

Abundance and production of particle-associated bacteria and its implication in a mangrove dominated estuary.

M-J. Gonsalves*, S. Nair, P.A. Loka Bharathi and D. Chandramohan

Microbiology Laboratory, National Institute of Oceanography, Goa, 403-004.

Council of Scientific and Industrial Research, India.

ABSTRACT: An annual study was carried out in the Mandovi, a mangrove dominated tropical estuary to assess the contribution of particle-associated bacteria (PAB) to the system's variability in terms of abundance and productivity. The suspended load, which was composed of inorganic mineral grains and allochthonous materials including mangrove litter, was high during the southwest monsoon season (sw monsoon). The ratio of organic:inorganic content of these particles was low during the pre- and post-monsoon seasons. The PAB abundance ranged from $1.0 \times 10^{10} \text{m}^{-2}$ to $22.8 \times 10^{10} \text{m}^{-2}$ and accounted for 4 to 94% of the total bacterial abundance. The variation in PAB abundance was due to their preferential colonization, which depended on the quantity and quality of the particles. The average annual PAB production was $214 \mu\text{g.C.m}^{-2}\text{d}^{-1}$ and contributed to an average of 35% of the total bacterial production. The primary productivity (PP) was 137, 14 and $163 \mu\text{g.C.m}^{-2}\text{d}^{-1}$ for the pre-monsoon, sw monsoon and post-monsoon periods respectively. The calculated maximum bacterial carbon demand (BCD) of PAB was 37% of the total BCD. On an annual basis <1% of PP contributed to PAB-BCD. It is suggested that the rest of the BCD was met from mangrove litter, and other allochthonous sources. PCA analysis showed that biotic parameters were predictors for ~ 50% of the variability and had a marked seasonality linked to salinity. The allochthonous sources contributed significantly to the structure of the biological community of this mangrove dominated estuary.

KEY WORDS: Particle-associated bacteria · Abundance · Production · Tropical mangrove estuary · Goa · India

INTRODUCTION

Estuaries are the most dynamic and biologically productive ecosystems on earth (Schlesinger 1997). Biological processes in the estuaries respond to changes in the quality and quantity of environmental inputs due to fluvial influence (Hubertz & Cahoon 1999, Russell et al. 2006). The autochthonous production as well as the imported organic and inorganic particles brings about the variation in the autotrophic and/or heterotrophic nature of the estuary (De Souza et al. 2003, Ram et al. 2003, Russell & Montagna 2007). Bacteria attached to these particles form an important constituent of the planktonic food web as they aggregate smaller particles and break down preferentially larger ones (Gorsky et al. 2000), and thus play a significant role in the mobilization of carbon. Activities of these associated bacteria are responsible in regulating the biogeochemical fate of particulate organic matter (Crump & Baross 2000, Crump et al. 1998). During the residence time of the particles, PAB may elaborate high enzymatic activities (Crump & Baross 2000, Crump et al. 1998, Smith et al. 1992), decompose the recalcitrant particles and improve the nutritional quality of the particles. Thus estuaries serve as important sites for the bacterial degradation of terrestrial and riverine organic matter associated with particles (Lee & Wakeham 1988). While PAB of temperate estuaries have been fairly well studied (Crump et al. 1998, Revilla et al. 2000), the data from tropical (Bano et al. 1997, De Souza et al. 2003) and sub-tropical (Ducklow & Shiah 1993) estuaries are scanty in spite of being ubiquitous. In the Indus river delta, Bano et al. (1997) reported that bacterial production associated with particles was carbon limited and could have a major impact on mangrove ecosystem structure and functioning and the production of economically important fish and shrimps. Study from tropical Zuari estuary situated on the west coast of India has shown that PAB were a significant component of the total bacterioplankton and the dynamics was determined by the availability of the substrates and the re-suspension of the sedimented material (De Souza et al. 2003). Mandovi and Zuari estuaries though adjacent to each other on the west coast of India, are influenced by different factors viz. a narrower channel, more of fresh water input and frequent navigation activities in the Mandovi as compared to that of the Zuari estuary. Thus, each estuarine environment exhibits a wide variation in their physical and chemical factors. One may expect PAB to be governed by different factors in the estuary. Even less understood is how monsoon driven wind would influence the bacterial process in a tropical estuary such as the Mandovi.

The Mandovi estuary, located in Goa on the West Coast of India, is fringed with extensive mangroves and opens into the Arabian Sea. The average density of mangroves in the Mandovi estuary is 461 trees ha⁻¹ and the litter yields in the Mandovi-Zuari estuarine system has been estimated to be 10.2 tonnes ha⁻¹ year⁻¹. A high runoff of relatively easily degradable organic matter and recalcitrant matter from the leaf litter and inorganic particles from terrestrial region comes into the estuarine system during the sw monsoon season. This input of litter may be retained in the estuary either by sinking or re-suspension (Crump et al. 1998). Thus, the Mandovi estuary receives autochthonous and large allochthonous inputs from different sources i.e. riverine discharge (particulate and dissolved matter) and mangrove leachate. Wafar et al. (1997) estimated particulate and dissolved organic carbon to be 498 and 876 mg.C.m⁻².d⁻¹ respectively in this estuarine system. The dissolved organic nitrogen and phosphorus released from the mangrove litter sustained the nutrient budget of the Mandovi-Zuari estuarine system (Wafar et al. 1997). The questions to be addressed in this context are, do bacteria colonize these particles and contribute to the nutrient budget and do these particles satisfy the bacterial carbon demand? In order to answer these questions, we estimated the abundance and production of PAB along with other biotic and abiotic factors that could be responsible in bringing the variations in the estuary.

