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

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ABSTRACT

Spatial and temporal variations in surface water phytoplankton pigment distribution in the Bay of Bengal were studied during the spring intermonsoon (SpIM, February-April) and the commencement of the summer monsoon (CSM, May-June), using pigment and diagnostic indices. The Prokaryotic pigment index (Prok_{DP}) was dominant at all the oceanic stations whereas the Flagellate pigment index (Flag_{DP}) was dominant at the near coastal stations. However, during the commencement of summer monsoon, an oscillation in the dominance of Prok_{DP} and Flag_{DP} was observed in the central oceanic bay, whereas flagellates and diatoms were dominant at the near coastal stations. This change in pigment pattern is possibly related to the influence of rainfall. Comparison of pigment data with microscopic cell counts indicated a significant relationship between the diatom pigment index (Diat_{DP}) and diatom abundance. However, the relationship between the dinoflagellate pigment index (Dino_{DP}) and dinoflagellate abundance was not significant. Studies coupling pigment composition analysis with microscopic analysis of phytoplankton in natural conditions should thus be a prerequisite in establishing valid biogeochemical and ecosystem models.

Keywords: phytoplankton; pigment; chlorophyll; oceanic; monsoon; Bay of Bengal

1. Introduction

Phytoplankton, the base of food webs in all water bodies, include diatoms, dinoflagellates, coccolithophores, green algae, cyanobacteria and other groups. All together, these marine phytoplankton are important contributors to global carbon fluxes; their contribution through photosynthetic carbon fixation, leads to the formation of ~45 gigatons of organic carbon per annum, of which 16 gigatons are exported to the ocean interior (Falkowski et al., 1998).

Qualitative and quantitative analysis of phytoplankton composition 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 μ m in size) in routine microscopic analysis. Pigment analysis using liquid 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). This method allows the quantification of over 50 phytoplankton pigments compared to other methods used to analyze chlorophyll (Jeffrey et al., 1997). Additionally, the composition of phytoplankton communities and their physiological status can also be inferred (Roy et al., 2006). The northern Indian Ocean has been studied using this approach on several occasions (Latasa and Bidigare, 1998; Barlow et al., 1999, 2008; Goericke et al., 2000; Roy et al., 2006). 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 focused on the Arabian Sea, whose characteristic features such as monsoon, upwelling and current patterns made it an area of interest, while its counterpart, the Bay of Bengal (BOB), remained unexplored.

The BOB, the eastern arm of the northern Indian Ocean is characterized by special features such as seasonal reversal of winds, surface currents and fresh water influx from the adjacent rivers controlling the stratification in the near-surface layers (Shetye et al., 1991,1993). 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 the contribution of specific phytoplankton groups to the total pigment pool. The objective of this study was to characterize the pigment composition in different regions of the BOB and to evaluate it in relation to the microscopic

cell counts of diatoms and dinoflagellates. This information will be important in developing applications of remote sensing in biogeochemical and ecosystem models.

2. Materials and Methods

Under the Indian Expendable Bathythermograph (XBT) Programme, surface water was collected with bucket on two transects [Chennai to Port Blair (CP) and Port Blair to Kolkata (PK)] (Fig. 1), from passenger ships plying along these transects. Samples were collected at one degree intervals from 22 stations on four occasions (February-March, April, April-May and May-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). For pigment analysis, seawater samples (5 L except station 22 where 2L) were filtered through 47-mm GF/F filters and stored in liquid nitrogen until 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 and the extracts were stored overnight at -20°C. 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). According to the standard practice, pigment indices were calculated (Table 1) following the method of Barlow et al. (2007). A linear regression between diagnostic pigments (DP) and total chlorophyll *a* showed a significant relationship ($r^2=0.91$, $n=84$, $p<0.01$). Thus, indices derived from DP were used 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 as Central Oceanic Bay (COB), Andaman Sea (AS), Northern Oceanic Bay (NOB) and the region influenced by river Hooghly, named as River Plume (RP). These regions were partitioned according to oceanic and coastal nature based on the bathymetry of the study area (Fig. 1).

Chlorophyll images were downloaded and processed (Level 3 MODIS, 4 km resolution). For the SpIM period, seasonal composite images were downloaded whereas for CSM, monthly images were downloaded. TRMM_3B42 data was downloaded for the grid area, Latitude 5⁰N-25⁰N, Longitude 78⁰E-95⁰E from Mirador data access (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. 2). The observational period was categorized into two seasons, spring intermonsoon (SpIM, Feb-May 2007) and commencement of the summer monsoon (CSM, May-June 2007).

Data analyses

Three samplings carried out during SpIM (February-May) were clubbed together and presented as SpIM, with standard deviation error bars whereas a single sampling was carried out during CSM.

The regional variation in community structure was determined by two-way ANOVA (alpha value 0.05). This was done separately for SpIM and CSM periods. Absolute values (Fucoxanthin, Peridinin, Alloxanthin + 19'-Butanoyloxyfucoxanthin + Chlorophyll *b* + 19'-Hexanoyloxyfucoxanthin, Zeaxanthin representing diatoms, dinoflagellates, flagellates and prokaryotes respectively) were used to perform two-way ANOVA.

Regression analysis was carried out between respective diagnostic pigment indices and microscopic counts of a) diatoms, b) total dinoflagellates, c) autotrophic dinoflagellates and d) autotrophic + mixotrophic dinoflagellates. In the regression analysis for diatoms, data for station number 22 was omitted as microscopic counts were not available.

3. Results

Total chlorophyll *a* (TChla) ranged from 0.013-0.681 mg/m³ during the SpIM period with minimum value at NOB and maximum at RP (Table 2). During CSM, TChla ranged from 0.001- 0.196 mg/m³ with minimum at AS and maximum at RP (Table 2). The lowest concentrations of pigments, close to the limit of quantification (LOQ), were generally associated with the CSM. The LOQ in this study was defined as signal (S) to noise (N) ratio (S/N=10) which is more widely used and acceptable in HPLC pigment analysis. These were experimentally determined by the method of serial dilutions for chlorophyll *a* and fucoxanthin which were in the order of 0.0002-0.0005 mg/m³. These were used as a reference to derive the LOQ for other marker pigments observed during these investigations as suggested by Hooker et al. (2005). However, it should be noted that the limit of detection (LOD) of the instrument is marginally lower than what was used in this analysis and typically represent (S/N=3-5). In most cases, the observed concentrations in the central BOB suggested extreme oligotrophic conditions.

