

Bacterioplankton dynamics in northern San Francisco Bay: Role of particle association and seasonal freshwater flow

*M. C. Murrell*¹

Department of Ocean Sciences, University of California, Santa Cruz, California 95064

J. T. Hollibaugh

Department of Marine Sciences, University of Georgia, Athens, Georgia 30602

M. W. Silver

Department of Ocean Sciences, University of California, Santa Cruz, California 95064

P. S. Wong

Department of Marine Sciences, University of Georgia, Athens, Georgia 30602

Abstract

Bacterioplankton abundance and metabolic characteristics were observed in northern San Francisco Bay, California, during spring and summer 1996 at three sites: Central Bay, Suisun Bay, and the Sacramento River. These sites spanned a salinity gradient from marine to freshwater, and sampling occurred during a period of seasonally declining river flow. The microbial measures included radio-labeled amino acid uptake (L-leucine, L-proline, L-serine), ectoenzyme activity (aminopeptidase and β -D-glucosidase), and bacterial abundance using 1- μ m filters to separate free from particle-associated bacteria. A seasonal decline in all bacterial metabolic measures was observed at all stations, suggesting that a system-wide variable may be important in controlling bacterial activity. One such variable is freshwater flow into the Bay (as a proxy for organic matter flux), which positively covaried with all metabolic measures. A sharp decline in particle-associated bacteria was also observed in Suisun Bay and the Sacramento River between July and August. This decline may have been due to combined effects of declining nutritive value of the aging particles and increasing grazing pressure by benthic filter feeders. Aminopeptidase activity was positively related with increasing salinity, and β -D-glucosidase was negatively correlated with increasing salinity, indicating a gradient in the relative quality of organic matter from carbohydrate-rich riverine to protein-rich oceanic material. Overall, Suisun Bay had the highest mean proportion of particle-associated bacteria (49%), followed by Sacramento River (36%) and Central Bay (11%). Particles were the sites of enhanced ectoenzyme activity but not amino acid incorporation. Bacteria may be actively dissolving the particulate organic matter, but their growth rates on particles are not significantly enhanced.

Estuaries are important sites for the bacterial degradation of terrestrial and riverine organic carbon (Lee and Wakeham 1988). In estuarine systems with low primary production and hence low in situ sources of organic carbon, this allochthonous carbon can also be important in supporting the estuarine food web via bacterial secondary production and trophic transfers in the microbial loop. Within an estuary, there are primarily four fates of this organic carbon: respiratory loss, incorporation into biomass, advection out of the system, and burial. A better understanding of the flow of carbon in es-

tuaries and the role of bacterioplankton is a continuing challenge and is ultimately critical for making accurate estimates of carbon flux to the coastal oceans.

The nature of bacteria-particle associations has been the topic of sustained interest for many years (Marshall 1976, 1984; Bitton and Marshall 1980; Wotton 1994). Particle surfaces often promote bacterial growth because they are sites of increased substrate concentration; however, particle attachment may also pose negative consequences to bacteria such as increased grazing pressure by filter-feeding organisms that cannot assimilate free-living bacteria. Also, especially in environments with significant anthropogenic inputs, particles are sites of adsorption of trace metals and organic compounds that may be toxic to bacteria.

One common feature of estuaries, especially when compared with oceanic environments, is a high abundance of both particles and particle-associated bacteria (e.g., Bell and Albright 1981; Bent and Goulder 1981; Cammen and Walker 1982; Iriberry et al. 1987). Two factors that affect bacteria-particle associations are the composition of the particles themselves and the fate of the particles in the environment. In estuaries, particles tend to be composed of bottom-resus-

¹ Present address: US EPA, Gulf Ecology Division, 1 Sabine Island Dr., Gulf Breeze, Florida 32561.

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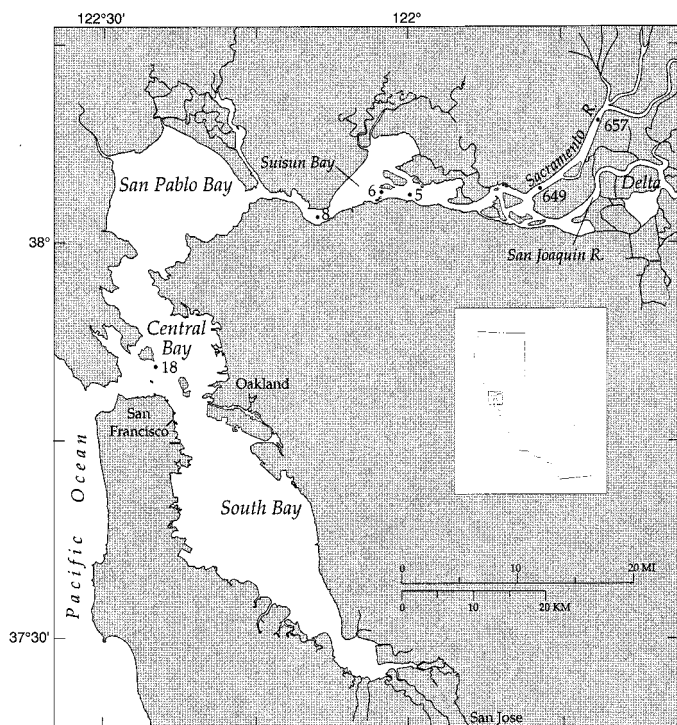


Fig. 1. Map of San Francisco Bay showing sampling locations.

pendent clays bound with organic matter, whereas in oceans particles are largely composed of detrital aggregates. Given their significant mineral content, estuarine particles tend to be less rich in organic compounds than are oceanic particles. The fate of particles is also quite different in estuarine and ocean environments. Particles tend to be retained in estuaries because of sinking and resuspension, whereas in the ocean particles tend to sink from the surface to deep waters. The estuarine and ocean environments also impose quite different fates on free-living bacteria. In estuaries, free-living bacteria tend to be advected from the system (as are dissolved and colloidal constituents), whereas in oceans free-living bacteria stay in the surface waters indefinitely. Because of these considerations, the ecological consequences of whether a bacterial cell is free living or particle associated may be very different in estuaries and oceans.

The purpose of this study was to investigate the patterns of bacterial activity in northern San Francisco Bay, a turbid estuarine environment, and to evaluate how the patterns relate to potential sources of dissolved and particulate organic carbon. We further wished to determine the relative abundance of free-living and particle-associated bacteria and whether there were measurable metabolic differences between these two assemblages. Such a difference would support the hypothesis that distinct selective pressures were acting on these two bacterioplankton communities.

Study site

Northern San Francisco Bay (Fig. 1) receives drainage from about 40% of California's land area via the Sacramento River and San Joaquin River systems. Seaward of the delta

region are Suisun and San Pablo Bays, which have narrow deep channels and broad shoal regions. Freshwater flows are highly seasonal, with high flows in winter and spring that decrease thereafter through the summer and fall. A typical annual range in freshwater outflow is $100\text{--}200\text{ m}^3\text{ s}^{-1}$ in summer to $1,000\text{--}1,500\text{ m}^3\text{ s}^{-1}$ in winter, with episodic peak flows as high as $10,000\text{ m}^3\text{ s}^{-1}$ during winter storms. About 80% of the annual influx of freshwater and sediment occurs during winter (Conomos and Peterson 1977; Conomos et al. 1979). The mean semidiurnal tidal range is about 2 m, and seawater intrudes up the bay approximately 60 km (into Suisun Bay), although this intrusion is heavily influenced by the magnitude of freshwater flow (Conomos et al. 1985). Phytoplankton production in this region is generally low ($39\text{ gC m}^{-2}\text{ yr}^{-1}$) because of high turbidity and heavy grazing by the Asian clam *Potamocorbula amurensis* (Alpine and Cloern 1992). Because of low in situ primary production and biomass, the bulk of the organic carbon input into this system appear to be of terrestrial and riverine origin (Jassby et al. 1993).

