Spatial variation of phytoplankton pigments along the southwest coast of India

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Abstract

Phytoplankton composition and abundance were studied along the southwestern Indian coast toward the end of the upwelling season in October 2004. Phytoplankton pigment analyses, complemented by limited microscopic counts, which were carried out to determine the community structure. Chlorophyll *a* was the most abundant of all pigments, followed by fucoxanthin. Zeaxanthin was abundantly found in the southern part of the study region (off Trivandrum), whereas fucoxanthin was the dominant marker pigment in the north (off Goa). The inferred shift in the community structure from a dominant picoplankton fraction and Prymnesiophytes to diatom-dominated microplankton toward the north is ascribed to differences in physico-chemical environment.

Keywords: Phytoplankton; prymnesiophytes; pigment; chlorophyll; zeaxanthin; picoplankton; west coast of India; Arabian sea

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1. Introduction

Accounting for approximately one-fourth of all plants in the world (Jeffery and Hallegraeaff, 1985), marine phytoplankton are important contributors to global carbon fluxes (Falkowski et al., 1998). Phytoplankton communities in the ocean comprise many different taxonomic groups, which together determine primary production and various trophic level interactions. Quantification of phytoplankton biomass and community composition is important for understanding the structure and dynamics of marine ecosystems.

Phytoplankton biomass is often quantified through chlorophyll *a* (Chl *a*), which has long been measured following spectrophotometric (Lorenzen, 1967) or Fluorometric (Holm-Hansen et al., 1965) methods. These methods suffer from spectral interference from other degradation products of chlorophyll (chlorophyllides, phaeophoribides and paheophytins). The application of High Performance Liquid Chromatography (HPLC)

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has been found to be more accurate and reliable for estimating not only Chl *a* but other pigments as well. This technique allows quantification of additional 50 phytoplankton pigments and carotenoids in marine phytoplankton (Wright et al., 1991; Jeffrey et al., 1997).

Phytoplankton identification and enumeration is usually done through microscopic examination. This procedure is time-consuming and also requires a high level of taxonomic skill. Moreover, smaller organisms such as picoplankton cannot be identified or counted with this approach. Alternatively, photosynthetic pigments can easily be studied to know the phytoplankton compostion and their physiological status. Most of these pigments (Table 1) have chemotaxonomic association. For example, fucoxanthin is considered to be a marker of diatoms; zeaxanthin, a marker of cyanobacteria; 19'-hexanoyloxyfucoxanthin of Prymnesiophyceae; alloxanthin and crocoxanthin of Cryptomonads; prasinoxanthin of prasinophytes; peridinin and chlorophyll c_2 of dinoflagellates (see Jeffrey et al., 1997, for details of signature pigments of various phytoplankton). However, it should be noted that marker pigments are not exclusive of any one group of algae. In natural environment pigment composition may well vary with prevailing light condition and photoadaptive state (Falkowski et al., 1991).

The semi-annual reversal of coastal currents in the Arabian Sea introduces a high degree of seasonality in the physico-chemical environment (Banse, 1959, 1968; Naqvi et al., 2000, 2006). The consequent strong biological response includes marked changes in the composition of phytoplankton, a subject of several previous studies (e.g., Subrahmanyan, 1959; Dehadrai and Bhargava, 1972; Devassy and Goes, 1988), all of which have been confined to microplankton, though. In general, phytoplankton has been found to be most abundant during the upwelling period that lasts from May-June to October-November. Diatoms constitute the bulk of microplankton exhibiting rich diversity. Dinoflagellates are the next abundant group, occasionally forming blooms sometimes associated with fish kills (Naqvi et al., 1998 and references therein), and in rare cases resulting in paralytic shellfish poisoning (Karunasagar et al., 1984). In September 2004 an incident of fish mortality also accompanied by the release of an obnoxious gas (presumably H_2S) from the sea that caused sickness to children led to a public health alarm around Trivandum (Lat. 8.5^{o}N). Investigations carried out just after

this incident suggested a bloom of holococolithophore and oxygen (O_2) depletion in the upwelled water to be the cause of fish mortality (Ramaiah et al., 2005). Such blooms, not recorded previously, are quite significant in the context of coastal ecosystem dynamics and biogeochemical processes. In this study we provide additional data on the plankton composition offshore of the sampling sites of Ramaiah et al. based on observations made approximately three weeks after the bloom. To our knowledge this is the first report on the spatial distribution of phytoplankton marker pigments along the Indian coast.

