

## *Chapter 3*

*Primary description of surface water  
phytoplankton pigment patterns  
in the Bay of Bengal*

### ***3.1. Introduction***

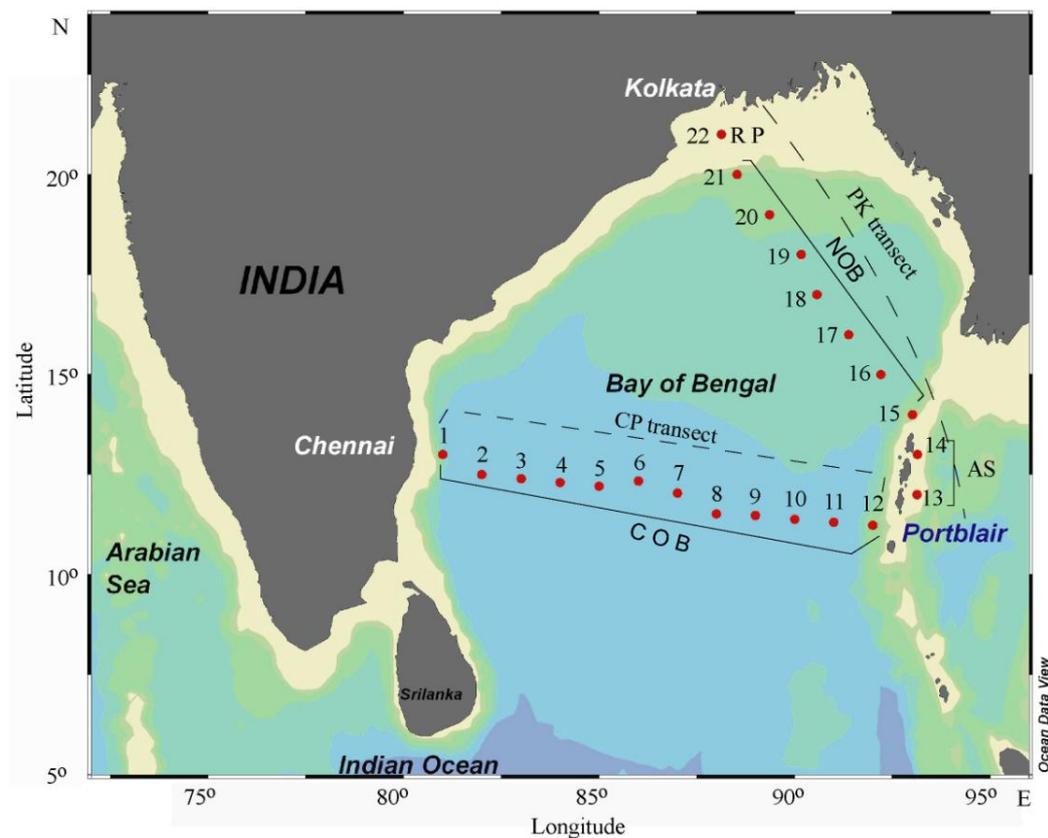
Phytoplankton, the microscopic floating plant life of all water bodies, include among their components: diatoms, dinoflagellates, coccolithophores, green algae, blue green algae and other groups. All together these marine phytoplankton are important contributors to global carbon fluxes. Qualitative and quantitative analysis of such groups is important to understand the community structure and dynamics of any ecosystem. To accomplish this, one approach is the identification and enumeration of phytoplankton through microscopic analysis. This approach is time-consuming and requires a high level of taxonomic expertise. There is also the risk of missing out smaller groups of phytoplankton (picophytoplankton,  $< 2\mu\text{m}$ ) in routine microscopic analysis. Pigment analysis using chromatography, is another approach considered as a powerful tool for characterization and monitoring of phytoplankton abundance and composition of field populations (Wright and Jeffrey 2006). The northern Indian Ocean has been studied using this approach on several occasions (Latasa and Bidigare 1998, Barlow et al. 1999, Goericke et al. 2000, Roy et al. 2006, Barlow et al. 2008). The seasonal pigment pattern of surface phytoplankton from the southern hemisphere was also studied in the recent past (Barlow et al. 2007). Most of these studies concentrated on the Arabian Sea as the study area. The characteristic features of the Arabian Sea, such as monsoon, upwelling and current patterns made it an area of interest, while its counterpart, the Bay of Bengal (BOB), has remained relatively unexplored.

The BOB, the eastern arm of the northern Indian Ocean is characterized by seasonal reversing of winds, currents influenced by monsoon and riverine flux causing the near-surface circulation to be thermohaline in nature. These features make the BOB a unique oceanic area and thus, understanding the spatio-temporal variations in the distribution of phytoplankton pigments in this area will provide novel information on contribution of specific groups to the total pigment pool. This information will be important in developing applications of remote sensing in biogeochemistry and ecosystem models.

### ***3.2. Materials and Methods***

#### ***3.2.1. Sampling strategy***

Under the Indian Expendable Bathythermograph (XBT) Programme, surface water was collected with bucket on two transects [Chennai-Port Blair (CP) and Port Blair-Kolkata (PK)] (Fig. 3.1), from passenger ships plying along these transects. Samples were collected at one degree intervals from 22 stations on 4 occasions, from February 2007 to June 2007. Surface water was collected for the enumeration and identification of micro-phytoplankton to the lowest possible taxonomic level using light microscopy, and for pigment analysis using High Performance Liquid Chromatography (HPLC).



**Fig. 3.1.** Study area showing station locations and regions (COB-Central Oceanic Bay, AS-Andaman Sea, NOB-Northern Oceanic Bay and RP- River plume) along Chennai–Port Blair (CP) and Port Blair–Kolkata (PK) transects.

### 3.2.2. Pigment analysis

Seawater (2-5 L) was filtered through 47 mm GF/F filters. The filters were stored in liquid nitrogen until further analysis in the shore laboratory. Phytoplankton pigments were extracted using 3 mL 95% acetone for 5 min in an ultrasonic bath filled with ice-water. The extracts were stored overnight at  $-20^{\circ}\text{C}$  for HPLC analysis. The entire extraction procedure was carried out in dim light conditions and at low temperature to minimize degradation of pigments. The HPLC analysis was carried out following the method of van Heukelem (2002).

