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Original Article

Seasonal variation in the coastal water phytoplankton communities and their environmental responses at upstream and downstream of the steep Naf River in the south-western Bay of Bengal

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Abstract: As a multinational river, the Naf River flows into the Bay of Bengal in the Indian Ocean, between the Cox's Bazar district of Bangladesh and the Rakhine state of Burma. In a multidisciplinary approach, several experiments were carried out to understand the seasonal diversity of the phytoplankton community structure. A total of four layers of water was sampled from four depths in the Naf River during monsoon (September) and winter (December) of 2016. 41 species of phytoplankton were identified, and 3 different dominant groups (Cyanobacteria, Diatoms, and Dinoflagellates) were found. Diatoms and cyanobacteria alone were found to be most prevalent. Higher species diversity was observed in the monsoon season, with *Synedra* sp. (1.84×10^5 cells L^{-1}, 18.76%) and winter with *Microcystis* sp. (1.41×10^5 cells L^{-1}, 17.74%), respectively. In monsoon, NO_3-N and PO_4-P were both higher than winter (450.9 and 34.4 µg L^{-1}, respectively) especially, at downstream Naf River. Moreover, high diversity indexes (richness) of phytoplankton were recorded along with these estuarine stations. Significant correlations ($P<0.01$) of nutrients with phytoplankton may liable behind these scenarios. An analysis of principal component analysis (PCA) and linear regression supported this correspondence. In the monsoon season, the concentration of Chlorophyll-α reached the highest level (165 µg L^{-1}) at a depth of 1.5 m, in Station-D. Cluster analysis based on the nutrient content of the Naf River was found two (upstream and downstream) mentionable zones during the winter and monsoon seasons. The results of the present study indicate that estuarine downstream areas are more productive than upstream areas of the Naf River at the southwest coastal zone of the Bay of Bengal.

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Introduction

Phytoplankton is the most diverse group of photosynthetic microorganisms, and are adapted to live partly or continuously in open seas, lakes, reservoirs, ponds, and river waters, where they contribute part or most of the organic carbon available to pelagic food webs (Reynolds, 2006; Barsanti and Gualtieri, 2006; Klais et al., 2017; Santana et al., 2017; Lee et al., 2018; Affe et al., 2019; Beak et al., 2019; Bhaskar et al., 2020). They are the agents of primary production and the key to the base of food chains and food webs, directly providing nutrients to zooplankton, fishes, and other aquatic animals (Shubert, 1984; Millman et al., 2005; Moser et al., 2017; Abonyi et al., 2018; Kim et al., 2019a; Bom and Colling, 2020).

Changes in phytoplankton dominance can affect whole aquatic ecosystems because they are the main source of food for many small fish and invertebrates (Dalelio et al., 2014; Maranon, 2015; Kim et al., 2019b; Park et al., 2020). The rapid changes in the abundance and composition of phytoplankton species as a result of environmental changes make them useful as aquatic bio-indicators for water quality monitoring

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(Plongon et al., 2016; Charalampous et al., 2018). Specific annual biological features in tropical rivers and reservoirs are expressed in plankton density and species composition (Palmer et al., 1977; Shubert, 1984; Washington, 1984; Pongswat et al., 2000; Nakajima et al., 2015; Shanks et al., 2017; Sai Elangovan et al., 2019). The key limiting nutrients for the growth of marine phytoplankton are sometimes considered to be phosphorus (P) and nitrogen (N), with an increase in their availability resulting in increased primary production rates and changes in population abundance, species richness, and species composition (Harvey, 1960; Berdalet et. al., 1996; Carter et al., 2005; Hobday et al., 2006; Choern et al., 2014; Beak et al., 2015; Spilling et al., 2015; Gherardi et al., 2016; Chan et al., 2020). Chlorophyll-α is regarded as a well-accepted index in this case for phytoplankton abundance and the population of primary producers in an aquatic environment (Camdevy'ren et al., 2005; Yoshikawa et al., 2017; Liu et al., 2020).

The Naf, a trans boundary river that forms Bangladesh's border with Myanmar, is one of the most important estuarine rivers in Bangladesh. The only coral island in County Saint Martin, the most southerly point, sits at the mouth of the Naf. The overall health of the Bay of Bengal ecosystem can also be affected by the discharge of nutrients from, and the productivity of, this river. Which, in return, directly affects the development of fish and fisheries in and around the river. The estuarine environment plays many economically significant roles in many ways. Though the estuarine area of Bangladesh has high faunal diversity, it has given the least importance due to a lack of sufficient research. Realizing the importance, several works pertaining to macrobenthos of coastal and estuarine waters of Bangladesh has been carried out (Hossain et al., 2009; Abu Hena et al., 2013; Asadujjaman et al., 2012; Noman et al., 2018). However, information on nutrients and primary producers, which act as a key platform of the Naf River estuary is yet untouched despite this river provides many ecological and economic services to the people of Bangladesh. Therefore, our study aimed

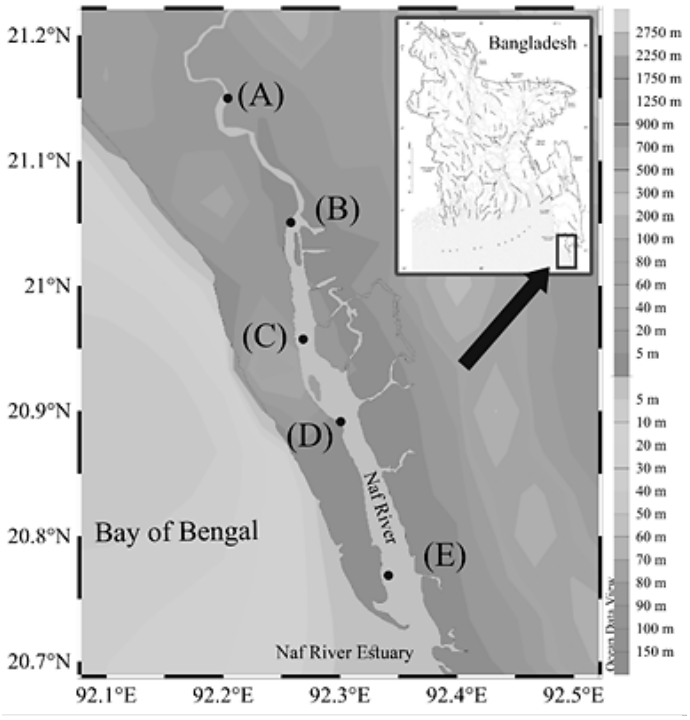

Figure 1. Location of the study area showing different stations in Naf River (Red-colored inset in the Map of Bangladesh indicates the position of Naf River).

to (i) investigate the seasonal and spatial distribution of phytoplankton biomass and their communities and (ii) the relationship between shifts in river nutrient levels and phytoplankton communities, species composition, and productivity.

Materials and Methods

Study area: The Naf River flows through the apex of the Bay of Bengal, roughly between latitudes 20°47'N and 92°28'E. A solid hydrodynamic regime is present in the flow. The prevailing southerly winds and swells and the convergence of northbound currents influence oceanographic conditions. The river's bed is sandy-muddy from the upper reaches to the lower reaches, where tidal water flows freely. Sampling for the present study was undertaken throughout the length of the Naf River, which runs from approximately 21°09'03"N 92°12'12"E to 20°44'32"N 92°21'40"E. It enters Bangladesh near Phalungkhali (Ukhiya Upazila) of Cox's Bazar district and joins the Bay of Bengal after Shah-prior-dweep (Teknaf Upazila) (Fig. 1). As Bangladesh and Myanmar are at odds over the Naf River on the coast, only five sampling stations

Table 1. Stations coordinates and estuary level in the study area.

