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Water quality parameters and phytoplankton characteristics in coastal mangrove ecosystem

Abstract

Studies on the water quality variable and phytoplankton characteristics in mangrove areas of Guangdong, China was carried out to determine the impact of changing water quality on phytoplankton and its cell size characteristics. Plankton samples were collected spatially from July to August 2018, at 23 selected sites across the mangroves. From the 630 taxa identified, diatoms constituted the highest number of species (420 species) and contributed 71.90% of the total phytoplankton abundance. Other important phyla were Cyanobacteria (16.35%, with 83 species) and Chlorophyta (10.70%, with 87species). Across all the sites, algal biomass was highest in the Coscinodiscophyceae compared to the other Classes of phytoplankton. Though the cell size range was 0.8-306µm, a greater proportion of phytoplankton was found within > 1000µm³ and 250 to <100µm³. The results of canonical correspondence analysis revealed that species with cell size <100µm³ were associated with increase electrical conductivity and nitrate concentration, while species with cell size >1000µm³ were associated with salinity and pH variation. The massive proliferation of these species, *Chaetoceros decipiens, Monoraphidium irregulare*, *Synechocystis* sp., and *Pseudanabaena limnetica* could become an indicator of nutrient enrichment. This study confirms that the Guangdong mangrove areas inhabit the richest phytoplankton community, and their cells size distributions are controlled by the water quality parameters.

Keywords: Algal biomass, cell-size dynamics, mangrove ecosystem, nutrients

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Aniefiok Ini Inyang, Monica Udeme Dan, Kokoette Sunday Effiong, Robert Udo Udo
Department of Marine Biology, Akwa Ibom State University, Ikot Akpaden, Nigeria

Correspondence: Aniefiok Ini Inyang, Department of Marine Biology, Akwa Ibom State University, Ikot Akpaden, Nigeria, Tel & Fax +234-904310-7269

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Introduction

Phytoplankton occupies an important component of the marine ecosystems, releasing oxygen during photosynthesis and aiding in the energy exchange process.[1] Its distribution and species abundance drive trophic state, productivity and energy flow in estuarine ecosystems.[2,3] According to Sin et al.,[4] phytoplankton community structure, composition, and species diversity in aquatic ecosystems are determined by several environmental variables. The spatial distribution of phytoplankton in the mangroves is widely affected by environmental variables such as water temperature, salinity, pH, electrical conductivity, turbidity, nutrient salts, and water calmness.[5,6] The influence of these factors on the phytoplankton community alters the species composition and diversity.[7,8] The mangrove ecosystem shows seasonal fluctuations among the environmental variables depending on regional rainfall, tidal inflow, and various abiotic and biotic processes.[9]

Usually, nutrient availability is considered as a key factor regulating phytoplankton composition, diversity, and structure, which reflects the environmental condition of the ecosystem.[6,10,11] Phytoplankton community and size composition are known to be related to nutrient availability.[12] Changes in phytoplankton community composition are usually accompanied by changes in phytoplankton size distributions, which possibly affect zooplankton community composition and structure.[12] Since phytoplankton plays a very important role in the marine ecosystems, the study of phytoplankton communities is very important in the mangrove ecosystem.[13-15] Recent studies have reviewed the factors regulating the diversity and community structure of phytoplankton in coastal ecosystems;[5,6,16-20] but idealistically, phytoplankton abundance and community composition in the coastal mangroves of northern part of South China Sea received little attention.[20] The coastal waters of Guangdong Province, China have received a large amount of nutrients and other pollutants from rivers and their watersheds[21] that can affect the spatial dynamic of the phytoplankton community structure.

Therefore, it is important to investigate the relationship between changes in nutrients concentration and phytoplankton community characteristics in coastal mangrove ecosystem of Guangdong Province, China. It's in this light that we elucidate the phytoplankton composition, cell-size dynamics, and biomass production across the mangroves during the rainy season.

Study area

The sampling was done at 23 different locations throughout the Guangdong mangrove forests, at latitude 20°12'-25°31'N and longitude 109°45'-117°20' E (Figure 1). The sampling sites were characterized by different species of true mangroves, such as *Acrostichum aureum*, *Excoecaria agallocha*, *Bruguiera gymnorrhiza*, *Kandelia obovata*, *Rizhophora stylosa*, *Lumnizera racemosa*, *Aegicera corniculatum*, *Avicennia marina*, and *Acanthus ilicifolius*, and non-true mangrove species e.g. *Pongamia pinnata*, *Hibiscus tiliaceus*, *Thespesia populnes*, *Herltiera littoralis*, *Cerbera manghas*, *Clerodendrum inerme*, and *Pluchea indica*.[6] Guangdong mangroves are influenced by semidiurnal to mixed tides all year round, with a tidal range of 0.8 m (neap tides) to 4.0m (spring tides). The average annual precipitation in the study region is ~2,000mm,[22] and about 85% of the total rainfall is concentrated in the wet season (April to September).

Materials and methods

Sampling procedures

The sample collection was carried out across the 23 sites inside the mangroves between July and August 2018. At each site, measurement of environmental variables and collection of plankton samples were obtained during flood tide.

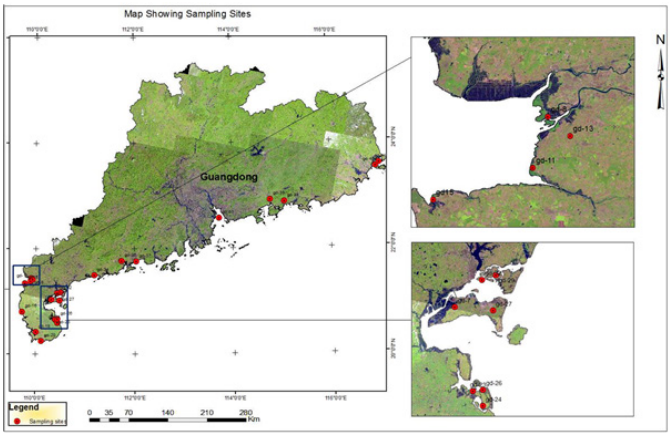

Figure 1 Map of the study area.