The remote-sensing images also indicated low range of chlorophyll *a* during SpIM and CSM (Fig. 3). The photo-pigment indices revealed the dominance of TChla_{TP} (total chlorophyll *a* to total pigment) over PPC_{TP} (photoprotective carotenoids to total pigments) and PSC_{TP} (photosynthetic carotenoids to total pigments) during SpIM and CSM periods (Figs. 4c and 5c). During SpIM period, dominance of PPC_{TP} over PSC_{TP} was observed at all the stations (Fig. 4c). However this pattern of dominance was altered during CSM at a few stations (Fig. 5c). An evaluation of the diagnostic pigment indices point out the dominance of Prok_{DP} followed by Flag_{DP} throughout the observed region during SpIM (Fig. 4a) except at the last station of PK. Diat_{DP} followed by Dino_{DP} were present in low proportions (Fig. 4a). However, during the CSM period, we could see oscillations in the dominance between Prok_{DP} and Flag_{DP} in the CP transect (Fig. 5a), whereas, in the PK transect, Flag_{DP} dominated in the Andaman Sea and Prok_{DP} was dominant in the northern oceanic Bay (Fig. 5a).

The changes in pigment patterns at the different regions were also studied. It was observed that during the SpIM period, all the regions except RP were dominated by Prok_{DP} followed by Flag_{DP} (Fig. 6a). In the RP region, Flag_{DP} was dominant followed by Prok_{DP} (Fig. 6a). The community structure observed during CSM was different than that observed during SpIM (Fig 6a, b). In the region AS, the community was dominated by Flag_{DP} followed by Prok_{DP} whereas the region RP was dominated by Diat_{DP} followed by Flag_{DP} (Fig. 6b). During CSM, the contribution of Dino_{DP} was high compared to that in the SpIM period (Fig 6a,b). Though regional variations in community structure were observed in both seasons (SpIM and CSM), the variation was statistically significant only during SpIM (Table 3).

Microscopic analysis of diatoms and dinoflagellates were used to quantify their cell abundance during SpIM and CSM period (Fig. 7a, b). During SpIM, dinoflagellates dominated and the maximum abundance was observed in the RP region (station 22) (Fig. 7a) whereas, diatoms were abundant during the CSM (Fig. 7b). Among the dinoflagellates, mixotrophic forms were dominant during the SpIM (Fig. 7c) and the CSM (Fig. 7d).

A linear regression between Diat_{DP} and microscopic cell counts of diatoms showed a significant relationship (Fig. 8a; $n = 79$, $p < 0.05$). However, fucoxanthin is also found in significant levels in Prymnesiophyceae, Chrysophyceae and Raphidophyceae (Jeffrey et al., 1997) and due to this reason the samples with Diat_{DP} above 0.05 but with diatom abundance less than 100 cells/L were checked for their level of ChlC3 and 19' But (two pigments found in high concentration in these 3 nanoflagellate groups

but not in diatoms). The concentration for ChlC3 and 19' But was found negligible. Thus, it had no effect on the above significant relationship between Diat_{DP} and microscopic cell counts of diatoms. Regression between Dino_{DP} and total dinoflagellate cell counts showed a non-significant relationship (Fig. 8b; $n=73$, $p=ns$). The correlation between Dino_{DP} and dinoflagellate cell counts did not improve even when the mixotrophic and heterotrophic forms were omitted from the total dinoflagellate counts (Fig. 8c, d).

4. Discussion

The phytoplankton biomass evaluated so far in the region (Radhakrishna et al., 1978, 1982; Bhattathiri et al., 1980; Devassy et al., 1983; Sarma and Aswanikumar 1991; Gomes et al., 2000; Kumar et al., 2002; Madhupratap et al., 2003) is based on fluorometer and spectrophotometer estimations and remote-sensing values. To the best of our knowledge, the present study, based on HPLC pigment characterization, is the first report from this area. The BOB is considered less productive as compared to the Arabian Sea, due to strongly stratified surface waters (Kumar et al., 2002). It has been observed that such stratified conditions support the dominance of prokaryotic groups (Chisholm, 1992; Cullen et al., 2002). Our observations also indicated the dominance of Prok_{DP} in the oceanic waters of the BOB (Figs. 4a and 5a). In a recent study from the Bay of Bengal, Hegde et al. (2008) observed that stratified conditions support the prevalence of *Trichodesmium*, which is a prokaryote with the ability to fix nitrogen. 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 contribution of *Prochlorococcus* sp. to the picoplankton group and this contribution was higher during SpIM compared to CSM (Fig. 4b and 5b). 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 PPCs in surface, low chlorophyll waters (Stuart et al., 1998; Gibb et al., 2000; Barlow et al., 2004). Our observations also indicate that PPCs tend to be high in surface waters during the SpIM period (Fig. 4c).

However, notable changes in the accessory pigments were observed with increase in the relative contribution of PSCs during the CSM period at a few stations of CP transect (Fig. 5c). 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. 6a, b) indicate their preference for nutrient-rich areas. Similarly, Diat_{DP} also showed a preference for nutrient-rich turbulent waters, being the dominant group at RP during the CSM (Fig. 6b). This change in community structure could be linked to the increased rainfall during this season (Fig. 2). 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_{DP} and diatom abundance was observed (Fig. 8a; $n=79$, $p<0.05$), in the case of Dino_{DP} versus dinoflagellate abundance, the relationship was not significant (Fig. 8b). 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.

5. Conclusions

Phytoplankton community structure in the Bay of Bengal is generally dominated by prokaryotes followed by flagellates with a low biomass of total chlorophyll *a*. Changes in the community structure were observed at the onset of the monsoon, indicating the influence of rainfall especially in near coastal regions like Andaman Sea and River plume. 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.

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Legends to figures

Fig. 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). Figure also represents the bathymetry of the study area.

Fig. 2. Rainfall (mm/hour) data for BOB for the period January- June 2007.

Fig. 3. Satellite-derived chlorophyll *a* concentration (mg/m^3) : (a) SpIM, 2007 (b) monthly image (June 2007) for CSM.

Fig. 4. Pigment indices for the SpIM period: (a) diagnostic indices (b) Tchla and DVChla/Tchla, (c) photo-pigment indices. See Table 1 for symbols and formulae.

Fig. 5. Pigment indices for the CSM period: (a) diagnostic indices (b) Tchla and DVChla/Tchla, (c) photo-pigment indices. See Table 1 for symbols and formulae.

Fig. 6. Percentage contribution of diagnostic indices to each region (COB, AS, NOB and RP) (a) SpIM and (b) CSM. See Table 1 for symbols.

Fig.7. Microscopic counts of diatoms and dinoflagellates (a) SpIM and (b) CSM and abundance of autotrophic, mixotrophic and heterotrophic dinoflagellate during (c) SpIM and (d) CSM.

Fig.8. Linear regression between : (a) diatom pigment index and diatom abundance, (b) dinoflagellate pigment index and dinoflagellate abundance, (c) dinoflagellate pigment index and autotrophic dinoflagellate abundance and (d) dinoflagellate pigment index and autotrophic + mixotrophic dinoflagellate abundance.

