

Intra-annual variations in phytoplankton biomass and its composition in the tropical estuary: Influence of river discharge

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Abstract

To examine the influence of river discharge on phytoplankton composition, time-series observations were conducted at upper, middle and lower Godavari estuary during 2009. The salinity variations in the estuary were depended on freshwater discharge and tidal exchange. River discharge brought a significant amount of nutrients, however it did not induce phytoplankton bloom due to severe light limitation driven by high turbidity. Enhanced phytoplankton biomass and abundance were associated with water column stratification during moderate discharge period, suggesting that water column stability is more important than nutrients to promote phytoplankton blooms. The contribution of diatoms to the total phytoplankton abundance increased with decrease in salinity and vice versa for blue-green algae. The relationship of phytoplankton abundance with salinity and nutrients suggests that low salinity and high N:P ratio favored growth of blue-green algae, whereas high salinity and low N:P/N:Si favored diatoms. This study suggested that discharge brought modification in phytoplankton composition.

Keywords Phytoplankton composition . river discharge . stratification . nutrients . estuary .

Estuaries, the transition zone between land and sea, are among the most productive marine ecosystems in the world (Bardarudeen et al., 1996; Gameiro et al., 2004). They receive land driven nutrients through rivers and modify them before delivering to the adjacent ocean (Ketchum et al., 1967). Phytoplankton constitutes the base of the food chain (Ananthan et al., 2008) and determines the organic carbon production in an estuary (Cloern, 1996). Among various abiotic factors in estuaries, fresh water discharge and tidal activities mainly control growth and abundance of inhabiting phytoplankton population (Cloern, 1996; Sin et al., 1999). Most of the estuaries are also known to severely light limited (Pennock, 1985; Lehman, 1992; Cole et al., 1992; Irigoien and Castel, 1997; Sarma et al., 2009). Land driven nutrients support rich phytoplankton production in estuaries (Crouzet et al., 1999; Nelli, 2005). However, it has been noticed that while nitrogen is limiting in the coastal waters, phosphorous is more limiting in fresh and estuarine waters due to biological uptake and chemical precipitation (Fisher et al., 1992; Nelli, 2005). In contrast, inorganic ammonium (NH_4^+) plays a significant role in the phytoplankton growth in polluted water (Crouzet et al., 1999). Even the river is not polluted, changes in river discharges are known to affect the salinity, nutrient load, and stratification as a resulting modification of estuarine and coastal biogeochemical processes, however, and the evidences are rather sparse (Van Bennekom et al., 1981; Admiraal et al., 1990).

Phytoplankton biomass accumulation and bloom formation in estuaries depends not only on their productivity, but also biotic factors such as grazing and abiotic factors such as washout, resuspension and deposition (Underwood and Kromkamp, 1999). Net increase in phytoplankton is only possible when net gain (through reproduction and recruitment) exceeds the losses due to death and grazing. As low river inputs usually associated with long water residence times that allow accumulation of phytoplankton and development of blooms (Gameiro et al., 2004). Phytoplankton communities consist of several taxonomic groups and the occurrence of various groups is depended on several physico-chemical characteristics of the water column (Roy et al., 2006). Seasonal variations in primary production are depend on the nutrient availability, light conditions and temperature (Jouenne et al., 2007). The species composition, biomass, relative abundance and their spatial and temporal distribution of this aquatic biota are an expression of the environmental health or biological integrity of a particular water body (EKWU and Sikoki, 2006). In order to understand the variability in marine ecosystem, it is necessary to quantify the phytoplankton biomass as well as community composition and examine the controlling factors on diversity of phytoplankton composition in the system.

Monsoonal estuaries are unique compared to other estuaries with reference to freshwater discharge pattern (Vijith et al., 2009; Sarma et al., 2009; 2012b). Discharge into the monsoonal estuaries is concentrated during short-span of time and entire estuary filled with freshwater with no vertical gradients in salinity. In contrast, monsoonal estuaries contain relatively high saline water with moderate stratification during no discharge period and high stratification is noticed during moderate discharge periods (Acharyya et al., 2012). Therefore, monsoonal estuaries experience wide range of salinity and stratification patterns within an annual cycle. Hence, all these changes determine the phytoplankton biomass and community composition in the monsoonal estuaries. Several studies have been conducted in the recent years in the Godavari estuary (Sarma et al., 2009; 2010; 2011; 2012; Acharyya et al., 2012) and also various estuaries in India about phytoplankton diversity, for instance, Cochin Back Waters (Madhu et al., 2007), Mahanadhi (Naik et al., 2009), Hooghly estuary (De et al., 1991) and Mandovi estuary (Pednekar et al., 2011). Decrease in biomass and abundance was found during the peak discharge period and increased during dry and moderate discharge periods in the Mahanadi and Hooghly estuaries. However, limited integrated studies have been conducted on phytoplankton composition and their relations to the environmental properties in the Indian estuaries. The purpose of this study is to investigate the taxonomic composition of estuarine phytoplankton assemblages and examine the influence of hydrographic properties on them in the largest monsoonal estuary, the Godavari, India.

The Godavari estuarine system is situated around 16°15' N and 82° 5' E covering 330 km². The Godavari is one of the largest rivers in India with a basin of 3.1×10⁵ km² and 25 tributaries and an annual discharge of 105 km³ (Rao et al., 1975). Among the 60 largest rivers in the world, Godavari River ranks 34th and 32nd in terms of catchment area and water discharge respectively (Ludwig et al., 1996; Gaillardet et al., 1999). Average annual rainfall in the Godavari river basin is 1512 mm y⁻¹, indicating dry basin climate. According to Central Pollution Control Board (CPCB, 1995), more than 75% of rainfall occurs during summer monsoon period (June–September) and it is the sole source of water in the Godavari River. This is the largest peninsular river, which traverses about 1480 km before opening into the Bay of Bengal. Godavari River serves freshwater for local agricultural activities and domestic use. Near the town Rajahmundry at Dowaleiswaram, the river flow to the estuary is controlled by a dam. After emerging from the dam, Godavari divides into two major distributaries. The eastward flowing major tributary is Gautami-Godavari, while the other flowing southwards is Vasistha-Godavari. The latter has relatively minor flow compared to the former tributary; hence, the sampling was conducted in the Gautami-Godavari. To understand phytoplankton composition, diversity and controlling factors in the Godavari estuary, three stations

have been selected representing freshwater zone (upper estuary), brackish water zone (middle estuary) and seawater zone (lower estuary) (Fig. 1). Samples were collected at monthly interval from January to December 2009 covering various discharge periods.

Temperature, salinity was measured using a portable Conductivity–Temperature–Depth profiler (CTD; SBE-19 plus, Sea- Bird Electronics, USA). Surface water samples were collected using a 5L Niskin bottle for chlorophyll-*a* (Chl-*a*), nutrients and Suspended Particulate Matter (SPM). Nutrients samples were analyzed following the standard spectrophotometric procedures (Grasshoff et al., 1983). The accuracies of nitrate+nitrite (NO₃+NO₂), ammonium, phosphate and silicate were ±0.2, 0.2, 0.1 and 0.2 µM, respectively. Nearly 150-500 ml of the water sample was filtered through glass fiber filter (GF/F; 0.7 µm; Whatman) and Chl-*a* retained on the filter was extracted with *N,N*-dimethyl formamide at 4° C in dark for 24h and fluorescence was measured using spectrofluorophotometer (Eclipse Varian Instruments, UK) following Suzuki and Ishimaru (1990). The signal frequency band (Fb) was recorded in the excitation and emission wavelength of 445 nm and 665 nm respectively. The analytical precision for Chl-*a* analysis was ±4%. All these analyses were completed within 12h of sampling at the shore-based laboratory established on the bank of the river. Discharge data were obtained from the Dam authorities at Dowleiswaram.

In order to determine phytoplankton composition, one liter of surface water sample was collected in a polythene bottle and fixed with 0.5% lugols iodine solution (Thronsen, 1978). Before identification, water samples were allowed to settle for 24 hours and the supernatant was decanted and sample concentrated to 10 ml. About 0.1 ml of concentrated sample were taken in a common glass slide and observed under BX51 OLYMPUS microscope (Olympus, Japan). Phytoplankton cell counts were observed under 400X magnification. Phytoplankton was identified following procedures given by earlier investigations (Santra, 1993; Tomas, 1996; Baker, 2012).

Diversity, dominance, evenness and richness of the data were under taken using software Primer 5.2.8 (Clark and Warwick, 1994).

Phytoplankton diversity (H') was calculated using the following equation

Shannon and Wiener Index $H' = -\sum (P_i \times \log (P_i))$ (Shannon and Weaver, 1998).

P_i is n/N . Where n is individual number of phytoplankton and N is total number of individuals of all of the phytoplankton in a sample.

Dominance of phytoplankton was calculated using the following equation

Index of dominance = $\sum (P_i^2)$ (Simpson, 1949).