2. Material and methods

Water samples were collected at a number of stations off the coast of Kerala (Fig. 1) that was affected by the above-mentioned obnoxious bloom and at few other stations along the southwest coast of India during a cruise of the coastal research vessel (CRV) *Sagar Sukti* during the period 2-10 October 2004. Niskin bottles (5-L) fixed on polyvinyl chloride (PVC)-coated hydrowire were used for sampling. Continuous profiles of salinity and temperature were also obtained at all stations except Sta. 13 using a Sea-bird Conductivity-Temperature-Depth (CTD) profiler (Sea Bird Electronics 25). Dissolved O_2 was measured immediately after collection following the Winkler procedure. Sub-samples for nutrients were deep-frozen for analysis in the shore laboratory performed soon after the cruise with a Skalar analyzer following standard methods (Grasshoff et al., 1983).

For the pigment analysis, samples were immediately filtered on a GF/F filter (pore size 0.7 μm) avoiding exposure of the filter paper to direct light and high temperature. The filter paper was stored in liquid nitrogen until analyzed in the shore laboratory as follows. The frozen filters were immersed in 3 mL of 95% acetone (v/v in deionized water) for extraction using a sonicator probe (5 sec, 25 kHz) under low light and temperature (4°C) followed by storage at -20°C for 4 hours. The extract was passed through a Teflon syringe cartridge (Millipore) having a glass fiber pre acrodisc filter (pore size 0.45 μm, diameter 25 mm) to remove the cellular debris. The clarified extract was collected in a 5 mL amber colour glass vial and placed directly into the temperature controlled (5°C) auto-sampler tray for the (HPLC) analysis.

Pigments were separated following a slight modification of the procedure of Van Hukelem (2002), which provides quantitative analysis of 20 pigments and qualitative analysis of several others. The HPLC system was equipped with an Agilent 1100 pump together with online degasser, an Agilent diode array detector connected via guard column to an Eclipse XDB C8 HPLC column (4.6x 150mm) manufactured by Agilent Technologies. The column was maintained at 60°C. Elution at a rate of 1.1 ml/minute was performed using a linear gradient program over 22 minutes with 5/95% and 95/5% of solvents B/A being the initial and final compositions of the eluant, where solvent B was methanol and solvent A was (70:30) methanol and 1 M ammonium acetate (pH 7.2) instead of 28 mM solution as recommended in the protocol. An isocratic hold on 95% B was necessary from 22-27 minutes for the elution of the last pigment (α- or β-carotene) at approximately 27 minutes. After returning to the initial condition (5% solvent B) by 31 minutes, the column was equilibrated for 5 minutes prior to next analysis. The eluting pigments were detected at 450 and 665 nm (excitation and emission) by the diode array detector. All chemicals used were of HPLC-grade, procured from E. Merck (Germany).

Commercially available standards obtained from DHI Inc (Denmark), were used for the identification and quantification of pigments. Solutions of chlorophyll *a,* chlorophyll *b,* chlorophyll c_2, chlorophyll c_3, fucoxanthin, peridinin, neoxanthin, diadinoxanthin, diatoxanthin, alloxanthin, violaxanthin, canthaxanthin, divinyl chlorophyll *a*, α-carotene, β-carotene and zeaxanthin were run in order to obtain calibration curves and absorption spectra and to determine detection limits. Identification was based on the retention time and peak shape, i.e. through fingerprint matching with known peak shape from the diode array spectra library created by running pure standard of individual pigments. The concentrations of the pigments were computed from the peak areas.

With the protocol followed, complete resolution and separation of α- and β-carotene could not be achieved; these pigments were, therefore, grouped together and treated as total carotenoids.

In order to know the contribution of each community, data reduction was performed and size structure indices were made (cf. Vidussi et al., 2001) for a few selected stations. Pigment data reduction was performed with the size structure correspondence presented

in Table 2. The diagnostic pigment (DP) is defined as the sum of seven pigments as follows:

DP= ZEA+ CHL *b* + ALLO+ 19'-HF+ 19' BF + FUC + PER.

The biomass proportion (BP) associated with each size class [picoplankton (<2 μm), nanoplankton (2-20 μm) and micropankton (20-200 μm)] is defined as,

BP_{pico}=(ZEA+CHL *b*)/DP

BP_{nano}= (ALLO + 19'-HF+19'BF)/DP

BP_{micro}= (FUC+PER)/DP

A linear regression analysis performed between DP and Chl *a* showed a significant correlation (r^2=0.84, n=21, $p<0.001$) indicating that DP can also act as a proxy of phytoplankton biomass. The existence of similar correlation between DP and Chl *a* has also been reported previously (Barlow et al., 2005; Vidussi et al., 2001). Thus, although the DP/Chl *a* ratio may change with variations in nutrient dynamics and prevailing light condition, the DP can still be used as a surrogate of phytoplankton biomass and for identifying general trends.

