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Bacterial Growth Efficiency, Respiration and Secondary Production and in the Cross River Estuary, Nigeria and the Near Coast

Antai, Ekpo Eyo[1], Asitok, Atim David[2], Paul, Akaninyene[3]

[1, 3]Institute of Oceanography, Nigeria

[2]Department of Microbiology, University of Calabar, Calabar, Nigeria

Abstract: ***Bacterial growth efficiency (BGE) can be a major factor in estimation of energy transfer within the food web, but its controlling factors are poorly understood. It also regulates measurements of bacterial production and respiration [BGE = BP/(BP + BR)]. BGE values in natural bacterial assemblage were determined over 12 months in a tropical estuary of Cross River and adjacent Gulf of Guinea. BGE values ranged from 20.80% to 52.40% with a mean value of 36.16% and correlated positively with bacterial production (BP) (r^2 = 0.676, p>0.001). Much of the BGE variation could be attributed to BP because BGE correlated poorly with BR (r^2 = 0.322, p<0.001) which had a conservative tendency. Variations of BGE will reflect the trophic status of the ecosystem and may be influenced by combination of factors associated with seasons. Therefore a constant value of BGE in assessing bacterial trophodynamics can be fraught with error.***

Keywords: Bacterial growth efficiency, bacterial production, bacterial respiration, DOC, Cross River estuary, Gulf of Guinea

1. Introduction

The study of bacteria in aquatic ecosystem is central to any ecological investigation since they are the most abundant and important biological components in the cycling of organic matter through incorporation of carbon into its body biomass and respiration. Among ecosystems, bacterial secondary production averages from 20 to 30% of primary production (1). Since bacterioplankton can be an important link between dissolved organic carbon and organism in the higher trophic levels (2, 3, 4) a lot of interest has been shown in their distribution and production.

The extent of the flow of organic carbon through the "microbial loop" remains largely uncertain because measurements of bacterial production are rarely undertaken with measurements of bacterial respiration. This limits the understanding of the role of bacteria in the carbon cycling in aquatic ecosystem (5). The relative importance of bacterial respiration and bacterial production are controlled by growth efficiency which determines what fraction of incorporated organic carbon is available to organisms in the higher trophic levels. Bacterial respiration can be constrained by the quality and quantity of dissolved organic carbon available for bacteria utilization (6) and its origin (7). Estimates of bacterial production and respiration would allow direct estimation of bacteria conversion of dissolved organic carbon into particulate organic carbon. Having shown a consistent co-variation of bacterial growth efficiency and productivity in aquatic ecosystem, it was suggested that a combination of availability of organic matter, nutrient and specific energetic demand of each water body may regulate bacteria growth efficiency (5). BGE is tightly associated with the physiological condition of bacteria and in this context may be an important sensitive pointer of the response of aquatic bacteria to their environment. Though there have been a number of studies on the mechanisms controlling the variability of BGE in aquatic ecosystems, a number of fundamental questions concerning the intrinsic factors controlling BGE remain unanswered.

The present study focuses on the measurement of bacterial production and respiration in the Gulf of Guinea, a tropical coastal ocean and to examine the seasonality of bacterial growth efficiency (BGE) in this ecosystem. Although studies have shown seasonality of bacterial metabolic activities in aquatic ecosystem (8, 21), few have highlighted seasonality in bacterial respiration rate and how such changes can influence the trophodynamics of bacterioplankton (8). This study will also examine the factors that regulates the variability or otherwise of BGE. This study is the first report on bacterial respiration (BR) and efficiency in the Cross River estuary and the Gulf of Guinea and is part of microbial ecology of the Cross river estuarine and near shore ecosystem. This study reports a 12 months survey at 2 stations near the mouth of the Cross River estuary and 1 strategic sampling stations in the Gulf of Guinea to measure primary production (PP), DOC, chlorophyll a, bacterial production (BP) and bacterial respiration that will be required to determine variations of bacterial growth efficiency and factors that may be responsible for such changes.