MATERIALS AND METHODS

Study site. The average water column depth of the Mandovi estuary is ~ 4m. It is influenced by tidal currents and is characterized by mixed semi-diurnal tides. Speed of propagation of both diurnal and semi-diurnal tides is about 6m/s, which remains unchanged over a distance of 40 km from the mouth (Shetye et al. 1995). The maximum current velocity during a tidal cycle is 1.1m/s (Pers. Comm. V. Sanil Kumar NIO, Goa). It is classified as a macrotidal estuary with spring tides > 2m (Shetye et al. 2007). The estuary is under the influence of the monsoon circulation and experiences annual recurrence of spells of heavy precipitation and is referred to as a “monsoonal” estuary. The estuary has a relatively low run off-season during November - May compared to the heavy run off period from June to October (Shetye et al. 1995). On an average, the volume of freshwater flowing through the Mandovi in a year exceeds the volume of the estuary by a factor of 40. Over 95% of the freshwater efflux occurs during June – October, implying that the water in the river channel is flushed out and renewed several times. Such

flushing turns the estuarine water limnetic from head to mouth (Shetye et al. 2007) during the monsoon period. The well defined seasons, viz. pre-monsoon (February – May), sw monsoon (June – September) and post-monsoon (October – January) brings about a marked variation in the biology of the system.

Sampling. The sampling station 15^o30.323N and 73^o52.430E was located ~12km from the mouth in the lower reaches of the estuary (Fig. 1). Monthly collection was done for one year during slack water. Surface and near-bottom water samples were collected using Niskin sampler and then transferred in acid cleaned polypropylene bottles and processed in the laboratory within 2 hours of sampling. For particle associated bacterial parameters the difference between total and the free-living bacterial parameters was considered. For total bacterial parameters we used water filtered through 220 µm mesh. To estimate free-living bacterial parameters we used water filtered through 3 µm pore size filter. To avoid clogging especially during the monsoon season when the water is highly turbid, the filter papers were changed or the volume of water filtered was reduced, depending on the extent of turbidity (visual observation or slow filtration). To minimize cell damage and the possibility of forcing some large cells through the filter pores, filtration was done at very low vacuum (<1kPa).

Physico-chemical parameters. Water temperature was recorded, pH (Orion 3 star) and salinity (AUTOSAL 8400 A) were measured. The particulate organic carbon (POC) and particulate organic nitrogen (PON) were determined using a Perkin Elmer Elemental CHN analyzer (Model 2400). The water samples were filtered through GF/D (Whatman) filter, dried at 60^oC and fumed with concentrated HCl for 24 h to remove carbonates prior to POC and PON analyses (Hedges & Stern 1984).

Particle parameters. The total number of particles present in the water sample was counted using a Coulter counter (TAIL Model) and the numbers was expressed volumetrically. Dry wt of 3 µm particles was estimated gravimetrically. Water samples were filtered through pre-weighed 3 µm pore size polycarbonate filters (Millipore) under low vacuum (<1kPa). The filters were then rinsed with 200 ml of membrane-filtered (0.22 µm) distilled water, dried to constant weight at 40^oC and weighed using a Metler balance (Model AE200) and expressed as percentage of total weight of particles (0.22 µm filter). For blanks, only 0.22 µm membrane filtered seawater

was used. Suspended particulate matter (SPM) was determined gravimetrically on pre-weighed GF/D (Whatman) filter as described by (Krey 1964). Filters, which were used for SPM, were then ignited at 450°C for 3h and re-weighed after cooling in order to determine the inorganic content of particles. The organic content was calculated by subtracting inorganic content values from SPM based on the loss on ignition method (LOI). This method is in use in many laboratories. Heiri et al. (2001) and others have sought to reduce the variability inherent to LOI. Barille-Boyer et al. (2003) have stated the various factors to be considered when calculating particulate organic matter using the LOI method.

Biological parameters

Primary production and chlorophyll *a* concentration. Primary production (PP) was measured by ¹⁴C assimilation method (Lohrenz et al. 1992). Water samples were collected in four 300-ml polycarbonate bottles from each depth. One ampoule of NaH¹⁴CO₃ (Board of Radiation and Isotope Technology, Mumbai with a specific activity of 185 kBq) was added to each bottle. Three bottles were considered for light and one for dark incubation. Incorporation of ¹⁴C was determined by filtering 100 ml of the sample from each bottle through GF/F filters (Whatman, USA). The filters were transferred to scintillation vials and exposed to HCl (0.5 N) fumes in vial. Liquid scintillation cocktail (5 ml) was added and the radioactivity measured in Packard 2500 TR liquid scintillation counter. PP rate was calculated as mg C m⁻³ d⁻¹, (UNESCO, 1994). Chl *a* concentrations were estimated fluorometrically with Turner Designs (USA) after filtration of 500 ml of water sample on Whatman GF/F glass fibre filters and extracted with 90% acetone following Parsons et al. (1984).

Bacteriological parameters

Particle – associated bacterial abundance and bacterial production. Samples for bacterial abundance were fixed in 2% (final concentration) formaldehyde. Bacterial abundance was enumerated by the acridine orange direct count (AODC) method (Hobbie et al. 1977). Counting was done in triplicate with 20 fields counted for each sample and bacterial numbers was expressed as numbers per litre.

Particle associated bacterial production (PABP) was estimated based on the difference between the whole water and the water filtrate after removing the 3-220 µm fraction in order to avoid conditions, which may reduce radiotracer exchange with the particles. Bacterial production

(BP) was estimated by measuring the rate of [methyl-³H] thymidine incorporation (Fuhrman & Azam 1982) by bacterial cells. Water samples (30 ml) were incubated for one hour with ³H-thymidine (specific activity = 52 Ci mM⁻¹, BARC, Mumbai) at a final concentration of 10 nM. The reaction was terminated with 2% neutral buffered formalin. The samples were then filtered through 0.22 µm polycarbonate filters (pre-soaked in 5% TCA), extracted with cold 5% TCA and rinsed with ethanol. The dried filters were then placed in scintillation vials and filled with 3 ml of dioxane-based scintillation cocktail (Sigma, USA). The samples were then radio-assayed using a Packard 2500 TR liquid scintillation counter. Thymidine incorporation rates were converted to bacterial carbon production (BCP) by using a conversion factor of 2.0 x 10¹⁸ cells produced mole⁻¹ thymidine incorporated (Iriberry et al. 1990) and a carbon conversion factor of 20 fg C cell⁻¹.

Data analysis. Before subjecting to statistical analysis the data were depth integrated. Spearman's correlation analyses was performed on the normalized data using statistical package Stat Soft Inc. STATISTICA for Windows version 6 to find out the relationship between PAB abundance, production and other environmental variables. The multivariate analysis – PCA was performed using PRIMER version 6 (Clarke & Warwick 1994). The variables were log (x + 1) transformed before performing PCA in order to reduce over domination of bacterial numbers with environmental parameters. Only those variables with a load of >0.1 were considered.