Other marker pigments were categorized into accessory photosynthetic pigments (APSP = PER + BUT + FUC + HEX + VIOLA + CHL*b*; Gibb et al, 2001) and accessory photo protective pigments [APPP = DIADINO + ALLO + DIATO + ZEA + TOTAL CAROTENEs (α+β)].

Microscopic examinations of phytoplankton were carried out to enumerate diatoms and dinoflagellates to partially evaluate the results of the pigment analysis.

3. Results

3.1. Hydrography

Hydrographic and chemical data collected during the cruise are presented in detail elsewhere (Kurian et al., submitted), and will only be discussed briefly here. Weak upwelling was found to occur everywhere in the study region. Surface temperature varied from 26.0 to 27.8°C, decreasing with depth to a minimum of 25.4° C at 25-30 m at near-shore stations (1-5); farther offshore, near-bottom temperature fell to 23.8°C at the

maximum depth of sampling (45 m, Sta. 12). Surface salinity ranged between 34.4 and 35.3, increasing with depth to a maximum of 35.8 in near-bottom waters. The upwelled waters were expectedly nutrient-enriched with the nitrate (NO_3^-) concentration varying from ~0 to 2.6 μM at the surface to a maximum of ~10 μM at depth (40 m, Sta. 14). However, at the two northernmost stations (15 and 17) where the near-bottom O_2 concentrations were, respectively, ~6 and 47 μM, the upwelled water was conspicuously NO_3^--depleted (< 2 μM), presumably due to the loss through denitrification that could have led to sulphate reduction just before our observations (cf. Naqvi et al., 2000). This is supported by the presence of high concentrations of ammonium (NH_4^+) in near bottom waters (maximum ~11 μM at 30 m, Sta. 17). Near-bottom O_2 levels were relatively higher at the southern stations (81-119 μM at depths exceeding 20 m), reflecting both the expected greater oxygenation of the upwelled water (Naqvi et al., 2006) and a weakening of the process of upwelling toward the south (see below).

3.2. Pigments

Chromatographic analysis revealed the presence of a wide range of pigments, exhibiting a clear spatial variability. Chl *a* and fucoxanthin were the two most abundant pigments, present at all sampling locations. There was considerable difference in the accessory pigment composition between stations located in the southern (2, 3, 5, 7, 8, 10 and 13) and the northern (15 and 17) portions of the observational area. Zeaxanthin (1-564 ng l^{-1}), 19'hexanoyloxyfucoxanthin (3-309 ng l^{-1}), and chlorophyll c1c2 (1-36 ng l^{-1}) were found to be more dominant at the southern stations. This indicates the proliferation of picoplankton (cyanobacteria) and prymnesiophytes in this region. It may be noted that stations 2, 3, 5, 10 and 13 were all located very close to the shore. At the northern stations, fucoxanthin became much more abundant with peridinin occurring occasionally, indicating the presence of diatoms and dinoflagellates.

Chl *a* was the most abundant of all pigments, but its concentration exhibited considerable spatial variability (Fig. 2). The highest concentration (8322 ng l^{-1}) was recorded just below the surface (depth 5 m) at Sta. 3, whereas the lowest (42.0 ng l^{-1}) occurred at surface of Sta. 11, further away from the coast along the same transect. Chl *a* concentrations were generally the highest at coastal stations reflecting eutrophic

conditions resulting from upwelling. Significant linear correlations were found between Chl *a* and the photosynthetic carotenoid and photosynthetic carotenoids and photo protective pigment (Fig. 3)

Fucoxanthin was the next most abundant pigment, occurring at all stations. This pigment together with peridinin is taxonomical biomarker for diatoms and dinoflagellates. Its concentration was the highest (1811 ng l^{-1}) at Sta. 17 where the Chl *a* concentration was 2262 ng l^{-1}. Fucoxanthin also has been reported to be one of the major accessory pigment in the central Arabian Sea with certain species of Phaeocystis being its potential source in this region (Latasa et al., 1998). Surface distribution of pigments for selected location is shown in Fig. 4.

A high surface value of APSP was recorded at Sta. 5 (1183 ng l^{-1}), which was located in the region of the above-mentioned holococolithophore bloom. Fucoxanthin was found to be the most abundant of all photosynthetic carotenoids, making the largest contribution to the APSP budget. Maximal concentrations of APSP were observed close to the sea surface. These pigments show peak absorption and are very active within the wavelength range 400-500 nm.

