INTERNATIONAL JOURNAL OF NATURAL AND APPLIED SCIENCES (IJNAS), VOL. 4, NOS.1& 2 (2009);P. 21 – 26, 1 TABLE, 3 FIGS.

# Bacterioplankton viability and production in the lower Cross River estuary, southeastern Nigeria.

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**ABSTRACT**

Temporal (12 months) and spatial dynamics of bacterioplankton abundance, production rate and viability based on cell integrity were determined in surface waters of the lower Cross River Estuary, South East Nigeria. Bacterial abundance was enumerated with epifluorescence microscopy, after staining with 4' 6-diamidino-2-phenylindole (DAPI). Bacterial abundance showed temporal and spatial variation ranging from 4.84 to 16.49 x $10^9$ cells $l^{-1}$. Bacterial production based on multiplying bacterial growth grate and abundance varied from 3.16 to10.69 $\mu gCl^{-1}h^{-1}$. A coupled relationship between chl *a* and bacterial abundance ($r^2$ = 0.906) is shown despite the in balance between bacteria and primary production. The proportion of viable bacterial cells varied from 52.4% to 77.3%, suggesting that bacteria in the Cross river estuary will constitute a significant active component of biological productivity.

## INTRODUCTION

The aquatic pelagic ecosystems are sustained mainly by a grazing food chain and microbial food chain. The microbial food chain basically recovers otherwise loss organic materials from sloopy feeding within the grazing food chain (Pomeroy and Weibe, 1993) and bacterial production is an important link between dissolved organic carbon, detritus and organisms of higher trophic levels. The bacteriovores are in turn consumed by protozoans and larger zooplankton (Wetzel, 1995). Bacterioplankton are known to have appreciable heterotrophic versatility (Egli, 1995) and constitute a significant component of total pelagic secondary production in most coastal plankton communities (Cole, 1988; Ducklow and Calson 1992, Ducklow, 1983). In most pelagic ecosystems the distribution, abundance and production of bacterioplankton are in coupled relationship with phytoplankton abundance (Bird and Kalff, 1984; Cole, 1988; Cho and Azam, 1990). Although many studies have shown strong phytoplankton-bacteria co-variation, others have reported weak or insignificant relationship between them (Findlay et al., 1991).

The estuarine waters of the Cross River and the adjoining Calabar and Great Kwa rivers are characterized by high standing stock of microbial biomass (Antai et al., 2003) and is highly productive. This study showed that bacteria contributed significantly to the energy flow in these environments.

However limited environmental and ecological studies have been undertaken in the estuary and there is no report to date on bacteria viability and the relationship between bacterial parameters and phytoplankton. The shores of the estuary are heavily lined with mangrove swamp forest infested with Nypa palms. This constitutes an important source of detrital material for bacterial attachment and utilization. This study reports bacterial abundance, production and viability (based on cell membrane integrity) of bacteria in relation to chlorophyll a concentration in lower cross river estuary

## MATERIALS AND METHODS

### Field site and sampling

The Cross River estuary is part of the Cross river basin situated approximately between longitudes $7^0$ and $10^0$E and latitudes $4^0$ and $8^0$N and is the largest estuary in Nigeria (Fig. 1). During the survey water samples were collected every month from February 2001 to January 2002 at 4 stations located between the mouth and mid-estuary. Samples were hand-collected just beneath the water surface using acid-washed 2 litre polyethylene sampling bottles.

