

## **Benthic-Pelagic Coupling Assessed Using Phytoplankton Marker Pigments: A Case Study From The Paradip Port, East Coast Of India**

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### **Abstract**

This study address the seasonal distribution of phytoplankton marker pigments (PMP) and the ratios (indicating freshness and fate) from water and surface sediments from the coastal port ecosystem (Paradip port, Odisha, east coast of India) and its utility in understanding phytoplankton pelagic-benthic linkages. Chlorophyll and PMP (for different groups and degradation) distribution revealed distinct seasonality, but the seasonal trend was different for water and sediments. High and low values were observed in the water column during inter-monsoons (fall/spring) and monsoons (southwest/northeast), respectively, whereas, in sediments, the reverse was recorded. However, the contribution of group specific PMP were different: fucoxanthin>chlorophyll b> zeaxanthin> alloxanthin>peridinin dominated in water and chlorophyll b>zeaxanthin >fucoxanthin> alloxanthin>peridinin in sediment. Selective grazing and stability of sedimentary PMP (fucoxanthin, diatoms PMP, is least stable than other groups) could contribute to such differences. Relatively high chlorophyll:pheopigment ratios in the water and low pheophorbide: pheophytin in sediments indicated the dominance of actively growing microalgae and chlorophyll degradation via chlorophyllidase pathway in the water but not in sediments. These findings suggest that (i) much of the phytoplankton (primarily diatoms) is lost due to herbivory before reaching bottom sediments, and (ii) pigment contribution is determined by selective grazing in water and PMP decay constants in sediments. Documenting such information will give new insights into ecosystem assessment and algal bloom research.

**Key words:** Phytoplankton; Pelagic-benthic linkages; Chlorophyll; Marker pigments; Pheopigments

## Introduction

Phytoplankton are accountable for about half of the global primary production and establish a base to the food web, which supports either directly or indirectly to the entire marine ecosystems (Jeffrey and Vesik 1997; Falkowski et al. 2004). Phytoplankton are represented by considerable diversity of algal groups with size ranging from 0.2 microns to several millimeters. All phytoplankton, by their photosynthetic activities, are a significant contributor to global carbon fluxes (Falkowski 1998). In the coastal area, benthic faunal changes are determined by the fluctuation of phytoplankton, which is major organic input (Zhang et al. 2015). Since phytoplankton are the principle element of biogeochemical cycles and food web dynamics, accessing phytoplankton biomass and its functional groups from pelagic and benthic environments plays a crucial role in environmental assessments.

Benthic-pelagic coupling is demonstrated as the exchange of energy, biomass, or inorganic nutrients between benthic and pelagic habitats. It plays a crucial part in the nutrient cycling and food web dynamics of aquatic ecosystems. Coastal and estuarine systems are strongly affected by anthropogenic activities, but the understating of organic matter and inorganic nutrient exchange between the benthic environment and water column are minimal (Griffiths et al., 2017). Therefore, knowledge of the phytoplankton composition and biomass from the pelagic ecosystem is essential to understand the linkage and dynamics (Mendes et al. 2011; Agirbas et al. 2015). The water column phytoplankton changes also fuel the benthic production (Graff and Rynearson 2011, Freiberg et al. 2011). Sedimentary pigments reflect phytoplankton production and its pathway in the water column and sediment. Hence, it is hypothesized that assessing the dynamics of phytoplankton marker pigments from sediments and water columns will improve the understanding of benthic-pelagic coupling, which is an essential process in the functioning of the ecosystem (Freiberg et al. 2011). Apart from the group-specific phytoplankton marker pigments, chlorophyll *a* and its degradation products (chlorophyllide *a* - Chlide *a*; pheophytin *a* - Pheo *a*; pheophorbide *a* - Pheid *a*;, and pyropheophytin *a* - pPheo *a*) often used as a diagnostic indicator for the fresh and degraded microalgal community, respectively (e.g. Wieking and Kröncke 2005; Pusceddu et al. 2009). Processes such as growth, grazing, cell sinking and senescence, photodegradation, fecal pellet sinking, physical mixing, and transport affect chl *a* and pheopigments concentrations in the euphotic zone. Hence assessing these pheopigments and their ratios will strengthen the understating of phytoplankton from the given ecosystem. So far several studies are either focused on the water column pigment distributions or sedimentary pigment biomarkers, including the Indian coasts (Ahel et al. 1996; Ston 2002; Lohrenz et al. 2003; Dyble and Moisander 2003; Henriksen et al. 2002; e.g., Chakraborty et al. 2011; Roy et al. 2011; Agirbas et al. 2017; Nunes et al. 2018; Naik et al. 2018,

Morata and Renaud. 2008; Reuss et al. 2010; Freiberg et al. 2011; Aneeshkumar and Sujatha 2012; Rasiq et al. 2016; Symczak-Zyla et al. 2008; Sañé et al. 2019). However, some microscopic studies indicated the persistence of benthic-pelagic coupling in bloom-forming diatoms (Patil and Anil 2008), diatom viability in sediments for several months (Anil et al. 2007), and phytoplankton pigment linkage between benthic and pelagic from the large shallow lake (Frieberg et al. 2011). The studies related to phytoplankton biomass and composition from the coastal sediment and its coupling between the water columns are limited to the best of our knowledge. This study expands the existing knowledge for the area in terms of distribution and dynamics of phytoplankton marker pigments, benthic-pelagic coupling, and the fate of ungrazed phytoplankton using marker pigments and the ratios indicating phytoplankton physiological status and the nature of the loss process.

## **Materials and Methods**

The present study was conducted in Paradip Port, which is one of the major seaport situated on the east coast of India (Fig. 1). It is an artificial lagoon type Port located at about 6.7 km south of Mahanadi River mouth in Odisha. This Port has a 190 m wide approach channel, protected by the northern and southern breakwaters with a length of 0.5 km and 1 km. Paradip Port exports and imports commodities like iron ore, manganese ore, coal, chrome ore, coking coal, petroleum, sulphuric acid, limestone. Paradip Port has 15 berths, three single point mooring, and 1 Ro-Ro jetty with the length from 50 m to 520 m and a well-maintained approach and entrance channel with the minimum depth of 17.1 m ([https://www.paradipPort.gov.in/Port\\_Brochure\\_Static.aspx](https://www.paradipPort.gov.in/Port_Brochure_Static.aspx)). Tides in this region are mixed semidiurnal type with an average tidal height of 1.87 m and 0.7 m during spring and neap tide, respectively. The average rainfall is 150 cm, with the maximum during active southwest monsoon SWM (June to August) and the little rainfall during FIM (retreating monsoon) from October to November. In general, the sediment texture is dominated by silt ( $59.0\% \pm 26.8\%$ ), followed by sand ( $37.3\% \pm 26.3\%$ ) and clay ( $2.8\% \pm 9.5\%$ ) during observation (Noyel and Desai, 2020).

## **Sampling strategy**

Water and sediment sampling was carried out from 12 fixed stations in the Paradip port (Fig. 1) on four different occasions representing different seasons for the period from 2014-2015, [8<sup>th</sup> - 15<sup>th</sup> August 2014 (SWM), 13<sup>th</sup> - 22<sup>nd</sup> December 2014 (NEM), 11<sup>th</sup> - 20<sup>th</sup> May 2015 (SIM), and 29<sup>th</sup> August to 8<sup>th</sup> September 2015 (FIM)]. Since September represent the transition between SWM and NEM seasons (Mukherjee et al. 2014) it is referred to as FIM in this study. During 2015 SWM, peak rainfall was observed during June to August 2015 and a decrease after that (Suppl. Fig. 1). All the

sampling was conducted between low-tide (1-1.5 m) and high-tide (2-3 m) in the morning. Additionally, during each sampling season, a 24h time series sampling (2h interval) at the center of the port (station no. 7, Fig. 1) was also carried out to assess the chl *a* and pheopigments variations due to tides (Suppl. Fig. 2-4. Water samples were collected using Niskin bottles (5 L) to measure environmental parameters such as salinity, dissolved oxygen, biological oxygen demand, phytoplankton pigments, and inorganic nutrients (nitrate, nitrite, ammonia, phosphate, and silicate). The water temperature was determined in situ using a multiparameter Sonde DS5X (Hydrolab). Salinity was measured with an Autosal (Guildline Autosal 8400B). Dissolved inorganic nutrients such as nitrate (NO<sub>3</sub>), phosphate (PO<sub>4</sub>), nitrite (NO<sub>2</sub>), ammonium (NH<sub>4</sub>), and silicate (SiO<sub>4</sub>) were analyzed by SKALAR SANplus ANALYSER. Dissolved oxygen (DO) and biological oxygen demand (BOD) were analyzed following standard methods (Parsons et al. 1984). For the pigment analysis, 250 to 500 ml of water samples were passed through GF/F filter (27mm; pore size 0.7 mm) and stored in at -80° C until analysis using high-performance liquid chromatography (HPLC). For the sediment pigment analysis, sediment samples were collected using Van Veen grab. The top 2cm surface sediment samples were collected using PVC corer with a diameter of 2.5 cm and stored in -80 °C till further analysis.

### **Phytoplankton pigment analysis from the water column**

The collected pigment samples were extracted by adding 3 to 4 ml of 90% acetone (V: V with the deionized water) to the frozen filter paper, which was sonicated using an ultrasonic probe (Labsonic U, B. Braun Biotech International, Leverkusen, Germany) for 10 sec at 20 kHz and kept on ice to prevent excessive heat. The extracts were then stored overnight at -20 °C for analysis using HPLC (Agilent Technology; Series 1200). Before analysis, all the extracts were filtered (Nylon filter membranes, 0.2 µm pore size) immediately before injection in the HPLC to remove cells and debris from sonicated filter paper. To prevent the photolysis of the samples, extraction and injection procedures were performed under dim light. Pigment analysis was performed following the method of Van Heukelem (2002) with a slight modification as detailed subsequently. The pigment extracts were injected into the HPLC system equipped with an Agilent 1200 series pump; an Agilent diode array detector connected using a reverse-phase C8 Column maintained with 60°C (150 X 4.6 mm, 3.5 mm particle size) with 3 phase linear solvent gradient program at a flow rate of 1.1 ml/min. The excitation and elution of the extracted pigments were detected at 450 and 665 nm. The quantitative and qualitative analysis of about 20 pigments, including pheopigments, was carried out using the commercially available pigment standards purchased from DHI Inc (Denmark) and Sigma–Aldrich (USA). Chlorophyll degradation products such as pheophytin and pheophorbide were also

calculated along with the other accessory pigments to understand the different season's ecosystem processes. Chlorophylls, carotenoids, and other chlorophyll breakdown products were quantified using the commercially available pigment standards purchased from DHI Inc (Denmark). Chlorophyllide, another degradation pigment, was not detected in any of the samples analyzed. Additionally, the following pigment ratios Chl *a*: total pheopigments (TPhe), pheophorbide: pheophytin (Pheid : Phe) were also considered for better interpretation. Among these, only the average ratio of Pheid: Phe ratio presented in this manuscript were taken from Sathish et al. (2020) and it is used only to compare with the data from the near bottom and surface sediment to delineate the factors influencing benthic-pelagic coupling.

### **Phytoplankton pigment analysis from the sediments**

Sediment samples (0.5 to 1 g) were transferred to the 15 ml amber colored polypropylene centrifuge tubes along with 4 ml of HPLC grade acetone. The mixture was then sonicated using an ultrasonic probe (Labsonic U, B. Braun Biotech International, Leverkusen, Germany) for 10 sec at 20 kHz and kept on ice to prevent excessive heat. After sonication, the mixture transferred to the deep freezer (-20 °C) for overnight incubation. Further, the sample mixture was centrifuged, filtered with a syringe filter (0.2 μ), and stored in the deep freezer until analysis. Quantitative analysis of sediment pigments was carried out using the HPLC system equipped with an Agilent 1200 series pump, and an Agilent diode array detector connected using a reverse-phase C18 Column (4.6 ×250 mm, 5.5 mm particle size) with 3 phase linear solvent gradient program was used. This gradient program was a modification of Wright et al. (1991) described by Chen et al. (2016) to enhance the separation of pheopigments. The excitation and elution of the extracted pigments were detected at 450 and 665 nm. The quantitative and qualitative analysis of chlorophylls, carotenoids, and chlorophyll degradation products was carried out using the commercially available pigment standards purchased from DHI Inc (Denmark) and Sigma–Aldrich (USA). Chlorophyllide, was not detected in any of the surface sediment samples as well. Pigments ratios Chl *a*: TPhe and Pheid: Phe were also calculated from analyzed sediment samples for better interpretation.

### **Statistical analysis**

ANOVA and followed by *post hoc* Tukey tests were performed using Statistica software to evaluate the spatial and temporal variations in the PMP's (both concentrations and contributions) and pheopigments ratios (Pheid:Phe and Chl *a*: TPhe). To evaluate the relationship between relative contribution (%) of PMP's and measured environmental parameters (temperature, salinity, DO, BOD, PO<sub>4</sub>, SiO<sub>3</sub>, NO<sub>2</sub>, NO<sub>3</sub> and NH<sub>4</sub>) redundancy analysis (RDA) was performed by using the

CANOCO 4.5 software (ter Braak and Smilauer 2002). For RDA, arcsine transformed data of relative contribution of PMP's were used. For surface water, PMP's and environmental parameters corresponding to surface water were used, whereas for NBW and sediment PMP's and environmental parameters corresponding to NBW were used. Before RDA, data was initially analyzed by detrended correspondence analysis. Since the most extended gradient length for the water column (Surface and NBW) and sediment was less than 3.0, RDA was performed.

