

# Significance of bacteria in the flux of organic matter in the tidal creeks of the mangrove ecosystem of the Indus River delta, Pakistan

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**ABSTRACT:** We studied bacterial biomass and production in 3 tidal creeks (Isaro, Gharo and Phitti Creeks) in the mangrove forests in the Indus River delta, Pakistan, to assess the significance of bacteria-mediated carbon fluxes in the creek ecosystem. Bacterial biomass, bacterial carbon production (BCP) and primary productivity (PP) were measured periodically for over a year during 1991–92. BCP was high, generally 50 to 300  $\mu\text{g C l}^{-1} \text{d}^{-1}$ . Despite such high BCP, bacterial abundance remained between  $1 \times 10^6 \text{ ml}^{-1}$  and  $4 \times 10^6 \text{ ml}^{-1}$  (20 to 80  $\mu\text{g C l}^{-1}$ ) indicating tight coupling between bacterial production and removal. Specific growth rates generally ranged from 1 to 7  $\text{d}^{-1}$  but the rate reached 24  $\text{d}^{-1}$  during a phytoplankton bloom, apparently a red tide, and this was an unprecedented growth rate for a natural assemblage. The abundance of attached bacteria exhibited a large variation, ranging from 4 to 92% (mean  $35 \pm 21\%$ ,  $n = 41$ ) in Isaro Creek and from 14 to 84% (mean  $37 \pm 28\%$ ,  $n = 10$ ) in Gharo Creek. Bacterial production due to attached bacteria was 73 to 96% of the total. Thus, a major fraction of BCP may have been directly available to metazoan grazers. BCP was generally much higher than net PP; the yearly integrated average BCP/PP for all sites was 2.0. Thus, the growth of bacteria, attached and free, probably represented the major pathway of the production of high quality (low C:N) biomass potentially available to the grazers. Average yearly integrated bacterial carbon demand (BCD), estimated conservatively by assuming a 30% growth efficiency for all sites, was 6.9 times net PP. Thus, the creek water columns were strongly and persistently net heterotrophic. Data integrated over the entire study period show that even if all phytoplankton production was utilized by bacteria it would satisfy only 7 to 20% of the BCD; the remaining 80 to 93% of BCD would be met by reduced carbon from other sources. Phytoplankton production was light limited due to high turbidity and, apparently, the majority of BCP could be supported by the input of mangrove detritus. Estimates of utilizable dissolved organic carbon (UDOC) in selected samples were 97 to 656  $\mu\text{g C l}^{-1}$ , indicating that in order to sustain the measured BCD ( $643 \pm 671 \mu\text{g C l}^{-1} \text{d}^{-1}$ ) the UDOC pool would turnover in <1 d to a few days. Limited data suggest that bacterial production was carbon rather than N or P limited, consistent with sustained high levels of inorganic N and P in the surface water. Since mangrove detritus provides most of the energy for bacterial production, which in turn is a significant source of high quality food for grazers, particularly via ingestion of attached bacteria, we predict that the ongoing destruction of mangrove forests in the Indus delta and elsewhere could have a major impact on mangrove ecosystem structure and functioning and the production of economically important fish and shrimp in mangrove creeks.

**KEY WORDS:** Bacteria · Organic matter · Bacterial production · Mangroves · Tidal creeks · Indus River delta

## INTRODUCTION

We studied the significance of bacterial processes in material and energy fluxes in the tidal creeks of the

Indus River delta as part of a multi-disciplinary study (PAKMER; Pakistan Mangrove Ecosystem Research). The Indus delta harbors large mangrove forests areally ranking fifth or sixth in the world. The supply of fresh-water to the Indus delta mangroves is quite limited and episodic because these mangroves are in an arid region (average rainfall  $\sim 200 \text{ mm yr}^{-1}$ ) and there is intense demand upstream on river water for agricul-

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tural irrigation (Ahmed 1992b, Harrison et al. 1994). The forests have shrunk drastically over the last several decades and the trees that remain are mainly small stunted *Avicennia marina* restricted to the banks of the better-flushed tidal channels (Snedaker 1984). A substantial fraction (~27%) of the forest area consists of tidal creeks and these could contribute significantly to the forest primary production and organic matter decomposition (for an overview of the ecosystem, see Snedaker 1984, Harrison et al. 1994).

Mangroves are amongst the most productive aquatic ecosystems (Mann 1972, Lugo & Snedaker 1974) and mangrove detritus supports rich fisheries in the creeks and in the adjacent coastal ocean. The material and energy fluxes in the mangrove ecosystems are distinctively shaped by the differences in the digestive capabilities of micro- and macrobiota. Metazoa cannot digest much of the lignocellulose-dominated mangrove detritus for lack of suitable digestive enzymes (Benner & Hodson 1985). Bacteria and fungi, on the other hand, commonly express ectoenzymes to hydrolyze the detritus to dissolved organic matter (DOM) which they can utilize. Also, leaf leachates and root exudates constitute a large and direct input into the DOM pool (Benner & Hodson 1985, Benner et al. 1986). Carbon exported from mangroves is important to bacteria (Healey et al. 1988). The DOM pool and the DOM-based microbial loop (Azam et al. 1983) may thus be a significant pathway of material and energy flows in the mangrove creek ecosystem. Bacterial biomass being N and P rich (bacterial C:N is <4 and C:P is 20; Lee & Fuhrman 1987, Fagerbakke et al. 1996), their presence would enrich the nutritionally poor mangrove detritus (C:N = 50 to 80) and produce high quality food for metazoa. In addition to phytoplankton production, bacterial growth may be a significant pathway of incorporating dissolved inorganic nitrogen and phosphorus (DIN and DIP) into biomass potentially available to grazers. Therefore, it was of interest to determine the relative production rates of phytoplankton and heterotrophic bacteria in this mangrove ecosystem.

In waters which receive little allochthonous organic matter input, heterotrophic bacterial production is ultimately limited by the supply of reduced carbon from primary production. The tidal creeks we studied had high turbidity which could limit light penetration and hence primary production (Harrison et al. 1994). Yet, bacterial carbon demand (BCD) need not be limited by the level of phytoplankton productivity because bacteria could use mangrove detritus in addition to phytoplankton production. We therefore hypothesized that BCD in mangrove creeks would exceed phytoplankton production, thus rendering the creeks net heterotrophic as well as making bacterial production the dominant

pathway for the incorporation of N and P into the biomass potentially available to protozoa and metazoa. Since free and attached bacteria are expected to have different trophic fates and transfer efficiencies to the higher trophic levels, it was of interest to determine the relative significance of the production of attached and free bacteria. In view of the large detritus load in the creeks, we hypothesized that a major fraction of bacterial biomass and production is particle associated.

There is no previous study addressing our hypotheses in the Indus delta mangrove ecosystem. Extensive studies of this nature have been done in Australian mangroves but these were mainly concerned with benthic microbial processes (Alongi 1988, 1992, Boto et al. 1989).

In order to test our hypotheses, we estimated the biomass and production rates of bacteria and phytoplankton in 3 tidal creeks and attempted to distinguish between the biomass and production rates of attached and free bacteria. We examined spatial variation in bacterial biomass and production during 2 transects in Isaro Creek. The scope of our study was limited to obtaining time series data to establish carbon budgets (and inferred nitrogen budgets) and did not evaluate the cause of variability in pools and rates, although some observations in this respect are noted. A companion paper (Harrison et al. 1997) considered the nutrient and phytoplankton dynamics at the same study sites.

