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# Phytoplankton Community Response to Seasonal Changes in Chlorophyll a and Nitrate-Phosphate Concentrations in a Tidal Blackwater River in Niger Delta

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### Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

### Article Information

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**Original Research Article** 

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### ABSTRACT

The study evaluated the seasonal abundance of standing stock of phytoplankton groups in a tidal blackwater system of the New Calabar River in Nigeria. The focus was on observable patterns in phytoplankton community structure as it relates to the variations in the supplies of production such as Chlorophyll *a* and growth-limiting nutrients such as Nitrate and Phosphate. The results showed there were significant seasonal variation in the cell counts of the dominant family groups such as Bacillariophyceae (70%:90%) and Chlorophyceae (3%:30%) to total phytoplankton between wet and dry season respectively. The results also produced a community structure composed of four groups with a consistent seasonal dominance order of Bacillariophyceae> Chlorophyceae> Cyanophyceae> Dinophyceae. The observation contrasts with studies of blackwater systems which has at varying levels other groups such Euglenophyceae, Charophyceae, Chrysophyceae, Cryptophyceae, Haptophyceae and Rhodophyceae. The three parameters of Nitrate, Phosphate and Chlorophyll *a* evaluated to explain the abundance in cell counts provide contrasting differences

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in their relationship to each of the phytoplankton groups between seasons. The results showed that seasonal changes in cell numbers were inversely related with changes in chlorophyll *a* concentration which suggests that it is not a reliable indicator of increase in cell numbers of the Phytoplankton groups within the study area. In comparison the Phosphate and Nitrate components had a moderate positive correlation with phytoplankton cell standing stock. This observation was interpreted as a probable effect of uptake and drawdown ratios of bioavailable nitrates and phosphate by phytoplankton in proportions that allow only marginal increase in Chlorophyll a production.

Keywords: Phytoplankton community; black water; nitrate-phosphate ratio; chlorophyll a.

#### **1. INTRODUCTION**

Phytoplankton are single celled microscopic plants that inhabit the pelagic zones of aquatic systems. These organisms are considered as keystone species [1,2,3], hence they determine the survivability of higher organisms in their habitat. In the food chain, Phytoplankton are the primary producers, meeting the nutrient and energy demand of higher organisms being able to reproduce rapidly and blossom extremely when conditions are favourable [4,5]. As a biological indicator of the aquatic health, reports by Onyema [6], Chellappa, et al. [7], and Nweze, et al. [8] have shown that their abundance, richness, diversity and distribution can be used for water quality assessment. In the Niger Delta studies of Phytoplankton community structure and standing stock have been conducted in several river systems [9,10,11]. Obienefo, et al. [12] examined the water quality and phytoplankton community of a stream system receiving municipal waste discharges in Rivers State; while Abowei, et al. [13] documented the temporal variations of the Phytoplankton of the Sombreiro river. Other studies such as Ebigwai, et al. [14] examined the physico chemical parameters and phytoplankton assemblages along spatial and temporal gradients in great Kwa River. Within the study area of the New Calabar River Nwadiaro and Ezefili [15] provided a preliminary checklist of the phytoplankton of the tidal portions while Erondu and Chindah [16] studied the variations in the physicochemical features and phytoplankton community in another section of the river. The current research is focused on how the patterns in phytoplankton abundance relates to the temporal variations in Chlorophyll a (Chl a) concentration and growthlimiting nutrients such as Nitrate and Phosphate.

#### 1.1 Study Area

The study area is located on the confluence between Bonny River and the New Calabar River systems as shown in Fig. 1.

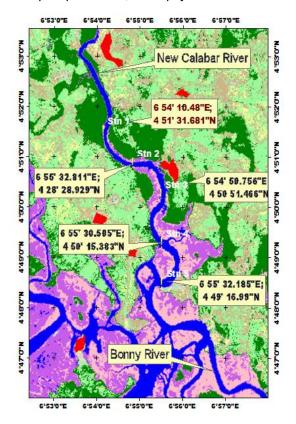


Fig. 1. Study area showing the location of stations and coordinates

The area of study is located between geographic coordinates 6° 54' 10.48"E; 4° 51' 31.681N upstream at station 1 and  $6^{\circ}$  55' 32.811" E and  $4^{\circ}$ 28' 28.929"N downstream at station 5. The study area is bounded by a mixture of landuse and landcover types such as mangroves, urban and freshwater swamp forest which has dominant species such as Annona senegalensis. Anthocleista vogelii, Elaeis guineensis; Harungana madagascariensis and Musanga cecropioides. The physicochemical characteristics of this study area has been well documented in a number of studies [17,18,19] showing the water to be a typical tidal blackwater in the rainy season to tidal clear water in the dry season. Typically pH range from 5.8 in the wet season to 7.5 in the dry season. Upstream a number of companies are establishing jetties along the banks of the river. The tide-dominated river distributes discharges from servicing companies that are located approximately 10 kilometers upstream.

