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CROBIAL UTILIZATION OF PARTICULATE ORGANIC CARBON IN NORTHERN SAN FRANCSICO BAY AND LINKS TO HIGHER TROPHIC LEVELS

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ABSTRACT

We examined bacterioplankton abundance and metabolic characteristics 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 ß-D-glucosidase), and bacterial abundance using 1μm filters to separate free from particle-associated bacteria. We observed a seasonal decline in all bacterial metabolic measures 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. We also observed a sharp decline in particle-associated bacteria in Suisun Bay and the Sacramento River between July and August. This may be 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 to increasing salinity and ß-D-glucosidase was negatively related to 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 proportion of particle-associated bacteria (avg. 49%), followed by Sacramento River (avg. 36%) and Central Bay (avg. 11%). Particles were the sites of enhanced ectoenzyme activity, but not amino acid incorporation. Therefore bacteria may be actively dissolving the particulate organic matter but their growth rates are not significantly enhanced on particles.

Key Words: Ecosystems and Ecology (0660); Bacteria (0165); Bioregion, Bay Area Delta (0235); Water Properties (2610); Environment-organism Interactions (0720)

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INTRODUCTION

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 including respiratory loss, incorporation into biomass, advection out of the system, and burial. A better understanding of the flow of carbon in estuaries 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 bacterial-particle associations has been the topic of sustained interest for many years (Marshall 1976, Bitton and Marshall 1980, Marshall 1984, 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 which may be toxic to bacteria.

One common feature of estuaries, especially when compared to oceanic environments, is a high abundance of both particles and particle-associated bacteria (Bell and Albright 1981, Bent and Goulder 1981, Cammen and Walker 1982, Iriberri et al. 1987, etc.). 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-resuspended clays bound with organic matter; while in oceans, particles are largely composed of detrital

aggregates. Given their significant mineral content, estuarine particles tend to be less organic-rich than oceanic particles. The fate of particles is also quite different in estuarine and ocean environments. Particles tend to be retained in estuaries due to 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); while in oceans, free-living bacteria stay in the surface waters indefinitely. Because of the above considerations, the ecological consequences of whether a bacterium 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.

MATERIALS AND METHODS

Study Site

Northern San Francisco Bay (Fig. 1) receives drainage from about 40% of California's land area via the Sacramento and San Joaquin River system. 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 and thereafter decreasing through the summer and fall. A typical annual range in freshwater outflow is 100-200

m^3s^{-1} in summer to 1000-1500 m^3s^{-1} in winter, with episodic peaks in flow as high as 10,000 m^3s^{-1} during winter storms. About 80% of the annual supply of freshwater and sediment occurs during winter (Conomos and Peterson 1977, Conomos et al. 1979). The mean semi-diurnal tidal range is about 2 meters and seawater intrudes up the bay on the order of 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 gC m^{-2} yr^{-1}) due to 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 sources to this system appear to be of terrestrial and riverine origin (Jassby et al. 1993).

Water Sampling and Processing

Samples were collected during monthly cruises aboard the USGS vessel R/V Polaris from April through 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 three sites were sampled: Central Bay (station 18), the Suisun Bay (station 8, 6, or 5), and the Sacramento River (station 649 or 657) (Fig. 1). Station numbers correspond to historical USGS sampling stations. The sampling location of the Suisun Bay station was dictated more by salinity than by geographic location as we were targeting our sampling to the estuarine turbidity maximum zone, which typically occurs in the 2-10 PSU salinity range. Basic hydrographic data including temperature, salinity, 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 μm 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 cm Hg) through a glass fiber filter with a nominal pore size of 1 μm (GF/C or equivalent) to remove particles and particle-associated bacteria. Aliquots of whole water and 1 μm fractionated water were used to determine bacterial abundance, amino acid incorporation, and ectoenzyme activity. An additional <0.2 μm fraction (for ectoenzyme activity only) was made by further filtering a portion of the 1 μm filtered water through a 0.2 μm cellulose acetate syringe filter. Samples for POC and 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).

Bacterial Cell Counts

Accurate counting of bacteria in these samples was made difficult because of the high abundance of particles, so a normally simple procedure required more effort and attention to detail. Samples were fixed with 2% final concentration borate buffered formaldehyde. Within one or two days 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 1250X (Hobbie et al. 1977). In order to minimize interference from suspended sediments, small volumes (1 or 2 ml) were filtered onto relatively large, 23 mm diameter filter area (Hoeffer filtration unit) and ca. 5 ml of the AO solution (50 μg ml^{-1} in 0.02 M NaP_2O_7, pH 9) were added to disperse bacteria evenly. A MSI backing filter (10 or 20 μm pore size) was used to further promote even dispersion. A minimum of 300 cells was counted per filter and at least 10 microscope fields (typically 20 to 40) were viewed. If the coefficient of variation between the duplicate counts was greater than 10%, then one or two additional samples were prepared and counted. We found that focusing on a small section of the microscope field improved our visual acuity in distinguishing bacteria from the

particles, so we typically scanned one-tenth or two-tenths of the reticule. Counting these small sections of the reticule took only a few seconds, which helped minimize cell fading caused by exposure to the intense illumination; it also forced us to scan more total fields of the slide and thus better account for 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.

Ectoenzyme Activity

Ectoenzyme activity was measured using fluorogenic substrate analogues that mimic naturally occurring substrates (Hoppe 1983). We used methyl coumarin amido leucine (MCA-Leucine) and methylumbelliferyl ß-D-glucoside (MUF-ß-D-glucoside). The fluorescent moieties, MCA and MUF, are similar (biphenyl) compounds which 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 ß-D-glucoside is similar to ß-D-glycosidic linkages of cellobiose and cellulose. Therefore, these substrates will be hydrolyzed by aminopeptidase and ß-D-glucosidase enzymes respectively.

For each assay, 5 ml of sample water were placed into a 15 ml polyethylene tube together with a saturating quantity of the fluorogenic substrate. We determined from preliminary experiments that 50 μM final concentration was sufficient for saturation. Stock solutions of the fluorogenic substrates were prepared in methyl cellosolve at 100X or 200X final concentration. Assays were conducted in duplicate (April through June) or triplicate (July to October) on whole water, <1 μm, and <0.2 μm fractions. Activity attributed to particle-associated bacteria was calculated as the difference between whole water and <1 μm fractions, while activity attributed to

free-living bacteria was calculated as the difference between <1 μm and the <0.2 μm fractions. The dissolved enzyme activity (<0.2 μm fraction) was considered a blank and was subtracted from the other treatments. Possible sources of dissolved enzymes include extracellular enzymes or membrane fragments that pass through a 0.2 μm filter yet still contain functioning enzymes. All incubations were conducted in the dark at *in situ* temperature for 1 to 3 hours (MCA-L-leucine) or ca 6 to 12 hours (MUF-ß-D-glucoside).