## MATERIAL AND METHODS

**Study sites.** The study area is located in a mangrove swamp in the western part of the Indus River delta. The sampling stations are in 3 inter-connected tidal creeks: Isaro Creek is connected with Gharo Creek which is connected with Phitti Creek which in turn opens into the Arabian Sea (Fig. 1). Logistic constraints restricted most sampling to Isaro and Gharo Creeks. Between January 1991 and January 1992 we sampled Isaro Creek 16 times and Gharo Creek 7 times. Phitti Creek was sampled only once. Two stations were selected in Isaro Creek, Isaro Main (IM) in the broader part of the creek and Isaro Branch (IB) in a small, narrow branch of the creek. Sampling in Gharo Creek was also done at 2 stations, one in the wide part of Gharo Creek called Gharo Main (GM) and the other in a side branch called Gharo Branch (GB).

Isaro and Gharo Creeks are both 3 to 9 m deep and 100 to 400 m wide. Salinity remained above 38‰ during the dry season but dropped to 32–35‰ during the rainy season (July–August). Current velocity (based on 2 current meter measurements) was 0.52 m s<sup>-1</sup> during the spring tide and 0.26 m s<sup>-1</sup> during the neap tide.

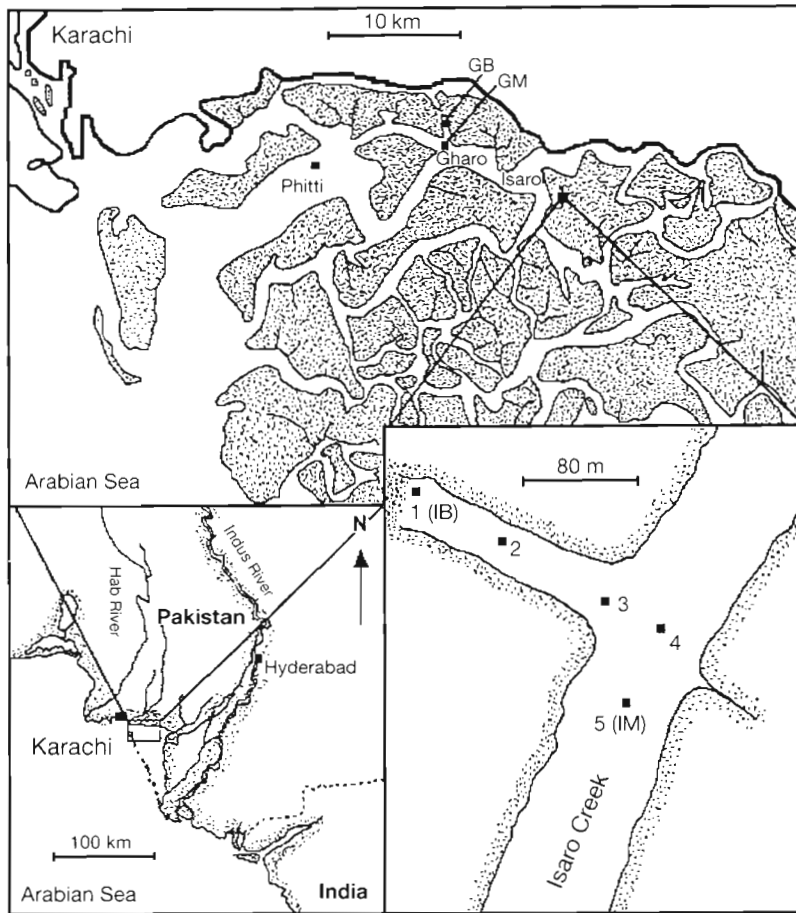


Fig. 1 Study area showing approximate position of sampling sites (■) in 3 tidal creeks, Isaro (IM: Isaro Main; IB: Isaro Branch), Gharo (GM: Gharo Main; GB: Gharo Branch) and Phitti Creeks, in the western part of the Indus River delta, Pakistan

Water movement during a 6 h flood or ebb spring tide was 5 to 11 km. Phitti Creek is the deepest of the 3 creeks, 8 to 20 m, with a salinity range of 33 to 38‰. This creek flushes completely with seawater. Current velocities were  $>1 \text{ m s}^{-1}$  during the ebb tide and  $<1 \text{ m s}^{-1}$  during the flood. All 3 creeks are lined by mangrove stands. IB is narrow and without an outlet; it is surrounded on 3 sides by mangrove stands and was likely to be most influenced by the input of mangrove exudates and detritus. The dominant species of mangrove at all study sites was *Avicennia marina* which is reportedly the most salt tolerant and, hence, this species has survived despite seasonally high salinities, up to 70‰ in summer, caused by the reduction in the flow of the Indus River into the estuary (Ahmed 1992a).

**Sampling.** Surface water samples were taken with an acid-washed insulated plastic container and bottom water samples were taken with a 5 l Niskin sampler. Temperature and oxygen profiles were determined with a YSI oxygen meter.

**Bacterial abundance.** Bacterial abundance was determined by the acridine orange direct count method (Hobbie et al. 1977). Surface and bottom water samples (10 ml) were fixed with borate-buffered 0.2  $\mu\text{m}$  filtered formalin at 2% final concentration and stored refrigerated until processing. Within a few days of collection 2 to 5 ml subsamples were stained with 0.01% final concentration acridine orange for 3 min and then filtered onto 0.2  $\mu\text{m}$  pore-size blackened Nuclepore polycarbonate membrane filters. Bacteria were counted by epifluorescence microscopy in 10 randomly selected fields. Attached bacteria were also estimated by counting 10 random fields in the same slide (Ducklow et al. 1985). A sonicator was not available, and therefore we could not dislodge the attached bacteria from particles. In order to roughly account for bacteria under the particles, we doubled our counts of the attached bacteria (Bent & Goulder 1981). Bacterial carbon was calculated from bacterial abundance by assuming a per cell carbon content of 20 fg (Lee & Fuhrman 1987).

**Bacterial carbon production (BCP).** Bacterial production was estimated from rates of  $^{14}\text{C}$ -leucine (Leu) incorporation into the protein fraction (Kirchman et al. 1985, Simon & Azam

1989). During the later part of the study (July 1991 to January 1992) a modified procedure (Smith & Azam 1992) was used because it was more economical and convenient. Surface and bottom water samples (1.5 ml) were incubated with 54 nM (final conc.) of  $^{14}\text{C}$ (U)-Leu in 2 ml Eppendorf tubes at *in situ* temperatures in the dark. All measurements were done in triplicate together with 1 blank. Blanks received 5% (final conc.) trichloroacetic acid (TCA) before adding Leu. Samples were incubated for known periods of time, always ~30 min, followed by the addition of TCA at 5% and bovine serum albumin (BSA) at 0.03% (w/v). Samples were centrifuged in a microcentrifuge at  $16000 \times g$  for 10 min. The supernatant was aspirated off and the pellet washed twice with 5% TCA. Liquid scintillation cocktail (1 ml) (Packard Opti-fluor) was added to each tube, and the tubes were placed in reusable scintillation vials and radioassayed in a liquid scintillation spectrometer. Bacterial protein production and bacterial carbon production were calculated according to Simon

& Azam (1989) assuming 2-fold isotope dilution. The method of Smith & Azam (1992) was compared with the filtration-based procedure with 3 water samples taken at each of IM and GM. Bacterial carbon production by the 2 methods gave the relationship ( $\pm 1$  SD): centrifugation/filtration =  $1.17 \pm 0.17$  ( $n = 6$ ).

