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Dominance of particle-attached bacteria in the Columbia River estuary, USA

Byron C. Crump[1,*], John A. Baross[1], Charles A. Simenstad[2]

[1]School of Oceanography, [2]School of Fisheries, University of Washington, Seattle, Washington 98195, USA

ABSTRACT: Particle-attached bacteria are a central component of the detrital food web of many turbid coastal and estuarine ecosystems. The Columbia River estuary, at the terminus of a 660 000 km^2 watershed in northwestern North America, is a turbid, partially mixed system that has a flushing time of 1 to 3 d. Several large, well-defined estuarine turbidity maxima (ETM) extend the residence time of both mineral and organic particles transported through the estuary. Water samples collected in the North Channel of the estuary every 2 h for 148 h (6 tidal cycles) in May 1995 were analyzed to determine the concentration and production of particle-attached and free-living bacteria, extracellular enzyme activity, turbidity, salinity, and particulate organic carbon (POC) concentration. The concentration of particle-attached bacteria, defined as those caught by a 3 µm filter, averaged 1.02×10^6 (SD = 1.00×10^6) cells ml^{-1} and correlated with turbidity and POC, and thus to some extent with the tidal cycle that maintains the ETM. The concentration of free-living bacteria was more constant, averaging 1.25×10^6 (SD = 0.4×10^6) cells ml^{-1}. Particle-attached bacterial carbon production, calculated from the rate of incorporation of ^{3}H-thymidine, averaged 1.61 (SD = 1.10) µg l^{-1} h^{-1}, accounted for 90% (SD = 9%) of total bacterial carbon production, and correlated with turbidity and POC. Extracellular enzyme activity, measured as the rate of hydrolysis of fluorescently labeled compounds, increased with turbidity and was predominantly associated with particles. Particle-attached bacteria probably account for most of the bacterial degradation of particulate organic material in the estuary, and the transfer of that material into the detrital food web. The hydrodynamics of the estuary contribute to the dominance of particle-attached bacteria by extending the residence time of particles in the ETM, and by quickly flushing free-living cells through the estuary, perhaps preventing the development of an estuarine population of free-living bacteria.

KEY WORDS: Columbia River · Particle-attached bacteria · Free-living bacteria · Estuarine turbidity maxima · Bacterial carbon production · Detrital food web

INTRODUCTION

Bacteria are the base of detrital food webs that recycle and consume dead and degraded organic matter. These food webs are particularly active in estuaries, thriving on the terrestrial and fluvial organic matter that passes through these systems to the ocean. In many estuaries a significant fraction of total bacterial carbon production is due to particle-attached bacteria (Bell & Albright 1981, Ducklow & Kirchman 1983, Iriberri et al. 1987, Kirchman & Ducklow 1987, Griffith et al. 1994), which are generally larger and more active per cell than free-living bacteria (Goulder 1977, Kirchman & Mitchell 1982, Unanue et al 1992). Particle-attached and free-living bacteria may be considered separate populations in estuaries because very different environmental factors control their contribution to the degradation of detritus and their roles in the detrital food web. Particle concentration and composition and the physical factors that control particle dynamics in estuaries have a strong influence over the concentration, rate of production, and the availability to grazers of particle-attached bacteria. In some highly turbid river estuaries like the Humber and the Tamar in England particle-attached bacteria have been found to dominate microbial activity (Bent & Goulder 1981,

*E-mail: bcrump@u.washington.edu

Plummer et al. 1987). Work on the Columbia River estuary, USA, found elevated microbial activity associated with estuarine turbidity maxima (ETM) (Baross et al. 1994) and that a significant fraction of this activity was associated with particles retained by a 20 µm screen (Crump & Baross 1996). Our understanding of the hydrodynamics of this system suggests that free-living bacteria and dissolved substances are rapidly flushed into and out of the estuary, but particles and particle-attached bacteria are retained in the estuary by ETM. For these reasons we hypothesized that most of the bacterial carbon production in the Columbia River estuary is due to particle-attached bacteria.

The Columbia River estuary (Fig. 1), in the northwestern United States, is a turbid, partially mixed system with a flushing time of 1 to 3 d (Neal 1972). Its hydrodynamics support large ETM at the upstream limit of the salinity intrusion in both of the main (North and South) channels (Jay 1994a, b). ETM result from net upstream flow in the lower layers of an estuary and act as particle trapping mechanisms that extend the residence time of negatively buoyant particles in the estuary over that of water, dissolved material, and neutrally buoyant particles. In the Columbia River estuary, particles in the ETM average 3 to 10% organic material by weight (Reed & Donovan 1994) and appear to undergo some microbial transformation in a sub-oxic environment (Prahl & Coble 1994). Water-column turbidity in the Columbia River estuary averages about 20 mg dry weight l^{-1}, higher than in the river or the coastal ocean, and can exceed 1000 mg l^{-1} in the ETM. *In situ* primary production (31×10^6 kg C yr^{-1}) is dwarfed by inputs of allochthonous organic material and river phytoplankton (21×10^7 kg C yr^{-1}) (Small et al. 1990). These conditions support a thriving detrital food web including detritivorous copepods and particle-attached bacteria that are most active when associated with the ETM (Baross et al. 1994, Simenstad et al. 1994a, Crump & Baross 1996).

The long-term goal of our research is to understand the role of bacteria in the degradation of detritus in the Columbia River estuary and ETM and the transfer of that material into the estuarine food web. The present study analyzes particle-attached and free-living bacterial activity in relation to salinity, turbidity and particulate organic carbon in the water column of the Columbia River estuary and discusses the detrital food web and its connection to the estuarine hydrodynamics that control particle cycling.

MATERIALS AND METHODS

Eulerian sampling series were conducted in May 1995 in the North Channel of the estuary at a location where the ETM passed below the anchored ship with every tide (Fig. 1). Samples were collected from about 1 m off the bed every 2 h during a 30 h and a 148 h sampling series using a high-volume, low-pressure pump coupled to a conductivity-temperature-depth (CTD) meter and an optical backscatter sensor (OBS) for measuring turbidity in relative OBS units (Simenstad et al. 1994b).

