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Relationship between N : P : Si ratio and phytoplankton community composition in a tropical estuarine mangrove ecosystem

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Abstract

The present work aims at understanding the importance of Brzezinski–Redfield ratio (modified Redfield ratio) as a determinant of natural phytoplankton community composition in a mangrove ecosystem. Even though this ecoregion has been reported to be mostly eutrophic, localised and anthropogenic influences often result in habitat variability especially with regard to nutrient concentrations at different parts of this ecosystem. Phytoplankton, an important sentinel in aquatic ecosystems may respond differently to such alterations in habitat thereby bringing about significant changes in the community composition. Results show that even though habitat variability does exist at our study area and varied on a spatial and temporal scale, the nutrient concentrations were intricately balanced that never became limited and complemented well with the concept of modified Redfield ratio. However, an integrative approach to study phytoplankton community involving microscopy and *rbc*L clone library and sequencing approach revealed that it was the functional traits of individual phytoplankton taxa that determined the phytoplankton community composition rather than the nutrient concentrations of the study area. Hence we conclude that the recent concept of functional traits and elemental stoichiometry does not remain restricted to controlled environment of experimental studies only but occur in natural mangrove habitat.

1 Introduction

Redfield was of the opinion that elemental compositions of phytoplankton were statistically uniform and variations in inorganic C : N : P ratios were primarily due to synthesis or decomposition of organic matter (1958). However, as different concepts of nutrient requirements and utilization by phytoplankton came into fray (Monod and Droop's model (Monod and Droop, 1968, 1983); resource-ratio theory (Tilman, 1982); variable-internal-stores model (Grover, 1991); the intermediate disturbance hypothesis (Sommer, 1995) the restrictive elemental theory of Redfield faced contradictions among biologists. Falkowski (2000) suggested that the changes in C : N : P stoichiometry from the proposed Redfield ratio of 106 : 16 : 1 was critical in understanding the role of phytoplankton in biogeochemistry. Thus, N : P ratio of 16 : 1 became a benchmark to differentiate N-limitation and P-limitation and was considered as a reference point for the upper limit of N : P in oceans (Falkowski, 1997; Tyrrell, 1999; Lenton and Watson, 2000). Increase in anthropogenic influences is expected to affect physical and chemical processes in aquatic ecosystems with a consequential effect on water biogeochemistry (Solomon et al., 2007; van de Waal et al., 2010). Thus, this rigid Redfield hypothesis for N : P ratio was challenged both with regard to elemental composition of phytoplankton and conditions of nutrient limitation (Broecker and Henderson, 1998). So it is possibly the plasticity of intrinsic elemental composition in phytoplankton and a complex balance between several biological processes including nitrogen fixation and denitrification that regulates N : P ratio in natural waters (Redfield, 1958; Falkowski, 2000).

In the past decades, fertilizer use and combustion of fossil fuel has significantly increased global nitrogen (N) pollution, especially in coastal aquatic habitats (Galloway et al., 2004). Internationally recognised organizations like the Ecological Society of America (Vitousek et al., 1997) and the Coastal Marine Team of the National Climate Change Assessment (Boesch et al., 2002; Scavia et al., 2002) have reported N pollution as one of the greatest consequences of human accelerated global change on the coastal ecosystems. Such increases in N loading can lead to eutrophication, a condition that would have both ecological and societal implications particularly with regard to fish and shellfish production, recreation and waste assimilation (Costanza et al., 1997) and eventually will affect ecosystem functioning (Nixon, 1995; Howarth et al., 2000; NRC, 2000). However, consensus of N loadings as the primary cause of eutrophication has often been debated and like lake ecosystems, phosphorus is also opined to regulate eutrophication and primary production in coastal areas and estuaries (Hecky and Kilham, 1988; Hecky, 1998; Hellstrom, 1998). To tide over this debate, Jaworski et al. (1972) suggested that the drivers in lake and estuarine ecosystems were different and hence separate parameters should be followed while working on estuarine and coastal environments. Interestingly, in that year, there were documentations of coastal eutrophication primarily brought about by N loadings due to increased human activity (Boesch, 2000). However, in the later part of 1990s, several mesocosm based experiment approaches and in situ studies established that it was total N rather than total P that primarily controlled eutrophication in estuarine systems.

Another important nutrient in natural aquatic ecosystems is silicate which is the primary constituent of diatoms, a major constituent of natural phytoplankton assemblages. Even though N loadings and P concentrations due to anthropogenic activity and detergent usage have increased, silicate levels have remained steadier through times (Gilpin et al., 2004). The N : Si ratio being main factor for diatom growth, increasing inorganic N : Si ratios may be due to Si limitation that may affect diatom growth. The relative cell counts and biomass of specific diatom taxon may get influenced as nutrient status alternates from N limitation to Si limitation (Davidson and Gurney, 1999). Thus, instead of traditional Redfield ratio of N : P as 16 : 1, modified Redfield ratio of N : P : Si as 16 : 1 : 15 (Brzezinski, 1985) is often used as a standard to understand nutrient limitation with respect to nitrogen, phosphorus or silicate for natural phytoplankton assemblages.

Mangrove ecosytems around South East Asia are largely eutrophic with high N loadings as most of these ecoregions border coastal areas (Talane-McManus et al., 2001). These habitats at the interface of land and ocean experiences changes in mixing conditions that would possibly alter physical and chemical properties of the habitat as well. Like other mangroves around the world, the Sundarbans mangrove ecoregion at the coastal fringes of West Bengal (India) and Bangladesh is affected by both the freshwater riverine influx of the Ganga–Brahmaputra–Meghna River system and marine water from the Bay of Bengal. Along the banks of these riverine systems, large human settlements and related anthropogenic factors contribute to high N loadings. Even in the early 1970s, it was reported that the Ganga–Brahmaputra River system is more polluted by human sewage and cattle waste than by industrial wastes, which would suggest higher N and P loadings (Basu et al., 1970; Gopalakrishnan et al., 1973). Different works have established this area to be largely eutrophic particularly with regard to nutrient concentrations that entails the Sundarbans mangrove area to be highly productive (Manna et al., 2010; Biswas et al., 2004). Thus, existence of mangrove ecosystem in the estuarine phase of tropical rivers can be a source as well as sedimentary sink for nutrients (Gonneea et al., 2004). So it becomes evident that the coastal area of West Bengal especially the Sundarbans mangrove region experiences huge N loadings especially from anthropogenic sources as well as from agricultural runoffs. Phytoplankton being an important sentinel to observe effects of multiple stressors is expected to be affected by this huge nutrient loadings and corresponding changes in the habitat. Even though the eutrophic status of the Sundarbans ecoregion is well reported, yet a specific effort to understand community composition of natural phytoplankton population as response to variations in Brzezinski–Redfield ratio of the habitat is not well documented.

Hence, the primary objective of this work was to determine whether the habitat of the study area reaches nutrient limited condition with respect to either of nitrogen, phosphorus or silicate. This would possibly provide us with information on whether modified Redfield ratio as an important driving factor for phytoplankton community composition also holds true for this apparently eutrophic mangrove ecoregion. In the present work we also tried to understand as to how phytoplankton community composition might shift under variable status of modified Redfield ratio under natural conditions. However, the change in community composition may not be only due to alterations in modified Redfield ratio but other physical and chemical parameters as well. Thus, this work will also allow us to envisage whether changes in a single stressor or a combination of stressors contribute to the changes in phytoplankton community composition in a mangrove dominated estuary.

2 Method

2.1 Study area

The study area was located in the south eastern part of Sagar Island, the largest island of Indian Sundarbans surrounded by River Hooghly in the north and west, Mooriganga estuary in the east and Bay of Bengal in south. The study area was selected at the confluence of a tidal creek (Chemaguri creek) and estuary (Mooriganga estuary) with closest proximity to the Bay of Bengal. Since the creek station [Station 1: 21°40′44.4″ N, 88°08′49.5″ E (Stn. 1)] opens into the estuary, the influence of freshwater will be more as compared to the estuarine station [Station 3: 21°40′40.6″ N, 88°09′19.2″ E (Stn. 3)] where influence of marine water will be more pronounced (Fig. 1). Thus, our study on these two stations would allow us to specifically understand the influences of freshwater and marine water on nutrient profiles and modified Redfield ratio. This work is part of a long term monitoring program that was initiated in 2010 at the Sundarbans (SBOTS – Sundarbans Biological Observatory Time Series) and continues till date.

2.2 Sample collection

Samplings were done onboard a motorised boat from February 2013 to January 2014 from both stations at bi weekly intervals. However, from June to September 2013, sampling efforts were reduced to monthly intervals due to inclement weather conditions caused by very high seasonal precipitation. Sample collections were restricted to surface waters because the euphotic depth in this area of Sundarbans largely tends to be less than 1 m. Abiotic variables like pH (pH meter, Eco testr), air and water temperature (Celsius thermometer), salinity (refractometer, ERMA, Tokyo), dissolved oxygen (DO meter, Eutech) and Secchi depth (Secchi disc) were measured by hand held instruments. In situ Secchi depth data were used to calculate Light Attenuation Coefficient of the habitat (K_t) (Holmes, 1970). Suspended Particulate Matter (SPM load) in this area and was measured under lab conditions (Harrison et al., 1997).