Materials and methods

Water sampling and processing—Samples were collected during monthly cruises aboard the U.S. Geological Survey (USGS) vessel R/V *Polaris* April–October 1996. We chose this period because of the predictable declining flow regime and to avoid sampling during highly variable rainfall and water flow conditions typical of winter months. On each date, samples were collected from three sites: Central Bay (Sta. 18), the Suisun Bay (Sta. 8, 6, or 5), and the Sacramento River (Sta. 649 or 657) (Fig. 1). Station numbers correspond to historical USGS sampling stations. The sampling location at the Suisun Bay station was dictated more by salinity than by geographic location; sampling was targeted to the estuarine turbidity maximum zone, which typically occurs in the 2–10 practical salinity units (PSU) salinity range. Basic hydrographic data including temperature, salinity, suspended particulate matter (SPM), and chlorophyll were collected by the USGS using standard methods (Table 1). The water was collected at the bow of the ship using an acid-cleaned polyethylene bucket and prescreened through a $160\text{-}\mu\text{m}$ mesh to remove large particles and zooplankton. Through all steps of processing, the sample water was handled as gently as possible to minimize dislodging bacteria from particles. A portion of the water was filtered by low vacuum ($<10\text{ cm Hg}$) through a glass fiber filter with a nominal pore size of $1\text{ }\mu\text{m}$ (GF/C or equivalent) to remove particles and particle-associated bacteria. Aliquots of whole water and $1\text{-}\mu\text{m}$ fractionated water were used to determine bacterial abundance, amino acid incorporation, and ectoenzyme activity. An additional $<0.2\text{-}\mu\text{m}$ fraction (for ectoenzyme activity only) was made by further filtering a portion of the $1\text{-}\mu\text{m}$ filtered water through a $0.2\text{-}\mu\text{m}$ cellulose acetate syringe filter. Samples for particulate organic carbon (POC) and dissolved organic carbon (DOC) were collected by filtering water through a GF/F glass fiber filter and retaining the filter and filtrate, respectively, as described elsewhere (Murrell et al. in prep.).

Table 1. Hydrographic conditions at the three sampling stations during the study period. Variables include temperature (Temp), salinity (Sal), chlorophyll (Chl), SPM, POC, and DOC.

Location	Date	Sta.	Temp. (°C)	Sal (PSU)	SPM (mg liter ⁻¹)	Chl (µg liter ⁻¹)	POC (µM)	DOC (µM)
Central Bay	3 Apr	18	12.7	27.5	7.8	7.1	80.9	133
	1 May	18	12.4	29.8	8.5	2.9	40.2	52.1
	12 Jun	18	12.5	30.3	16	3.8	64.2	74.4
	17 Jul	18	16.9	29.2	13	2.4	44.6	83.6
	13 Aug	18	15.5	31.6	9.1	2.7		118
	11 Sep	18	15.4	31.4	5.3	3.0	39.7	81.2
	16 Oct	18	16.5	29.9	6.2	2.4	29.4	61.4
Suisun Bay	3 Apr	8	14.7	4.8	50	2.4	104	296
	1 May	6	18.6	2.9	18	4.3	64.4	155
	12 Jun	6	21.1	1.8	55	1.9	116	138
	17 Jul	6	20.6	4.4	94	0.7	197	148
	13 Aug	5	22.7	3.7	33	1.4	77.8	148
	11 Sep	6	20.0	7.8	40	1.5	100	127
	16 Oct	5	18.9	5.8	14	1.8	67.4	132
Sacramento River	3 Apr	649	14.2	0	18	2.1	53.0	2,196
	1 May	649	18.4	0	19	2.9	38.1	127
	12 Jun	657	21.0	0	12	2.1	49.8	116
	17 Jul	657	21.6	0	21	1.7	98.9	153
	13 Aug	657	23.3	0	10	1.7	49.6	125
	11 Sep	657	20.4	0	20	1.7	80.8	143
	16 Oct	657	18.6	0	11	2.1	65.5	115

Bacterial cell counts—Accurate counting of bacteria in these samples was difficult because of the high abundance of particles; a normally simple procedure required more effort and attention to detail. Samples were fixed with 2% final concentration borate-buffered formaldehyde. Within 1–2 d of sample collection, duplicate aliquots were stained with acridine orange (AO), filtered onto black 0.2-µm polycarbonate filters, mounted on microscope slides, and counted using a Zeiss Axiophot epifluorescence microscope at $\times 1,250$ (Hobbie et al. 1977). To minimize interference from suspended sediments, small volumes (1 or 2 ml) were filtered onto a relatively large 23-mm-diameter filter area (Hoeffler filtration unit), and ~ 5 ml of the AO solution (50 µg ml⁻¹ in 0.02 M NaP₂O₇, pH 9) was added to disperse bacteria evenly. A Micron Separation Inc. (MSI) backing filter (10 or 20 µm pore size) was used to further promote even dispersion. A minimum of 300 cells were counted per filter, and at least 10 microscope fields (typically 20–40) were viewed. If the coefficient of variation between the duplicate counts was $>10\%$, then one or two additional samples were prepared and counted. By focusing on a small section of the microscope field, visual acuity for distinguishing bacteria from the particles was improved; 10–20% of the reticule was typically scanned. Counting these small sections of the reticule took only a few seconds, which helped minimize cell fading caused by exposure to the intense illumination; thus, more total fields of the slide could be scanned to give a more accurate picture of the variation in cell dispersion on the filter surface. Bacteria were enumerated in whole and 1-µm-fractionated samples, corresponding to total and free-living bacteria, respectively; particle-associated bacterial abundances were calculated by the difference of those estimates.

Bacterial dispersion and enumeration—To investigate bacterial dispersion patterns on the filter surface, cell counts were distributed into 10 equally sized bins, and adherence to a Poisson distribution was evaluated by calculating a coefficient of dispersion (Sokal and Rohlf 1995). A coefficient of dispersion (CD; the ratio of the variance of bin counts to the mean bin count) near unity suggests that bacteria were randomly distributed on the filter surface, values >1 suggest clumping, and values <1 suggest repulsion.

The dispersion of the bacteria on the filter surface appeared to conform to a Poisson distribution. Including all samples counted ($n = 146$), the mean (\pm SE) CD was 1.10 ± 0.05 . There was very little difference in counts between whole water samples ($CD = 1.09 \pm 0.07$, $n = 72$) and the <1 -µm-fractionated samples ($CD = 1.10 \pm 0.08$, $n = 74$). Particle-associated bacteria (whole water samples) were initially expected to have a more clumped distribution on the filter surface, but in general particles were very small (<5 µm), and large clumps of bacteria attached to particles were rarely observed. Assuming that the Poisson distribution criterion was satisfied and given a minimum of 600 cells per sample (duplicate counts of 300 cells per count), a coefficient of variation of about 4% was estimated. The uncertainty can be higher for particle-associated bacteria because their abundance was determined indirectly as the difference in abundance between whole and 1-µm-fractionated samples. When the difference between the two direct counts is small (hence the number of particle-associated bacteria is small), then the compounded variance of the difference will be relatively large.

Ectoenzyme activity—Ectoenzyme activity was measured using fluorogenic substrate analogues that mimic naturally

occurring substrates (Hoppe 1983): methyl coumarin amido (MCA) leucine and methylumbelliferyl (MUF) β -D-glucoside. The fluorescent moieties MCA and MUF are similar (biphenyl) compounds that are covalently bonded to parent substrate compounds and only become fluorescent when the bonds are broken. The bond between MCA and L-leucine is peptide like, whereas the bond between MUF and β -D-glucoside is similar to β -D-glycosidic linkages of cellobiose and cellulose. Therefore, these substrates will be hydrolyzed by aminopeptidase and β -D-glucosidase enzymes, respectively.

For each assay, 5 ml of sample water was placed into a 15-ml polyethylene tube with a saturating quantity of the fluorogenic substrate. Preliminary experiments indicated that 50 μ M final concentration was sufficient for saturation. Stock solutions of the fluorogenic substrates were prepared in methyl cellosolve at 100 \times or 200 \times final concentration. Assays were conducted in duplicate (April–June) or triplicate (July–October) on whole water and <1- μ m and <0.2- μ m fractions. Enzyme activity attributed to particle-associated bacteria was calculated as the difference in activity between the whole water and the <1 μ m fractions, and activity attributed to free-living bacteria was calculated as the difference in activity between the <1- μ m and the <0.2- μ m fractions. The dissolved enzyme activity (<0.2- μ m fraction) was considered a blank, and the values were subtracted from those for the other treatments. Possible sources of dissolved enzymes include extracellular enzymes or membrane fragments that pass through a 0.2- μ m filter yet still contain functioning enzymes. All incubations were conducted in the dark at in situ temperature for 1–3 h (MCA-L-leucine) or ~6–12 h (MUF- β -D-glucoside).