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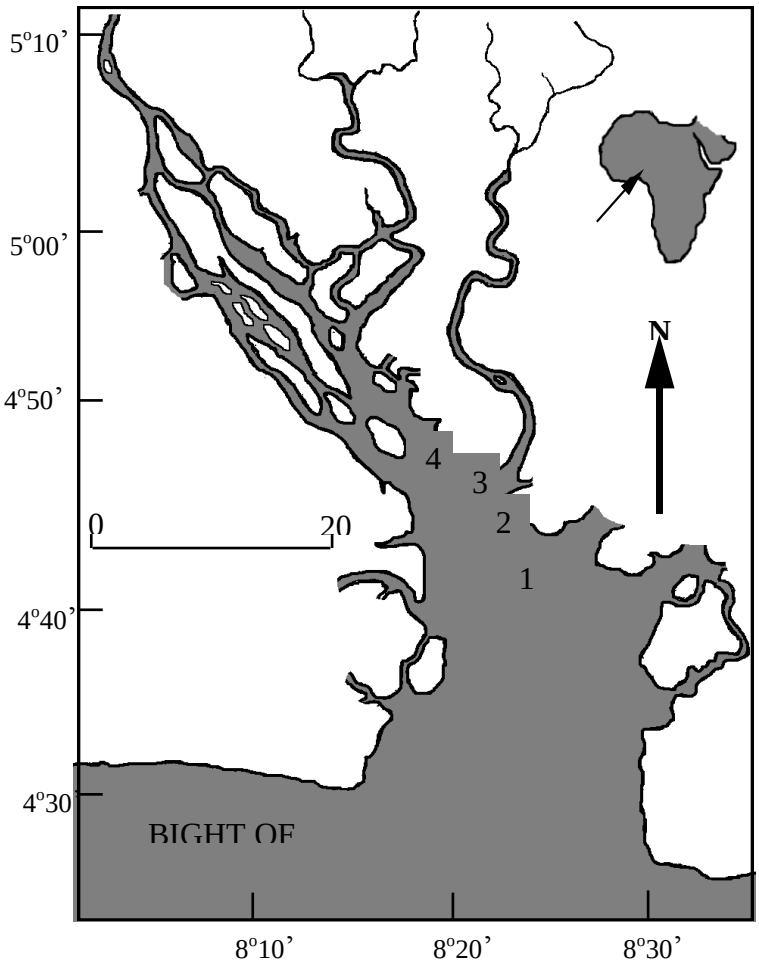


Fig. 1: Map of Cross River estuary showing sampling locations

**Chlorophyll *a* and Bacterial Abundance**

To measure Chlorophyll *a* concentration, 150 ml of the estuarine water sample was filtered onto 25 mm GF/F filters, then measure spectrophotometerically after extraction with acetone as suggested by Parson et al. (1992)

For bacterial count, 10 ml water samples were fixed with formaldehyde (2% final conc.) and refrigerated. Abundance was determined by epifluorescence microscopy on samples stained with 4' 6-diamidino-2-phenylindole (DAPI) at 0.1µg $L^{-1}$ final stain concentration, incubated for 7 mins in darkness and filtered onto 0.2µm pore size pre-blackened nuclepore filter and counted with epifluorescence microscope (Porter and Feig, 1980). More than 200 cells were counted for each sample.

**Bacterial Growth rate and Production**

Bacterioplankton growth rate in the surface water was determined using a 1:10 dilution-incubation technique. The water sample was first passed through a 5µm pore size filter to remove grazers. The 5µm filtrate was thereafter diluted 10 times with 0.2 µm filtered sterilized water from the same source. Bacteria growth rates were calculated from the increase of bacterial abundance over a 12 h incubation at 28°C with subsamples taken every 3 h. The regression line for logarithm of bacterial abundance vs time was consistently significant for all stations throughout the study period.

Bacterial cell production was calculated by multiplying bacterial abundance by growth rate at every station (Moriaty, 1990). The cell to carbon conversion was based on an average cell volume of 0.1µm$^3$ determined microscopically and a specific gravity of 1.1 and a carbon content of 22% of the wet weight (Bratbak and Dundas, 1984; Simon and Azam, 1989), using a conversion factor of 2.42 x $10^{-14}$ gC $cell^{-1}$. The cells were mostly rod shaped and within a size range of 0.45-0.50µm x 0.7-1.0µm giving a biovolume of 0.071 – 0.130µm$^3$.

**Cell Viability**

Bacteria viability in the water body was estimated using mixed green and red dyes (Live/Dead BacLight viability kit; Molecular Probe Inc., Oregon, USA). The mixed dye differentiates live (green fluorescent) and dead (red fluorescent) cells which is based on the membrane integrity (Molecular Probe Inc. 1993). Bacterial cells in the samples were incubated with the mixed dyes for 15 min at 28°C and filtered through sudan black stained nuclepore filter (0.2µm pore size) and counted with epifluorescence microscope.