All samples were filtered gently by floating a plastic 100 ml filter tower (Millipore) fitted with a 3 µm polycarbonate filter (Poretics) on 1 l of sample water in a 2 l beaker. Large particles settled to the bottom of the beaker and therefore did not clog the filter. Water

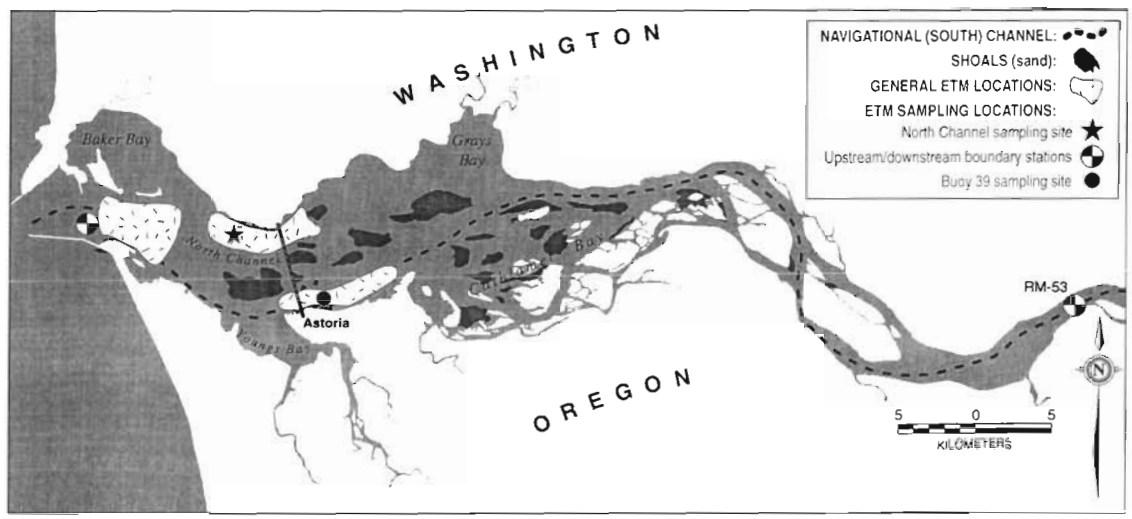

Fig. 1. Columbia River estuary (USA) with location of sampling site in the North Channel and extent of the estuarine turbidity maxima (ETM)

flowed up through the filter into the filter tower as the tower sank and was drawn out of the tower with a pipette.

Bacterial carbon production in filtered and unfiltered samples was determined by measuring the incorporation of methyl-tritiated thymidine (TdR; Fuhrman & Azam 1982) (20 nM final conc. at 64 Ci $mmol^{-1}$) into the cold TCA insoluble fraction in four 5 ml subsamples incubated with constant agitation at 12°C (conversion factors: 2×10^{18} cells mol^{-1} TdR, 25 fg carbon $cell^{-1}$; Bell 1993). Duplicate incubations were terminated at 0 and 60 min with 0.25 M NaOH (final conc.; Zweifel et al. 1995). Particle-attached bacterial carbon production was calculated by subtracting the filtered water production from the unfiltered water production.

Bacteria concentrations were determined from formaldehyde-fixed (2% final conc.) filtered and unfiltered samples collected during the 30 h series and the first 84 h (4 tidal cycles) of the 148 h series with direct counts using a Zeiss UEM epifluorescent microscope (Hobbie et al. 1977, Porter & Feig 1980). Unfiltered water samples were stained for 3 min with acridine orange (AO, 1 drop of 1 mg ml^{-1} solution ml^{-1} sample), diluted with a Triton X-100 detergent solution (1 drop 0.5% solution ml^{-1} sample), sonicated for 8 to 10 min (260 W, 12.7 mm diam. tip, samples in ice bath 1 to 2 cm from tip), filtered onto a black-stained 0.2 µm polycarbonate filter, destained with 0.5 ml isopropanol to remove the excess AO (Zimmermann & Meyer-Reil 1974), and finally stained with DAPI for 10 min. AO staining prevented nonspecific staining by DAPI so that cells attached to particles were much easier to distinguish from nonspecifically stained particulate material. Cells were enumerated in 30 fields per filter. Sonication and Triton X-100 broke up larger particles and released some cells from particles, giving a more even distribution of cells on the filter, and making the cells easier to identify and count (Velji & Albright 1986). We did not detect any loss of cells due to sonication or destaining with isopropanol. Bacteria concentrations in 3 µm filtered water samples were measured using the same procedure except the samples were not diluted with Triton X-100 solution or sonicated. Filters were not destained with isopropanol after staining with DAPI, and therefore non-nucleoid-containing bacteria, if present, were probably counted as cells (Zweifel & Hagström 1995). Particle-attached bacteria concentration was calculated by subtracting concentration in filtered water from concentration in unfiltered water. Average thymidine incorporation per cell was calculated for particle-attached and free-living bacteria from the bacterial concentration and the rate of thymidine incorporation per liter.

Methodological error for bacteria concentration and rate of thymidine incorporation was determined as 95% confidence intervals as described by Zar (1984). Confidence intervals for bacterial concentrations were calculated for the mean number of cells per field from direct counts of 30 fields per filter. Confidence intervals for rates of thymidine incorporation were calculated for the slopes of the lines described by 4 measurements of tritiated thymidine incorporation versus time of incubation. Confidence intervals were propagated through the calculation of thymidine incorporation rate per cell (Taylor 1982).

Extracellular enzyme activity was estimated in 10 sets of filtered and unfiltered samples selected to represent different turbidity regimes. For each sample, separate 30 ml subsamples were incubated with constant agitation for 2 h with 10 µM (final conc.) MCA-L-leucine (7-amido-4-methyl coumarin-L-leucine), MUF-D-glucoside (methylumbelliferyl-β-D-glucoside), and MUF-cellobioside. The degradation of these fluorescently labelled substrates was measured every 30 min by combining 2.7 ml of each incubation with 0.3 ml of borax buffer (50 mM boric acid, 29 mM sodium borate, pH 10) and reading the fluorescence with a Turner fluorometer fitted with a Wratten 7-60 excitation filter (320 to 390 nm) and an Omega 441 BP11 (interference) emission filter (400 to 500 nm) and calibrated with water from a control incubation containing no labeled substrate (Hoppe 1983, Vetter & Deming 1994).