Plankton sampling and laboratory procedures

The phytoplankton samples were collected during flooding tide by vertically hauling within the depth of 1m, using plankton net of 0.045mm size. Approximately 90 L of water was filtered at each station, and the net was rinsed before new sampling to avoid contamination of the new sample by the previous one. The collected phytoplankton samples in 300mL plastic bottles were preserved with neutral Lugol's solution of 10% for further analysis. Further analysis and identification were conducted as described by Inyang and Wang.[6] The phytoplankton photographs were taken using Olympus DP73 digital cameras at the magnification level of 400X and 200X. All organisms, unicells, filaments, trichomes, or coenobia were counted as a single algal unit and recorded as cells.ml^{-1}. At least 500cells were counted at each station. The cell count was converted from cells. ml^{-1} of phytoplankton into cells/liter of cell density according to the equation:

$$\text{Cell density (Cell.L}^{-1}) = \frac{(A \times 1000)V}{L} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where;

A = Average number of cells count in the S-R counting chamber

V = volume of plankton concentrates (50mL)

L = Volume of original water filtered in a liter (90L).

The phytoplankton species identification up to the species level was based on the morphological structure of the cell, using the following literatures[23-26] and with the following noted website, algaebase.com (ab), World Register of Marine Species (WoRMS), Protist information server of Japan (http://protist.i.hosei.ac.jp), microscopyview.com and Nordic Microalgae. However, the phytoplankton taxonomy was carried out using.[27]

Algal biovolume determination

During the counting process, each species was allocated to size classes following Olenina et al.,[28] The descriptions of the cell volume are based on the measures of the size of the species and the adaptation of the shapes to geometric shapes as described by Olenina et al.,[28] and, Sun and Liu.[29] The individual biovolumes of the different counting units were then multiplied with their respective abundance to get the biovolume per L, according to the equation:

Biovolume taxon ($\mu m^3.L^{-1}$) = abundance (L^{-1}) x VCU x 10^{-9} …… (2)

Where; VCU = volume of counting unit (μm^3)

From the biovolume data, the biomass (wet weight) was juxtaposed by rough assumption of a plasma density of 1 g.cm^{-3} according to Cen:[30]

10^6 $\mu m^3.L^{-1}$ (biovolume) = 1 $\mu g.L^{-1}$ (wet weight) …….. (3)

The biovolume classes adopted in this work is given below:

Very small = < 100μm^3; Small = 100 – 250μm^3; Medium-sized = 250 – 1000μm^3; Large and very large = > 1000 μm^3.[31,32]

Measurement of water quality variables

At each site, in-situ measurement of water quality parameters such as water temperature, pH, salinity, electrical conductivity (EC), turbidity, and total dissolved solids (TDS) were measured using a Quanta® Water Quality Monitoring System (Hydrolab Corporation, USA) during flood water level within the depth of 1m. 300mL of surface water was collected using sampling plastic bottles for analysis of nutrients (phosphate, nitrate, nitrite and silicate). The water sample was filtered using 0.22 µm Whatman® GF/F filters before analysis. Within 2–3hrs after sampling, nitrate (NO_3-N), nitrite (NO_2-N), phosphate (PO_4-P), and silicate (SiO_3-Si) were analyzed using a SKALAR auto-analyzer (Skalar Analytical B.V. SanPlus, Holland). Chlorophyll *A* analysis was carried out according to HELCOM[33] using a SHIMADZU Spectrophotometer (UV-1700-Japan) and calculated as follows:

$$Cv = \frac{10^3.e.A(665K)}{83.V.L} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where;

C*v* = Chl. *A* concentration, mg/m^3,

e = volume of ethanol, cm^3

A(665K) = absorbence at 665nm (the peak) minus the absorbence at 750nm after correction by the cell-to-cell blank, L = length of the curvette, (cm), V = water volume filtered, dm^3, 83 = absorption coefficient in 96% ethanol.

Rainfall data was acquired from the Global Precipitation Climatology Project (GPCP) during the southwest monsoon period (April to September, 2018). The total value of July and August precipitation data was used in the analysis.

Data analysis

Statistical analysis was carried out with the aid of SPSS (version 22.0), Canoco 5, PAST v. 3, and PRIMER v.7. To estimate the significant factor among the water quality parameters in the mangrove forests, PCA plot[34] was carried out via Canoco 5 software (Houston, TX, USA) on the eleven water quality variables. The variation in the water quality variables between the sites was studied by ANOVA (one-way) with the Duncan test at a probability level of 5%, comparing the mean value of variable with significant F between the sites. Before this analysis, all the water quality variables were transformed with Log(X + 1) (except pH) and tested for normality test through kurtosis and skewness statistical tests ($p < 0.001$).

The phytoplankton community structure was analyzed using non-parametric multivariate tools, found in PRIMER v. 7 (PRIMER-E Ltd, Roborough, Plymouth, UK) software package in accordance with Clarke and Warwick,[35] Clarke and Gorley.[36] Before multivariate analysis, all species that occurred in less than five sites and/or

that never surpassed, at least in one sample, 0.5% of abundance were discarded to reduce the influence of rare taxa. Meanwhile, all the multivariate analysis (Cluster, SIMPER, and metric Multi-Dimensional Scaling) was based on the Log(X + 1) pre-transformed original data matrix. The SIMPER routine was performed to calculate the average similarity of the samples of each site, but also the average dissimilarity of all pairs of inter-site samples based on the grouping factor generated by the cluster analysis.

However, for better visualization of the Bray-Curtis similarity matrix in a two-dimensional plot, a non-metric Multi-Dimensional Scaling (nMDS) ordination was performed. The distances between pairs of samples in the plot reflect their relative dissimilarity in species composition and abundance. Meanwhile, the nMDS ordination plot was tested for significant differences between groups of samples, using one-way analysis of similarities (ANOSIM),[32] in PRIMER v. 7 (PRIMER-E Ltd, Roborough, Plymouth, UK).

To assess the relationships between nutrients and phytoplankton cell sizes, canonical correspondence analysis (CCA) using PAST v. 3 and Pearson's correlation analysis (SPSS 22.0) were employed.