Richness was calculated using the following equation

Margalef Index $D' = S-1/\log N$ (Margalef, 1957).

Where S = number of species and N = number of individuals.

Evenness was calculated using the following equation (Pileou, 1966)

Evenness index $J' = H'/\log S$ (Pileou, 1966)

H = Shannon Index of general diversity; S = Number of species.

Seasonal and spatial variations in phytoplankton were examined by hierarchical cluster analysis using the Bray-Curtis similarity index as an estimate of similarity among months and stations using Primer 5.2.8 software. Canonical Correspondence Analysis (CCA) was used to assess associations between phytoplankton and environmental variables (using past3 software). The matrix with biotic data was constructed only with species abundant in at least one sample. Correlation coefficient (Spearman) among physicochemical and biological parameters was computed using the statistical software (Statistica 5.0), to understand the relation between environmental properties and phytoplankton composition. Grapher (version 3) was used for the 2 dimensional graphical representations of the data.

The monthly mean river discharge into the Godavari estuary varied from near zero during No Discharge Period (hereafter called as NDP) (January to May) to $<120 \text{ m}^3 \text{ s}^{-1}$ during Moderate Discharge Period (MDP) (June, October-December) and $>500 \text{ m}^3 \text{ s}^{-1}$ during Peak Discharge Period (PDP) (July to September; Fig. 2). The water temperature during the study period varied between 25.9 and 32.4° C in the estuary and minimum was observed during the winter while maximum in summer (Fig. 2). The salinity in the upper estuary ranged from 0.05 to 16.9 whereas 0.1 to 30.9 and 0.1 to 33.4 in the middle and lower estuary respectively (Fig. 2). SPM varied between 22.0 and 145 mg. l^{-1} and maximum was observed with PDP (Fig. 2).

Ammonium concentrations in the entire estuary ranged between 0.16 and 2.98 μM with relatively high concentration in the upper estuary and decreased towards lower estuary. Relatively higher concentrations were noticed during NDP and PDP (Fig. 3). The peak in nitrate, phosphate and silicate concentrations was associated with initial pulse of discharge and relatively higher in the upper and decreased towards lower estuary. Significant increase in nitrate concentrations was observed associated with PDP up to 115.1 μM in the upper estuary while it was $\sim 20\text{-}40 \mu\text{M}$ in the middle and lower estuary (Fig. 3). The mean concentration of Dissolved Inorganic Nitrogen (DIN) (Nitrate + Nitrite + Ammonium), Dissolved Inorganic Phosphate (DIP) and Dissolved Inorganic

Silicate (DISi) were comparatively higher in the upper estuary (27.3 ± 36 , 1.8 ± 1.9 and 43.7 ± 28 μM), than middle (11.7 ± 14 , 1.1 ± 0.9 , 17.9 ± 17 μM) and lower estuary (6.7 ± 6 , 0.9 ± 0.3 , 16.5 ± 19.6 μM) respectively.

Higher concentration of phytoplankton biomass (in terms of Chl-*a*) (mean 6.9 ± 4 ; 0.5 - 20.9 mg m^{-3}) was observed in the study area during MDP associated with strong stratification and mesohaline conditions, whereas it was low during NDP (mean 4.4 ± 4.7 ; 0.2 - 17.8 mg m^{-3}) and PDP (mean 4.6 ± 3 , 1.3 - 10.5 mg m^{-3}) (Fig. 4). During onset of discharge (June) and decreased phase of discharge (October), Chl-*a* showed marked increase in the entire estuary (10.8 ± 9.0 and 8.6 ± 2 mg m^{-3}) compared with NDP (1.8 ± 1 mg m^{-3} during March). The phytoplankton abundance was higher during MDP ($4.5 \pm 5.8 \times 10^5$ cells. l^{-1} ; Fig. 4) followed by NDP ($2.8 \pm 2 \times 10^5$ cells. l^{-1}) and PDP ($1.2 \pm 0.73 \times 10^5$ cells. l^{-1}). The highest abundance of phytoplankton was noticed during October in the upper and middle estuary (16.8×10^5 cells. l^{-1}) and decreased significantly towards lower estuary (24×10^4 cells l^{-1}) due to influx of fresh water associated with nutrients in former region. Five major classes were recorded in this study, including diatoms (58 species), dinoflagellates (7 species), green algae (31 species), blue-green algae (16 species) and silicoflagellates (1 species).

The river Godavari is the tropical and monsoonal estuary as the fresh water discharge into the estuary is determined by the monsoonal precipitation (Vijith et al., 2009; Sarma et al., 2009). The discharge peaks during monsoon period (PDP), when discharge per day exceeds volume of the estuary due to heavy monsoonal precipitation. During this period, entire estuary turns into a freshwater lake with no vertical salinity gradient (Vijith et al., 2009; Acharyya et al., 2012; Sridevi et al., 2015). In contrast, intense stratification is generally associated with fresh water discharge in the estuaries located in America and Europe (Christopher et al., 2002). During NDP, Godavari estuary is dominated by seawater due to the drying of upstream river, Whereas MDP shows strong vertical stratification due to salinity gradients (Sridevi et al., 2015). Year 2009 received about 22% less rainfall from that of long term means averaged all over India, which was the highest recorded negative rainfall departure since last 20 years (<http://www.imd.gov.in/section/nhac/dynamic/endseasonreport09.pdf>). Indian Meteorological Department (IMD) recognized year 2009 as a drought-hit year, which recorded “highest mean annual temperature over the country since 1901” (<http://www.imd.gov.in/doc/warm2009.pdf>) (Acharyya et al., 2012). Consistent with meagre rainfall pattern, the length of zero discharge periods of year 2009 (193 days) in the Godavari river estuary was far above than 2008 (119 days) and 2007 (118 days) (Acharyya et al., 2012). In addition to this, the mean annual average discharge of Godavari estuary in 2009 was the lowest ($218 \text{ m}^3 \text{ s}^{-1}$) since

last 22 years and was about one fourth of the long term average of 934 m³ s⁻¹. Godavari River is dammed at several locations along its course for irrigation and domestic consumption of freshwater that prevents seasonality in discharge and facilitates concentrated flow during a short span of time when water levels exceed the capacity of dam reservoir. As a result salinity of the estuary varies between near zero and ~34 and magnitude of dilution with seawater decreases with distance from mouth. During NDP, the entire estuary was dominated by high salinity with mean of 13.4±3.8, 26.3±5, and 31.8±3 in the upper, middle and lower estuary respectively (Fig. 2). The amplitude of tide varies between 0.8 and 1.2 m during neap and spring periods, respectively and seawater intrusion into the estuary was observed until 50 kms from the mouth of the estuary (Sridevi et al., 2015). During PDP, no significant spatial salinity gradients were observed within the estuary with mean of 0.08±0.03, 0.06±0.01 and 1.4±2 in the upper, middle and lower estuary respectively due to enormous amount of freshwater discharge (Fig. 2). On the other hand, significant spatial gradient (0.82±1.3, 8.7±8, and 19.3±11 in the upper, middle and lower estuary respectively) was observed during MDP. A marked salinity gradient of 18.5±2.3 was found in the study area between upper and lower estuary during both NDP and MDP and it was minimal during PDP (1.3±2; Fig. 2). Discharge also brought significant amount of SPM to the estuary. The SPM concentrations during the NDP were <60 mg l⁻¹ and has been increased to >100 mg l⁻¹ during PDP (Fig. 2). Sarma et al. (2009) found that the depth of light penetration was >3 m during NDP and has been shallowed to <0.1 m during the PDP that severely influences the phytoplankton biomass.

Upstream river, ground water, atmospheric transport and benthic fluxes act as major sources of nutrients in shallow coastal waters (Nowicki and Nixon, 1985; Malone et al., 1988; Paerl, 1997; Conley, 2000). Due to storage of water in the dam reservoir for a long period (~6-8 months) during NDP, organic matter in the reservoir undergoes significant modification in terms of concentration and composition (Sarma et al., 2011; 2012). In present study, enhanced nutrients concentrations of DIN, DIP and DISi were observed in the estuary during PDP associated with discharge. Relatively higher concentrations were observed in the upper (63.3±48, 2.9±3.9 and 45.2±22 µM), than middle (33.3±12, 1.1±0.4, 29.3±15.9 µM) and lower estuary (14.9±5, 1.0±0.2, 26.9±5 µM respectively) (Fig. 3). Comparatively lower concentration of nutrients were found during NDP in the entire estuary with mean concentrations of DIN, DIP and DISi were 3.6±2, 1.6±0.7 and 57.1±25.9 µM and 2.9±1, 0.8±0.3 and 20.3±28 µM in the upper and lower estuary respectively (Fig. 3). Nutrients concentrations decreased from upper estuary to lower estuary due to dilution or removal, both biologically and chemically, during estuarine mixing (Fig. 3). In addition to nutrient concentrations, their ratios also showed significant variations with reference to space and time. Relatively high N:P

