

**ECOLOGICAL STUDIES OF THE SPATIAL AND TEMPORAL DISTRIBUTION OF PHYTOPLANKTON
AT HIGH TIDE WITHIN THE CROSS RIVER ESTUARY, SOUTH-EASTERN NIGERIA**

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Authors' contributions

This work was collaboratively undertaken among both authors. Author OPN designed the study, performed the statistical analysis and managed the literature searches. Author AEM wrote the protocol, wrote the first draft of the manuscript and managed the analyses of the study. The authors read and approved the final manuscript.

ABSTRACT

This study investigated the species composition, abundance, distribution and seasonal occurrences of microalgae (phytoplankton) in the Cross River Estuary. Water samples for physicochemical parameters and microalgae analysis were collected monthly at high tide for six (6) months from three stations within the study area. Water samples were collected using twenty liters (20L) plastic bucket, filtered through 55 μ m plankton net, preserved in four percent (4%) formaldehyde and were stained with 3 ml Lugol's iodine in the laboratory prior to analysis. Data from this study were analysed using ANOVA, descriptive statistics and regression analysis. A total of 41 species of microalgae belonging to five (5) taxonomic groups were identified. The groups of microalgae identified during this study were arranged according to their species richness and followed the order of dominance: Bacillariophyceae > Chlorophyceae > Dinophyceae > Cyanophyceae > Euglenophyceae. There was no significant difference in the abundance of microalgae within the three stations ($P > 0.05$) but there was a significant difference in the abundance of microalgae between the two seasons ($P < 0.05$). Diversity, during this study, is considered to be very low. The abundance of microalgae significantly correlated with the concentrations of silicates ($r = 0.763$, $P < 0.05$), nitrates ($r = 0.724$, $P < 0.05$), and phosphates ($r = 0.834$, $P < 0.05$). Diversity, during this study, is considered to be very low. Besides nutrients, the composition and abundance of microalgae is to a greater extent affected by tide.

Keywords: Microalgae, Physicochemical parameters, Cross River Estuary, Tide.

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INTRODUCTION

Plankton are the most important fragment of the aquatic ecosystem. They are microscopic organisms that swim freely, in the aquatic ecosystem, drifting in the surface in the direction of current, wind and/or wave. Coastal and estuarine systems are extremely sensitive to eutrophication which leads to changes in plankton composition in the area.

Phytoplankton are primary producers, they constitute the starting point of energy transfer (Ekwu and Sikoki, 2006) while the zooplankton creates a link between the primary producers and consumers of the aquatic ecosystem (Shikha and Malik, 2015). Due to various sources of anthropogenic and natural input of materials including nutrients, (Azma and Anis, 2016, Dai, *et al.*, 2014), urbanized estuaries are particularly vulnerable to pollution (Akpan, 2015) which can affect the abundance and distribution pattern of plankton species.

Nutrients like nitrogen and phosphorus affects algae, protozoa and bacteria which serve as prey of zooplankton, indirectly affecting zooplankton survival (Marine Bio Conservation Society, 2017). The phytoplankton community consists of both toxic and non-toxic species, some toxic algal species have negative effects on egg production, hatching success and total productive output of zooplankton grazers (Barreiro, *et al.*, 2007).

MATERIALS AND METHODS

Study Area

Cross River Estuary is located at Latitude 4⁰30'N and 5⁰15'N and Longitude 8⁰00'E and 8⁰30'E (Fig. 1). The vegetation is noticeably dominated by nypa palm (*Nypa frutican*) and mangroves; red mangrove (*Rhizophora racemosa*), and white mangrove (*Avicennia africana*). It passes through many communities. Many anthropogenic activities going on within and around the Cross River Estuary include dredging, fishing, boating, navigation, and swimming, to mention but a few. This aquatic body receives effluent discharges from many industries and residential houses sited close to it. The climate is characterized by a long wet season from April to November and a dry season from November ending to March with a mean annual rainfall of about 2000mm (Akpan and Ofem, 1993). Air temperature generally range from 22°C in the wet season to 35°C in the dry season, with a relative humidity above 60% at all seasons (Akpan and Ofem, 1993). Its major tributaries are the Calabar River, Great Kwa River and the Akpa Yafe (Akpan, 2015).