Time point subsamples (typically 1 ml) were removed from the incubation tubes and placed into screw-capped 13 X 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 we sampled multiple time points (up to 5) and determined that fluorescence increased linearly during the first several hours. Consequently, in later experiments we took only initial and final subsamples. By measuring the change in fluorophore concentration over time in each treatment, we avoided variability due to treatment-specific differences in fluorescence quench. Samples were kept in the dark at room temperature until fluorescence was read within one or two days. 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 UV lamp, a 300-400nm excitation filter and a 410-600nm 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), which are briefly outlined here. We routinely used 4,5-[^3H]-L-leucine (L-leucine), but for the last three months of the study, we did additional assays for

incorporation of 5-[^{3}H]-L-proline (L-proline) and 3-[^{3}H]-L-serine (L-serine). For each amino acid, two sets of duplicate whole water samples and one set of duplicate 1 μm fractionated water samples were placed in 15 ml polyethylene tubes and spiked with the appropriate radio-labeled amino acid (5 Ci mmol^{-1}, 100nM final concentration). After a 1-2 hour incubation period at *in situ* temperature, one set of whole water samples was filtered onto 0.45 μm pore size Millepore HA filters and the other set onto 1 μm polycarbonate filters to capture activity attributed to total bacteria or only particle-associated bacteria, respectively. The third treatment (water samples which were 1 μm filtered prior to incubation) were filtered onto 0.45 μm pore size Millepore HA filters. This treatment provided another measure of free-living bacterial incorporation that we used for comparison to rates calculated by difference. 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, unlabelled sample water, iced 5% TCA and iced 80% ethanol to precipitate proteins and rinse away unincorporated label. Filters were placed in scintillation vials and allowed to air dry for one 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, the vials were vortexed and assayed for radioactivity with a Beckman liquid scintillation counter equipped with an external standard to determine quench and calculate counting efficiency.

Conversion of L-leucine incorporation into bacterial production used 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 1986).

From August to October, we measured incorporation rates of two additional amino acids: L-proline and L-serine. Our choice of using 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). We incubated samples of whole water and <1 μm fractionated water in BIOLOG plates and after ca. 1 week incubation time, scored the plates by the intensity of the color development of the tetrazolium violet indicator using a Bio Rad microtitre plate reader. We then ranked each substrate by the difference in color development between whole water and <1 μm treatments. L-proline and L-serine were ranked 4th and 10th respectively (2nd and 3rd among amino acids) out of 95 substrates. For comparison, L-leucine ranked 31st (6th among amino acids). We reasoned that 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 $m^3 s^{-1}$ in the beginning of April decreasing to 100 $m^3 s^{-1}$ by October. (Fig. 2). Flow during 1996 was similar to the 40 year average, suggesting that our study period represented a typical seasonal progression in the North Bay. Chlorophyll concentrations were highest in Central Bay, averaging 3.5 $\mu g\ l^{-1}$ over the study period and exceeded 5 $\mu g\ l^{-1}$ only in April (Table 1). The Suisun Bay and Sacramento River stations both had average chlorophyll concentrations of 2.0 $\mu g l^{-1}$, with only slightly elevated levels evident in April and May but never exceeding 5 $\mu g\ l^{-1}$. Suspended

sediment loads were generally highest in the Suisun Bay, followed by the River station and lowest in Central Bay (Table 1).

Bacterial Abundance

During this study, total bacterial abundance averaged 3.4 X 10^6 cells ml^{-1} (range 2.4 to 4.7) with no clear spatial or temporal trends (Fig. 3). Across all stations and dates, particle-associated abundance averaged 31% (range 3 to 86 %) of total abundance. Suisun Bay had the highest percentage of particle-associated bacteria averaging 45% (range 6 to 86%), the Sacramento River site was intermediate averaging 33% (range 3 to 64%), and the Central Bay site had the lowest fraction, averaging 13% of total abundance (range 3 to 25%). At both 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). Averaging data before, after this shift, the particle-associated bacteria fraction dropped from 68% to 16% in the Suisun Bay, and from 52% to 11% in the River.

L-leucine Incorporation and Bacterial Production

L-leucine incorporation (Fig. 4) over all dates and stations averaged 304 pmol $l^{-1}hr^{-1}$. Central Bay had the highest average rates (396 pmol $l^{-1}hr^{-1}$), but also the most variable, ranging from 121 to 677 pmol $l^{-1}hr^{-1}$. In Central Bay, there was an evident seasonal decline in L-leucine incorporation except for a peak in September. L-leucine incorporation averaged 257 pmol $l^{-1}hr^{-1}$ at Suisun Bay (range: 143 to 528) and 260 pmol $l^{-1}hr^{-1}$ at the Sacramento River (range: 127 to 439). While 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 $\mu gC\ l^{-1}d^{-1}$ and averaged 35 $\mu gC\ l^{-1}d^{-1}$ across all stations and dates.

Central Bay typically had the highest bacterial production, averaging 61 μgC $l^{-1}d^{-1}$, while Suisun Bay and Sacramento River sites were similar, each averaging 21 μgC $l^{-1}d^{-1}$. In general, our bacterial production estimates agree with previously published estimates from this (Hollibaugh and Wong 1996) and other systems (review: Ducklow and Carlson 1992). However due to the uncertainties with the conversion factors used to calculate bacterial secondary production, we will focus our interpretation on the incorporation rate data.

Particle-associated L-leucine incorporation averaged 30% of the total over all stations and dates, which was 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. 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, 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 evidenced by the strong correlations between the different measures (Table 2). L-proline incorporation was similar to that for L-leucine, averaging 193 pmol $l^{-1}hr^{-1}$ (range 36 to 535), while L-serine was consistently higher, averaging 451 pmol $l^{-1}hr^{-1}$ (range 165 to 988). The downward seasonal trend in total incorporation was evident although we only have measurements for the last three 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.

Aminopeptidase Activity

Across all stations and dates, bacterial aminopeptidase activity averaged 59 nmol $l^{-1}hr^{-1}$ (range: 6 to 206 nmol $l^{-1}hr^{-1}$, Fig. 6). Similar to amino acid incorporation, total aminopeptidase activity was higher in Central Bay (average: 75 nmol $l^{-1}hr^{-1}$) and more variable (6 to 206) than activity at the other two sites. The Suisun Bay and River stations had similar aminopeptidase activity, averaging 50 and 52 nmol $l^{-1}hr^{-1}$ respectively. There was also evidence of a seasonal decline in aminopeptidase activity from April to October; the largest shift occurred between June and July.

Particle-associated aminopeptidase 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 aminopeptidase activity, averaging 75% of total, despite having the lowest particle-associated bacterial abundance. The Suisun Bay and River stations also had particle-associated aminopeptidase activities (65% and 57% respectively), which 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.