### *3.2.3. Pigment calibrations and estimation of the analytical detection limit*

Pigment calibration standards were purchased from Sigma-Aldrich Company and DHI Water and Environment (Hørsholm, Denmark). At least 6-7 replicate injections were run for each standard. Single point calibrations were made except for chlorophyll *a* for which multipoint calibrations were used and corresponding response factors were determined. The analytical detection limit (ADL) which is typically given by a signal to noise ratio (S/N) = 3-5 was experimentally determined by the technique of serial dilutions, which were in the order of 0.0002-0.0005 mg m<sup>-3</sup>. Injections of a mix standard were routinely done along with a set of samples to check the accuracy of the response factors and changes in the retention time. Pigment identification in samples was done manually by comparing HPLC in-line diode array detector spectra with those of standards and published spectra. The method followed gave a good response for tropical and subtropical waters with analytical separations of monovinyl chlorophyll *a* from divinyl chlorophyll *a* forms.

### *3.2.4. Data analyses*

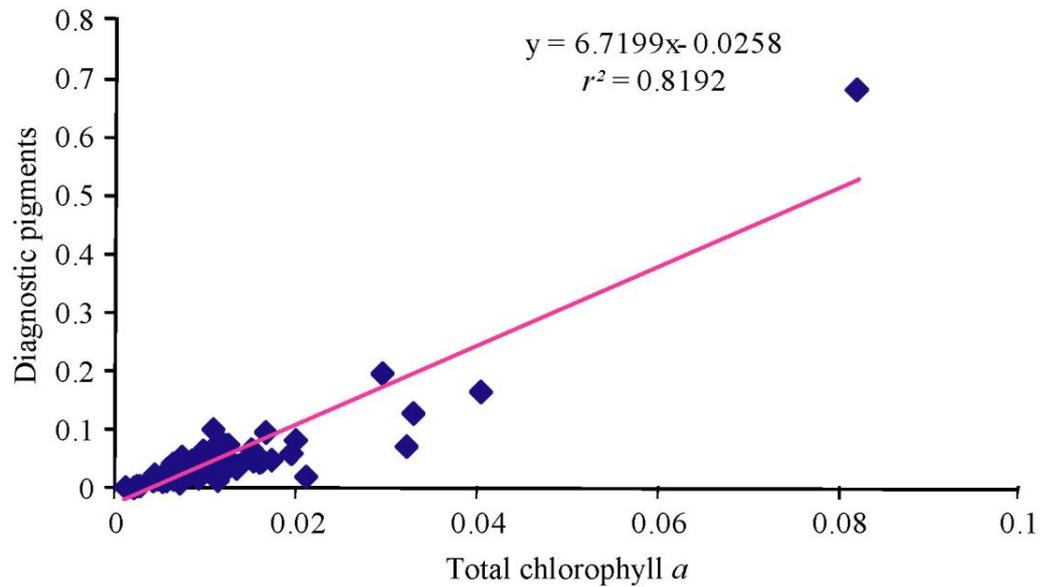
Grouping of pigments permits the formulation of variables which is often useful in photo-physiological studies (Bidigare et al. 1987) and have been presented in detail in Table 3.1. For example, the pool of accessory non-chlorophyll *a* pigments are useful in remote-sensing investigations (Trees et al. 2000). Carotenoids are functionally classified as photosynthetic (PSC) and non-photosynthetic (mainly photoprotective,

PPC) (Bidigare et al. 1990, Babin et al. 1996). The PSC pigments include fucoxanthin and the 19'-hexanoyloxyfucoxanthin pigments, and are prominent in regions of high productivity (Barlow et al. 2002). The PPC group includes zeaxanthin, diadinoxanthin and diatoxanthin. This group is dominant in surface waters with low chlorophyll and can account for up to 70% of the total pigment pool (Gibb et al. 2001).

The ratios that can be derived from these pooled variables, for e.g., PSC/TChla, are dimensionless and have the advantage of automatically scaling the comparison of results from different areas and pigment concentrations. The diagnostic pigment (DP) criteria was introduced by Claustre (1994) to estimate a pigment derive analog to the f- ratio (the ratio of new production to total production). The use of DP was extended by Vidussi et al. (2001) and more recently by Barlow et al. (2007) to derive size-equivalent pigment indices which roughly correspond to the biomass proportions of pico, nano and microplankton. Thus, DP can be used to understand the biomass structure of an area purely based on pigment sums and ratios.

**Table 3.1.** The pigment symbol, names, formulae and taxonomic groups designation (Jeffrey and Vesk 1997) for diagnostic pigment sums and pigment indices (Barlow et al. 2007) including some from Vidussi et al. (2001)\*.