Samples for nutrient analysis were collected separately in 125 mL HDPE amber bottles and fixed with neutral formalin immediately after collection to a final concentration of 4 % (*v*/*v*). The fixation was mainly done to reduce photochemical microbial activity on Dissolved Organic Matter (DOM) that may result in release of Dissolved Organic Nitrogen (DON) from humic acid, a major constituent of DOM (Dell' Anno et al., 1999). Precautions were taken to minimise any photo-oxidation of the samples by keeping the samples in specifically designed dark boxes during transportation. Samples for phytoplankton cell counts were collected in 125 mL amber coloured bottles and fixed with 4 % formalin.

The sample collection for molecular work was restricted to the estuarine station because this station was located at the confluence of creek and estuary with pronounced marine water inflow. Since phytoplankton population at this area was dominated by diatoms (Bhattacharjee et al., 2013; Samanta and Bhadury, 2014), the phytoplankton diversity is expected to be more at the estuarine station as chromophytic algae (diatoms) mostly tend to be euryhaline. Moreover, since the phytoplankton population has already been worked out extensively (Samanta and Bhadury, 2014), molecular work was much restricted and focussed mainly in understanding whether the basic phytoplankton population has undergone any major shift on a temporal and inter annual scale. Thus, to keep a continuation with previous works, sample collections for molecular work were restricted to 18 October 2012 (post monsoon), 22 March 2013 (pre monsoon) and 1 August 2013 (monsoon). During sample collection for molecular work, 2 L surface water from the estuarine station was collected separately and kept without fixation.

2.3 Nutrient analysis

On transportation to laboratory, the water samples for nutrient analysis were filtered through 0.45 µ nitrocellulose filter paper (Rankem) within 24 h of collection to minimise microbial oxidation. Dissolved nitrate (Finch et al., 1998), ortho-phosphate (Strickland and Parsons, 1972) silicate (Turner et al., 1998) and ammonia (Liddicoat et al., 1975) concentrations were measured spectrophotometrically in a UV-Vis spectrophotometer (U2900, Hitachi Corporation). The concentration of each nutrient was calculated from standard curves. The molar concentrations of each nutrient were used to determine molar ratios for N : P, Si : P, N : Si and were extrapolated for N : P : Si ratio (Redfield–Brzezinski ratio).

2.4 Microscopic study of phytoplankton population

In the laboratory, samples for microscopic enumeration and cell counts were gravity settled (24 h) and phytoplankton cell counts were performed by drop count method in triplicates (Verlancer and Desai, 2004). Cell count data were extrapolated to 1000 mL both for total phytoplankton as well as for individual species. Phytoplankton genera were identified using different monographs and they belonged to three main groups, i.e., diatoms, dinoflagellates and green algae (Desikachary, 1959, 1987; Tomas, 1997). Analysis of phytoplankton data were done on the basis of percentage contribution, species diversity index (H') (as relative abundance of species dependence function), and species evenness (J) (as function of equality degree in genera abundance).

2.5 Determination of cellular biovolumes and carbon content

Cellular biovolumes and surface area were determined for the dominant phytoplankton taxa following the specific geometric shapes and formulae proposed by Hillebrand et al. (1999). Subsequently, cellular carbon contents were deduced following Menden Deuer and Lessard (2000). Unlike cell count data, for biovolume estimation live specimen were taken into consideration as application of preservative often result in shrinkage of cell dimensions, that would lead to false estimation of cellular biovolumes (Wetzel and Likens, 1991). Cell dimensions for individual taxon were noted to calculate the biovolume and for each individual taxon based on published literatures (Hillebrand et al., 1999). Previous works have reported that conversions of linear datasets to cellular biovolumes have largely remained limited as determination of third dimension is often assumptive. Keeping in view the MacDonald–Pfitzer diminutive hypothesis (MacDonald, 1869; Pfitzer, 1869), measurements of at least five individual cells for each taxon were recorded and mean values were considered for each dimension to determine the biovolumes. Cellular biovolumes were converted for cellular carbon content to understand the seasonal and spatial patterns (Menden Deuer and Lessard, 2000) that may possibly occur due to changes in nutrient status of the habitat.

2.6 Statistical analysis

Principal Component Analyses (PCA) were performed to statistically establish habitat variability of both the stations (creek (Station 1) and estuarine station (Station 3)) by considering environmental parameters as variables (STATISTICA 7.0). The datasets for each variable were $\log(x+1)$ transformed to reduce the scale variability and were used as the input data. Similar PCA plot of the cases (months) were performed to understand if any seasonal pattern was present with respect to diversity of habitat. In order to envisage the relationship between phytoplankton and environment, the datasets were condensed into two matrices, one representing the species abundance for each site and the other one for environmental variables. A Canonical Correspondence Analysis (CCA) was applied for each station so as to represent not only the species composition variation pattern but also the relationships between species and each environmental variable for each of the creek (Station 1) and estuarine stations (Station 3) (Ter Braak and Prentice, 1988). Biotic variables were represented by abundances of individual phytoplankton taxa determined from microscopic analysis. Environmental variables included physical parameters like air temperature (AT), water temperature (WT), pH, salinity, tide, light attenuation coefficient (LA Coeff), SPM load (SPM) and chemical parameters like nitrate, phosphate, silicate, ammonia, dissolved oxygen (DO) along with molar ratio of nutrients (DIN–DIP, DIN–DSi, DSi–DIP). Like PCA, all variables were $\log(x+1)$ transformed and the significances of CCAs were analysed by running Monte-Carlo tests on 499 permutations under a reduced model ($P < 0.05$). The first two significant canonical roots were used to develop the diagram using CanoDraw. The canonical factor loadings show the correlation between variables and determine the importance of each environmental species in determining the phytoplankton (numbers in CCA plots represent individual species) variability of the study area.

2.7 Environmental DNA extraction and PCR amplification of *rbc*L gene fragment

On transportation to laboratory, 2 L water sample specifically collected for molecular study was filtered through 0.45 µm nitrocellulose filter paper (47 mm diameter; Rankem) using a vacuum pump. The filters were subsequently stored at −20 °C until DNA extraction. Environmental DNA extraction from filters followed by PCR amplification of *rbc*L gene fragments and subsequent steps were followed as detailed previously by Samanta and Bhadury (2014).

2.8 Cloning and sequencing

After purification, the PCR products were cloned using pGEM-T Easy vector system (Promega, Madison, WI, USA) following manufacturer's instructions. Plasmid DNA containing inserts was sequenced with SP6 primer in an ABI Prism 3130 Genetic Analyzer based on BigDye Terminator chemistry. The clone library abbreviations used for 18 October 2012, 22 March 2013 and 1 August 2013 are Stn3_Oct_12_, Stn 3_Mar13_and Stn3_Aug13_ respectively.

2.9 Sequence analysis and molecular phylogeny

Chromatograms were manually checked in BioEdit v7.0 (Carlsbad, CA, USA) for any ambiguity or error before undertaking further downstream analysis. The DNA sequences were subsequently translated into amino acid sequences by the online Transeq software (http://www.ebi.ac.uk/emboss/transeq) and then compared with published *rbc*L sequences from GenBank, EMBL, DDBJ, and PDB using blastp tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Uncultured environmental and cultured phytoplankton *rbc*L amino acid sequences that overlapped with the sequences generated in this study based on blastp validation (only top ten blastp hits were included) were aligned using Clustal Omega (Dublin, Ireland). The alignment file generated was manually checked in Seaview v4.0 for any error or ambiguity. On verification, a phylogenetic tree was constructed using Neighbor-joining method in MEGA version 6 (Saitou and Nei, 1987; Tamura et al., 2011). Bootstrap test (1000 replicates) was performed to get the best topology of consensus tree with the value $> 50\,\%$ significant branching (Felsenstein, 1985). The sequences generated as part of this study have been submitted to GenBank and their accession numbers are from KJ720820–KJ720885.

3 Results

3.1 Hydrological features of the habitat

The general environmental and hydrological properties of the study area were typical for tropical estuarine area where the entire sampling period was categorised as pre monsoon (February–June 2013), monsoon (July–October 2013) and post monsoon (November 2013–January 2014). Spatial differences in water temperature were observed with the mean water temperature of the estuarine station ($29.39 \pm 4.28\,^{\circ}$C) being higher than the creek station ($28.42 \pm 3.23\,^{\circ}$C) (Fig. 1a). The pH largely remained around 8, which decreased slightly during monsoon due to precipitation events (Fig. 1b). In contrast, mean salinity at the estuarine station (15.71 ± 7.32) was higher as compared to the creek station (11.67 ± 7.23) with drastic fluctuations between pre monsoon, monsoon and post monsoon (Fig. 1c). Fluctuations in SPM load and Light Attenuation Coefficient (K_t) were similar, suggesting their interdependence in decreasing the euphotic depth of the habitat. Dissolved Oxygen (DO) levels gradually decreased from pre monsoon to monsoon that again began to increase with the advent of post monsoon (Table 1).

