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Seasonal Variability of Phytoplankton Accessory Pigments (Chlorophyll-b and Chlorophyll-c) in the Calabar River Estuary: South Eastern Nigeria

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Seasonal variability of phytoplankton accessory pigments in the Calabar River estuary was studied during the period, July to October, which is a transition period between the rainy season and the dry season. Water samples were collected at Calcemco Jetty. The samples were analyzed for the concentration of chlorophyll-b and Chlorophyll-c using spectrophotometric method. A significant positive correlation was obtained between the concentration of chlorophyll b and chlorophyll c in low tide neap ($r > 0.998$, $n = 4$, $p = 0.05$) and in low tide spring ($r > 0.886$, $n = 4$, $p = 0.05$). Correlations were also positive between the concentration of chlorophyll b and chlorophyll c in high tide neap ($r > 0.75$, $n = 4$, $p = 0.05$) and high tide spring ($r > 0.675$, $n = 4$, $p = 0.05$). The concentration at low tide during spring was significantly higher than that of high tide during neap. Apart from spring tide, when maximum values occurred in September, chlorophyll-c values attained maximum in October as found for chlorophyll-a and chlorophyll-b. There was no significant difference in concentration of chlorophyll b between high and low tide and between neap and spring tide but low tide concentrations were relatively higher than high tide concentrations. Similarly, concentrations at neap tide were relatively higher than concentrations at spring tide.

Key Words: Phytoplankton accessory pigments, Calabar river.

INTRODUCTION

Phytoplankton is photosynthetic free-floating plants living in the water column, whose movements are more or less dependent on current[1-3]. Phytoplankton organisms fix solar energy by photosynthesis using carbon dioxide, nutrients and trace metals.

According to Akpan[3], all autotrophic phytoplankton contains pigments, which are responsible for the trapping of solar energy needed for photosynthesis. These pigments include chlorophylls, carotenoids, phycobyllins

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and phaeopigments[1]. Phytoplankton usually shows a wide range of sizes: nano-plankton (cells, 20 ppm), micro-plankton (cell between 20 and 200 pm), mesoplankton, macroplankton and megaplankton[1,3].

Phytoplankton pigments have been found to vary widely from one algal group to another and composition of various pigments have also been found to varies within a particular algal group[4].

Natural and anthropogenically-induced perturbations in the aquatic environment have been found to affect the concentration of phytoplankton pigment in an estuary system for example, Bannister[5] reported that mineral nutrition affects the pigment content of algae. In their study on deficiencies of iron and magnesium. Talling and Rzoska[6] observed. The pronounced effect on chlorophyll synthesis due to the concentration magnesium and iron. Nitrogen starvation in algae leads to loss of phycocyanin[3]. Pollution affects the phytoplankton population and pigments level within a given aquatic system. Eddy[7] attributed the low concentration of phytoplankton pigment in the Qua Iboe river estuary to the pollution of the river by oil spills and industrial waste. Akpan and Frank[8] found that spent engine oil adversely affects the phytoplankton pigments of the Cross river system. Eddy *et al.*[9] also found that phytoplankton pigments can be adversely affected by physicochemical and hydrographic condition of the water. In Cross-river estuary, Akpan and Offem[10] have attributed the increased in chlorophyll and pheopigment concentration in water column to high turbulence due to wave and tidal actions. It has been found that although light and availability of nutrients are the dominant factors that regulate phytoplamkton concentrations in most esturies, herbivore grazing may be important in other cases[2,10]. Other factors[9,11] that affects phytoplankton pigments are salinity, grazing activities, light and temperature of the water, pH, *etc*.

The influence of season on physicochemical parameters of water and on the phytoplankton pigment composition of an estuary has been extensively studied. Hallegraeeff[12] reported that in coastal stations off Sydney (New South Walles, Australia), a sudden increase in chlorophyll a (up to 280 mg/m^3) was observed during May, July, September, January and February as compared to other months. In East African coastal water, Holden and Green[13] investigated pigments distribution during three annual cycles at Port Hacking in August, September, October (early spring) and in December, January, February (Summer) and observed a strong positive correlation between chlorophyll peak and intrusion of nutrient-rich slope water into the continental shelf. In Cross river estuary, Akpan[3] found higher values of pigments concentration in the dry season compare to the rainy season. Similar observations have been made in other rivers including Oshun river in Nigeria[8,14].