## RESULTS

**Bacterial Abundance and Production**

Total bacterial abundance in the estuary ranged from 4.84 x $10^9$ at station 2 (April) to 16.49 x $10^9$ cells $l^{-1}$ at station 3 (January) and there was no significant differences among stations.Mean seasonal bacterial distribution at the 4 stations is presented in Fig. 2. Bacterial abundance showed temporal variation but the seasonal variation was limited to a range of less than $10^1$(Table 1).

Bacterial specific growth rate varied from 0.035 to 0.142 $h^{-1}$ with a mean of 0.09 $h^{-1}$. Bacterial growth rate had it peaks in April, May and June during the wet season and did not show a good correlation with chlorophyll a concentration. Bacterial production in the Cross River estuary was consistently higher during the wet season than the dry season. It varied from 3.16 at station 4 (December) to 10.69 µg$Cl^{-1}h^{-1}$ at station 3 (May) (Table 1). Bacterial production did not show good correlation with bacterial abundance ($r^2$= 0.45) and may have been greatly influenced by the growth rate.

**Table 1. Bacterial parameters and chlorophyll *a* concentration in the lower Cross River**

| Sampling Month | Station | Bacterial abundance (x $10^6$ cells $ml^{-1}$) | Bacterial growth rate ($h^{-1}$) | Bacterial carbon production (µg C $l^{-1}$ $h^{-1}$) | Chlorophyll *a* (µg C $l^{-1}$) |
|---|---|---|---|---|---|
| February | 1 | 14.32 | 0.049 | 7.59 | 18.5 |
| 2001 | 2 | 13.31 | 0.041 | 6.28 | 11.2 |
| | 3 | 16.07 | 0.039 | 6.27 | 24.3 |
| | 4 | 15.4 | 0.045 | 6.93 | 15.8 |
| March | 1 | 7.31 | 0.045 | 3.29 | 5.9 |
| | 2 | 7.83 | 0.042 | 3.29 | 6.2 |
| | 3 | 8.22 | 0.051 | 4.19 | 8.5 |
| | 4 | 8.16 | 0.049 | 4.02 | 9.3 |
| April | 1 | 5.07 | 0.142 | 72 | 4.7 |
| | 2 | 4.84 | 0.137 | 6.63 | 3.9 |
| | 3 | 5.24 | 0.131 | 6.86 | 4.2 |
| | 4 | 5.11 | 0.14 | 7.15 | 4.5 |
| May | 1 | 7.11 | 0.125 | 8.89 | 5.4 |
| | 2 | 6.94 | 0.132 | 9.16 | 5.1 |
| | 3 | 7.08 | 0.137 | 10.69 | 6.2 |
| | 4 | 7.14 | 0.13 | 9.28 | 6.3 |
| June | 1 | 6.33 | 0.103 | 6.52 | 5.2 |
| | 2 | 5.82 | 0.118 | 6.87 | 5.4 |
| | 3 | 6.52 | 0.11 | 7.17 | 6.1 |
| | 4 | 6.04 | 0.115 | 6.95 | 5.6 |
| July | 1 | 6.84 | 0.093 | 6.36 | 5.8 |
| | 2 | 6.81 | 0.098 | 6.67 | 6.2 |
| | 3 | 6.97 | 0.103 | 7.18 | 5.9 |
| | 4 | 6.89 | 0.095 | 6.55 | 5.8 |
| August | 1 | 10.03 | 0.061 | 6.12 | 10.4 |
| | 2 | 9.68 | 0.064 | 6.2 | 8.3 |
| | 3 | 9.03 | 0.059 | 5.33 | 8.1 |
| | 4 | 10.11 | 0.06 | 6.07 | 9.9 |
| September | 1 | 13.28 | 0.064 | 8.5 | 13.8 |
| | 2 | 13.14 | 0.061 | 8.02 | 12.9 |
| | 3 | 13.92 | 0.07 | 9.74 | 13.2 |
| | 4 | 13.74 | 0.067 | 9.21 | 14.7 |
| October | 1 | 7.83 | 0.073 | 5.72 | 6.3 |
| | 2 | 7.94 | 0.067 | 5.32 | 7.2 |
| | 3 | 8.16 | 0.071 | 5.79 | 7.4 |
| | 4 | 8.23 | 0.066 | 5.43 | 7.4 |
| November | 1 | 6.94 | 0.075 | 5.21 | 6.7 |
| | 2 | 6.82 | 0.081 | 5.52 | 7.2 |
| | 3 | 7.41 | 0.072 | 5.34 | 7.4 |
| | 4 | 6.89 | 0.077 | 5.31 | 7.4 |