ß-D-glucosidase Activity

ß-D-glucosidase activity, measured from June to October (Fig. 7), was much lower than aminopeptidase activity, averaging 2.9 nmol $l^{-1}hr^{-1}$ (range: 0.3 to 6.8 nmol $l^{-1}hr^{-1}$), but overall the

two measures were significantly correlated (Table 2). This pattern of low ß-D-glucosidase activity relative to aminopeptidase is consistent with results found in studies that measured both simultaneously (Smith et al. 1992, Karner and Herndl 1992, Karner and Rassoulzadegan 1995, Amy et al. 1996). Consistent with aminopeptidase and amino acid incorporation, ß-D-glucosidase activity underwent a clear seasonal decline at all stations; the sharpest declines occurred between June and July in Central Bay and Suisun Bay stations, and after August in the Sacramento River. In contrast to L-leucine incorporation and aminopeptidase activity, average ß-D-glucosidase activity was highest in the Sacramento River, (5.1 nmol $l^{-1}hr^{-1}$), followed by Suisun Bay (2.1 nmol $l^{-1}hr^{-1}$) and Central Bay (1.4 nmol $l^{-1}hr^{-1}$).

Particle-associated ß-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 ß-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 ß-D-glucosidase activity dropped from 69% to 15% in Central Bay, 87% to 62% in Suisun Bay and from 72% to 43% in the Sacramento River.

Bacteria vs. Environmental Variables

In general, salinity and temperature correlated poorly with the microbial metabolic measures except for the negative correlation between salinity and glucosidase activity (>1 μm, whole water), a result that reflects the dominance of glucosidase activity in freshwater of the Sacramento River (Table 3). Chlorophyll correlated positively with whole water L-leucine and

aminopeptidase activity, not with ß-D-glucosidase, and negatively with particle-associated L-serine incorporation. There were few correlations between microbial variables and DOC or POC. Interestingly, free-living (<1 μm) 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 μm and whole water (total) fractions corresponding to the fractions for which we have direct estimates of activity and abundance. The whole water fraction consistently had higher per cell rates than the <1 μm fraction indirectly suggesting that particle-associated bacteria were more metabolically active than free-living bacteria. This trend is much more obvious with ectoenzymes than with amino acid incorporation measures. We tested the statistical significance of these differences in the analysis of covariance (ANCOVA).

In order to compare the activities of free-living and particle-associated bacteria, we performed ANCOVA on log-transformed cell-specific activities as dependent variables with 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 and hence zero freshwater content.

The results are presented in Table 5 as an ANCOVA for each of the five metabolic measures in which we tested for effects of fractionation (free living versus total) and station location on the cell-specific metabolic rates. The interaction between the main effects was non-significant for all metabolic measures, so we removed this term from the model. The freshwater

flux covariable was highly significant ($P < 0.02$) for all but the L-leucine analysis, which was marginally significant ($P < 0.09$). In general, the amino acid incorporation rates did not show significant differences between fractions or stations. This 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 our sample size, which is quite small for L-proline and L-serine. In contrast, ANCOVA on aminopeptidase and ß-D-glucosidase show highly significant fractionation effects and that whole water samples had statistically higher metabolic rates.

DISCUSSION

Temporal Trends

The most consistent and somewhat surprising trend in this dataset 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 (except for L-serine >1 μm) and sometimes negative correlations with temperature suggest otherwise (Table 3). Next, one might expect that organic carbon concentration would strongly correlate with bacterial metabolism, but the lack of such correlations (Table 3) suggest 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 (to a greater or lesser extent), this implies that 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 strongly correlated with bulk metabolic measures (Table 3). We have assumed that freshwater flow serves as a reasonable proxy for organic carbon flux into this system, and that this flux is a limiting resource to the

bacterioplankton (Jassby et al. 1993). That was our justification for using freshwater flux as a covariable in the analysis of covariance (Table 5). However, it is important to note that bacterioplankton metabolism may not be responding to freshwater flow, per se, but to variables which 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 to the bacterioplankton. These temporal changes in the quality of the organic matter would not be reflected in bulk DOC measurements. Also noteworthy is that the average annual wind velocity in this region typically reaches a maximum in the mid-summer 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 particulate organic carbon to the water column and the amount of exchange with adjacent, organic-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, Pedros-Alio and Brock 1983, Laanbroek and Verplanke 1986, Iriberri 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, while relatively little sediment is delivered to, or exported from, the system during summer (Conomos and Peterson 1977). Therefore, these particles may become colonized by bacteria early in the season and 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 a bacterium to reside.

A shift in mode from particle-associated to free-living may be an active 'de-colonization' process or a more passive process whereby differential mortality favors free-living over particle-associated bacteria. An active mechanism has been theorized in 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, de-colonization 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 may be negligible during low flow conditions. For example, water residence times in Suisun Bay can exceed 30 days during summer (Walters et al. 1985) and thus far exceed typical bacterial generation times of 1 to 2 days (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 1994) communities and may affect bacterioplankton. For example, Werner and Hollibaugh (1993) showed that *P. amurensis* can ingest and assimilate free-living bacteria, though with relatively low efficiency. While it has not been measured, it is likely that *P. amurensis* would be more efficient at ingesting particle-associated bacteria than free-living bacteria.

Spatial Trends

Superimposed on the above temporal trends are spatial differences among the stations, differences that 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 enhanced in Central Bay over the Suisun and Sacramento River stations (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 C/N ratios (Murrell et al. in prep) in Central Bay than at the other two sites. Interestingly, ß-D-glucosidase activity showed the opposite spatial trend, being high in the Sacramento River, intermediate in Suisun Bay, and low in the Central Bay, hence the negative correlation with salinity (Table 3). This ß-D-glucosidase pattern suggests that bacteria further upstream were better able to utilize cellulose than those downstream, a result consistent with the observed gradient in carbohydrate concentration in this system (Murrell et al. in prep).

Substrate Incorporation vs. 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 on the bacterial community. Ectoenzyme activity, on the other hand, measures cell-surface expression of ectoenzymes and, as such, measures the potential of the bacterioplankton community to hydrolyze polymeric substrates. This is a crucial microbial process 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, they tend to correlate (Somville and Billen 1983, Somville 1984, Vives Rego et al. 1985, Rosso and Azam 1987, Karner et al. 1992, Muller-Niklas 1994) as they do in this study (L-leucine and aminopeptidase, Table 2). This 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 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 non-bacterial sources from fungi (Priest 1984, Unanue 1993), eukaryotic plankton (Chrost 1991) or dissolved enzymes adsorbed onto clay particles (Wetzel 1991) may be important. This may, in part, explain the high fractionation of ectoenzymes on particles (Tables 5), and result in overestimating the per-cell ectoenzyme rates of the particle-associated bacteria

(Table 4). However, it is clear that bacteria associated with enzymatically active particles, regardless of the origin of the enzyme, may be able to exploit this microenvironment 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. This 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 compared to the ubiquitous free-living bacteria (Alldredge and Youngbluth 1985, Alldredge et al. 1986). In contrast, estuaries are environments rich in small particles (mostly < 20 μm, as in the present study [Murrell, unpub.]) and the contribution of particle-associated bacteria is often very significant both in terms of abundance and activity (Goulder 1977, Bent and Goulder 1981, Bell and Albright 1981, Cammen and Walker 1982, Plummer et al. 1987, Crump and Baross 1996, this study, and many others).

