi et al., 2012).

The diagnostic pigment (DP) criteria derived from different combinations of marker pigments (Table 1), roughly corresponds to the biomass proportions of various size groups (pico-, nano- and micro-) of phytoplankton (Vidussi et al., 2001; Barlow et al., 2007). The sum of PPCs, PSCs, total chlorophyll *b* and total chlorophyll *c* is taken as total accessory pigments (TAcc), which are useful in remote sensing studies (Trees et al., 2000). The sum of TAcc and total chlorophyll *a* provides the index of total pigment (Tpig), whereas the total photosynthetic pigment (PSP) is the sum of PSCs, total chlorophyll *a*, total chlorophyll *b* and total chlorophyll *c* (Table 1). The biomass proportion of various phytoplankton size groups are derived from different combinations of diagnostic pigments (Vidussi et al., 2001; Uitz et al., 2006), where the pigment indices roughly correspond to the biomass proportion factors of pico- (pPF), nano- (nPF) and micro-(mPF) phytoplankton (see Table 1), respectively (Roy et al., 2011).

The photopigment indices generally reflect the fractions of chlorophylls and carotenoids in the total pigment (Barlow et al., 2007). Both groups of carotenoid pigments, ie., PPCs and PSCs are useful in the photo-physiological studies (Bidigare et al., 1996). The photoprotection index (PI) is the ratio between the PPCs and total chlorophyll *a* (Griffith et al., 2010; Moreno et al., 2012). A high PI indicates an oligotrophic condition (open ocean, stratified waters, etc), whereas low PI values represent productive (e.g, well mixed coastal waters, fronts etc) waters (Moreno et al., 2012). The major photopigment ratios are symbolised as  $TChl\ a_{TP}$ ,  $PSC_{TP}$ ,  $PPC_{TP}$ , and  $TAcc_{TP}$  (Table 1).

## 2.5. Statistical analysis

Student's t-test (two tailed) was performed to find out the statistical difference in the means of major environmental variables between the GoM and the PB. The parametric paired t-test was performed to compare the seasonal data of both the GoM and the PB. Pearson correlation was carried out between the DP and major physico-chemical and biological parameters. Canonical

Correspondence Analysis (CCA) was performed using CANOCO 4.5 (Ter Braak and Smilauer, 2002) to find out the relationship between biomass of various PFGs and environmental variables. The CCA is an ordination method to reveal correlations between communities and environmental factors.

### 3. Results

#### 3.1. Hydrography

Although the atmospheric conditions over the GoM and the PB remained similar, the PB was relatively warmer (av.  $30.4 \pm 0.7^\circ\text{C}$ ) and low saline (av.  $34.9 \pm 0.6$ ) than the GoM (av.  $29.7 \pm 1.1^\circ\text{C}$  & av.  $35.4 \pm 0.4$ ) during SWM (Figs.2a & c). The SST and SSS showed significant spatial variation ( $P < 0.05$ ) in these two areas during SWM (Tables 4 & 5). Likewise, significant differences in SST ( $P < 0.01$ ) and SSS ( $P < 0.05$ ) were also apparent in the GoM (av.  $26.6 \pm 0.49^\circ\text{C}$  & av.  $28.5 \pm 4.7$ ) and the PB (av.  $25.5 \pm 0.6^\circ\text{C}$  & av.  $26.3 \pm 4$ ) during the NEM (Fig. 2b & d). During SWM, the SPM did not show much variation in the two basins (Tables 4 & 5), but there was a significant variation ( $P < 0.01$ ) during NEM as evident from the SPM values in the PB (av.  $19.23 \pm 3 \text{ mg L}^{-1}$ ) and the GoM (av.  $1.47 \pm 0.74 \text{ mg L}^{-1}$ ; Fig. 2e & f). Among inorganic nutrients (Figs. 3a-d), the concentration of nitrate ( $\text{NO}_3^-$ ) did not show significant variation ( $P > 0.05$ ) between the PB (av.  $0.84 \pm 0.55 \mu\text{M}$ ) and the GoM (av.  $0.74 \pm 0.47 \mu\text{M}$ ) during SWM (Table 5). However, phosphate ( $\text{PO}_4^{3-}$ ) showed significant variation ( $P < 0.05$ ) between the GoM (av.  $0.46 \pm 0.24 \mu\text{M}$ ) and the PB (av.  $0.28 \pm 0.18 \mu\text{M}$ ; Table 5). During NEM the concentration of  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  did not show significant differences between the GoM and the PB (Table 5).

#### 3.2. Phytoplankton pigment concentrations

A total of nine marker pigments were identified in the GoM during SWM (Table 4). The major pigments were zeaxanthin, fucoxanthin, peridinin, chlorophyll c3, chlorophyll b, chlorophyll c2, diadinoxanthin,  $\beta$ -carotene and alloxanthin. There was a marked spatial variation in chlorophyll a ( $0.25 - 1.45 \text{ mg m}^{-3}$ ) in the GoM (Fig. 4a) with an exceptionally high chlorophyll a ( $30.3 \text{ mg m}^{-3}$ ; not included in the calculation of seasonal mean, pigment ratios and indices) at station 11, which also contained high zeaxanthin ( $10.7 \text{ mg m}^{-3}$ ), chlorophyll b ( $1.8 \text{ mg m}^{-3}$ ), and  $\beta$ -Carotene ( $0.54 \text{ mg m}^{-3}$ ). Among other marker pigments, fucoxanthin (av.  $0.23 \pm 0.24 \text{ mg m}^{-3}$ ) and peridinin (av.  $0.2 \pm 0.16 \text{ mg m}^{-3}$ ) were the major components in the southern GoM (Fig. 4b & c), whereas zeaxanthin was dominant towards the northern region (Fig.4d). However, there was so much distinct variations in chlorophyll a (av.  $0.65 \pm 0.25 \text{ mg m}^{-3}$ ) in the PB during the SWM (Fig. 4a), where a total of eight pigments were identified, including zeaxanthin (av.  $0.41 \pm 0.22 \text{ mg m}^{-3}$ ), peridinin (av.  $0.16 \pm 0.12 \text{ mg m}^{-3}$ ), fucoxanthin (av.  $0.08 \pm 0.02 \text{ mg m}^{-3}$ ), chlorophyll c2 (av.  $0.07 \pm 0.03 \text{ mg m}^{-3}$ ), chlorophyll c3 (av.

$0.03 \pm 0.04 \text{ mg m}^{-3}$ ) and diadinoxanthin (av.  $0.03 \pm 0.01 \text{ mg m}^{-3}$ ). Concentration of zeaxanthin was relatively high ( $1.1 \text{ mg m}^{-3}$ ) in the coastal waters (station 20) of the southern PB (Fig. 4d).