<b>Symbol</b>	<b>Pigment</b>	<b>Designation of phytoplankton groups</b>
Chla	Chlorophyll <i>a</i> (plus allomers and epimers)	
Chlb	Chlorophyll <i>b</i>	Chlorophytes
Chlc1	Chlorophyll <i>c</i> <sub>1</sub>	
Chlc2	Chlorophyll <i>c</i> <sub>2</sub>	
Chlc3	Chlorophyll <i>c</i> <sub>3</sub>	
Chlidea	Chlorophyllide <i>a</i>	
DVChla	Divinyl chlorophyll <i>a</i>	Prochlorophytes
DVChlb	Divinyl chlorophyll <i>b</i>	Prochlorophytes
All	Alloxanthin	Cryptophytes
But	19'-Butanoyloxyfucoxanthin	Crysophytes
Caro	ββ-Carotene + βε-carotene	
Diad	Diadinoxanthin	
Diato	Diatoxanthin	
Fuc	Fucoxanthin	Diatoms
Lut	Lutein	
Hex	19'-Hexanoyloxyfucoxanthin	Prymnesiophytes
Per	Peridinin	Dinoflagellates
Viol	Violaxanthin	
Zea	Zeaxanthin	Cyanobacteria
	<b>Pigment sum</b>	<b>Formula</b>
TChla	Total chlorophyll <i>a</i>	Chla + DVChlab + Chlidea
Chlbc	Sum of chlorophyll <i>b</i> and <i>c</i>	Chlb + Chlc <sub>1</sub> + Chlc <sub>2</sub> + Chlc <sub>3</sub>
PPC	Photoprotective carotenoids	All + Caro + Diad + Diato + Lut + Viol + Zea
PSC	Photosynthetic carotenoids	But + Fuc + Hex + Per
TPig	Total pigments	TChla + Chlbc + PPC + PSC
DP	Diagnostic pigments	All + But + Chlb + Fuc + Hex + Per + Zea
	<b>Pigment index</b>	<b>Formula</b>
DVChla/Tchla	Divinyl chlorophyll <i>a</i> to total chlorophyll <i>a</i>	DV Chla/TChla
TChla <sub>TP</sub>	Total chlorophyll <i>a</i> to total pigments	TChla/TPig
PPC <sub>TP</sub>	Photoprotective carotenoids to total pigment	PPC/TPig
PSC <sub>TP</sub>	Photosynthetic carotenoids to total pigment	PSC/TPig
Diat <sub>DP</sub>	Diatom proportion of DP	Fuc/DP
*Dino <sub>DP</sub>	Dinoflagellate proportion of DP	Per/DP
Flag <sub>DP</sub>	Flagellate proportion of DP	(All + But + Chlb + Hex)/DP
Prok <sub>DP</sub>	Prokaryote proportion of DP	Zea/DP

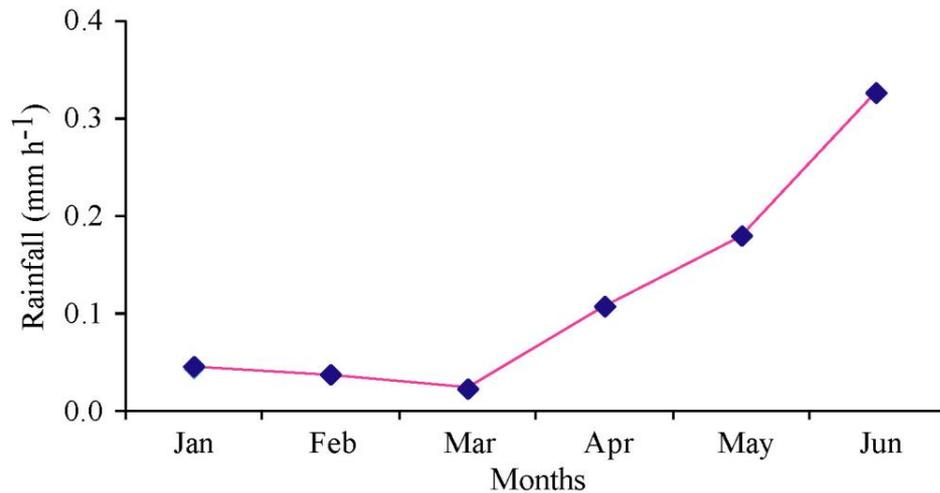


**Fig. 3.2.** Linear regression between total chlorophyll *a* and diagnostic pigments.

A linear regression between total chlorophyll *a* and diagnostic pigments (DP) showed a significant relationship ( $r^2 = 0.8192$ ,  $n = 85$ ,  $p < 0.001$ ) (Fig. 3.2). Thus, DP was used as an index to understand the pattern of community dominance in the sampling area.

In order to see the regional variation in pigment patterns with respect to the sampled seasons, the two transects in the study area, CP and PK, were partitioned into different regions: Central Oceanic Bay (COB), Andaman Sea (AS), Northern Oceanic Bay (NOB) and the region influenced by river Hooghly, referred to here as River Plume (RP). These regions were partitioned according to oceanic and coastal nature based on the bathymetry of study area (Fig. 3.1). TRMM\_3B42 data was downloaded for the grid area, Latitude 5°N-25°N, Longitude 78°E-95°E from Mirador data access ([http://mirador.gsfc.nasa.gov/collections/TRMM\\_3B42\\_006.shtml](http://mirador.gsfc.nasa.gov/collections/TRMM_3B42_006.shtml)) to

obtain rainfall data and to follow changes in the monsoon during the observed period (Fig. 3.3).