RESULTS

Water column characteristics

Abiotic variables

The range in abiotic variables for the three seasons is given in Table 1. Water temperature of the Mandovi estuary ranged from 26.0 to 33.0 °C. Salinity showed wide variation ranging from 0 (June) during the sw monsoon season to a high value of 36 (May) during the pre-monsoon season. Suspended load was recorded throughout the study period with a high load of 1.4 g.m⁻² during the sw monsoon season. The major component of the SPM was inorganic in nature and increased with increase in the quantity of SPM (p < 0.001, r = 0.94). These inorganic particles were mainly clay minerals like montmorillite, illite, kaolinite, chlorite, gibbsite and goethite. Among these, kaolinite and chlorite were dominant and at times reached as high as 45% (data not shown). The annual variation in the POM:SPM ratios was between 0.02 and 0.77.

Although the SPM showed seasonal variation, the particle number and the percentage of weight per volume of particle of size between >3 and $<220 \mu\text{m}$ did not show marked variability with season. The particle number ranged from 3.7 to $12.7 \times 10^9 \text{m}^{-2}$ exclusive of the value of the month of October of $137 \times 10^9 \text{m}^{-2}$. The percentage wt/vol of the $>3\mu\text{m}$ fraction during all the months ranged from 22% in June to 70% in December. On an annual basis, organic carbon of the particles ranged from 0.8 to 17.9mg m^{-2} , with the pre-monsoon month of March having the lowest and the monsoon month of August the highest value. Organic nitrogen content of particles showed monthly variation. The seasonal average was 0.6 , 1.3 and 0.7mg.m^{-2} during the pre-monsoon, sw monsoon and post-monsoon, respectively. As in the case of organic carbon, organic nitrogen was high during the monsoon season. In general, the particles POC:PON ratio ranged from 4.5 to 7.4 during pre-monsoon, 4 to 7.2 during sw monsoon and 4.4 to 18.1 during post-monsoon season. Principal component analysis aggregated the above abiotic variables into the second (PC2) and third (PC3) component only. The variables were salinity, SPM and inorganic content for PC2 and explained ca. 25% of the variation. In addition to the variables in PC2, PC3 had particle number and nature of the particle (POC & PON) which explained ca. 10% of the variability (Table 2).

Biotic variables

Chl *a* ranged from 111 to $165 \mu\text{g.C m}^{-2}$ during the pre-monsoon, 1.8 to $74 \mu\text{g.C m}^{-2}$ during the monsoon and 116 to $379 \mu\text{g.C m}^{-2}$ during the post-monsoon season (Fig. 2). PP varied from a minimum of 62.5 , 2.6 and $59.5 \mu\text{g.C.m}^{-2}.\text{d}^{-1}$ to a maximum of 183.1 , 36.5 and $230.6 \mu\text{g.C.m}^{-2}.\text{d}^{-1}$ during the pre-monsoon, sw-monsoon and post-monsoon seasons, respectively (Fig. 2). The PP value was 137 , 14 and $163 \mu\text{g.Cm}^{-2}.\text{d}^{-1}$ for the pre-monsoon, sw monsoon and post-monsoon periods respectively. PCA analyses showed that PP was one of the variables in PC1. However, Chl *a* was a component of PC2 (Table 2).

The total bacterial abundance was of an order of 10^{10}m^{-2} . In the study site, it varied from 0.9 to $2.4 \times 10^{10} \text{m}^{-2}$ during the pre-monsoon, 0.08 to $9.2 \times 10^{10} \text{m}^{-2}$ during the sw monsoon and 0.9 to $2.7 \times 10^{10} \text{m}^{-2}$ during the post-monsoon (Fig. 3). On an annual basis, the average particle-associated density accounted for 65% of the total bacterial community. The average PAB seasonal load was $1.1 \times 10^{10} \text{m}^{-2}$, $3.5 \times 10^{10} \text{m}^{-2}$ and $8.2 \times 10^{10} \text{m}^{-2}$ during the pre-monsoon, monsoon and post-monsoon seasons respectively. The trend of the distribution of PAB

community was similar to that of the total community (Fig. 3). Likewise, when the total bacterial production increased the PAB production also increased. Annually the total production ranged from 140 to 2280 $\mu\text{g.C.m}^{-2}.\text{d}^{-1}$. The contribution of PAB production to the total bacterial production (TBP) varied from 13 to 79%, and ranged from 66 – 863 $\mu\text{g.C.m}^{-2}.\text{d}^{-1}$ (Fig. 4). The seasonal average of PAB production was 137.3, 210.8 and 293.6 $\mu\text{g.C.m}^{-2}.\text{d}^{-1}$ during the pre-monsoon, sw monsoon and post-monsoon seasons respectively. The ratios of PABP: PP were 1.0, 15.2 and 1.8 for the pre-monsoon, sw monsoon and post-monsoon period, respectively (Table 3). PCA analysis showed that the biological components were responsible for 47.9% of the variability. Particle number and nature of the particle along with biological variables in PC3 accounted for about 83.7% of the variation (Table 2) in the estuary.

DISCUSSION

In an estuary, suspended matter determines habitats and the structure of the microbial community (Chen et al. 2005). The source, nature and composition of the particles in the suspended matter affect the microbial processes. In the Mandovi estuary, the SPM and the particles (>3 to <220 μm), were dominated by inorganic fractions and showed seasonal variation. When compared to the adjacent Zuari estuary, the quantity of SPM is twice that recorded in the Zuari estuary. Sixty percent of these particles were rich in organic carbon (De Souza et al. 2003) unlike those in Mandovi. Though the factors contributing to efflux of water to both the estuaries are similar, the nature of fluvial inputs and the watershed area determines the nature of the particles. A recent study on fluvial inputs into the Mandovi estuary (Mesquita & Kaisary 2007) has shown that considerable inputs from anthropogenic activities, mainly in the form of dissolved/ particulate manganese and iron are brought in by wind borne transport during the non-monsoon months and by river runoff during the sw monsoon from mining rejects in the vicinity. This was reconfirmed in our study as the particles showed the presence of the clay mineral grains like montmorillite, illite, kaolinite and chlorite, gibbsite and goethite. This is further corroborated by the low POM:SPM ratios encountered in this study. The recalculated POM based on the method suggested by Barille-Boyer et al. (2003) did not bring about a significant change in the POM:SPM ratio. As suggested by Barille-Boyer et al. (2003), it is essential that a factor for this estuary is calculated and not directly apply available factors, as qualitative and quantitative mineralogy may differ with estuaries.

