Elucidating the variation of phytoplankton pigments in estuarine ecosystem

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Abstract

Phytoplankton pigments were used to study the community structure and phytoplankton size class in Goa's estuaries. The study revealed that fucoxanthin and chlorophyll a were the most dominant pigments. The correlation of diagnostic pigments (DP) and chl a correlated positively in both estuaries (Mandovi: $R^2 = 0.703$, $P < 0.01$; Zuari: $R^2 = 0.892$, $P < 0.01$), suggesting that DP can serve as a proxy to measure phytoplankton biomass. Results showed that with DP and biomass proportion, phytoplankton size class (picoplankton, nanoplankton and microplankton) can be derived. Picoplankton biomass was highest during pre-monsoon season, while microplankton biomass was high during monsoon season. The high abundance of microplankton may support planktivorous fishery productivity.

Keywords

Biomass proportion; Diagnostic pigments; HPLC analysis; Mandovi estuary; Phytoplankton size classes; Zuari estuary

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1. Introduction

The estuary is a region where marine and riverine environments meet and a rapid exchange of materials including nutrients between sediments and water takes place. An estuary is one of the most productive zones in the marine ecosystem, which forms an excellent nursery ground for a variety of commercially important fishes (Gameiro et al., 2004). Phytoplankton are the dominant primary producer in the marine ecosystem and support organic production (Bharathi et al., 2022). The characteristics of the phytoplankton community reflect the quality of estuarine waters due to their adaptive response to changes in environmental factors such as light availability, temperature, salinity, nutrients, and pH as well as biological factors like grazing (Patil and Anil, 2011). Various other studies have also shown that the strong relationship between the abundance of phytoplankton in estuarine ecosystems and the quality of estuarine waters is due to their ability to respond rapidly to environmental changes (Hilligsoe et al., 2011; Paerl and Justic, 2013; Tao et al., 2020; Chilton et al., 2021).

Generally, phytoplankton groups are broadly classified into different functional types based on their physiological characteristic and ecological role (Le Quéré et al., 2005). There are a number of techniques that have been used to quantify the different size classes of phytoplankton (Nair et al., 2008). Some of the common methods used to identify the different size classes of phytoplankton are microscopic analysis, flow cytometry, size-fractionated filtration, and the analysis of phytoplankton pigments (Wollschläger et al., 2015; Chase et al., 2020). Of these, the most commonly used technique to quantify the abundance and identify the community structure of phytoplankton is microscopic method (Soares et al., 2011).

However, the identification and enumeration of phytoplankton by microscopic method are laborious and require a high degree of taxonomic knowledge (Roy et al., 2006). Moreover, this method does not allow the identification or counting of microscopic organisms, such as the picoplankton group. Therefore, to overcome this limitation, the flow cytometry technique is used to identify picoplankton (Ning et al., 2021). However, with this method also there is a disadvantage as it is restricted to a specific size range (the upper limit is typically 15–20 µm), making it difficult to obtain data on the larger size class of phytoplankton (Van Dijk et al., 2010). Some researchers have even used remote sensing method to analyse phytoplankton size class, but there are challenges and limitations, particularly in an estuarine environment. One of the major challenge is the complex and dynamic nature of the estuarine environment which affects the accuracy and reliability of remote sensing data (Twardowski et al., 2007; Kudela et al., 2015; Mouw et al., 2017).

The pigments possess unique characteristic which is useful in studying the community structure of phytoplankton due to their functional attributes. Studies showed that the pigment composition in water sample reflects the taxonomic composition of the phytoplankton, while the presence or absence of certain marker pigments can be used to describe the phytoplankton community composition in the aquatic ecosystems (Jeffrey et al., 1999; Thrane et al., 2015). Alloxanthin, chlorophyll *b*, fucoxanthin, 19-hexfucoxanthin, and 19-but-fucoxanthin, peridinin and zeaxanthin as some of the major diagnostic pigments (DP) for Bacillariophyta, Cryptophyta, Chlorophyta, Haptophyta, Dinophyta, and Cyanophyta (Paerl et al., 2003; Barlow et al., 2007). The distinct attributes of the pigments and their association with a particular group of phytoplankton have made them an ideal diagnostic tool in phytoplankton studies (Roy et al., 2015). As a result, pigment analysis is considered one of the best techniques for mapping the phytoplankton community in terms of their abundance and composition.

The Mandovi and Zuari estuaries are two major estuarine systems located on India's west coast in Goa that flow into the Arabian Sea. These estuaries are highly dynamic and ecologically rich, shaped by a pronounced monsoonal cycle that drives significant seasonal variations in both hydrological and biological parameters. Salinity ranges from 0 to 5 psu during monsoon season and it goes up to 30 psu during dry/summer season, while temperature varies between 24°C and 32°C, with cooler waters observed during the monsoon due to rainfall and cloud cover, and peak temperatures occurring in the dry season (Shetye et al., 2007; Vijith et al., 2009; Pednekar et al., 2014; Anand et al., 2014). During the monsoon season, heavy freshwater inflow causes drastic changes in salinity, nutrient distribution, and tidal currents, while the dry season brings seawater intrusion and more stable conditions. Dissolved oxygen (DO) levels fluctuate seasonally, reaching up to 7 to 8 mg L^{-1} during the monsoon, when freshwater input is high, while it decreases to 4 to 5 mg L^{-1} during the pre-monsoon season due to reduced mixing and increased respiration rates (Verlecar et al., 2006).

Similarly, nutrients concentration also shows seasonal variation. The study by Pednekar et al. (2014) showed that during the monsoon, nutrients level increases due to land runoff, with nitrate ranging from 5 to 15 μM, phosphate from 0.5 to 2 μM, and silicate between 10 and 30 μM, while the nutrients level decreases during the dry season, even though tidal mixing help sustain the productivity in both estuaries throughout the year. According to studies, these estuaries are dominated by diverse phytoplankton groups, such as diatoms and dinoflagellates, whose distribution and seasonal abundance are greatly affected by the changes in physico-chemical factors (de Senerpont Domis et al., 2013; Patil and Anil, 2015; Ramakrishnan and Veronica, 2022). Studies also showed that phytoplankton blooms frequently occur during both monsoon and non-monsoon seasons, which is mainly nutrient-driven (Ramakrishnan and Veronica, 2022; Patil and Sathish, 2023).

Considering the significance of phytoplankton in marine ecosystem functioning, understanding the characteristic and the community structure of phytoplankton is important. In view of this, we investigated the spatio-temporal variation of phytoplankton pigments with respect to community structure in the estuarine waters of Goa, India. During this study, we evaluated the variation in the distribution of phytoplankton pigments, the spatio-temporal variation in the Mandovi and Zuari estuaries, and the pigments derived phytoplankton size class from various locations.

2. Material and methods

2.1 Study sites and field sampling

The samplings were carried out from different locations of the Mandovi (15.49°N to 15.50°N and 73.81°E to 73.92°E) and Zuari (15.34°N to 15.40°N and 73.86°E to74.0°E) estuaries, central west coast of India, Goa (Figure 1). Monthly sample collection was done from January–December 2021. The sampling stations were designated as M1 (Upper Section), M2 (Middle Section), and M3 (Lower Section/Mouth of the estuary) in the Mandovi estuary, whereas Z1 (Upper Section), Z2 (Middle) and Z3 (Lower/Mouth of the estuary) were the three sampling stations in the Zuari estuary. A clean bucket was used to collect the surface water samples. From each station, samples were collected in triplicate along with other hydrological data.

2.2 Physical and chemical parameter

Physico-chemical parameters data such as temperature, salinity, dissolved oxygen and inorganic nutrients were collected from the surface water sample. Surface water temperature and salinity were recorded by a digital thermometer (TM 120) and a refractometer (Atago). Dissolved oxygen (DO) was measured by Winkler's method. Nutrients were estimated with the help of an autoanalyzer (Skalar San ^{plus} analyzer) as per standard protocol (Strickland and Parsons, 1972).