The concentration of suspended particulate matter (SPM) in unfiltered samples was measured by pressure filtering 2 to 4 l of sample onto pre-weighed 90 mm diameter polycarbonate filters and correcting the gravitational particle weight for the salt content using a neutron activation analysis of chloride (Prahl & Coble 1994) and the assumption of constant salt composition for seawater (OPEN University 1989). Particulate organic carbon (POC) was measured in unfiltered samples filtered from a known volume onto a precombusted (450°C for 2 h) GF/F filter. Filters were fumed with concentrated HCl for 4 h to remove any inorganic carbonate, oven-dried at 60°C overnight, and cut in half. The POC and particulate nitrogen content of the packaged filter was determined by high temperature combustion using a Carlo Erba 1500 CNS analyzer setup and calibrated with the acetanilide standard as described by Verardo et al. (1990).

RESULTS

Concentrations of free-living bacteria varied from 0.61×10^9 to 2.42×10^9 cells l^{-1}, with a mean of 1.25×10^9 (SD = 0.40×10^9, n = 54) cells l^{-1}, and were negatively correlated with salinity (Table 1, Fig. 2). Particle-attached bacteria concentrations varied from $<2.60 \times 10^6$ (detection limit) to 5.10×10^9, with a mean of $1.02 \times$

Table 1. Spearman rank correlation coefficients (ρ), number of paired comparisons (n), and p-values for relationships between turbidity (OBS and SPM), POC, and salinity, and free-living and particle-attached bacterial concentration, production, and ^{3}H-thymidine (TdR) incorporation per cell. Calculations with bacterial production (l^{-1}) were made for all samples, freshwater samples (salinity <1), and estuarine water samples (salinity <1). Highly significant correlations ($p < 0.001$) are presented in bold type

Spearman correlation matrix	OBS			SPM			POC			Salinity		
	ρ	n	p	ρ	n	p	ρ	n	p	ρ	n	p
Free-living bacteria												
Concentration	0.226	52	–	0.270	47	–	0.090	44	–	−0.419	52	<0.005
Production l^{-1}	−0.123	88	–	−0.002	71	–	−0.115	69	–	**0.507**	**88**	**<0.001**
Fresh	0.217	19	–	0.052	17	–	0.133	16	–			
Estuarine	−0.15	68	–	0.188	52	–	0.350	51	–	0.178	68	–
TdR incorporation $cell^{-1}$	−0.183	52	–	0.231	47	–	−0.133	44	–	**0.612**	**52**	**<0.001**
Particle-attached production												
Concentration	0.443	51	<0.002	**0.505**	**45**	**<0.001**	0.575	42	<0.02	−0.094	51	–
Production l^{-1}	**0.425**	**88**	**<0.001**	**0.545**	**71**	**<0.001**	**0.593**	**69**	**<0.001**	0.293	88	<0.02
Fresh	0.337	19	–	0.228	17	–	0.106	16	–			
Estuarine	**0.652**	**68**	**<0.001**	**0.567**	**52**	**<0.001**	**0.694**	**51**	**<0.001**	**−0.506**	**68**	**<0.001**
TdR incorporation $cell^{-1}$	−0.161	47	–	−0.166	41	–	−0.096	38	–	0.385	47	<0.01
SPM	**0.761**	**69**	**<0.001**									
POC	**0.806**	**67**	**<0.001**	**0.862**	**67**	**<0.001**						
Salinity	−0.320	88	<0.005	0.051	69	–	−0.135	67	–			

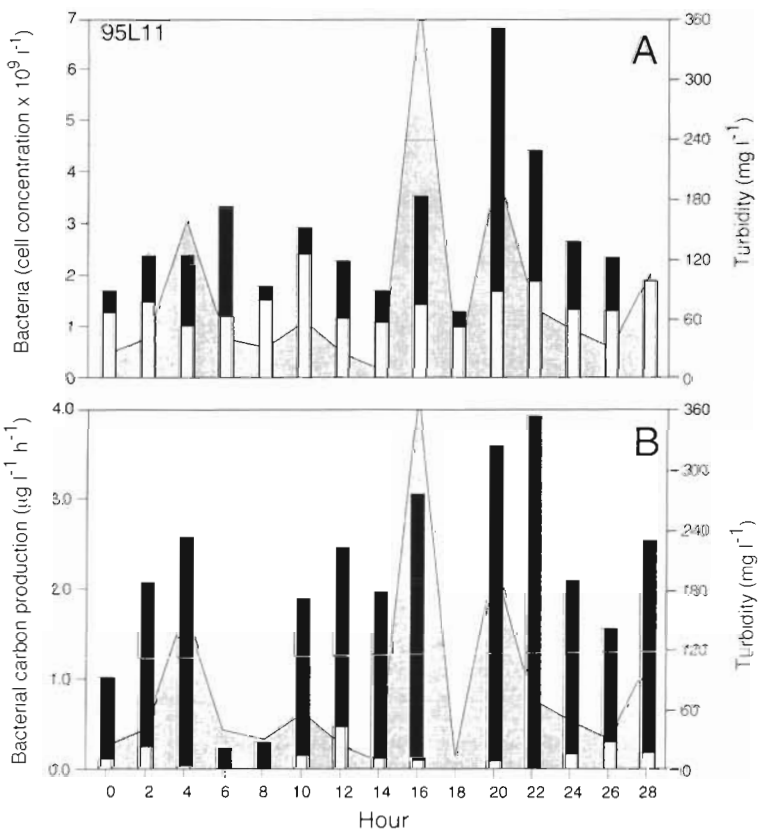

Fig. 2. Bacterial (A) concentration and (B) carbon production, and turbidity (solid line, shaded below for clarity) in near-bottom samples collected every 2 h during a 28 h sampling series in the North Channel of the Columbia River estuary. The open areas of the columns represent free-living bacteria and the shaded areas represent particle-attached bacteria (>3 µm)

10^9 (SD = 1.00×10^9, n = 52) cells l^{-1}, and were positively correlated with turbidity (OBS and SPM) and POC (Table 1, Fig. 2).