## Results

### Environmental parameters

The results revealed that the measured environmental parameter showed a distinct seasonality. Water temperature ranged from 25.2 to 30.4 °C with the highest temperature during FIM ( $29.7 \pm 0.4$  °C). The water column remained cooler during NEM (25.2 to 26.9 °C) and warmer during SWM (19.1 to 28.6) and SIM (27.4 to 28.8 °C). Salinity variations were wide (25.6 to 33.9), with the highest (33.8 to 33.9) salinity observed during SIM followed by NEM (28.9 to 32.6) and FIM (28.1 to 31.5). The lowest salinity was found during SWM (25.6 to 28.7). Higher salinity was observed in the NBW compared to surface water during all seasons, suggesting the freshwater influence in the port area. ANOVA revealed an insignificant difference ( $p > 0.05$ ) in salinity and temperature between surface and NBW. DO concentration ranged from 2.6 to 5.6  $\mu\text{g l}^{-1}$  with the maximum concentration ( $5.2 \pm 0.4$   $\mu\text{g l}^{-1}$ ) in the surface water during SWM. There was no considerable difference in the averaged DO concentrations during NEM, FIM, and SIM and ranged between 4.0 to 4.7  $\mu\text{g l}^{-1}$ . ANOVA revealed a significant difference ( $p < 0.001$ ) in DO concentrations for surface and NBW, except during NEM. BOD values ranged from negligible to 2.7  $\mu\text{g l}^{-1}$  with the maximum and minimum demand during FIM (0.6 to 2.7  $\mu\text{g l}^{-1}$ ) and SWM (0 to 0.8  $\mu\text{g l}^{-1}$ ), respectively (Fig. 2).

$\text{NO}_3$  concentrations were high during FIM (1.2 to 14.7  $\mu\text{M}$ ) compared to other seasons and during the rest of the seasons (SWM, NEM, and FIM) magnitude of the averaged values (4.0 to 4.8  $\mu\text{M}$ ) was same.  $\text{NO}_2$  concentration ranged from 0.4 to 3.3 with the maximum and minimum during SIM ( $1.8 \pm 0.2$   $\mu\text{M}$ ) and SWM ( $0.9 \pm 0.2$   $\mu\text{M}$ ) respectively.  $\text{PO}_4$  concentrations ranged up to 2.8  $\mu\text{M}$  with the higher and lowest values during SIM (2.2 to 3.0  $\mu\text{M}$ ) and FIM (0 to 0.5  $\mu\text{M}$ ), respectively. Highest  $\text{SiO}_4$  levels were observed during FIM (8.1 to 134.9  $\mu\text{M}$ ) and SIM (52.3 to 100.0  $\mu\text{M}$ ) compared to NEM (31.8 to 106.4  $\mu\text{M}$ ) and SWM (13.7 to 27.0  $\mu\text{M}$ ). Interestingly,  $\text{SiO}_4$  concentrations ( $71.1 \pm 41.9$   $\mu\text{M}$ ) were relatively higher in the NBW compared to surface water ( $60.3 \pm 37.6$   $\mu\text{M}$ ) during FIM. There was no significant difference ( $p > 0.05$ ) in  $\text{PO}_4$ ,  $\text{NO}_3$ ,  $\text{NO}_2$  and  $\text{SiO}_4$  values between surface and NBW (Fig. 3).

### **Phytoplankton pigment concentrations**

Altogether 16 pigments were observed in the water column, and 11 pigments were observed in the surface sediment samples. Chl *a*, Chl *b*, Fuco, Zea, Allo, Peri, Phe *a*, and Pherd *a* were the primary pigments observed in the water column and sediment. The results indicated a distinct seasonality in the distribution of biomass (chl *a*) and marker pigments (for different groups and degradation) from the sediment and overlying water column. However, the seasonal trend in the surface and NBW was the same but not with the sediment. In the surface and NBW, chl *a* ranged between 0.1 to 8.8  $\mu\text{g l}^{-1}$ . The higher values were observed during spring (1.1 to 3.8  $\mu\text{g l}^{-1}$ ) and fall (0.7 to 3.1  $\mu\text{g l}^{-1}$ ) inter-monsoons compared to the southwest (0.2 to 0.7  $\mu\text{g l}^{-1}$ ) and northeast (0.1 to 1.6  $\mu\text{g l}^{-1}$ ) monsoons. The chl *a* in sediments ranged between 0.01 to 0.2  $\mu\text{g g}^{-1}$  and the higher values were observed during southwest (0.03 to 0.14  $\mu\text{g g}^{-1}$ ) and northeast (0.02 to 0.24  $\mu\text{g g}^{-1}$ ) monsoons compared to fall (0.01 to 0.05  $\mu\text{g g}^{-1}$ ) and spring (0.03 to 0.10  $\mu\text{g g}^{-1}$ ) inter-monsoons (Fig. 4 and Table. 1). Next to chl *a*, Fuco, a marker pigment of diatom, was the dominant accessory pigment with average of all stations ranged between 0.07 to 0.60  $\mu\text{g g}^{-1}$  in the water column and ranging from 0.01 to 0.13  $\mu\text{g g}^{-1}$  in the sediment. High Fuco concentrations were observed in the water column during fall and spring inter-monsoons (average for all stations, 0.29 to 0.60  $\mu\text{g l}^{-1}$ ), and low values during southwest and northeast monsoons (average 0.07 to 0.13  $\mu\text{g l}^{-1}$ ) and the reverse trend was observed in the sediments. Moreover, significantly higher concentrations of Chl *b*, followed Zea, Allo, and Peri, which are considered as marker pigments for chlorophyte, cyanobacteria, cryptophytes and dinoflagellates, respectively, were observed during inter-monsoons (fall and spring) compared to monsoons (southwest and northeast; Table 1). Interestingly, 19' hexafucoxanthin and prasinoxanthin, which are marker pigments for prymesiophytes and prasinophytes, were present on most of the occasions, but the contribution was negligible in the water column and was not detected in the sediments. Phe *a*, and Pherd *a* are the primary and secondary breakdown product of the Chl *a*. The average concentration of Phe *a*, and Pherd *a* in the water column ranged between 0.02 to 0.14  $\mu\text{g l}^{-1}$ , whereas in the sediments, the ranges for former and latter were 0.15-1.26 and 0.06 to 0.32  $\mu\text{g g}^{-1}$ , respectively. Two-way ANOVA of all the detected pigments revealed significant variations ( $p < 0.001$ ) between seasons but not between the stations, and two hourly sampling (i.e. no tidal effect).

### **Phytoplankton marker pigment contributions**

Group specific phytoplankton marker pigments such as Chl *b* (chlorophytes), Fuco (diatom), Zea (cyanobacteria), Allo (cryptophytes), Peri (dinoflagellates), Pras (prasinoxanthin) and 19' Hexa (prymesiophytes) showed distinct seasonal and depth variations (among the surface, NBW and sediment) in the contributions. However, the percentage contribution varied between the water

column depth and sediment. Among the PMP's, Fuco, Chl *b* and Zea were dominant marker pigments in both water column and sediment. In water column, Fuco (Avg. 31.9 to 69.2 %) was the dominant marker pigment followed by the Chl *b* (Avg. 10.4 to 34.4 %) and Zea (Avg. 4.3 to 19.9 %) whereas in sediment Chl *b* (Avg. 30 to 38.1 %) contribution was higher followed by Zea (Avg. 24.2 to 30.2 %) and Fuco (Avg. 14 to 27 %). Fuco contributions in the surface water ranged between the 18.3 to 70.7 % with the maximum contributions during SWM ( $52.3 \pm 7.9$  %) and SIM ( $43.8 \pm 10.2$  %) compared to FIM ( $40.3 \pm 13.5$  %) and NEM ( $31.9 \pm 9.7$  %) whereas in NBW, Fuco contributions ranged between 21.9 to 80.1 % with the higher contribution during spring ( $61.7 \pm 4.1$  %) and fall ( $69.2 \pm 10.2$  %) inter-monsoons compared to SWM ( $55.3 \pm 7.8$  %) and NEM ( $37.6 \pm 15.2$  %). In sediment, Fuco contributions ranged between 6.9 to 46.1 % with maximum contribution during SWM ( $27 \pm 9.4$  %) and NEM ( $17.7 \pm 4.5$  %) compared to SIM ( $16.2 \pm 6.1$  %) and FIM ( $14 \pm 4.3$  %). Next to Fuco, Chl *b* was the most dominant pigment in the water column whereas in sediment, Chl *b* was the dominant PMP. In water column, Chl *b* ranged between 2.9 to 49.3 % with the maximum contribution during NEM (2.9 to 49.3 %) and SWM (12.8 to 35.4 %) compared to SIM (10.6 to 32.6 %) and FIM (5 to 27.2 %) whereas in sediment Chl *b* ranged between 13.6 to 62.9 % with the higher contributions during FIM (27.1 to 47.4 %) and SIM (19.3 to 62.9 %) compared to NEM (13.6 to 58.7 %) and SWM (20.8 to 41.4 %). The contribution of Zea, the third highest contributor, in the water column ranged between 0 to 38.9 % with the maximum contributions during NEM (2.8 to 38.9 %) and FIM (0 to 38.4 %) compared to SWM (4.3 to 15.7 %) and SIM (3.5 to 18.9 %) whereas in sediment it ranged between 14.1 to 41.3 % with higher contribution during NEM (18.3 to 40.5 %) and SIM (17.6 to 41.3 %) compared to SWM (15.8 to 35.9 %) and FIM (14.1 to 35.7 %). The contribution of other PMP were minimal and notable but not similar between water column and sediment. For instance, Peri and Allo contributions were higher in the sediment (Avg. Peri; 5.7 to 9.5 % and Allo; 7.8 to 16.6 %) compared to water column (Avg. Peri; 0.8 to 7.7 % and Allo; 2.4 to 6.2 %). In the water column Allo contribution was maximum during SIM (2.8 to 11.6 %) and SWM (2.2 to 12.8 %) and minimum during NEM (0 to 9.2 %) and FIM (1.1 to 8.2 %) whereas in sediment it was maximum during SWM (7.1 to 24.0 %) and FIM (8.2 to 22.5 %) compared to SIM (3.3 to 23 %) and NEM (0 to 12.6 %). In the case of Peri, contribution was higher during SIM (0 to 17.1 %) and FIM (0 to 12 %) compared to SWM (0 to 8.3 %) and NEM (0 to 10.3 %) in the water column whereas in sediment it ranged between 2.9 to 15 % with maximum contribution during NEM (2.9 to 15 %) and SIM (3.5 to 12.4 %). While 19 hexa (Avg. 1.2 to 6.4 %) and Pras (Avg. 2.8 to 5.5 %) were the least contributor and were detected only in water column. Their maximum contributions was observed during NEM and FIM (only Pras). Two way ANOVA of PMP revealed significant ( $p < 0.01$ ) variations between the sampling seasons (except for Peri and



Pras) and verticals (i.e., among the surface water, NBW and sediment) but the variation was insignificant ( $p > 0.05$ ) between the sampling stations and two hourly sampling (i.e. no tidal effect).

### **Chlorophyll a and Pheopigment ratios**

In this study, chl *a*:total phaeopigments (TPhe) and Pheophorbide:pheophytin ratios were considered as the former and latter indicates the micro-algal status and nature of the degradation pathway respectively. Chl *a*: TPhe ratio was high in the water column (average 3.95 to 9.17) compared to sediment (0.11 to 0.17) (Fig. 5). In surface water, the maximum of the average was observed during SIM ( $9.17 \pm 3.57$ ) followed by SWM ( $9.00 \pm 3.59$ ) and FIM ( $8.46 \pm 3.76$ ). The lowest value was observed during NEM ( $5.59 \pm 5.02$ ). Whereas in NBW, the maximum Chl *a*:TPhe ratio ( $6.28 \pm 2.75$ ) was observed during NEM while the minimum ( $3.95 \pm 2.26$ ) was observed during SWM (Fig. 6). Chl *a*: TPhe ratio from the surface sediments were observed  $< 0.17$  round the year. ANOVA results indicate there was no significant ( $p > 0.05$ ) seasonal or spatial variation in the Chl *a*: TPhe ratio from the water column as well as from the sediment. Pheid: Phe ratios were also high in the water column (average 0.67 to 1.39) compared to surface sediment (0.40 to 0.76) (Fig. 7). Box and whiskers plot (Fig. 7) indicating the higher Pheid:phe median values observed in the NBW (0.89 to 1.17) compared to surface water (0.55 to 0.72) except during NEM season (surface water- 1.04; NBW- 0.62). Whereas in sediment median values are much lower, (0.24 to 0.39) compared to water column depths. Variation between the quartiles were higher in the NBW compared to surface water during all the seasons while in sediment large variations observed only during SWM. Statistical outliers observed in the surface water (during SWM, NEM and SIM) and sediment (FIM, NEM and SIM) due to some stations with higher Pheid: Phe ratios. In surface water, Pheid: Phe ratio was high during NEM ( $1.39 \pm 1.33$ ) followed by SIM ( $1 \pm 1.02$ ), SWM ( $0.99 \pm 0.78$ ) and FIM ( $0.71 \pm 0.45$ ), while in NBW highest Pheid: Phe ratios were observed during SIM ( $1.24 \pm 0.5$ ) and SWM ( $1.19 \pm 0.66$ ) compared to FIM ( $0.88 \pm 0.35$ ) and NEM ( $0.67 \pm 0.46$ ). Pheid: Phe ratio in the surface sediment showed the maximum ( $0.76 \pm 0.59$ ) during SWM and the minimum ( $0.40 \pm 0.44$ ) during NEM (Fig. 7). ANOVA results suggested that there was no significant ( $p > 0.05$ ) spatial and temporal (seasonal and two hourly) variations in Pheid: Phe ratio between the stations or seasons.