Time point subsamples (typically 1 ml) were removed from the incubation tubes and placed into screw-capped 13- \times 100-mm borosilicate glass tubes with 5 ml of alkaline buffer (0.05 M glycine, 0.2 M NH_4OH , pH 10.3). The alkaline pH of the buffer maximized the fluorescent yield of the fluorophore and effectively stopped further enzymatic activity. In early incubation experiments, subsamples were taken at multiple time points (up to five), and fluorescence was found to increase linearly during the first several hours. Consequently, in later experiments only initial and final subsamples were evaluated. By measuring the change in fluorophore concentration over time in each treatment, variability due to treatment-specific differences in fluorescence quench was avoided. Fluorescence quench among samples was negligible based on standard addition experiments (data not shown). Samples were kept in the dark at room temperature until fluorescence was read within 1–2 d. Repeated measures showed that the fluorescence was stable for several days. Fluorescence was read on either a Turner Model 111 or a Turner Designs Model TD-700 using a near-ultraviolet lamp, a 300–400-nm excitation filter, and a 410–600-nm emission filter. The fluorometer was calibrated each time with pure MUF and MCA solutions in alkaline buffer.

Radiolabel incorporation—Amino acid incorporation was determined using ^3H -labeled substrates following methods of Hollibaugh and Wong (1992). We routinely used 4,5- ^3H -L-leucine (L-leucine), but for the last 3 months of the study, we did additional assays for incorporation of 5- ^3H -L-pro-

line (L-proline) and 3- ^3H -L-serine (L-serine). For each amino acid, two sets of duplicate whole water samples and one set of duplicate 1- μ m fractionated water samples were placed in 15-ml polyethylene tubes and spiked with the appropriate radio-labeled amino acid (5 Ci mmol^{-1} , 100 nM final concentration). After a 1–2-h incubation period at in situ temperature, one set of whole water samples was filtered onto 0.45- μ m pore size Millepore HA filters, and the other set was filtered onto 1- μ m polycarbonate filters to capture activity attributed to total bacteria or only particle-associated bacteria, respectively. The third treatment (water samples that were 1- μ m filtered prior to incubation) were filtered onto 0.45- μ m pore size Millepore HA filters. This treatment provided another measure of free-living bacterial incorporation that was used for comparison with rates calculated by the difference method. Blank incorporation of substrate was routinely measured on additional duplicate samples by filtering immediately after adding substrate. Blanks were typically <5% of total incorporation and were not subtracted.

The filters were sequentially rinsed with iced unlabeled sample water, iced 5% trichloroacetic acid, and iced 80% ethanol to precipitate proteins and rinse away unincorporated label. Filters were placed in scintillation vials and allowed to air dry for 1 to several days, and the Millepore HA filters were dissolved with 1 ml of ethyl acetate. Scintillation cocktail (Aquasol II, New England Nuclear) was added to each vial, and the vials were vortexed and assayed for radioactivity with a liquid scintillation counter (Beckman) equipped with an external standard to determine quench and calculate counting efficiency.

Conversion of L-leucine incorporation into bacterial production was accomplished using an empirically derived molar L-leucine conversion of 0.323 cells amol^{-1} for Central Bay, 0.172 cells amol^{-1} for Suisun Bay and Sacramento River (Hollibaugh and Wong 1996), and 20 fg C cell^{-1} cellular carbon content (Lee and Fuhrman 1987).

From August to October, incorporation rates of two additional amino acids, L-proline and L-serine, were measured. The choice of L-proline and L-serine was based on results from a separate metabolic analysis using BIOLOG microtitre plates (data not shown) that were used to characterize the capacity of the microbial community to utilize each of 95 substrates as a sole carbon source (Garland and Mills 1991). Samples of whole water and <1- μ m fractionated water were incubated in BIOLOG plates, and after ~1 week the plates were scored by the intensity of the color development of the tetrazolium violet indicator using a microtiter plate reader (Bio Rad). Each substrate was then ranked by the difference in color development between whole water and <1- μ m treatments. L-Proline and L-serine were ranked 4th and 10th, respectively (2nd and 3rd among amino acids), of 95 substrates. For comparison, L-leucine ranked 31st (6th among amino acids). The large metabolic differences in L-proline and L-serine utilization between particle-associated and free-living bacteria might also be evident in short-term substrate incorporation experiments.

Results

Hydrographic conditions—The study period spanned a period of waning seasonal freshwater flow from 1500 $\text{m}^3 \text{s}^{-1}$

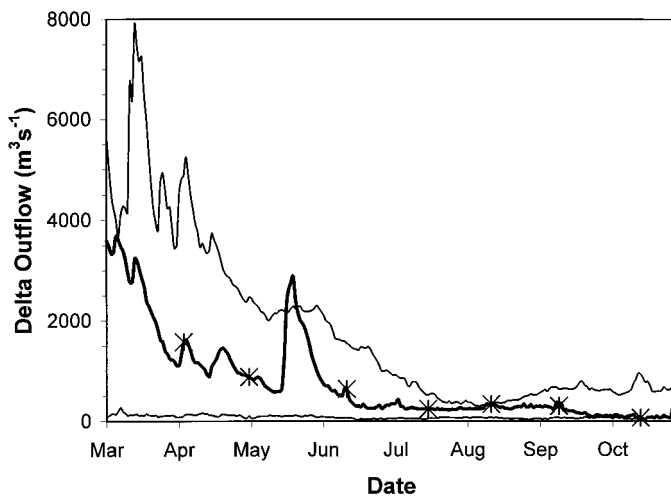


Fig. 2. Delta outflow from the San Joaquin and Sacramento Rivers during the 1996 study period (dark line) compared with the 95th percentile confidence intervals of the 40-yr average (light lines). Flow estimated by the California Department of Water Resources DAYFLOW algorithm.

in the beginning of April to $100 \text{ m}^3 \text{ s}^{-1}$ by October (Fig. 2). Flow during 1996 was similar to the 40-yr average, suggesting that the study period represented a typical seasonal progression in the North Bay. Chlorophyll concentrations were highest in Central Bay, averaging $3.5 \mu\text{g liter}^{-1}$ over the study period and exceeded $5 \mu\text{g liter}^{-1}$ only in April (Table 1). The Suisun Bay and Sacramento River stations both had average chlorophyll concentrations of $2.0 \mu\text{g liter}^{-1}$, with only slightly elevated levels evident in April and May but never exceeding $5 \mu\text{g liter}^{-1}$. Suspended sediment loads were generally highest in the Suisun Bay, followed by the Sacramento River station, and lowest in Central Bay (Table 1).

Bacterial abundance—During this study, total bacterial abundance averaged $3.4 \times 10^6 \text{ cells ml}^{-1}$ (range, $2.4\text{--}4.7 \times 10^6 \text{ cells ml}^{-1}$) with no clear spatial or temporal trends (Fig. 3). Across all stations and dates, particle-associated abundance averaged 31% (range, 3–86%) of total abundance. Suisun Bay had the highest percentage of particle-associated bacteria, averaging 45% (range, 6–86%), the Sacramento River site was intermediate, averaging 33% (range, 3–64%), and the Central Bay site had the lowest fraction, averaging 13% of total abundance (range, 3–25%). At both the Suisun Bay and Sacramento River stations, there was a downward shift in particle-associated bacteria between July and August that persisted through October (Fig. 3). The average particle-associated bacteria fraction dropped from 68% to 16% in the Suisun Bay and from 52% to 11% in the Sacramento River.

