

## 6. Bacterial Association with Particles: Aggregation to Dissolution

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### Introduction

Particles are all those biogeochemical entities encompassing the microscopic matter, both living and non-living. Smaller particles including microaggregates, phytoplankton, fecal pellets, organic debris and clay mineral particles (Alldredge, 1989) lead to the formation of aggregates in aquatic media due to their coagulative nature. They also include organisms, like bacteria, protozoans and metazoans, which are components of aggregates in aquatic environments (Grieser, 1988; Eisma, 1992; Laybourne-Parry et al., 1992; Rogerson and Laybourne-Parry, 1992a,b; Kies 1995; Zimmermann and Kausch, 1996), each with their own communities of attached microorganisms in various nutritional states. The “organic” particles include viruses (0.02 – 0.03  $\mu\text{m}$ ), bacteria (>0.22-1  $\mu\text{m}$ ), picoplankton (<5  $\mu\text{m}$ ), polysaccharide strings and sheets (3 to >100  $\mu\text{m}$ ) and detritus (0.5 – 2500  $\mu\text{m}$ ). “Inorganic” comprise of clay minerals (200-1000  $\mu\text{m}$ ) aluminosilicates (200-1000 nm) and iron colloids (100 – 500 nm) or a combination of any of these. They are present as solitary particles or aggregates of different types of particles in the processes of the turnover, decomposition and sinking flux of both organic and inorganic matter in aquatic systems. Physical aggregation process of these particles may be enhanced by exudates, exopolymers and products of cell lysis generated by attached microorganisms which increase the stickiness of colliding particles and form a mucilaginous matrix in which many components become embedded (Alldredge, 1989). Aggregated materials are mostly in the turbid zone where the freshwater in the river mixes with seawater (Kies, 1995). In estuarine waters, phytoplankton, plankton detritus and heterotrophic consumers form an obvious source of suspended particles (Laane, 1982). Flocculation is an important process in controlling the settling velocities of particles and during the tidal cycles there may be changes in the particle size distribution in estuaries (Dyer, 1986). In addition, abundance and size of aggregates undergo considerable seasonal (Kies, 1995; Zimmermann and Kausch, 1996) and spatial variation (Kies, 1995).

Colloids, which range in size of 0.001-1mm represent 10 to 50% of total ‘dissolved’ organic carbon (DOC) in seawater (Ogawa and Ogura, 1992; Benner et al., 1992; Guo et al., 1994) and may coagulate to form larger particles (Wells and Goldberg, 1993). Kepkays’ (1994) review on coagulation has brought out that bubbling may enhance coagulation of submicron particles, suggesting that coagulation rates may be high in surface waters. Alldredge et al. (1993) suggest that polysaccharide-rich, transparent exopolymer particles (TEP) are formed by coagulation of submicron particles.

They found that TEP of size dimension 3-100  $\mu\text{m}$  are abundant (28-5000 particles  $\text{ml}^{-1}$ ) in coastal waters off California. These authors also suggested that aggregates might stick to phytoplankton cells to form larger particles called marine snow that enhance the sinking flux of organic carbon. However, Smith et al. (1992) and Ploug et al. (1999) found that once the bacteria colonize the aggregate they solubilize and remineralize the aggregate thus reducing vertical fluxes.

### Bacterial association with particles

Bacteria colonize marine aggregates where they grow and are preyed upon (Kiorboe et al., 2002). Bacteria typically occur on aggregates in concentrations that are orders of magnitude higher than in the ambient water (Caron, 1987; Caron et al., 1986; Turley and Mackie, 1994). They are the “microchip” of the vast oceans realms, be it in the deep ocean vents or the pelagic ecosystems; bacteria attached to particles are able to utilize not only the particulate organic compounds that make up the particles but also the dissolved compounds concentrated on particle surface. The organisms living in and on aggregates form an important part of the plankton ecosystem, aggregating small particles and breaking down preferentially larger ones (Jackson et al., 1987; Gorsky et al., 2000). The nature of particles and their adsorption capacities determine the magnitude of bacterial colonization (Hoppe, 1984). By the sedimentation of marine particles, which serve as

simple vehicles for sinking fluxes, attached bacteria reach less favorable deeper waters. However, in tidally driven shallow waters frequent resuspension of particles will expose attached bacteria to fluctuating radiation and salinity with the possibility of impairing their physiological functions (Barry and Wainwright, 1997; Kitano et al., 1986). With newly emerging views, marine particle aggregates are seen as complex microcosms within which material and energy flows are controlled by interactions among different microbes and their environments (Kiorboe et al., 2001; Long and Azam, 2001; Shanks and Edmondson, 1990). Several studies show that bacterial colonization of particle surfaces and subsequent biofilm formation are controlled by a variety of factors like hydrodynamics, surface chemistry, physiology and genotype of the cell (Grossart et al., 2003a).