ratios were observed during PDP and was decreased from upper estuary (41.4 ± 24.3) to lower estuary (16.4 ± 7), whereas these ratios were significantly lower during NDP (2.2 ± 0.9 and 3.7 ± 1.6 at upper and lower estuary respectively) (Fig. 5). Such wide variations in nutrient ratios can be attributed to variable sources of nutrients to the estuary. Sarma et al. (2010) observed that anthropogenic nutrients such as fertilizers are the major source of nutrients to the estuary during discharge period whereas ground water and benthic exchange during NDP. Since nitrogen rich fertilizers, such as urea and diammonium phosphate is used in this region, N:P ratios were significantly higher than Redfield ratio of 16. As a result of fertilizer usage ammonium concentrations were always $>1 \mu\text{M}$ in the estuary during entire study period. On the other hand, high silicate concentrations were found in the entire study period at the upper estuary and the higher concentration were found only during discharge period in the middle and lower estuary. The N:Si ratio was high during PDP at the upper estuary (1.5 ± 0.8) and close to global mean values of 1 and it was decreased towards lower estuary (0.5 ± 0.09) (Fig. 5). On the other hand, N:Si ratios were lower during NDP (0.1 ± 0.03 and 0.4 ± 0.3 at upper and lower estuary respectively). From the nutrient data set, there is no evidence of nutrients (DIN, DIP and DISi) limitation, but N:Si:P (stoichiometric nutrient balance) ratio's suggesting that both phosphate and silicate may be a limiting (relative limitation) nutrients during NDP. Therefore the variations in concentrations and ratios of nutrients, brought by the river discharge, would have significant impact on phytoplankton community structure in Godavari Estuary.

Phytoplankton community being highly sensitive and quickly responds towards environmental changes, they are good indicator of trophic status (Manna et al., 2010). River discharge has a significant impact on the salinity, stability of water column (Narasimha Rao et al., 2001; Acharyya et al., 2012), light penetration, SPM load (Sarma et al., 2009) and nutrient concentrations (Sarma et al., 2010) in the Godavari estuary. Earlier studies showed that river discharge has significant impact on phytoplankton biomass in the Godavari estuary (Sarma et al., 2009; Acharyya et al., 2012). In present study, it was noticed that low phytoplankton biomass and abundance associated with peak discharge due to high SPM, and low light penetration whereas relatively higher biomass and abundance was noticed during MDP and NDP. Similar kind of results was observed in Mahanadi and Hooghly estuaries (De et al., 1991; Naik et al., 2009). On the other hand, river discharge not only affect phytoplankton biomass but also functional groups of phytoplankton community. Diatoms abundance varied from 0.2 to 7.4×10^5 (cells. l^{-1}) and their abundance decreased from upper to lower estuary during the study period. Overall diatoms dominated throughout the year in the entire estuary and contributed from 11 to 100% to the total abundance in the entire estuary (Fig. 4). Diatoms consistently dominated group (except October in

upper estuary; December in middle estuary; April in down estuary) which could well thrive in widely changing hydrological conditions (Mani, 1992; Tiwari and Nair, 1998; Rajasegar et al., 2000; Gopinathanm et al., 2001; Gowda, 2001; Senthilkumar et al., 2002) while other phytoplankton groups exhibit in different abundance according to their salinity tolerance ranges. The dominance of diatoms particularly during NDP was also observed in other monsoonal estuaries viz. Kaduviyar estuary (Vengadesh Perumal et al., 2009), Cochin Back waters (Madhu et al., 2007), Mandovi estuary (Pednekar et al., 2011). Dinoflagellates abundance ranged from 0 to 0.5×10^5 cells. l^{-1} . In upper estuary <5% contributed by dinoflagellates during MDP, whereas they were found throughout the year in the middle and lower estuary (Fig. 4). Dinoflagellates were not very abundant phytoplankton community in the Godavari estuary as they mostly prefer oligotrophic conditions; hence, they fail to survive in the eutrophic waters by competing with diatoms (Menzel et al., 1963; Cushing, 1989). Besides diatoms and dinoflagellates, green algae and blue green algae were commonly found in study area during the discharge period, compared to NDP due to changes in salinity. Green algae were observed during the study period with abundance, ranged from 0 to 7.5×10^5 . Green algae presented throughout the year in the upper estuary and contributed almost 50% to the total abundance during MDP (December) and the least during NDP. High abundance of green algae was found at middle and lower estuary during MDP due to the influx of fresh water (Fig. 4). Blue-green algae were identified during the study period and their abundance, ranged between 0 and 14.8×10^5 . Blue-green algae was found throughout the year at upper estuary and shown the highest percentage (~90%) during MDP (October) and the lowest percentage during NDP. On the other hand, blue-green algae were found at middle and lower estuary throughout the discharge period (Fig. 4).

The contribution of diatoms was decreased during PDP and MDP and was replaced by blue-green algae in the upper and lower estuaries. The exceptionally high abundance of green algae and blue-green algae during the discharge period (both PDP and MDP) were caused by the intense freshwater influx. Naik et al. (2009) also observed that the high abundance of blue-green algae during moderate discharge period in Mahandi estuary. The condition and the affinity of these groups with high nutrient concentration favor their development, instead of the development of other photosynthesizing organisms (Graham and Wilcox, 2000). River discharge brought significant changes not only in phytoplankton abundances but also in species composition. We attempted here to assess the trophic state of the Godavari estuary on the basis of phytoplankton species composition at various discharge scenarios.

Overall, diatoms contributed (68-100%) to the total abundance during NDP (except April in lower estuary) in the entire estuary but showed significant difference with reference to taxa in the estuary. For instance, *Melosira moniliformis* and *Thalassiosira anguste-lineate* dominated in the upper whereas *Chaetoceros lorenzianum* and *Nitzschia closterium* in the middle and *Chaetoceros messanensis*, *Nitzschia closterium* and *Skeletonema costatum* were dominant in lower estuary. Cochin back waters were also dominated by *Skeletonema* spp. and *Nitzschia* spp. during NDP (Madhu et al., 2007). With reference to phytoplankton taxa, middle and lower estuary dominated by similar taxa due to high salinity (≥ 20), which was significantly higher compared to upper estuary (≤ 15). In blue-green algae, *Merismopedia glauca* was dominant in the upper and middle estuary whereas *Trichodesmium erythraeum* found abundant in the lower estuary. Green algae showed significant difference with reference to space. *Chlorococcum humicola* and *Pediastrum tetras* were abundant at the upper estuary whereas *Chlorococcum humicola*, *Eudorina* spp. and *Ulothrix variabilis* were dominant at middle while *Echinospherella* spp. at the lower estuary.

During MDP, phytoplankton abundance was dominated by blue-green algae, green algae and diatoms. Among the blue-green algae, *Cylindrospermopsis* sp. dominated at the upper estuary, whereas *Anabaena* spp. and *Nostoc carneum* in the middle and *Aphanocapsa* spp. at the lower estuary. In the green algae, *Pediastrum simplex*, *Scenedesmus acutus* and *Sphaerocystis* spp. dominated in the upper estuary, *Chlorella vulgaris* at middle and *Oocystis submarina*, *Pediastrum simplex*, *Triploceras gracile* and *Volvox aureus* in the lower estuary. In contrast, diatoms contribution decreased during MDP with *Nitzschia longissima*, *Thalassiosira eccentrica* and *Melosira moniliformis* were abundant in the upper estuary, *Leptocylindrus danicus*, *Melosira moniliformis* and *Synedra ulna* in the middle and *Leptocylindrus danicus*, *Skeletonema costatum*, *Thalassionema nitzschioides* in the lower estuary. Madhu et al. (2007) also notice the dominance of *Synedra* spp. during low saline water condition in Cochin back waters.

In upper and middle estuary, the phytoplankton abundance showing the lowest density during PDP. The higher river flow supported mainly of nano phytoplankton ($< 20 \mu\text{m}$), fast growing functional groups, which were able to maintain biomass even in high flushing conditions. In addition to this, several large phytoplankton, such *Pinnularia* spp., which normally dwell on the sediment surface, were also noticed in the water column that could be due to re-suspension of sediment by heavy flow of water (Costa et al., 2009). Venkataramana et al. (2017) noticed Polychaete species in the water column, which dwell in the sediments, during the PDP. Among blue-green algae, *Nostoc carneum* and *Oscillatoria subbrevis* were abundant in the entire estuary whereas *Thalassiosira*

eccentrica, *Coscinodiscus granii*, *Leptocylindrus danicus* and *Nitzschia longissima* dominated in the diatom group. Relatively less spatial variability found compared to MDP and NDP due to fast flowing of waters. Therefore, low phytoplankton biomass and abundance were driven by high SPM and low flushing time.