L-Leucine incorporation and bacterial production—L-Leucine incorporation (Fig. 4) over all dates and stations averaged $304 \text{ pmol liter}^{-1} \text{ h}^{-1}$. Central Bay had the highest average rates ($396 \text{ pmol liter}^{-1} \text{ h}^{-1}$) but they were also the most variable, ranging from 121 to $677 \text{ pmol liter}^{-1} \text{ h}^{-1}$. In Central Bay, there was an evident seasonal decline in L-

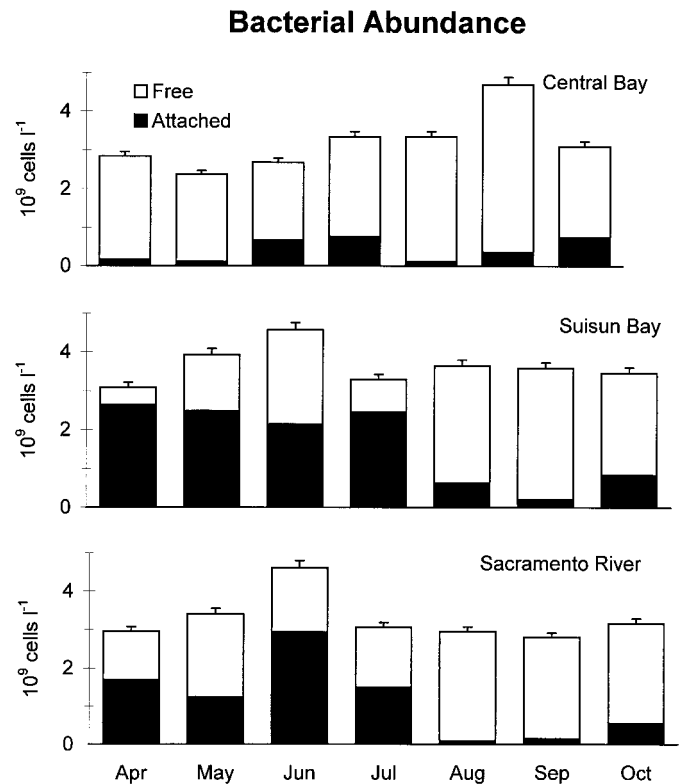


Fig. 3. Bacterial abundance in San Francisco Bay from April through October 1996 for Central Bay, Suisun Bay, and Sacramento River stations. Stacked bars represent abundance of particle-associated (solid) and free-living (open) bacteria. Error bars are standard errors of total.

leucine incorporation, except for a peak in September. L-Leucine incorporation averaged $257 \text{ pmol liter}^{-1} \text{ h}^{-1}$ at Suisun Bay (range, $143\text{--}528 \text{ pmol liter}^{-1} \text{ h}^{-1}$) and $260 \text{ pmol liter}^{-1} \text{ h}^{-1}$ at the Sacramento River (range, $127\text{--}439 \text{ pmol liter}^{-1} \text{ h}^{-1}$). Although there was also a seasonal decline evident at these two lower salinity sites, it was least evident at Suisun Bay.

Bacterial secondary production, calculated from L-leucine incorporation rates (Fig 4, right axis), ranged from 16 to $105 \mu\text{gC liter}^{-1} \text{ d}^{-1}$ and averaged $35 \mu\text{gC liter}^{-1} \text{ d}^{-1}$ across all stations and dates. Central Bay typically had the highest bacterial production, averaging $61 \mu\text{gC liter}^{-1} \text{ d}^{-1}$, whereas Suisun Bay and Sacramento River sites were similar, each averaging $21 \mu\text{gC liter}^{-1} \text{ d}^{-1}$. In general, the bacterial production estimates agree with previously published estimates from this (Hollibaugh and Wong 1996) and other systems (for a review, see Ducklow and Carlson 1992). However, because of the uncertainties with the conversion factors used to calculate bacterial secondary production, the interpretation is based on the incorporation rate data.

Particle-associated L-leucine incorporation averaged 30% of the total over all stations and dates, a value similar to abundance fractionation. Station-specific particle-associated L-leucine incorporation averaged 26% in Central Bay, 42% in the Suisun Bay, and 22% in the Sacramento River. The seasonal decline from April to October was evident in the particle-associated fraction (Fig. 4, solid bars) at all stations.

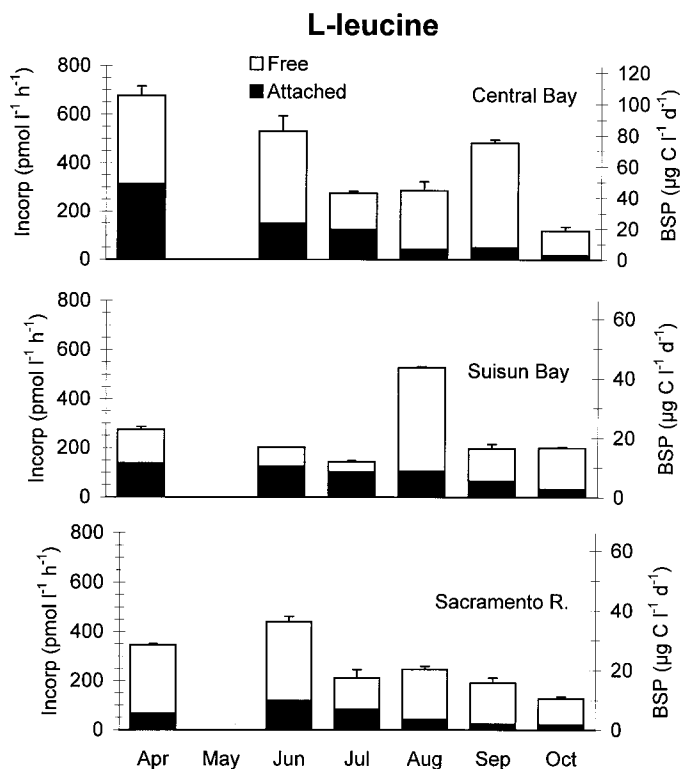


Fig. 4. L-Leucine incorporation and bacterial secondary production (BSP, right axis). Legend as in Figure 3.

There was a downward shift in particle-associated L-leucine incorporation at all stations between July and August, a shift similar to that observed in bacterial abundances in Suisun Bay and the Sacramento River (Fig. 4). Averaging data before and after this shift, the particle-associated L-leucine incorporation dropped from 40% to 13% in Central Bay, from 61% to 23% in Suisun Bay, and from 28% to 15% in the Sacramento River.

L-Proline and L-serine incorporation—In general, the incorporation patterns for L-proline and L-serine were remarkably similar to those for L-leucine (Fig. 5), as demonstrated by the strong correlations between the different measures (Table 2). Incorporation of L-proline was similar to that of L-leucine, averaging $193 \text{ pmol liter}^{-1} \text{ h}^{-1}$ (range, 36–535 $\text{pmol liter}^{-1} \text{ h}^{-1}$), whereas incorporation of L-serine was consistently higher, averaging $451 \text{ pmol liter}^{-1} \text{ h}^{-1}$ (range, 165–988 $\text{pmol liter}^{-1} \text{ h}^{-1}$). The downward seasonal trend in total incorporation was evident, although measurements were available only for the last 3 months of the study (Fig. 5). The fractionation patterns for all amino acids were also very similar to each other, with the highest particle-associated incorporation in Suisun Bay, followed by Sacramento River and Central Bay.

Amino peptidase activity—Across all stations and dates, bacterial amino peptidase activity averaged $59 \text{ nmol liter}^{-1} \text{ h}^{-1}$ (range, 6–206 $\text{nmol liter}^{-1} \text{ h}^{-1}$; Fig. 6). Similar to amino acid incorporation, total amino peptidase activity was higher in Central Bay (average, $75 \text{ nmol liter}^{-1} \text{ h}^{-1}$) and more var-

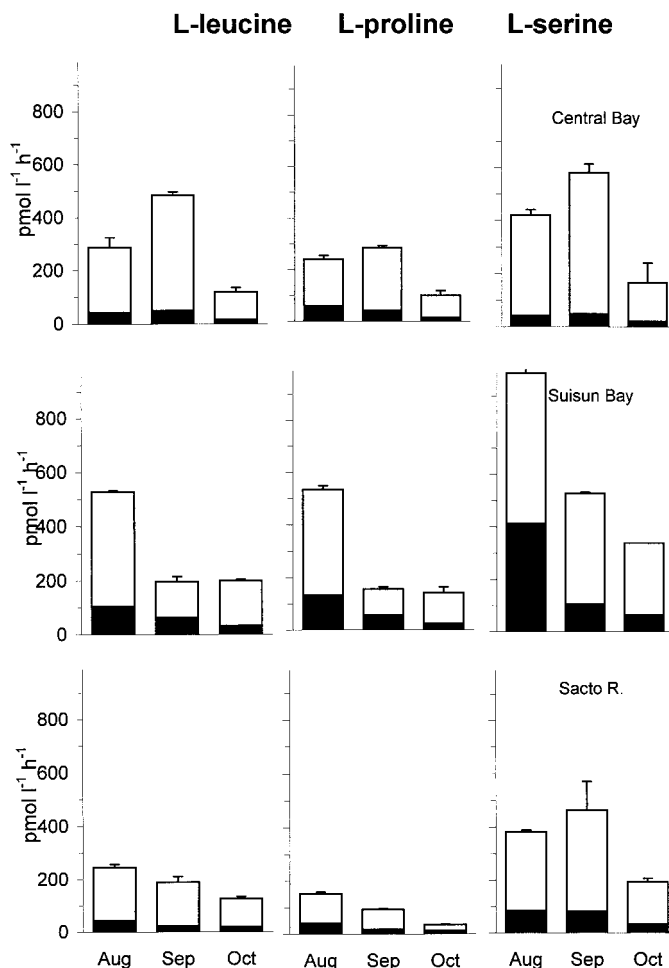


Fig. 5. L-Leucine, L-proline, and L-serine incorporation. Legend as in Figure 3.