## Factors affecting bacterial attachment/detachment to aggregates

Bacterial populations on aggregates are caused by complex activities. Various factors like bacterial motility and nature of the substrate govern colonization rates on particles. Kiorboe et al. (2002) found motile bacteria to rapidly colonize aggregates, especially those enriched with organic substrates faster than non-enriched aggregates, whereas non-motile bacteria do not. A significant fraction of pelagic bacteria are motile (Fenchel, 2001; Grossart et al., 2001; Mitchell et al., 1995), and simulation models have shown that motility and chemosensing capability are important for colonization, even of sinking aggregate (Jackson, 1989; Kiorboe and Jackson, 2001). Fuerst (1995) found, most *Cytophaga* spp to be motile by means of gliding and a majority of Planctomycetales produce small, flagellated swarmer cells that probably are involved in the colonization of new surfaces. Equipped with characteristics that allow attachment to surfaces, some bacteria belonging to the *Cytophaga-Flavobacterium-Bacteriodes* group and the order Planctomycetales are also found to be associated with particles (DeLong et al., 1993). In addition Kiorboe et al. (2002) also found that flow enhances colonization rates. Freely swimming bacteria was found to encounter an aggregate in <1 day at typical upper –ocean aggregate concentrations which is faster than even starving bacteria burn up their reserves, and bacteria may therefore rely solely on aggregates for food. They pointed out that the encounter rate between aggregates and free bacteria is independent of processes on the aggregate. Once on the aggregates, after a typical residence time of about 3 h, the bacteria may detach as described later.

Thus there is a rapid exchange between attached and free bacteria. However, Kiorboe et al. (2002) pointed out that the equilibrium bacterial abundances on aggregates as a result of colonization and detachment might be governed by biological interactions other than colonization and detachment. Several studies on the succession of microbes on aggregates are reported (Grossart and Simon, 1998; Knoll et al., 2001; Ploug and Grossart, 1999; Unanne et al., 1998; Worner et al., 2000). Succession of microbial populations on particles depends on inter- and intraspecific interactions and the environmental conditions (Dang and Lovell, 2000; Okabe et al., 2002). An example of this is that of *Streptococcus mutans* and *Lactobacillus casei* which form a mixed, stable community under glucose limitation at pH of 7.0. However, lowering of pH to 4.8 results in a dramatic reduction of *S. mutans* cell numbers (Bowden and Hamilton, 1989). Kiorboe et al. (2002) suggested that inter- and intraspecific interactions among bacteria and between, bacteria and their predators may be more important than colonization in governing the population dynamics of bacteria on natural aggregates as these interactions among bacteria may modify the “decision” to attach or detach by, the release of signal molecules (“quorum sensing”) and antibiotic substances. Quorum sensing appears to regulate swarming behavior in some pathogenic bacteria (Eberl et al., 1999). Gram et al. (2002) reported that some of the strains isolated from marine particles release signal molecules. In addition, a large fraction of bacterial isolates from marine particles are known to display antagonistic activities towards other bacteria (Martinez et al., 1996), which suggests that inhibition among attached bacteria can also affect the development of microbial communities on particles (Grossart et al., 2003a). Such interactions may affect the population dynamics of an ecosystem substantially by significantly influencing community composition and activities of attached bacteria and thus regulating the biogeochemical fate of particulate organic matter in the ocean (Long et al., 2003). Surface-active chemicals also influence microbial attachment, and cellular adsorption is mediated by cell surface hydrophobicity, bacterium –substratum charge interactions, surface roughness and surface free energy (Vanhaeke et al., 1990, Van Loosdrecht et al., 1990, Whitekettle, 1991). For example pathogenic strains of *Vibrio* express chemotactic behavior toward specific mucous surfaces of fish depending on mucous chemistry, temperature and other parameters (Bordas et al., 1998). Other heterotrophic bacteria are also able to react very specifically to changes in environmental conditions. Mueller (1996) showed that the specific rate coefficients for adsorption of two *Pseudomonas* species increased significantly when the organisms

were starved. During that time, desorption probability was zero, demonstrating that not only attachment but also detachment probability may be strongly dependent on time and changes in the environment.