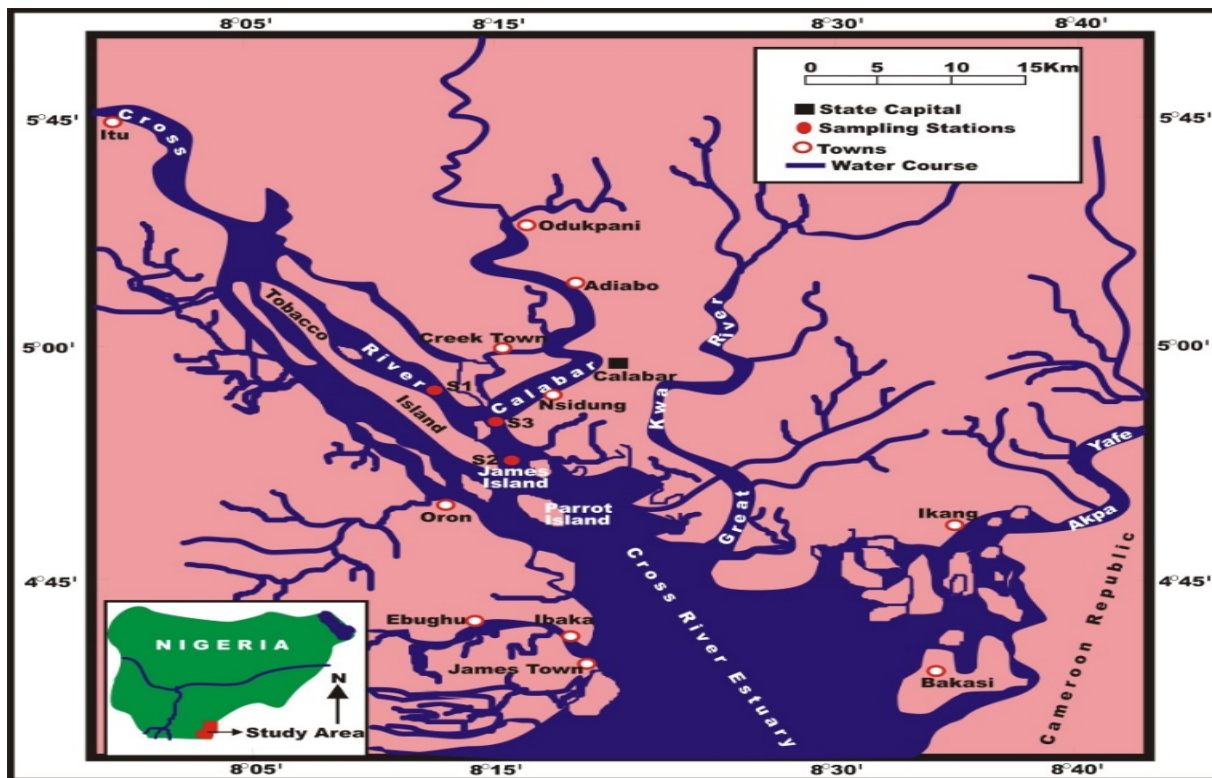


FIG. 1: Map of Cross River Estuary showing the Sampling Stations

Sampling Stations and Samples Collection

Three sampling stations (1-3) were chosen along the shoreline of the Estuary. The coordinates of each station were obtained using Geographic Positioning System (GPS) as shown in (Table 1). Water samples for this study were collected from each station for six (6) months. Samples were collected once every month at high tide from the different stations using a 20 liters bucket and was filtered through a 55µm mesh size plankton net. The filtrates were transferred into 20 ml properly labeled sterile plastic containers and fixed with 4 percent formaldehyde.