iable ($6\text{--}206 \text{ nmol liter}^{-1} \text{ h}^{-1}$) than activity at the other two sites. The Suisun Bay and Sacramento River stations had similar amino peptidase activity, averaging 50 and 52 $\text{nmol liter}^{-1} \text{ h}^{-1}$, respectively. There was also evidence of a seasonal decline in amino peptidase activity from April to October; the largest shift occurred between June and July.

Particle-associated amino peptidase activity was generally higher than comparable abundance and amino acid incorporation measurements, averaging 65% of total for all stations and dates. Of the three sites, Central Bay had the highest particle-associated amino peptidase activity, averaging

Table 2. Pearson product-moment correlations among log-transformed metabolic measures.

	L-proline	L-serine	Amino pepti- dase	β -D-glucosi- dase
L-leucine	0.90**	0.88**	0.59**	0.21
L-proline		0.82**	0.22	-0.11
L-serine			0.44	0.30
Amino peptidase				0.69**

** $P < 0.01$.

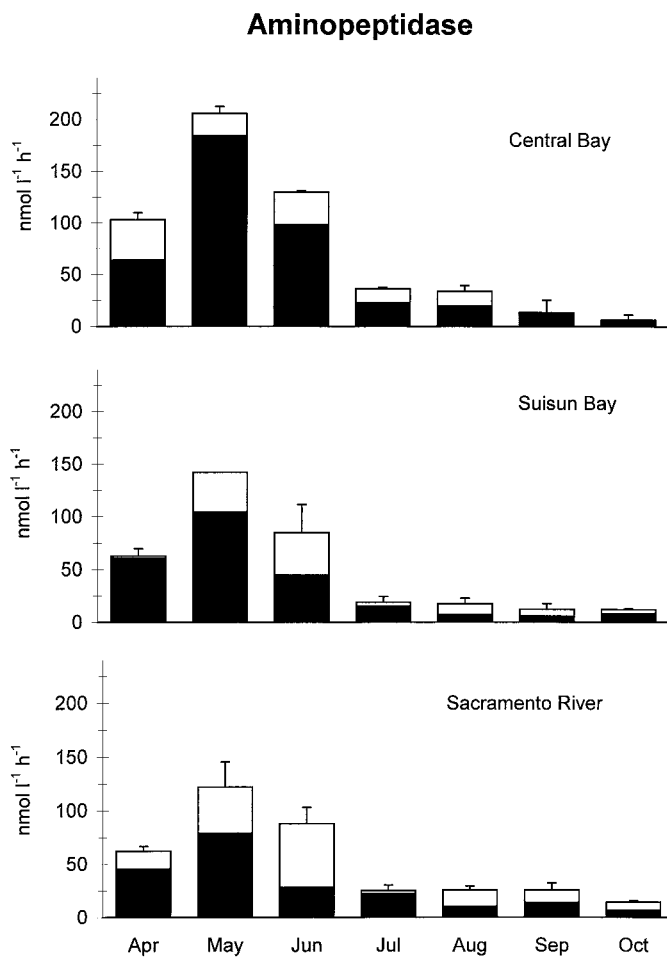


Fig. 6. Aminopeptidase activity. Legend as in Figure 3.

75% of total, despite having the lowest particle-associated bacterial abundance. The Suisun Bay and Sacramento River stations also had particle-associated aminopeptidase activities (65% and 57%, respectively) that were generally higher than comparable abundance and amino acid incorporation. There was a subtle shift in particle-associated aminopeptidase activity between July and August, as with bacterial abundance and L-leucine incorporation measures. Averaging data before and after the shift, particle-associated L-leucine incorporation dropped from 76% to 50% in Suisun Bay and from 64% to 47% in the Sacramento River.

β-D-glucosidase activity—*β-D-glucosidase* activity, measured from June to October (Fig. 7), was much lower than aminopeptidase activity, averaging 2.9 nmol liter⁻¹ h⁻¹ (range, 0.3–6.8 nmol liter⁻¹ h⁻¹), but overall the two measures were significantly correlated (Table 2). This pattern of low *β-D-glucosidase* activity relative to aminopeptidase activity is consistent with results found in studies in which both were measured simultaneously (Karner and Herndl 1992; Smith et al. 1992; Karner and Rassoulzadegan 1995; Amy et al. 1997). Consistent with aminopeptidase activity and amino acid incorporation, *β-D-glucosidase* activity underwent a clear seasonal decline at all stations; the sharpest

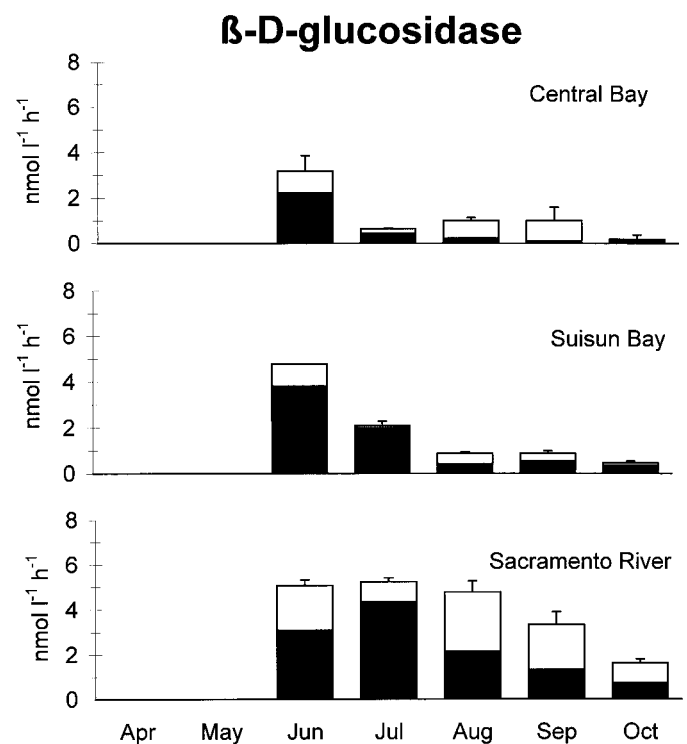


Fig. 7. *β-D-Glucosidase* activity. Legend as in Figure 3.

declines occurred between June and July at the Central Bay and Suisun Bay stations and after August in the Sacramento River. In contrast to L-leucine incorporation and aminopeptidase activity, average *β-D-glucosidase* activity was highest in the Sacramento River (5.1 nmol liter⁻¹ h⁻¹), followed by Suisun Bay (2.1 nmol liter⁻¹ h⁻¹) and Central Bay (1.4 nmol liter⁻¹ h⁻¹).

Particle-associated *β-D-glucosidase* activity averaged 56% of the total for all stations and dates, which was higher than comparable measures of bacterial abundance and L-leucine incorporation but similar to fractionation of aminopeptidase activity. Of the three sites, Suisun Bay had the highest particle-associated *β-D-glucosidase* activity, averaging 72% of total. The Central Bay and Sacramento River stations had particle-associated aminopeptidase activity of 42% and 54%, respectively. As with the other measures, a consistent seasonal decline in the particle-associated fraction between July and August was evident at all stations. Averaging data before and after this shift, the particle-associated *β-D-glucosidase* activity dropped from 69% to 15% in Central Bay, from 87% to 62% in Suisun Bay, and from 72% to 43% in the Sacramento River.