Table 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 one index from Vidussi et al. (2001)*.

Symbol	Pigment	Designation of phytoplankton groups
Chla	Chlorophyll <i>a</i> (plus allomers and epimers)	
Chlb	Chlorophyll <i>b</i>	Chlorophytes
Chlc1	Chlorophyll <i>c</i> ₁	
Chlc2	Chlorophyll <i>c</i> ₂	
Chlc3	Chlorophyll <i>c</i> ₃	
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	Cynobacteria
	Pigment sum	Formula
TChla	Total chlorophyll <i>a</i>	Chla + DVChlab + Chlidea
Chlbc	Sum of chlorophyll <i>b</i> and <i>c</i>	Chlb + Chlc1 + Chlc2 + Chlc3
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
	Pigment index	Formula
DVChla/Tchla	Divinyl Chlorophyll <i>a</i> to total chlorophyll <i>a</i>	DV Chla/Tchla
TChla _{TP}	Total chlorophyll <i>a</i> to total pigments	TChla/TPig
PPC _{TP}	Photoprotective carotenoids to total pigment	PPC/TPig
PSC _{TP}	Photosynthetic carotenoids to total pigment	PSC/TPig
Diat _{DP}	Diatom proportion of DP	Fuc/DP
*Dino _{DP}	Dinoflagellate proportion of DP	Per/DP
Flag _{DP}	Flagellate proportion of DP (excluding dinoflagellates)	(All+But+Chlb+Hex)/DP
Prok _{DP}	Prokaryote proportion of DP	Zea/DP

Table 2

Range of total chlorophyll a (mg/m^3) in the different regions (COB, AS, NOB and RP) of Bay of Bengal during SpIM and CSM periods.

Sampling seasons	Regions			
	COB	AS	NOB	RP
SpIM	0.018-0.064	0.023-0.082	0.013-0.165	0.681
CSM	0.002-0.067	0.001-0.015	0.009-0.057	0.196

Table 3

Two-way ANOVA to evaluate the variation in community structure in the different regions in Bay of Bengal during SpIM and CSM periods.

	SpIM		CSM	
	<i>df</i>	<i>p-value</i>	<i>df</i>	<i>p-value</i>
Community	3	0.0736	3	0.4756
Regions	3	0.0382	3	0.0929