Name of the station	Location of the station	Coordinates		Estuary level	Total length of the Naf River in Bangladesh along with the study area coverage
		N	E		
A	PhalungKhali	21°8'60"	92°12'13"	Lower estuary	63 Km (44 km; 70%)
B	Silkhali	21°3'5"	92°15'23"	Lower-mid estuary	
C	Dhumdumia	20°57'26"	92°16'3"	Mid estuary	
D	Teknaf	20°53'23"	92°17'57"	Upper-mid estuary	
E	Dakhkhin Para	20°46'8"	92°20'30"	Upper estuary	

from the mouth to the head of the Naf River estuary stations were chosen. All of the stations were chosen from various estuary elevations. Levels of the Naf River estuary were named according to stations' pattern (Table 1). Each station was sampled in triplicate fashion at four depths in the vertical water column (0, 1.5, 3, and 6 m) except in stations B and C (water depth was less than 6 m).

Sample collection and preservation: Water samples were collected using a 1L Kemmerer type water sampler (Model: Wildco-3-1200-E35) during Monsoon and Winter, 2016. Sub-samples of water were then taken for phytoplankton, Chlorophyll-α, and nutrient analysis. A country boat was used to collect the water samples throughout the study period. Water samples collected for phytoplankton analysis were passed through plankton net (125 µm). Filtrates were carefully decanted into plastic sampling bottles up to a standard volume of 50 ml. Thereafter, phytoplankton samples were preserved in 5% formalin for the qualitative and quantitative analyses and species identification. 200 ml of the collected water sample was filtered using filter paper (Whatman GF/F, 0.45 µm, diameter 47 mm) *in situ* for Chlorophyll-α and nutrient content analysis. The filtrates and filtered water samples were then immediately brought back to the laboratory, kept frozen, and stored at -20°C until analysis. Analysis of Chlorophyll-α (µg L^{-1}) and nutrient (NO_3-N and PO_4-P) content (µg L^{-1}) were performed using HACH (Model DR 6600) at the laboratory of Fisheries and Marine Science, Noakhali Science and Technology University.

Sample extraction and analysis of Chlorophyll-α: The filters were cut into small pieces and placed in a 50 ml centrifuge tube, then 15 ml of 90% acetone was added and allowed to stand overnight in a refrigerator. These were then centrifuged at room temperature for 10 min at 3,000 RPM. The supernatants were decanted into a 50 mm path length spectrophotometer cuvette. The methods employed for algal absorption measurements and calculations are described by Parsons et al. (1984).

Phytoplankton enumeration, identification, and diversity indexes: For each sub-sample, 1 ml from every 50 ml the preserved sample was transferred to a 1 ml capacity Sedgwick-Rafter counter, and phytoplankton was counted using a Luminous microscope (XSZ21-05DN, China). Phytoplankton was counted using the formula proposed by Stirling (1985), with the resultant count expressed as the number of cells per L of water (cells L^{-1}). The total number of phytoplankton present in the collected sample was calculated by the following formula:

$$N = \frac{n \times v}{V} \times 1000$$

Where N = total number of phytoplankton cells per liter of water filtered, n = average number of phytoplankton cells in 1 ml of plankton sample, v = the volume of phytoplankton concentrates (ml), and V = the volume of total water filtered (L). Phytoplankton was identified (mostly to species level) based on morphology, using taxonomic keys of Prescott (1962) and referring to the monographs of Yamazi (1979), Hustedt (1985), and Sahu et al. (2013). Identification and measurement of the phytoplankton were carried out using a light microscope equipped with eyepiece graticules. Shannon-Wiener diversity index (Shannon and Weaver, 1949) was calculated by using the following respective formulae:

$$H = -\sum_{i=1}^{S} Pi\ Ln\ Pi$$

Where, S is total number of species in a sample, Pi = Proportion (Pi=n_i/N) of total sample individuals

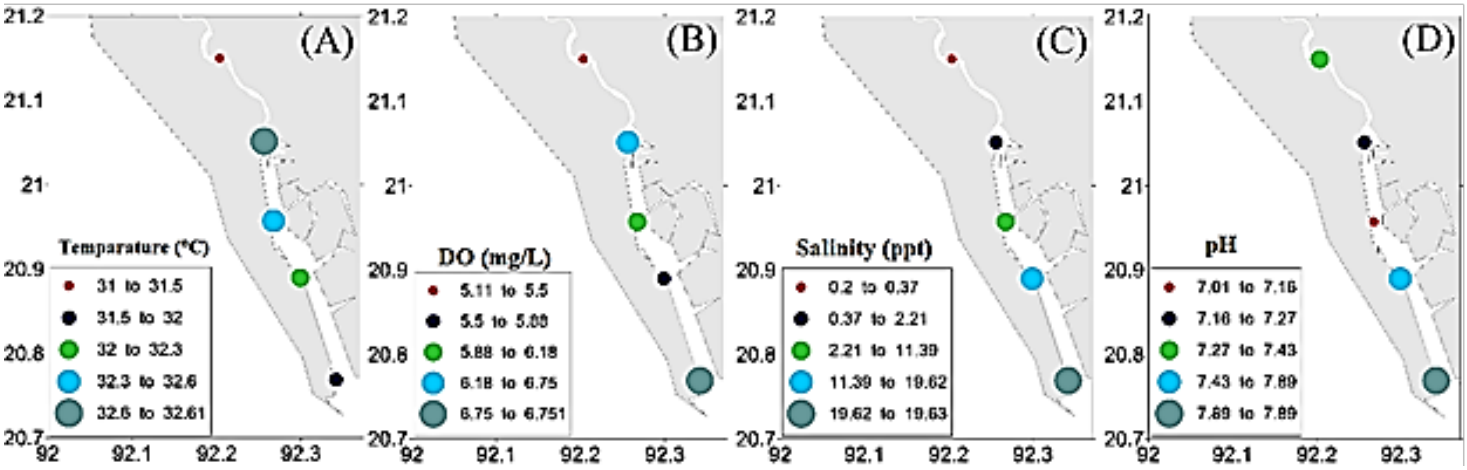

Figure 2. Environmental parameters of sampling stations in study area.

belonging to the i^{th} species, N = Total number of individuals of all the species and n_i = number of individuals belonging to the i^{th} species. Margalef's species richness (Gleason, 1922) or Margalef's index was done by:

$$D = \frac{S-1}{\ln N}$$

Where, D = Margalef's index, S = Number of species in the sample, *ln* = log-normal, and N = Total number of individuals in the sample. And evenness index (Pielou, 1967) of phytoplankton species evenness or equality was counted by $J = H/l_n S$; where, J = Evenness index, H = Shannon-Weiner index value, *ln* = log normal, *S* = Total number of species in sample.

Statistical approaches and data demonstrations: A t-test was run using IBM (SPSS, version 20, 2011) to assess the change in Chlorophyll-α concentrations between Monsoon (September) and Winter (December). Statistical comparisons of different diversity indices were performed using Paleontological Statistics (PAST version 3.11) (Hammer et al., 2001). Surfer (version 12) was used for average surface demonstration of all tabulated and examined parameters. The concentration of different parameters was shown through a line graph by Excel stats software and contour color map by Ocean Data View (ODV 2018). Cluster analysis (MCA) was performed after using the Pearson Coefficient by Multivariate Statistical Package Software (Kovach, 1998). PCA (Principal Component Analysis) plot was also deployed accordingly. Linear regressions were performed by Microsoft Excel (v2016) software. SPSS (v25) was used for the Pearson correlation and covariance. However, data were demonstrated in graphs based on significant relationships accordingly.

Results

Naf River hydrology: The Naf River demonstrated high temperature at the mid and low across the upstream region (Fig. 2A). However, Dissolved Oxygen (DO; mg L^{-1}), salinity (ppt), and pH were high at the downstream region near the Naf River's estuarine area. Low DO (Fig. 2B) and salinity (Fig. 2C) were observed at the upstream area and low pH was found in the mid of the river (Fig. 2D).