Abundance and activity fractionation—A fractionation step was required to estimate activity and abundance of the free-living and particle-associated bacterial communities. For the radiolabel incorporation methods, samples could be fractionated either pre- or postincubation, whereas with ectoenzyme assays, only preincubation fractionation was possible. To explore whether prefractionation disrupted the natural bacterial associations and consequently disrupted the metabolic processes, an additional <1- μ m prefractionated

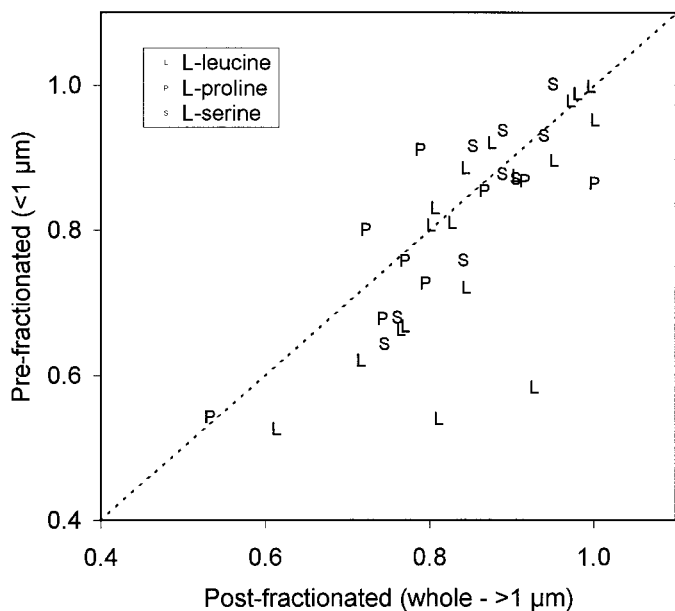


Fig. 8. Comparison free-living bacterial activity using two fractionation methods. Data were log-transformed and normalized by substrate. L = L-leucine; P = L-proline; S = L-serine.

treatment was included in all radiolabel incorporation experiments. Incorporation patterns of this $<1\text{-}\mu\text{m}$ treatment were compared with the difference in values between the two postfractionated treatments (whole water filtered onto $0.45\text{-}\mu\text{m}$ or $1\text{-}\mu\text{m}$ filters) by testing for a linear relationship with a slope of 1. Although there was considerable scatter about the regression line, the agreement between the two methods was quite good (Fig. 8). The regression was significant ($R^2 = 0.59$), with a slope of 1.00 ± 0.14 and an intercept of -0.04 ± 0.12 (mean \pm SE). These values were not significantly different from 1 and zero, respectively, suggesting that the two methods were equally valid in this study.

The ability to prefractionate samples without bias is important for subsequent analysis for two reasons. First, for analysis of per-cell metabolism by analysis of covariance (ANCOVA), differences between the prefractionated $<1\mu\text{m}$ treatment (i.e., free-living) and the whole water treatment were evaluated, and any fraction effect was attributed to particle-associated bacteria. This approach was preferred because direct estimates of bacterial abundance were available for these fractions. Use of the $>1\text{-}\mu\text{m}$ postfractionated activities would have necessitated use of the indirect cell abundance estimates (the difference between whole water and $<1\text{-}\mu\text{m}$ fractions) to normalize the metabolic rates, and there would have been no way to control for the resulting compounded error. Second, using the prefractionated $<1\text{-}\mu\text{m}$ treatments makes the amino acid incorporation and ectoenzyme assays more directly comparable to each other because the samples were subjected to the same exact handling protocol. Comparison of the postfractionated treatments (whole water vs. $>1\text{ }\mu\text{m}$) was preferred for bulk characterizations because these samples required less manipulation and were handled identically until the end of the incubation period. Therefore, the convention of using the prefractionated treat-

ment was adopted for cell-specific calculations (ANCOVA), using the postfractionated treatments for displaying the bulk rates (Figs. 4, 5) or using both for correlations with environmental variables (Table 3).

Bacteria versus environmental variables—In general, salinity and temperature correlated poorly with the microbial metabolic measures except for the negative correlation between salinity and β -D-glucosidase activity ($>1\text{ }\mu\text{m}$, whole water), a result that reflects the dominance of β -D-glucosidase activity in freshwater of the Sacramento River (Table 3). Chlorophyll correlated positively with whole water L-leucine and aminopeptidase activity, not with β -D-glucosidase, and negatively with particle-associated L-serine incorporation. There were few correlations between microbial variables and DOC or POC. Free-living ($<1\text{ }\mu\text{m}$) bacterioplankton abundance correlated negatively with SPM, DOC, and freshwater flow. Of the environmental variables, freshwater flow appears to be most strongly linked to bacterioplankton metabolism.

Cell-specific activities—Cell-specific amino acid and ectoenzyme activities are shown in Table 4 for the $<1\text{-}\mu\text{m}$ and whole water (total) fractions corresponding to the fractions for which there are direct estimates of activity and abundance. The whole water fraction consistently had higher per cell rates than did the $<1\text{-}\mu\text{m}$ fraction, indirectly suggesting that particle-associated bacteria were more metabolically active than are free-living bacteria. This trend is much more obvious with ectoenzyme activities than with amino acid incorporation measures. The significance of these differences was tested with an ANCOVA.

To compare the activities of free-living and particle-associated bacteria, an ANCOVA was performed with log-transformed cell-specific activities as dependent variables and freshwater flow index as the covariate. The freshwater flow index (I) was calculated as

$$I = F*(35 - S)/35$$

where F is delta outflow, S is salinity, and 35 is the approximate salinity of full-strength seawater (i.e., zero freshwater content).

The results are presented in Table 5 as an ANCOVA for each of the five metabolic measures that were tested for effects of fractionation (free living vs. total) and station location on the cell-specific metabolic rates. The interaction between the main effects was nonsignificant for all metabolic measures, so this term was removed from the model. The freshwater flux covariable was highly significant ($P < 0.02$) for all but the L-leucine analysis, for which it was marginally significant ($P < 0.09$). In general, the amino acid incorporation rates did not show significant differences between fractions or stations. This finding suggests either that there was no difference in the ability of free-living and particle-associated bacteria to incorporate amino acids or that the differences were not resolvable with the small sample size for L-proline and L-serine. In contrast, the ANCOVA of aminopeptidase and β -D-glucosidase showed highly significant fractionation effects, and whole water samples had significantly higher metabolic rates.

Table 3. Pearson product-moment correlation coefficients between bacterial metabolic (log-transformed) and abundance measures versus environmental variables.

Measure	<i>n</i>	Salinity	Temp	SPM	Chl	POC	DOC	Flow index
L-leucine								
<1 μm	18	0.36	0.01	-0.56*	0.41	-0.41	-0.55*	-0.47
>1 μm	18	0.11	-0.23	0.31	0.45	0.32	0.08	0.30
Whole water	18	0.33	-0.39	-0.30	0.60**	-0.28	0.01	0.14
L-proline								
<1 μm	9	0.16	0.25	0.11	0.00	-0.02	0.18	0.29
>1 μm	9	0.07	0.29	0.52	-0.20	0.35	0.37	0.23
Whole water	9	0.30	0.10	0.30	0.01	0.05	0.15	0.05
L-serine								
<1 μm	9	-0.15	0.42	0.43	-0.23	0.37	0.50	0.55*
>1 μm	9	-0.52	-0.74*	0.76*	-0.69*	-0.62	0.75*	0.72*
Whole water	9	-0.13	0.37	0.59	-0.28	0.48	0.54	0.45
Aminopeptidase								
<1 μm	21	-0.15	-0.02	-0.14	0.37	-0.15	-0.04	0.34
>1 μm†	21	0.15	-0.54*	-0.03	0.54*	-0.09	0.17	0.49*
Whole water	21	0.03	-0.40	-0.06	0.52*	-0.12	0.13	0.51*
β-D-glucosidase								
<1 μm	15	-0.15	0.11	-0.48	0.33	-0.46	-0.18	0.40
>1 μm†	15	-0.63*	0.48	0.40	-0.28	0.46	0.50	0.72**
Whole water	15	-0.54*	0.40	0.22	-0.11	0.31	0.46	0.69**
Bacterial abundance								
<1 μm	21	0.40	0.05	-0.46*	0.08	-0.40	-0.55**	-0.61**
>1 μm†	21	-0.49*	0.20	0.52*	-0.17	0.40	0.46*	0.66**
Whole water	21	-0.17	0.38	0.13	-0.14	0.03	-0.09	0.13

* $P < 0.05$; ** $P < 0.01$.