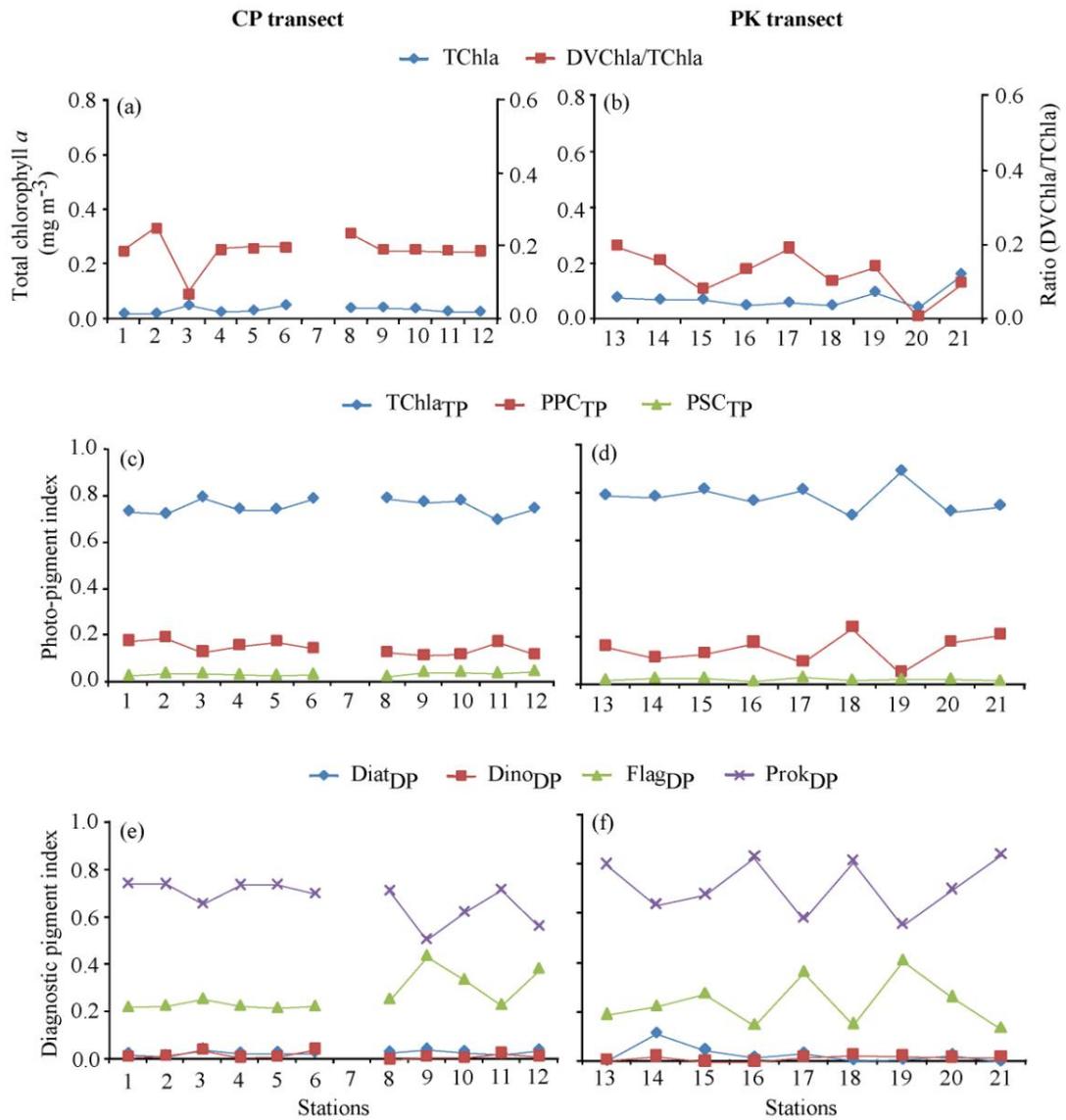
**Fig. 3.3.** Rainfall (mm h<sup>-1</sup>) data for the BOB during the period January-June 2007.

The observational period was categorized into two seasons, spring intermonsoon (SpIM, Feb-April 2007) and commencement of the summer monsoon (CSM, May-June 2007).