In most tropical rivers, river discharges is controlled by the seasonal changes in rainfall regimes, during the wet season, when rainfall dominates, river discharge increases with resultant decrease in the residence time of water and changes in phytoplankton pigments distribution[3]. In Cross river estuary, Akpan *et al.*[15] observed four fold increase in water depth during the wet season compared to the dry season. Similarly, Lowenberg and Kunzel[16] have observed a marked increase in river discharge of Cross river from a mean value of 879 $m^3 s^{-1}$ during the dry season to 2,553 $m^3 s^{-1}$ during the wet season.

Because of their ecological and economic (productivity) importance, accurate quantitative data on phytoplankton pigments is frequently required. Four approaches are usually applicable in the analysis of phytoplankton pigment. These include, spectrophotometric method, flouremetric methods and automated estimation method. The first spectrophotometric method for algal pigments in seawater was described by Richard and Thompson in 1952. Some improvements to this method were suggested by Parsons and Strickland[17] and new spectrophotometric equations were given by Jeffrey and Humphrey[11].

The flourometric method is based on the use of the turner fluorometer. Modified methods for the simultaneous determination of chlorophyll-a, b and c have been developed in recent times[17,18].

Automated estimation method is neither highly accurate nor precise. However, it has the important advantage over other methods in which it can be used to monitor chlorophyll concentrations while a vessel is underway or in vertical profiles pumped samples[19].

Thin layer chromatography is currently in use as a method for measuring phytoplankton pigments. It involves two phases: stationary and mobile phase and the separation mechanism is by partitioning. Application of this method to identification of degradation products of chlorophyll has gained some measure of success[20].

Chlorophyll is the most important pigment present in algae. Chlorophyll-a, which is the most abundant of all algal chlorophyll, is present in all species[3] and constitutes *ca.* 1-2 % of dry weight of phytoplanktonic algae[1]. Chlorophyll-a may be determined down to 1 mg^{-3} by absorption spectrophotometry[17] or to a fiftieth of this amount by fluorometry[1]. The different forms of chlorophyll in algae include, chlorophyll-a, chlorophyll-b, chlorophyll-c (chlorophyll-c_1 and chlorophyll-c_2), chlorophyll-d and chlorophyll-e. Apart from chlorophyll-a, all other algal chlorophylls are considered as accessory or secondary pigment because only chlorophyll-a functions as photosynthetic pigment.

Carotene is another algal pigment. Carotenes are the main lipid content of algae. Several studies on carotenes have shown that carotenoids are less affected by harsh environmental conditions and should relate more to cell biomass than chlorophyll[4,10].

The importance of algal pigment in an estuary system has long been research upon. Chlorophyll-a measurement gives an estimate of primary productivity and extent of pollution of an aquatic environment[3]. In contrast to chlorophyll-a, studies shown that carotenoids are less affected by harsh environmental conditions and as such should relate more to cell biomass[10] than chlorophyll-a hence the concentration of carotenoid in an estuary can also serve as a pollution indicator. The concentration of chlorophyll-a, carotenoid and phaeopigment has been used as indicator of algal biomass[20].

Physiological conditions of phytoplankton may be predicted from the ratio of chlorophyll-a to other pigments in the estuary[18,21]. Fish production can be correlated with pigments data[22] since the major source of fish food is phytoplankton whose density is directly proportional to the concentration of pigments in a given estuary.

EXPERIMENTAL

The study area was the Calabar river estuary, which takes its source[3] from the Oban in South Eastern Nigeria, located between longitude 7º 49' and 9º 28' East and latitude 4º 49' and 5º 56' north covering an estimated area of 2300 km^2. The width of the river is reported to be 1.5 km at its mouth and the depth of the river is also reported to be in the range of 7-10 km recorded during high tide and 5-8 km during low tide[23].

The climate of the area is influenced by the southwest wind, which blows from the Atlantic Ocean and the dry dusty northeast winds which come from the Sahara desert. Sampling was done twice a month from July to October 2003. Water samples were collected from the surface of the river at a point called Calcemco Jetty, which extends far into the river and is located at *ca.* 10 km from the river mouth. Samples were taken during low tide and high tide for both spring tide (SP) and neap tide (NP).