### **Relationship between environmental parameters and PMP contribution**

Redundancy analysis was performed to understand the relationship between environmental variables and group specific PMP's contributions (Fig. 8). Temperature, salinity, PO<sub>4</sub>, NO<sub>2</sub> are the major variables significantly influencing the PMP's from the water column and sediment. In surface water biplot, axis 1 and 2 explained 69.1 and 91.8 % variance associated with the marker pigments and

environmental variables, respectively. Fuco positioning with the temperature and low salinity, SiO<sub>4</sub> and NO<sub>3</sub> suggested that the warmer and low salinity conditions favoured diatoms during inter-monsoons (fall and spring) and SWM by utilizing SiO<sub>4</sub> and NO<sub>3</sub>. Chl *b* and 19' Hexa positioned with the high salinity and low temperature conditions suggesting the cooler temperature, and high salinity during NEM favoured chlorophytes and prymnesiophytes. Peri and Allo positioned with the high temperature, Chl *a*, NO<sub>2</sub> suggesting that these conditions during inter-monsoons coincided with the higher dinoflagellates and cryptophytes. Zea and Pras was positioned with the high NO<sub>3</sub>, SiO<sub>4</sub> and low PO<sub>4</sub> and NH<sub>3</sub> values and is mainly due to the increased contributions of cyanobacteria and prasinophytes during FIM and NEM.

In NBW biplot, axis 1 and 2 explained 94.4 and 97.4 % variance associates with the marker pigments and environmental variables, respectively. Relationship with the PMP contributions and environmental variables in NBW is different compared to the surface water (except for Fuco). Like surface water, Fuco positioned with the high temperature and Chl *a* concentrations suggesting that the warmer conditions during inter-monsoons favoured phytoplankton biomass, particularly diatoms.. On the other hand high PO<sub>4</sub>, NO<sub>2</sub> and NO<sub>3</sub> and low SiO<sub>4</sub> concentrations favoured the next dominant pigment Chl *b* during NEM and SWM. While the third dominant pigment Zea and the least contributed pigment Pras dominance during NEM coincided with high PO<sub>4</sub>, NH<sub>3</sub> and DO values and lower temperature and Chl. Positioning of Peri with high SiO<sub>4</sub> and low PO<sub>4</sub>, NO<sub>2</sub> and NO<sub>3</sub> concentrations suggesting their role in favouring dinoflagellates during FIM and NEM while the 19 hexa and Allo showed the insignificant relationship with the environmental variables in NBW.

In sediment biplot, axis 1 and 2 explained 57.3 and 84.7 % variance associates with the marker pigments and environmental variables, respectively. Relationship trend between sediment PMP contributions and environmental variables is different compared to surface water and NBW. The contribution of the most dominant PMP Chl *b* was positioned with the high salinity and SiO<sub>4</sub> and lower NH<sub>3</sub> and DO values indicated their influence on the chlorophytes dominance during inter-monsoons (fall and spring). The increase in Fuco contribution during SWM and NEM seasons could be due the prevalence of high DO, Chl *a*, NH<sub>3</sub> values and lower salinity, Chl *a*, SiO<sub>4</sub>, and NO<sub>2</sub> concentrations.. The notable contribution of Allo was coincided with the high temperature and low PO<sub>4</sub>, DO and Chl *a* suggesting their role in favouring cryptophytes during SWM and FIM. The Zea and Peri contributions positioned with the environmental settings (high salinity, Chl *a*, NO<sub>2</sub> and PO<sub>4</sub> values and low temperature) coincided with higher cyanobacteria and dinoflagellates during NEM and SIM.

## Discussion

The present study elucidates the usefulness of phytoplankton marker pigments (such as chlorophylls, carotenoids, and pheopigments) to understand the benthic-pelagic coupling from the Paradip port ecosystem. The measured environmental variables showed a distinct seasonal variation, and this was also reflected in the distribution of phytoplankton biomass and group specific marker pigments (Fig. 2, 3 and 8). For instance, (i) water temperature followed the classic radiation pattern and so with the phytoplankton community (Fig. 8; Sarma et al. 2013) i.e. flagellates dominated in cooler and diatoms in warmer during NEM and FIM, respectively, (ii) seasonal freshening of surface water (during SWM) coincided with low biomass, (iii) seasonal influx of high inorganic nutrients (Sundaray et al. 2006) coincided with high phytoplankton biomass. The nutrient concentrations were not depleted in the study area throughout the year (except phosphate during FIM; Figs. 3 and 8). Generally, low water movement and current effects from the inner port area of Ceuta port (North Africa) and Tolo Harbour (Hong Kong) explain that the port structure negatively influences the benthic community (Guerra-García and García-Góme 2003) and induced algal blooms (Wong and wong 2004), respectively. Paradip port, which is considered as a semi-enclosed body, also supports this assumption of relatively higher nutrient concentrations and restricted circulations. Though Paradip port handles large volume of cargo, the influence of anthropogenic activities on the ecosystem (particularly on nutrient concentrations and phytoplankton biomass) is much less compared to some of the major ports along the Indian coasts. For instance, the nutrient concentrations and phytoplankton (biomass and composition) are much less than the other major ports along the Indian coasts (Sathish et al. 2020). Further, the statistically no significance between the stations also signifies nearly homogenous (except at few instances during inter monsoons) phytoplankton pigment distribution during the study period (Fig. 4). Nevertheless, these findings on spatio-temporal variations agree (only distribution trends) with previous studies conducted during 2011-12 (Mohapatra and Panda, 2017).

Previous microscopic studies from Paradip port (Mohapatra and Panda, 2017) as well as from the east coast of India (Goudaand Panigrahy 1989; Goudaand Panigrahy 1996; Madhupratap et al. 2003; Panigrahy et al. 2006; Paul et al. 2008; Naik et al. 2008; Chakraborty et al. 2011; Baliarsingh et al. 2012) suggest that diatom and dinoflagellates are the dominant phytoplankton groups from the water column. However, the information on the smaller or other taxonomic groups of phytoplankton is lacking. In our study, the distribution and dynamics of sedimentary pigments did not match the dynamics of phytoplankton pigments in surface water and NBW. PMP observed from the sediment could be the recent settlement of phytoplankton due to the high sedimentation and resuspension

(Ananth and Sundar, 1990). The chlorophyll *a* distribution indicates that the Paradip port waters are more productive during inter-monsoons (fall and spring) compared to the monsoons (southwest and northeast), but pigment markers from the surface sediment exhibit the reverse trend i.e. high and low during monsoons and inter-monsoons respectively (Fig. 3). This could be due to the termination followed by sedimentation of water column phytoplankton chlorophyll *a* to the sediment towards the end of inter-monsoons.

However, the contribution of the major phytoplankton marker pigments was different between the surface and near bottom waters as well as between those and sediments (Table 1 and Figs. 5). The high concentrations of Fuco, chl *b* and Zea in the water column indicates the dominance of diatom, chlorophytes and cyanobacteria (Jeffrey and Vesk 1997). Diatom was the dominant group during warmer seasons, except during NEM (i.e. winter) season. Higher diatom contribution observed in the NBW compare to the surface water (Fig 5) was likely attributable to their ability to adapt in low light conditions (Lionard et al., 2005). Additionally, relatively higher Pheid:phe ratio also suggests the prevalence of herbivory over the degradation due to senescence in the NBW compared to surface water (except during NEM). Apart from the diatom, chlorophytes followed by cyanobacteria are other dominant group with the higher contribution during NEM. These groups, which proliferated under relatively saline and high concentrations of macronutrients (PO<sub>4</sub>, NO<sub>3</sub> and SiO<sub>4</sub>), showed a significant depth wise (between surface and NBW stations) and seasonal variations. PMP's such as Pras, Per, and 19 Hexa, which indicates the presence of prasinophytes, dinoflagellates, and prymnesiophytes respectively (Jeffrey and Vesk 1997), were the least contributors (Table 1 and Fig. 5) and also showed a significant difference between surface and NBW. Since, in all seasons, the water column is well-mixed as evidenced from the same water temperature, salinity, and DO between the surface and bottom waters, the light availability and the biological activity (as evidenced from BOD data and pheophorbide: pheophytin ratio) could influence the variability in PMP relative contribution, chl *a* and its degradation between surface and bottom water. In surface sediment, higher contributions of Chl *b*, Zea, Fuco and Allo indicated the dominance of chlorophytes, cyanobacteria, diatom and cryptophytes, respectively. Chl *b* and Zea were major contributors followed by Fuco and Allo. Fuco, Pras and 19 Hexa contributions decreased compared to the water column, whereas the Zea, Allo, Chl *b* and peridinin concentrations increased markedly. Higher Zea and Allo concentrations during monsoons (SWM and NEM) coincided with the higher sediment chlorophyll *a* concentration. The variations in pigment concentrations indicates increase in the relative contributions of chlorophytes, cyanobacteria, cryptophytes and dinoflagellates as well as a decrease in the diatom. Marker pigments representing

prymnesiophytes, and prasinophytes are negligible and it could be existed in the below the detection limits (Table 1; Figs. 4 and 5). The relative contribution PMP for phytoplankton groups indicates that the organic matter in the sediment was majorly from the chlorophytes followed by cyanobacteria, diatoms, cryptophytes and dinoflagellates (Fig. 5).

The diatom dominance in the water column was not reflected in the sediment, and the possible reasons are delineated in this study. Some recent studies suggested that the nano and picophytoplankton might be crucial in fueling organic matters to the benthic community (Chen et al. 2016). The dominance of chlorophytes (chl b) in the sediment can be explained by the following (i) selective grazing by zooplankton on diatom and other groups decreases the percentage contribution (ii) much of the settled diatom could be grazed by benthic invertebrates (iii) marker pigments of diatom are least stable than marker pigments of chlorophytes and cyanobacteria. Bianchi et al. (2000) observed comparable decay constant (0.04-0.07 day<sup>-1</sup>) for both Chl a and Fuco in laboratory sediment microcosms. Meanwhile Schüller et al. (2015) reported the decay rate constant  $k$  of phytoplankton pigments in sediment cores from New Zealand fjords, which suggested that Allo, Zea and Chl b were less degradable than Chl a:  $k$  (y-1)= -0.004 for Chl a, -0.001 for Chl b, -0.0002 for Zea, +0.0001 for Allo, where negative and positive value means pigments decay and accumulate within the core, respectively. Previous studies have pointed out that chl b, leutin, Zea and allo are more chemically stable than Fuco, which attributed to the presence of 5,6 epoxides in Fuco (Repeta and Gagosian 1987). This would also be consistent with the result that the contribution by Fuco was lower and the contribution by Zea was higher in the sediments, compared to those in the water column. In spite of the smaller Allo contributions in the water column, elevated levels in the sediment could be due to the higher stability of Allo than others as suggested by Schüller et al. (2015). Additionally, several studies also reported the influence of microphytobenthos on the phytoplankton group contributions from the shallow coastal benthic ecosystems (Macintyre et al. 1996; Hardison et al. 2013; Semcheski et al. 2016). In this study, even though Paradip port is a shallow coastal water (depth 6.3 to 21.1m; Fig. 4), microphytobenthos may not influence the outcome of this study significantly as the biomass distribution showed distinct variations with respect to the season as well as between water and also the contribution of active microalgae in sediment is negligible compared to overlying waters as evidenced by substantially low chl *a* to total pheopigment ratios (Fig. 6). Phytoplankton in aquatic ecosystems undergoes several losses (mortality) processes (e.g., grazing, sedimentation, cell senescence, and viral lysis), and therefore, it is important to determine the fate of phytoplankton from the sediment and overlying water column. In such cases, PMP and chlorophyll degraded pigments (such as pheopigments) can be used to

understand the chlorophyll breakdown (ChlB) pathway, which will lead to a better understanding of the benthic-pelagic coupling from the given ecosystem. To the best of our knowledge, more in-depth information on chl *a* breakdown is available for leaf senescence in land plants compared to algae (Scheer, 2012). In the ChlB pathway, pheophorbide formation is an important step, formed either via chlorophyllide or pheophytin. Some studies reported that the initial ChlB-pathway induced by grazing and senescence are not the same, i.e., chlorophyll-chlorophyllide-pheophorbide (CCP) and chlorophyll-pheophytin-pheophorbide (CPP) in the former and latter case, respectively (Satoh and Hama 2013; Hu et al. 2013; Sathish et al. 2020). Dephytylation of chl *a* by chlorophyllase activity during cell senescence was widely accepted as the only pathway until the discovery of (i) the role of pheophytinase, a chloroplast-located enzyme, and (ii) the involvement of chlorophyllase activity in the conversion of chlorophyll into chlorophyllide when leaf cells are disrupted (e.g., during grazing/chewing) or when chlorophyllase is genetically mislocalized to chloroplasts. In phytoplankton, the enzymatic activities involved in chlorophyll breakdown varied significantly among the species and were confirmed through grazing studies. For instance, (i) ChlB-pathway under grazing-pressure is species-specific and the grazer's feeding habits (i.e., selective grazing) and, (ii) under grazing-pressure the pheophorbide concentration were higher and also the maximum pheophorbide:pheophytin ratio was observed in diatoms or diatom dominated mixed assemblage (>1 to 40) than other tested autotrophic flagellates (0.1 to <1) suggesting a potential proxy for determining nature of breakdown pathway. In such cases, pheophorbide to pheophytin (Pheid: Phe) ratios can be used to determine the dominant chlorophyll breakdown pathway and the fate of phytoplankton in the given ecosystem. In this study, pool of chlorophyll degraded pigments is mainly derived from the pheophytin and pheophorbide, which can be attributed to the presence of CCP or CPP pathway, even with the absence of chlorophyllide.