### 2. METHODOLOGY

Water was collected in the wet season (June, July August) and dry season months (December 2015, January and February 2016) from five stations at 1 kilometer apart. Collections were 20-Madhurima Gayen cm below the surface with a 2-L discrete sampling bottle closed by a messenger along a gradient of 5 km. The collection was hand-mixed and a 250-ml subsample removed. The subsample was treated with 5 ml of lugol solution. Lugol solution both preservers the samples and stains the organisms facilitating later laboratory work. Preserved samples were placed on ice, returned to the Laboratory and stored in the dark. The laboratory procedure involved concentrating the 250 ml subsample to 15 ml by allowing the sample to settle. From the concentrated sample 1-ml subsamples were pipetted individually to a Sedgewick-Rafter counting chamber using Stempel pipettes. Microscopic counts were made of all the cells for each slide for each station. The procedure was repeated for three subsamples from each sample. Identifications were made to genus and species where possible. The abundance of each taxon was recorded and used to compute the number of cells per litter (cells/liter).

Chlorophyll a samples which were taken from near surface (20 cm) were stored in ice and in the laboratory were filtered with a 5 -micrometer Millipore membrane filter through vacuum filtration. The filtrate was transferred to a test tube containing 90% acetone. After cooling for 24 hours the sample was macerated in a grinder and centrifuged for 15 minutes after which MgCO<sub>3</sub> was added to the tubes. The optical density of the resultant supernatant was measured on spectrophotometer at the wavelength of 663 nm and 750 nm using acetone as a reference. Between the readings the sample was transferred to 25 µl of 2N HCl and centrifuged for one minute. The absorbance was again measured at the previous wavelengths of 650 and 750 nm. Chlorophyll a (µg/l) were calculated as referenced in APHA [20].

Phosphate in the water sample was determined with APHA 4500-PC test method; a colorimetric method based on the formation of a yellow complex under acidic condition in the presence of Vanadium with the intensity of the yellow colour being proportional to Phosphate concentration. The sample was analyzed at a 470 wavelength of nm with UV-Spectrophotometer - DR2000. Nitrate in the water samples was determined by Cadmium Reduction Method (APHA 4500-NO3-E) using UV-Spectrophotometer \_ DR2000 at а wavelength of 543 nm.

Statistical tools in SAS JMP software such as ANOVA, Students t-test, regression and correlation were used to evaluate variation in seasonal abundance among the phytoplankton groups and also relationships between Chl *a*, Phosphate and Nitrate to the abundances for each group.

### 3. RESULTS

### 3.1 Seasonal Dynamics

### 3.1.1 Phytoplankton

Figs. 2 to 5 show within season variation among the phytoplankton groups in the wet season (June, July August) and dry season months (December 2015, January and February 2016). In Fig. 2 the standing crop of the Bacillariophyceae ranged from 34,000 and 95,000 cells/L ( $61.6 \pm 24.8$ ) in June 2015; 12,000 and 87,000 cells/L ( $35.8 \pm 30.3$ ) in July 2015 and 12,000 and 78,000 cells/L ( $47.8 \pm 29.1$ ) in August 2015. The abundance values in Fig. 2 for the dry season Bacillariophyceae ranged between 56,000 and 87,000 cells/L ( $73.2 \pm 12.5$ ) in December 2015; 5000 and 76,000 cells/L ( $24.8 \pm$ 29.3) in January 2016 and 6000 and 45,000 cells/L ( $21 \pm 17.5$ ) in February 2016.

In Fig. 3 the wet season standing abundance of the Chlorophyceae ranged from 3000 and 21,000 cells/L (11.4  $\pm$  6.88) in June 2015; 2000 and 14,000 cells/L (5.6  $\pm$ 4.98) in July 2015 and 4000 and 17000 cells/L (9.8  $\pm$ 5.81) in August 2015. In the dry season the abundance of the Chlorophyceae ranged from 5000 and 65,000 cells/L (23.4  $\pm$  26.2) in December 2015; 4000 and 25000 cells/L (14.2  $\pm$  9.86) in January 2016; and 9000 and 14000 cells/L (11.8  $\pm$  2.28) in February 2016. In Fig. 4 the wet season standing abundance for Cyanophyceae ranged from 5000 and 12000 (7.8  $\pm$  3.11) in June 2015; 2000 and

12000 cells/L (6.8  $\pm$  4.15) in July 2015 and 3000 and 5000 cells/L (5.4  $\pm$  3.78) in August 2015. The dry season abundance of the Cyanophyceae ranged from 7000 and 21,000 cells/L (15  $\pm$  6.04) in December 2015; 0 and 54,000 cells/L in January 2016; and 2000 and 31,000 cells/L in February 2016. In Fig 5 the wet season standing abundance for the Dinophyceae ranged from 0 and 3000 cells/L  $(1.2 \pm 1.30)$  in June 2015; 0 and 1000 cells/L  $(0.2 \pm 0.45)$  in July 2015 and 0 and 1000 cells/L  $(0.2 \pm 0.45)$  in August 2015. The dry season abundance of the Dinophyceae ranged from 0 and 7000 cells/L in December 2015; 0 and 6000 cells/L in January 2016 and 0 and 1000 cells/L in February 2018.