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3.2 Distribution of nutrients and habitat variability

Dissolved Inorganic Nitrogen (DIN) was constituted by both nitrate and ammonia as these are the two assimilatory forms of nitrogen that are mainly utilised by phytoplankton populations. At the estuarine station, nitrate concentrations showed an irregular pattern with maximum values during peak monsoon period (July–September 2013) that gradually receded during early post monsoon (October–November 2013). However, there was a gradual increasing trend in the subsequent late post monsoon period (December 2013–January 2014) (Fig. 2a). Ammonia concentrations largely remained low and sometimes reached below detection limits (Fig. 2b). Phosphate concentrations were much lower (1–7.9 µM) compared to nitrate (30.88–65.08 µM) and silicate (6.22–44.75 µM) although it never reached below detection limits. Phosphate concentrations were maximum in pre monsoon (May–June 2013) which did not fluctuate significantly in the subsequent monsoon and post monsoon periods (Fig. 2c). Unlike both nitrate and phosphate, silicate concentrations began to increase from monsoon period (August 2013) and continued to increase during post monsoon with almost a 7-fold increase in concentration (6.22 µM (July 2013)–44.75 µM (January 2014)) (Fig. 2d). Thus, variations in nutrient concentrations were independent of each other and showed different temporal patterns. The profiles of all the nutrients showed similar temporal patterns at the creek station as well with slight variations. The annual mean nitrate level at creek station was lower (45.31 µM) as compared to the estuarine station (47.07 µM) and were similar for phosphate concentrations. In contrast, mean annual silicate concentration at creek station (21.13 µM) was comparatively higher to that of the estuarine station (19.39 µM). However, these differences were statistically not significant and hence it suggests that although temporal variations were evident, spatial differences in habitat were less pronounced. The N : P ratio ranged from 9.38 (November 2013) to 33.23 (February 2013) at the creek station, which largely remained below the proposed Redfield ratio of 16 : 1 (Fig. 3a). This pattern persisted in the estuarine station as well where the N : P ratio was relatively higher than the creek station and ranged from 6.93

(April 2013) to 35.65 (February 2013) (Fig. 3b). On the other hand, variations in N : Si ratio at the creek station was less pronounced (0.95 (December 2013)–9.8 (February 2013)) (Fig. 3a) as compared to the estuarine station [1.18 (January 2014)–14.03 (July 2013)] (Fig. 3b).

Seasonally, nutrient concentrations for both nitrate and phosphate were found to be maximum during monsoon, although highest silicate concentrations were recorded during post monsoon period. As mentioned earlier, ammonia represents a key component of the dissolved inorganic nitrogen pool. However, unlike other nutrients ammonia was not detected during most of the sampling period, suggesting the apparent absence or very low levels of ammonia concentrations. Thus, nitrate primarily accounted for the bulk of inorganic nitrogen in our study area. Seasonal nitrate level between stations largely remained same whereas ammonia levels at the creek station was twice to that of the estuarine station in pre monsoon and vice versa during post monsoon (Fig. 2b). Even though ammonia levels fluctuated between stations, low concentrations of the same did not affect the total dissolved inorganic nitrogen pool of the habitat. Accordingly, seasonal estimates of N : P ratio showed that it mostly remained below the proposed Redfield ratio of 16N : 1P, although it equalled the 16 : 1 ratio during pre monsoon at creek station (Fig. 3a). This was indicative for a weakly nutrient limited condition as per the proposed Redfield ratio. However, during this period ammonia concentrations were significantly high as compared to other seasons, which may have accounted for the high N : P ratio, rather than due to phosphate limitation. Seasonal N : P : Si ratio (Table 1) also remained well below the limited condition ratio of 16 : 1 : 15 thereby confirming that neither of the basic elements of the habitat indicated nutrient limitation.

3.3 Principal Component Analysis (PCA)

In an attempt to elucidate inter-relationship among variables of the habitat, Principal Component Analysis (PCA) was performed after considering the datasets for abiotic variables and nutrient profiles. The PCA plots were done after considering Factor 1 and Factor 2 (F1 v/s F2) that cumulatively explained 67.15 and 60.15 % of cumulative variance of the dataset at the creek station and estuarine station respectively (Supplement data I). At the creek station, pH, tidal status and ammonia with high positive factor loadings clustered together with salinity and dissolved oxygen (DO) which had positive loadings along F1 but negative loading along F2 (Fig. 4a). This suggests the apparent inter dependence of these variables with similar temporal trends. However, the lengths of vector indicate that even though tidal effects were correlated with pH, salinity, DO and ammonia levels, it was not the sole factor responsible for bringing out temporal alterations of these variables. In contrast, water temperature (WT) and air temperature (AT) grouped together very closely with high factor loadings. This would mean that water temperature was solely regulated by air temperature. On the other hand, nitrate, phosphate and dissolved inorganic nitrogen (DIN) all grouped together in the opposite quadrat to that of tidal status, indicative of freshwater sources as the major factor that determined the temporal trends of these parameters. Finally, SPM load, Light attenuation Coefficient and Silicate concentrations grouped together in a different quadrat with negative loadings for F1 but positive loadings with F2. The close proximity and length of the vectors suggest that they were significantly interdependent on each other for the temporal variations in the fluctuation patterns during the entire sampling period.

Similarly, at the estuarine station grouping of abiotic variables largely remained similar to that of creek station although they clustered in different quadrats of the PCA plot (Fig. 4b). Thus, as expected both air and water temperature grouped together ammonia and tidal status tended to group together with intermediate factor loadings and vector lengths that suggested tides as an important component in determining the temporal patterns of ammonia concentrations. The nutrient pattern (nitrate, phosphate, DIN) largely remained similar with that of the creek station aligning in a diagonally opposite quadrat to that of tidal status. This also held true with regard to silicate, SPM load and Light Attenuation Coefficient.

Projection of the cases for these PCA plots (February 2013–January 2014) clearly allowed us to demarcate seasonal habitat diversification at both the creek and estuarine stations. As evident from Fig. 5a, months representing monsoon and post monsoon periods clearly separated out in the PCA plot. However, during late pre monsoon (April–June 2013) and early monsoon (July 2013), the representative months overlapped. This would indicate towards seasonal transitions when temperature variations were not very significant. Similarly, at the estuarine station the seasonal separation for habitat heterogeneity was more prominent as compared to the creek station (Fig. 5b).

3.4 Phytoplankton community composition

The phytoplankton community was composed mainly of diatoms with intermittent occurrence of green algae (Chlorophyceae) and dinoflagellates (Dinophyceae). Thus, the entire phytoplankton community was composed of forty six species of diatoms belonging to twenty seven families and seventeen orders. The green algal population was represented by two species representing Hydrodictyaceae that comes under the order Sphaeropleales. The dinoflagellate taxa belonged to three different orders, each being the type specimen for the representative family (Table 2). Among diatoms, Naviculales was maximally presented with eight species belonging to six different families. The second largest representative order was Bacillariales with seven different species belonging to the family Bacillariaceae. The other dominant order was Thalassiosirales with six different genera belonging to three distinct families viz. Stephanodiscaceae, Thalassiosiraceae and Skeletonemataceae (Table 2).

Analyses of the temporal trend in phytoplankton functional groups showed that at the creek station the bulk of the diatom population was largely constituted by pennate diatoms with a gradual increase of centric species during post monsoon (Fig. 6a and b). Interestingly, dinoflagellate population remained restricted to the estuarine station and was consistently present during pre monsoon with a gradual increasing trend of centric diatom population (Fig. 6b). Seasonally, centric species at estuarine station was more abundant during pre monsoon, although with seasonal progression pennate taxa began to flourish at the estuarine station (Fig. 6b).

Estimates of biotic indices revealed that phytoplankton species diversity was more at Station 1 (1.039 (June 2013) to 2.90 (April 2013)) as compared to Station 3 (0.912 (December 2013) to 2.431 (February 2013)). Interestingly, at both stations diversity was maximum during the pre monsoon period (Table 3). Species evenness being a measure of the relative contributions of individual species to the total population, varied from 0.814 (May 2013) to 0.953 (August 2013) at Station 1 and 0.382 (November 2013) to 0.976 (May 2013) at Station 3 respectively. (Table 3). Thus, a decrease in the species evenness indicated the possibility of single species dominance which was clearly evident when species like *Thalassiosira* sp. and *Navicula* sp. accounted for about 40–50 % of the total population during post monsoon (Table 3). Results further show that the phytoplankton population at station 1 was mostly dominated by *Nitzschia* sp. (Table 3). Another dominant species in our study area was *Thalassiosira* sp. with ubiquitous presence during the entire study period from pre monsoon to post monsoon at both stations (Bhattacharjee et al., 2013). This was the most abundant species at station 3 and persistently remained the same for most part of our sampling efforts (Table 3). Monthly data from station 3 showed that in September 2013 (monsoon) *Navicula* sp. began to dominate the population and made up to 26.9 % of the population. In the subsequent months, this trend persisted and accounted for 53.68 % of the population in November 2013. The significant presence of *Navicula* sp. was also recorded in December 2013 although it was not the most abundant species and made up to 23.22 % of the total phytoplankton population. Thus, based upon cell count data, four major taxa (*Thalassiosira* sp., *Navicula* sp., *Nitzschia* sp., *Skeletonema costatum*) were identified as the primary contributors to the total phytoplankton abundance at our study area.

3.5 Analysis of eukaryotic phytoplankton community by *rbc*L clone library approach

In total 66 *rbc*L clones were sequenced that showed significant identity (97–100 %) at the amino acid level with published cultured and uncultured eukaryotic *rbc*L amino acid sequences of Type ID chromophytic algal groups available in databases (Gen-

Bank/EMBL/DDBJ). Bacillariophyceae like *rbc*L sequences (> 84 % of total 64 *rbc*L type ID clones) dominated clone libraries in the present study.