2.3 Taxonomy

In the laboratory, a sub-sample of water sample (~250 mL) was collected in amber-coloured plastic bottles and preserved in 2 mL of Lugol's iodine solution (Santhanam et al., 1987). The samples were concentrated, up to 5–10 mL, by siphoning them through a glass tube with a 10 μm mesh at one end. Phytoplankton cells were counted in the Sedgewick-Rafter counting chamber and identified under an inverted light microscope (Olympus IX73). The species of phytoplankton were identified with the standard taxonomic keys (Tomas, 1997). This data was used as a reference to study the correlation between marker pigments and phytoplankton groups.
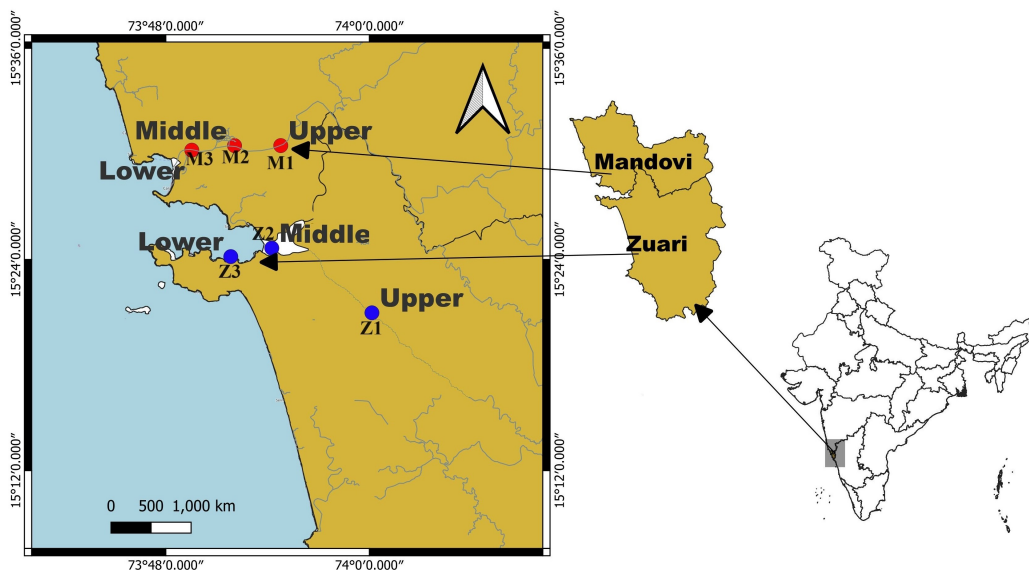
Figure 1. Study area showing the sampling locations from the Mandovi and Zuari estuary, Goa.

2.4 Pigment analysis

To analyse the characteristic of phytoplankton pigments, the other sub-sample of surface seawater (500–1000 mL) was filtered through GF/F (Whatman) filter paper and stored at −20°C until analysis. To identify and characterise the phytoplankton pigments, high-performance liquid chromatography (Shimadzu prominence i-series LC 2030) was used. The phytoplankton pigments were quantified as per the Van Heukelem and Thomas (2001) method. Pigments were extracted in three to five millilitres of ice-cold 100% methanol (HPLC grade, Merck) overnight at −20°C. The cellular debris was eliminated by filtering through a Teflon syringe filter (0.2 µm). After 0.2 µm syringe filtration, the sample was mixed in a 9:1 ratio with a buffer containing 28 mM aqueous tetrabutyl ammonium acetate (TBAA; AR Grade, TCI) at pH 6.5 and methanol.

HPLC was calibrated with pigments standard from DHI (Denmark). A methanol-based reversed-phase linear gradient solvent system was utilized for the analysis, involving a binary gradient elution where solvent A (70:30, methanol and 28 mM TBAA at pH 6.5) and solvent B was 100% methanol. The gradient increased from 5% to 95% solvent B over 22 minutes, followed by a hold at 95% solvent B until 29 minutes. The injection volume varied from 300 to 500 µL, with a solvent flow rate maintained at 1.1 mL min^{-1}. Marker pigments concentration was determined by analysing the peak areas of the standard. The eluting pigments were detected at wavelengths of 450 and 665 nm. In all the analyses, HPLC-grade solvents (Merck) were used. The HPLC system was equipped with a photodiode array (PDA) detector and a Shim Pack GIST C8 column (4.6 mm diameter, 3.0 µm pore size). The time taken to analyse one sample was approximately 40 minutes.

2.5 Biomass quantification of different phytoplankton size classes

The different size class of phytoplankton was recorded and the data reduction approach was used to ascertain the contribution of each phytoplankton group (Vidussi et al., 2001). Accordingly, the concentration of marker pigments was converted to biomass proportion of the phytoplankton size class with the help of diagnostic pigment. The following formula was used to calculate the sum of all diagnostic pigment (ΣDP) and biomass proportion (BP) of different size classes of phytoplankton (picoplankton, < 2 µm; nanoplankton, 2–20 µm; and microplankton 20–200 µm (Roy et al., 2006; Chowdhury and Biswas, 2023).

$$\Sigma DP_w = 1.41[fuco] + 1.1[peri] + 1.27[19-hf] \\ + 0.35[19-bf] + 0.60[allo] + 1.01[chl\, b] \quad (1) \\ + 0.86[zea]$$

$$BP_{Micro} = (1.41[fuco] + 1.1[peri])/\Sigma DP_w \quad (2)$$

$$BP_{Nano} = (0.60[allo] + 1.27[19-hf] \\ + 0.35[19-bf])/\Sigma DP_w \quad (3)$$

$$BP_{Pico} = (0.86[zea] + 1.01[chl\, b])/\Sigma DP_w \quad (4)$$

The distribution pattern of accessory photosynthetic pigments (APSP) (Peridinin (peri) + 19′ Butanoyloxyfucoxanthin (bf) + Fucoxanthin (fuco) + 19′ Hexanoyloxyfucoxanthin (hf) + Violaxanthin (viol) + Chlorophyll *b* (chl

b) and accessory photoprotective pigment (APPP) (Diadinoxanthin (diad) + Alloxanthin (allo) + Diatoxanthin (diat) + Zeaxanthin (zea) + Total carotenes ($\alpha + \beta$)) was also determined (Gibb et al., 2001).

2.6 Statistical analysis

The environmental data presented are mean values ± standard deviation (SD). Dendrogram clustering was performed in PRIMER software (ver. 7) to analyse the phytoplankton size class based on the diagnostic pigments (DP). The correlation between diagnostic marker pigments and the distribution of phytoplankton community was carried out in IBM SPSS statistical software (ver. 23). Pearson correlation analysis was performed to understand the correlation between different environmental parameters (Temperature, salinity, nitrate, nitrite, phosphate, and silicate) and phytoplankton size group (Micro, nano and pico) (PAST analytical software, Version 4.03). The ANOVA calculation was performed in MS Excel 2016 to test the significant difference in pigment composition present in each sampling station of the Mandovi and Zuari estuaries.

3. Results

3.1 Hydrography

The analysis of environmental parameters revealed that sea surface temperatures in the Mandovi and Zuari estuaries ranged from 26 to 34°C. In the Mandovi estuary, the maximum temperature was recorded in May at 33.12 ± 0.004°C in the upper section, while the minimum was observed in November at the middle section, measuring 26.43 ± 0.002°C. In contrast, the highest temperature in the Zuari estuary was 33.67 ± 0.115°C during April month at the lower section, and the minimum temperature 26.73 ± 0.21°C was also noted in November (Figure 2a,b).

Salinity levels in both estuaries varied from 0 to 36 psu, peaking in May (35.8 ± 0.24 psu) in the lower section of the Mandovi estuary, while in the Zuari estuary highest salinity was observed in the middle section (33.96 ± 0.058 psu). Notably, salinity was significantly lower in June in the middle section (0.1 ± 0.001 psu) of the Mandovi estuary, while in the Zuari estuary, the lowest salinity was recorded in the upper section (0.3 ± 0.002 psu) (Figure 2c,d). The results indicated that in both estuaries, salinity was lowest during the monsoon month of June compared to the months of April to May (summer).