PCA analysis also showed that the inorganic particles were one of the main components of PC2, which brought about a variation of 25% in the estuary. The characteristics of suspended matter in this estuary appear to be influenced by anthropogenic sources and are dominated by organo-mineral aggregates. Such dominance of organo-mineral aggregates with variable amounts of inorganic fraction (avg. 89%) has been reported in the Scheldt estuary (Chen et al. 2005). In Mandovi, inorganic particles were dominating due to dilution of organic matter (Hopkinson et al. 1998). However, the possibility of these particles to be exclusively inorganic is remote as mangrove litters are a source of particles in the $>3 - <220 \mu\text{m}$ range in this estuary. Occurrences of higher numbers of inorganic particles associated with organic matter than the inorganic particles alone have been reported in estuaries like Danube and San Francisco Bay (Hoch et al. 1995, Berger et al. 1996). On an annual basis, except for the months of March and August, organic carbon of the particles ranged from 2.6 to 7.8 mg m^{-2} with an overall high value during the sw monsoon season. This increased organic content of particles may be due to the shallow nature of the sampling site (4 m), leading to enrichment of organic matter from sediments via re-suspension by the tidal currents. Additional inputs from land runoff and riverine sources occur during this period. The abundance of PAB in the Mandovi estuary was 2 - 4 times more than that reported in temperate waters (Berger et al. 1996, Hoch et al. 1995) and the adjacent Zuari estuary (De Souza et al. 2003). However, it is lower than the highly organic rich Hudson and Indus river delta estuaries (Sanudo-Wilhelmy & Taylor 1999, Bano et al. 1997). Though the quantitative POM content of the particles was low, its quality was high with a POC:PON ratio of 5.5 to 7.3. A ratio from 5 to 20 reflects that the particles contain decaying mangrove litter (Wafar et al. 1997). The low ratio could also be due to high value of nitrogen content in the particles from the large inputs of ammonium either from fertilizers (Sarma et al. 2001) or re-suspension of sediments (Sardesai & Sundar 2007). The possibility of nutrients adsorbing to these inorganic particles cannot be eliminated (Holmboe & Kristensen 2002). In the Mandovi, the residence time of water is about 5-6 days during the sw monsoon season (Qasim & Wafar 1990). The degradation of organic matter will therefore be slow during the sw monsoon season due to relatively short residence time of resources. Thus, being enriched, these particles can serve as sites for bacterial colonization rather degradation especially during sw monsoon season. In our study, PAB formed a high percentage of total bacteria suggesting that the bacteria preferred these particles as a niche for colonization. The density of bacteria on

particles could depend mainly on the organic content (quality) and the number of particles (quantity). PCA also supported this inference, that the number of particles and their organic content could influence the variability of the estuary (Table 2).

PAB production was low in Mandovi estuary. Various carbon conversion factors for BP have been reported for estuarine/ coastal ecosystems because of intra (20 to 77%) and inter (32 to 83%) variability. Fukuda et al. (1998) concluded from their study that a factor of 30.2 ± 12.3 fg C cell⁻¹ would be applicable for coastal bacterial assemblages. Earlier studies on estuaries from Indian regions (Bhaskar & Bhosle 2008) have adopted 11 fg C cell⁻¹, a value lower than that of the open ocean (Garrison et al. 2000) while Ram et al. (2007) used a conversion factor of 20 fg C cell⁻¹ based on Lee & Fuhrman (1987). In this study a carbon conversion factor of 20 fg cell⁻¹ was used as it falls within the range of 12.3-30.2 fg C cell⁻¹ given by Fukuda et al. (1998) and also to enable comparison of our values with similar studies in Indian estuaries. The contribution by PABP to the TBP was 35% which was low when compared to that of 60% in the Zuari estuary (De Souza et al. 2003) in spite of the PAB abundance being in the same order of 10⁹l⁻¹. PAB production was also low when compared to reported values of Loire estuary (803 µg.l⁻¹d⁻¹) in France (Ducklow & Shiah 1993) and tidal creeks of Indus River Delta (900 µg.l⁻¹d⁻¹) in Pakistan (Bano et al. 1997). Earlier studies (Simon et al. 1990, Smith et al. 1992, Grossart & Simon 1998) on aggregate-associated bacterial production rates have been measured by pooling several aggregates. However, Ploug & Grossart 1999 and Grossart & Ploug 2000 have shown high variability in production rates among aggregates of same and different sizes of particles due to diffusion coefficient, as exchange of solutes like radiotracers is reduced when aggregates are pooled and not in continuous suspension. In our study, though the aggregates were not pooled, the variability in number and weight per volume was low. It appears that the particles in the Mandovi estuary are relatively uniform in size and low in phytoplankton content as the chl *a*:POC ratio was low. Hence, the low POM and nature of the organic matter would have affected the growth rate (Murrell et al. 1999), which resulted in the low BP values observed in this estuary. The integrated particle-associated bacterial productivity was up to 1.8 times higher than that of PP. These values were higher than values reported for the temperate region (Williams 1981, Cole et al. 1988), but were closer to that reported for the Indus river delta (Bano et al. 1997).

Particles are known to be preferential sites for bacterial colonization (Grossart & Ploug 2000). The cumulative carbon flux into bacteria/BCD depends on the bacterial growth efficiency (BGE). BGE is calculated based on production and respiration rates. Since particle associated bacterial respiration was not measured in this study, the BGE value was obtained using two methods. In the first method we used the equation of Grossart & Ploug (2000) and in the second method we took the reported BGE values from the literature and used it for calculation of BCD.