Relations between measured environmental variables and phytoplankton assemblages during different discharge period were explored using Canonical Correlation Analysis (CCA). A total of 28 dominant species (17 diatoms, 1 Dinoflagellate, 3 green algae and 7 blue-green algae) and 9 environmental variables were used for the analysis (Table 1). The result of the analysis clearly showed that the variation in the phytoplankton community was mainly affected by the discharge influenced changes in the environmental condition in the Godavari estuary. Eigenvalue of axis 1 ($\lambda = 0.722$), 2 ($\lambda = 0.625$) and 3 ($\lambda = 0.322$) explained 71% of the relation between species and environmental data (Table 1). The first CCA axis, separated the no discharge and discharge samples (Fig. 6). Variation on the first CCA axis was related to the NDP with high positive loading of salinity. For instance diatoms such as *Asterionella* spp., *Chaetoceros* spp., *Guinardia* spp., *Skeletonema* spp., *Thalassionema* spp., *Thalassiothrix* spp., and *Peridinium* spp. occurred during the NDP in middle and lower esturine regions. Nitrate, N:Si and N:P were negatively loaded on the first axis characterising the discharge related changes in the Godavari estuary. CCA axis 2 integrated the discharge and silicate, and the dominant phytoplankton were *Merismopedia* spp., *Melosira* spp., *Fragillaria* spp.. In axis 3, discharge showed positive relation with *Pediastrum* spp. Therefore, the CCA analysis confirmed that the variation in river discharge associated changes in the environment is the primary factors effecting phytoplankton distribution in Godavari River estuary.

The species diversity depends mostly upon the water column stability, nutrient load, zooplankton grazing (Carstensen et al., 2007). High diversity during MDP in the lower estuary (1.1) was contributed by diatoms whereas green algae in the upper estuary (1.0). The phytoplankton community was very rich in species diversity during MDP and PDP (1.0 and 0.9) due to influx of fresh water and nutrients into the estuary (Fig. 7). In contrast, less diversity was noticed during NDP (0.7) due to high water residence time (>30 days). Dominance index indicated that the community was dominated by fewer numbers of species in comparison to the total number of individuals. The high dominance was found during NDP (0.4) due to stable water column favored accumulation of phytoplankton and contrasting to this was found during the discharge period (0.2) (Fig. 7). Sharp decline in diversity index was observed during NDP associated with high phytoplankton abundance as the majority of phytoplankton was dominated by three species *Chaetoceros lorenzianum*,

Chaetoceros messanensis and *Nitzschia closterium*. Evenness of the species was high during both MDP (0.8) and PDP (0.8) than NDP (0.6) due to high flow of waters made phytoplankton to distribute evenly during former period (Fig. 7). Species richness, expressed as simple relation between number of species and number of individuals, was high during NDP (1.4) and MDP (1.6) than the PDP (1.2). Such high richness was caused by enhanced tidal influence and high discharge from upper estuary brought new marine and freshwater dominant species to the estuary respectively during NDP and MDP (Fig. 7).

To understand similarity between months in upper, middle and lower estuaries, clusters have been made with abundance of all taxa, and these clusters showed ~20% similarity in each cluster. In upper estuary, similarity among the sampling period, based on the phytoplankton composition, showed three groups (Fig. 8a). The largest group was formed consists of MDP and NDP (January to March, November and December, except July and September) while other groups consists of discharge period (August, October) as one and transition period between NDP to PDP as another (May and June). Middle estuary formed as four groups consist of NDP and MDP (January to May, June and November), and the other two groups consist of the discharge period (July-September and December) whereas October month formed as separate group when high phytoplankton abundance was observed (Fig. 8a). Three groups were found in the lower estuary and were separated mostly with reference to discharge pattern. One group consists of completely NDP and MDP (January to May, June and December except July) and the other two consists of PDP and MDP (August to November) (Fig. 8a). Therefore, the phytoplankton composition in the estuary were mainly controlled by the discharge pattern.

In order to examine the similarity in the phytoplankton composition within the estuary with reference to the discharge, the similarity index plot was shown in figure 8b and showed three groups at 40% level. The largest group was formed consists of PDP at all three locations. The second group consists of upper and middle estuary during MDP. The third group consists of NDP at all three stations and MDP at lower estuary. This study reveals that entire estuary responded similarly during PDP whereas upper and middle estuary behaved similarly during MDP whereas middle and lower estuary showed similarity during NDP.

This study demonstrated that the taxa composition of the phytoplankton community changes quickly due to sharp variations in environmental variables. The rapid nutrient inputs with different ratios are of considerable importance since they can modify the phytoplankton community structure. Rapid changes in salinity due to fresh water flushing also influence dramatic changes in

phytoplankton composition (either for group or genus) as it is evident in the taxonomic data. In order to examine the same, the relationship of phytoplankton abundance, biomass and groups were correlated with hydrographic properties (Table 2).

A significant negative relation was observed between salinity and nitrate/N:P in upper, middle and lower estuaries. It is indicating that land driven discharge is main source of nitrate to Godavari estuary. In upper estuary, blue-green algae showed inverse correlation with salinity ($r^2=0.7$; $p<0.01$) and positive relation with nitrate/N:P ($r^2=0.8$; $p <0.01$) suggesting that low salinity and nitrate rich waters are favorable to grow. As a result the upper estuary was dominated by blue-green algae during PDP and MDP. In the middle and lower estuaries, green algae showed significant inverse relation with salinity. It is suggesting that the occurrence of green algae in the middle and lower estuaries, was mainly controlled by changes in salinity (Table 2).

The summary of the physical and chemical conditions during different discharge periods and biological responses were given in figure 9. This study suggested that different physical conditions were encountered by Godavari estuary due to variable discharge patterns such as salt content, water column stability, flushing rates, suspended load and light penetration depth etc. Since the river discharge is a significant source of nutrients, including terregeneous and artificial (fertilizers), to the system, their concentrations vary with discharge pattern. It has been noticed that not only the nutrient concentrations, but also their ratios also varied during different discharge periods. Despite high nutrient levels during discharge period, the highest phytoplankton biomass and abundance were noticed during MDP, suggesting that stratified water column with brackish salinity conditions are conducive for phytoplankton growth. High phytoplankton diversity and evenness in species was noticed during PDP and MDP, whereas high dominance and high species richness was found during NDP. Though diatoms were dominant (>50%) during the entire study period, however its contribution was relatively lower during MDP when blue-green algae contribution increased significantly. Therefore, discharge brought significant modification in salinity, flushing time and suspended load that changed the chemical composition of nutrients, and together had significant impact on the phytoplankton composition in the Godavari estuary. In terms of phytoplankton composition, the entire estuary responded similarly during the PDP while upper and middle estuaries behaved alike during MDP and, middle and lower estuaries were comparable during NDP. Therefore, this study suggests that modifications in the discharge either due to natural, variations in the intensity of monsoon due to climate change, or artificially due to the construction of more dams

and re-routing the river channels, will bring significant modifications in the plankton community that may be even irreversible.

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Table 1 Correlation of environmental variables and phytoplankton taxa with axes. Numbers in bold are indicating high correlation between environmental variables/ phytoplankton species with axes

	Variables	Code	Group	Axis 1	Axis 2	Axis 3
				$\lambda=0.72202$	$\lambda=0.62567$	$\lambda=0.32232$
Environmental variables	Discharge	Dis		-0.4	-0.4	0.5
	Salinity	Sal		0.8	0.7	-0.3
	SPM	SPM		-0.3	-0.2	-0.1
	Ammonium	Amm		0.1	0.1	-0.1
	Nitrate	Nit		-0.5	-0.1	0.1
	Phosphate	Pho		-0.2	-0.2	0
	Silicate	Sil		-0.2	-0.5	0.1
	N:Si	N:Si		-0.6	0.2	0.1
	N:P	N:P		-0.5	-0.2	0.1
Phytoplankton species	<i>Asterioniella</i> spp.	Ast-D	Diatom	0.8	0.5	0.0
	<i>Cheatoceros</i> spp.	Cha-D	Diatom	1.3	1.0	-0.5
	<i>Coscinodiscus</i> spp.	Cos-D	Diatom	0.2	-0.1	0.2
	<i>Cyclotella</i> spp.	Cyc-D	Diatom	0.7	0.0	-0.8
	<i>Fragellaria</i> spp.	Fra-D	Diatom	0.0	-0.8	0.6
	<i>Guinardia</i> spp.	Gui-D	Diatom	1.0	0.6	-0.3
	<i>Leptocylindrus</i> spp.	Lep-D	Diatom	-0.1	-1.4	-0.5
	<i>Melosira</i> spp.	Mel-D	Diatom	-0.2	-0.8	0.9
	<i>Navicula</i> spp.	Nav-D	Diatom	0.2	-0.3	-0.4
	<i>Nitzschia</i> spp.	Nit-D	Diatom	0.4	0.3	1.2
	<i>Odentella</i> spp.	Ode-D	Diatom	0.4	-0.4	3.3
	<i>Paralia</i> spp.	Par-D	Diatom	0.7	0.1	0.7
	<i>Skeletonema</i> spp.	Ske-D	Diatom	1.1	0.8	-0.6
	<i>Synedra</i> spp.	Syn-D	Diatom	-0.3	-1.3	-0.2
	<i>Thalassiosira</i> spp.	Ths-D	Diatom	0.1	-0.3	0.6
	<i>Thalassionema</i> spp.	Thn-D	Diatom	0.3	0.5	-0.2
	<i>Thalassiothrix</i> spp.	Tht-D	Diatom	0.9	0.7	-0.8
	<i>Peridinium</i> spp.	Per-Di	Dinoflagellate	0.7	0.4	-0.4
	<i>Actinotenium</i> spp.	Act-G	Green algae	-0.4	-0.9	6.8
	<i>Chlorella</i> spp.	Chl-G	Green algae	-0.1	-2.3	-1.5
	<i>Pediastrum</i> spp.	Ped-G	Green algae	-0.1	-0.8	0.6
	<i>Anabena</i> spp.	Ana-B	Blue-green algae	-1.0	-0.3	-0.8
	<i>Aphanocapsa</i> spp.	Aph-B	Blue-green algae	-0.3	-0.3	1.3
	<i>Cylindrospermopsis</i> spp.	Cyl-B	Blue-green algae	-2.1	1.3	-0.4
	Unidentified Blue-green algae	UIC-B	Blue-green algae	-0.7	-0.3	0.6
	<i>Merismopedia</i> spp.	Mer-B	Blue-green algae	-0.2	-0.6	0.1
	<i>Nostoc</i> spp.	Nos-B	Blue-green algae	-1.5	0.7	0.0
	<i>Trichodesmium</i> spp.	Tri-B	Blue-green algae	1.2	1.0	0.0
	Variance			30.8	26.7	13.8