4. Discussion

Oceanic phytoplankton comprise of many different groups, which makes visual interpretation of the data sometimes complicated, necessitating data reduction. The size structure provides useful insights into ecosystem functioning as the larger phytoplankton are known to be generally dominant in nutrient-rich, productive waters whereas smaller phytoplankton are more abundant under oligotrophic conditions.

The biomass proportions derived from the marker pigments and DP as defined above indicate that the picoplankton community contributed significantly to the biomass structure in the southern-most part of our study region where a bloom of holococolithophore is reported to have occurred about 3 weeks earlier (Ramaiah et al., 2005). In contrast, microplankton appear to be most abundant in the northern transect. For the whole sampling area, the order of abundance was microplankton (38.6%), picoplankton (32.7%) and nanoplankton (24.6%).

Picoplankton communities are generally dominated by prochlorophyte and cyanobacteria, which are common in tropical oceans and most likely, represent systems associated with regenerated production (Claustre, 1994). The greater abundance of picoplankton especially at the surface at the southern stations as compared to the northern stations probably reflects the difference in macronutrient availability. Upwelling, which brings about nutrient enrichment of near-surface waters along the west coast of India during and just after the southwest monsoon, begins and ends a little earlier in the south than in the north. With the period of our observations being close to the end of the upwelling period, this process was therefore more developed at the northern-most stations, supporting larger biomass of microplankton. A significant contribution was also seen from the nanoplankton community except at Sta. 4.

The abundance of diatoms, confirmed by microscopic analysis (see below), at Stations 15 and 17 is intriguing given the low levels of NO_3^- and high concentrations of NH_4^+. Our results are in conflict with the belief that diatoms generally utilize NO_3^- (usually the most abundant form in the upwelling systems) and the presence of NH_4^+ in high concentrations, which has an inhibitory effect on NO_3^- uptake, may not favour their growth (Dugdale et al., 2002).

The correlation between the microscopically determined diatom cell density and fucoxanthin associated with diatoms was reasonably good, if a few outliers were ignored (Fig. 5). Deviations of the pigment-derived data from the microscopic data may occur because some of the pigments could be commonly present in different algal classes; in fact, the pigment composition may sometimes vary within species, physiological condition and photoadptaive states (Falkowski et al., 1991). Nevertheless, our results seem to provide a reasonably good account of phytoplankton composition that is consistent with the hydrography.