2. Material and Methods

The study area, the Cross River Estuary, Nigeria lies approximately between latitude $4^0$30' N and $5^0$15'N and between longitude $8^0$00'E and $8^0$40'E. The estuary has already been described by Akpan (1993) and it discharges into the Gulf of Guinea. It is the largest estuary along the West African coast and the fringes of the estuary are lined with mangroves and nypa palm. The river basin covering an estimated area of 54,000km^2 is rich in clay material and is situated within the tropical rain forest. The mineral rich catchment area in combination with the dense vegetation

and torrential rainfall pattern typical of the area plays a remarkable role in the biogeochemical cycling of organic and inorganic nutrient in the estuary. The climate in the area is characterized by long wet season from April to October and dry season from November to March. Water temperature ranges from 23.5^{0}C during wet season to 29^{0}C in dry season.

Surface water samples were collected with clean 5 litre polyethylene sampling bottles at 3 sampling stations (St 1 and St 2 – estuarine and St 3 - coastal) every month from October 2009 to September 2010. The study area is within the Nigerian inland and continental shelf South East of Nigeria and has a maximum depth of 22 metres (Fig. 1). 200 ml water samples for Chl.*a* were immediately filtered through a pre-combusted Whatman GF/F filters (45^{o}C, 2h) and adjusted to pH 2 with 6 N HCL. Chlorophyll *a* was extracted with 96% ethanol, and was measured by spectrophotometry [9]. Dissolved organic carbon samples were analysed using platinium-catalysed combustion followed by infrared detection of CO_2 (Shimadzu TOC – 5000, Shimadzu, Columbia, (USA).

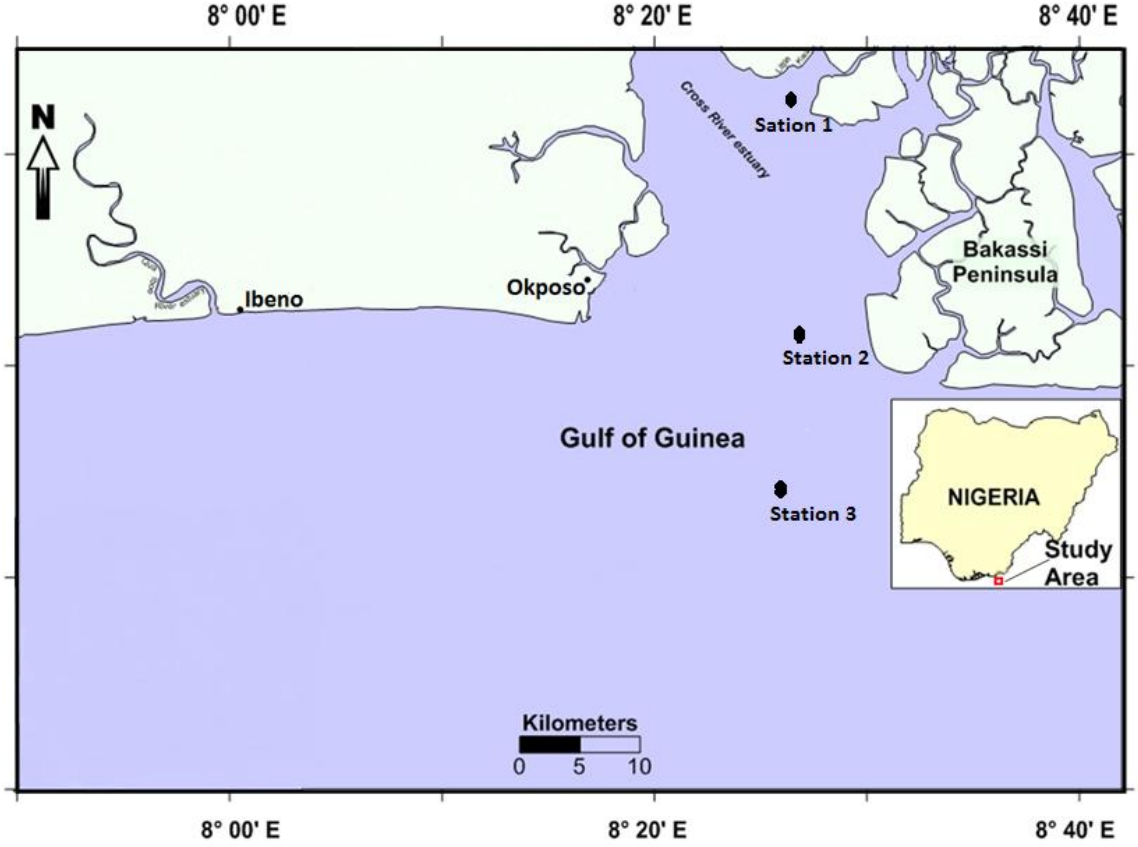

Figure 1: Map of the study area in the sampling stations in the Cross River estuary and the Gulf of Guinea