## Grazing and Mortality

Although natural marine particles are important nursery ground for heterotrophic bacteria, they can also be risky niches for the attached bacterial population (Grossart, et al., 2003a). Marine particles house a rich bacterivorous protozoan fauna (Buswell et al., 1997; Caron, 1987) and are ingested by larger zooplankton and fish (Green and Dagg, 1997; Grossart et al., 1998, Kiorboe, 2000, Rickard et al., 2002). Many microbial ecologists reported attachment by many species directly to particles, either to use the abundant attached bacterial community as a food source or to feed on the locally enhanced concentration of free bacteria that are strained more efficiently by attached than by free flagellates (Caron, 1987, Artolozaga et al., 2000, Grossart et al., 2003a, Kiorboe et al., 2003, Christensen-Dalsgaard and Fenchel, 2003). Indeed, bacterial abundance on natural marine particle aggregates is often lower than the theoretical growth limit. Thus Shanks and Edmondson (1990) suggested that mortality of attached bacterial population caused by grazing is an important factor regulating bacterial population dynamics on the aggregates. Hence, microbial dynamics on marine particle aggregates will require coupling bacterial growth dynamics and predator-prey interactions (Kiorboe et al., 2003). Such predator-prey interactions may render bacterial abundances on aggregates independent of aggregate size (Kiorboe, 2001). According to Kiorboe et al. (2002) these interactions also depend on the density and fractal nature of natural aggregates.

## Characteristics of bacteria associated with particles

Bacterial communities on natural aggregates consist of assemblages of many species (Kiorboe et al., 2002). A number of studies have shown that bacteria attached to particles may be phylogenetically different from free-living bacteria (Bidle and Fletcher, 1995, Crump et al., 1999, DeLong et al., 1993, Rath et al., 1998). Free-living and particle-associated bacterial assemblages are perceived to be metabolically and phylogenetically distinct (Riemann and Winding, 2001). However, Hollibaugh et al. (2000) observed similarities as well as dissimilarities between the composition of free-living and attached bacterial assemblages in samples collected at different

locations in San Francisco Bay. They suggested the degree of phylogenetic overlap to be dependent on the particle origin. Riemann and Winding (2001) found that there was no consensus in the interplay between free-living and attached bacterial communities. They suggested that free-living and particle-associated bacterial communities should not be perceived as separate entities but as interacting assemblages, where the extent of phylogenetic overlap is dependent on the nature of the particulate matter. There are selective forces present in particle microenvironments that gradually drive community succession towards a phylogenetic composition that differs from that of the surrounding water.

## Importance of particle associated bacteria (PAB)

### Transport

Particles play a significant role as transport vehicles, as microorganisms associated with them sink at rates, which are potentially several orders of magnitude higher than a single unattached cell. Biogenic aggregates >0.5 mm in diameter (Fowler and Knauer, 1986) are responsible for the vertical flux of nutrients and transport of attached bacteria to the deep sea floor. Contribution by PAB to total bacterial production in the deep waters is about 48% (Turley and Stutt, 2000). Macroscopic organic aggregates in the ocean as well as in lakes are decomposed by microorganisms.

Traditionally marine aggregates were regarded to play a role in the vertical transport of POM. Now their abundance may indicate the presence of a more complex food web in pelagic waters by influencing the heterotrophic turnover and recycling of detrital material to higher trophic levels of the aquatic food web (Kiorboe et al., 2002). Caron et al. (1982) thought that aggregates are important loci of microbial activity in the water column, similar to the much larger oceanic 'marine snow' aggregates. Marine snow aggregates in the ocean being important exporters of organic material, are also sites of elevated microbial activity. Some pelagic flagellates colonize particles, such as marine snow, graze on the marine snow-associated bacteria and, thus have an impact on the dynamics of the attached microbial communities (Kiorboe et al., 2004). In the Columbia River estuary, the key step in the transfer of detrital organic matter into the food web was the direct consumption of particles and particle-attached bacteria by metazoans (Crump et al., 1998). Through the ingestion of the lake snow aggregates, a rapid and efficient transfor mation of protein rich microbial biomass to higher trophic levels

could be mediated providing a short - cut between the 'microbial loop' (Azam et al., 1983) and the top levels of the classical food chain. Particularly, where the abundance of aggregates is high as in the littoral zone of lakes and the ocean, they may contribute a substantial portion of food to juvenile fish. Bacterivory and viral infection is known to lead to the release of labile DOM which has been regarded as a short-circuit of the microbial loop (Fuhrman, 1992; Nagata and Kirchman, 1992), where organic carbon is intensively recycled via a cyclic flow of matter within a DOM - bacteria loop. At a community level, bacterial recycling of organic matter may significantly increase the ratio of secondary production relative to primary production (Fuhrman, 1992) by a mechanism called network virtual amplification (Higashi et al., 1993).