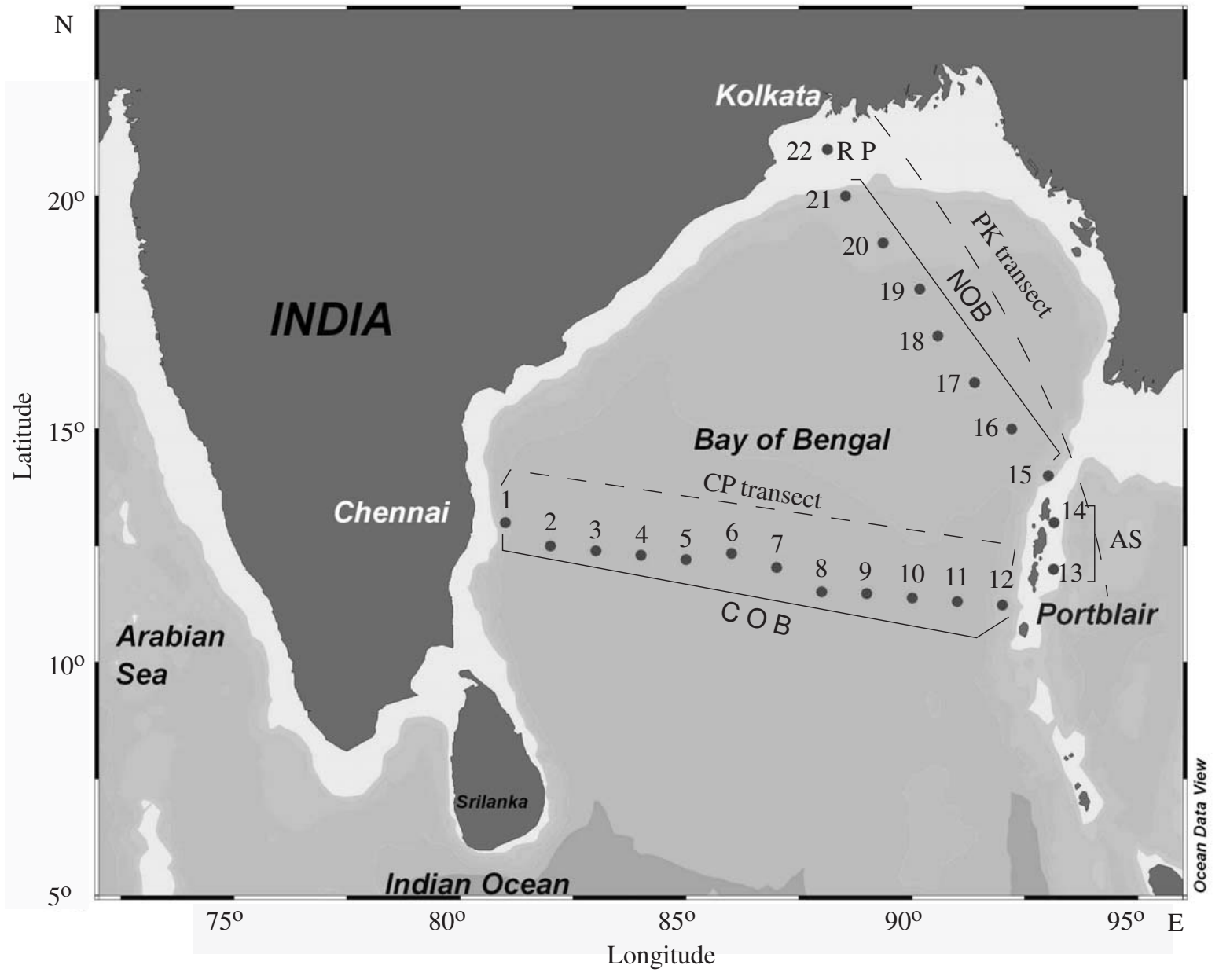


Fig. 1

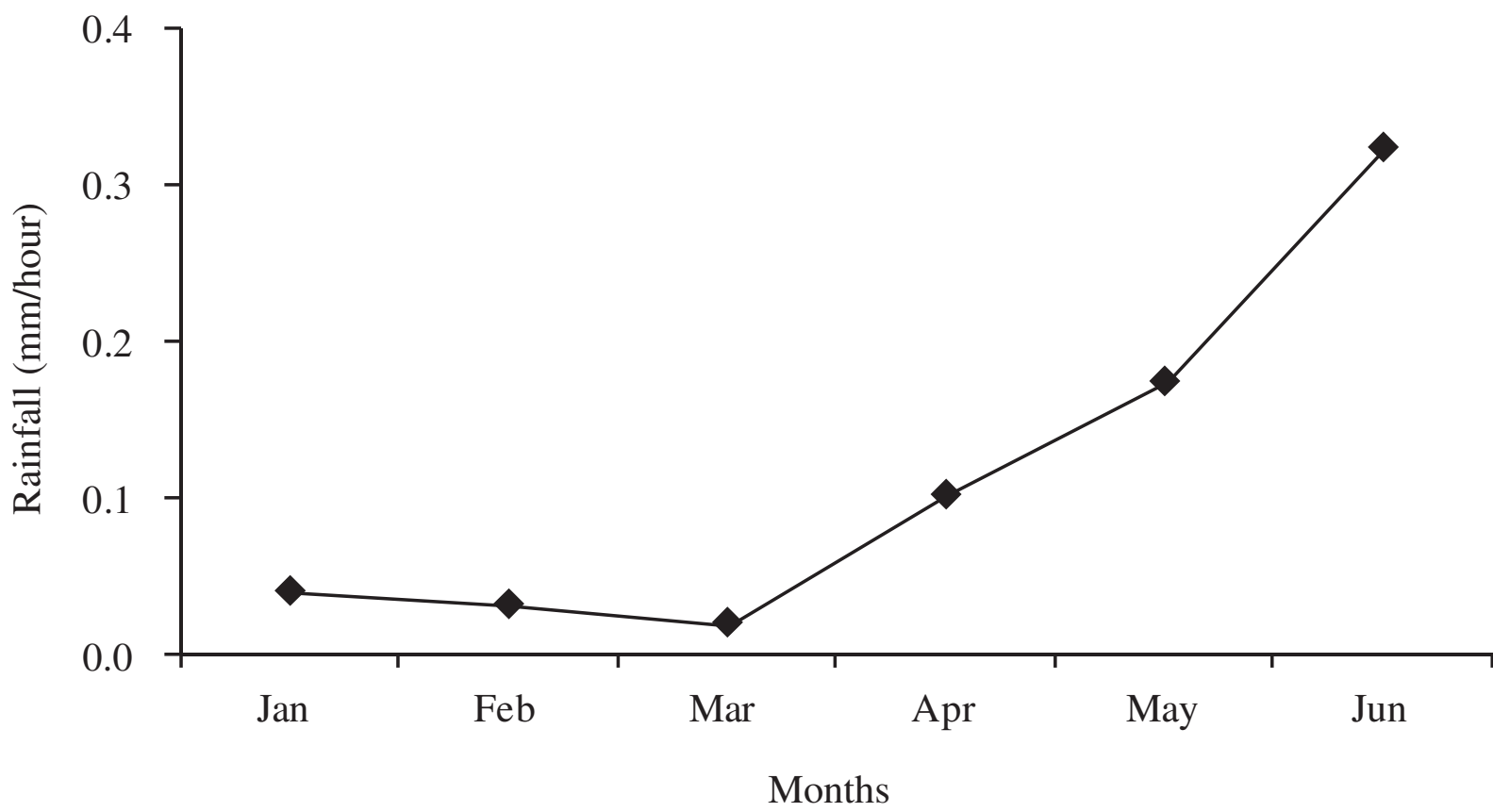
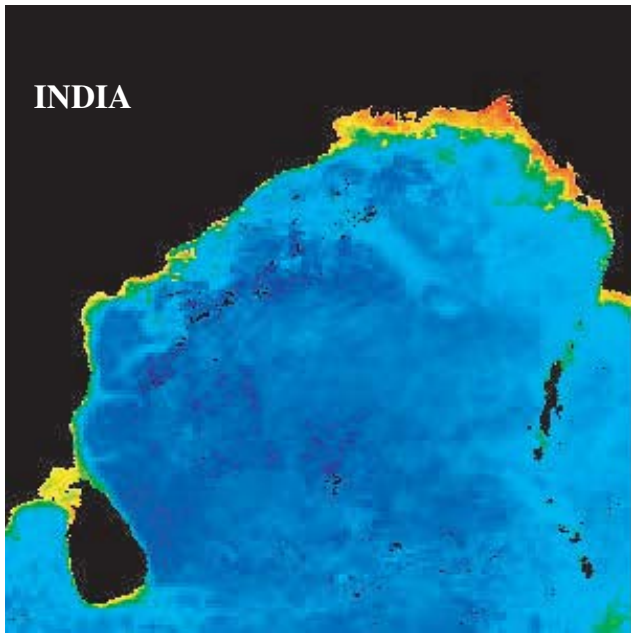


Fig. 2

(a)



(b)