Results

Spatial variation in environmental parameters

he spatial distribution of the environmental variables across the mangroves is presented in Table S1 (supplementary material). The univariate statistics of the environmental variables and their normality test are given in Table 1. The test results confirm the normal distribution of the data (pH, tds, EC, turbidity, salinity, water temperature, reactive phosphate, and silicate) with a confidence coefficient of 95% ($p < 0.05$). Moreover, the results of the ANOVA and post hoc (Duncan) tests ($p < 0.05$) provided in Table S2 (supplementary material) confirmed that all the environmental variables were significantly different across the sampling sites due to the differences in the catchment characteristics. As shown in Table S3 (supplementary material), precipitation has a small positive impact on nitrate, EC, turbidity, and chlorophyll *A* concentration, but a significant impact on the water temperature ($p < 0.05$). Chlorophyll *A* concentration varied greatly across the sampling sites, with a mean value of 1.82 mg.m^{-3}. Its highest value was 13.53 mg.m^{-3} (gd-11), while its lowest value was 0.14 mg.m^{-3} (gd-20). It was noted that the chlorophyll *A* concentration was higher across the Leizhou Peninsula (i.e. gd-2 to gd-29) than in other places in the mangroves.

Table 1 Descriptive statistics and normality test ($p < 0.05$) of the physiochemical variable measured across the mangrove forest (n = 23)

Variables	pH	Tds (ppt)	Salinity	Turb. (FTU)	Tem (°C)	EC (µg/sm)	Phosp. (mg/L)	Nitrate (mg/L)	Nitrite (mg/L)	Silicate (mg/L)	Chl. *A* (mg/m³)
Min	7.47	2.04	2.15	9.20	12.79	86.90	0.00	0.00	0.00	1.01	0.14
Max	8.71	22.02	22.82	204.00	34.49	9702.00	2.50	2.00	1.75	3.04	13.53
Mean	8.04	11.26	13.44	56.84	29.24	3472.78	1.09	0.09	0.14	2.16	1.90
Stand. Dev.	0.39	6.14	7.46	42.42	4.32	2022.02	1.05	0.42	0.47	0.41	2.80
Variance	0.15	37.70	55.69	1799.14	18.63	4088562.14	1.11	0.17	0.22	0.17	7.84
Skewness	**-0.03**	**-0.68**	**-0.67**	**-0.34**	**-0.66**	**-0.22**	**0.13**	**3.43**	**3.26**	**0.37**	**1.77**
Kurtosis	**-1.23**	**-1.13**	**-1.14**	**0.64**	**1.61**	**-0.08**	**-1.86**	**11.38**	**9.72**	**0.24**	**3.99**

Principal component analysis (PCA) given in Figure 2 produced four principal components that cumulatively explained 88.18% of water quality variations between the sites, with axis1 (42.41%) and axis 2 (21.80%) with eigenvalues of 0.42 and 0.21 respectively. The total variation was 67.05% with Tau value as 0.51 at the eigenvalues of 1 of the variation between the sites. Meanwhile, variables such as, EC, temperature, turbidity, salinity/tds, nitrite, and silicate contributed significantly to the water quality variability in the mangroves. Also, phosphate and nitrate contributed averagely.

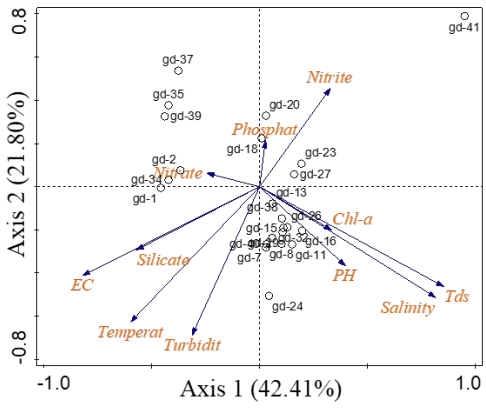

Figure 2 The PCA biplots of the environmental variables in the mangrove ecosystems {EC. electrical conductivity, Tds. total dissolve solids, Chl. *A* chlorophyll *A*}.

Phytoplankton taxa and biomass distribution

A total of 630 taxa belonging to 13 Classes of phytoplankton were identified across the 23 studied sites, Table S4 (supplementary material). The present distribution of the identified taxa was classified into 5 groups: rare (32.22% of the total taxa identified), less common (38.73%), common (18.25%), abundant (5.08%) and very abundant (5.71%). Meanwhile, the following species were reported most common and abundant species in the mangroves, *Coscinodiscopsis jonesiana*, *Cyclotella meneghiniana*, *Skeletonema costatum*, *Bacillaria paxillifer*, *Nitzschia palea*, and *Navicula cryptonella* (Figure 3). Spatially, diatoms (with 420 species) represented 71.90% of the total phytoplankton biomass. Other groups recorded were Cyanophyta (16.35%, with 83 species including 24 new taxa) and Chlorophyta (10.70%, with 87 species including 37 new taxa); Dinopyta, Euglenophyta, Charophyta, Orcharophyta, and Cryptophyta constituted < 0.5% of the total phytoplankton abundance, Figures S1&S2 (supplementary material). The spatial distribution of phytoplankton Classes found in the mangroves are given in Figure 4a. Diatom – Mediophyceae such as *Chaetoceros* sp., *C. meneghiniana*, *Thalassiosira mala*, *T. leptopus*, *T. minicosmica,* and *S. costatum* were the main predominant species found across the Leizhou Peninsula (gd-7, 15, 23, 24, 26, 27, 29). A shift from the Mediophyceae dominating assemblage to the Bacillariophyceae dominating assemblage was observed across gd-1, 8, 20, 32, 38 & 40. Precisely, *Asterionellopsis glacialis*, *N. longissima* var. *reversa,* and *Thalassionema nitzschioides* predominated gd-20; *N. sigma, N.*

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palea, and *N. cryptonella* predominated gd-8; and *N. brevissima*, *N. clausii*, and *Cocconeis placentula* predominated gd-32, 38 & 40. High abundance of Coscinodiscophyceae was only observed at gd-13 & 15, where *C. jonesiana* proliferates the surface water. Cyanophyta such as the periphytic taxa (*Oscillatoria* sp.) and planktonic taxa (*Merismopedia* sp., *Synechoccus* sp., *Pseudanabaena limnetica* & *Anabaenopsis elenkinii*) proliferate the surface water across gd-2, 16 & 18. Also, planktonic cyanobacteria such as *Aphanocapsa* sp., *Microcystic* sp., *Chroccocus* sp. & *Synechocystis* sp. proliferate the surface water at site gd-34, 35, 37 & 41(Figure 4a).