TABLE 1

Sampling Stations And Coordinates

Stations	COORDINATES	
	LATITUDE	LONGITUDE
Station 1	4°54'56.52" N	8°14'28.52" E
Station 2	4°54'16.44" N	8°15'56.96" E
Station 3	4°52'48.96" N	8°15'48.95" E

Samples Analysis

Water temperature, pH, dissolve oxygen (DO), Nitrate and phosphate of the river were measured in situ from January 2017 to June 2017 and sampling was done on a monthly basis in triplicate. Temperature was measured using a mercury in glass thermometer. pH was measured using Jenway pH meter. DO, Nitrate and Phosphate were determined by methods described by APHA/AWWA and WEF (1995). Other parameters such as Salinity (ppm), Silicate (mg/l) and Ammonium (mg/l) were measured in the Institute of Oceanography Laboratory, University of Calabar, Calabar. Samples for Phytoplankton studies were equally carried out in the Laboratory, the samples were allowed to settle after which they were stained with 3 ml Lugol's iodine. 1 ml each of the homogenate samples were dropped on a microscopic slide and viewed under a compound microscope at 80, 100 and 200X magnifications using the X10 magnification lens. Identification was done using different taxonomic keys by Newell and Newell (1966), Maochlan (1983) and Ward and Whipple (1959). Ecological index (Margalef's and Simpson's Indices) of phytoplankton identified during this study was determined according to Ogbeibu (2005).

Data Analysis

The data collected were put through descriptive statistic for mean, standard deviation and range values. Analysis of Variance (ANOVA) was applied to test for significant difference, effect with probability of $P=0.05$ was regarded significant. Regression analysis (r) was used to ascertain the relationship between the physico-chemical parameters and the abundance of phytoplankton in the different sampling stations.

Results and Discussion

Microalgae species identified during the study

Forty-one species of microalgae, ranging from 27 species of Bacillariophyceae, six species of Chlorophyceae, four (4) species of Dinophyceae, three (3) species of Cyanophyceae and a species of Euglenophyceae, were identified and arranged in their order of species richness .

The taxonomic listing, distribution and abundance of the microalgal species identified during this study are shown in Table 2.

Table 2
Taxonomic listing, distribution and abundance of the microalgal species identified during the study

S/N	Family	Species	Stations			Total
			Station1 Density /ml	Station2 Density /ml	Station3 Density /ml	
	Bacillariophyceae					
1		<i>Actinocyclus sp.</i> Ehrenberg	-	-	1	1
2		<i>Amphipleura sp.</i> Kützing	-	2	-	2
3		<i>Amphiprora sp.</i> Ehrenberg	7	2	4	13
4		<i>Aulacodiscus orientalis.</i> Ehrenberg	1	-	-	1
5		<i>Bacillaria paradoxa.</i> Gmelin	-	-	1	1
6		<i>Biddulphia aurita.</i> (Lyngbye) Brébisson	11	88	-	99
7		<i>Biddulphia sinensis.</i> Greville	87	21	58	166
8		<i>Chaetoceros sp.</i> Ehrenberg	4	-	-	4
9		<i>Coscinodiscus sp.</i> Ehrenberg	39251	10628	10460	60339
10		<i>Cyclotella cumta.</i> (Kützing) Brébisson	223	230	506	959
11		<i>Cylindrotheca closterium.</i> (Ehrenberg) Reimann & Lewin	40	1	234	275
12		<i>Cymbella sp.</i> Agardh	2	1	58	61
13		<i>Ditylum brighwelli.</i> (T.West) Grunow	115	9	47	171
14		<i>Ditylum thermalis</i>	2595	333	731	3659
15		<i>Gomphonema acuminatum.</i> Ehrenberg	2	-	1	3
16		<i>Gyrosigma acuminatum</i> (Ehrenberg) Hassall	33	132	451	616
17		<i>Melosira granulata</i> Agardh	3	-	3	6
18		<i>Navicula sp.</i> Bory	5	-	51	56
19		<i>Nitzschia sp.</i> Hassall	1	86	12	99
20		<i>Pinnularia sp.</i> Ehrenberg	433	5	120	558
21		<i>Pleurosigma estuarii.</i> Smith	40	16	67	123
22		<i>Skeletonema costatum.</i> (Greville) Cleve	53922	29669	78803	162394
23		<i>Stauroneis sp.</i> Ehrenberg	1	1	1	3
24		<i>Stephanodiscus astrea.</i> (Kützing) Grunow	4	-	383	387
25		<i>Surirella sp.</i> (Kützing) Turpin	1216	778	53	2047
26		<i>Tabellaria sp.</i> Ehrenberg ex Kützing	6	3	32	41
27		<i>Triceratium sp.</i> Ehrenberg	6	74	3	83
						232167
	Chlorophyceae					
28		<i>Actinastrum sp.</i> Lagerheim	-	1	-	1
29		<i>Eudorina elegans.</i> Ehrenberg	1	-	1	2
30		<i>Pediastrum sp.</i> Meyen	9	2	36	47
31		<i>Scenedesmus sp.</i> (Lagerheim) Chodat	18	2	156	176
32		<i>Staurastrum sp.</i> Meyen ex Ralfs	4	3	153	160
33		<i>Tribonema vulgare.</i> Pascher	5	2	1	8
						394
	Dinophyceae					
34		<i>Ceratium furca</i> (Ehrenberg) Claparède et Lachmann	1	58	145	204
35		<i>Ceratium fusus</i> (Ehrenberg) Dujardin	1	56	138	195
36		<i>Peridinium bipes</i> Ehrenberg	13	2	3	18
37		<i>Gymnodinium breve</i> Stein	-	-	2	2
						419
	Cyanophyceae					
38		<i>Aphanothece sp.</i> Nägeli	1	-	-	1
39		<i>Microcystis pulverea</i> Kützing	1	-	-	1
40		<i>Oscillatoria rubescens</i> De Candolle ex Gomont	-	3	-	3
						5
	Euglenophyceae					
41		<i>Phacus acus.</i> Ehrenberg	1	1	-	2
						2
	Total (N)		90863	42209	92715	232987
	No. of Species (S)		35	29	32	
	Margalef's Index(d)		2.87	2.72	2.71	
	Simpson's Diversity Index (D)		0.46	0.56	0.74	

