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ORIGINAL RESEARCH ARTICLE

Characterization of phytoplankton size-structure based productivity, pigment complexes (HPLC/CHEMTAX) and species composition in the Cochin estuary (southwest coast of India): special emphasis on diatoms

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Abstract Seasonal studies on size-fractionated phytoplankton productivity (biomass and primary production), marker pigments, and species composition and abundance were carried out in the Cochin estuary (CE), located on the southwest coast of India, to identify the critical environmental factors that control the consistent preponderance of diatoms. The overall results of the study showed a significant contribution of small-sized phytoplankton, specifically nanophytoplankton (2—20 μm), to the total chlorophyll *a* and primary production in the estuary, regardless of seasons. Diatoms constituted the major phytoplankton taxa, showed an exceptional seasonal scale increase in numerical abundance during the post-southwest monsoon. The relative increase in fucoxanthin (biomarker of diatoms) over other marker pigments substantiated the numerical dominance of diatoms throughout the sampling periods. This is the first study in the CE in which phytoplankton marker pigments have been detected and elucidated the seasonality of functional groups based on HPLC/chemotaxonomy analytical

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approaches. The prevalence of high $Diat_{DP}$ and diatom chlorophyll *a* equivalent (estimated by CHEMTAX), further confirmed the preponderance of diatoms in the CE, despite the intermittent dominance of cyanophytes and cryptophytes (monsoon period). The consistent increase in SPM levels (> 25 mg L^{-1}), established at all sampling stations, indicated that the water column turbidity might be one of the significant environmental factors hindering the growth of large-sized phytoplankton (ca. >20 μm) in the CE even if the system invariably holds high inorganic nutrients, irrespective of seasons.

1. Introduction

Estuaries are one of the most productive coastal water ecosystems worldwide as it receives surplus amounts of inorganic nutrients from various terrestrial sources and thus, accelerates the tremendous growth and biomass accumulation of diverse phytoplankton communities. The inorganic forms of nitrogen (N), phosphorus (P), and silica (Si) are recognised as the essential macro-nutrients triggering the growth and abundance of phytoplankton in coastal inland waters (Cloern, 2001; Malone et al., 1996). Normally, N is reported to be the prime limiting nutrient of phytoplankton in marine ecosystems (Ryther and Dunsten, 1971), whereas P in freshwater ecosystems (Schindler, 1977). In estuaries, nutrient availability is apparently influenced by the prevailing flow patterns, enabled mainly by the river flow and terrestrial runoff, and the tidal exchange of seawater normally diluting the nutrient levels. Therefore, the tidal dynamics play a vital role in regulating the estuarine biological productivity (particularly phytoplankton production), water quality, and material transport and dispersion (Cloern et al., 2014). Furthermore, phytoplankton growth in the coastal/estuarine systems is very much dependent on a suit of physical factors, i.e., water column turbidity, vertical mixing, changes in flushing and residence time, altered optical properties, etc. (Nixon, 1986; Paerl et al., 2007). In river-dominated estuaries, the prevalence of high water column turbidity critically limits phytoplankton growth by increasing light attenuation in the euphotic zone (Cloern, 1987). A numerical modeling study has revealed that estuarine phytoplankton productivity drastically decreases when the SPM level rises from 10 to 100 mg L^{-1} (Peterson and Festa, 1984).

Since the phytoplankton community quickly responds to anthropogenic pressures, particularly to nutrient loadings and toxin inputs, it has been recognized as one of the sensitive biological indicators of eutrophication in coastal waters (Coutinho et al., 2012; Paerl et al., 2003). Phytoplankton synthesizes organic carbon by photosynthesis and plays vital roles in coastal production, nutrient cycling, and pelagic food web dynamics (Paerl et al., 1998; Pinckney et al., 2001; Wetz and Paerl, 2008). The phytoplankton photosynthesis (*primary production*) is primarily enabled by certain chemicals in chloroplasts, known as photosynthetic pigments or photopigments (chlorophylls and carotenoids), which usually functions as the indicators of the physiological status of each phytoplankton functional group (PFG), for example, chlorophytes, cryptophytes, cyanophytes, haptophytes, bacillariophytes, dinophytes, etc. (Reynolds, 1997). Among photosynthetic pigments, chlorophyll *a* (chl *a* hereafter) has been used as a proxy of phytoplankton biomass for several years as they are the major light-harvesting pigments (Richards and Thompson, 1952). The coastal phytoplankton community habitually exhibits a distinctive temporal scale variability in its chl *a* content, pigment complexes, primary production rates, and species abundance and diversity as per the prevailing hydrological changes (Paerl et al., 2003). The large-sized phytoplankton tends to grow in nutrient-rich coastal waters (Chisholm, 1992; Gibb et al., 2001; Zhu et al., 2009) and are mostly comprised of larger (>20 μm) diatoms and dinoflagellates (Patil and Anil, 2015; Sarthou et al., 2005). The availability of surplus dissolved inorganic silica (DSi) causes the consistent proliferation of diatoms in coastal waters in addition to the availability of N and P (Conley and Malone, 1992; Officer and Ryther 1980). When DSi becomes the limiting factor for phytoplankton growth, there is a shift from diatoms to non-siliceous algae (D'Costa and Anil, 2010). Diatoms are thus recognized as a biological indicator of acceptable water quality in coastal waters and good food sources for filter-feeding zooplankton, benthic invertebrates, larval fishes, etc. (Pearl et al., 2003).

Each PFG has its own characteristic photosynthetic (marker) pigments or pigment suits, which signifies the corresponding trophic status for a given area (Barlow et al., 2008). Some of these marker pigments are designated as diagnostic pigments (DP) for specific PFGs, for example, fucoxanthin for diatoms, peridinin for dinoflagellates, zeaxanthin for cyanophytes, alloxanthin for cryptophytes, etc. (Table 1). However, overlapping can be evident between these groups for sharing certain pigments (Barlow et al., 2008; Wright and Jeffrey, 2006). The introduction of high-performance liquid chromatography (HPLC) has been enabled oceanographers for the effective detection and quantification of phytoplankton marker pigments; which ultimately provide holistic information about the physiological/ecological status of individual phytoplankton functional communities (Jeffrey et al., 1997; Wright and Jeffrey, 2006). The HPLC based pigment study is not only a faster and reliable analytical tool, particularly for analyzing a large number of samples but also it requires less taxonomic knowledge than the conventional microscopic phytoplankton identification (Lewitus et al., 2005; Wright et al., 1996; Wright and Jeffrey, 2006).

Table 1 Abbreviations, names and selected taxonomic designations (Jeffrey et al., 1997) for chlorophylls, carotenoids, pigment sums and indices (* excluding dinoflagellates).

Abbreviations	Pigment	Designation
Chl *a*	Chlorophyll *a*	
Chl *c2*	Chlorophyll *c2*	
Chl *c3*	Chlorophyll *c3*	
Chl *b*	Chlorophyll *b*	Chlorophytes
DV chl *a*	Dininyl chlorophyll *a*	Prochlorophytes
Allo	Alloxanthin	Cryptophytes
Diad	Diadinoxanthin	
Fuco	Fucoxanthin	Diatoms (major)
Hex	19'Hexanoyloxy fucoxanthin	Prymnesiophytes
Per	Peridinin	Dinoflagellates
Zea	Zeaxanthin	Cyanobacteria
β - Car	β - Carotene	
But	19'Butanoyloxy fucoxanthin	Chrysophytes
Viol	Violoxanthin	
Pigment sum		*Formula*
TChl *a*	Total chlorophyll *a*	Chl *a* + DV Chl *a* + Chlide *a*
PPCs	Photoprotective carotenoids	Allo+Diad+Vio+Zea+β-Car
PSCs	Photosynthetic carotenoids	But+Fuco+Hex+Per
DP	Diagnostic pigments	PSC+Allo+Zea+TChl *b*
PSP	Photosynthetic pigments	PSC+T Chl *a*
TP	Total pigments	TChl *a* +TAcc
Pigment index		*Formula*
PI	Photoprotection index	PPC/TChl *a*
$Diat_{DP}$	Diatom proportion of DP	Fuc/DP
$^{*}Flag_{DP}$	Flagellate proportion of DP	(All+But+Chlb+Hex)/DP
$Prok_{DP}$	Prokaryote proportion of DP	Zea/DP

The Cochin estuary (CE hereafter), one of the largest estuarine systems located on the southwest coast of India, is comprised of a shallow (2—7 m depth) network of backwaters; receives an enormous quantity of freshwater from 7 major rivers seasonally (Qasim, 2003). The estuary has been subjected to multiple pressures from anthropogenic activities for the last 3—4 decades (Gupta et al., 2009; Martin et al., 2008), and eventually, the system has been transformed into a net heterotrophic system (Thottathil et al., 2008) from a positive net ecosystem (Qasim et al., 1969; Qasim, 2003). Geographically, the CE is located in a tropical regime, and hence, hydrological properties of this inland water body are highly dynamic due to the strong influence of monsoonal (southwest monsoon) rainfall and associated terrestrial runoff (John et al., 2020; Madhu et al., 2007; Qasim et al., 2003). Approximately 60 % of the total rainfall received over this region is during the southwest monsoon (June—September) period; it usually begins in late May or early June and ends in late September or sometime in early October (Revichandran et al., 2012; Qasim, 2003). With the onset of monsoon and within the course of a few weeks, the entire hydrology of the CE undergoes remarkable changes due to the huge freshwater inflow (Shivaprasad et al., 2013); subsequently gets transformed into a typical freshwater system (Menon et al., 2000; Qasim 2003). The CE, in general, sustained high levels of inorganic nutrients (nitrate, phosphate, and silicate), irrespective of seasons (Madhu et al., 2017; Martin et al., 2008; Sankaranarayanan and Qasim, 1969), and these nutrient enrichments often happen via two major sources, i.e., point source (domestic and industrial wastes) from the terrestrial origin (Devi et al., 1983; Sankaranarayanan and Qasim, 1969) and non-point source from riverine and terrestrial runoff (Balachandran et al., 2003; Madhu et al., 2010a and 2010b; Martin et al., 2008). There are more than 1.6 million people live on the banks of the CE that spread across three districts across Kerala state, resulting in incessant input of an enormous quantity of domestic wastes (~227.2 million litres per day) into the estuary (Remani et al., 2010). The FVCOM hydrodynamic modeling studies (coupled with the Lagrangian particle module) showed variable water residence time (spatio-temporally) in the CE, wherein longer residence time prevailed during the PRM (up to 90 days in southernmost regions) compared to the shorter residence time of MN (25 days) and PM (30 days) periods (John et al., 2020). The middle-east part (near station 8) of the CE usually receives surplus amounts of industrial effluents from various small-scale/large-scale industries, located on the banks of Periyar river, related to fertilizers, pesticides, chemicals, and allied industries and which frequently discharge untreated wastewaters (Martin et al., 2012).