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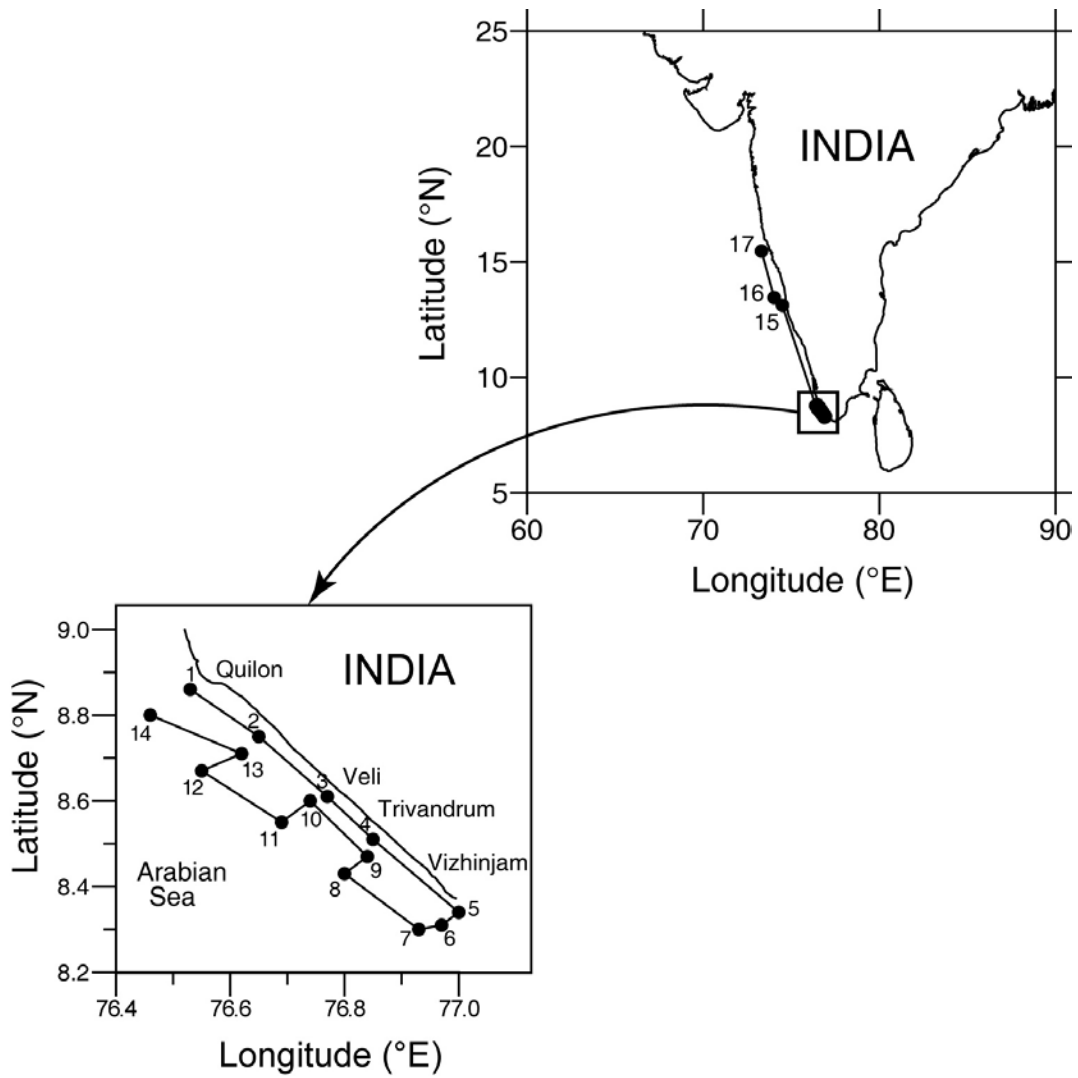
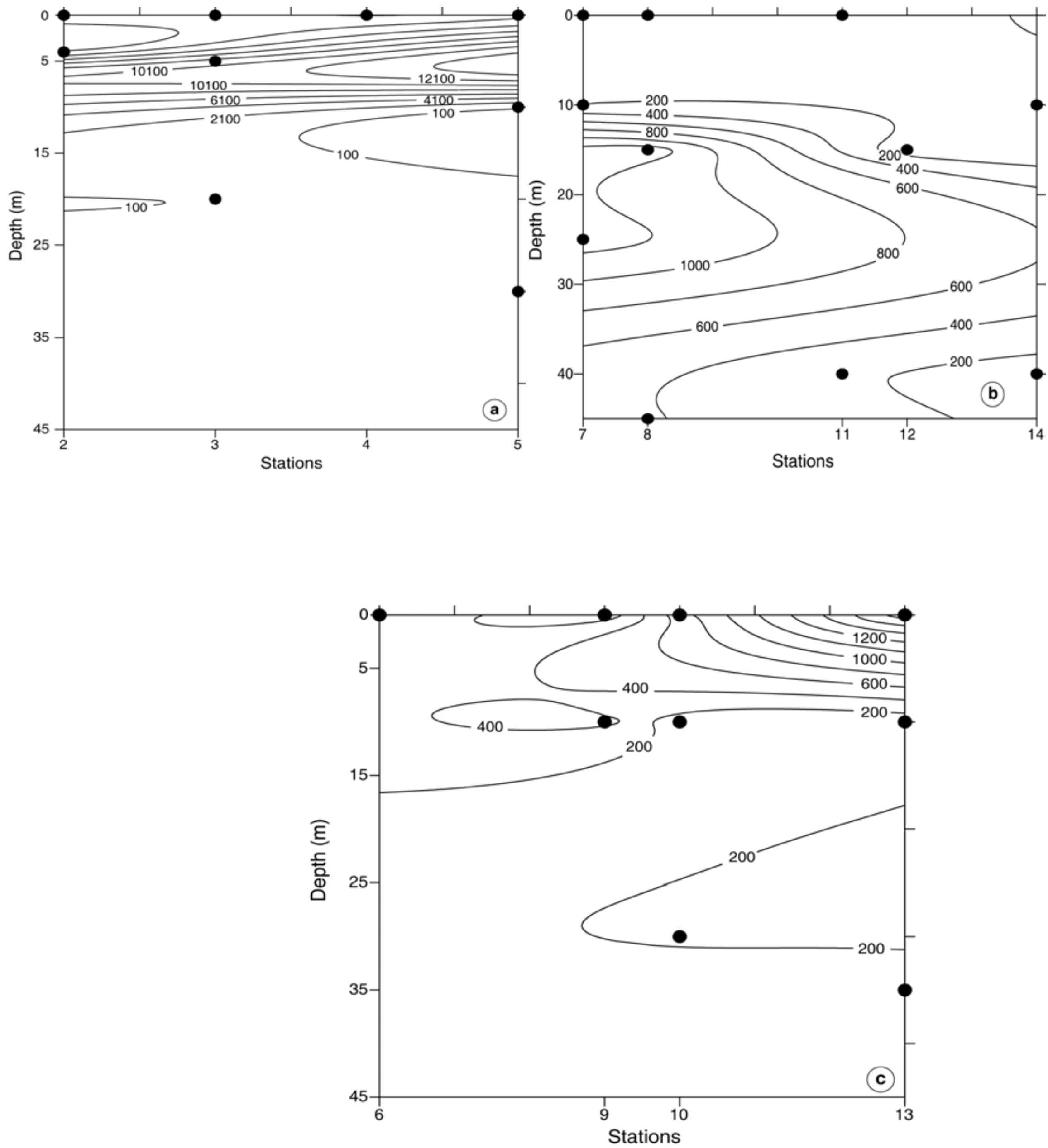
Fig. 1.