Estimation of primary production

Primary production was measured using in-situ incubations just beneath the water surface at every sampling station using $NaH^{14}CO_3$ [10, 11]. Two transparent bottles and another containing dichlorophenol dimethylurea (DCMU) as control were incubated for 4 hours. After incubation, the samples were collected on GF/F filters and rinse properly with 0.1mol/L HCL. These were placed in vials and scintillants added before measuring radioactivity using liquid scintillation counter. Carbon fixed in the DCMU control bottles were subtracted from those in the experimental bottles [12].

Bacterial production and abundance

BP was determined by using radioactive tritiated thymidine incorporation method [8]. With this method [Methyl-^{3}H] thymidine (final conc. = 10 nM; specific activity 3.1 to 3.3 TBq mmol^{-1}; New England Nuclear, USA) was added in triplicate 10 ml samples. Samples were incubated for 30 min at ambient temperature and fixed with formaldehyde (2 % final conc.). Bacteria were collected on 0.2µm Milipore filter and washed 5 times with 2ml ice cold 5% trichloroacetic acid. The filter was then placed in scintillation vials with 8ml of scintillant and subsequently tritiated thymidine incorporation was assayed. All samples were corrected for abiotic adsorption of radioisotope by subtracting the radioactivity in a formaldehyde killed control. Rates of [^{3}H] TdR incorporation into bacterial DNA were converted to bacterial production using a thymidine conversion factor of 2.0 x 10^{18} cells produced mol^{-1} thymidine incorporated [13]. Bacterial cell to carbon conversion was based on an average cell volume of 0.1 µm^3 determined microscopically and a specific gravity of 1.1 and a carbon content of 22% of the wet weight [14, 15], using the conversion factor of 2.42 x 10^{-14}gC cell$^{-1.}$ The cells were mostly rod shaped and within a size range of 0.45 – 0.58µm x 0.6-0.9µm giving a mean bio-volume of 0.079 – 0.24µm$^{3.}$ Water samples for bacterial counts were preserved with buffered formalin (2% final conc.) and stored at 4^{o}C until analysed. The bacterial samples were stained with 4'6' – diamidino-2-phenlindole (DAPI) and filtered unto pre-blackened Nuclepore filters (pore size, 0.2µm) [16] and

counted under epifluorescence microscope (Olympus BH-2). Between 12 and 20 fields were observed in order to count at least 300 cells [17]. The coefficient of variation ranged from 2.3 to 11.2%.

Bacterial respiration and growth efficiency

60 ml BOD bottles were filled to overflowing as soon as the water samples were collected from each sampling station. Three bottles were fixed immediately with Winkler reagents and twelve other bottles were incubated soon after in the dark at ambient temperature. Another set of 15 bottles were filled with 0.8µm filters water sample at low vacuum at each sampling station and treated as described above. Three BOD were fixed with Winkler reagent after 12, 24 and 36 h incubation. Concurrently 1 BOD bottle was analysed for bacterial abundance as described above. Dissolved oxygen was determined by a spectrophotometric modification of the Winkler method (18, 19). The rate of oxygen consumption (0_2 L^{-1} d^{-1}) was determined by plotting oxygen against time (0, 12, 24, 36 h). This was converted to carbon respired (µg C L^{-1} h^{-1}) by using a respiratory quotient of 1 (20). Bacterial growth efficiency (BGE) was calculated as BGE= [BP/(BP + BR)] x 100, where BP (bacterial production) was determined using ^{3}H thymidine incorporation as described above and BR (bacterial respiration) was calculated from the time-course incubations also described above.

3. Results and Discussion

Chlorophyll *a*, DOC and Primary production

Chl *a* concentrations were higher at the estuarine stations (ST-1 and ST-2) than at the coastal station (ST-3) (Fig. 2). At all the stations higher concentrations were noticed during the dry season (December to March). Primary production showed a similar trend with Chl a in the study. The primary productivity at the estuarine station was higher (20.37 – 186.32 µg $CL^{-1}d^{-1}$) when compared with the coastal stations (19.77 – 151.38 µg $CL^{-1}d^{-1}$), although the primary production rates did not differ significantly between the stations ($p < 0.001$). DOC concentrations in the water column were generally high. They were clearly higher at ST-1 (3.01 – 14.02 mg l^{-1}) and ST-2 (1.89 – 11.34 mg l^{-1}) than ST-3 (1.41 – 9.11 mg l^{-1}). Peak values were also observed during the dry season.