During NEM also, the GoM exhibited a more or less similar pattern in the distribution of chlorophyll *a* ( $0.17\text{-}2.8 \text{ mg m}^{-3}$ ) with higher concentrations ( $>2 \text{ mg m}^{-3}$ ) towards the northern bay, near Mandapam (Fig.5a). Out of the 10 marker pigments identified in the GoM during this period, fucoxanthin was the dominant pigment (av.  $0.9 \pm 1.1 \text{ mg m}^{-3}$ ) which showed wide variation ( $0.02$  to  $3.9 \text{ mg m}^{-3}$ ). An exceptionally high value of fucoxanthin ( $>3 \text{ mg m}^{-3}$ ) was observed in the northern GoM (Fig. 5b) near Mandapam (stations 11 and 13), whereas zeaxanthin, chlorophyll *c2*, diadinoxanthin, 19'hexanoyloxyfucoxanthin, chlorophyll *c3*, violaxanthin, alloxanthin and divinyl chlorophyll *a* were also present in very low levels (Table 4). The peridinin ( $>1 \text{ mg m}^{-3}$ ) was high at a few locations (stations 4, 11 & 14) in the GoM (Fig. 5c). Similarly, an unusual rise in divinyl chlorophyll *a* ( $3.1 \text{ mg m}^{-3}$ ) was also detected at location 13, where fucoxanthin ( $3.15 \text{ mg m}^{-3}$ ) and chlorophyll *c2* ( $1.43 \text{ mg m}^{-3}$ ) were also high. The PB waters did not show such an increase in chlorophyll *a* (av.  $0.81 \pm 0.40 \text{ mg m}^{-3}$ ) during NEM (Fig. 5a). Eight marker pigments were detected from the area with zeaxanthin (av.  $0.26 \pm 0.09 \text{ mg m}^{-3}$ ) as predominant (Fig. 5d) followed by fucoxanthin (av.  $0.22 \pm 0.12 \text{ mg m}^{-3}$ ; Fig. 5b) and peridinin (av.  $0.12 \pm 0.29 \text{ mg m}^{-3}$ ; Figs.5c) and traces of diadinoxanthin, 19-hexanoyloxyfucoxanthin, chlorophyll *c3*, alloxanthin and chlorophyll *c2* (Table 4).

### 3.3. Phytoplankton pigments and size structure

The distribution of diagnostic pigment (DP) was almost uniform in the PB during SWM (av.  $0.65 \pm 0.21 \text{ mg m}^{-3}$ ) and NEM (av.  $0.64 \pm 0.27 \text{ mg m}^{-3}$ ) monsoon periods, but showed significant seasonal variation in the GoM (Tables 4). The PPCs and PSCs showed seasonality only in the GoM (Table 4). The mean Tpig was high in the GoM during NEM (av.  $3.27 \pm 2.65 \text{ mg m}^{-3}$ ) than SWM (av.  $1.62 \pm 0.81 \text{ mg m}^{-3}$ ), whereas it was  $<2 \text{ mg m}^{-3}$  in the PB (Table 4). The mean TAcc and PSP were also high in the GoM during the NEM than during the SWM (Table 4). The phytoplankton size structure estimated from these pigments showed that mPF was high in the GoM both during SWM (av.  $60.52 \pm 24.11\%$ ) and NEM (av.  $58.47 \pm 28.13\%$ ). However, there was a distinct increase in the pPF (av.  $63.1 \pm 16.6\%$ ) over mPF (av.  $36.9 \pm 16.6\%$ ) in the PB during SWM, while both mPF (av.  $49.9 \pm 31.1\%$ ) and pPF (av.  $45.03 \pm 17.3\%$ ) were more or less similar during NEM.

### 3.4. Phytoplankton functional groups (PFGs)

The CHEMTAX analysis identified four major PFGs (diatoms, dinoflagellates, cyanobacteria and cryptophytes) contributing to the chlorophyll *a* biomass in the GoM and the PB (Fig. 6). During SWM, diatoms were the major contributor (av.  $43.5 \pm 26.1\%$ ) of total chlorophyll *a* (av.  $0.4 \pm 0.36 \text{ mg}$

$\text{m}^{-3}$ ) in the GoM (Fig. 6a), followed by cyanobacteria (av.  $0.24 \pm 0.31 \text{ mg m}^{-3}$ ), dinoflagellates (av.  $0.14 \pm 0.11 \text{ mg m}^{-3}$ ) and cryptophytes (av.  $0.03 \pm 0.09 \text{ mg m}^{-3}$ ). In contrast, chlorophyll *a* in the PB was mainly contributed by cyanobacteria (av.  $0.31 \pm 0.14 \text{ mg m}^{-3}$ ; av. 51.2%), followed by dinoflagellates (av.  $0.18 \pm 0.13 \text{ mg m}^{-3}$ ; av. 27.7%), diatoms (av.  $0.09 \pm 0.05 \text{ mg m}^{-3}$ ; av. 15.8%) and cryptophytes (av.  $0.04 \pm 0.06 \text{ mg m}^{-3}$ ; av. 5.3%). During NEM also, chlorophyll *a* in the GoM was mainly contributed by diatoms (av.  $0.51 \pm 0.28 \text{ mg m}^{-3}$ ; av. 53.7%), whereas it was cyanobacteria (av.  $0.63 \pm 0.36 \text{ mg m}^{-3}$ ; av. 76.9%) in the PB (Fig. 6d).

### 3.5. Pigment indices and ratios

The PPC:PSC ratio was high in the PB during both SWM (av.  $2.48 \pm 1.82$ ) and NEM (av.  $1.33 \pm 0.83$ ), while it was low in the GoM during SWM (av.  $1.13 \pm 0.88$ ) and NEM (av.  $0.70 \pm 0.62$ ). The PI values were also high in the PB (Table 6) both during SWM (av.  $0.71 \pm 0.41$ ) and NEM (av.  $0.37 \pm 0.16$ ). The TChl  $a_{TP}$  showed relatively high seasonality in the GoM than in the PB (Table 6). Unlike TChl  $a_{TP}$ , the TAcc $_{TP}$ , was greater (av.  $0.58 \pm 0.08$ ) in the PB during NEM (Table 6).

### 3.6. Influence of environmental parameters on PFGs

Linear regression analysis adopted for examining the influence of environmental variables (temperature, salinity, SPM, nitrate and phosphate) on DP did not reveal any significant relationship, except for a significant positive correlation ( $P < 0.01$ ) with chlorophyll *a* in the GoM (Table 7). Canonical Correspondence Analysis (CCA) to investigate the environmental (temperature, salinity, SPM, nitrate and phosphate) influence on PFGs in the GoM indicated that diatoms were influenced by phosphate, salinity, SST and SPM during SWM (Fig. 7a). The high cyanobacterial biomass in the PB coincided with an increase in SST and low phosphate levels (Fig. 7a). During NEM, diatoms and dinoflagellates in the GoM exhibited positive correlation with salinity and SST (Fig. 7b), whereas in PB, cyanobacterial biomass increased with turbidity (Fig. 7a).

## Discussion

Even though the GoM and the PB are adjacent bays along the southeast coast of India, both exhibited different hydrographical (Rao et al., 2008; Anjusha et al., 2013) as well as biological (Roa et al., 2008; Sridhar et al., 2010; Jyothibabu et al., 2013; Jagadeesan et al., 2013) characteristics. The submerged island chain, the 'Adam's Bridge' located between the two water bodies form a major barrier preventing the inter-basin exchange of water mass (Murty and Verma, 1964; Rao et al., 2011). This region, the confluence zone of the AS and the BoB between India and Sri Lanka in the midst of Adams Bridge is known as the Indo-Sri Lanka Channel (ISLC), has a shallow canal permitting a restricted exchange of waters (Rao et al., 2011). Since the southern GoM is opening to



the southeastern AS, the hydrographic property of the GoM has a more resemblance to the eastern Arabian Sea (Rao et al., 2008; Anjusha et al., 2013). Likewise, an opening of northern PB to the BoB through the Palk Strait enables the entry of low saline waters, especially during NEM (Shankar & Shetye, 1997; Rao et al., 2011). Thus, the seasonal reversal in the circulation (Shankar et al., 2002) seems to have a great influence on the hydrographical (Rao et al., 2008) as well as biological (Sridhar et al., 2008; Anjusha et al., 2013; Jagadeesan et al., 2013) properties of both the GoM and the PB.