Dissolved oxygen (DO) levels ranged from 3 to 7 mg L^{-1} in both the estuaries. The highest concentration was recorded in July at the upper sections (6.96 ± 0.722 mg L^{-1}) in the Mandovi and 6.53 ± 0.056 mg L^{-1} in the Zuari estuary respectively. However, DO levels dropped significantly in the month of September in the Mandovi estuary in the upper section (4.05 ± 0.527 mg L^{-1}), while it was in the middle section (3.64 ± 0.125 mg L^{-1}) during the month of April in the Zuari estuary (Figure 2e,f).

The concentration of inorganic nutrients increased significantly in the upper sections of both the Mandovi and Zuari estuaries compared to the middle and lower sections. During the southwest monsoon season, especially in July, the Mandovi estuary showed a considerably higher average concentration of nitrate (15.3 ± 1.282 µM), nitrite (1.04 ± 0.160 µM), and phosphate (1.92 ± 0.060 µM) than other months (Figure 3a,c,e,g). Similarly, in the Zuari estuary, the average concentrations in July were elevated, with nitrate at 16.29 ± 0.59 µM, nitrite at 1.88 ± 0.04 µM, and phosphate at 1.86 ± 0.05 µM (Figure 3b,d,f,h). Such substantial increase in nutrients level during the southwest monsoon can be attributed to tidal mixing, agricultural runoff from nearby farmlands, and wastewater discharge, etc. In contrast, the concentration of inorganic nutrients was notably lower during the pre-monsoon season (February to May). The nutrients concentration was lowest in the month of February at the lower section of both estuaries. In Mandovi estuary, nitrate was 0.663 ± 0.03 µM, nitrite at 0.033 ± 0.007 µM, and phosphate at 0.042 ± 0.004 µM, Similarly, in Zuari estuary, nitrate was 1.28 ± 0.19 µM, nitrite (0.116 ± 0.03 µM), and phosphate (0.333 ± 0.02 µM), (Figure 3).

The silicate concentration showed great variation between the months in both Mandovi and Zuari estuaries (Figure 3g,h). The highest silicate concentration was 59.53 ± 1.21 µM in the Mandovi estuary at the upper section during the month of June to July. Similarly, the highest concentration was 23.54 ± 3.005 µM at the upper section of the Zuari estuary during the month of August to September. Conversely, the concentration of silicate was lowest during the pre-monsoon season, particularly in April, in the lower section of the Mandovi estuary (9.57 ± 0.055 µM), whereas it was 2.82 ± 0.235 µM in the Zuari estuary.

3.2 Pigments

The results of HPLC analysis showed several key pigments from both Mandovi and Zuari estuaries, in addition to the predominant chlorophyll *a* and fucoxanthin pigments. The pigments were peridinin (peri), 19′ hexanoyloxyfucoxanthin (19-hf), diadinoxanthin (diad), alloxanthin (allo), zeaxanthin (zea), chlorophyll *b* (chl *b*), prasinoxanthin (prasi), and β-carotene (β-car) (Figure 4). These pigments displayed distinct spatial variability in both estuaries. The pigments concentration in both the estuary across the months were statistically significant (P < 0.05; One-way ANOVA).