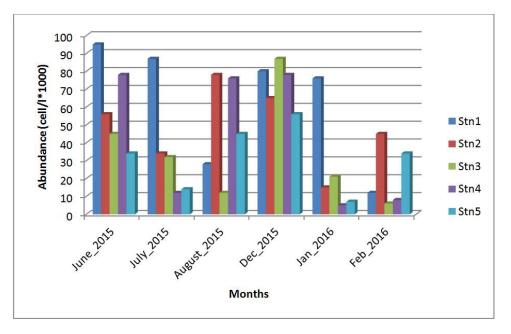


Fig. 2. Variation of abundance of Bacillariophyceae in the wet and dry season

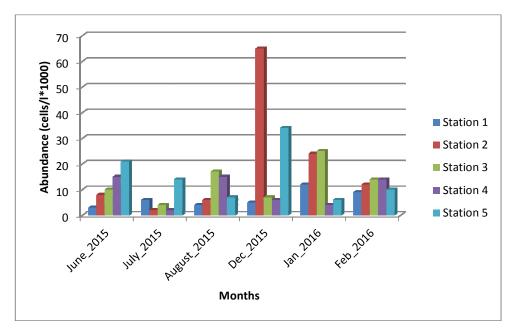


Fig. 3. Variation of abundance of Chlorophyceae in the wet and dry season

Onwuteaka and Choko; ARRB, 21(6): 1-19, 2017; Article no.ARRB.38456

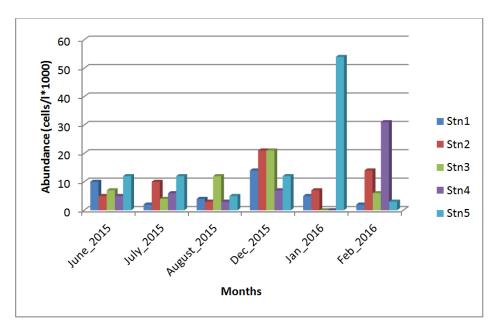


Fig. 4. Variation of abundance Cyanophyceae in the wet and dry season

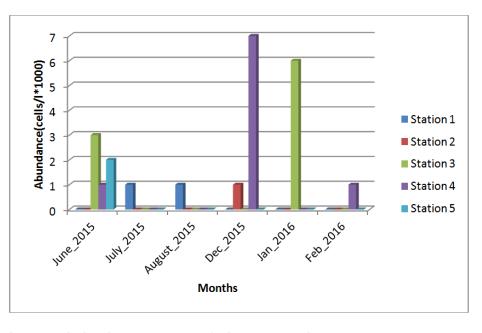
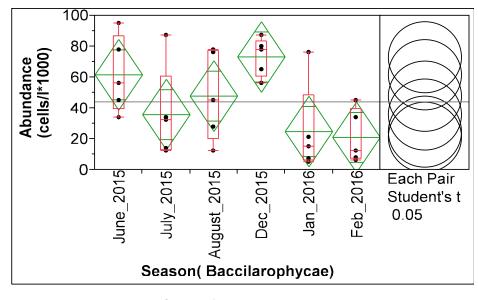


Fig. 5. Variation in abundance of Dinophyceae in the wet and dry season

Fig. 6 shows the abundance values of Bacillariophyceae with mean diamonds that are above and below the grand mean respectively. The overlap of the mean diamonds which represents the confidence intervals provides the evidence of the differences between the group means at the given confidence level. The summary statistics of the Students t analysis shows a significant difference between the group of January and February 2016 and the group of June, August and December 2015. A significant difference was also observed between July 2015 and December 2015.

Fig. 7. shows the abundance values of Chlorophyceae with mean diamonds that are above and below the grand mean respectively. The overlap of the mean diamonds which represents the confidence intervals provides the first evidence that the difference between the group means were not significantly different at the given confidence level. The summary statistics of the Students t analysis shows that the p value of <0.05 supports the fact that the

significant differences was not substantive between the variance estimates among the seasons. However there is a significant difference between December 2015 and January 2016 due to the non-overlap of the Confidence interval as indicated by the mean diamonds.