In the post monsoon clone library (Stn3_Oct_12_), 28 of 30 clones (> 93 %) were Bacillariophyceae like sequences (97–100 %) whereas 1 clone (Stn3_Oct12_Clone43) showed sequence identity with Cryptophyceae like *rbc*L sequences. Another clone (Stn3_Oct12_Clone69) separated out as a divergent lineage in the phylogenetic tree and showed only 89 % sequence identity with reported *rbc*L like amino acid sequences available in databases. On the other hand, in the pre monsoon clone library (Stn 3_Mar13_), 24 out of 30 *rbc*L clones (80 %) showed sequence identity with cultured and uncultured Bacillariophyceae like *rbc*L sequences (97–100 % at amino acid level) whereas 5 clones and 1 clone showed significant identity with published *rbc*L sequences of Cryptophyceae and Haptophyceae. For the monsoon clone library (Stn3_Aug13_), even though 6 *rbc*L clones were sequenced, only 4 clones were taken into consideration for phylogenetic analyses as 2 clones showed sequence identity with published type IA/B *rbc*L uncultured marine phototrophic eukaryotic sequences. Thus, out of the 4 clones, 2 clones showed identity with Bacillariophyceae like sequences whereas other 2 showed identity with Cryptophyceae like sequences (Supplement data II).

A phylogenetic tree based on the NJ approach revealed that in case of Bacillariophyceae clade, 4 different major subclades were observed. The largest subclade consisted of 19 clones, of which 10 clones represented post monsoon (Stn3_Oct12_), 7 clones represented pre monsoon (Stn3_Mar13_) and 2 clones were representatives of monsoon samples (Stn3_Aug13_) with close phylogenetic affiliation with *Amphora montana* TCC477 (Acc. No. AGG86629) and *A. caribaea* (Acc. No. AHX02804) like *rbc*L sequences, representing the order Thalassiophysales.

The second largest subclade consisted of 16 clones representing both pre monsoon and post monsoon populations showing phylogenetic affiliation with cultured *Halamphora coffeaeformis* (Acc. No. AHX02824) like *rbc*L sequences belonging to the order Naviculales. The other major subclade consisted of 11 clones (Stn3_Oct12_, Stn3_Mar13_) that clustered with *rbc*L sequences of cultured diatoms, namely *Minutocellus polymorphus* CCMP497 (Acc. No. AEB91188), *Leyanella arenaria* R23 (Acc. No. AEP69260), *Papiliocellulus simplex* CS431 (Acc. No. AEB91250) from Cymatosirales and several uncultured *rbc*L sequences previously reported from Gulf of Mexico (West Florida Shelf), Monterey Bay (California), northern South China Sea, Daya Bay (China) as well as from Chemaguri creek and Mooriganga estuary of Indian Sundarbans.

Finally, 7 clones from both pre monsoon (Stn3_Mar13_) and post monsoon (Stn3_Oct12_) grouped together with *rbc*L sequences of cultured centric diatoms like *Thalassiosira minima* (Acc. No. ABF60355), *Discostella stelligera* (Acc. No. ABF60389), *Thalassiosira nodulolineata* (Acc. No. ABF60345), *Lithodesmium intricatum* (Acc. No. AEB91298) belonging to the order Thalassiosirales and several uncultured *rbc*L sequences previously targeted from both Chamaguri creek and Mooriganga estuary of Indian Sundarbans. Three clones (Stn3_Mar13_Clone 15, Stn3_Mar13_Clone06 and Stn3_Oct12_Clone58) separated out and clustered only with clones previously recorded from Chemaguri creek, Sundarbans suggesting that these sequences are novel in nature and possibly unique to this estuarine mangrove habitat (Supplement data II).

The second dominant clade in the phylogenetic tree was represented by 8 clones that grouped with Cryptophyceae like *rbc*L sequences. Out of 8 *rbc*L clones, 5 clones were from pre monsoon library (Stn3_Mar13_), 3 clones represented monsoon clone library (Stn3_Aug13_) whereas 1 clone was from post monsoon period (Stn3_Oct12_) showing close phylogenetic affiliation with *rbc*L sequence of *Teleaulax* sp. TUC-2 (Acc. No. BAD42424) and other uncultured *rbc*L sequences previously reported from various coastal ecosystems such as the L4 site of English Channel, Monterey Bay (California), northern South China Sea as well as from Chemaguri creek and Mooriganga estuary of Indian Sundarbans. The *rbc*L sequence of *Dinophysis fortii* also clustered in this group although this taxon does not contain type ID RuBisCO. A single clone (Stn3_Mar13_Clone64) clustered with *rbc*L sequences of *Phaeocystis globosa* (Acc. No. AFV93448), *P. pouchetii* (Acc. No. BAF80671) and uncultured *rbc*L sequences from English Channel, L4 site, Monterey Bay (California) as well as Sundarbans mangrove ecoregion that represented Haptophyceae population in this area as evident from phylogenetic tree (Supplement data II).

3.6 Variations in cellular biovolumes and C content of dominant taxa

As mentioned in the previous section, cellular biovolumes and carbon contents were determined for dominant phytoplankton taxa (*Skeletonema costatum*, *Thalassiosira* sp., *Navicula* sp. and *Nitzschia* sp.). Interestingly, spatial patterns were evident as centric diatom taxa had higher biovolumes and C content (*S. costatum*, *Thalassiosira* sp.) in estuarine station (Station 3) relative to creek station (Station 1). An opposite pattern was observed for pennate diatom taxa (*Navicula* sp. and *Nitzschia* sp.) in both the stations (Table 4). Some seasonal changes were also observed where a gradual increase in C content was recorded both the pennate taxa from pre monsoon to post monsoon through monsoon in station 1. However, cellular C content for *S. costatum*, *Thalassiosira* sp. and *Nitzschia* sp. showed a gradual decreasing trend from pre monsoon to post monsoon. Even though both spatial and temporal changes were observed, spatial variations were more pronounced. The cellular C content for *Thalassiosira* sp. changed by 40–45 % between stations, being more pronounced in *Nitzschia* sp. that altered by 4.5–62 % between the two stations on a seasonal basis. A plot between nutrient concentrations and cellular C contents of dominant phytoplankton species in the creek (*Navicula* sp., *Nitzschia* sp. and *Thalassiosira* sp.) (Fig. 7a–c) and estuarine stations (*Thalassiosira* sp. and *Nitzschia* sp.) revealed that there was an apparent increasing trend in cellular C content during periods of high N : P ratio at the estuarine station with no such general pattern at the creek station (Fig. 7d and e).

3.7 Relationship between species composition and environmental variables

CCA (Canonical Correspondence Analyses) were performed for each of the creek and estuarine stations to explain the relationship between species assemblages and selected environmental variables. Physical parameter like salinity and light attenuation coefficient along with chemical parameters like orthophosphate, silicate and molar ratio of DIN–DIP and DIN–DSi largely explained the variation in phytoplankton assemblage in both stations. However, with respect to other environmental parameters, the importance of each variable was spatially different.

In creek station (Station 1), the first two significant canonical roots explained 52.3 % of the observed variance within the dataset (Fig. 8a, Table 5). The 1st canonical root separated species (explained 33.8 % of variance) mainly on the basis of nutrient status of the habitat whereas the 2nd canonical root (18.5 % of variance) distinguished species on the basis of nutrient molar ratio (negatively) and physical parameters. Species like *Cyclotella* sp. (15), *Pinnularia* sp. (31), *Leptocylindrus* sp. (29), *Cocconeis* sp. (21), *Cymbella* sp. (28), *Gyrosigma* sp. (6) and *Triceratium* sp. (30) grouped together with nitrate and silicate as well as SPM load and Light attenuation coefficient. This would suggest that under high nutrient availability these species tend to proliferate under low light conditions brought about by increased light attenuation because of high SPM load. In the other quadrat rare species like *Bollerochea* sp. (3), *Cerataulina* sp. (4) and more abundant species like *Thalassionema* sp. (13) and *Thalassiothrix* sp. (14) showed a preference for conditions of high ammonia where molar ratio of DIN–DIP and DIN–DSi seemed to play regulatory role as well. Apparently since these species had an affinity towards ammonia, the other nutrients seem to play an indirect but less important role in determining the contribution of these species to the phytoplankton community composition. The dominant species of the study area like *Thalassiosira* sp. (2), *Amphora* sp. (19), *Campylodiscus* sp. aligned very closely with temperature, suggesting the preference towards higher temperature. However, the lengths of vectors for air (AT) and water temperature (WT) indicate temperature as a less important variable in determining the phytoplankton community composition as compared to other environmental variables at the creek station. Finally, abundant species like *Nitzschia* sp. (7), *Navicula* sp. (9), *Skeletonema costatum* (24), *Bacillaria* sp. (26) along with other species clustered together in a direction opposite to pH and tide, suggesting that their abundance were more influenced by freshwater inflow and seasonal precipitation rather than marine water brought about by tidal actions.