Table 2 Rank correlation matrix (Spearman's) of phytoplankton biomass and abundance with physical and chemical parameters in the Godavari estuary. ***= $p < 0.001$, **= $p < 0.01$ and *= $p < 0.09$. (In the box top, middle and lower numbers denotes for upper, middle and lower estuaries)

	Abundance	Biomass	Diatoms	Dino flagellates	Green algae	Blue-green algae	Salinity	NH ₄ ⁺	NO ₃ ⁻	NO ₂ ⁻	PO ₄ ⁻	SiO ₄ ⁻
Diatoms	**0.7 ***0.9 ***0.9	0.1 0.3 0.1	1 1 1									
Dino flagellates	0.1 0.1 0.3	0 0.2 0.2	-0.1 0 0.1	1 1 1								
Green algae	*0.6 0.2 -0.1	-0.3 0.1 0.3	0.2 0.1 -0.1	0.4 -0.1 -0.1	1 1 1							
Blue-green algae	0.5 0.3 -0.5	0.1 *0.6 0	0.1 0.1 **0.7	0 0.2 -0.2	0.3 *0.6 0.4	1 1 1						
Salinity	-0.2 0.2 *0.6	-0.1 -0.1 *-0.5	0.5 0.2 0.3	0 0.3 *0.5	-0.2 ***-0.8 *-0.5	**0.7 -0.4 -0.3	1 1 1					
NH₄⁺	-0.1 0.2 -0.4	*0.7 -0.4 -0.1	-0.3 0.2 *-0.6	0.3 -0.1 -0.1	-0.5 0 -0.3	-0.4 *-0.5 *0.5	-0.1 0.2 -0.1	1 1 1				
NO₃⁻	0.3 -0.4 **0.7	0.4 -0.1 *0.5	-0.3 -0.4 -0.5	0 -0.2 -0.3	0.1 0.5 0.5	**0.8 0.3 0.3	***-0.9 ***-0.8 ***-0.9	0.3 -0.2 0.2	1 1 1			
NO₂⁻	0.4 -0.4 -0.2	-0.1 -0.1 -0.2	0.1 -0.3 -0.2	0.1 -0.5 0.2	0.5 0.2 -0.1	*0.7 0 0.3	-0.5 -0.3 0	-0.4 -0.1 0.2	0.4 0.2 0	1 1 1		
PO₄⁻	-0.3 0 -0.1	*0.5 0 0.5	0 0.2 -0.1	0.2 *-0.6 -0.3	-0.5 0.5 **0.7	-0.2 0.1 0.4	0.3 *-0.6 *-0.6	**0.7 -0.1 -0.1	0 0.3 0.4	-0.5 *0.7 -0.2	1 1 1	
SiO₄⁻	-0.3 -0.3 -0.1	0 -0.1 0.4	0.1 -0.1 0	-0.3 -0.4 0.2	-0.4 *0.6 -0.4	-0.5 0 -0.2	0.5 *-0.6 -0.2	0.1 0.3 0.5	-0.3 *0.5 0.3	*-0.5 *0.7 0.2	0.1 *0.5 -0.3	1 1 1
N:P	0.4 -0.3 *-0.7	0.2 -0.2 0.3	-0.3 -0.4 *-0.5	-0.1 0.3 0	0.3 0.1 0	***0.8 0 0.3	***-0.9 -0.4 *-0.5	0 0.1 0.4	***0.9 *0.6 **0.7	*0.7 -0.3 0.3	-0.4 -0.4 -0.2	-0.4 0.1 *0.5

Legend to figures

Figure 1: Figure showing station locations in the Godavari estuary

Figure 2: Monthly distribution of water temperature, salinity and SPM in the Godavari estuary at upper (left panel), middle (middle panel) and lower (right panel) estuary. Thick line on the top panel represents river discharge.

Figure 3: Monthly distribution of nitrate, ammonium, phosphate, silicate in the Godavari estuary at upper (left panel), middle (middle panel) and lower (right panel) estuary. Thick line on the top panel represents river discharge

Figure 4: Monthly distribution of phytoplankton biomass (Chl-*a*), abundance and relative abundance in the Godavari estuary at upper (left panel), middle (middle panel) and lower (right panel) estuary. Thick line on the top panel represents river discharge. ND indicates no data.

Figure 5: Monthly distributions of dissolve inorganic nitrogen to phosphate ratios (N:P) and dissolved inorganic nitrogen to silicate ratios (N:Si) in the Godavari estuary at upper (left panel), middle (middle panel) and lower (right panel) estuary. Thick line on the top panel represents river discharge

Figure 6: CCA bi-plot showing relationship between the environmental parameters and phytoplankton composition at Godavari estuary. Numbers are indicating months (1 to 12: January to December), Symbols are indicating discharge periods (dot: No discharge period, Triangle: Peak discharge period and Square: Moderate discharge period) and colours are indicating sampling location (Black: Upper estuary, Red: Middle estuary and Green: Lower estuary). The codes of the species and environmental variables are the same as in Table 1.

Figure 7: (a) Diversity index, (b) dominance index, (c) Evenness index and (d) Richness index during different discharge period in the Godavari estuary

Figure 8: (a) Dendrogram showing similarity of monthly variation in phytoplankton abundance at upper (top panel), middle (middle panel) and lower estuaries (bottom panel). (b) Dendrogram showing similarity in the entire estuary in phytoplankton abundance. In this U, M and L represents upper, middle and lower estuaries respectively. Whereas ND, MD and PD represent no discharge, moderate discharge and peak discharge periods.

Figure 9: Schematic diagrams showing different physical, chemical conditions and biological responses during different discharge patterns in the Godavari estuary. Dia. indicates diatoms, Dino. indicates dinoflagellates, Gre. indicates green algae and Blu. indicates blue-green algae. The total number of samples per peak discharge, moderate discharge and no discharge periods are 9, 12 and 15 respectively.