Bacterial abundance and production

Bacterial abundance in the study area ranged from 0.86 to 9.14 x 10^9 cells L^{-1} and showed strong seasonal trend (Fig. 3) and the estuarine stations had higher numbers than the coastal location. Variations of bacterial production determined in the various stations were highly significant (f = 8.58, df = 35 and p<0.001) (Fig 4). At the estuarine stations BP varied from 9.82 to 98.47 µg C $l^{-1}d^{-1}$ (CV = 38%.) and was generally higher during the dry season. BP at the coastal station varied from 7.15 to 77.23 µg C $l^{-1}d^{-1}$ (CV =31%)

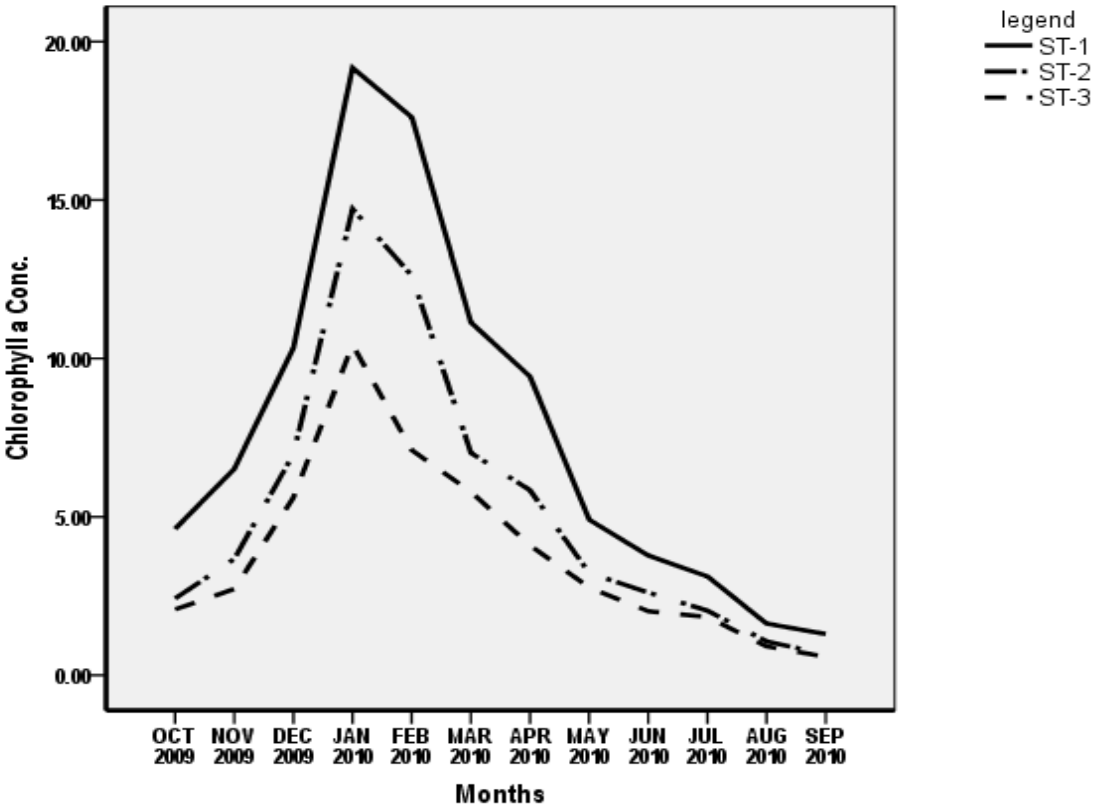

Figure 2: Temporal variation of Chlorophyll a (µg L^{-1}) at the Cross River estuary (ST-1 and ST-2) and the Gulf of Guinea (ST-3)