Earlier studies have revealed that the BoB is a prominent region for the formation of warm pools (Sengupta and Ravichandran, 2001; Rao et al., 2002). The SST over the BoB is always  $>28^{\circ}\text{C}$  during summer, which triggers convection in the near surface waters (Gadgil et al., 1984). The SST recorded over the GoM (av.  $29.6 \pm 1.1^{\circ}\text{C}$ ) and the PB (av.  $30.4 \pm 0.7^{\circ}\text{C}$ ) are also comparable, indicating a possible occurrence of convection in this region as well. Since the PB is shallow and semi-enclosed, the surface waters are much warmer than the GoM (Anjusha et al., 2013). However, a restricted flow through the Pamban Pass or the ISLC carries low saline waters to the nearshore regions in the GoM as well as entry of high saline waters to the southern parts of the PB (Fig. 2c). The cool and low saline waters in the PB was probably brought by the BoB through the Palk Strait (Shetye et al., 1996), while the minor fresh water plumes along the western margin of the PB could be due to fresh water inputs from rivers such as Vaigai, Vaishali and Valryar (Chandramohan et al., 2001).

The phytoplankton in the GoM was found to sustain diverse marker pigments than in the PB (Table 4). The predominance of zeaxanthin in the PB signifies the abundance of cyanobacteria, an autotrophic picoplanktonic community proliferating in oligotrophic waters (Chisholm, 1992; Veldhuis et al., 2005). Normally, zeaxanthin is an indicator of cyanobacterium, *Synechococcus* or *Prochlorococcus*, (Gibbs et al., 2000; Chisholm, 1992). The non-detectability of divinyl chlorophyll *a*, an exclusive biomarker of prochlorophytes, suggests the absence of *Prochlorococcus* in the PB. The *synechococcus* are normally found to grow in the surface waters of the tropical and subtropical seas enriched in nitrate ( $>0.5\mu\text{M}$ ; Chisholm,1992). Nitrate can suppress the growth of *Prochlorococcus* (Moore et al., 1995), as it essentially prefers ammonia or urea (Chisholm,1992). Since nitrate concentrations in the PB and GoM were  $>0.5\mu\text{M}$ , it is probable that the growth of *Prochlorococcus* was suppressed. Therefore, the abundance of zeaxanthin in the PB could be due to the dominance of *Synechococcus*. The high fucoxanthin (av.  $0.88 \pm 1.13\text{ mg m}^{-3}$ ) and peridinin (av.  $0.44 \pm 0.66\text{ mg m}^{-3}$ ) in the southern GoM suggest that diatoms and dinoflagellates are the dominant microplankton community during SWM (Figs. 4b&c) as observed in the nutrient rich eastern Arabian Sea (Parab et al., 2006; Roy et al., 2006), whereas the presence of zeaxanthin in the northern GoM indicates the blooming of cyanobacteria. The presence of cool, high saline and

turbid waters (Fig. 2), rich in inorganic  $\text{PO}_4^{3-}$  and  $\text{NO}_3^-$  (Fig. 3a & c) in the southern GoM could be due to the intrusion of AS upwelled waters (Anjusha et al., 2013). The abundance of diatoms and dinoflagellates in the southern GoM are further evidences to this observation (Sawant and Madhupratap 1996; Habeebrehman et al., 2008). The upwelled waters are normally rich in diatoms due to the nutrient enrichment (Tont, 1976; Malone, 1980). Since  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  were present in moderate levels (Table 4), this would have enhanced the growth of diatoms in the GoM.

The distribution of chlorophyll *a* reveals that the GoM is more productive than the PB (Fig. 4a & 5a). A very high value of chlorophyll *a* ( $30.3 \text{ mg m}^{-3}$ ) at station 11 in the northern GoM was associated with very high zeaxanthin ( $10.7 \text{ mg m}^{-3}$ ), chlorophyll *b* ( $1.8 \text{ mg m}^{-3}$ ) and  $\beta$ -carotene ( $0.54 \text{ mg m}^{-3}$ ). The high zeaxanthin in this bloom (Fig. 3b) is apparently due to the abundance of cyanobacterial population (Wright and Jeffrey, 2006; Roy et al., 2011). The bloom was identified to be *Trichodesmium erythraeum* Ehrenberg, a filamentous non-heterocystus N-fixing cyanobacteria, common in the oligotrophic tropical waters (Carpenter, 1983). An increase in zeaxanthin and chlorophyll *a* due to *Trichodesmium erythraeum* Ehrenberg bloom has been reported previously in the AS (Parab et al., 2006; Roy et al., 2011; Naik et al., 2011). The CHEMTAX results also confirms that ~90% of chlorophyll *a* in the bloom was derived from the cyanobacteria (*Trichodesmium erythraeum* Ehrenberg).

It has been shown that in tropical waters micro-algal communities accumulate more PPCs to mitigate the photo-oxidative damage of cells caused by ultraviolet radiation (Barlow et al., 2007; Moreno et al., 2012). During SWM, the PPC:PSC ratio was much higher in the PB (av.  $2.48 \pm 1.82$ ) than in the GoM (av.  $1.13 \pm 0.88$ ), mostly facilitated by zeaxanthin. The high zeaxanthin and increased photoprotection index (PI) in the PB indicates its oligotrophic condition as compared to the GoM. The substantial increase in SPM levels in the PB during the NEM suggests a turbid condition (Fig. 2f). It is established that the PB is a trap of suspended sediments ( $\sim 0.448 \times 10^6 \text{ m}^{-3}$ ) entraining from the BoB (Sridhar et al., 2008), which even lead to the formation of sand banks (Chandramohan et al., 2001; Jyothibabu et al., 2013). This turbidity and cloud cover (Yapa, 2000; Bindu and Muniyandi, 2006) during NEM can reduce the light penetration and hence, the growth of large phytoplankton (diatoms and dinoflagellates). The recent flow cytometric measurements (Jyothibabu et al., 2013) have revealed the predomination of photosynthetic cyanobacterial community in the PB, comprised of *Synechococcus*. Therefore, increase in zeaxanthin and chlorophyll *a* in the PB are probably caused by the proliferation of *Synechococcus* community, as they have a tendency to proliferate in tropical waters containing optimum nitrate (Osion et al., 1990; Landry et al., 1996).

Alternately, a drop in the PPC:PSC ratio in the GoM (especially during NEM), could be due to the increase in fucoxanthin and peridinin. PSCs are generally high in productive waters that support the proliferation of larger phytoplankton (Malone 1980; Barlow et al., 2002). The significant relationship ( $P < 0.01$ ) with chlorophyll *a* reveals that DP can be an indicator of phytoplankton biomass in the GoM. However, it was not valid for the PB as the relationship between DP and chlorophyll *a* did not show much significance ( $P > 0.05$ ).

## **Conclusion**

The present study on the distribution of phytoplankton marker pigments forms the first report on the phytoplankton ecology of the two adjacent marine ecosystems (GoM and PB) along the southeast coast of India. The hydrographic properties are found to be responsible for the growth of different phytoplankton communities in these two regions. The study could identify a dominant autotrophic prokaryotes in the PB and a microplankton (diatoms and dinoflagellates) community in the GoM. The high photopigment indices and ratios in the PB compared to that in the GoM are indicative of an environmental stress in the PB. The warm surface waters (during SWM) and increased water column turbidity (during NEM), seem to limit the growth of large phytoplankton in the PB. On the contrary, dynamic conditions could have favoured a dominant microphytoplankton community in the GoM.