In the upper section of the Mandovi estuary, the concentrations of peri (0.092 ± 0.002 mg m^{-3}), allo (0.173 ± 0.021 mg m^{-3}), zea (0.417 ± 0.001 mg m^{-3}), chl *b* (0.958 ± 0.107 mg m^{-3}), and β-car (0.0974 ± 0.001 mg m^{-3}) were significantly higher in the month of May (Figure 4a). However, the highest concentration of peri (0.218 ± 0.005 mg m^{-3}), diad (0.256 ± 0.002 mg m^{-3}), allo (0.145 ± 0.004 mg m^{-3}), zea (0.265 ± 0.144 mg m^{-3}), and chl *b* (0.803 ± 0.281 mg
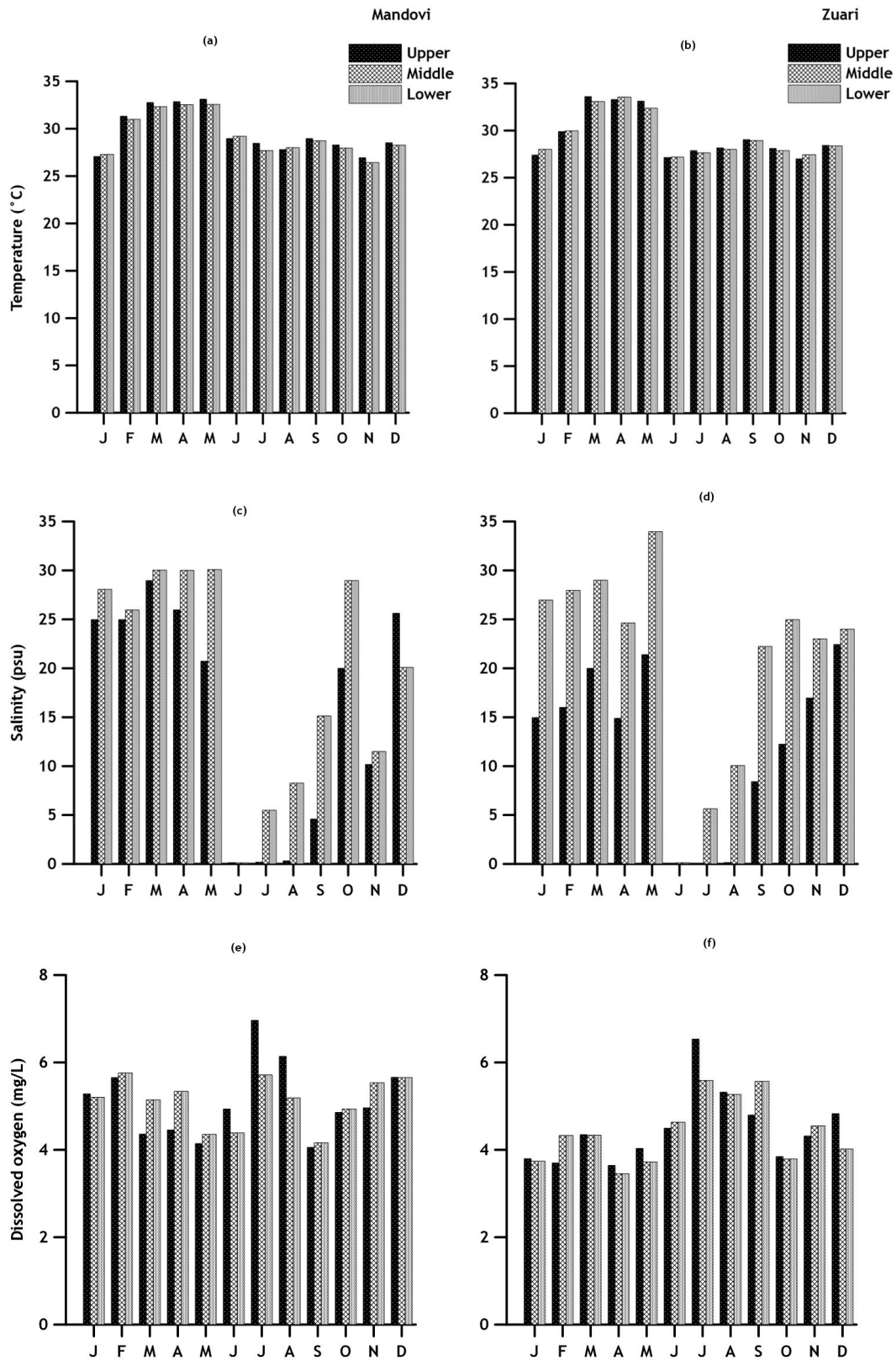
Figure 2. Variation of physio-chemical parameters in the Mandovi and Zuari estuaries (temperature (a–b), salinity (c–d) and dissolved oxygen (e–f).
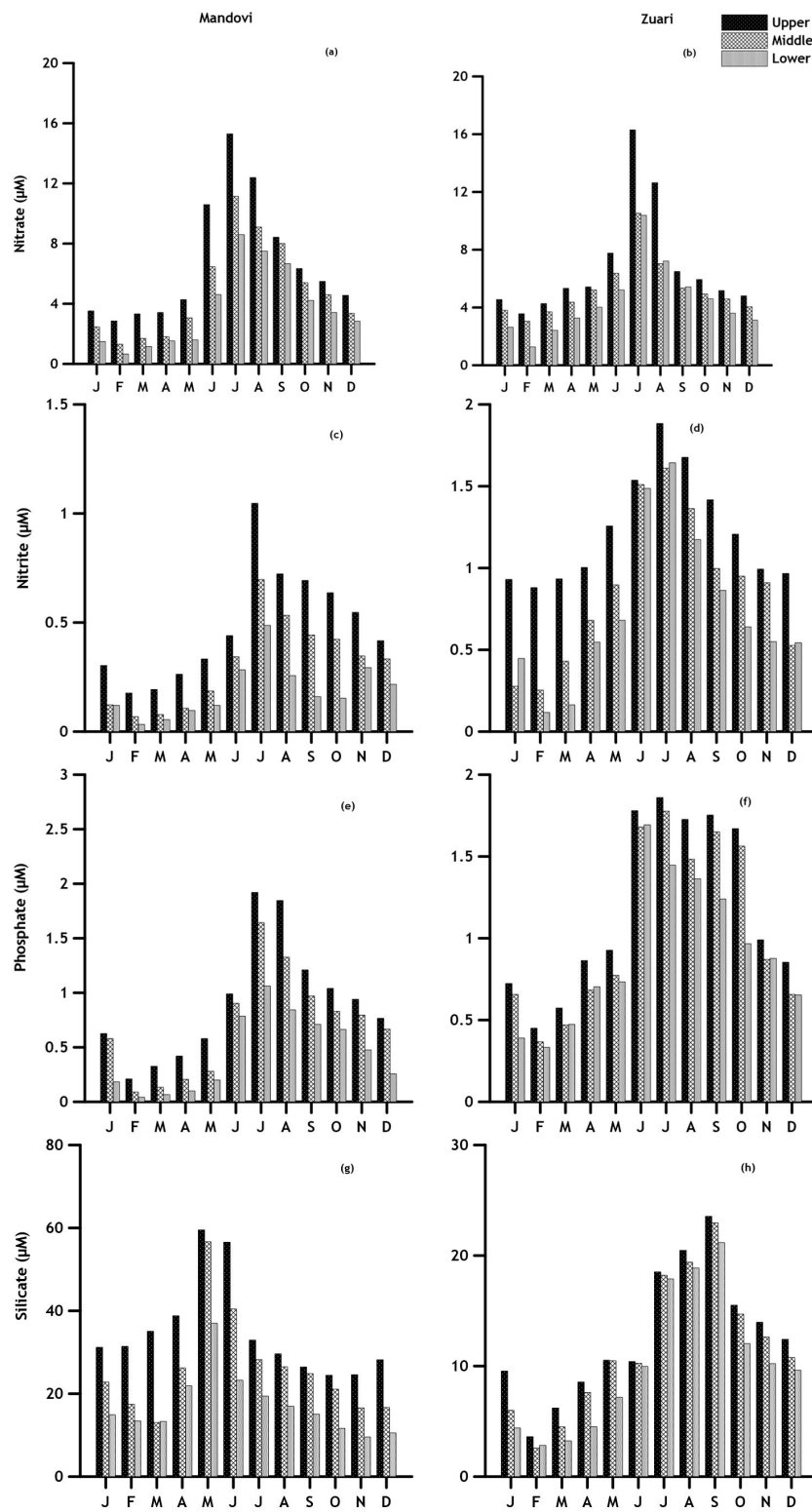
Figure 3. Variation of concentration of dissolved inorganic nutrients in the Mandovi and Zuari estuaries (nitrate (a–b), nitrite (c–d), phosphate (e–f) and silicate (g–h).
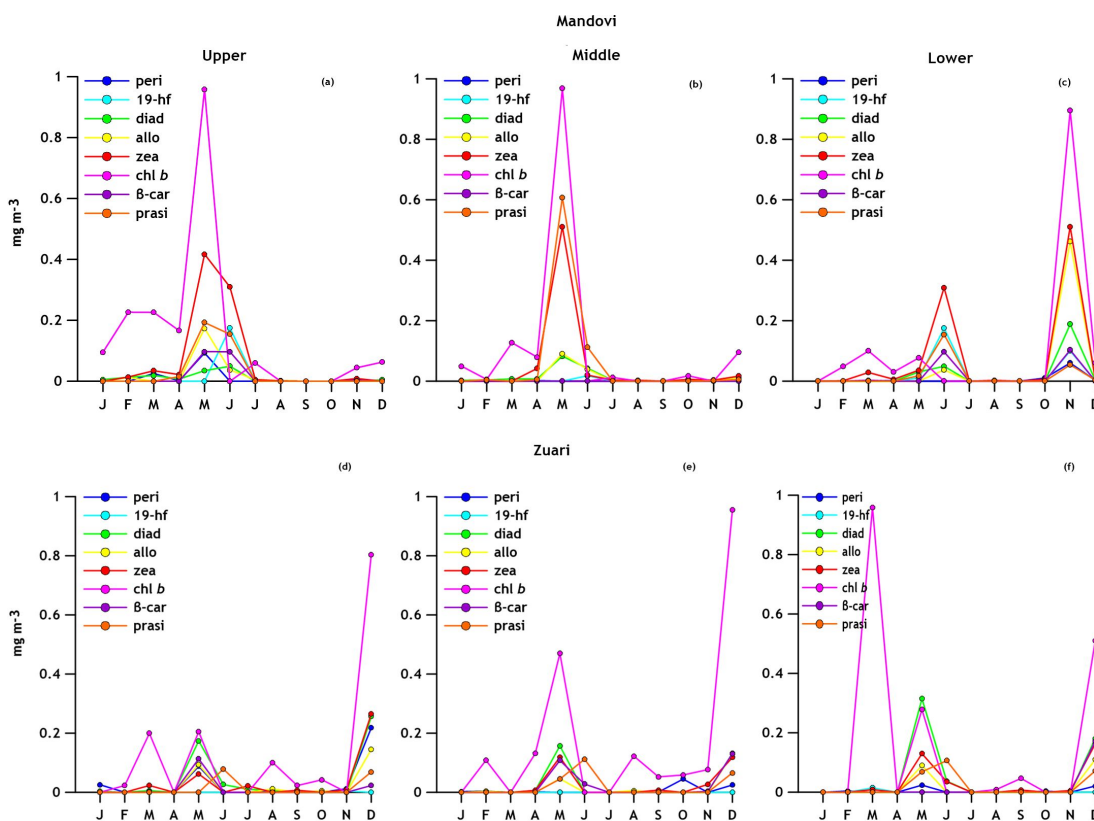
Figure 4. Monthly variation of dominant pigments present in the Mandovi and Zuari estuarine waters.

m^{-3}) was recorded during December in the upper section of Zuari estuary (Figure 4d).

The concentration of pigments like diad (0.082 ± 0.006 mg m^{-3}) and zea (0.511 ± 0.001 mg m^{-3}) peaked during May in the middle section of both estuaries (Figure 4b,e). On the other hand, the concentration of peri was significantly elevated in both Mandovi (0.002 ± 0.0 mg m^{-3}) and Zuari (0.044 ± 0.211 mg m^{-3}) estuaries during October. It was also observed that allo, chl *b*, and prasi concentrations were considerably higher in May (0.090 ± 0.004) mg m^{-3}), March (0.101 ± 0 mg m^{-3}), and May (0.607 ± 0.007 mg m^{-3}) in the middle section of Mandovi estuary (Figure 4b), while significant increase in these pigments – allo (0.13 ± 0.001 mg m^{-3}), chl *b* (0.382 ± 0.08 mg m^{-3}), and β-car (0.131 ± 0 mg m^{-3}) was noted in the middle section of the Zuari estuary during December (Figure 4e).