Riverine nutrients patterns:

Vertical distributions of abiotic factors: The concentration of NO_3-N was approximately 10 times higher than PO_4-P, with both increasing towards the estuary (supplementary file 1). The highest concentration of NO_3-N (450.9 µg L^{-1}) was recorded in monsoon at the surface of Station-D (Fig. 3C) while the lowest (66.4) was at the surface of Station-A in winter (Fig. 3G). Monsoon and winter also showed temporal variations for all the variables, with a decrease in NO_3-N, PO_4-P, from 287.18 and 23.53 µg L^{-1} in Monsoon to 223.90 and 20.60 µg L^{-1} in Winter, respectively (Table 1). The highest average concentration of NO_3-N was recorded (Table 1) in Monsoon at 6 m depth (374 µg L^{-1}) whereas the lowest was in Winter at the surface layer (209.32 µg L^{-1}). The PO_4-P concentration ranged from 19.06 to 23.07 µg L^{-}

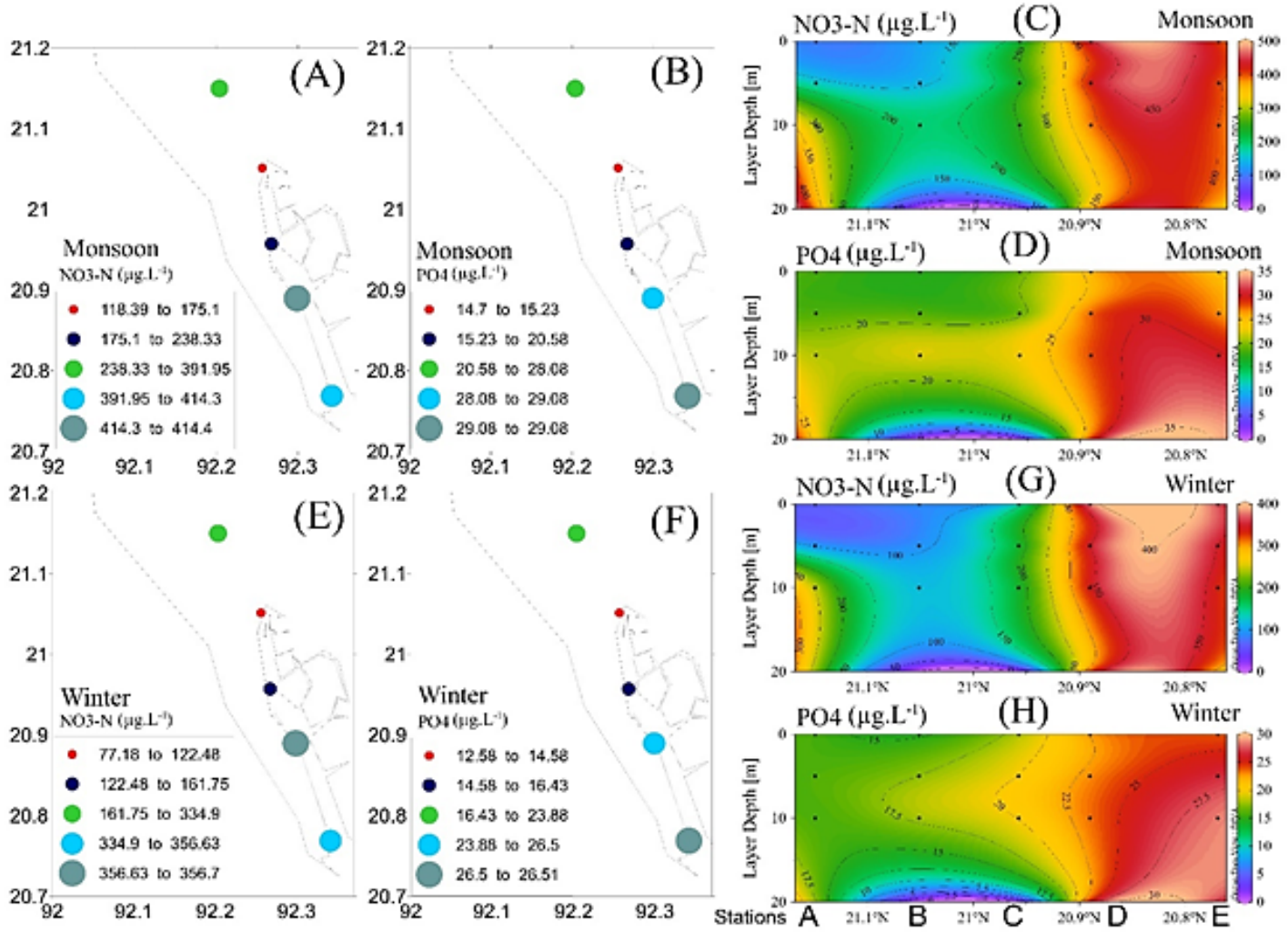

Figure 3. Average distributions of biotic and abiotic factors at Naf the River.

^{1} in winter and 21.14 to 29.39 µg L^{-1} in Monsoon (Table 1). PO_4-P concentration was highest (34.4 µg L^{-1}) in Monsoon at 6 m (Fig. 3D) and the lowest (14.7 µg L^{-1}) was in winter at the surface of Station-B (Fig. 3H).

Horizontal distributions of abiotic factors: The average concentrations of NO_3-N and PO_4-P in Monsoon were 287.18 and 23.53 µg L^{-1} while in winter they were 233.90 and 20.6 µg L^{-1}, respectively. The highest average concentration of NO_3-N was recorded in Monsoon at Station-D (414.3 µg L^{-1}) whereas the lowest was in winter at Station-B (102.91 µg L^{-1}). The average PO_4-P concentration ranged from 16.43 to 29.08 µg L^{-1} at Station-A in Winter and Station-E in Monsoon respectively. Especially, Station-D always showed the highest concentrations for NO_3-N compared to all depths during both seasons (Fig. 3A, E). On the other hand, Station-E demonstrated the highest concentrations for PO_4-P during both seasons accordingly (Fig. 3B, E).

Distribution of biotic and abiotic factors:

Horizontal profile: The mean NO3-N, PO4-P, and chlorophyll-α concentrations in Monsoon were 287.18, 23.53, and 0.85 µg L^{-1}, respectively, while in winter they were 233.90, 20.6 and 0.52 µg L^{-1}, respectively (Table 1). Mean phytoplankton cell density varied seasonally (Table 1). Spatial changes in the phytoplankton biomass (herein chlorophyll-α) were similar with phytoplankton cell density and yielded the highest value at Station-D in both seasons (Fig. 4A-B and E-F).

Vertical patterns: Phytoplankton cell density decreased sharply with an increase in depth. The concentration of chlorophyll-α slightly increased for