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 that one is sampling. In San Francisco Bay, for example, particles are largely composed of mineral grains bound together with organic matter. In Suisun Bay, the abundance of particles in the size range of 2 to 25 μm is ca. 5 X 10^9 l^{-1} with the vast majority being at smaller end of the size spectrum (Murrell, unpub.). In

contrast, Riley and colleagues (review: Riley 1970) described comparably-sized detrital particles in ocean waters which were composed of carbohydrate-rich "aggregates" and protein-rich "flakes", and which were several orders of magnitude scarcer (2.5 to 29 X 10^4 l^{-1}) than San Francisco Bay particles. So comparing a 5 ml sample from each environment (a typical volume used for bacterial metabolic measures) one would in effect be sampling ca. 25 million relatively organic-poor San Francisco Bay particles or from 125 to 1450 relatively organic-rich "Riley" particles. If San Francisco Bay particles had only 1% of the organic content of "Riley" particles, they would still represent a carbon resource at least two orders of magnitude higher than "Riley" particles.

A central question remains. Are particle-associated bacterial communities distinct from free-living 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 growth that is more rapid. Per-cell metabolic measures often suggest more active bacteria on marine snow, however this is often 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 are more metabolically active than free-living bacteria (Kirchman and Mitchell 1982, Pedrós-Alió and Brock 1983, Iriberri et al. 1987, Unanue et al. 1992), and other studies which find little or no consistent differences (Goulder 1976, Cammen and Walker 1982, Ducklow and Kirchman 1983). Perhaps the most conclusive evidence to date is the unique sequence profiles found in cloned ribosomal RNA genes extracted from marine snow communities compared to 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 suggested little or no differences between free-living and particle-associated bacteria (Hollibaugh et al., in prep). This is consistent with our finding from this study that free-living and particle associated bacteria had no statistical differences in growth rates 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. Perhaps this conclusion is not surprising given that 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, most covarying significantly with freshwater flux. We observed a sharp temporal decline in particle-associated bacteria 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 related to increasing salinity, and ß-D-glucosidase was negatively related to increasing salinity; a pattern which 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 was no statistical difference in per-cell amino acid incorporation rates, suggesting no measurable difference in intrinsic growth in these two micro-environments. On the other hand, per-cell ectoenzyme rates suggest that particle-

associated bacteria were statistically much more active than free-living bacteria. However, the contribution from particle-bound, non-bacterial enzymes may cause some of this difference. Overall, in San Francisco Bay, particles are clearly important sites for attachment and growth of bacteria, but do not necessarily harbor faster growing bacteria compared to free-living bacteria.

SUMMARY

This research was motivated by a desire to better understand the role of bacterioplankton in the northern San Francisco Bay ecosystem. Examples of pertinent questions include how abundant they are, how fast they grow, where they grow, what they use as substrates for growth, and whether they are a significant resource for higher trophic levels. Answers to these questions help reveal the underlying environmental controls on their abundance and growth. These are topics of ongoing interest and significance to ecologists because they are fundamental to the functioning of aquatic systems.

The central conclusions from this study were that a large proportion of bacterial abundance and activity was particle associated, that this proportion underwent a consistent shift late in the summer, and that bacterial activity declined over the season. The seasonal changes in bacterioplankton strongly correlated with the seasonal decline in freshwater flow, suggesting that bacterioplankton production may be limited by the supply of organic matter from freshwater sources.

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Table 1. Hydrographic conditions at the three sampling stations during the study period. Variables include temperature (°C), salinity (PSU), chlorophyll (μg l^{-1}), suspended particulate matter (mg l^{-1}), particulate organic carbon (μM), and dissolved organic carbon (μM).

Location	Date	Stn	Temp	Sal	SPM	Chl	POC	DOC
Central Bay	3-Apr	18	12.7	27.5	7.8	7.1	nd	133
	1-May	18	12.4	29.8	8.5	2.9	40.2	52.1
	12-June	18	12.5	30.3	16	3.8	64.2	74.4
	17-July	18	16.9	29.2	13	2.4	44.6	83.6
	13-Aug	18	15.5	31.6	9.1	2.7	nd	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	nd	263
	1-May	6	18.6	2.9	18	4.3	64.4	155
	12-June	6	21.1	1.8	55	1.9	116	138
	17-July	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
Sacto. R.	3-Apr	649	14.2	0	18	2.1	nd	2196
	1-May	649	18.4	0	19	2.9	38.1	127
	12-June	657	21.0	0	12	2.1	49.8	116
	17-July	657	21.6	0	21	1.7	98.9	172
	13-Aug	657	23.3	0	10	1.7	49.6	125
	11-Sep	657	20.4	0	20	1.7	80.8	137
	16-Oct	657	18.6	0	11	2.1	65.5	143

Table 2. Pearson product-moment correlations among log-transformed metabolic measures. Statistical significance is noted at the $P < 0.05$ (*) and $P < 0.01$ (**) level.

	L-proline	L-serine	Amino-peptidase	ß-D-glucosidase
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 **

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

	Salinity	Temp	SPM	Chl	POC	DOC	Flux
L-leucine, <1 µm (n=18)	0.36	0.01	-0.56 *	0.41	-0.22	-0.55 *	-0.47
L-leucine, >1 µm	0.11	-0.23	0.31	0.45	0.10	0.08	0.30
L-leucine, whole water	0.33	-0.39	-0.30	0.60 **	-0.13	0.01	0.14
L-proline, <1 µm (n=9)	0.16	0.25	0.11	0.00	0.23	0.18	0.29
L-proline, >1 µm	0.07	0.29	0.52	-0.20	0.36	0.37	0.23
L-proline, whole water	0.30	0.10	0.30	0.01	0.23	0.15	0.05
L-serine, <1 µm (n=9)	-0.15	0.42	0.43	-0.23	0.19	0.50	0.55 *
L-serine, >1 µm	-0.52	0.74 *	0.76 *	-0.69 *	-0.03	0.75 *	0.72 *
L-serine, whole water	-0.13	0.37	0.59	-0.28	0.17	0.54	0.45
Amino-peptidase, <1 µm (n=21)	-0.15	-0.02	-0.14	0.37	-0.01	-0.04	0.34
Amino-peptidase, >1 µm [a]	0.15	-0.54 *	-0.03	0.54 *	-0.01	0.17	0.49 *
Amino-peptidase, whole water	0.03	-0.40	-0.06	0.52 *	-0.09	0.13	0.51 *
ß-D-glucosidase, <1 µm (n=15)	-0.15	0.11	-0.48	0.33	-0.22	-0.18	0.40
ß-D-glucosidase, >1 µm [a]	-0.63 *	0.48	0.40	-0.28	0.02	0.50	0.72 **
ß-D-glucosidase, whole water	-0.54 *	0.40	0.22	-0.11	0.09	0.46	0.69 **
Bacterial abundance, <1 µm (n=21)	0.40	0.05	-0.46 *	0.08	-0.05	-0.55 **	-0.61 **
Bacterial abundance, >1 µm [a]	-0.49 *	0.20	0.52 *	-0.17	0.05	0.46 *	0.66 **
Bacterial abundance, whole water	-0.17	0.38	0.13	-0.14	0.00	-0.09	0.13

* $p < 0.05$, ** $p < 0.01$

[a] Microbial measures used for correlation calculated by difference between whole water and < 1 µm fractions.