Grossart and Ploug's equation for bacterial respiration (BR) is,

$$BR = 1.57 \times BP^{0.86}$$

The BR value was calculated to be 159, and accordingly the BGE was estimated to be 57%. The annual BCD for PAB was estimated to be 373 mg.C.m⁻² (Table 3).

Very few studies are available on BGE of PAB (Grossart & Ploug 2000, Ploug & Grossart 1999). Ploug & Grossart (1999) reported 0.35 ± 0.1 BGE for aggregates of >0.7mm. Grossart & Ploug (2000) showed how BGE varied with the age of the aggregate from 0.45 ± 0.04 for 1-3 day old aggregates to 0.23 ± 0.06 and 0.04 ± 0.01 for 7 and 14 day old aggregates, respectively. Benner & Hodson (1985) found lower bacterial growth yield of 5 to 20% for mangrove particulate detritus and 30% for mangrove leachates on long-term incubations. This low growth yield was presumably due to structural complexity of the detritus (Bano et al. 1997). All these studies have shown the variability of BGE depending on the age of the aggregates and that the assumption that BGE increases with BP may not hold good for estuaries with high allochthonous inputs from terrestrial sources. Thus, BGE of aggregates can range from 3 to 49% with an average of 24% (Grossart & Ploug 2000). Further, the reported range of BGE of the total bacterioplankton in the Mandovi estuary was from 22 to 31% (Ram et al. 2007). As the Mandovi estuary has inputs from mangrove detritus and a significant amount of particles were inorganic, we assume that the growth efficiencies for PAB could be within a lower range. Generally, low BGE values have been reported for mangrove detritus. Hence, based on the above rationale we used an average value of 24% for calculating the BCD for PAB. BCD for PAB was thus calculated to be 891 mg.C.m⁻²d⁻¹, which is more than twice that calculated by using the equation. For estimating the total annual BCD of Mandovi, we have used the published BGE value of 27% (Ram et al. 2007) and the calculated BCD was 2427 mg.C.m⁻²d⁻¹. This value is within the reported range for the Indus river delta by Bano et al. (1997). The calculated BCD for PAB ranged from 15 to 37% of the total BCD in the Mandovi estuary. The total BCD was 23

times more than the PP carbon which was much less than that reported for Zuari estuary. Lignell (1990) has observed that phytoplankton exudates in the estuaries provide less than half the BCD. BCD of PAB was 3.6 and 8.5 times higher than PP which means that 0.1 and 0.4 % of PP could be contributed to the BCD. Therefore, it is possible that the remaining BCD was met from mangrove litter, and other allochthonous sources. Since the BCD of PAB is high in the Mandovi, the turnover is faster on the particles compared to long turnover times of aggregates >0.5mm (Simon et al. 1990, Smith et al. 1992).

PCA showed that 47.9% of the variation in the estuary is governed by the biotic variables. The number and nature of particles (PC3) increased the variability by 10.3%. This suggests that apart from biotic parameters, the number and nature of particles also effect variation in the estuary. From the present study it can be deduced that, the flux of organic carbon in the mangrove dominated Mandovi estuary is governed by PAB, which, in turn is dependent more on the input of quantity and quality of the particles from the adjacent mangroves and river run off for preferential colonization than on phytoplankton carbon alone.

Acknowledgements. This work was sponsored by the Office of Naval research, Washington, USA. The authors thank the anonymous referees for their valuable suggestions, which contributed to the improvement of the manuscript. The authors express their thanks to C.T. Achuthankutty for reviewing this manuscript and for his valuable comments. This is NIO contribution no:xxxx

LITERATURE CITED

- Bano N, Nisa M-U, Khan N, Saleem M, Harrison PJ, Ahmed SI, Azam F (1997) Significance of bacteria in the flux of organic matter in the tidal creeks of the mangrove ecosystem of the Indus river delta, Pakistan. *Mar Ecol Prog Ser* 157:1-12
- Barille-Boyer AL, Barille L, Masse H, Razet D, Heral M (2003) Correction for particulate organic matter as estimated by loss on ignition in estuarine ecosystems. *Estuar Coast Shelf Sci* 58:147-153.
- Bhaskar PV, Bhosle NB (2008) Bacterial production, glucosidase activity and particle-associated carbohydrates in Dona Paula bay, west coast of India. *Estuar Coast Shelf Sci* 80:413-424

- Benner R, Hodson RE (1985) Microbial degradation of the leachable and lignocellulosic components of leaves and wood from *Rhizophora mangle* in a tropical mangrove swamp. *Mar Ecol Prog Ser* 23:221-230
- Berger B, Hoch B, Kavka G, Herndl G (1996) Bacterial colonization of suspended solids in the River Danube. *Aquat Microb Ecol* 10:37-44
- Chen MS, Wartel S, Eck BV, Maldegem DV (2005) Suspended matter in the Scheldt estuary. *Hydrobiol* 540:79-104
- Clarke KR, Warwick RM (1994) Change in marine communities: an approach to statistical analysis and interpretation. Plymouth Marine Laboratory, Plymouth, p 1-4
- Cole JJ, Findlay S, Pace ML (1988) Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Mar Ecol Prog Ser* 43:1-10
- Crump BC, Baross JA (2000) Characterization of the bacterially-active particle fraction in the Columbia River estuary. *Mar Ecol Prog Ser* 206:13-22
- Crump BC, Baross JA, Simenstad CA (1998) Dominance of particle-attached bacteria in the Columbia River estuary, USA *Aquatic Microbial Ecol* 14:7-18
- De Souza MJBD, Nair S, Loka Bharathi PA, Chandramohan D (2003) Particle-associated bacterial dynamics in a tropical tidal plain Zuari estuary, India. *Aquat Microb Ecol* 33:29-40
- Ducklow HW, Shiah FK (1993) Bacterial production in estuaries. In: Ford TG (ed) *Aquatic Microbiology: An ecological approach*. Blackwell Scientific Publications, Boston, p 261-287
- Fuhrman JA, Azam F (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. *Mar Biol* 66:109-120
- Fukuda R, Ogawa H, Nagata T, Koike I (1998) Direct determination of carbon and nitrogen contents of natural bacterial assemblages in marine environments. *Appl Environ Microbiol* 64:3352-3358
- Garrison DL, Gowing MM, Hughes MP, Campbell L, Caron DA, Dennett MR, Shalapyonok A, Olson RJ, Landry MR, Brown SL, Lui HB, Azam F, Steward GF, Ducklow HW, Smith DC (2000) Microbial food web structure in the Arabian sea: a US JGOFS study. *Deep Sea Res II* 47:1387-1422
- Gorsky G, Picheral M, Stemmann L (2000) Use of the underwater video profiler for the study of aggregate dynamics in the north Mediterranean. *Estuar Coast Shelf Sci* 50:121-128