Even though the CE exhibited a distinct seasonal heterogeneity in hydrochemical properties, the phytoplankton chl *a* appeared to be consistently high (> 10 mg m^{-3}) almost year-round, except during the peak monsoon months (Devassy and Bhattathiri, 1974; Menon et al., 2000; Qasim 2003). Previous studies have documented that a large fraction (>70 %) of chl *a* biomass and primary production was contributed by the nanophytoplankton (2—20 μm) community (Madhu et al., 2010b; Qasim, 1974), mostly comprised of smaller diatoms, e.g., *Skeletonema costatum, Navicula directa, Cylindrotheca closterium, Thalassiosira subtilis*, etc. (Madhu et al., 2017; Menon et al., 2000; Qasim et al., 2003). In CE, most of the phytoplankton ecological studies for the last few decades were restricted to the spatio-temporal distribution patterns of chl *a* biomass (either spectrophometry or fluorometry) and species composition and abundance (Menon et al., 2000; Qasim, 2003). However, studies that include size-structure (pico-, nano- and micro-) based phytoplankton productivity and also marker pigments of major functional communities were remained meager in the CE. According to previous reports, the phytoplankton community in the CE was found to be dominated by diatoms, irrespective of seasons (Madhu et al., 2017; Menon et al., 2000; Qasim et al., 2003) and recognised as the vital component of the prevailing planktonic food web as primary producers (Jyothibabu et al., 2006; Madhu et al., 2007; Qasim 2003). However, so far, there are no ample pigment-based studies (by HPLC) to substantiate the dominance of diatoms in the CE except the conventional microscopy-based qualitative assessment (Madhu et al., 2017; Menon et al., 2000). The present study is, therefore, aimed to examine the environmental factors which are likely controlling the consistent preponderance of diatoms in the CE and also its significant contribution to the overall phytoplankton productivity using multivariate analytical procedures. The size-fractionated phytoplankton productivity measurements (biomass and primary production) were executed to assess the relative contribution of each size group (pico-, nano- and micro-) to the total chl *a* content and primary production rates in the estuary with respect to the prevailing hydrological changes. The seasonal dynamics and succession patterns of major phytoplankton taxonomic groups, including species, were evaluated using the conventional taxonomy (microscopy) and chemical taxonomy (HPLC/CHEMTAX) methods.

2. Material and methods

2.1. Study area and sampling strategy

The study was conducted at the central region of the CE, a microtidal (amplitude ~1 m), semidiurnal inland water body (depth range, 4—15 m), located on the southwest coast of India (Lat. 09°30′—10°10′N and Lon. 76°15′—76°25′ E). Two permanent inlets, i.e., Cochin (450 m width) and Azhikode (250 m width), connect the backwaters to the Arabian Sea. The constant mixing with seawater through tidal exchanges via these two inlets has given backwaters the characteristics of a tropical estuary (Balchand and Nair, 1994). There are seven major rivers draining into the estuary. Among them, the Periyar river is the largest one, having maximum discharge and play a vital role on salinity distribution, especially in the central region (Madhupratap, 1987). The regional climate is warm and humid during the pre-and post-southwest monsoon periods and a long (ca. 4 months) rainy (monsoon) season during the southwest monsoon period (Qasim, 2003). The Cochin metropolitan city is situated on the banks of this estuary in the proximity of the Cochin inlet. Therefore, the study region incessantly receives a large quantity of industrial and domestic wastes from various sources (Gupta et al., 2009; Martin et al., 2008).

Seasonal field observations and samplings were conducted in the CE during the pre-monsoon (4th May 2010), monsoon (6th July 2009), and post-monsoon (28th October 2009) periods using a mechanized powerboat. A total of 8 sampling stations were fixed in the estuary (Figure 1) based on the prevailing salinity patterns (i.e., marine, brackish water, and freshwater zones) that prevalent during the pre-and post-phases of monsoon. The stations 1 to 3 were fixed on the south of the Cochin inlet (wherein station 1 was at the Muvattupuzha river mouth region); stations 4 and 5 were located near to the Cochin inlet, and the stations 6 to 8 were positioned in the northern part of the estuary, where Periyar river joins.

2.2. Enviornmental variables

A portable CTD profiler (Sea-Bird SBE19) was deployed at each station to retrieve the temperature and salinity. Water samples were collected from the near-surface (0.5 m depth) using a 5 litre Niskin sampler for estimating hydrochemical and biological (phytoplankton) parameters. The suspended particulate matter (SPM, mg L^{-1}) in the water sample was determined gravimetrically using pre-weighed (pre-combusted) Millipore membrane filters (nominal pore size, 0.45 μm; 45 mm dia), which were subsequently dried at 70°C for about 24 hours and re-weighed (according to APHA 2005). For the estimation of inorganic nutrients (Nitrate — NO_3^-, phosphate — PO_4^{3-} and silicate — SiO_3^-), water samples collected from the near-surface waters were filtered through GF/C Whatman filters (nominal pore size 1.2 μm) and analyzed within 3 to 4 hours after the collection using a UV-VIS Spectrophotometer (Model-1650PC, Shimadzu) following standard colorimetric techniques (Grasshoff et al.,1983).

2.3. Size-fractionated phytoplankton biomass (chl *a*) and primary production

The estimation of size-fractionated phytoplankton biomass (chl *a*) was carried out by the sequential filtering of 1 L of near-surface (0.5 m depth) water, initially through 20 μm Nytex® mesh and subsequently through 2 and 0.2 μm cellulose nitrate filters (0.45 mm dia). The cells retained by the 20 μm sieve are the microplankton (>20 μm), which later concentrating on GF/F (nominal pore size 0.7 μm) filters, whereas those retained by 2 and 0.2 μm filters constitute the nano- (2—20 μm) and pico- (<2 μm) phytoplankton, respectively with a minimal vacuum (<150 mm Hg). After filtration, pigments were extracted in 90% acetone after keeping 24 h in the dark at 4°C and pigment concentration

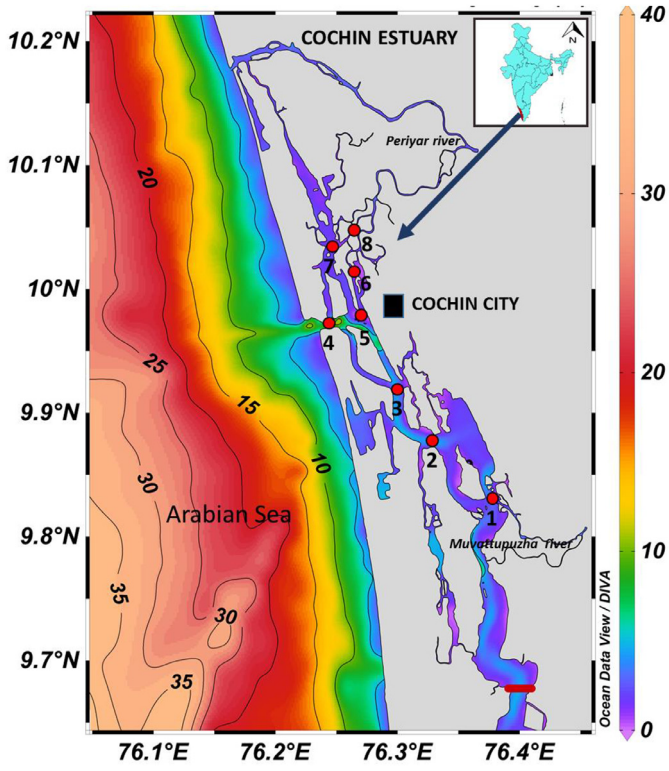

Figure 1 Map of Cochin estuary and details of sampling stations.

was measured fluorometrically (Fluorometer, Model-7200, Turner Designs®).

The primary production rate was estimated by simulated *in situ* method (deck incubation) using the ^{14}C technique (Parsons et al., 1984). Water samples collected from the near-surface were immediately passed through a 200 μ Nytex® mesh to remove large-sized zooplankton and transferred to 300-ml capacity biological oxygen demand (BOD) bottles (three light bottles and one dark bottle). Each bottle was inoculated with 1 ml of $NaH^{14}CO_3$ (activity 5 μC) solution and incubated for 6 h starting at noon. At the end of incubations, samples were sequentially filtered through 20 μm Nytex® mesh and 2 and 0.2 μm cellulose nitrate filters. The filters were used for subsequent analysis in a liquid scintillation counter (Wallac 1409, DSA-Perkin Elmer-USA) after treatment with HCl fumes to remove inorganic carbon. The primary production rate was calculated according to the equation described in the protocol (Parsons et al., 1984).

2.4. Phytoplankton species composition and abundance

For qualitative and quantitative studies of phytoplankton, 2 litres of water sample was taken from near-surface and fixed with few drops of acid Lugol's iodine solution. After the settling and siphoning procedure (Utermöhl, 1958), 1 ml of the aliquot of the sample was taken in a Sedgewick-Rafter counting cell in duplicate under the inverted microscope (OLYMPUS, CK-30) for identifying and counting phytoplankton cells mostly larger than 10 μm (Tomas, 1997).