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#### Table 1

Abbreviations, names and selected taxonomic designations (Jeffrey et al, 2005) for chlorophylls, carotenoids, pigment sums and indices.

#### Table 2

Input pigment:chlorophyll *a* ratios for the GoM and the PB for southwest monsoon and northeast monsoon

#### Table 3

Output pigment:chlorophyll *a* ratios for the GoM and the PB for southwest monsoon and northeast monsoon

#### Table 4

The mean ( $\pm$ SD) values for major environmental parameters, chlorophyll *a*, major marker pigments and pigment sum in the GoM and the PB during southwest monsoon and northeast monsoon ('-' not detected).

#### Table 5

P-value of t-test results of the mean of major environmental parameters in the GoM and the PB between seasons (southwest monsoon and northeast monsoon) and area (GoM and PB). '\*\*' significant at 0.05 level; '\*\*\*' significant at 0.01 level.

#### Table 6

The mean (plus the standard deviation) values for phytoplankton pigment indices and ratios in the GoM and the PB during southwest monsoon and northeast monsoon

#### Table 7

Regression coefficient of diagnostic pigments (DP) with major environmental parameters in the GoM and the PB during southwest monsoon and northeast monsoon ('\*' significant at 0.05 level; '\*\*\*' significant at 0.01 level).

#### Figure 1

Study area and sampling locations.

#### Figure 2

Distribution of SST ( $^{\circ}$ C), SSS and SPM ( $\text{mg L}^{-1}$ ) in the GoM and the PB during southwest monsoon and northeast monsoon.

Figure 3

Distribution of Nitrate ( $\text{NO}_3\text{-}\mu\text{M}$ ) and phosphate ( $\text{PO}_4^{3-}\ \mu\text{M}$ ) in the surface waters of the GoM and the PB during southwest monsoon and northeast monsoon.

Figure 4

Distribution of chlorophyll *a* ( $\text{mg m}^{-3}$ ) and major marker pigments in the surface waters of the GoM and the PB during southwest monsoon.

Figure 5

Distribution of chlorophyll *a* ( $\text{mg m}^{-3}$ ) and major marker pigments in the surface waters of the GoM and the PB during northeast monsoon.

Figure 6

Relative contribution of chlorophyll *a* of various phytoplankton functional groups (diatoms, dinoflagellates, cyanobacteria and cryptophytes) in the GoM and the PB during southwest monsoon and northeast monsoon.

Figure 7

Canonical correspondence analysis (CCA) biplots of phytoplankton functional groups (PFGs) and major environmental variables (Temperature, Salinity, SPM, Nitrate -  $\text{NO}_3$ , Phosphate -  $\text{PO}_4^{3-}$ ); in the GoM and the PB during (A) southwest monsoon and (B) northeast monsoon. The following abbreviations were used for different PFGs: *Diat*, *Diatoms*; *Dino*, *Dinoflagellates*; *Cyano*, *Cyanobacteria*; *Crypto*, *Cryptophytes*.

Table 1.

Abbreviations	Pigment	Designation
Chl <i>a</i>	Chlorophyll <i>a</i>	
Chl <i>c</i> 2	Chlorophyll <i>c</i> 2	
Chl <i>c</i> 3	Chlorophyll <i>c</i> 3	
Chl <i>b</i>	Chlorophyll <i>b</i>	Chlorophytes
DV chl <i>a</i>	Divinyl chlorophyll <i>a</i>	Prochlorophytes
Allo	Alloxanthin	Cryptophytes
Diad	Diadinoxanthin	
Fuco	Fucoxanthin	Diatoms (major)
Hex	19' Hexanoyloxy fucoxanthin	Prymnesiophytes
Per	Peridinine	Dinoflagellates
Zea	Zeaxanthin	Cyanobacteria
β -Car	B- Carotene	
But	19' Butanoyloxy fucoxanthin	Haptophytes (major)
Viol	Violaxanthin	
<i>Pigment sum</i>		<i>Formula</i>
TChl <i>a</i>	Total chlorophyll <i>a</i>	Chl <i>a</i> +DVChl <i>a</i> +Chlide <i>a</i>
PPCs	Photoprotective carotenoids	Allo + Diad + Vio + Zea + β-Car
PSCs	Photosynthetic carotenoids	But+Fuco + Hex + Per
DP	Total diagnostic pigments	PSC + Allo+ Zea + TChl <i>b</i>
TAcc	Total accessory pigments	PPC+PSC+TChl <i>b</i> +TChl <i>c</i>
PSP	Photosynthetic pigments	PSC+T Chl <i>a</i>
TP	Total pigments	TChl <i>a</i> +TAcc
<i>Pigment index</i>		<i>Formula</i>
PI	Photoprotection index	PPC/TChl <i>a</i>
TChl <i>a</i> <sub>TP</sub>	Total chlorophyll <i>a</i> to total pigments	TChl <i>a</i> /TP
PPC <sub>TP</sub>	Photoprotective carotenoids to total pigments	PPC/TP
PSC <sub>TP</sub>	Photosynthetic carotenoids to total pigments	PSC/TP
TAcc <sub>TP</sub>	Total accessory pigments to total pigments	TAcc/TP
pPF	Picophytoplankton proportion factor	(Zea+Chl <i>b</i> )/DP
nPF	Nanophytoplankton proportion factor	(Hex+But+Allo)/DP
mPF	Microphytoplankton proportion factor	(Fuco+Peri)/DP

Table 2.

Groups	Per	Fuco	Diad	$\beta$ Car	Allo	Zea	Chl <i>b</i>	Chl <i>c2</i>
<i>GoM - SWM</i>								
Dinoflagellates	1.063	0	0	0	0	0	0	0.037
Cyanobacteria	0	0	0	0	0	0.348	0	0
Diatoms	0	0.755	0.030	0	0	0	0	0
Cryptophytes	0	0	0	0	0.229	0	0	0
<i>GoM - NEM</i>								
Dinoflagellates	1.063	0	0	0	0	0	0	0.037
Cyanobacteria	0	0	0	0	0	0.048	0	0
Diatoms	0	0.159	0.030	0	0	0	0	0
Cryptophytes	0	0	0	0	0.042	0	0	0.077
<i>PB - SWM</i>								
Dinoflagellates	0.747	0	0.241	0	0	0	0	0.523
Chlorophytes	0	0	0	0.050	0	0.326	0.017	0
Cyanobacteria	0	0	0	0	0	0.680	0	0
Diatoms	0	1.020	0.448	0	0	0	0	0
Cryptophytes	0	0	0	0	0.042	0	0	0.077
<i>PB - NEM</i>								
Dinoflagellates	0.747	0	0.241	0	0	0	0	0.523
Cyanobacteria	0	0	0	0	0	0.175	0	0
Diatoms	0	0.755	0.448	0	0	0	0	0
Cryptophytes	0	0	0	0	0.229	0	0	0.174

Table 3.