Bacterial carbon production (µg l^{-1} h^{-1}) in unfiltered samples was always much higher than in 3 µm filtered samples and tended to peak at or around the maximum ETM turbidities (Figs. 2 & 3). Particle-attached bacterial carbon production ranged from 0.13 to 4.50 µg l^{-1} h^{-1}, with a mean of 1.61 µg l^{-1} h^{-1} (SD = 1.10, n = 90), and represented on average 90% (SD = 9%) of total bacterial production. Particle-attached bacterial carbon production was positively correlated with turbidity (OBS and SPM) and with POC, but free-living bacterial carbon production was not (Table 1, Fig. 4a).

There was a marginally significant positive correlation between salinity and particle-attached bacterial carbon production (Table 1). This relationship was driven by low bacterial carbon production in freshwater samples (Fig. 4a). When freshwater samples were excluded, there was a significant negative correlation between salinity and both particle-attached bacterial carbon production and turbidity (Table 1). This was because the highest measured turbidities and attached bacterial carbon production rates occurred at intermediate salinities (Fig. 4b).

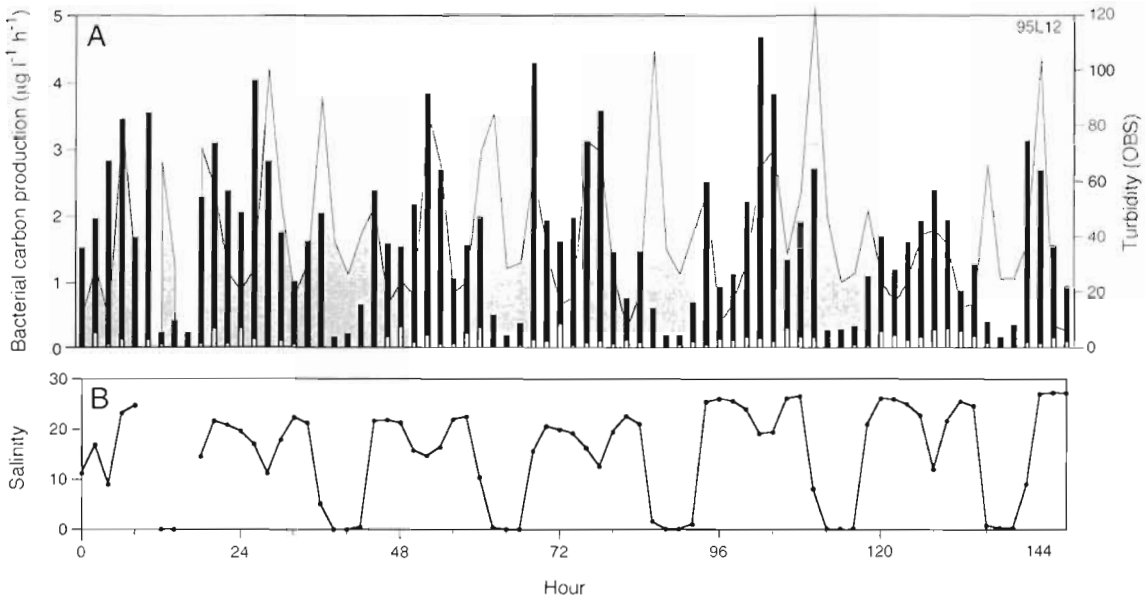

Fig. 3. (A) Bacterial carbon production and turbidity (solid line, shaded below for clarity), and (B) salinity in near-bottom samples collected every 2 h during a 148 h sampling series in the North Channel of the estuary

Free-living bacterial carbon production in estuarine water (salinity >1) varied from 0.01 to 0.49 µg l^{-1} h^{-1} with a mean of 0.16 (SD = 0.10, n = 68) µg l^{-1} h^{-1}. In freshwater samples (salinity <1) free-living bacterial carbon production varied from undetectable to 0.11 µg l^{-1} h^{-1} with a mean of 0.04 (SD = 0.03, n = 20) µg l^{-1} h^{-1}. Thus free-living bacterial carbon production was on average 4 times higher in the estuary than in the river. Particle-attached bacterial carbon production in estuarine water varied from 0.59 to 4.51 µg l^{-1} h^{-1}, with a mean of 1.99 (SD = 0.93, n = 68) µg l^{-1} h^{-1}, and correlated well with turbidity (OBS and SPM), POC, and particle-attached bacterial concentration. In freshwater samples, particle-attached bacterial carbon production varied from 0.13 to 0.60 µg l^{-1} h^{-1}, with a mean of 0.28 (SD = 0.14, n = 20) µg l^{-1} h^{-1}, and did not correlate with turbidity or POC.

Thymidine incorporation per cell for free-living bacteria averaged 2.8×10^{-9} (SD = 3.2×10^{-9}, n = 54) pmol TdR $cell^{-1}$ h^{-1}. Thymidine incorporation per cell for particle-attached cells was 10 to 100 times higher (Fig. 5), averaging 4.79×10^{-8} (SD = 4.17×10^{-8}, n = 47). In estuarine water (salinity >1) specific growth rates averaged 0.15 (SD = 0.16, n = 46) d^{-1} for free-living bacteria and 2.6 (SD = 1.7, n = 37) d^{-1} for particle-attached bacteria.

The rate of extracellular enzymatic hydrolysis of MCA-L-leucine, MUF-D-glucoside and MUF-cellobioside increased with turbidity in unfiltered samples, and was greatly reduced in filtered samples (Fig. 6). Hydrolysis of MCA-L-leucine was less reduced by 3 µm filtration than hydrolysis of the carbohydrate compounds.