- Grossart HP, Simon M (1998) Bacterial colonization and microbial decomposition of limnetic organic aggregates (lake snow). *Aquat Microb Ecol* 15:127-140
- Grossart H-P, Ploug H (2000) Bacterial production and growth efficiencies: Direct measurements on riverine aggregates. *Limnol Oceanogr* 45:436-445
- Hedges JJ, Stern JH (1984) Carbon and nitrogen determinations of carbonate-containing solids. *Limnol Oceanogr* 29:657-663
- Heiri O, Lotter AF, Lemcke G (2001) Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. *J Paleolimnol* 25:101-110
- Hobbie JE, Daley RJ, Jasper S (1977) Use of Nucleopore filters for counting bacteria by fluorescence microscopy. *Appl Environ Microbiol* 33:1225-1228
- Hoch BM, Berger B, Kavka G, Herndl GJ (1995) Remineralization of organic matter and degradation of the organic fraction of suspended solids in the River Danube. *Aquat Microb Ecol* 9:279-288
- Holmboe N, Kristensen E (2002) Ammonium adsorption in sediments of tropical mangrove forest (Thailand) and a temperate Wadden Sea area (Denmark). *Wetlands Ecol and Manag* 10:453-460
- Hopkinson CS, Buffam I, Hobbie J, Vallino J, Perdue M, Eversmeyer B, Prahel F, Covert J, Hodson R, Moran MA, Smith E, Baross J, Crump B, Findlay S, Foreman K (1998) Terrestrial inputs of organic matter to coastal ecosystems: an intercomparison of chemical characteristics and bioavailability. *Biogeochem* 43:211-234
- Hubertz ET, Cahoon LB (1999) Short-term variability of water quality parameters in two shallow estuaries of North Carolina. *Estuar* 22:814-823
- Iriberry J, Unanue M, Ayo B, Barcina I, Egea L (1990) Bacterial production and growth rate estimation from (super(3)H) thymidine incorporation for attached and free-living bacteria in aquatic systems. *Appl Environ Microbiol* 56:483-487
- Krey I (1964) Die mittlere Tiefenverteilung von seston Mikrobiomasse und detritus in mordlichen Nordatlantik. *Kieler Meeresforsch* 20:18-29
- Lee S, Fuhrman JA (1987) Relationships between biovolume and biomass of naturally derived marine bacterioplankton. *Appl Environ Microbiol* 53:298-1303

- Lee C, Wakeham S (1988) Organic matter in seawater: Biogeochemical process, In: Chemical oceanography, v. 9. Academic Press p 1-51
- Lignell R (1990) Excretion of organic carbon by phytoplankton: its relation to algal biomass, primary productivity and bacterial secondary productivity in the Baltic Sea. *Mar Ecol Prog Ser* 68:85-99
- Lohrenz SE, Knauer GA, Asper VL, Tuel M, Michaels AF, Knap AH (1992) Seasonal variability in primary production and particle flux in the northwestern Sargasso Sea: U.S. JGOFS Bermuda Atlantic Time-Series Study. *Deep Sea Res* 39:1373-1391
- Mesquita AM, Kaisary S (2007) Distribution of iron and manganese. In: Shetye SR, Kumar MD, Shankar D. (eds) *The Mandovi and Zuari estuaries*. Research Publishing services, Chennai, India p 99-104
- Murrell MC, Hollibaugh JT, Silver MW, Wong PS (1999) Bacterioplankton dynamics in northern San Francisco Bay: Role of particle association and seasonal freshwater flow. *Limnol Oceanogr* 44:295-308
- Parsons TR, Maita Y, Lalli CM (1984) *A manual of chemical and biological methods for seawater analysis* Pergamon, Oxford p 173
- Ploug H, Grossart H-P (1999) Bacterial production and respiration in suspended aggregates – a matter of the incubation method. *Aquat Microb Ecol* 20:21-29
- Qasim SZ, Wafar MVM (1990) Marine resources in the tropics. *Resour Manag Optimization* 7:141-169
- Ram ASP, Nair S, Chandramohan D (2003) Bacterial growth efficiency in the tropical estuarine and coastal waters of Goa, Southwest coast of India. *Microb Ecol* 53:591-599
- Ram ASP, Nair S, Chandramohan D (2007) Bacterial growth efficiency in the tropical estuary: Seasonal variability subsidized by allochthonous carbon. *Microb Ecol* 45:88-96
- Revilla M, Iriarte A, Madariaga I, Orive E (2000) Bacterial and phytoplankton dynamics along a trophic gradient in a shallow temperate estuary. *Estuar Coast Shelf Sci* 50:297-313
- Russell MJ, Montagna PA, Kalke RD (2006) The effect of freshwater inflow on net ecosystem metabolism in Lavaca Bay, Texas. *Estuar Coast Shelf Sci* 68:231–244
- Russell MJ, Montagna PA (2007) Spatial and temporal variability and drivers of net ecosystem metabolism in Western Gulf of Mexico Estuaries. *Estuar and Coasts* 30:137-153