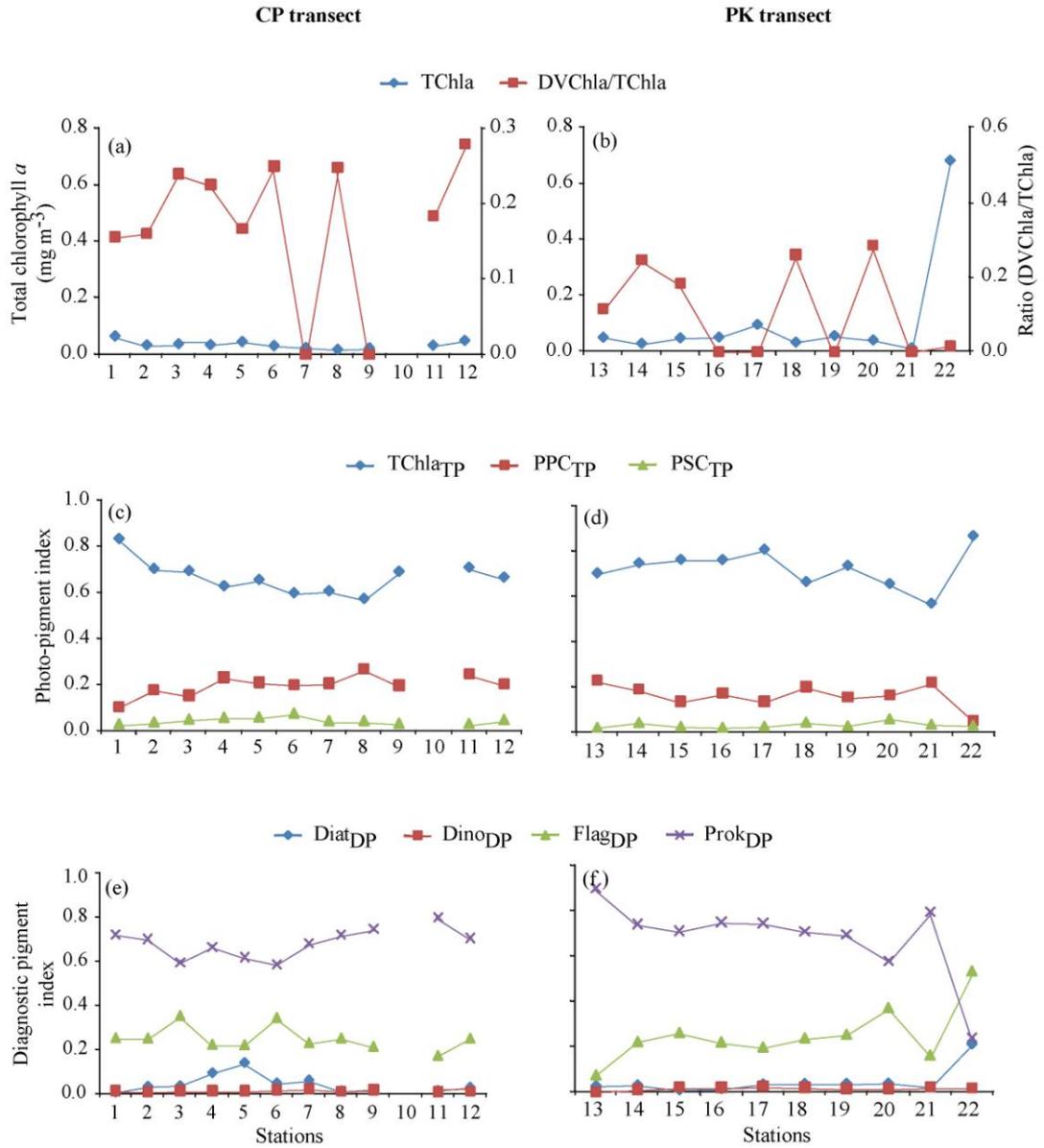
### **3.3. Results**

#### *3.3.1. The Analytical Detection Limit and total chlorophyll a concentrations*

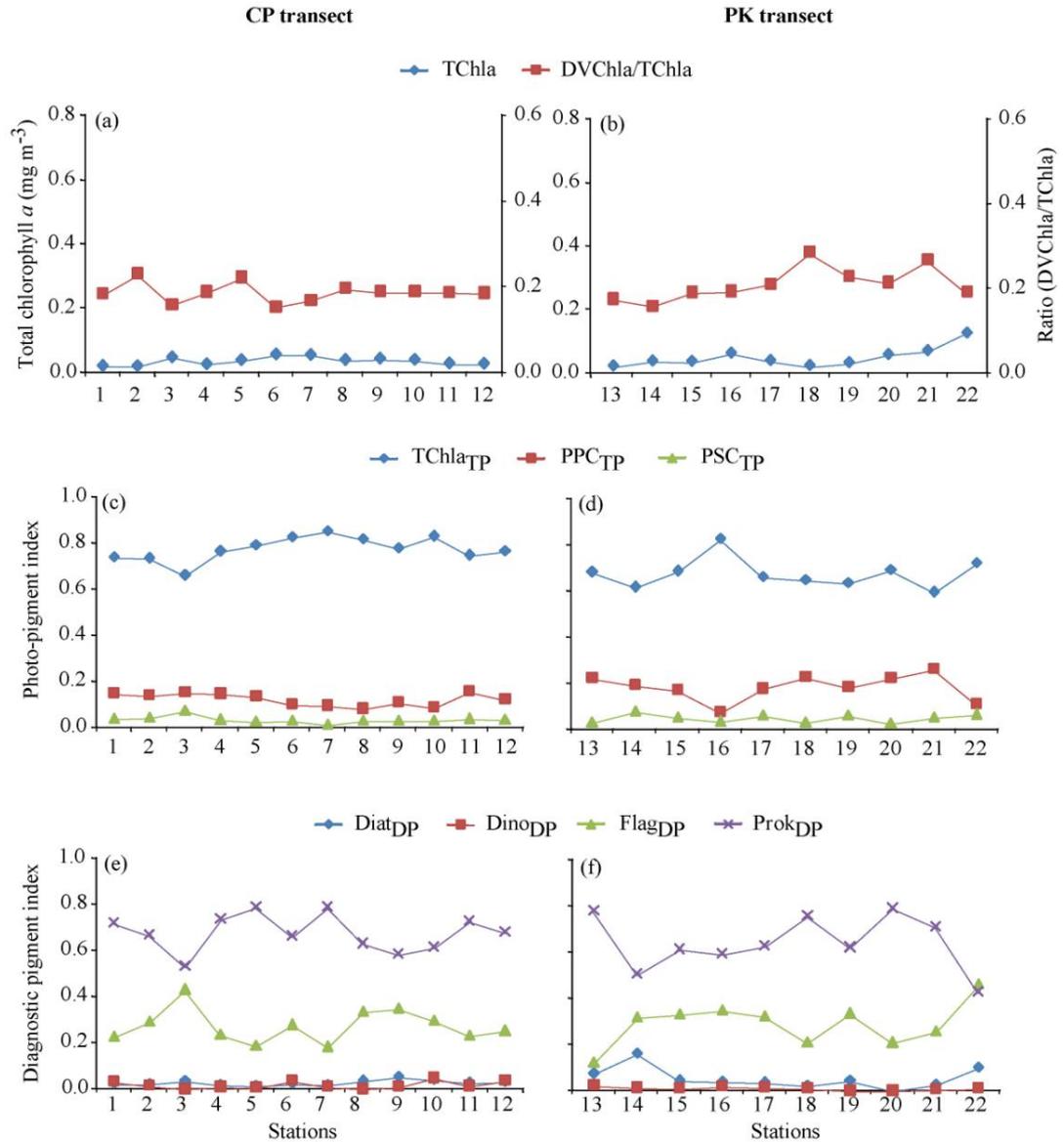
The Analytical Detection Limit (ADL) in this study (the S/N ratio) = 10. Total chlorophyll *a* (TChla) ranged from 0.002-0.067 mg m<sup>-3</sup> at the CP transect and 0.001-0.681 mg m<sup>-3</sup> at the PK transect (Figs. 3.4-3.7a,b, Table 3.2). The lowest concentrations of pigments, close to the ADL, were generally associated with the CSM (Fig. 3.7a,b).