Table 4. Cell-specific bacterial metabolic measures, 10^{-20} mol cell^{-1} h^{-1}, except amino-peptidase, 10^{-18} mol cell^{-1} h^{-1}

Location	Date	L-leucine		L-proline		L-serine		Am-pep		ß-D-gluc	
		<1 µm	*Tot*	*<1 µm*	*Tot*	*<1 µm*	*Tot*	*<1 µm*	*Tot*	*<1 µm*	*Tot*
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**
Sacto. 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**

Table 5. ANCOVA results of log-transformed cell-specific metabolic measures testing effects of fractionation, station location and the flow index covariable.

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

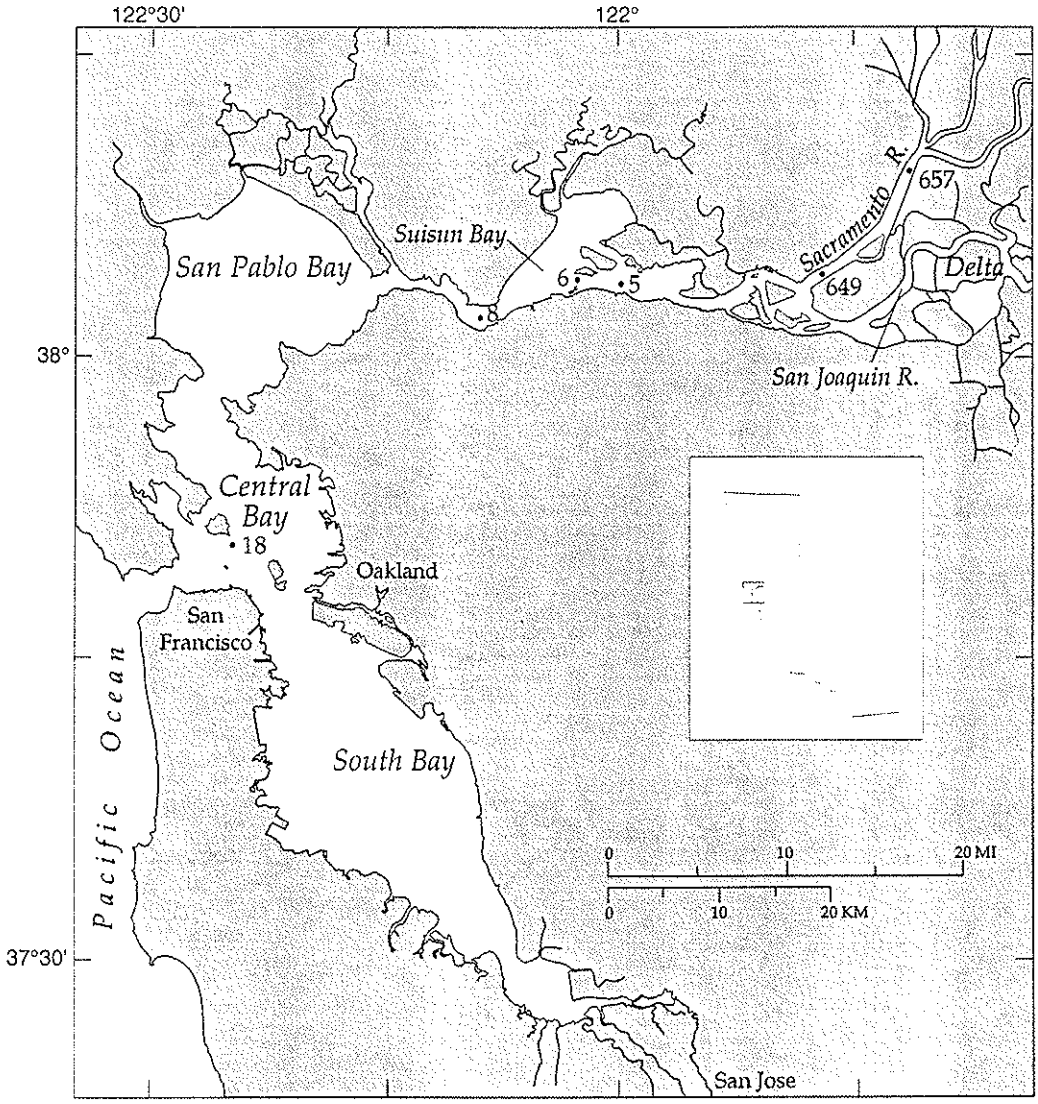

Figure 1. Map of northern San Francisco Bay showing sampling locations.