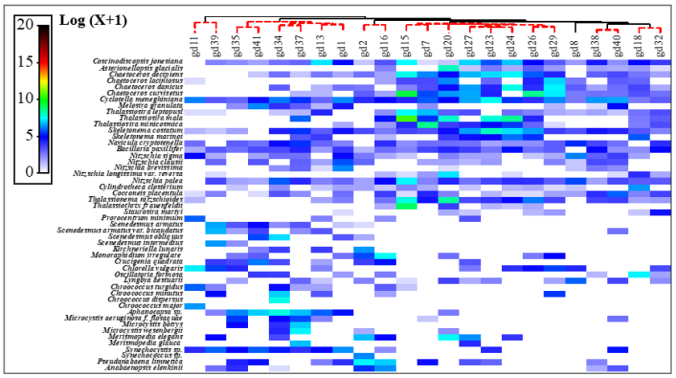

Figure 3 Shade plot, a visual representation abundances of a total of 50 phytoplankton species out of 710 phytoplankton taxa across different stations during wet season with spectrum scale intensity proportional to the Log X + 1 abundance. The dendrogram shows species clustering using standard agglomerative methods of calculating group average, with white denotes the absence of the species.

The phytoplankton wet biomass fluctuated across the mangroves, with a mean value of 2.23 x 10^4 mgL^{-1}, Figure S3 (supplementary material), and ranged from 3.80 x 10^3 mgL^{-1} to 8.40 x 10^4 mgL^{-1}. It was significantly different (t = 2.16, p = 0.05) across the mangroves. The algal biomass for all stations was higher in the Coscinodiscophyceae compared to the other Classes of phytoplankton (Figure 4b). Meanwhile, there was an increased biomass of Mediophyceae (gd-7, 20 & 29); Bacillariophyceae (gd-8, 11, 16 & 35); Chlorophyceae (gd-34 & 41); Trebouxiophyceae (gd-11), and Cyanophyceae (gd-2).

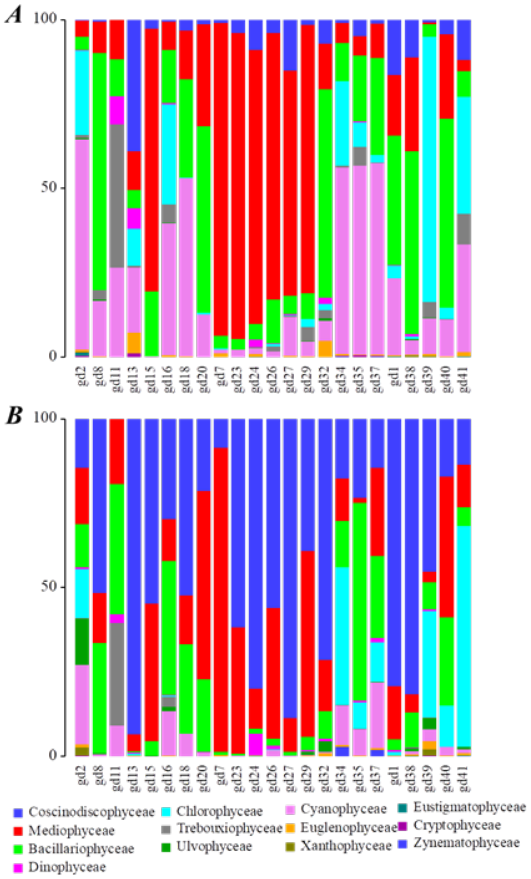

Figure 4 Spatial distribution of Algal abundance (***A***) and Biomass (***B***) at different phytoplankton Classes.

Phytoplankton size distribution

The cell size range was 0.8-306 µm and was grouped into four different size volumes, Figure 5. The phytoplankton class size of < 100 µm³ were mainly predominated by *Scenedesmus* sp., *Monoraphidium* sp., *Selenastrum* sp., *Crucigenia* sp., *Merimopodia* sp., *Chroccocus* sp., *Microcystis* sp., *Synechococcus* sp., *Aphanocapsa* sp., *Kirchneriella* sp., *T. mala*, *N. closterium* f. *minutissima*, *N. atomus*, *Leptocylindrus minimus*, etc, which were recorded across gd-2, 15, 16, 34, 35, 37, 39 & 41. Their distribution and proliferation in the mangroves were associated with high EC and nutrient salts. Class size 100-250 µm³ was maximally represented at gd-11. *C. vulgaris*, *N. palea*, *C. major*, *C. turgidus*, and *Scenedesmus* sp. were the predominant species of this class. However, these two class size were mainly freshwater taxa that were added to the mangroves via river water input and occurred only at low salinity sites. The class size 250-1000 µm³ was maximally represented by *C. curvisetus* at gd-26. Other species that make up this class include *P. limnetica*, *H. tenerrima*, *S. marinoi*, *N. transitans*, *N. cryptotenella*, *N. brevissima*, etc. The class size > 1000 µm³ constitute the greater proportion of phytoplankton assemblage in the mangroves. Its distribution fluctuated across the mangroves but was mainly represented across the Leizhou Peninsular. Species, such as *Coscinodiscopsis* sp., *Chaetoceros* sp., *C. meneghiniana*, *Thalassiosira* sp., *S. costatum*, *O. sinensis*, *B. paxillifer*, *N. longissima* var. *reversa*, *Thalassionema* sp., *Thalassiothrix* sp., *O. formosa*, *L. aestuarii*, etc predominated this class. Generally, class size > 1000 µm³ constituted the largest phytoplankton biomass.