2.5. Photosynthetic pigments

One to two liters of water samples were filtered onto Whatman GF/F filters (nominal pore size 0.7 μm; 45 mm diameter). Subsequently, the filters were frozen and kept at −20°C until the analysis. During the time of analysis, the frozen filters kept in the deep freezer were taken out and disrupted for 30 seconds in 4 ml of 90% acetone using a sonicator (Model 100, Fischer Scientific) at 4°C, and extracted by passing through 0.2 μm polycarbonate membrane filters (25 mm diameter). Subsequently, the pigment extracts (400 μL) were injected into the HPLC system (Agilent 1100) using a reverse-phase C8 column (150 × 4.6 mm, 3.5 mm particle size) with a three-phase solvent gradient at a flow rate of 1.1 mL/min (van Heukelem, 2002). The absorption spectra of the individual pigments were measured at two wavelengths 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 (RT) against authentic standards (Table 1). The HPLC system was calibrated with standard pigments obtained commercially from DHI-Institute for Water and Environment, Denmark.

2.6. CHEMTAX calculations

The chl *a* contribution from each PFG was estimated from the ratio of marker pigments to total chl *a* using a mathematical software (matrix-factorization programme) called CHEMical TAXonomy or CHEMTAX (Mackey et al., 1996; Wright et al., 1996). The initial pigment ratios of major phytoplankton classes (diatoms, dinoflagellates, cyanophytes and cryptophytes) were obtained from the

Table 2 Seasonal input pigment:chlorophyll *a* ratios for the Cochin estuary.

Groups	Per	Fuco	Diad	Allo	β Car	Zea	Chl *b*	Chl *c2*
PRM								
Dinoflagellates	1.06	0	0	0	0	0	0	0.04
Cryptophytes	0	0	0	0.23	0	0	0	0.08
Cyanobacteria	0	0	0	0	0.02	0.35	0	0
Diatoms	0	0.76	0.03	0	0	0	0	0
MN								
Dinoflagellates	1.06	0	0	0	0	0	0	0.04
Cryptophytes	0	0	0	0.23	0	0	0	0.08
Chlorophytes	0	0	0	0	0	0.01	0.26	0
Cyanobacteria	0	0	0	0	0.02	0.35	0	0
Diatoms	0	0.76	0.03	0	0	0	0	0
PM								
Dinoflagellates	0.75	0	0.24	0	0.02	0	0	0.53
Cryptophytes	0	0	0	0.19	0	0	0	0.17
Chlorophytes	0	0	0	0	4.66	0.118	0.569	0
Cyanophytes	0	0	0	0	0.20	0.68	0	0
Diatoms	0	1.02	0.45	0	0.03	0	0	0

Table 3 Seasonal output pigment:chlorophyll *a* ratios for the Cochin estuary.

Groups	Per	Fuco	Diad	Allo	β Car	Zea	Chl *b*	Chl *c2*
PRM								
Dinoflagellates	0.51	0	0	0	0	0	0	0.02
Cryptophytes	0	0	0	0.18	0	0	0	0.06
Cyanobacteria	0	0	0	0	0.02	0.29	0	0
Diatoms	0	0.23	0.01	0	0	0	0	0
MN								
Dinoflagellates	0.51	0	0	0	0	0	0	0.02
Cryptophytes	0	0	0	0.17	0	0	0	0.06
Chlorophytes	0	0	0	0	0	0.01	0.21	0
Cyanobacteria	0	0	0	0	0.02	0.37	0	0
Diatoms	0	0.18	0.02	0	0	0	0	0
PM								
Dinoflagellates	0.29	0	0.09	0	0.01	0	0	0.21
Cryptophytes	0	0	0	0.14	0	0	0	0.13
Chlorophytes	0	0	0	0	0.51	0.11	0.01	0
Cyanophytes	0	0	0	0	0.10	0.36	0	0
Diatoms	0.00	0.40	0.06	0	0.01	0	0	0

literature (Schlüter et al., 2011), and the concentrations of major pigments detected in the present study 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 functional community with respect to their chlorophyll *a* content (Eker-Develi et al., 2012; Llewellyn et al., 2005).

2.7. Pigment indices and ratios

The diagnostic pigment (DP) was estimated as the sum of seven selected biomarker pigments as given in Table 1 (Barlow et al., 2007; Vidussi et al., 2001). The sum of photoprotective carotenoids (PPCs) and photosynthetic carotenoids (PSCs) were estimated from the corresponding marker pigments mentioned in Table 1 (Trees et al., 2000). The total photosynthetic pigment (PSP) was taken as the sum of PSCs, total chlorophyll *a*, total chlorophyll *b* and total chlorophyll *c* (Table 1). Both groups of carotenoid pigments, i.e., PPCs and PSCs were useful in the photo-physiological studies (Barlow et al., 2008). The photoprotection index (PI) was calculated as the ratio between the PPCs and total chl *a* (Griffith et al., 2010; Moreno et al., 2012). A high PI value indicates an oligotrophic condition (e.g., open ocean, stratified waters, etc.), whereas low PI represents productive (e.g., well-mixed coastal waters, fronts, etc.) waters (Moreno et al., 2012). Major phytoplankton taxonomic groups such as diatoms, small flagellates, and prokaryotes were identified based on the

indices using DPs, and the indices symbolizing these groups were designated as $Diat_{DP}$, $Flag_{DP}$, and $Prok_{DP}$ (Table 1).

2.8. Statistical analysis

The hierarchical cluster analysis (HCA) and non-metric multidimensional scaling (NMDS) were applied to determine the similarity of sampling sites based on phytoplankton species composition and abundance. For identifying the best-adapted phytoplankton species for the prevailing environmental conditions, BV-STEP analysis was adopted (PRIMER-v.6, Plymouth Routines in Multivariate Ecological Research, Plymouth Marine Laboratory, Plymouth, UK; Clarke and Gorley, 2006). Afterward, the best-adapted phytoplankton species identified by BV-STEP analysis were correlated with environmental variables using RDA analysis (CANOCO software). In addition, RDA analysis was also performed to find out the influence of environmental variables on chl *a* and primary production rates of various phytoplankton size groups as well as chl *a* equivalents (CHEMTAX) of various PFGs (diatoms, dinoflagellates, cryptophytes, cyanophytes, etc).

3. Results

3.1. Environmental parameters

Generally, the southwest coast of India has predominantly two distinct seasons considering the precipitation and temperature: a cold rainy season (southwest monsoon; ~4 month duration) and a warm, dry season (pre-and-post southwest monsoons) for the rest of the year (Qasim, 2003). Weather in and around the Cochin was warm and dry during the PRM and PM periods compared to the MN period. The present study has shown the prevalence of warm and well-mixed waters across the study region during the PRM (av. 32.4 ± 0.6°C) and PM (av. 30.7 ± 0.7°C) periods compared to the cold (av. 27.4 ± 0.9°C) stratified waters of the MN period (Figure 2a). The estuary sustained consistently high SPM levels (av. > 25 mg L^{-1}) in the surface waters, irrespective of seasons, along with a distinctive spatial variation (Figure 2b). A distinct seasonal scale (mean) increase in SPM was prevalent during the PRM (av. 41.3 ± 17 mg L^{-1}) with a maximum value (73.2 mg L^{-1}) recorded at station 5. A well-distinct spatially varied salinity conditions (i.e., oligohaline-mesohaline-euhaline) occurred during the PRM (7.1—30.8) and PM (2.3—25.1) periods. On the other hand, the overall study region was found to be oligohaline (up to 1.6) during the MN period (Figure 2c). The major inorganic nutrients such as NO_3, PO_4^3 and SiO_4 showed a distinctive spatio-temporal variation, wherein NO_3 (av. 26.1±7.1 μM) and PO_4^3 (av. 4.3 ± 2.4 μM) were noticeably high almost throughout the study region during the MN (Figure 2d and e). Similar to SPM distribution, consistently high silicate concentrations (>20 μM) were detected all over the sampling stations (except station 4), regardless of seasons (Figure 2f). However, from a seasonal perspective, the estuary sustained considerably high silicate levels (av. 63.4 ± 2.35 μM), especially during the PM period compared to the PRM (av. 40.2 ± 17.2 μM) and the MN (av. 23.2 ± 5.6 μM) periods.

3.2. Size-fractionated phytoplankton biomass and primary production

The size-structure based phytoplankton productivity estimations showed a clear-cut spatio-temporal variation in total chl *a* and primary production (PP hereafter) in the surface waters of the CE (Figure 3a and 4a). The seasonal mean of both chl *a* (av. 13.1 ± 8.2 mg m^{-3}) and PP higher (av. 856 ±1149 mg C m^{-3} d^{-1}) were remarkably high during the PM. However, a consistent and moderately higher chl *a* (av. 9.5 ± 2 mg m^{-3}) and PP (av. 657 ± 282 mg C m^{-3} d^{-1}) were recorded during the PRM. By contrast, the estuary sustained remarkably low chl *a* (av. 4.2 ± 3 mg m^{-3}) and PP (av. 12.3 ± 5.8 mg C m^{-3} d^{-1}) during the MN period. Even though, major contribution (80—90%) of chl *a* and PP in the CE was derived from the nanophytoplankton community, irrespective of seasons, there was a distinct spatio-temporal variation in the quantitative contribution from this size group (Figure 3c and 4c). The chl *a* and PP derived from microphytoplankton and picophytoplanktoon did not show noticeable spatio-temporal variation in the estuary (Figures 3 and 4). However, a slight increase in microphytoplankton PP was discernible compared to picophytoplankton (Figure 4b).

3.3. Phytoplankton marker pigments

A widespread dominance of fucoxanthin (biomarker of diatoms) was evident all over the study region, irrespective of seasons (Figure 5). The seasonal mean of fucoxanthin was much higher during the PM (av. 9 ± 16.9 mg m^{-3}) compared to the PRM (av. 1.04 ± 0.46 mg m^{-3}) and MN (av. 0.57 ± 0.16 mg m^{-3}) periods. The whole study region, especially stations 1 to 5 sustained remarkably high fucoxanthin levels (> 6 mg m^{-3}) during the PM period. Besides fucoxanthin, an intermittent (seasonal) increase in peridinin (biomarker of dinoflagellates) was observed during the PM and PRM periods. By contrast, moderate levels of zeaxanthin and alloxanthin were detected in most of the sampling stations during the MN period (Figure 5b and 5c). Similarly, a nominal increase in chlorophyll *c2* (chl *c2*) and diadinoxanthin was detected at certain stations, regardless of seasons, wherein chl *c2* was seemingly high during the PM (av.1.4 ± 1.5 mg m^{-3}), particularly at stations 1 to 5. Other marker pigments such as pheophorbides, β-carotenoids and 19'-hexanoyloxyfucoxanthin were also detected in low levels during the PM period.