The seawater was filtered through a glass fibre filter of pore size, 0.5 m. As the seawater filtration continues, a few drops of a suspension of magnesium carbonate were added to prevent acidity on the filtrate. The filtrate was placed in a 15 mL centrifuge tube. Acetone (15 mL) was added and the tube was shaken properly and allowed to stand overnight in a dark refrigerated place. The supernatant was decantated into 10 cm path length spectrophotometer and the absorbance at wavelength of 750, 665, 647, 630, 510 and 480 nm. Each absorbance was corrected by subtracting the 750 nm reading from the 664, 647 and 630 nm absorptions. The 510 nm absorbance was corrected by subtracting two times the 750 nm absorbance from that of 550 nm while the 480 nm absorbance was corrected by subtracting three times the 750 nm absorbance from the absorbance the absorbance at 480 nm. The amount of chlorophyll-a (C_a), chlorophyll-b (C_b), chlorophyll-c (C_c) and caroteinoids (C_p) were calculated from the formula,

$$C_b = 21.03E_{647} - 5.43E_{664} - 2.66E_{630}$$
$$C_c = 24.52E_{630} - 1.67E_{664} - 7.60E_{647}$$

where E_{647}, E_{630} and E_{664} are the corrected absorbance at 647, 630 and 664 nm wavelength, respectively and C_b and C_c are the concentration of chlorophyll-b and c, respectively in mg/mL. The concentration of chlorophyll/m^3 is given by the equation, mg chlorophyll/m^3 = (cv)/10U. Where v is the volume of acetone added (5 mL). U is the volume of the sample (250 mL).

RESULTS AND DISCUSSION

Seasonal variability in the concentration of chlorophyll b during the study period is presented in Fig. 1. The concentrations of chlorophyll-b were ranged from 0.00 mg/m^3 (August) to 3.22 mg/m^3 (September) during high tide in spring. Low tide concentrations spring ranged from 1.02 mg/m^3 (August) to 10.86 mg/m^3 (October).

Neap tide concentrations were observed to range from 1.17 mg/m^3 (August) to 4.66 mg/m^3 (October) in high tide and from 1.20 mg/m^3 (July) to 12.04 mg/m^3 (October) in low tide.

There was no significant difference in concentration of chlorophyll b between high and low tide and between neap and spring tide ($p > 0.05$) but low tide concentrations were relatively higher than high tide concentrations. Similarly, concentration at neap tide was relatively higher than concentrations at spring tide.

A significant positive correlation was obtained between the concentration of chlorophyll-b and chlorophyll-c in low tide neap ($r > 0.998$, $n = 4$, $p = 0.05$) and in low tide spring ($r > 0.886$, $n = 4$, $p = 0.05$). Correlations were also positive between the concentration of chlorophyll-b and chlorophyll-c in high tide neap ($r > 0.75$, $n = 4$, $p = 0.05$) and high tide spring ($r > 0.675$, $n = 4$, $p = 0.05$).

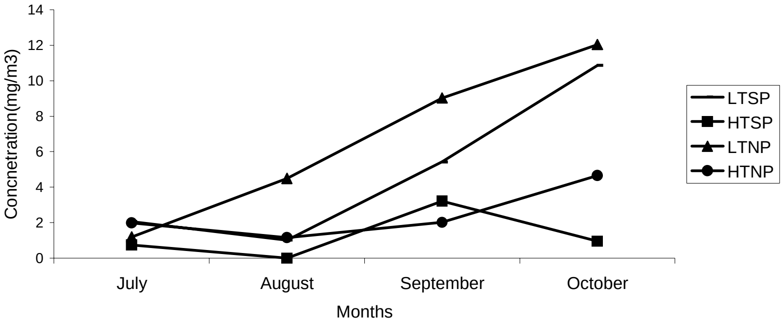

Fig. 1. Seasonal variability in chorophyll-b during the various tidal regimes

When the observed concentration of chlorophyll b was compared with values of chlorophyll a obtained by Eddy[7] on the Calabar river, a positive correlation was observed during neap tide at high and low tides and during spring low tide. Chlorophyll-b is a characteristics pigment in the green algae[18,24] and is found in *Prosinophycae, Euglenophycae* and *Chlorophycae*[4,15,21,25].