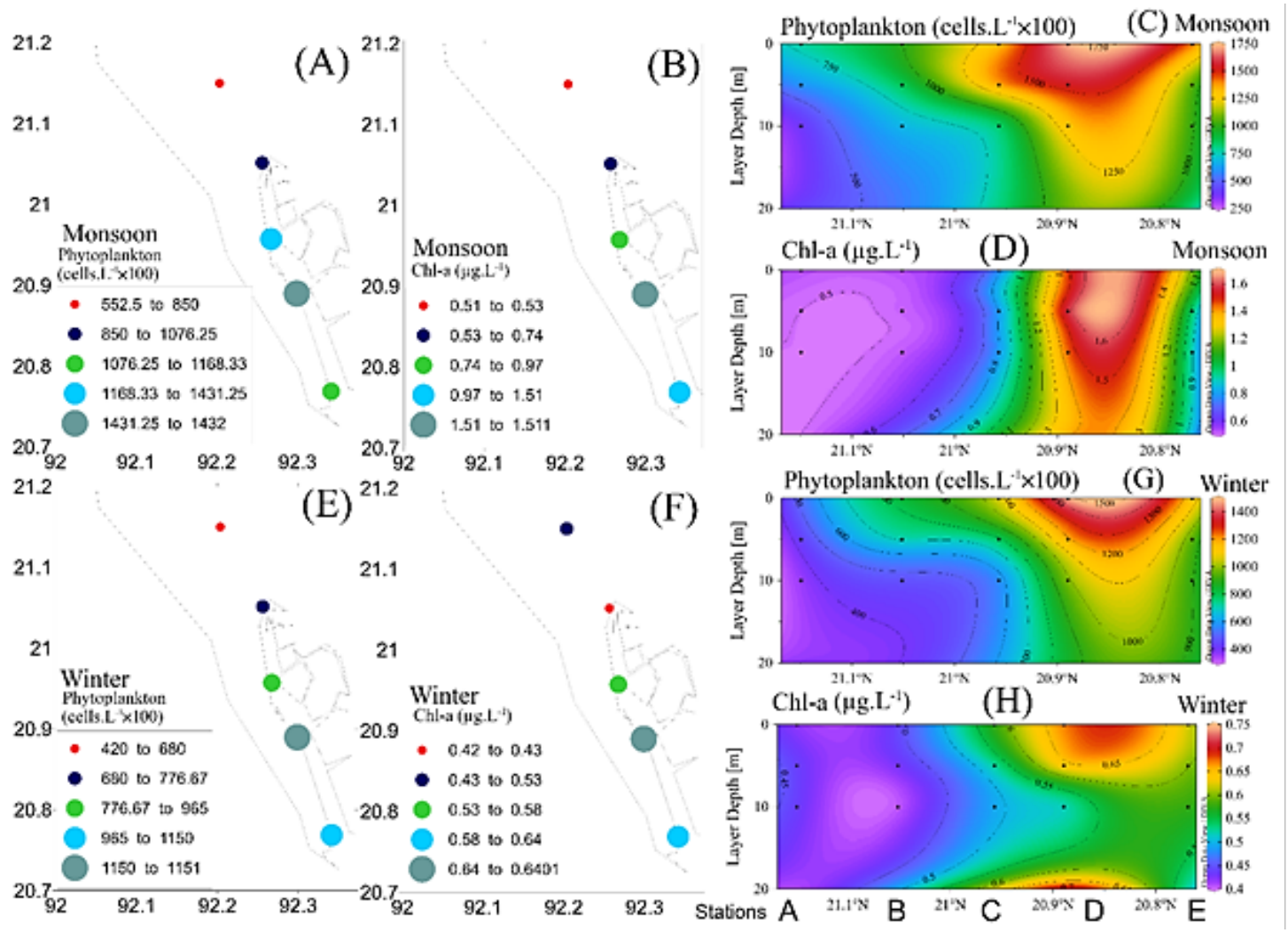

Figure 4. Vertical distribution of biotic and abiotic factors at the Naf River.

both months when comparing the surface to the deepest sample (6 m), with a decrease in the middle layers (1.5 and 3 m). Monsoon and Winter also showed temporal variations for all the variables, with a decrease in chlorophyll-α and phytoplankton from 0.88 µg L^{-1} and 9.93×10^5 cells L^{-1} in Monsoon to 0.53 µg L^{-1} and 7.03×10^5 cells L^{-1} in Winter, respectively. The average chlorophyll-α concentration was the highest in Monsoon at the depth of 6 m (0.91 µg L^{-1}) whereas the lowest was in Winter at the depth of 3 m (0.48 µg L^{-1}) (Table 1).

The distribution of phytoplankton abundance was similar to that of biomass showing the highest and the lowest values in the northeastern and southwestern part of the Naf River at station D and A, respectively, both in Monsoon (Fig. 4C-D) and Winter (Fig 4G-H). With an increase in profundity, phytoplankton cell density decreased dramatically. When comparing the surface to the deepest sample (6 m), the concentration of chlorophyll-α increased marginally for both months, with a decrease in the middle layers (1.5 and 3 m). With all the variables, Monsoon and Winter also showed temporal differences, with a reduction in chlorophyll-α and phytoplankton from 0.88 µg L^{-1} and 9.93×10^5 cells L^{-1} in Monsoon to 0.53 µg L^{-1} and 7.03×10^5 cells L^{-1} in Winter. The average concentration of chlorophyll-α was highest in Monsoon at a depth of 6 m (0.91 µg L^{-1}) while the lowest concentration was 3 m (0.48 µg L^{-1}) in Winter (Table 1).

Species distribution: During the current analysis, a total of 41 phytoplankton taxa belonging to three separate classes (Cyanobacteria, Diatoms, and Dinoflagellates) were found in which Cyanobacteria

Table 2. Group-wise lists of phytoplankton species identified from the Naf River with average cell density (Cells/L) of Monsoon and Winter, 2016 during the present study (Bold=Highest concentrations).

Groups	Species Identified	Monsoon	Winter	Monsoon %	Winter %
Cyanobacteria	***Microcystis* sp.**	**1.3×10^5**	**1.41×10^5**	**13.18**	**17.74**
	Trichodesmium erythraeum	3.25×10^4	2.96×10^4	3.31	3.72
Diatoms	*Amphora* sp.	2×10^3	2.67×10^3	0.20	0.34
	Bacillaria paxillifer	2.08×10^4	1.18×10^4	2.11	1.49
	Bacteriastrum hyalinum	2×10^3	1.25×10^3	0.20	0.16
	Bellerochea malleus	8.25×10^3	9.67×10^3	0.84	1.22
	Corataulina pelagica	1.5×10^4	2.03×10^4	1.53	2.55
	Chaetoceros didymus	1×10^3	-	0.10	0.00
	Coscinodiscus centralis	3.25×10^3	4.25×10^3	0.33	0.53
	Cyclotella sp.	1.33×10^4	1.38×10^4	1.36	1.74
	Diploneis weissflogii	9.67×10^3	5×10^3	0.98	0.63
	Eucampia zodiacus	6.25×10^3	5.25×10^3	0.64	0.66
	Grammatophora marina	2.67×10^4	2.06×10^4	2.71	2.59
	Lithodesmium undulatum	7.75×10^3	4.67×10^3	0.79	0.59
	Navicula sp.	3.47×10^4	3.18×10^4	3.53	3.99
	Paralia sp.	2.92×10^3	2.42×10^3	0.30	0.30
	Planktoniella sol	1.58×10^4	1.80×10^4	1.60	2.26
	Pleurosigma sp.	3.30×10^4	1.56×10^4	3.36	1.96
	Pseduonitzschia pungens	1.50×10^3	5.00×10^2	0.15	0.06
	Rhizosolenia alata	5.02×10^4	3.64×10^4	5.11	4.58
	Rhizosolenia castracanei	3.50×10^3	2.50×10^3	0.36	0.31
	Rhizosolenia crassispina	1×10^4	7×10^3	1.02	0.88
	Rhizosolenia imbricata	2.38×10^4	2.22×10^4	2.43	2.79
	Rhizosolenia setigera	1.88×10^4	4.08×10^3	1.92	0.51
	Skeletonema costatum	3.00×10^3	4.75×10^3	0.31	0.60
	Stephanopyxis turris	4.67×10^3	5.00×10^2	0.48	0.06
	Surirella sp.	3.50×10^3	7.50×10^2	0.36	0.09
	Synedra sp.	**1.84×10^5**	1.09×10^5	**18.76**	13.66
	Thalassionema nitzschioides	1.13×10^5	6.63×10^4	11.50	8.33
	Thalassiosira rotula	7.42×10^3	8.50×10^3	0.76	1.07
	Thalassiothrix fraunfeldii	1.38×10^5	**1.21×10^5**	14.04	**15.20**
	Triceratium favus	2.26×10^4	1.79×10^4	2.30	2.25
	Triceratium reticulatum	3.00×10^3	1.75×10^3	0.31	0.22
	Triceratium robertsianum	1.33×10^3	-	0.14	0.00
Dinoflagellates	*Ceratium macroceros*	1×10^3	1.50×10^3	0.10	0.19
	Ceratium trichoceros	8.33×10^3	**1.91×10^4**	0.85	**2.40**
	Ceratium tripos	**1.03×10^4**	1.28×10^4	**1.04**	1.61
	Ornithocercus steinii	4.83×10^3	6.58×10^3	0.49	0.83
	Prorocentrum maximum	3.17×10^3	6.75×10^3	0.32	0.85
	Prorocentrum scutellum	1×10^3	4.50×10^3	0.10	0.57
	Protoperidinium steinii	1×10^3	3.75×10^3	0.10	0.47
Total	41	8.90×10^5	7.23×10^5	100.00	100.00

belonged two species (*Microcystis* sp. and *Trichodesmium erythraeum*), Dinoflagellates belonged seven and Diatoms belonged the remaining 32 species. Diatoms were the most common, followed by Dinoflagellates and Cyanobacteria, at all stations in both months. All of the 41 species reported were present in the Monsoon, while 2 species of Diatom (*Chaetoceros didymus* and *T. robertsianum*) were absent in the winter (Table 2).