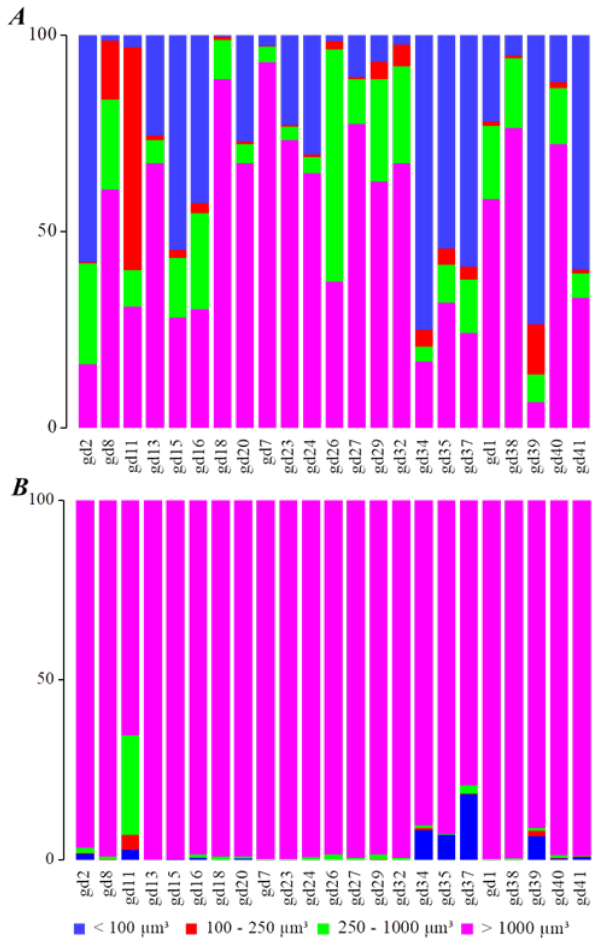

Figure 5 Phytoplankton size volume distribution: (***A***) size class abundance and (***B***) biomass distribution.

Multivariate analysis of the phytoplankton community structure

The hierarchical clustering amongst phytoplankton abundance at 50% similarity showed four groups; each was clearly associated with a specific site (Figure 6). The clustering dendrogram displayed a strong inter-relation with high similarities within groups of similar species on specific sites (Table 2). Diatoms formed the highest percentage contribution in a cluster 'b & c' compared to other phytoplankton groups, with the occurrence of *C. meneghiniana*, *C. decipiens*, *S. costatum*, *C. curvisetus*, *C. danicus*, *N. palea*, *C. jonesiana, T. minicosmica*, T. *leptopus, T. mala*, and *T. nitzschioides* predominating the Leizhou Peninsular (Figure 6). Periphytic and planktonic cyanobacteria (*Aphanocapsa* sp. and *Synechocystis* sp. respectively) with other diatom species (*C. meneghiniana*, *C. jonesiana* and *N. palea*) contributed to the intra-site species similarity within cluster 'd' which is associated with low salinity and high EC. Within cluster 'a', species such as *P. limnetica*, *M. irregular*, *M. elegans*, and *C. meneghiniana* contributed to the intra-site species similarity and were associated with high EC and nutrient salts.

Table 2 SIMPER analysis. The most contributing species of intra-site similarities are reported with their average similarity (Av. Sim) and percentage of contribution to the average intra-group similarity (%)

Group a		**Group b**	
Species	**SIM (%)**	***Species***	**SIM %**
Pseudanabaena limnetica	22.67	*Chaetoceros decipiens*	10.72
Monoraphidium irregulare	18.43	*Cyclotella meneghiniana*	9.69
Merismopedia elegans	15.84	*Skeletonema costatum*	8.23
Cyclotella meneghiniana	13.6	*Thalassionema nitzschioides*	7.89
Skeletonema costatum	8.36	*Thalassiosira mala*	7.71
Chaetoceros decipiens	5.84	*Chaetoceros curvisetus*	7.56
Chaetoceros curvisetus	5.84	*Thalassiosira minicosmica*	7.43
Nitzschia palea	5.84	*Chaetoceros danicus*	6.76
Coscinodiscopsis jonesiana	3.58	*Odontella sinensis*	4.68
Total	**100**	**Total**	**70.67**
Av. Sim	**57.19**	**Av. Sim**	**65.52**
Group c		Group d	
Species	**SIM %**	Species	**SIM %**
Cyclotella meneghiniana	12.95	*Synechocystis sp.*	22.16
Skeletonema costatum	12.02	*Cyclotella meneghiniana*	18.41
Chaetoceros curvisetus	10.56	*Aphanocapsa sp.*	15.85
Nitzschia palea	10.48	*Coscinodiscopsis jonesiana*	7.77
Coscinodiscopsis jonesiana	10.21	*Nitzschia palea*	7.38
Chaetoceros danicus	8.51	*Skeletonema costatum*	6.68
Chaetoceros decipiens	5.80	*Chlorella vulgaris*	4.60
Thalassiosira leptopus	5.72	*Pseudanabaena limnetica*	3.46
Thalassionema nitzschioides	5.62	*Chaetoceros decipiens*	1.88
Chaetoceros laciniosus	3.99	*Microcystis botrys*	1.82
Total	**85.86**	**Total**	**90.01**
Av. Sim	**57.72**	**Av. Sim**	**48.42**

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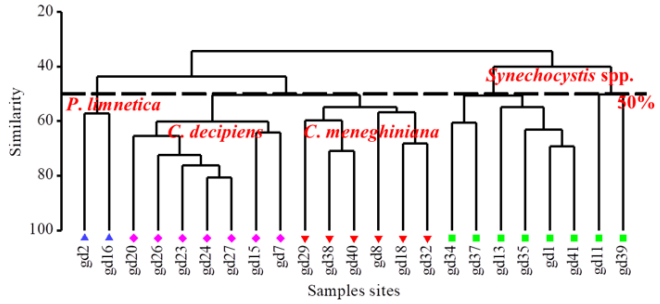

Figure 6 Hierarchical clustering of dendrogram showing dominant species in various clusters (group-average linking 23 phytoplankton samples of Bray Curtis similarities calculated on Log (X+1) transformed abundance).