Groups	Per	Fuco	Diad	B-Car	Allo	Zea	Chl <i>b</i>	Chl <i>c2</i>
<i>GoM - SWM</i>								
Dinoflagellates	0.506	0	0	0	0	0	0	0.018
Cyanobacteria	0	0	0	0	0	0.258	0	0
Diatoms	0	0.319	0.026	0	0	0	0	0
Cryptophytes	0	0	0	0	0.221	0	0	0
<i>GoM - NEM</i>								
Dinoflagellates	0.506	0	0	0	0	0		0.018
Cyanobacteria	0	0	0	0	0	0.046		0
Diatoms	0	0.306	0.058	0	0	0		0
Cryptophytes	0	0	0	0	0.038	0		0.069
<i>PB - SWM</i>								
Dinoflagellates	0.397	0	0.032	0	0	0	0	0.155
Chlorophytes	0	0	0	0.087	0	0.519	0.011	0
Cyanobacteria	0	0	0	0	0	0.542	0	0
Diatoms	0	0.474	0.104	0	0	0	0	0
Cryptophytes	0	0	0	0	0.038	0	0	0.069
<i>PB - NEM</i>								
Dinoflagellates	0.386	0	0.057	0	0	0	0	0.153
Cyanobacteria	0	0	0	0	0	0.161	0	0
Diatoms	0	0.343	0.203	0	0	0	0	0
Cryptophytes	0	0	0	0	0.163	0	0	0.124

Table 4.

Abbreviation	SWM		NEM	
	GoM	PB	GoM	PB
Temperature (°C)	29.6 ± 1.1	30.4 ± 0.7	26.6 ± 0.49	25.5 ± 0.6
Salinity	35.4 ± 0.4	34.9 ± 0.6	28.5 ± 4.7	26.3 ± 4.0
NO <sub>3</sub> (µM)	0.74 ± 0.47	0.84 ± 0.55	0.50 ± 0.32	0.48 ± 0.46
PO <sub>4</sub> (µM)	0.46 ± 0.24	0.28 ± 0.18	0.24 ± 0.12	0.31 ± 0.18
SPM (mg L <sup>-1</sup> )	8.33 ± 5.04	9.1 ± 7.12	1.47 ± 0.74	19.23 ± 3.00
Chl <i>a</i> (mg m <sup>-3</sup> )	0.79 ± 0.41	0.62 ± 0.24	1 ± 0.63	0.81 ± 0.40
DVChl <i>a</i> (mg m <sup>-3</sup> )	-	-	0.22 ± 0.80	-
Chl <i>b</i> (mg m <sup>-3</sup> )	0.12 ± 0.46	-	-	-
Chl <i>c</i> 2 (mg m <sup>-3</sup> )	0.09 ± 0.10	0.07 ± 0.03	0.24 ± 0.34	0.09 ± 0.04
Chl <i>c</i> 3 (mg m <sup>-3</sup> )	0.09 ± 0.11	0.03 ± 0.04	0.09 ± 0.10	0.10 ± 0.04
β-Caro (mg m <sup>-3</sup> )	0.04 ± 0.14	0.01 ± 0.02	-	-
Allo (mg m <sup>-3</sup> )	0.01 ± 0.03	-	-	0.02 ± 0.02
But (mg m <sup>-3</sup> )	-	-	-	-
Diad (mg m <sup>-3</sup> )	0.07 ± 0.05	0.03 ± 0.01	0.07 ± 0.04	0.04 ± 0.02
Fuco (mg m <sup>-3</sup> )	0.23 ± 0.24	0.08 ± 0.02	0.88 ± 1.13	0.22 ± 0.12
Hex (mg m <sup>-3</sup> )	-	-	0.08 ± 0.07	0.04 ± 0.04
Peri (mg m <sup>-3</sup> )	0.20 ± 0.16	0.16 ± 0.12	0.40 ± 0.66	0.12 ± 0.29
Zea (mg m <sup>-3</sup> )	0.18 ± 0.11	0.41 ± 0.22	0.29 ± 0.10	0.26 ± 0.09
Tpig (mg m <sup>-3</sup> )	1.62 ± 0.81	1.41 ± 0.41	3.27 ± 2.65	1.69 ± 0.61
DP (mg m <sup>-3</sup> )	0.63 ± 0.36	0.65 ± 0.21	1.66 ± 1.59	0.64 ± 0.27
PSP (mg m <sup>-3</sup> )	1.97 ± 1.66	0.96 ± 0.37	2.91 ± 2.68	1.37 ± 0.58
TAcc (mg m <sup>-3</sup> )	0.83 ± 0.46	0.79 ± 0.25	2.06 ± 1.82	0.88 ± 0.29
TChl <i>a</i> (mg m <sup>-3</sup> )	1.39 ± 1.22	0.62 ± 0.24	1.21 ± 0.86	0.81 ± 0.40
PPC (mg m <sup>-3</sup> )	0.25 ± 0.09	0.45 ± 0.22	0.36 ± 0.10	0.32 ± 0.11
PSC (mg m <sup>-3</sup> )	0.43 ± 0.39	0.23 ± 0.13	1.36 ± 1.61	0.37 ± 0.28

Table 5

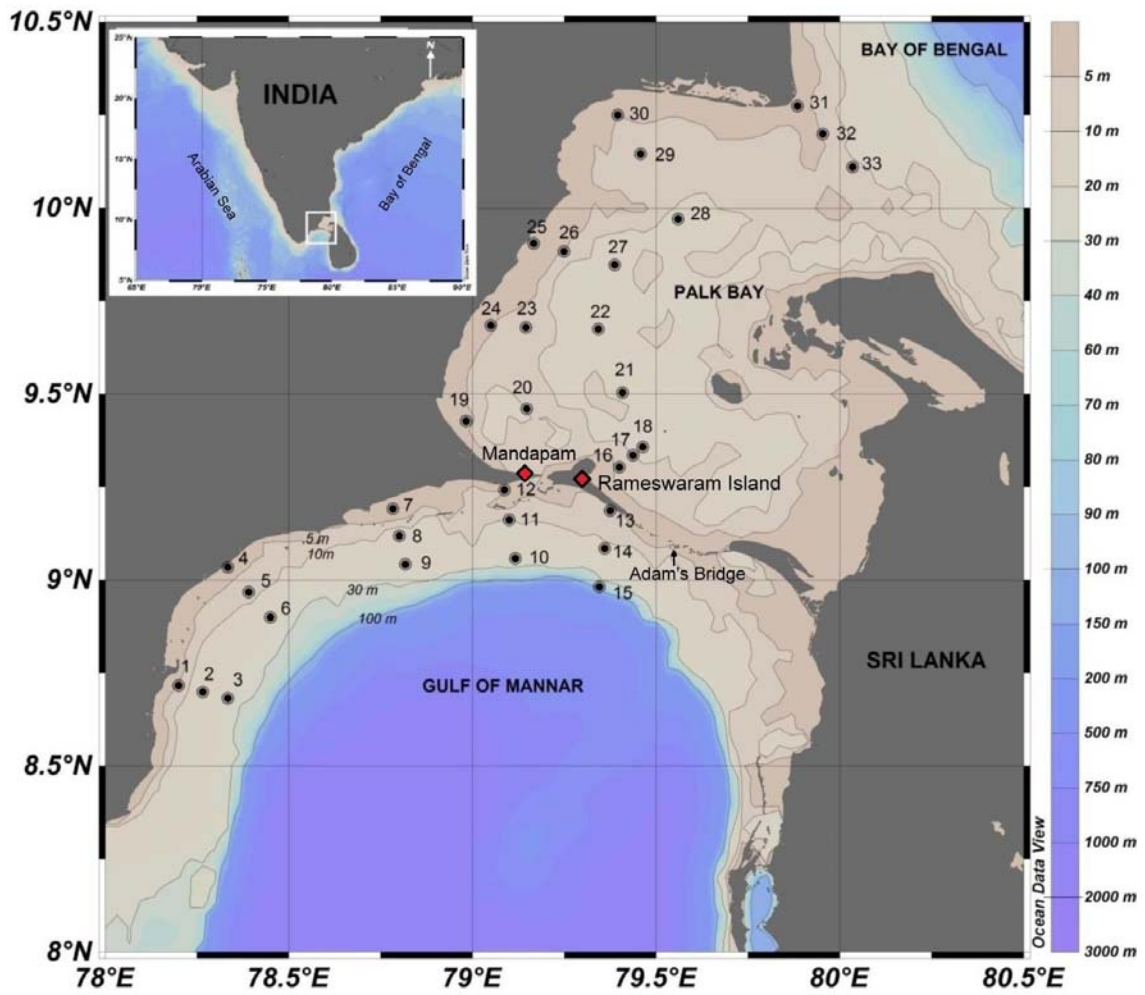
Parameters	Between seasons (SWM/NEM)		Between area (GoM/PB)	
	GoM	PB	SWM	NEM
Temperature (°C)	<0.0001**	0.0002**	0.0245*	<0.0001**
Salinity	<0.0001**	0.0002**	0.0314*	0.0147*
SPM (mg L <sup>-1</sup> )	0.0002**	0.0006**	0.9801	<0.0001**
Nitrate (µM)	0.1298	0.0386*	0.5943	0.4369
Phosphate (µM)	0.006**	0.4485	0.0165*	0.2278
Chl a (mg m <sup>-3</sup> )	0.5416	0.1108	0.1622	0.3954