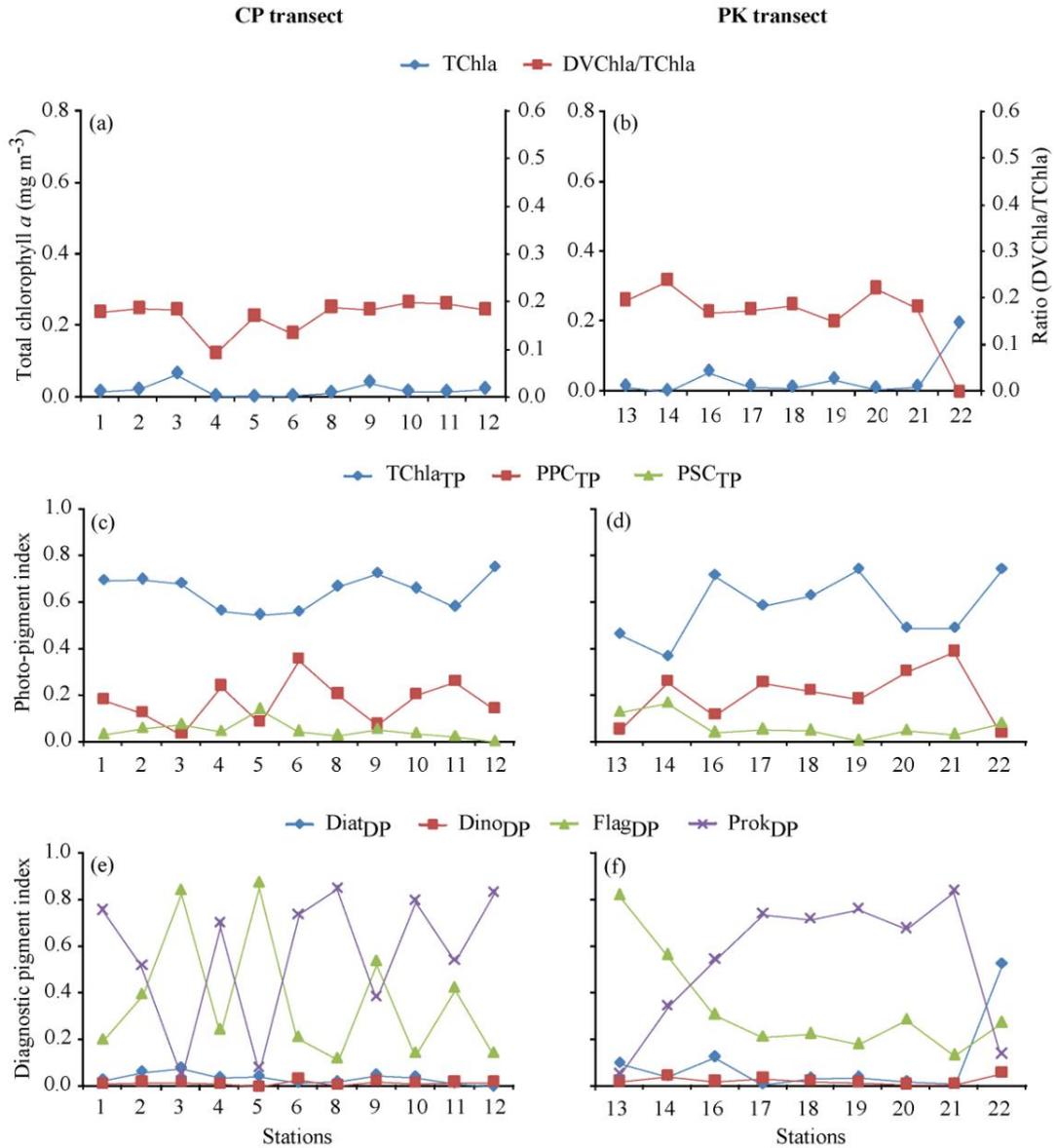
**Fig. 3.4.** Pigment indices for the period February-March 2007 in the Chennai-Port Blair (CP) and Port Blair-Kolkata (PK) transects. (a,b) Total chlorophyll  $a$  ( $\text{mg m}^{-3}$ ) and DVChla/TChla, (c,d) photo-pigment indices and (e,f) diagnostic pigment indices. Description of symbols and formulae in Table 3.1.



**Fig. 3.5.** Pigment indices for the period April 2007 in the Chennai-Port Blair (CP) and Port Blair-Kolkata (PK) transects. (a,b) Total chlorophyll *a* (mg m<sup>-3</sup>) and DVChla/TChla, (c,d) photo-pigment indices and (e,f) diagnostic pigment indices. Description of symbols and formulae in Table 3.1.



**Fig. 3.6.** Pigment indices for the period April-May 2007 in the Chennai-Port Blair (CP) and Port Blair-Kolkata (PK) transects. (a,b) Total chlorophyll  $a$  ( $\text{mg m}^{-3}$ ) and DVChla/TChla, (c,d) photo-pigment indices and (e,f) diagnostic pigment indices. Description of symbols and formulae in Table 3.1.



**Fig. 3.7.** Pigment indices for the period May-June 2007 in the Chennai-Port Blair (CP) and Port Blair-Kolkata (PK) transects. (a,b) Total chlorophyll  $a$  ( $\text{mg m}^{-3}$ ) and DVChla/TChla, (c,d) photo-pigment indices and (e,f) diagnostic pigment indices. Description of symbols and formulae in Table 3.1.

**Table 3.2.** Minimum and maximum total chlorophyll *a* ( $\text{mg m}^{-3}$ ) in Chennai-Port Blair (CP) and Port Blair-Kolkata (PK) transects during the period February-June 2007.