| | | | | | |
|---|---|---|---|---|---|
| December | 1 | 5.93 | 0.069 | 4.09 | 7.2 |
| 2002 | 2 | 5.67 | 0.058 | 3.29 | 6.5 |
| | 3 | 6.04 | 0.055 | 3.32 | 5.1 |
| | 4 | 5.96 | 0.053 | 3.16 | 7.4 |
| January | 1 | 16.22 | 0.038 | 6.16 | 15.7 |
| 2002 | 2 | 15.84 | 0.035 | 5.54 | 10.5 |
| | 3 | 16.49 | 0.043 | 7.09 | 12.4 |
| | 4 | 16.37 | 0.039 | 6.38 | 14.2 |

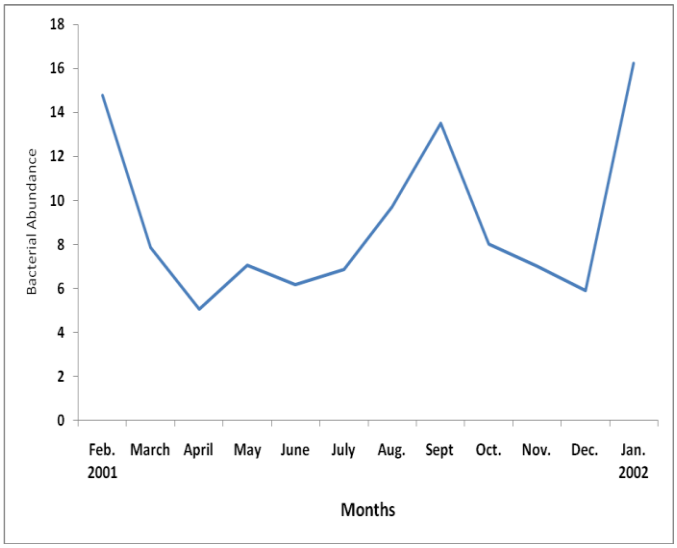


Fig. 2. Mean seasonal bacterial (x $10^6$ cells $ml^{-1}$) distribution in the lower Cross River estuary

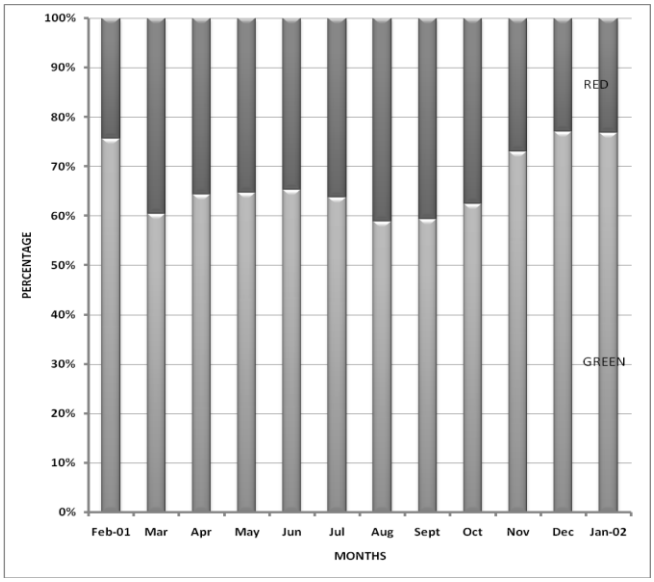


Fig. 3. Mean distribution of viable (green cells) and dead (red cells) bacteria based on membrane integrity in the lower Cross River.