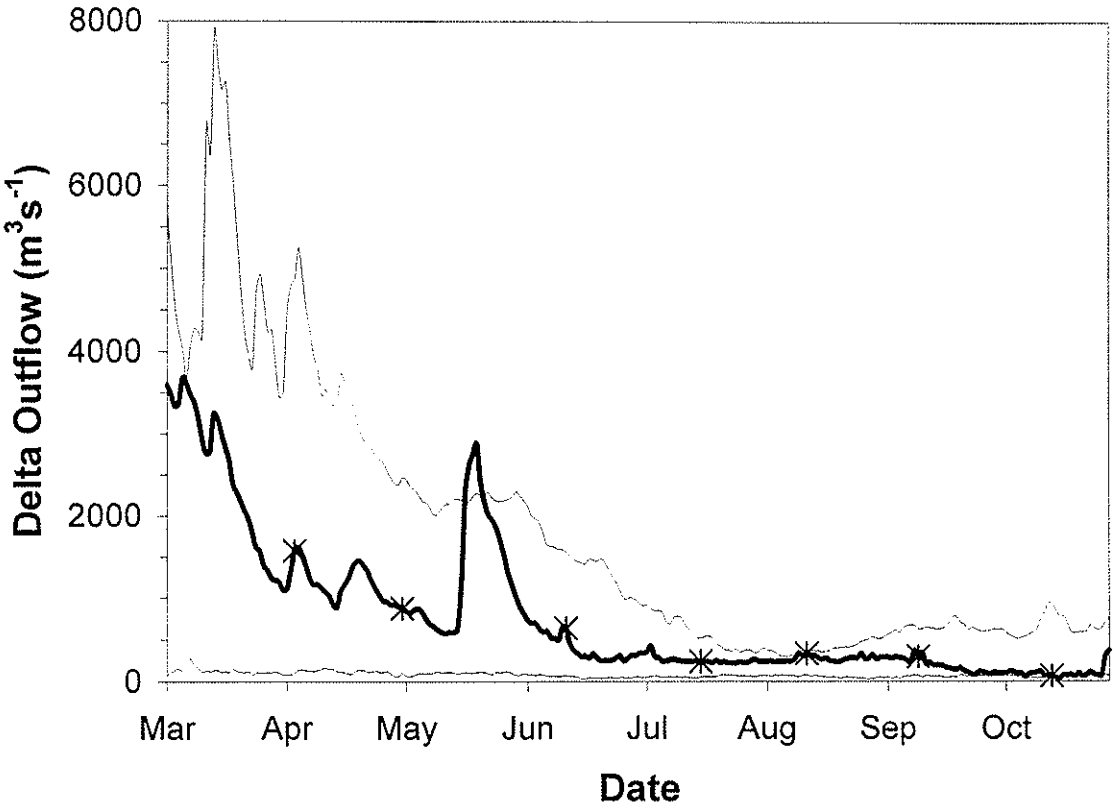

Figure 2. Delta outflow from the San Joaquin and Sacramento Rivers during the 1996 study period (dark line) compared to the 95th percentile confidence intervals of the 40 year average (light lines). Sampling dates denoted by the asterisks. Flow estimated by the California Department of Water Resources DAYFLOW algorithm.

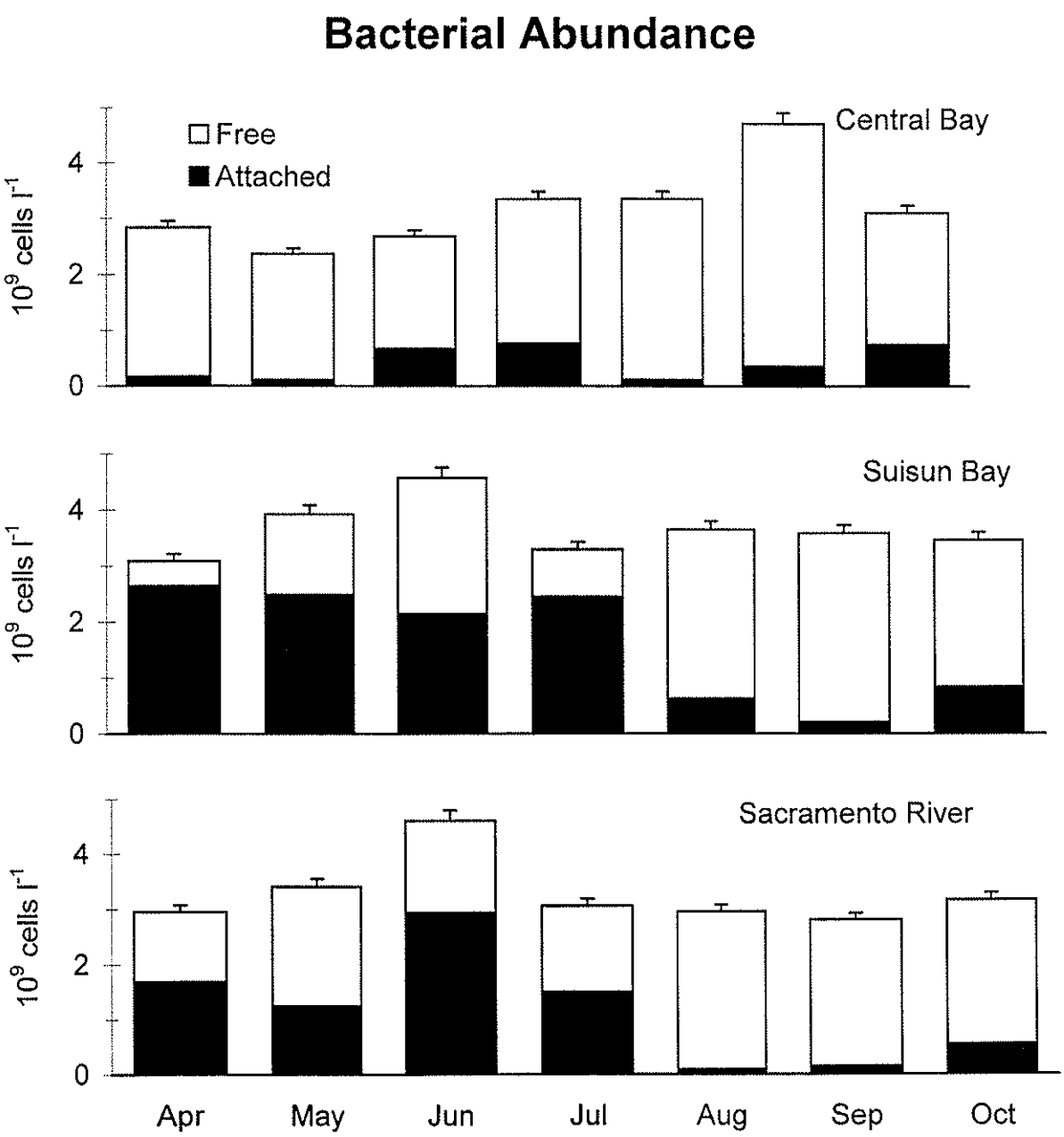

Figure 3. Bacterial abundance in San Francisco Bay from April through October 1996 for Central Bay, Suisun Bay, and Sacramento River stations. Stacked bars represent abundance of particle-associated (solid) and free-living (open) bacteria. Error bars are standard errors of total.