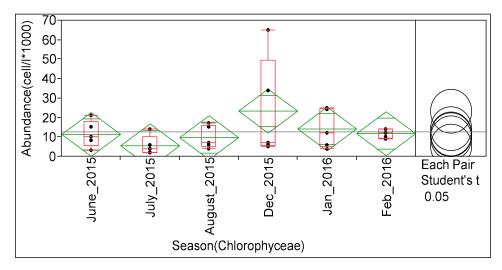


#### **Connecting Letters Report**

Level				Mean
Dec_2015	А			73.2
June_2015	А	В		61.6
August_2015	А	В	С	47.8
July_2015		В	С	35.8
Jan_2016			С	24.8
Feb_2016			С	21.0

Levels not connected by same letter are significantly different

### Fig. 6. One-way ANOVA of wet and dry season season's total phytoplankton abundance

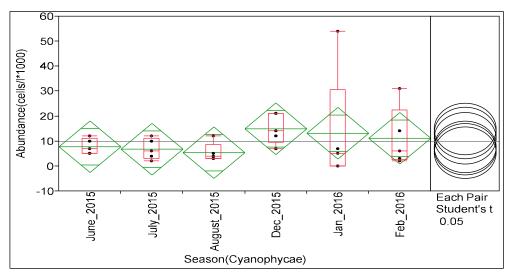


Level			Mean
Dec_2015	А		23.4
Jan_2016	А	В	14.2
Feb_2016	А	В	11.8
June_2015	А	В	11.4
August_2015	А	В	9.8
July_2015		В	5.6

#### **Connecting Letters Report**

Levels not connected by same letter are significantly different

### Fig. 7. Analysis of Variance of Chlorophyceae abundance in the wet and dry season



#### **Connecting Letters Report**

Level		Mean
Dec_2015	А	15.0
Jan_2016	А	13.2
Feb_2016	А	11.2
June_2015	А	7.8
July_2015	А	6.8
August_2015	A	5.4

Levels not connected by same letter are significantly different.

#### Fig. 8. Analysis of Variance of Cyanophyceae abundance in the wet and dry season

Fig. 8. shows the abundance values of Cyanophyceae with mean diamonds that are above and below the grand mean respectively. The overlap of the mean diamonds which represents the confidence intervals provides the evidence of no significant differences (p= 0.05) between the group means at the given confidence level. Similarly in Fig. 9 are shown the abundance values of Dinophyceae with mean diamonds that are above and below the grand mean. The overlap of the mean diamonds which represents the confidence intervals provides evidence of no significant differences (p= 0.05)

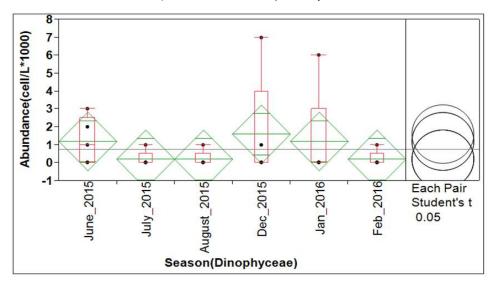
between the group means at the given confidence level.

#### 3.1.2 Phytoplankton composition

Figs. 10 to 13 present the wet and dry season bivariate charts of the relative abundance of each phytoplankton family in relation to total phytoplankton. In Fig. 10 the contribution of Bacillariophyceae to total phytoplankton in the wet and dry season were 70% and 90% ( $r^2 = 0.69$  and 0.91) respectively. In Fig. 11 the Chlorophyceae contributed 3% and 30% ( $r^2$ 

=0.03 and 0.3) to total phytoplankton in the wet and dry season respectively; the contribution of the Cyanophyceae (Fig. 12) to total phytoplankton was 7% and 9% ( $r^2$  =0.07 and

0.09) in the wet and dry season; while the Dinophyceae (Fig. 13) contributed 2% and 1% ( $r^2$  =0.02 and 0.02) in the wet and dry season respectively.



#### **Connecting Letters Report**

Level		Mean
Dec_2015	A	1.6
June_2015	A	1.2
Jan_2016	A	1.2
July_2015	A	0.2
August_2015	A	0.2
Feb_2016	А	0.2

Levels not connected by same letter are significantly different

Fig. 9. Analysis of Variance of Dinophyceae abundance in the wet and dry season

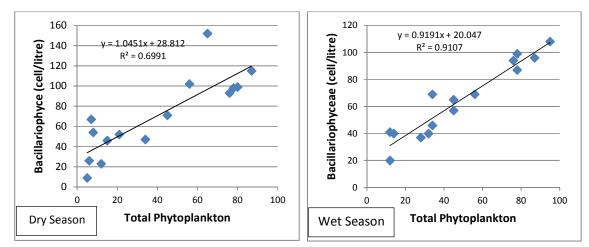


Fig. 10. Regression coefficient of relative abundance of Bacillariophyceae to total phytoplankton

Onwuteaka and Choko; ARRB, 21(6): 1-19, 2017; Article no.ARRB.38456

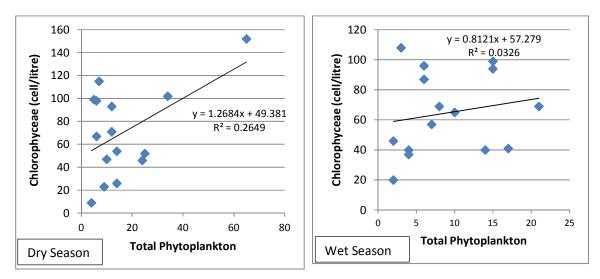


Fig. 11. Regression coefficient of relative abundance of Chlorophyceae to total phytoplankton