**Chlorophyll a concentration**

Chlorophyll a distribution in the Cross River showed clear seasonal trend. Higher concentrations were noted during the dry season particularly in January and February. Chlorophyll a concentration varied from 0.8 in June (Station 3) to 24.5 µg C $l^{-1}$ in February (Station 1) (Table 1) The temporal variation showed a good correlation with bacterial abundance ($N_o$) and was statistically significant ($P<0.001$) and is expressed as:

$N_o = 0.62(Chl_a) + 1.132$ ($r^2 = 0.963$, n = 48)

**Viability of bacterial cell**

The proportion of green stained cells (viable) was high in Cross River estuary. It varied from 52.4% in April (wet season) to 77.3% in January (dry season) (fig.3). The seasonality was obvious. The mean live cells were 69.5%. The sum green and red cells showed strong correlation with DAPI stain counts and was statistically significant ($r^2 = 0.783$, $P<0.001$).

## DISCUSSION

The distribution of chlorophyll a in the Cross River estuary showed marked seasonal variation. Their distribution influenced the distribution of bacterial abundance with obvious correlation between phytoplankton and bacterial abundance. This relationship was not extended to bacterial specific growth rate ($r^2 = 0.432$ ). Extraneous organic material input especially during the wet season can disrupt the phytoplankton-growth rate relationship. Bacteria can derive nutrient from terrigenous organic material thereby having growth rate which will exceed the threshold of available DOC from phytoplankton. Therefore in the Cross River the high growth rates noticed during the wet season may be influenced by organic material inputs from storm run-off.

Bacteria-phytoplankton relationship is well documented in a variety of waters (Bird and Klaff, 1984; Cole et al., 1988; Cho and Azam 1990; Naganuma and Muira, 1997). This relationship is based on the fact that bacteria utilize photosynthetic exudates as DOC for growth. It is also known that bacteria is involve in remineralization of nutrient

necessary for phytoplankton growth thereby associating with phytoplankton through positive feed back thus the coupled relationship. This relationship exists even against the imbalance where bacterial respiration exceed primary production (del Giorgio et al., 1997) and in coastal waters that may derive significant portion of allochthonous organic matter (Naganuma and Muira 1997; Webster et al., 1996).

**Bacterial production**

Bacterial production rate showed poor correlation with bacterial abundance ($r^2$ = 0.233) and chlorophyll concentration ($r^2$ = 0.174). The production rates may have been influenced by bacterial specific growth rate. Bacterial production rate in this study varied from 3.16 in December to 10.69 $\mu gCl^{-1}h^{-1}$ in May and is comparable to the upper ranges of 0.02 – 6.25 $\mu gCl^{-1}h^{-1}$ estimated for various cross-pelagic systems (Cole et al. 1988). The rate of bacterial production in the Cross River exceed primary production (1.75 – 4.86 $\mu gCl^{-1}h^{-1}$) in the water column (unpublished data). This shows an imbalance between bacterial production and primary productivity in the water column. This discrepancy may be explained by allochthonous input of terrigenous dissolved organic matter. The Cross River water system is very turbid and these materials are derived from the fringing mangrove swamp and rain forest and these could be a good source of material for bacterial production. Opsahl and Banner (1997) showed that terrigenous DOM is subject to microbial utilization. This imbalance will contradict the view that bacterial secondary production is 20 – 30% of primary production

In Cross River estuary, most of pelagic bacteria are viable and their rate of production compared to primary production indicates that they depend on terrigenous DOM in addition to photosynthetically produced DOC for growth.

**Bacterial viability**

The proportion of viable cells (52.4 to 77.3%) in the Cross river estuary are high and within the range (49.3 to 75.1%) determined by Naganuma and Miura (1997) at different locations within Seto Inland sea. The determination of viability based on cell integrity is high compared with others based on specific cell metabolic activities like response to nalidixic acid (39.8%, Kogure et al., 1980), respiration (25%, Dutton et al., 1986). This non-specific method may serve as a better alternative to metabolically specific methods. The application of this method will however call for a clear definition live and dead cells. A situation of metabolically dead but membrane active cells may occur in the natural environment as highlighted by Naganuma and Miura (1997).