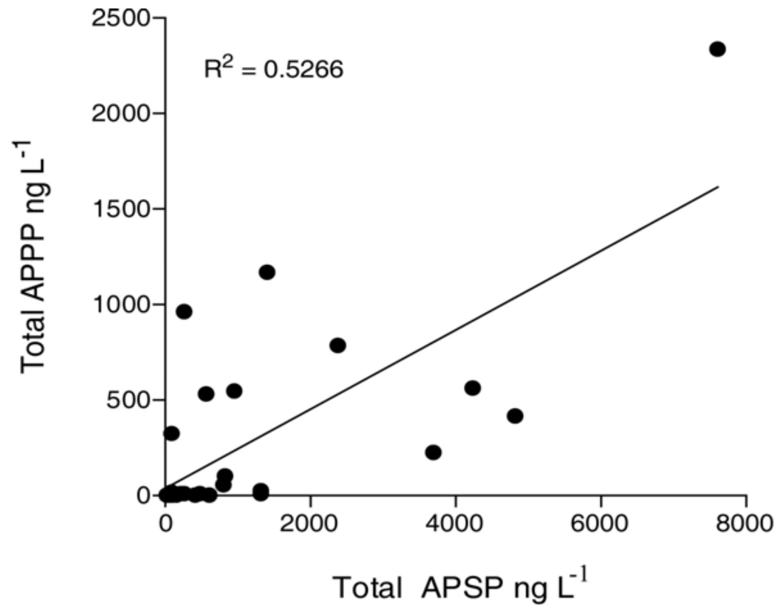
Station locations: Cruise track indicating the sampling locations along the southwest coast of India.

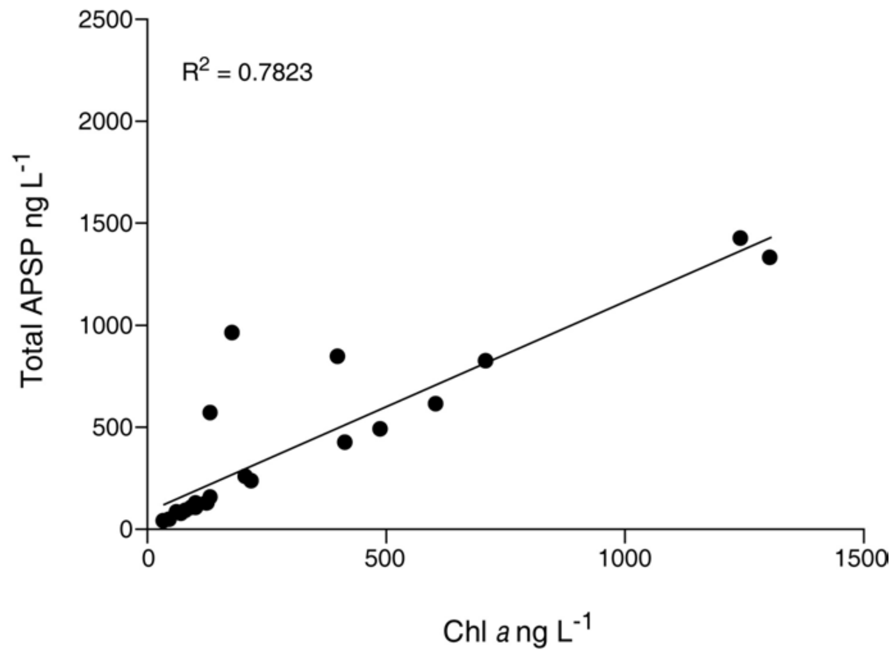
Fig. 2.