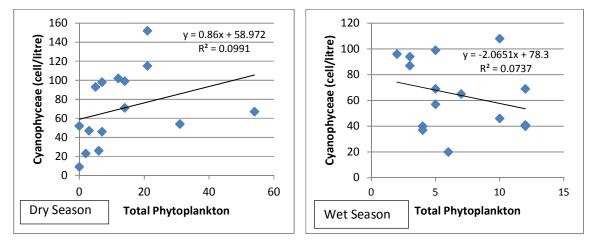


Fig. 12. Regression coefficient of relative abundance of Cyanophyceae to total phytoplankton

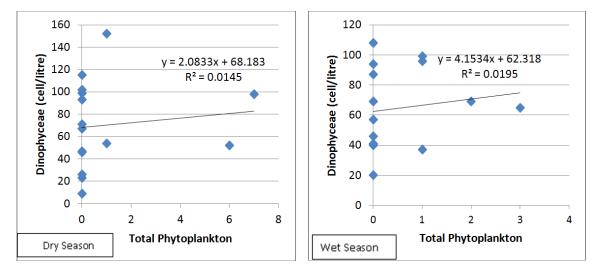


Fig. 13. Regression coefficient of relative abundance of Dinophyceae to total Phytoplankton

### 3.1.3 Physicochemistry

Figs. 14 to 16 show the concentrations of Chlorophyll *a* (Chl *a*), Nitrate and Phosphate during the wet season months of June, July and August 2015. In Fig. 14 the concentration of Chl *a* ranged from 12.4 and 30.2  $\mu$ g/l (18.6 ± 7.31) in June 2015; 12.4 and 29  $\mu$ g/l (20.9 ± 7.27) in July

2015 and 10.2 and 31  $\mu$ g/l (16.8 ± 8.36) in August 2015; and range from 7.3 and 23  $\mu$ g/l (14.78 ± 6.36) in December 2015; 12.7 and 15.6  $\mu$ g/l (13.76 ±1.21) in January 2016; and 12.1 and 22.1  $\mu$ g/l (17.42 ± 4.31) in February 2016. In Fig. 15 the Phosphate concentration ranged from 0.4 and 0.5 mg/l in June 2015; 0.4 and 0.5 mg/l in July 2015 and 0.2 and 0.5 mg/l in August 2015;

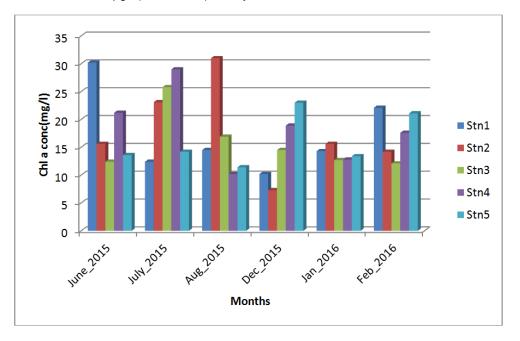


Fig. 14. Chlorophyll a concentration (µg/I) in the wet and dry season

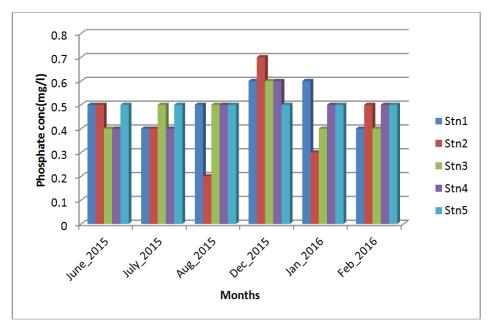


Fig. 15. Phosphate concentration (mg/l) in the wet and dry season

and in dry season ranged from 0.5 and 0.7 mg/l in December 2015, 0.3 and 0.6 mg/L in July 2016 and 0.4 and 0.5 mg/l in February 2016.

In Fig.16 the wet season Nitrate concentration ranged from 0.2 and 1.5 mg/l in June 2015; 0.2 and 1.2 mg/l in July 2015 and 0.4 and 0.9 mg/l in August 2015 and in the dry season values for Nitrate ranged from 0.2 and 0.4 mg/l in December 2015; 0.2 and 1.2 mg/l in January 2016 and 0.2 and 0.4 mg/l in February 2016.

Fig. 17. shows the concentration values of Phosphate (mg/l) with mean diamonds that are above and below the grand mean respectively. The overlap of the mean diamonds which represents the confidence intervals provides evidence of the differences between the group means at the given confidence level. The summary statistics of the Students t analysis shows a significant difference exists only in the concentration values obtained in December 2015 as illustrated by the SAS JMP connecting letter report.