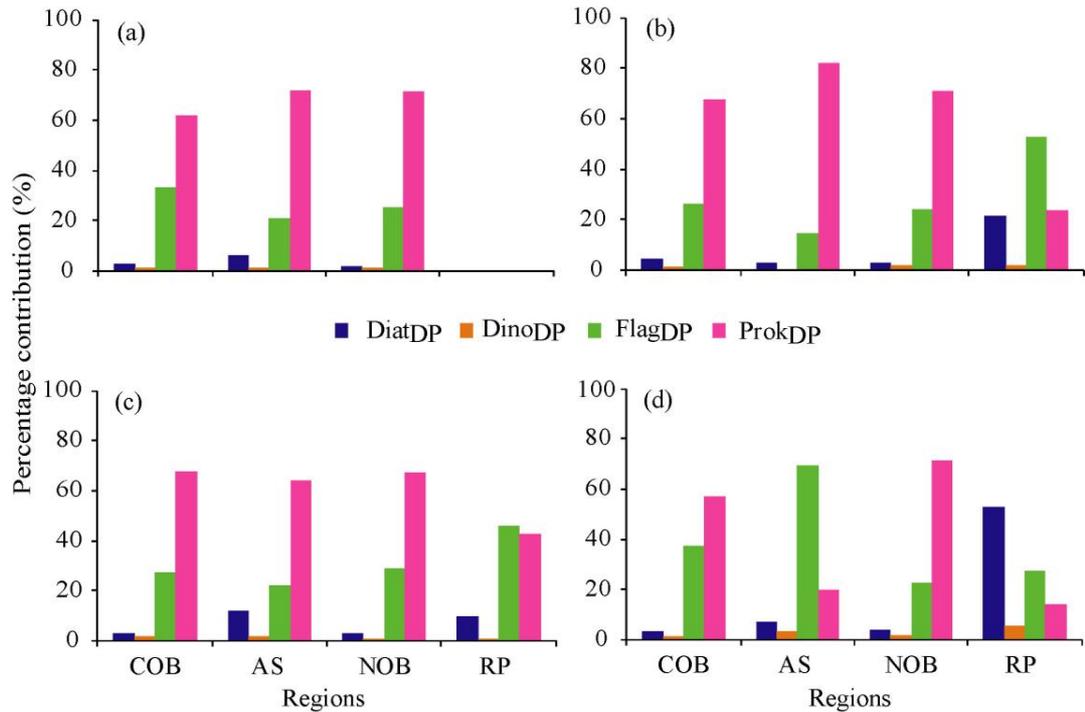
<b>Sampling period</b>	<b>CP transect</b>	<b>PK transect</b>
February-March 2007	0.020-0.051	0.047-0.165
April-2007	0.018-0.064	0.013-0.681
April-May 2007	0.020-0.055	0.023-0.128
May-June 2007	0.002-0.067	0.001-0.196

### 3.3.2. Photo-pigment indices

The photo-pigment indices revealed the dominance of  $\text{TCh}_{\text{TP}}$  (Total chlorophyll *a*/total pigments) over  $\text{PPC}_{\text{TP}}$  (Photoprotective carotenoids/total pigments) and  $\text{PSC}_{\text{TP}}$  (Photosynthetic carotenoids/total pigments) whereas  $\text{PPC}_{\text{TP}}$  dominance was observed over  $\text{PSC}_{\text{TP}}$  in all the observations at CP and PK transects (Fig. 3.4-3.7c,d).

### 3.3.3. Diagnostic pigment indices

An evaluation of the diagnostic pigment indices point out the dominance of  $\text{Prok}_{\text{DP}}$  followed by  $\text{Flag}_{\text{DP}}$  throughout the observed region during SpIM (Feb-May 2007), except at the last station of PK (station 22) (Fig. 3.4-3.6e,f).  $\text{Diat}_{\text{DP}}$  followed by  $\text{Dino}_{\text{DP}}$  were present in low proportions. However, during the period CSM (May-June 2007), we could see oscillations in the dominance between  $\text{Prok}_{\text{DP}}$  and  $\text{Flag}_{\text{DP}}$  in the CP transect (Fig. 3.7e), whereas, in the PK transect,  $\text{Flag}_{\text{DP}}$  dominated in the AS and  $\text{Prok}_{\text{DP}}$  was dominant in the NOB (Fig. 3.7f).

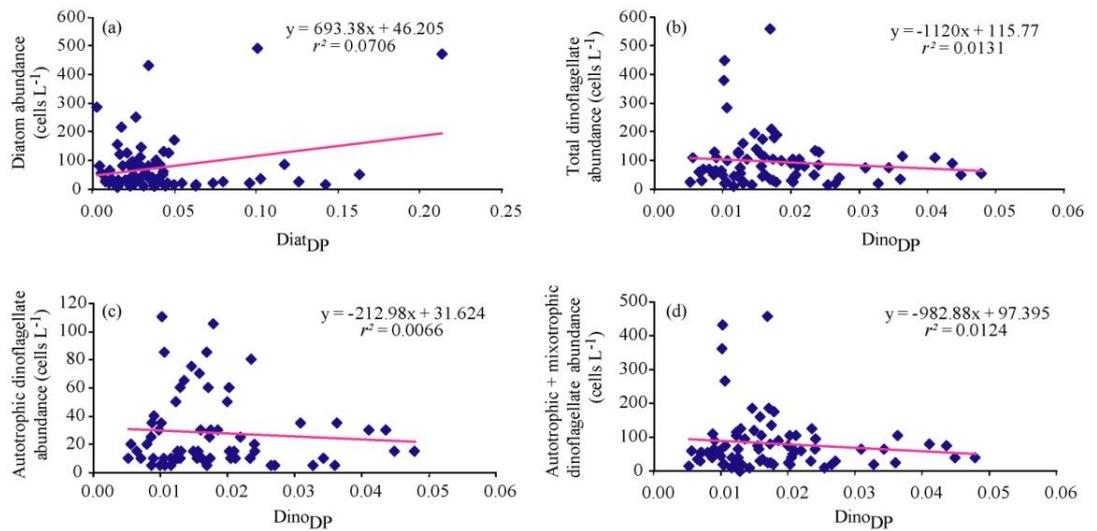


**Fig. 3.8.** Percentage contribution of diagnostic pigment indices in each region (COB, AS, NOB and RP) in (a) February-March, (b) April, (c) April-May and (d) May June. Description of diagnostic pigment indices in Table 3.1.

#### 3.3.4. Changes in pigment patterns in different regions

Throughout the observation period, the COB and NOB regions were dominated by Prok<sub>DP</sub> followed by Flag<sub>DP</sub>, whereas a decrease in the percentage contribution of Prok<sub>DP</sub> and an increase in Flag<sub>DP</sub> was observed during CSM, as compared to the SpIM (Fig. 3.8a-d). The AS showed a pattern similar to COB and NOB except for the fact that Flag<sub>DP</sub> dominated over Prok<sub>DP</sub> during CSM. In the RP region, diagnostic pigment indices for all groups (Prok<sub>DP</sub>, Flag<sub>DP</sub>, Diat<sub>DP</sub> and Dino<sub>DP</sub>) exhibited a completely different pattern compared to the other three regions (COB, AS and NOB)

(Fig. 3.8a-d). The Flag<sub>DP</sub> diagnostic pigment index was dominant during the SpIM observations (Fig. 3.8a-c), whereas the Diat<sub>DP</sub> index was dominant during the CSM (Fig. 3.8d). A considerable increase in Dino<sub>DP</sub> was also observed during SpIM at the RP region (Fig. 3.8d).