Spatial variability of chlorophyll a (ng L_1). (a) Chlorophyll a distribution in inshore waters (2, 3, 4 and 5). (b) Chlorophyll a distribution in off shore waters along the same transects (7, 8, 11 and 14). (c) Chlorophyll a distribution at the middle transects (6, 9, 10 and 13). The black dots represent the sampling points.

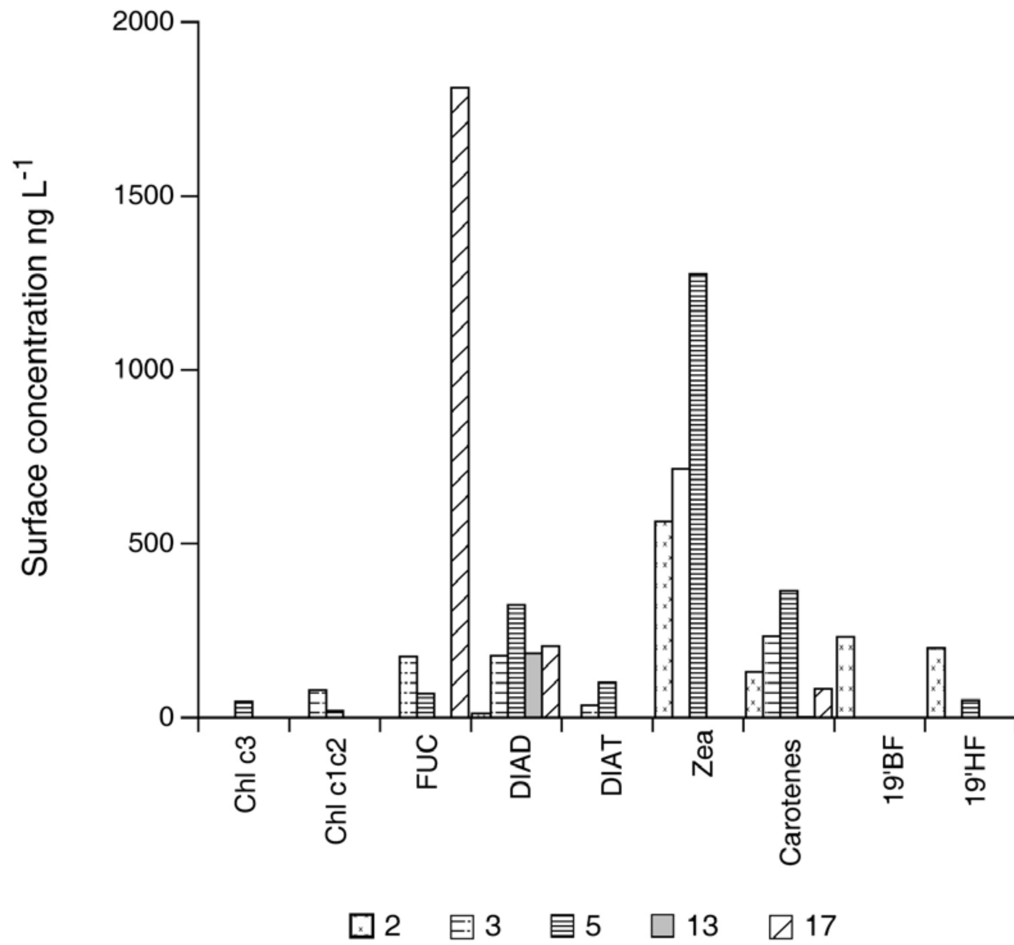
Fig. 3.