Figure 1: Figure showing station locations in the Godavari estuary

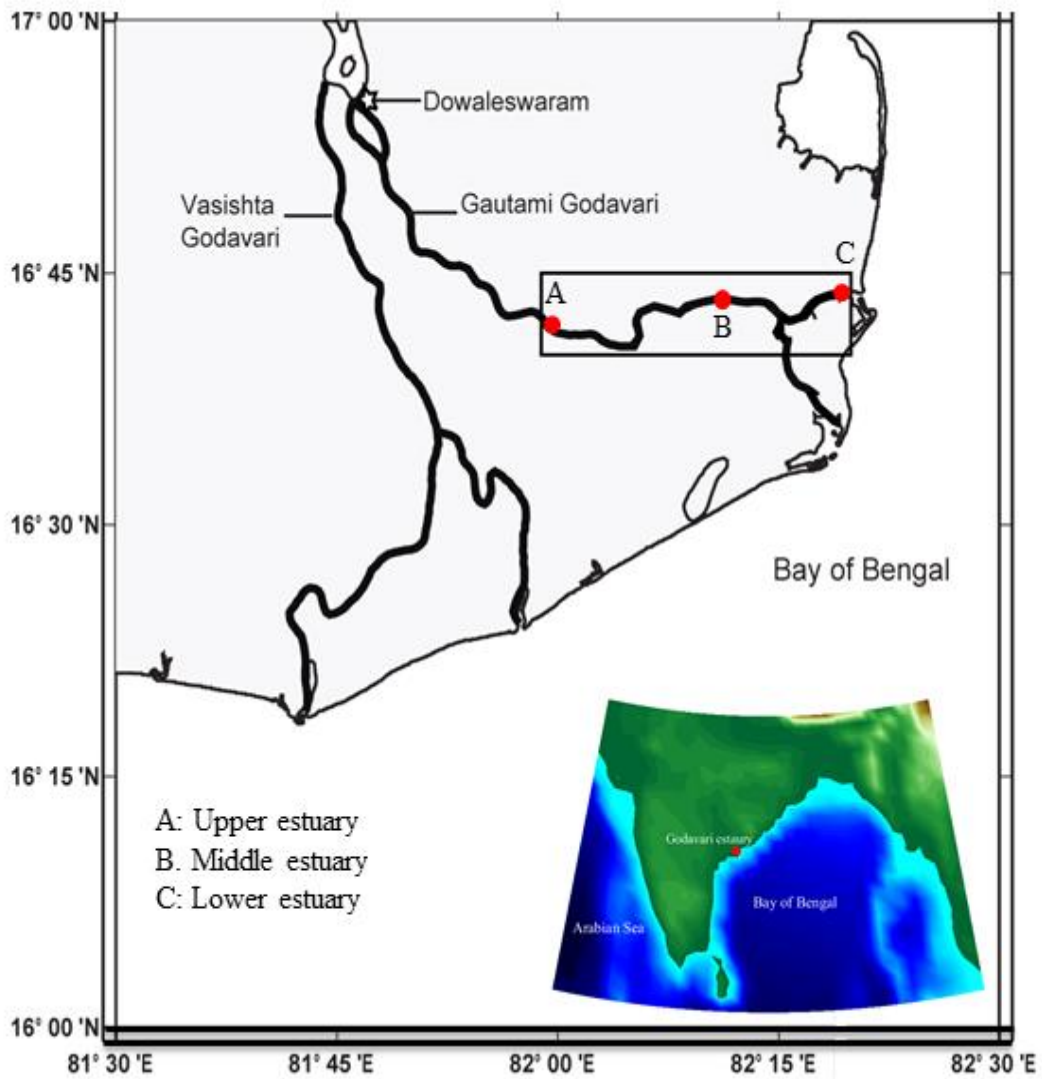


Figure 2: Monthly distribution of water temperature, salinity and SPM in the Godavari estuary at upper (left panel), middle (middle panel) and lower (right panel) estuary. Thick line on the top panel represents river discharge.

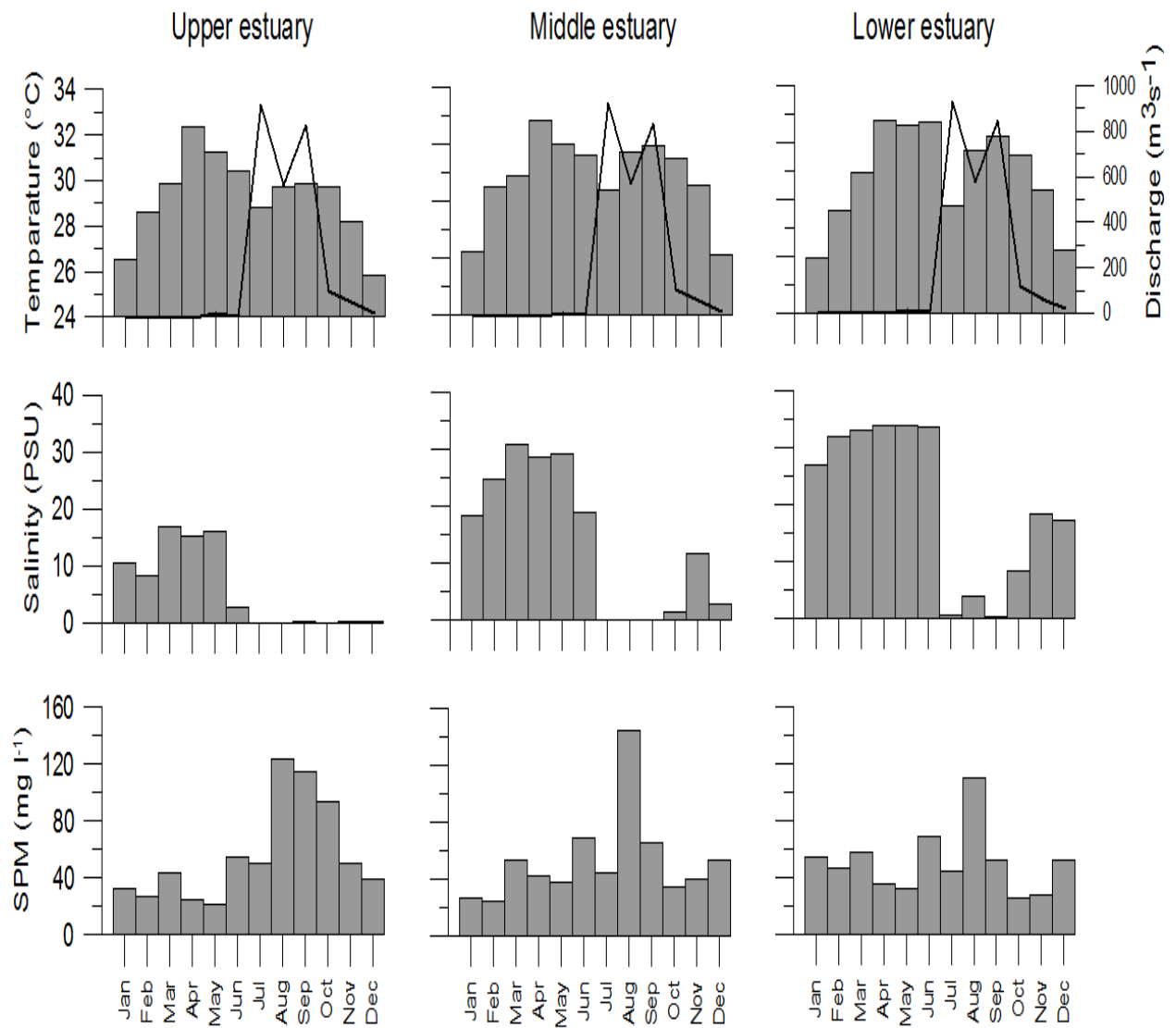


Figure 3: Monthly distribution of nitrate, ammonium, phosphate, silicate in the Godavari estuary at upper (left panel), middle (middle panel) and lower (right panel) estuary. Thick line on the top panel represents river discharge.