Degradation rates vary abundantly among the marker pigments (Leavitt and Hodgson 2001, Schüller et al. 2015) and between the different habitats (Hurley and Armstrong 1990). Pigment degradation in the water column is rapid compared to the sediment (Leavitt and Hodgson 2001, and references therein). In different habitats (i.e., fresh and marine water), pigments degradation mainly depend on the photo oxidation, selective grazing, senescence, sinking rate (different species of living and dead cells) and microbial decay, cell lysis, and enzymatic metabolism during senescence (Louda et al. 1998; Reuss and Conley 2005; Fietz et al. 2005 and reference within). Subsequently, phytoplankton chlorophyll (cells and detritus matters) settled on the surface sediment, degrade more rapidly (Bianchi et al. 2000), and less rapidly (Hurley and Armstrong 1990; Yacobi et al. 1991) during oxic and anoxic conditions, respectively depending on the life strategies of phytoplankton,

burrowing invertebrates, and the physical condition of water (Leavitt and Carpenter 1989; Yacobi and Ostrovsky 2012). Oxic conditions ( $4.0$  to  $4.9 \mu\text{g l}^{-1}$ ) in NBW and increasing Chlorophyll degradation products from the surface sediment obtained in the present study support this statement. Also, the degradation of carotenoids highly depends on the chemical structure, the number of oxygen atoms, and the presence of 5,6-epoxy groups (Schüller et al. 2015, and references within). Perhaps, the decay constant ( $K^a$ ) of Chlorophyllide *a* ( $-0.007 \pm -0.007 \text{ year}^{-1}$ ) is lower than that of chlorophyll *a* ( $-0.004 \pm -0.003 \text{ year}^{-1}$ ), pheophorbide *a* ( $-0.003 \pm -0.001 \text{ year}^{-1}$ ) and pheophytin *a* ( $-0.003 \pm -0.003 \text{ year}^{-1}$ ; Schüller et al. 2015) i.e., it would be transformed faster than other degradation products, so only Chlorophyllide *a* was not detected in our samples. Hence chlorophyll and pheopigments ratios can be a good indicator to understand the fate of phytoplankton.

The present study indicates that the Pheid: Phe ratio did not exhibit significant variations between the seasons and stations, but the variations between the water column and sediments were apparent. Additionally, the station data were averaged and presented in this study considering enclosed port systems and well-mixed water columns. Low and high Pheid: Phe ratios (average) in the sediment ( $0.40$  to  $0.76$ ) and water column ( $0.67$  to  $1.39$ ) suggested that the contribution by CCP is smaller (or CPP is larger) to Pheid in sediments than that in water-columns. Meanwhile, the ratios indicate active cell destruction via grazing in the water column compared to sediment, which could be the reason for the lower contribution of diatom (fuco) and cryptophytes (allo) from the sediment (Fig. 7) when assuming that zooplankton feed selectively on them. Chlorophyll : TPhe (total pheopigments), an indicator of the freshness of organic matter deposited in the benthos (Morata et al. 2011) or actively growing community (Grippio et al. 2009; Allison et al. 2013), did not reveal any significant difference between the season. Higher Chlorophyll: TPhe values in the surface water and NBW ( $3.95$  to  $9.17$ ) compared to surface sediment ( $0.11$  to  $0.17$ ), indicating the presence of actively growing microalgal community in the water column and the organic matters in the sediment was not fresh (Fig. 6). In a parallel investigation, multivariate index of trophic state (TRIX) indicated good water quality in near-bottom water and high organic carbon load in the sediment might resulted in the stressed environment (Noyel and Desai 2020). Taking these into consideration, it is assumed that (i) much of the phytoplankton (mostly diatoms) is lost due to herbivory before reaching bottom sediments, and (ii) given low Pheid: Phe ratios in sediment samples, the effect of benthic grazing on PMPs will not be significant and PMP decay constants will determine pigment contribution. Generally, in shallow systems, the autochthonous and allochthonous supply do contribute to the pool of degraded pigments. Therefore the additional knowledge on the degree of suspended organic matter degradation and terrestrial material contribution would have strengthened the findings. Since

Paradip port is a sea port the magnitude of salinity variation in the port (even during peak monsoon) is very less compared to other estuarine ports eg. Cochin port (5.3 to 22.6; Satish et al. 2020) or Marmugao port (3.8 to 36.2) where salinity variations is large. This is because the Mahanadi estuary is located north of the port and therefore the influence of river water entering the port will be minimal. Considering the minimal riverine flux (as evidenced from salinity data), and the presence of the (i) lowest pheopigment concentration compared to estuarine and other seaports (up to  $6.07 \text{ mg m}^{-3}$ ; Sathish et al. 2020), (ii) active growing phytoplankton as evidence from high chlorophyll: pheopigment ratios and (iii) the distinct seasonality in chlorophyll distribution (with lowest ( $<1 \text{ mg m}^{-3}$ ) and highest ( $1.5\text{-}2.5 \text{ mg m}^{-3}$ ) during monsoon and inter-monsoon, respectively) it is assumed that the phytoplankton marker pigments in this study are mostly of autochthonous origin. These findings suggest that the knowledge on phytoplankton marker pigments combined with the chlorophyll degradation products from the benthic and pelagic environment will better understand the benthic-pelagic coupling, which will be helpful in ecosystem assessment and algal bloom research.

## **Conclusion**

This study concludes that the distribution of chlorophyll and PMP (for different groups and degradation) from Paradip port revealed a distinct seasonality. Still, the seasonal trends between water and sediment are not the same. Pheophorbide to pheophytin ratio indicated that the contribution by CCP is smaller (or CPP is larger) to Pheid in sediments than that in water-columns when a lower ratio is found in sediments than water samples. The contribution of actively growing microalgae was high in the water column but negligible in sediment, suggesting organic input was not fresh. It is assumed that (i) much of phytoplankton is lost due to herbivory in the water column before reaching bottom sediments, and (ii) PMP decay constants determine pigment contribution. Documenting the phytoplankton (biomass and composition), chlorophyll breakdown products, and key environmental variables will be helpful in ecosystem assessment and algal bloom research.

## **Acknowledgments**

We are grateful to the Director of CSIR–National Institute of Oceanography for his support and encouragement. We thank Dr. S.S. Sawant, and Mr. K. Venkat for co-ordinating Port Biological Baseline Survey (PBBS) at Paradip port under the above project and the project staff, who were involved in the PBBS, for their help during sampling. We are also thankful to the anonymous reviewers for their constructive suggestions. First author also acknowledges Council of Scientific



and Industrial Research (CSIR) for the Senior Research Fellowship. This is an NIO contribution, No. ....

**Ethical approval:** No animal testing was performed during this study.

**Consent to Participate:** Not applicable

**Consent to Publish:** Not applicable

**Author's contribution:** Sathish K: Port sampling, sample analysis, data interpretation and statistical analysis; JS Patil: Original concept, Port sampling, data interpretation, manuscript elaboration and supervision; AC Anil: Manuscript elaboration and also co-supervised all works including acquisition of funds and project administration.

**Funding:** This work was supported by DG Shipping, Ministry of Shipping, Govt. of India funded project Ballast Water Management Program-India (GAP 2429).

**Competing Interests:** No conflicts of interest have been declared by the authors.

**Availability of Data and material:** All data concerned to the study are included in this manuscript and some are also provided as supplementary. However it is not applicable for material availability.

## Reference

- Agirbas E, Feyzioglu AM, Kopuz U, Llewellyn CA (2015) Phytoplankton community composition in the south-eastern Black Sea determined with pigments measured by HPLC-CHEMTAX analyses and microscopy cell counts [J]. *J Mar Biol Assoc UK* 95(1): 35–52.
- Agirbas E, Sahin A, Aytan U, Koral S, Fidan D (2017) Seasonal changes in phytoplankton size classes (PSC) derived from HPLC pigment data along the South-Eastern Black Sea [J]. *Turkish J Fish Aquat Sc* 17: 1449-1465
- Ahel M, Barlow RG, Mantoura RFC (1996) Effect of salinity gradients on the distribution of phytoplankton pigments in a stratified estuary [J]. *Mar Ecol Prog Ser* 143: 289–295
- Allison GJ, Wagner ME, McAllister M., Ren AKJ, Snyder RA (2013) Sand bottom microalgal production and benthic nutrient fluxes on the northeastern Gulf of Mexico nearshore shelf [J]. *Gulf Caribb Res* 25, 1-8.
- Ananth, PN, Sundar, V (1990). Sediment budget for Paradip port, India. *Ocean and Shoreline Management* [J] 13(1), 69–81. [https://doi.org/10.1016/0951-8312\(90\)90013-8](https://doi.org/10.1016/0951-8312(90)90013-8)
- Aneeshkumar N, Sujatha CH (2012) Biomarker pigment signatures in Cochin back water system – A tropical estuary south west coast of India [J]. *Est Coast Shelf Sci* 99: 182–190
- Anil AC, Mitbavkar S, D'Silva MS, Hegde S, D'Costa PM, Meher SS, Banerjee D (2007) Effect of ageing on survival of benthic diatom propagules [J]. *J Exp Mar Biol Ecol* 343: 37–43.

- Baliarsingh SK, Sahu BK, Srichandan S, Sahu KC (2012) Seasonal Variation of Phytoplankton Community in Navigable Channel of Gopalpur Port, East Coast of India: A Taxonomic Study [J]. *Int J Modern Bot* 2(3): 40–46.
- Bianchi TS, Johansson B, Elmgren R (2000). Breakdown of phytoplankton pigments in Baltic sediments: effects of anoxia and loss of deposit-feeding macrofauna [J]. *J. Exp. Mar. Biol. Ecol* 251,161–183.
- Chakraborty P, Acharyya T, Babu PVR, Bandyopadhyay D (2011) Impact of salinity and pH on phytoplankton communities in a tropical freshwater system: An investigation with pigment analysis by HPLC [J]. *J Environmental Monit* 13(3): 614–620.
- Chen J, Oseji O, Mitra M, Waguespack Y, Chen N (2016) Phytoplankton Pigments in Maryland Coastal Bay Sediments as Biomarkers of Sources of Organic Matter to Benthic Community [J]. *J Coast Res* 32(4): 768–775
- Dyble J, Moisander PIAH (2003) Phytoplankton Photopigments as Indicators of Estuarine and Coastal Eutrophication [J]. *BioScience* 53(10): 953–964
- Falkowski PG (1998) Biogeochemical Controls and Feedbacks on Ocean Primary Production [J]. *Science* 281: 200–206
- Falkowski PG, Katz ME, Knoll AH, Quigg A, Raven JA, Schofield O, Taylor FJR (2004) The evolution of modern eukaryotic phytoplankton [J]. *Science* 305: 354–360
- Fietz S, Sturm M, Nicklisch A (2005). Flux of lipophilic photosynthetic pigments to the surface sediments of Lake Baikal [J]. *Glob Planet Change* 46, 29–44.
- Freiberg R, Nõmm M, Tõnno I, Alliksaar T, Nõges T, Kisand A (2011) Dynamics of phytoplankton pigments in water and surface sediments of a large shallow lake [J]. *Estonian J Earth Sciences* 60: 91–91.
- Gouda R, Panigrahy RC (1996) Ecology of phytoplankton in coastal waters off Gopalpur, east cost of India [J]. *Ind J Mar Sci* 25: 81-84.
- Gouda R, Panigrahy RC (1989) Diurnal variation of phytoplankton in Rushikulya estuary, east coast of India [J]. *Ind J Mar Sci* 18: 246-250.
- Graff JR, Ryneerson TA (2011) Extraction method influences the recovery of phytoplankton pigments from natural assemblages [J]. *Limnol Oceanogr* 9(4): 129-139.
- Griffiths JR, Kadin M, Nascimento FJA, Tamelander T, Törnroos A, Bonaglia S, Bonsdorff E, Brüchert V, Gårdmark A, Järnström M, Kotta J, Lindegren M, Nordström MC, Norkko A, Olsson J, Weigel B, Žydelis R, Blenckner T, Niiranen S, Winder M (2017) The importance of benthic–pelagic coupling for marine ecosystem functioning in a changing world [J]. *Global Change Biology*, 23(6): 2179–2196.
- Grippo MA, Flegger JW, Condrey R, Carman KR (2009) High benthic microalgal biomass found on Ship Shoal, north-central Gulf of Mexico [J]. *Bull Mar Sci* 84: 237-256.
- Guerra-García JM, García-Gómez JC (2005) Assessing pollution levels in sediments of a harbour with two opposing entrances. Environmental implications [J]. *J Environ Management*, 77: 1-11.