The observed variation in the concentration of chlorophyll-b may be attributed to the changes in green algae composition between high and low tide and between spring and neap tide. Akpan[3] stated that chlorophyll-b showed significant positive correlation with phytoplankton density in the Cross river estuary which implies that the importance of chlorophyll-b in the phytoplankton density of an estuary cannot be over emphasized. Seasonal variability in the concentration of chlorophyll-b may be due to the fact that as wet season progresses from July to October with increasing amount of rainfall, the growth of green algae is increasingly favourable tending to a maximum in October hence the concentration of chlorophyll-b is expected to response in proportion to the amount of rainfall. The slight drop in the concentration of chlorophyll-b during August in low tide neap and in low and high tide spring is due to August break, a period which is often characterized by a reduction in the amount of rainfall[9]. During rainy season, there is an increasing dilution of the river depending on the amount of precipitation. Dilution reduces the concentration of pollutants and thus algae growth. Apkan *et al.*[15] stated that in the Calabar river estuary, the concentration of ammonium and other are low. Therefore, the effect of rainfall, which varies between high tide and low tides within the same season, may influence river discharge, dilution of nutrients and resuspension of sediment. This explained the difference in the concentration of chlorophyll-b within the various tidal regimes.

Seasonal variability in the concentration of chlorophyll-c is presented in Fig. 2.

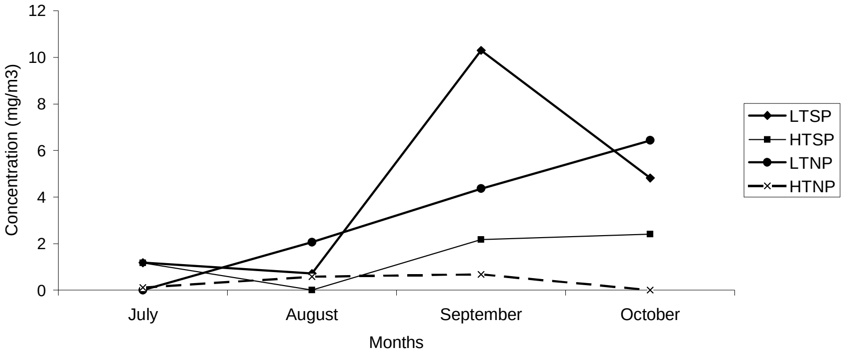

Fig. 2. Seasonal variability of phytoplankton chlorophyll-c

The concentration of chlorophyll-c ranged from 0.00 (August) to 2.41 mg/m^3 (October) during high tide spring and from 0.00 (October) to 0.68 mg/m^3 (September) during high tide neap. During low tide, spring tide concentrations were observed to vary from 0.72 (August) to 10.30 mg/m^3 (September) while neap tide concentrations vary from 0.00 (July) to 6.43 mg/m^3 (October).

Generally, spring tide concentrations were higher than neap tide concentration and low tide concentrations were relatively higher than high tide concentrations. Lower concentrations were measured in July, August and October at low tide. When these concentrations were compared with values of chlorophyll-a reported by Eddy *et al.*[26], the ratio of chlorophyll a to chlorophyll-c varied from 0.6 mg/m^3 in July during spring, to infinity in August and October during neap tide.

There was a strong positive correlation between the concentration of chlorophyll-c and chlorophyll-b in low tide neap ($r > 0.998$, $n = 4$, $p = 0.05$) and in low tide spring ($r > 0.886$, $n = 4$, $p = 0.05$). Correlation between the concentration of chlorophyll-b and chlorophyll-c at high tide were also positive.

Chlorophyll-c is found in brownish algae, which includes the *Cryptophyceae, Dinophyceae, Rhaphidophyceae, Chrysophyceae, Haptophyceae, Bacciloriaphyceae, Xanthophyceae* and *Phaeophyceae*[12]. Changes in concentration of chlorophyll has been found to be mainly associated with changes in algal composition[26].

Chlorophyll-c concentration was relatively higher during spring than neap tide. The concentration at low tide during spring was significantly higher than that of high tide during neap. Apart from spring tide, when maximum values occurred in September, chlorophyll-c values attained maximum in October as found for chlorophyll-a and chlorophyll-b.

The distribution of chlorophyll-c during high tide, shows abnormal respond to the changing estuaries dynamic and environmental factors. In contrast, the relationship was almost linear during neap in low tide. Akpan[3] found the dominance of diatom in the phytoplankton of the Cross river estuary. Similar reports were given by Moses[22] and Nawa[27]. It is therefore, implies that the seasonal changes concentration of chlorophyll-c in Calabar river estuary is expected to follow changes in diatom biomass. Other factors responsible for the variation of concentration of chlorophyll-c are: (1) River discharge which influences salinity distribution, residual circulation, turbidity and density-stratification and nutrient distribution. (2) Tidal current speed, which affects vertical mixing and horizontal dispersion. (3) Surface insulation and (4) Temperature and wind speed.