**Fig. 3.9.** Linear regression between (a) diatom diagnostic pigment and diatom abundance, (b) dinoflagellate diagnostic pigment and total dinoflagellate abundance, (c) dinoflagellate diagnostic pigment and autotrophic dinoflagellate abundance, and (d) dinoflagellate diagnostic pigment and autotrophic + mixotrophic dinoflagellate abundance.

A linear regression between Diat<sub>DP</sub> and microscopic cell counts of diatoms showed a significant relationship ( $r^2 = 0.0706$ ,  $n = 80$ ,  $p < 0.02$ ) (Fig. 3.9a), whereas Dino<sub>DP</sub> against dinoflagellate cell counts showed a non-significant relationship ( $r^2 = 0.0131$ ,  $n = 74$ ,  $p = ns$ ) (Fig. 3.9b). The correlation between Dino<sub>DP</sub> and dinoflagellate cell counts did not improve even when the mixotrophic and heterotrophic forms were omitted from the total dinoflagellate counts (Fig. 3.9c-d).

### 3.4. Discussion

The phytoplankton biomass evaluated so far in the region (Radhakrishna et al. 1978, Bhattathiri et al. 1980, Radhakrishna et al. 1982, Devassy et al. 1983, Sarma and Aswanikumar 1991, Gomes et al. 2000, Prasanna Kumar et al. 2002, Madhupratap et al. 2003) is based on fluorometer and spectrophotometer estimations and remote-sensing values. The present study, based on HPLC pigment characterization, is the first report from this area. In this study, the pigment composition was characterized and evaluated in relation to the microscopic cell counts of diatoms and dinoflagellates. The BOB is considered less productive as compared to the Arabian Sea, due to strongly stratified surface waters (Prasanna Kumar et al. 2002). It has been observed that such stratified conditions support the dominance of prokaryotic groups (Chisholm 1992, Cullen et al. 2002). The observations also indicated the dominance of Prok<sub>DP</sub> in the oceanic waters of the BOB (Fig. 3.4-3.7e,f). In a recent study from the BOB, Hegde et al. (2008) observed that stratified conditions support the prevalence of *Trichodesmium*, which is a prokaryote with the ability to fix nitrogen. Thus, this prokaryote will have a substantial input in new production in stratified waters. In the Baltic Sea, it has been observed that nitrogen fixation by diazotrophs leads to the transfer of newly fixed nitrogen to picoplanktonic organisms and supports the microbial foodweb (Ohlendieck et al. 2000). In the present work, the DVChla/TChla ratio indicated the dominance of *Prochlorococcus* sp. among the picoplankton group (Fig. 3.4-3.7a,b). The metabolic properties of *Prochlorococcus* (Vaulot and Partensky 1992, Casey et

al. 2007, Martiny et al. 2009a) give them a flexible metabolism and the ability to assimilate nitrate and nitrite (Martiny et al. 2009b). Hence, *Prochlorococcus* can assimilate newly fixed nitrogen by micro-prokaryotes like *Trichodesmium* and maintain its dominance in oceanic waters.

Earlier studies on accessory pigments from tropical latitudes demonstrated the greater presence of PPC<sub>TP</sub> in surface, low chlorophyll waters (Stuart et al. 1998, Gibb et al. 2000, Barlow et al. 2004). The observations from this study also indicate that PPC<sub>TP</sub> tend to be high in surface waters during the SpIM period (Fig. 3.4-3.7c,d). However, notable changes in the accessory pigments were observed with increase of PSCs during CSM period at few stations of CP transect (Fig. 3.7c). Similar changes were observed in the Atlantic, Pacific, Gulf of Oman and Arabian Sea (Trees et al. 2000, Veldhuis and Kraay 2004). They observed that the change in community structure is a physiological response to the changing environment thereby resulting in changes in accessory pigments. In the present work, the change in accessory pigments during CSM might be due to the responses of the community to the changing environment influenced by rainfall. This indicates that environmental and meteorological conditions may alter phytoplankton dynamics through a chain of linked processes. These variations in accessory pigments in turn are likely to affect the optical properties of phytoplankton which has implications for ocean colour remote-sensing (Sathyendranath et al. 2005).

The second dominant group was the flagellates and their dominance in the AS and RP near coastal regions (Fig. 3.7f) indicate their preference for nutrient-rich coastal waters. Similarly, Diat<sub>DP</sub> also showed a preference for nutrient-rich turbulent waters, being the dominant group at RP during the CSM (Fig. 3.7f). This change in community structure could be linked to the increased rainfall during this season (Fig. 3.3). Thus a more significant change in community structure can be expected as rainfall reaches its peak. Comparison of diagnostic pigments and microscopic cell counts indicates that though a significant relationship between Diat<sub>DP</sub> and diatom abundance was observed (Fig. 3.9a), it is pertinent to note that this significance was arrived at even without considering the contribution of haptophytes, which also contain fucoxanthin. In the case of Dino<sub>DP</sub> versus dinoflagellate abundance (Fig. 3.9b-d) the relationship was not significant. This suggests that peridinin as a marker pigment did not work well for the dinoflagellate population in the region. In view of this, further research comparing the HPLC pigment composition of dinoflagellates with live cell abundances (to eliminate artifacts due to preservatives) should be considered.

Summarizing the results, phytoplankton community structure in the BOB was dominated by Prok<sub>DP</sub> followed by Flag<sub>DP</sub> with a low biomass of TChla, during the study period. Changes in the pigment pattern were observed at the onset of the monsoon, indicating the influence of rainfall especially in near coastal regions like AS and RP. Comparative studies between microscopic counts and diagnostic pigment

indices suggest coupling pigment composition analysis with microscopic analysis of natural assemblages to establish valid biogeochemical and ecosystem models. Notably, the components of dinoflagellate communities could be missed by pigment analysis alone.