- Hardison AK, Canuel EA, Anderson IC, Tobias CR, Veuger B, Waters MN (2013) Microphytobenthos and benthic macroalgae determine sediment organic matter composition in shallow photic sediments [J]. *Biogeosciences*, 10(8): 5571–5588.
- Henriksen P, Riemann BO, Kaas H, Sørensen HM, Sørensen HL (2002) Effects of nutrient-limitation and irradiance on marine phytoplankton pigments [J]. *J Plank Res*, 24: 835–858.
- Hu X, Ayumi T, Ryouichi T (2013) Simple extraction methods that prevent the artifactual conversion of chlorophyll to chlorophyllide during pigment isolation from leaf samples [J]. *Plant Methods* 9(1): 19.
- Hurley JP, Armstrong DE (1990) Fluxes and transformations of aquatic pigments in Lake Mendota, Wisconsin [J]. *Limnol Oceanogr* 35: 384–398.
- Jeffrey SW, Vesk M (1997) Introduction to marine phytoplankton and their pigment signatures. In: Jeffrey SW, Mantoura RFC, Wright SW, (eds) *Phytoplankton pigments in oceanography* [M]. SCOR-UNESCO, Paris, 37–84.
- Leavitt PR, Carpenter SR (1989) Effects of sediment mixing and benthic algal production on fossil pigment stratigraphies [J]. *J. Paleolimnol* 2: 147-158.
- Leavitt PR, Hodgson DA (2001) Sedimentary pigments. In: Smol JP, Birks, HJB, Last WM (eds) *Tracking environmental change using lake sediments. Volume 3: Terrestrial, algal, and siliceous indicators* [M]. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 295-325
- Lionard, M, Azémar F, Boulétreau S, Muylaert, K., Tackx M, Vyverman, W (2005) Grazing by meso- and microzooplankton on phytoplankton in the upper reaches of the Schelde estuary (Belgium/The Netherlands) [J]. *Estuar. Coast. Shelf Sci* 64, 764-774.
- Lohrenz SE, Carroll CL, Weidemann AD, Tuel M (2003) Variations in phytoplankton pigments, size structure and community composition related to wind forcing and water mass properties on the North Carolina inner shelf [J]. *Cont Shelf Res* 23:1447–1464.
- Louda JW, Li J, Liu L, Winfree MN, Baker EW (1998) Chlorophyll degradation during senescence and death [J]. *Organic Geochemistry* 29: 1233–1251.
- Macintyre HL, Miller DC, Geider RJ (1996) Microphytobenthos: The ecological role of the "secret garden" of unvegetated, shallow-water marine habitats. II. Role in sediment stability and shallow-water food webs [J]. *Estuaries* 19(2): 202–212.
- Madhupratap M, Gauns M, Ramaiah N, Prasanna Kumar S, Muraleedharan PM, de Souza SN, Sardesai S, Muraleedharan U (2003) Biogeochemistry of Bay of Bengal: physical, chemical, and primary productivity characteristics of the central and western Bay of Bengal during summer monsoon 2001 [J]. *Deep-Sea Res II*, 50: 881–886.
- Mendes CR, Sá C, Vitorino J, Borges C, Tavano Garcia VM, Brotas V (2011) Spatial distribution of phytoplankton assemblages in the Nazaré submarine canyon region (Portugal): HPLC-CHEMTAX approach [J]. *J Mar Syst* 87(1): 90–101.
- Mohapatra KR, Panda RC (2017) Spatiotemporal variation of water quality and assessment of pollution potential in Paradip port due to port activities [J]. *Ind J Geo Mar Sci* 46(07): 1274–1286.

- Morata N, Renaud PE (2008) Sedimentary pigments in the western Barents Sea: A reflection of pelagic–benthic coupling? [J]. *Deep-Sea Res II*, 55: 2381– 2389.
- Morata N, Poulin M, Renaud PE (2011) A multiple biomarker approach to tracking the fate of an ice algal bloom to the sea floor [J]. *Polar Biol* 34, 101–112.
- Mukherjee A, Shankar D, Fernando V, Amol P, Aparna SG, Fernandes R, Michael GS, Khalap ST, Satelkar NP, Agarvadekar Y, Gaonkar MG, Tari AP, Kankonkar A, Vernekar S (2014) Observed seasonal and intraseasonal variability of the East India Coastal Current on the continental slope J. *Earth Syst. Sci.* 123(6): 1197–1232
- Naik RK, Damare S, Yapa KKAS, Costa PMD, Roy R (2018) First report of toxic *Prorocentrum rathymum* (Dinophyceae) and its pigment composition from coastal waters of the eastern Arabian Sea [J]. *Ind J Geo Mar Sci* 47: 645–652.
- Naik S, Acharya BC, Mohapatra A (2009) Seasonal variations of phytoplankton in Mahanadi estuary, east coast of India [J]. *Ind J Mar Sci* 38(2): 184–190.
- Noyel V, Desai DV (2020) Spatio-temporal variation in the macrobenthos of Paradip port, east coast of India [J]. *Curr Sci* 119(1): 68-82
- Nunes S, Latasa M, Gasol JM, Estrada M (2018) Seasonal and interannual variability of phytoplankton community structure in a Mediterranean coastal site [J]. *Mar Ecol Prog Ser* 592: 57–75.
- Panigrahy RC, Mishra S, Sahu G, Mohanty AK (2006) Species composition and diversity of surf zone phytoplankton of Gopalpur coast, East coast of India [J]. *J Mar Biol Assoc India* 8(1983): 1–15.
- Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis [M]. Pergamon Press, New York.
- Patil JS, Anil AC (2008) Temporal variation of diatom benthic propagules in a monsoon-influenced tropical estuary [J]. *Cont Shelf Res* 28: 2404–2416.
- Paul JT, Ramaiah N, Sardessai S (2008) Nutrient regimes and their effect on distribution of phytoplankton in the BOB [J]. *Mar Environ Res*, doi:10.1016/j.marenvres,05.007.
- Pusceddu A, Antonio D, Mauro F, Roberto D (2009) Quantity and bioavailability of sediment organic matter as signatures of benthic trophic status [J]. *Mar Ecol Prog Ser*, 375: 41–52.
- Rasiq KT, Kurian S, Karapurkar SG, Naqvi SWA (2016) Sedimentary pigments and nature of organic matter within the oxygen minimum zone (OMZ) of the Eastern Arabian Sea (Indian margin) [J]. *Estuar Coast Shelf Sci* 176: 91–101.
- Repeta DJ, Gagosian RB Carotenoid diagenesis in recent marine sediments-I. The Peru continental shelf (15°S, 75°W) [J]. *Geochimica Et Cosmochimica Acta* 51: 1001-1009.
- Reuss N, Leavitt PR, Hall RI, Bigler C, Hammarlund D (2010) Development and application of sedimentary pigments for assessing effects of climatic and environmental changes on subarctic lakes in northern Sweden [J]. *J Paleolimnol* 43: 149–169.
- Reuss N, Conley DJ (2005) Effects of sediment storage conditions on pigment analyses [J]. *Limnol Oceanogr Methods* 3:477–487.

- Roy R, Pratihary A, Narvenkar G, Mochemadkar S, Gauns M, Naqvi S (2011) Estuar Coast Shelf Sci The relationship between volatile halocarbons and phytoplankton pigments during a *Trichodesmium* bloom in the coastal eastern Arabian Sea [J]. Estuar Coast Shelf Sci 95: 110–118.
- Sañé E, Valente A, Fatela V, Cabral MC, Beltrán C, Drago T (2019) Assessment of sedimentary pigments and phytoplankton determined by CHEMTAX analysis as biomarkers of unusual upwelling conditions in summer 2014 off the SE coast of Algarve [J]. J Sea Res 146: 33-45.
- Sarma, VVSS, Krishna MS, Viswanadham, Rao GD, Rao VD, Sridevi B, Kumar BSK, Prasad VR, Subbaiah CV, Acharyya T, Bandopadhyay D (2013) Intensified oxygen minimum zone on the western shelf of BOB during summer monsoon: influence of river discharge [J]. J. Oceanogr 69(1): 45–55.
- Sathish K, Patil, JS, Anil, AC (2020) Phytoplankton chlorophyll-breakdown pathway: Implication in ecosystem assessment. J. Environ Manage 258, 109989
- Satoh Y, Hama T (2013) Stepwise alteration from fluorescent to non- fluorescent chlorophyll derivatives during early diagenesis of phytoplankton in aquatic environments [J]. J Exp Mar Biol Ecol 449: 36–44.
- Scheer H., (2012) Chlorophyll breakdown in aquatic ecosystems [J]. Proc. Natl. Acad. Sci 109 (43), 17311-17312.
- Schüller SE, Bianchi TS, Li X, Allison MA, Savage C (2015) Historical Reconstruction of Phytoplankton Composition in Estuaries of Fiordland, New Zealand: the Application of Plant Pigment Biomarkers [J]. Estuar Coast 38(1): 56–71.
- Semcheski MR, Egerton TA, Marshall HG. (2016) Composition and diversity of intertidal microphytobenthos and phytoplankton in Chesapeake Bay [J]. Wetlands 36(3): 483–496.
- Ston J (2002) Pigment composition in relation to phytoplankton community structure and nutrient content in the Baltic Sea [J]. Oceanologia 44: 419–437.
- Sundaray SK, Panda UC, Nayak BB, Bhatta D (2006) Multivariate statistical techniques for the evaluation of spatial and temporal variations in water quality of the Mahanadi river-estuarine system (India) - A case study [J]. Environ Geochem Health, 28(4): 317–330.
- Symczak-Zyla M, Kowalewska G, Louda JW (2008) The influence of microorganisms on chlorophyll a degradation in the marine environment [J]. Limnol Oceanogr, 53: 851–62.
- ter Braak CJF, Smilauer P (2002) CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5) [M]. <http://www.canoco.com/>
- Van Heukelem L (2002) HPLC phytoplankton pigments: sampling, laboratory methods, and quality assurance procedures [M]. In: Mueller J, Fargion G (Eds.), Ocean Optics Protocols for Satellite Ocean Color Sensor, NASA Technical Memorandum 2002–2004. 3(2): 16, 258–268.
- Wieking G, Kröncke I (2005) Is benthic trophic structure affected by food quality? The Dogger Bank example [J]. Mar Biol 146: 387–400.
- Wong CK, Wong CK (2004) Study of phytoplankton characteristics in Tolo Harbour, Hong Kong , by HPLC analysis of chemotaxonomic pigments [J]. J Appl Phycol 16: 469–476.

- Wright SW, Jeffrey SW, Mantoura RFC et al. (1991) Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton [J]. *Mar Ecol Prog Ser* 77: 183–196.
- Yacobi YZ, Mantoura RFC, Llewellyn CA (1991) The distribution of chlorophylls and carotenoids and their breakdown products in Lake Kinneret (Israel) sediments [J]. *Freshwater Biol* 26: 1–10.
- Yacobi YZ, Ostrovsky I (2012) Sedimentation of phytoplankton: role of ambient conditions
- Zhang Q, Warwick RM, McNeill CL, Widdicombe CE, Sheehan A, Widdicombe S (2015) An unusually large phytoplankton spring bloom drives rapid changes in benthic diversity and ecosystem function [J]. *Prog Oceanogr* 137: 533–545.

## Figure legends

Figure 1. Location of the study region and the sampling stations. 1. Boat basin, 2. Area adjacent to fertilizer berth, 3. Fertilizer berth II, 4. North Quay II, 5. Central Quay III, 6. Central Quay I, 7. Turning circle, 8. East Quay II, 9. North Quay I, 10. Coal berth I, 11. Coal berth II, and 12. Stone pitching side. Star symbol indicates location of time series station.

Figure 2. Seasonal variations in the measured environmental variables from the surface and near bottom (NBW) waters. SWM – southwest monsoon, NEM - northeast monsoon, FIM – fall inter-monsoon, SIM – spring inter-monsoon. Bars represent mean value for the 12 stations and the error bars indicate standard deviations from 12 stations.

Figure 3. Seasonal variations in the nutrient concentrations from the surface and near bottom (NBW) waters. SWM – southwest monsoon, NEM - northeast monsoon, FIM – fall inter-monsoon, SIM – spring inter-monsoon. Bars represent mean value for the 12 stations and the error bars indicate standard deviations reflecting the differences among stations.

Figure 4. Spatial and seasonal variations in the concentrations of phytoplankton marker pigments from the overlying waters (surface and near bottom - NBW) and sediments. SWM – southwest monsoon, NEM - northeast monsoon, FIM – fall inter-monsoon, SIM – spring inter-monsoon.

Figure 5. Bar graphs showing the spatial and seasonal variations in the contribution (%) of phytoplankton marker pigments for the overlying waters (surface and near bottom - NBW) and sediments. SWM – southwest monsoon, NEM - northeast monsoon, FIM – fall inter-monsoon, SIM – spring inter-monsoon.

Figure 6. Seasonal variations in the chlorophyll *a* to total pheopigment ratios from the surface and near bottom waters. SWM – southwest monsoon, NEM - northeast monsoon, FIM – fall inter-monsoon, SIM – spring inter-monsoon. Bars represent mean value for the 12 stations and the error bars indicate standard deviations reflecting the differences among stations.

Figure 7. Seasonal variations in the pheophorbide to pheophytin ratios from the surface and near bottom waters. Bar plot represent (a) station wise data and (b) mean value for the 12 stations and the error bars indicate standard deviations reflecting the differences among stations for each season. SWM – southwest monsoon, NEM - northeast monsoon, FIM – fall inter-monsoon, SIM – spring inter-monsoon. Bars represent the mean value for the 12 stations and the error bars indicate standard deviations reflecting the differences among stations. \* Numbers in the parenthesis represent values for outliers.