## Nutrition

The PAB may improve nutritional quality of detrital particles for grazing by bacteriovores. Bacteria attached to a particle (>1mm) are larger prey and thus may be more important than unattached bacteria for bacteriovores (Ferguson and Rublee, 1976). Particle-attached bacteria can have a very different role in a food web compared to free-living bacteria because they may be directly grazed by larger metazoans, bypassing consumption by protozoan grazers and 'short-circuiting' the microbial loop (King et al., 1980; Baross et al., 1994). This appears to be the case in the Columbia River estuary where epibenthic copepods were found to feed directly on particle-attached bacteria (Simenstad et al., 1994). Also, the consumption of aggregated colloids by metazoan zooplankton may provide a direct pathway that channels colloidal carbon up to zooplankton (Nagata and Kirchman, 1997). Larger aggregates like marine snow are also a food source for large particle feeders such as fish and zooplankton (Alldredge, 1979; Urrere and Knauer, 1981; Larson and Shanks, 1996). Microaggregates (phytoplankton colonized by microorganisms) in turn actively interact with free-living bacteria and particle feeders by providing them with labile substrates (Azam and Cho, 1987; Herndl, 1988) and particles with high nutritional value (Chrost, 1991). Indeed, bacteria are now known as the major inputs sources to the organic carbon reservoir in seawater as they not only substantially mediate the production of semi-labile and refractory DOM but also add carbon to the pool by their own mortality (Nagata and Kirchman, 1999). Attached bacterial activity may play a key role in the breakdown and dissolution of particulate detritus in estuarine waters (Anesio et al., 1999). Activities of attached bacteria on decomposition of fecal pellets (Jacobsen and Azam, 1984); seagrass (Peduzzi and Herndl, 1991) and marine snow (Smith et al., 1992) lead to the release

more DOC than they assimilate. Some of these authors also suggest that colonizing bacteria may serve as 'baby machines' thus becoming a source of free bacteria. Hence, degrading matter can be a source of both dissolved nutrients as well as free bacteria in an ecosystem (Anesio et al., 2000).

## Effect on the surrounding environment

Attached bacterial growth is dependent on the specific micro-niche created by marine particles (Baty et al., 2000; Kiorboe et al., 2001; Simon et al., 2002; Sternberg et al., 1999). Attached bacteria are also responsible in the modification of their surrounding microenvironment, thereby affecting the behavior and physiology of other microbes. Other studies indicate increased bacterial growth in the presence of another strain of bacterium due to production of probiotics (Madigan et al., 2000). Moreover, bacteria from marine particle aggregates produce communication signals – the acylated homoserine lactones (AHLs) that are involved in quorum sensing (Gram et al., 2002). These communication signals govern bacterial phenotypic traits like biofilm formation, exoenzyme and antibiotic production (Fuqua and Greenberg, 2002). This production of AHLs by particle-colonizing bacteria strongly indicates the importance of intra- and interspecific interactions in the control of microbial dynamics on particle surfaces. Communication and regulation via AHL production has proved to be more useful for attached bacteria than for free-living bacteria as particles provide physically more stable platforms whereupon the chemicals can act. Besides, the formation of microcolonies or multiple cell layers on particle surfaces as well as formation of biofilms with an extensive exopolymer matrix aid in better chemical communication among bacteria (Decho, 1990). In addition, formation of multiple cell layers or dense bacterial communities on particles may also facilitate plasmid exchange among the attached bacteria (Licht et al., 1999), which will lead to the evolution of a genetically and functionally diverse attached bacterial community.

In general particle-associated bacteria play an important role in the trophic dynamics of an ecosystem (Fig. 1)

## Bacterial role in aggregation formation and dissolution

In aquatic systems particles are important components in the turnover, decomposition and sinking flux of both organic and inorganic matter and elements (Simon et al., 2002). Both DOC and particles can be found throughout the water column,

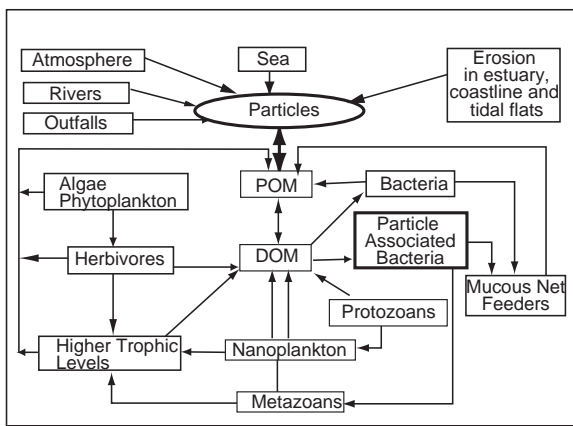


Fig 1: Importance of particles and particle associated bacteria in trophic dynamics

but may vary greatly in composition and quantity at different depths (Williams, 2000). Organic particles offer a niche that invites specialization. Rates of degradation of the organic matter depend on several factors like the size and lability of the aggregates (Kiorboe et al., 2002). Degradation of organic matter in the microbial food web is a mixture of physical and enzymatic processes resulting in products that can be absorbed by osmotrophic organisms (Zweifel et al., 1996). This complex has been the concern of several studies aimed at explaining the distribution of organic matter (Zweifel et al., 1996; Sundh, 1992; Taylor and Joint, 1990).