At the surface and 1.5 m depth at Station-D, the highest 21 species were found in Monsoon and Winter, respectively. The lowest 7 species were found in both months at 3 m depth and 6 m depth at Station-A as well as at Station-A (6 m) and Station-B (l0 m) in winter, respectively (Fig. 5). *Synedra* sp., (Monsoon 18.76 and Winter 13.66%; Diatom), *Thalassiothrix fraunfeldii* (Monsoon 14.04 and Winter 15.20%; Diatom), and *Microcystis* sp. were the organisms with the highest cell densities (Monsoon 13.18%; Cyanobacteria; Winter 17.74%) (Table 2). The single species with the highest cell density in Monsoon was *Synedra* sp. (1.84×10^5 cells L^{-1}), while *Microcystis* sp.

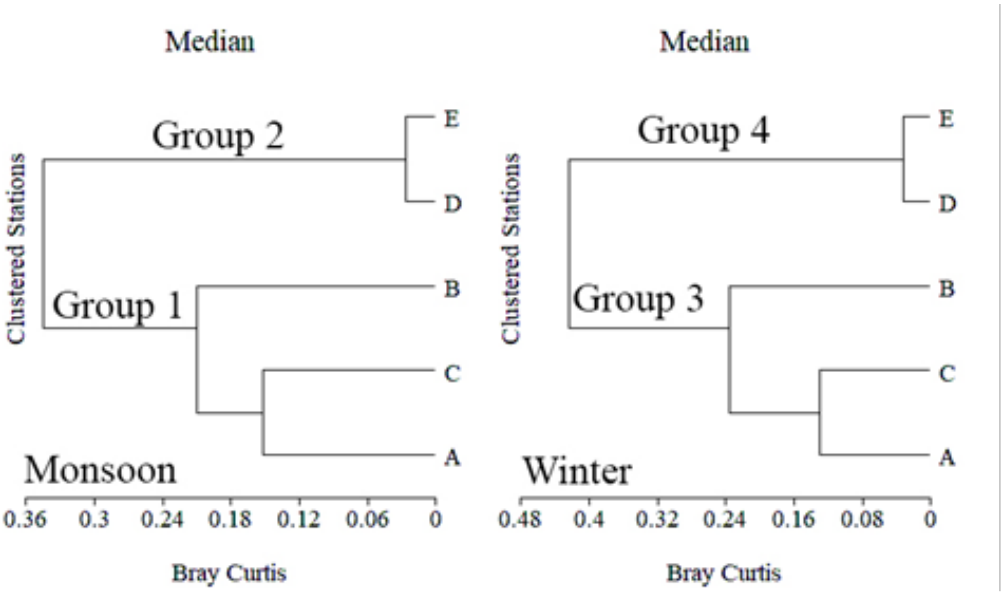

Figure 5. Cluster analysis of sampling stations after considering nutrients as variables.

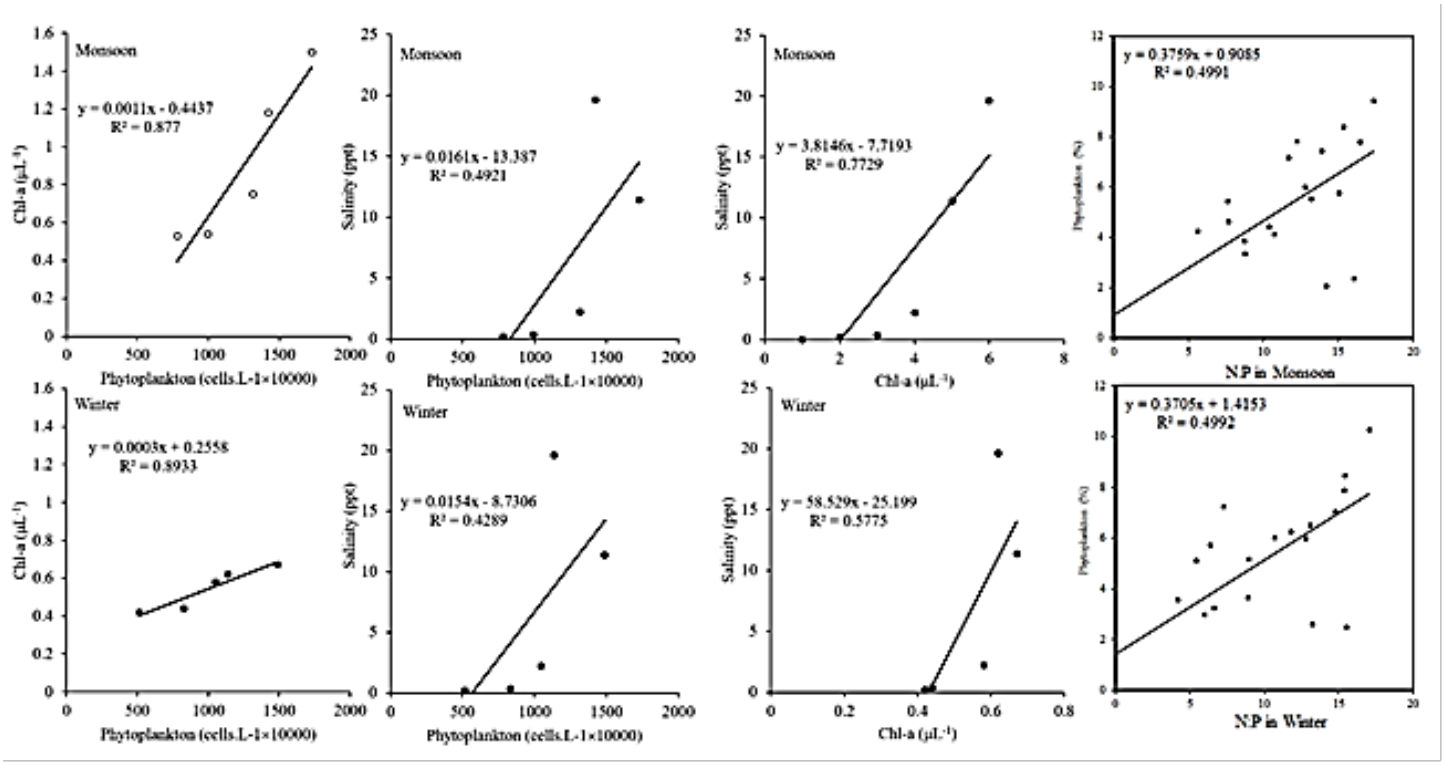

Figure 6. Linear regressions among biotic factors and its correspondences with physical parameters of the Naf River.

obtained the highest position in Winter (1.41×10^5 cells L^{-1}).