3.4. Phytoplankton pigment indices and ratios

A notable increase (seasonal mean) in DP was evident in the estuary during the PM (av. 14.89 ± 17.67 mg m^{-3}) compared to the PRM (av. 1.81 ± 1.48 mg m^{-3}) and MN (av. 1.43 ± 0.68 mg m^{-3}) periods. The fucoxanthin constituted a major fraction (>90%) of DP, irrespective of seasons, particularly during the PM and PRM periods. However, during the MN period, zeaxanthin contributed ~50% (mean) of DP apart from fucoxanthin. The PPCs and PSCs were also showed a clear-cut spatio-temporal variation, wherein a remarkable increase in PPCs (av. 0.95 ± 0.71 mg m^{-3}) over PSCs (av. 0.57 ± 0.16 mg m^{-3}) was evident during the MN period.

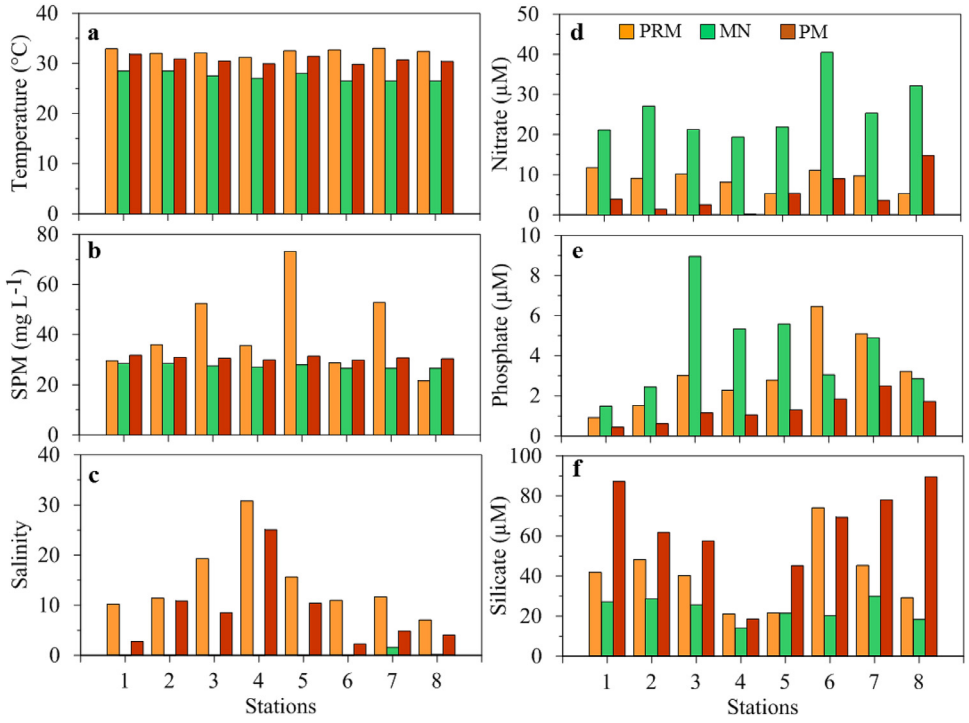

Figure 2 Seasonal distribution of major physicochemical parameters in the surface waters.

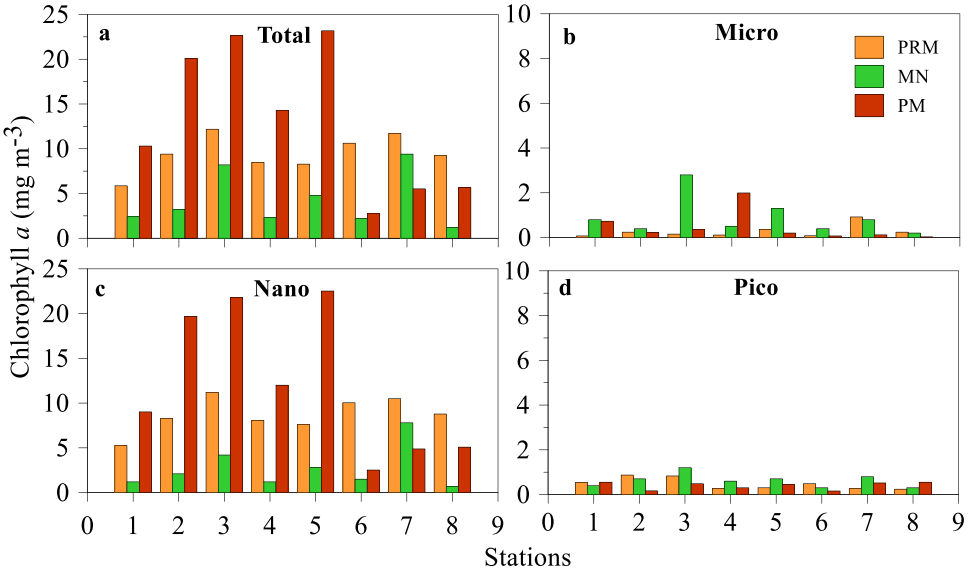

Figure 3 Seasonal distribution of size-fractionated chlorophyll *a* (mg m^{-3}) in the surface waters.

By contrast, a noticeable increase in PSCs was detected during the PM (av. 14.48 ± 17.47 mg m^{-3}) period. The total photosynthetic pigments (PSP) exhibited a marked increase during the PM (av. 28.42 ± 25.98 mg m^{-3}), particularly at stations 2 to 4, located near the Cochin inlet. The seasonal mean of PI values were distinctly high in the estuary during the MN period (av. 0.24 ± 0.19) compared to other two sampling periods (Table 4).

The diagnostic pigment indices clearly showed an increase in $Diat_{DP}$ (>0.5) in all sampling stations, regardless of seasons, which indicated the preponderance of diatoms (>50% of the total population) over other taxonomic groups. A distinctive seasonal scale increase in $Diat_{DP}$ evident especially during the PM (av. 0.96 ± 0.05) and PRM (av. 0.72 ± 0.29) periods apparently revealed the predominance of diatoms at 96% and 72%, respectively, of the total phytoplankton abundance. However, a marked increase in $Prok_{DP}$ (0.52–0.7) prevalent across the study region (mainly at stations 1, 4 and 7) during the MN period denotes the dominance (50–70%) of prokaryotes. The $Flag_{DP}$ was consistently low (<1) throughout the study region, irrespective of seasons, which signifies the

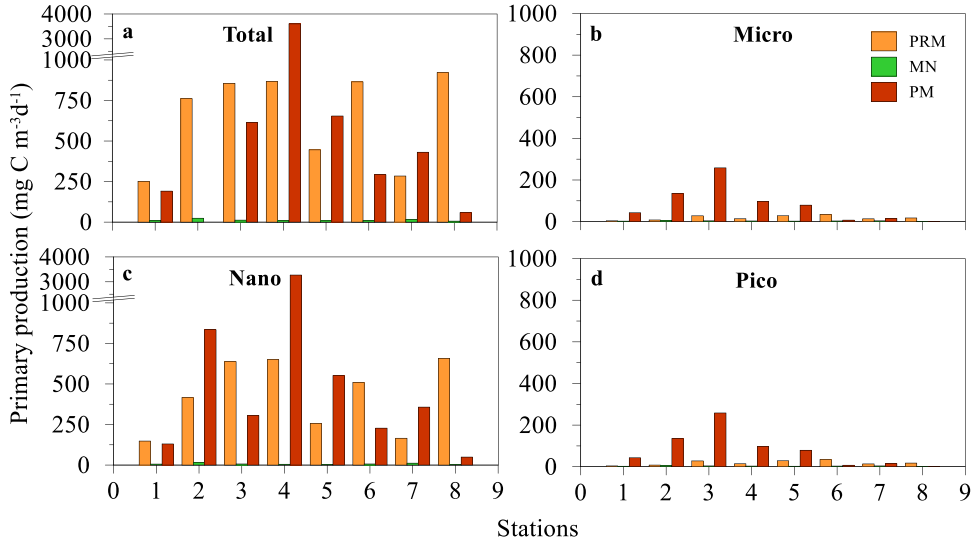

Figure 4 Seasonal distribution of size-fractionated primary production (mgC m^{-3} d^{-1}) in the surface waters.

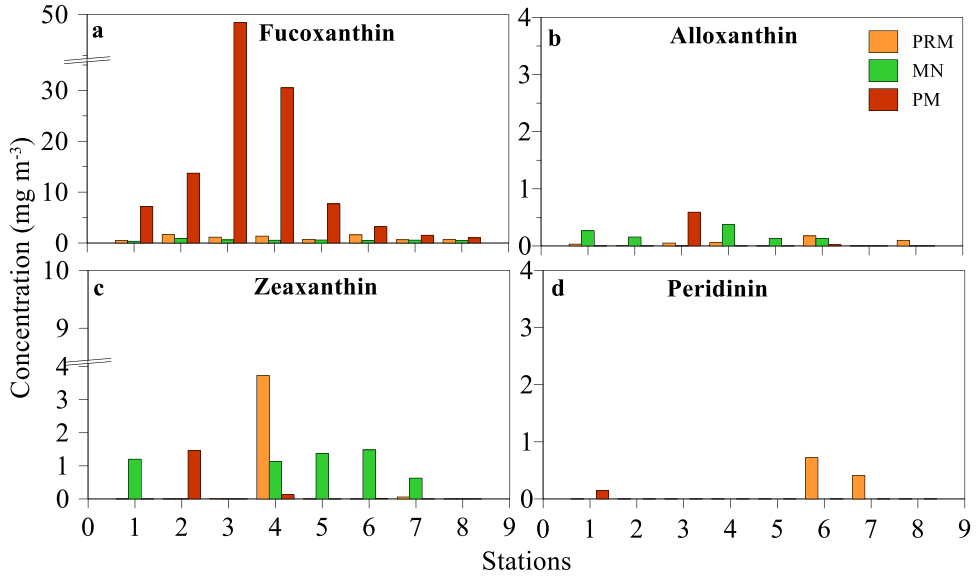

Figure 5 Seasonal distribution of major phytoplankton marker pigments (mg m^{-3}) in the surface waters.

low abundance of small flagellates (<10%), compared to diatoms.