**Concentration-dependence of leucine incorporation.** Leu incorporation was measured at a range of added concentrations in Isaro Creek in July 1991. We wanted to determine the leucine pool turnover time (Azam & Hodson 1981) as well as  $K_m + S_n$  and  $V_{max}$  (Wright & Hobbie 1965). We added 8 to 130 nM Leu to determine the concentration which would maximize the participation of exogenous leucine in protein synthesis (to minimize isotope dilution; Simon & Azam 1989). In order to estimate the kinetic parameters  $V_{max}$ ,  $t/f$  and  $K_m + S_n$ , the incorporation data was plotted as  $[A]$  versus  $t/f$ , where  $[A]$  is the concentration of leu added, and  $f$  is the fraction of the added label incorporated in time  $t$ .

**Bacterial carbon demand (BCD).** This was calculated on the basis of BCP by assuming a carbon assimilation efficiency of 30% (Bjørnsen & Kuparinen 1991) but a range of 10 to 30% was used in some cases (see 'Discussion').

**BCP of attached and free bacteria.** During 2 transects, on September 18 and December 2, 1991, subsamples were filtered through 0.6  $\mu\text{m}$  Nuclepore filters and the bacteria passing the filters were considered free. Filtrates and unfiltered subsamples were incubated with Leu to measure BCP, as above. BCP in the  $>0.6 \mu\text{m}$  fraction was calculated as the difference between the BCP of total and 0.6  $\mu\text{m}$  filtered samples.

**Specific growth rate.** The assemblage-average bacterial specific growth rate ( $\mu$ ) was calculated as:  $\mu = [\ln(B_0 + P) - \ln B_0]/T$ , where  $B_0$  was initial bacterial carbon,  $P$  was bacterial carbon production and  $T$  was incubation time for the  $^{14}\text{C}$ -leucine incorporation assay.

**Utilizable dissolved organic carbon (UDOC).** This was measured essentially by the bacterial carbon yield method of Ammerman et al. (1984). Briefly, creek surface water samples were filtered through 0.6  $\mu\text{m}$  Nuclepore filters to eliminate or reduce the abundance of bacterivorous protozoa and 50 ml of the filtrate was incubated at  $25 \pm 5^\circ\text{C}$  for 1 to 4 d. Bacteria in the filtrate were counted at the beginning ( $T_0$ ) and periodically during the incubation. The yield of bacteria was calculated as the difference between the maximum cell count during the incubation and that at  $T_0$ . Samples which became contaminated with protozoa were discarded.

**Chlorophyll a (chl a) and primary production (PP).** Chl a was measured spectrophotometrically in samples collected on GF/F filters and extracted with 90% ace-

tone (Parsons et al. 1984). PP was measured by the  $^{14}\text{C}$  method (Steeemann Nielsen 1952, Parsons et al. 1984) and described by Harrison et al. (1997). Areally integrated values for the entire water column were calculated for comparison with areally integrated BCP and BCD.

## RESULTS

**Physicochemical parameters.** Water temperature ranged from 28 to  $34^\circ\text{C}$  in summer and 20 to  $22^\circ\text{C}$  in winter (Fig. 2). Oxygen concentration ranged from 5.6 to  $7.0 \text{ mg l}^{-1}$  and salinity ranged from 37 to 41‰ in Isaro Creek and 36 to 43‰ in Gharo Creek during our sampling (not shown). In most cases there was no difference in surface and near-bottom temperature, salinity and dissolved oxygen concentration, indicating that the water column was well mixed.

**Bacterial abundance, chl a and carbon pools.** Generally, bacterial abundance ranged from 1 to  $4 \times 10^6 \text{ ml}^{-1}$  in Isaro Creek (mean  $2.7 \pm 1.6 \times 10^6 \text{ ml}^{-1}$ ,  $n = 60$ ; Fig. 3A) and 1 to  $3 \times 10^6 \text{ ml}^{-1}$  in Gharo creek (mean  $2.1 \pm 0.7 \times 10^6 \text{ ml}^{-1}$ ,  $n = 21$ ; Fig. 4A). Bacterial abundances in the surface and the near-bottom water samples were quite similar, which indicates that the water columns were well mixed. Chl a ranged from 1 to  $8 \mu\text{g l}^{-1}$  (mean  $3.6 \pm 2.6 \mu\text{g l}^{-1}$ ,  $n = 57$ ) in Isaro Creek (Fig. 3B), except during phytoplankton blooms on February 13 and June 5, 1991 (18 and  $41 \mu\text{g l}^{-1}$ , respectively). Chl a in Gharo Creek ranged from 1 to  $3.5 \mu\text{g l}^{-1}$  (mean  $2.5 \pm 1 \mu\text{g l}^{-1}$ ,  $n = 17$ ; Fig. 4B) and values in the surface and the near-bottom samples were also similar in most cases. The ratio of bacterial carbon to phytoplankton carbon ( $C_b/C_p$ ; not shown) generally ranged from 0.1 to 0.5. In IM, on February 13, bacterial abundance was very low,  $0.3$  to  $0.4 \times 10^6 \text{ ml}^{-1}$  (Fig. 3A). For comparison, these abundances are in the lower part of the range of bacterial abundances in the oligotrophic waters of the North Pacific central gyre (Cho & Azam 1990). Interestingly, the low counts occurred

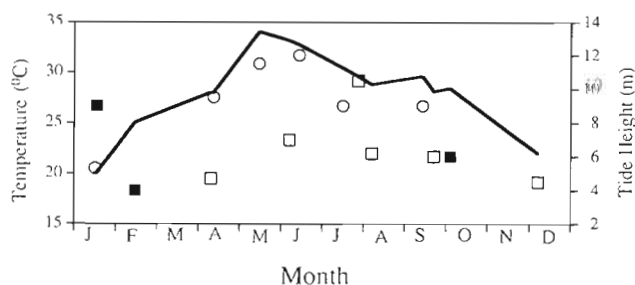


Fig. 2. Surface water temperature (solid line) and tidal height at sampling time in Isaro Creek during 1991. (O) Spring tide; (□) neap tide; (■) between spring and neap tide

during a phytoplankton bloom, apparently a red tide, with surface chl *a* of  $18 \mu\text{g l}^{-1}$  (Fig. 3B) and at a time when bacterial production ( $255 \mu\text{g C l}^{-1} \text{d}^{-1}$ ; Fig. 3C) and  $\mu$  ( $24 \text{d}^{-1}$ ; Fig. 3D) were very high. Thus, there must have been highly efficient removal of bacterial biomass possibly through intensive grazing and/or phage-induced lysis (not measured).