In the lower section, diad (0.188 ± 0.11 mg m^{-3}), allo (0.462 ± 0.001 mg m^{-3}), zea (0.511 ± 0.114 mg m^{-3}), chl *b* (0.716 ± 0.299 mg m^{-3}), and β-car (0.103 ± 0.006 mg m^{-3}) were significantly high during the month of November in the Mandovi estuary (Figure 4c), while in the Zuari estuary the concentration of allo (0.11 ± 0.114 mg m^{-3}), zea (0.163 ± 0.007 mg m^{-3}), and β-car (0.172 ± 1.004 mg m^{-3}) was higher in the lower section during December (Figure 4f). Furthermore, 19-hf (0.014 ± 0.610 mg m^{-3}) and chl *b* (0.575 ± 0.064 mg m^{-3}) showed an increase dur-ing March, and the highest concentration of peri (0.024 ± 0.001 mg m^{-3}) and diad (0.315 ± 0.074 mg m^{-3}) was recorded during the month of May (Figure 4f).

The results showed that chlorophyll *a* and fucoxanthin are the predominant pigments and their distribution var-ied significantly in both estuaries (Figure 5). Chlorophyll *a* concentration was higher in the lower section of both estuaries. In the Mandovi estuary, the highest concentra-tion of chlorophyll *a* was recorded at the lower section during the months of June (1.725 ± 0.472 mg m^{-3}) and November (2.658 ± 0.667 mg m^{-3}), whereas in the Zuari estuary, it was in the month of December (3.35 ± 0.002 mg m^{-3}). Similarly, the concentration of fucoxanthin was comparatively higher in the lower section during the month of November (0.698 ± 0.001 mg m^{-3}) and in middle sec-tion during the month of May (0.669 ± 0.114 mg m^{-3}) in the Mandovi estuary (Figure 5), whereas in the Zuari estu-ary, fucoxanthin was higher in the month of May at lower region (1.447 ± 0.001 mg m^{-3}) and in the upper section (0.812 ± 0.264 mg m^{-3}) during the month of December. On the other hand, fucoxanthin concentration in the Zuari estuary was comparably lower than chlorophyll *a* during monsoon season, however, no such trend was observed in the Mandovi estuary. Pearson correlation between fucox-anthin and chlorophyll *a* pigment showed a strong positive correlation in both the Mandovi (0.604, P < 0.05) and Zuari
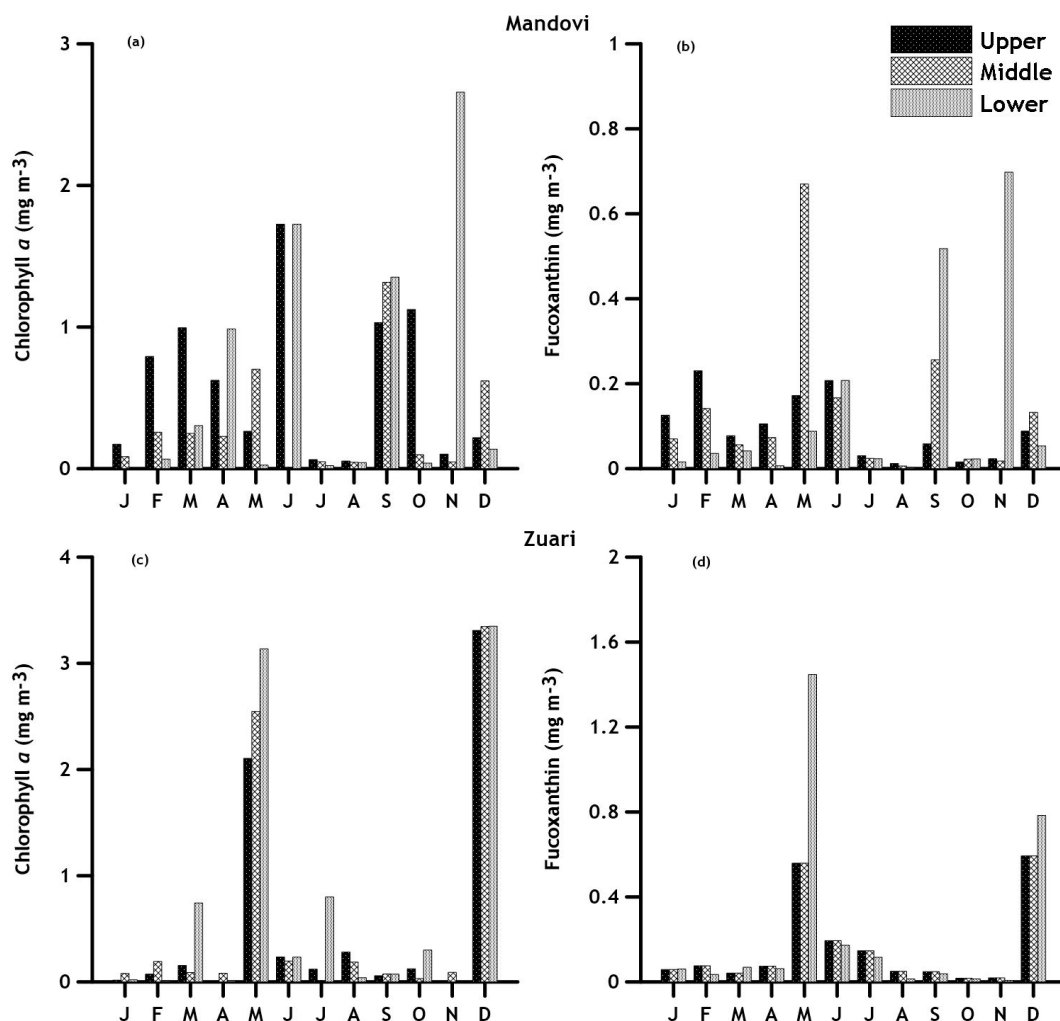
Figure 5. Concentration of chlorophyll *a* and fucoxanthin pigments in the Mandovi estuary (a–b) and in the Zuari estuary (c–d), Goa.

(0.930, P < 0.01) estuaries. This indicates that major contribution of phytoplankton biomass will be predominantly from the diatom group because fucoxanthin is the marker pigment of diatom.

The distribution of accessory photosynthetic pigments (APSP) and accessory photoprotective pigments (APPP) showed a variation between the two estuaries (P < 0.05). The level of APSP and APPP was higher in the lower section of the Mandovi estuary during November, while in the Zuari estuary, they were significantly higher in the upper section during December (Figure 6). Chlorophyll *b*, fucoxanthin, and peridinin were the major pigments of APSP, while diadinoxanthin, zeaxanthin, and diatoxanthin were the major APPP pigments.