3.5. Phytoplankton abundance and diversity

A distinctive spatio-temporal variation in phytoplankton total abundance, composition and species diversity was discernible in the surface waters of the CE during the study period. In general, a profound increase in total phytoplankton abundance (> 1500 × 10^3 cells L^{-1}) was prevailed during the PM period, especially at stations 1 to 5 (Figure 6a). Numerically, diatoms constituted the predominant taxa (52—99 % of the total abundance) in almost all sampling stations, irrespective of seasons. During the PRM, along with dominant diatoms (av. 79.5 ± 15.9%), certain dinoflagellates were also encountered (~30—40 % of total abundance), specifically at stations 6 to 8. The study region characteristically showed a station-specific dominance of various diatom species during this season, for example, *Skeletonema costatum* (at stations 3 to 5), *Cyclotella* sp. (at stations 1 to 3), *Cylindrotheca closterium* (at stations 6 to 8), etc. (Figure 8). However, during the MN period, a substantial decrease in total phytoplankton abundance (av. 43.03 × 10^3 cells L^{-1}) was widespread across the study region (Figure 6a). *S. costatum* was the most dominant species during this period, especially at stations 3 to 5 (>70% of total abundance), which was followed by *Thalassiosira subtilis* (st. 1 and 2), *Leptocylindrus danicus* (st. 8), *Navicula* species (st. 1), *N. closterium* (st. 6) and *Nitzschia longissima* (st. 7). Besides diatoms, a moderate abundance of some chlorophyte species, e.g., *Scenedesmus* sp., *Agmenellum* sp., was also observed at certain stations. During the PM period, phyto-

Table 4 The mean values (± SD) for major environmental parameters, chlorophyll *a*, major marker pigments, pigment sums, pigment indices and ratios during monsoon, post-monsoon and premonsoon ('—' not detected).

Parameters	Seasons		
(Abbreviations)	Monsoon	Post-monsoon	Pre-monsoon
Temperature (°C)	27.38 ± 0.88	30.68 ± 0.69	32.35 ± 0.58
SPM (mg L^{-1})	24.98 ± 16.65	25.75 ± 13.12	41.25 ± 16.97
Salinity	0.23 ± 0.56	9.52 ± 7.02	14.63 ± 7.49
NO_3 (μM)	26.09 ± 7.14	5.23 ± 5.19	8.75 ± 2.46
PO_4 (μM)	4.32 ± 2.38	1.36 ± 0.68	3.16 ± 1.82
SiO_3 (μM)	24.38 ± 4.17	64.60 ± 22.70	40.23± 17.2
Chl *a* (mg m^{-3})	4.0 ± 1.27	12.45 ± 9.19	4.25 ± 2.16
Chl *b* (mg m^{-3})	—	0.13 ± 0.36	—
Chl c2 (mg m^{-3})	0.24 ± 0.21	1.41 ± 1.53	0.79 ±0.66
Chl c3 (mg m^{-3})	—	0.08 ± 0.16	—
β-Caro (mg m^{-3})	0.02 ± 0.06	0.23 ± 0.16	—
Allo (mg m^{-3})	0.13 ± 0.13	0.07 ± 0.21	0.03 ± 0.06
Diad (mg m^{-3})	0.07 ± 0.07	0.25 ± 0.34	0.05 ± 0.09
Fuco (mg m^{-3})	0.57 ± 0.16	9.0 ± 16.92	1.04 ± 0.46
Hex (mg m^{-3})	—	0.22 ± 0.57	0.09 ± 0.25
Peri (mg m^{-3})	—	0.02 ± 0.05	0.14 ± 0.28
Zea (mg m^{-3})	0.73 ± 0.65	0.18 ± 0.52	0.47 ± 1.32
Tpig (mg m^{-3})	5.77 ± 1.69	23.92 ± 24.41	6.86 ± 3.71
DP (mg m^{-3})	1.43 ± 0.68	9.63 ± 17.78	1.77 ± 1.47
PSP (mg m^{-3})	4.82 ± 1.40	23.18 ± 23.68	6.31 ± 2.85
TChl *a* (mg m^{-3})	4.25 ± 1.43	13.94 ± 10.10	5.03 ± 2.38
PPC (mg m^{-3})	0.95 ± 0.71	0.74 ± 0.92	0.55 ± 1.39
PSC (mg m^{-3})	0.57 ± 0.16	9.24 ± 17.47	1.27 ± 0.56
PI	0.25 ± 0.19	0.05 ± 0.05	0.08 ± 0.18
PPC:PSC	1.91 ± 1.61	0.16 ± 0.32	0.43 ± 1.02
$Diat_{DP}$	0.54 ± 0.35	0.75 ± 0.42	0.74 ± 0.28
$Prok_{DP}$	0.62 ± 0.08	0.02 ± 0.04	0.09 ± 0.26
$Flag_{DP}$	0.07 ± 0.07	0.04 ± 0.04	0.08 ± 0.18

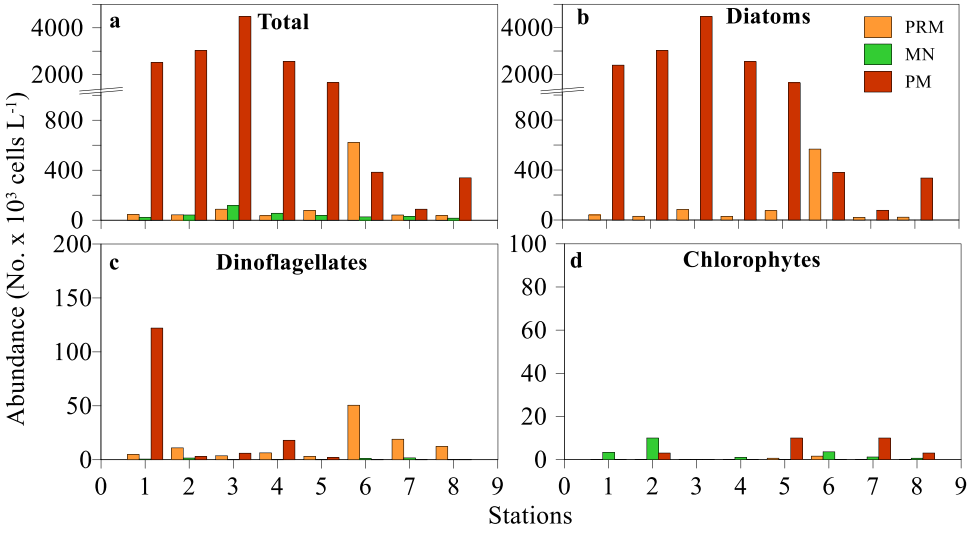

Figure 6 Seasonal distribution of total phytoplankton abundance and major taxonomic groups in the surface waters.

plankton taxa was mainly dominated by *Skeletonema costatum* (77—98.9%), except station 6. *Navicula distans* was the dominant diatom species (45.7%) encountered at station 6. Similar to total abundance, the phytoplankton species diversity (*H'*) also exhibited a distinct spatio-temporal variation in the estuary, wherein maximum species diversity (seasonal mean) was apparent during the PM (0.62) period, compared to the PRM (1.7) and MN (2.5) periods.

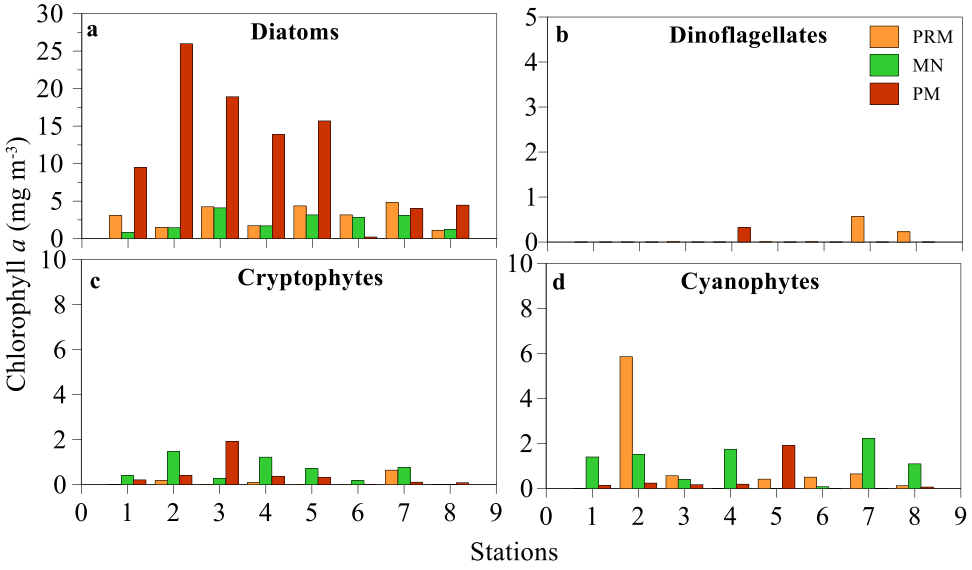

Figure 7 Seasonal distribution of chlorophyll *a* equivalents of major PFGs (calculated by CHEMTAX) in the surface waters.