**Bacterial production.** BCP at all 4 stations varied more than an order of magnitude. The rates generally ranged from 50 to  $300 \mu\text{g C l}^{-1} \text{d}^{-1}$  (mean  $219 \pm 196 \mu\text{g l}^{-1} \text{d}^{-1}$ ,  $n = 54$ ; Fig. 3C) in Isaro Creek and 50 to  $150 \mu\text{g l}^{-1} \text{d}^{-1}$  in Gharo Creek (mean  $102 \pm 46 \mu\text{g l}^{-1} \text{d}^{-1}$ ,  $n = 20$ ; Fig. 4C). The values were somewhat higher in IB than in IM. Concurrent peaks, on June 5, occurred in IB and IM with BCP values of 664 and  $900 \mu\text{g l}^{-1} \text{d}^{-1}$ . Bacterial abundance and production rate are comparable to

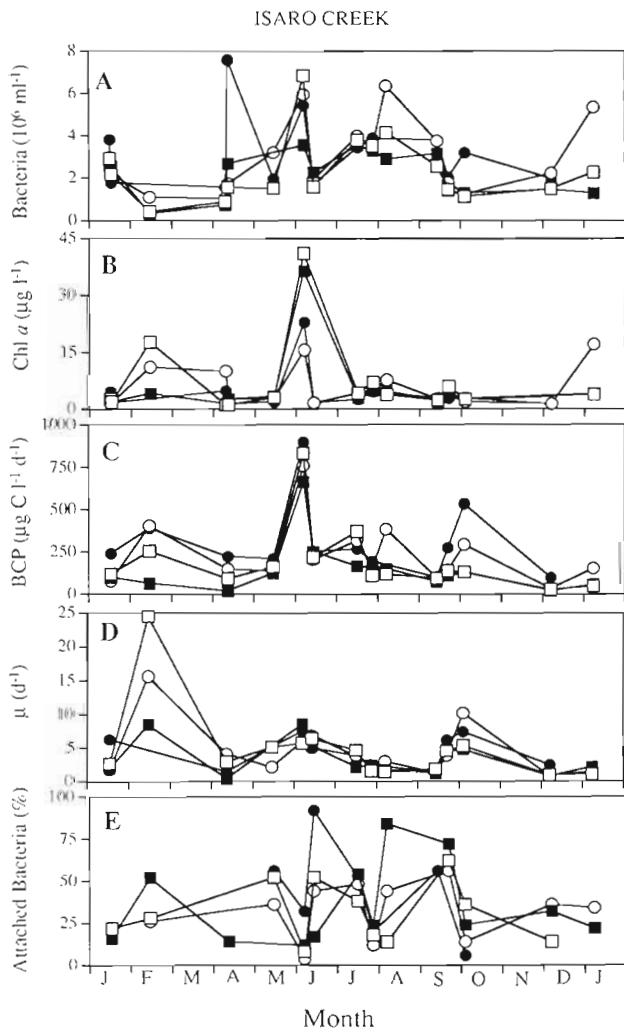


Fig. 3. Measured variables in Isaro Main (IM) surface ( $\square$ ) and bottom ( $\blacksquare$ ) and Isaro Branch (IB) surface ( $\circ$ ) and bottom ( $\bullet$ ) from January 1991 to January 1992. BCP: bacterial carbon production

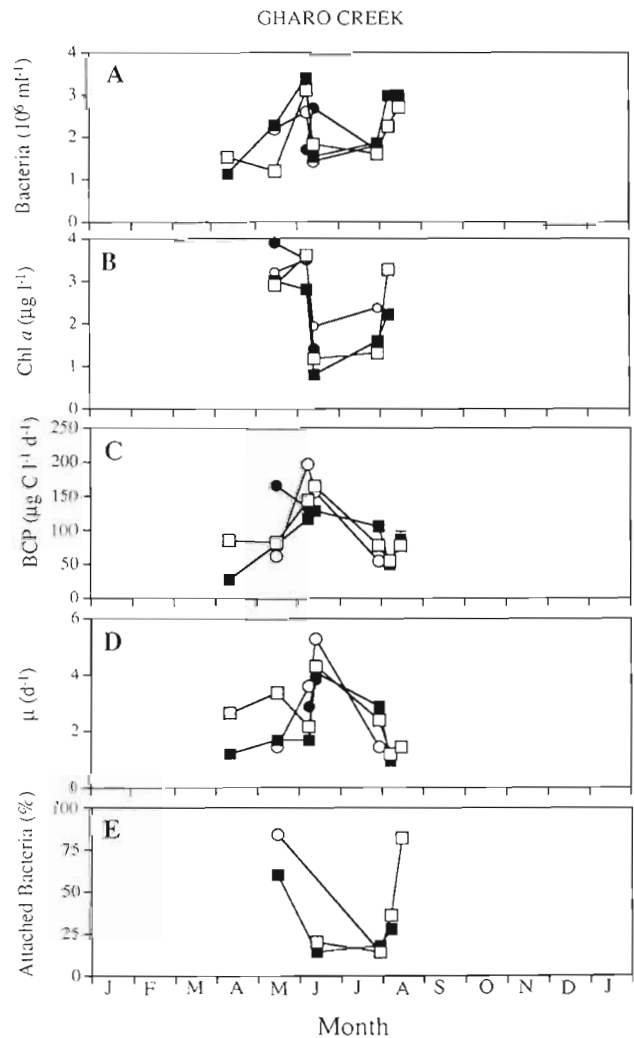


Fig. 4. Measured variables in Gharo Main (GM) surface ( $\square$ ) and bottom ( $\blacksquare$ ) and Gharo Branch (GB) surface ( $\circ$ ) and bottom ( $\bullet$ ) from April 1991 to August 1991

those in the Hudson River estuary, USA, a strongly heterotrophic ecosystem (Findlay et al. 1991).

**Specific growth rates.** Specific growth rates ( $\mu$ ) in both Isaro and Gharo Creeks were very high. In Isaro Creek the assemblage-averaged specific growth rates generally ranged from 2 to  $7 \text{d}^{-1}$  (Fig. 3D). Peaks in  $\mu$  occurred both in IM and IB at the time of a phytoplankton bloom, on February 13, when the values of  $\mu$  were  $24 \text{d}^{-1}$  (IM) and  $15.6 \text{d}^{-1}$  (IB). As stated above, these peaks coincided with low bacterial abundances. During a second and larger phytoplankton bloom, on June 5, both bacterial abundance and production were the highest measured in this study and  $\mu$  was  $\sim 7 \text{d}^{-1}$ . Thus, in contrast to the first bloom, this bloom was accompanied by a large population of rapidly growing bacteria (although not as rapidly as during the first bloom). In Gharo Creek, no major phytoplankton

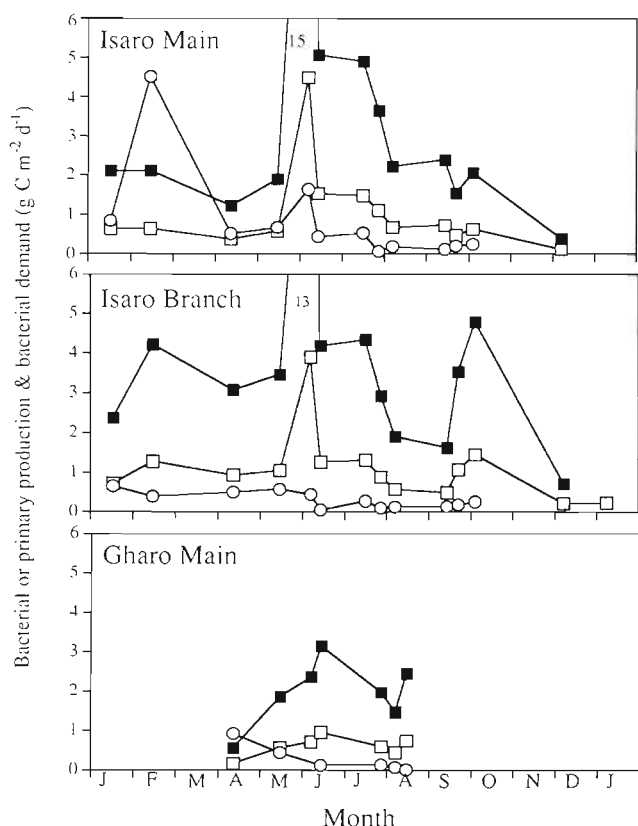


Fig. 5. Depth-integrated primary production (O), bacterial production (□) and bacterial carbon demand assuming 30% growth efficiency (■) at IM, IB and GM stations

blooms were recorded (chl *a* was  $\sim 1$  to  $3.5 \mu\text{g l}^{-1}$ ) nor any large increases in  $\mu$ , which generally ranged from 1 to  $3 \text{ d}^{-1}$  (Fig. 4D). Bacterial assemblages in Phitti Creek, sampled only once, showed modest  $\mu$  of 0.4 to  $1.6 \text{ d}^{-1}$ .