† Microbial measures used for correlation calculated by difference between whole water and <1-μm fractions.

Discussion

Temporal trends—The most consistent and somewhat surprising trend in this data set was the temporal decline in bacterial amino acid incorporation and ectoenzyme activity (Figs. 4–7). One might expect that this trend was simply a metabolic response to seasonal changes in temperature; however, the lack of strong positive correlations (except for L-serine >1 μm) and the sometimes negative correlations with temperature suggest otherwise (Table 3). Next, one might expect that organic carbon concentration would be strongly correlated with bacterial metabolism, but the lack of such correlations (Table 3) suggests that standing crop measures of carbon resources are poor predictors of bacterioplankton dynamics in this system. Because the temporal decline was evident at all sites and with all metabolic measures, a system-wide variable may be dominating the observed patterns. One such variable may be freshwater flow, which also declined over the study period and was strongly correlated with bulk metabolic measures (Table 3). Freshwater flow has been considered a reasonable proxy for organic carbon flux into this system, and this flux was considered a limiting resource for the bacterioplankton (Jassby et al. 1993), which was the justification for using freshwater flux as a covariate in the ANCOVA (Table 5). However, bacterioplankton metabolism may not be responding to freshwater flow per se but to variables that covary with freshwater flow. One possibility is

that particulate organic matter delivered during high flow periods (i.e., during the winter and spring immediately prior to this study) may become progressively degraded over the summer and thus be a diminishing resource for the bacterioplankton. These temporal changes in the quality of the organic matter would not be reflected in bulk DOC measurements. Also, the average annual wind velocity in this region typically reaches a maximum in the midsummer months and begins to decline by August (Conomos et al. 1985). This decline in average wind velocity later in the season may reduce the supply of resuspended POC to the water column and the amount of exchange with adjacent organic-matter-rich marshes.

Another striking and consistent temporal trend was the decline in particle-associated bacterial abundance in Suisun Bay and the Sacramento River between July and August. Such seasonal changes have been observed in a variety of other systems (Bent and Goulder 1981; Cammen and Walker 1982; Pedrós-Alió and Brock 1983; Laanbroek and Verplanke 1986; Iriberry et al. 1987; Unanue et al. 1992), although the timing, magnitude, and likely cause of the shifts differ. In San Francisco Bay, this shift may be linked to the timing of sediment delivery. About 80% of the annual sediment load is delivered to San Francisco Bay during the winter months, but relatively little sediment is delivered to or exported from the system during summer (Conomos and Peterson 1977). Therefore, these particles may become colo-

Table 4. Cell-specific bacterial metabolic measures. Values are $\times 10^{-20}$ mol cell $^{-1}$ h $^{-1}$, except for aminopeptidase (Ampep), which is $\times 10^{-18}$ mol cell $^{-1}$ h $^{-1}$.

Location	Date	L-leucine		L-proline		L-serine		Ampep		β-D-glucosidase	
		<1 μm	Total	<1 μm	Total	<1 μm	Total	<1 μm	Total	<1 μm	Total
Central Bay	3 Apr	14	24					15	40		
	1 May							9.6	87		
	12 Jun	20	20					16	51	48	140
	17 Jul	5.2	8.3					5.3	14	7.7	25
	13 Aug	6.2	8.6	5.2	7.2	11	13	4.4	14	6.1	36
	11 Sep	7.4	10	4.2	6.0	12	12	0.23	6.5	1.0	22
	16 Oct	2.4	3.9	2.5	3.2	3.1	5.3	0.51	3.7	21	11
	Average	9.2	12	3.9	5.5	8.5	10	7.2	31	17	47
Suisun Bay	3 Apr	5.9	8.9					4.8	23		
	1 May							26	44		
	12 Jun	1.8	4.4					17	22	15	110
	17 Jul	2.9	4.4					4.3	7.7	49	76
	13 Aug	14	14	5.8	15	27	27	3.5	5.3	5.9	29
	11 Sep	4.5	5.5	2.8	4.4	9.9	15	2.0	4.7	4.1	28
	16 Oct	3.0	5.8	2.9	4.1	6.0	9.8	1.6	4.3	6.5	18
	Average	5.4	7.2	3.8	7.7	14	17	8.5	16	16	53
Sacramento River	3 Apr	2.7	12					13	25		
	1 May							20	40		
	12 Jun	14	9.5					36	25	98	150
	17 Jul	8.5	6.9					2.0	14	97	220
	13 Aug	9.3	8.3	8.2	5.2	16	13	5.4	9.4	34	190
	11 Sep	8.1	6.8	4.5	3.3	20	17	4.5	11	23	140
	16 Oct	2.2	4.0	1.0	1.1	3.6	6.1	2.8	6.0	35	80
	Average	7.4	7.9	4.6	3.2	13	12	12	19	57	160

nized by bacteria early in the season and may go through a successional sequence similar to that observed in phytoplankton detritus (Biddanda and Pomeroy 1988), marine snow (Muller-Niklas et al. 1994), and fecal pellets (Pomeroy and Diebel 1980; Pomeroy et al. 1984). As the season progresses, particles may become depleted of labile organic material and hence they may become a less desirable place for bacteria to reside.

A shift in mode from particle association to free living may be an active decolonization process or a more passive process whereby differential mortality favors free-living over particle-associated bacteria. An active mechanism has been proposed for oceanic systems based on the observation that the abundance of marine-snow-associated bacteria decreased with increasing depth (Azam and Smith 1991; Turley and Mackie 1994). In this case, decolonization provides a means for bacteria to avoid sinking to the deep sea. In contrast, the particle trapping properties of estuaries provide a passive mechanism for the net accumulation of particle-associated bacteria and the net dispersion of free-living bacteria (Painchaud and Therriault 1989). This net advantage conferred to particle-associated bacteria may be especially important during high flow conditions when dispersion of free-living bacteria is expected to be high. However, this advantage may be negligible during low flow conditions. For example, water residence times in Suisun Bay can exceed 30 d during summer (Walters et al. 1985) and therefore far exceed typical bacterial generation times of 1–2 d (Hollibaugh and Wong 1996). Therefore, the seasonal reduction in freshwater flow

may cause a net increase in free-living bacterial abundance consistent with our observations in Suisun Bay and the Sacramento River but cannot explain the concomitant decrease in particle-associated bacterial abundance.

In addition to the aging of particulate organic matter, benthic grazing may contribute to the observed temporal decrease in particle-associated bacteria. Over the summer, benthic grazers may have a cumulative grazing impact on particle-associated bacteria. In San Francisco Bay, the Asian clam, *Potamocorbula amurensis*, has reached extraordinarily high densities since its introduction in 1986. *P. amurensis* has been linked to fundamental changes in phytoplankton (Alpine and Cloern 1992) and zooplankton (Kimmerer et al. 1994) communities and may affect bacterioplankton. For example, Werner and Hollibaugh (1993) showed that *P. amurensis* can ingest and assimilate free-living bacteria, although with relatively low efficiency. Although ingestion of particle-associated bacteria has not been measured in *P. amurensis*, this process is that probably more efficient than ingestion of free-living bacteria.

Spatial trends—Superimposed on the above temporal trends are spatial differences among the stations, which vary depending on the metabolic measure. To the extent that bacterial metabolism reflects the ambient organic substrates available, these results imply that the quality of the organic material may differ among the stations. On any given date, L-leucine incorporation and aminopeptidase activity were greater in Central Bay than in Suisun Bay and the Sacra-

Table 5. ANCOVA results of log-transformed cell-specific metabolic measures testing effects of fractionation, station location, and the flow index covariable. Columns in the table include the degrees of freedom (df), *F*-statistic (*F*) and probability (*P*) of a Type I error.