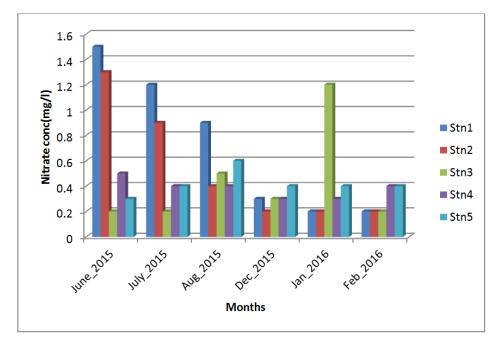
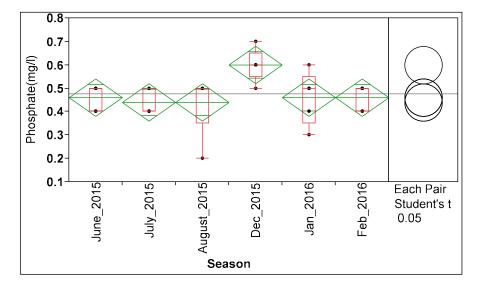


Fig. 16. Nitrate concentration (mg/l) in the wet and dry season



Level			Mean
Dec_2015	А		0.60
June_2015		В	0.46
Jan_2016		В	0.46
Feb_2016		В	0.46
July_2015		В	0.44
August_2015		B	0.44

#### **Connecting Letters Report**

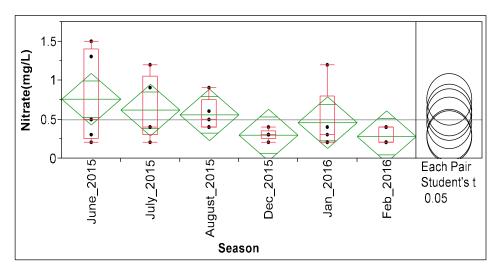
Levels not connected by same letter are significantly different

#### Fig. 17. Analysis of variance of phosphate concentration (mg/l) by season

Fig. 18. shows the concentration values of Nitrate (mg/l) with mean diamonds that are above and below the grand mean respectively. The overlap of the mean diamonds which represents the confidence intervals provides evidence of the differences between the group means at the given confidence level. The summary statistics of the Students t analysis shows a significant difference between June 2015 and February 2016 as illustrated by the SAS JMP connecting letter report.

Fig. 19. shows the one way analysis of Chlorophyll a (Chl a) concentration (mg/l) by season. The diamond box and ring in the student's t section of the chart represent each group's Chl a concentration, occupying different positions in the chart with respect to the grand mean. The evidence from the overlapping confidence Intervals and the grand mean confirm the lack of significant difference between the months and season. This similarity was illustrated by the SAS JMP connecting letter report which showed that no pair of means was significantly different.

Figs. 20 to 23 shows the bivariate fit of the relationship of abundance of phytoplankton families to nitrate-phosphate ratio. In Fig. 20. the bivariate fit for Bacillariophyceae indicated that the nitrate-phosphate ratio influenced abundance by 22% and 33% ( $r^2 = 0.22$  and 0.33) in the dry and wet season respectively. In Fig. 21. the nitrate-phosphate ratio indicated an influence on abundance of Chlorophyceae by 6% and 25%  $(r^2 = 0.06 \text{ and } 0.25)$  in the dry and wet season respectively; for Cyanophyceae Fig. 22 indicated that the nitrate-phosphate ratio influenced the abundance by 11% and 4% ( $r^2 = 0.11$  and 0.04) in the dry and wet season respectively; while in Fig. 23. for Dinophyceae the nitrate-phosphate ratio indicated an influence of 2% and 7% ( $r^2$ = 0.02, 0.07) on the abundance in the dry and wet season respectively.

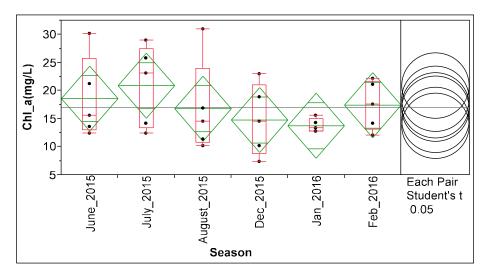


Level			Mean
June_2015	А		0.76
July_2015	Α	В	0.62
August_2015	А	В	0.56
Jan_2016	А	В	0.46
Dec_2015	А	В	0.30
Feb_2016		В	0.28
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**Connecting Letters Report** 

Levels not connected by same letter are significantly different.

#### Fig. 18. Analysis of variance of nitrate concentration (mg/l) in the wet and dry season



#### **Connecting Letters Report**

	Mean
А	20.90
А	18.60
А	17.42
А	16.80
А	14.78
А	13.76
	A A A

Levels not connected by same letter are significantly different

### Fig. 19. Oneway analysis of Chlorophyll a concentration (mg/l) in the wet and dry season

Figs. 24 to 27 show the regression plots of Chlorophyll a (mg/l) to abundance of Phytoplankton groups in the study. In Fig. 24 Chl a has a low negative relationship with the abundance of Bacillariophyceae ( $r^2 = -0.11$  and -0.25) in the dry and wet season respectively. Similarly in Fig. 25 Chl *a* has a low negative (inverse) relationship with the abundance of Chlorophyceae in the dry season ( $r^2 = -0.23$ ) but a moderate negative (inverse) relationship with

abundance in the wet season ( $r^2 = -0.78$ ). In Fig. 26 the Cyanophyceae is shown with a low negative (inverse) relationship with the abundance in the dry season ( $r^2 = -0.16$ ) and a moderate negative (inverse) relationship with the abundance ( $r^2 = 0.5$ ) in the wet season. For the Dinophyceae, Fig. 27 shows a low negative (inverse) relationship with the abundance ( $r^2 = -0.09$  and  $r^2 = -0.19$ ) in both the dry and wet season respectively.