In the estuarine station (Station 3), first two canonical roots explained 51.7 % of the cumulative variance. Unlike Station 1, here neither of the canonical roots can be considered for explaining any specific property of the habitat (Fig. 8b, Table 5). However, species specific alignments remained independent of salinity suggesting that even though it was an important property of the habitat, the species were not affected which would suggest the euryhaline nature of the phytoplankton community. There were two major groups of which the first group composed of 11 taxa that showed positive response towards condition of high silicate availability under low light conditions (high K_t) where species like *Cyclotella* sp. (6), *Thalassionema* sp. (7) and *Gyrosigma* sp. (8) similar response to that of the creek station. The other important taxa in this group were *Coscinodiscus* spp. (1, 27), *Nitzschia* spp. (3, 9), *Bacillaria* sp. (5) and the exclusively marine species (*Ditylum brightwelli* (10)) even though their alignments to environmental variables were different from the creek station. The other major cluster constituted of 10 taxa of which 4 pennate taxa namely *Navicula* sp. (14), *Fragilaria* sp. (21), *Nitzschia* sp. (23) and *N. longissima* (25) aligned with pH as was observed for Station 1, suggesting pH of habitat as the primary determinant of their abundance. Likewise, *Amphiprora* sp. (16) and *Pleurosigma* sp. (15) showed a preference towards conditions of high DIN–DSi that was similar to what was observed from CCA plot of Station 1.

If we consider CCA plots as indicative representation of species specific responses to environmental conditions, only ten species showed almost similar response at both stations. Among them all taxa other than *Cyclotella* sp. were pennate which would suggest that pennate population were restrictive in their response to environmental conditions thereby making them less adaptive to diversity and temporal changes of habitat as compared to centric phytoplankton population of our study area.

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4 Discussion

The hydrological parameters of the study area especially with respect to water temperature and salinity were typical for tropical coastal estuary with distinct seasonal patterns. The low spatial variations in salinity profiles at both stations confirm the apparent inter connection between these stations with greater influence of marine water in the estuarine habitat. In contrast, the drop in salinity and consequent “freshening up” during periods of seasonal precipitation was more evident at the creek station that implied the greater proximity of freshwater sources at creek station. Thus, with respect to salinity levels, these two habitats largely represent transitional water affected by tidal actions that cannot be segregated as distinctly freshwater or estuarine in nature. The pH was weakly alkaline which is expected for a tidally influenced estuary.

Nutrient concentrations fluctuated on a temporal scale primarily with regard to nitrate, phosphate and silicate concentrations. Such high concentrations of major nutrients apparently seem to be an important property not only for Sundarbans but for other mangrove ecosystems located across South East Asia (Talane-McManus et al., 2001). Works from different estuaries have suggested that the concentration of nutrients like nitrate and silicate are often several times higher than the receiving coastal water as was found for Amazon River (Edmond et al., 1981), Missisippi River (Dortch and Whitledge, 1992; Rabalais et al., 1996) and in the freshwater region of Chesapeake Bay (Ward and Twilley, 1986; Fisher et al., 1988). However, unlike other estuaries, ecoregions at the land ocean interface like the Sundarbans mangrove show comparatively more temporal variations due to localised influences like agricultural runoffs, non point discharges from anthropogenic sources and aquacultural farms. The influences of runoffs can be testified from the findings that DIN (nitrate and ammonia) levels were maximum during monsoon when localised runoffs were higher as compared to other seasons due to heavy rainfall (Biswas et al., 2010; Manna et al., 2010; Chaudhuri et al., 2012). Ammonia concentrations were often not detected with a gradual decreasing trend for oxygen. Previous works have shown that crenarchaeal *amo*A gene abundance is quite high in this area that therefore would possibly utilize oxygen to enzymatically convert ammonia to nitrite through nitrification (Dayal, 2013). The low availability of ammonia seems to contradict the general concept of eutrophication that is prevalent with regard to the habitat of the Sundarbans mangrove ecoregion.

Results further show that not only nutrients fluctuated on a temporal scale, but the molar ratio of nutrients like N : P (Redfield ratio), N : Si changed as well. Apparently, although the nutrient levels varied significantly on a monthly basis, seasonally the nutrient levels largely remained below the proposed Redfield ratio of 16 : 1 suggesting that neither N- nor P-limitation was prevalent at our study area. In monsoon there was a slight increase in N : P ratio which was mainly because of excess nitrogen inputs due to anthropogenic activities and non point discharges.

Multivariate analysis of abiotic variables (PCA) not only revealed the inter relationship between the variables but plot of the cases further established the existence of well demarcated seasonal patterns of the habitat at both stations. Thus, seasonal diversity of habitat at our study area was well established, a condition that further questions the general perception of the Sundarbans mangrove ecoregion to be eutrophic. Rather we would like to opine that nutrient concentrations were high at this area which may become eutrohic if nitrogen and phosphorus loadings remain unmonitored and continues to increase in recent future, primarily due to anthropogenic activity.

Analysis of the phytoplankton community revealed that it was largely composed of similar taxa at both stations, further supporting our opinion of transitional water of this area. Comparisons of microscopic observations suggest that through the years, even though the basic the phytoplankton population were similar, inter annual variations were significant in the phytoplankton community composition as revealed from some previous work from this area (Bhattacharjee et al., 2013). However, such conclusions may be premature as further cloning effort may provide us with information on cryptic phytoplankton diversity from this area as was revealed by a recent work undertaken from this area (Samanta and Bhadury, 2014). The dominance of diatom in the phytoplankton community has been well established through both microscopy and molecular approach. Observation of dinoflagellate taxa was highly restrictive and appeared only in periods when temperature and salinity profiles favoured their growth and proliferation. Thus, *Prorocentrum micans*, a bloom forming species was recorded in our sampling efforts only once on when maximum water temperature (39 °C) was recorded in the study area (5 April 2013). Sequencing efforts also showed the presence of *Phaeocystis* like *rbc*L sequences, another bloom forming haptophyte which has been reported from previous studies (Bhattacharjee et al., 2013; Samanta and Bhadury, 2014).

The *rbc*L clone library work although preliminary in nature, strongly support our findings of microscopic studies, suggesting that the eukaryotic phytoplankton population were represented by similar taxa, although temporal and inter annual variations were evident. Temporal patterns were clearly observable where a less dominant order Naviculales (Samanta and Bhadury, 2014) that made up only 4 % of the clone library in 2010–2011 started to dominate the clone libraries in 2012–2013. However, database search separated out Naviculales in the present study where some new sequences were added as *Halamphora* spp., which segregated our clones with *Amphora* like sequences under Naviculaes and Thalassiophysales, previously grouped only under Thalassiophysales (Stepanek and Kociolek, 2014). The grouping of *Halamphora* spp. and *Amphora* spp. like sequences in the same cluster indicate that the amino acid sequences specific to *rbc*L gene did not resolve Naviculales and Thlassiophysales as a separate clade. However, even though clone library work reported Thalassiophysales to dominate the phytoplankton community, very few *Amphora* spp. were observed microscopically possibly because of the small size of these taxa (9–15 µm (Krasske, 1932); 12–20 µm (Stepanek and Kociolek, 2011)). Interestingly, significant sequence identity were observed with both Mooriganga estuary and Chemaguri creek uncultured samples, a finding that further testifies our observation of transitional water at our study area. The *rbc*L sequences also showed significant identity with those reported from South China Sea and Daya Bay, China, and other habitats like the coastal and oceanic water of Monterey Bay, California and English Channel (L4 site), suggesting the ubiquitous existence of eukaryotic phytoplankton communities across a wide range of habitat. The detection of novel *rbc*L sequences as revealed by phylogeny indicates the presence of unique phytoplankton taxa that may have adapted themselves to this ecosystem. One such new species, a centric diatom *Thalassiosira sundarbana* has been recently identified and described from this ecosystem (Samanta and Bhadury, 2015).

Even though observable seasonal variability of habitat was found, it can be said that the eukaryotic phytoplankton community was more resilient to both spatial and temporal changes in response to the general water quality of the habitat. It was further revealed through sequencing efforts that inter annual changes were low in the eukaryotic phytoplankton community (Bhattacharjee et al., 2013; Samanta and Bhadury, 2014) which would indicate strong environmental filtration. In other words, the environment exerts its influence in selecting specific traits that are shared by phylogenetically related species which complements the habitat of our study area (Webb et al., 2002).

As observed in CCA, the responses of individual taxa to different variables indicate that the physiological requirement of phytoplankton population largely regulated their proliferation in a habitat, rather the habitat imparting any effect on the population. In the previous section it has been established that the habitats of either station did not vary much and were transitional water affected by tidal influences. However, there was distinct seasonal diversity of the environmental conditions of the habitat as was revealed from PCA plot of cases, although the phytoplankton community did not show such well defined seasonal patterns. Studies have shown that interactive effects between temperature and light (Novak and Brune, 1985), salinity (Cho et al., 2007), nutrient concentrations (Maddux and Jones, 1964) can shift the optimum temperature for growth thereby altering species specific responses. In both the CCA plots, even though the vector length of salinity made it an important variable of the habitat, as none of the species showed high loadings in that quadrat, we presume that the phytoplankton community was largely composed of mesohaline to euryhaline taxa and none were polyhaline in nature. This is further corroborated from the biogeographic distribution data of *rbc*L clone library work in the present study.The apparent adaptability of the phytoplankton community to salinity variations were further established from biogeographic distribution, as revealed from clone library data. Thus, in disagreement with general idea about Sundarbans, we would propose that it is the functional trait and corresponding elemental stoichiometry of phytoplankton species/groups that determined the phytoplankton community composition at our study area (Ho et al., 2003; Quigg et al., 2003; Klausmeier et al., 2004; Arrigo, 2005) rather than eutrophication and related changes in the habitat.