**Attached bacteria.** Attached bacteria were highly variable during different sampling periods (Figs. 3E & 4E); at times they became dominant, ranging from 4 to

92% (mean  $35 \pm 21\%$ ,  $n = 41$ ) in Isaro Creek and 14 to 84% (mean  $37 \pm 28\%$ ,  $n = 10$ ) in Gharo Creek. There was no clear seasonal pattern.

**Primary production.** This was measured regularly in Isaro and Gharo Creeks and once in Phitti Creek. The detailed data are presented by Harrison et al. (1997). Except for 2 peaks of 4.5 and  $1.65 \text{ g C m}^{-2} \text{ d}^{-1}$  on February 13 and June 5, respectively, in IM, the depth-integrated values in IM and IB ranged from 0.1 to  $0.8 \text{ g C m}^{-2} \text{ d}^{-1}$  (Fig. 5). The IB station showed much less pronounced peaks in PP than IM. At GM, PP ranged from 0.005 to  $0.9 \text{ g C m}^{-2} \text{ d}^{-1}$ . PP was limited by light penetration (Harrison et al. 1994) at all stations at all times.

**Relationship of PP with BCP and BCD.** In most samples BCP was greater than net PP (Fig. 5). Yearly integrated BCP/PP averaged for all sites except Phitti Creek was 2.0 (Table 1). BCD calculated by assuming a carbon assimilation efficiency of 10 or 30% was compared with PP to estimate BCD/PP (Table 1); this ratio for the entire data set (except Phitti Creek) ranged from 2.3 to 9.4 (assuming 30% growth efficiency), or 6.9 to 28.2 (assuming 10% growth efficiency).

**Time-course of leucine incorporation.** Whether the time course was linear during our  $\sim 0.5 \text{ h}$  incubations was determined on 1 occasion in IM and IB. The uptake of leucine incorporation was linear for at least 1.2 h (Fig. 6A).

**Concentration-dependence of leucine incorporation.** Simon & Azam (1989) recommended 20 nM Leu addition for marine samples, since they found that label incorporation into protein was maximum at or below that concentration. We considered that in eutrophic waters in this study, higher leucine additions may be necessary (Riemann & Azam 1992). In our routine sampling, we had arbitrarily chosen to add 54 nM leucine and we wanted to test whether the label incorporation rates at 54 nM approached  $V_{\text{max}}$ . In all our samples, label incorporation was submaximal at 20 nM, but  $V_{\text{max}}$  was approached at 30 to 57 nM. The

Table 1. Integrated yearly bacterial carbon production (BCP), bacterial carbon demand (BCD, assuming C assimilation efficiency range of 30 to 10%), primary production (PP), bacterial N production/phyto. N production (assuming bacterial C:N = 4 and phytoplankton C:N = 7) and bacterial C respiration/PP. Data from Phitti Creek are not included in average

Location	BCP ( $\text{g C m}^{-2} \text{ yr}^{-1}$ )	BCD (30–10%) ( $\text{g C m}^{-2} \text{ yr}^{-1}$ )	PP ( $\text{g C m}^{-2} \text{ yr}^{-1}$ )	BCP/PP	BCD/PP	Bact. N prod./ phyto. N prod.	Bact. C resp./PP
Isaro Main	293	977–2930	425	0.7	2.3–6.9	1.2	1.6–6.2
Isaro Branch	355	1183–3550	126	2.8	9.4–28.2	4.9	6.6–25.4
Gharo Main	225	750–2250	111	2.0	6.8–20.3	3.5	4.7–18.3
Gharo Branch	116	387–1160	42	2.8	9.2–27.6	4.8	6.4–24.9
Phitti (only 1 sampling)	204	680–2040	7.5 Cloudy day	27	90–272	40.6	64–245
Average	247	824–2470	176	2.0	6.9–20.8	3.6	4.8–18.7

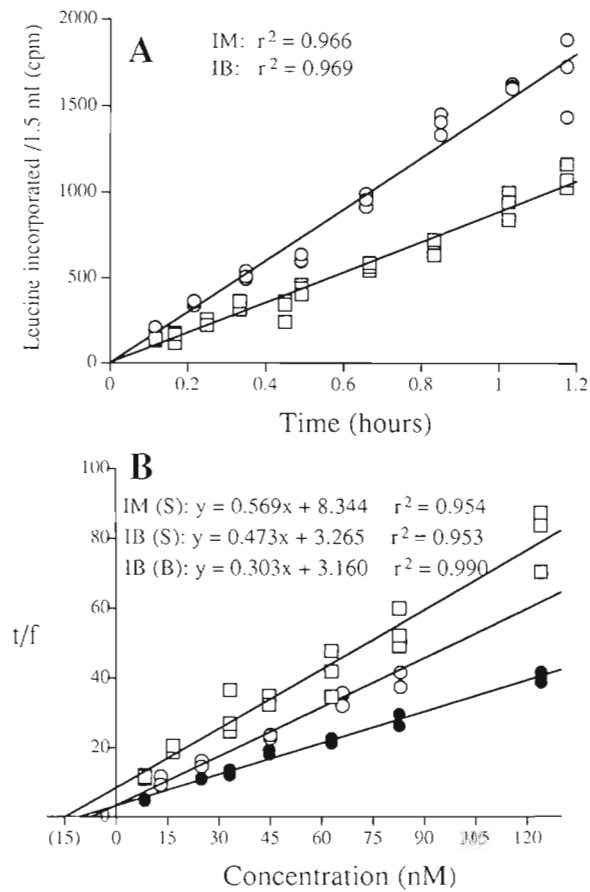


Fig. 6. (A) Incorporation of  $^{14}\text{C}$ -leucine as a function of time in waters of IM ( $\square$ ) and IB ( $\circ$ ); (B) Wright-Hobbie plots for bacterial uptake of  $^{14}\text{C}$ -leucine in IM surface ( $\square$ ), IB surface ( $\circ$ ) and IB bottom ( $\bullet$ ).  $f$ : fraction of the added label incorporated in time  $t$

uptake data showed an excellent fit to the Wright-Hobbie formulation ( $r^2 = 0.95$  to  $0.99$ ; Fig. 6B) without any evidence of multiphasic kinetic patterns in the concentration range used (Azam & Hodson 1981). Whether other low  $K_m$  and high  $V_{\text{max}}$  uptake systems existed in the population cannot be determined because we used a relatively narrow range of concentration.  $V_{\text{max}}$  was determined from the kinetic plots and ranged from  $1.5$  to  $2.8 \text{ nmol l}^{-1} \text{ h}^{-1}$ , equivalent to BCP rates of  $112$  to  $208 \text{ } \mu\text{g C l}^{-1} \text{ d}^{-1}$ . Leucine pool turnover rates were rapid. The  $K_m + S_n$  values, and hence the maximum estimates of  $S_n$ , were only  $8$  to  $15 \text{ nM}$ . Thus, the leucine pool was highly dynamic and was kept at a low level by rapid utilization by bacteria and possibly fungi as well.