Phytoplankton diversity: Phytoplankton cell density showed a steady increase from Station-A to Station-D for both Monsoon and Winter, accompanied by a small decrease in Station-E. The cell density in Monsoon was higher than in Winter, while the phytoplankton cell density during the study period was higher in the surface layer than the other river layers. A similar trend from Station-A to Station-E was also seen in the number of species described. The highest cell density (1.73×10^7 cells L^{-1}) recorded was at the surface layer of Station-D in Monsoon and the lowest (3.6×10^5 cells L^{-1}) was at a depth of 3 m in the month of Winter at Station-A. This showed a correlation with the concentration of NO_3-N that also

Table 3. Station wise calculated phytoplankton diversity index (H= Shannon, R= Margalef, and J= Evenness) of different layers (depth) during the study period.

Month	Indices	Depth (m)	A	B	C	D	E
Monsoon	H	0m	1.73	1.76	1.87	**1.97**	1.89
		5m	1.58	1.58	1.83	1.83	1.89
		10m	1.58	1.64	1.76	1.96	1.74
		20m	1.63	-	-	1.81	1.64
	R	0m	0.6	0.65	0.94	**1.21**	0.93
		5m	0.49	0.54	0.84	0.99	0.77
		10m	0.44	0.55	0.67	1.17	0.65
		20m	0.5	-	-	0.95	0.6
	J	0m	0.51	0.49	0.38	0.33	0.39
		5m	0.54	0.49	0.42	0.35	0.47
		10m	**0.61**	0.52	0.48	0.34	0.47
		20m	0.57	-	-	0.36	0.47
Winter	H	0m	1.68	1.66	1.71	2.08	1.98
		5m	1.6	1.57	1.79	**2.09**	2
		10m	1.59	1.56	1.71	1.95	1.92
		20m	1.46	-	-	1.86	1.86
	R	0m	0.5	0.48	0.83	1.16	1.12
		5m	0.56	0.48	0.73	**1.23**	1.07
		10m	0.44	0.44	0.62	0.95	0.84
		20m	0.44	-	-	0.78	0.84
	J	0m	0.59	0.59	0.37	0.38	0.36
		5m	0.5	0.53	0.46	0.37	0.39
		10m	**0.61**	0.6	0.5	0.41	0.45
		20m	0.54	-	-	0.46	0.43

showed a comparable trend with the stations (Fig. 5).

Different indices of diversity were also tested on current data (Table 3). In winter, the diversity of Shannon-Wiener (H) ranged from a maximum of 2.089 at Station-D (1.5 m) to a minimum of 1.462 at Station-A (6 m), while the minimum was 1.578 at Station-A (3 m) at Monsoon and a maximum of 1.972 at Station-surface D's layer (Table 3). The highest Margalef (D) index in winter was 1.234 at Station-D (1.5 m) and the lowest was 0.436 at Station-B. (3 m). The maximum index of Margalef (D) for Monsoon was 1.21 for Station-D (0 m), while the minimum was 0.4418 for Station-A at 1.5 m depth (Table 3). The maximum evenness or equitability (J) of the species observed was 0.613 at Station-A (3 m) in Winter, while the minimum was 0.3265 at Station-D (0 m) in Monsoon. During this study period, no consistent pattern of evenness was observed (Table 3).

Environmental correlations among parameters: Stations can be divided into ecological zones geographically, i.e., upstream (St. A, B and C) and downstream (St. D and E) of the river Naf. Cluster (Fig. 5) analysis of both seasons reveals that after taking nutrients as a component, the sampled stations were divided into two sub-divisions, such as upstream (Groups 1 and 3) and downstream (Groups 2 and 4). In both seasons, on average, downstream stations displayed the highest concentration of all parameters (Fig. 5). Linear regressions in both seasons (Fig. 6) showed a strong positive relationship between chlorophyll-α and phytoplankton. Salinity also showed significant positive linearity with them, particularly during the monsoon with chlorophyll-α (Fig. 6). Besides, the N:P ratio demonstrated a significant positive relationship with the phytoplankton percentage (Fig. 6). Phytoplankton biomass showed strong positive linearity, especially in winter with phosphate and in Monsoon with nitrate, however, cell density showed weak linear relationship with nutrients in both seasons (Fig. 7).

PCA is an appropriate way to display a dataset's variety and clear patterns (Pitchaikani and Lipton, 2017). Clustered nutrients and phytoplankton (green circle) showed close relationships between them (Fig.

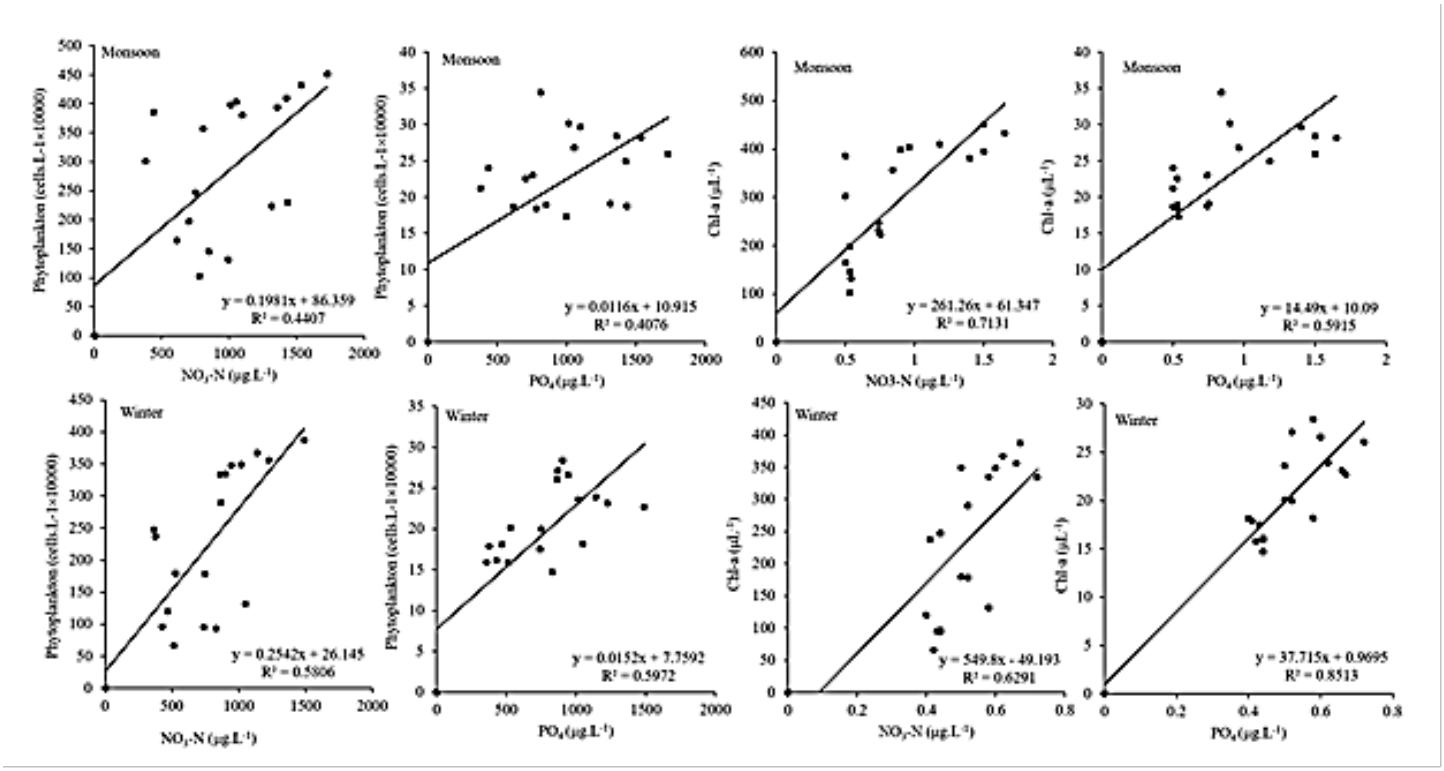

Figure 7. Linear regressions of biotic factors with nutrients of the Naf River.