3.6. Phytoplankton functional group dynamics in terms of Chl *a*

The relative contribution of chl *a* estimated from the major PFGs (diatoms, dinoflagellates, chlorophytes, cyanophytes and cryptophytes) using the CHEMTAX calculations revealed a distinct spatio-temporal variation (Figure 7a—d). More than 70% of chl *a* was found to be derived exclusively from diatoms, irrespective of seasons. The chl *a* equivalent from diatoms were substantially high during the PM (av. 11.59 ± 8.66 mg m^{-3}) period compared to the PRM (av. 3.01 ±1.42 mg m^{-3}) and MN (av. 2.3 ±1.15 mg m^{-3}) periods. The cyanophytes constituted the second predominant taxonomic group in the study region on the basis of chl *a* contribution, which was more evident during the MN period (av. 1.06 ± 0.82 mg m^{-3}), especially at stations 2, 4 and 7. Besides, a moderate contribution of chl *a* from cryptophytes was also evident during the MN (av. 0.63 ± 52 mg m^{-3}) and PM (av. 0.43 ± 62 mg m^{-3}) periods. The chl *a* equivalents of dinoflagellates and chlorophytes were found to be minimum (<0.5 mg m^{-3}) at certain stations; restricted during the PRM and MN periods, respectively.

3.7. Environmental influence on phytoplankton community structure

The seasonality in phytoplankton community structure was examined using phytoplankton species abundance data (4th root transformed) by adopting multivariate similarity (Bray-Curtis, PRIMER 6) analysis (Clarke and Gorley, 2006). Resultantly, two distinct groups or assemblages of sampling stations (at 30% similarity) were formed, in which group I was comprised of stations sampled in PRM and group II was formed of stations sampled both in MN and PM periods (Figure 9a). This was further confirmed by the non-metric multidimensional (NMDS) plot (stress 0.17) (Figure 9b). The seasonal abundance of dominant phytoplankton species, estimated using BV STEP analysis, was represented in the bubble plots (Figure 10), which revealed a conspicuous dominance of *S. costatum* in all sampling locations, regardless of seasons. A distinct numerical dominance of *S. costatum* (~20 × 10^3 cells L^{-1}) was evident in most of the sampling stations (1 to 5) during the PM period. The bubble plots have revealed the seasonal occurrence of certain species; for example, *Cyclotella* sp. and *C. closterium* (diatoms) and *Alexandrium* sp. and *Prorocentrum ovatum* (dinoflagellates) were abundant during the PRM (Figure 10). Similarly, *T. subtilis, Nitzschia longissima, Navicula distans* (diatoms) occurred during both MN and PM periods and *Scenedesmus* sp. (chlorophyte) was observed only during the MN period.

The RDA plots, depicting the interrelationships between the environmental variables and phytoplankton parameters (biomass, primary production and species composition) showed remarkable temporal scale correlation trends (Figures 11a—c). The nanophytoplankton chl *a* and PP showed a positive correlation with salinity, SPM, temperature and silicate during the productive periods (PRM and PM), whereas during MN, PO_4 and NO_3 appeared to be the major environmental influencing factors for micro-and picophytoplankton size fractions (Figure 11a). During MN and PM periods, species such as *S. costatum, Nitzschia longissima, Navicula distans, N. directa, T. subtilis, Prorocentrum gracile* showed a positive correlation to NO_3 and SiO_4 (Figure 11b). On the other hand, species such as *T. mobiliensis, Cylindrotheca closterium, Alexandrium* sp., *Protoperidium ovatum, Cyclotella* sp., were exhibited a positive correlation to a suite of environmental variables (e.g., salinity, SPM, temperature, and phosphate) during the PRM (Figure 11b). The RDA plot showing the interrelationships between environmental variables and chl *a* equivalents of major phytoplankton functional groups (CHEMTAX calculations) revealed distinct seasonal correlation patterns (Figure 11c), wherein SiO_3 showed a positive relationship with diatoms (all stations) and cryptophytes (st. 3, 4 and 7) during the most productive period (PM). The dinoflagellates showed a positive relationship with

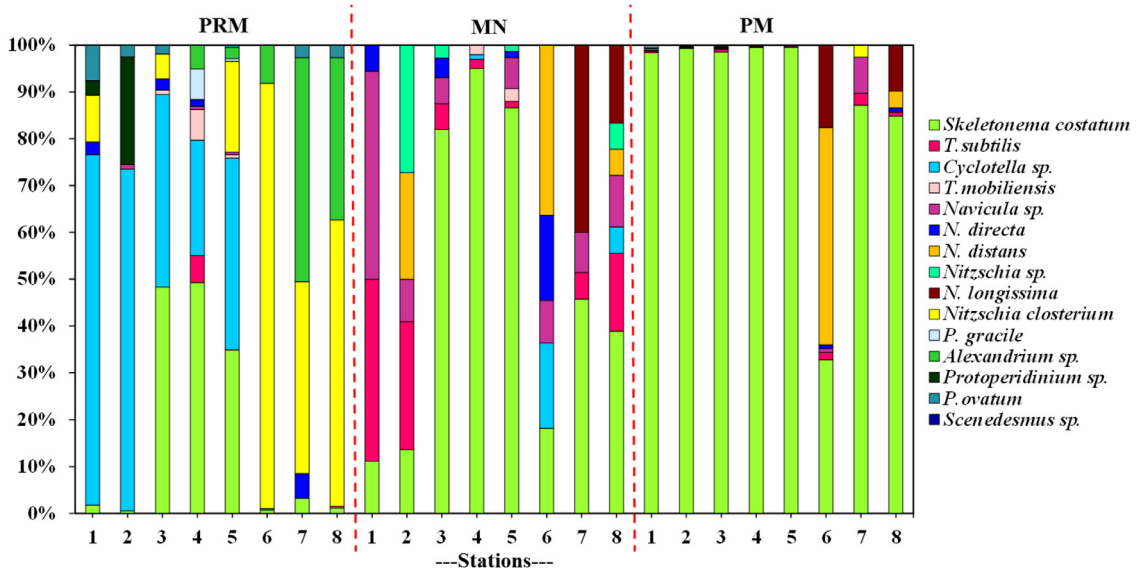

Figure 8 Seasonal percentage abundance of major phytoplankton species (by BV STEP analysis) in the surface waters.

SPM, salinity, and temperature during the PRM, whereas the cyanophytes were found to be influenced by PO_4 and NO_3.

4. Discussion

Recent studies have documented a progressive water quality deterioration of CE due to the tremendous increase in nutrient inputs and concomitant eutrophication processes (Gupta et al., 2009; Lallu et al., 2014; Madhu et al., 2017; Martin et al., 2008). High nutrient loadings (CPCB report, 1996; Sankaranarayanan and Qasim, 1969) and subsequent phytoplankton growth enabled the CE into a system of positive net ecosystem production since long back (Qasim et al., 1969; Qasim, 2003). However, as per recent reports, the CE has been transformed into a system of negative net ecosystem production because of the alarming increase in organic enrichments caused by the diverse human activities (Gupta et al., 2009; Thottathil et al., 2008).

The prevalence of warm (av. 32.4 ± 0.6) and high salinity surface waters (7.1–30.8) in the CE during the PRM period obviously indicated the strong influence of neritic incursion due to the tidal activity (Shivaprasad et al., 2013). Similarly, a drastic increase in inorganic nutrients, particularly SiO_4 (av. 40.2 ± 17.2 μM), PO_4^3 (av. 3.2 ± 1.2 μM), and NO_3 (av. 8.8 ± 2.5 μM) prevalent during this period along with high SPM (av. 41.3 mg L^{-1}) disclosed the well-mixed state of the estuary (Vinita et al., 2017). Since the present study did not accomplish the analysis of inorganic NH_4, the actual N:P ratio of the estuary could not be estimated to explain the seasonal scale variability in nutrient stoichiometry. According to earlier studies, DIN in the CE was chiefly constituted (~60%) by NH_4, especially during the PRM period (Madhu et al., 2010b, Martin et al., 2010). The proximate regions of stations 6 to 8 were reported to sustain high NH_4 levels (>100 μM), resultant of frequent industrial/domestic discharges (Miranda et al., 2008). The prevalence of weak flushing and longer residence time (15–23 days) usually causes a considerable increase in nutrient levels in the east-central zone (stations 6–8) of the CE (John et al., 2020; Lallu et al., 2014). Regarding phytoplankton growth patterns, nanophytoplankton community was reported to contribute a significant portion of total chl *a* (av. 92 ± 2.7%) and primary production during the PRM and, therefore, the dominant diatoms prevalent during this period may be belonged to the nanophytoplankton size-category. The occurrence of diverse diatom species in moderate abundance, for example, *Cyclotella* sp. (st. 1 to 5), *S. costatum* (st. 3 to 5), and *C. closterium* (st. 6 to 8), pointed out the spatially variable growth patterns of diatoms (Figure 8). The enhanced fucoxanthin levels (av. 1.04 ± 0.46 mg m^{-3}) have confirmed the apparent dominance of diatoms (av. 79.49 ± 15.9%) during this period, and it was further corroborated by the microscope-based phytoplankton composition data (Figure 6).