3.3 Phytoplankton size class
The quantification of biomass proportion of each size class of phytoplankton from different locations of both the estuaries showed that microplankton biomass was highest in all the locations. According to the size class defined by the diagnostic pigment analysis (DPA), the accessory pigments contribution of different size classes of phytoplankton were – picoplankton (0.2–2 μm) (38%), nanoplankton (2–20 μm) (−5%), and microplankton (20–200 μm) (57%) in the Mandovi estuary. Similarly, in the Zuari estuary, the distribution of picoplankton was 30%, nanoplankton was 2% and the largest contribution was from microplankton (68%). Microplankton and picoplankton were the predominant size class of phytoplankton in both estuaries, while the abundance of nanoplankton group in both estuaries was very low (2–5%). The overall contribution of microplankton was between 57 and 68% in both estuaries, compared to 30 and 38% of picoplankton (Figure 7). The correlation analysis showed that diagnostic pigments (DP) from the Mandovi and Zuari estuaries were positively correlated with phytoplankton size class or total phytoplankton biomass (Mandovi: R^2 value = 0.703 and Zuari: R^2 value = 0.892).
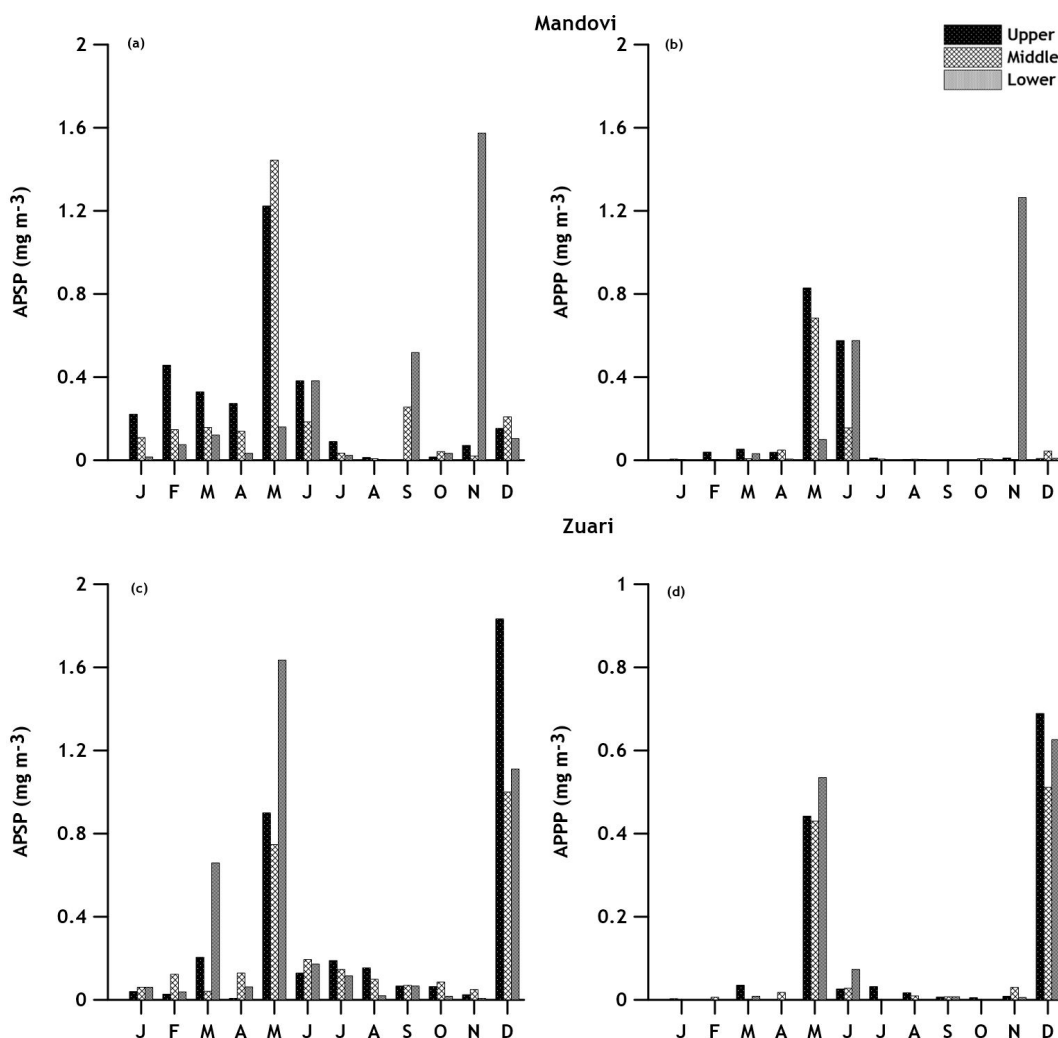
Figure 6. The concentration of accessory photosynthetic pigments (APSP) and accessory photoprotective pigments (APPP) in the Mandovi (a–b) and Zuari (c–d) estuarine waters of Goa.

3.4 Phytoplankton community structure

The microscopic study of phytoplankton diversity and distribution showed a seasonal variation in both the estuary. Bacillariophyta, Cyanophyta, Chlorophyta, Dinophyta, and Chrysophyta were the major phytoplankton groups observed during the study. The results showed that the species diversity was high in the lower section of both the estuary. Species like *Cyclotella striata*, *Skeletonema costatum*, *Thalassionema nitzschioides*, *Actinoptychus senarius*, *Paralia sulcata*, *Striatella* sp., *Synedra* spp., *Synendropsis* spp., *Dinophysis miles*, *Heterodinium milneri*, *Oxitoxum* spp., *Pyrodinium* spp. were the predominant diatoms and dinoflagellates species found during all the months in Zuari estuary. On the other hand, *Skeletonema costatum*, *Thalassionema nitzschioides*, *Nitzschia longissima*, and *Pleurosigma normanii*, *Asteromphalus* spp., *Detonula* spp., *Planktoniella sol*, *Amphora* spp., and *Pinnularia* spp. were observed in the Mandovi estuary.

The clustering of diagnostic marker pigments showed a clearly delineating phytoplankton groups based on pigment specificity (Figure 8). The clustering was similar in both the estuaries. These observations were supported by the correlation analysis (Table 1). The analysis showed that specific diagnostic marker pigments positively correlated with a specific group of phytoplankton in both estuaries. The Bacillariophyta, Dinophyta, and Cyanophyta exhibited a strong positive correlation with marker pigment, such as fucoxanthin correlated to diatom (Mandovi: $R^2 = 0.745$, $P < 0.01$; Zuari: $R^2 = 0.838$, $P < 0.01$), peridinin with dinophyta (Mandovi: $R^2 = 0.783$, $P < 0.01$; Zuari: $R^2 = 0.872$, $P < 0.01$), and zeaxanthin with cyanophyta (Mandovi: $R^2 = 0.744$, $P < 0.01$; Zuari: $R^2 = 0.815$, $P < 0.01$). The strong and positive correlation between the marker pigments and phytoplankton groups strongly supports the view that marker pigments such as fucoxanthin, peridinin and zeaxanthin could be reliably used to deter-
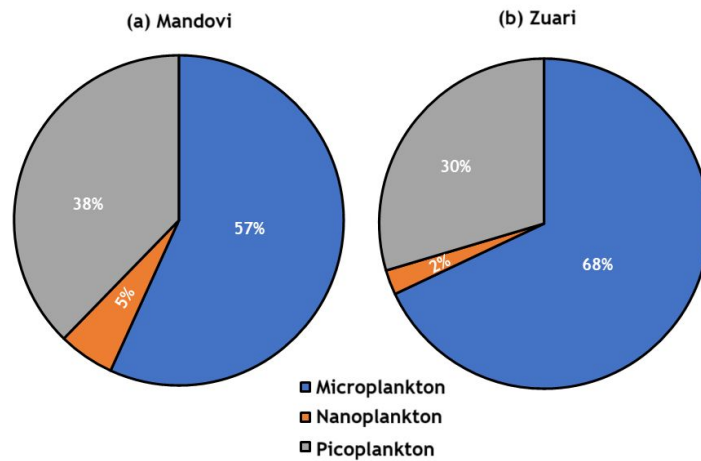
Figure 7. The overall biomass proportion of phytoplankton size class distribution in Mandovi estuary (a) and Zuari estuary (b).
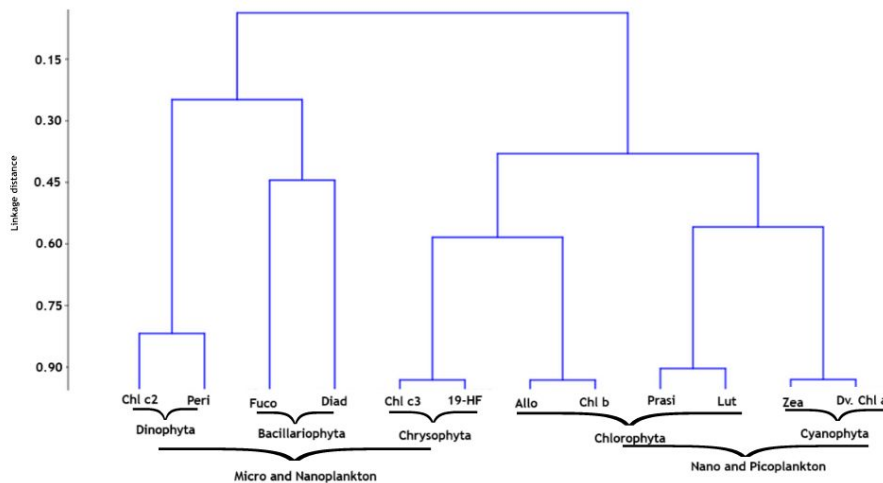