Bacteria attached to particles were able to utilize not only the organic compounds that made up the particle, but also dissolved organic compounds concentrated at the particle surface (Jannasch and Pritchard, 1972). Dissolved organic matter (DOM) represents a major part (>90%) of the organic carbon pool in seawater, and plays important roles in oceanic biogeochemistry.

The DOM pool consists of about 50% refractory and 50% semi-labile DOM in the upper oceans (Williams and Druffel, 1987) and plays important roles in global and regional biogeochemistry. Bacteria have 100 times the uptake potential of phytoplankton and are therefore potential key players in rapidly consuming dissolved nutrients from patches and transferring them into the food web (Nicholas and Walling, 1998). Although bacteria are responsible for assimilating most of the DOC in aquatic ecosystem, yet the fluxes of DOC through bacteria include a wide variety of compounds derived from unknown sources and composition (Azam et al., 1993). Changes in DOC composition rather than concentration may drive bacterial production and other metabolic processes (Kirchman et al., 1991; Sondergaard et al., 1995). In the Hudson River, detritus from aquatic vascular plants supplies dissolved organic carbon capable of supporting high bacterial secondary production

(Findlay et al., 1992) perhaps compensating for a nearly 90% decline in phytoplankton production since the invasion of zebra mussels (Caraco et al., 1997). The ability of vascular plant matter to support bacterioplankton growth varies with the degradability of the detritus, which can be predicted by differences in cellulose and lignin content as well as detrital C:N ratios (Moran and Hodson, 1989).

Particle-associated microflora may probably be responsible for most of the bacterial degradation of POM. They may obtain nutrients by degrading solid organic matter using extracellular hydrolytic enzymes –exoenzymes (Chrost, 1991). Bacterivorous flagellates may indirectly affect aggregate degradation, since grazing may lead to higher bacterial growth rates and better substrate utilization due to a more efficient nutrient regeneration (Gude, 1986).

Gram et al. (2002) reported that AHLs production by bacteria on marine snow is important for quorum sensing and regulation of physiological functions such as exoenzyme synthesis (Fuqua and Greenberg, 2002). It has been demonstrated in the case of marine snow that enzymes associated with the particles hydrolyze much more particulate organic carbon than is required by PAB, possibly providing nutrients for free-living bacteria (Delong et al., 1993). Moreover, there is accumulating evidence that attached bacteria, despite their lower yield, exhibit higher extracellular enzymatic activity (Kirchman, 1983; Hoppe et al., 1988; 1993; Karner and Herndl, 1992; Smith et al., 1992). This was supported by Delong et al., (1993) who found that PAB were phylogenetically distinct from FLB and were able to degrade a wide range of polymeric compounds. In the Columbia River estuary, extracellular enzyme activity was detected principally on particles suggesting that particulate organic material was the primary polymeric food source for bacteria in the estuary. The relatively high activity of particle-attached bacteria and their close association with particles suggested that they produced most of these enzymes and were therefore, probably responsible for most of the bacterial degradation of POC in the estuary (Crump et al., 1998). It is presumed that by hydrolysis and diffusion PAB create a zone around themselves that is enriched in DOC (Martinez et al., 1996). The role of 'microbial loop' including microzooplankton is likely to be particularly important in rapidly recycling nutrients above the thermocline. This is because bacteria attached to particles and larger phytoplankton cells are likely to sink out of the mixed layer faster than the free-living (motile) bacteria (Wangersky, 1984; Kirchman et al., 1982). In contrast to bacteria that may be attached to surfaces, freely suspended bacterioplankton probably compete for

ambient DOM that is released by a variety of processes, including the lysis and leakage of phytoplankton (Nagata, 2000). Quantifying the rate at which bacteria colonize aggregates is key to understanding microbial turnover of aggregates. They cause aggregates to solubilize and remineralize, apparently at high rates (Alldredge and Cohen, 1987; Ploug and Grossart, 2000; Ploug et al., 1999). These aggregates may be important sources of DOM for free bacteria and that solute plumes trailing behind sinking aggregates may be important sites of bacterial growth. Thus seepage of DOM from degrading aggregates is known to affect microbial processes even outside the aggregates (Grossart and Simon, 1998; Kiorboe and Jackson, 2001; Smith et al., 1992). Even if microbial processes on aggregates account for only a small fraction of the total microbial activity in the water column (Alldredge and Gotschalk, 1990; Turley and Mackie, 1994). Hence, the bacterial activities on aggregates are important to understand the role of marine aggregates in an ecosystem.