Table 6

Indices/ratios	SWM		NEM	
	GoM	PB	GoM	PB
PI	0.42 ± 0.24	0.71 ± 0.41	0.45 ± 0.34	0.37 ± 0.16
TChl a <sub>TP</sub>	0.48 ± 0.09	0.47 ± 0.09	0.40 ± 0.08	0.52 ± 0.10
PPC <sub>TP</sub>	0.20 ± 0.10	0.31 ± 0.11	0.19 ± 0.14	0.18 ± 0.06
PSC <sub>TP</sub>	0.22 ± 0.12	0.15 ± 0.05	0.32 ± 0.17	0.19 ± 0.10
PPC:PSC	1.13 ± 0.88	2.48 ± 1.82	0.70 ± 0.62	1.33 ± 0.83
TAcc <sub>TP</sub>	0.52 ± 0.09	0.53 ± 0.09	0.60 ± 0.08	0.48 ± 0.10
pPF	35.36 ± 25.85	63.09 ± 16.61	34.02 ± 25.41	45.03 ± 17.32
nPF	4.12 ± 14.36	-	7.5 ± 7.2	8.96 ± 10.3
mPF	60.52 ± 24.11	36.91 ± 16.61	58.47 ± 28.13	49.88 ± 31.1

Table 7

Parameters	SWM		NEM	
	GoM	PB	GoM	PB
Temperature (°C)	0.174)	0.041	0.159	0.101
Salinity	0.179	0.473*	0.239	0.164
SPM (mg L <sup>-1</sup> )	0.361	0.213	0.114	0.331
Nitrate (µM)	0.117	0.095	0.117	0.114
Phosphate (µM)	0.296	0.022	0.318	0.241
Chl a (mg m <sup>-3</sup> )	0.998**	0.302	0.705**	0.469*





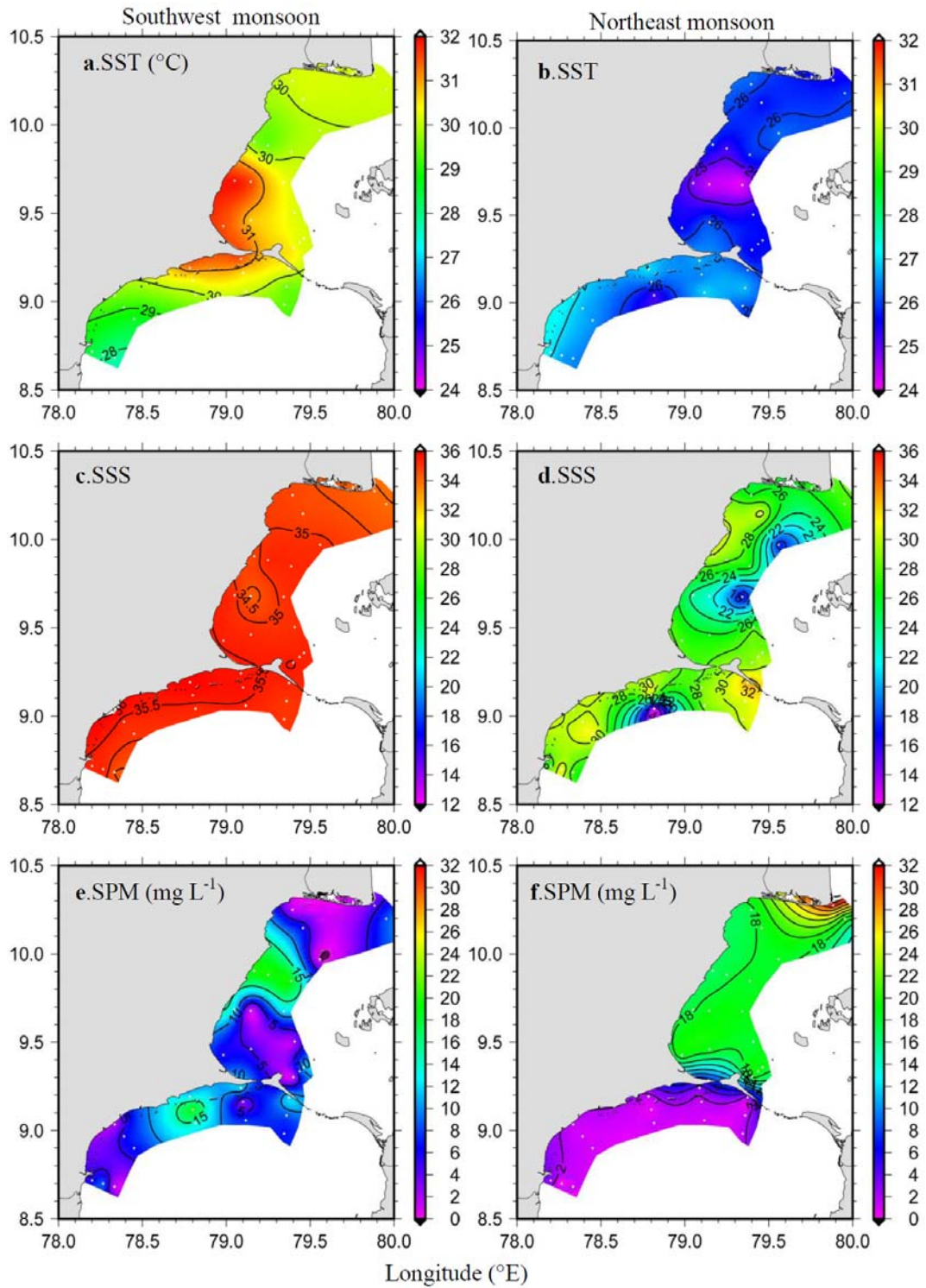


Figure 2.