As a further testimony to our opinion, our observations show that there was an increase in cellular biovolume for centric taxa (*Skeletonema costatum*, *Thalassiosira* sp.) by 2.6–45 % (Table 4) at the estuarine station as compared to the creek station. This was possibly because under such conditions of fluctuating nutrient at our study stations, larger sized diatom species with increased nutrient storage capacity in vacuoles (Pahlow et al., 1997) are better adapted to this environment, a phenomenon pertaining to "luxury consumption." Moreover, the nutrient profile especially with respect to N : P ratio was intricately balanced and complied well with the theory of Redfield ratio, although at times it did reach the benchmark of 16 : 1. However, the consistencies of populations with no large and sudden change especially with regard to HAB (Harmful Algal Bloom) forming species indicate towards the adaptability to fluctuations in N : P ratio. This complements well with previous works where phytoplankton growth has been demonstrated to occur over a wide range of N : P ratios, ranging from 5 to 34 (Geider and La Roche, 2002). The wide range of environmental N : P ratios in which phytoplankton can grow is a reflection of the highly variable elemental stoichiometry of phytoplankton species/groups. Thus we would agree with the idea that the canonical Redfield N : P ratio of 16 is not a universal biochemical optimum, but instead represents an average of species-specific N : P ratios (e.g. Klausmeier et al., 2004).

5 Conclusion

In conclusion we can say that even though nutrient concentrations at our study area were high, the general perception of Sundarbans being eutrophic does not hold true at our study area. As per the Oslo–Paris convention (OSPAR, 2009), eutrophication is not only about nutrient concentrations but includes other features like bloom formation and hypoxia which was not observed in our study area. The water quality with respect to Redfield ratio and phytoplankton community do not indicate eutrophication, the persistent high N loadings due to anthropogenic and localized influences can render eutrophication of habitat in the near future. Since the recent concept of functional traits and elemental stoichiometry holds true for phytoplankton population of this area, an increase in N loadings may promote selected group of phytoplankton, thereby decreasing diversity of this area. Since this trait based approach has not been much worked out for phytoplankton in this part of the world, especially under natural conditions, we would focus in understanding how individual species isolated from this ecoregion may possibly respond to higher N loadings through experimental approach in our future work. This would possibly allow us to predict "true eutrophication" by analyzing the phytoplankton community composition of the Sundarbans mangrove ecoregion. Such predictive analyses will be helpful in implementing necessary measures to sustain and conserve the water quality and minimize the incidence of eutrophication of our study area and Sundarbans as a whole.

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Table 1. Seasonal profiles of hydrological parameters at the creek (Station 1) and estuarine (Station 3) from the study area during February 2013–January 2014.

Parameters	Seasons					
	Pre Monsoon		Monsoon		Post Monsoon	
	Station 1	Station 3	Station 1	Station 3	Station 1	Station 3
Air Temperature (°C)	34.18 ± 4.05	35.33 ± 4.29	34.19 ± 3.34	33.91 ± 2.94	26.72 ± 4.28	26.52 ± 4.23
Dissolved Oxygen (mg L^{-1})	6.74 ± 0.94	7.33 ± 1.19	4.91 ± 1.12	4.52 ± 0.86	4.93 ± 0.35	5.32 ± 0.39
Secchi Depth (m)	0.28 ± 0.03	0.35 ± 0.12	0.23 ± 0.07	0.19 ± 0.03	0.12 ± 0.01	0.11 ± 0.02
Light Attn Coeff [K_t] (m^{-1})	6.22 ± 1.43	4.13 ± 0.22	7.24 ± 2.68	7.44 ± 0.44	12.07 ± 2.4	13.09 ± 0.12
SPM Load (mg L^{-1})	215.76 ± 98.51	116.26 ± 30.14	204.9 ± 129.94	163.93 ± 81.64	396.78 ± 37.82	331.8 ± 194.85
Brzezinski–Redfield ratio (N : P : Si molar ratio)	16.03 : 1 : 3.78	13.57 : 1 : 5.63	11.68 : 1 : 2.46	15.55 : 1 : 5.09	12.36 : 1 : 10.66	12.86 : 1 : 10.89

Table 2. Taxonomic classification of phytoplankton taxa recorded from microscopic observations.

Class	Order	Family	Genus/Species	Habitat
Bacillariophyceae	Coscinodiscales	Coscinodiscaceae	*Coscinodiscus* sp.	both
		Hemidiscaceae	*C. excentricus*	both
			C. radiatus	both
			Actinocyclus sp.	estuary
	Surirellales	Surirellaceae	*Surirella* sp.	both
			Campylodiscus sp.	both
	Bacillariales	Bacillariaceae	*Nitzschia* sp.	both
			N. longissima	both
			N. sigma	creek
			Bacillaria sp.	creek
			Bacillaria paxillifer	creek
			Cylindrotheca closterium	both
			C. fusiformis	both
	Achnanthales	Cocconeidaceae	*Cocconeis* sp.	both
	Thalassiosirales	Stephanodiscaceae	*Cyclotella* sp.	both
		Thalassiosiraceae	*Cyclotella stylorum Planktoniella* sp.	estuary
		Skeletonemataceae	*Thalassiosira* sp.	estuary
			Thalassiosira pseudonana	both
			Skeletonema costatum	both
				both
	Thalassionematales	Thalassionemataceae	*Thalassionema nitzschoides*	both
			Thalassiothrix frauenfeldii	both
	Naviculales	Pleurosigmataceae	*Gyrosigma* sp.	both
		Diploneidaceae	*Pleurosigma* sp.	both
		Naviculaceae	*Diploneis* sp.	both
		Amphipleuraceae	*Navicula* sp.	both
		Stauroneidaceae	*Amphiprora* sp.	both
		Pinnulariaceae	*Amphiprora ornata*	creek
			Stauroneis sp.	both
			Pinnularia sp.	both

Table 2. Continued.

Class	Order	Family	Genus/Species	Habitat
	Lithodesmiales	Lithodesmiaceae	*Ditylum brightwelli*	estuary
	Triceratiales	Triceratiaceae	*Triceratium* sp. *Odontella* sp.	estuary estuary
	Cymbellales	Gomphonemataceae Cymbellaceae	*Gomphonema* sp. *Cymbella* sp.	both creek
	Chaetocerotales	Chaetocerotaceae	*Chaetoceros* sp.	estuary
	Thalassiophysales	Catenulaceae	*Amphora* sp.	both
	Fragilariales	Fragilariaceae	*Fragilaria* sp. *Synedra* sp.	creek creek
	Paraliales	Paraliaceae	*Paralia* sp.	estuary
	Rhizosoleniales	Rhizosoleniaceae	*Rhizosolenia* sp. *Guinardia* sp.	creek creek
	Leptocylindrales	Leptocylindraceae	*Leptocylindrus* sp.	creek
	Hemiaulales	Hemiaulaceae Bellerocheaceae	*Eucampia* sp. *Cerataulina* sp. *Bellerochea* sp.	estuary creek creek
Chlorophyceae	Sphaeropleales	Hydrodictyaceae	*Pediastrum* sp. *Pediastrum simplex*	creek creek
Dinophyceae	Prorocentrales	Prorocentraceae	*Prorocentrum micans*	estuary
	Gonyaulacales	Ceratiaceae	*Ceratium furca*	estuary
Noctilucophyceae	Noctilucales	Noctilucaceae	*Noctiluca* sp.	estuary

Table 3. Monthly variation in biotic indices (species diversity index (SDI), species evenness) and percentage contributions of dominant phytoplankton taxa to the total population.

Habitat	Months	SDI (H')	Species evenness (J)	Dominant taxa	% contribution
Creek Station (Station 1)	Feb 2013	2.388329	0.931141	*Thalassiosira* sp.	18.18
	Mar 2013	2.183669	0.941422	*Coscinodiscus* sp.	14.7
	Apr 2013	2.90061	0.952731	*Nitzschia* sp.	17.92
	May 2013	1.5823	0.813619	*Thalassiosira* sp.	28.57
	Jun 2013	1.039719	0.946393	*Nitzschia* sp.	50
	Jul 2013	1.519241	0.847905	*Nitzschia* sp.	38.46
	Aug 2013	1.320887	0.952818	*Nitzschia* sp.	37.5
	Sep 2013	1.961535	0.851884	*Nitzschia* sp.	33.33
	Oct 2013	1.827268	0.849497	*Nitzschia* sp.	32.69
	Nov 2013	1.89009	0.820856	*Nitzschia* sp.	31.15
	Dec 2013	1.805341	0.868186	*Thalassiosira* sp.	28.57
	Jan 2014	2.017607	0.876236	*Nitzschia* sp., *Cocconeis* sp.	21.88 21.88
Estuarine station (Station 3)	Feb 2013	2.431162	0.94784	*Cocconeis* sp.	16.67
	Mar 2013	2.030347	0.937659	*Thalassiosira* sp.	15.65
	Apr 2013	1.773626	0.898732	*Thalassiosira* sp.	17.11
	May 2013	2.02208	0.975546	*Thalassiosira* sp.	16
	Jun 2013	1.772132	0.95521	*Thalassiosira* sp.	20.31
	Jul 2013	1.744656	0.922132	*Thalassiosira* sp.	36.5
	Aug 2013	1.670166	0.932138	*Thalassiosira* sp.	33.33
	Sep 2013	2.006196	0.87127	*Navicula* sp.	26.9
	Oct 2013	1.992505	0.958192	*Skeletonema costatum*	23.09
	Nov 2013	0.987546	0.3825	*Navicula* sp.	53.68
	Dec 2013	0.911885	0.392518	*Thalassiosira* sp.	43.7
	Jan 2014	1.549657	0.962856	*Nitzschia* sp., *Thalassiosira* sp.	28.57 28.57

Table 4. Seasonal variation in cellular biovolume, surface area and total carbon content of dominant phytoplankton taxa recorded from both the stations.