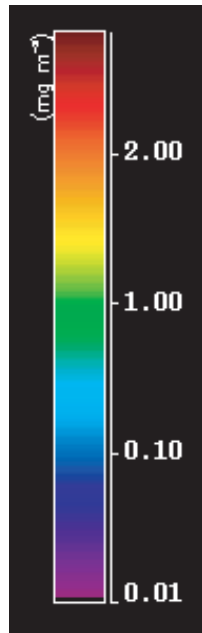
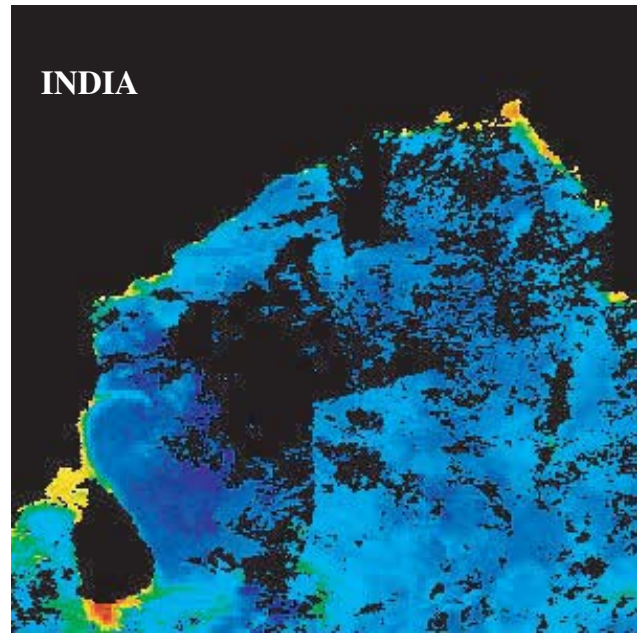
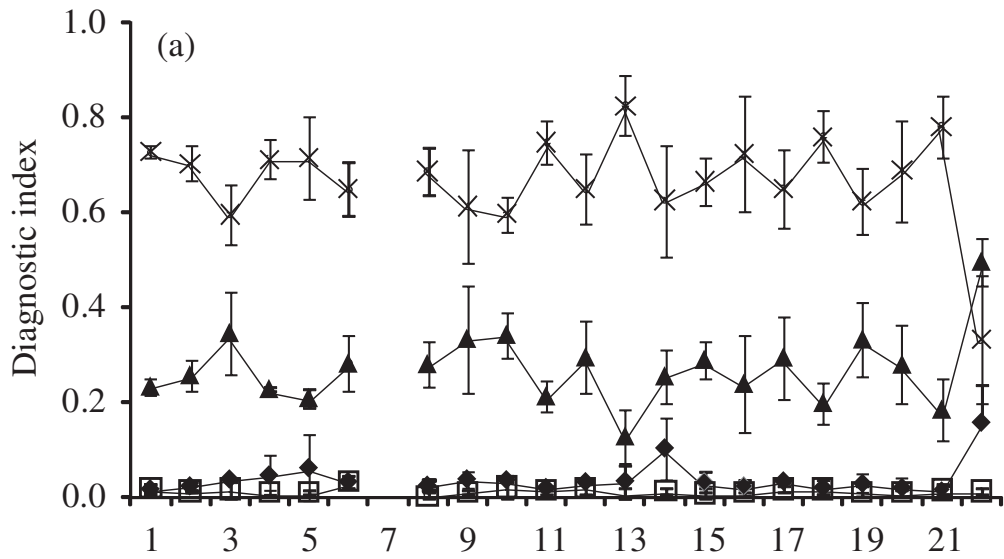
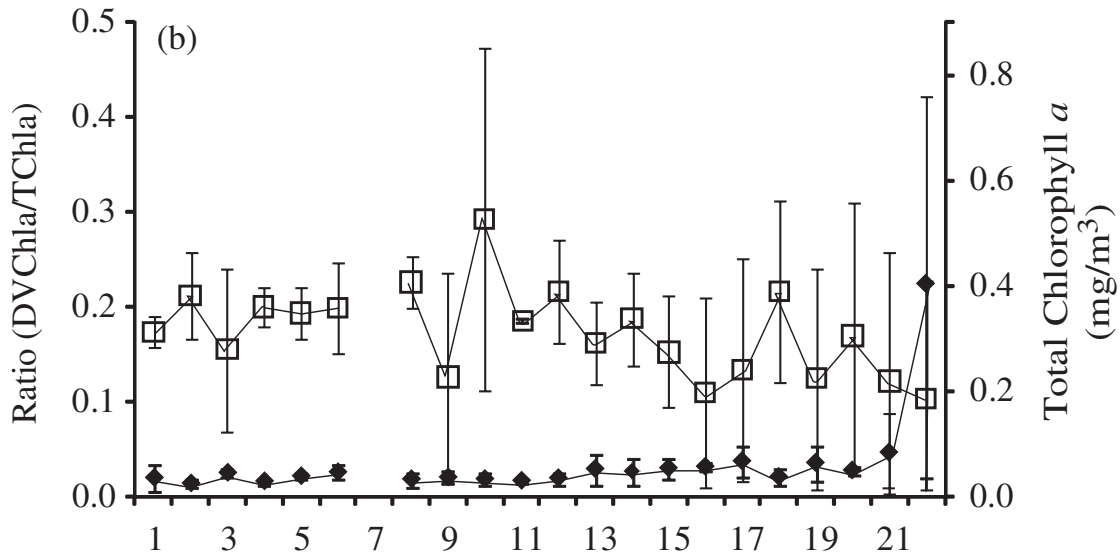