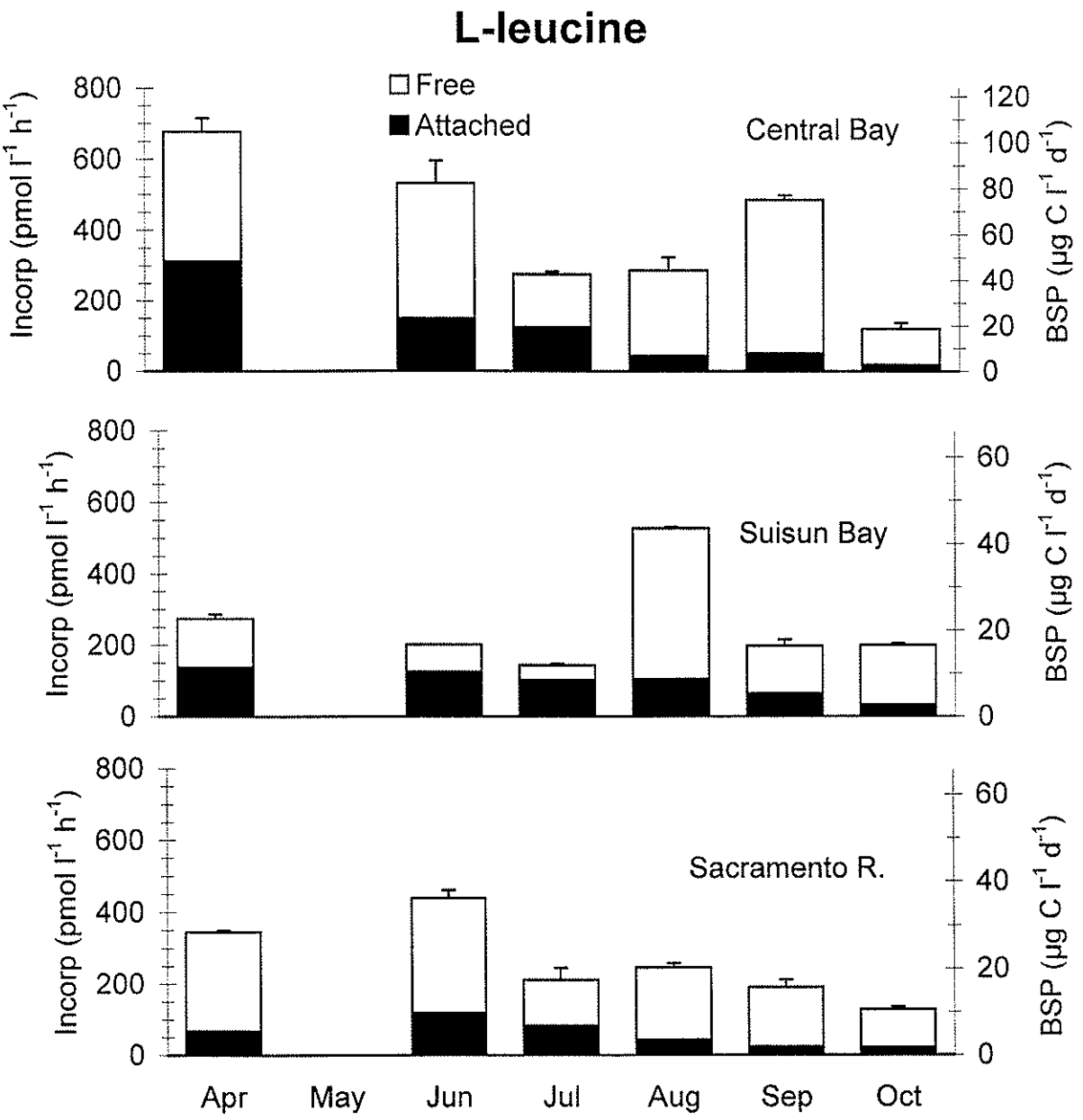

Figure 4. L-leucine incorporation and bacterial secondary production (BSP, right axis). Legend as in Figure 3.

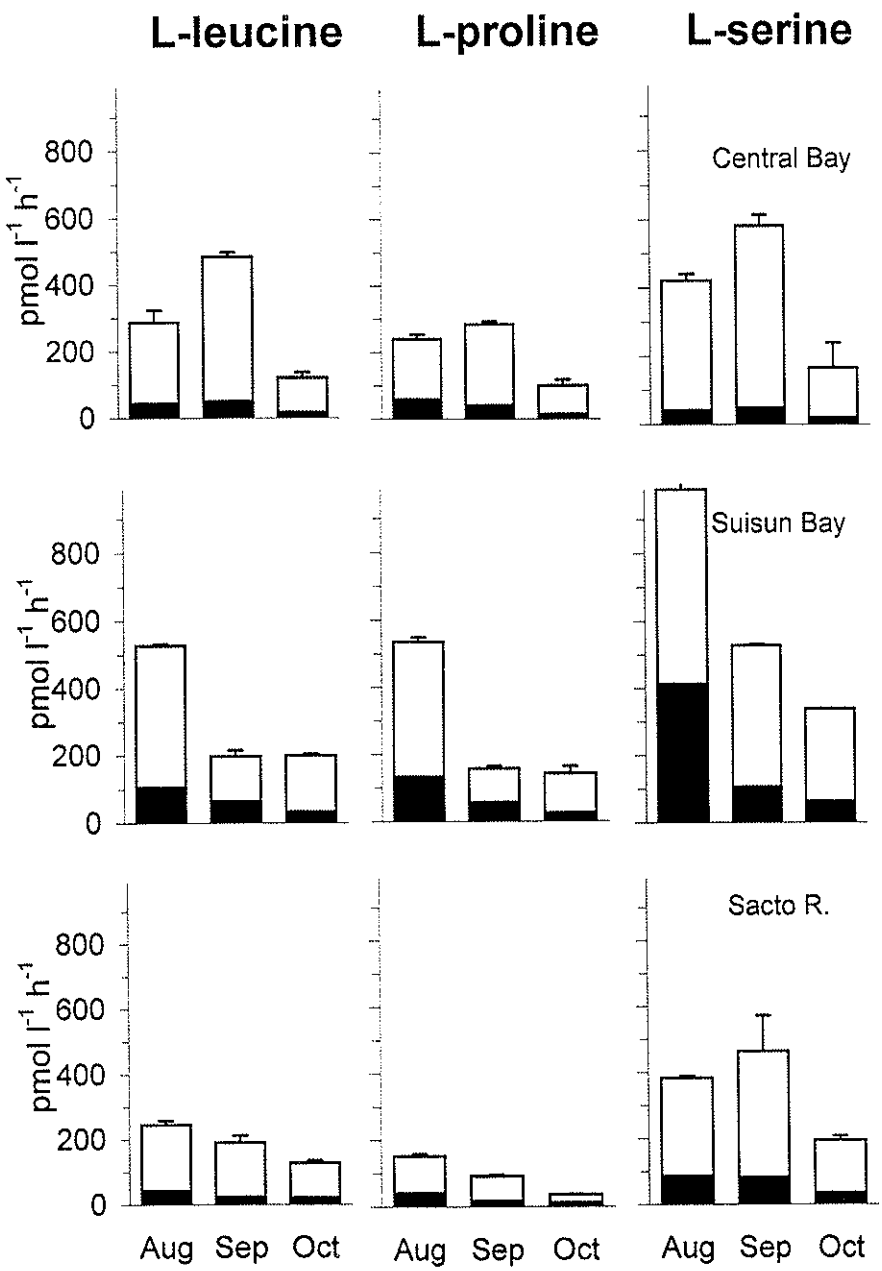

Figure 5. L-leucine, L-proline, and L-serine incorporation. Legend as in Figure 3.