Figure 8. Redundancy analysis biplot to understand the relationship between the measured environmental variables and relative contribution of phytoplankton marker pigments (–Fuco – Fucoxanthin; chl b – chlorophyll b, zea – zeaxanthin, allo – alloxanthin, peri – peridinin, pras – prasinoxanthin, 19 hex – 19' hexafucoxanthin) for (a) surface water, (b) near-bottom water and (c) surface sediments. In case of sediments, environmental variables corresponding to near bottom waters were used.

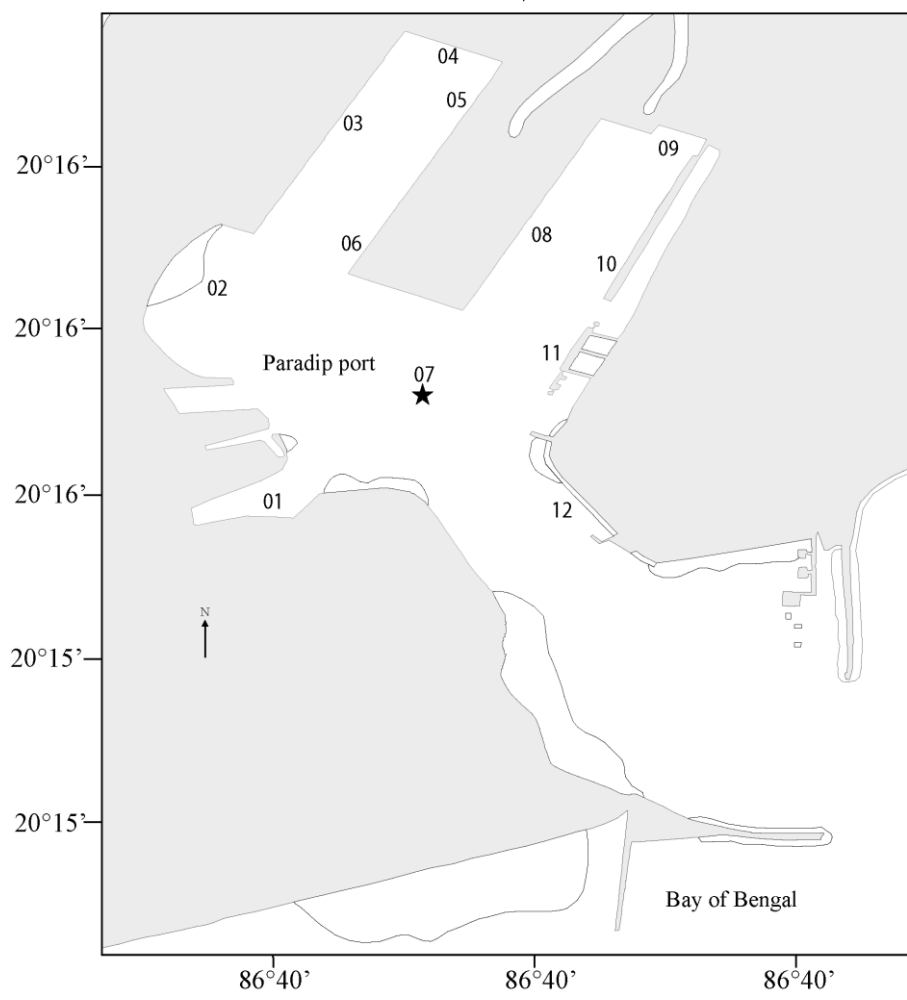
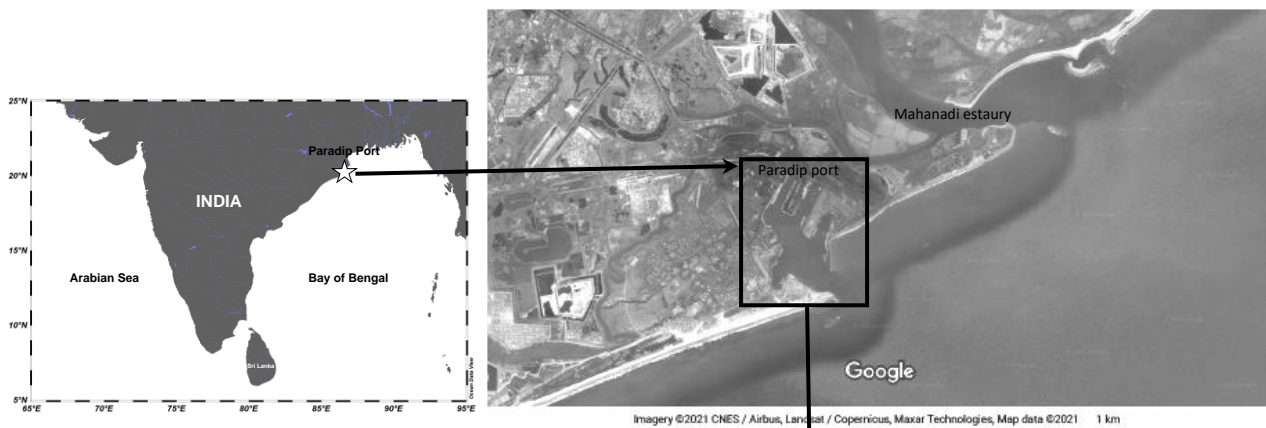


Fig. 1



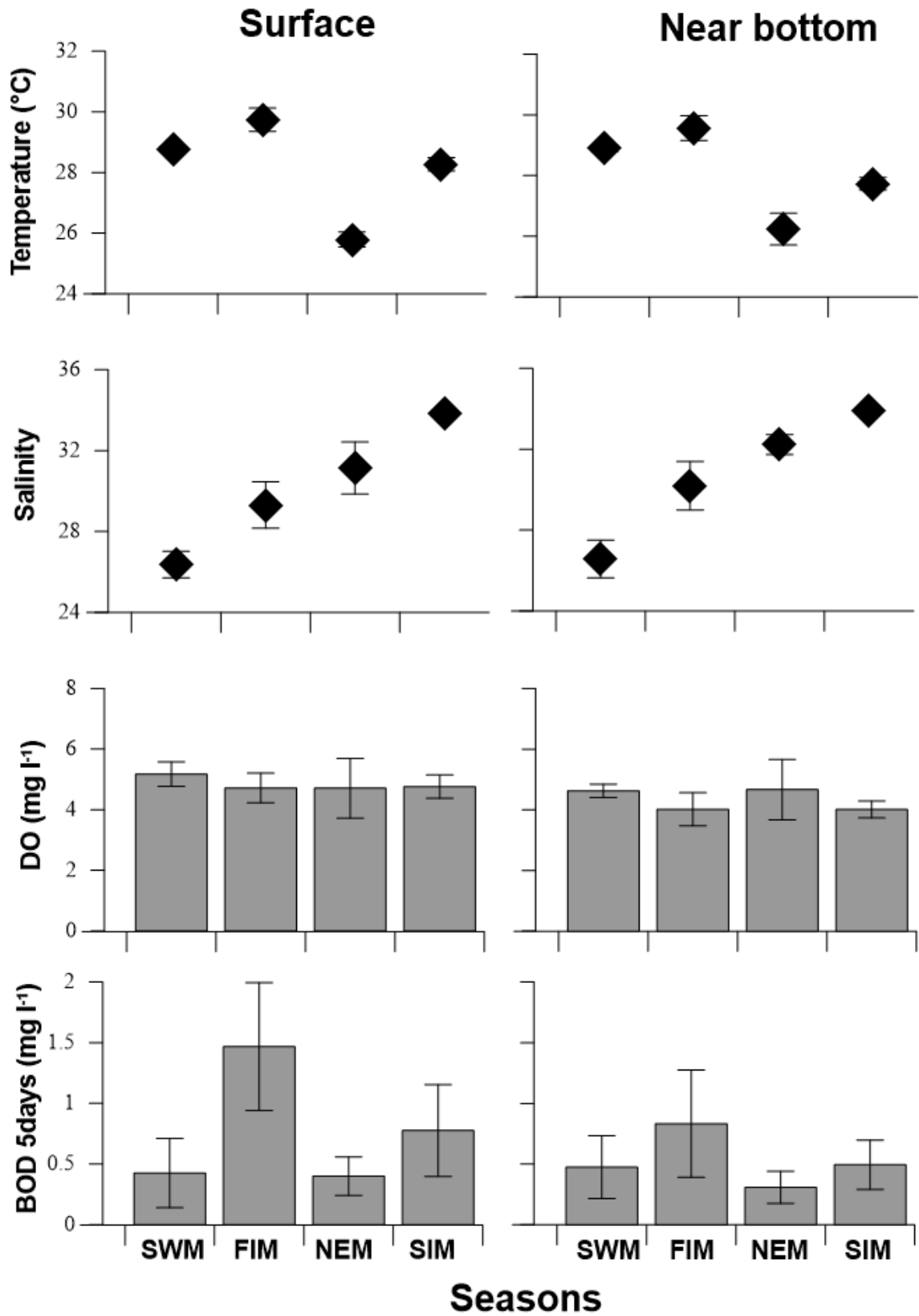


Fig. 2.

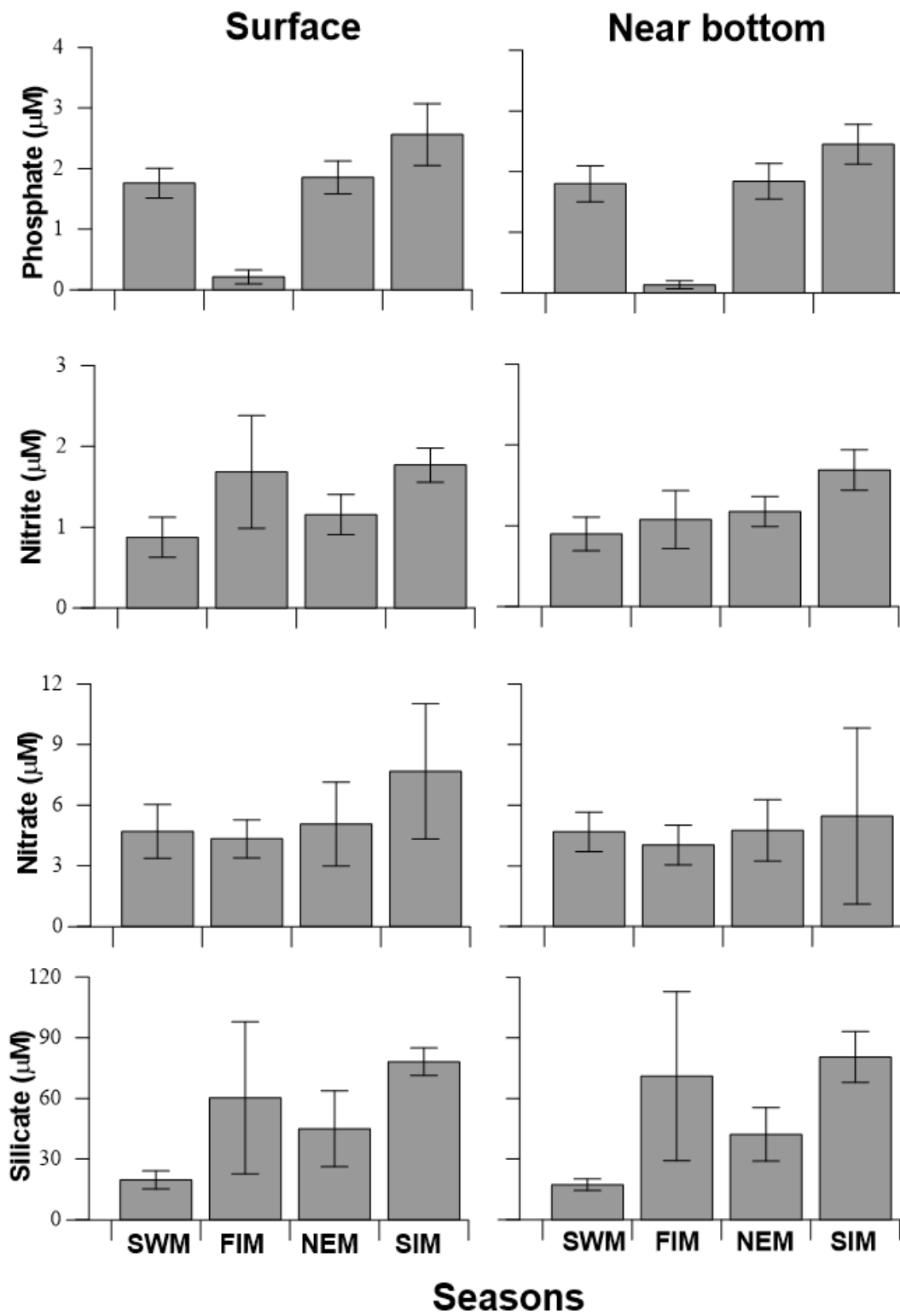


Fig. 3.