**Utilizable DOC.** Bacterial growth was followed in seawater batch cultures on 3 sampling dates (Table 2) to determine bacterial yield supportable by  $0.6 \text{ } \mu\text{m}$  filtered seawater and thus the pool of dissolved utilizable organic matter. Bacterial yield ranged from  $1.5$  to  $9.9 \times$

$10^{-6} \text{ ml}^{-1}$ , which corresponds to a BCD range of  $97$  to  $656 \text{ } \mu\text{g C l}^{-1}$  (assuming  $30\%$  growth efficiency). We found that sometimes even in those samples which showed no protozoan contamination, bacterial counts decreased after the initial increase. This decline could be due to phage attack or mortality due to nutrient stress. Bacterial mortality could have caused an underestimation of UDOC estimates. Our estimates of UDOC are therefore conservative, because we assumed a high growth yield and because of possible underestimation of BCD due to bacterial mortality.

**Isaro transect.** Two transects on September 18 and December 2, 1991, were made in Isaro Creek to examine the mesoscale variability ( $0.1$  to a few km) of bacterial parameters. Stns 1 and 2 were surrounded on 3 sides by mangrove stands and Stns 3 to 5 were in the broader portion of the main creek (Fig. 1). Stns 1 and 5 were the same as IB and IM stations, respectively, in the seasonal sampling. During the transect, bacterial abundance ranged from  $1.4$  to  $2.6 \times 10^{-6} \text{ ml}^{-1}$  (Fig. 7A) and showed a general decrease from Stn 1 to Stn 5. During this transect,  $\mu$  for the bacterial assemblages was much higher during September ( $4$  to  $6 \text{ d}^{-1}$ ) than in December (generally  $1$  to  $2 \text{ d}^{-1}$ ; Fig. 7C). Depth-integrated bacterial production was high at all stations compared to primary production (Fig. 7E). The ratio of BCD/PP ranged from  $8$  to  $18$ . The highest BCD/PP ratio was at IB (Stn 1) and this ratio showed a general decrease along the transect. Attached bacteria ( $>0.6 \text{ } \mu\text{m}$ ) for all 5 stations in both transects ranged from  $32$  to  $74\%$  (mean  $53 \pm 11\%$ ; Fig. 8A). By the difference between the total and  $0.6 \text{ } \mu\text{m}$ -filtered leucine incorporation measurements,  $73$  to  $96\%$  (mean  $87 \pm 11\%$ ) leucine incorporation was by the fraction which was retained by the  $0.6 \text{ } \mu\text{m}$  Nuclepore filter (Fig. 8B). Production due to free bacteria was a minor fraction ( $4$  to  $27\%$ , mean  $12 \pm 10\%$ ) of the total. Specific growth rate of  $>0.6 \text{ } \mu\text{m}$  bacteria ranged from  $6$  to  $10$  and  $1.9$  to

Table 2. Yield of bacteria and utilizable dissolved organic matter (UDOM, calculated from bacterial yield assuming  $30\%$  carbon assimilation efficiency) with  $\pm 1 \text{ SD}$  ( $n = 3$  where SD is given) in  $0.6 \text{ } \mu\text{m}$  filtered surface water from Isaro Creek

Stn	Sampling date	Yield of bacteria ( $10^9 \text{ l}^{-1}$ )	UDOM ( $\mu\text{g C l}^{-1}$ )
1	Mar 1990	8.8	578
3	Mar 1990	4.4	290
5	Jul 1991	$9.9 \pm 0.1$	$656 \pm 5$
1	Sep 1991	$4.2 \pm 0.6$	$278 \pm 41$
2	Sep 1991	$3.0 \pm 0.8$	$196 \pm 52$
3	Sep 1991	2.3	152
4	Sep 1991	$1.5 \pm 0.3$	$97 \pm 18$
5	Sep 1991	3.6	240

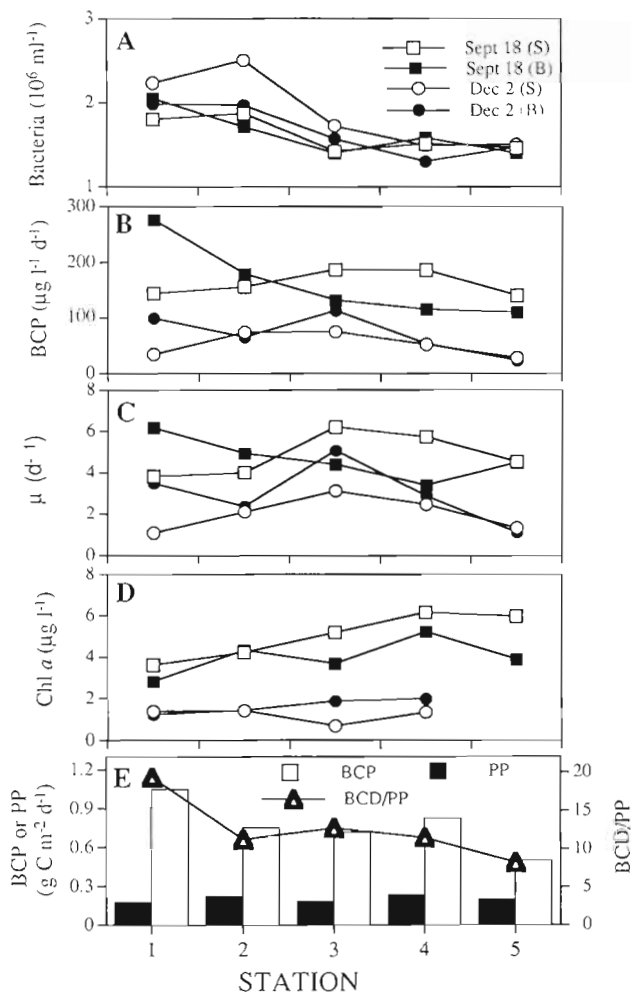


Fig. 7. (A) Bacterial abundance, (B) bacterial carbon production (BCP), (C) specific growth rate and (D) chl  $a$  during 2 transects on September 18 and December 2, 1991, in Isaro Creek and (E) depth-integrated BCP, phytoplankton production (PP) and bacterial carbon demand/PP (BCD/PP), measured only on September 18, 1991

$8 \text{ d}^{-1}$  in the September 18 and December 12 transects, respectively (Fig. 8B). Production due to attached bacteria may have been overestimated to an unknown extent because some free bacteria may have been retained by the  $0.6 \mu\text{m}$  Nuclepore filter (we would probably have reduced the overestimation by using  $1 \mu\text{m}$  Nuclepore filters, but we did not have these available to us at that time).