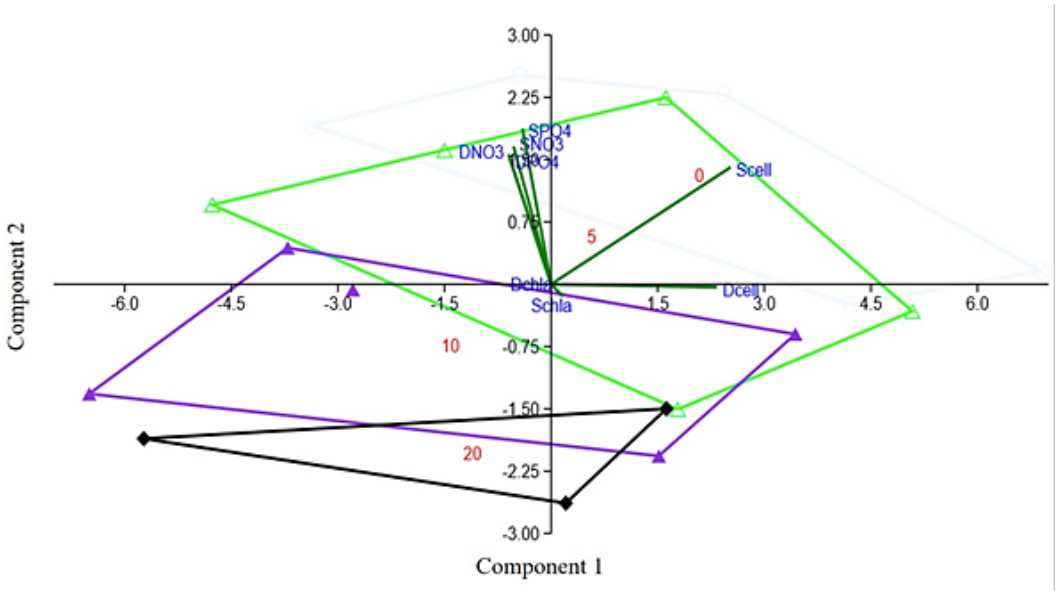

Figure 8. PCA plot clarifies the highest plankton diversity with relation to nutrients (SPO4; Concentration of PO4 in Monsoon (Monsoon), DPO4; Concentration of PO4 in Winter (Winter), SNO3; Concentration of NO3 in Monsoon, SNO3; Concentration of NO3 in Winter, Schla; Concentration of Chlorophyll-α in Monsoon, Dchla; Concentration of Chlorophyll-α in Winter, Scell; Plankton density in Monsoon, Dcell; Plankton density in Winter) in the Naf River.

8). The extracted PCA 1 showed a variance of 66.840% from the Naf River (Table 4). In Monsoon, the PCA plot showed a close relationship between nutrient concentration and plankton density from the Naf River water depth of 0 m (Fig. 8). The extracted PCA 1 showed a variance of 66.84 % from the Naf River (Table 4). A paired sample t-test was used to test if there was a statistical difference between the mean chlorophyll-α concentrations during Monsoon and Winter. Significant findings of the individual sample t-test {t (17) = -4.664, P = 0.0005} suggest a significant difference between Monsoon (M = 0.8772,

Table 4. Eigen analyses of the significant principal components of different parameters.

Component	PCA 1		PCA 2	
Eigen value	6.684		2.118	
Percentage of variance	66.84		33.16	
	Factor loadings			
Depth	0.269		0.922	
Station	0.901		-0.078	
Seasons	**Monsoon**	**Winter**	**Monsoon**	**Winter**
NO3-N	0.913	0.944	0.2	0.115
PO4-P	0.883	0.929	0.466	0.22
Chlorophyll-α	0.866	0.842	-0.255	-0.277
Density	0.602	0.733	-0.711	-0.581

Table 5. Pearson correlation between nutrients and plankton diversity of the Naf River.

		Phytoplankton	Chl- α	NO_3-N	PO_4
Monsoon	Phytoplankton	1			
	Chl- α	0.86**	1		
	NO3-N	0.66**	0.84**	1	
	PO4	0.64**	0.77**	0.87**	1
		Phytoplankton	Chl- α	NO_3-N	PO_4
Winter	Phytoplankton	1			
	Chl- α	0.86**	1		
	NO3-N	0.76**	0.79**	1	
	PO4	0.77**	0.92**	0.84**	1

SD = 0.39873, N = 18) and Winter (M = 0.5250, SD = 0.09996, N = 18) in chlorophyll-α concentrations. The mean decrease was 0.35, with the gap between the mean of 0.51 to 0.19 having a 95% confidence interval. In different months, Pearson's correlation (Table 5) between water depth, plankton density, and nutrients showed positive important correspondences with each other (P>0.01). In both seasons, strong positive relationships (r = 0.86) between phytoplankton and chlorophyll-α showed the accuracy of phytoplankton data (Table 5). Nutrients have shown a more important seasonal association with chlorophyll-α than the corresponding mean density of phytoplankton.

Discussion

Environmental influences on phytoplankton diversity: The Naf River showed similar zonal patterns of seasonally high phytoplankton and chlorophyll concentrations, particularly near the estuarine zone. A steady increase in all the variables was observed in both months as it shifted in a seaward direction (i.e., from station-A-E). The concentration of these variables may be diluted by constant freshwater inflows at the upstream stations. Besides, domestic wastewater runoff (e.g., agricultural fertilizers) will effectively increase the concentration of nutrients (Shen et al., 2008). Also, salinity changes were observed from upstream to downstream of the river Naf. It may also contribute to the development of phytoplankton (Li et al., 2017). In both seasons, substantial linear regression between salinity and chlorophyll-α was also reported.

It was found that coastal rivers compared to riverine waters are enriched with a high number of phytoplankton species in Bangladesh (Islam and Aziz, 1977; Hoque et al., 1999; Sarker et al., 2018). These are common scenarios along the coast due to high salinity and nutritional enrichment (Rahman et al., 2013). The substantial linearity of salinity with chlorophyll-α and phytoplankton in both seasons supported the present study. On the other hand, during both seasons, the Naf River displayed medium phytoplankton diversity compared to other coastal

Table 6. Reported phytoplankton diversity from other places.

Type	Reported Rivers	Reported taxa	References
Coastal Rivers	Kirtankhola	5	Sharif et al. 2017
	Meghna Estuary	8	Sharif et al. 2017
	Buragauranga	10	Ahmed et al. 2010
	Karnafully	13	Hosen et al. 2019
	Meghna	17	Ahsan et al. 2012
	Meghna	23	Sharif et al. 2017
	Reju Khal	27	Iqbal et al. 2017
	Shibsha	31	Shah et al. 2008
	Meghna	40	Flura et al. 2018
	Naf	**41**	**This Study**
	Meghna Estuary	50	Sarker et al. 2016
	Mathamuhuri	91	Hoque et al. 1999
	Rupsha-Pashur	97	Rahman et al. 2013
	Bhola-Baleswar	110	Rahman et al. 2013
	Karnafully	111	Islam and Aziz 1977
	Khalpatua-Arpangachia	122	Rahman et al. 2013
Inland Rivers	Padma	17	Ahsan et al. 2012
	Buriganga	27	Ferdous et al. 2012
	Padma	29	Ahmed and Alfasane 2004
	Padma	35	Rahman and Huda 2012
	Turag	35	Khatun and Alam 2020
	Tetulia	39	Ahsan et al. 2012
	Dhepa	52	Ara et al. 2018
	Jamuna	54	Alam et al. 2014
	Padma	54	Haque et al. 2019
	Padma	60	Flura et al. 2016
	Shitalakhya	62	Islam and Huda 2016

rivers. These differences can influence the bulk availability of nutrients, among other coastal areas (Hossain et al., 2017).