Fig. 3

Figure(s) Diat_{DP} ◆ Dino_{DP} □ Flag_{DP} ▲ Prok_{DP} ✕



TChla ◆ DVChla/TChla □



TChla_{TP} ◆ PPC_{TP} □ PSC_{TP} ▲

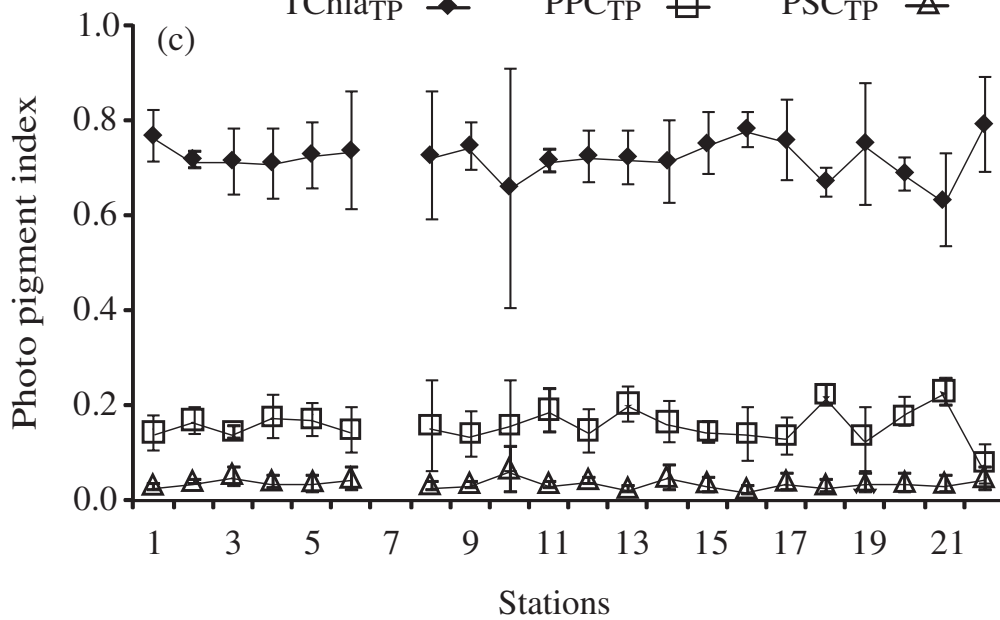


Fig. 4

Figure(s) Diat_{DP} ◆ Dino_{DP} □ Flag_{DP} ▲ Prok_{DP} ✕

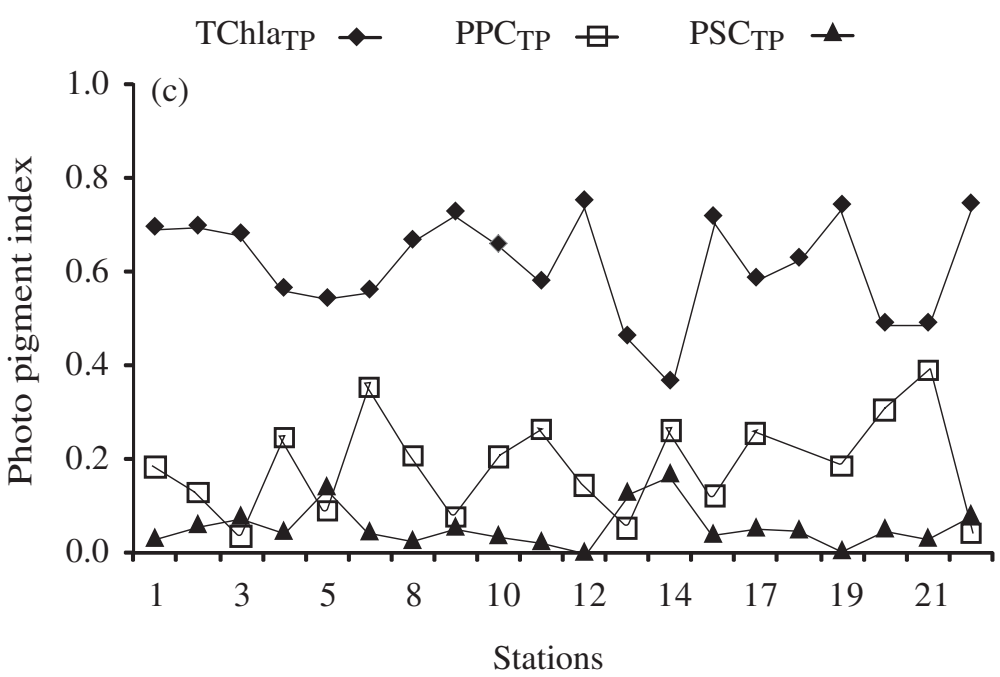
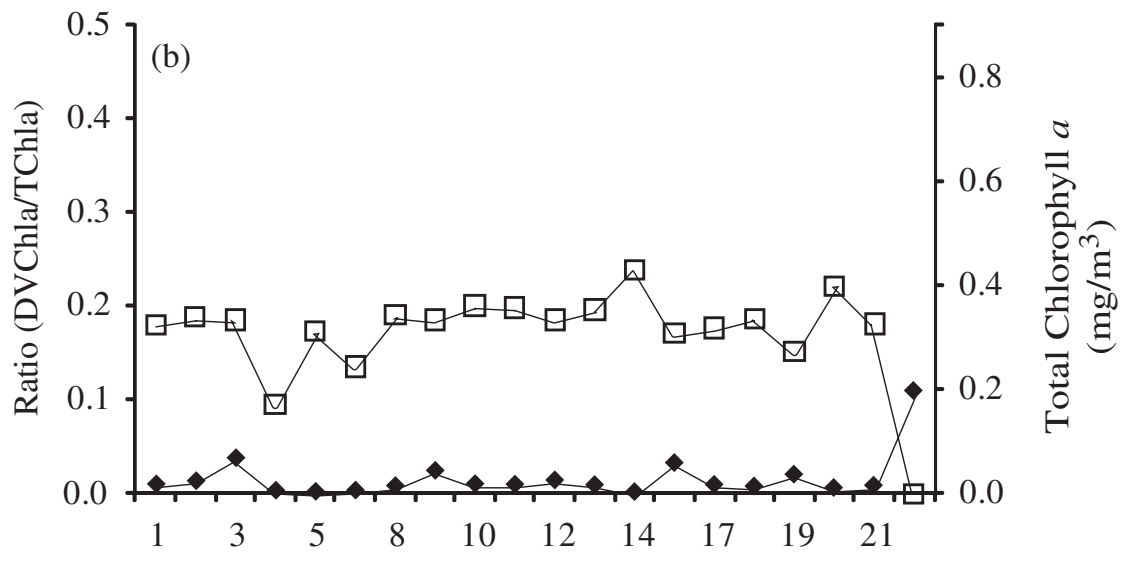
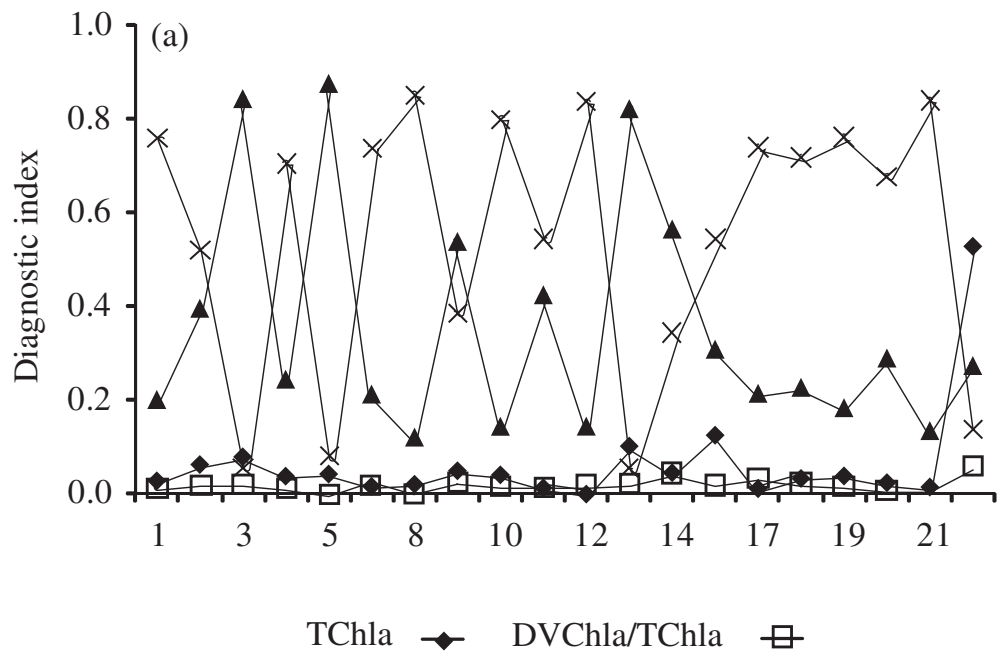


Fig. 5

Figure(s)