## DISCUSSION

### Food web significance of bacterial production

In view of the low nutritional quality of the mangrove detritus, the production of phytoplankton and bacterial

biomasses probably represented the main pathways for the synthesis of high quality (low C:N) biomass potentially available to the grazers in the mangrove creek ecosystem. It is noteworthy, then, that BCP was generally substantially higher than net PP (average BCP/PP was 2.0; Table 1). Since the C:N ratio of bacteria is  $\sim 4$  (Lee & Fuhrman 1987) and that of phytoplankton is 6 to 7 (Harris 1986) the bacteria:phytoplankton N production ratio would be even higher than the C production ratio. Thus, bacterial production may be an important pathway of the synthesis of high quality biomass in our study area.

We did not study the trophic fate of bacterial production. Heterotrophic microflagellates were present at  $10^3$  to  $10^4 \text{ ml}^{-1}$ , abundances which are typical of coastal ocean surface waters. However, we did not measure their grazing on bacteria. Viruses could have lysed some of the bacterial production. We counted viruses, by transmission electron microscopy, in 2 samples from Isaro Creek in January 1992 and found abundances on the order of  $3 \times 10^6 \text{ ml}^{-1}$  (these may be underestimates since some viruses may have gone unnoticed by being adsorbed on particles); however, the rate of phage-induced mortality of bacteria was not determined. Ingestion of attached bacteria is a potentially important pathway for the transfer of bacterial production to the higher trophic levels. The percentage of attached bacteria (Figs. 3E & 4E) in the seasonal study was highly variable, ranging from 4 to 92% in Isaro Creek. In the 2 Isaro Creek transects, discussed earlier, the abundance of attached bacteria was 32 to 74% and their production was 73 to 96% of the total (Fig. 8; but these may be overestimates, as discussed). Depending on the size of the particles to which bacteria were attached, this bacterial production could be directly available to a variety of detritivores (Lawrence et al. 1993, Crump & Baross 1996). Such transfer of bacterial production to the higher trophic levels is probably quite important because of the direct nature of the transfer. Further, the high variability in percentage of attached bacteria is significant because it could cause variability in transfer pathways, i.e. whether the transfer is direct or via protozoa. In view of the significance of bacterial production as a food source, future studies should determine the trophic fate(s) of bacterial production and its significance for food web structure and functioning in the creek ecosystems.

### Carbon fluxes

BCD is a useful measure of cumulative carbon flux into bacteria. However, it is difficult to quantify, on the basis of BCP measurements, because of the uncertainty in bacterial growth yield. Earlier studies as-



sumed growth yield to be 50% but a number of recent studies found much lower growth yields. Most literature values are within 50%, generally 10 to 30%, for a variety of coastal and oceanic environments. Linley & Newell (1984) found that the growth yield of bacteria utilizing detritus decreases with increasing C:N. Since the C:N of mangrove leaf litter was very high (~80; S. King unpubl.), we expected the bacterial growth yield for our samples to be quite low. Bjørnsen (1986) found a growth yield of 30% for open-ocean bacteria. Tranvik & Höfle (1987) and Tranvik (1988) found values of 26% in clear and humic lakes while Zweifel et al. (1993) found a range of 11 to 53% for coastal seawater samples. Smith et al. (1995) estimated yields of 9 to 17% for bacterial growth in a diatom bloom in a mesocosm. Generally, low values have been reported for growth on mangrove detritus. Benner & Hodson (1985) found bacterial growth yield on mangrove leachates was 30% for long-term incubations and, in another experiment, 2-fold higher for short-term incubations (Benner et al. 1986). However, mangrove particulate detritus was used at lower growth yields of 5 to 20% (Benner & Hodson 1985) presumably due to structural complexity of the detritus. In view of this literature, we considered it appropriate to use, for bacteria utilizing mangrove particulates and leachates, a wide range of yield values, 10 to 30%, thus covering the values found in most studies. This results in a 3-fold range of our BCD estimates, but sets reasonable upper and lower limits on the significance of bacteria in carbon fluxes.

Even our minimum BCD estimates show that the mangrove creek waters were persistently and highly net heterotrophic systems. At all stations and most sampling times, the net PP accounted for only a small fraction of BCD. Data integrated over the entire study period shows that even if all PP was used by bacteria, it would satisfy only 7 to 20% of the BCD; thus 80 to 93% of BCD would be met by reduced carbon from other sources. These percentages would be even higher if some of the phytoplankton production was used, as it most probably was, by organisms other than the heterotrophic bacteria. Assuming bacterial growth yield of 10 to 30%, we can also calculate bacterial carbon respiration. Average respiration, integrated over the entire study period and for all sites, would be 824 to 2470 g C m<sup>-2</sup> yr<sup>-1</sup> or 3 to 12 times the rate of CO<sub>2</sub> fixation by the water-column phytoplankton. Our estimates of respiration were similar to respiration in the Hudson River estuary (Findlay et al. 1991, Howarth et al. 1992). We note, parenthetically and in concurrence with Jahnke & Craven

(1995), that since respiration is the major fate of the organic matter taken up by bacteria, its direct measurement should be an important goal of the studies of bacteria-mediated carbon fluxes in aquatic ecosystems.

The mangrove trees probably supplied most of the BCD not met by the phytoplankton, through leaf litter, leaf leachates and root exudates. Additional organic matter could have been derived via the Indus River, however most upstream river water has been diverted for agricultural irrigation. Whether the river input of organic matter is indeed insignificant in the carbon budget of the mangrove creeks needs to be addressed in future studies. Benthic productivity and organic matter utilization were not examined and therefore their contributions to the water-column carbon dynamics are unknown. Kristensen et al. (1992) measured benthic metabolism in Isaro Creek and found that only 0.06 g C m<sup>-2</sup> d<sup>-1</sup> carbon was mineralized, via sulfate reduction, which was <0.1% of our estimates of carbon mineralization in the water column.

#### Controls on bacteria-mediated carbon flux

There was a very tight coupling between bacterial production and removal. Specific growth rates gener-

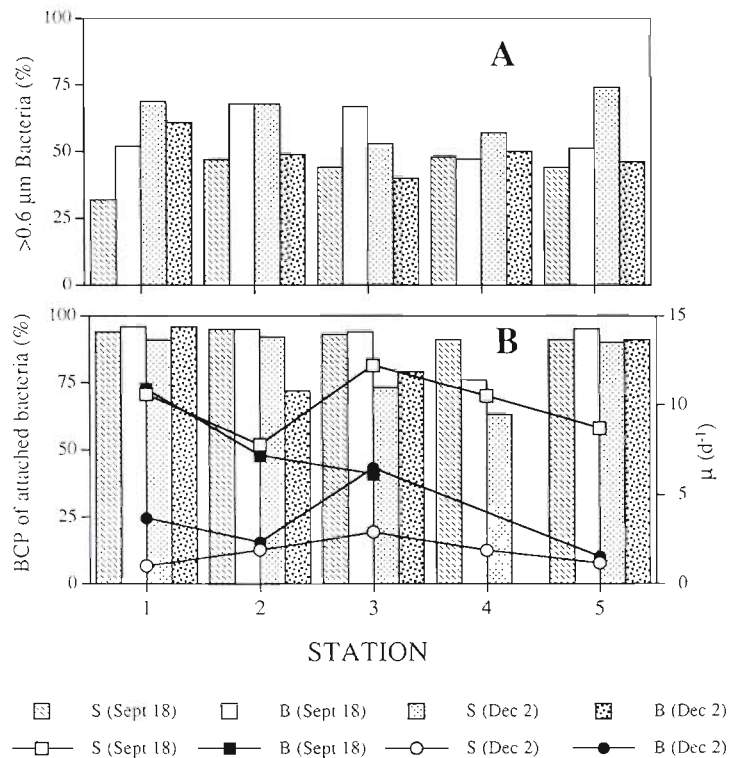


Fig. 8. (A) Percent bacterial abundance and (B) production (BCP, bars) and specific growth rate ( $\mu$ , lines) of attached (>0.6  $\mu$ m fraction) bacteria during 2 transects in Isaro Creek