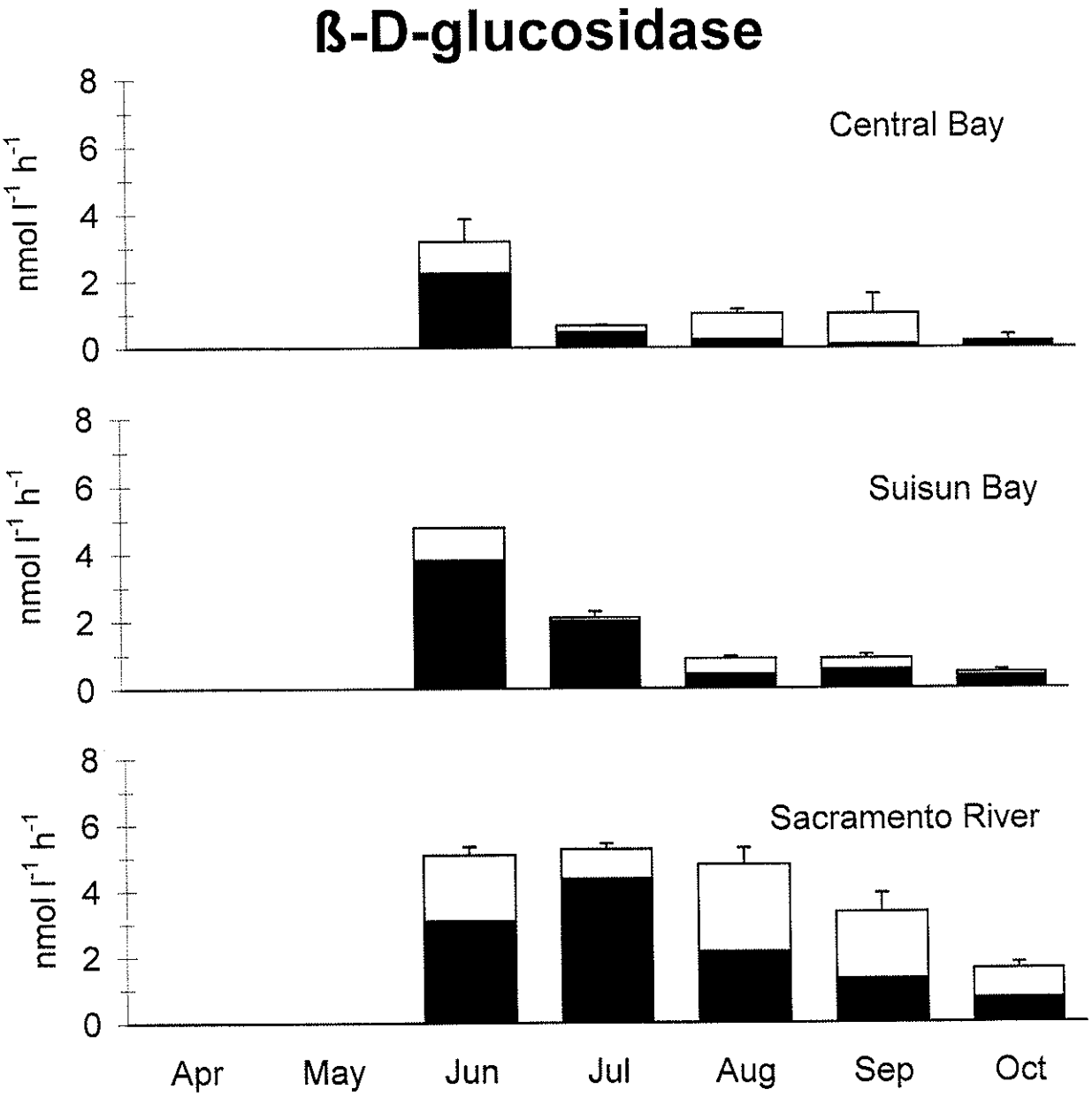

Figure 6. Amino-peptidase activity. Legend as in Figure 3.

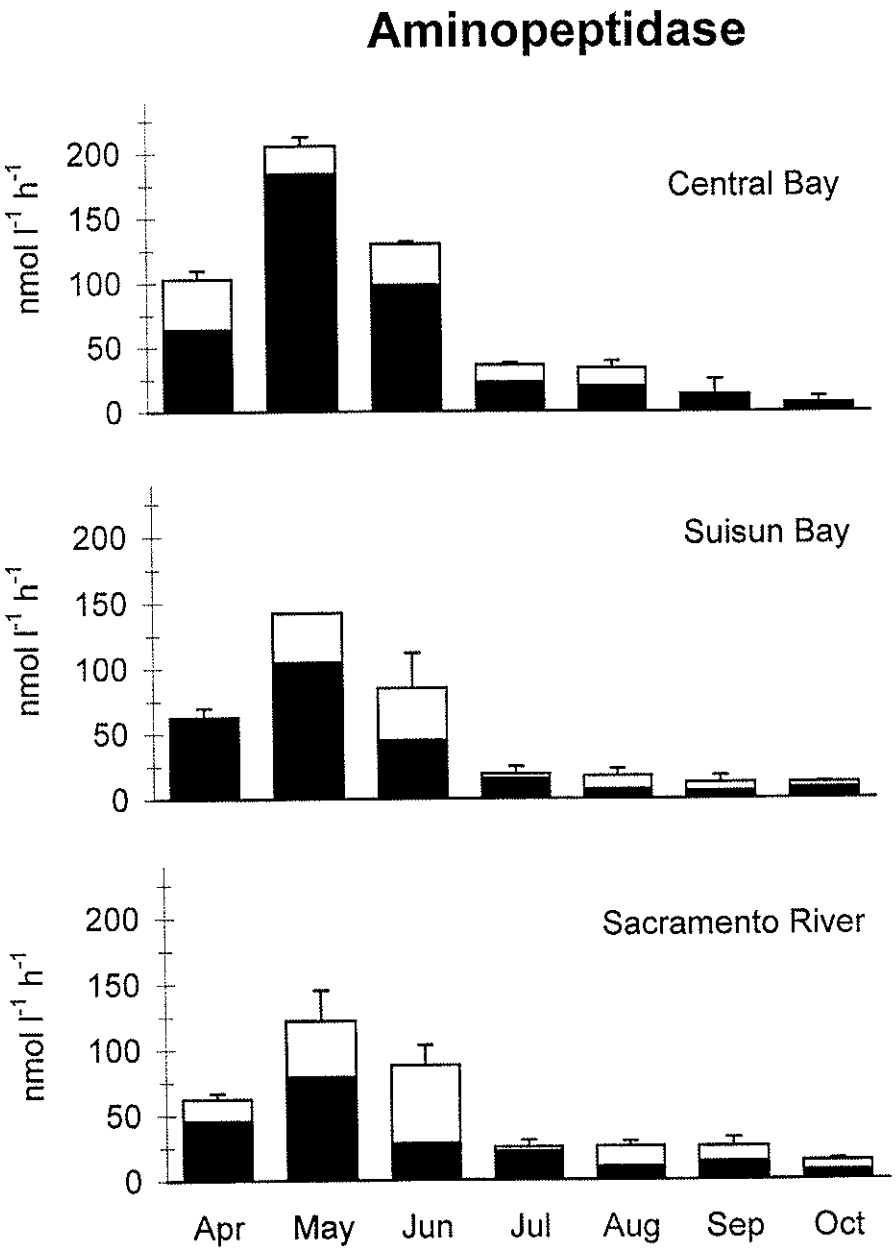

Figure 7. ß-D-glucosidase activity. Legend as in Figure 3.