During the MN period, the drastic decrease in phytoplankton chl *a* (av. 4.2 ± 3 mg m^{-3}) and abundance (av. 43.07 ± 31.7 × 10^3 cells L^{-1}) prevalent in the study region revealed the profound influence of massive freshwater inputs from the rivers. A conspicuous salinity drop (<2) evident in whole the study region, along with the substantial increase in NO_3 and PO_4, indicated the significant influence of monsoon induced river discharge and terrestrial runoff (Revichandran et al., 2012; Shivaprasad et al.,2013). The upper reaches of the central CE, wherein Periyar and Muvattupuzha rivers join, are reported to be supplied by large amounts of inorganic nutrients during the MN period (Lallu et al., 2014; Madhu et al., 2007; Martin et al., 2008; Sankaranarayan and Qasim, 1969). Although SPM levels remained comparatively low (av. 25 ± 16.7 mg L^{-1}) in the estuary during the MN period, the existing level (≥ 25 mg L^{-1}) was sufficient to limit the water column irradiance required for the phytoplankton growth (Cloern, 1987). The prevalence of low daily solar radiation (<350 ly day^{-1}) being received in and around the Cochin region, especially in MN period, due to the heavy cloud cover and incessant rainfall, always limits phytoplankton growth (Madhu et al.,

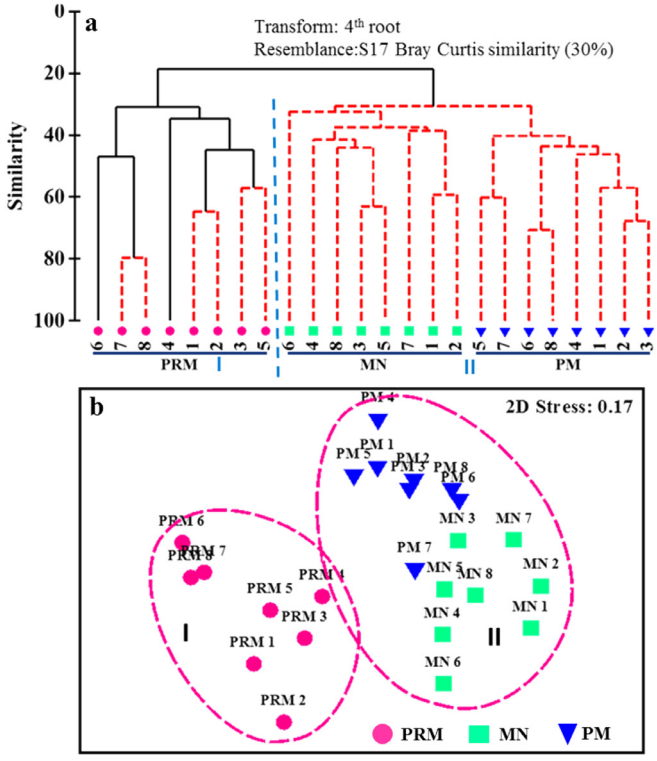

Figure 9 Representation of station locations (seasonal scale) based on Bray Curtis similarity using phytoplankton abundance and composition data; a) Dendrogram, b) NMDS ordination.

2007 and 2010a). During this period, the remarkable seasonal scale decrease in phytoplankton growth, particularly primary production rate (av. 12 ± 5.8 mgC m^{-3} d^{-1}), was found to be mainly contributed by nanophytoplankton community (ca. 61.6%). Even though the whole study region was appeared to be freshwater dominated during the MN period, the preponderance of diatoms prevailed in the study region indicated its varied salinity tolerances. The obvious dominance of *S. costatum*, particularly at stations 3 to 5 (>80% of total abundance), implies that this centric diatom species is well adapted to low salinity waters as well, despite its marine origin (Castillo et al., 1995; Naik et al., 2010). In addition, dominance of *T. subtilis* and *Navicula* sp. observed at the river mouth stations (1 and 2) indicated the spatial scale species diversity of diatoms. As far as the marker pigments are concerned, the relatively similar concentration of zeaxanthin (av. 0.73 ± 0.65 mg m^{-3}) and fucoxanthin (av. 0.57 ± 0.16 mg m^{-3}) prevalent in most of the sampling stations indicated the numerical dominance of both cyanophytes and diatoms. Also, the marginal increase in alloxanthin (av. 0.13 ± 0.13 mg m^{-3}) detected in many of the sampling stations signifies the co-occurrence of cryptophytes along with cyanophytes and diatoms (Figure 6). The CHEMTAX derived chl *a* equivalents of cyanophytes and cryptophytes apparently substantiated the above mentioned statement (Figures. 7c and d).

The remarkable seasonal scale increase in chl *a* (av. 13.1 ± 8.2 mg m^{-3}) and primary production (av. 855.6 ± 1148.8 mgC m^{-3} d^{-1}) recorded during the PM period implies that the estuary is conducive for the substantial growth of phytoplankton, especially nanophytoplankton community. The conspicuous decrease in NO_3 and PO_4 in the surface waters can be related to the influence of neritic waters and also the excessive utilization of nanophytoplankton community. The notable increase in fucoxanthin (av. 14.2 ± 17 mg m^{-3}), corresponding to chl *a*, indicates the substantial growth of diatoms in the overall study region. Furthermore, the tremendous increase in the abundance of *S. costatum* (77.3—98.9% of the total abundance), evident in all sampling stations, except station 6, suggests that this particular diatom species could be well adapted to wide salinity ranges, i.e., oligohaline, mesohaline, polyhaline and euhaline, as reported by previous studies (Huang et al., 2004; Naik et al., 2010).

The tropical and sub-tropical estuaries usually exhibit high phytoplankton growth rates year-round (except during the peak monsoon months) because of the prevailing high levels of irradiance and inorganic nutrients (Costa et al., 2009; Navas et al., 2020; Nittrouer et al., 1995; Qasim, 2003), compared to the temperate estuaries (Gocke et al., 2001; Soria-Piriz et al., 2017). However, a few of the tropical estuaries often exhibit low phytoplankton biomass, despite having high nutrients, because of the prevalence of high water column turbidity due to the enhanced suspended sediments (Burford et al., 2008; Cloern, 1987). According to previous reports, the CE usually sustains relatively high SPM year-round, derived from diverse sources (Qasim 2003; Madhu et al., 2017). The tidal activity is reported to enhance sediment re-suspension and concomitant turbidity increase in the CE, especially during the non-monsoon months, mainly near the proximity of inlets (John et al., 2020; Vinita et al., 2017). By contrast, during the monsoon months, the prevalence of enormous river discharge leads to a remarkable increase in SPM levels of allochthonous origin (Madhu et al., 2010a; Qasim 2003). The present study has also shown a similar hydrological scenario, in which consistently high SPM levels were recorded near the proximity of the inlets, especially during the PRM and PM periods, which suggests the profound influence of tidal forcings (Vinita et al., 2017).

Generally, the variations in chl *a* and marker pigments are found to be associated with the overall changes of phytoplankton functional composition (Barlow et al., 2007; Platt et al., 2005). The pigment indices and ratios estimated using chl *a* and marker pigments can provide information on phytoplankton productivity and physiological status of functional communities (Barlow et al., 2008;Trees et al., 2000; Veldhuis and Kraay, 2004). In the present study, the significant increase in $Diat_{DP}$, and also diatom chl *a* equivalents estimated using CHEMTAX calculations substantiated the clear-cut dominance of diatoms, irrespective of seasons. The noticeable increase in PI (>0.2) during the MN period, estimated from PPCs and total chl *a*, supported the prevalence of low phytoplankton productivity due to the prevailing monsoon induced environmental perturbations (i.e., shorter water residence time and enhanced water column turbidity and cloud cover). However, low PI values (<0.1) perceived during the PRM and PM periods, signified the high productive conditions of the estuary (Moreno et al., 2012). The noticeable decline in the overall phytoplankton growth evident during the MN period can be linked to the prevailing low irradiance and river-induced high SPM levels, despite the enhanced nutrient levels (Madhu et al.,

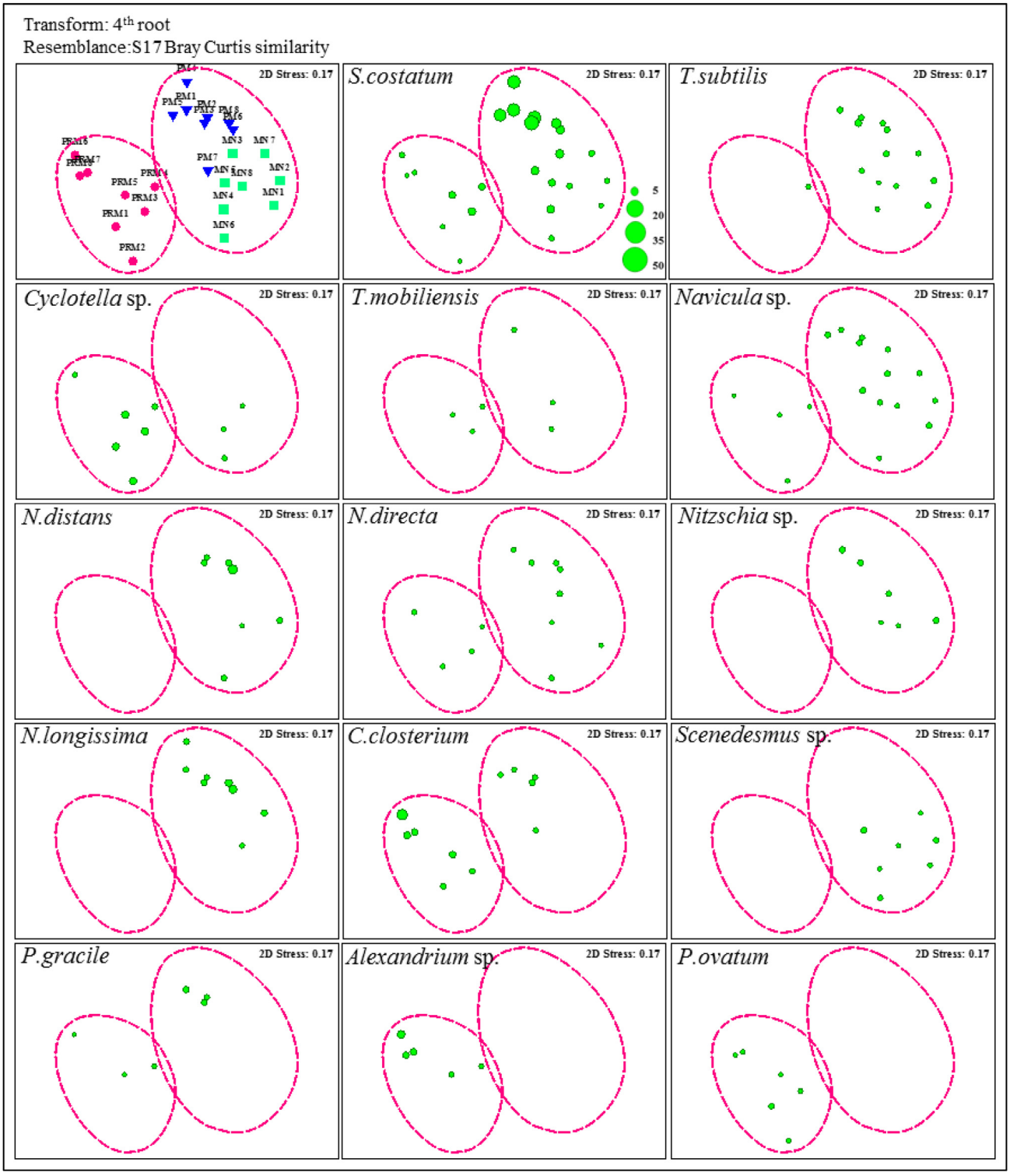

Figure 10 Bubble plot representation of major phytoplankton species (BV STEP analysis) in the surface waters superimposed in the NMDS ordination.