Seasonal influences on biotic diversity: There are major variations in the diversity of phytoplankton and other environmental parameters in the Naf River between two seasons. Seasonal variances in the sampled data were evaluated and verified accordingly by PCA and t-test. The extracted PCA 1 showed a variance of 66.84% from the Naf Flow. Similar findings were also recorded by Mannar Gulf, India (Pitchaikani and Lipton, 2017). In this study, high phytoplankton abundance was observed in the monsoon after winter, especially near the Naf River estuary. The sampled nutrients are correspondingly strong during the monsoon as well. These were similar to the Bangladesh Eastern Coast study (Maheshkhali Channel) (Jewel et. al., 2002) and found a maximum NO_3-N concentration in Monsoon, ranging from 0.8 to 3.0 mg L^{-1}, when phytoplankton population cell density peaks were also found. The causes of elevated nitrate levels may be freshwater inflow and terrestrial run-off during the monsoon seasons, as well as the oxidation of ammonia from nitrogen to nitrite (Hanninen et al., 2000; Rajasegar, 2003). Additionally, due to the bulk availability of necessary nutrients, post-monsoon was also recorded with high phytoplankton along coastal rivers (Sharif et al., 2017). Besides, during winter, the index of Shannon and Margalef (D) was also strong, associated with the highest heterogeneity or stability of this structure and maximum species richness of the phytoplankton group (Pielou, 1967). It was comparable to the Meghna River findings in the monsoon season because of the flow of ambient nutrients (Hossain et al., 2017).

Cyanobacteria affect the food chain in aquatic

environments by producing toxic blooms and are typically considered to be a hazard, with increases in their abundance commonly correlated with changes in nutrient levels (Marshall, 2009). High cyanobacteria concentrations in both seasons and high nutrient concentrations, respectively, were also observed in the current research. Phytoplankton succession is primarily affected by the interactions and seasonal cycling of physical, biological, and chemical factors such as temperature, grazing, and/or nutrients (Sommer et al., 1986). Overall, during both seasons, high substantial positive linearity and positive associations of phytoplankton and chlorophyll-α with Naf nutrients assisted these phenomena. Nutrients play a big proactive role in biotech development in this region because they are the most productive ecological system in Bangladesh (Noman et al., 2018).

Responses of phytoplankton to nutrients: Nutrients are the main contributor to the water body's production of phytoplankton (Sun et al., 2010). In both seasons, especially in winter, chlorophyll-α was found to correlate significantly with phosphate and then nitrate in the Naf River. During these seasons, it can influence biotic development (Yuan et al., 2014). The link between phytoplankton and nutrients was also confirmed by Pearson. Diatoms can thrive in turbulent water due to the nutrient richness of the water body (Thomas et al., 1995). It may be responsible for the dominance of phytoplankton contributed by diatoms near the estuarine region of Naf, especially at station D. Potentially, estuarine vegetation often affects the diversity of planktons along the coastal zone (Jiang et al., 2015). Due to the input of nutrients, especially from the bottom, the diversity of phytoplankton and its concentration are high across the downstream of the Naf River in each case.

Zonal impact on phytoplankton: Phytoplankton densities increased with nutrient content, while salinity, temperature, and water depth decreased from place to place in coastal waters (Jiang et al., 2015). These theoretically create an ecological zone in the body of water (Li et al., 2017). Therefore, it is possible to divide the interrelation between parameters into two ecological zones geographically, i.e. upstream and downstream of the Naf River. Cluster analysis was suggested as a useful method for marking ecological zonation within the study area after considering nutrients as variables (Deng et al., 2008). There are two referential zones according to the latest cluster analysis: Group 1 (upstream) and Group 2 (downstream) during the monsoon, and Group 3 (upstream) and Group 4 (downstream) during the winter. In addition, the zone-specific Pearson correlation with positive and negative correspondence also substantially differentiates the zonal features.

***Upstream of Naf river*:** The diversity of phytoplankton in the upstream of the Naf River was found to be poor with less richness and evenness. This situation could be responsible for nutrient limitations (Guo et al., 2014). Except for the temperature, on average, DO, salinity and pH were found very low across this area. In addition to a strong negative correlation of phosphate with phytoplankton in the monsoon and with chlorophyll-α an in the winter, corresponding correlations have been observed. Significant portions of primary productivity may be restricted by phosphate, which can cause phytoplankton to decrease here (Wang et al., 2003). In addition, a major limiting factor for the growth of diatoms was low nutritional levels (Wei et al., 2017). The Naf River phytoplankton is largely respectably dominated by diatoms and cyanobacteria. The combined effect of environmental scarcity (Marshall, 2009) is responsible for low concentrations of phytoplankton across this area.

***Downstream of Naf river*:** The downstream Naf River had a rather distinctive estuarine features, then the rest of the study region. High nutrient concentrations and other environmental parameters have been found in this area. Nutrients, especially nitrate, can act as a precursor, i.e. diatom and cyanobacteria, for dominant phytoplankton (Gong et al., 2003). This phenomenon was also confirmed in both seasons by the strong positive association of nitrate with phytoplankton. Turbulence has been documented to provide phytoplankton nutrients (Estrada and Berdalet, 1997), and there is growing evidence that turbulence directly affects dinoflagellate physiology (Juhl et al., 2000).

The Brackish portion of the Naf River (St. D and E) is a possible turbulent site due to the Bay of Bengal estuarine link. By enriching nutrients across this area, it can potentially increase the diversity of phytoplankton (Juhl and Latz, 2002). In summer and autumn, further sampling is recommended to consider the phytoplankton group structure in the Naf River all year round.

Conclusions

Strong species richness and abundance have been shown by the Naf River ecosystem. Diatoms are dominant in the group, i.e. During the Monsoon, *Synedra* sp., and *Thalassiothrix fraunfeldii* while cyanobacteria, i.e., *Microcystis* sp. in winter. The density of phytoplankton cells increased from upstream to downstream, while also demonstrating an inverse association with the sampling depth. Productive downstream has been found with high nutrient abundances that intrigue the diversity and richness of phytoplankton along the Naf River estuarine region. Therefore, this study has provided a framework on which future research can construct, promoting a deeper understanding of the Naf River and its management. The limitations of the present analysis were, however, the sampling data in autumn and summer. In order to imagine the phytoplankton group structure of the Naf River along the north-western Bay of Bengal, more projects should be introduced covering certain seasonal data deficiencies with larger areas.

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Supplementary file 1

Average values of Nutrients (NO_3-N and PO_4-P (µg/L)) and Phytoplankton cell density (cells/L×10^5) along with Chlorophyll-*a* concentration (µg/L) of the present study in the Naf River.

	Seasons	September	NO_3-N	PO_4-P	Chlorophyll-*a*	Cell Density
Depth wise	**Monsoon**	0	263.57	21.14	0.90	**12.49**
		5	274.96	22.28	0.88	10.98
		10	307.52	25.08	0.83	8.43
		20	**374.00**	**29.37**	**0.91**	7.82
		Average	305.01	24.47	0.88	9.93
	Winter	0	209.32	19.06	**0.55**	**10.05**
		5	214.84	20.66	0.53	8.18
		10	246.05	21.22	0.48	6.57
		20	**286.90**	**23.70**	**0.55**	7.03
		Average	239.28	21.16	0.53	7.96
Station wise	**Monsoon**	A	238.33	20.58	0.51	5.53
		B	157.85	19.60	0.53	8.50
		C	233.47	20.30	0.74	11.68
		D	**414.30**	28.08	1.51	**14.31**
		E	391.95	**29.08**	**0.97**	10.76
		Average	287.18	23.53	0.85	10.16
	Winter	A	161.75	16.43	0.42	4.20
		B	102.91	16.77	0.43	6.80
		C	163.30	19.43	0.53	7.77
		D	**356.63**	23.88	0.64	**11.50**
		E	334.90	**26.50**	**0.58**	9.65
		Average	223.90	20.60	0.52	7.98