Diat_{DP} ■ Dino_{DP} ■ Flag_{DP} □ Prok_{DP} ■

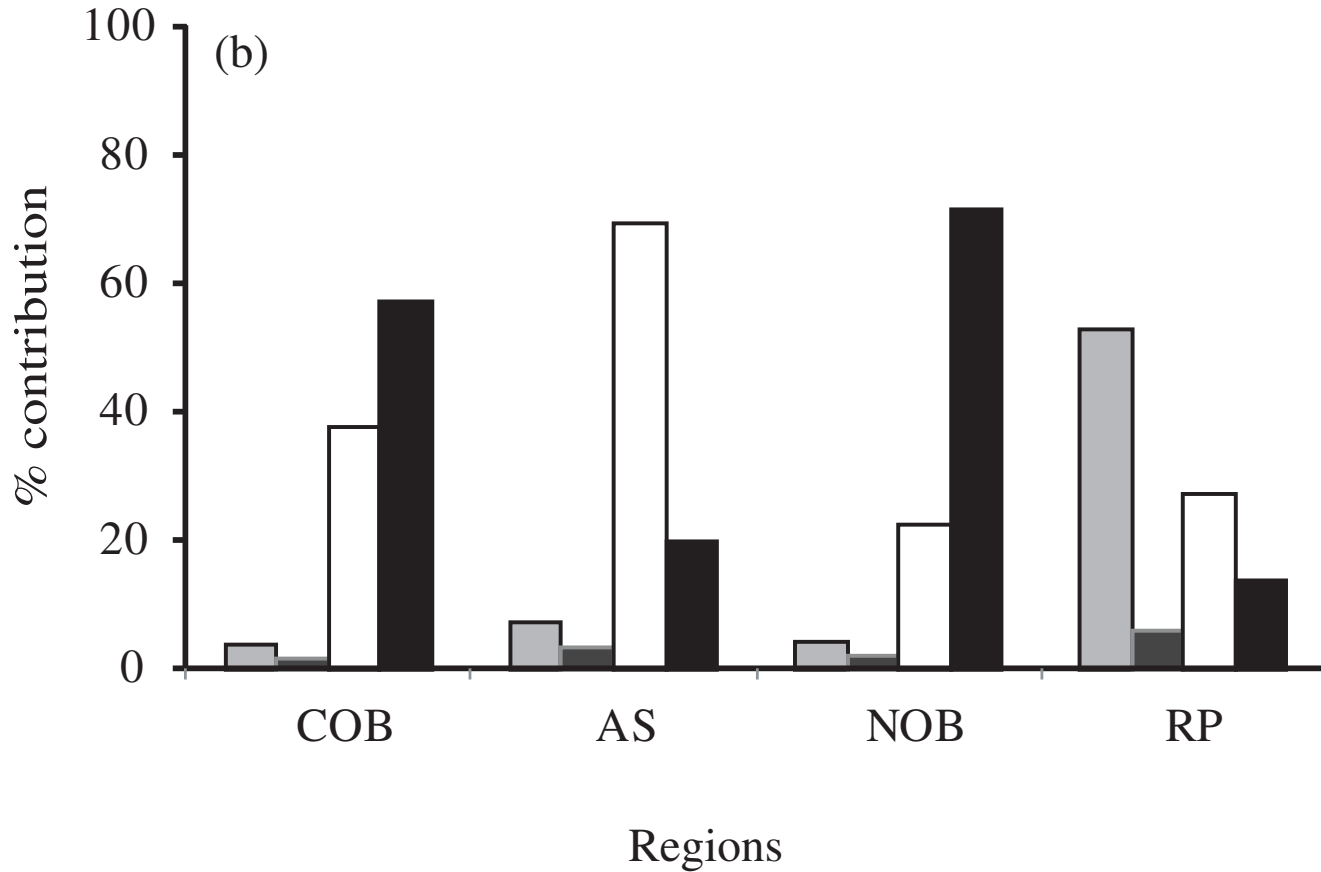
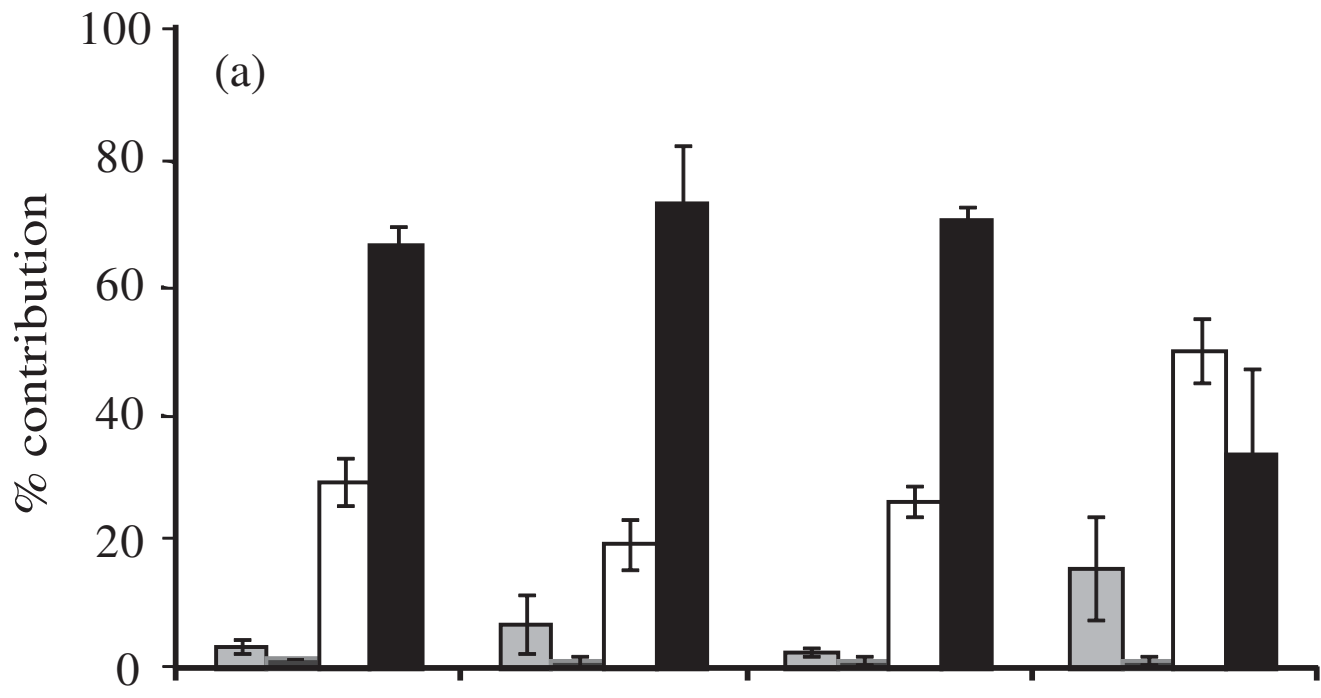