- Sanudo-Wilhelmy SA, Taylor GT (1999) Bacterioplankton dynamics and organic carbon partitioning in the lower Hudson River estuary. *Mar Ecol Prog Ser* 182:17-27
- Sardessai S, Sundar D (2007) Variability of nitrate and phosphate. In: Shetye SR, Kumar MD, Shankar D. (eds) *The Mandovi and Zuari estuaries*. Research Publishing services, Chennai, India p. 59-66
- Sarma VVSS, Kumar MD, Manerikar M (2001) Emission of carbon dioxide from a tropical estuarine system, Goa, India *28*:1239-1242
- Schlesinger WH (1997) *Biogeochemistry: an analysis of global change*, 2nd edition. San Diego, California: Academic Press, p. 588
- Shetye SR, Gouveia AD, Singbal SY, Naik CG, Sundar D, Michael GS, Nampoothiri G (1995) Propagation of tides in the Mandovi-Zuari estuarine network. *Proc-Indian-Acad-Sci-Earth-Planet-Sci* 104:667-682
- Shetye SR, Michael GS, Vishwas CP (2007) Mixing and intrusion of salt. In: Shetye SR, Kumar MD, Shankar D (eds) *The Mandovi and Zuari estuaries*. Research Publishing Services, Chennai, p. 49-58
- Simon M, Alldredge AL, Azam F (1990) Bacterial carbon dynamics on marine snow. *Mar Ecol Prog Ser* 65:205-211
- Smith DC, Simon M, Alldredge AL, Azam F (1992) Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. *Nature* 359:139-142
- Wafar S, Untawale AG, Wafar MVM (1997) Litter fall and energy flux in a mangrove ecosystem. *Estuar Coast Shelf Sci* 44:111-124
- Williams PJJ (1981) Incorporation of microheterotrophic processes into the classical paradigm of the planktonic food web. *Kieler Meeresforsch.* 5:1-28

Table 1: Range of water column characteristics in the Mandovi estuary.

Parameters/Seasons▶ ↓	Pre-monsoon	SW-monsoon	Post-monsoon
Temperature ($^{\circ}\text{C}$)	29.5-33.0 (31.1)*	26.0-32.0 (28.3)	28.0-32.5 (30.5)
pH	5.9-7.9 (7.3)	5.9-7.9 (6.9)	6.1-8.1 (7.5)
Salinity	29-36 (32.0)	0-10 (4.0)	17-31 (25.0)
SPM ($\text{g}\cdot\text{m}^{-2}$)	0.03-0.70 (0.22)	0.62-2.53 (1.37)	0.05-0.29 (0.19)
Inorganic ($\text{g}\cdot\text{m}^{-2}$)	0.01-0.69 (0.21)	0.57-2.46 (1.33)	0.02-0.26 (0.15)
POM: SPM	0.03-0.77 (0.29)	0.02-0.07 (0.04)	0.11-0.70 (0.28)
Particle wt (%)	44-64 (52)	22-56 (42)	37-70 (52)
Particle nos. $\times 10^9\text{m}^{-2}$	5.1-12.7 (8.1)	3.7-11.6 (6.3)	6.5-137.0 (40.2)
POC ($\text{mg}\cdot\text{m}^{-2}$)	0.8-7.8 (3.3)	2.6-17.9 (7.6)	3.5-6.6 (5.3)
PON ($\text{mg}\cdot\text{m}^{-2}$)	0.2-1.6 (0.6)	0.6-2.5 (1.3)	0.2-1.3 (0.7)
POC:POM	0.07-0.36 (0.17)	0.04-0.40 (0.18)	0.14-0.17 (0.16)

*Mean values in parenthesis

Table 2. Principal component analysis of variables

PC	Eigenvalues	% Variation	Cum. % variation
1	12.8	47.9	47.9
2	6.81	25.5	73.4
3	2.74	10.3	83.7
Eigenvectors (coefficients in the linear combinations of variables making up PC's)			
Variables	PC1	PC2	PC3
Salinity	-0.031	0.357	-0.311
SPM	-0.049	-0.505	-0.277
Organic	0.001	-0.071	-0.056
Inorganic	-0.037	-0.624	-0.401
POM:SPM	0.027	0.004	-0.025
Particle no.	-0.012	0.085	-0.115
Particle wt.	0.004	0.055	0.056
POC	0.046	-0.061	0.114
PON	0.035	-0.081	0.202
POC:PON	0.011	0.030	-0.083
Chl a	-0.026	0.330	-0.491
PP	0.107*	0.286	-0.391
Total counts	0.262	-0.061	-0.135
TC-PAB	0.255	-0.050	-0.140
TBP	-0.184	0.024	-0.165
PABP	-0.154	-0.014	-0.327
POC:POM	0.664	0.063	0.122

*Variables with a load of >0.1 are considered

Table 3. Annual production of particle-associated bacteria (PABP), primary production (PP) and bacterial carbon demand (BCD). Values are depth integrated ($\text{mg.C.m}^{-2}.\text{d}^{-1}$).

Method	BR	PABP	BGE (%)	BCD	PP	(PABCD/TBCD*)/PP (%)
1	159	214	57	373	105	0.15
2	-	214	24	891	105	0.35

*TBCD-total bacterial carbon demand= $2427 \text{ mg.C.m}^{-2}.\text{d}^{-1}$, BR- bacterial respiration, BGE- bacterial growth efficiency