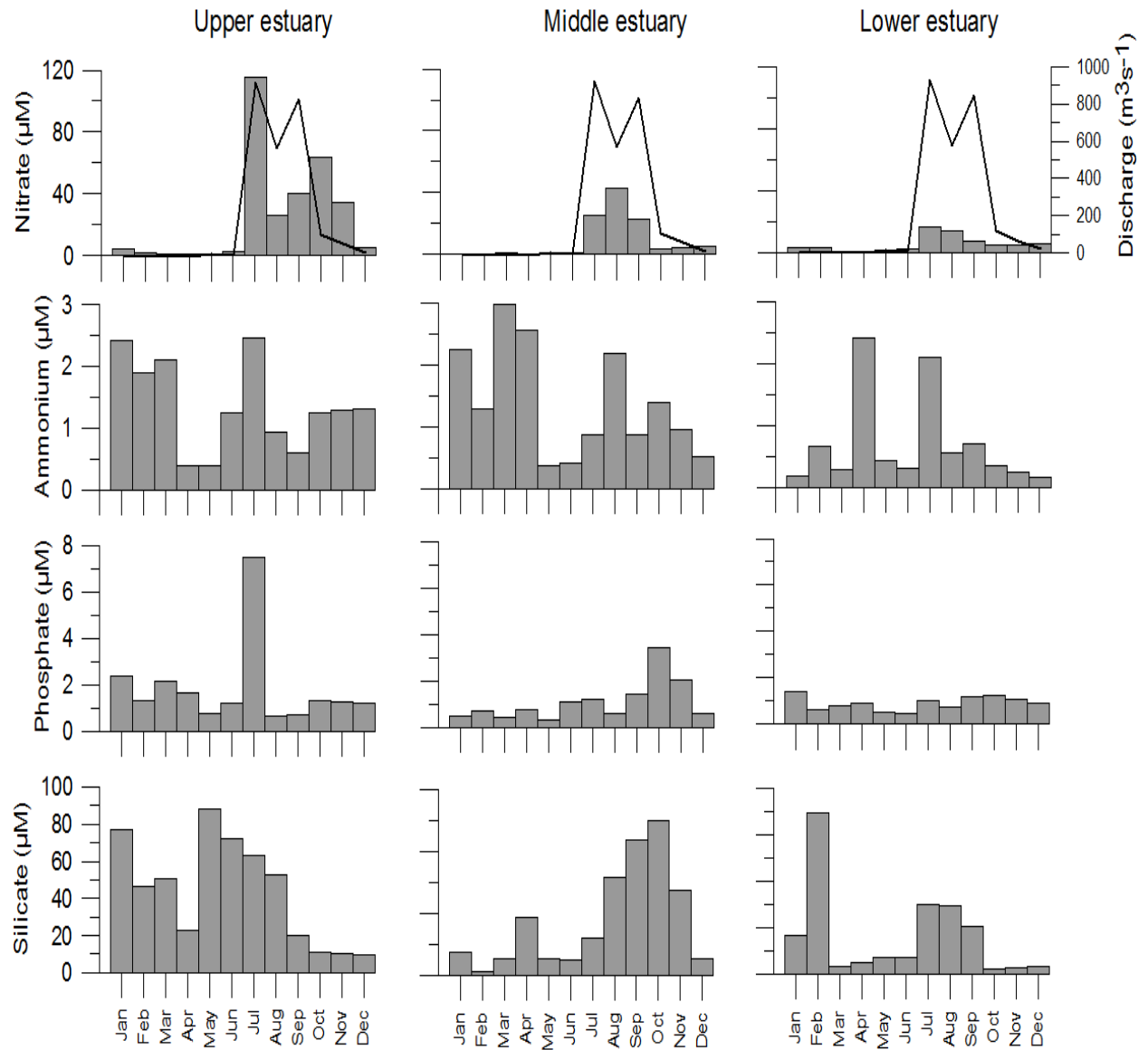


Figure 4: Monthly distribution of phytoplankton biomass (Chl-*a*), abundance and relative abundance in the Godavari estuary at upper (left panel), middle (middle panel) and lower (right panel) estuary. Thick line on the top panel represents river discharge. ND indicates no data.

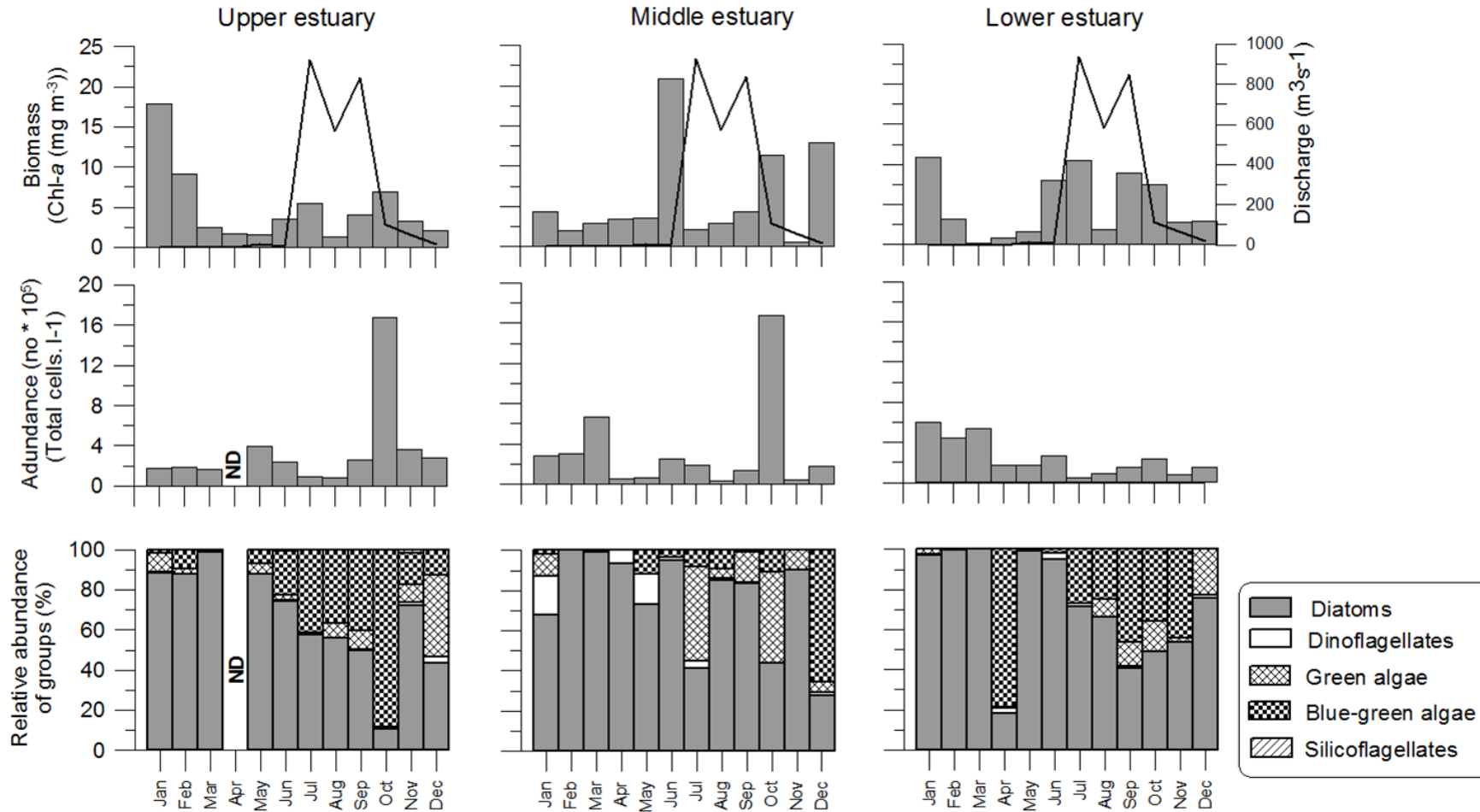


Figure 5: Monthly distributions of dissolve inorganic nitrogen to phosphate ratios and dissolved inorganic nitrogen to silicate ratios in the Godavari estuary at upper (left panel), middle (middle panel) and lower (right panel) estuary. Thick line on the top panel represents river discharge.



Figure 6: CCA bi-plot showing relationship between the environmental parameters and phytoplankton composition at Godavari estuary. Numbers are indicating months (1 to 12: January to December), Symbols are indicating discharge periods (dot: No discharge period, Triangle: Peak discharge period and Square: Moderate discharge period) and colours are indicating sampling location (Black: Upper estuary, Red: Middle estuary and Green: Lower estuary). The codes of the species and environmental variables are the same as in Table 1.