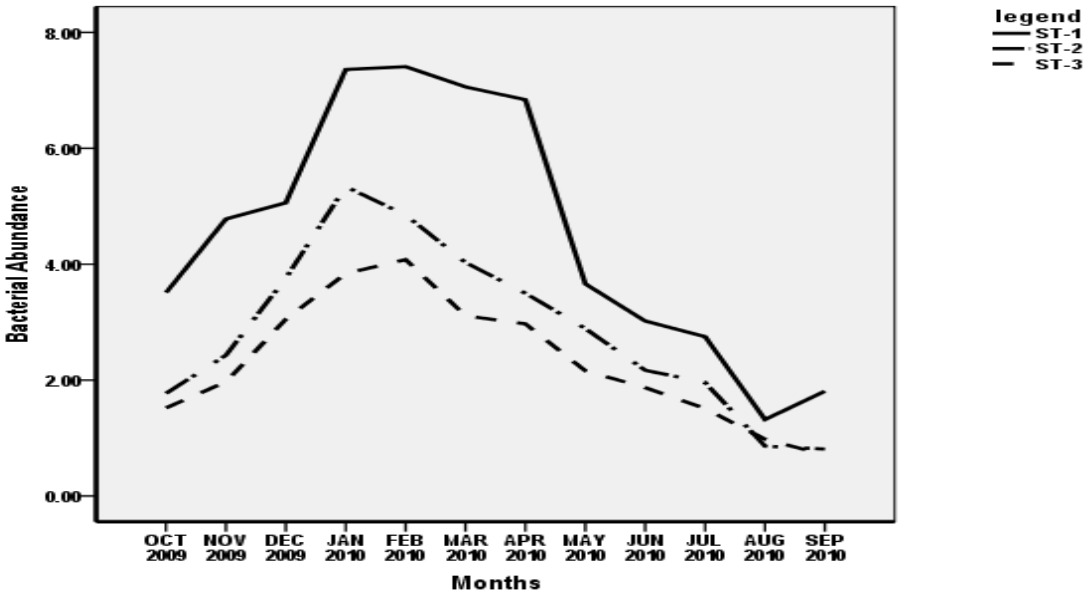

Figure 3: Temporal variation of Bacterial abundance (x 10^9 cells L^{-1}) at the Cross River estuary (ST-1 and ST-2) and the Gulf of Guinea (ST-3)

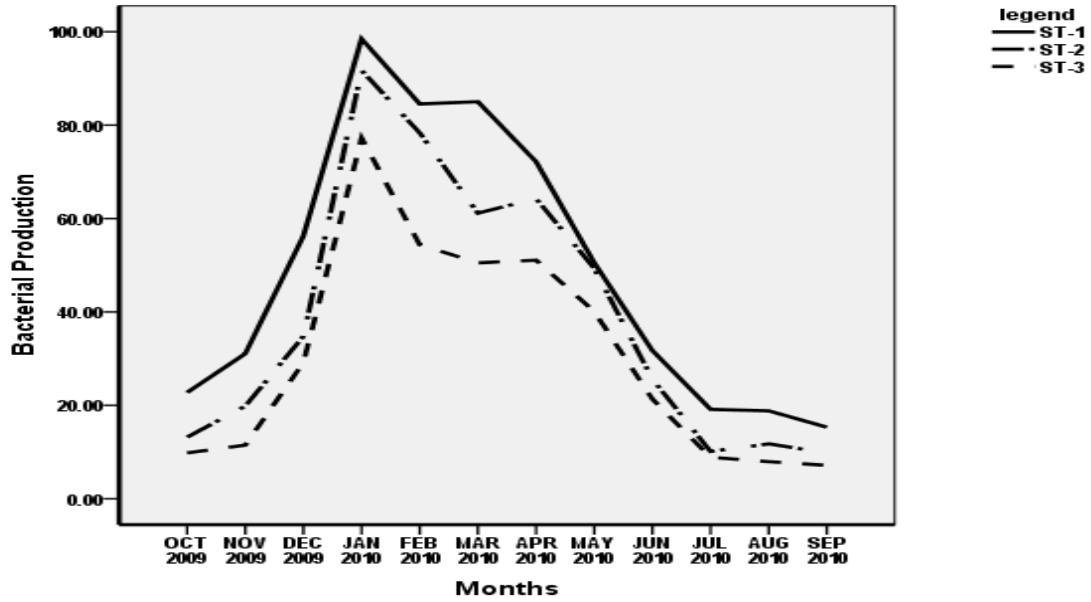

Figure 4: Variation of Bacterial carbon production (µg C $L^{-1}d^{-1}$) at the Cross River estuary (ST-1 and ST-2) and the Gulf of Guinea (ST-3)