The results of MDS ordinations of samples based on Log (x + 1) transformed abundance data are shown in Figure 7. By superimposing the sites based on similarity tests, 4 main groups have been formed in the mangroves. It becomes apparent that the groups differ in community composition, as respective sites group together. This is supported by the significantly lower stress factor of the ordination (0.14). Group site 'b & c' was dominated by planktonic diatom species whose class size ranged from 250 to < 1000 µm³. They were mainly marine residents, confirming the presence of neritic-rich water biota in the mangroves. In contrast, the group site 'd' was dominated by planktonic and periphytic cyanobacteria whose class size was < 100 µm³. They were mainly fresh-water residents and occupied the eastern part of the Guangdong coast. The inter-site dissimilarity (> 60%) between 'b & d' and 'c & d' was mainly caused by marine diatoms and planktonic cyanobacteria respectively. The ANOSIM test (global R = 0.825; p = 0.1) and pairwise tests (Table S4) further explain the similarity level in species composition and distribution among the sites, and between the various groups.

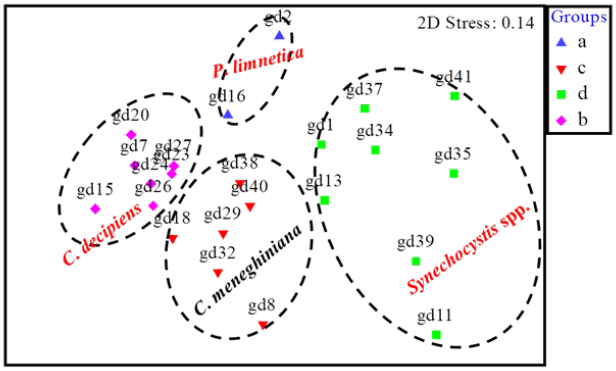

Figure 7 MDS ordinations of the phytoplankton samples based on Log (X+1) transformed abundance data and Bray-Curtis similarities showing dominant species in various clusters.

The relationship between environmental variables and phytoplankton taxa

The relationship between environmental parameters and the phytoplankton characteristics was modeled using the CCA ordination plot and Pearson's correlation analysis. Here, a relationship was established between phytoplankton class size and the environmental parameters (Table 3). The statistical relationships between cell abundance of phytoplankton taxa and environmental variables are shown in Figure 8 and Table S5. The canonical axes of phytoplankton taxa/environmental variables significantly accounted for 68.88% (*pseudo*-F = 0.662, $p < 0.002$), which axis 1 and 2 explained 51.35% and 17.53% of the variation respectively. This explains the fact that the phytoplankton taxa were significantly related to environmental variables on each axis. Furthermore, the occurrence and distribution of small-celled species of green algae, cyanobacteria, and diatoms were positively influenced by EC and nitrate. Most of these taxa were riverine assemblages and constituted the main food source for copepod zooplankton. The distribution of large-celled size diatoms and dinoflagellates was positively affected by pH, salinity concentration, and nutrients. Meanwhile, phytoplankton has special ecophysiological traits and each species tends to become dominant when growth conditions match its specific requirements. In our study, *A. glacailis*, *T. leptopus*, *T. mala*, *T. minicosmica*, *C. jonesiana*, *Chaetoceros* sp., *S. costatum*, *T. nitzschioides*, *M. irregular*, *S. obliquus*, and *P. limneticus* could become an indicator for nutrient enrichments as they become dominant. Specifically, *T. mala* formed a negative correlation with nutrients, while *T. leptopus*, *S. costatum*, *T. nitzschioides*, *M. wesenbergii*, *M. botrys*, *M. elegans*, *M. irregular*, *S. obliquus* and *P. limnetica* showed a significant positive correlation with nutrients. The massive proliferation of these taxa was mainly affected by nutrient fluctuations than were closely related to temperature changes.

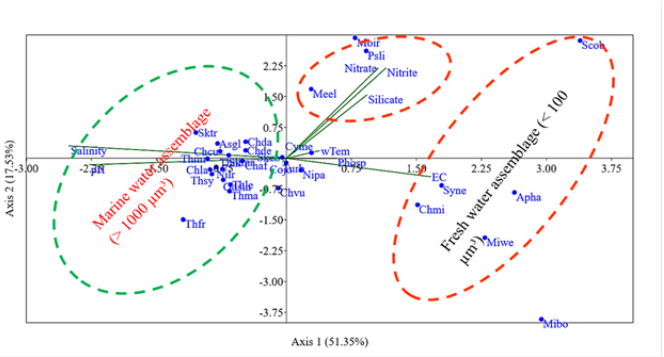

Figure 8 CCA showing the relationship between phytoplankton assemblages, class size and environmental variables.

Table 3 Results of Pearson's correlation coefficient between physicochemical variables and phytoplankton taxa in the mangrove forest

Phytoplankton taxa	Codes	pH	Sali.	Turb	Tem	EC	Phos	Nitra	Nitri	Silic
Diatom										
C. jonesiana	Cojo	0.152	0.367	-0.135	-0.258	0.124	-0.023	-0.312	-0.313	-0.155
A. glacialis	Asgl	0.332	0.286	-0.281	-0.347	-0.378	-0.175	-0.196	-0.200	0.079
C. decipiens	Chde	**0.447***	**0.579****	-0.097	0.011	-0.188	-0.071	-0.140	-0.151	-0.342
C. danicus	Chda	**0.495***	**0.473***	0.086	-0.061	-0.236	-0.075	-0.155	-0.173	-0.034
C. curvisetus	Chcu	**0.573****	**0.575****	-0.320	0.091	-0.337	-0.117	-0.116	-0.133	-0.347
C. meneghiniana	Cyme	0.056	0.240	-0.157	0.283	0.260	-0.001	-0.111	-0.108	0.016

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Table 3 Continued...