Taxa	Shape	Seasons								
		Pre monsoon			Monsoon			Post monsoon		
		V (mm^3)	A (mm^2)	C content (pg C)	V (mm^3)	A (mm^2)	C content (pg C)	V (mm^3)	A (mm^2)	C content (pg C)
Station 1 (Chemaguri creek)										
Skeletonema sp.	Cylinder + 2 half spheres	0.00106	0.659	23729.82	0.00112	0.697	24909.27	0.001076	0.669	24045.1
Thalassiosira sp.	Cylinder	0.0000115	0. 00202	441.04	0.0000104	0.00183	403.65	0.000011	0.00193	424.1
Navicula sp.	Prism on elliptic base	0.0000381	0.0116	1267.05	0.0000406	0.0124	1340.02	0.0000441	0.0134	1441.29
Nitzschia sp.	Prism on parallelogram	0.0000016	0.000784	77.59	0.0000018	0.000872	86.08	0.0000019	0.00096	90.28
Station 3 (Mooriganga estuary)										
Skeletonema sp.	Cylinder + 2 half spheres	0.00152	1.2	32598.99	0.001154	0.910506	25574.27	0.001088	0.858	24281.2
Thalassiosira sp.	Cylinder	0.0000216	0. 00454	768.52	0.0000205	0.0043	733.93	0.0000196	0.004116	705.47
Navicula sp.	Prism on elliptic base	0.0000289	0.0068	993.23	0.0000312	0.00733	1062.55	0.0000266	0.00625	923.25
Nitzschia sp.	Prism on parallelogram	0.0000018	0.00081	125.61	0.0000022	0.00099	102.72	0.000002	0.0009	94.45

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Table 5. Eigenvalues and percentage of cumulative explained variance by temporal Canonical Correspondence Analysis (CCA) in creek (Station 1) and estuarine (Station 3) stations.

Axes	Eigenvalues		Cumulative percentage variance	
	Station 1	Station 3	Station 1	Station 3
1	0.381	0.794	33.8	34.2
2	0.208	0.408	52.3	51.7
3	0.156	0.346	66.1	66.6
4	0.111	0.256	76.0	77.6

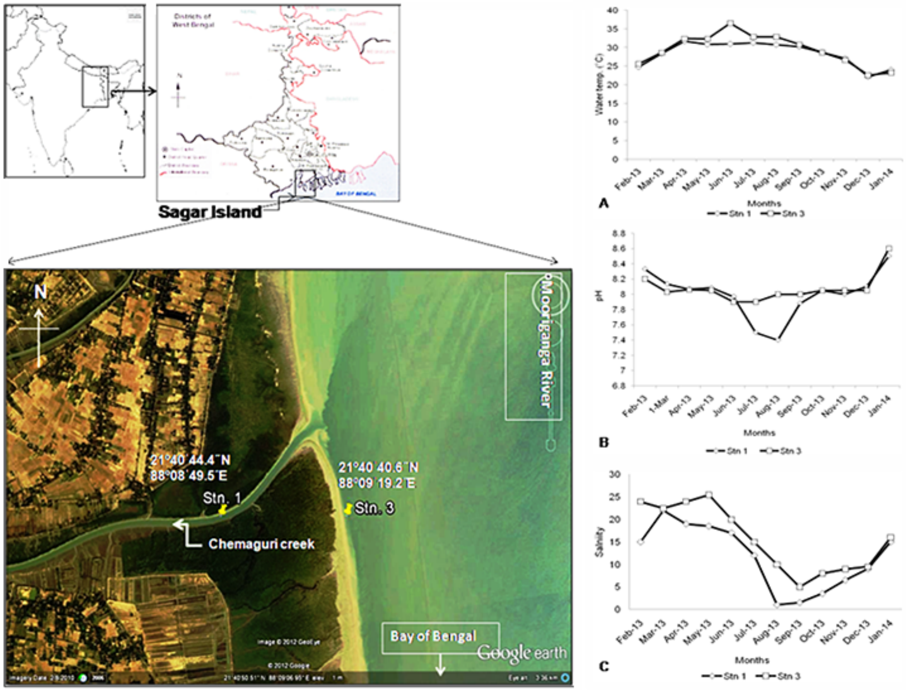

Figure 1. Map of the study area showing both the creek (Stn. 1) and estuarine (Stn. 3) stations along with monthly data of **(a)** water temperature, **(b)** pH and **(c)** salinity.

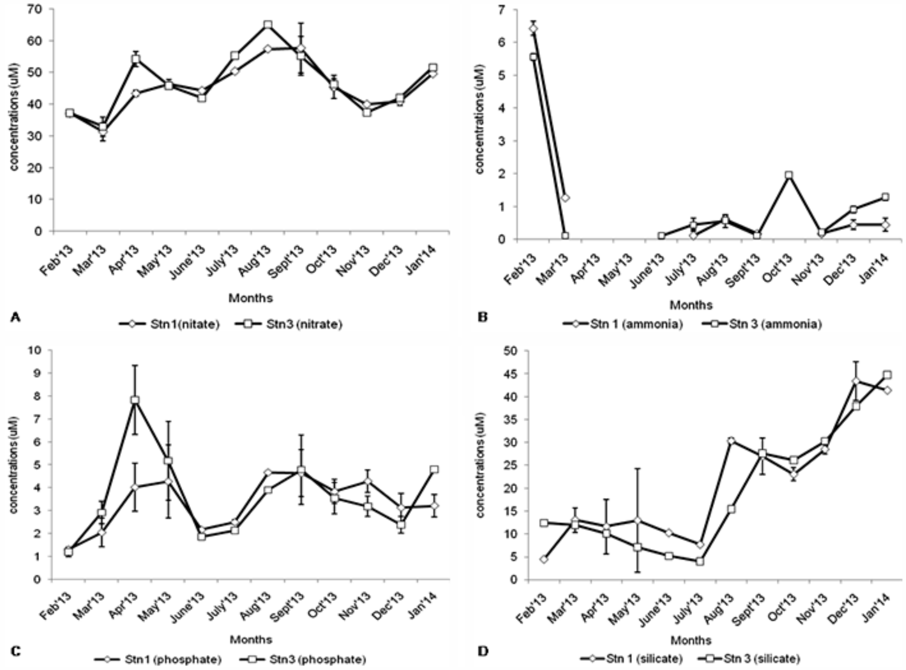

Figure 2. Monthly variations in **(a)** nitrate, **(b)** ammonia, **(c)** phosphate and **(d)** silicate concentrations at the two sampling stations.

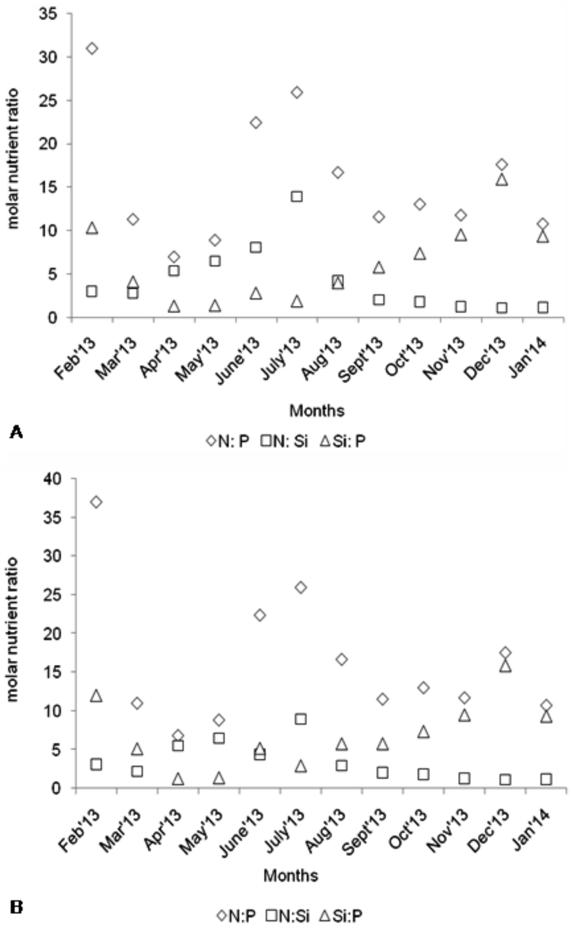

Figure 3. Monthly variations in nutrient molar ratios (N : P, Si : P, N : Si) at the creek **(a)** and estuarine **(b)** stations.