Bacterial Respiration and Growth efficiency

Bacterial respiration and growth efficiency followed the seasonal trend exhibited by bacterial production and abundance. BR varied from higher rates of 22.32 to 116.48 µg $CL^{-1}d^{-1}$ from October to January (dry season) and the rate decreased to 17.68 to 93.36 µg $CL^{-1}d^{-1}$ from May to July (wet season). The relationship between bacterial respiration and production was highly significant (r^2 = 0.805, $p<0.001$) (Fig. 5) at all the sampling stations. BGE within the study area varied from 20.80 to 52.40% with an average of 36.16% and there was no significant difference in BGE within the sampling stations. Coefficient of variation in BGE over the study period was not as high (CV= 17%) as in other measured parameters (Fig. 6) BGE showed strong positive relationship with BP at all the sampling stations (r^2 = 0.676, $p<0.001$) (Fig. 7). Although BP and BR which are the components for BGE calculation co-varied throughout the study (Fig. 5), BR showed a more conservative behavior and did not correlate strongly with BGE (Fig 8).

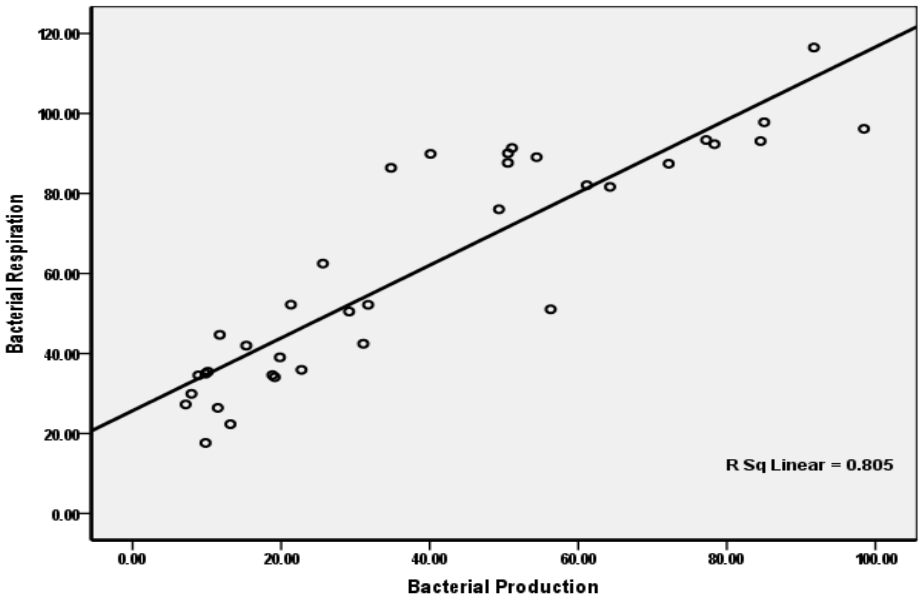

Figure 5: Relationship between bacterial respiration and bacterial production in all the sampled areas of Cross River estuary and Gulf of Guinea