Figure 8. Group clustering of phytoplankton size class based on the diagnostic pigments from both the estuaries. Bracket indicate the groupings.
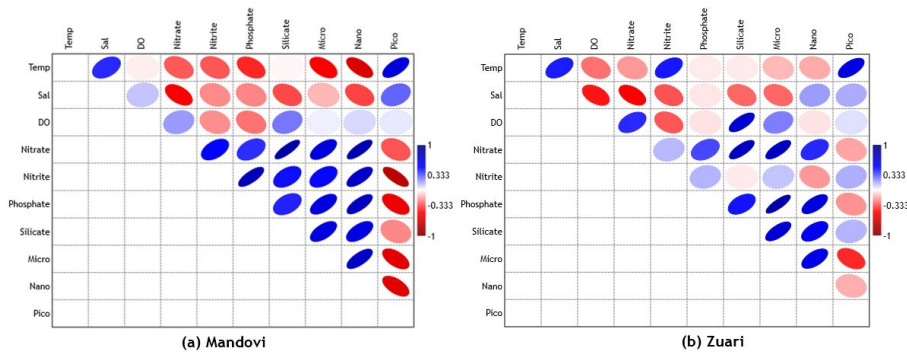

Figure 9. The correlation between physicochemical parameters and phytoplankton size class of Mandovi and Zuari estuarine waters of Goa. Only significant correlation (P < 0.05) is presented here, with correlation values ranging from +1 (dark blue) to −1 (dark red) (Temp: Temperature; Sal: Salinity; DO: Dissolved oxygen; Micro: Microplankton; Nano: Nanoplankton; Pico: Picoplankton).

Table 1. Correlations between the diagnostic marker pigments (Fuco: Fucoxanthin; Peri: Peridinin; Zea: Zeaxanthin; and Lut: Lutin) and the phytoplankton groups (Bacillariophyta (Dia), Dinophyta (Dino), Cyanophyta (Cyano), and Chlorophyta (Chloro).

				MANDOVI					
Parameter	Dia	Dino	Cyano	Chloro	Fuco	Peri	Zea	Lut	Allo
Dia	1								
Dino	0.276	1							
Cyano	0.467	−0.105	1						
Chloro	−0.270	0.052	−0.300	1					
Chryso	−0.596	−0.509	−0.172	0.393					
Fuco	0.745**	0.221	0.033	−0.151	1				
Peri	0.349	0.783**	−0.008	0.103	0.624*	1			
Zea	0.316	−0.105	0.744**	−0.022	0.206	−0.002	1		
Lut	0.491	0.004	0.266	−0.173	0.566*	0.773**	0.440	1	

				ZUARI					
Parameter	Dia	Dino	Cyano	Chloro	Fuco	Peri	Zea	Lut	Allo
Dia	1								
Dino	−0.183	1							
Cyano	0.149	−0.278	1						
Chloro	0.381	−0.029	−0.657*	1					
Chryso	0.070	0.034	−0.347	0.354					
Fuco	0.838**	0.121	−0.089	−0.327	1				
Peri	−0.342	0.872**	−0.224	−0.412	0.808**	1			
Zea	−0.351	0.156	0.815**	−0.436	0.917**	0.950**	1		
Lut	0.064	−0.260	−0.256	0.222	−0.188	−0.168	−0.188	1	

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level.

mine the specific group of the phytoplankton community.

3.5 Correlations between environmental parameters and phytoplankton size community

The correlation between phytoplankton size community and environmental parameters was analysed by Pearson correlation analysis. The dissolved inorganic nutrients showed a significant effect on the abundance of phytoplankton size class ($P < 0.05$). Microplankton size class in the Mandovi and Zuary estuaries showed a positive correlation with nitrate (Mandovi $R^2 = 0.629$, $P < 0.05$; Zuari $R^2 = 0.727$, $P < 0.01$) and silicate (Mandovi $R^2 = 0.364$, $P < 0.05$; Zuari $R^2 = 0.672$, $P < 0.05$) (Figure 9). Nanoplankton size class also showed a positive correlation with the inorganic nutrients such as nitrate (Mandovi $R^2 = 0.800$, $P < 0.01$; Zuari $R^2 = 0.423$, $P < 0.05$), nitrite (Mandovi $R^2 = 0.711$, $P < 0.01$), phosphate (Mandovi $R^2 = 0.742$, $P < 0.01$; Zuari $R^2 = 0.636$, $P < 0.05$), and silicate (Mandovi $R^2 = 0.603$, $P < 0.05$; Zuari, $R^2 = 0.541$, $P < 0.05$) (Figure 9). The strong positive correlation by both microplankton and nanoplankton showed their affinity for nutrient-rich water, which normally occurs during monsoon and post-monsoon season. The biomass growth corresponded with the availability of inorganic nutrients particularly with nitrate during these seasons.

Picoplankton, on the other hand, showed a strong negative correlation with all the dissolved inorganic nutrients in both the estuaries, while showing a positive correla-

tion with surface water temperature and salinity ($P < 0.05$) (Figure 9). The negative correlation between nutrients and picoplankton indicated that picoplankton growth is not affected much by the availability of nutrients because of their small cell size. As a result, they can thrive better in oligotrophic condition. The correlation results further showed that the biomass growth of picoplankton could be affected by temperature and salinity. We observed higher biomass growth of picoplankton under a warmer condition during the pre-monsoon season.

4. Discussion

The pigments are useful chemotaxonomic proxies for determining phytoplankton group (de Vargas et al., 2015; Kramer et al., 2019; Chandrasekhararao et al., 2022). Since phytoplankton responds swiftly to environmental changes, it can be a valuable tool for evaluating the alterations in aquatic environments (Fietz et al., 2005). In the present study, the chromatographic data provided a comprehensive characterization of pigments, which have been used as marker pigments to investigate the variation in phytoplankton community structure in the estuarine waters of Goa, while we identified five predominant groups from the microscopic studies. Out of these, Bacillariophyta (diatom) were the most dominant group constituting 74% to 77% of the total population.

The chromatographic data revealed that fucoxanthin, peridinin, zeaxanthin, diadinoxanthin, and chlorophyll *a* were the most dominant and frequently occurring phyto-
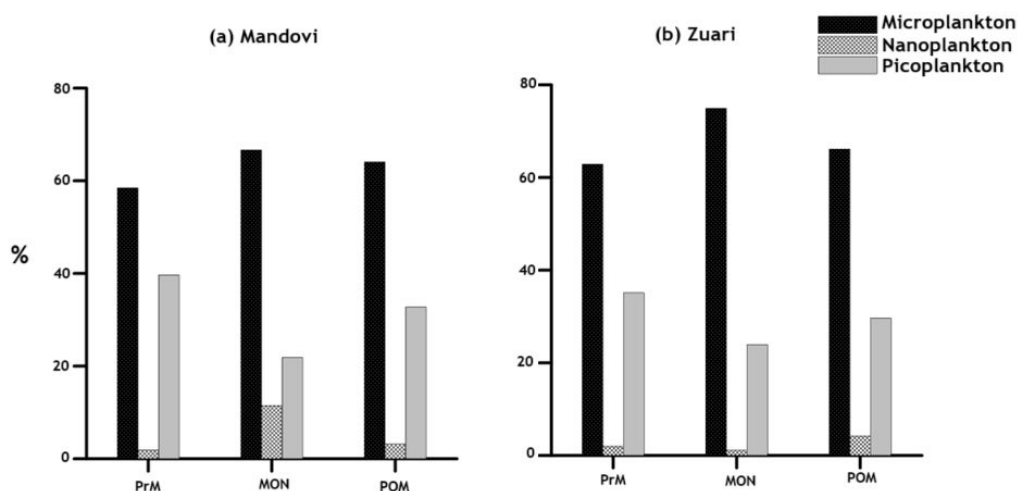
Figure 10. Seasonal variation in the biomass proportion of phytoplankton size classes in Mandovi (a) and Zuari (b) estuarine waters of Goa, during PrM (Pre-monsoon, February to May), MON (Monsoon, June to September), and POM (Post-monsoon, October to January) seasons.

plankton pigments in both the estuaries. The predominance of diatoms and dinoflagellates was shown by a high concentration of fucoxanthin and peridinin pigments in both estuaries, while the presence of taxonomic significance of cyanophytes and, chlorophytes was indicated by the pigments like zeaxanthin, and chlorophyll *b*. A similar observation has been reported by Roy et al. (2006), Chai et al. (2016), and Paul et al. (2021). We also observed that marker pigments such as fucoxanthin, peridinin and zeaxanthin strongly correlated with phytoplankton like diatom, dinoflagellates and cyanobacteria.