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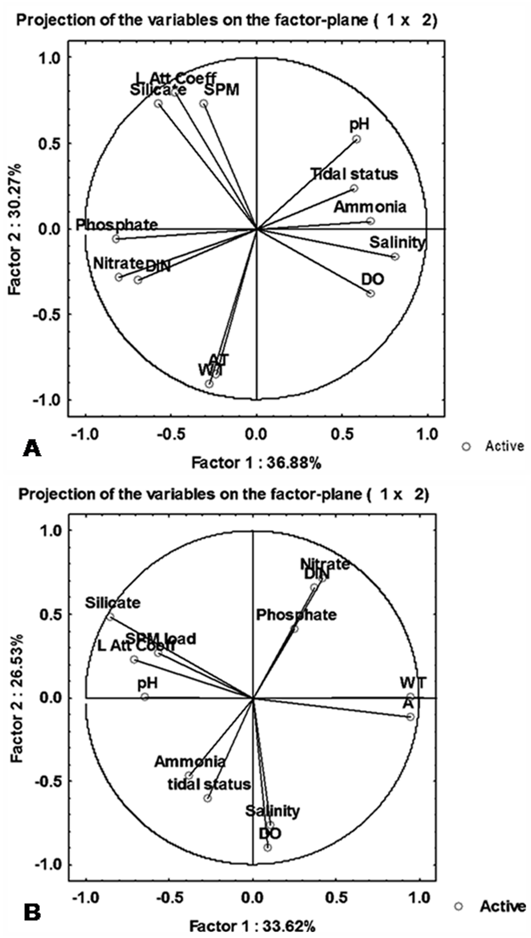

Figure 4. PCA plots between environmental variables to understand the inter relationship among abiotic variables at the creek **(a)** and estuarine **(b)** stations.

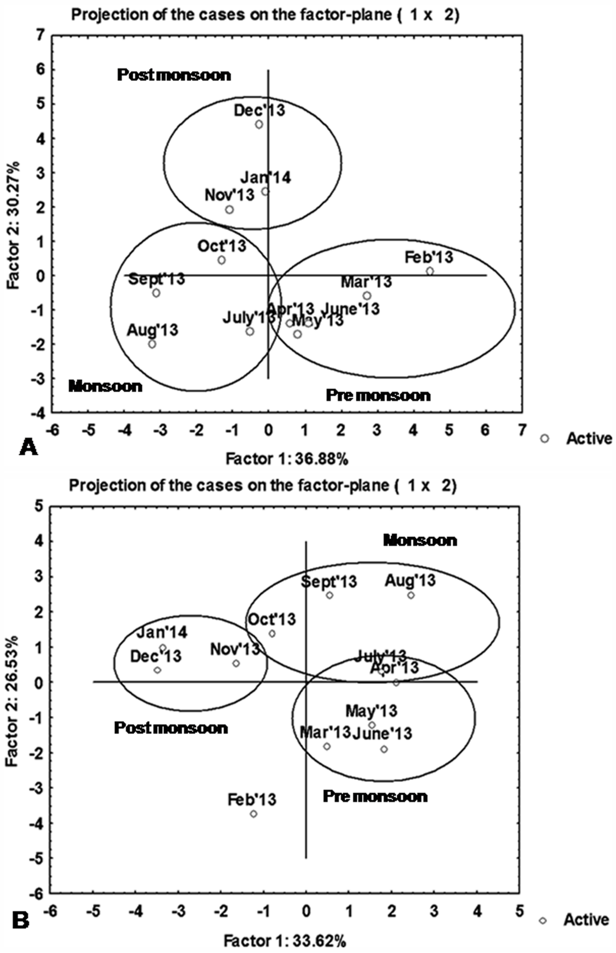

Figure 5. PCA plots of cases (months) to understand seasonal habitat variability at the creek **(a)** and estuarine **(b)** stations.

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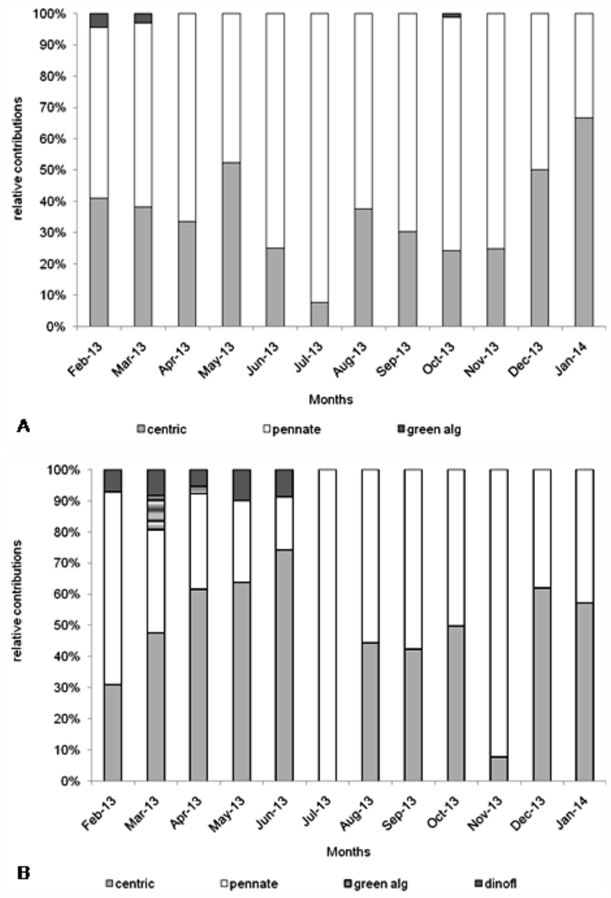

Figure 6. Monthly variations in the relative contributions of different phytoplankton classes to the total population at the creek **(a)** and estuarine **(b)** stations.

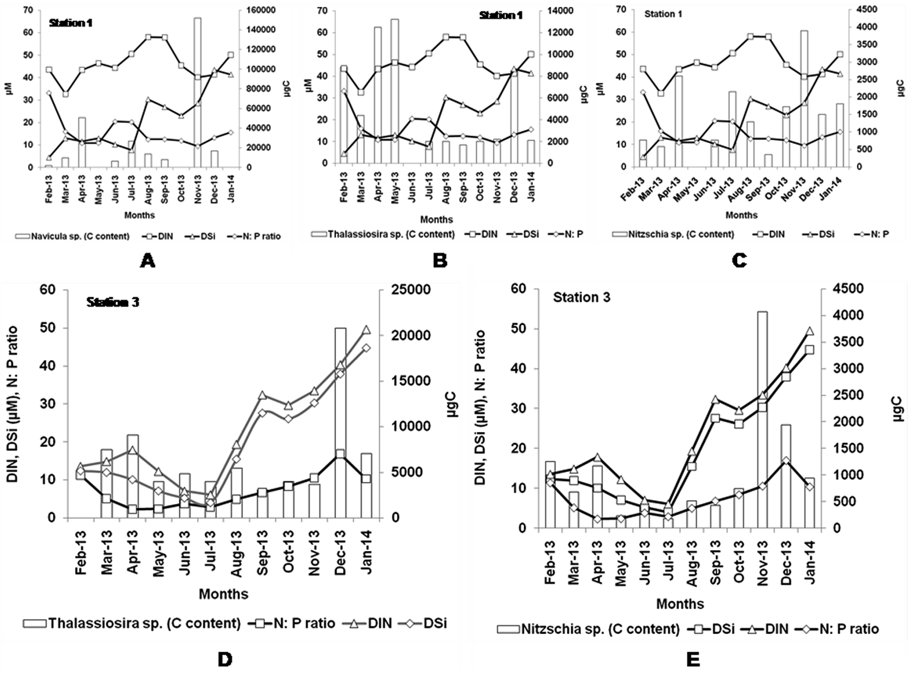

Figure 7. Monthly variations in total carbon content as responses to nutrients (DIN, DSi) and Redfield ratio (N : P) for dominant phytoplankton taxa at Station 1 **(a–c)** and Station 3 **(d, e)** respectively.

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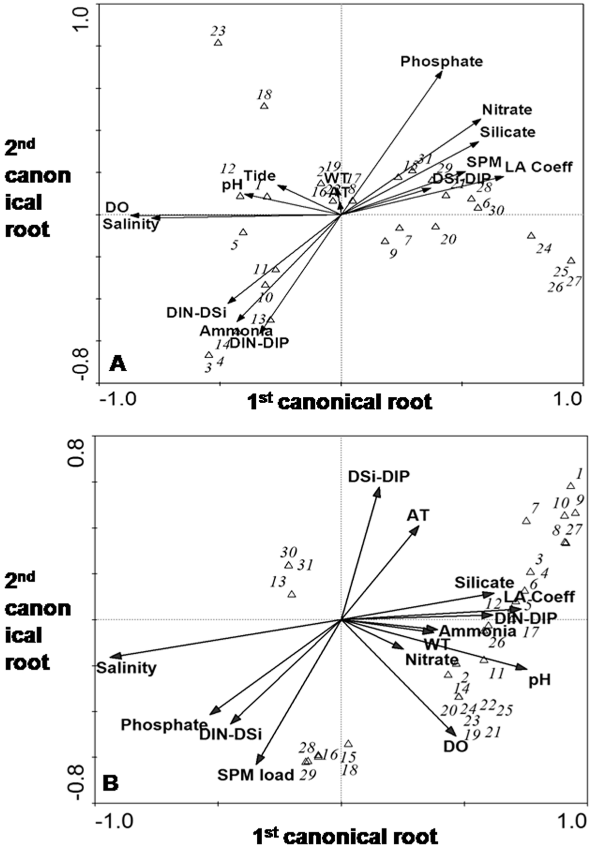

Figure 8. Orthogonal projections of canonical correspondence analyses between phytoplankton species (open triangles) and environmental variables (arrows) at the creek **(a)** and estuarine **(b)** stations. Numbers represent individual taxon as mentioned in the manuscript.