Particles harbor dense microbial communities (Caron et al., 1982; Grossart and Simon, 1998), suggesting that they are important sites for processes like production, decomposition and nutrient recycling in the water column (Alldredge and Silver, 1988; Simon et al., 2002). Organic particles and the surrounding "phycospheres" (Azam and Ammerman, 1984; Bell and Mitchell, 1972; Bowen et al., 1993) and "detritospheres" (Biddanda and Pomeroy, 1988) serve as micropatches of concentrated substrates that attract pelagic bacteria (Azam, 1998; Kiorboe et al., 2001) especially those having chemotactic senses (Blackburn et al., 1998; Blackburn and Fenchel, 1999). About 40 to 70% of marine bacteria are motile (Grossart et al., 2001), many of which have chemotactic behavior (Blackburn et al., 1998; Blackburn and Fenchel, 1999). This behavior allows them to cluster around patches of DOM or rapidly colonize POM (Fenchel, 2001; Kiorboe et al., 2002). For example, the chemotactic bacteria grow 50% faster when clustering within nutrient patches around detrital particles (Blackburn et al., 1997). Grossart et al. (2003a) inferred that motility and foraging strategies could be important adaptations of heterotrophic bacteria and Archaea to gradients of DOM and POM in the ocean. A well-documented but very often overlooked fact is that there is a continuous conversion of DOM to POM in an ecosystem just like the metabolic pathways of our own beings- the catabolic and anabolic. This mechanism is bacterially mediated as seen above. Our vast oceans serve as a source, as well as a sink of both dissolved and particulate matter.

## PAB in Tropical Mandovi-Zuari estuaries

The nature of bacteria-particle associations have been the topic of sustained interest for many years (Bitton and Marshall, 1980; Wotton, 1994; Murrell et al., 1999; De Souza et al., 2003; Selje and Simon, 2003). Associated bacteria are usually found in low abundance (10%) compared with the free-living bacteria in most open aquatic ecosystems (Kirchman, 1983; Simon, 1987), although in some cases they reach up to 50-60% of the total biomass (Pedros Alio and Brock, 1983; Becquevort et al., 1998). Like many other estuarine ecosystems, the Zuari and Mandovi estuary on the West coast of India exhibited a high load of suspended matter (De Souza, 2000). These estuaries are influenced by mangroves and the waters of the Arabian Sea. Here the particle-associated bacteria were those that were  $> 3\mu\text{m}$  as often seen in other such studies (Kirchman and Mitchell, 1982; Simon, 1985; Crump et al., 1998; Hollibaugh et al., 2000). Particles form a significant part of this estuarine system. The bacterial abundance and variation on particles in Zuari and Mandovi estuaries was higher than in the temperate waters. The PAB density ranged from  $10^7$  to  $10^{10}\text{ l}^{-1}$ . The minimum and maximum densities were recorded during the pre-monsoon and post-monsoon period. This was about 2-5 fold higher than that, reported in temperate waters (Berger et al., 1996; Cammen and Walker, 1982). Similarly there was a large magnitude of variation in the bacterial abundance within the estuary compared to those in temperate estuaries (Ducklow, 1982; Kondratiff and Simmons, 1985; Hoch et al., 1995). Crump et al. (1998) had observed variability in particle-associated bacteria every 2 h during a 28 h sampling series in North Columbia River estuary. In the Mandovi and Zuari estuaries, the variation in abundance of PAB is much more i.e. by a factor of 100. In temperate estuaries, bacterial abundance seemed to be limited by one or more factors. Though the waters from the adjacent areas influenced the Mandovi and Zuari estuaries, this variable did not influence the bacterial parameters. In spite of salinity being a variable factor in the Zuari estuary (Fig. 2), it did not have an influence on the variation in the abundance of bacteria (De Souza, 2000).

There was a significant difference in abundance between the Mandovi and Zuari stations. The percentage contribution of PAB to the total bacteria was from 66 to 81% at Mandovi, 27 to 56% at Zuari. In these estuaries, bacterial numbers were not related to suspended load or particle numbers suggesting that factors other than the availability of colonization sites regulate the bacteria abundance on particles (Friedrich et al., 1999). In the study, the observed