The high concentration of chlorophyll *a* (phytoplankton biomass) during monsoon season corresponded with the high abundance of diatom observed under the microscope in both the Mandovi (1.1×10^4 cells L^{-1}) and Zuari estuaries (1.7×10^4 cells L^{-1}). This observation is in accordance with the study of Figueiras et al. (2014), wherein they showed that the phytoplankton biomass (concentration of chlorophyll *a*) is affected by the abundance of diatom. During this study, a clear spatio-temporal variation of phytoplankton was found with the distribution of pigments, which was supported by the microscopic study and correlation analysis. The high concentration of chlorophyll *a* in the Zuari estuary during December was possibly due to the high abundance of microplankton, especially diatoms.

Furthermore, the correlation between chlorophyll *a* and DP was strongly significant ($R^2 = 0.912$; $P < 0.01$), which indicates that DP can reliably estimate the biomass of phytoplankton. With the help of DP, we could derive the biomass proportion and determine the phytoplankton size class. Earlier studies have also reported a similar observation of a strong correlation between DP and chlorophyll *a*, wherein they showed that because of such correlation, DP can be used as a proxy to measure phytoplankton biomass (Vidussi et al., 2001; Barlow et al., 2005). Therefore, the DP

come as a useful tool to estimate the biomass proportion of each phytoplankton size classes.

Important eco-biogeochemical processes such as nutrient cycling, light availability, mixing and stratification influence the size structure of phytoplankton species in the marine ecosystem and the proportion of biomass obtained from pigments could indicate the phytoplankton's size structure (Turner et al., 2021; Vesna Flander et al., 2021). In the present study, the microplankton size fraction is composed mainly of diatom and dinoflagellates species. Their biomass proportion (BP_{Micro}) was 57% in the Mandovi, while it was 68% in the Zuari. The contribution of microplankton was highest during the south west monsoon season. This was positively correlated with the prevailing environmental factors (Mandovi $R^2 = 0.629$, $P < 0.05$; Zuari $R^2 = 0.727$, $P < 0.01$). During this period, the characteristic of seawater in both estuaries underwent a significant change, such as a decline in salinity due to high precipitation and freshwater discharge, reduced light intensity, and nutrient enrichment from land runoff and resuspension of sediment (Shetye et al., 1995; Anand et al., 2014). The study of the interaction between physicochemical factors and the biotic components indicated that microplankton were the most affected by the concentration of nutrients and their abundance is a key indicator of nutrient-rich waters.

In this present study, the contribution of the nanoplankton group in the overall phytoplankton community structure was found to be low, only 5% in the Mandovi estuary and 2% in the Zuari estuary (Figure 7). They belonged to chlorophyta and chrysophyta groups, and formed the third size fraction of the phytoplankton community. Their abundance during pre-monsoon season in both the Mandovi and Zuari estuaries was very low, which was probably affected by salinity or the activity of the grazers. Previous

studies also showed that the low abundance of nanoplankton in the estuarine waters has been due to active grazing by the ciliates and flagellates (Jyothibabu et al., 2006; Madhu et al., 2008). Conversely, the biomass proportion of the nanoplankton size class was higher during the monsoon and post-monsoon seasons. The high abundance of nanoplankton during monsoon and post-monsoon indicated that their abundance is associated with low salinity and high nutrient conditions (Figure 10).

Conversely, the abundance of picoplankton was high during pre-monsoon months in both the estuary (Figure 10). They constituted the second most dominant size fraction of the phytoplankton community and comprised mainly of unicellular prochlorophyta and cyanobacteria species. The per cent contribution of biomass proportion of the picophytoplankton group was 38% and 30% in the Mandovi and Zuari estuaries, respectively. During this season salinity ranged from 27–31, and temperature from 31–32.5°C, but the inorganic nutrient were quite low (NO_3^- – 1.3–3.2 µM; NO_2^- – 0.23–1.2 µM; PO_4^{3-} – 0.4–0.51 µM; SiO_4^{4-} – 17–24 µM) in both the Mandovi and Zuari estuaries.

The results showed that the biomass proportion of picoplankton was mainly affected by temperature rather than by nutrients. This suggested that the nutrient requirement of the cell is correlated to their cell size, and thus, picoplankton thrive better in nutrient-poor conditions than other phytoplankton-sizes class. Supporting our observation, the study by Mouriño-Carballido et al. (2016) showed highly productive picoplankton growth in an oligotrophic condition, while Rajaneesh et al. (2018) showed that the growth of picoplankton increases with the increase in temperature and light level. The increase in picoplankton biomass proportion coincided with the increase in temperature as observed from February to May. This positive correlation between picoplankton and temperature was significant. Similar effects of environmental factors such as nutrients, salinity and temperature have been observed on other size class phytoplankton, like nano- and microplankton, in earlier studies as well (Rekik et al., 2015; Zhao et al., 2016; Pulina et al., 2017; Chowdhury and Biswas, 2023). Furthermore, studies have showed that changes in salinity can lead to shifts in species composition, while warmer waters tend to favour smaller phytoplankton size class such as picoplankton (Marañón et al., 2012; Wang et al., 2019; Gomez and Souissi, 2020). Similar observations were reported from the estuaries of south west coast as well by Roy et al. (2016) and Rajaneesh et al. (2018).

The presence of large-sized phytoplankton such as diatom indicates nutrient-rich and productive water, which supports the marine aquatic food chain and fisheries resources (Smetacek et al., 1998; Huete-Ortega et al., 2010; Shankar et al., 2019). A marine food web is greatly influenced by phytoplankton size class, while the phytoplankton community structure is largely affected by the vari-

ation in salinity and nutrient concentration (Dutta et al., 2016; Saifullah et al., 2016; Bharathi et al., 2022). It is apparent that the community structure and composition of phytoplankton have a significant impact on the trophic structure of a marine ecosystem. Studies have shown that small-sized phytoplankton support longer food chain that is characterised by carnivorous fishery, while shorter food chain is supported by large-sized phytoplankton like diatoms (Albin et al., 2022; Chowdhury and Biswas, 2023). Therefore, the high cell density of large sized microplankton observed in these estuaries may have a positive impact on fisheries recruitment and stock development. However, any change in phytoplankton biomass production as a result of climate change event or other anthropogenic activities may negatively impact the fishery resources.

5. Conclusion

The highly dynamic nature of an estuarine ecosystem is often reflected by the variability in physicochemical properties such as light, temperature, salinity and nutrients, etc. Such variations in the ecosystem might change the concentration of pigments; yet, the current study demonstrated that the use of diagnostic pigments in studying the dynamics of phytoplankton community and their function in the estuarine ecosystem can reliably estimate this community structure and biomass in a relatively short time with a discernibly reliable data. Although salinity gets affected during monsoon due to the influx of freshwater, receiving large amount of nutrients as a runoff from the adjacent areas seemingly support the productivity of microplankton. Considering the high biomass production of microplankton, the productivity of the planktivorous fishery could be higher in these estuaries than the carnivorous fishery.

Acknowledgments

Conflict of interest

None declared.

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