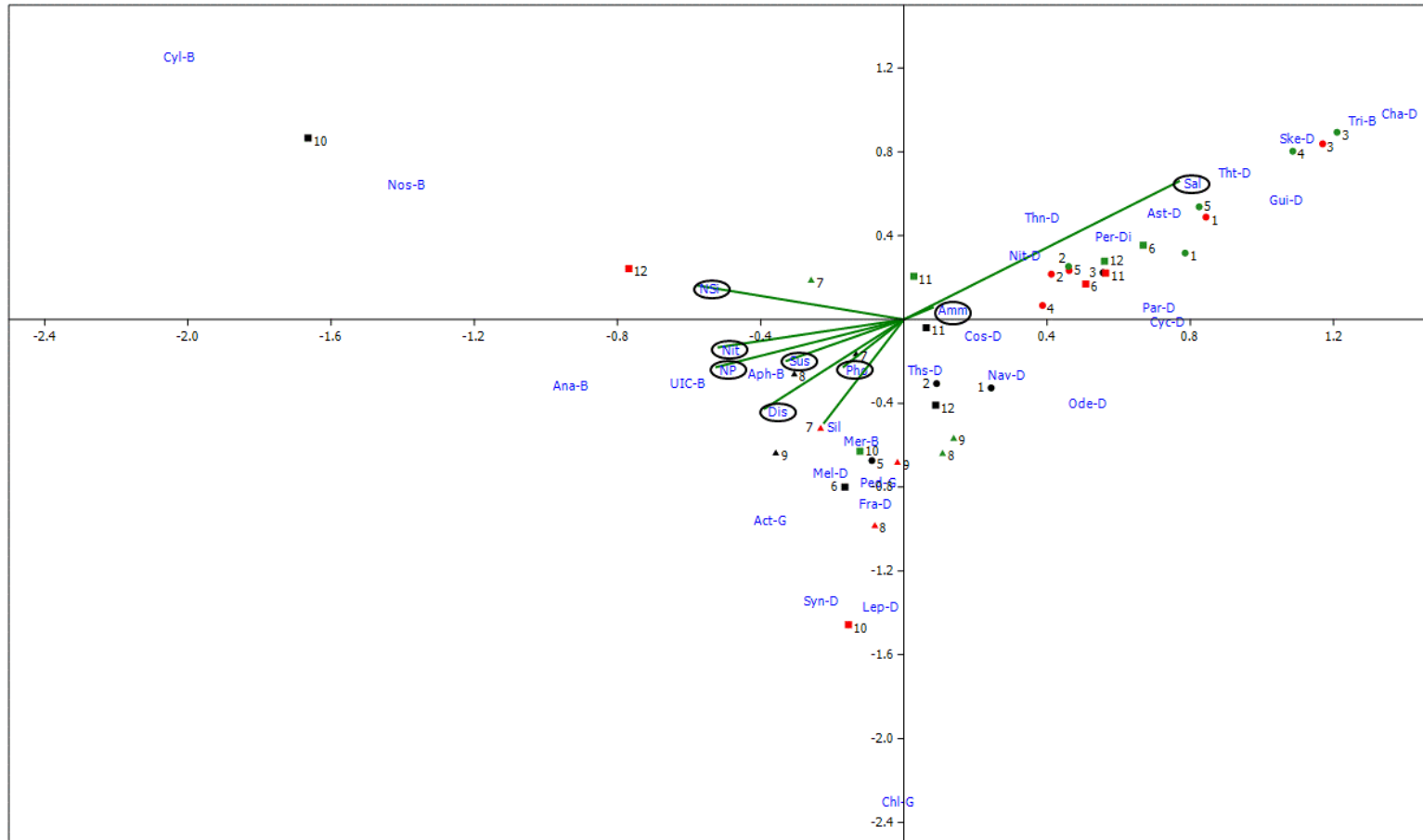


Figure 7: (a) Diversity index, (b) dominance index, (c) Evenness index and (d) Richness index during different discharge period in the Godavari estuary.

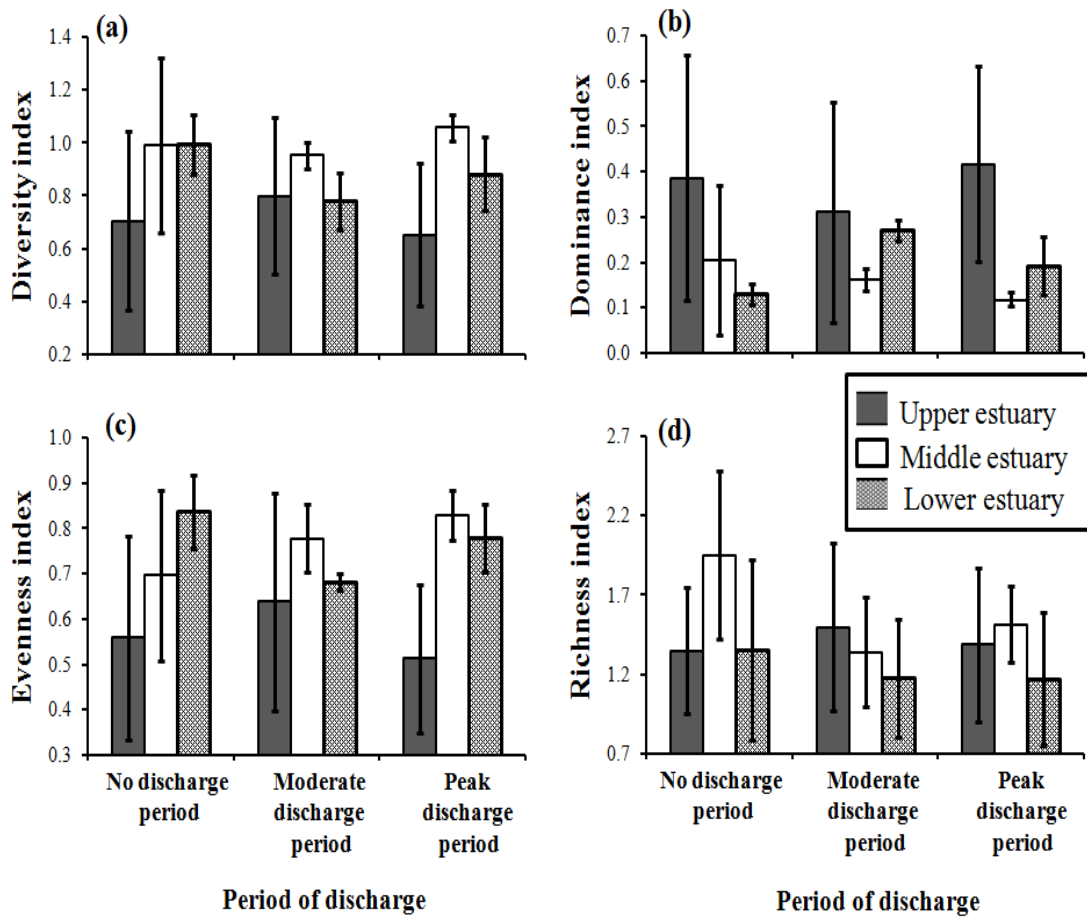


Figure 8: (a) Dendrogram showing similarity of monthly variation in phytoplankton abundance at upper (top panel), middle (middle panel) and lower estuaries (bottom panel). (b) Dendrogram showing similarity in the entire estuary in phytoplankton abundance. In this U,M and L represents upper, middle and lower estuaries respectively. Whereas ND, MD and PD represent no discharge, moderate discharge and peak discharge periods.

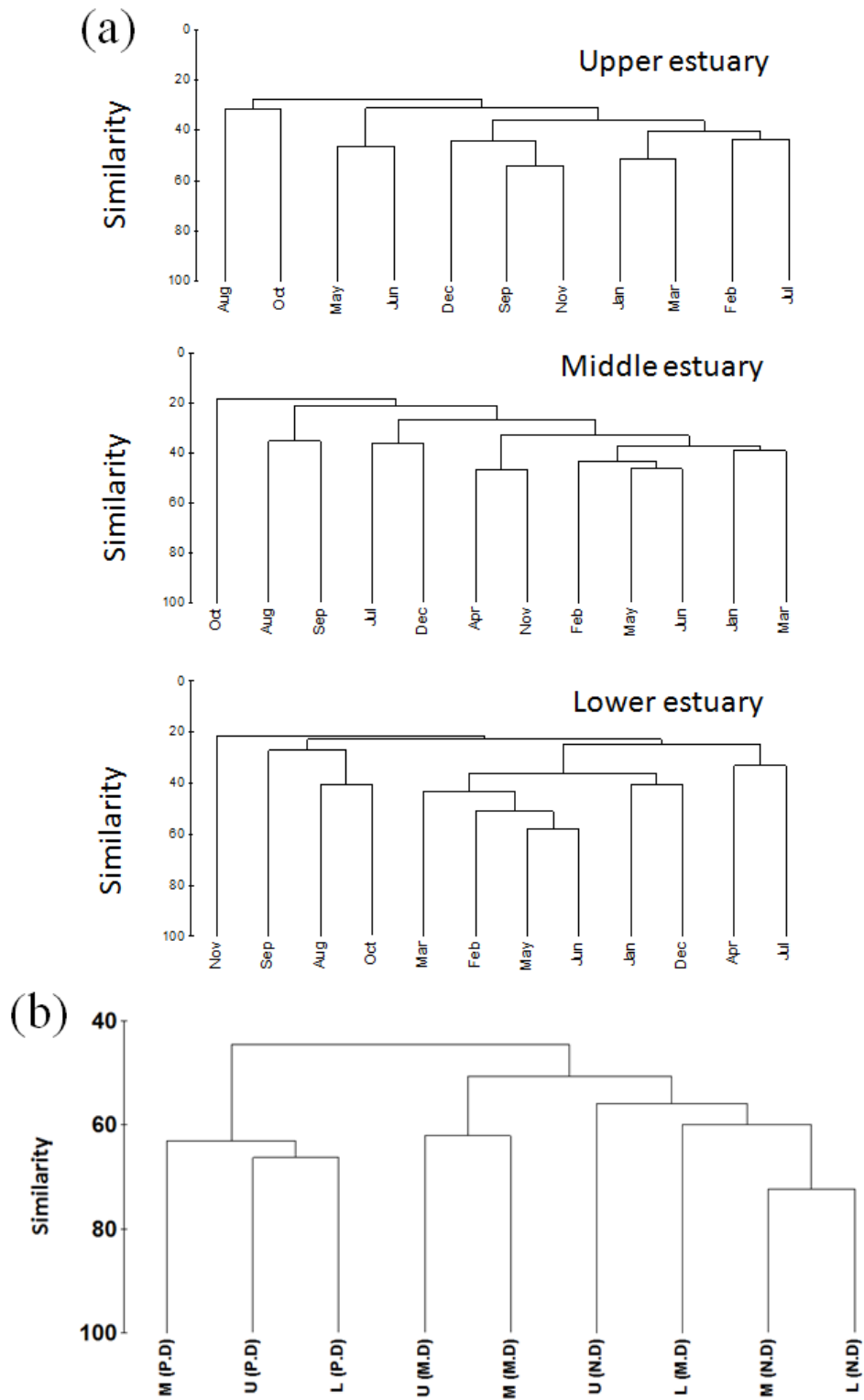


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