Bacillariophyceae (diatoms) was the most prevalent group of microalgae and similar records have been observed in Nigerian coastal waters by Essien-Ibok (2013) in Mbo River, Eyo *et al.* (2013) in the Great Kwa River and Akpan (2015) in Cross River. *Skeletonema costatum* was the highest in abundance and occurrence. It occurred in all the three Stations sampled and constituted 162,394 (69.7 percent) out of the total abundance of 232,992. This was followed by *Coscinodiscus sp.* with the numerical abundance of 60,339 (25.9 percent). Diatoms were more in Stations one and three and this may be attributed to higher concentrations of silicates at these Stations as stated by Ekwu and Sikoki (2006). The Chlorophyceae were more abundant in Station three during the rainy season. This can be linked to decreased salinity due to fresh water intrusion during the season. The Dinophyceae were more abundant at Station three than in the other Stations. Cyanophyceae recorded only five individuals while Euglenophyceae was represented by an individual organism throughout the sampling period. In this study, a strong correlation existed between silicates and the abundance of microalgae.

The present study shows a variant in order of dominance from those of Tiseer, *et al.* (2008) and Eyo, *et al.* (2013). Diatoms were the most dominant group of microalgae while euglenoids were the least and this might be due to low concentrations of nitrate and phosphate during this study at high tide. The distribution of microalgae in this study can be linked to high tide with increase in nutrient level and organic pollution. Davis and Ugwumba (2013) reported that the distribution and high abundance of diatoms shows they can thrive even with increased anthropogenic inputs at high tide. Population fluctuations are driven by the relationship between the internal population processes and the external environmental factors (Blauw *et al.*, 2012, Brazell, 2009).

“Tides cause a pattern of stabilization-destabilization in circulation that results in the highest rates of primary production in many estuarine, coastal, oceanic and frontal environments” (Zimmermann, 2013). “The destabilization period occurs during flood and ebb tides resulting in the replenishment of nutrients to the photic zone from more nutrient-rich underlying waters” (Davis and Ugwumba, 2013).

Density and abundance of microalgae

The density and abundance of microalgae during this study was very high when compared with other studies in Nigerian coastal waters. The recorded densities and abundance is due to the high abundance of diatoms showing their resilience to anthropogenic input and high turbidity at high tide. They were at their peak during the dry seasons when the nutrient concentrations were high. This and the presence of these harmful species can be attributed to lack of rainfall during the dry season and tide which brings in water from the sea and increases anthropogenic inputs.

Diversity index

Station one had the highest Margelef’s index (d), followed closely by Stations two and three while the highest value for Simpson’s diversity index (D) was recorded in Station three, then Stations two and one. The species diversity was low and the microalgal species identified during this study were evenly distributed within the three sample stations. This can be due to increased turbidity caused due to tidal turbulence within the estuary.