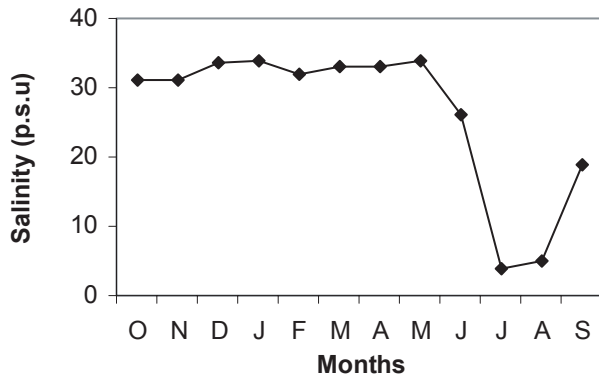


Fig.2 Salinity variations in the Zuari estuary

organic content of the bottom waters was high which may be due to the bottom-resuspended clays, bound with organic matter. Although organic carbon concentration is expected to correlate to bacterial activity, in these estuaries the organic matter concentration was not related to the bacterial abundance. In both of these estuaries, particles rich in abundance of inorganic content drastically reduced the colonization as also reported by Berger et al., (1996); Hoch et al., (1995).

ecologically sound (low stress). However, during the pre-monsoon season, when the salinity values are the highest, the productivity rates were relatively lower especially in the bottom waters. Lack of large variation in this tropical estuary may be due to the absence of marked temperature differences and seasonal planktonic blooms. Although there was no pronounced variation in abundance and productivity in the Zuari waters, there was an increase in abundance and productivity from pre-monsoon, monsoon to post-monsoon (Fig. 3 & 4).

The increase in bacterial numbers during the southwest monsoon season was due to input of easily utilizable organic matter from terrestrial region or the re-suspension of organic matter from the sediment to the water column. As the season progressed from the pre-monsoon period, the particles tended to become organically less labile with concomitant increase in hydrolytic enzymatic activities during the southwest and post monsoon. In line with previous reports (Crump et al., 1998, Selje and Simon, 2003), PAB in Zuari estuary are involved in the decomposition processes, as they usually appear to have high enzymatic activities.

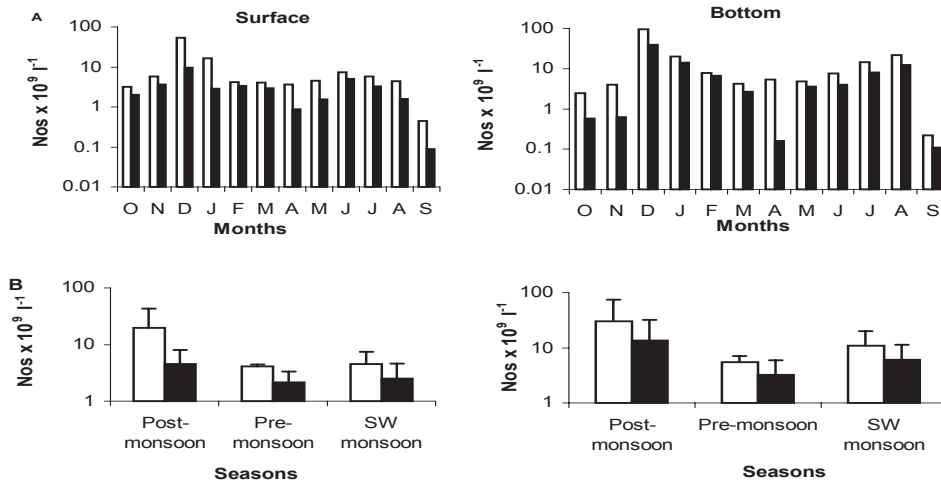


Fig 3. Monthly (A) and seasonal mean of 4 months variation along with standard deviation (B) in total and particle-associated bacterial (PAB) counts in surface and bottom waters - Total bacteria (open bars) - PAB (solid bars).

The rate of bacterial production observed in the Zuari estuary was high. The contribution of carbon production rate by PAB to the total bacterial carbon production was 38% at Mandovi while at the Zuari estuary it was 64%. Such high bacterial production was observed in Loire estuary Ivory Coast and in a tropical mangrove tidal creek of Indus River Delta in Pakistan (Ducklow and Shiah, 1993, Bano et al., 1997). This high productivity throughout the year indicated that PAB contributed significantly to high production of the estuary, which apparently is

Although the bacterial production was high during the SW monsoon months, the bacterial standing stock was non-parallel to the production rate in the estuary. As population of bacteria in an ecosystem is controlled mainly either by substrate availability (bottom up control) or by grazers (top down) and higher growth rate of particle-associated bacteria their size was larger than the free-living bacteria in the Zuari estuary. Although ingestion by grazers per sec was not measured in the Zuari waters, the rate of bacterial production and the standing stock did reflect grazing pressure on the PAB. As PAB abundance and