Phytoplankton taxa	Codes	pH	Sali.	Turb	Tem	EC	Phos	Nitra	Nitri	Silic
Diatom										
T. leptopus	Thle	**0.437***	**0.445***	0.198	0.097	-0.309	-0.133	-0.318	-0.324	**-0.520***
T. mala	Thma	0.337	0.334	-0.170	-0.180	-0.105	0.022	-0.233	-0.237	**-0.427***
T. minicosmica	Thmi	**0.586****	**0.450***	0.028	-0.083	-0.191	-0.306	-0.247	-0.251	-0.389
S. costatum	Skco	0.267	0.390	-0.304	-0.202	-0.054	-0.197	-0.271	-0.301	-0.209
T. nitzschioides	Thni	**0.521***	**0.597****	-0.035	-0.046	-0.250	-0.125	-0.333	-0.338	-0.373
T. frauenfeldii	Thfr	0.266	0.298	-0.140	-0.036	-0.058	0.112	-0.123	-0.125	**-0.471***
C. laciniosus	Chla	**0.549****	**0.431***	-0.095	0.138	-0.325	-0.066	-0.237	-0.241	-0.134
C. affinis	Chaf	0.365	0.344	-0.071	-0.033	-0.112	-0.328	-0.253	-0.257	-0.259
S. marinoi	Skma	0.349	0.227	-0.274	**-0.634****	-0.287	-0.092	-0.223	-0.227	-0.005
S. tropicum	Sktr	**0.451***	0.403	-0.308	**-0.479***	-0.166	-0.104	-0.187	-0.191	0.000
O. sinensis	Odsin	**0.513***	**0.471***	0.026	0.081	-0.175	-0.285	-0.292	-0.297	**-0.635****
N. longissima var. reversa	Nilr	0.337	-0.001	-0.002	-0.402	**-0.533****	-0.118	-0.150	-0.153	0.053
N. palea	Nipa	0.182	0.141	-0.052	-0.034	0.157	-0.126	-0.319	-0.326	-0.331
T. synedriforme	Thsy	0.330	.371	-0.165	-0.069	0.127	-0.032	-0.155	-0.158	-0.365
Green algae										
M. irregulare	Moir	-0.185	-0.001	-0.240	0.004	0.019	0.041	**0.459***	**0.451***	0.395
C. vulgaris	Chvu	0.160	0.125	0.202	-0.007	0.016	-0.021	-0.243	-0.247	-0.203
S. obliquus	Scob	-0.403	**-0.506***	0.188	0.147	**0.505***	0.012	0.381	**0.416***	**0.445***
Cyanobacteria										
Aphanocapsa sp.	Apha	**-0.674****	**-0.806****	-0.013	0.040	**0.526****	0.030	0.084	0.118	0.182
M. elegans	Meel	-0.076	-0.114	-0.219	-0.302	-0.303	-0.128	0.292	0.262	0.060
P. limnetica	Psli	-0.270	-0.097	-0.244	0.091	0.020	-0.130	**0.558****	**0.553****	0.231
C. minutus	Chmi	-0.320	-0.233	0.096	0.242	0.247	-0.014	-0.144	-0.147	0.185
M. botrys	Mibo	**-.0500***	**-0.601****	-0.241	-0.101	0.387	0.189	-0.113	-0.115	-0.062
M. wesenbergii	Miwe	-0.405	**-0.491***	-0.284	-0.041	0.148	0.146	0.055	0.044	0.011
Synechocystis sp.	Syne	**-0.613****	**-0.622****	0.012	0.157	**0.570****	-0.024	-0.005	0.026	0.092

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed). {EC. electrical conductivity, Sali. salinity, Turb. turbidity, Tem. water temperature, Phosp. phosphate, Nitra. Nitrate, Nitri. Nitrite, Silic. Silicate}.

Discussion

Mangroves usually maintain relatively high phytoplankton production due to naturally available nutrients from the surrounding mangrove litters which sustain the ecological balance between phytoplankton productivity and diversity.[37,38] According to Tanaka and Choo,[39] nutrient out-welling from the mangrove swamp affected the phytoplankton distribution. Meanwhile, nutrient inputs from anthropogenic activities into the mangroves often offset its fragile ecological balance, resulting in eutrophication. In this study, higher phytoplankton densities (Figure S4) coincide with higher phosphate availability, illustrating the relationship between phytoplankton and environmental changes, especially those related to eutrophication. Moreover, the pca biplot analysis has revealed the main nutrient salt that impacted the phytoplankton community structure in the mangroves. Studies have shown that high phytoplankton density in mangrove estuaries is generally controlled by phosphate and nitrogen concentration.[5,6,13,34,40] The spatial fluctuation of nutrient concentrations in the mangroves could be due to the monsoonal intrusion of rainfall along with land runoff[16,41] from urban areas, agricultural farmlands, waste water discharge from aquaculture facilities,[6,39,40] influxes from the sediments,[39,43] oxidation of ammonia and biodegradation of detrital material,[16] and utilization by the mangrove plants[43] and algal cells.[5] Generally, nitrate, phosphates, salinity, and turbidity have a positive correlation with the abundance and distribution of phytoplankton in tropical ecosystems.[5,14,44]

Species dominance and richness in the mangroves depend on environmental variables that would provide a competitive advantage to the specific group. Under natural conditions, diatoms normally contribute the highest percentage compared to other phytoplankton groups.[5,6,34] In this study, diatoms dominated the phytoplankton community (71.90%) but cyanobacteria and green algae formed only 16.35% and 10.70% respectively of the total phytoplankton when environmental variables favored them. Dinoflagellates formed <0.5% of the total phytoplankton species. Diatoms dominated the phytoplankton composition in mangrove estuaries and were influenced by increase pH, salinity, and silicate concentration,[6,34,45] whereas cyanobacteria and green algae were influenced by low salinity and high EC and nutrients concentration.[6] As indicated by the PCA analysis, turbidity, EC, salinity, water temperature, and nitrite were the main factors influencing the phytoplankton community structure in the mangroves. Meanwhile, the inputs of freshwater showed impact on the salinity gradient, which, together with the increased water turbidity, subsequently caused a decline in diatom abundance and favored cyanobacteria and green algae increase. In fact, salinity has been reported as a major factor associated with shifts in plankton community structure in the mangroves.[5,6,14,16,31,44,46]