Variable	df	<i>F</i>	<i>P</i>
L-leucine			
Fraction	1	2.583	0.118
Station	2	2.281	0.119
Flow index	1	3.095	0.088
Error	31		
L-serine			
Fraction	1	0.853	0.372
Station	2	2.763	0.100
Flow index	1	24.561	<0.001*
Error	13		
L-proline			
Fraction	1	0.888	0.363
Station	2	1.128	0.353
Flow index	1	7.38	0.018*
Error	13		
β -D-glucosidase			
Fraction	1	27.672	<0.001*
Station	2	18.952	<0.001*
Flow index	1	18.521	<0.001*
Error	25		
Aminopeptidase			
Fraction	1	14.329	0.001*
Station	2	3.938	0.028*
Flow index	1	22.47	<0.001*
Error	37		

* $P < 0.05$.

mento River (Figs. 4, 6). Therefore, bacteria were likely exposed to organic substrates with higher labile content in Central Bay than at the other two sites. Such a result is consistent with the generally higher chlorophyll (Table 1) and lower carbon:nitrogen ratios (Murrell et al. in prep.) in Central Bay than at the other two sites. β -D-Glucosidase activity showed the opposite spatial trend; it was high in the Sacramento River, intermediate in Suisun Bay, and low in the Central Bay, hence the negative correlation with salinity (Table 3). This β -D-glucosidase pattern suggests that bacteria further upstream were better able than those downstream to utilize cellulose, a result consistent with the observed gradient in carbohydrate concentration in this system (Murrell et al. in prep.).

Substrate incorporation versus ectoenzyme activity—Amino acid incorporation and ectoenzyme activity measure different processes; however, they are both metabolic indices. Amino acid incorporation integrates bacterial activities, including membrane transport and translational assembly of proteins. Therefore, this measure is often considered a proxy for intrinsic growth, assuming a constancy of protein content and amino acid composition in the bacterial community. Ectoenzyme activity, however, measures cell-surface expression of ectoenzymes and, as such, measures the potential for the bacterioplankton community to hydrolyze polymeric

substrates. This microbial process is crucial because it breaks down polymers into smaller units that only then can be transported across the cell membrane. If bacterioplankton rapidly adapt to the surrounding environment, then ectoenzyme activity may reflect the recent substrate environment to which the bacteria have been exposed. Because both approaches measure aspects of bacterial metabolism, it is not surprising that, when measured simultaneously, the results are correlated (Somville and Billen 1983; Somville 1984; Vives Rego et al. 1985; Rosso and Azam 1987; Karner et al. 1992; Muller-Niklas et al. 1994) as they do in this study (L-leucine and aminopeptidase, Table 2). This finding implies a certain degree of coupling between enzymatic hydrolysis of polymers and uptake of hydrolysis products (Hoppe et al. 1988), although this coupling may not always be tight (Smith et al. 1992).

We have assumed that ectoenzyme activity in northern San Francisco Bay is primarily of bacterial origin. This assumption is supported by the similar trends in ectoenzyme (Figs. 6, 7) and L-leucine incorporation rates (Fig. 4) and the positive correlations between the measures seen in Table 2. However, nonbacterial sources from fungi (Unanue et al. 1993), eukaryotic plankton (Chrost 1990), or dissolved enzymes adsorbed onto clay particles (Wetzel 1991) may be important. This adsorption may, in part, explain the high fractionation of ectoenzymes on particles (Table 5) and may result in overestimation of the per-cell ectoenzyme rates of the particle-associated bacteria (Table 4). However, bacteria associated with enzymatically active particles, regardless of the origin of the enzyme, may be able to exploit this micro-environment enriched with hydrolysis products and consequently grow faster than free-living bacteria.

Role of bacterial attachment—The importance of bacterial attachment to surfaces has been addressed in a variety of studies originating from batch culture studies that demonstrated that bacterial growth rates were positively related to the available surface area for attachment. These experiments led to the conclusion that most bacterial activity in the sea must occur on particles (Zobell 1943). This view has been refuted by those who have shown that virtually all oceanic bacteria are free living (Wiebe and Pomeroy 1972; Azam and Hodson 1977). The dominance of free-living bacteria in the ocean is further supported by studies of bacteria associated with large, rare marine detrital aggregates (marine snow), which contribute at most only a few percent of the bacterial activity as compared with the ubiquitous free-living bacteria (Alldredge and Youngbluth 1985; Alldredge et al. 1986). In contrast, estuaries are rich in small particles (mostly $< 20 \mu\text{m}$, as in the present study, Murrell unpubl.), and the contribution of particle-associated bacteria is often very significant in terms of both abundance and activity (e.g., Goulder 1976; Bell and Albright 1981; Bent and Goulder 1981; Cammen and Walker 1982; Plummer et al. 1987; Crump and Baross 1996; this study).

Because the term *particle* encompasses such a variety of possible materials and sizes, it is important to be clear about the quality and abundance of particles being sampled. In San Francisco Bay, for example, particles are largely composed of mineral grains bound together with organic matter. In Sui-

sun Bay, the abundance of particles in the size range of 2–25 μm is ca. $5 \times 10^9 \text{ liter}^{-1}$, with the vast majority at the smaller end of the size range (Murrell unpubl.). In contrast, Riley and colleagues (for review, see Riley 1970) described comparably sized detrital particles in ocean waters. These particles were composed of carbohydrate-rich aggregates and protein-rich flakes and were several orders of magnitude scarcer ($2.5\text{--}29 \times 10^4 \text{ liter}^{-1}$) than the San Francisco Bay particles. For example, in a 5-ml sample from each environment (a typical volume used for bacterial metabolic measures) there would be 25 million relatively organic-matter-poor San Francisco Bay particles or 125–1,450 relatively organic-matter-rich “Riley” particles. If San Francisco Bay particles had only 1% of the organic content of the Riley particles, they would still represent a carbon resource at least two orders of magnitude greater than the Riley particles.

A central question remains: are particle-associated bacterial communities distinct from free-living bacterial communities? This question has been addressed in a wide range of aquatic habitats, but there appears to be no clear consensus. For example, marine-snow-associated bacteria are generally larger than free-living bacteria, suggesting more rapid growth. Per-cell metabolic measures often suggest more active bacteria on marine snow; however, this difference often is not statistically significant (Alldredge et al. 1986; Smith et al. 1992). Results from estuarine, coastal, and freshwater environments are mixed, with several studies showing that particle-associated bacteria are larger and more metabolically active than free-living bacteria (Kirchman and Mitchell 1982; Pedrós-Alió and Brock 1983; Iriberry et al. 1987; Unanue et al. 1992) and other studies in which few or no consistent differences were found (Goulder 1976; Cammen and Walker 1982; Ducklow and Kirchman 1983). The most conclusive evidence to date is the unique sequence profiles found in cloned ribosomal RNA genes extracted from marine snow communities when compared with free-living bacteria (Delong et al. 1993). This phylogenetic distinction between bacterioplankton assemblages may also be evident in estuarine environments, at least during some times of the year (Bidle and Fletcher 1995; Noble et al. 1997).

However, in San Francisco Bay, a similar molecular approach showed little or no differences between free-living and particle-associated bacteria (Hollibaugh et al. in prep.). This finding is consistent with the results of the present study in that free-living and particle-associated bacteria were not significantly different in growth rate, based on per-cell amino acid incorporation rates. It is also consistent with our (unquantified) observation that particle-associated bacteria and free-living bacteria appear very similar in size and general morphology. San Francisco Bay particles are so small that each individual particle may not be a significant resource to a bacterium. Instead, bacteria may gain an advantage by rapidly shifting from free-living to particle-associated modes of existence.

Conclusions—All bacterial activity indicators declined during the study period, and most covaried significantly with freshwater flux. A sharp temporal decline in particle-associated bacteria was observed in Suisun Bay and the Sacramento River. This decline may be due to combined effects

of loss of nutritive value of particles, a decrease in advection of free-living bacteria, and cumulative benthic grazing pressure. Aminopeptidase activity was positively correlated with increasing salinity, and β -D-glucosidase was negatively correlated with increasing salinity, a pattern that suggests a gradient in the quality of available organic matter from more carbohydrate rich in the river to more protein rich in the ocean. Although particle-associated bacteria were on average more active than free-living bacteria, there were no significant differences in per-cell amino acid incorporation rates, suggesting no measurable differences in intrinsic growth in these two microenvironments. Ectoenzyme activity rates suggest that particle-associated bacteria were much more active than free-living bacteria. However, the contribution from particle-bound nonbacterial enzymes may cause some of this difference. In San Francisco Bay, particles are clearly important sites for attachment and growth of bacteria but do not necessarily harbor bacteria that grow more quickly than do free-living bacteria.

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