Seasonal variations in Microalgae abundance

At 5% level of significance, there was no significant difference in the abundance of microalgae within the three (3) stations but there was a significant difference in the

abundance of microalgae within the two seasons. This means that microalgae, during this study, was evenly distributed within the three sampled stations but the abundance was higher during the dry season than in the rainy season.

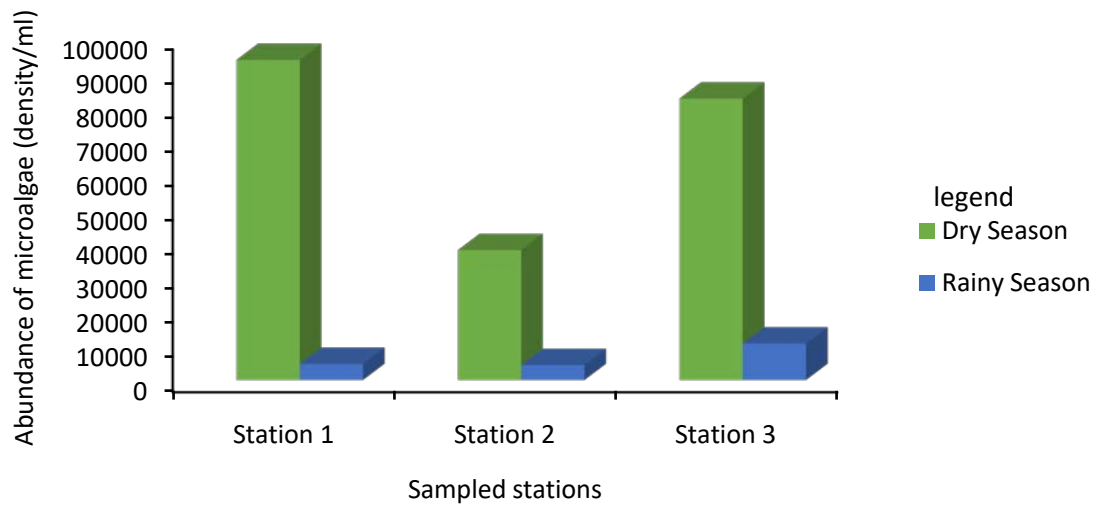


FIG. 1. Seasonal variations in microalgal abundance during the two seasons

Physicochemical parameters

The mean values and Ranges of each parameter is represented in (Table 2). The temperature value was from 29.56 ± 0.73 and 30.72 ± 0.75 , best microalgal growth is between 20°C and 30°C . pH was within the range of 6.54 ± 0.59 and 6.58 ± 0.57 and diatoms can thrive even at pH lower than 7.6. Dissolved oxygen ranged between 4.50 ± 0.73 and 5.97 ± 1.27 , the decrease in concentration of dissolved oxygen during the rainy season might be due to the input of oxygen demanding waste. Salinity concentrations was higher during the dry season (7.45 ± 3.57) while rainfall and the inflow of freshwater from the river caused a significant drop in salinity (1.94 ± 1.86). Nitrates was almost insignificant ranging from 0.23 ± 0.17 in the dry season to 0.56 ± 0.16 in the wet season. the slight increase in nitrate concentrate during the rainy season might be due to surface runoff from farms where fertilizers are used.

Diatoms thrive with increased silicate concentrations, silicate ranged from 2.13 ± 0.59 during the rainy season to 4.15 ± 0.79 during the dry season. Ammonium ranged between 0.067 ± 0.52 and 3.08 ± 2.25 during this study, green algae and cyanobacteria prefer ammonium to nitrate so their low concentration might be the reason behind the low species as well as density of green algae and cyanobacterial cells identified during this study. Phosphates being the primary limiting factor in phytoplankton growth ranged from 0.81 ± 0.58 (wet season) to 1.18 ± 0.67 (dry season). Increased phytoplankton abundance during the dry season might be due to the slight increase in phosphate during the season.