Phytoplankton size distribution as a function of nutrient availability in the study area has been observed. However, nutrient enrichment alters phytoplankton community composition and distribution in coastal waters.[47] No consistent model relationship between eutrophy and phytoplankton size distributions was established, though Walters et al.[48] and Kiorboe[49] reported that increased nutrient concentrations increased relatively larger cells in phytoplankton populations. Kimor[50] and Huszar et al. suggested that small phytoplankton cells will dominate under conditions of eutrophication. In this study, the dominance of small size taxa was marked by average to a high concentration of phosphate in the water. Generally, the spatial distribution of phytoplankton class sizes across the study area was found to be characterized by nutrient characteristics and salinity/

freshwater gradient. The result of CCA exhibits that algal biomass of different sizes is associated differently with the environmental variables. The test of significance using the Monte Carlo test on CCA highlights the linear relationship between phytoplankton species-environmental variable relations ($p < 0.05$), (Table S6). The eigenvalues and the percentages of variance on axis 1 are found to be higher than axis 2.[22,51] We found that taxon biomass of a size range of 250 to > 1000µm³ correlated positively significantly with pH and salinity, and negatively with nutrients. Meanwhile, the taxon biomass of size range of < 250µm³ correlated positively significantly with EC and nutrients. This class size formed the main food source of copepod during this period. Based on this study, we discover that the class size/nutrient relationship in mangroves is influenced by salinity/freshwater gradient and nutrients.

Phytoplankton richness across the mangrove was $7.31 \geq d \leq 20.63$ indicating a healthy ecosystem (Table S6). However, the positive significant correlation that exists between Simpson's diversity index (1-λ), Shannon diversity index and species richness ($p < 0.01$) indicated that the variation of species diversity in the mangroves coincide with species richness.[40] Comparing the species diversity in this study with other mangroves (Table 4), it's observed that the species diversity at the Guangdong mangrove ecosystems remains richest. Rare and less common taxa constitute the greatest proportion of algal assemblage in the mangroves and may need better conservation practices.

Tables 4 Phytoplankton abundance, diversity and biomass at different mangrove ecosystem

Estuary/region	Phytoplankton Abundance/ Species Richness	Chlorophyll *A*	Biomass (wet weight)	Source
Matang mangrove estuary, Malaysia	NA	< 20 µg.L^{-1} (neap tide); 80 µg.L^{-1} (spring tide)	NA	Tanaka and Choo (2000)
Godavari mangrove estuary, India	NA	12.49 µg.L^{-1}	NA	Tripathy et al., (2005)
Pichavaram mangrove waters, India	1400 - 292,000 cells.L^{-1}; 750 - 321,000 cells.L^{-1}; 400 - 297,000 cells.L^{-1} in different locations	0.24 - 69.82 µg.L^{-1}; 0.20 - 105.60 µg.L^{-1}; 0.20 - 94.80 µg.L^{-1} in different locations	NA	Rajkumar et al., (2009)
Kaduviya estuary, Nagapattinam, India	28,797 - 62,900 cells.L^{-1}; 14,135-74,697 cells.L^{-1} in different locations	3.4 - 9.3 µg.L^{-1}	NA	Perumal et al., (2009)
Sundarbans mangrove forest, Bangladesh	36 species	NA	NA	Aziz et al., (2012)
Kuantan, Pahang, Malaysia	71 species; 7.0 x 10^{4} cells.L^{-1}	NA	NA	Mohammad-Noor et al., (2013)
Mangrove estuary, Philippines	61 species	0.02 - 2.6 µg.L^{-1}	NA	Canini et al., (2013)
Sundarbans mangrove estuaries, Bangladesh	3.755 x 10^{3} - 10^{5} cells.L^{-1}	0.24 - 3.11 µg.L^{-1}	NA	Rahman et al., (2013)
Kuala Nyalau river estuary, Malaysia	113,000 - 147,000 cells.L^{-1}	0.12 - 0.18 mg.m^{-3}	NA	Saifullah et al., (2014)
Eastern Obolo mangrove estuary, Nigeria	Dry season: 84 species, 6959 algal unit; Wet season: 84 species, 4027 algal unit	Dry season: 0.002 - 0.01 mg.L^{-1}, Wet season: 0.002 - 0.02 mg.L^{-1}	NA	Effiong et al., (2016)
South China Sea mangrove ecosystem along Guangdong coast	Wet season: 671 species, 12.56 x 10^{7} cells.L^{-1}	Wet season: 0.14 - 13.53 mg.m^{-3}	Wet season 5.13 x 10^{5} mg.L^{-1}	This study

Conclusion

This study elucidated that the tropical phytoplankton community structure in the mangroves were mainly shaped by environmental fluctuations associated with the rainy season. Diatoms formed the most dominant group, contributing to 71.90% of the total phytoplankton and generating the largest algal biomass in the mangrove ecosystems. Cyanobacteria and green algae formed the next largest group of phytoplankton in the mangroves. The main driving factors influencing the spatial dynamics of phytoplankton species were rainfall, tidal mixing, nutrients, water temperature, turbidity, and EC. The phytoplankton community structure in the study area was characterized by an indicator species associated with catchment characteristics. *C. meneghiniana*, *S. costatum*, *Chaetoceros* sp., *N. palea*, *C. jonesiana*, *T. nitzschioides*, and *Thalassiosira* spp. were dominant in the Leizhou Peninsular characterized by high salinity, pH, and average nutrients. There was a shift in dominance from diatoms to *P. limneticus*, *M. irregularis*, *Microcysris* sp., *S. obliquus*, *Aphanocapsa* sp., *Chrooccocus* sp., etc. at the site where salinity and pH were low while EC and nutrients remained high. An algae cell size/nutrient relationship was established in the mangroves. The small-sized taxa, which were mainly of fresh-water origin, were controlled by high nutrients and EC, whereas the large-sized species were mainly characterized by moderate nutrients, high salinity, and pH. Diatoms and some periphytic cyanobacteria tend to form the bulk of the biomass in this category. Biodiversity community structures revealed that the study area maintains the highest species richness and diversity among the mangrove ecosystems in the world. Further studies are needed to model the selection of phytoplankton species for various environmental conditions and the cell size/nutrient association in the mangrove ecosystems.

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Declaration of competing interest

The author declares that there is no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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