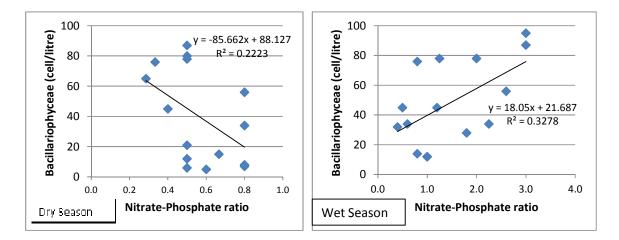


Fig. 20. Regression coefficient of Nitrate-Phosphate to abundance of Bacillariophyceae

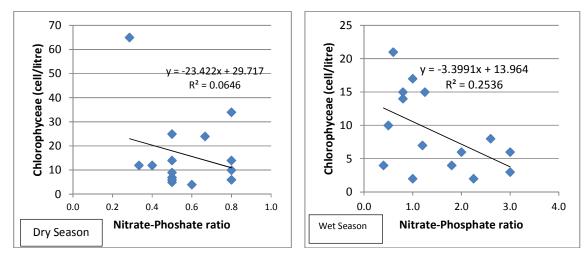


Fig. 21. Regression coefficient of Nitrate-Phosphate to abundance of Chlorophyceae

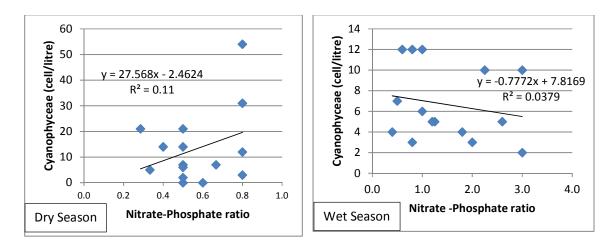


Fig. 22. Regression coefficient of Nitrate-Phosphate to abundance of Cyanophyceae

Onwuteaka and Choko; ARRB, 21(6): 1-19, 2017; Article no.ARRB.38456

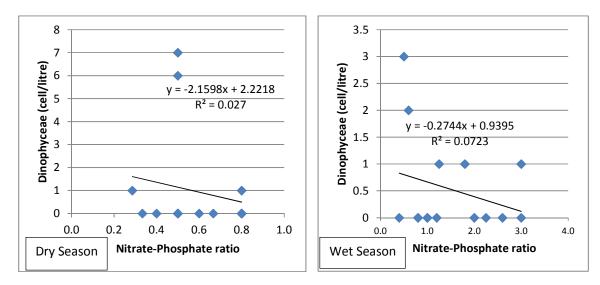


Fig. 23. Regression coefficient of Nitrate-Phosphate to abundance of Dinophyceae

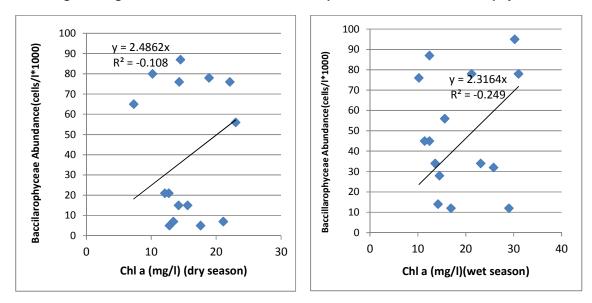


Fig. 24. Regression coefficient of Chl *a* to abundance of Bacillariophyceae (dry and wet season)

#### 4. DISCUSSION

The evidence from the study showed a nonlinear relationship between total phytoplankton and individual groups of phytoplankton. Significant seasonal variation were observed in the cell counts of the dominant family groups such as Bacillariophyceae (70%:90%) and Chlorophyceae (3%:30%) to total phytoplankton between wet and dry season. The lack of a similar significance in the abundance of Cyanophyceae and Dinophyceae in relation to total phytoplankton clearly suggests the complex relationship between community descriptors and environmental drivers. The non-linear findings collaborate studies [21-25] which suggest that fluctuations in phytoplankton groups rather than total phytoplankton are best estimators of phytoplankton standing stock and community structure. The consistent pattern of community structure in wet and dry season which occurred decreasing order of importance in of Bacillariophyceae Chlorophyceae > Cyanophyceae > Dinophyceae is in sharp contrast to community structure in studies of blackwater river systems [26,27]. Evidence from