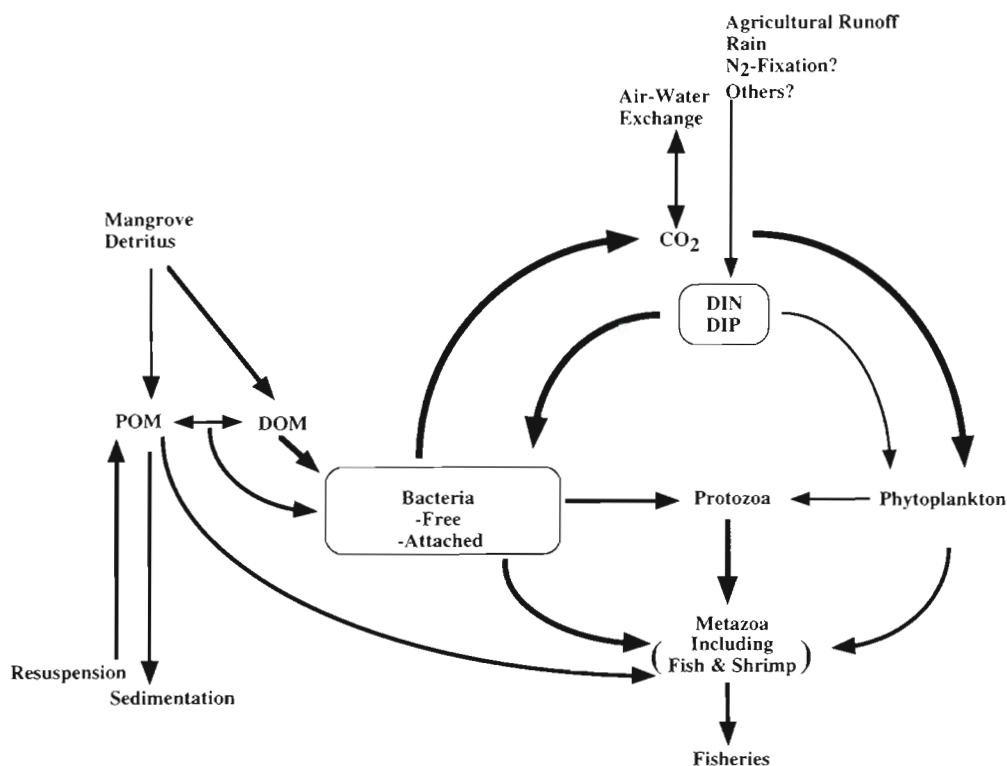


Fig. 9. Conceptual model of carbon fluxes in the Indus River delta mangrove tidal creeks. The model incorporated our finding of major carbon fluxes mediated by heterotrophic bacteria in the creek ecosystem. POM, DOM: particulate and dissolved organic matter; DIN, DIP: dissolved inorganic nitrogen and phosphorus

ally ranged from 1 to 7 d<sup>-1</sup>. Assemblage-average specific growth rate as high as 24 d<sup>-1</sup> was found in one instance. Remarkably, despite such rapid growth, bacterial abundances generally remained within narrow ranges and at relatively modest levels of 1 to 4 × 10<sup>6</sup> ml<sup>-1</sup>. These observations indicate a tight coupling in the carbon flow through the microbial loop.

Bacterial yield of the seawater cultures was on the order of 1.5 to 9.9 × 10<sup>6</sup> bacteria ml<sup>-1</sup> (Table 2) or ~30 to 200 μg C l<sup>-1</sup>. If we assume a carbon assimilation efficiency of 30% then the UDOC pool would be 97 to 656 μg C l<sup>-1</sup> (8 to 55 μM). As mentioned before, the bacterial yield method may have underestimated the UDOC if significant bacterial mortality had occurred due to virus attack (Fuhrman & Suttle 1993, Fuhrman & Noble 1995). We cannot express these UDOC levels as a percentage of the total DOC since there are no DOC measurements in our study area. However, our UDOC values are comparable to those in the Savannah River site (USA), where the DOC concentration was 3 to 5 mg l<sup>-1</sup>, and in the Okefenokee Swamp (USA), where the DOC concentration was 32 to 39 mg l<sup>-1</sup> (Moran & Hodson 1990). In order to sustain the measured BCD (643 ± 671 μg C l<sup>-1</sup> d<sup>-1</sup>) the UDOC pool would have to turn over in less than a day to a few days. If the UDOC

pool was significantly underestimated due to viral mortality of bacteria then the actual turnover times would be longer than we estimate. Consistent with rapid UDOC turnover, we found that the <sup>14</sup>C-leucine added at 5 nM had an assimilation turnover time of ~5 h. Assuming a 70% assimilation efficiency for leucine utilization (Carlucci et al. 1986) the leucine pool turnover time due to assimilation + respiration would be ~3.5 h. Fuhrman (1987) found comparable turnover times for amino acids in the Long Island Sound (USA) waters. Since amino acids are amongst the most readily utilizable UDOC components, the total UDOC pool turnover time would be longer than 3.5 h and this is consistent with our estimates of UDOC turnover times of <1 d to a few days.

Limited data suggest that bacterial production in our study area may have been limited by the supply of energy rather than N or P. Enrichment of creek water samples with 10 μM ammonium or phosphate did not significantly enhance bacterial protein production rate (not shown). This is consistent with the observation that ammonium and phosphate concentrations in the water column were generally quite high, greater than 1 μM (Harrison et al. 1997). It would thus appear that the introduction of N and P into the particulate phase

via bacterial growth was limited by the supply of energy, much of which was derived from mangrove carbon.

### A conceptual model of material and energy flow

Fig. 9 highlights the significance of the input of mangrove detritus as providing the majority of the reduced carbon needed to channel N and P from the dissolved phase into the particulate phase, in the form of bacterial biomass, where it becomes available to the protozoa and metazoa. The C:N ratio of natural assemblages of marine bacteria has been reported to be 3.8 (Lee & Fuhrman 1987). Using average bacterial carbon production for the 3 creeks, we estimated that N assimilation into bacteria in the creek water columns would be on the order of  $100 \text{ g N m}^{-2} \text{ yr}^{-1}$ . What fraction of the reduced carbon and assimilated N is passed on to the higher trophic levels will depend partly on the types of organisms feeding on bacteria and their abilities to digest and assimilate bacterial biomass. Further, the utilization of the bacterial biomass will depend on whether bacteria are attached to the particles or whether they are free (discussed above). The focus of the conceptual model for the mangrove creeks in Fig. 9 is that bacteria are the main conduit for channeling N and P into the particle-based food web leading up to the higher trophic level animals, including the shrimp and the fish of commercial importance. The primary producers, being light limited, play a lesser role in introducing N and P into the biomass. However, the energy needed to channel N into the microbial biomass appears to be derived largely from the mangrove productivity. This highly simplified model predicts that the destruction of the mangrove forests, now occurring, could have a major impact on the ecosystem structure and functioning as well as the production of economically important fish and shrimp in the Indus delta mangroves.

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