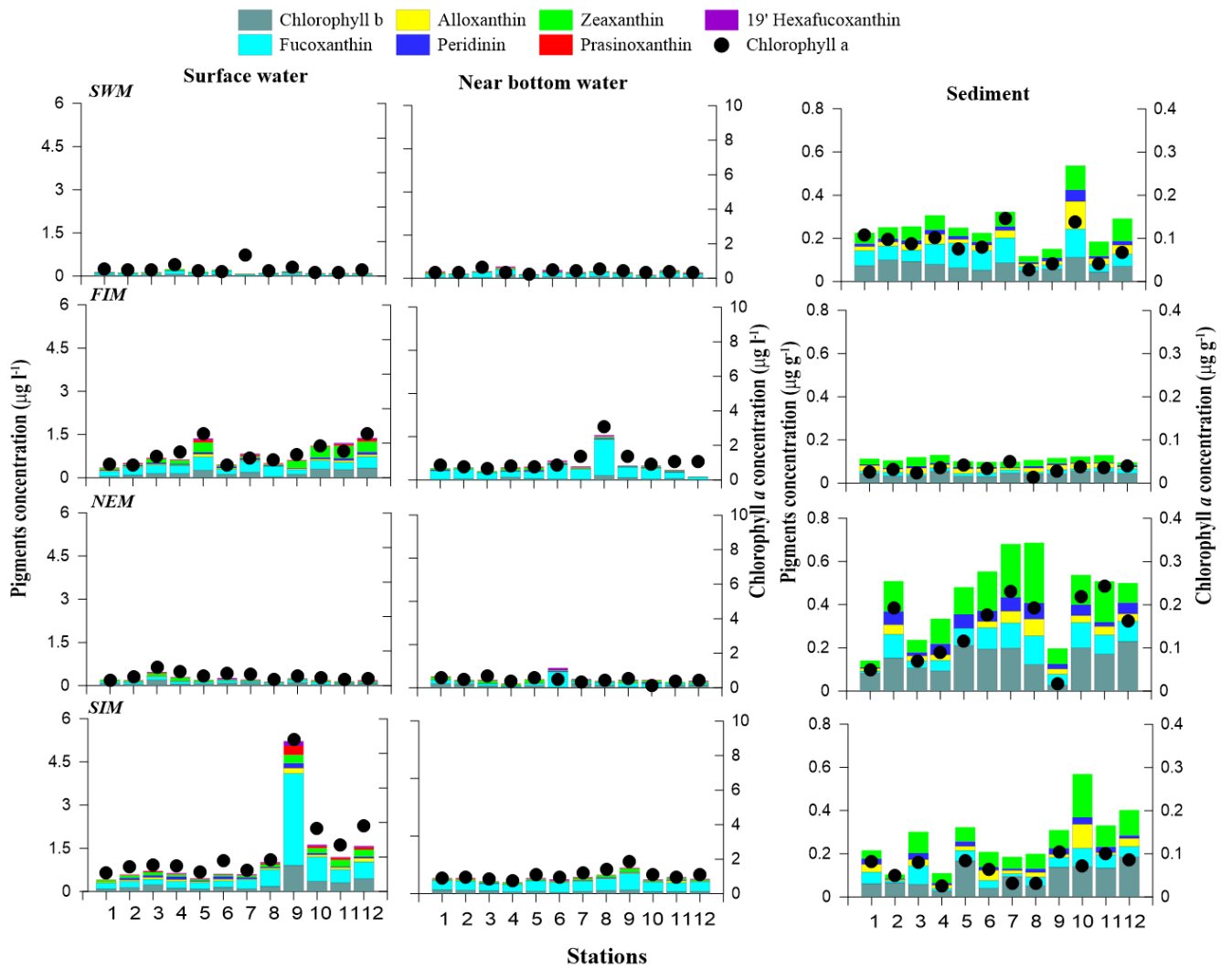


Fig. 4.

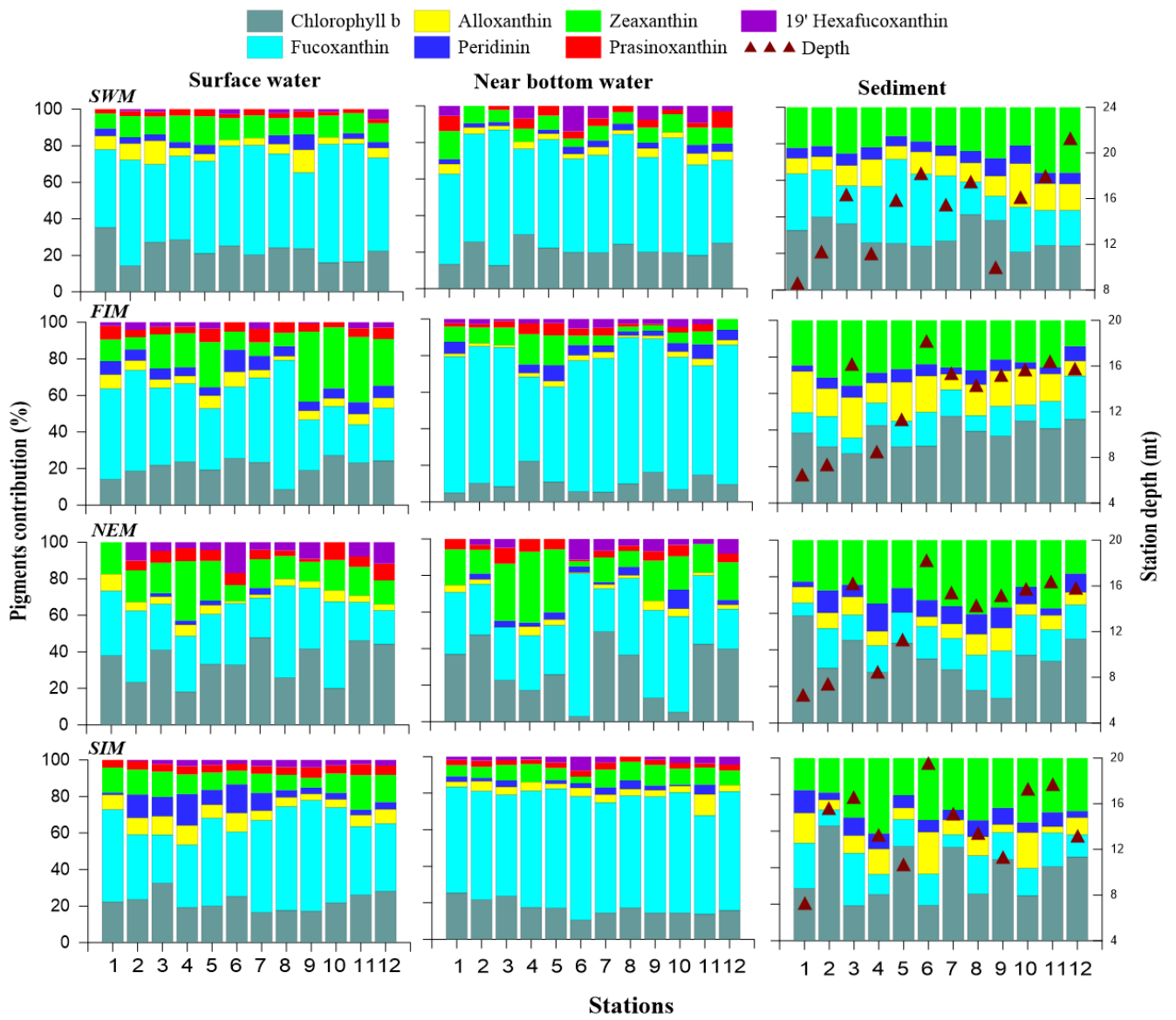


Fig. 5.

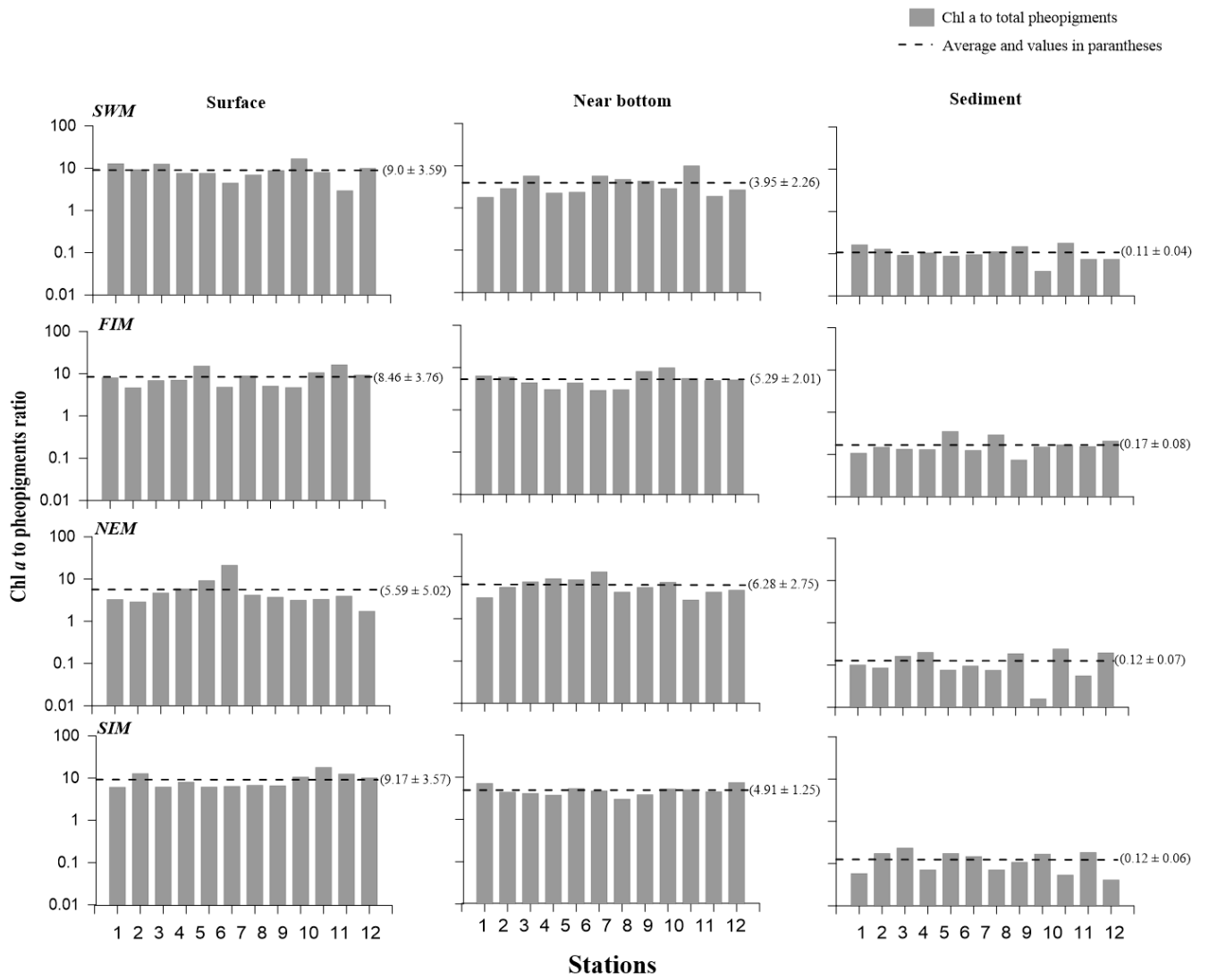


Fig. 6.

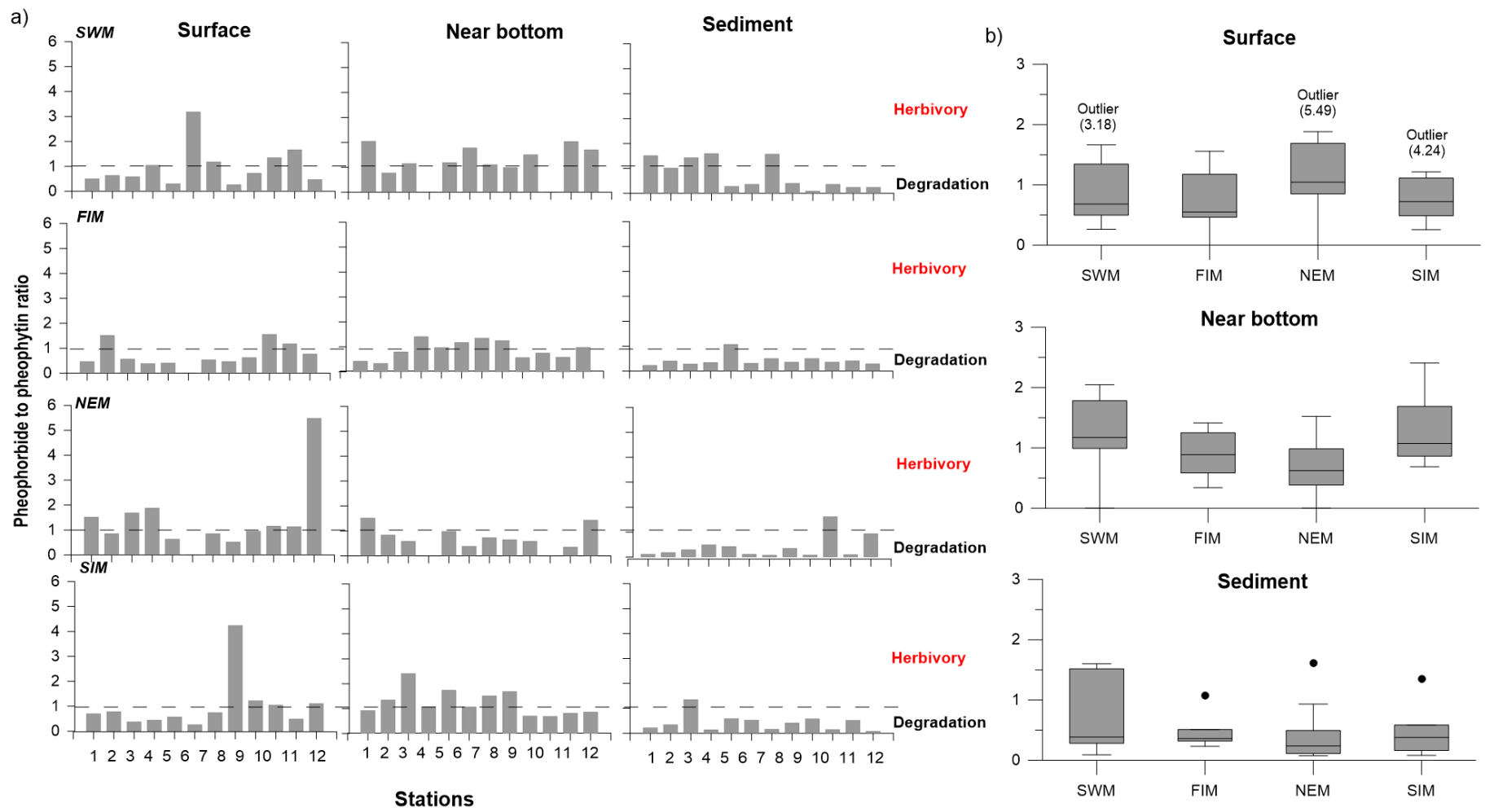


Fig. 7.