2010a; Qasim 2003). Usually, nutrient-enriched coastal waters support the growth of large-sized phytoplankton, microphytoplankton in particular, formed of diatoms and dinoflagellates (Lionard et al., 2008; Sarthou et al., 2005). However, the relative increase in nanophytoplankton chl *a* can be assumed to be due to the prevailing hydrological peculiarities. Previous reports have documented light-limited phytoplankton growth in the CE, especially in the water column, because of the year-round increase in SPM (Madhu et al., 2017; Qasim 2003). The present study also showed a consistently high SPM (>25 mg L^{-1}), especially during the PRM (Figure 2b). Therefore, the drastic decrease

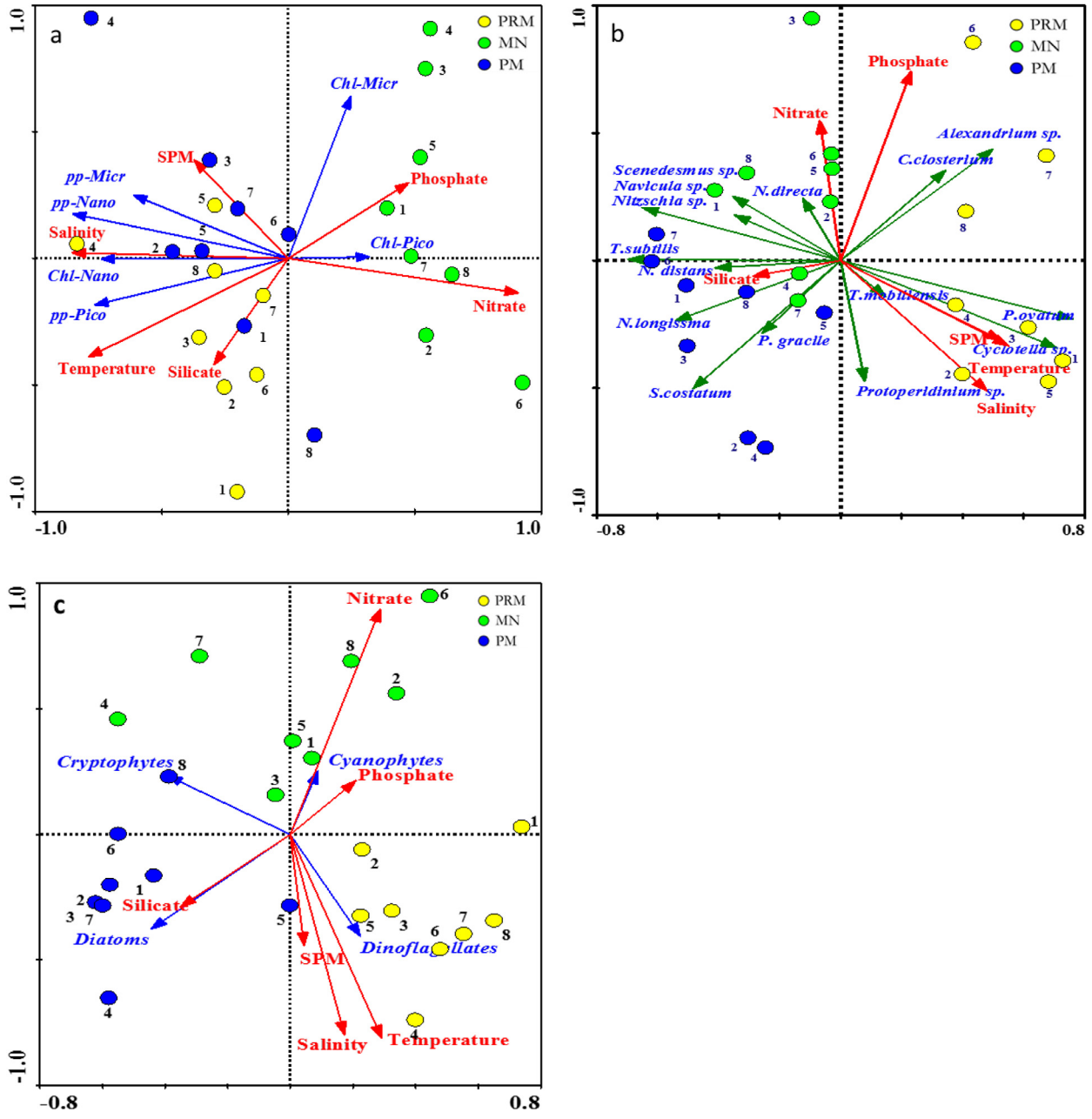

Figure 11 RDA plots depicting the inter-relationships of environmental variables between a) size-fractionated phytoplankton biomass and primary production, b) phytoplankton dominant species (BV STEP), c) chlorophyll *a* equivalents of major PFGs (CHEMTAX).

in phytoplankton growth, in terms of chl *a* and primary production, evident during the PRM, compared to the PM, was due to the tide induced enhancements in water column SPM, in spite of the prevalence of high nutrients and longer residence time (John et al., 2020; Vinita et al., 2017). Apart from the environmental influences, high grazing rates of the zooplankton community (both meso-and microzooplankton) also reported causing a substantial reduction in phytoplankton standing stock in the CE, especially during the PRM period (Jyothibabu et al., 2006; Vineetha et al., 2015).

The positive correlations established between the SPM and phytoplankton variables (chl *a* and primary production), pointed out the substantial influence of SPM on nanophytoplankton growth during the PM and PRM periods along with salinity, temperature and silicate (Figure 11a). The environmental factors, which usually control the phytoplankton growth and abundance in the CE are diverse due to their dynamic nature (Menon et al., 2000; Qasim 2003). Therefore, it can be presumed that no single factor is responsible for the overall growth and distribution of the phytoplankton community in the CE. The NMDS plots, based on phytoplankton species composition and abundance data, revealed the formation of two major clusters among the sampling stations at 30% similarity, i.e., I (PRM) and II (MN

and PM) (Figure 9). Generally, the phytoplankton species composition showed a close resemblance between the MN and PM periods than PRM (Figure 8). The RDA plots depicting the environmental influence on phytoplankton productivity, species composition and chl *a* equivalents of major PFGs (CHEMTAX) showed definite interrelationship patterns (Figure 11a-c). A suit of environmental parameters (e.g., salinity, SPM, temperature and silicate) found to influence the nanophytoplankton growth during the productive periods (PRM and PM), whereas PO_4 and NO_3 found to influence the phytoplankton biomass, especially for micro-and picophytoplankton fractions, during the MN period (Figure 11a). The RDA plots depicting the environmental influence on dominant phytoplankton species (BV STEP analysis) have shown the prevalence of positive correlation of SiO_3 and NO_3 with dominant phytoplankton species, mostly diatoms (*S. costatum, Nitzschia longissima, Navicula distans, N. directa, T. subtilis*) during the MN and PM periods. On the other hand, during the PRM, salinity, SPM, temperature and PO_4 were found to influence the growth and abundance of dominant phytoplankton species such as *T. mobiliensis, Cylindrotheca closterium, Alexandrium* sp., *Protoperidium ovatum, Cyclotella* sp. (Figure 11b).

Recent flowcytometry based picophytoplankton studies have shown a remarkable contribution of *Synechococcus* community to the total phytoplankton biomass in the CE, wherein phycoerythrin-rich (PE-rich) and phycocyanin-rich (PC-rich) strains of *Synechococcus* were reported to be abundant during the non-monsoon and monsoon periods, respectively (Mohan et al., 2016; Rajaneesh et al., 2015). However, previous size-structure based productivity estimations have documented considerably low chl *a* (<10% of the total) from picophytoplankton community compared to the nanophytoplankton chl *a* (Madhu et al., 2010b and 2017). The present study also supports the result in which chl *a* from picophytoplankton was consistently low (<5%) in the CE. These results, thus, clearly revealed the fact that even though the CE is favourable for the proliferation of small-sized phytoplankton because of the occurrence of high water column turbidity, the prevailing environmental conditions are not conducive for the substantial growth of picophytoplankton because of the prevalence of high nutrient levels (Chisholm, 1992; Iriarte and Purdie, 1994).

5. Conclusion

The present study has explicitly revealed the preponderance of diatoms in the CE, predominantly encompassed by small-sized species (mostly <20 μm), irrespective of seasons, even though the estuary persisted a distinctive spatio-temporal hydrological discrepancy. The relative dominance of fucoxanthin, enhanced diatom chl *a* equivalents (by CHEMTAX calculations) and also the consistent increase in $Diat_{DP}$ substantiated the preponderance of diatoms, proved by the microscope based qualitative examinations. The substantial contribution of nanophytoplankton to the total chl *a* and primary production revealed the significant role of the smaller phytoplankton community (mostly diatoms) towards the structuring and functioning of the planktonic food web and associated estuarine processes in the CE. Among diatoms, consistent numerical domination of *S. costatum* occurred throughout the study region, regardless of seasons, indicated its wide hydrological adaptability, particularly to salinity. The prevalence of enhanced water column turbidity due to the consistent increase in SPM was found to be one of the major influencing factors for the stable growth of smaller diatoms in the estuary. The characterization of marker pigments using HPLC, the first-time study from this region, could reveal not only the predominance of diatoms in the CE but also brought out the information regarding the distribution patterns of small-sized (<10 μm) phytoplankton taxonomic groups such as cyanophytes and cryptophytes.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: CSIR National Institute of Oceanography, Goa, India.

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