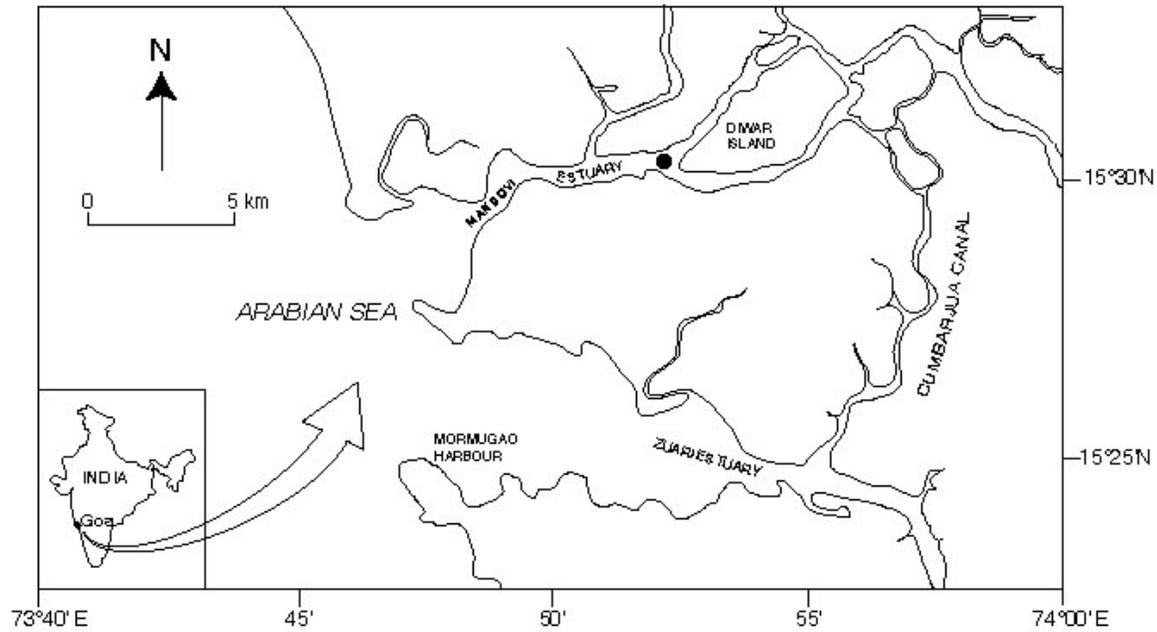


Fig. 1. Map showing the sampling site in the Mandovi estuary on the west coast of India.

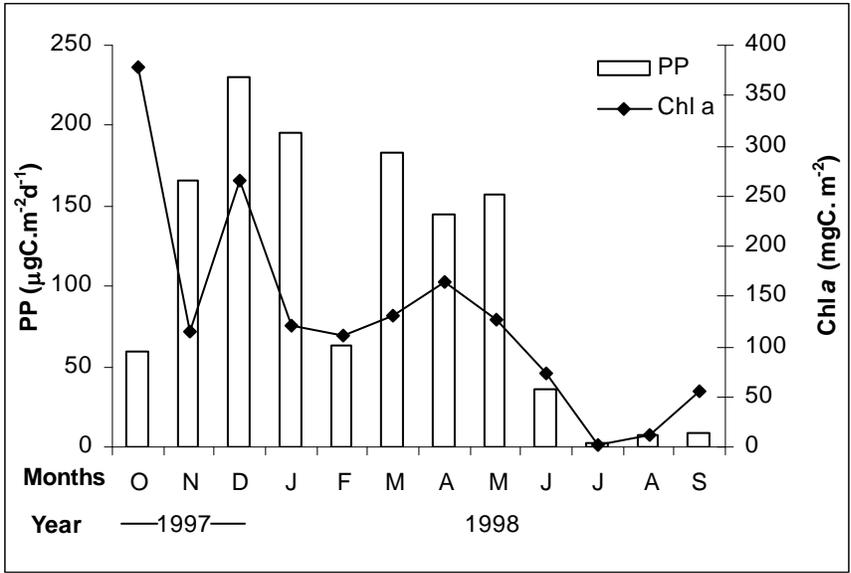


Fig. 2. Monthly variation in primary productivity and Chl a

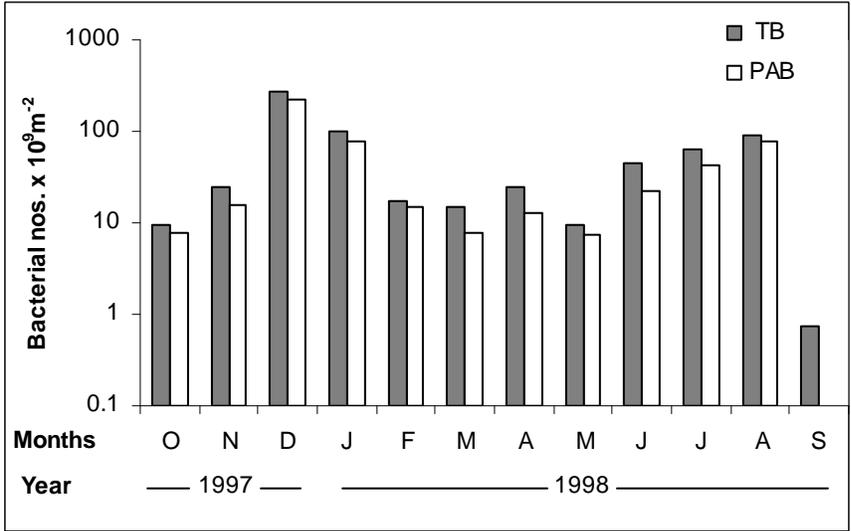


Fig. 3. Annual variation in the total and particle-associated bacterial abundance in the Mandovi estuary. TC - total counts, PAB - particle-associated bacteria.

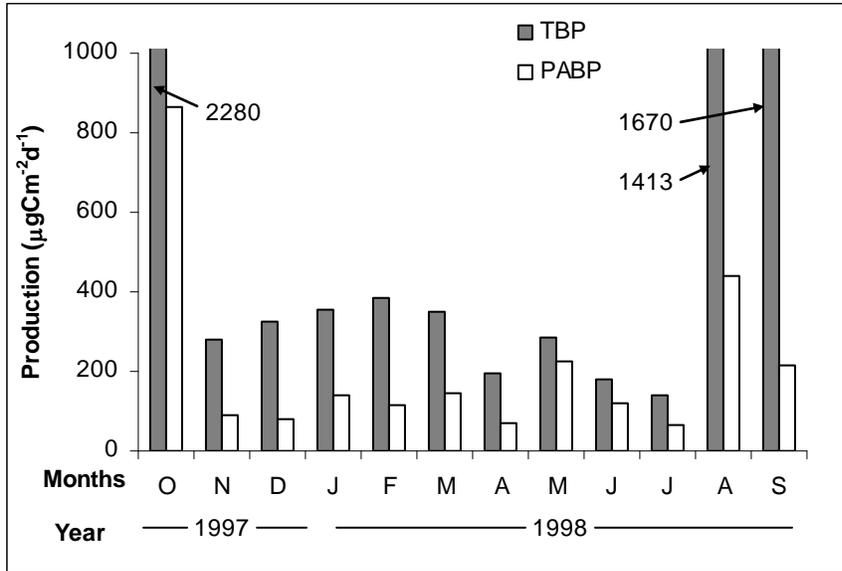


Fig. 4. Annual variation in the total bacterial production (TBP) and particle-associated bacterial production (PABP).