DISCUSSION

Bacterial carbon production

An average of 90% of the bacterial carbon production in the water column of the Columbia River estuary was due to particle-attached bacteria. This was true not only in the ETM, where total bacterial carbon production was enhanced, but also in less turbid estuarine water and freshwater (Figs. 2 & 3). Other river estuaries where attached bacteria dominate total bacterial activity in the water column, such as the Humber and the Tamar in England, are generally more turbid than the Columbia River estuary (Goulder 1977, Bent & Goulder 1981, Plummer et al. 1987, Uncles & Stephens 1993). It appears that in the Columbia River estuary, the fast flushing time and the presence of ETM particle trapping combine to create conditions where particle-attached bacteria thrive on trapped particulate organic material while free-living bacteria are washed out of the system too quickly to establish an active estuarine community. Painchaud & Therriault (1989) describe such systems as accumulative for particle-attached bacteria and dispersive for free-living bacteria.

The relative contribution of particle-attached bacteria to bacterial carbon production in coastal and estuarine systems is variable and depends on the concentration and growth rate of both particle-attached and free-living bacteria (Griffith et al. 1990, Unanue et al. 1992). For example, in the upper Chesapeake Bay, particle-attached bacterial production was highest during the summer months, but the relative contribution of

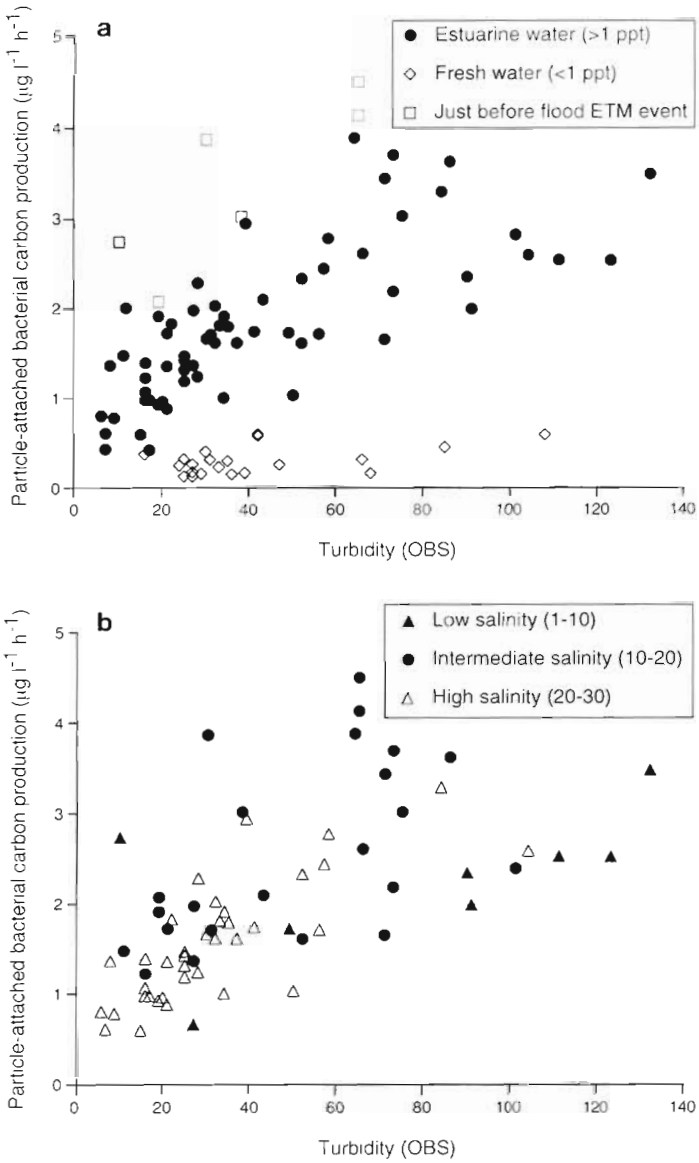

Fig. 4. Particle-attached bacterial production versus turbidity (OBS) for (a) all data from Figs. 2 & 3 and (b) data for samples with estuarine salinity

particle-attached bacteria to total bacterial production was highest in winter, when free-living bacterial production was lowest (Griffith et al. 1994). In spring and summer, the Columbia River estuary had high particle-attached bacterial carbon production and low free-living bacterial carbon production (Crump & Baross 1996, this study). A seasonal study has not yet been completed to determine if similar results also occur during the late fall and winter.

The assumption of a single average value of carbon per cell in the calculation of bacterial carbon production may have introduced some bias to the results. Since particle-attached bacterial cells are generally thought to be larger than free-living bacteria, estimates of bacterial carbon production may be relatively low for particle-attached bacteria and relatively high for free-living bacteria. Estimates of bacterial carbon production also assumed that the supply of tritiated thymidine to all bacterial cells was the same during the incubations. However, the supply of thymidine to bacteria on the inside of particles may have been limited, giving an underestimate of production by particle-attached bacteria. Another potential source of error was that some of the particle-attached cells could have detached from particles while passing through the plankton pump or while the particles were being filtered, giving an underestimate of particle-attached bacterial carbon production and concentration and an overestimate of free-living bacterial carbon production and concentration. No significant correlation was found between free-living and particle-attached bacterial concentrations or production (Table 1), suggesting that the pump and the 3 µm filtration method did not cause many particle-attached cells to release from their particles and enter the free-living fraction. Nevertheless, our estimates of particle-attached bacterial concentration and production should be considered conservatively low, and estimates of free-living bacterial concentration and production should be considered conservatively high.

There are many other reports where particle-attached and free-living bacteria were separated by filtration after samples were incubated with radiolabeled compounds (Hodson et al. 1981, Cammen & Walker 1982, Kirchman & Mitchell 1982, Iriberri et al. 1987). This procedure could cause attached bacterial cells to become dislodged from particles or, conversely, free-living cells to attach to particles during the period of incubation, particularly if samples were agitated during incubation as they were in the present study. Moreover, vacuum filtration of samples after incubation could cause particle-attached bacteria to dislodge and pass through the filter. These problems were reduced in the present study by gently filtering samples prior to incubation. We assumed that passing bacterial cells through a 3 µm polycarbonate filter did not affect their growth rate. Although this was not tested, similar experiments with less turbid water samples from the continental shelf off the coast of Georgia, USA (Griffith et al. 1990), and from the North Pacific (Crump unpubl.) demonstrated that ^{3}H-TdR incorporation rates were not significantly reduced when samples were filtered to remove particle-attached bacteria. This suggests that the growth rate of bacteria was not greatly reduced by passing them through a filter.