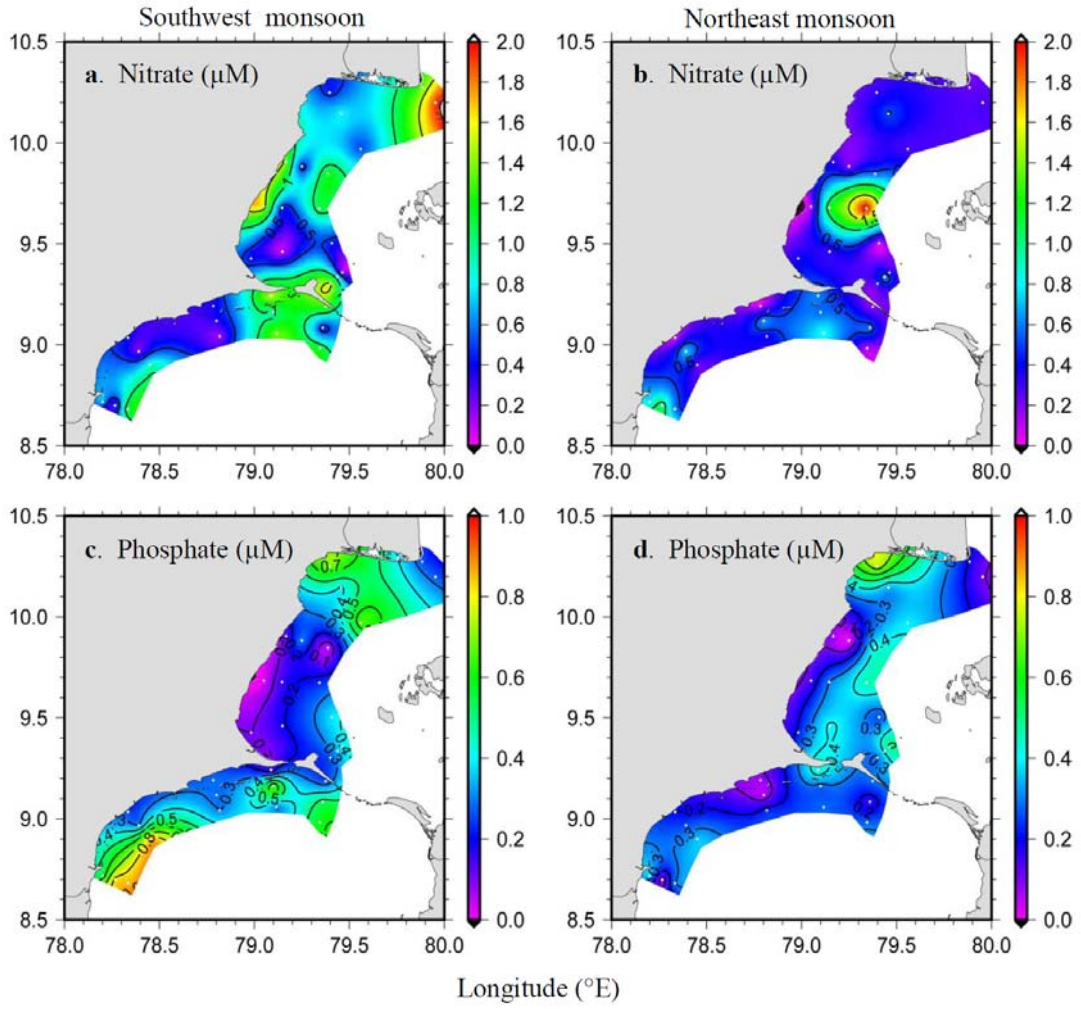


Figure 3.

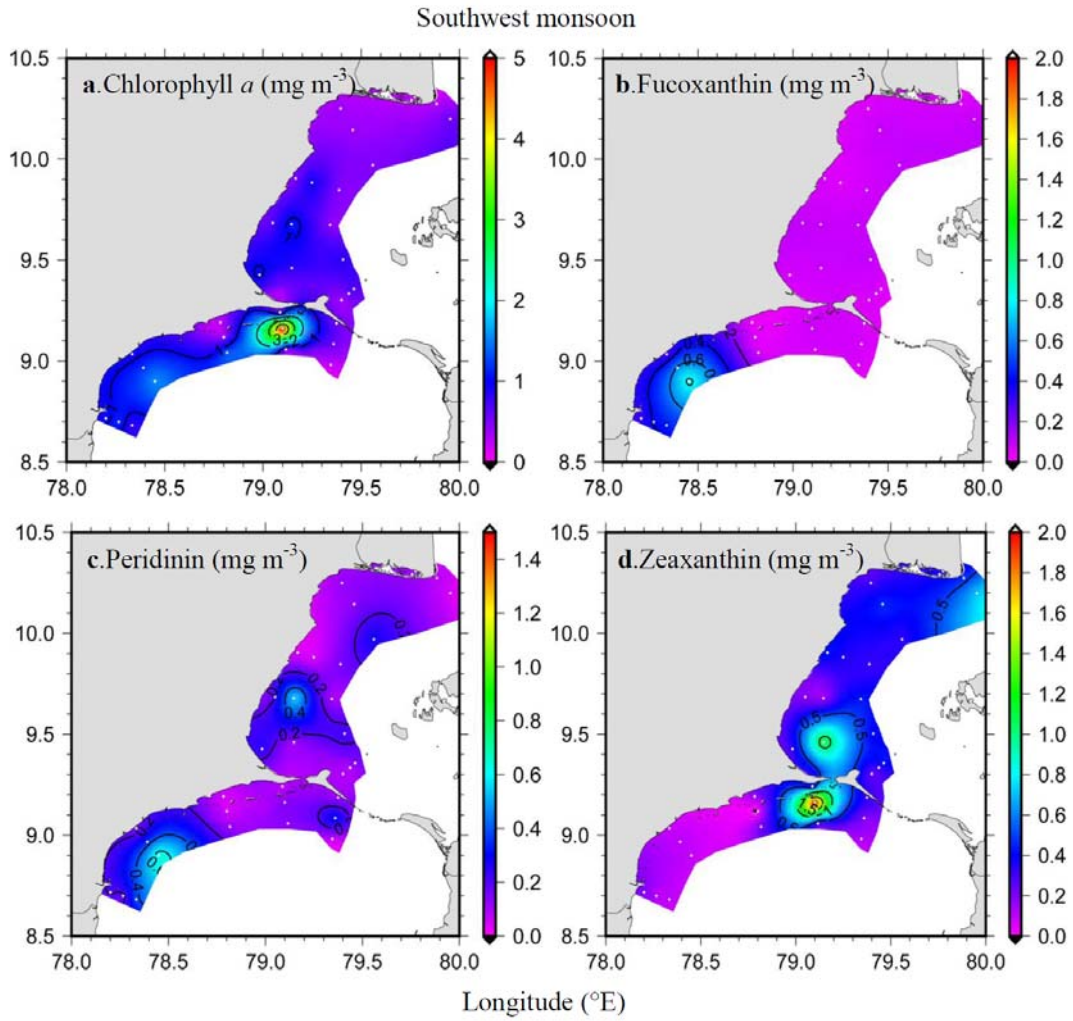


Figure 4.