Bacteria in the Cross River estuary will constitute a significant active component of the aquatic ecosystem and will depend on the phytoplankton and terrigenous organic input to sustain the high level of production and viable bacterial population determined in this study.

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**REFERENCES**

Antai, E.E. and Asitok, A. (2007) Changes in C:N ratio in mangrove dominated Calabar River. *Afri. J. Pollution and Environ. Health,* 11: 56-65

Bird, D.F. and Klaff, C.A. (1984). Empirical relationships between bacterial abundance and chlorophyll concentration in fresh and marine waters *Can. J. Fish Aquat. Sci.,* 41: 1015-1023

Bratbak, G. and Dundas, I. (1984). Bacterial dry matter content and biomass estimations. *Appl. Environ. Microbiol*., 48:755-757

Cho, B.C. and Azam, F. (1990). Biogeochemical significance of bacterial biomass in the ocean's eutrophic zone. *Mar. Ecol. Prog. Ser*., 63:253-259

Cole, J.J., Finlay, S. and Pace, M.L. (1988). Bacterial production in fresh and saltwater ecosystem; a cross system overview. *Mar. Ecol. Prog. Ser.,* 43: 1-10

Del Giorgio, P.A, Cole, J.J and Cimbleris, A. (1997). Respiration rate in bacteria *Bioscience* 33: 494-501

Ducklow, H.W. (1983). Production and fate of bacteria in the ocean exceed phytoplankton production in unproductive aquatic systems. *Nature* 385: 148-151

Ducklow, H.W. and Carlson, C.A. (1992). Oceanic bacteria production. In:Advances in Microbial Ecology. (Marshal, K.C. Ed.), Plenum Press, New York : 113-181

Dutton, R.J., Bitton, G. and Koopman, B. (1986). Application of a direct microscopic method of the determination of active bacteria in lakes. *Water Res.,* 20: 1461-1464

Elgi, T. (1995). The ecological and physiological significance of the growth of heterotrophic microorganisms with mixtures of substrates. In: Advances in Microbial Ecology (J.G. Jones ed), Plenum Press, New York: 305-386.

Finlay, S., Pace, M.L., Lintus, D. and Howe, K. (1992). Bacterial metabolism of organic carbon in the tidal freshwater Hudson Estuary. *Mar. Ecol. Prog. Ser.*, 89:147-153

Kogure, K., Shimidu, U. and Tada, N. (1980) .Distribution of viable marine bacteria in neritic seawater around Japan. Molecular Probe, Inc . Fluorescent probes for assessing cell viability. *Bio Probes* (New products and applications from Molecular Probes, Inc.) 18: 1-2

Moriaty, D.J.W. (1990). Technique for estimating bacterial growth rates and production of biomass in aquatic environments. p. In: Method in Microbiology (Grigorova, R and Norris J.R. Eds.)*Techniques in Microbial Ecology*, Academic Press, San Diego. 22 :211-234.

Naganuma, T. and Miura, S. (1997). Abundance, production and viability of bacterioplankton in Seto Inland Sea, Japan. *J. Oceanogr.* 53:435-442

Opsahl, S and Benner, R. (1997). Distribution and cycling of terrigenous dissolved organic matter in the ocean. *Nature*, 386:480-482

Parson, T.R. Maita,Y and Lalli, C.M. (1992). A manual of chemical and biological methods for seawater analysis. Pergamon Press, New York.

Pomeroy, L.R. and Wiebe, W.J. (1993). Energy sources for microbial food webs, *Mar. Microb. Food Webs*, 7:101 -118

Porter, K.G. and Feig, Y. (1980). The use of DAPI for identifying and counting aquatic microflora. *Limnol Oceanogr*. 25:943-948

Webster, I.T., Norquay, S.J., Ross, F.C. and Wooding, R.A. (1996). Solute exchange by convection within estuarine sediments. *Estuar. Coast Shelf Sci.*,42:171-183

Wetzel, R.G. (1995) Death, detritus and energy flow in aquatic ecosystems. *Freshwater Biology*, 33:83-89.