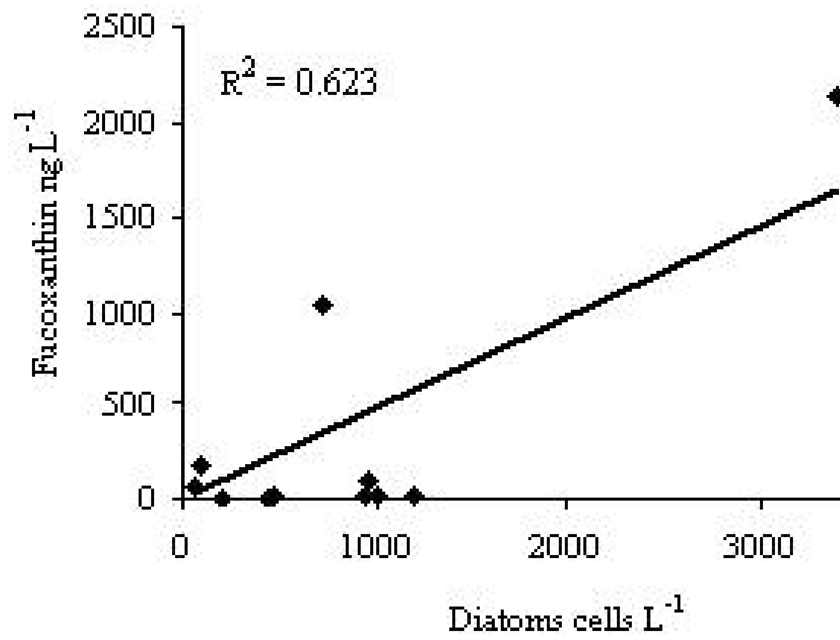
Relationship between photosynthetic and photoprotecting carotenoid pigments; relationship between Chl a and photosynthetic carotenoids.

Fig. 4.



Representative surface distribution of individual pigments at stations 2, 3, 5, 13, and 17.

Fig. 5.



Comparison of diatoms cell density obtained from microscopic data and specific marker pigment fucoxanthin obtained from HPLC.

Table 1
Chemotaxonomic relation used in the study of phytoplankton taxonomy.
(Gibb et al., 2001; Jeffrey et al., 1997).

Pigment	Abbreviation	Occurrence
Chlorophyll a	CHL*a*	Total algal biomass (including cyanobacteria)
Chlorophyll b	CHL*b*	Chlorophytes, prasinophytes
Chlorophyll c1c2	CHL*c2*	Diatoms, prymnesiophytes, cryophytes, dinoflagellates
Chlorophyll c3	CHL*c3*	Prymnesiophytes, cryophytes
Alloxanthin	ALLO	Cryptophytes
19’-Butanoyloxyfucoxanthin	BUT	Chrysophytes, prymnesiophytes
Diadinoxanthin	DIADINO	Diatoms, dinoflagellates, prymnesiophytes, chrysophytes
Diatoxanthin	DIATO	Diatoms, dinoflagellates, prymnesiophytes, chrysophytes
Divinyl chlorophyll a	DvCHL*a*	Prochlorococcus sp.
Fucoxanthin	FUC	Diatoms, prymnesiophytes, chrysophytes
19’-hexanoyloxyfucoxanthin	HEX	Prymnesiophytes
Lutein	LUT	Chlorophytes, prasinophytes
Peridinin	PER	Autotrophic dinoflagellates
Violaxanthin	VIO	Chlorophytes, prasinophytes
Prasinoxanthin	PRAS	Prasinophytes
Zeaxanthin	ZEA	Cyanobacteria, Prochlorococcus sp.

Table 2

Size structure correspondence used for the performing data reduction. For further details see Vidussi et al. (2001).

Pigments	Taxonomic Significance	Size (μm)	References
Zeaxanthin	cyanobacteria	<2	1,2,5
Divinyl- chlorophyll *a*	and prochlorophytes	<2	3,4,5
Chlorophyll *b*	green flagellates and prochlorophytes	<2	6,7,8,9
19’hexanoyloxyfucoxanthin	chromophytes and nanoflagellates	2-20	10,11,12,13,14
19’butanoyloxyfucoxanthin	chromophytes and nanoflagellates	2-20	15,16
Alloxanthin	cryptophytes	2-20	14,17
Fucoxanthin	diatoms	>20	10,11,13,18
Peridinin	dinoflagellates	>20	18,19,20

References are 1, Gieskes et al .(1988); 2, Guillard et al.(1985); 3, Goericke and Repeta (1992); 4, Gieskes and Kraay (1983 a); 5,Chisholm et al. (1988); 6, Partensky et al (1993); Moore et al . (1995); 8, Jeffrey (1976); 9, Simon et al . (1994); 10, Bjornland and Liaaen-Jensen (1989); 11, Hooks et al (1998); 12, Arpin et al (1976); 13, Wright and Jeffrey (1987); 14, Jeffery and Vesk (1997); 15, Andersen et al (1993); 16, Bjorland et al. (1989); 17 , Gieskes and Kraay (1983 b); 18, Kimor et al. (1987); 19, Johassen et al.(1974); and 20, Jeffrey et al .1975.