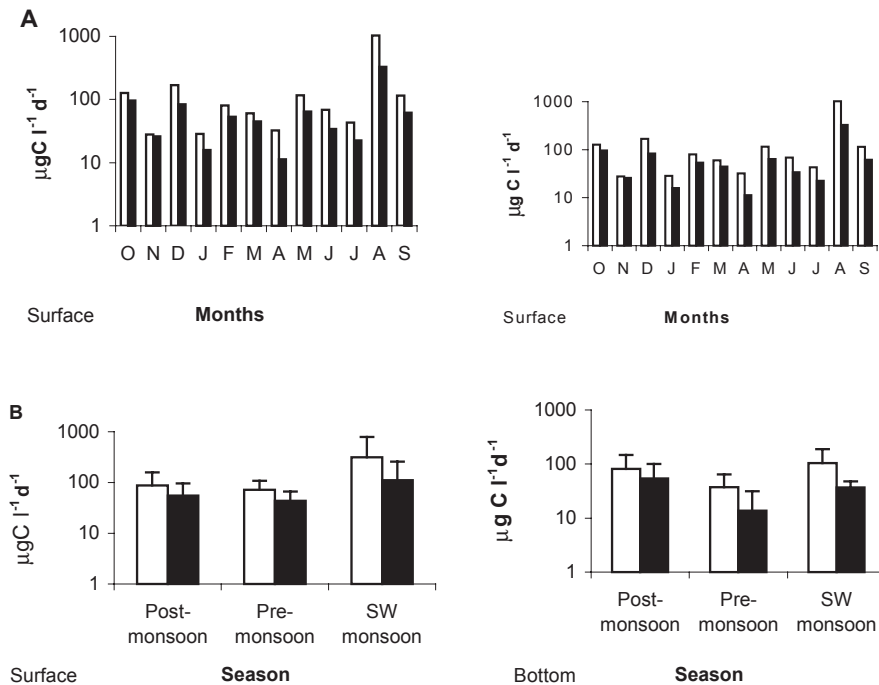


Fig 4. Monthly (A) and seasonal mean of 4 months variation along with the standard deviation (B) in total and particle-associated bacterial production (PABP) rates in surface and bottom waters. - Total bacterial production (open bars) - PABP (solid bars).

production formed a significant portion of the microbial biomass in the Zuari waters, the bacterial carbon demand was expected to be high. The carbon from PP (assuming 10% exudates) was not sufficient to meet the bacterial carbon demand except during the pre-monsoon season (Table 1) (De Souza et al., 2003). Hence, it is suggested that the excess carbon requirement was met from allochthonous sources as reported by Bano et al. (1997).

Table 1: Seasonal variation in particle associated bacterial carbon demand (BCD) based on bacterial growth efficiency (BGE) of 26 and 10% and primary production (PP). Values are depth integrated ( $\text{mg C m}^{-2} \text{d}^{-1}$ ). Asterisk indicates reported BGE values in the Zuari waters

Season	PP	BCD ( $26^* - 10^*$ )	BCD/PP
Pre-monsoon	1022	438-1140	0.4-1.1
SW monsoon	594	1142-2970	1.9-5.0
Post-monsoon	717	842-2190	1.2-3.1

## Future prospects

In future, research on particle-associated/attached bacteria could focus on their role in the aquatic system versus their free-living counterparts. The rates and mechanisms of turnover of these bacteria in the dissolution and aggregation of particles in food web dynamics and global biogeochemical cycles of the oceans need to be understood better. Quantitative data on the role of these groups need to be amassed

for a better feel on their ecological might. Although caution should be thrown while extending imaginations but with the modern era of molecular methods and new evolving techniques there are compelling reasons to think that, since particles are ubiquitous in nature so it is quite possible that the PAB may have the same position in nature. They should be looked for in the extreme environments to answer some interesting questions concerning the adaptability of these groups to environments such as the Antarctic and the deep oceans; microbial ecologists have shown bacteria to be mediators between the POM and DOM conversion, finally serving as a link to higher trophic levels. Nonetheless, this issue is by no means clear-cut as there are several environmental factors, autotrophic and heterotrophic pathways and physiology of the organisms, which play a role on the activities of this population. More so in tropical regions where temperature and seasons have a role to play on ecosystem dynamics, studies on how bacteria coordinate their activities using different chemical clues will help to increase our knowledge on their behaviors like niche colonization, enzyme production or nutrient acquisition. With the understanding of such factors, models should give us an holistic perspective into the controls involved in population dynamics of bacteria on aggregates and evaluate their biological potential in bacterioplankton ecology. Scientific endeavours will throw light on circumstances that these organisms play in short-circuiting the microbial loop and recycling of organic carbon. The PAB throw open new horizons to the biotechnological world not



only serving as exoenzyme 'generators' but also as potential sources of chemical agents like antibiotics. They may also mediate the degradations/ conversion/ biotransformation of many toxic compounds. Equipped with capacities as carriers of various molecules PAB are good tools for various scientific application which could throw open new horizons for medical and industrial explorations.

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