these studies indicates the presence Euglenophyceae, of the Charophyceae, Chrysophyceae, Cryptophyceae, Haptophyceae and Rhodophyceae within the community. In other studies [10,11,23,24,25] there was the dominance the Euglenophyceae, of and Cyanophyceae over the Bacillariophyceae depending on seasonal dynamics. The three parameters of Nitrate, Phosphate and Chlorophyll a examined in this study to explain the abundance of cell counts provide contrasting differences in their relationship to each of the phytoplankton groups between seasons. In contrast to the higher cell counts of Bacillariophyceae and Chlorophyceae to total phytoplankton in the dry season, the Nitrate: Phosphate (N:P) ratio was a better estimator of standing cell counts for the wet season. The wet season relationship of cell counts to N:P ratio was also higher for Dinophyceae despite the lack of seasonal differences in the abundance of Cyanophyceae and Dinophyceae to total phytoplankton. This agrees with the studies [12,28] which show that nitrate and phosphate in combination with other interacting factors such as temperature exert various influences over phytoplankton cell abundance or bio-volume. The estimation of cell counts by Chlorophyll a was an inverse relationship for all the phytoplankton groups and was higher in the wet season in a descending order Chlorophyceae of > Bacillariophyceae Cvanophyceae > > Dinophyceae. The disparity between the N:P

ratio and Chlorophyll a in their relationship to phytoplankton cell counts is indicative of the more relative importance of Nitrogen and Phosphorous to increases in cell counts over chlorophyll a. Chlorophyll a in this study was shown to have a negative relationship to abundance of cell counts. The relationship of Chlorophyll a to phytoplankton cell abundance has been evaluated in many studies [29-37]. Among these studies there is lack of agreement of a linear concurrence in the relationship between ChI a and phytoplankton standing stock or community structure. In this study the seasonal change in cell numbers did not coincide closely with the chlorophyll a concentration which suggests that it is not a reliable indicator of increase in cell numbers of phytoplankton groups within the study area. This observation is plausibly a reflection of uptake and drawdown ratios of bioavailable nitrates and phosphate by phytoplankton in proportions that allow only marginal increase in Chlorophyll a production. This inference is supported by the low correlations phosphate nitrate. between (r=0.1665; 0.2434) and Chl a. Thus for this blackwater system the study observed that phosphate and nitrate have important implications in the spatial and temporal variations of phytoplankton cell standing stock than Chl a. The lack of shift of the community to Cyanophyceae (blue-greens) dominance also reflects the low nutrient regeneration and recycling of nitrates and phosphates.

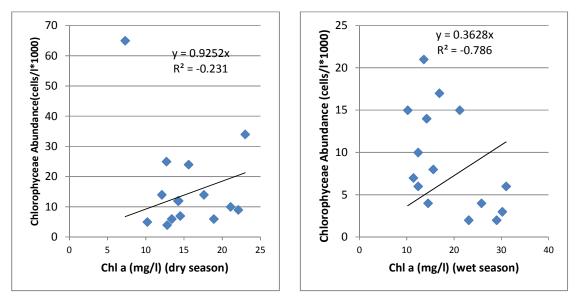


Fig. 25. Regression coefficient of Chl a to abundance of Chlorophyceae (dry and wet season

Onwuteaka and Choko; ARRB, 21(6): 1-19, 2017; Article no.ARRB.38456

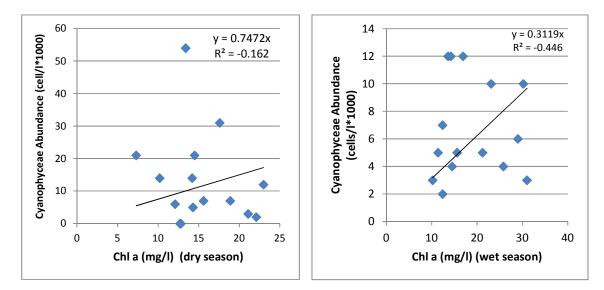
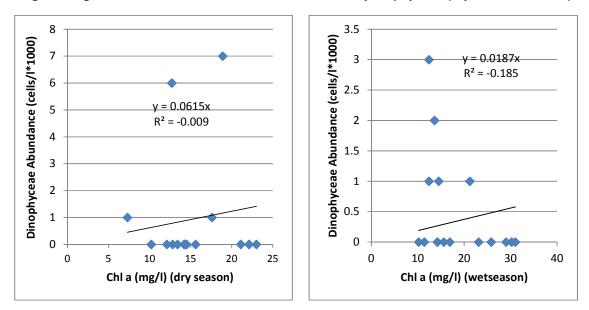


Fig. 26. Regression coefficient of Chl a to abundance of Cyanophyceae (dry and wet season)





### 5. CONCLUSION

The study has shown evidence that suggest Nitrate-Phosphate concentrations as better estimators of phytoplankton standing stock abundance in comparison with Chlorophyll *a* in this blackwater system of the New Calabar River.

### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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