During spring, river discharge is high especially during high tide while retention time of water is low. According to Alpine *et al.*[28] when river discharge is high and residence time of water is low, growth rates of plankton are insufficient to maintain estuarine populations. Such observations have also been reported in most estuaries including the Cross river estuary[10]. San Francisco Bay[28] and Columbia river[20].

Lowenberg and Kunzel[16] stated that the mean river discharge in the Cross river changed from 879 in January to 2533 m^3/s in July. River discharge reduces the concentration of chlorophyll-c through its influence on estuarine dynamic. Such dynamic could include salinity distribution and penetration. River discharge is normally high during spring tide and increases salinity which significantly chlorophyll-c concentration[28]. According to Eddy *et al.*[26], most diatoms disappeared from waters at high salinity and although diatoms do not constitute the complete assemblage in either fresh or brackish water, they demonstrate the general trend for most fresh water cells to rupture and lose their chlorophyll on encountering even low salinities. The variation in salinity of the calabar river has been reported by Eddy *et al.*[9] and the values were found to range from 70 to 80 % during high tide and 73 to 80 % during spring tide. In neap, the values ranged from 62 to 75 % and from 72 to 87.5 % for high and low tides, respectively. There was no significant difference in the concentration and seasonal variability of salinity during the various tidal regimes. In spring, values decrease from July to August, with the minimum value in August and a sharp increase in October. A similar observation was found in low tide for both spring and neap except that the minimum value in neap was observed in September. The seasonality in salinity was attributed to an increase in freshwater input and river discharge. Eddy[7] also stated that changes in estuaries salinity due to seasonal regimes of river discharge and precipitation. Therefore, observed seasonality in the concentration of chlorophyll-c partly influenced by the salinity of the water even as similar trends have been reported in other estuaries[20].

Residual circulation, turbidity and density stratification are also consequences of river discharge. Circulation and mixing can change chlorophyll concentration and influence community composition over short (hourly) time scales, tidal currents display and mixed water masses, generate surface convergences and cause vertical mixing. Over long time scales, tidal currents cause horizontal dispersion that transports phytoplankton resident in the deeper channels mixed with those resident over the adjacent shallow[6].

Forth-nightly neap-spring tide causes daily to monthly variation in the rate of horizontal dispersion and vertical mixing[28]. Cumulative multiple regression analysis shows that daily solar radiation input and light extinction in the water column could account for about 75 % of the variability in chlorophyll concentration in a given estuary[20]. Reports from Mt. Saint

Helens shows that volcanic ash and mud can lower the concentration of chlorophyll and other pigments by reducing the photozone in the estuary[20].

Nutrient availability may exert a significant role in controlling the concentration of chlorophyll-c and variation of their distribution between low spring and neap tide and between low and high may also account for the changing concentration of chlorophyll-c. Variation in concentration between high and low tide resulting from distribution of nutrients may be due to dilution effect. During high tide the volume of water increases but decreases during low tide, such inter tidal changes has effect of varying the concentration of chlorophyll-c through dilution which is excessive during high tide.

Herbivorous grazing activity which differs significantly during spring, neap, high and low tide can also account for the variation in concentration of chlorophyll-c between the various tidal regimes and phytoplankton density from April to September and ascribed it grazing activity while Akpan[2] stated that the low concentration of chlorophyll in the Cross river system due to herbivorous grazing activity. However, this may be less significant in the Calabar river estuary[3].

Wind driven resuspension sustains high population of phytoplankton (hence chlorophyll) in the water column where as reduced turbulence enhance vertical flux of diatoms to the bethos[29]. The dominate of diatoms in Calabar river had earlier been discussed. Therefore it follows that variation in wind driven resuspension between spring and neap tide; because it determines diatom population in the water hence chlorophyll-c.

Conclusion

The study was aimed at studying the seasonality of phytoplanton accessory pigment in the Calabar river estuary. It has been found that the concentration of these assesory pigment in the estuary changes with season and with tide. Certain factors including environmental ans estuarine factors have been found to be responsible for the seasonal variability of the concentration of phytoplankton assesory pigment in the estuary. These pigments, though not needed for photosynthesis are strong indicators of the phytoplankton biomass in the estuary and since the biomass affects the productivity of the estuary, it becomes necessary to conclude that these accessory pigments are indicators for the productivity of the Colabar river estuary.

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