Table 2
 Variation in mean values of physicochemical parameters in each sampling station

Parameters	Station 1	Station 2	Station 3
Temperature (°C)	30.60 ± 0.92	30.00 ± 1.10	29.85 ± 0.75
pH	6.48 ± 0.52	6.66 ± 0.72	6.58 ± 0.50
DO(mg/l)	5.20 ± 1.57	5.62 ± 1.14	4.88 ± 1.13
Salinity(ppm)	4.90 ± 3.99	4.76 ± 4.44	4.39 ± 4.17
Nitrate(mg/l)	0.40 ± 0.28	0.42 ± 0.23	0.39 ± 0.25
Silicate(mg/l)	3.25 ± 1.29	2.91 ± 1.21	3.20 ± 1.46
Ammonium(mg/l)	1.83 ± 2.21	1.95 ± 2.20	1.85 ± 2.01
Phosphate(mg/l)	0.60 ± 0.80	0.69 ± 0.76	0.61 ± 0.79

Microalgal abundance had a significant positive relationship with phosphate ($r = 0.834$, $P < 0.05$) and silicate ($r = 0.763$, $P < 0.05$) but had a significant negative relationship with nitrate ($r = 0.724$, $P < 0.05$) (Fig. 2). This means that, during this study, about 69% of the variation in abundance of microalgae was due to concentration of phosphate, 58% was due the concentration of silicate while 52% was due to the concentration of nitrate.