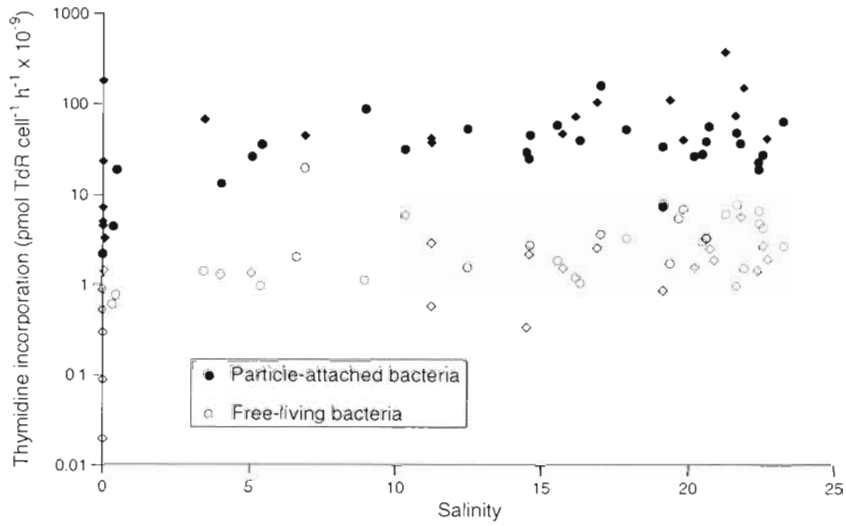

Fig. 5. Average thymidine incorporation rate per cell for all samples from the 28 h sampling series and the first 4 d of samples from the 148 h sampling series. Methodological error (95% confidence intervals) represented 61% (SD = 24%) of the values for circles and >100% for diamonds

Bacterial concentration

Concentrations of particle-attached bacteria in coastal and estuarine systems are variable, but often correlate with measurements of particle concentration, as in the Humber estuary (Bent & Goulder 1981), the Bay of Fundy, Canada (Cammen & Walker 1982), the coast of Spain (Unanue et al. 1992), and the turbidity maximum zone of the St. Lawrence estuary, Canada, but not in the relatively less turbid freshwater and saltwater zones of the St. Lawrence estuary (Painchaud & Therriault 1989). In the Columbia River estuary the concentration of particle-attached bacteria was widely variable, and was correlated with turbidity (OBS and SPM) and POC (Table 1). Free-living bacterial concentration in the Columbia River estuary was constant throughout the system, and, except in some highly turbid samples, was about the same or greater than particle-attached bacterial concentration.

The average free-living bacterial doubling time in samples with estuarine salinity was 223 h [SD = 190, n = 47, assuming 2×10^{18} cells produced mol^{-1} TdR incorporated (Fuhrman & Azam 1982), doubling time = ln2/specific growth rate], which is much longer than the flushing time of the estuary (about 1 to 3 d; Neal 1972). This suggests that free-living bacteria were native to river and coastal waters and simply washed in and out of the estuary. The average doubling time of the estuarine population of particle-attached bacteria, however, was 9.4 h (SD = 7.5, n = 37), which is less than the flushing time of the estuary, and potentially much less than the residence time of particles trapped in the ETM. Particles are thought to accumulate in the Columbia River estuary during neap tides and to disperse on spring tides when shear forces are greater and salt water is washed out of the estuary. So, parti-

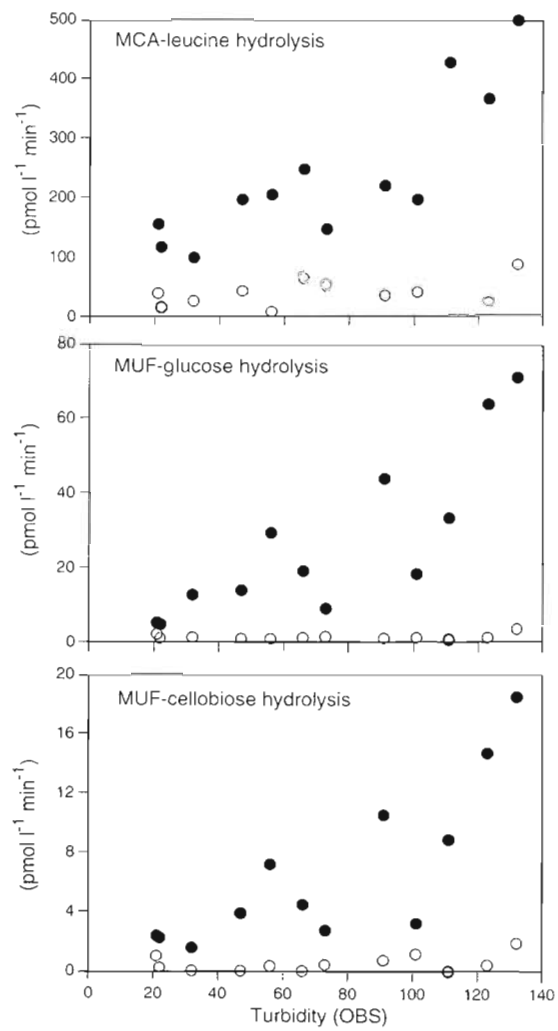

Fig. 6. Hydrolysis rates of fluorescently labelled substrates in untreated water samples (●) and 3 µm filtered water samples (○) plotted against turbidity of samples (OBS)

cles trapped in the ETM potentially remain in the estuary for 2 to 4 wk or longer if they are not washed out during the spring tide (D. J. Reed pers. comm.). This is enough time for an estuarine population of particle-attached bacteria to develop before being washed out of the system. Therefore, it is likely that this population is composed of bacterial types native to the estuary and adapted for growth on particulate organic material under estuarine conditions.

Thymidine incorporation per cell

Specific thymidine incorporation rates were much higher for particle-attached bacteria than for free-living bacteria in both freshwater and estuarine samples (Fig. 5). This result has also been found in many other coastal and estuarine systems from measurements of the maximum incorporation rate of radioactive organic compounds (Hodson et al. 1981, Cammen & Walker 1982, Kirchman & Mitchell 1982, Kirchman & Ducklow 1987). An important caveat regarding these calculations is that they produce an average thymidine incorporation rate per cell. Individual thymidine incorporation rates probably varied over a large range within both groups of bacteria. It is possible that a large percentage of free-living cells were inactive, and that the active free-living and particle-attached cells incorporated thymidine at the same rate. But if this were the case, active particle-attached cells would far outnumber active free-living cells.