Fig. 6

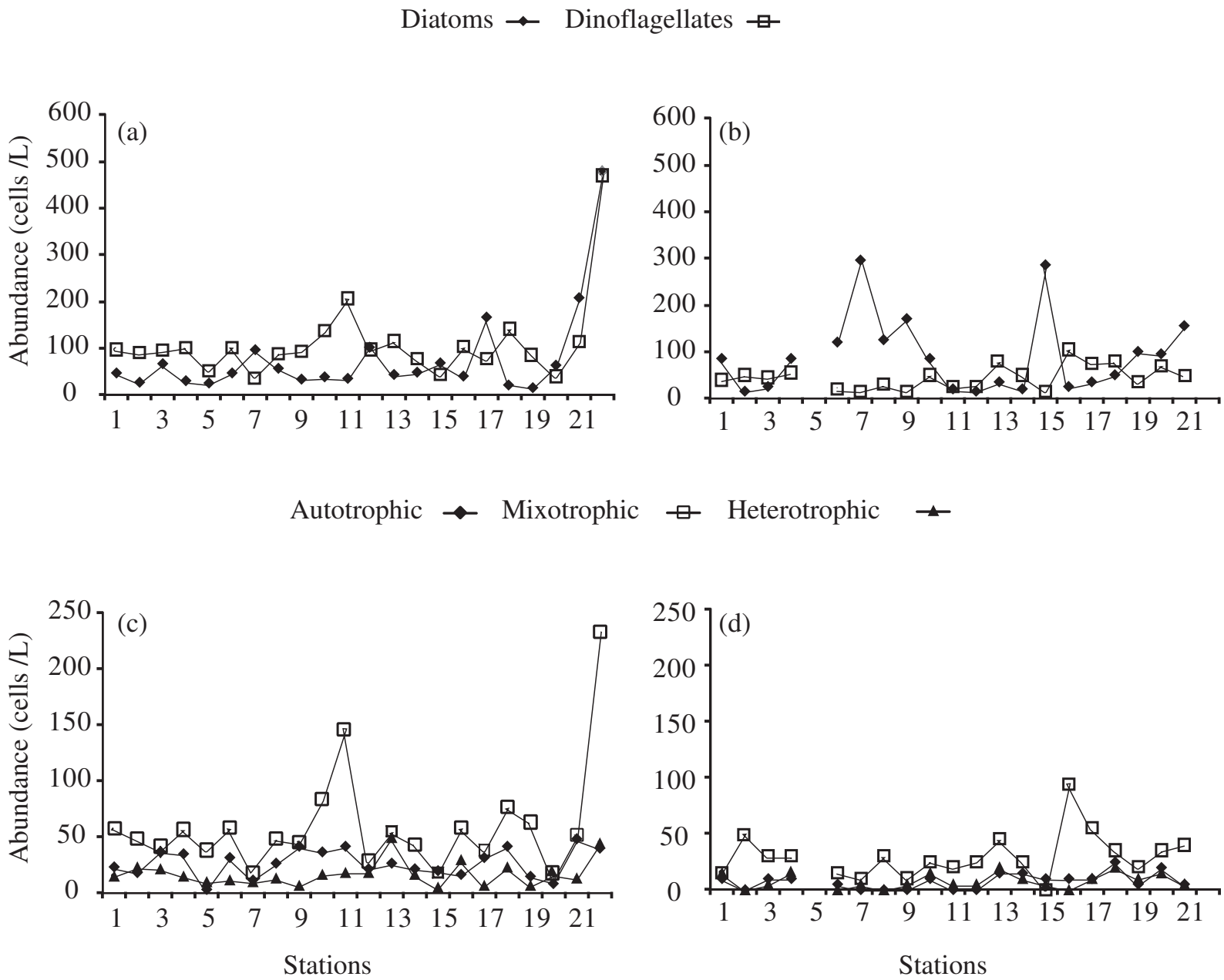


Fig. 7

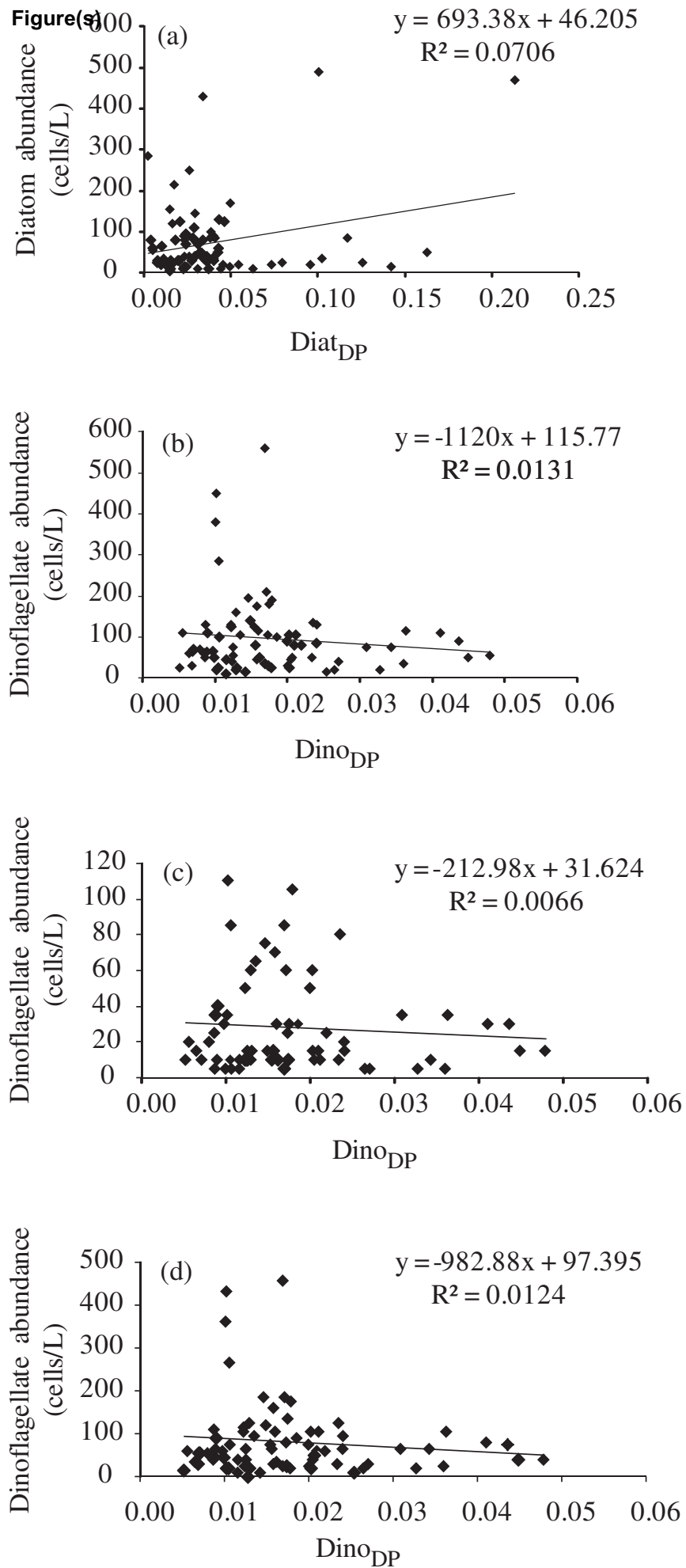


Fig. 8