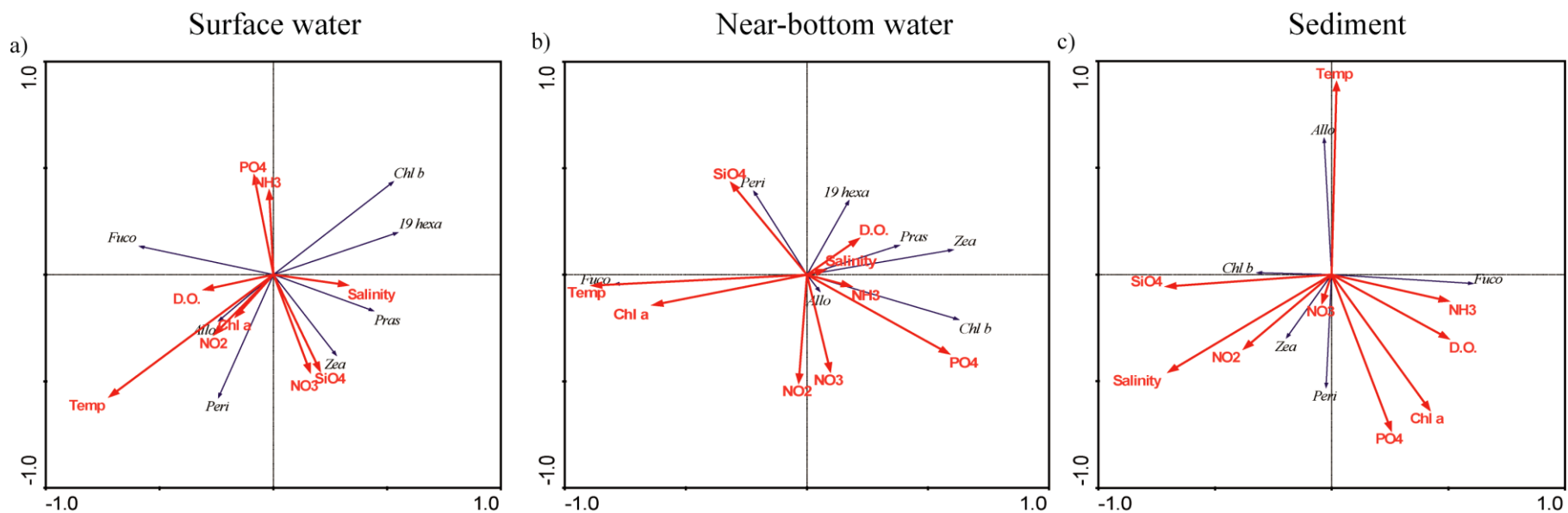


Fig. 8.

Table 1. Seasonal variations in the concentrations of chlorophylls, carotenoids and pheopigments from the surface water, near bottom water (NBW) and surface sediments. Chl values from water (surface and NBW) and sediments are in L<sup>-1</sup> and g<sup>-1</sup> dry weight, respectively. Note SWM – southwest monsoon; NEM –northeast monsoon; FIM – fall inter-monsoon; SIM – inter-monsoon; PSC – photosynthetic carotenoids and PPC – photoprotective carotenoids.

| Pigments             | Average and range of pigment concentrations |                  |                  |                   |                  |                  |                  |                  |                  |                  |                  |                    |
|----------------------|---------------------------------------------|------------------|------------------|-------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|--------------------|
|                      | SWM                                         |                  |                  | FIM               |                  |                  | NEM              |                  |                  | SIM              |                  |                    |
|                      | Surface                                     | NBW              | Sediment         | Surface           | NBW              | Sediment         | Surface          | NBW              | Sediment         | Surface          | NBW              | Sediment           |
| <b>chlorophyll</b>   |                                             |                  |                  |                   |                  |                  |                  |                  |                  |                  |                  |                    |
| Chl <i>a</i> (µg)    | 0.38 (0.20-0.69)                            | 0.42 (0.25-0.65) | 0.08 (0.03-0.15) | 1.42 (0.72-2.57)  | 1.13 (0.67-3.08) | 0.03 (0.01-0.05) | 0.56 (0.32-1.04) | 0.56 (0.12-1.63) | 0.15 (0.02-0.24) | 2.54 (1.08-8.8)  | 1.10 (0.77-1.86) | 0.07 (0.03-0.11)   |
| Chl <i>b</i> (µg)    | 0.03 (0.01-0.07)                            | 0.05 (0.02-0.12) | 0.07 (0.05-0.11) | 0.18 (0.04-0.34)  | 0.05 (0.01-0.16) | 0.04 (0.03-0.06) | 0.08 (0.03-0.20) | 0.08 (0.01-0.15) | 0.15 (0.03-0.23) | 0.26 (0.09-0.91) | 0.10 (0.05-0.14) | 0.10 (0.03-0.19)   |
| Chl <i>c</i> (ng)    | 3.1 (0.7-8.4)                               | 7.6 (2.7-14.3)   | 0                | 22.4 (11.2-42.9)  | 48.2 (10.6-263)  | 0                | 16.5 (2.1-39.3)  | 20.9 (8.5-33.9)  | 0                | 79.4 (0.0-740.8) | 25.7 (11.9-55.7) | 0                  |
| <b>carotenoids</b>   |                                             |                  |                  |                   |                  |                  |                  |                  |                  |                  |                  |                    |
| <b>PSC</b>           |                                             |                  |                  |                   |                  |                  |                  |                  |                  |                  |                  |                    |
| 19BUT (ng)           | 2.6 (0-5.2)                                 | 10.9 (0-23.1)    | 0                | 27.0 (0-94.2)     | 7.2 (0-18.9)     | 0                | 0.6 (0-7.2)      | 1.8 (0-6.0)      | 0                | 18.2 (0.0-77.1)  | 7.0 (0.0-17.7)   | 0                  |
| Fuc (µg)             | 0.07 (0.05-0.12)                            | 0.13 (0.07-0.19) | 0.07 (0.02-0.13) | 0.29 (0.17-0.46)  | 0.37 (0.08-1.25) | 0.02 (0.01-0.02) | 0.07 (0.03-0.12) | 0.13 (0.05-0.54) | 0.08 (0.01-0.13) | 0.60 (0.19-3.19) | 0.34 (0.24-0.58) | 0.05 (0.01-0.09)   |
| 19Hexa (ng)          | 1.6 (0-5.8)                                 | 12.3 (0-40.5)    | 0                | 18.2 (0-46.8)     | 15.4 (0-38.4)    | 0                | 14.9 (0-44.7)    | 15.3 (0-78.1)    | 0                | 38.6 (0.0-208)   | 15.6 (0.0-38.3)  | 0                  |
| Per (ng)             | 4.7 (0-13.9)                                | 6.2 (0-13.1)     | 17.8 (7.7-52.6)  | 49.6 (25.5-91.8)  | 20.4 (0-39.5)    | 6.2 (3.6-8.3)    | 2.3 (0-8.9)      | 8.7 (0-28.0)     | 44.0 (4.1-74.8)  | 68.9 (4.9-174)   | 14.5 (0.0-30.2)  | 19.4 (4.2-31.4)    |
| <b>PPC</b>           |                                             |                  |                  |                   |                  |                  |                  |                  |                  |                  |                  |                    |
| Allo (µg)            | 0.01 (0.00-0.02)                            | 0.01 (0-0.02)    | 0.03 (0.01-0.20) | 0.04 (0.01-0.1)   | 0.01 (0.00-0.02) | 0.02 (0.01-0.03) | 0.01 (0.00-0.02) | 0.01 (0-0.02)    | 0.03 (0-0.08)    | 0.07 (0.03-0.19) | 0.03 (0.02-0.07) | 0.03 (0.01-0.11)   |
| B-car (ng)           | 5.7 (1.4-18.3)                              | 5.3 (2.0-9.9)    | 0                | 23.5 (4-49.4)     | 11.6 (2.0-32.5)  | 0                | 16.5 (10.4-29.3) | 12.2 (0-25.3)    | 0                | 61 (20-185)      | 19.1 (8.8-58.2)  | 0                  |
| Diad (ng)            | 7.7 (4.5-17.2)                              | 17.0 (5.6-28.3)  | 19.9 (5.3-63.1)  | 42.4 (0-108.1)    | 45.1 (13.3-159)  | 5.8 (2.3-9.2)    | 13.6 (3.9-24.3)  | 12.1 (4.5-33.0)  | 20.0 (2.9-37.1)  | 85.3 (32.1-362)  | 30.9 (19.8-48.5) | 12.6 (0.0-20.9)    |
| Diat (ng)            | 1.7 (0-2.9)                                 | 8.9 (0-20.4)     | 50.6 (16.2-143)  | 15.2 (0-28.5)     | 5.5 (2.0-14.2)   | 23.8 (15.6-35.6) | 6.5 (1.7-14.9)   | 5.0 (0-18.4)     | 105 (14.1-208)   | 28.9 (8.5-114)   | 10.0 (5.7-16.2)  | 54.5 (14.3-130.3)  |
| Lut (ng)             | 4.0 (2.3-8.3)                               | 11.3 (4.9-27.3)  | 96.5 (0-402.6)   | 39.4 (10.6-91.5)  | 8.1 (2.5-15.5)   | 47.5 (23.1-65.6) | 17.8 (2.4-49.7)  | 19.4 (5.2-37.5)  | 151 (26.2-244)   | 29.4 (13.0-72.3) | 12.6 (0.0-18.7)  | 151.3 (34.1-442.1) |
| Viola (ng)           | 3.0 (0.0-5.1)                               | 19.6 (3.7-41.3)  | 4.7 (0-12.8)     | 28.2 (9.1-51.8)   | 14.9 (0-25.6)    | 3.4 (0-5.7)      | 10.7 (0-20.1)    | 14.5 (0-37.6)    | 7.9 (0.0-13.7)   | 43.8 (15.1-185)  | 28.3 (18.9-47.4) | 9.3 (0.0-55.9)     |
| Zea (µg)             | 0.02 (0.01-0.04)                            | 0.02 (0.01-0.04) | 0.06 (0.03-0.12) | 0.18 (0.03-0.43)  | 0.04 (0.01-0.08) | 0.03 (0.01-0.04) | 0.04 (0.02-0.10) | 0.05 (0.02-0.10) | 0.14 (0.03-0.28) | 0.12 (0.04-0.28) | 0.05 (0.02-0.10) | 0.08 (0.02-0.20)   |
| Neo (ng)             | 3.7 (0-8.7)                                 | 19.7 (6.8-40.2)  | 10.1 (2.6-24.1)  | 37.5 (8.3-86.3)   | 5.9 (0-13.9)     | 5.7 (2.1-11.5)   | 9.7 (0-26.6)     | 9.1 (0-28.6)     | 17.2 (4.1-26.8)  | 67.5 (12.8-341)  | 25.3 (8.9-52.7)  | 15.2 (4.9-42.9)    |
| Pras (ng)            | 4.1 (2.0-8.7)                               | 10.3 (0-23.2)    | 0                | 42.7 (20.3-99.7)  | 15.6 (0-30)      | 0                | 12.9 (0-30.2)    | 12.4 (0-23.7)    | 0                | 62.2 (17.1-310)  | 16.7 (10.0-24.6) | 0                  |
| <b>Deg product</b>   |                                             |                  |                  |                   |                  |                  |                  |                  |                  |                  |                  |                    |
| Pheo <i>a</i> (µg)   | 0.02 (0.01-0.04)                            | 0.049 (0-0.07)   | 0.51 (0.13-0.99) | 0.11 (0.04-0.18)  | 0.11 (0.09-0.19) | 0.15 (0.06-0.21) | 0.06 (0.03-0.1)  | 0.05 (0-0.09)    | 1.26 (0.30-4)    | 0.14 (0.06-0.22) | 0.11 (0.06-0.21) | 0.57 (0.15-1.92)   |
| Pheide <i>a</i> (µg) | 0.02 (0.01-0.09)                            | 0.078 (0-0.15)   | 0.32 (0.05-0.8)  | 0.07 (0.000-0.12) | 0.11 (0.03-0.26) | 0.06 (0.04-0.07) | 0.08 (0-0.20)    | 0.04 (0-0.11)    | 0.27 (0.05-0.56) | 0.14 (0.05-0.67) | 0.13 (0.06-0.24) | 0.15 (0.04-0.25)   |