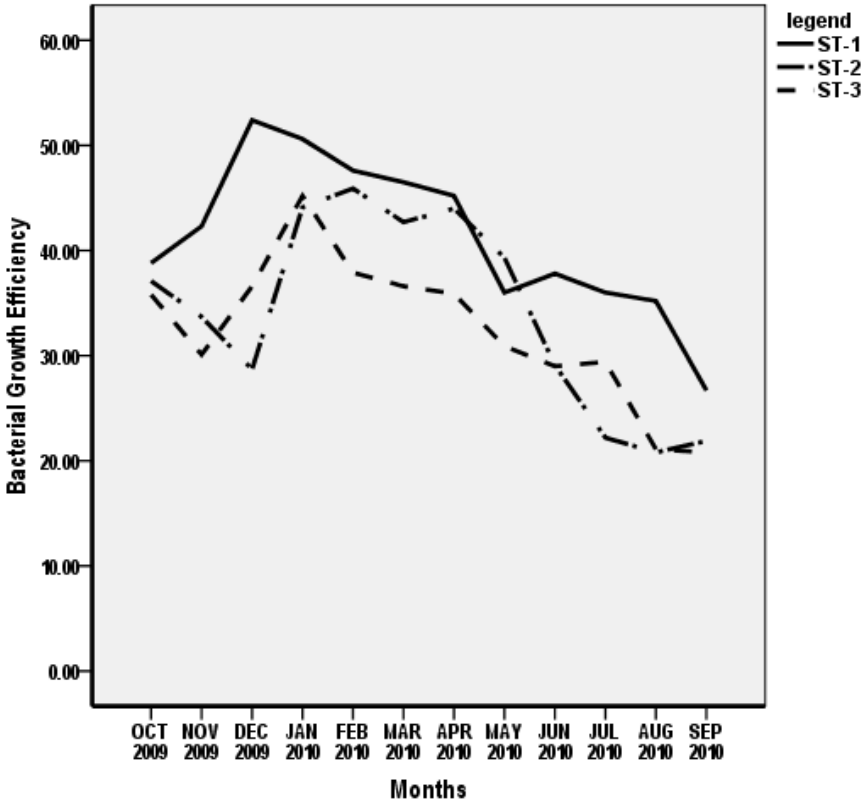

Figure 6: Variations in Bacterial growth efficiency (%) at the Cross River estuary (ST-1 and ST-2) and the Gulf of Guinea (ST-3)

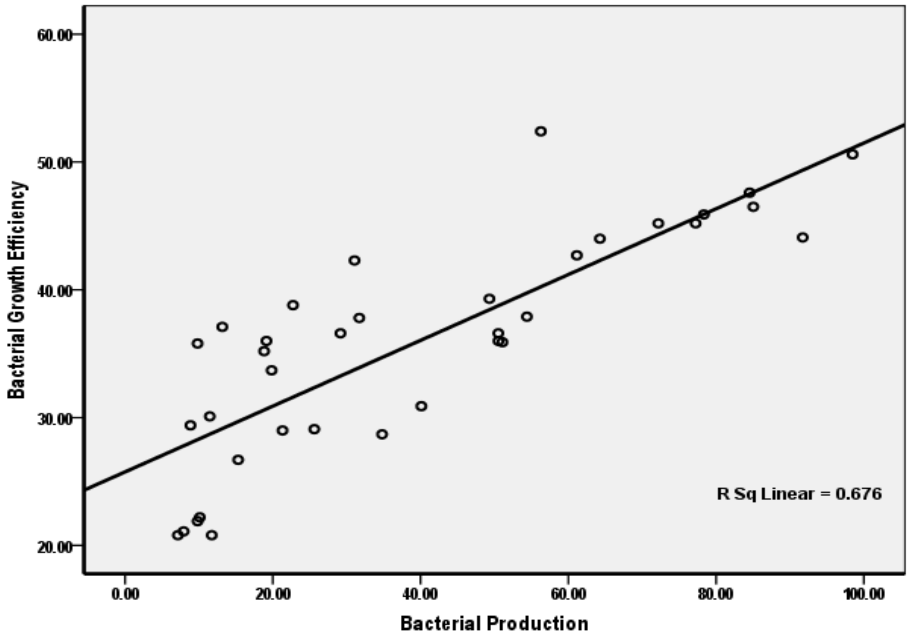

Figure 7: Relationship between bacterial growth efficiency and bacterial production in all the sampled areas of Cross River estuary and Gulf of Guinea