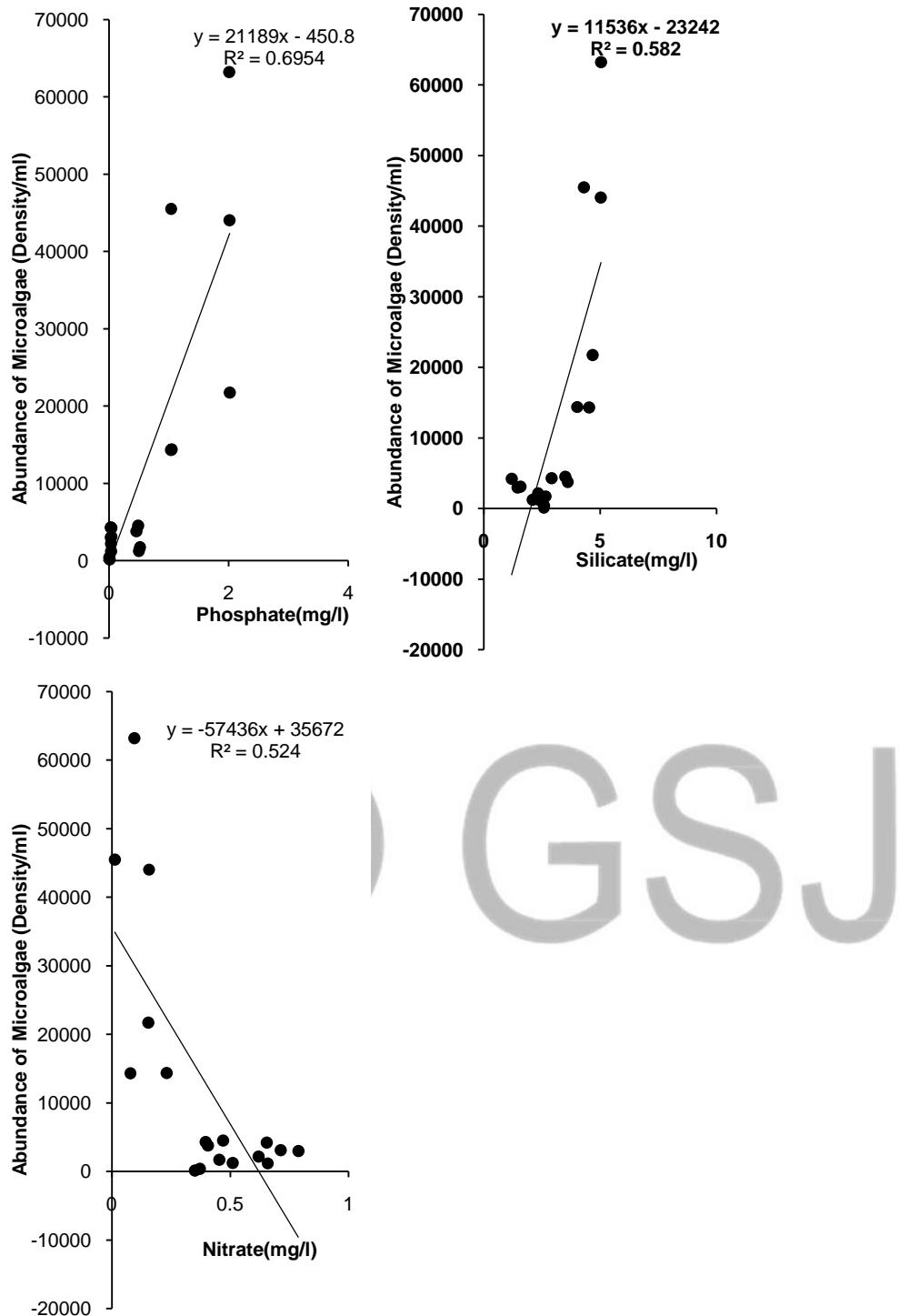


FIG. 2: Relationships between the abundance of microalgae phosphate, silicate and nitrate

Discussion of the physicochemical parameters

Temperature

Growth in phytoplankton is at its maximum when temperature is within the range of 20 °C and 30 °C. The highest temperature of 32 °C was observed during the dry season and the lowest 29 °C in the rainy season, these fluctuations in temperature as observed during this study might be due to the weather condition at the time of sampling.

pH

Relative rate of photosynthesis changes with pH and diatoms can thrive the best at lower pH when compared to dinoflagellates which thrives best with increased pH (Chen *et al.*, 1994). There was no seasonal variation in the hydrogen ion concentrations during this study.

Dissolved oxygen

The concentrations of dissolved oxygen dropped during the rainy season, this can be attributed to the input of oxygen demanding wastes from the river. As observed during this study, the abundance of microalgae increased with increase in dissolved oxygen concentration.

Salinity

Due to salt water intrusion from the ocean, salinity was at its peak during the dry season while during the rainy season, the inflow of freshwater from the rivers and rainfall led to the dilution of the Cross River Estuary leading to a significant drop in salinity. *Skeletonema costatum* has been reported to grow between salinities of 0 to 30 ppm (Balzano *et al.*, 2011).

Nitrate and Ammonium

These are the most nitrogen sources for phytoplankton growth. Nitrogen is a limiting factor for phytoplankton growth and nitrate concentrations were very low during this study. This might be the reason the abundance of other phytoplankton groups was insignificant when compared to diatoms. The concentrations of ammonium were high in the dry season, a trend of decreasing nitrate uptake with increasing ammonium concentrations shows ammonium is preferred especially in green algae and cyanobacteria.

Phosphates

These are the primary factor limiting growth in microalgae and were within the same range in the three Stations sampled. Increase in phosphorus leads to increased total phytoplankton biomass.

Silicates

Increase in silicate concentrations leads to increase in diatoms. Low concentration of silicates during the rainy season might be attributed to dilution from rainfall (Akpan & Offem, 1993), this might be the reason behind the significant decrease in the abundance of diatoms as observed during this study.

Summary

This study has shown that high tide as well as nutrient concentrations has a great influence on the composition and distribution of microalgae. Diatoms were the most abundant and are good indicators of organic pollution. During and following high tide, there is a shortage of light into the estuary due to increased turbidity and phytoplankton mixing which in turn leads to decreased photosynthesis but the diatoms can adapt and thrive even with these changes. This might be the reason why the abundance of the other families was insignificant when compared with that of the diatoms and increased concentrations of *Skeletonema costatum* and *Coscinodiscus sp.* (diatoms) can impact the aquatic ecosystem

adversely. Also, Salinity in estuaries are raised by the intrusion of salt water from the ocean at high tide and is diluted with the inflow of freshwater from rivers and rainfall.

Conclusion

Although no bloom was recorded during this study, our environments are changing and the dynamics of abundance and distribution of these organisms may change subsequently in line with alterations in our environment. *Skeletonema Costatum* and *Coscinodiscus sp.* (diatoms) were the most abundant and are potentially harmful, causing discolouration of the water body, oxygen depletion, can form oily film on the water surface and may produce chemicals that are similar to phosphorus and nitrogen thereby blocking important biochemical pathways in algae.

Recommendations

“So, to capture these natural changes,”

- i. High resolution monitoring is required and can be a helpful step towards the detection and prediction of the long-term response of coastal microalgae to our ever changing environmental conditions.
- ii. Further studies should be carried out on the effects of tide (high and low tides) on our water bodies.

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