Specific thymidine incorporation rates in estuarine water samples (salinity >1) were significantly higher than in freshwater samples for both groups of bacteria (Mann-Whitney test, free-living $p < 0.00001$, attached $p < 0.00001$). The methodological error associated with these rates was often quite high after propagating methodological error from bacterial carbon production and bacterial concentration. However, the trend in the particle-attached bacteria data (Fig. 5) suggests that riverine particles undergo changes when mixed into the estuary that affect bacterial carbon production rates. These changes may involve some form of interaction between riverine particles and particles either generated in the estuary or particles that have resided in the estuary for sufficient time to acquire an estuarine bacterial fauna. These estuarine particles may host faster-growing bacteria, giving a higher average rate of production when combined with riverine particles. Flocculation has been shown to make some organic material more available to bacteria (Tranvik & Sieburth 1989). It is possible that flocculation of dissolved organic material and aggregation with other particles in brackish water promotes bacterial colonization and growth. Another possibility is that organic material available to heterotrophic bacteria could be more labile in the estuary than in the river, accelerating bacterial growth rates. For example, freshwater phytoplankton are a major source of organic matter to the estuary (Small et al. 1990) and have been observed microscopically to be a significant component of ETM particles (Crump unpubl. results). Although these phytoplankton can survive at low salinities (Jackson et al. 1987), they probably perish at higher salinities and provide fresh, labile organic material to bacteria in the estuary.

Turbidity

Although there is a correlation between particle-attached bacterial carbon production and turbidity, variability in the relationship and the outlying values give indications of the heterogeneity of particles in this system (Fig. 4a). First, samples with relatively low bacterial carbon production per unit turbidity were collected in freshwater (salinity <1) at the end of strong ebb tides, suggesting that a shift occurred between fresh and estuarine water that affects bacterial production rates. Second, samples that showed relatively high bacterial carbon production occurred just prior to peak turbidity of flood ETM, as though particles supporting unusually high bacterial carbon production were resuspended first by the incoming tide, followed by the resuspension of less bacterially active particles. These very bacterially active particles were probably organic rich and had a relatively low density and therefore were the last to settle out after resuspension events, putting them in position to be resuspended early during the next tide. Finally, the relationship between bacterial carbon production and turbidity appeared to diminish at high turbidity (Fig. 4a). At times when ETM were most turbid they often contained resuspended sand grains and bits of wood that added to the overall turbidity of the samples, but did not host an active bacterial population based on microscopic examination and direct measurements of ^{3}H-TdR incorporation by rapidly settling particles (data not shown). These particles may have been responsible for the variation in bacterial carbon production estimates at the higher turbidities.

In May 1992 another study documented that less than 50% of the bacterial carbon production in the ETM of the Columbia River estuary was associated with particles retained by a 20 µm screen (Crump & Baross 1996), implying that particles that host the active population of bacteria both in and outside the ETM were between 3 and 20 µm. *In situ* observation with a video camera (data not shown) and particle analyses in the lab (Reed & Donovan 1994) have shown that many particles in ETM are larger, rapidly sinking

aggregates composed of smaller particles. These 'macroaggregates' are common in estuaries (Kranck 1973, Eisma & Li 1993, Li et al. 1993, Fennessy et al. 1994, ten Brinke 1994), are formed by collision and adhesion of smaller particles (Eisma 1986), and are thought to be trapped in ETM due to increased sinking rate (Kranck 1981, Reed & Donovan 1994). Aggregates are also known to be sites of intense biological activity (Alldredge & Silver 1988, Muschenheim et al. 1989, Zimmermann & Kausch 1996). It is possible that small, bacterially active particles in the Columbia River estuary are incorporated and retained in the ETM by aggregation with other ETM particles, and our sampling procedure causes disaggregation into individual particles and more cohesive 'microaggregates'.

Salinity

The highest levels of particle-attached bacterial carbon production were found at intermediate salinities (Fig. 3), and appeared to be due in part to the coincidence of intermediate salinities and elevated turbidity (Fig. 4b). High-turbidity ETM, with associated high bacterial carbon production, were most often found at intermediate salinities. Lower-turbidity samples with unusually high bacterial carbon production were also found at these intermediate salinities. In freshwater and at higher salinities, turbidity and particle-attached bacterial carbon production were reduced. A similar result was found at higher salinities in the plume of the Hudson river estuary, USA, where particle-attached bacterial activity was correlated with POC concentration but not with salinity (Ducklow & Kirchman 1983).

First difference analysis (Table 2) showed that changes in salinity over 2 h periods did not correlate with changes in particle-attached bacterial carbon production in estuarine water. Changes in turbidity over those same 2 h periods, however, did correlate with changes in particle-attached bacterial carbon production. So, other than the difference in particle-attached bacterial carbon production between fresh and estuarine water (discussed earlier), there did not appear to be a direct relationship between salinity and particle-attached bacterial carbon production. The relationship with salinity is instead a product of the cycling of particles by the hydrodynamics of the estuary because ETM tend to be located around the head of the salt wedge at intermediate salinities, and the strength of resuspension events increases with the velocity of water moving over the bed and the extent of mixing between freshwater and saltwater.

The positive correlation between free-living bacterial carbon production and salinity was driven by low production estimates in freshwater samples (Table 1). Bacterial activity is often greater at intermediate salinity in estuaries than in freshwater or seawater (Griffith et al. 1990, Chin-Leo & Benner 1992). Ducklow & Kirchman (1983) found free-living bacterial activity to be negatively correlated with salinity in the Hudson River plume at high estuarine and coastal water salinities and suggested that bacterial activity was enhanced by high levels of organic matter in the estuary. In the Columbia River estuary, inputs of allochthonous organic matter and freshwater phytoplankton are much greater than *in situ* primary production, so free-living bacterial activity at estuarine salinity may have been enhanced by the release of organic matter from particles or from dying freshwater phytoplankton.