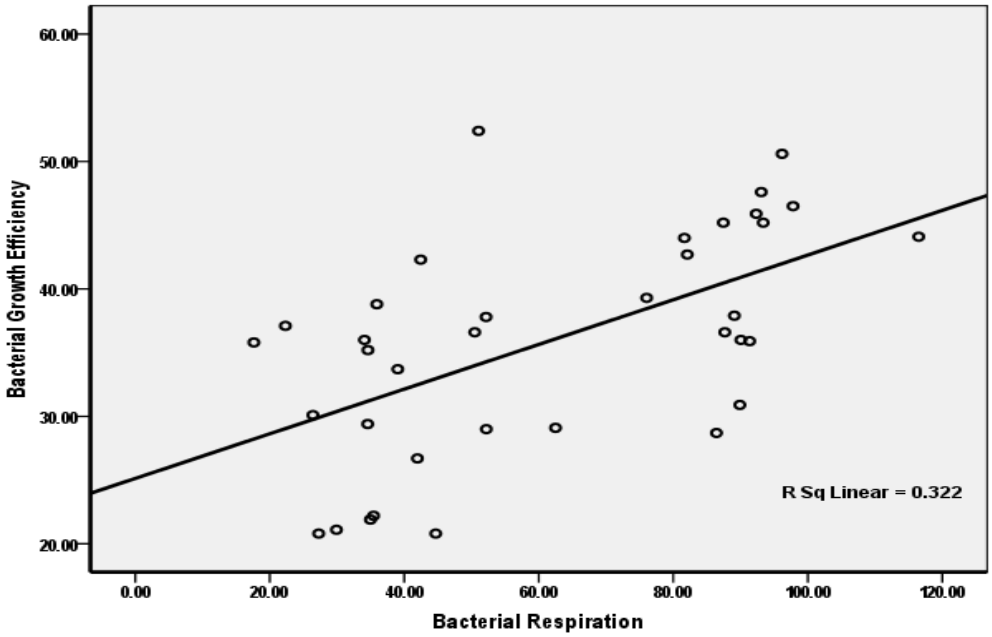

Figure 8: Relationship between Bacterial growth efficiency and bacterial respiration in all the sampled areas of Cross River estuary and Gulf of Guinea

To understand the functioning of any aquatic ecosystem and its trophodynamics, the knowledge of BGE is necessary. In this study the use of filtration method that retains the nutrient and constituents of the sampled environment tens to overcome the bias associated with the dilution method. The method adopted therefore in this study will represent as close as possible in situ measurement of natural situation.

The consistent monthly sampling regime adopted in The Cross river estuary and its near coast showed a distinct seasonal pattern in bacterial parameters with higher rates during the dry season. This is consistent with earlier studies in the Gulf of Guinea (21). BP showed a clear distributional pattern - increasing inward from the coast. This pattern was not noticed with BR and BGE

BP and BR needed for the calculation of BGE co-varied strongly (r^2 = 0.805) and responded similarly to temporal conditions. In the gulf of Guinea PP, DOC and BP showed strong correlation, suggesting that organic carbon from primary producers may be a major source of liable organic carbon in this area (21). Another important source of organic carbon in the area may be litters from mangrove lining the fringes of this estuary. This however has not masked the temporal variation of DOC in this water body, the input from this source may not be very significant and one

credible reason may be that organic carbon from this source may be refractory and not readily available for bacterial uptake.

BP and BR varied about the same magnitude and this is contrary to results obtained in Hudson River where BR were constrained and showed lower variability compared to BP (22) and in experimental manipulation (23). It was shown that in the Cross River estuary bacteria were largely active (52.4 to 77.3%) (23), indicating that although all bacterial cells were respiring, a good proportion of the cells were actively dividing and accumulating biomass. Temporal variations in BR and BP rates were significant but appropriate estimates will require repeated measurements over time and space. In this study, season seems to exert a strong influence on the variability because higher rates of BR and BP were noticed during the dry season. Seasonality of BR has been reported other studies (25, 26, 22). During the wet season the Cross River estuary and the Gulf of Guinea will receives large amount of particulate material with flood water. This will reduce photosynthetic activity and also introduce refractive organic material from terrigenous sources. This may explain the sharp decrease in bacterial activities noticed during this period.

Variations of BGE during the study were within the ranges noticed in other studies (25, 26). In the study area temperature remained relatively unchanged 23.5 to 29^{o}C and will therefore have little or no effect on the variability of BGE contrary to some findings in temperate regions (27, 28). Although the positive significant relationship between BGE and DOC (r^2 =0.711; $p<0.001$) and BGE and BP (r^2 =0.676; $p<0.001$) with clear seasonal trend makes the pattern predictable, this does not explain the intrinsic seasonal mechanism that may be responsible for such variations. This will suggest intensive measurements of biological and physico-chemical parameters over an extended period which has not been addressed in this study. There is also the need to explore if BGE is a system dependent parameter that can only be calculated and applied to specific ecosystem as the need arises.

In this study the value of BGE is influenced strongly by the values of BP. Other studies have also shown that BGE can be strongly expressed as a function of BP (29, 22) since BR showed a more conservative tendency. Again, a shift in the proportion of active bacterial population will result in significantly higher BP and will increase the dependence of BGE on BP. The low variability of BGE in the estuary and the coastal water could be explained by significant positive relationship BP and BR. The variation in the BGE reflects the trophic status of the ecosystem and may be influenced by combination of factors associated with seasons. The variations noticed in BGE support the suggestions that the use of constant BGE in accessing bacterial trophodynamics and food web interactions in aquatic ecosystems may be, for the most part inaccurate (30, 29, 22).

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