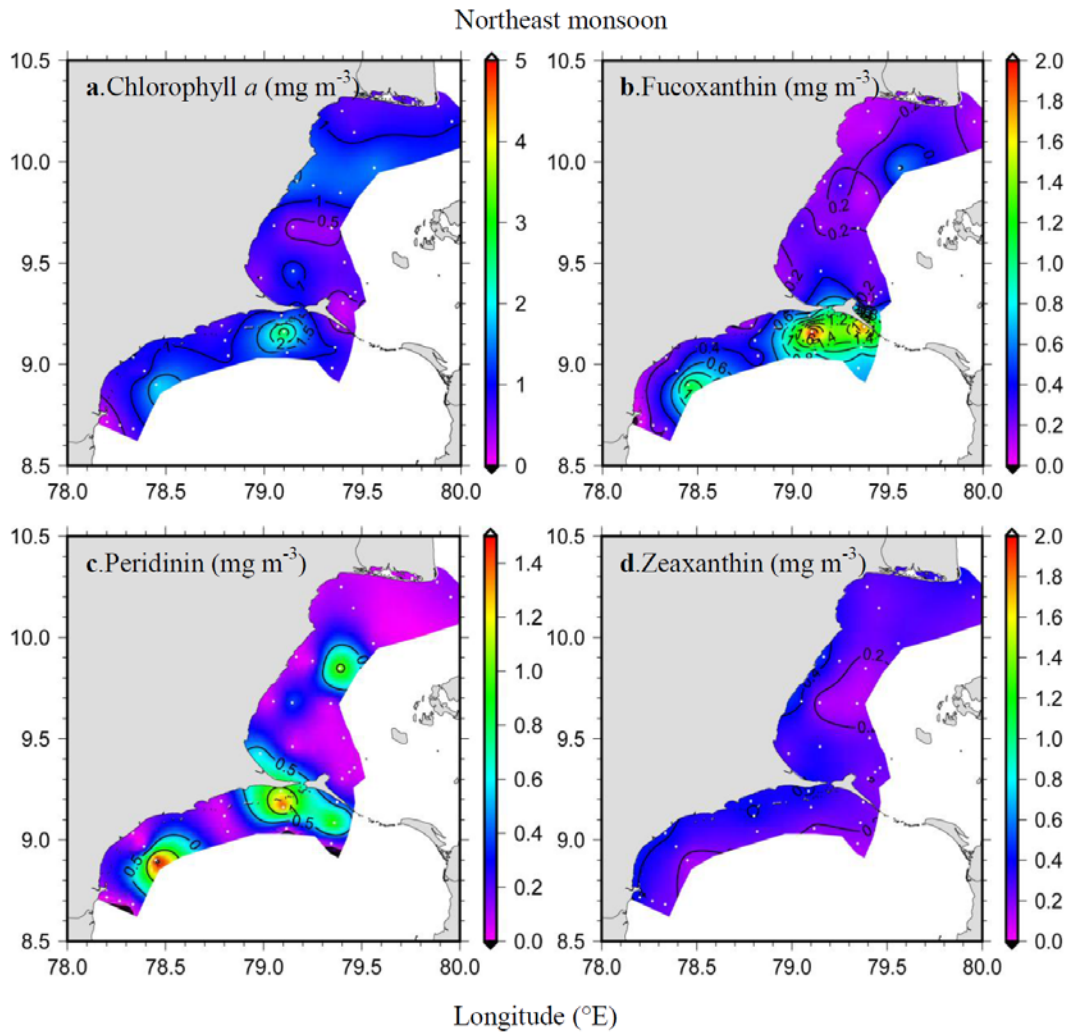


Figure 5.

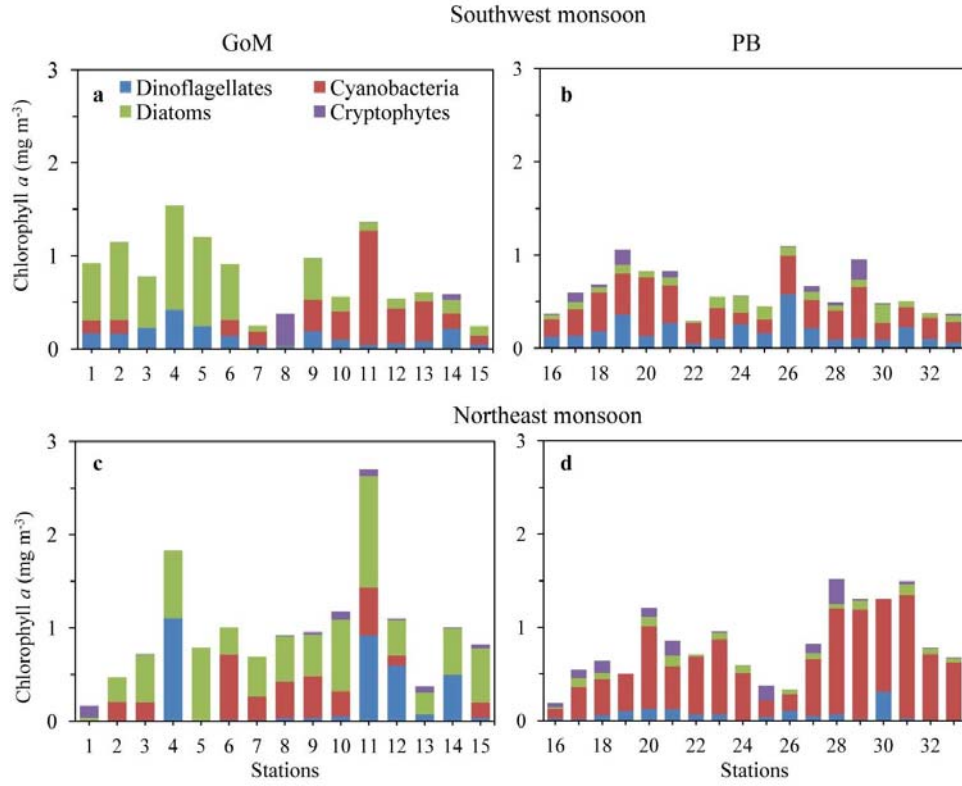


Figure 6.

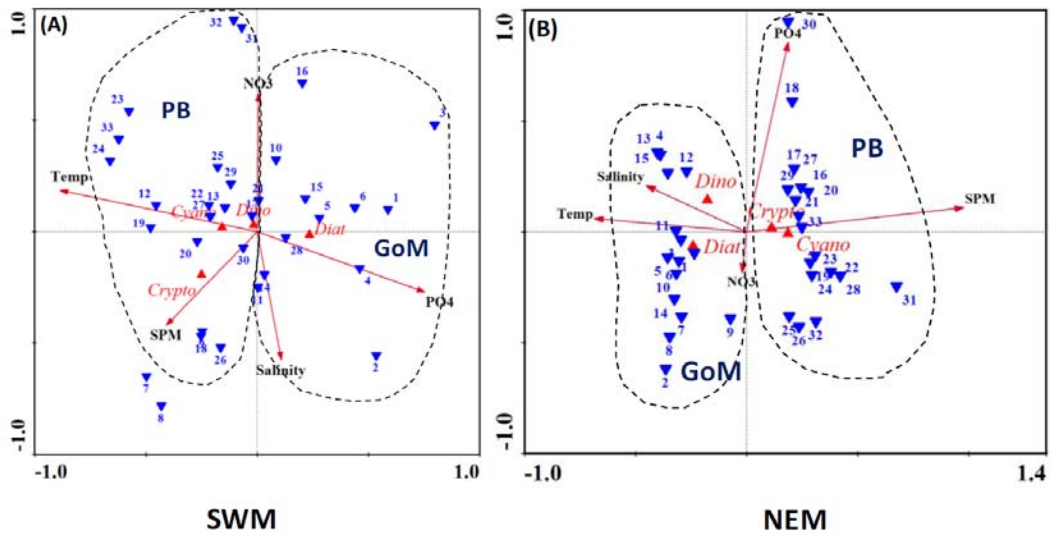


Figure 7