Degradation of detritus

As a central component of the detrital food web, bacteria degrade insoluble organic polymers into compounds small enough for transport into the cell using

Table 2. Spearman rank correlation coefficients (ρ), number of paired comparisons (n), and p-values for relationships between the magnitude of change (first differences) in bacterial and physical data over 2 h intervals. Highly significant correlations ($p < 0.001$) are presented in bold type

Spearman correlation matrix	Δ(salinity)			Δ(OBS)			Δ(SPM)			Δ(attached production)		
	ρ	n	p	ρ	n	p	ρ	n	p	ρ	n	p
Δ(free-living production)												
All samples	0.275	84	<0.02	−0.037	83	–	−0.096	66	–	−0.008	88	–
Brackish	0.236	64	–	−0.179	63	–	−0.248	43	–			
Δ(attached production)												
All samples	−0.076	84	–	**0.620**	**83**	**<0.001**	**0.682**	**66**	**<0.001**			
Brackish	−0.272	64	<0.05	**0.578**	**63**	**<0.001**	**0.691**	**43**	**<0.001**			
Δ(OBS)	−0.262	83	–									
Δ(SPM)	−0.153	62	–	**0.862**	**61**	**<0.001**						

degradative enzymes either attached to their membranes or released into the environment (Hollibaugh & Azam 1983, Hoppe 1983, 1986). In the turbidity maxima region of the Elbe Estuary, Germany, proteolytic enzyme activity was found to correlate with the concentration of SPM and dissolved free amino acids (Bernat et al. 1994). In the Columbia River estuary, extracellular enzyme activity was detected principally on particles (Fig. 6), 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 suggest that they produced most of these enzymes and therefore were probably responsible for most of the bacterial degradation of POC in the estuary.

It is possible that cell-free enzymes could have stuck to the 3 µm filters, giving an underestimate of non-particle-associated enzyme activity. Low-binding polycarbonate filters were used to reduce this possible source of error. If some percentage of particle-free enzymes were captured by the filter, we would expect to see a relationship between particle-attached and free-living enzyme activity. However, there was no such relationship. Moreover, extracellular enzyme activity increased with turbidity, further supporting the conclusion that most of the active enzymes were attached to particles.

A rough estimate of POC turnover time by particle-attached bacterial decomposition can be made using the rate of bacterial carbon production and the concentration of POC in individual samples (Ducklow et al. 1985). Assuming a relatively high carbon conversion efficiency of 50% (g carbon produced g^{-1} carbon consumed), conservatively long estimates of average turnover time of POC due to bacterial decomposition were 71 d (SD = 38, n = 51) in estuarine water samples and 355 d (SD = 119, n = 15) in freshwater. Using a 10% conversion efficiency, turnover times decrease to 8 d (SD = 4, n = 51) in estuarine water and 39 d (SD = 13, n = 15) in freshwater. The extent to which particle-attached bacteria alter the flux of allochthonous detrital organic carbon through the estuary will depend on how long that carbon remains in the estuary, which cannot be estimated at this point. However, as previously discussed, the fraction of allochthonous organic material that is trapped in the ETM is thought to remain in the estuary on average 2 to 4 wk, or long enough for a significant fraction to be decomposed by particle-attached bacteria.

Enzymes associated with marine snow have been shown to hydrolyze much more POC than is required by particle-attached bacteria, possibly providing food for free-living bacteria (Smith et al. 1992). If this is true for particles in the ETM of the Columbia River estuary, then the turnover time of POC may be more rapid than calculated above. The fact that we did not detect enhanced free-living bacterial activity in turbid water samples suggests that freshly hydrolyzed dissolved organic matter (DOC) was not leaving the particles or that it was not being taken up by free-living bacteria. However, ETM are very dynamic in this system, and free-living bacteria probably do not remain in the ETM long enough to detectably change in their growth rate. It is more likely that DOC released from ETM particles is mixed into the estuary and contributes to the general enhancement we see in estuarine free-living bacterial carbon production over that in freshwater (Figs. 2 & 3).

Detrital food web

Particle-attached bacteria can have a very different role in a food web than 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. 1994a), and metazoans known to be capable of feeding directly on free-living bacteria such as larvaceans (King et al. 1980) are not present. The dominance of particle-attached bacteria in the Columbia River estuary suggests that the contribution of bacteria to the estuarine food web is mediated by particle dynamics. Bacteria are a high quality food source, and when they are attached to particles they increase the quality of detrital material as food (Heinle et al. 1977). Detritivorous metazoans may therefore be the principle consumers of bacterial biomass in this system (Simenstad et al. 1994a).

Up to 75% of the primary consumption by metazoans in the Columbia River estuary can be attributed to estuarine zooplankton (Jones et al. 1990, Simenstad et al. 1990). Due to low *in situ* primary production, allochthonous organic material is thought to be the dominant food source for these grazers (Simenstad et al. 1990). The system supports thriving populations of the copepods *Eurytemora affinis* and *Coullana canadensis* (Simenstad et al. 1994a). In spring 1992, a species of the rotifer *Keratella* was also found to be abundant at all salinities in the estuary with concentrations as high as 2200 l^{-1} (Crump & Baross 1996). These organisms are capable of consuming detritus (Heinle et al. 1977, Bogdan et al. 1980, Starkweather 1980) and were found to be concentrated in the ETM where they may take advantage of the high concentration of food particles (Morgan 1993, Simenstad et al. 1994a, Crump

& Baross 1996). Preliminary experiments demonstrated grazing on particle-attached bacteria by *E. affinis* and *C. canadensis* (Simenstad et al. 1994a). In low salinity waters of the Elbe Estuary 50 to 75% of the rotifers were found to be associated with suspended aggregate particles (Zimmermann & Kausch 1996). This may also be the case in the Columbia River estuary where the concentration of rotifers correlated with SPM and bacterial carbon production (Crump & Baross 1996). During our study, direct consumption of particles and particle-attached bacteria by metazoans was potentially the key step in the transfer of detrital organic matter into the food web of the Columbia River estuary.

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